HANDBOOK OF U.S. COLORANTS

FOODS, DRUGS, COSMETICS, AND MEDICAL DEVICES

DANIEL M. MARMION

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Foods, Drugs, Cosmetics, and Medical Devices

Third Edition

DANIEL M. MARMION

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To my wife and best friend, Annette

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PREFACE TO THE THIRD EDITION

In the seven years since *U.S. Colorants for Foods, Drugs, and Cosmetics* was first revised, a great many important changes have taken place in the field of color additives—several new products have been added to the lists of permitted colorants, a few have been delisted, the identity, status, and permitted areas of use of others have been redefined, specifications for many have been modernized, analytical technology has improved, and some of the old-time manufacturers and suppliers of color additives have either gone out of the business altogether, or have been absorbed by competitors. The purpose of this third edition is to document these changes.

In preparing this revision, the basic structure of the book was kept the same. However, because of the large number of colorants now listed for use in medical devices, the book's name was revised slightly to more accurately reflect its content.

The purpose of the *Handbook of U.S. Colorants: Foods, Drugs, Cosmetics and Medical Devices* is the same as that of the previous

editions; to serve as a manual on color additives that is useful to all who are concerned with them in any way. I am sincerely grateful to everyone who helped me toward this goal.

DANIEL M. MARMION

Orchard Park, New York
July 1991

PREFACE TO THE FIRST EDITION

Because of their widespread use and economic importance and the frequent controversies centered around them, much has been written about the colorants used in foods, drugs, and cosmetics. Unfortunately, what has been written is widely distributed throughout the literature. What follows is an attempt to gather together as much of this information as possible. Hopefully, this collection will serve as a manual for those who manufacture colorants, regulate their use, incorporate them into their products, study their effects, or consume the myriad of articles in which they are found. No such manual exists now.

The colorants considered here are, for the most part, only those now in use in the United States. A small number of recently delisted colorants are discussed, either because they were delisted after this work was published or because it was felt that they might still exist in products on the market and could still be of some interest. A few others not used in the United States are considered in certain analytical discussions, because their similarity to U.S. colorants might make the procedures adaptable to American products.

This handbook is divided into three parts. *Part A* provides a general background of color additives and includes information on their history and regulation, lists of currently permitted colorants, their description, properties, areas of use, specifications, and other items of interest. *Part B* deals with colorant analysis. The treatment is extensive, because the purity requirements imposed on color additives have generated a vast number of procedures. Most are given in detail; however, a few of the less important ones are summarized in the bibliographies following the various sections. Topics covered include identification, strength, moisture, metals, insolubles, inorganic salts, and colored as well as colorless impurities. *Part C,* including the resolution of mixtures and the analysis of commercial products, is somewhat of a potpourri designed to give the reader enough of a background to be able to deal with the nearly infinite number of possible situations with which he or she might be confronted.

Throughout this work, the nomenclature is what is commonly employed in connection with color additives. Although many of the terms may appear unorthodox, they are from the jargon of the industry and will be familiar to people working in the field.

DANIEL M. MARMION

Buffalo, New York
January 1979

HANDBOOK OF U.S. COLORANTS

PART A

HISTORY, REGULATION, DESCRIPTION, AND USE

1

HISTORY: COLORANTS IN USE TODAY

Color is as common in our environment as the air about us. In fact, it is so prevalent, we are not always aware just how much we rely on it. Color is important as a means of identification, as a method of judging quality, and for its basic esthetic value. Consequently, it is no wonder that for centuries it has played a prominent role in three of the items most important to man—food, medicine, and physical appearance.

History is filled with accounts of the widespread use of color as an additive. Paintings in Egyptian tombs dating as far back as 1500 B.C. depict the making of colored candy. The first-century Roman historian Pliny the Elder tells us that wine was artificially colored four centuries before the birth of Christ. We know, too, that spices and condiments have been colored for at least 500 years, and that paprika, turmeric, and saffron have been used to color foods for centuries. An edict issued in Paris in 1396 that forbade the coloring of butter suggests another early and perhaps improper application of color additives.

The use of colorants in drugs undoubtedly has as long a history, considering that color has been associated with disease and its treatment

since antiquity. Many such practices are documented in Egyptian papyri.

The use of colorants in cosmetics has probably been more widespread and certainly better documented than their use in either foods or drugs. Archeologists have evidence that Egyptian women used green ore of copper as an eye shadow as early as 5000 B.C. Egyptians also used henna to dye their hair, carmine to redden their lips, and kohl (an antimony compound) to blacken their eyebrows, lids, and lashes. Thousands of years ago it was common practice for Indians to tint their faces yellow with saffron and to dye their feet red with henna. In similar times, Chinese women used vegetable extracts to dye their feet, their cheeks, and the tips of their tongues, and men and women in Asia Minor smeared their faces with litmus and marshmallow. It is said that Babylonians shaded their lips with red lipstick in the belief that the color would prevent demons from entering their body through their mouths. The ancient Romans colored their faces with white lead and chalk, and their hair and beards with blue and gold dyes. The Old Testament tells us that Jezabel "painted her face with stibic stone", probably Sb_2S_3 (4 Kgs. 9, 30–31).

Until the middle of the nineteenth century, all the colorants used in foods, drugs, and cosmetics were obtained from natural sources including animals, vegetables, and minerals. Then, in 1856, Sir William Henry Perkin discovered the first synthetic organic dyestuff, mauve, and soon a host of new and different colorants was added to the artist's palette. These new colorants were available in a wider variety of shades, were stronger and more permanent than natural organic dyes and, in the opinion of some, were safer for use in foods than many of the mineral colorants in use at the time.

Europeans began to employ these colorants to tint foods almost immediately, and soon used them to color drugs and cosmetics, too. French wines, for example, were dyed with fuchsine, a triphenylmethane dye, as early as 1860. The United States first legalized the use of synthetic organic dyes in foods by an act of Congress that authorized the addition of coloring matter to butter (August 2, 1886). The second such recognition came some 10 years later when on June 6, 1896, Congress officially recognized coloring matter as a legitimate constituent of cheese. By 1900, Americans were eating a wide variety of artificially colored products, including ketchup, jellies, cordials, butter, cheese, ice cream, candy, sausage, noodles, and wine. The use of artificial colorants in drug and cosmetic products was also on the rise.

The rapid growth in the use of color additives was prompted not just by the availability of these new dyestuffs, but by the numerous changes that were taking place in food technology, as well. The development of cheap food substitutes such as margarine for butter, jellied glucose for jam, soft drinks for fruit juices, etc., created needs for colorants where previously there had been none. The increased use of food preservatives, refrigeration, canning, and large-scale food processing—all of which tended to alter the natural color of food products—demanded that something be done to restore the normal appearance of food products. The national distribution of foods and the year-round availability of some that were once considered seasonal made it desirable to establish a uniform appearance for products whose color was dependent on the time of the year they were produced or the location from which they were obtained. The invention of so many new and varied dyestuffs could not have been more timely.

This lavish use of color additives was soon recognized as a threat to the public's health. Of particular concern was the fact that substances known to be poisonous were often incorporated into foods and that dyes were frequently used to hide poor quality, to add weight or bulk to certain products, and to pass off imitation foods as real.

Some of these practices were dishonest but not inherently dangerous. For example, flour, pastry and pasta were often colored yellow to conceal dirt and to make these products appear to have a higher egg content than they really had; ordinary oranges were injected with red dye to give them the look of blood oranges; green watermelons were similarly treated to make them look ripe; old meat was colored to make it appear fresh; the skins of lemons and oranges were dyed to give them the appearance of coming from a particular part of the country; and jams and jellies were tinted to make them seem to contain more fruit than they really did. In London about 1900, the addition of yellow colorant to skimmed and watered milk was so common that housewives often refused to buy whole, unadulterated milk because they thought it looked unnatural. The frequency with which the color of certain foods were altered with dyestuffs was so great that dye manufacturers began to offer mixtures of colorants with names like "egg substitute", "mustard color", "beer", "pie filling", and "raspberry color".

Other early uses of colorants were criminal and often deadly. In 1820, for example, Fredrick Accum reported the demise of a woman who

frequently ate pickles while at her hairdresser—pickles that had been colored green with copper sulfate. A Manchester tea shop is said to have been found stocked with copper arsenite, lead chromate, and indigo for dyeing used tea leaves for resale. Cheese rinds and cayenne pepper were often colored with red lead (Pb_3O_4), and copper acid orthoarsenite was once found in apple tarts. Before the advent of synthetic organic dyes, candy was generally shaded with a variety of mineral pigments including red lead, lead chromate, vermilion (HgS), and lead carbonate. One survey taken in Boston in 1880 showed that 46% of all candy examined contained one or more mineral pigments, chiefly lead chromate. Perhaps the classic horror story of the time is that of the druggist who in 1860 gave a caterer copper arsenite to use for making a green pudding for a public dinner. Two people died as a result.

As disturbing as the above misuses of colorants were, so too was the fact that often little or no control was exercised over the purity of the colors added to foods, and that often dyes found unsatisfactory for coloring textiles were sometimes deliberately channeled into food products. Abuses like these were the subject of a paper delivered by Dr. E. Ludwig to the International Congress of Medicine in Budapest in August, 1909, in which he discussed the examination of some 200 food samples gathered in a suburb of Vienna in the 1870s. Dr. Ludwig reported that about 90% of the samples were found to contain arsenic, which was traceable to the colorant used to dye them. The particular colorant was an otherwise unsalable mother liquor recovered during the manufacture of magenta; it contained 8% arsenic.

Toward the end of the 1800s, concern began to mount both here and abroad over some of the dangerous and deceptive ways colorants were being used in foods, and so a number of legal steps were taken to right these wrongs. In the late 1890s and early 1900s, for example, several European countries including Austria, Italy, Germany, France, and Belgium wrote laws that either outlawed the use of certain colorants in foods, or limited the colorants that could be used to certain specified dyestuffs. At about the same time, state and municipal governments in the United States moved to control the use of colors in foods, too. New York and North Carolina banned the adition of "injurious colors" to foods. Minnesota and North Carolina forbade the use of all coal-tar dyes in all foods, and North Dakota and Wyoming declared foods and beverages adulterated if they contained aniline or any other coal-tar color. Colorado and

Wisconsin outlawed the use of artificial colors in sausages, South Dakota forbade the coloring of oleomargarine, and Virginia banned the use of colorants in cakes, crackers, candy, and ice cream. Numerous states including Arkansas, California, Connecticut, Iowa, Minnesota, Missouri, New Jersey, New York, Pennsylvania, Tennessee, Wisconsin, and Wyoming prohibited the use of artificial colorants in vinegar. California, Oklahoma, Pennsylvania, Utah, and Wisconsin all wrote laws forbidding the use of colors in milk, and California prohibited their use in cream as well. Some 46 states wrote laws which prohibited the use of poisonous colors in candy.

American food manufacturers moved, too, to stop the misuse of colors in food. One step in this direction was the circular issued on February 1, 1899 by the executive committee of the National Confectioners' Association that enumerated 21 coal-tar colorants which the committee considered to be harmful and therefore unfit for adding to foods.

Eventually, though, it became obvious that the individual efforts of industry and state and local governments were just not enough to curb the misuse of colorants in foods, and that some form of national government control was needed if the public's health was to be protected. The beginning of this control in the United States was probably 1862—the year Congress established the Department of Agriculture (USDA) and its Division of Chemistry, later renamed the Bureau of Chemistry.

In 1883, Dr. Harvey Wiley took charge of the Bureau and set as one of his major goals the protection of the purity of the nation's food supply. Dr. Wiley believed that this protection could only be effectively provided by the Federal Government, and so he began a 20-year effort to convince Congress to pass appropriate legislation. (The first of many such bills was proposed in 1886, but failed to obtain Congressional approval.)

In his quest for safer food, Wiley first turned his attention to the chemicals then used as preservatives. He began a serious study of them in 1902, establishing his famous "hygienic table", a place where a group of young men popularly known as his "poison squad" agreed to eat all their meals under his supervision so that he could systematically study the effects of various preservatives on their digestion and health.

When The Appropriations Act of 1900 for the Department of Agriculture provided the Bureau with funds to investigate the relationship of color additives to health and to establish rules for controlling their addition to foods, Wiley focused his attention on them. A result of the

Bureau's investigation was a series of Food Inspection Decisions (FIDs) issued by the Secretary of Agriculture. One (FID 4[3c], published August 6, 1904) declared a food as adulterated "if it be colored, powdered or polished with intent to deceive or to make the article appear of better quality than it really is". Another exempted fabricated confections from this adulteration proviso, except when the candy contained a colorant that might lead the consumer to believe that a naturally colored ingredient was present, when in fact it was not. This regulation made it necessary, for example, to declare the presence of such substances as imitation chocolate on the product's label (FID 29, September 27, 1905). Food Inspection Decision 39 (May 1, 1906) took the first real step toward controlling the use of unsafe colorants in foods by stopping the importation of macaroni colored with Martius Yellow.

The publicity that Wiley's "poison squad" created, the release in 1904 of Bureau of Chemistry Bulletin 84 describing the "squad's" findings, the FIDs issued from 1904 to 1906 regarding food colors, the public outrage produced by the publication in 1906 of Upton Sinclair's novel *The Jungle* (an expose of the Chicago meat packing industry), and other events of the time finally induced Congress to draft and pass the Food and Drug Act of 1906 and encouraged President Roosevelt to sign it into law on June 30th.

The Food and Drug Act of 1906, popularly known as the "Wiley Act" because of Dr. Wiley's many efforts to obtain its passage, really only banned the addition of "poisonous" colors to confectionery products and prohibited the addition of colorants to foods for the purpose of concealing inferiority. Wiley, though, saw the Act as a mandate for the Bureau to generally control the practice of adding colors to food, and set out to do just that. Since neither he nor his staff knew much about the dyes that were being used in foods at the time, he sought help outside the Bureau, and in the summer of 1906 hired Dr. Bernhard C. Hesse as a consultant. Hesse, born in East Saginaw, Michigan in 1869, had degrees in both pharmacy and chemistry, and was an expert on the German dye industry.

His principal assignment with the Bureau was to determine what colorants, if any, could be safely added to food, and what restrictions should be placed on their use. It was not his job to concern himself with either the ethics of using color additives or the dietetic aspects regarding their use.

Hesse began his work by examining the chemistry and the physiology of the 695 colorants then known to be in use throughout the world for coloring foods, and by reviewing the laws of numerous countries and states that controlled their use. He found that these colors were manufactured by 37 different firms, that very few of the colorants had ever been tested for their effects on health, and that most of the testing that had been conducted was meaningless because either the true chemical identities of the colorants that were tested were unknown, or because the procedures used for testing them were naive.

Hesse learned, too, that of the 80 food colorants sold in 1907 in the United States, 30 of them had never been tested and therefore their safety was simply unknown, 26 had been tested but the results were contradictory, eight were considered to be harmful by most experts, and the remaining 16 were deemed to be more or less harmless.

After much study, Hesse and the Bureau concluded that coal-tar dyes should not be used in foods indiscriminately. They also concluded that only specific colorants that were proved to be safe should be permitted in foods, that this list of permitted colorants should be kept as short as possible, and that each batch of colorant intended for use in food should be tested and certified as pure before allowing its use.

Armed with these principles, and taking into consideration recommendations such as those of the National Confectioners' Association, the current needs of industry, and suggestions by German and American dye manufacturers, Dr. Hesse proposed the following seven colorants for use in food products in the United States (FID 76, July 13, 1907):

Original Name	*Current Name*
Amaranth	*
Ponceau 3R	*
Orange I	*
Erythrosine	FD&C Red No. 3
Naphthol Yellow S	Ext. D&C Yellow No. 7
Light Green SF Yellowish	*
Indigo Disulfo Acid, Sodium Salt	FD&C Blue No. 2

*No longer permitted in foods, drugs or cosmetics in the United States.

Food Inspection Decision 77, issued September 25, 1907 established procedures for certification of these colorants, and made it clear that each batch of dye had to be certified separately.

With legislation now in place for regulating colors, Hesse, Wiley, and the Bureau turned their attention to the problem of developing an adequate supply of the recommended colorants, pure enough for use in food. A survey of the dyes commercially available at the time showed most to be unacceptable, many containing as much if not more impurity or impurities than main component. So a call went out to industry to provide better products. Response was swift. A few respondents continued to preach that any and all colorants should be permitted in foods, and that it was up to the Bureau to prove individual colorants unsafe before forbidding their use. Some agreed with the concept of limiting the colorants allowed in food to a specific few, but balked at the seven chosen, claiming that they did not meet industry's needs, since they could not be used to produce all the shades required by the food industry, and since none of them were suitable for coloring fats and oils. Most argued that it was technically impossible to produce colorants of the purity proposed and that it was unnecessary for food colors to be that pure anyway, because most were used at such low levels their purity was irrelevant. One firm though, H. Kohnstamm and Co. of New York City, took up the challenge and began to work with the Bureau to produce colorants with the highest purity possible.

Kohnstamm's approach was to buy the best colorants they could obtain from Germany, purify them, test the resultant products as thoroughly as possible, then submit samples to the Bureau for evaluation. By November, 1907, Hesse had a sample of Light Green SF Yellowish from Kohnstamm that he felt he could approve for use in food, and so, on April 1, 1908, it became the first official batch of United States certified food color. By mid-summer 1908, Kohnstamm had submitted samples of all seven colorants which Hesse believed to be of acceptable quality. On January 26, 1909, Kohnstamm announced to the trade that it was ready to accept orders for commercial quantities of all seven colorants.

In May, 1908, the firm of Schoellkopf, Hartford and Hanna of Buffalo, New York began to manufacture specially purified lots of the seven colorants for certification and sale through its National Aniline Company. Schoellkopf, who at that time was the largest and most successful manufacturer of coal-tar colors in the United States, had an advantage over

Kohnstamm since they, unlike Kohnstamm, made their own dyes and thus could control colorant purity by starting with the finest raw materials available and using the best process conditions then known for making the dyes. By 1909, Schoellkopf and Kohnstamm were in direct competition in the food color business. In August, 1909, Kohnstamm published its first price list for certified colors, offering them as mixtures with Kohnstamm trade names. In September of that same year, Schoellkopf began advertising the seven individual colorants for sale under their technical names.

In November, 1909, the Board of Food and Drug Inspection, which at that time handled all questions regarding the regulation and enforcement of the Food and Drug law, ruled that the Bureau of Chemistry had to prove that a colorant was unsafe before it could forbid its use in foods. In effect, the Board rejected Hesse's and Wiley's bid for mandatory certification, angering both men as well as H. Kohnstamm and Co., who had all worked so hard and long to develop the seven colorants recommended by the Bureau in the belief that they and only they would be permitted in food. Food Inspection Decision 117 issued May 3, 1910, somewhat softened this blow by officially recommending the use of certified colors, using language that led many to believe that certification of colors additives was mandatory, and led most to believe that certification was to their advantage. FID 117, aggressive advertising by both Kohnstamm and Schoellkopf, plus new regulations issued by Illinois, Iowa, Idaho, and Nevada mandating the use of only certified colorants within their states, produced dramatic improvement in the sale of certified colors. Before long, the Board had received enough requests from jobbers who wished to repack already certified colorants under their own labels that it issued FID 129 (November 21, 1910) authorizing repacking and outlining a procedure to be followed for recertification.

In 1910, Hesse completed an official report of his work for the Bureau. This report was issued on February 10, 1912 as "U.S. Department of Agriculture, Bureau of Chemistry—Bulletin No. 147. Coal-Tar Colors Used in Food Products". With his work now completed, Hesse resigned his government post effective June 1, 1911. On March 15, 1912, Wiley, too, resigned, thus ending an era in the history of the Bureau, and in food color legislation.

Soon after Hesse published his list of seven colorants, pleas began to pour in to expand it. Some of the requests were based on reasonable

arguments. The National Association of Manufacturers of Soda Water Flavors, for example, proposed adding Tartrazine to the list because they believed that Naphthol Yellow S did not produce a satisfactory fast color in acidulated fruit syrups such as lemonade, and because they were convinced that Naphthol Yellow S imparted a bitter taste to these products. There were numerous complaints, too, that Light Green SF Yellowish was unavailable (because of World War I) and that it was not stable to light anyway, and that Indigo Disulfonic Acid, too, was unstable to light, acids, and air. Margarine manufacturers pushed to have oil-soluble colors added to the list since none of the permitted colorants dissolved in oily foods and since the trade in general did not like to use the natural oil-soluble colorant, annatto.

In response to the appeals of the trade, testing was begun in 1915 to find dyes that were both safe and suitable for coloring oil-based foods. As a result of this testing, Sudan I, Butter Yellow, Yellow OB, and Yellow AB were added to the list of permitted colorants in 1918 (FID 175, April 23). However, when the majority of the workers at the T. Willard Ready Co. of Chicago who packaged Sudan I and Butter Yellow for distribution to margarine manufacturers came down with severe contact dermatitis from handling the colorants, both were promptly delisted (FID 180, June 7, 1919).

By the early 1920s, several new firms, including Warner-Jenkinson Co. of St. Louis, Wm. Stange and Co. of Chicago, and Bates Chemical Co. of Lansdowne, Pa., joined in the manufacture of certified colors. At about the same time, the Bureau began seizing shipments of color additives on the grounds that they were not on the "list", or that they contained excessive amounts of diluents, arsenic, or other colorants, or that they were presented as certified colorants when in fact they were not. Prosecution on these grounds proved difficult because of the voluntary nature of the certification program and because of the earlier ruling by the Board of Food and Drug Inspection that the Bureau had to prove that colorants were dangerous before it could legally remove them from the marketplace.

During the 1920s, the number of colorants on the "list" continued to grow. At National Aniline's suggestion, Guinea Green B was added on July 10, 1922 (FID 184). On April 9, 1927, Fast Green FCF was added too (FID 207). Fast Green FCF was unique in that it was the first colorant extensively studied for safety at the direction and expense of the firm that

wanted it listed, Warner-Jenkinson Co. In 1929, Brilliant Blue FCF was added at the request of National Aniline, and Ponceau SX and Sunset Yellow were added at the request of Warner-Jenkinson Co. Also during the 20s, two more states passed legislation requiring that only certified colorants be used in food products sold in their states—North Dakota in 1923, and Minnesota in 1925.

In 1927, the responsibility for policing the Food and Drug Act of 1906 was taken away from the Bureau of Chemistry and given to the newly formed Food, Drug and Insecticide Administration which, in 1930, was renamed the Food and Drug Administration, commonly called the FDA. (The FDA continues to enforce the laws pertaining to color additives; however, control of the FDA was transferred from the U.S. Department of Agriculture to the Federal Security Agency (FSA) in 1940. The FSA became the Department of Health, Education, and Welfare in 1953, then the Department of Health and Human Services in 1980).

In 1933, the many shortcomings of the 1906 Act prompted the FDA to recommend to President Franklin D. Roosevelt that the Act be completely revised. This launched a five-year legislative battle which culminated in the writing of the Food, Drug and Cosmetic Act of 1938, which was signed into law on June 25th of that same year.

This new law resulted in a number of important changes for color additives. It made their certification mandatory, made it clear that this certification had to be on a batch-wise basis, and that the entire cost of the certification program had to be borne by industry. It provided for the addition of any coal-tar colorant to the list of permitted colorants, as long as the colorant proved harmless and suitable for use in foods. The 1938 Act branded a food as adulterated if it contained anything but a duly certified colorant, allowed manufacturers to add harmless diluents to colorants, and extended the FDA's control to the coloring of drugs and cosmetics, too. The Act also instituted specific, formal procedures for developing regulations for color additives, and for dealing with controversial issues pertaining to them. Under the 1938 Act, the Secretary of Agriculture, at his or her own initiative, or in response to a request from a substantial portion of industry, could, with at least thirty days notice, schedule a public hearing to deal with matters specified in the notice. If any new rule or order resulted from the hearing, the rule or order could not take effect in less than ninety days from its publication, unless the FDA could prove that an emergency existed that required immediate

action. Parties affected by any proposed new regulations were allowed to file for exception to them, and if their request for exception was denied, they were allowed to appeal to the Federal Court of Appeals for the circuit in which they resided. Thus, a system for judicial review of new regulations was established.

An announcement for the first such hearing was issued on January 4, 1939, with the hearing to take place on February 6th. At the hearing, the FDA proposed that the fifteen colors certifiable under the 1906 Act be listed for use under the new law, and recommended that two new colors be added to this list—Orange SS, and the dipotassium salt of Naphthol Yellow S. The FDA also proposed a new systematic method for naming certified colorants. The reason for the new nomenclature was to clearly differentiate between a certified colorant and a technical-grade colorant with the same chemical structure but a lower level of purity.

All seventeen colorants that the FDA recommended for use in foods were listed. The result of the FDA's proposal for the development of new nomenclature for colorants was the creation of three categories of coal-tar dyes, and a method for naming each. The three categories created were:

1. FD&C Colorants: Those certifiable for use in coloring foods, drugs, and cosmetics, in general.
2. D&C Colorants: Dyes and pigments considered safe in drugs and cosmetics ingested or used in direct contact with mucous membranes.
3. Ext. D&C Colorants: Those colorants that, because of their oral toxicity, were not certifiable for use in products intended for ingestion, but were considered safe for use in products externally applied.

Based on the new nomenclature, a red colorant intended for use in foods, drugs, and cosmetics, for example, would be called FD&C Red No. 1. The "FD&C" indicating its intended use, the "Red" designating its hue, and the "No. 1" distinguishing it from other red colorants that could be used for the same purposes.

Also at this first hearing, details were worked out regarding the labeling of certified colorants and the records that would have to be kept. Diluents that could be added to colorants were identified, and procedures for requesting certification were also established.

On September 14, 1939, Oil Red XO was added to the list of permitted colorants as FD&C Red No. 32. At about the same time, the FDA resumed

pharmacological retesting of food colorants on the grounds that many of them had not been tested since the turn of the century, and the standards of pharmacology had changed greatly since then. The new tests undertaken were far more sophisticated than any that had been conducted before.

During the forties, the war effort and new Federal regulations that requried the FDA to batch-certify insulin and penicillin seriously taxed the FDA's limited resources, forcing it to terminate its retesting program, and to generally turn its attention away from colorants. Although new evidence surfaced at that time that cast doubts on the safety of FD&C Yellow Nos. 3 and 4 and FD&C Green No. 2, the FDA did not take this data seriously and did little to investigate these matters further. Meanwhile, the sales of certified colors grew steadily from 2.2 to 4.5 millions pounds per year, FD&C Violet No. 1 was added to the list of colorants permitted in foods (January, 1950), and all seemed well with color additives. Then events occurred that changed the picture dramatically.

In the fall of 1950, FDA field offices in both Pittsburgh and Kansas City received complaints that a number of children had developed abdominal pains and diarrhea after eating orange-colored "Trick or Treat" candy that had been manufactured by the Sweet Candy Company of Salt Lake City, Utah. Samples of the suspect candy were promptly gathered up and sent to Washington for evaluation, and when volunteers there ate some of the candy and experienced the same illnesses the children complained of, FDA officials consulted with the manufacturer in an attempt to determine the exact cause of the problem. After a thorough investigation which eliminated the more common explanations for such illnesses such as employees of the manufacturer having contagious diseases, or the candy being bacteriologically contaminated, the FDA focused its attention on the composition of the candy and found that it contained an abnormally high amount of colorant—1.2–1.8% FD&C Orange No. 1. The Orange No. 1 was from a properly certified lot, and the amount that the candy contained was legal, but the level of colorant was unusually high and, in the FDA's opinion, could explain why the children and FDA volunteers who ate the candy became ill.

When volunteers became similarly sick after consuming small amounts of the colorant itself, the FDA's concern increased substantially since FD&C Orange No. 1 was one of the three food colorants most

widely used at that time. The FDA immediately ordered a review of all pharmacological data then on file pertaining to Orange No. 1, but its pharmacologists soon found that the data available was insufficient to prove whether the colorant was safe or toxic. Although the FDA did not consider the candy incident serious enough to ban the use of Orange No. 1, the Administration did order a complete retesting of all FD&C colorants, and directed that the study start with Orange No. 1.

Testing began on FD&C Orange No. 1 and FD&C Yellow No. 6 in June, 1951, and on FD&C Red Nos. 2 and 4 and FD&C Yellow No. 5 later that same year. The new tests were far more thorough than any previously undertaken and were based on more rigorous standards of pharmacology. The new studies included two-year chronic oral toxicity experiments on rats similar to those performed in the 1930s, but this time as much as 2% of the total diet fed to the animals was colorant rather than the 0.1% fed during the tests performed earlier.

In the case of FD&C Orange No. 1, additional experiments were conducted using dogs fed colorant at levels of 0.2% and 1.0% of their total diet. In 1952, a subcutaneous injection study with rats was inaugurated in an effort to determine Orange No. 1's carcinogenicity, and in 1953 another study with dogs was added to ascertain the colorant's cathartic properties.

While all this testing was going on, another event was taking place that would also have long-range effects on color additives—the hearings of the House Select Committee to Investigate the Use of Chemicals in Food Products. The chairman of the Committee was James Delaney of New York, and the Committee eventually became known as the "Delaney Committee". The hearings, which took place between 1950 and 1953, soon focused the nation's attention on the numerous chemicals then being added to foods, including colorants, and the adverse publicity that these hearings created eventually raised the public's concern regarding the safety of the country's food supply. The Halloween Candy incident served to add to the consumer's anxiety over color additives in particular.

During the hearings, several eminent scientists testified that there was a possibility that some food colorants might be carcinogens, and that little testing had been done to prove whether or not this was true. Partly because of this testimony, the FDA began to consider the carcinogenicity of colorants by looking for tumors in the animals being used

for the Orange No. 1 and Red No. 32 studies, and by adding the above-mentioned subcutaneous injection test to their study of FD&C Orange No. 1.

In early 1953, results of the new round of animal testing began to trickle in. The findings indicated that FD&C Yellow Nos. 5 and 6 were reasonably safe, but the news for FD&C Red No. 32 and FD&C Orange No. 1 was not good. In the case of FD&C Orange No. 1, nearly every animal fed higher levels of colorant showed some sort of adverse effect ranging from simple loss in weight to death. The results of the study confirmed that Orange No. 1 was an acute toxin, and clearly indicated that the colorant had significant potential for causing chronic injury. The results for FD&C Red No. 32 were even worse, since pharmacologists were unable to find any no-effect level in the preliminary sub-acute feeding study that they could use as a guide to establish the dosage levels to use in the chronic tests. Chronic tests with rats were undertaken anyway, using feeding levels of 0.25% and 0.1% of the animals' diet. All 48 rats fed at the 0.25% level died within 20 weeks; 16 of the 24 animals fed at the 0.1% level died within 26 weeks. All of the control animals were alive and healthy at the end of the year. In a separate study with dogs, animals fed only 0.04% of their diet as colorant lost half their weight in 124 to 148 days.

Since 1938, some in the Administration had interpreted the Food, Drug and Cosmetic Act to mean that a colorant could be certified as long as it could be shown to be safe under conditions of normal use; the colorant did not have to be harmless per se. Most in the FDA, though, believed that the Act really meant that if tests indicated that a color additive could cause harm in man or animal under any conditions or at any level, its use in food should be forbidden. Because the majority within the FDA subscribed to this latter way of thinking, the Administration set out to delist both FD&C Orange No. 1 and FD&C Red No. 32. Removing Red No. 32 from the market did not seem to pose a great problem since little of it was sold anyway, and since its only real use was in coloring the skins of oranges grown in Texas and Florida. Delisting Orange No. 1, though, would be a much more serious step because thousands of pounds of it were used each year to color numerous products including soft drinks, candy, baked goods, and processed meats.

In response to the FDA's move to delist these colorants, industry cried "foul", charging the Administration with being too harsh in its inter-

pretation of the law on the grounds that the colorants could probably be used safely at some level and that, therefore, the FDA need only limit the amount of these colorants used in foods, not delist them altogether. The FDA countered that it had no authority to set limits on the amount of colorants that could be used in foods, and that even if it did, setting limits would require the FDA to be continually performing many difficult and time-consuming analyses in order to ensure that these levels were not exceeded, a task that the FDA was very reluctant to undertake.

Because of the growing pressure on color additives, those who manufactured them banded together in 1953 and formed the Certified Color Industry Committee (CCIC). The major concern of the Committee at the time was not really the delisting of FD&C Red No. 32, or even FD&C Orange No. 1, but the belief that delisting these colors for the reasons given would set a precedent that would eventually lead to the delisting of all color additives since, in the opinion of the CCIC, if someone looked long and hard enough, a test could likely be found that would show every color to be unsafe under at least some condition. Future events would prove that the Committee's fears were well founded.

In late 1953, an announcement appeared in the *Federal Register* that a hearing would take place on January 19, 1954 to consider the delisting of FD&C Red No. 32 and FD&C Orange No. 1 as well as FD&C Orange No. 2, which the FDA now considered unsafe for use in food products, too. After the hearing, little happened until December 30, 1954 when another FDA notice appeared in the *Federal Register* recommending that the three colors in question be delisted for use in foods, and relisted as Ext. D&C colorants. The CCIC and the Florida and Texas orange growers (the users of Red 32) immediately began to fight this recommendation but, in spite of their efforts, an order removing the three colors from the FD&C list and placing them on the Ext. D&C list appeared in the *Federal Register* on November 16, 1955. The order was to take effect in 90 days.

The delisting of FD&C Red No. 32 would create a serious problem for Florida and Texas orange producers because they believed that they needed to color the skin of their fruit in order for it to appear acceptable to the public, and Red 32 was the only colorant available at the time with which to do the job. In an effort to save Red No. 32 from delisting, the Florida Citrus Exchange lobbied the government on behalf of the farmers, and eventually a compromise bill was written which allowed for the

continued certification and use of Red No. 32 for coloring orange skins for three more years on the assumption that a suitable replacement could be found within that period of time.

Meanwhile, the Certified Color Industry Committee challenged the delisting of all three colorants in court, again attacking the "harmless per se" principle and again arguing that the FDA should be forced to set limits on the use of colorants and not allowed to delist them altogether.

In the midst of the controversy over Red No. 32 and Orange Nos. 1 and 2, the CCIC's worst fears came true when the FDA announced on September 18, 1956 that it now had data that indicated that FD&C Yellow Nos. 1, 2, 3, and 4 were not totally harmless and that these colorants would have to be delisted, too. Yellows 3 and 4 were the only oil-soluble colors left for coloring margarine, butter, and other fatty foods, so a new crisis was at hand. Naturally, the CCIC objected to these proposed delistings, and again took to the courts to prevent them.

The threat of losing FD&C Yellow Nos. 3 and 4 was partially offset by the recent availability of a new food colorant, synthetic β-carotene. Hoffmann LaRoche, Inc., who had developed the process for making the product, advertised it as a natural colorant that was naturally safe—a colorant that did not need to be regulated by the FDA since it was not a coal-tar dye. The FDA countered that although β-carotene did indeed exist in nature, this was no guarantee that its factory-produced analogue was safe since it could contain impurities that might need monitoring. The FDA argued further that other synthetic colorants such as indigo and alizarine also existed in nature but still required certification, so synthetic β-carotene should require certification too. The CCIC openly sided with the FDA on this issue since they feared that any colorant that did not require certification would have an unfair advantage over their products which did.

Continued argument over the β-carotene issue pointed to yet another weakness in the existing law. By this time, it was obvious to all that new regulations for color additives were sorely needed. The problem now was that the FDA and industry each viewed the need with a different sense of urgency, and each wanted any new legislation slanted in their favor.

The Food and Drug Administration craved a law that was more realistic and that allowed it some latitude in enforcing it. The FDA did not want new rules that would saddle it with additional time-consuming responsibilities that could be difficult to perform (such as policing the

level of colorants in foods), nor did it want any changes in the law that might jeopardize the public's health. The FDA was willing to work toward the development of new regulations, but was in no great hurry to see them in place.

Because of the recent delistings and the threat of more to come, most manufacturers and users of color additives believed that if the existing law was not revised as quickly as possible their businesses would die, so they wanted immediate action. They particularly wanted new rules that would relist colors previously delisted on the grounds that most would never have been delisted if the FDA had had the authority to set limits on their use in the first place.

On January 30, 1957, the CCIC took the first significant step towards new legislation by submitting a draft bill to the FDA for its consideration. The FDA was not satisfied with the draft, mainly because it retained the term coal-tar colorant, and because it failed to provide the FDA with guidelines for setting limits on the use of colorants considered to be of limited safety.

Based on the FDA's objections, the CCIC revised its bill and had the revision submitted to the House of Representatives on July 30, 1957 as HR 8945. The revision proved more acceptable to the FDA, but the Administration still refused to support its passage because it did not address the issue of who was responsible for testing colorants for safety, and because it contained a grandfather clause that required the FDA to continue to list current colors at levels and for uses then extant, until it could be proved that the colorants were harmful as used.

From this point on, things began to go increasingly badly for the color additive industry. On December 15, 1958 the Supreme Court settled the issue regarding FD&C Red No. 32 and FD&C Orange Nos. 1 and 2 once and for all by ruling unanimously that these colorants must be delisted because, in the Court's opinion, the existing law indeed did not allow the FDA to set limits on the use of nonharmless food colorants.

On February 6, 1959 a final order appeared in the *Federal Register* delisting FD&C Yellow Nos. 1, 2, 3, and 4 as of May 6th.

The next major blow came on April 15, 1959 when the FDA announced that it intended to delist seventeen D&C colorants including nearly every colorant then important in the formulation of lipstick. This move brought the cosmetic industry and their trade organization, the Toilet Goods Association (TGA) into the argument on the side of the CCIC.

In June of 1959, the Department of Health, Education and Welfare (HEW), not wishing to be burdened with any industry-sponsored legislation, submitted its own proposal to Congress (House of Representatives, HR 7624; Senate, S 2197). After much haggling, mostly about the need for a clause that addressed the potential carcinogenicity of colorants, Congress sent a compromise version of HEW's proposal to President Eisenhower, who signed it into law on July 12, 1960 (Public Law 86–618). This legislation was to become popularly known as the Color Additives Amendments of 1960.

Basically, the Amendments provided a much-needed breathing spell. For one thing, they allowed for the continued use of existing color additives pending the completion of investigations needed to ascertain their suitability for listing as permanent colorants. Equally as important, they authorized the Secretary of Health, Education, and Welfare to establish limits of use for colorants, thus eliminating the controversial harmless-per-se interpretation formerly employed. A special provision, commonly known as the Delaney clause, specifically directed the Secretary not to list a color additive for any use if that colorant could be shown to induce cancer in humans or animals. Another feature of the Color Additives Amendments gave the FDA jurisdiction over all color additives by eliminating any distinction under the law between coal-tar colors and any other color additives, regardless of their source or method of manufacture. The Amendments addressed the issue of the need to certify colorants such as β-carotene by empowering the Secretary to decide which colors must be certified and which could be exempted from certification, based on their relationship to public health. Unfortunately for the CCIC, the 1960 Amendments did not relist previously delisted colorants as it so desperately wanted.

Under provisions of the new law, the producers and consumers of color additives were obliged to provide any and all scientific data and analytical methodology needed to obtain permanent listing of a color additive. Because of the expense involved, testing was started on only those colors that were of economic importance and, consequently, many previously certifiable colors were eventually delisted by default. The deadline or closing date for providing the required data has been extended several times by the Secretary, using powers granted to him by the Amendments. Most colorants are now permanently listed; those that are not continue to be listed provisionally.

Since passage of the Amendments, little of any great significance has happened in the field of color additives. A few new colors have been added to the lists of permitted colorants, a few old standbys have been delisted, and there has been more emphasis placed on the use of colorants derived from natural products such as beets and grapes. The Certified Color Industry Committee (CCIC) has been replaced by the Certified Color Manufacturers Association (CCMA), a few old-time manufacturers of colorants have gone out of the business, but there really have been no new crises to equal those of the 1950s.

Probably, the only major change in the law since 1960 came with the passage of the Medical Device Amendments of 1976 (Public Law 94–295), which created a fourth category of color additive by mandating the separate listing of colorants for use in medical devices if the color additive in them comes in direct contact with the body for a significant period of time. This has resulted in the listing of several new colorants, particularly for use in tinting contact lenses.

Colorants currently in use and their status are shown in Tables 1.1–1.4. These lists are accurate as of June 1, 1991 but are subject to change at any time by both addition and deletion. A chronological history of synthetic certifiable food colors is given in Table 1.5. Diluents permitted in colorants used in foods and drugs are shown in Tables 1.6 and 1.7.

The regulations regarding color additives can be found in Title 21 of *The Code of Federal Regulations,* Parts 70 to 82. Changes in these regulations are published in the *Federal Register.** Additional information as to what colorants can be used and the regulations pertaining to them can be obtained from the Food and Drug Administration, Division of Colors and Cosmetics, 200 C St., S.W., Washington, D.C. 20204.

**The Code of Federal Regulations* is usually revised yearly. The *Federal Register* is published daily, Monday through Friday, except on official holidays. Both documents are published by the Office of the Federal Register, National Archives and Records Administration, Washington, D.C. 20408, and both are distributed through the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

TABLE 1.1 Colorants permitted in foods

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
	Subject to Certification	
FD&C Blue No. 1	42090	
FD&C Blue No. 2	73015	
FD&C Green No. 3	42053	
FD&C Red No. 3	45430	
FD&C Red No. 40	16035	
FD&C Yellow No. 5	19140	
FD&C Yellow No. 6	15985	
Citrus Red No. 2	12156	Skins of oranges that are not intended or used for processing only; 2.0 ppm max., based on the weight of the whole fruit
Orange B	19235	Sausage and frankfurter casings or surfaces only; 150 ppm max., based on the weight of the finished product
	Exempt from Certification	
Annatto extract	75120	
β-Apo-8'-carotenal	40820	Maximum—15 mg/lb of solid or semisolid food, or pint of liquid food
Canthaxanthin	40850	Maximum—30 mg/lb of solid or semisolid food, or pint of liquid food
Caramel		
β-Carotene	75130 (Natural) 40800 (Synthetic)	
Carrot oil		
Cochineal extract; Carmine	75470	
Corn endosperm oil		Chicken feed only
Dehydrated beets (beet powder)		
Dried algae meal		Chicken feed only
Ferrous gluconate		Ripe olives only
Fruit juice		
Grape color extract		Nonbeverage food only
Grape skin extract		Beverages only
Paprika		
Paprika oleoresin		
Riboflavin		

(continued)

TABLE 1.1 *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Saffron	75100	
Synthetic iron oxide	77491 77492 77499	Dog and cat food only; 0.25% (w/w) max.
Tagetes meal and extract	75125	Chicken feed only
Titanium dioxide	77891	1% (w/w) Maximum in finished food
Toasted partially defatted cooked cottonseed flour		
Turmeric	75300	
Turmeric oleoresin	75300	
Ultramarine blue	77007	Salt for animal feed only; 0.5% (w/w) max.
Vegetable juice		

[a]No color additive or product containing one can be used in the area of the eye, in surgical sutures, or in injections, unless so stated. Also, no colorant can be used to color foods for which standards of identity have been promulgated under Section 401 of the Federal Food, Drug and Cosmetic Act, unless the use of added color is authorized by the standard. Colorants without restrictions can be used for coloring foods generally, in amounts consistent with good manufacturing practice.

TABLE 1.2 Colorants permitted in drugs

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
	Subject to Certification	
FD&C Blue No. 1	42090	
FD&C Blue No. 2	73015	Nylon sutures for use in general surgery only; 1% (w/w) max. Ingested drugs only
FD&C Green No. 3	42053	
FD&C Red No. 3	45430	Ingested drugs only. (Listed provisionally for other uses).
FD&C Red No. 4	14700	Externally applied drugs only
FD&C Red No. 40	16035	
FD&C Yellow No. 5	19140	
FD&C Yellow No. 6	15985	

TABLE 1.2 ***(Continued)***

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
D&C Blue No. 4	42090	Externally applied drugs only
D&C Blue No. 9	69825	Cotton and silk sutures (including those for ophthalmic use) only; 2.5% (w/w) max. Drugs in general.
D&C Green No. 5	61570	Nylon 66 and Nylon 6 nonabsorbable surgical sutures only; 0.6% (w/w) max.
D&C Green No. 6	61565	Externally applied drugs only
D&C Green No. 8	59040	Externally applied drugs only; 0.01% (w/w) max.
D&C Orange No. 4	15510	Externally applied drugs only
D&C Orange No. 5	45370:1	Ingested mouthwashes and dentifrices only. Externally applied drugs only; 5 mg/daily dose of drug max.
D&C Orange No. 10	45425:1	Externally applied drugs only
D&C Orange No. 11	45425	Externally applied drugs only
D&C Red No. 6	15850	Drugs in general. Combined total of D&C Red No. 6 and D&C Red No. 7 not more than 5 mg/daily dose of drug
D&C Red No. 7	15850:1	Same as D&C Red No. 6
D&C Red No. 17	26100	Externally applied drugs only
D&C Red No. 21	45380:2	
D&C Red No. 22	45380	
D&C Red No. 27	45410:1	
D&C Red No. 28	45410	
D&C Red No. 30	73360	
D&C Red No. 31	15800:1	Externally applied drugs only
D&C Red No. 33	17200	Ingested drugs, other than mouthwashes and dentifrices; 0.75 mg/daily dose of drug max. Externally applied drugs , mouthwashes and dentifrices
D&C Red No. 34	15880:1	Externally applied drugs only
D&C Red No. 36	12085	Ingested drugs, other than mouthwashes and dentifrices; 1.7 mg/daily dose of drug max. if taken continuously for less than one year; 1.0 mg/daily dose of drug max. if taken continuously for longer than one year. Externally applied drugs

(continued)

TABLE 1.2 Colorants permitted in drugs *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
D&C Red No. 39	13058	Externally applied quarternary ammonium germicides only; 0.1% (w/w) max.
D&C Violet No. 2	60725	Externally applied drugs only
D&C Yellow No. 7	45350:1	Externally applied drugs only
D&C Yellow No. 8	45350	Externally applied drugs only
D&C Yellow No. 10	47005	
D&C Yellow No. 11	47000	Externally applied drugs only
Ext. D&C Yellow No. 7	10316	Externally applied drugs only
	Exempt from Certification	
Alumina	77002	
Aluminum powder	77000	Externally applied drugs only (1)
Annatto extract	75120	(1)
Bismuth oxychloride	77163	Externally applied drugs only (1)
Bronze powder	77440	Externally applied drugs only (1)
Calcium carbonate	77220	
Canthaxanthin	40850	Ingested drugs only
Caramel		
β-Carotene	75130 (Natural) 40800 (Synthetic)	(1)
Chromium-cobalt-aluminum oxide		Polyethylene sutures for use in general surgery only; 2% (w/w) max.
Chromium hydroxide green	77289	Externally applied drugs only (1)
Chromium oxide greens	77288	Externally applied drugs only (1)
Cochineal extract; Carmine	75470	
Copper powder	77400	Externally applied drugs only (1)
Dihydroxyacetone		Externally applied drugs intended solely or in part for imparting color to the human body only
Ferric ammonium citrate		With pyrogallol in plain and chromic catgut sutures for use in general and ophthalmic surgery only; 3% (w/w) max. total citrate–pyrogallol complex
Ferric ammonium ferrocyanide		Externally applied drugs only (1)
Ferric ferrocyanide	77510 77520	Externally applied drugs only (1)

TABLE 1.2 *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Guanine	75170	Externally applied drugs only (1)
Logwood extract	75290	Nylon 66, Nylon 6, and silk nonabsorbable sutures for use in general and ophthalmic surgery only; 1.0% (w/w) max.
Mica	77019	Dentifrices and externally applied drugs only (1)
Potassium sodium copper chlorophyllin	75810	Dentifrices only; 0.1% max.
Pyrogallol	76515	With ferric ammonium citrate in plain and chromic catgut sutures for use in general and ophthalmic surgery only; 3% (w/w) max. total citrate–pyrogallol complex
Pyrophyllite		Externally applied drugs only
Synthetic iron oxide	77491 77492 77499	5 mg/day (as Fe) in drugs that are ingested
Talc	77019	
Titanium dioxide	77891	(1)
Zinc oxide	77947	Externally applied drugs only (1)

[a]No color additive or product containing one can be used in the area of the eye, in surgical sutures or in injections unless so stated. Colorants without restrictions can be used for coloring drugs generally, in amounts consistent with good manufacturing practice.

(1)May also be used in those drugs intended for use in the area of the eye.

TABLE 1.3 Colorants permitted in cosmetics

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
	Subject to Certification	
FD&C Blue No. 1	42090	
FD&C Green No. 3	42053	
FD&C Red No. 4	14700	External use only
FD&C Red No. 40	16035	

(continued)

TABLE 1.3 Colorants permitted in cosmetics *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
FD&C Yellow No. 5	19140	
FD&C Yellow No. 6	15985	
D&C Blue No. 4	42090	External use only
D&C Brown No. 1	20170	External use only
D&C Green No. 5	61570	
D&C Green No. 6	61565	External use only
D&C Green No. 8	59040	External use only; 0.01% (w/w) max.
D&C Orange No. 4	15510	External use only
D&C Orange No. 5	45370:1	5.0% (w/w) max. in lipstick and other lip cosmetics; ingested mouthwashes and dentifrices; externally applied cosmetics
D&C Orange No. 10	45425:1	External use only
D&C Orange No. 11	45425	External use only
D&C Red No. 6	15850	
D&C Red No. 7	15850:1	
D&C Red No. 17	26100	External use only
D&C Red No. 21	45380:2	
D&C Red No. 22	45380	
D&C Red No. 27	45410:1	
D&C Red No. 28	45410	
D&C Red No. 30	73360	
D&C Red No. 31	15800:1	External use only
D&C Red No. 33	17200	Cosmetic lip products only; 3% (w/w) max. Mouthwashes, dentifrices and externally applied cosmetics only
D&C Red No. 34	15880:1	External use only
D&C Red No. 36	12085	Cosmetic lip products only; 3% (w/w) max. Externally applied cosmetics only
D&C Violet No. 2	60725	External use only
D&C Yellow No. 7	45350:1	External use only
D&C Yellow No. 8	45350	External use only
D&C Yellow No. 10	47005	
D&C Yellow No. 11	47000	External use only
Ext. D&C Violet No. 2	60730	External use only
Ext. D&C Yellow No. 7	10316	External use only
	Exempt from Certification	
Aluminum powder	77000	External use only (1)
Annatto	75120	(1)

TABLE 1.3 ***(Continued)***

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Bismuth citrate		Hair dyes for scalp only; 0.5% (w/v) max.
Bismuth oxychloride	77163	(1)
Bronze powder	77440	(1)
Caramel		(1)
Carmine	75470	(1)
β-Carotene	75130 (Natural) 40800 (Synthetic)	(1)
Chromium hydroxide green	77289	External use only (1)
Chromium oxide greens	77288	External use only (1)
Copper powder	77400	(1)
Dihydroxyacetone		Externally applied cosmetics intended solely or in part for imparting color to the human body only
Disodium EDTA-copper		Shampoos only
Ferric ammonium ferrocyanide		External use only (1)
Ferric ferrocyanide	77510 77520	External use only (1)
Guaiazulene		External use only
Guanine	75170	(1)
Henna	75480	Hair dyes only, not near eye
Lead acetate		Hair dyes for scalp only; 0.6% (w/v) max. as Pb
Manganese violet	77742	(1)
Mica	77019	(1)
Potassium sodium copper chlorophyllin	75810	Dentifrices only; 0.1% max. (2)
Pyrophyllite		External use only
Silver		Fingernail polish only; 1% max.
Synthetic iron oxides	77491 77492 77499	(1)
Titanium dioxide	77891	(1)
Ultramarine blue	77007	External use only (1)

(continued)

TABLE 1.3 Colorants permitted in cosmetics *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Ultramarines (green)	77013	External use only (1)
(pink)	77007	
(red)	77007	
(violet)	77007	
Zinc oxide	77947	(1)

[a]No color additive or product containing one can be used in the area of the eye, in surgical sutures or injections unless so stated. Colorants without restrictions can be used for coloring cosmetics generally, in amounts consistent with good manufacturing practice.

(1)May also be used in cosmetics intended for use in the area of the eye.

(2) Can only be used in combination with certain substances.

TABLE 1.4 Colorants permitted in medical devices

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
	Subject to Certification	
[Phthalocyaninato(2-)] copper	74160	Polypropylene sutures, polybutester nonabsorbable sutures for use in general and ophthalmic surgery, polybutylene terephthalate monofilament nonabsorbable sutures for general and ophthalmic surgery, and polymethylmethacrylate monofilament used as supporting haptics for intraocular lenses; 0.5% (w/w) max. Contact lenses
D&C Blue No. 6	73000	Polyethylene terephthalate sutures for general surgical use, 0.2% (w/w) max.; plain or chromic collagen absorbable sutures for general surgical use, 0.25% (w/w) max.; plain or chromic collagen absorbable sutures for ophthalmic surgical use, 0.5% (w/w) max.; polypropylene sutures for general

TABLE 1.4 ***(Continued)***

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
		surgical use, 0.5% (w/w) max.; polydioxanone synthetic absorbable sutures for ophthalmic and general surgical use, 0.5% (w/w) max.
D&C Green No. 6	61565	Polyethylene terephthalate sutures for general and ophthalmic surgery, 0.75% (w/w) max.; polyglycolic acid sutures for general and ophthalmic surgery with diameter greater than U.S.P. size 8–0, 0.1% (w/w) max.; polyglycolic acid sutures for general and ophthalmic surgery with diameter not greater than U.S.P. size 8–0, 0.5% (w/w) max.; poly(glycolic acid-co-trimethylene carbonate) sutures for general surgery, 0.21% (w/w) max.
		Contact lenses, 0.03% (w/w) max.
D&C Red No. 17	26100	Contact lenses only
D&C Violet No. 2	60725	Polyglactin 910 (glycolic-lactic acid polyester) synthetic absorbable sutures for general and ophthalmic surgery, 0.2% (w/w) max.; polydioxanone synthetic absorbable sutures for use in general and ophthalmic surgery, 0.3% (w/w) max.; polymethylmethacrylate intraocular lens haptics, 0.2% (w/w) max.
		Contact lenses
D&C Yellow No. 10	47005	Contact lenses only
	Exempt from Certification	
1,4-Bis[4-(2-methacryl-oxyethyl) phenyl-amino] anthraquinone		Contact lenses only
1,4-Bis[(2-methylphenyl)-amino]-9,10-anthracenedione		Contact lenses only

(continued)

TABLE 1.4 Colorants permitted in medical devices *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Carbazole violet	51319	Contact lenses only
Chlorophyllin-copper complex, oil soluble		Polymethylmethacrylate bone cement only; 0.003% (w/w) max.
Chromium-cobalt-aluminum oxide	77343	Contact lenses only
Chromium oxide greens	77288	Contact lenses only
C.I. Vat Orange 1	59105	Contact lenses only
7,16-Dichloro-6,15-dihydro-5,9,14,18-anthrazinetetrone	69825	Contact lenses only
2-[[2,5-Diethoxy-4-[(4-methylphenyl)-thiol]phenyl]azo]-1,3,5-benzenetriol		To mark soft (hydrophilic) contact lenses with the letters R and L only; 1.1×10^{-7} g/lens max.
16,23-Dihydrodinaphtho-[2,3-a:2',3'-i]naphth [2',3':6,7]indolo [2,3-c]carbozole-5,10,15,17,22,24,-hexone	70800	Contact lenses only
N,N'-(9,10-Dihydro-9,10-dioxo-1,5-anthracene-diyl)bisbenzamide	61725	Contact lenses only
16,17-Dimethoxydi-naphtho [1,2,3-cd:3',2',1'-1m]perylene-5,10-dione	59825	Contact lenses only
4-[(2,4-Dimethylphenyl)-azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol- 3-one		Contact lenses only
6-Ethoxy-2-(6-ethoxy-3-oxobenzo[b]thien-2(3H)-ylidene)benzo-[b]thiophen-3(2H)-one	73335	Contact lenses only
Iron oxides	77491	Contact lenses only
Phthalocyanine green	74260	Contact lenses only

TABLE 1.4 *(Continued)*

Food and Drug Administration Official Name	Colour Index Number	Uses and Restrictions[a]
Poly(hydroxyethyl methacrylate)-dye copolymers		Contact lenses only
Titanium dioxide	77891	Contact lenses only

[a]Colorants can only be used in those Medical Devices cited. Colorants without specific restrictions regarding quantity can be used in amounts not to exceed the minimum reasonably required to accomplish the intended coloring effect.

TABLE 1.5 Chronological history of synthetic food colors in the United States.

Year Listed for Food Use	Common Name	FDA Name	Colour Index Number	Year Delisted	Currently Permitted in Food
1907	Ponceau 3R	FD&C Red No. 1	16155	1961	No
1907	Amaranth	FD&C Red No. 2	16185	1976	No
1907	Erythrosine	FD&C Red No. 3	45430	—	Yes
1907	Orange I	FD&C Orange No. 1	14600	1956	No
1907	Naphthol Yellow S	FD&C Yellow No. 1	10316	1959	No
1907	Light Green SF Yellowish	FD&C Green No. 2	42095	1966	No
1907	Indigotine	FD&C Blue No. 2	73015	—	Yes
1916	Tartrazine	FD&C Yellow No. 5	19140	—	Yes
1918	Sudan I	—	12055	1919	No
1918	Butter Yellow	—		1919	No
1918	Yellow AB	FD&C Yellow No. 3	11380	1959	No
1918	Yellow OB	FD&C Yellow No. 4	11390	1959	No
1922	Guinea Green B	FD&C Green No. 1	42085	1966	No
1927	Fast Green FCF	FD&C Green No. 3	42053	—	Yes
1929	Ponceau SX	FD&C Red No. 4	14700	1976	No
1929	Sunset Yellow FCF	FD&C Yellow No. 6	15985	—	Yes
1929	Brilliant Blue FCF	FD&C Blue No. 1	42090	—	Yes

(continued)

TABLE 1.5 ***(Continued)***

Year Listed for Food Use	Common Name	FDA Name	Colour Index Number	Year Delisted	Currently Permitted in Food
1939	Naphthol Yellow S potassium salt	FD&C Yellow No. 2	10316	1959	No
1939	Orange SS	FD&C Orange No. 2	12100	1956	No
1939	Oil Red XO	FD&C Red No. 32	12140	1956	No
1950	Benzyl Violet 4B	FD&C Violet No. 1	42640	1973	No
1959	Citrus Red No. 2	Citrus Red No. 2	12156	—	Yes
1966	Orange B	Orange B	19235	—	Yes
1971	Allura Red AC	FD&C Red No. 40	16035	—	Yes

TABLE 1.6 Diluents permitted in color additives for food use

Diluent	Restrictions
General Use	
Castor oil	500 ppm max. in the finished food
Dioctylsodium sulfosuccinate	9 ppm max. in the finished food
Inks for Marking Food Supplements in Tablet Form, Gum, and Confectionery	
Castor oil	500 ppm max. in the finished food
Dioctylsodium sulfosuccinate	9 ppm max. in the finished food
Alcohol, SDA-3A	No residue
n-Butyl alcohol	No residue
Cetyl alcohol	No residue
Cyclohexane	No residue
Ethyl cellulose	
Ethylene glycol monoethyl ether	No residue
Isobutyl alcohol	No residue
Isopropyl alcohol	No residue
Polyoxyethylene sorbitan monooleate (polysorbate 80)	
Polyvinyl acetate	
Polyvinylpyrrolidone	
Rosin and rosin derivatives	
Shellac, purified	

TABLE 1.6 *(Continued)*

Diluent	Restrictions
Inks for Marking Fruit and Vegetables	
Castor oil	500 ppm max. in the finished food
Dioctylsodium sulfosuccinate	9 ppm max. in the finished food
Acetone	No residue
Alcohol, SDA-3A	No residue
Benzoin	
Copal, Manila	
Ethyl acetate	No residue
Ethyl cellulose	
Methylene chloride	No residue
Polyvinylpyrrolidone	
Rosin and rosin derivatives	
Silicon dioxide	No more than 2% of the ink solids
Terpene resins, natural	
Terpene resins, synthetic	
For Coloring Shell Eggs	
Castor oil	500 ppm max. in the finished food
Dioctylsodium sulfosuccinate	9 ppm max. in the finished food
Alcohol, denatured, formula 23A	
Damar gum (resin)	
Diethylene glycol distearate	
Ethyl cellulose	
Ethylene glycol distearate	
Japan wax	
Limed rosin	
Naphtha	
Pentaerythritol ester of fumaric acid-rosin adduct	
Polyethylene glycol 6000	
Polyvinyl alcohol	
Rosin and rosin derivatives	
Miscellaneous Special Uses	
Castor oil	500 ppm max. in the finished food
Dioctylsodium sulfosuccinate	9 ppm max. in the finished food
Polyvinylpyrrolidone	In or as food-tablet coatings, 0.1% max. in the finished food

TABLE 1.7 Diluents permitted in color additives for drug use

Diluent	Restrictions
Injested Drugs—General Use	
Castor oil	
Dioctylsodium sulfosuccinate	
Alcohol, specially denatured	
Cetyl alcohol	
Isopropyl alcohol	In color coatings for pharmaceutical forms, no residue
Polyoxyethylene (20) sorbitan monostearate (Polysorbate 60)	
Polyoxyethylene (20) sorbitan tristearate (Polysorbate 65)	
Polysorbate 80	
Polyvinyl-pyrrolidone	
Sorbitan monooleate	
Sorbitan monostearate	
Sorbitan triolease	
Special Use—Inks for Branding Pharmaceutical Forms	
All diluents listed above under: *Injested Drugs—General Use*	
All diluents listed in Table 6 under: *Inks for Marking Food Supplements in Tablet Form, Gum, and Confectionery*	
Ethyl lactate	
Polyoxyethylene sorbitan monolaurate (20)	
Externally Applied Drugs	
All diluents listed above under: *Injested Drugs—General Use*	
Benzyl alcohol	
Ethyl cellulose	
Hydroxyethyl cellulose	
Hydroxypropyl cellulose	

2

AREAS OF USE

With all the problems associated with the manufacture, sale, and use of color additives, it is easy to ask why we bother with them at all. The answer to this question is not simple and is not the same in all cases.

Basically, we add color to products to make them recognizable and pleasing to the consumer so that he or she will buy them. The reason for coloring any particular item depends on a number of factors as explained below.

COLORING FOOD

Most staple foods such as meat, white bread, potatoes and other vegetables, and most fruits are not artificially colored since their natural appearance is perfectly acceptable. Those foods that are, are colored because they have no natural color of their own, because their natural color was destroyed or drastically altered as a result of processing or storage, or because their color varies greatly with the season of the year or their geographic origin. Thus, colorant added to such foods is intended

to make them appear the way the customer wants and expects them to appear.

What consumers want in the color of a food depends in turn on a variety of things, including their cultural background, their past experiences, their desire for color coordination, esthetic appeal, local customs, fads, and so on. For such reasons, we have green beer on St. Patrick's day, orange and black confections on Halloween, and red and green ones at Christmas time. This, too, is why a Texas red hot sold in the South is often colored quite differently than one sold in the North, why Midwesterners prefer butter with a deep yellow color, and why on birthdays the decorations on a boy's cake are blue and those on a girl's are pink.

What consumers want and expect in the color of a food depends too on just how well established the color of that food is and just how closely its color is associated with its quality. Most likely we would not buy black cabbage, green rice, or purple milk, for example, since none of these items meets our standards of identity for these foods. Also, because of color, we see green grapefruit and bananas as immature, we are generally suspicious of anything but the most brilliant red beef, and we shun excessively brown or spotted produce in favor of the brightest, most uniformly colored products available. The colors of some foods, in fact, are so well fixed in our minds that they serve as reference standards when speaking of certain hues—lemon yellow, eggshell white, cherry red, chocolate brown, and pea green, to mention just a few.

Colorless Foods

The major use of color additives in food is in products containing little or no color of their own. These include many liquid and powdered beverages, gelatin desserts, candies, ice creams, sherbets, icings, jams, jellies, and snack foods. Without the addition of color to some of these—gelatin desserts and soft drinks, for example—all flavors of the particular product would be colorless, unidentifiable, and probably unappealing to the consumer.

Process and Storage Difficulties

Often the process used to prepare a food results in the formation of a color in the product, the depth of which depends largely on the time, temperature, pH, air exposure, and other parameters experienced during pro-

cessing. Here again, it is deemed necessary to supplement the color of the product to ensure its uniformity from batch to batch. Items that fall into this category include certain beers, blended whiskies, brown sugars, table syrups, toasted cereals, and baked goods.

The storage of food can also be a problem because natural pigments often deteriorate with time due to exposure to light, heat, air, and moisture or because of interaction of the components of the product with each other or with the packaging material. The color of maraschino cherries, for example, fares so poorly with storage that they are routinely bleached then artificially colored.

Regional and Seasonal Problems

The problems of the dairy and citrus fruit industries are typical of those encountered with products produced in different areas of the country or at different times of the year.

Consider the growing of oranges. In many parts of the United States, the soil and weather conditions are such that chlorophyll continuously forms in the fruit as well as in the leaves of the trees; the result is mature oranges that are substantially greener than the same variety of orange produced in regions of the country with different growing conditions. Florida Valencia oranges, for example, mature in the latter part of March when the weather is favorable to the development of chlorophyll, which is produced in such quantities in the fruit peel that it eventually turns pale and green. In fact, most varieties of Florida oranges tend to be green, suggesting immaturity, even though they contain the proper ratio of solids to acid for fully nutritious, mature fruit.

The necessity of coloring these oranges to make them comparable in appearance and thus as commercially acceptable as naturally orange-colored fruit from other areas of the country was recognized years ago and began on a commercial scale about 1934. Today the peels of those (and only those) oranges not intended for processing continue to be dyed where necessary. The percentage of the total crop colored varies from year to year and depends largely on the weather.

The problems associated with dairy products are even more complex. Approximately 90% of the yellow color in milk is due to the presence of β-carotene, a fat-soluble carotenoid extracted from feed by cows. As is well known, summer milk is more yellow than winter milk. This is largely due, of course, to seasonal feeding practices in which cows

grazing on lush green pastures in the spring and summer months consume much higher levels of carotenoids than do cows barn-fed on hay and grain in the fall and winter. The problem is further complicated since various breeds of cows and even individual animals differ in the efficiency with which they extract β-carotene from feed and in the degree to which they convert it into colorless vitamin A. The differences in the color of milk are more obvious in products made from milk fat, since here the yellow color is concentrated. Thus, unless standardized through the addition of colorant, products like butter and cheese show a wide variation in shade and in many cases appear unsatisfactory to the consumer. In addition to standardizing the color of butter and certain yellow cheeses by the addition of yellow colorants, it is frequently necessary to use various amounts of blue or green colorants when making gorgonzola, nuworld, provolone, blue, and various other cheeses in order to neutralize the yellow of the curd used to prepare them.

Other products whose natural color varies enough to make standardization of their color desirable include the shells of certain kinds of nuts, the skins of red and sweet potatoes, and ripe olives.

Miscellaneous Uses

Inks used by inspectors to stamp the grade or quality on meat must, by law, be made from food-grade colors. Dyes used in packaging materials that come in direct contact with a food must also be food-grade or, if not, it must be established that no part of the colorant used migrates into the food product. Pet foods, too, if colored, must contain only those colorants recognized by the FDA as suitable for the purpose.

COLORING DRUGS

Compared with the food and cosmetic industries, pharmaceuticals are a minor though important consumer of colorants. Originally, dyes were used in drugs to make them more appealing to the consumer by adding color to otherwise colorless products, by masking unsatisfactory natural colors, and by standardizing the appearance of drugs whose color varied from batch to batch as a consequence of the manufacturing process, a difference in the color of the raw materials used, or both. Some drugs, of

course, contain added color for cosmetic purposes, as in the case of the skin-tone dyes added to certain creams and ointments used to treat disorders such as acne.

Although colors are still added to drugs for these purposes, the major use of colorants in pharmaceuticals currently is to provide the manufacturer with a simple means of identifying his products so that they are not inadvertently mixed during production and shipment. Since no industry-wide standards exist for coloring drugs, each manufacturer has been free to develop and use the in-house scheme that best fits his needs. Many such codes have been devised and so today the same product frequently appears on the market under several color forms. (The *Physicians' Desk Reference* contains colored photographs of tablets and capsules as an aid in identifying drugs.)

Coloring Cosmetics

The reasons for using color additives in cosmetics are perhaps more obvious than the reasons for their use in either foods or drugs. Products such as aftershave lotions, hair tonics, and soaps contain additives purely for esthetic reasons. In many cases, though, the colorant is a major, functional part of a cosmetic, often comprising half of its total weight. Some cosmetics, including eyebrow pencils, nail polishes, and rouges, are really little more than colorants mixed with one or more materials that serve simply as binders, vehicles, or diluents to give the product desirable application properties.

Compared with foods and drugs, cosmetics usually contain much higher amounts of colorants. Although foods and drugs seldom contain more than a few to several hundred parts per million (ppm) of colorant, cosmetics often contain several percent.

COLORING MEDICAL DEVICES

Color additives are routinely added to a number of kinds of medical devices. Some which are frequently colored are surgical sutures, surgical cements, and contact lenses.

Sutures are usually colored to make them more visible during surgery and, depending on the application, during removal of the suture after the

sutured area has healed. Surgical cements, too, are colored to make them more visible during use.

Colorants are used in contact lenses for several reasons. The simplest use is to mark lenses with the letters "L" and "R" in order to distinguish one lens from another. Initially, hard lenses were lightly tinted to aid in recognition and handling. These lenses were tinted throughout the whole of the lens and the overlap of color onto the iris usually did not significantly change its color. This practice was somewhat carried over into the manufacture of soft lenses where the area of the lens in juxtaposition to the iris was tinted but the overlapping peripheral area was left clear. In some instances, this type of lens did alter eye color. More recently, much darker soft lenses containing opaque inorganic oxide pigments have been developed for the specific purpose of changing the apparent color of the iris. The effect of contact lenses on eye color varies widely with the patient and the type of lens used. Any effect obtained can generally be enhanced further by the use of appropriate eye make-up. The advertising literature of the various companies who offer the different types of lenses usually contains statements regarding results that can be expected. Some lenses are said to simply enhance the color of lightly colored eyes. Others are said to add sparkle to grey or blue eyes. Still others supposedly change brown eyes to blue, green or aqua.

Except for contact lenses, beauty is usually not a major consideration when adding color to medical devices, and there is no official color scheme that dictates what colorant to add to any particular product.

3

REGULATIONS GOVERNING USE

The rules governing color additives are complex and constantly changing, so it is difficult for any discussion of them to be complete and accurate. What follows, then, is a presentation of the principles involved in colorant regulation in the hope that it will provide some insight into the kinds of problems associated with their manufacture and use. The points discussed apply to all colorants intended for use in the United States, regardless of whether they are produced domestically or abroad.

LISTED AND PROVISIONALLY LISTED COLORANTS

From a legal standpoint, colorants can be divided into two groups—those listed for use and those provisionally listed. Listed additives are colors that have been sufficiently evaluated to convince FDA of their safety for the application intended. These colorants are also known popularly as

"permanently" listed colorants,* a misnomer since they in fact can be delisted for sufficient cause. Provisionally listed colorants, on the other hand, are dyes and pigments that are not considered unsafe but that nevertheless have not undergone all the tests required by the Color Additives Amendments of 1960 to establish their eligibility for "permanent" listing. Currently, these colors can still be used in those applications in which they were used prior to enactment of the 1960 amendments, unless newer temporary regulations restrict their use further. The status of these colorants is reviewed about once each year and, if sufficient reason exists and if the manufacturers or consumers of these colors request it, their provisional listing status is extended pending completion of the required scientific investigations.

CERTIFIED COLORANTS AND COLORANTS EXEMPT FROM CERTIFICATION

A further distinction between color additives is made relative to whether there is requirement for FDA certification. In general, only synthetic organic colorants are now subject to certification, whereas natural organic and inorganic colorants, such as turmeric and titanium dioxide, are not. The exemption from certification for a particular colorant holds whether the colorant is truly obtained from natural sources or is synthetically produced, as in the case of natural and synthetic β-carotene.

If a color requires certification prior to its sale, an appropriate size representative sample of each batch, along with a request for certification must be submitted to the FDA, Color Certification Branch, to see if it conforms to the specifications and other conditions established for it. The formats to use when requesting the certification of various kinds of colorants are shown in Figs. 3.1–3.4. The charge for certification of a straight colorant, a lake of a straight colorant, or a repack of either, is $0.25 per pound of the batch, with a minimum charge of $160. The charge for the certification of other repacks is: $25 minimum, plus $0.06 per pound for each pound over 100 pounds, for batches weighing less

*To develop and properly evaluate a new color additive and obtain "permanent" listing status for it is a tedious task that could take from 5–7 years, depending upon how the colorant is to be used. A suggested procedure to follow to obtain listing for a colorant can be found in Appendix D.

REQUEST FOR CERTIFICATION OF
A BATCH OF STRAIGHT COLOR ADDITIVE

Date .....

Division of Color Technology
HFF–430, Center for Food Safety and Applied Nutrition.
Food and Drug Administration
200 C St. S.W.
Washington, D. C. 20204

In accordance with the regulations promulgated under the Federal Food, Drug, and Cosmetic Act, we hereby make application for the certification of a batch of straight color additive.

Name of color .....
(As listed in 21 CFR Part 74)

Batch number .....
(Manufacturer's number)

Batch weigh ..... pounds

Batch manufactured by .....

..... at .....
(Name and address of actual manufacturer)

How stored pending certification .....
(State conditions of storage, with kind
and size of containers, location, etc.)

Certification requested of this color for use in .....

.....
(State proposed uses)

Required fee, $ .... (drawn to the order of Food and Drug Administration.)

The accompanying sample was taken after the batch was mixed in accordance with 21 CFR80.22 and is accurately representative thereof.

(Signed) .....

by .....

.....
(Title)

Figure 3.1.

REQUEST FOR CERTIFICATION OF A BATCH OF COLOR ADDITIVE MIXTURE

Date .....

Division of Color Technology
HFF–430, Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.
Washington, D. C. 20204

In accordance with the regulations promulgated under the Federal Food, Drug, and Cosmetic Act, we hereby make application for the certification of a batch of color additive mixture.

Name of mixture .....
(Manufacturer's trade name)

Batch number .....
(Manufacturer's number)

Weight of batch ..... pounds

Volume of batch ..... (If liquid) gallons

Batch manufactured by .....

Constituents of the mixture:

1. Color(s). (List separately each color and each lot number.)

Name of color as certified — Lot number

.....
.....

Quantity used (in pounds) — *Obtained from*

.....
.....

2. List of diluents. (List separately each diluent.)

Name of diluent

.....
.....

Quantity used

By weight — By volume (*if liquid*)

.....
.....

Batch mixed as follows .....
(Describe in detail)

How stored pending certification .....
.....
(State conditions of storage, with kind and size of containers, location, etc.)

Certification requested for use in .....
.....
(State proposed uses)

Required fee, $ .... (drawn to the order of Food and Drug Administration).

The accompanying sample was taken after the batch was mixed in accordance with 21 CFR 80.22 and is accurately representative thereof.

(Signed) .....
By .....
.....
(Title)

Figure 3.2.

REQUEST FOR CERTIFICATION OF A BATCH OF COLOR ADDITIVE LAKE.

Date .....

Division of Color Technology
HFF–430, Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.
Washington, D.C. 20204

In accordance with the regulations promulgated under the Federal Food, Drug, and Cosmetic Act, we hereby make application for the certification of a batch of color additive lake.

Name of color .....

Batch number .....
(Manufacturer's number)

Batch weigh ..... pounds

Name of color used .....

Quantity ..... pounds

Lot number .....
(When certification of the lake for use in foods is requested)

Precipitant used .....

Substratum used.....

Quantity ..... pounds

Batch manufactured by .....
at .....
(Name and address of actual manufacturer)

How stored pending certification .....
.....
(State conditions of storage, with kind
and size of containers, location, etc.)

Certification requested of this color for use in .....
.....
.....
(State proposed uses)

Required fee, $ .... (drawn to the order of Food and Drug Administration).

The accompanying sample was taken after the batch was mixed in accordance with 21 CFR 80.22 and is accurately representative thereof.

(Signed) .....
By .....
.....
(Title)

Figure 3.3.

REQUEST FOR CERTIFICATION OF A REPACK OF A BATCH OF CERTIFIED COLOR ADDITIVE

Date .....

Division of Color Technology
HFF–430, Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.
Washington, D.C. 20204

In accordance with the regulations promulgated under the Federal Food, Drug, and Cosmetic Act, we hereby make application for the certification of a batch of color additive repack.

Name of color .....
(As listed in regulations and as certified; or repacker's name, if a mixture)

Original lot number .....

Certified color content .....

This color obtained from .....

Batch number .....

Batch weigh ..... pounds

How stored pending certification .....
.....
(State conditions of storage, with kind and size of containers, location, etc.)

Certification requested for use in .....
.....
.....
(State proposed uses)

Required fee, $.... (drawn to the order of Food and Drug Administration).

The accompanying sample was taken after the batch was mixed in accordance with 21 CFR 80.22 and is accurately representative thereof.

(Signed) .....
By .....
.....
(Title)

Figure 3.4.

than 1000 pounds; $79 plus $0.02 per pound for each pound over 1000 pounds, for batches weighing more than 1000 pounds. If the batch is found satisfactory, a lot number is assigned to it and a certificate of certification is issued. (Sample certificates issued for food colors and for nonfood colors are shown in Figs. 3.5–3.6.) These certificates are valid so long as the regulations pertaining to the storage, packaging, labeling, distribution, and use of the lot are strictly adhered to.

SPECIFICATIONS

Most colorants in use today have specifications that must be met before they can be sold. In the case of the provisionally listed colors, these specifications are only temporary in that they will undoubtedly be revised if and when the colorants are removed from the provisional lists. Specifications for a synthetic aromatic organic dye, a synthetically produced natural colorant and an inorganic pigment, are given as examples in the text that follows. Specifications for other colorants can be found in Appendix A.*

FD&C Red No. 40

FD&C Red No. 40 shall conform to the following specifications and shall be free from impurities other than those named to the extent that such other impurities may be avoided by good manufacturing practice:

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—Not more than 14.0%.
Water-insoluble matter—Not more than 0.2%.
Higher sulfonated subsidiary colors (as sodium salts)—Not more than 1.0%.

*In an article published in 1926 (see Bibliography section) W. C. Bainbridge gave the specification for food colors at that time as follows:

1. Arsenic content must be less than 1/700,000.
2. Must be free from heavy metals according to the authorized test.
3. Must be structurally true to type.
4. The allowable amounts of contamination with other dyes varies between 1.5% and 3%.
5. Decomposed dyes and other organic impurities must be reduced to a minimum.
6. Insoluble matter must in no case exceed 0.3%.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
WASHINGTON, D.C. 20204

Mr. John Doe
Divisional Vice President
The XYZ Chemical Company
22 Industrial Drive
Cincinnati, OH 45237

LOT NO: AF0000

DATE: AUG-18-90

FEE: $987.00

CIN-DO

COLOR ADDITIVE CERTIFICATE

THE BATCH OF COLOR ADDITIVE DESCRIBED BELOW IS HEREBY CERTIFIED TO YOU. THE USE OF THIS COLOR IS SUBJECT TO THE TERMS, CONDITIONS AND RESTRICTIONS SET FORTH IN THE FEDERAL FOOD, DRUG AND COSMETIC ACT AND THE REGULATIONS THEREUNDER.

NAME OF COLOR	FD&C YELLOW No. 5 ALUMINUM LAKE
BATCH NUMBER	123-X
QUANTITY IN POUNDS	3,948.000

CERTIFIED % TOTAL COLOR - 36

CERTIFIED FOR USE IN:

Foods, Drugs & Cosmetics - in amounts consistent with current good manufacturing practice. The name of the color additive MUST appear on the label of the final product.

Heinz J. Eiermann

FOR THE
COMMISSIONER OF FOOD AND DRUG.

1 900000 FORM FDA 3000-A (4/86) 0000

Figure 3.5.

DEPARTMENT OF HEALTH, EDUCATION, & WELFARE
FOOD AND DRUG ADMINISTRATION
WASHINGTON D. C. 20204

LOT NO. K -0000

DATE September 7, 1990

25.00

Mr. John Doe
Divisional Vice President
The XYZ Chemical Company
22 Industrial Drive
Cincinnati, OH 45237

COLOR ADDITIVE CERTIFICATE

The batch of Color Additive described below is hereby certified to you. The use of this color is subject to the terms, conditions, and restrictions set forth in the Federal Food, Drug and Cosmetic Act and the regulations thereunder.

Name of Color	Your Batch	Quantity in Lbs	Cert. % Pure Color	Certified For Use In
D&C Orange Blend	xyz	96	42	Externally Applied Cosmetics

cc: CIN-DO

Heinz J. Eiermann

FOR THE
COMMISSIONER OF FOOD AND DRUGS

FD-CO-6 (6/64)

Figure 3.6.

Lower sulfonated subsidiary colors (as sodium salts)—Not more than 1.0%.
Disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl) azo]-8-(2-methoxy-5-methyl-4-sulfophenoxy)-2-naphthalene-sulfonic acid—Not more than 1.0%.
Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid (Schaeffer's salt) —Not more than 0.3%.
4-Amino-5-methoxy-o-toluenesulfonic acid—Not more than 0.2%.
Disodium salt of 6,6'-oxybis(2-naphthalenesulfonic acid)—Not more than 1.0%.
Lead (as Pb)—Not more than 10 ppm.
Arsenic (as As)—Not more than 3 ppm.
Total color—Not less than 85.0%.

β-Apo-8'-Carotenal

β-Apo-8'-carotenal shall conform to the following specifications:

Physical state—Solid.
One percent solution in chloroform—Clear.
Melting point (decomposition)—136–140°C (corrected).
Loss of weight on drying—Not more than 0.2%.
Residue on ignition—Not more than 0.2%.
Lead (as Pb)—Not more than 10 ppm.
Arsenic (as As)—Not more than 1 ppm.
Assay (spectrophotometric)—96–101%.

Titanium Dioxide

Titanium dioxide shall conform to the following specifications:

Lead (as Pb)—Not more than 10 ppm.
Arsenic (as As)—Not more than 1 ppm.
Antimony (as Sb)—Not more than 2 ppm.
Mercury (as Hg)—Not more than 1 ppm.
Loss on ignition at 800°C (after drying for 3 hr at 105°C)—Not more than 0.5%.
Water-soluble substances—Not more than 0.3%.

Acid-soluble substances—Not more than 0.5%.
Titanium dioxide—Not less than 99.0% after drying for 3 hr at 105°C.
Lead, arsenic, and antimony—Determined in the solution obtained by boiling 10 *g* of the titanium dioxide for 15 min in 50 mL of 0.5 *N* hydrochloric acid.

In addition to individual specifications, general specifications have been written for provisionally listed certifiable colors:

	Maximum Percent
FD&C Colors	
Lead	0.001
Arsenic (as As_2O_3)	0.00014
Heavy metals (except lead and arsenic) (precipitated as sulfides)	Trace
Mercury	0.0001
D&C and Ext. D&C Colors	
Lead	0.002
Arsenic (as As_2O_3)	0.0002
Heavy metals (except lead and arsenic) (precipitated as sulfides)	0.003
Colors that are barium salts—Soluble barium in dilute HCl (as $BaCl_2$)	0.05

The limit of 1 ppm mercury placed on colors intended for use in foods was established by a letter from the Acting Director of the Division of Colors and Cosmetics to the certified color manufacturers on February 9, 1970. This action was the first step taken to replace the somewhat nebulous "heavy metals" specifications previously used with concrete limits for specific metals.

USE RESTRICTIONS

There are numerous restrictions on the use of color additives. They cannot, for example, be employed to deceive the public by adding weight or bulk to a product or by hiding quality. In addition, special permission is needed to use colorants or products containing them in the area of the

eyes, in injections, in surgical sutures, and in foods for which standards of identity have been promulgated under Section 401 of the Federal Food, Drug and Cosmetic Act.

Other restrictions pertaining to the areas of use and the quantities of colorants allowed in products are specified in regulations for particular additives. Citrus Red No. 2, for example, can only be used to color the skins of oranges not intended for processing, whereas pyrophyllite can be used only to color drugs that are to be externally applied. A special case of the restricted use of a colorant is that of FD&C Red No. 4. Although it is designated as an FD&C colorant (implying that it can be generally used in foods), its use is now limited to coloring externally applied drugs and cosmetics only. FD&C Red No. 4 can no longer be used to color foods and ingested drugs at all. So many limitations have crept into the system that the designations FD&C, D&C, and Ext. D&C no longer have their original meaning.

The amount of a color additive allowed in a product depends on both the colorant and the article being colored. For example, TiO_2 when used to color foods cannot exceed 1% by weight of the food product. On the other hand, there is no numerical limit set on its use in the coloring of ingested or externally applied drugs. Similarly, ultramarine blue may be used to color salt intended for animal feed, but not in amounts exceeding 0.5% by weight of the salt. When numerical limits for the use of colorants are not specified, the amount allowed is controlled by "good manufacturing practice"—an ill-defined term that in effect says that you can not use more of a colorant in a product than is needed to achieve the desired effect. Today, the excessive use of colorants is rarely a problem since manufacturers are not likely to waste costly additives and, at the same time, run the risk of making their products appear unnatural.

INTERNATIONAL CONTROL

Attempts have been made to regulate color additives on an international basis. The European Economic Community (EEC) has prepared lists of colorants which they believe are suitable for use by member countries. The effect of this action is sure to be felt as the EEC becomes more established as part of the world economy.

Also important is the work of the Joint FAO/WHO Expert Committee on Food Additives. In 1955 at a conference of the Food and Agriculture

Organization of the United Nations and the World Health Organization, the two groups recommended that they collect and disseminate information on food additives. A joint committee was formed, and through its efforts a number of important publications have been issued regarding the identity, purity, and toxicological evaluation of color additives. Copies of these reports are available from WHO Publications Center USA, 49 Sheridan Ave., Albany, NY 12210 or the United Nations Bookshop, New York, NY 10017.

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4

CERTIFIED COLORS

Presently, all certified colors are factory-prepared materials belonging to one of several different chemical classes. Although a few such as D&C Blue No. 6 (indigo) are known to exist in nature, certified colors owe their commercial importance to man's ability to produce them synthetically.

Because of the starting materials used in their manufacture in the past, certified colors were once known as *coal-tar dyes.* Today, since most of the raw materials used in their preparation are obtained from petroleum, this term no longer applies.

Compared to noncertified color additives, certified colors are a cheaper, brighter, more uniform, and better characterized group of dyestuffs with higher tinctorial strengths and a wider range of hues. They are available singly ("primary colors") and in admixture with other certified colors ("secondary mixes"). Most are sold in various forms, including powders, granules, pastes, solutions and dispersions, and as lakes. Most

are also available as-is, or mixed with salt, or sugar or some other approved solvent or diluent, depending on the colorant and its intended use.

By properly blending the available primary colorants, a nearly infinite number of shades can be prepared. Examples of some mixes useful for coloring foods are shown in Table 4.1.

TABLE 4.1 Representative secondary mixes

	Parts by Weight					
Shade[a]	FD&C Blue No. 1	FD&C Blue No. 2	FD&C Red No. 3	FD&C Red No. 40	FD&C Yellow No. 5	FD&C Yellow No. 6
Strawberry			5	95		
Black (licorice)	36			22		42
Egg yellow					85	15
Cinnamon	5			35	60	
Lime green	3				97	
Mint green	25				75	
	33				67	
Orange						100
				25	20	55
					84	16
Grape	20			80		
		8.2		91.8		
Black cherry	5			95		
Chocolate	10			45	45	
	8			52	40	
Tea, root beer, or cola	8			52	40	
	5			25	70	
Butterscotch	3			22	57	18
	2			24	74	
	1.5		8.5		90	
Caramel	6		21		64	9
Peach				60		40
Raspberry	5		75			20
Cheddar cheese					55	45

[a]Obviously, more than one combination of colorants can be used to produce a particular shade. The mixture to use depends on the effect desired and the product to be colored.

CHEMICAL CLASSIFICATIONS

Azo colors comprise the largest group of certified colorants. They are characterized by the presence of one or more azo bonds (—N=N—) and are synthesized by the coupling of a diazotized primary aromatic amine to a component capable of coupling, usually a naphthol. Certifiable azo colors can be subdivided into four groups: insoluble unsulfonated pigments, soluble unsulfonated dyes, insoluble sulfonated pigments, and soluble sulfonated dyes.

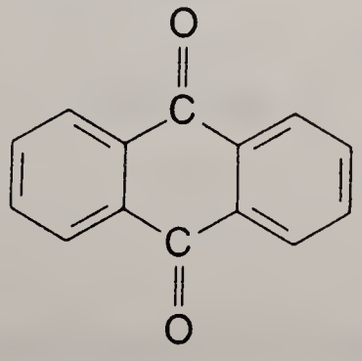
D&C Red No. 36 is an unsulfonated pigment. It contains no groups capable of salt formation, and is thus insoluble directly on coupling. Its chlorine group *ortho* to the azo group results in a sterically hindered molecule with low solubility and excellent light stability.

The unsulfonated dyes Citrus Red No. 2 and D&C Red No. 17 are insoluble in water but soluble in aromatic solvents.

Insoluble sulfonated pigments are made from colorants that contain a sulfonic acid group that is easily converted into an insoluble metal salt. In most cases, the sulfonic acid group is *ortho* to the diazo further reducing the solubilizing characteristics of the sulfonic grouping. The shade of these products is affected by the metal incorporated into the molecule and the physical characteristics of the colorants. D&C Red Nos. 7 and 34 are insoluble sulfonated pigments.

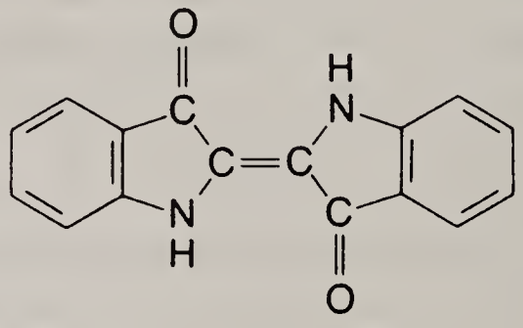
The soluble azo dyes contain one or more sulfonic acid groups. Their degree of water solubility is determined by the number of sulfonic groups present and their position in the molecule. FD&C Red No. 40 and D&C Orange No. 4 belong in this class.

Anthraquinone colorants all contain the following structure:

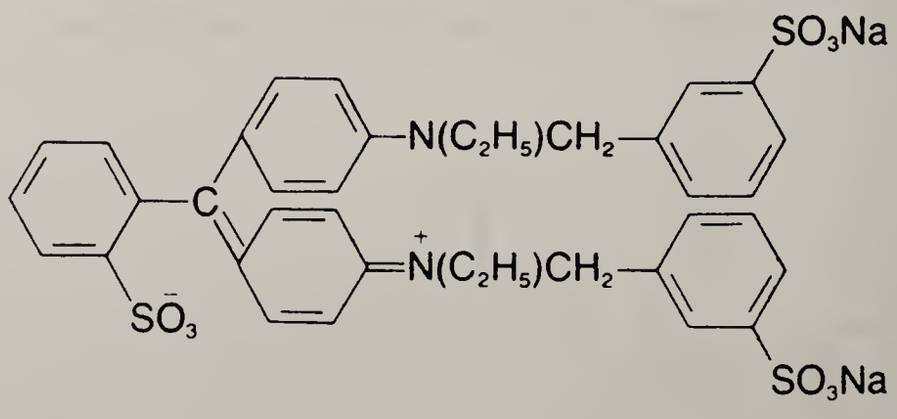


Included in this grouping are D&C Green No. 5, a water-soluble sulfonate, D&C Green No. 6, an unsulfonated water-insoluble compound, and D&C Violet No. 2, a water-insoluble hydroxyanthranquinone. Anthraquinone color additives, in general, are light stable and have good physical and chemical properties for use in cosmetics.

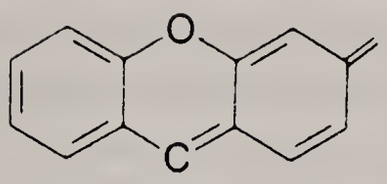
There are three color additives of the indigoid type, including D&C Blue No. 6 (indigo, an insoluble pigment), FD&C Blue No. 2 (the water-soluble disodium sulfonate derivative of indigo), and D&C Red No. 30 (an insoluble thioindigoid). All are related to indigo which has the following structure:



Three dyes are triaryl- or triphenylmethanes. Each, like FD&C Blue No. 1, consists of three aromatic rings attached to a central carbon atom. All are water-soluble, anionic, sulfonated compounds. FD&C Blue No. 1 has the following structure:



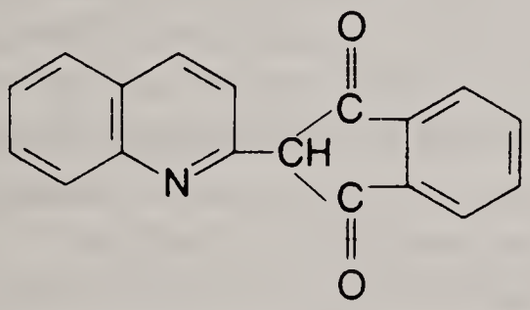
The second largest group of color additives are the xanthenes, which are characterized by the following structure:



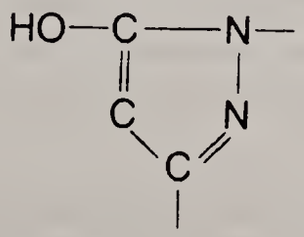
Xanthene colors can be either acidic or basic. Acid xanthenes are known to exist in two tautomeric forms. The phenolic type, or "fluorans", are free-acid structures such as D&C Orange No. 10 and D&C Red No. 21. Most have poor water solubility. In contrast to these, the quinoids or xanthenes are usually the highly water-soluble sodium salt counterparts of the fluorans such as D&C Orange No. 11 and D&C Red No. 22.

Presently, there are no certifiable basic xanthene colorants.

Two of the remaining colorants on the list of certifiables are quinolines; the solvent-soluble D&C Yellow No. 11, and its water-soluble sulfonated derivative, D&C Yellow No. 10. Both are derived from quinaldine by condensation with phthalic anhydride. D&C Yellow No. 11 has the following structure:



Two others—FD&C Yellow No. 5 and Orange B—are pyrazolones that contain the following common group:



The pyrazolones may also be classified as azo dyes since each contains an —N=N— group.

One nitro dye (Ext. D&C Yellow No. 7), one pyrene colorant (D&C Green No. 8), and one phthalocyanine dye ([Phthalocyaninato (2-)] copper) complete the list of certifiable colors.

The certifiable colors in use today are shown on pp. 92–118.

LAKES

Lakes are a special kind of color additive prepared by precipitating a soluble dye onto an approved insoluble base or substratum. In the case of D&C and Ext. D&C lakes, this substratum may be alumina, blanc fixe, gloss white, clay, titanium dioxide, zinc oxide, talc, rosin, aluminum benzoate, calcium carbonate, or any combination of two or more of these materials. Currently, alumina is the only substratum approved for manufacturing FD&C lakes.

FD&C lakes were first approved for use in 1959. Today, they are the most widely used type of lake. To make one, an alumina substrate is first prepared by adding sodium carbonate or sodium hydroxide to a solution of aluminum sulfate. Next, a solution of certified colorant is added to the resulting slurry, then aluminum chloride is added to convert the colorant to an aluminum salt, which then adsorbs onto the surface of the alumina. The slurry is then filtered, and the cake is washed, dried, and ground to an appropriate fineness—typically 0.1–40 μm.

Lakes are available with pure dye contents ranging from less than 1% to more than 40% and with moisture levels of 6–25%. Typical FD&C lakes contain 10–40% pure dye and 15–25% moisture. Typical use levels are 0.1–0.3%. Lakes are marketed as-is, or mixed with other lakes or approved diluents, or dispersed in various edible vehicles such as hydrogenated vegetable oil, coconut oil, propylene glycol, glycerin, or sucrose syrup, or dispersed in other approved media that make the mixtures appropriate for printing food wrappings, for marking capsules, for incorporating into health products that come into direct contact with the skin, and so on.

Lakes are insoluble in most solvents, although some bleeding or leaching may be observed in solvents in which the unlaked dye is soluble. FD&C lakes are insoluble in water in the pH range of 3.5–9.0, but outside this range, the lake substrate tends to dissolve releasing the captive dye.

Properties of lakes that enhance their usefulness include their opacity, their ability to be incorporated into products in the dry state, their relative insolubility, and their superior stability toward heat and light. Such properties have made possible the more effective and more efficient preparation of candy and tablet coatings, and often eliminate the need to remove moisture from "dry" products before coloring them. Lakes have also made possible the coloring of certain products that, because of their nature, method of preparation, or method of storage, cannot be colored with ordinary color additives.

Since there are no solvent-soluble FD&C colors, FD&C lakes have proven particularly valuable for coloring water-repelling foods such as fats, gums, waxes, and oils, and for coloring food-packaging materials including lacquers, containers, plastic films, and inks from which soluble dyes would be quickly leached. Similarly useful applications have been found for D&C and Ext. D&C lakes in their respective areas of application.

Unlike dyes that color objects through their adsorption or attachment from solution to the material being colored, lakes, like other pigments, impart color by dispersing them in the medium to be colored. As a consequence of this pigment-like character, both the shade and the tinctorial strength of lakes are highly dependent on the conditions used in their manufacture as well as their physical properties, including their particle size and crystal structure.

Some specific products in which lakes are used include icings, fondant coatings, sandwich cookie fillings, cake and doughnut mixes, decorative sugar crystals, coated and compression tablets (candy or pharmaceutical), hard candy, candy wafers, chewing gums, wax coatings for cheeses, yogurts, dry beverage bases, dessert powders, snack foods, spice mixes, canned and semi-moist burger-type pet foods, lotions, creams, toothpastes, nail polishes, face powders, lipsticks, printing inks, plastic films, decorative coatings, can linings, meat trays, produce containers, and margarine tubs.

PROPERTIES

To fully appreciate the properties of the color additives in use today, it is helpful to first outline the requirements of a good colorant. In doing

this, though, one must realize that since the potential areas and conditions of use for most additives are so numerous, it is next to impossible to define the perfect colorant and even more difficult to produce such a product. Nevertheless, it is generally recognized that at least the following criteria must be met if a colorant is to be useful.

1. It must be safe at the levels used and under the conditions used.
2. It must not impart any offensive property to a product.
3. It must be stable.
4. It must be nonreactive with the products and containers in which it is used.
5. It must be easy to apply to products.
6. It should be cheap.
7. It should have a high tinctorial strength.

The degree of safety required of a color additive is obviously dependent on the areas and frequency of use intended. Realistically, the toxicity of an Ext. D&C color used in hand soaps, rouges, and other products applied to the surface of the body ought not cause as much concern as the toxicity of a D&C colorant used to color drugs that are to be ingested. On the other hand, no stone can be left unturned in proving the safety of an FD&C colorant intended for use in our food supply.

For the sake of this discussion, we presume that all colors permitted in the United States are nontoxic when used as the law allows. It is important to note, though, that not all colors considered "safe" in this country are considered as such in other parts of the world, and vice versa. The reasons for this vary but are frequently related to the ground rules employed in testing them. Here in the United States, for example, it is believed that when studying a colorant's toxicity it should be tested in a manner analogous to the conditions under which it will be used. Consequently, since it is most important that food colors be safe when ingested, animal-feeding studies play a key role in their evaluation. By contrast, scientists in other parts of the world often place a great deal of emphasis on the effects of injecting a solution of the proposed colorant under the skin of test subjects. Understandably, since the mechanisms involved in these tests are so different, the conclusions drawn from them have often also been different and have resulted in the establishment of

lists of permitted colors more or less on national or regional bases. Although it is not always clear which school of thought is right when decisions are reached regarding the toxicity of a colorant, it is certain that the failure to be aware of and to understand the reasoning used to make these decisions has more than once caused undue public concern over the safety of color additives in use in the United States. The publicity often given to unscientific and inconclusive independent studies of the toxicity of colors has simply added to the confusion.

Offensive properties that can be transferred to a product by a colorant include taste and odor, whether it is the taste or odor of the colorant itself or of trace impurities in the colorant that have extremely low taste or odor thresholds. In the case of foods and drugs, this is not likely to be much of a problem, since the amount of colorant used in such products is usually low. However, in the case of highly colored cosmetics including lipsticks, face powders, rouges, and other substances used in the area of the mouth and nose, the problem is at least potentially more serious.

An even more serious problem can result from the instability of a colorant, whether it is inherent instability or instability caused by reaction of the dyestuff with a product or a product's container. Generally, color additives have shown excellent stability when stored in the dry state. For example, most certified food colors show little degradation after storage periods of 15 years or more. Unfortunately, the stability of a colorant stored neat is no guarantee of its stability in a product. Consequently, use tests must still be performed and on an individual product/colorant basis.

Many factors can and indeed do contribute to the instability of colorants. Trace metals, for example, including zinc, tin, aluminum, iron, and copper are known to cause fading of some additives. Azo dyes in particular are troublesome in this regard in that they often react with food cans and at a rate proportional to their concentration, causing corrosion of the container and a corresponding loss in the food's dye content. Some colors lack stability in retorted protein foods, whereas others are attacked by reducing and oxidizing agents, including certain invert sugars, aldehydes, and peroxides as well as ascorbic acid, which is a flavor antioxidant. Acid dyes are frequently incompatible with the quaternary salts used in various cosmetics.

Light, of course, is the enemy of all coloring agents, and color additives are no exception. As in the case of the general or overall stability of color additives, the stability of a colorant toward light, either neat or in solution, is not necessarily the same as its stability toward light in a product. Various ingredients, including aldehydic flavors, reducing sugars, and perfume oils, are known to enhance the effects of light on some colorants, whereas, ironically, others prove to be more light stable in a product than alone. Several methods are used to minimize the effects of light on colorants in products, including packaging in light-proof containers, the incorporation of ultraviolet (UV) absorbers into the products, the use of color lakes, and the careful selection of the other ingredients used in the product. No precaution, of course, is better than choosing the correct color for the job in the first place. In general, the resistance to light of dyestuffs now in use as color additives decreases in the order: quinoline-anthraquinone-triphenylmethane-azo-fluoran and pyrene.

The pH value must also be considered when choosing a colorant, since not all of them can be used at all pH values. FD&C Red No. 3, for example, precipitates from acid solution whereas FD&C Green No. 3 turns blue under alkaline conditions. Lakes often show amphoteric properties, with both acids and alkalis tending to solubilize the inorganic substrate releasing free colorant. Other colors exhibit less drastic yet important pH-related changes in their properties, including shifts in shade, variations in shelf life, changes in solubility, and loss of tinctorial strength.

The ease with which a colorant can be applied to a product or, for that matter, the ability to use a colorant for a particular application at all is a function of both the colorant's structure and the product's matrix. Unfortunately, there are no universally useful colorants, and a compromise must almost always be made in their design and selection. The water-soluble FD&C colors, for example, which are so very useful in water-based foodstuffs—including soft drinks and gelatin desserts—are of only limited value in fatty foods (except as the lakes), since the same functional groups that render these dyes water soluble also limit their fat and solvent solubility. Analogous problems exist in the use of D&C and Ext. D&C colorants, whether they are pigments, dyes, or lakes. Thus, it is important in choosing a colorant or, for that matter, in developing a new colorant to seriously consider the application properties needed based on the uses it will be put to.

The other properties most desirable in a color additive—low cost and high tinctorial strength—are, for the most part, closely related. The tinctorial strength or coloring power of a dyestuff determines the amount and thus the cost of the colorant that must be added to a product to achieve a particular effect. A colorant's tinctorial strength is an inherent property of its chemical structure and cannot be changed, although maximum use can be made of it by selecting the physical form, vehicle, and conditions under which it is used.

The cost per pound of the dyestuff is determined like that for any other product by the cost of the raw materials, equipment, and labor needed to produce it, as well as the supply and demand of the colorant. To these expenses must be added the additional cost needed to ensure the ultrahigh purity required of such colorants as well as the cost of certification. All these factors combine to make certified color additives far more expensive than typical technical dyestuffs. The saving feature, of course, is that in most cases relatively little colorant is needed to achieve the desired depth of shade in a product, and thus the cost of the colorant adds relatively little to the cost of the finished product.

Properties of a number of colorants are shown in Tables 4.2–4.12. Most of the values given were gleaned from the literature and, in general, refer to commercial colorants and not pure compounds. Since the composition of certified colorants can vary substantially with regard to the amounts of pure dye, salt, moisture, subsidiary dyes, trace metals, and so on that they contain, and since the properties of color additives are affected by their composition, care must be taken in using these tables.

PRODUCTION AND USE

The pounds of each colorant certified by the FDA over the past few years can be found in Table 4.13. The primary FD&C colors obviously dominate this picture, since they alone account for 80% or more of the total number of pounds of colorant certified during any one year.

Certified colorants are added to only about 10% of our total food supply. The major areas in which they are used are shown in Tables 4.14 and 4.15. The picture Table 4.14 presents is not complete in that it neither accounts for the pounds of color exported and sold to jobbers, nor reflects the usage of the relatively new FD&C Red No. 40; nevertheless, it

provides a good indication of current practice. Based on the maximum color concentrations shown in these tables and the total annual production of food in each food category, the total certified food color that might be ingested per person per year is estimated to be 0.043 lb. Based on recent annual colorant production figures and current total population, this figure is closer to 0.024 lb/year—a trivial amount when compared to other items consumed per person per year. See Table 4.16.

TABLE 4.2 Water solubility of FD&C colors

		2°C		25°C		60°C	
Federal Name	Common Name	g/100mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal
FD&C Blue No. 1	Brilliant Blue FCF	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Blue No. 2	Indigotine	0.8	1.0	1.6	2.1	2.2	2.9
FD&C Green No. 3	Fast Green FCF	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Red No. 3	Erythrosine	9.0	11.7	9.0	11.7	17.0	22.1
FD&C Red No. 4	Ponceau SX	4.7	6.1	11.0	14.3	11.0	14.3
FD&C Red No. 40	Allura Red	18.0	23.4	22.0	28.6	26.0	33.8
FD&C Yellow No. 5	Tartrazine	3.8	4.9	20.0	26.0	20.0	26.0
FD&C Yellow No. 6	Sunset Yellow FCF	19.0	24.7	19.0	24.7	20.0	26.0

TABLE 4.3 Alcohol solubility of FD&C colors

	100% Alcohol				75% Alcohol				50% Alcohol				25% Alcohol			
	25° C		60° C		25° C		60° C		25° C		60° C		25° C		60° C	
Federal Name	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal
FD&C Blue No. 1	0.15	0.20	0.15	0.20	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Blue No. 2			0.008	0.009	0.07	0.09	0.07	0.09	0.30	0.39	0.35	0.46	0.50	0.65	0.60	0.78
FD&C Green No. 3	0.01	0.01	0.01	0.01	10.0	13.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Red No. 3			0.01	0.01	0.6	0.78	0.80	1.04	1.0	1.3	1.0	1.3	8.0	10.4	8.0	10.4
FD&C Red No. 4			0.01	0.01	0.30	0.39	0.40	0.53	1.0	1.3	1.1	1.4	1.4	1.8	2.0	2.6
FD&C Red No. 40	0.001	0.001	0.05	0.07	0.2	0.26	0.9	1.17	1.3	1.69	5.5	7.15	9.5	12.4	22.0	28.6
FD&C Yellow No. 5			0.001	0.001	1.1	1.4	1.2	1.56	4.0	5.2	8.4	10.9	12.0	15.6	17.0	22.1
FD&C Yellow No. 6			0.001	0.001	0.3	0.39	0.3	0.39	3.0	3.9	4.0	5.2	10.0	13.0	15.0	19.5

TABLE 4.4 Glycerine solubility of FD&C colors

	100% Glycerine				75% Glycerine				50% Glycerine				25% Glycerine			
	25° C		60° C		25° C		60° C		25° C		60° C		25° C		60° C	
Federal Name	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal
FD&C Blue No. 1	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Blue No. 2	1.0	1.3	1.0	1.3	1.0	1.3	1.0	1.3	1.0	1.3	1.5	2.0	1.0	1.3	1.5	2.0
FD&C Green No. 3	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Red No. 3	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	16.0	20.8	16.0	20.8	14.0	18.2	19.0	24.7
FD&C Red No. 4	5.8	7.54	5.8	7.54	4.2	5.46	4.2	5.46	4.2	5.46	4.2	5.46	6.0	7.8	6.0	7.8
FD&C Red No. 40	3.0	3.9	8.0	10.4	4.5	5.85	8.8	11.5	12.0	15.6	14.0	18.2	20.0	26.0	20.0	26.0
FD&C Yellow No. 5	18.0	23.4	18.0	23.4	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Yellow No. 6	20.0	26.0	20.0	26.0	18.0	23.4	18.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0

TABLE 4.5 Propylene Glycol Solubility of FD&C colors

	100% Glycol				75% Glycol				50% Glycol				25% Glycol			
	25° C		60° C		25° C		60° C		25° C		60° C		25° C		60° C	
Federal Name	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal
FD&C Blue No. 1	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Blue No. 2	0.1	0.13	0.1	0.13	0.4	0.52	0.4	0.52	0.4	0.52	0.4	0.52	0.6	0.78	2.0	2.6
FD&C Green No. 3	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Red No. 3	20.0	26.0	20.0	26.0	15.6	20.3	15.6	20.3	6.6	8.6	9.2	12.0	6.6	8.6	9.4	12.2
FD&C Red No. 4	2.0	2.6	2.0	2.6	1.6	2.1	1.6	2.1	2.6	3.38	2.6	3.38	4.4	5.7	4.4	5.7
FD&C Red No. 40	1.5	2.0	1.7	2.2	2.0	2.6	3.2	4.1	7.5	9.8	10.0	13.0	18.0	23.4	22.0	28.6
FD&C Yellow No. 5	7.0	8.1	7.0	9.1	10.4	13.5	13.0	16.9	12.4	16.1	20.0	26.0	20.0	26.0	20.0	26.0
FD&C Yellow No. 6	2.2	2.86	2.2	2.86	2.2	2.86	2.6	3.38	7.0	9.1	12.8	16.6	20.0	26.0	20.0	26.0

TABLE 4.6 pH Stability of FD&C colors

Federal Name	Common Name	pH = 3	pH = 5	pH = 7	pH = 8
FD&C Blue No. 1	Brilliant Blue FCF	Slight fade after 1 week	Very slight fade after 1 week	Very slight fade after 1 week	Very slight fade after 1 week
FD&C Blue No. 2	Indigotine	Appreciable fade after 1 week	Appreciable fade after 1 week	Considerable fade after 1 week	Fades completely
FD&C Green No. 3	Fast Green FCF	Slight fade after 1 week	Very slight fade after 1 week	Very slight fade after 1 week	Slight fade and appreciably bluer
FD&C Red No. 3	Erythrosine	Insoluble	Insoluble	No appreciable change	No appreciable change
FD&C Red No. 4	Ponceau SX	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Red No. 40	Allura Red	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 5	Tartrazine	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 6	Sunset Yellow FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change

TABLE 4.7 Stability of FD&C colors in the presence of various acids

Federal Name	Common Name	10% Citric Acid	10% Acetic Acid	10% Malic Acid	10% Tartaric Acid
FD&C Blue No. 1	Brilliant Blue FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Blue No. 2	Indigotine	Completely faded after 1 week	Completely faded after 1 week	Considerably faded after 1 week	Considerably faded after 1 week
FD&C Green No. 3	Fast Green FCF	No appreciable change	No appreciable change	Slight fade after 1 week	Slight fade after 1 week
FD&C Red No. 3	Erythrosine	Insoluble	Insoluble	Insoluble	Insoluble
FD&C Red No. 4	Ponceau SX	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Red No. 40	Allura Red	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 5	Tartrazine	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 6	Sunset Yellow FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change

TABLE 4.8 Stability of FD&C colors in the presence of various alkalis

Federal Name	Common Name	10% Sodium Bicarbonate	10% Sodium Carbonate	10% Ammonium Hydroxide	10% Sodium Hydroxide
FD&C Blue No. 1	Brilliant Blue FCF	Slight fade after 1 week	Fades completely	Considerable fade	Fades completely
FD&C Blue No. 2	Indigotine	Fades completely	Fades completely	Fades completely	Yellower
FD&C Green No. 3	Fast Green FCF	No appreciable change	Considerable fade and appreciably bluer	Considerable fade and appreciably bluer	Fades completely
FD&C Red No. 3	Erythrosine	No appreciable change	Slight fade after 1 week	Slight fade after 1 week	Fades completely
FD&C Red No. 4	Ponceau SX	Slight fade after 1 week	No appreciable change	No appreciable change	Slightly yellower
FD&C Red No. 40	Allura Red	Slightly bluer after 1 week	Appreciably bluer after 1 week	Appreciably bluer after 1 week	Much bluer after 1 week
FD&C Yellow No. 5	Tartrazine	No appreciable change	No appreciable change	No appreciable change	Considerable fade after 1 week
FD&C Yellow No. 6	Sunset Yellow FCF	No appreciable change	No appreciable change	No appreciable change	Slight fade after 1 week

TABLE 4.9 Stability of FD&C colors in the presence of various sugars

Federal Name	Common Name	10% Cerelose	10% Dextrose	10% Sucrose	10% Cerelose in 2.5% Citric Acid
FD&C Blue No. 1	Brilliant Blue FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Blue No. 2	Indigotine	Considerable fade after 1 week	Considerable fade after 1 week	Slight fade after 1 week	Considerable fade after 1 week
FD&C Green No. 3	Fast Green FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Red No. 3	Erythrosine	No appreciable change	No appreciable change	No appreciable change	Insoluble
FD&C Red No. 4	Ponceau SX	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Red No. 40	Allura Red		No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 5	Tartrazine	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 6	Sunset Yellow FCF	No appreciable change	No appreciable change	No appreciable change	No appreciable change

TABLE 4.10 Stability of FD&C colors in other media

Federal Name	Common Name	1% Sodium Benzoate	1% Ascorbic Acid	25 ppm Sulfur Dioxide	250 ppm Sulfur Dioxide
FD&C Blue No. 1	Brilliant Blue FCF	No appreciable change	Slight fade after 1 week	No appreciable change	Very slight fade after 1 week
FD&C Blue No. 2	Indigotine	Slight fade after 1 week	Considerable fade after 1 week	Fades completely	Fades completely
FD&C Green No. 3	Fast Green FCF	No appreciable change	Slight fade after 1 week	No appreciable change	Very slight fade after 1 week
FD&C Red No. 3	Erythrosine	Very slight fade after 1 week	Insoluble	Insoluble	Insoluble
FD&C Red No. 4	Ponceau SX	No appreciable change	Considerable fade after 1 week	No appreciable change	No appreciable change
FD&C Red No. 40	Allura Red	No appreciable change	No appreciable change	No appreciable change	No appreciable change
FD&C Yellow No. 5	Tartrazine	No appreciable change	Appreciable fade after 1 week	Appreciable fade after 1 week	Appreciable fade after 1 week
FD&C Yellow No. 6	Sunset Yellow FCF	No appreciable change	Considerable fade after 1 week	Appreciable fade after 1 week	Appreciable fade after 1 week

TABLE 4.11 Solubilities of D&C and Ext. D&C colorants

	H_2O	Glycerol	MeOH	EtOH	Petroleum Jelly
D&C Blue No. 4	S	S	S	S	C
D&C Blue No. 6	IU	D	I	I	D
D&C Blue No. 9	IU	ID	Ia	I	D
D&C Brown No. 1	S	S	S	SS	IE
D&C Green No. 5	S	S	S	SS	IE
D&C Green No. 6	I	Ia	SS	SS	M
D&C Green No. 8	SF	SSF	SSF	SSF	Ia
D&C Orange No. 4	S	S	S	M	IE
D&C Orange No. 5	IB	SS	S	M	D
D&C Orange No. 10	IB	SS	S	M	D
D&C Orange No. 11	Similar to those of FD&C Red No. 3				
D&C Red No. 6	S	S	SS	Ia	I
D&C Red No. 7	I	D	Ia	Ia	D
D&C Red No. 17	I	SS	SS-M	SS	S
D&C Red No. 21	IBF	Da	SS	SS	D
D&C Red No. 22	SF	SF	SF	SF	IE
D&C Red No. 27	IB	Da	SS	SS	D
D&C Red No. 28	S	S	S	S	IE
D&C Red No. 30	IU	D	I	I	I
D&C Red No. 31	M	SS	SS	SS	I
D&C Red No. 33	S	S	SS	SS	I
D&C Red No. 34	I	I	Ia	I	D
D&C Red No. 36	I	D	Ia	Ia	D
D&C Red No. 39	Ia	M	M-S	S	I
D&C Violet No. 2	I	Ia	SS	SS	S
D&C Yellow No. 7	IBF	SSF	SF	SS	D
D&C Yellow No. 8	SF	SF	SF	M	IE
D&C Yellow No. 10	S	S	M	SS	I
D&C Yellow No. 11	I	SS	S	S	S
Ext. D&C Violet No. 2	S	S	SS	SS	I
Ext. D&C Yellow No. 7	S	S	M	SS	I

ABBREVIATIONS FOR TABLES 4.11 AND 4.12

a—May bleed or stain, very sparingly soluble.

B—Insoluble in water, soluble in aqueous alkaline solution.

C—Practically insoluble, but useful in nearly neutral or slightly acid emulsions.

D—Practically insoluble, but may be dispersed by grinding and homogenizing; solid mediums (waxes) should be softened or melted before or during the grinding.

d—Hue becomes duller or darker.

E—Practically insoluble in the fatty acid, oil, or wax, but useful in coloring slightly alkaline aqueous emulsions.

F—Solution usually fluorescent.

(continued)

TABLE 4.11 ***(Continued)***

Toluene	Stearic Acid	Oleic Acid	Mineral Oil	Mineral Wax	Et_2O	Me_2CO	AcOBu
I	C	C	C	C	I	Ia	I
Ia	D	D	D	D	I	I	I
Ia	D	D	D	D	I	I	I
I	IE	IE	IE	IE	SS	SS	I
I	IE	IE	IE	IEW	I	SS	I
S	M	M	M	M	SS	SS	S
I	Ia	Ia	I	I	Ia	Ia	Ia
I	IE	IE	IE	IE	I	Ia	I
I	D	D	D	D	M	S	I
I	D	D	D	D	M	S	I
	Similar to those of FD&C Red No. 3						
I	I	I	I	I	I	Ia	I
I	D	D	D	D	I	Ia	I
S	S	S	S	S	SS	SS	M
I	D	D	D	D	M*	S	I
I	IE	IE	IE	IE	Ia	SS	I
I	D	D	D	D	Ia	SS	I
I	IE	IE	IE	IE	Ia	SS	I
Ia	D	D	D	D	Ia	Ia	Ia
I	I	I	I	I	I	Ia	Ia
I	I	I	I	I	I	I	I
I	D	D	D	D	I	D	D
I	D	D	D	D	I	Ia	D
Ia	I	SS	I	Ia	S	S	SS
S	S	S	S	SW	SS	SS	S
I	D	D	D	D	SS*	S	I
I	IE	IE	IE	IE	Ia	kla	I
I	I	I	I	I	Ia	SS	I
S	S	S	S	S	S	S	S
I	I	I	I	I	I	SS	I
I	I	I	I	I	I	M	I

l—Insoluble.

J—Tends to thicken or gel the solution.

k—Turns brownish in hue.

L—Turns orange in hue.

M—Moderately soluble (< 1%).

m—Turns scarlet in hue.

p—Dye precipitated as heavy-metal salt or color acid.

r—Turns redder in hue.

S—Dissolves (solubility ≥ 1%).

SS—Sparingly soluble (< 0.25%).

sl—Slightly.

U—In alkaline-reducing vats a soluble leuco compound forms.

TABLE 4.12 Fastness properties of D&C and Ext. D&C colorants

	Light	10% AcOH	10% HCl	10% NaOH
D&C Blue No. 4	3	5	5	4
D&C Blue No. 6	6	7I	5I	L6U
D&C Blue No. 9	7	7I	5I	6IU
D&C Brown No. 1	3	5	5	6sly
D&C Green No. 5	5	5	5	5
D&C Green No. 6	4	5L	5I	6I
D&C Green No. 8	2	I	I	5
D&C Orange No. 4	5	5	5	2m
D&C Orange No. 5	2	4aI	4I	Sr
D&C Orange No. 10	2	4aI	4I	Sr
D&C Orange No. 11	Similar to D&C Red No. 3			
D&C Red No. 6	5	5	4	4d
D&C Red No. 7	6	5I	4I	5I
D&C Red No. 17	3	5L	4Id	5I
D&C Red No. 21	2	3I	3I	5Sr
D&C Red No. 22	2	2py	1py	5
D&C Red No. 27	2	3I	3	5Sr
D&C Red No. 28	3	2p	4p	6
D&C Red No. 30	6	7I	I	6IU
D&C Red No. 31	5	5	4	5
D&C Red No. 33	5	6	3z	5
D&C Red No. 34	4	5I	4	4I
D&C Red No. 36	6	6I	4d	4d
D&C Red No. 39	2	Sv	Sv	6Sx
D&C Violet No. 2	4	5I	5I	5I
D&C Yellow No. 7	2	I	I	S6
D&C Yellow No. 8	3	3p	3p	6
D&C Yellow No. 10	3	5	5	4r
D&C Yellow No. 11	2	I	5I	Iw
Ext. D&C Violet No. 2	5	5	5	5
Ext. D&C Yellow No. 7	4	5	5	5

v—Turns violet in hue.
W—Not fast to prolonged storage in some waxes.
w—Becomes tinctorially weaker.
x—Turns yellow in hue.
y—Turns yellower in hue.
z—Hazy or cloudy.
*—Practically colorless.
1—Very poor fastness.
2—Poor fastness.
3—Fair fastness.
4—Moderate fastness.
5—Good fastness.
6—Very good fastness.
7—Excellent fastness.

(continued)

TABLE 4.12 ***(Continued)***

0.9% Physiol Salt Soln.	5% $FeSO_4$	5% Alum	Oxidizing Agents	Reducing Agents
6	4	4	2	1
I	I	I	6	U
I	I	I	6	U
6	p	p	3	1
5	4	4	3	2
I	I	I	3	2
6	4d	4d	3	3
6	Jp	Jp	3	3
I	I	I	3	3
I	I	I	3	3
Similar to D&C Red No. 3				
6	p	p	3	1
I	4Id	4I	3	1
I	4Id	4I	3	1
I	Id	4I	4	4
6	3d	2y	4	4
1	I	I	4	4
6	z	p	4	4
I	I	I	5	u
6	p	p	3	1
6	4	4	3	1
I	I	I	3	1
I	4d	4	3	1
I	4aId	I	3	3
6I	4I	4I	2	1
I	I	I	3	3
6	zp	p	3	3
6	z	4	2	5
I	I	I	2	5
6	4z	4	3	2
6	zd	4	3	3

PERMITTED COLORANTS

The identities shown for the following colorants are those assigned by the Food and Drug Administration, as they appear in the Code of Federal Regulations (21 CFR 70–82). Often, the name is not the best, reflecting certain inconsistencies in the system used to arrive at them, but they are the official FDA designations and thus are given here. The structures shown are, in general, taken from the Colour Index (CI) and represent

TABLE 4.13 Pounds of color additives certified by FDA during fiscal year

	1970	1971	1972	1973	1974	1975
FD&C—Primaries						
FD&C Blue No. 1	83,309	92,928	94,796	121,108	159,135	158,539
FD&C Blue No. 2	39,974	43,391	39,218	63,135	88,581	84,840
FD&C Green No. 3	5,005	7,082	5,864	4,049	5,180	9,157
FD&C Red No. 2	1,463,753	1,283,367	729,461	982,528	902,812	1,377,944
FD&C Red No. 3	154,288	166,744	238,658	228,436	285,567	337,144
FD&C Red No. 4	23,352	21,289	28,640	15,912	28,261	35,037
FD&C Red No. 40	—	263	892,282	565,354	729,359	788,147
FD&C Violet No. 1	8,897	3,200	66,684	35,953	Delisted	Delisted
FD&C Yellow No. 5	956,681	1,033,464	1,092,724	1,030,987	1,289,878	1,391,325
FD&C Yellow No. 6	939,641	1,016,456	887,444	1,011,164	995,813	1,084,284
	3,674,900	3,668,184	4,075,771	4,058,626	4,484,586	5,266,417
FD&C—Lakes						
FD&C Blue No. 1	32,433	24,303	34,279	47,198	67,141	57,637
FD&C Blue No. 2	6,553	11,021	9,988	18,890	34,118	35,817
FD&C Green No. 3	379	114	—	—	—	—
FD&C Red No. 2	53,229	50,676	22,110	34,888	50,041	40,776
FD&C Red No. 3	102,160	110,165	120,690	182,913	169,692	181,712
FD&C Red No. 40	—	—	9,463	27,351	33,482	32,966
FD&C Violet No. 1	14,970	16,591	20,925	17,400	Delisted	Delisted
FD&C Yellow No. 5	291,750	331,989	391,972	432,346	492,764	502,832
FD&C Yellow No. 6	108,837	111,079	139,358	172,622	188,267	235,610
	610,311	655,938	748,785	936,508	1,035,505	1,087,350
D&C—Primaries						
D&C Blue No. 6	514	2,095	1,847	252	3,042	234
D&C Blue No. 9	—	—	—	—	—	—
D&C Green No. 5	3,492	2,053	1,307	4,331	13,432	18,670
D&C Green No. 6	1,385	3,954	1,400	1,989	2,475	—
D&C Green No. 8	15,086	4,879	4,998	8,112	11,793	7,529
D&C Orange No. 4	—	873	1,616	3,827	4,284	1,024
D&C Orange No. 5	8,046	4,342	5,959	7,082	3,932	5,034
D&C Orange No. 10	—	2,430	—	—	1,529	525
D&C Orange No. 17	—	—	546	—	2,500	6,161
D&C Red No. 6	—	—	—	—	—	—
D&C Red No. 8	516	545	778	—	2,073	978
D&C Red No. 10	—	—	—	—	516	—
D&C Red No. 17	304	—	10	84	413	707
D&C Red No. 19	2,830	709	2,991	2,750	6,281	4,272
D&C Red No. 21	7,028	4,957	3,165	3,390	8,559	4,378
D&C Red No. 22	3,310	4,935	361	2,386	3,182	3,918
D&C Red No. 27	—	—	953	367	1,935	—
D&C Red No. 28	511	1,747	601	708	1,439	922
D&C Red No. 30	—	—	—	—	638	—
D&C Red No. 31	868	869	—	—	—	—
D&C Red No. 33	2,341	1,284	1,324	956	2,666	1,732
D&C Red No. 36	4,749	4,172	4,161	6,067	7,919	6,858
D&C Red No. 37	516	1,193	547	2,134	—	1,223
D&C Red No. 39	2,024	—	—	2,000	—	—
D&C Violet No. 2	2,821	1,264	1,648	2,030	500	—
D&C Yellow No. 7	—	400	1,200	400	476	1,988

TABLE 4.13 ***(Continued)***

1976[a]	1977	1978	1979	1980	1981	1982
148,112	184,115	184,332	228,782	174,874	194,663	184,595
79,215	98,936	85,030	88,083	86,980.4	117,724.9	83,911.7
6,438	4,111	5,945	5,967	3,593	7,061	4,942
239,257	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
363,875	548,557	429,522	432,750	397,553	432,898.8	420,873.2
4,169	7,822[b]	6,668[b]	20,329[b]	7,714[b]	6,937.8[b]	16,462.1[b]
1,500,760	1,520,648	1,799,690	2,304,231	1,885,393	2,083,308	1,748,552.3
Delisted	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
1,543,764	1,165,528	1,541,179	1,497,622	1,377,164	1,426,584.8	1,456,047.7
1,081,714	991,347	1,071,148	1,288,352	1,071,536	1,242,066.2	1,154,590.1
4,967,304	4,521,064	5,123,514	5,866,116	5,004,807.4	5,511,184.5	5,069,974.1
49,839	96,821	74,227	99,316	75,317	54,440.9	84,625.9
39,911	78,584	57,225	97,889	55,357	68,275.5	88,018.6
—	—	—	—	—	—	—
3,871	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
262,948	269,784	314,178	398,895	181,301	271,612.2	327,655
81,975	71,206	101,206	115,145	85,863	61,923.7	107,037.1
Delisted	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
558,851	727,776	643,280	631,010	681,077	475,447.4	432,885.5
189,885	447,108	241,442	389,167	392,221.3	362,186.5	322.299
1,187,280	1,691,279	1,431,558	1,731,422	1,471,136.3	1,293,886.2	1,362,521.1
1,257	1,836	217	—	—	—	—
66	—	—	—	—	—	—
6,191	3,124	10,210	8,938	5,965	4,501	4,609.1
2,960	660	3,220	440	800	4,006	600
11,095	10,153	7,730	12,075	11,923	17,761	13,694
474	2,691	3,598	4,593	6,233	8,022	6,302.5
8,324	4,889	3,010	4,616	3,805	2,043	2,398
—	687	575	—	—	—	—
654	1,500	14,290	2,809	17,448	7,760	7,026
—	543	1,410	—	689	—	696
957	454	701	500	985	2,026	483
1,600	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
—	1,316	172	—	1,851	—	—
4,526	2,400	1,359	3,423	3,395	1,736.5	1,633.1
7,826	2,825	4,197	784	909	2,633	4,609
4,341	2,857	1,853	1,865	2,954	2,582	922
615	1,731	2,441	2,902	—	4,133	—
—	—	676	—	—	592	—
—	2,416	371	—	—	—	—
—	—	—	294	833	—	—
6,491	4,469	9,139	4,142	3,986	9,724.9	6,665
3,024	3,839	4,260	2,671	5,936	6,364	3,544
—	586	1,160	1,354	625	—	—
—	1,720	—	500	—	—	—
6,574	971	—	—	666	100	100
924	—	—	—	—	660	—

(continued)

TABLE 4.13 Pounds of color additives certified by FDA during fiscal year *(Continued)*

	1970	1971	1972	1973	1974	1975
D&C Yellow No. 8	4,548	1,581	3,861	2,367	6,400	6,100
D&C Yellow No. 10	4,458	4,376	9,009	14,125	15,787	17,508
D&C Yellow No. 11	1,200	2,179	2,000	1,971	3,450	511
	66,547	50,837	50,282	67,328	105,221	90,272
D&C—Lakes						
D&C Blue No. 1	2,429	2,370	457	—	1,951	2,417
D&C Blue No. 4	—	705	—	—	—	—
D&C Green No. 5	—	—	—	—	—	—
D&C Green No. 6	—	—	—	—	—	—
D&C Green No. 8	—	—	—	—	—	—
D&C Orange No. 4	628	3,887	1,335	4,147	—	2,238
D&C Orange No. 5	7,563	9,360	3,095	5,667	3,177	12,459
D&C Orange No. 17	12,220	16,206	9,164	14,904	21,405	12,560
D&C Red No. 2	693	—	1,483	1,665	—	522
D&C Red No. 3	6,477	5,743	7,812	9,116	4,819	10,891
D&C Red No. 6	9,192	12,739	20,747	32,850	34,732	17,003
D&C Red No. 7	22,605	2,817	20,929	32,961	49,150	37,665
D&C Red No. 8	6,300	—	—	1,864	3,202	644
D&C Red No. 9	27,860	39,091	35,101	65,391	52,398	83,338
D&C Red No. 10	2,056	2,667	1,308	4,520	4,809	7,390
D&C Red No. 11	1,875	1,685	1,030	1,886	5,666	4,010
D&C Red No. 12	2,166	2,663	2,640	3,257	8,048	5,879
D&C Red No. 13	2,017	1,393	746	4,268	4,365	5,264
D&C Red No. 19	12,607	7,376	5,590	16,655	14,553	10,387
D&C Red No. 21	14,049	17,600	6,971	18,680	13,889	17,887
D&C Red No. 27	1,701	7,216	7,617	9,337	6,622	10,280
D&C Red No. 30	6,608	20,209	7,070	11,092	13,501	31,921
D&C Red No. 33	—	—	—	—	—	—
D&C Red No. 34	867	1,551	3,195	2,756	13,048	7,863
D&C Red No. 36	570	2,709	—	3,216	959	4,111
D&C Yellow No. 5	20,811	13,460	25,829	14,868	30,201	22,454
D&C Yellow No. 6	5,636	2,674	2,245	4,253	7,016	2,702
D&C Yellow No. 10	2,054	774	1,592	1,104	6,566	1,355
	168,984	174,895	165,956	264,457	300,077	311,240
Ext. D&C—Primaries						
Ext. D&C Green No. 1	256	389	294	559	1,777	1,981
Ext. D&C Violet No. 2	—	—	—	—	—	782
Ext. D&C Yellow No. 1	4,141	8,769	8,404	9,869	10,685	15,613
Ext. D&C Yellow No. 7	1,032	—	1,046	1,853	—	—
	5,429	9,158	9,744	12,281	12,462	18,376
Ext. D&C—Lakes						
Ext. D&C Yellow No. 7	1,764	1,275	1,555	1,516	1,395	730
	1,764	1,275	1,555	1,516	1,395	730
Other Colorants						
Citrus Red No. 2	4,612	—	2,300	1,638	496	12,172
Orange B	34,017	28,653	20,409	31,211	19,043	31,161
[Phthalocyaninato(2-)] Copper	524	—	—	—	154	—
	39,153	28,653	22,709	32,849	19,693	43,333

TABLE 4.13 ***(Continued)***

1976[a]	1977	1978	1979	1980	1981	1982
3,800	2,800	4,985	7,982	3,993	2,641	9,131.3
13,913	18,751	46,283	32,453	49,193	50,500.9	68,609.9
5,326	4,906	1,553	4,121	2,497	3,300	2,200
90,938	78,124	123,410	96,462	124,686	131,086.3	133,222.9
884	—	927	1,250	822	—	559
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
—	—	—	—	—	—	—
1,000	1,000	1,693	938	2,348	2,242	1,645
6,633	4,669	1,335	1,575	1,837	1,850	—
24,015	27,191	34,910	11,420	19,459	13,722.5	18,381.2
—	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
8,199	6,590	13,473	7,818	9,494	7,983.6	7,085.4
36,731	49,811	56,348	40,206	26,293	48,925	52,016.1
35,060	52,875	85,589	107,928	98,371	99,297.7	145,428.4
1,686	3,623	—	3,886	—	1,970	1,864
54,058	87,050	109,060	93,284	82,855	69,107.2	66,243.2
1,025	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
2,751	Delisted	Delisted	Delisted	Delisted	Delisted	Delisted
4,257	2,908	Delisted	Delisted	Delisted	Delisted	Delisted
7,621	1,256	Delisted	Delisted	Delisted	Delisted	Delisted
14,272	13,217	10,555	15,314	11,687	22,450.7	14,395.6
18,061	3,643	3,250	8,519	2,489	2,651	1,559.2
7,283	2,176	1,175	6,951	3,351	5,471	10,616
18,108	22,040	27,706	33,217	30,368	19,249	29,495
475	—	1,356	3,048	—	—	3,655
7,023	—	627	6,781	5,376	11,111	4,955
—	—	—	1,596	—	—	—
12,395	27,014	17,498	20,418	17,005	13,819	9,001.1
5,866	2,460	3,520	—	4,042	—	1,653.5
7,289	4,449	22,018	25,840	38,757	66,311	55,058.1
274,692	311,972	391,040	389,989	354,554	386,150.7	423,610.8
—	Delisted	Delisted	—	Delisted	Delisted	Delisted
884	—	407	942	1,408	488	2,424
10,578	7,170	Delisted	—	Delisted	Delisted	Delisted
2,102	—	2,488	—	1,953	—	1,540
13,564	7,170	2,895	942	3,361	488	3,964
2,427	—	—	—	—	—	—
2,427	—	—	—	—	—	—
1,752	—	—	—	—	881	668
29,045	38,909	17,788	—	—	—	—
—	—	—	—	77.3	—	—
30,797	38,909	17,788	0	77.3	881	668

(continued)

TABLE 4.13 Pounds of color additives certified by FDA during fiscal year *(Continued)*

	1983	1984	1985	1986	1987	1988	1989	1990
FD&C—Primaries								
FD&C Blue No. 1	180,920.5	260,417	225,053.7	217,019	242,766.1	280,125.5	302,876.6	233,418.0
FD&C Blue No. 2	136,042.7	101,223.4	95,647.2	84,746.9	153,220.6	127,355.8	127,631.2	93,336.5
FD&C Green No. 3	3,488	3,597	5,414	4,697.4	5,863	5,742	4,051	7,493.0
FD&C Red No. 3	464,230.8	241,264.7	276,849.5	252,543.1	275,170	268,087.8	258,042.8	182,596.3
FD&C Red No. 4	12,450.5	9,515.8	16,742.9	16,072	15,562.9	10,943	19,461	26,840.7
FD&C Red No. 40	1,885,628.5	2,630,578	2,081,636	2,695,696.3	2,085,857.2	2,648,533.4	2,628,978.8	2,595,719.8
FD&C Yellow No. 5	1,470,615.2	1,620,540.3	1,126,618.7	1,514,154.7	1,510,484.5	1,664,087.4	1,575,481.3	1,642,914.1
FD&C Yellow No. 6	1,086,580.8	1,535,050.3	1,154,239.6	1,600,961.6	1,064,356.1	1,654,579.9	1,537,026.4	1,606,996.8
	5,239,957	6,402,186.5	4,982,201.6	6,385,891	5,353,280.4	6,659,454.8	6,453,549.1	6,389,315.2
FD&C—Lakes								
FD&C Blue No. 1	83,757	75,127.3	84,741.6	77,035	86,405.1	85,498.5	121,538.9	70,016.9
FD&C Blue No. 2	74,198	90,314.3	62,594.9	81,169.8	120,389	88,269.6	88,698.4	77,047.6
FD&C Green No. 3	—	—	—	—	—	—	—	—
FD&C Red No. 3	302,086.3	163,857.6	218,184.5	209,124.4	202,028.6	227,688.5	222,850.2	43,581.7
FD&C Red No. 40	103,373	224,182.4	261,894.6	293,798.6	281,200	232,867.4	300,217	407,369.8
FD&C Yellow No. 5	536,574.1	643,392.4	544,099.5	677,286.7	708,309.1	839,439.2	717,142.9	793,778.8
FD&C Yellow No. 6	379,530.3	484,160	382,503.9	350,352.4	442,406.9	526,258.1	462,380.6	602,936.6
	1,479,518.7	1,681,034	1,554,019	1,688,766.9	1,840.738.7	2,000,021.3	1,912,828	1,994,731.4

D&C—Primaries								
D&C Blue No. 4	—	—	—	—	—	—	22	—
D&C Blue No. 6	120.5	—	550	—	—	—	—	—
D&C Blue No. 9	—	—	—	—	—	—	3.8	—
D&C Green No. 5	3,413	7,654	3,233	3,352	2,438	994	6,160.2	4,129.0
D&C Green No. 6	500	1,735	500	800	1,095	1,716	1,417	1,175.0
D&C Green No. 8	12,161	21,659	17,151	18,036	21,780	28,626.5	32,995.3	38,277.9
D&C Orange No. 4	7,961.3	5,631.5	4,294.2	9,322.4	3,808.6	9,821.4	16,633.2	5,043.0
D&C Orange No. 5	306	1,174	1,741	7,465	2,569	2,499	1,713	—
D&C Orange No. 10	—	—	—	—	—	—	—	—
D&C Orange No. 17	2,191	—	1,390	4,625	—	2,402	Delisted	Delisted
D&C Red No. 6	354	288	2,483	442	638.4	700.6	—	3,803.5
D&C Red No. 7	—	—	—	110	110.2	154.3	176.4	568.1
D&C Red No. 8	976	—	514	905	1,317	1,108	Delisted	Delisted
D&C Red No. 9	—	—	—	464	1,000	—	Delisted	Delisted
D&C Red No. 17	1,507	2,705	685	1,928	2,619	2,084	2,662	4,681.0
D&C Red No. 19	441	1,365	1,607.5	2,094.5	100.3	—	Delisted	Delisted
D&C Red No. 21	5,201	584	5,875	7,560	8,205	3,034	4,749	5,582.0
D&C Red No. 22	4,948	4,071	3,791	785	5,208	—	—	6,431.0
D&C Red No. 27	286	4,390	1,529	3,318	250	2,145.2	1,492	4,318.0
D&C Red No. 28	3,293	3,939	5,559	5,253	3,301.4	2,913.2	1,544.6	7,910.0
D&C Red No. 30	66	—	1,542	2,426	3,228	3,513	5,511	7,226.1
D&C Red No. 31	—	—	—	—	—	—	—	—
D&C Red No. 33	7,446.6	18,810.5	2,450	9,438.5	8,461.6	14,378.9	6,381.5	14,969.3
D&C Red No. 34					—	—	33.1	—
D&C Red No. 36	3,641	6,413	2,552	8,735	5,230	2,274	5,614	6,644.5

(continued)

TABLE 4.13 Pounds of color additives certified by FDA during fiscal year (Continued)

	1983	1984	1985	1986	1987	1988	1989	1990
D&C—Primaries *(cont)*								
D&C Red No. 37	—	—	—	—	—	—	Delisted	Delisted
D&C Red No. 39	—	—	—	—	—	—	—	—
D&C Violet No. 2	—	—	309	2,218.5	2,354	2,906	1,512	1,529.0
D&C Yellow No. 7	—	1,102	—	—	—	1,100	—	250.0
D&C Yellow No. 8	5,392.8	4,402.4	5,209.1	2,753.6	1,200	3,550	2,051.4	2,992.0
D&C Yellow No. 10	46,536.7	20,082.6	75,805	25,360.6	42,268.6	38,650	76,430.4	43,933.4
D&C Yellow No. 11	3,200	2,900	1,840	3,626.5	2,809	1,597	2,778	1,800.0
	109,941.9	108,906	140,609.8	121,018.6	119,991.1	126,167.1	169,879.9	161,262.8
D&C—Lakes								
D&C Blue No. 1	500	—	—	—	—	615	660	—
D&C Green No. 5	—	—	—	—	—	—	—	—
D&C Green No. 6	—	—	—	—	—	—	—	—
D&C Green No. 8	—	—	—	—	—	—	—	—
D&C Orange No. 4	1,530	1,241	—	—	3,094	1,152	—	4,002.0
D&C Orange No. 5	—	2,785	3,417	2,949	5,526	1,722	9,162	1,309.0
D&C Orange No. 17	6,113.6	1,082.7	1,598.6	3,106.9	1,018.7	2,041.8	Delisted	Delisted
D&C Red No. 3	10,631.7	10,565.2	4,378.8	7,921.7	5,555.3	11,449.3	15,949.8	5,633.8
D&C Red No. 6	69,052.9	81,095	107,243.3	88,808	91,873.2	90,797.6	126,911.7	115,961.4
D&C Red No. 7	119,542.2	168,121.2	165,870.5	191,375.8	133,047.3	156,283.7	178,252.6	218,198.4
D&C Red No. 8	—	—	—	—	—	—	Delisted	Delisted
D&C Red No. 9	35,334.4	24,570.9	29,746.5	42,611	12,238.2	2,629	Delisted	Delisted
D&C Red No. 19	3,394.9	5,600	9,352.1	17,449	3,711	—	Delisted	Delisted
D&C Red No. 21	4,676.7	5,848.6	7,500	15,968.6	10,348.4	9,524.2	16,519.7	18,835.6

D&C Red No. 22	—	—	—	1,853	—	—	—	1,050.0
D&C Red No. 27	6,428	23,932	24,095	40,627	39,752	43,472.2	58,554.8	83,327.7
D&C Red No. 28	—	165	165	330	557	594.6	1,080.6	1,679.0
D&C Red No. 30	32,983.9	43,461.1	49,546	20,230	26,265.1	36,371.1	37,864.9	42,554.2
D&C Red No. 31	—	—	—	—	—	795	—	—
D&C Red No. 33	830	1,130	1,325	726	2,590	2,322	1,050	4,121.0
D&C Red No. 34	12,789	3,340	11,686	12,336	10,647	4,228	4,192	3,326.0
D&C Red No. 36	—	—	—	1,473	704	—	—	—
D&C Yellow No. 5	8,679.4	16,380.5	9,841.9	9,371.3	20,229.4	17,174.6	11,749.9	10,129.5
D&C Yellow No. 6	2,788.8	3,486.3	1,879	1,459	1,946.5	2,500.9	3,829.6	6,224.5
D&C Yellow No. 10	70,477.6	58,117.4	72,157.7	71,493.5	84,892.8	86,633.3	71,791.7	82,753.7
	385,753.1	450,921.9	499,802.4	530,088.8	453,995.9	470,306.3	537,569.3	599,105.8
Ext. D&C—Primaries								
Ext. D&C Violet No. 2	2,555	5,133	2,396	5,393.3	2,864	8,471	7,722.2	4,940.0
Ext. D&C Yellow No. 7	1,746	724	—	967	900	9,752	1,547	400.0
	4,301	5,857	2,396	6,360.3	3,764	18,223	9,269.2	5,340.0
Other Colorants								
Citrus Red No. 2	3,843.1	—	771.8	3,183.3	2,551.3	4,614.4	—	—
Orange B	—	—	—	—	—			—
[Phthalocyaninato (2-)] Copper	27	481	94	2,209	264	101	6	214.5
	3,870.1	481	865.8	5,392.3	2,815.3	4,715.4	6	214.5

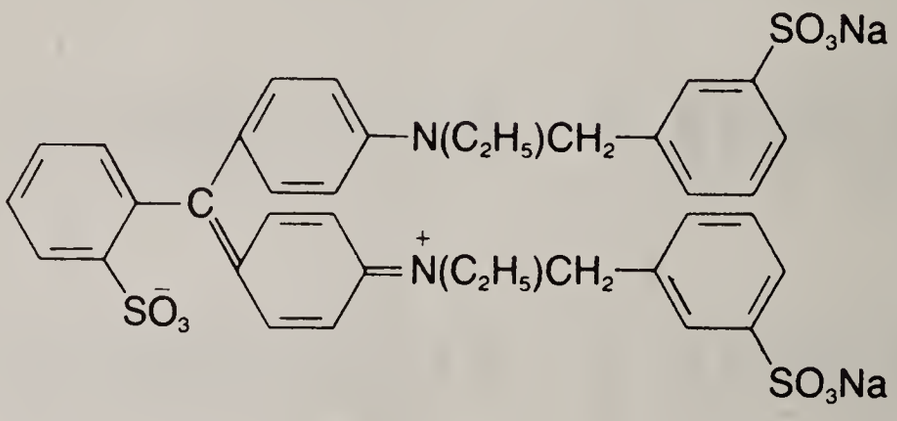
[a]In 1976, the Federal fiscal year was changed to end in September rather than June. Consequently, the figures shown for 1976 only, represent fifteen consecutive months of certification.

[b]Delisted for ingested use.

each colorant's principal component. Chemical Abstracts Service (CAS) and European Economic Community (EEC) identification numbers are also included.

FD&C Blue No. 1

Synonyms: Brilliant Blue FCF; CI Food Blue 2 (42090).
CAS Reg. No.: 2650-18-2.
Chemical Structure:



Identity: Principally the disodium salt of ethyl[4-[*p*-[ethyl(*m*-sulfobenzyl)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*m*-sulfobenzyl)ammonium hydroxide inner salt with smaller amounts of the isomeric disodium salts of ethyl[4-[*p*-[ethyl (*p*-sulfobenzyl)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](*p*-sulfobenzyl) ammonium hydroxide inner salt and ethyl[4-[*p*-[ethyl(o-sulfobenzy)amino]-α-(o-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene](o-sulfobenzyl)ammonium hydroxide inner salt.
Empirical Formula: $C_{37}H_{34}N_2O_9S_3Na_2$.
Molecular Weight: 792.84.
Dye Classification: Triphenylmethane.
Manufacturing Process: Condense benzaldehyde-o-sulfonic acid with α-(*N*-ethylanilino)-*m*-toluenesulfonic acid ("benzylethylaniline sulfonic acid").

FD&C Blue No. 2

Synonyms: Indigotine, Indigo Carmine; CI Food Blue 1 (73015).
CAS Reg. No.: 860-22-0.

TABLE 4.14 Pounds of primary colors used in foods, drugs, and cosmetics[a]

Category	FD&C Blue No. 1	FD&C Blue No. 2	FD&C Green No. 3	Orange B	FD&C Red No. 2	FD&C Red No. 3	FD&C Red No. 4	FD&C Violet No. 1	FD&C Yellow No. 5	FD&C Yellow No. 6	Total
Candy, confections	6,632	2,499	124	0	67,637	11,665	0	1,459	59,903	52,770	202,689
Beverages	15,800	2,375	301	0	282,695	1,056	0	985	78,933	181,292	563,437
Dessert powders	3,270	1,659	14	0	62,363	8,616	0	0	59,961	51,622	187,505
Cereals	843	99	0	0	15,558	1,421	0	0	52,496	35,464	105,881
Maraschino cherries	597	0	98	0	8,104	3,469	11,308	0	5,644	4,830	34,050
Pet food	1,473	6,764	0	0	67,058	1,023	0	1,278	101,743	23,226	202,565
Bakery goods	3,680	673	7	0	43,522	9,560	0	369	77,885	42,203	177,899
Ice cream, sherbet, dairy products	2,599	179	7	0	29,697	621	0	45	35,048	23,868	92,064
Sausage	647	0	0	16,890	36,084	4,970	0	0	6,502	99,605	164,698
Snack foods	305	0	0	0	3,623	766	0	2	18,456	11,409	34,561
Meatstamping inks	11	0	0	0	12	10	0	2,223	15	0	2,271
Miscellaneous	5,345	1,990	1,298	0	46,219	18,200	398	1,134	44,841	29,134	148,559
Subtotal (food use)	41,202	16,238	1,849	16,890	662,572	61,377	11,706	7,495	541,427	555,423	1,916,179
Pharmaceuticals	3,250	593	220	0	21,179	12,168	1,186	347	17,275	15,938	72,156
Cosmetics	397	30	27	0	3,417	903	630	96	3,125	2,148	10,773
Totals	44,849	16,861	2,096	16,890	687,168	74,448	13,522	7,938	561,827	573,509	1,999,108

[a]Figures represent sales for the first 9 months of 1967, but do not include exports or sales to jobbers and other manufacturers. Some of the colorants permitted then are no longer in use. It can be assumed, though, that substitute colorants are used at about the same concentrations.

TABLE 4.15 Major categories of processed food manufactured using certified colors and levels of color used

Category	Level of Color Used	
	Range (ppm)	Average (ppm)
Candy and confections	10–400	100
Beverages (liquid and powdered)	5–200	75
Dessert powders	5–600	140
Cereals	200–500	350
Maraschino cherries	100–400	200
Pet foods	100–400	200
Bakery goods	10–500	50
Ice cream and sherbets	10–200	30
Sausage (surface)	40–250	125
Snack foods	25–500	200
Miscellaneous (nuts, salad dressing, gravy, spices, jams, jellies, food packaging, etc.)	5–400	—

TABLE 4.16 Approximate annual per capita consumption of some common items in the United States

Certified Color Additives[a]	0.023 lb
Food[b]	1393 lb
Sugar and other sweeteners[c]	120 lb
Salt[d]	8.5 lb
Alcohol[e]	17.1 lb
2.56 gal of distilled spirits	
1.84 gal of wine	
25.95 gal of beer	
Aspirin[f]	0.064 lb
Cigarettes[g]	4138

[a]Calculated by dividing the average number of pounds of Primary FD&C colorants certified by FDA in the years 1970–1990 by a population of 227.5 million.

[b]Average for the years 1960–1980 as reported in *Food Consumption Prices and Expenditures 1960–1980,* Statistical Bulletin Number 672, Economic Research Service, U.S. Department of Agriculture.

[c]*Ibid.* Excludes sugar used in the production of canned and frozen fruits, canned fruit juices, canned vegetables, and unskimmed sweetened condensed milk.

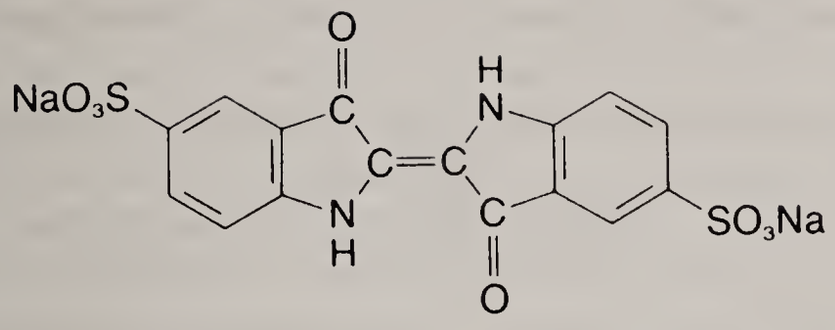
[d]FENNER, S. FDA Consumer, March 1980, pp. 2–7. Includes salt obtained from processed foods as well as salt that occurs naturally in foods and some drinking waters.

[e]First Special Report to the U.S. Congress on Alcohol & Health, U.S. Department of Health, Education and Welfare, 1971. 1970 figures; based on the drinking-age population—those 15 and older.

[f]TAYLOR, F. FDA Consumer, Dec. 1980/Jan. 1981, pp. 12–17.

[g]Average number consumed by people 18 and older during the years 1960–1979 as reported in *Smoking, Tobacco and Health,* U.S. Department of Health and Human Services.

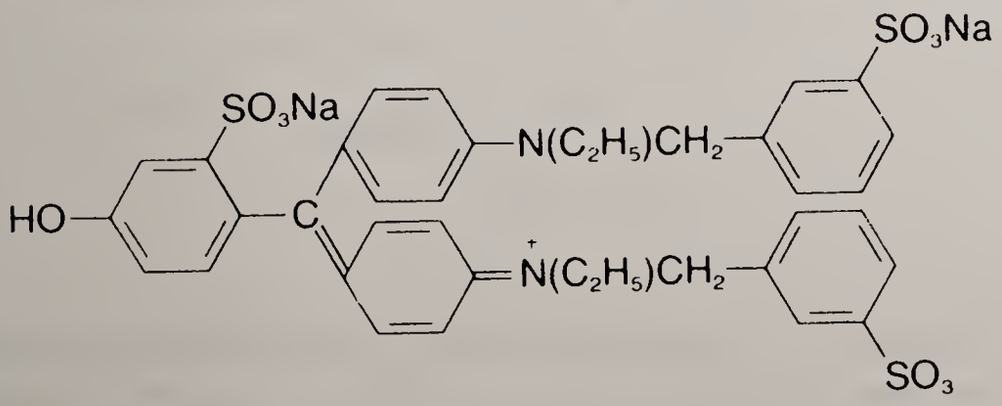
EEC No.: E 132.
Chemical Structure:



Identity: Principally the disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid (CAS Reg. No. 860-22-0) with smaller amounts of the disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo- 1H-indole-5-sulfonic acid (CAS Reg. No. 54947-75-0) and the sodium salt of 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid (CAS Reg. No. 605-18-5).
Empirical Formula: $C_{16}H_8N_2O_8S_2Na_2$.
Molecular Weight: 466.36.
Dye Classification: Indigoid.
Manufacturing Process: Sulfonation of indigo.

FD&C Green No. 3

Synonyms: Fast Green FCF; CI Food Green 3 (42053).
CAS Reg. No.: 2353-45-9.
Chemical Structure:



Identity: Principally the inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl [(3-sulfophenyl) methyl] amino] phenyl] (4-hydroxy-2-sulfophenyl) methylene] -2,5-cyclohexadien-1-ylidene] -3-sulfobenzenemethanaminium hydroxide (CAS Reg. No. 2353–45–9); with smaller amounts of the isomeric inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl [(3-sulfophenyl) methyl] amino] phenyl] (4-hydroxy-2-sulfophenyl) methylene] -2, 5-cyclohexadien-1-ylidene]-4-sulfobenzenemethanaminium hydroxide; of *N*-ethyl-*N*-[4-[[4-[ethyl [(4-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene] -2,5-cyclohexadien-1-ylidene] -4-sulfobenzenemethanaminium hydrox ide and of *N*-ethyl-*N*-[4-[[4-[ethyl[(2-sulfophenyl)methyl]amino] phenyl] (4-hydroxy-2-sulfophenyl) methyl-ene]-2, 5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminiumhydroxide.

Empirical Formula: $C_{37}H_{34}N_2O_{10}S_3Na_2$.

Molecular Weight: 808.86

Dye Classification: Triphenylmethane.

Manufacturing Process: Condense *p*-hydroxybenzaldehyde-o-sulfonic acid with α-(*N*-ethylanilino)-*m*-toluenesulfonic acid.

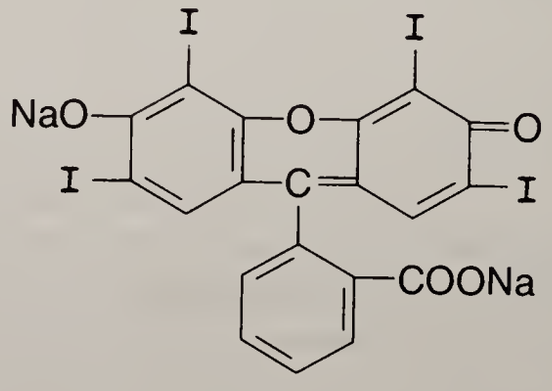
FD&C Red No. 3

Synonyms: Erythrosine, Erythrosine Bluish; CI Food Red 14 (45430).

CAS Reg. No.: 16423-68-0.

EEC No.: E 127.

Chemical Structure:

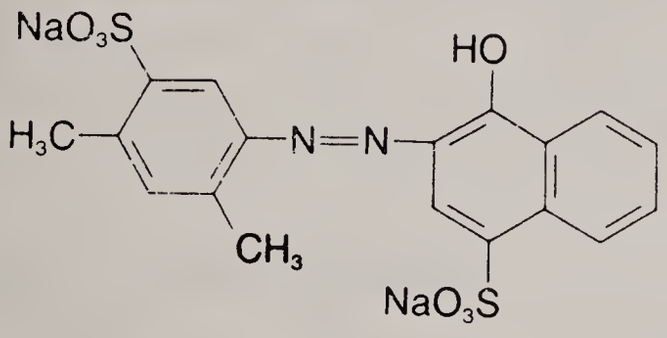


Identity: Principally the monohydrate of 9(o-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3H-xanthen-3-one, disodium salt, with smaller amounts of lower iodinated fluoresceins.

Empirical Formula: $C_{20}H_6O_5I_4Na_2$.
Molecular Weight: 879.86.
Dye Classification: Xanthene.
Manufacturing Process: Iodination of fluorescein (D&C Yellow No. 7).

FD&C Red No. 4

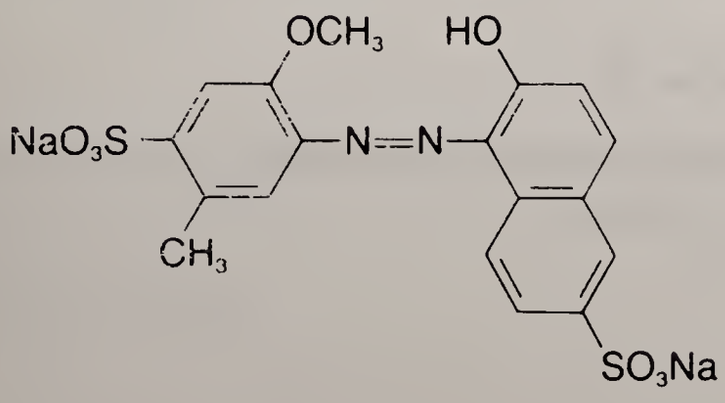
Synonyms: Ponceau SX; CI Food Red 1 (14700).
CAS Reg. No.: 4548-53-2.
Chemical Structure:



Identity: Principally the disodium salt of 3-[(2,4-dimethyl-5-sulfophenyl)azo]-4-hydroxy-1-naphthalenesulfonic acid.
Empirical Formula: $C_{18}H_{14}N_2O_7S_2Na_2$.
Molecular Weight: 480.43.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 1-amino-2,4-dimethylbenzene-5-sulfonic acid with 1-naphthol-4-sulfonic acid.

FD&C Red No. 40

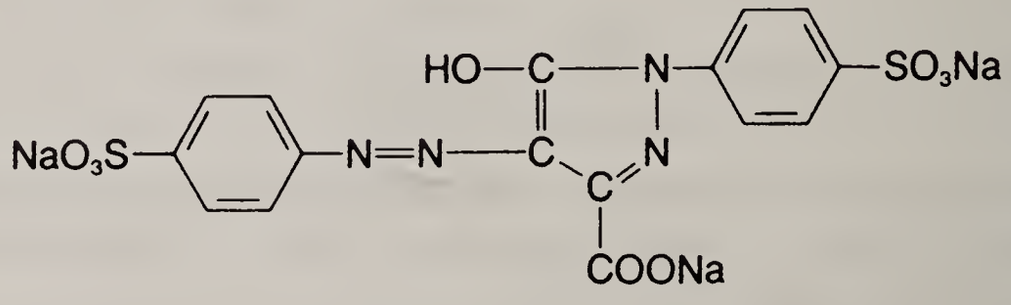
Synonyms: Allura Red; CI Food Red 17 (16035).
CAS Reg. No.: 25956-17-6.
Chemical Structure:



Identity: Principally the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid.
Empirical Formula: $C_{18}H_{14}N_2O_8S_2Na_2$.
Molecular Weight: 496.43.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 5-amino-4-methoxy-2-toluenesulfonic acid with 6-hydroxy-2-naphthalenesulfonic acid.

FD&C Yellow No. 5

Synonyms: Tartrazine; CI Food Yellow 4 (19140).
CAS Reg. No.: 1934-21-0.
EEC No.: E 102.
Chemical Structure:

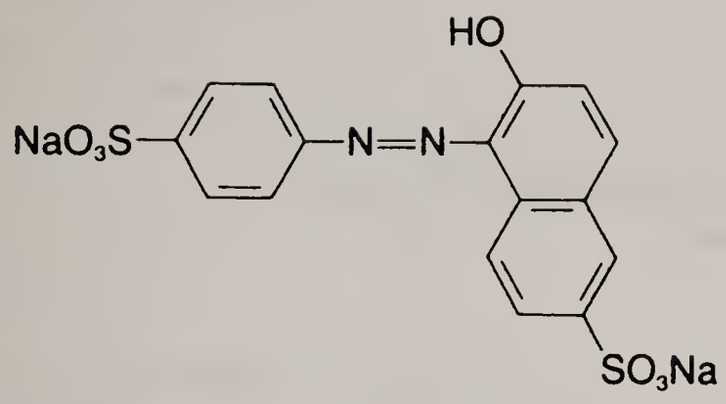


Identity: Principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenyl-azo]-1*H*-pyrazole-3-carboxylic acid.
Empirical Formula: $C_{16}H_9N_4O_9S_2Na_3$.
Molecular Weight: 534.37.
Dye Classification: Pyrazolone.
Manufacturing Processes: (a) Condense phenylhydrazine-*p*-sulfonic acid with oxalacetic ester, couple the product with diazotized sulfanilic acid, then hydrolyze the ester with sodium hydroxide or (b) condense phenylhydrazine-*p*-sulfonic acid with dihydroxytartaric acid.

FD&C Yellow No. 6

Synonyms: Sunset Yellow; CI Food Yellow 3 (15985).
CAS Reg. No.: 2783-94-0.
EEC No.: E 110.

Chemical Structure:



Identity: Principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid (CAS Reg. No. 2783-94-0). The trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid (CAS Reg. No. 50880-65-4) may be added in small amounts.

Empirical Formula: $C_{16}H_{10}N_2O_7S_2Na_2$.

Molecular Weight: 452.37.

Dye Classification: Monoazo.

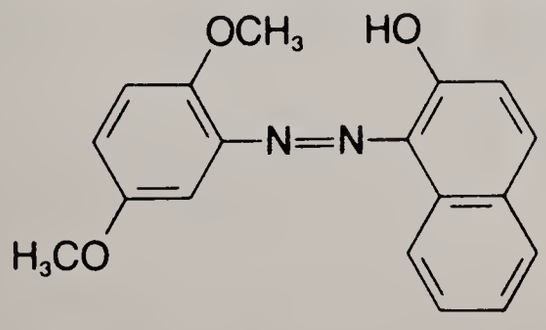
Manufacturing Process: Couple diazotized sulfanilic acid with 2-naphthol-6-sulfonic acid.

Citrus Red No. 2

Synonyms: CI Solvent Red 80 (12156).

CAS Reg. No.: 6358-53-8.

Chemical Structure:



Identity: Principally 1-(2,5-dimethoxyphenylazo)-2-naphthol.

Empirical Formula: $C_{18}H_{16}N_2O_3$.

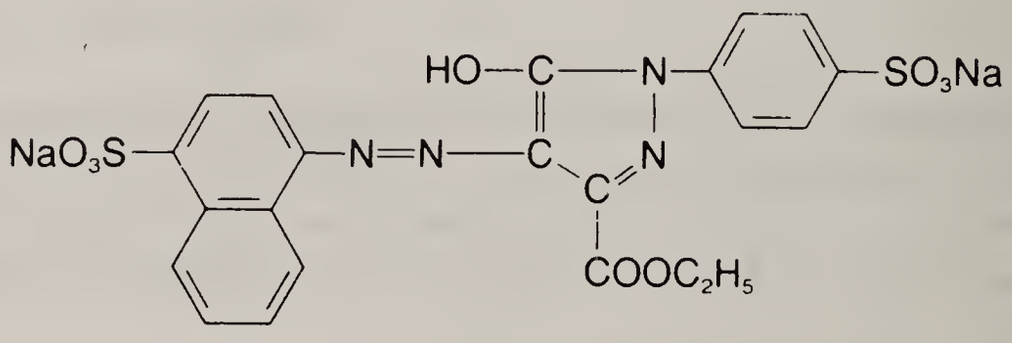
Molecular Weight: 308.34.

Dye Classification: Monoazo.

Manufacturing Process: Couple diazotized 2,5-dimethoxyaniline with 2-naphthol.

Orange B

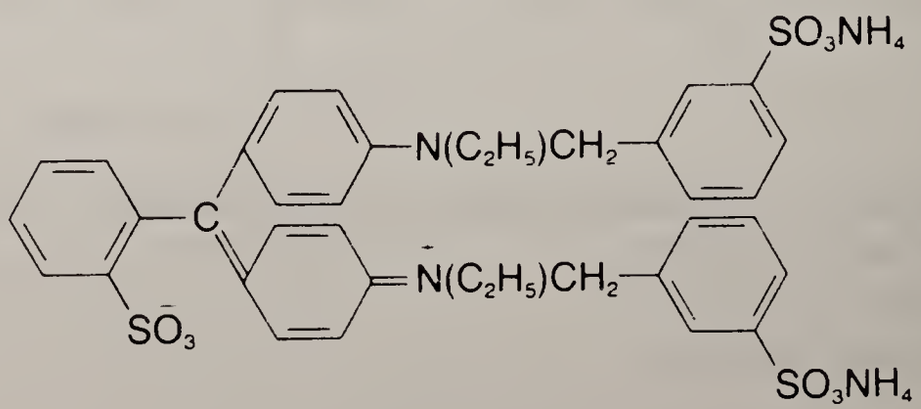
Synonyms: CI Acid Orange 137 (19235).
Chemical Structure:



Identity: Principally the disodium salt of 1-(4-sulfophenyl)-3-ethyl-carboxy-4-(4-sulfonaphthylazo)-5-hydroxypyrazole.
Empirical Formula: $C_{22}H_{16}N_4O_9S_2Na_2$.
Molecular Weight: 590.50.
Dye Classification: Pyrazolone.
Manufacturing Process: React phenylhydrazine-*p*-sulfonic acid with the sodium derivative of diethyl hydroxymaleate; partially hydrolyze, to remove one ethyl group; then couple with diazotized naphthionic acid.

D&C Blue No. 4

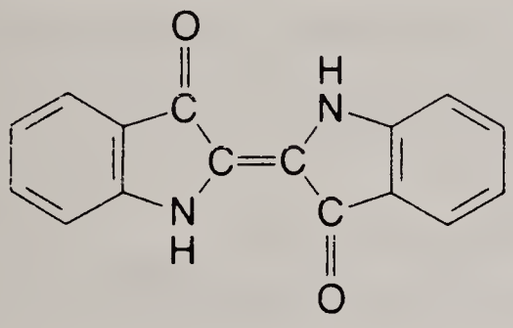
Synonyms: Alphazurine FG, Erioglaucine; CI Acid Blue 9 (42090).
CAS Reg. No.: 6371-85-3.
Chemical Structure:



Identity: NH_4 Salt corresponding to FD&C Blue No. 1.
Empirical Formula: $C_{37}H_{42}N_4O_9S_3$.
Molecular Weight: 782.96.
Dye Classification: Triphenylmethane.
Manufacturing Process: Same as FD&C Blue No. 1.

D&C Blue No. 6

Synonyms: Indigo; CI Vat Blue 1 (73000).
CAS Reg. No.: 482-89-3.
Chemical Structure:

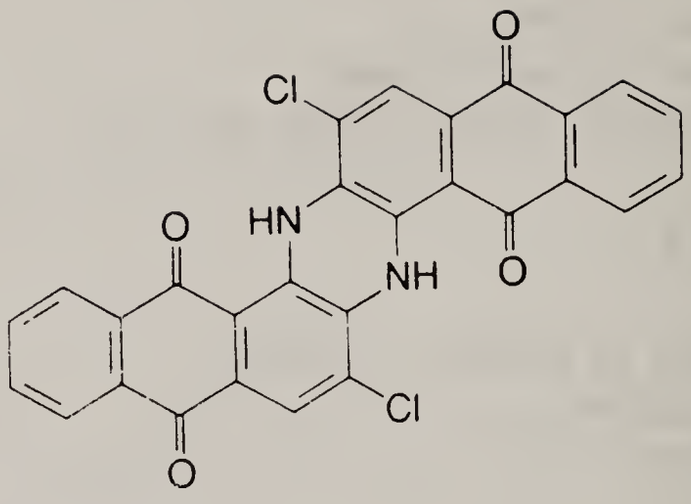


Identity: Principally [$\Delta^{2,2'}$-biindoline]-3,3'-dione.
Empirical Formula: $C_{16}H_{10}N_2O_2$.
Molecular Weight: 262.27.
Dye Classification: Indigoid.
Manufacturing Processes: (a) Convert *N*-phenylglycine into pseudoindoxyl by fusion with sodium amide (or sodium and a current of ammonia) in the presence of a mixture of potassium and sodium hydroxides and sodium cyanide. Oxidize the pseudoindoxyl with air. (b) Convert phenylglycine-o-carboxylic acid[*N*-(carboxymethyl)-anthranilic acid] into indoxylic acid by fusion with alkalis and follow by air oxidation in alkaline solution.

D&C Blue No. 9

Synonyms: Indanthrene Blue, Carbanthrene Blue; CI Vat Blue 6 (69825).
CAS Reg. No.: 130-20-1.

Chemical Structure:



Identity: Principally 7,16-dichloro-6,15-dihydro-5,9,14,18-anthrazinetetrone.
Empirical Formula: $C_{28}H_{12}N_2O_4Cl_2$.
Molecular Weight: 511.32.
Dye Classification: Anthraquinone vat.
Manufacturing Process: Chlorinate indanthrene.

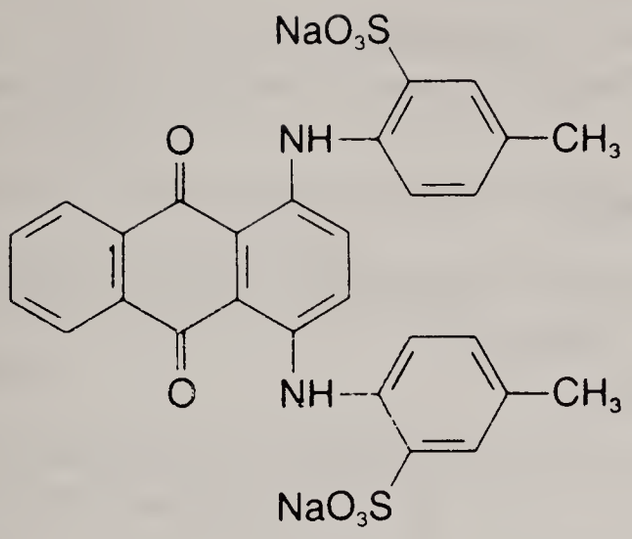
D&C Brown No. 1

Synonyms: Resorcin Brown; CI Acid Orange 24 (20170).
CAS Reg. No.: 1320-07-6.
Identity: A mixture of the sodium salts of 4[[5-[(dialkylphenyl)azo]-2,4-dihydroxyphenyl]azo]-benzenesulfonic acid. The alkyl group is principally the methyl group.
Empirical Formula: $C_{20}H_{17}N_4O_5SNa$.
Molecular Weight: 448.43.
Dye Classification: Diazo.
Manufacturing Process: Couple diazotized sulfanilic acid and diazotized crude xylidine with resorcinol.

D&C Green No. 5

Synonyms: Alizarine Cyanine Green F; CI Acid Green 25 (61570).
CAS Reg. No.: 4403-90-1.

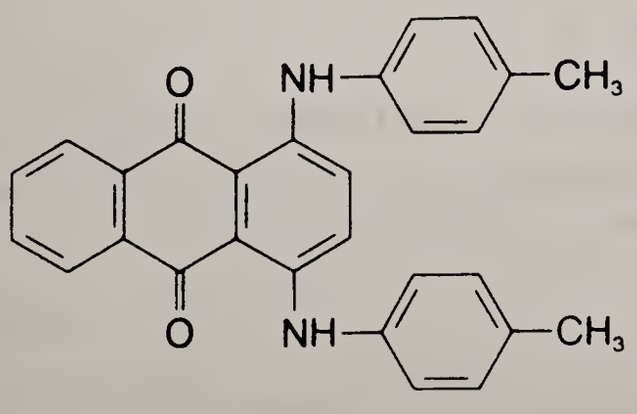
Chemical Structure:



Identity: Principally the disodium salt of 2,2'-[(9,10-dihydro-9,10-dioxo-1,4-anthracenediyl)diimino]bis[5-methyl-benzenesulfonic acid].
Empirical Formula: $C_{28}H_{20}N_2O_8S_2Na_2$.
Molecular Weight: 622.59.
Dye Classification: Anthraquinone.
Manufacturing Process: Condense leucoquinizarin with *p*-toluidine, then sulfonate.

D&C Green No. 6

Synonyms: Quinizarin Green SS; CI Solvent Green 3 (61565).
CAS Reg. No.: 128-80-3.
Chemical Structure:

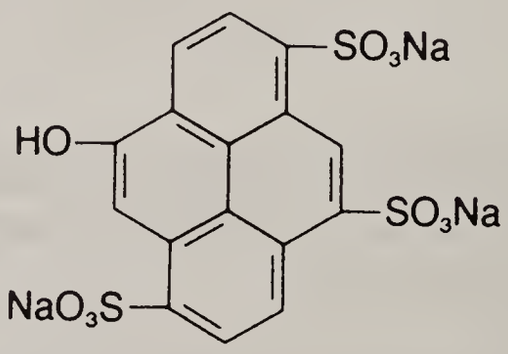


Identity: Principally 1,4-bis[(4-methylphenyl)amino]-9,10-anthracenedione.

Empirical Formula: $C_{28}H_{22}N_2O_2$.
Molecular Weight: 418.50.
Dye Classification: Anthraquinone.
Manufacturing Process: Condense leucoquinizarin with *p*-toluidine.

D&C Green No. 8

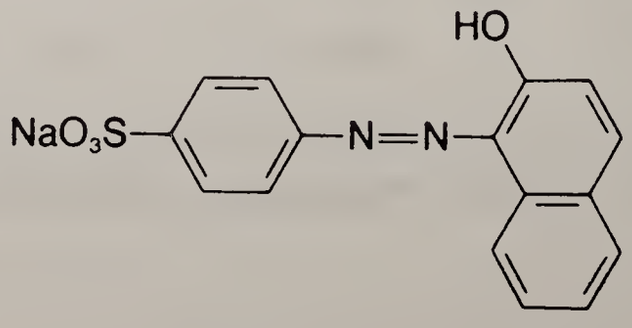
Synonyms: Pyranine Concentrated; CI Solvent Green 7 (59040).
CAS Reg. No.: 6358-69-6.
Chemical Structure:



Identity: Principally the trisodium salt of 8-hydroxy-1,3,6-pyrenetrisulfonic acid.
Empirical Formula: $C_{16}H_7O_{10}S_3Na_3$.
Molecular Weight: 524.39.
Dye Classification: Pyrene.
Manufacturing Process: Sulfonate pyrene to tetrasulfonic acid, salt out with sodium chloride, hydrolyze in sodium hydroxide solution, add formic acid, and salt out with sodium chloride.

D&C Orange No. 4

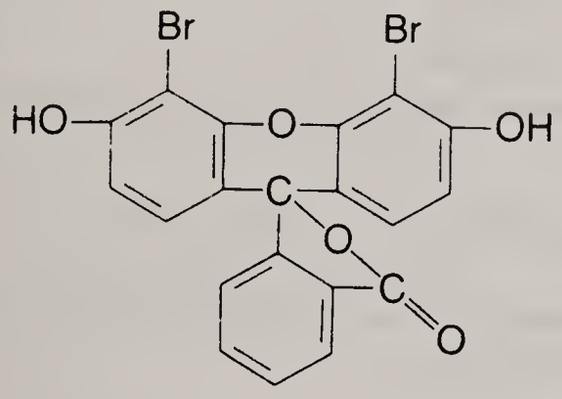
Synonyms: Orange II; CI Acid Orange 7 (15510).
CAS Reg. No.: 633-96-5.
Chemical Structure:



Identity: Principally the sodium salt of 4-[(2-hydroxy-1-naphthalenyl) azo]benzenesulfonic acid.
Empirical Formula: $C_{16}H_{11}N_2O_4SNa$.
Molecular Weight: 350.33.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized sulfanilic acid with 2-naphthol.

D&C Orange No. 5

Synonyms: Dibromofluorescein; CI Solvent Red 72 (45370:1).
CAS Reg. No.: 596-03-2.
Chemical Structure:

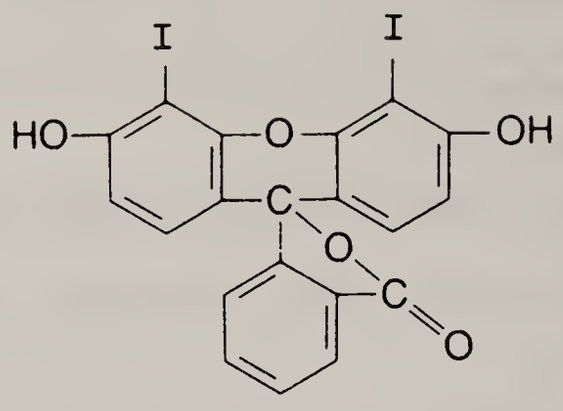


Identity: A mixture consisting principally of the sodium salt of 4',5'-dibromofluorescein (CAS Reg. No. 596-03-2) plus smaller amounts of 2',4',5'-tribromofluorescein (CAS Reg. No. 25709-83-5) and 2',4',5',7'-tetrabromofluorescein (CAS Reg. No. 15086-94-9).
Empirical Formula: $C_{20}H_{10}O_5Br_2$.
Molecular Weight: 490.11.
Dye Classification: Fluoran.
Manufacturing Process: Brominate fluorescein (D&C Yellow No. 7).

D&C Orange No. 10

Synonyms: Diiodofluorescein; CI Solvent Red 73 (45425:1).
CAS Reg. No.: 38577-97-8.

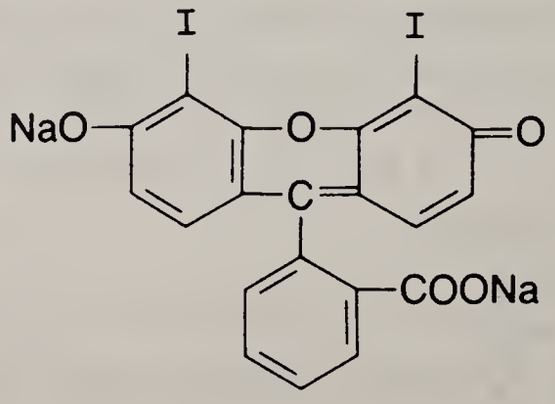
Chemical Structure:



Identity: A mixture consisting principally of 4',5'-diiodofluorescein, 2',4',5'-triiodofluorescein, and 2',4',5',7'-tetraiodofluorescein.
Empirical Formula: $C_{20}H_{10}O_5I_2$.
Molecular Weight: 584.10.
Dye Classification: Fluoran.
Manufacturing Process: Iodinate fluorescein (D&C Yellow No. 7).

D&C Orange No. 11

Synonyms: Erythrosine Yellowish Na; CI Acid Red 95 (45425).
CAS Reg. No.: 33239-19-9.
Chemical Structure:

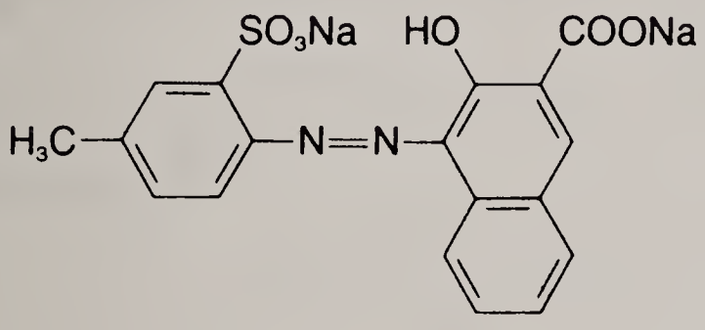


Identity: A mixture consisting principally of the disodium salts of 4',5'-diiodofluorescein, 2',4',5'-triiodofluorescein and 2',4',5',7'-tetraiodofluorescein.
Empirical Formula: $C_{20}H_8O_5I_2Na_2$.

Molecular Weight: 628.07.
Dye Classification: Xanthene.
Manufacturing Process: Convert D&C Orange No. 10 to the Na salt.

D&C Red No. 6

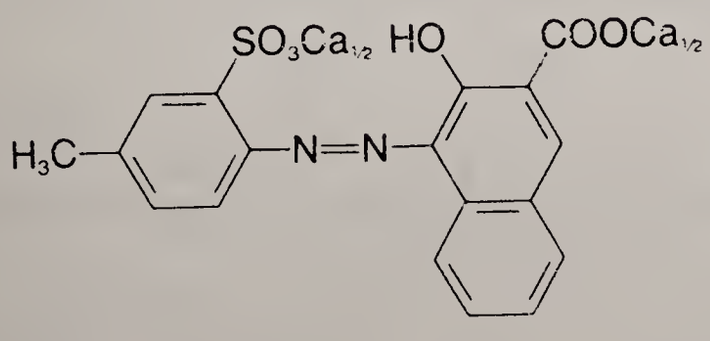
Synonyms: Lithol Rubin B; CI Pigment Red 57 (15850).
CAS Reg. No.: 5858-81-1.
Chemical Structure:



Identity: Principally the disodium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl)azo]-2-naphthalenecarboxylic acid.
Empirical Formula: $C_{18}H_{12}N_2O_6SNa_2$.
Molecular Weight: 430.35.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 6-amino-*m*-toluenesulfonic acid with 3-hydroxy-2-naphthoic acid.

D&C Red No. 7

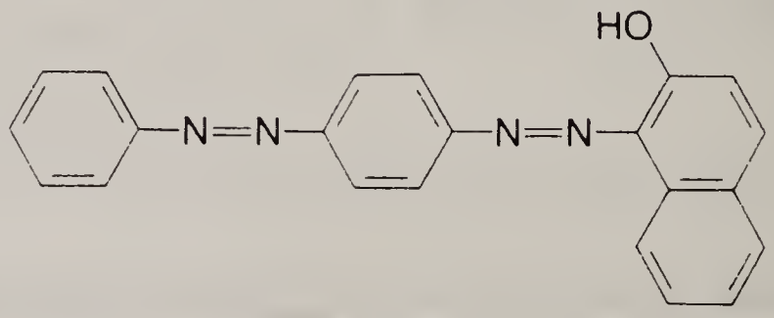
Synonyms: Lithol Rubin B Ca; CI Pigment Red 57:1 (15850:1).
CAS Reg. No.: 5281-04-9.
Chemical Structure:



Identity: Principally the calcium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl)azo]-2-naphthalenecarboxylic acid.
Empirical Formula: $C_{18}H_{12}N_2O_6SCa$.
Molecular Weight: 424.45.
Dye Classification: Monoazo.
Manufacturing Process: Heat D&C Red No. 6 with $CaCl_2$.

D&C Red No. 17

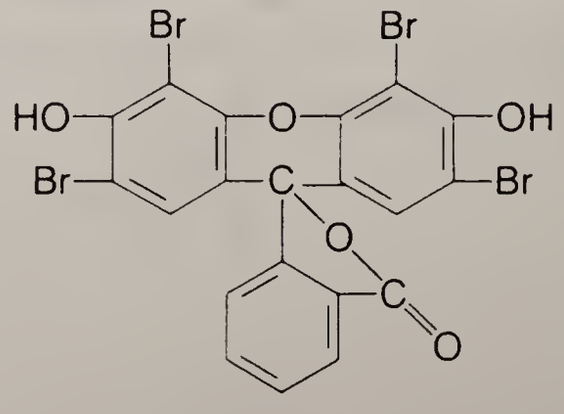
Synonyms: Toney Red, Sudan III; CI Solvent Red 23 (26100).
CAS Reg. No.: 85-86-9.
Chemical Structure:



Identity: Principally 1-[[4-(phenylazo)phenyl]azo]-2-naphthalenol.
Empirical Formula: $C_{22}H_{16}N_4O$.
Molecular Weight: 352.40.
Dye Classification: Diazo.
Manufacturing Process: Couple diazotized aminoazobenzene with 2-naphthol.

D&C Red No. 21

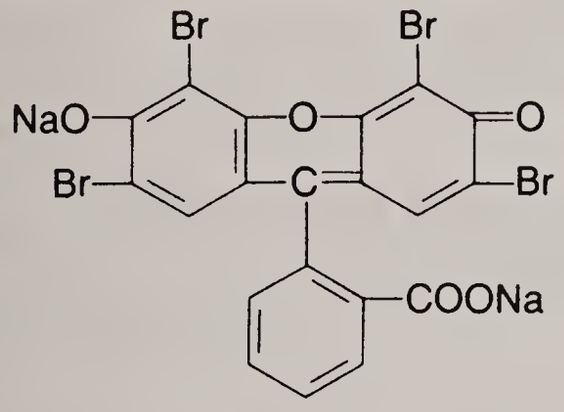
Synonyms: Tetrabromofluorescein; CI Solvent Red 43 (45380:2).
CAS Reg. No.: 15086-94-9.
Chemical Structure:



Identity: Principally 2',4',5',7'-tetrabromofluorescein (CAS Reg. No. 15086-94-9); may contain smaller amounts of 2',4',5'-tribromofluorescein (CAS Reg. No. 25709-83-5) and 2',4',7'-tribromofluorescein (CAS Reg. No. 25709-84-6).
Empirical Formula: $C_{20}H_8O_5Br_4$.
Molecular Weight: 647.90.
Dye Classification: Fluoran.
Manufacturing Process: Brominate fluorescein (D&C Yellow No. 7).

D&C Red No. 22

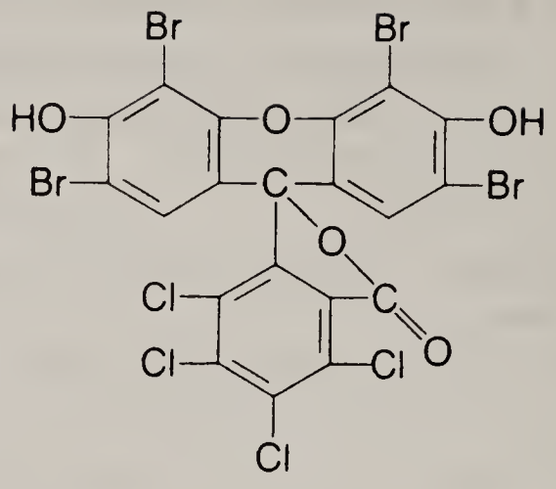
Synonyms: Eosin Y; CI Acid Red 87 (45380).
CAS Reg. No.: 17372-87-1.
Chemical Structure:



Identity: Principally the disodium salt of 2',4',5'7'-tetrabromofluorescein; may contain smaller amounts of the disodium salts of 2',4',5'-tribromofluorescein and 2',4',7'-tribromofluorescein.
Empirical Formula: $C_{20}H_6O_5Br_4Na_2$.
Molecular Weight: 691.87.
Dye Classification: Xanthene.
Manufacturing Process: Convert D&C Red No. 21 to the Na salt.

D&C Red No. 27

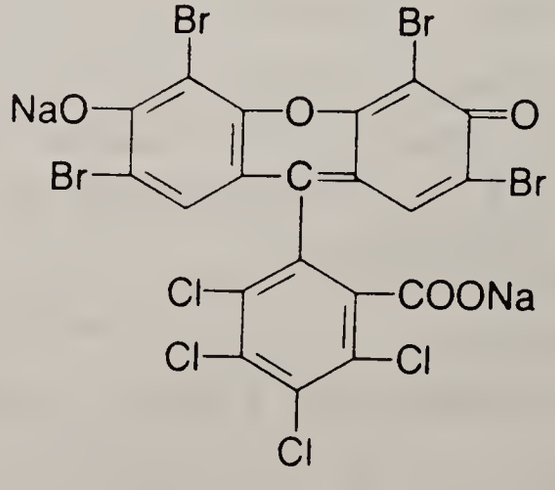
Synonyms: Tetrabromotetrachlorofluorescein; CI Solvent Red 48 (45410:1).
CAS Reg. No.: 13473-26-2.
Chemical Structure:



Identity: Principally 2',4',5',7'-tetrabromo-4,5,6,7-tetrachlorofluorescein.
Empirical Formula: $C_{20}H_4O_5Cl_4Br_4$.
Molecular Weight: 785.69.
Dye Classification: Fluoran.
Manufacturing Process: Condense resorcinol with tetrachlorophthalic anhydride, then brominate.

D&C Red No. 28

Synonyms: Phloxine B; CI Acid Red 92 (45410).
CAS Reg. No.: 18472-87-2.
Chemical Structure:

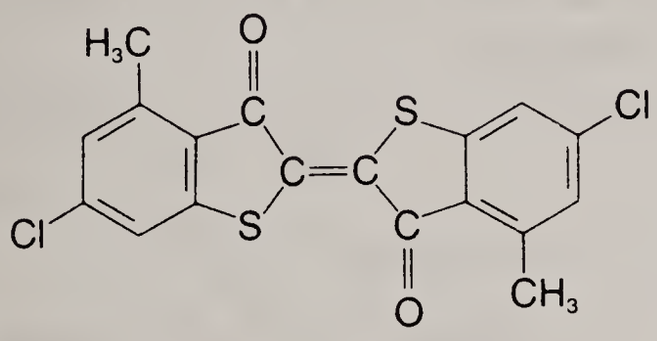


Identity: Principally the disodium salt of 2',4',5',7'-tetrabromo-4,5,6,7-tetrachlorofluorescein.
Empirical Formula: $C_{20}H_2O_5Cl_4Br_4Na_2$.
Molecular Weight: 829.65.

Dye Classification: Xanthene.
Manufacturing Process: Convert D&C Red No. 27 to the Na salt.

D&C Red No. 30

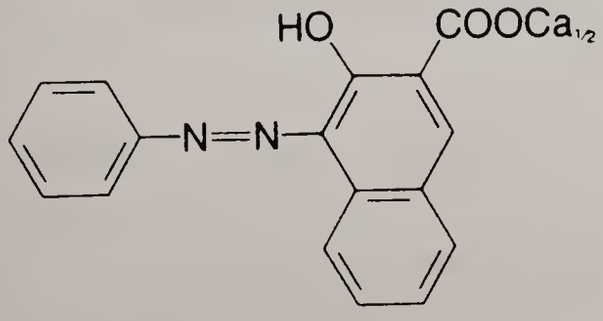
Synonyms: Helindone Pink CN; CI Vat Red 1 (73360).
CAS Reg. No.: 2379-74-0.
Chemical Structure:



Identity: Principally 6-chloro-2-(6-chloro-4-methyl-3-oxobenzo[*b*]thien-2(3*H*)-ylidene)-4-methyl-benzo[*b*]thiophen-3(2*H*)-one.
Empirical Formula: $C_{18}H_{10}O_2S_2Cl_2$.
Molecular Weight: 393.31.
Dye Classification: Indigoid.
Manufacturing Process: Oxidize 6-chloro-4-methyl-thioindoxyl, or chlorinate 4,4'-dimethylthioindigo.

D&C Red No. 31

Synonyms: Brilliant Lake Red R; CI Pigment Red 64:1 (15800:1).
CAS Reg. No.: 6371-76-2.
Chemical Structure:

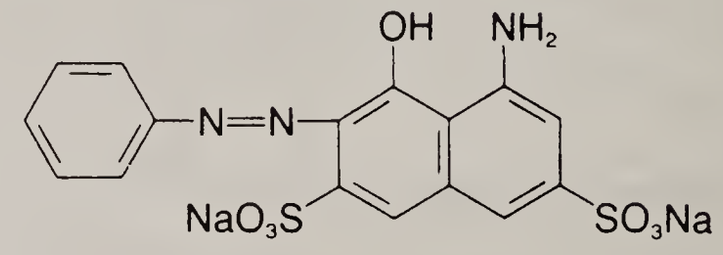


Identity: Principally the calcium salt of 3-hydroxy-4-(phenylazo)-2-naphthalenecarboxylic acid.

Empirical Formula: $C_{17}H_{11}N_2O_3Ca_{1/2}$.
Molecular Weight: 311.33.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized aniline with 3-hydroxy-2-naphthoic acid and then convert to the Ca salt.

D&C Red No. 33

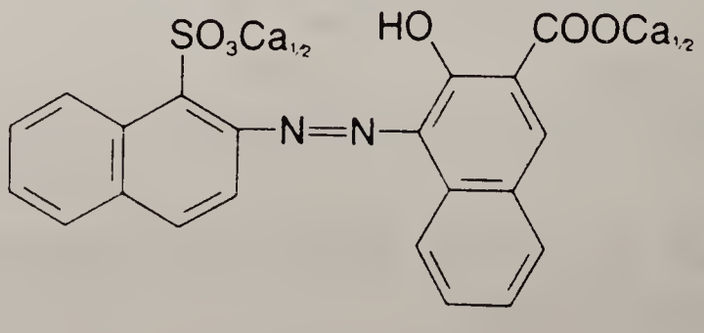
Synonyms: Acid Fuchsine; CI Acid Red 33 (17200).
CAS Reg. No.: 3567-66-6.
Chemical Structure:



Identity: Principally the disodium salt of 5-amino-4-hydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid.
Empirical Formula: $C_{16}H_{11}N_3O_7S_2Na_2$.
Molecular Weight: 467.39.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized aniline with 8-amino-1-naphthol-3,6-disulfonic acid in alkaline solution.

D&C Red No. 34

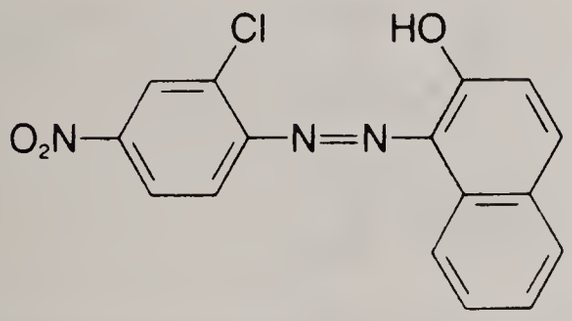
Synonyms: Deep Maroon, Fanchon Maroon, Lake Bordeaux B; CI Pigment Red 63:1 (15880:1).
CAS Reg. No.: 6417-83-0.
Chemical Structure:



Identity: Principally the calcium salt of 3-hydroxy-4-[(1-sulfo-2-naphthalenyl)azo]-2-naphthalenecarboxylic acid.
Empirical Formula: $C_{21}H_{12}N_2O_6SCa$.
Molecular Weight: 460.48.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 2-naphthylamine-1-sulfonic acid with 3-hydroxy-2-naphthoic acid and then convert to the Ca salt.

D&C Red No. 36

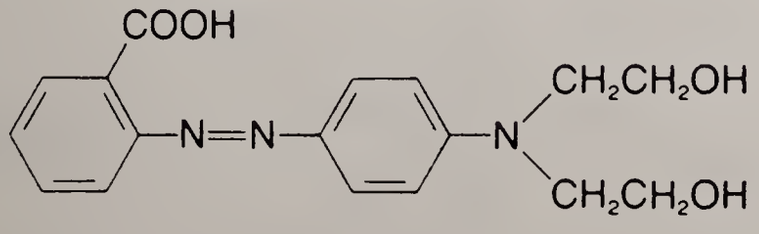
Synonyms: Flaming Red; CI Pigment Red 4 (12085).
CAS Reg. No.: 2814-77-9.
Chemical Structure:



Identity: 1-[(2-Chloro-4-nitrophenyl)azo]-2-naphthalenol.
Empirical Formula: $C_{16}H_{10}N_3O_3Cl$.
Molecular Weight: 327.73.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 2-chloro-4-nitroaniline with 2-naphthol.

D&C Red No. 39

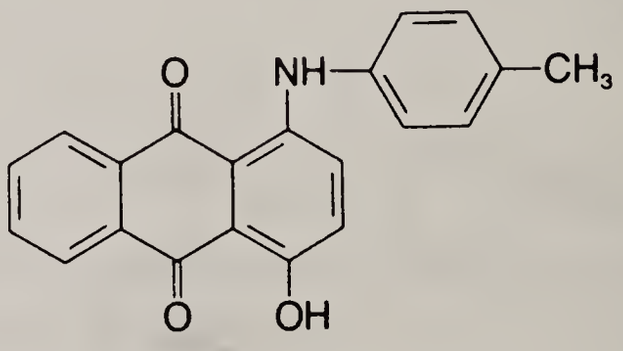
Synonyms: Alba Red; CI Pigment Red 100 (13058).
CAS Reg. No.: 6371-55-7.
Chemical Structure:



Identity: o[*p*-(β,β'-Dihydroxy-diethylamino)phenylazo]-benzoic acid.
Empirical Formula: $C_{17}H_{19}N_3O_4$.
Molecular Weight: 329.36.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized anthranilic acid with 2,2'-(phenylimino)diethanol.

D&C Violet No. 2

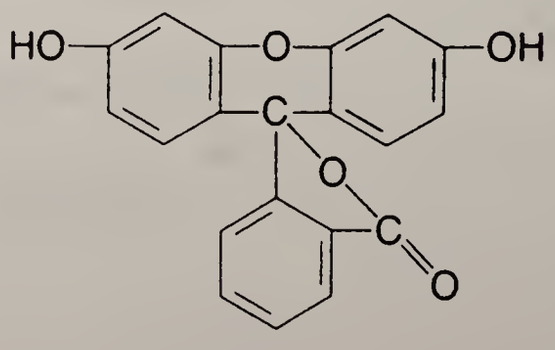
Synonyms: Alizurol Purple SS; CI Solvent Violet 13 (60725).
CAS Reg. No.: 81-48-1.
Chemical Structure:



Identity: Principally 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthracenedione.
Empirical Formula: $C_{21}H_{15}NO_3$.
Molecular Weight: 329.36.
Dye Classification: Anthraquinone.
Manufacturing Process: Condense quinizarin with *p*-toluidine, or condense 1-hydroxy-4-halogenoanthraquinone with *p*-toluidine.

D&C Yellow No. 7

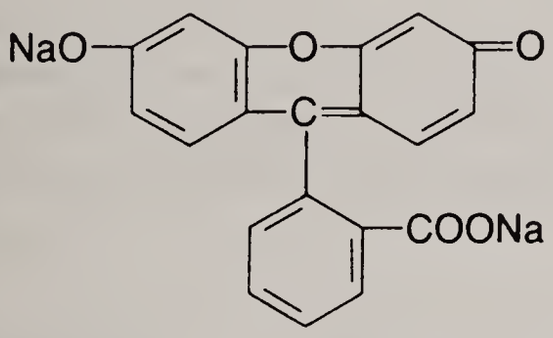
Synonyms: Fluorescein; CI Solvent Yellow 94 (45350:1).
CAS Reg. No.: 2321-07-5.
Chemical Structure:



Identity: Principally fluorescein.
Empirical Formula: $C_{20}H_{12}O_5$.
Molecular Weight: 332.31.
Dye Classification: Fluoran.
Manufacturing Process: Condense resorcinol with phthalic anhydride in the presence of $ZnCl_2$ or H_2SO_4.

D&C Yellow No. 8

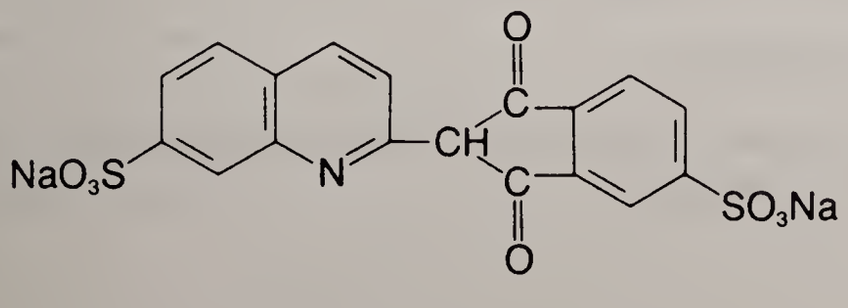
Synonyms: Uranine; CI Acid Yellow 73 (45350).
CAS Reg. No.: 518-47-8.
Chemical Structure:



Identity: Principally the disodium salt of fluorescein.
Empirical Formula: $C_{20}H_{10}O_5Na_2$.
Molecular Weight: 376.28.
Dye Classification: Xanthene.
Manufacturing Process: Convert D&C Yellow No. 7 to the Na salt.

D&C Yellow No. 10

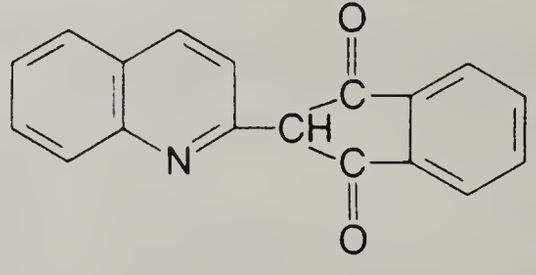
Synonyms: Quinoline Yellow; CI Acid Yellow 3 (47005).
CAS Reg. No.: 8004-92-0.
EEC No.: E 104.
Chemical Structure:



Identity: A mixture of the sodium salts of the mono- and disulfonic acids of 2-(2-quinolinyl)-1*H*-indene-1,3 (2*H*)-dione consisting principally of the sodium salts of 2-(2,3-dihydro-1,3-dioxo-1*H*-indene-2-yl)-6-quinolinesulfonic acid and 2-(2,3-dihydro-1,3-dioxo-1*H*-indene-2-yl)-8-quinolinesulfonic acid with lesser amounts of the disodium salts of the disulfonic acids of 2-(2-quinolinyl)-1*H*-indene-1,3(2*H*)-dione (CAS Reg. No. 8004-92-0).
Empirical Formula: $C_{18}H_9NO_8S_2Na_2$.
Molecular Weight: 477.38.
Dye Classification: Quinoline.
Manufacturing Process: Sulfonate D&C Yellow No. 11.

D&C Yellow No. 11

Synonyms: Quinoline Yellow SS, Quinoline Yellow Spirit Soluble; CI Solvent Yellow 33 (47000).
CAS Reg. No.: 8003-22-3.
Chemical Structure:

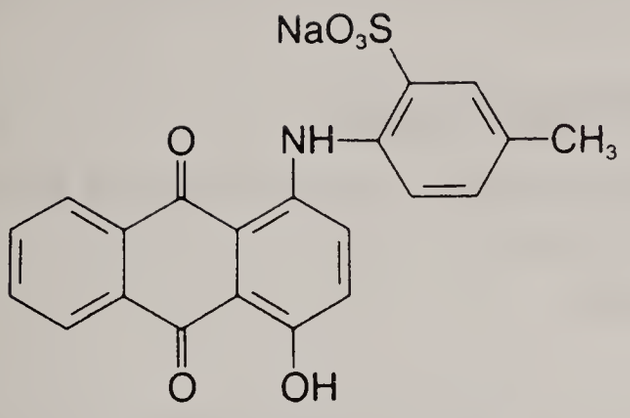


Identity: Principally 2-(2-quinolyl)-1,3-indandione.
Empirical Formula: $C_{18}H_{11}NO_2$.
Molecular Weight: 273.29.
Dye Classification: Quinoline.
Manufacturing Process: Condense quinaldine with phthalic anhydride in the presence of $ZnCl_2$.

Ext. D&C Violet No. 2

Synonyms: Alizarine Violet; CI Acid Violet 43 (60730).
CAS Reg. No.: 4430-18-6.

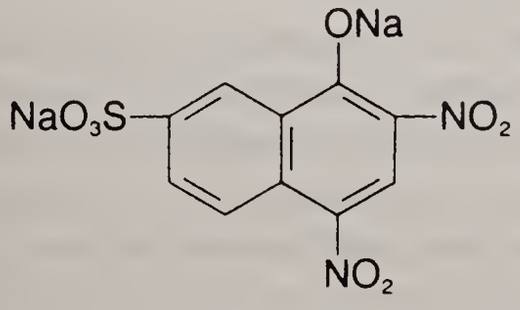
Chemical Structure:



Identity: Principally the monosodium salt of 2-[(9,10-dihydro-4-hydroxy-9,10-dioxo-1-anthracenyl)amino]-5-methylbenzesulfonic acid.
Empirical Formula: $C_{21}H_{14}NO_6SNa$.
Molecular Weight: 431.40.
Dye Classification: Anthraquinone.
Manufacturing Process: Sulfonate D&C Violet No. 2 and then convert to the sodium salt.

Ext. D&C Yellow No. 7

Synonyms: Naphthol Yellow S; CI Acid Yellow 1 (10316).
CAS Reg. No.: 846-70-8.
Chemical Structure:

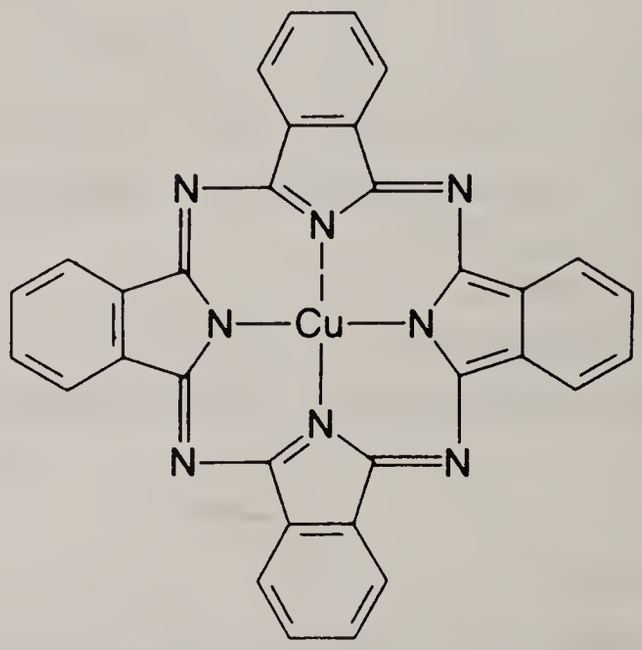


Identity: Principally the disodium salt of 8-hydroxy-5,7-dinitro-2-naphthalenesulfonic acid.
Empirical Formula: $C_{10}H_4N_2O_8SNa_2$.
Molecular Weight: 358.19.
Dye Classification: Nitro.

Manufacturing Process: Nitrate the di- or trisulfonic acids of 1-naphthol or the nitroso compound of the 2,7-disulfonic acid.

[Phthalocyaninato(2-)] Copper

Synonyms: Copper Phthalocyanine; CI Pigment Blue 15 (74160).
CAS Reg. No.: 147-14-8.
Chemical Structure:



Identity: [Phthalocyaninato(2-)] copper.
Empirical Formula: $C_{32}H_{16}CuN_8$.
Molecular Weight: 576.08.
Dye Classification: Phthalocyanine.
Manufacturing Processes: (a) Heat phthalonitrile with cuprous chloride at 180-200° C. (b) Heat phthalic anhydride, phthalimide, or phthalamide with a copper salt and urea, cyanoguanidine or *p*-toluenesulfonamide and cuprous (or cupric) chloride in the presence of ammonium molybdate or arsenic oxide (phthalic anhydride/urea process).

5

COLORANTS EXEMPT FROM CERTIFICATION

By law, the Commissioner of Food and Drug has the authority to exempt particular color additives from the batch-certification procedure whenever he believes that, because of their nature, certification is not needed to protect the public health (see p. 21). This chapter deals with those additives.

Although "exempt" colorants need not be certified prior to their sale, they are subject to surveillance by FDA to ensure that they meet current government specifications and that they are used in accordance with the law. Specifications for exempt colorants are given in Appendix A. Restrictions pertaining to their use are discussed in Chapters 1–3.

With the passage of the 1960 amendments, all exempt colorants then in use were provisionally listed pending completion of the studies needed to obtain their "permanent" listing. Since that time, most of them as well as several completely new colors have achieved this status. Exempt color additives now in use and their status are shown in Tables 1.1–1.4.

Exempt colorants are made up of a wide variety of organic and inorganic compounds representing the animal, vegetable, and mineral

kingdoms. Some, like β-carotene and zinc oxide, are essentially pure factory-produced chemicals of definite and known composition. Others, including annatto extract, cochineal extract, caramel and beet powder are mixtures obtained from natural sources and have somewhat indefinite compositions. Many of the additives included in Tables 1.1–1.4 are relatively unimportant as colorants and are only classified as such because of the loose definition of a color additive given in the 1960 amendments. Only the more important of the colorants are considered in detail here.

In general, exempt colorants have less coloring power than certified colorants and thus have to be used at higher levels. Some—particularly those of plant origin—tend to be less stable, more variable in shade, and therefore more complicated to use than certified colorants, and are more likely to introduce undesirable flavors and odors into the products in which they are incorporated. Also, depending on their nature and origin, exempt colorants can vary substantially in composition from batch to batch, are more likely to be contaminated with undesirable trace metals, insecticides, herbicides, and bacteria such as *Salmonella,* and can be more difficult to obtain in steady supply compared with certified colorants.

Exempt colorants are inherently neither more nor less safe than their certified cousins. However, some see them as having been obtained from nature ("natural") and, as such, imagine them as less of a health hazard than certified colorants. In fact, they, like all color additives, are fabricated products.

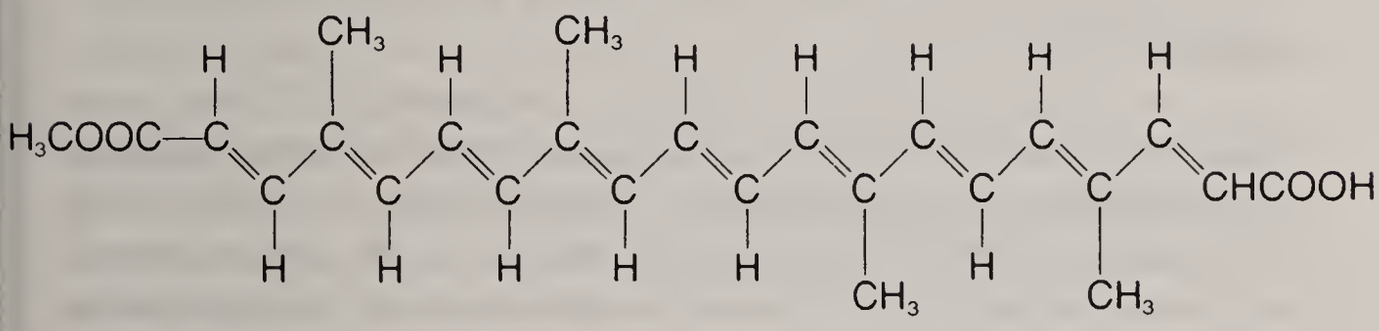
In the descriptions of exempt colorants which follow, Colour Index (CI), Chemical Abstract Service (CAS), and European Economic Community (EEC) identification numbers are included where known.

ANNATTO EXTRACT

The annatto tree (*Bixa orellana)* is a large, fast-growing shrub cultivated in tropical climates, including parts of South America, India, East Africa, and the Caribbean. The tree produces large clusters of brown or crimson capsular fruit containing seeds coated with a thin, highly colored resinous coating or mark that serves as the raw material for preparation of the colorant known an annatto extract (CAS Reg. No. 8015-67-6).

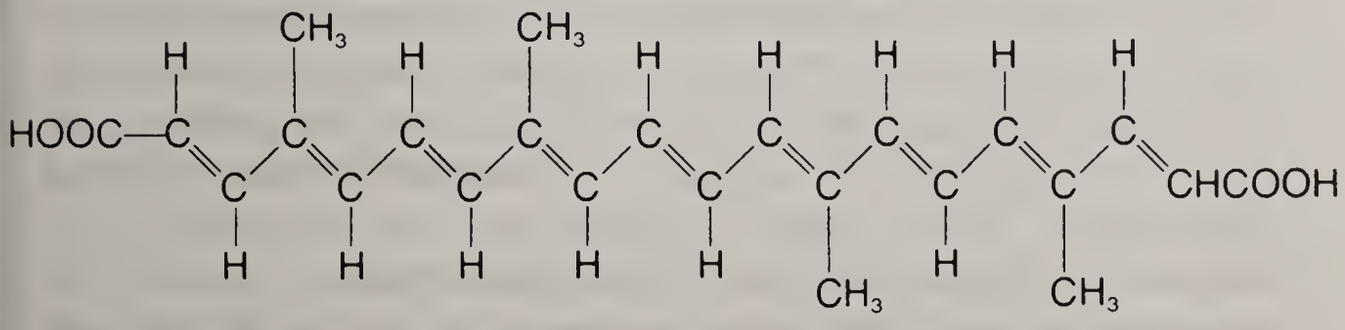
The colorant is prepared by leaching the annatto seeds with an extractant prepared from one or more approved, food-grade materials taken from a list that includes various solvents, edible vegetable oils and fats, and alkaline aqueous and alcoholic solutions. Depending on the use intended, the alkaline extracts are often treated with food-grade acids to precipitate the annatto pigments, which in turn may or may not be further purified by recrystallization from an approved solvent. Annatto extract is one of the oldest known dyes, used since antiquity for the coloring of food, textiles, and cosmetics. It has been used in the United States and Europe for over 100 years as a color additive for butter and cheese.

The chief coloring principle found in the oil or fat extracts of annatto seeds is the carotenoid bixin (CI Natural Orange 4, CI No. 75120, EEC No. E 160b), which is the monomethylester of the dicarboxylic acid norbixin:



BIXIN: $C_{25}H_{30}O_4$ (mw 394.51)

The major colorant in alkaline aqueous extracts is norbixin:



NORBIXIN: $C_{24}H_{28}O_4$ (mw 380.48)

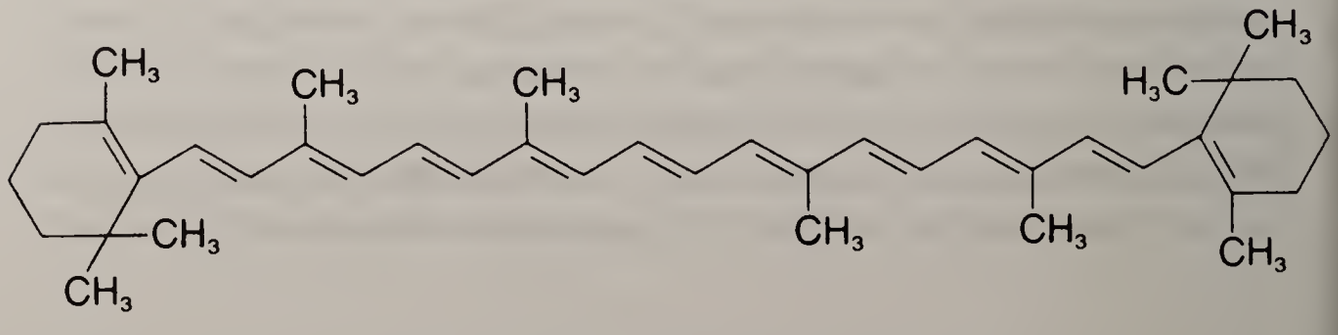
Annatto extract is sold in several physical forms, including dry powders, propylene glycol/monoglyceride emulsions, oil solutions and suspensions, and alkaline aqueous solutions containing anywhere from 0.1–30% active colorant calculated as bixin or norbixin, as appropriate. It is used in products at levels of 0.5–10 ppm as pure color, resulting in hues

ranging from butter-yellow to peach, depending on the type of color preparations employed and the product colored. Annatto extract's chief use is in foods such as butter, margarine, processed cheeses, non-dairy creamers, cooking oils, salad dressings, cereals, ice cream, ice cream cones, sausage casings, bakery goods, snack foods, and spices. It is often used in combination with turmeric.

The chemistry and performance of annato extract is essentially that of bixin, a brownish-red crystalline material that melts at 198°C. It is moderately stable toward light and has good stability toward oxidation, change in pH, and microbiological attack. Bixin is very stable toward heat up to 100°C, fairly stable at 100–125°C, and unstable above 125°C, where it tends to form 13-carbomethoxy-4,8-dimethyltridecahexane-oic acid.

β-CAROTENE

β-Carotene is an isomer of the naturally occurring carotenoid, carotene (CI Food Orange 5, CI No. 40800, CI Natural Yellow 26, CI No. 75130, CAS Reg. No. 7235-40-7, EEC No. E 160a). It is the pigment largely responsible for the color of various products obtained from nature including butter, cheese, carrots, alfalfa, and certain cereal grains. The colorant is synthetically produced from acetone, using the process developed in the 1950s by Hoffmann-LaRoche Inc., which results in the formation of the optically inactive all-*trans* form. It is this synthesis that made β-carotene so important in the history of the use of color additives since it was one of the first "natural" colorants synthetically produced on a commercial scale and the one that eventually raised the question as to whether factory-produced analogues of natural colorants should require certification by the FDA such as "coal-tar dyes" do, and whether such compounds could continue to be referred to as "natural colors." This controversy eventually led to the creation of the category of colorants called "colorants exempt from certification."



β-CAROTENE: $C_{40}H_{56}$ (mw 536.89)

β-Carotene forms reddish-violet platelets that melt in the range 176–182°C. It is insoluble in water, ethanol, glycerine, and propylene glycol, and only slightly soluble in boiling organic solvents such as ether (0.05%), benzene (0.2%), carbon disulfide (1%), and methylene chloride (0.5%). Its solubility in edible oils is about 0.08% at room temperature, 0.2% at 60°C, and 0.8% at 100°C. β-Carotene is sensitive to alkali and very sensitive to air and light, particularly at high temperatures. Pure, crystalline β-carotene remains unchanged for long periods of time when stored under CO_2 below 20°C but is almost completely destroyed after only 6 weeks when stored in air at 45°C. Vegetable fat and oil solutions and suspensions are quite stable under normal handling conditions. β-Carotene is a rarity among color additives in that it is one of the few with nutritional value since it is converted biologically by humans into vitamin A; 1 g of β-carotene = 1,666,666 USP units of vitamin A.

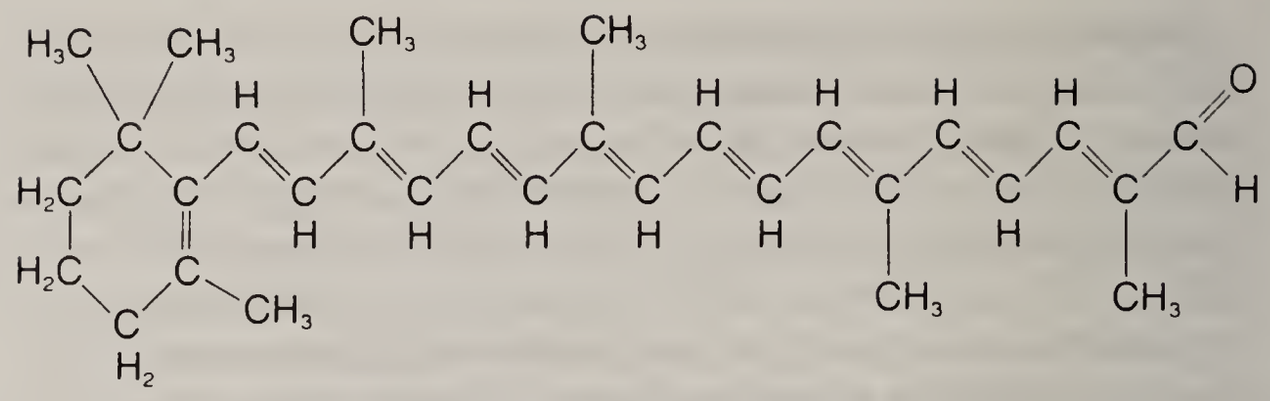
β-Carotene is marketed as dry crystals packed under nitrogen, as a dry water-dispersible powder containing about 1% β-carotene, dextrin, gum acacia, partially hydrogenated vegetable oil, sucrose, sodium ascorbate, and *dl*-alpha tocopherol, as liquid and semisolid suspensions in edible oils including vegetable, peanut, and butter oils, as water-dispersible beadlets composed of colorant plus vegetable oil, sugar, gelatin, and carbohydrate, and as emulsions.

The colorant is used at levels ranging from 2–50 ppm as pure color to shade margarine, shortening, butter, cheese, baked goods, confections, ice cream, eggnog, macaroni products, soups, juices, and beverages. Its chief advantages over other colorants are its nutritional value and its ability to duplicate natural yellow to orange shades.

β-APO-8′-CAROTENAL

This colorant (CI Food Orange 6, CI No. 40820, CAS Reg. No. 1107-26-2, EEC No. E 160e) is an aldehydic carotenoid widely distributed in nature; it is isolated from numerous items, including spinach, oranges, grass, tangerines, and marigolds. It is synthetically produced as the crystalline all-*trans* stereoisomer, which is a purplish-black powder that melts (with decomposition) in the range 136–140°C (corrected).

β-Apo-8′-carotenal has provitamin activity with 1 g of the colorant equal to 1,200,000 IU of vitamin A. Like all crystalline carotenoids, it



β-APO-8′-CAROTENAL: $C_{30}H_{40}O$ (mw 416.65)

slowly decomposes in air through oxidation of its conjugated double bonds and thus must be stored in sealed containers under an atmosphere of inert gas, preferably under refrigeration. Also like other carotenoids β-apo-8′-carotenal readily isomerizes to a mixture of its *cis* and *trans* stereoisomers when its solutions are heated to about 60°C or exposed to ultraviolet light.

In general, its solubility characteristics are similar to those of β-carotene except that it is slightly more soluble in the usual solvents. In addition, because of its aldehydic group, β-apo-8′-carotenal is slightly soluble in polar solvents such as ethanol. Its solubility in various solvents is:

Solvent	Solubility at 24°C in Weight Percent
Vegetable oils	0.7–1.5
Orange oil	1.5–2.0
Ethanol	~0.2
Propylene glycol	Trace
Cyclohexane	~0.7
Chloroform	>1.0

Vegetable-oil solutions of the colorant are orange to red, depending on their concentration. Aqueous dispersions range in hue from orange to orange-red.

β-Apo-8′-carotenal is sold as a dry powder, as 1–1.5% vegetable oil solutions, as 20% suspensions in vegetable oil, as 2–4% solutions in a mixture of monoglycerides and *dl*-α-tocopherol, and as 10% dry beadlets. The vegetable-oil suspensions are purplish-black fluids at room temperatures that set to thick pastes when refrigerated. The dry beadlets

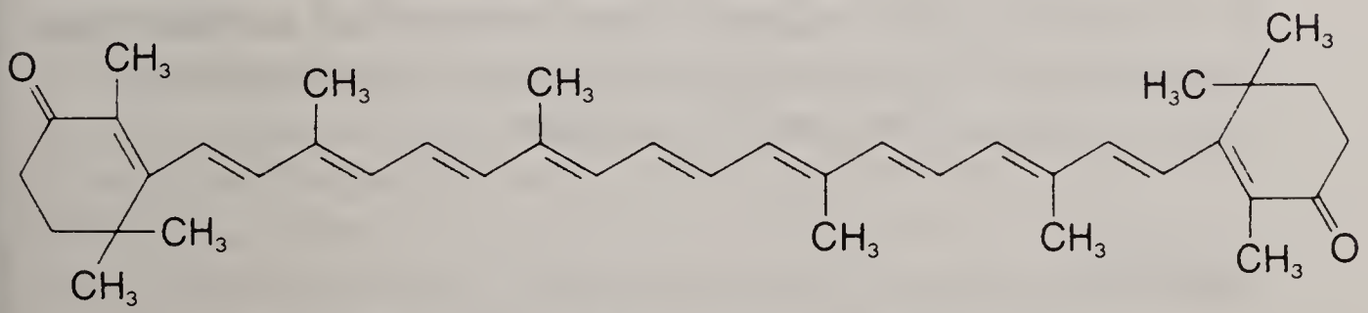
are colloidal dispersions of colorant in a matrix of gelatin, vegetable oil, sugar, starch, and antioxidants.

β-Apo-8′-carotenal is used wherever an orange to reddish-orange shade is desired. The dry beadlets are water-dispersible and can be used to color aqueous-based foods and beverages such as juices, fruit drinks, soups, jams, jellies, and gelatins. The vegetable-oil solutions and suspensions are most useful in fat base or fat-containing foods including processed cheese, margarine, salad dressings, fats, and oils. Use levels typically range within 1–20 ppm as pure color.

CANTHAXANTHIN

The newest of the synthetically produced carotenoid color additives, canthaxanthin (β-carotene-4,4′-dione, CAS Reg. No. 514-78-3), became commercially available about 1969. Its CI designation is Food Orange 8, CI No. 40850. Its EEC designation is E 160g.

Unknown until 1950 when F. Haxo isolated it from an edible mushroom (*Cantharellus cinnabarinus*), canthaxanthin has since been identified in sea trout, algae, daphnia, salmon, brine shrimp, and several species of flamingo. Crystalline canthaxanthin is prepared synthetically from acetone or β-ionone using procedures similar to those used for β-carotene and β-apo-8′-carotenal.



CANTHAXANTHIN: $C_{40}H_{52}O_2$ (mw 564.85)

Canthaxanthin crystallizes from various solvents as brownish-violet, shiny leaves that melt with decomposition at 210°C. As is the case with carotenoids in general, the crystals are sensitive to light and oxygen and, when heated in solution or exposed to ultraviolet light or iodine, form a mixture of *cis* and *trans* stereoisomers. Consequently, crystalline canthaxanthin should be stored under inert gas at low temperatures. Unlike

its cousin carotenoid colorants β-carotene and β-apo-8′-carotenal, canthaxanthin has no vitamin A activity. It is chemically stable at pH 2–8 (the range normally encountered in foods) and unaffected by heat in systems with a minimal oxygen content.

The solubility of canthaxanthin in most solvents is low compared with β-carotene and β-apo-8′-carotenal. Some representative values follow:

Solvent	Solubility at 25°C in Weight Percent
Vegetable oils	0.02
Orange oil	2.0
Ethanol	Insoluble
Acetone	0.03
Propylene glycol	Trace
Benzene	0.2
Chloroform	10

Oil solutions of canthaxanthin are red at all concentrations. Aqueous dispersions are orange or red depending on the type of emulsion prepared.

Besides as a dry powder, canthaxanthin is commercially available as a water-dispersible, dry beadlet composed of 10% colorant, gelatin, vegetable oil, sugar, starch, antioxidants, and preservatives. Canthaxanthin is used at levels of 5–60 ppm as pure color to produce a tomato red. The colorant is useful in coloring tomato products such as tomato soup, spaghetti sauce, and pizza sauce, Russian and French dressings, fruit drinks, sausage products, and baked goods.

CARAMEL

Officially, "The color additive caramel is the dark-brown liquid or solid material resulting from the carefully controlled heat treatment of the following food-grade carbohydrates: dextrose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof, or sucrose." Practically speaking, caramel is burned sugar.

Caramel (CI Natural Brown 10, EEC No. E 150) is most often made from liquid corn syrup with a reducing sugar content of 60% or more,

expressed as dextrose. Sucrose (cane sugar) is less frequently used as the starting material because of its relatively high cost and because of process difficulties often encountered when using it since, after inversion, the dextrose and levulose present react at different rates, making the burning process difficult to control, sometimes resulting in a product inferior to that made from corn sugar. In most cases a small amount of an approved acid, alkali, or salt is used to expedite the reaction and to obtain products with specific properties for specific applications.

To prepare the colorant, the liquid corn sugar and the appropriate reactants are cooked at about 250°F for several hours or until the proper tinctorial power has been obtained. The product is then filtered and stored cool to minimize further caramelization. Often it is drum- or spray-dried to produce free-flowing powders containing 5% or less moisture.

Because of the many variables in ingredients and process conditions involved in the manufacturing of caramel, its exact chemical composition is unknown. Caramel coloring is freely soluble in water and insoluble in most organic solvents. Its solubility in solutions containing 50-70% alcohol varies with the type of caramel. In concentrated form the colorant has a distinctive burned taste that is unnoticeable at the typical levels of use. The specific gravity of caramel coloring syrups ranges from 1.25 to 1.38, whereas the total solids content varies from 50% to 75%. The pH of the acid-proof caramels used for carbonated beverages and acidified solutions is normally 2.8-3.5. Most bakers' caramels, which are a less refined grade of colorant used for cookies, cakes, bread, and so on, have a higher pH due to differences in their manufacturing processes.

In aqueous solution, caramel coloring exhibits colloidal properties, with the particles carrying small positive or negative electrical charges, depending on the method used in its manufacture and the pH of the product being colored. The nature of this charge is most important in using caramel since it must be the same as that of the product it is added to, or else mutual attraction will occur causing flocculation or precipitation. A good soft drink caramel should carry a strong negative charge and have an isoelectric point at pH = 1.5 or less. Beer caramel usually has a positive charge.

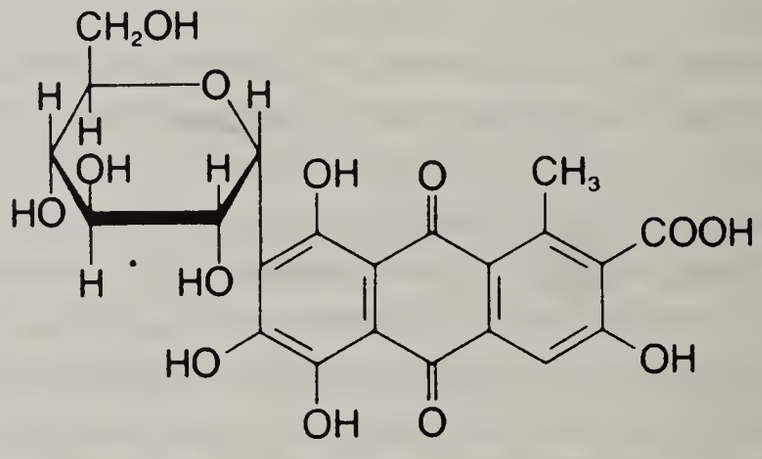
75–85% of the caramel produced in the United States is used in soft drinks, particularly root beers and colas. Caramel is also used extensively to standardize the hue of blended whiskeys, liqueurs, wines, and beer. Other uses include the coloring of baked goods, syrups, preserves, can-

dies, pet foods, gravies, canned meat products, soups, condiments, vinegars, dark sugars, cough syrups, and pharmaceuticals. Where the use of liquid coloring is impractical, such as in cake mixes and other dry products, powdered caramel is added. Typical use levels are high (0.1-30%), but the colorant is relatively inexpensive and shows good stability in most products.

Shades that can be produced using caramel colorants range from delicate yellows, to reds, to the darkest browns.

CARMINE; COCHINEAL EXTRACT

Among the more interesting of the color additives in use today are cochineal extract and its related colorant, carmine. They are interesting not only because of their characteristics, but also because of the part



CARMINIC ACID: $C_{22}H_{20}O_{13}$ (mw 492.39)

their source, cochineal, played in the political and economic history of the New World and those who settled in it.

Cochineal extract (CI Natural Red 4, CI No. 75470, EEC No. E 120) is the concentrated solution obtained after removing the alcohol from an aqueous-alcoholic extract of cochineal, which is the dried bodies of the female insect *Coccus cacti (Dactylopius coccus costa)*, a variety of shield louse. The coloring principle of the extract is believed to be carminic acid, an hydroxyanthraquinone linked to a glucose unit, comprising approximately 10% of cochineal and 2–4% of its extract.

Carmine (CAS Reg. No. 1390-65-4) is the aluminum or calcium-aluminum lake on an aluminum hydroxide substrate of the coloring principle (again, chiefly carminic acid) obtained by the aqueous extraction of cochineal. Carmine is normally 50% or more carminic acid.

The cochineal insect lives on a species of cactus, *Nopalea coccinelliferna,* and was once known only in Mexico. The Aztecs cultivated it for its color value and often exacted it as tribute. It is believed that Cortez found native Mexicans using cochineal when he arrived there in 1518 and at first believed it to be kermes, an ancient dyestuff widely used in Europe at the time. The eventual discovery that cochineal was in fact a new colorant, and one 10 times stronger than kermes, gave the Spaniards an exclusive on what was to become an important and lucrative article of commerce that they jealously controlled until Mexico finally freed itself from Spain. By the end of the 16th century, as much as 500,000 lb of cochineal were being shipped from Mexico to Spain each year —a rather astounding figure considering that it requires about 70,000 hand-gathered insects to make a single pound of cochineal. Numerous attempts were made to raise the cochineal beetle in other areas of the world, but most failed due partly to the specialized climates needed for its cultivation and partly to the Spaniards' doggedness in guarding what they considered a good thing. In spite of these obstacles, cochineal was eventually produced elsewhere, including the Canary Islands, Spain, the East and West Indies, Palestine, and parts of Central and South America. The cochineal trade peaked about 1870 then declined rapidly due to the introduction of synthetic colors in 1856.

Cochineal extract is typically acid (pH = 5–5.3) and has a total solids content of about 6%. It frequently contains sodium benzoate as a preservative. Cochineal extract varies in shade from orange to red, depending on pH. It is insoluble in typical solvents including water, glycerine, and propylene glycol but can be dispersed in water. It exhibits good stability toward light and oxidation but poor stability toward pH and microbiological attack. Its tinctorial strength is only moderate. Use levels range from 25–1000 ppm.

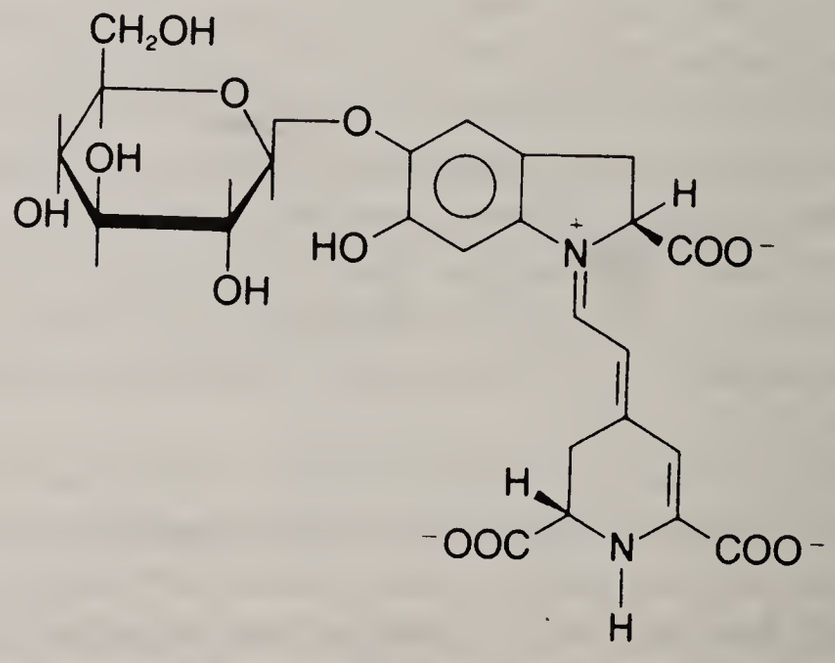
Carmine is a pigment and thus exhibits little solubility in most solvents. Since it is also an aluminum lake, it can be solubilized by strong acids and bases that cause degradation of the substratum and release of the color.

Both colorants are useful for producing pink shades in retorted meat products, candy, confections, aperitif alcoholic and soft drinks, cider, vinegar, yogurts, ice creams, baked goods, jams, jellies, rouge, eye shadow, and pill coatings.

DEHYDRATED BEETS (BEET POWDER)

This color additive is defined as "a dark red powder prepared by dehydrating sound, mature, good quality, edible beets."

Beet roots contain both red pigments (betacyanins) and yellow pigments (betaxanthins), known collectively as betalains. Generally, the betacyanin content of beets far exceeds that of the betaxanthins. Of the betacyanins present, 75–95% is betanin (EEC No. E 162), making it the principle pigment in beet colorant.



BETANIN
(CAS Reg. No. 7659-95-2)

Although many factors influence the actual quantity of pigment present in beet tissue, the average amount has been estimated as 1000 mg/100 g of total solids, or 120 mg/100 g of fresh weight.

Beet extract is also used as colorant. Extract is sold as either a concentrate prepared by evaporating beet juice under vacuum to a total solids content of 40–60%, or as a powder made by spray-drying the concentrate. Both products usually contain ascorbic or citric acid as a stabilizer, and

a preservative such as sodium propionate. On a dry-weight basis, beet extract typically contains between 0.4 and 1.0% betanin, 80% sugar, 8% ash, and 10% crude protein.

Beet colorant readily dissolves in water and water-based products. It is reasonably stable when used from pH 4 to pH 7, and it is adequately light stable. However, beet colorant does degrade readily at temperatures as low as 50°C, particularly when exposed to air or light. It is most stable to heat in the range of pH 4.0–5.0. Because of the carbohydrates present in beet colorant, it tends to carry the natural flavor of beets.

Alone, beet colorant produces hues resembling raspberry or cherry. When used in combination with water-soluble annatto, strawberry shades result.

Beet colorant is best used in foods with short shelf lives that do not require high or prolonged heat treatment. When heat treatment is necessary, degradation of the colorant is minimized by adding it after the heat treatment, or as near the end of the heating cycle as possible. Beet colorant has been used successfully to color such products as hard candies, yogurts, ice creams, salad dressings, ready-made frostings, cake mixes, meat substitutes, powdered drink mixes, gravy mixes, marshmallow candies, soft drinks, and gelatin desserts. Typically, the colorant is added at levels of 0.1–1%, based on the weight of the final product.

GRAPE COLOR EXTRACT; GRAPE SKIN EXTRACT

Grape color extract (EEC No. E 163) is an aqueous solution of anthocyanin grape pigments made from Concord grapes or a dehydrated water soluble powder prepared from the aqueous solution. The aqueous solution is prepared by extracting the pigments from precipitated lees produced during the storage of Concord grape juice. It contains the common components of grape juice, namely anthocyanins, tartrates, malates, sugars, and minerals, etc., but not in the same proportion as found in grape juice. The dehydrated water soluble powder is prepared by spray drying the aqueous solution containing added malto-dextrin.

The purple color of Grape color extract is due to the presence of water-soluble pigments, mainly the 3-mono- and 3,5-di-glucosides of malvidin, delphinidin and cyanidin, and their acylated derivatives. Colorant stability is greatest below pH 4.5. Colorant intensity increases as pH falls. Grape color extract is stable to light and at temperatures adequate

for canning most fruit. It is affected by oxygen, and by SO_2 concentrations greater than 150 ppm, and by metal ions, especially tin, iron and aluminum which can complex with anthocyanins to produce a bluer color. Complexation can be controlled somewhat by the addition of metal sequestrants such as pyrophosphates, EDTA, citrate, etc. Ascorbic acid appears to improve color stability by acting as an oxygen scavenger.

Grape color extract is used to color such products as bakers jams, non-standard jellies and preserves, sherbets, ices, pops, raspberry, grape and strawberry yogurts, gelatin desserts, canned fruit, fruit sauces, candy and confections, and bakery fillings and toppings. Typical use levels are 0.05 to 0.8%, based on the weight of the finished product.

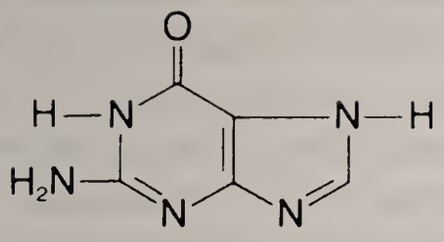
Grape skin extract (enocianina) is a purplish-red liquid prepared by the aqueous extraction (steeping) of the fresh deseeded marc remaining after grapes have been pressed to produce grape juice or wine. It contains the common components of grape juice namely, anthocyanins, tartaric acid, tannins, sugars, minerals, etc., but not in the same proportions as found in grape juice. During the steeping process, sulfur dioxide is added and most of the extracted sugars are fermented to alcohol. The extract is concentrated by vacuum evaporation, during which practically all of the alcohol is removed. A small amount of sulfur dioxide may be present.

Typically, Grape skin extract has a specific gravity of 1.13 g/mL at 20°C, a solids content of 28–32° Brix (±3°), a pH of 3.0, and a color strength as anthocyanin of about 1.25% (as measured at 520 nm in pH 3.0 citrate buffer). Grape skin extract is also available as spray-dried powders with color values three to four times those of the liquid.

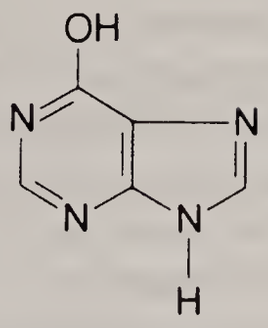
The properties and uses of Grape skin extract are similar to those of Grape color extract.

GUANINE (PEARL ESSENCE)

Guanine (CI Natural White 1, CI No. 75170), is the crystalline material obtained from fish scales and consists principally of the two purines, guanine (CAS Reg. No. 73-40-5) and hypoxanthine (CAS Reg. No. 68-94-0). The guanine content of the colorant varies from 75% to 97%, whereas the hypoxanthine content ranges from 3% to 25%, depending on the particular fish and tissue from which the crystals are derived.



GUANINE: $C_5H_5N_5O$ (mw 151.13)



HYPOXANTHINE: $C_5H_4N_4O$ (mw 136.11)

Guanine is obtained from various fish including menhaden, herring, and alewives. To prepare the colorant, scales are scraped from the fish, levigated, and washed with water, and then made into one or more commercial forms, depending on the intended end use. Typically, guanine is supplied as a paste or suspension in water, castor oil, or nitrocellulose. Guanine is not a colorant in the strict sense but instead is used to produce iridescence in a product.

The hue of the colorant varies greatly with the amount and type of pigment found in the fish scales. Carotenoids produce reds and yellows, melanin results in blacks, and combinations of guanine and melanin produce greens and blues. Only when guanine is found alone is the product silvery or pearly white.

Guanine is used in lipsticks, nail polishes, and eye makeup.

PAPRIKA AND PAPRIKA OLEORESINS

Paprika is the deep red, sweet, pungent powder prepared from the ground, dried pod of mild capsicum (*Capsicum annum*). It is one of the two principal kinds of red pepper; the other is cayenne pepper or cayenne. Paprika is produced in large quantities in Hungary and is also available from many warm-climate areas, including Africa, Spain, and the American tropics. The chief classifications of paprika are Hungarian paprika, which has the pungency and flavor characteristics of that produced in Hungary (Rosenpaprika and Koenigspaprika), and the Spanish paprika (pimenton, pimiento), which has the characteristics of paprika produced in Spain.

Paprika oleoresin (EEC No. E 160c) is the combination of flavor and color principles obtained by extracting paprika with any one or a combination of approved solvents: acetone, ethyl alcohol, ethylene dichlor-

ide, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene. Depending on their source, paprika oleoresins are brown-red, slightly viscous, homogeneous liquids, pourable at room temperature, and containing 2–5% sediment.

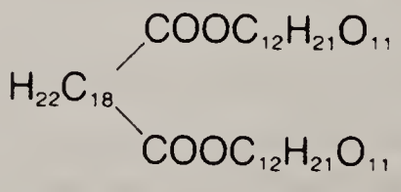
The oleoresins are available in various standardized forms in which 1 lb of oleoresin is equal to 10–30 lb of paprika. Paprika oleoresins are typically standardized by dilution with vegetable oil or mono- or diglycerides.

Paprika and its oleoresin are approved for use in foods in general where its application as a color additive frequently overlaps its use as a spice. Both products have good tinctorial strength and are used at levels of 0.2–100 ppm to produce orange to bright-red shades.

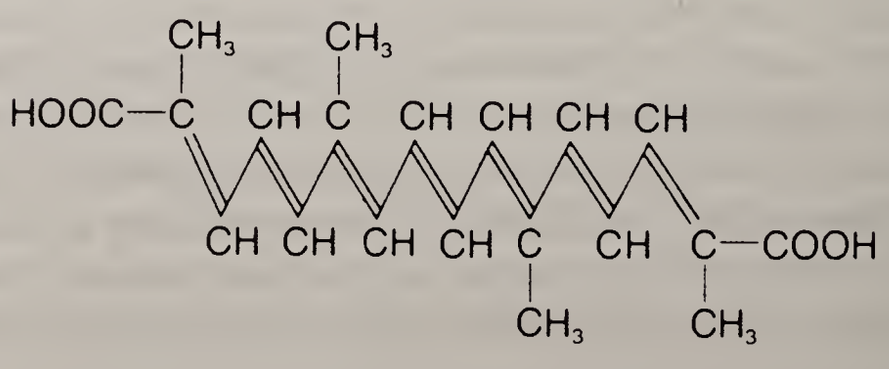
SAFFRON

Saffron, known also as CI Natural Yellow 6 (CI No. 75100), *safran, crocine, crocétine,* and *crocus,* is the dried stigma of *Crocus sativus,* a plant indigenous to the Orient but also grown in North Africa, Spain, Switzerland, Greece, Austria, and France. It is a reddish brown or golden yellow odoriferous powder having a slightly bitter taste. The stigmas of approximately 165,000 blossoms are required to make 1 kg of colorant.

The coloring principles of saffron are crocin and crocetin.



CROCIN: $C_{44}H_{64}O_{26}$ (mw 1,008.97)



CROCETIN: $C_{20}H_{24}O_4$ (mw 328.41)

Crocin is a yellow-orange glycoside that is freely soluble in hot water, slightly soluble in absolute alcohol, glycerine, and propylene glycol, and insoluble in vegetable oils. Crocin melts with decomposition at about 186°C and has absorption maxima in methanol at about 464 nm and 434 nm.

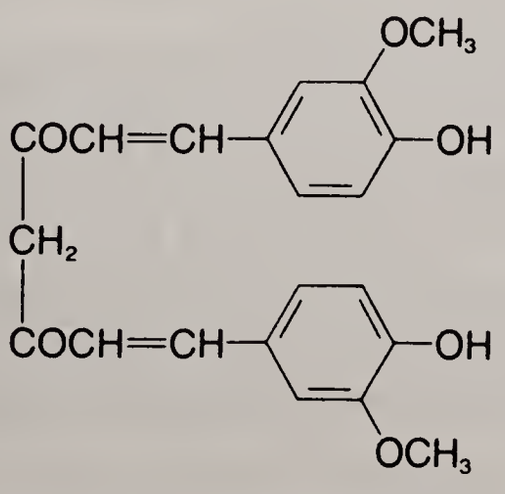
Crocetin is a dicarboxylic acid that forms brick-red rhombs from acetic anhydride that melt with decomposition at about 285°C. It is very sparingly soluble in water and most organic solvents but soluble in pyridine and similar organic bases as well as in dilute sodium hydroxide.

As a food colorant, saffron shows good overall performance. In general, it is stable toward light, oxidation, microbiological attack, and changes in pH. Its tinctorial strength is relatively high, resulting in use levels of 1–260 ppm.

TURMERIC AND TURMERIC OLEORESIN

Turmeric (CI Natural Yellow 3, CI No. 75300, EEC No. E 100) is the dried and ground rhizome or bulbous root of *Curcuma longa,* a perennial herb of the Zingiberaceae family native to southern Asia and cultivated in China, India, South America, and the East Indies. It is a yellow powder with a characteristic odor and a sharp taste.

Turmeric oleoresin is the combination of flavor and color principles obtained from turmeric by extracting it with one or a combination of the



CURCUMIN: $C_{21}H_{20}O_6$ (mw 368.39)

following solvents: acetone, ethyl alcohol, ethylene dichloride, hexane, isopropyl alcohol, methyl alcohol, methylene chloride, and trichloroethylene.

The principal coloring matter in turmeric and its oleoresin is curcumin (1,6-heptadiene-3,5-dione, 1,7-bis[4-hydroxy-3-methoxy phenyl], CAS Reg. No. 458-37-7), an orange-yellow, crystalline powder, insoluble in water and ether but soluble in ethanol and glacial acetic acid. It has a reported melting point of 180–183°C.

Turmeric is available in various powdered forms, some containing as much as 90–95% curcumin, and as suspensions in a variety of carriers, including edible vegetable oils and fats, and mono- and diglycerides, most containing 2–6% curcumin. Turmeric oleoresin is most often sold as solutions in propylene glycol with or without added emulsifying agents, typically containing 20–25% curcumin. Both products exhibit poor to moderate stability to light, oxidation, and change in pH but good tinctorial strength. Turmeric is typically used at levels of 0.2–60 ppm, whereas use levels for its oleoresin are 2–640 ppm. Both are used alone or in combination with other colorants such as annatto to shade pickles, mustard, spices, margarine, ice creams, cheeses, pies, cakes, candies, soups, cooking oils, and salad dressings. Turmeric and its oleoresin produce bright yellow to greenish-yellow shades, and are often used as replacements for FD&C Yellow No. 5.

CHROMIUM HYDROXIDE GREEN

This color additive is principally hydrated chromic sesquioxide, $Cr_2O_3 \cdot XH_2O$ (CI Pigment Green 18, CI No. 77289, CAS Reg. No. 12182-82-0, Veridian, Guignet's Green).

It is prepared by pasting potassium or sodium dichromate with three times its weight of boric acid, roasting the mixture at 500°C in a muffle furnace in an oxidizing atmosphere, then hydrolyzing the melt with water and superheated steam. The product is then dried and ground.

Chromium hydroxide green is a more bluish and brilliant green than chromium oxide greens. It is quite transparent, has good strength and excellent stability. It is used in eye makeup and soap.

CHROMIUM OXIDE GREENS

Chromium oxide greens is principally chromic sesquioxide, Cr_2O_3 (CI Pigment Green 17, CI No. 77288, CAS Reg. No. 1308-38-9). It is usually prepared by one of two methods:

1. Fuse potassium dichromate and boric acid, drown the product in water, and then dry it at high temperature.
2. Precipitate chrome alum with sodium hydroxide, then roast the chromous hydroxide; extract, wash and then dry it at a high temperature.

Cr_2O_3 is a yellowish (sage) green pigment. It has good strength and opacity, and excellent stability. It is used in eye makeup and soap.

SYNTHETIC IRON OXIDE

This colorant is one or a combination of various synthetically prepared iron oxides, including the hydrated forms. The naturally occurring oxides are unacceptable as color additives because of the difficulties frequently encountered in purifying them.

Iron oxide (EEC No. E 172) is recognized under various names, including CI Pigment Black 11 and CI Pigment Browns 6 and 7 (CI No. 77499), CI Pigment Yellows 42 and 43 (CI No. 77492), and CI Pigment Reds 101 and 102 (CI No. 77491). The chemical composition and hence the empirical formula of the colorant varies greatly with the method of manufacture used but can generally be represented as $FeO \cdot XH_2O$, $Fe_2O_3 \cdot XH_2O$, or some combination thereof. Most are made from copperas (ferrous sulfate, $FeSO_4 \cdot 7H_2O$). The commonly used forms are the yellow hydrated oxides, (ochre) and the brown, red, and black oxides.

The yellow oxides are prepared by precipitating hydrated ferric oxide from a ferrous salt using an alkali, followed by oxidation. The shades obtained range from light lemon yellow to orange, depending on the conditions used for the precipitation and oxidation. Yellow oxides contain about 85% Fe_2O_3 and 15% water of hydration.

Brown oxides are manufactured either by blending mixtures of the red, yellow, and black oxides or by precipitation of an iron salt with alkali followed by partial oxidation of the precipitate. The result is a mixture of red Fe_2O_3 (CAS Reg. No. 1309-37-1) and black Fe_3O_4 (CAS Reg. No. 1309-38-2) ($FeO \cdot Fe_2O_3$).

Red iron oxides are usually prepared by calcining the yellow oxides to form Fe_2O_3. The shade of the red oxide depends on the characteristics of the original yellow pigment, and the conditions of calcination and ranges from light to dark red. The product is 96–98.5% Fe_2O_3.

The black oxides are prepared by the controlled precipitation of Fe_3O_4 (treat $FeSO_4 \cdot 7H_2O$ with NaOH and O_2) to form a mixture of ferrous and ferric oxides.

Iron oxides are stable pigments insoluble in most solvents but usually soluble in hydrochloric acid. Those not soluble in HCl can be fused with potassium hydrogen sulfate ($KHSO_4$) and then dissolved in water.

The major use of iron oxide as a colorant is in cosmetics, particularly eye makeup and face powders. It is also permitted in dog and cat food at levels not exceeding 0.25% by weight of the finished food, and in drugs.

TALC

Talc, CI Pigment White 26, CI No. 77019, CAS Reg. No. 14807-96-6, is finely powdered, native, hydrous magnesium silicate ($3MgO \cdot 4SiO_2 \cdot H_2O$, "soapstone") sometimes containing a small amount of aluminum silicate. It is produced in many parts of the world, including France, Italy, India, and the United States. The typical composition of USP talc is:

Silicon dioxide (SiO_2)	60.13%
Magnesium oxide (MgO)	32.14%
Calcium oxide (CaO)	0.39%
Aluminum oxide (Al_2O_3)	1.84%
Ferric oxide (Fe_2O_3)	0.15%
Acid solubles	<2.0%
Water solubles	<0.1%
Loss on ignition	4.90%
Lead (Pb)	<5 ppm
Arsenic (As)	<1 ppm

Theoretically, talc is a pure white, odorless, unctuous powder rated as among the softest materials available, assigned a hardness of No. 1–1.5 on the Mohs Mineralogical Scale. Actually, it is a white-gray powder possessing varying amounts of softness and slip, depending on its origin. The best grades of talc are very white crystalline powders with a lamellar structure, a greasy feel, and a particle size of 74 μm or less. Micronized talcs are often 40 μm or less in size. The specific gravity of talc is about 2.70.

TITANIUM DIOXIDE

Titanium dioxide (TiO_2, MW 79.90, Titanic Earth, CI Pigment White 6, CI No. 77891, CAS Reg. No. 13463-67-7, EEC No. E 171) is the whitest, brightest pigment known today, with a hiding power four to five times greater than that of its closest rival, zinc oxide.

Titanium dioxide exists in nature in three crystalline forms: anatase, brookite, and rutile, with anatase as the commonly available form. Anatase has a high refractive index (2.52) and excellent stability toward light, oxidation, changes in pH, and microbiological attack. Titanium dioxide is virtually insoluble in all common solvents.

Only synthetically prepared TiO_2 can be used as a color additive. It is permitted in foods at levels up to 1% and is used to color such products as confectionary panned goods, cheeses, and icings. It is also widely used in tableted drug products and in numerous cosmetics such as lipsticks, nail enamels, face powder, eye makeup, and rouges, in amounts consistent with good manufacturing practice.

The colorant's chief disadvantages are its inability to blend well with the other ingredients usually found in powder formulations, its tendency to produce blue undertones, and its ability to catalyze the oxidation of perfumes.

ULTRAMARINES

The ultramarines are synthetic, inorganic pigments of somewhat indefinite composition. Basically, they are sodium aluminosulfosilicates with crystal structures related to the zeolites and empirical formulas that can be approximated as $Na_7Al_6Si_6O_{24}S_3$. They are intended as the duplicate of the colorants produced from the naturally occurring semiprecious gem lazurite (*Lapis lazuli*). Their color is believed due to polysulfide linkages in a highly resonant state.

Ultramarines are manufactured by the heat-treating and then very slow cooling of various combinations of kaolin (China clay), silica, sulfur, soda ash, and sodium sulfate plus a carbonaceous reducing agent such as rosin or charcoal pitch. The formulation of ingredients, temperature, time, cooling rate, subsequent treatment, and other variables determines the resultant color. Firing temperatures range from 700–800°C, whereas firing times vary from a few to as many as 150 hr.

The basic product of the ignition is Ultramarine Green (CI Pigment Green 24, CI No. 77013). This is converted into Ultramarine Blue (CI Pigment Blue 29, CI No. 77007) by further heat treatment in the presence of sulfur, or into Ultramarine Violet (CI Pigment Violet 15, CI No. 77007) by heating with 5% ammonium chloride for 4 days at 200–250°C. Ultramarine Violet is converted into Ultramarine Red (CI No. 77007) by treating it with gaseous hydrochloric acid at 70–200°C for 4 hr or by reacting it with gaseous nitric acid at higher temperatures.

Ultramarines are insoluble in water and organic solvents but soluble in acids, which cause their discoloration and the liberation of hydrogen sulfide. They have excellent permanency and resistance to alkali but poor tinting and hiding power.

Ultramarine Blue is used in salt intended for animal feed (≤0.5% w/w). All ultramarines are used in the cosmetic field in such products as mascara, eyebrow pencils, and soaps.

ZINC OXIDE

Of all the white pigments used in the cosmetic field, zinc oxide ranks among the most important. Although it does not have the hiding power of colorants such as titanium dioxide, zinc oxide has certain advantages, including its brightness, ability to provide opacity without blue undertones, adhesiveness or "stick," and therapeutic properties, as it is mildly antiseptic and has drying and healing effects on the skin.

Zinc oxide (MW 81.37; CI Pigment White 4, CI No. 77947, CAS Reg. No. 1314-13-2) is a white or yellowish white amorphous, odorless powder with pH = 6.95–7.37. It is practically insoluble in water but soluble in dilute acetic acid, mineral acids, ammonia, ammonium carbonate, and alkali hydroxides.

As a colorant, zinc oxide is used in face powders, rouges, and eye makeups at levels of 5–30%.

MISCELLANEOUS COLORANTS

Other colorants not requiring certification have been defined in the Code of Federal Regulations. Most of these are of only minor to moderate importance and have only limited usage.

Alumina—A white, odorless, tasteless, amorphous powder consisting essentially of aluminum hydroxide, $Al_2O_3 \cdot XH_2O$.

Aluminum powder—CI Pigment Metal 1, CI No. 77000, EEC No. E 173. Finely divided particles of aluminum prepared from virgin aluminum. It is free from admixture with other substances.

Bismuth citrate—The synthetically prepared crystalline salt of bismuth and citric acid, principally $BiC_6H_5O_7$.

Bismuth oxychloride—CI Pigment White 14, CI No. 77163. A synthetically prepared white or nearly white amorphous or finely crystalline, odorless powder consisting principally of BiOCl. Bismuth oxychloride is synthetic pearl essence. It is used in lipstick, nail polish, eye makeup, and other cosmetics to produce a lustrous, pearly effect. See Guanine.

Bronze powder—CI Pigment Metal 2, CI No. 77400. A very fine metallic powder prepared from alloys consisting principally of virgin electrolytic copper and zinc with small amounts of the virgin metals aluminum and tin. It contains small amounts of stearic or oleic acid as a lubricant.

Calcium carbonate—CI Pigment White 18, CI No. 77220, EEC No. E 170. A fine, white, synthetically prepared powder consisting essentially of precipitated calcium carbonate, $CaCO_3$.

Carrot oil—The liquid or the solid portion of the mixture, or the mixture itself obtained by the hexane extraction of edible carrots (*Daucus carota* L.) with subsequent removal of the hexane by vacuum distillation. The resultant mixture of solid and liquid extractives consists chiefly of oils, fats, waxes, and carotenoids naturally occurring in carrots.

Chlorophyllin-copper complex, oil soluble—The chlorophyllin is obtained by extraction from a mixture of fescue and rye grasses. The chlorophyll is acid-treated to remove chelated magnesium which is replaced with hydrogen, which in turn is replaced with copper. This mixture is diluted to 5% concentration with a mixture of palm oil, peanut oil, and hydrogenated peanut oil.

Chromium-cobalt-aluminum oxide—CI Pigment Blue 36, CI No. 77343, CAS Reg. No. 68187-11-1. A blue-green pigment obtained by calcining a mixture of chromium oxide, cobalt carbonate, and aluminum oxide. It may contain small amounts (<1% each) of oxides of barium, boron, silicon, and nickel.

Copper powder—CI Pigment Metal 2, CI No. 77400. A very fine free-flowing metallic powder prepared from virgin electrolytic copper. It contains small amounts of stearic or oleic acid as a lubricant.

Corn endosperm oil—A reddish brown liquid composed chiefly of glycerides, fatty acids, sitosterols, and carotenoid pigments obtained by isopropyl alcohol and hexane extraction from the gluten fraction of yellow corn grain.

Dihydroxyacetone—This colorant is 1,3-dihydroxy-2-propanone.

Disodium EDTA-copper—Disodium [[*N*,*N*′-1,2-ethanediylbis[*N*-(carboxymethyl)glycinato]](4–)-*N*,*N*′,O,O′,O^{N},O$^{N'}$]cuprate (2–).

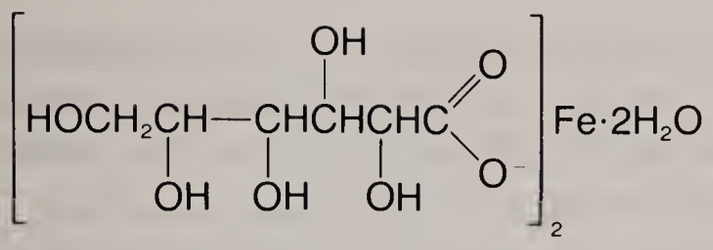
Dried algae meal—A dried mixture of algae cells (genus *Spongiococcum,* separated from its culture broth), molasses, cornsteep liquor, and a maximum of 0.3% ethoxyquin. The algae cells are produced by suitable fermentation, under controlled conditions, from a pure culture of the genus *Spongiococcum.*

Ferric ammonium citrate—A mixture of complex chelates prepared by the interaction of ferric hydroxide with citric acid in the presence of ammonia. The chelates occur in brown and green forms, are deliquescent in air, and are reducible by light.

Ferric ammonium ferrocyanide—The blue pigment obtained by oxidizing under acidic conditions with sodium dichromate the acid-digested precipitate resulting from mixing solutions of ferrous sulfate and sodium ferrocyanide in the presence of ammonium sulfate. The oxidized product is filtered, washed, and dried. The pigment consists principally of ferric ammonium ferrocyanide with small amounts of ferric ferrocyanide and ferric sodium ferrocyanide.

Ferric ferrocyanide—CI Pigment Blue 27, CI No. 77510. The color additive ferric ferrocyanide is a ferric hexacyanoferrate pigment characterized by the structural formula $Fe_4[Fe(CN)_6]_3XH_2O$, which may contain small amounts of ferric sodium ferrocyanide and ferric potassium ferrocyanide.

Ferrous gluconate—Fine yellowish gray or pale greenish yellow powder or granules having a slight odor resembling that of burned sugar. One gram dissolves in about 10 mL of water with slight heating. It is practically insoluble in alcohol. A 1:20 solution is acid to litmus.



FERROUS GLUCONATE: $C_{12}H_{22}FeO_{14}\cdot 2H_2O$ (mw 482.17)

Fruit juice—The concentrated or unconcentrated liquid expressed from mature varieties of fresh, edible fruits; or a water infusion of the dried fruit.

Guaiazulene—Principally 1,4-dimethyl-7-isopropyl-azulene.

Henna—CI Natural Orange 6, CI No. 75480. The dried leaf and petiole of *Lawsonia alba* Lam (*Lawsonia inermis* L.).

Lead acetate—The trihydrate of the lead salt of acetic acid; $Pb(OOCCH_3)_2\cdot 3H_2O$.

Logwood extract—A reddish brown-to-black solid material extracted from the heartwood of the leguminous tree *Haematoxylon campechianum.* The active colorant substance is principally hematein. The latent coloring material is the unoxidized or leuco form of hematein called *hematoxylin.* The leuco form is oxidized by air.

Manganese violet—CI Pigment Violet 16, CI No. 77742. A violet pigment obtained by reacting phosphoric acid, ammonium dihydrogen orthophosphate, and manganese dioxide at temperatures above 450°F. The pigment is a manganese ammonium pyrophosphate complex having the approximate formula: $Mn(III)NH_4P_2O_7$.

Mica—CI Pigment White 20, CI No. 77019. A white powder obtained from the naturally occurring mineral, muscovite mica, consisting predominantly of a potassium aluminum silicate, $K_2Al_4(Al_2Si_6O_{20})(OH_4)$ or, alternatively $H_2KAl_3(SiO_4)_3$. Mica may be identified and semi-quantitatively determined by its characteristic X-ray diffraction pattern and by its optical properties.

Poly(hydroxyethyl methacrylate)-dye copolymers. The color additives formed by reacting one or more of the following reactive dyes with poly(hydroxyethyl methacrylate), so that the sulfate group (or groups) or chlorine substituent of the dye is replaced by an ether linkage to poly(hydroxyethyl methacrylate). The dyes that may be used alone or in combination are

1. Reactive Black 5 [2,7-naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6-bis((4-((2-(sulfooxy)ethyl) sulfonyl) phenyl) azo)-tetrasodium salt] (CAS Reg. No. 17095-24-8).

2. Reactive Blue 21 [copper, (29*H*, 31*H*-phthalocyaninato(2-)-N^{29},N^{30}, N^{31},N^{32})-,sulfo ((4-((2-(sulfooxy)ethyl)sulfonyl)phenyl) amino) sulfonyl derivs] (CAS Reg. No. 73049-92-0).
3. Reactive Orange 78[2-naphthalenesulfonic acid, 7-(acetyl-amino)-4-hydroxy-3- ((4- ((2-sulfooxy) ethyl) sulfonyl) phenyl) azo)-] (CAS Reg. No. 68189-39-9).
4. Reactive Yellow 15 [benzensulfonic acid, 4-(4,5-dihydro-4-((2-methoxy-5-methyl-4-((2-(sulfooxy)ethyl)sulfonyl)phenyl) azo)-3-methyl- 5-oxo-1*H*-pyrazol-1-yl)-] (CAS Reg. No. 60958-41-0).
5. Reactive Blue No. 19 [2-anthracene-sulfonic acid, 1-amino-9,10-dihydro-9,10-dioxo-4-((3-((2-(sulfooxy)ethyl)sulfonyl) phenyl) amino)-, disodium salt] (CAS Reg. No. 2580-78-1).
6. Reactive Blue No. 4[2-anthracenesulfonic acid, 1-amino-4-(3-((4,6-dichloro-s-triazin-2-yl) amino)-4-sulfoanilino)-9,10-dihydro-9,10-dioxo, disodium salt] (CAS Reg. No. 4499-01-8).
7. C.I. Reactive Red 11[5-((4,6-dichloro-1,3,5-triazin-2-yl)amino-4-hydroxy-3-((1-sulfo-2-naphthalenyl)azo)-2, 7-naphthalene-disulfonic acid, trisodium salt] (CAS Reg. No. 12226-08-3).
8. C.I. Reactive Yellow 86[1,3-benzenedisulfonic acid, 4-((5-aminocarbonly-1-ethyl-1,6-dihydro-2-hydroxy-4-methyl-6-oxo-3-pyrindinyl) azo)-6-(4,6-dichloro-1,3,5-triazin-2-yl) amino)-, disodium salt] (CAS Reg. No. 61951-86-8).
9. C.I. Reactive Blue 163 [triphenodioxazinedisulfonic acid, 6,13-dichloro-3, 10-bis((4-((4,6-dichloro-1,3,5-triazin-2-yl)amino) sulfophenyl)amino)-, tetrasodium salt] (CAS Reg. No. 72847-56-4).

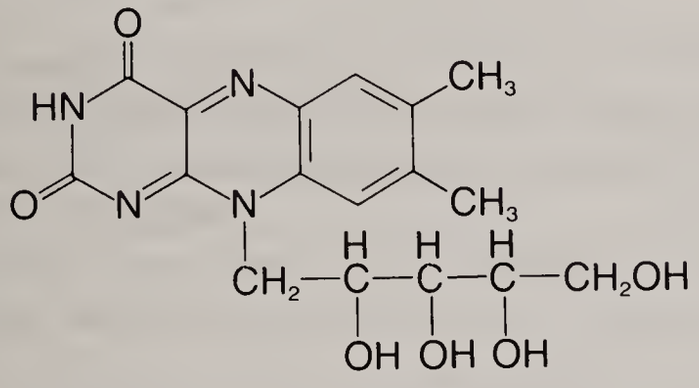
Potasssium sodium copper chlorophyllin (chlorophyllin-copper complex)—A green-black powder obtained from chlorophyll by replacing the methyl and phytyl ester groups with alkali and replacing the magnesium with copper. The source of the chlorophyll is dehydrated alfalfa.

Pyrogallol—This colorant is 1,2,3-trihydroxybenzene.

Pyrophyllite—A naturally occuring mineral substance consisting predominantly of a hydrous aluminum silicate, $Al_2O_3 \cdot 4SiO_2 \cdot H_2O$, intimately mixed with lesser amounts of finely divided silica, SiO_2. Small amounts (usually <3%) of other silicates, such as potassium aluminum silicate, may be present. Pyrophyllite may be identified and

quantitatively determined by its characteristic X-ray powder-diffraction pattern and by its optical properties.

Riboflavin—A yellow or orangish yellow crystalline powder having a slight odor. It melts at about 280°C, and its saturated solution is neutral to litmus. When dry, it is not affected by diffused light, but when in solution, light induces deterioration. One gram dissolves in about 3000–20,000 mL of water, depending on the internal crystalline structure. It is less soluble in alcohol than in water. It is insoluble in ether and in chloroform but is very soluble in dilute solutions of alkalies. A solution of 1mg in 100 mL of water is pale greenish yellow by transmitted light and has an intense yellowish green fluoresence that disappears on the addition of mineral acids or alkalies.



RIBOFLAVIN: $C_{17}H_{20}N_4O_6$ (mw 376.37)

Silver—The color additive silver (EEC No. E174) is a crystalline powder of high-purity silver prepared by the reaction of silver nitrate with ferrous sulfate in the presence of nitric, phosphoric and sulfuric acids. Polyvinyl alcohol is used to prevent the agglomeration of crystals and the formation of amorphous silver.

Tagetes meal and extract—Tagetes (Aztec marigold) meal is the dried, ground flower petals of the Aztec marigold (*Tagetes erecta* L.) mixed with not more than 0.3% ethoxyquin. Tagetes extract is a hexane extract of the flower petals of the Aztec marigold. It is mixed with an edible vegetable oil, or with an edible vegetable oil and a hydrogenated edible vegetable oil, and not more than 0.3% ethoxyquin. It may also be mixed with soy flour or corn meal as a carrier.

Toasted partially defatted cooked cottonseed flour—This product is prepared by delinting and decorticating food-quality cottonseed. The meats are screened, aspirated, and rolled; moisture is adjusted, the

meats heated, and the oil expressed; the cooked meats are cooled, ground, and reheated to obtain a product varying in shade from light to dark brown.

Vegetable juice—The concentrated or unconcentrated liquid expressed from mature varieties of fresh, edible vegetables.

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APPENDIX A

COLORANT SPECIFICATIONS

The specifications given here are based on the April, 1990 edition of the Code of Federal Regulations (21 CFR) and revisions to 21 CRF that appeared in the Federal Register through June, 1991. All are maxima unless indicated otherwise.

In addition to the requirements specified, color additives must be free of all impurities other than those named to the extent that such impurities can be avoided by current good manufacturing practice.

In most instances, specific analytical methods are not cited for determining the individual properties of color additives. This, of course, can lead to controversy since each method normally has a different precision and accuracy associated with it. To minimize problems, close contact between industry and government is essential to ensure that each is using comparable analytical technology.

FD&C Blue No. 1

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15.0%, total.

Water-insoluble matter—0.2%.
Leuco base—5%.
Sum of *o*-, *m*-, and *p*-sulfobenzaldehydes—1.5%.
N-Ethyl-*N*-(*m*-sulfobenzyl)sulfanilic acid—0.3%.
Subsidiary colors—6.0%.
Chromium (as Cr)—50 ppm.
Arsenic (as As)—3 ppm.
Lead (as Pb)—10 ppm.
Total color—85.0% min.

FD&C Blue No. 2 (for Coloring Surgical Sutures)

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
Water-insoluble matter—0.4%.
Isatin-5-sulfonic acid—0.4%.
Isomeric colors—18%.
Lower sulfonated subsidiary colors—5%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Total color—85% min.

FD&C Blue No. 2 (for Coloring Food and Ingested Drugs)

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
Water-insoluble matter—0.4%.
Isatin-5-sulfonic acid—0.4%.
5-Sulfoanthranilic acid—0.2%.
Disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid—18%.
Sodium salt of 2-(1,3-dihydro-3-oxo-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid—2%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

FD&C Green No. 3

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
Water-insoluble matter—0.2%.
Leuco base—5%.
Sum of 2-,3-,4-formylbenzenesulfonic acids, sodium salts—0.5%.
Sum of 3- and 4-[[ethyl(4-sulfophenyl)amino]methyl]benzenesulfonic acid, disodium salts—0.3%.
2-Formyl-5-hydroxybenzenesulfonic acid, sodium salt—0.5%.
Subsidiary colors—6%.
Chromium (as Cr)—50 ppm.
Arsenic (as As)—3 ppm.
Lead (as Pb)—10 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

FD&C Red No. 3

Volatile matter (at 135°C) and chlorides and sulfates (calculated as the sodium salts)—13%, total.
Water-insoluble matter—0.2%.
Sodium iodide—0.4%.
Unhalogenated intermediates—0.1%, total.
Triiodoresorcinol—0.2%.
2(2′,4′-Dihydroxy-3′,5′-diiodobenzoyl) benzoic acid—0.2%.
Monoiodofluoresceins—1.0%.
Other lower iodinated fluoresceins—9.0%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Total color—87.0% min.

FD&C Red No. 4

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—13%, total.
Water-insoluble matter—0.2%.

5-Amino-2,4-dimethyl-1-benzenesulfonic acid, sodium salt—0.2%.
4-Hydroxy-1-naphthalenesulfonic acid, sodium salt—0.2%.
Subsidiary colors—2%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—87% min.

FD&C Red No. 40

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—14.0%, total.
Water-insoluble matter—0.2%.
Higher-sulfonated subsidiary colors (as sodium salts)—1.0%.
Lower-sulfonated subsidiary colors (as sodium salts)—1.0%.
Disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl) azo]-8-(2-methoxy-5-methyl-4-sulfophenoxy)-2-naphthalenesulfonic acid—1.0%.
Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid—0.3%.
4-Amino-5-methoxy-*o*-toluenesulfonic acid—0.2%.
Disodium salt of 6,6′-oxybis(2-naphthalenesulfonic acid)—1.0%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Total color—85.0% min.

FD&C Yellow No. 5

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—13%, total.
Water-insoluble matter—0.2%.
4,4′-[4,5-Dihydro-5-oxo-4-[(4-sulfophenyl)hydrazono]-1*H*-pyrazol-1,3-diyl]bis[benzenesulfonic acid], trisodium salt—1%.
4-[(4′,5-Disulfo[1,1′-biphenyl]-2-yl)hydrazono]-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, tetrasodium salt—1%.
Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl) hydrazono]-1*H*-pyrazole-3-carboxylate, disodium salt—1%.

Sum of 4,5-dihydro-5-oxo-1-phenyl-4-[(4-sulfophenyl) azo]-1*H*-pyrazole-3-carboxylic acid, disodium salt, and 4,5-dihydro-5-oxo-4-(phenylazo)-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, disodium salt—0.5%.

4-Aminobenzenesulfonic acid, sodium salt—0.2%.

4,5-Dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, disodium salt—0.2%.

Ethyl or methyl 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylate, sodium salt—0.1%.

4,4′-(1-Triazene-1,3-diyl)bis[benzenesulfonic acid], disodium salt—0.05%.

4-Aminoazobenzene—75 ppb.

4-Aminobiphenyl—5 ppb.

Aniline—100 ppb.

Azobenzene—40 ppb.

Benzidine—1 ppb.

1,3-Diphenyltriazene—40 ppb.

Lead (as Pb)—10 ppm.

Arsenic (as As)—3 ppm.

Mercury (as Hg)—1 ppm.

Total color—87% min.

FD&C Yellow No. 6

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—13%.

Water-insoluble matter—0.2%.

Sodium salt of 4-aminobenzenesulfonic acid—0.2%.

Sodium salt of 6-hydroxy-2-naphthalenesulfonic acid—0.3%.

Disodium salt of 6,6′-oxybis[2-naphthalenesulfonic acid]—1%.

Disodium salt of 4,4′-(1-triazene-1,3-diyl)bis[benzenesulfonic acid]—0.1%.

Sum of the sodium salt of 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid and the sodium salt of 4-[(2-hydroxy-1-naphthalenyl) azo]benzenesulfonic acid—1%.

Sum of the trisodium salt of 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid and other higher sulfonated subsidiaries—5%.

4-Aminoazobenzene—50 ppb.
4-Aminobiphenyl—15 ppb.
Aniline—250 ppb.
Azobenzene—200 ppb.
Benzidine—1 ppb.
1,3-Diphenyltriazene—40 ppb.
1-(Phenylazo)-2-naphthalenol—10 ppm.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—87% min.

FD&C Lakes

Must be prepared from previously certified FD&C colors.
Soluble chlorides and sulfates (as sodium salts)—2.0%.
Inorganic matter, insoluble in hydrochloric acid—0.5%.

Citrus Red No. 2

Volatile matter (at 100°C)—0.5%.
Water-soluble matter—0.3%.
Matter insoluble in carbon tetrachloride—0.5%.
Uncombined intermediates—0.05%.
Subsidiary dyes—2.0%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Total color—98% min.

Orange B

Volatile matter (at 135°C)—6.0%.
Chlorides and sulfates (calculated as the sodium salts)—7.0%, total.
Water insoluble matter—0.2%.
1-(4-Sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone and 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone—0.7%.
Naphthionic acid—0.2%.
Phenylhydrazine-*p*-sulfonic acid—0.2%.
Trisodium salt of 1-(4-sulfophenyl)-3-carboxy-4-(4-sulfonaphthylazo)-5-hydroxypyrazole—6.0%.

Other subsidiary dyes—1.0%.
Lead (as Pb)—10 ppm.
Arsenic (as As)— 1 ppm.
Total color—87.0% min.

D&C Blue No. 4

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
Water-insoluble matter—0.2%.
Leuco base—5%.
Sum of *o*-, *m*-, and *p*-sulfobenzaldehydes, ammonium salts—1.5%.
N-Ethyl-*N*-(*m*-sulfobenzyl) sulfanilic acid, ammonium salt—0.3%.
Subsidiary colors—6%.
Chromium (as Cr)—50 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

D&C Blue No. 6

Volatile matter (at 135°C)—3%.
Matter insoluble in *N*,*N*-dimethylformamide—1%.
Isatin—0.3%.
Anthranilic acid—0.3%.
Indirubin—1%.
Lead (as Pb)—10 ppm.
Mercury (as Hg)—1 ppm.
Arsenic (as As)—3 ppm.
Total color—95% min.

D&C Blue No. 9

Volatile matter (at 135°C)—3%.
Matter extractable by alcoholic HCl (0.1 mL of concentrated hydrochloric acid per 50 mL of 95% ethyl alcohol)—1%.
2-Aminoanthraquinone—0.2%.
Organically combined chlorine in pure dye—13.0%-14.8%.

Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Total color—97% min.

D&C Brown No. 1

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—16%, total.
Water-insoluble matter—0.2%.
Sulfanilic acid, sodium salt—0.2%.
Resorcinol—0.2%.
Xylidines—0.2%.
Disodium salt of 4[[5-[(4-sulfophenyl)azo]-2,4-dihydroxyphenyl]azo] benzenesulfonic acid—3%.
Monosodium salt of 4[[5-[(2,4-dimethylphenyl)azo]-2,4-dihydroxy-phenyl]azo]benzenesulfonic acid—29% min., 39% max.
Monosodium salt of 4[[5-[(2,5-dimethylphenyl)azo]-2,4-dihydroxy-phenyl]azo]benzensulfonic acid—12% min., 17% max.
Monosodium salt of 4[[5-[(2,3-dimethylphenyl)azo]-2,4-dihydroxy-phenyl]azo]benzenesulfonic acid—6% min., 13% max.
Monosodium salt of 4[[5-[(2-ethylphenyl)azo]-2,4-dihydroxy-phenyl] azo]benzenesulfonic acid—5% min., 12% max.
Monosodium salt of 4[[5-[(3,4-dimethylphenyl)azo]-2,4-dihydroxy-phenyl]azo]benzenesulfonic acid—3% min., 9% max.
Monosodium salt of 4[[5-[(2,6-dimethylphenyl)azo]-2,4-dihydroxy-phenyl]azo]benzenesulfonic acid—3% min., 8% max.
Monosodium salt of 4[[5-[(4-ethylphenyl)azo]-2,4-dihydroxy-phenyl] azo]benzenesulfonic acid—2% min., 8% max.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—84% min.

D&C Green No. 5 (for Coloring Surgical Sutures)

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—20%, total.
Water-insoluble matter—0.2%.
1,4-Dihydroxyanthraquinone—0.2%.

2-Amino-*m*-toluenesulfonic acid—0.2%.
Subsidiary colors—5%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Total color—80% min.

D&C Green No. 5 (for Coloring Drugs and Cosmetics)

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—20%, total.
Water-insoluble matter—0.2%.
1,4-Dihydroxyanthraquinone—0.2%.
Sulfonated toluidines—0.2%.
p-Toluidine—0.0015%.
Sum of monosulfonated D&C Green No. 6 and Ext. D&C Violet No. 2—3%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—80% min.

D&C Green No. 6

Volatile matter (at 135°C)—2.0%.
Water-soluble matter—0.3%.
Matter insoluble in carbon tetrachloride—1.5%.
p-Toluidine—0.1%.
1,4-Dihydroxyanthraquinone—0.2%.
1-Hydroxy-4-[(4-methylphenyl) amino]-9,10-anthracenedione—5.0%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—96.0% min.

D&C Green No. 8

Volatile matter (at 135°C)—15%.
Water-insoluble matter—0.2%.
Chlorides and sulfates (calculated as sodium salts)—20%.

Trisodium salt of 1,3,6-pyrenetrisulfonic acid—6%.
Tetrasodium salt of 1,3,6,8-pyrenetetrasulfonic acid—1%.
Pyrene—0.2%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—65% min.

D&C Orange No. 4

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—13%, total.
Water-insoluble matter—0.2%.
2-Naphthol—0.4%.
4,4′-[Diazoamino]-dibenzenesulfonic acid—0.1%.
Sulfanilic acid, sodium salt—0.2%.
Subsidiary colors—3%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—87% min.

D&C Orange No. 5

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—10%.
Insoluble matter (alkaline solution)—0.3%.
4′,5′-Dibromofluorescein—50–65%.
2′,4′,5′-Tribromofluorescein—30–40%.
2′,4′,5′,7′-Tetrabromofluorescein—10%.
Sum of 2′4′- and 2′,5′-dibromofluoresceins—2%.
4′-Bromofluorescein—2%.
Fluorescein—1%.
Phthalic acid—1%.
2-(3,5-Dibromo-2,4-dihydroxybenzoyl) benzoic acid—0.5%.
Brominated resorcinol—0.4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Orange No. 10

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—8%.
Insoluble matter (alkaline solution)—0.5%.
Phthalic acid—0.5%.
2-[3′,5′-Diiodo-2′,4′-dihydroxybenzoyl] benzoic acid—0.5%.
Fluorescein—1%.
4′-Iodofluorescein—3%.
2′,4′-Diiodofluorescein and 2′,5′-diiodofluorescein—2%.
2′,4′,5′-Triiodofluorescein—35%.
2′,4′,5′,7′-Tetraiodofluorescein—10%.
4′,5′-Diiodofluorescein—60-95%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—92% min.

D&C Orange No. 11

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—8%.
Water-insoluble matter—0.5%.
Phthalic acid—0.5%.
2-[3′,5′-Diiodo-2′,4′-dihydroxybenzoyl] benzoic acid, sodium salt—0.5%.
Fluorescein, disodium salt—1%.
4′-Iodofluorescein, disodium salt—3%.
2′,4′-Diiodofluorescein and 2′,5′-diiodofluorescein—2%.
2′,4′,5′-Triiodofluorescein—35%.
2′,4′,5′,7′-Tetraiodofluorescein, disodium salt—10%.
4′,5′-Diiodofluorescein, disodium salt—60–95%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—92% min.

D&C Red No. 6

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—10%, total.
Ether-soluble matter—Passes test (see p. 403).
2-Amino-5-methylbenzenesulfonic acid, sodium salt—0.2%.
3-Hydroxy-2-naphthalenecarboxylic acid, sodium salt—0.4%.
3-Hydroxy-4-[(4-methylphenyl)azo]-2-naphthalenecarboxylic acid, sodium salt—0.5%.
p-Toluidine—15 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 7

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—10%, total.
Ether-soluble matter—Passes test (see p. 403).
2-Amino-5-methylbenzenesulfonic acid, calcium salt—0.2%.
3-Hydroxy-2-naphthalenecarboxylic acid, calcium salt—0.4%.
3-Hydroxy-4-[(4-methylphenyl)azo]-2-naphthalenecarboxylic acid, calcium salt—0.5%.
p-Toluidine—15 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 17

Volatile matter (at 135°C)—5%.
Matter insoluble in both toluene and water (the color additive is mixed in toluene and the resultant residue is isolated and mixed with water to obtain the matter insoluble in both toluene and water)—0.5%.
2-Naphthol—0.2%.
1-(Phenylazo)-2-naphthol—3%.

1-[[2-(Phenylazo)phenyl]azo]-2-naphthalenol—2%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Chlorides and sulfates (calculated as sodium salts)—3%.
Aniline—0.2%.
4-Aminoazobenzene—0.1%.
Total color—90% min.

D&C Red No. 21

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—10%, total.
Insoluble matter (alkaline solution)—0.5%.
Phthalic acid—1%.
2-(3,5-Dibromo-2,4-dihydroxybenzoyl) benzoic acid—0.5%.
2′,4′,5′,7′-Tetrabromofluorescein, ethyl ester—1%.
Brominated resorcinol—0.4%.
Fluorescein—0.2%.
Sum of mono- and dibromofluoresceins—2%.
Tribromofluoresceins—11%.
2′,4′,5′,7′-Tetrabromofluorescein—87% min.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 22

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—10%, total.
Water-insoluble matter—0.5%.
Disodium salt of phthalic acid—1%.
Sodium salt of 2-(3,5-dibromo-2,4-dihydroxybenzoyl) benzoic acid—0.5%.
2′,4′,5′,7′-Tetrabromofluorescein, ethyl ester—1%.
Brominated resorcinol—0.4%.
Sum of disodium salts of mono- and dibromofluoresceins—2%.

Sum of disodium salts of tribromofluoresceins—25%.
Disodium salt of 2′,4′,5′,7′-tetrabromofluorescein—72% min.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 27

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—10%, total.
Insoluble matter (alkaline solution)—0.5%.
Tetrachlorophthalic acid—1.2%.
Brominated resorcinol—0.4%.
2,3,4,5-Tetrachloro-6-(3,5-dibromo-2,4-dihydroxybenzoyl) benzoic acid —0.7%.
2′,4′,5′,7′-Tetrabromo-4,5,6,7-tetrachlorofluorescein, ethyl ester—2%.
Lower halogenated subsidiary colors—4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 28

Sum of volatile matter (at 135°C) and halides and sulfates (calculated as sodium salts)—15%, total.
Insoluble matter (alkaline solution)—0.5%.
Tetrachlorophthalic acid—1.2%.
Brominated resorcinol—0.4%.
2,3,4,5-Tetrachloro-6-(3,5-dibromo-2,4-dihydroxybenzoyl) benzoic acid —0.7%.
2′,4′,5′,7′-Tetrabromo-4,5,6,7-tetrachlorofluorescein, ethyl ester—2%.
Lower halogenated subsidiary colors—4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

D&C Red No. 30

Volatile matter (at 135°C)—5%.
Chlorides and sulfates (calculated as sodium salts)—3%.
Matter soluble in acetone—5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 31

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—10%, total.
Aniline—0.2%.
3-Hydroxy-2-naphthoic acid, calcium salt—0.4%.
Subsidiary colors—1%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—90% min.

D&C Red No. 33

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—18%, total.
Water-insoluble matter—0.3%.
4-Amino-5-hydroxy-2,7-naphthalenedisulfonic acid, disodium salt—0.3%.
4,5-Dihydroxy-3-(phenylazo)-2,7-naphthalenedisulfonic acid, disodium salt—3.0%.
Aniline—25 ppm.
4-Aminoazobenzene—100 ppb.
1,3-Diphenyltriazene—125 ppb.
4-Aminobiphenyl—275 ppb.
Azobenzene—1 ppm.
Benzidine—20 ppb.
Lead (as Pb)—20 ppm.

Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—82% min.

D&C Red No. 34

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
2-Amino-1-naphthalenesulfonic acid—0.2%.
3-Hydroxy-2-naphthoic acid—0.4%.
Subsidiary colors—4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

D&C Red No. 36

Volatile matter at 135°C—1.5%.
Matter insoluble in toluene—1.5%.
2-Chloro-4-nitrobenzenamine—0.3%.
2-Naphthalenol—1%.
2,4-Dinitrobenzenamine—0.02%.
1-[(2,4-Dinitrophenyl)azo]-2-naphthalenol—0.5%.
4-[(2-Chloro-4-nitrophenyl)azo]-1-naphthalenol—0.5%.
1-[(4-Nitrophenyl)azo]-2-naphthalenol—0.3%.
1-[(4-Chloro-2-nitrophenyl)azo]-2-naphthalenol—0.3%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—95% min.

D&C Red No. 39

Volatile matter (at 100°C)—2.0%.
Matter insoluble in acetone—1.0%.
Anthranilic acid—0.2%.
N,*N*(β-β′-Dihydroxy-diethyl)aniline—0.2%.

Subsidiary colors—3.0%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Total color—95.0% min.

D&C Violet No. 2

Volatile matter (at 135°C)—2.0%.
Matter insoluble in both carbon tetrachloride and water—0.5%.
p-Toluidine—0.2%.
1-Hydroxy-9,10-anthracenedione—0.5%.
1,4-Dihydroxy-9,10-anthracenedione—0.5%.
Subsidiary colors—1.0%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Total color—96.0% min.

D&C Yellow No. 7

Sum of water and chlorides and sulfates (calculated as sodium salts)—6%, total.
Matter insoluble in alkaline water—0.5%.
Resorcinol—0.5%.
Phthalic acid—0.5%.
2-(2,4-Dihydroxybenzoyl)benzoic acid—0.5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—94% min.

D&C Yellow No. 8

Sum of water and chlorides and sulfates (calculated as sodium salts)—15%, total.
Matter insoluble in alkaline water—0.3%.
Resorcinol—0.5%.
Phthalic acid—1%.
2-(2,4-Dihydroxybenzoyl)benzoic acid—0.5%.

Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

D&C Yellow No. 10

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%.
Matter insoluble in both water and chloroform—0.2%.
Total sulfonated quinaldines, sodium salts—0.2%.
Total sulfonated phthalic acids, sodium salts—0.2%.
2-(2-Quinolinyl)-1*H*-indene-1,3 (2*H*)-dione—4 ppm.
Sum of sodium salts of the monosulfonates of 2-(2-quinolinyl)-1*H*-indene-1,3 (2*H*)-dione—75% min.
Sum of sodium salts of the disulfonates of 2-(2-quinolinyl)-1*H*-indene-1,3 (2*H*)-dione—15%.
2-(2,3-Dihydro-1,3-dioxo-1*H*-indene-2-yl)-6,8-quinolinedisulfonic acid, disodium salt—3%.
Diethyl ether soluble matter other than that specified [using added 2-(2-quinolinyl)-1*H*-indene-1,3 (2*H*)-dione for calibration]—2 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

D&C Yellow No. 11

Volatile matter (at 135°C)—1%.
Ethyl alcohol-insoluble matter—0.4%.
Phthalic acid—0.3%.
Quinaldine—0.2%.
Subsidiary colors—5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—96% min.

D&C Lakes

Ether extracts—0.5%.
Soluble chlorides and sulfates (as sodium salts)—3.0%.
Intermediates—0.2%.

Ext. D&C Violet No. 2

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—18%, total.
Water-insoluble matter—0.4%.
1-Hydroxy-9,10-anthracenedione—0.2%.
1,4-Dihydroxy-9,10-anthracenedione—0.2%.
p-Toluidine—0.1%.
p-Toluidinesulfonic acids, sodium salts—0.2%.
Subsidiary colors—1%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—80% min.

Ext. D&C Yellow No. 7

Sum of volatile matter (at 135°C) and chlorides and sulfates (calculated as sodium salts)—15%, total.
Water-insoluble matter—0.2%.
1-Naphthol—0.2%.
2,4-Dinitro-1-naphthol—0.03%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—85% min.

Ext. D&C Lakes

Ether Extracts—0.5%.
Soluble chlorides and sulfates (as sodium salts)—3.0%.
Intermediates—0.2%.

[Phthalocyaninato(2-)] Copper

Volatile matter (at 135°C)—0.3%.
Salt content (as NaCl)—0.3%.
Alcohol-soluble matter—0.5%.
Organic chlorine—0.5%.
Aromatic amines—0.05%.
Lead (as Pb)—40 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color—98.5% min.

Alumina

Acidity or alkalinity: agitate 1 g of colorant with 25 mL of water and filter; the filtrate shall be neutral to litmus paper.
Matter insoluble in dilute HCl—0.5%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Mercury (as Hg)—1 ppm.
Aluminum oxide (Al_2O_3)—50% min.

Aluminum Powder

Fineness—100% shall pass through a 200-mesh screen and 95% shall pass through a 325-mesh screen.
Mercury—1 ppm.
Arsenic—3 ppm.
Lead—20 ppm.
Aluminum—99% min.

Annatto Extract (and Pigments Precipitated Therefrom)

Arsenic (as As)—3 ppm.
Lead (as Pb)—10 ppm.
Solvent residue—no more than that permitted for corresponding solvent in spice oleoresins.

β-Apo-8′-Carotenal

Physical state—solid.
1% Solution in chloroform—clear.
Melting point (decomposition)—136–140°C (corrected).
Loss of weight on drying—0.2%.
Residue on ignition—0.2%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Assay (spectrophotometric)—96–101%.

Bismuth Citrate

Mercury (as Hg)—1 ppm.
Arsenic (as As)—3 ppm.
Lead (as Pb)—20 ppm.
Volatile matter—1%.
Bismuth citrate—97% min.

Bismuth Oxychloride

Volatile matter—0.5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Bismuth oxychloride—98% min.

Bronze Powder

Stearic or oleic acid—5%.
Cadmium (as Cd)—15 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Aluminum (as Al)—0.5%.
Tin (as Sn)—0.5%.
Copper (as Cu)—70% min.; 95% max.

Zinc (as Zn)—30%.
Maximum particle size 45 μ—95% min.
Al, Zn, Sn, and Cu content shall be based on the weight of the dried powder after thorough washing with ether.

Calcium Carbonate

Loss on drying (200°C for 4 hr)—2.0%.
Acid insolubles—0.2%.
Heavy metals—30 ppm.
Lead—10 ppm.
Fluoride—50 ppm.
Arsenic—3 ppm.
Magnesium and alkali salts—1.0%.
Barium—no green color when a platinum wire is dipped in a 2.5% acidified sample solution and held in a nonluminous flame.
Assay (dry basis)—98.0–100.5%.

Canthaxanthin

Physical state—solid.
1% Solution in chloroform—complete and clear.
Melting range (decomposition)—207–212°C (corrected).
Loss on drying—0.2%.
Residue on ignition—0.2%.
Total carotenoids other than transcanthaxanthin—5%.
Lead—10 ppm.
Arsenic—3 ppm.
Mercury—1 ppm.
Assay—96–101%.

Caramel

Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—0.1 ppm.

Carmine

Volatile matter (135°C for 3 hr)—20.0%.
Ash—12.0%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Carminic acid—50.0% min.
Viable *Salmonella* microorganisms—none.

β-Carotene

Physical state—solid.
1% Solution in chloroform—clear.
Loss on drying—0.2%.
Residue on ignition—0.2%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Assay (spectrophotometric)—96–101%.

Carrot Oil

Hexane—25 ppm.

Chlorophyllin-Copper Complex, Oil Soluble

Moisture—0.5%.
Nitrogen—0.2–0.3%.
Total copper—0.2–0.4%.
Free copper—200 ppm.
Lead—20 ppm.
Arsenic—5 ppm.
Sulfated ash—2.5%.
Total color—4.5–5.5%.

Chromium-Cobalt-Aluminum Oxide

Chromium (as Cr_2O_3)—34–37%.
Cobalt (as CaO)—29–34%.

Aluminum (as Al_2O_3)—29–35%.
Lead (as Pb)—30 ppm.
Arsenic (as As)—3 ppm.
Total oxides of Al, Cr, and Co—97% min.
Lead and arsenic shall be determined in the solution obtained by boiling 10 g of the colorant for 15 min. in 50 mL of 0.5 *N* HCl.

Chromium Hydroxide Green

Water-soluble matter—2.5%.
Chromium (as Cr_2O_3) in 2% NaOH extract—0.1% (based on sample weight).
Boron (as B_2O_3)—8%.
Total volatile matter at 1000°C—20%.
Cr_2O_3—75% min.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.

Chromium Oxide Greens

Chromium (as Cr_2O_3) in 2% NaOH extract—0.075% (based on sample weight).
Arsenic (as As)—3 ppm.
Lead (as Pb)—20 ppm.
Mercury (as Hg)—1 ppm.
Cr_2O_3—95% min.

Cochineal Extract

pH (at 25°C)—5.0-5.5.
Protein ($N \times 6.25$)—2.2%.
Total solids—5.7–6.3%.
Methyl alcohol—150 ppm.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.

Carminic acid—1.8% min.
Viable *Salmonella* microorganisms—none.

Copper Powder

Stearic or oleic acid—5%.
Cadmium (as Cd)—15 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Copper (as Cu)—95% min.
Maximum particle size 45 μm—95% min.

Corn Endosperm Oil

Total fatty acids—85% min.
Iodine value—118–134.
Saponification value—165–185.
Unsaponifiable matter—14%.
Hexane—25 ppm.
Isopropyl alcohol—100 ppm.

Dehydrated Beets (Beet Powder)

Volatile matter—4%.
Acid-insoluble ash—0.5%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Mercury (as Hg)—1 ppm.

Dihydroxyacetone

Volatile matter (at 34.6°C for 3 hr at ≤30 mm Hg pressure)—0.5%.
Residue on ignition—0.4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Iron (as Fe)—25 ppm.
1,3-Dihydroxy-2-propanone—98% min.

Disodium EDTA-Copper

Total copper—13.5% min.
Total (ethylenedinitrilo)tetraacetic acid—62.5% min.
Free copper—100 ppm.
Free disodium salt of (ethylenedinitrilo)tetraacetic acid—1.0%.
Moisture—15%.
Water-insoluble matter—0.2%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Ferric Ammonium Citrate

Iron (as Fe)—14.5–18.5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Ferric Ammonium Ferrocyanide

Oxalic acid or its salts—0.1%.
Water-soluble matter—3%.
Water-soluble cyanide—10 ppm.
Volatile matter—4%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Nickel (as Ni)—200 ppm.
Cobalt (as Co)—200 ppm.
Mercury (as Hg)—1 ppm.
Total iron (as Fe) corrected for volatile matter—33-39%.

Ferric Ferrocyanide

Water-soluble cyanide—10 ppm.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Nickel (as Ni)—200 ppm.
Cobalt (as Co)—200 ppm.
Mercury (as Hg)—1 ppm.
Oxalic acid—0.1%.
Water-soluble matter—3%.
Volatile matter—10%.
Total iron (as Fe) corrected for volatile matter—37–45%.

Ferrous Gluconate

Assay (as $C_{12}H_{22}FeO_{14}$, dried basis)—95.0% min.
Loss on drying (105°C for 4 hr)—6.5–10%.
Arsenic (as As)—3 ppm.
Chloride—700 ppm.
Ferric iron—2%.
Lead—10 ppm.
Mercury—3 ppm.
Oxalic acid—passes test.
Reducing sugars—passes test.
Sulfate—0.1%.

Grape Color Extract

Pesticide residues—not more than permitted in or on grapes by regulations promulgated under section 408 of the Federal Food, Drug, and Cosmetic Act.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.

Grape Skin Extract (Enocianina)

Same as for Grape Color Extract.

Guaiazulene

Melting point—30.5–31.5°C.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Mercury (as Hg)—1 ppm.
Total color—99% min.

Guanine

Guanine—75% min.
Hypoxanthine—25%.
Ash (ignition at 800°C)—2%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Assay (total purines)—96% min.

Henna

Shall contain no more than 10% of plant material from *Lawsonia alba* Lam. (*Lawsonia inermis* L.) other than the leaf and petiole, and shall be free from admixture with material from any other species of plant.
Moisture—10%.
Total ash—15%.
Acid-insoluble ash—5%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Lead Acetate

Water-insoluble matter—0.02%.
pH (30% solution, weight to volume at 25°C)—4.7–5.8.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Lead acetate—99% min.

Logwood Extract

Volatile matter (at 110°C)—15%.
Sulfated ash—20%.
Hematein—5–20%.
Lead (as Pb)—70 ppm.

Arsenic (as As)—4 ppm.
Mercury (as Hg)—3 ppm.

Manganese Violet

Ash (at 600°C)—81% min.
Volatile matter (at 135°C for 3 hr)—1%.
Water-soluble substances—6%.
pH of filtrate of 10 g of color additive (shaken occasionally for 2 hr with 100 mL of freshly boiled distilled water)—4.7–2.5.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.
Total color (based on Mn content of as-is sample)—93% min.

Mica

Fineness—100% shall pass through a 100-mesh screen.
Loss on ignition at 600–650°C—2%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.

Paprika Oleoresin

Solvent residue—no more than that permitted for the corresponding solvent in spice oleoresins.

Potassium Sodium Copper Chlorophyllin (Chlorophyllin–Copper Complex)

Moisture—5.0%.
Nitrogen—5.0%.
pH of 1% solution—9-11.
Total copper—4-6%.
Free copper—0.25%.
Iron—0.5%.
Lead (as Pb)—20 ppm.

Arsenic (as As)—5 ppm.
Ration, absorbance at 405 nm to absorbance at 630 nm—3.4–3.9.
Total color—75% min.

Pyrogallol

Melting point—130–133°C.
Residue on ignition—0.1%.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Pyrophyllite

Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Lead and arsenic shall be determined in the solution obtained by boiling 10 g of pyrophyllite for 15 min in 50 mL of 0.5 *N* HCl.

Riboflavin

Assay (as $C_{17}H_{20}N_4O_6$, dry basis)—98.0–102.0%.
Specific rotation, $[\alpha]_D^{25}$(dry basis)—between −112° and −122°.
Loss on drying—1.5%.
Lumiflavin—passes test.
Residue on ignition—0.3%.

Silver

Lead (asPb)—10 ppm.
Arsenic (as As)—5 ppm.
Mercury (as Hg)—1 ppm.
Silver (as Ag)—99.9% min.

Synthetic Iron Oxide (for Dog and Cat Food)

Arsenic (as As)—5 ppm.
Lead (as Pb)—20 ppm.
Mercury (as Hg)—3 ppm.

Synthetic Iron Oxide (for Cosmetics or Ingested or Topically Applied Drugs)

Arsenic (as As)—3 ppm.
Lead (as Pb)—10 ppm.
Mercury (as Hg)—3 ppm.

Tagetes (Aztec Marigold) Meal and Extract

Tagetes meal shall be free from admixture with other plant material from *Tagetes erecta* L. and from plant material or flowers of any other species of plant.

Tagetes extract shall be prepared from tagetes petals meeting the above-mentioned specification and, in addition, shall conform to the following requirements:

Melting point—53.5–55.0°C.
Iodine value—132–145.
Saponification value—175–200.
Acid value—0.60–1.20.
Titer—35.5–37.0.
Unsaponifiable matter—23.0–27.0%.
Hexane residue—25 ppm.

All determinations except the hexane residue shall be made on the initial extract of the flower petals (after drying in a vacuum oven at 60°C for 24 hr) prior to the addition of oils and ethoxyquin; hexane determination shall be made on the color additive after addition of vegetable oils, hydrogenated vegetable oils and ethoxyquin.

Talc

Loss on ignition (at red heat to constant weight)—5.0%.
Acid-soluble substances as sulfate—2.0%.
Reaction and soluble substances—0.1%.
Water-soluble iron—passes test.
Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.

Lead and arsenic shall be determined in the solution obtained by boiling 10 g of talc for 15min. in 50 mL of 0.5 *N* hydrochloric acid.

Titanium Dioxide

Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Antimony (as Sb)—2 ppm.
Mercury (as Hg)—1 ppm.
Loss on ignition at 800°C (after drying for 3 hr at 105°C)—0.5%.
Water-soluble substances—0.3%.
Acid-soluble substances—0.5%.
TiO_2 (After drying for 3 hr at 105°C)—99.0% min.
Lead, arsenic, and antimony shall be determined in the solution obtained by boiling 10 g of the colorant for 15 min in 50 mL of 0.5 *N* hydrochloric acid.

Toasted Partially Defatted Cooked Cottonseed Flour

Arsenic (as As)—0.2 ppm.
Lead (as Pb)—10 ppm.
Free gossypol—450 ppm.

Turmeric Oleoresin

Solvent residue—no more than that permitted for the corresponding solvent in spice oleoresins.

Ultramarine Blue (for Coloring Salt Intended for Animal Feed)

Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Mercury (as Hg)—1 ppm.

Ultramarines (for Coloring Externally Applied Cosmetics)

Lead (as Pb)—20 ppm.
Arsenic (as As)—3 ppm.
Mercury (as Hg)—1 ppm.

Zinc Oxide

Zinc oxide (as ZnO)—99% min.
Loss on ignition at 800°C—1%.
Cadmium (as Cd)—15 ppm.
Mercury (as Hg)—1 ppm.
Arsenic (as As)—3 ppm.
Lead (as Pb)—20 ppm.

APPENDIX B

SOME DOMESTIC SUPPLIERS OF COLOR ADDITIVES

Beatrice Foods Co., Special Products Division, 156 W. Grand Ave., P.O. Box 749, Beloit, WI 53511 (800-328-7517; 608-365-5561). Annatto, turmeric, beet color, blends of FD&C Yellow No. 5 and FD&C Yellow No. 6.

BIOCON (US) Inc., 518 Codell Dr., Lexington, KY 40509 (800-458-7831; 606-269-6351). Annatto, carmine lake, cochineal, turmeric, paprika, grape-skin extract, beet extract, and beet powder.

Chr. Hansen's Laboratory, Inc., 9015 West Maple St., Milwaukee, WI 53214 (414-476-3630). Beet powder, annatto, turmeric, and cochineal.

Colorcon, Inc., Moyer Blvd., West Point, PA 19486 (215-699-7733). Certified FD&C Lakes.

Crompton & Knowles Corporation, 1595 MacArthur Blvd, Mahwah, NJ 07430 (201-818-1200). Certified FD&C and D&C colorants, carmine lake, annatto, and beet powder.

D. D. Williamson & Company, Inc., P.O. Box 6001, Louisville, KY 40206 (502-895-2438). Caramel colors.

Hilton-Davis, 2235 Langdon Farm Rd., Cincinnati, OH 45237 (513-841-4000). Certified FD&C and D&C colorants, iron oxides, and inorganic colorants.

H. K. Color Group, 155 Helen St., South Plainfield, NJ 07080 (201-769-1122). Certified D&C colorants, carmine lake, titanium dioxide, and iron oxides.

Hoffmann-LaRoche, Inc., 304 Kingsland St., Nutley, NJ 07110 (201-235-5000). β-Carotene, β-apo-8′-carotenal, and canthaxanthin.

Kalsec, Inc., P.O. Box 511, Kalamazoo, MI 49005 (616-349-9711). Paprika, turmeric, and annatto.

Mearl Corporation, 41 East 42nd St., New York, NY 10017 (212-573-8500). Natural and synthetic Pearl Essence.

Meer Corporation, 9500 Railroad Ave., North Bergen, NJ 07047 (201-861-9500). Beet powder, annatto, paprika, turmeric, henna, and grape-skin extract.

Miles Laboratories, Inc., Biotech Products Division, P.O. Box 932, Elkhart, IN 46515-0932 (800-348-7414; 219-264-8716). Annatto.

Pfizer, Inc., 235 East 42nd St., New York, NY 10017 (212-573-2323). Calcium carbonate, synthetic iron oxides and talc.

Pylam Products Company, Inc., 1001 Stewart Ave., Garden City, NY 11530 (800-645-6096; 516-222-1750). Certified FD&C and D&C colorants.

Rona Pearl, Inc., P.O. Box 81, Bayonne, NJ 07002 (201-437-0800). Natural and synthetic pearl pigments, titanium dioxide, mica- and talc-base colorants.

Sethness Products Co., 2367 W. Logan Blvd., Chicago, IL 60647 (312-235-6400). Caramel colors.

Smith Chemical & Color Company, Inc., 104-20 Dunkirk St., Jamaica, NY 11412 (718-454-9400). Synthetic iron oxides, ultramarine blue, chromium oxide, mica, talc, zinc oxide, and calcium carbonate.

Sun Chemical Corporation, Pigments Division, 441 Tompkins Ave., Staten Island, NY 10305 (718-981-1600). Certified FD&C Lakes, certified D&C colorants, synthetic iron oxides, manganese violet, ultramarine blue, chromium hydroxide green, chromium oxide greens, and titanium dioxide.

Warner-Jenkinson, 2526 Baldwin St., St. Louis, MO 63106 (314-889-7600). Certified FD&C and D&C colorants, carmine lake, titanium dioxide, annatto, turmeric, and beet color.

Welch Foods, Inc., Westfield, NY 14787 (716-326-3131). Grape-color extract.

Whittaker, Clark & Daniels, Inc., 1000 Coolidge St., South Plainfield, NJ 07080 (201-561-6100). Certified FD&C and D&C colorants, calcium carbonate, chrome oxide greens, mica, talc, titanium dioxide, ultramarines, and zinc oxide.

APPENDIX C

GLOSSARY

NOTE: Some of the following terms have broader meanings than those stated. The definitions given here are as the terms relate to color additives in particular.

ADULTERATE—To render impure, spurious, or inferior by adding extraneous or improper ingredients.

AREA OF THE EYE—That area enclosed within the circumference of the supraorbital ridge and the infraorbital ridge, including the eyebrow, the skin below the eyebrow, the eyelids and the eyelashes, and conjunctival sac of the eye, the eyeball, and the soft areolar tissue that lies within the perimeter of the infraorbital ridge.

BATCH—An homogeneous lot of color additive or color additive mixture produced by an identified production operation, which is set apart and held as a unit for the purpose of obtaining certification of such quantity.

BATCH NUMBER—The number assigned to a batch of colorant by the person who requests certification of the batch.

BLEED—Leaching of an impurity or minor constituent from a dyed article or a solid dye.

BLOWOUT—Procedure (or its result) whereby solid colorant is dispersed onto a moist absorbent surface to detect impurities or a physical mixture of colorants.

BRIGHTNESS—The attribute of a color that classifies it as equivalent to some member of the series of achromatic (neutral) color perceptions ranging from very dim to very bright or dazzling. Analogous to "value" in the Munsell system of color notation.

CARCINOGEN—A cancer-producing substance.

CERTIFICATION—The submission of a sample of color additive to the Food and Drug Administration and, after subsequent analysis, the issuance of a certificate of acceptance or "certification."

CHROMA—In the Munsell system of color notation, that quality of color by which we distinguish a strong one from a weak one; the intensity of a distinctive hue; color intensity.

COAL-TAR DYE—An erroneous name often used to describe certifiable colors in the belief that they are still derived from coal tar.

COLOR—That aspect of visual perception by which an observer distinguishes differences between two structure-free fields of view of the same size and shape, such as may be caused by differences in the spectral composition of the radiant energy concerned in the observation.

COLOR ADDITIVE—A dye, pigment, or other substance synthesized, extracted, isolated, or otherwise derived from a vegetable, animal, mineral, or other source and that, when added or applied to a food, drug, cosmetic, or the human body or any part thereof, is capable of imparting color, either alone or through reaction with another substance.

COLORANT—A substance such as a dye or pigment that colors or modifies the color of something else; a color additive.

COSMETIC—Articles intended to be rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering appearance; articles (except soap) intended for use as a component of any such articles.

DELANEY CLAUSE—That portion of the Color Additive Amendments of 1960 that forbids the use in foods, drugs, and cosmetics of any color

additive that can be shown by reasonable tests to cause cancer in man or other animals.

DILUENT—Any component of a color-additive mixture that is not itself a color additive and has been intentionally mixed therein to facilitate the use of the mixture in coloring foods, drugs, or cosmetics or in coloring the human body.

DRAW-DOWN—Samples used to judge undertone and masstone, prepared by spreading a blob of pigment onto a white backing with a single stroke of a blade.

DRUG—Articles recognized in the official *United States Pharmacopeia,* official *Homeopathic Pharmacopeia* of the United States, or official *National Formulary,* or any supplement thereto; articles intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals; articles (other than food) intended to affect the structure or any function of the body of man or other animals; articles intended for use as a component of any articles specified above, but not including devices or their components, parts, or accessories.

DYE—A chemical compound that is capable of imparting color and that is soluble in the vehicle in which it is applied.

EXCIPIENT—An inert substance used as a diluent or vehicle.

FLASHING—The visible effect of individual colors in a color blend separately dissolving when the blend is added to a solvent.

FOOD—Articles (including chewing gum) used for food or drink for man or other animals; items used for components of any such article.

HIDING POWER—The opacity of a colored film, usually measured by observing the amount of black transmitted through equal film thicknesses of color when the colored dispersion is drawn down on a sheet of checkered black-and-white paper.

HOMOLOGOUS COLORS—A series of colorants with similar chemical structures that differ only in their chain lengths or in the number of substituent groups they contain.

HUE—In the Munsell system of color notation, the name of a color. That quality by which we distinguish one color family from another, such as red from yellow or green from blue or purple.

INTERMEDIATE—A compound from which a colorant is directly or indirectly synthesized.

ISOMERIC COLORS—Colorants with the same empirical formula but different structural forms.

LAKE—A pigment prepared by precipitating a soluble dye onto an insoluble reactive or adsorptive substratum or diluent.

LISTED COLORANTS—Popularly called "permanently" listed colorants. Those colorants that have been sufficiently evaluated to convince the Food and Drug Administration of their safety for the application intended. See PROVISIONALLY LISTED COLORANTS.

LOT NUMBER—The identifying number or symbol assigned by the Food and Drug Administration to a batch of color additive after certification.

MASSTONE—The color (without regard to background) of a thick layer of a pigment incorporated into a vehicle.

MEDICAL DEVICE—An instrument, apparatus, implement, machine, contrivance, implant, invitro reagent, or other similar or related article, including any component, part or accessory, which is:

- recognized in the official National Formulary, or the United States Pharmacopeia, or any supplement to them;
- intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals; or
- intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its principal intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its principal intended purposes.

MIXTURE—A color additive made by mixing two or more straight colorants, or one or more straight colorants and one or more diluents, or both. A Secondary colorant.

MUTAGEN—An agent that causes the production of a mutation, i.e., a change in the character of a gene that is perpetuated in subsequent divisions of the cell in which it occurs.

NATURAL COLORANT—One obtained from natural sources; not man-made.

OLEORESIN—The mixture of color and flavor principles obtained from a spice or herb by extracting it with one or more selected solvents and then removing the solvent.

OPACITY—The quality or state of being opaque (i.e., impenetrable by light).

PIGMENT—A colored or white chemical compound that is capable of imparting color and is insoluble in the solvent in which it is being applied. That which is a pigment in relation to one solvent may be a dye in relation to another solvent.

PLATING—The process by which powdered colorant is uniformly deposited onto the surface of a particulate substrate by dry mixing.

POUR-OUT—The process (or its result) whereby a dye solution or dispersion is uniformly spread as a broad streak on a flat uncolored surface for evaluation.

PROVISIONALLY LISTED COLORANTS—Colorants that are not considered unsafe but that nevertheless have not undergone all the tests required by the Color Additives Amendments of 1960 to establish their eligibility for listing. See LISTED COLORANTS.

PURE COLOR (DYE)—The amount of color contained in a color additive, exclusive of any intermediate, diluent, substratum, or other substance.

SATURATION—That attribute of a color perception that determines the degree of its difference from the achromatic (neutral) color perception most resembling it.

SECONDARY COLORANT—A mixture (q.v.).

SHADE—Hue.

STRAIGHT COLOR—A single colorant or a lake of a single colorant.

SUBSIDIARY COLORS—A structural variant of a dye in which the variation is the position, number, and/or chain length of substituent groups.

SUBSTRATUM—The substance on which the pure color in a lake is extended.

TERATOGEN—An agent that causes abnormal development.

TINCTORIAL STRENGTH—A measure of the potential coloring power of a dye.

TONER—An organic pigment containing no substratum or diluent.

UNDERTONE—Color of a thin layer of a pigment incorporated into a vehicle and drawn down on white paper, or color of a tint of the pigment, sometimes as viewed by transmitted light.

VALUE—In the Munsell system of color notation, the lightness of a color. That quality by which we distinguish a light color from a dark one.

APPENDIX D

A GUIDE FOR OBTAINING THE LISTING BY THE FDA OF A PROPOSED NEW COLOR ADDITIVE

There is no formal set of rules to follow that will ensure that the Food and Drug Administration will list a new color additive for use in foods, drugs, cosmetics, and/or medical devices. However, at least the following points must be considered if a reasonable chance for success is expected.

To begin with, it should be determined early that any colorant considered for development will do the job expected of it, i.e., that it will readily dye those things that need to be dyed, that it will produce the desired shade, that it has high tinctorial power, is easy to apply, and is reasonably stable to light, heat, moisture, acids, alkalis, sugars, flavors, product matrices, product containers, etc.

Next, the literature should be consulted to determine if there are any health or environmental hazards associated with the colorant, the raw materials used to prepare it, the chemicals used to prepare them, any waste products from the manufacturing process, or any closely related colorants or intermediates. Similar consideration should be given to the hazards associated with any subsidiary colorant, isomeric colorant, degradation product, or any other organic or inorganic impurity (metals,

salts, etc.) that could be present as a result of the process conditions, or the raw materials, or the equipment used in the colorant's manufacture, its storage, or its formulation into products.

Then, using an authentic pure laboratory sample (so as not to be confused by the properties of impurities!) prepared by the proposed process, screening tests should be performed to answer key questions regarding the colorant, such as: What is its oral toxicity (LD_{50})? Is it a carcinogen, a mutagen, a teratogen, or fetal toxin? What is its dermal toxicity? What effect does it have on skin, eyes, and mucous membranes? Is it an allergen? An aquatic toxin? What is its metabolic fate? Does it biodegrade and are its degradation products safe? Does it bioconcentrate? Would the presence of a likely impurity change the picture any?

Good analytical methods should then be developed that can be used to establish beyond reasonable doubt both the purity and the identity of the raw materials and the finished colorant. In developing the methods, due consideration should be given to the use of traditional techniques as well as the latest tools available. Some of the methods should be screening in nature (high-performance liquid chromatography, thin-layer chromatography, etc.) capable of detecting impurities not originally considered. Where practical, more than one technique should be developed for determining a property or impurity. Appropriate standards should be obtained for calibrating the methods, the precision and accuracy of each method should be well established, and the methods, standards, and results should be thoroughly documented.

Once a colorant has been selected for development, a pilot-plant quantity of it should be prepared by the proposed procedure. Then, using the best available methods, the colorant and the raw materials used to prepare it should be analyzed for strength and any anticipated impurities, and appropriate screening tests should be performed to determine the toxicity of the raw materials and the toxicity and stability of the color additive.

At this time, a summary should be prepared describing the process to be used to manufacture the colorant and its intermediates (including the equipment and raw materials); the criteria by which the quality of the raw materials and the finished product will be judged (proposed specifications); the methods of analysis to be used; the proposed packaging, labeling, and storage of the product; the toxicity studies to be undertaken on the colorant, its intermediates and any potentially toxic impurities that

could be found in the colorant; the stability studies planned for the colorant alone and in representative product matrices; the proposed areas and levels of its use; its anticipated annual consumption; its metabolic fate, its impact on the environment; and any other pertinent information available. This summary should then be discussed in detail with the appropriate FDA personnel, and their approval of the proposed program or some improved version of it should be obtained.

Next, calculate the amount of raw materials needed to establish their identity, purity, and toxicity and to prepare the amount of colorant needed for similar tests and for retain-samples. Then, obtain at least twice these quantities, preferably from the same lot. Document their source and their method of preparation as well as their identity, purity, and homogeneity.

Calculate the amount of colorant needed to perform the required use and stability tests (both alone and in product matrices), to determine the colorant's toxicity and to provide appropriate retain-samples. Then, prepare at least twice this quantity using the approved, documented method of preparation. Homogenize the colorant, pack it appropriately, then sample, analyze, and document each container using approved and documented methods for sampling and analysis.

Finally, select a competent, appropriate, reputable organization to perform the toxicity and stability studies, establish testing protocols, and clear these plans with the FDA. In the case of feeding studies, details that must be considered include: the storage and periodic analysis of the raw dyestuff; the purity, consistency and nutritional value of the colorless feed; the method of preparation, storage, analysis, and auditing of the colored and blank feed; the number and nature of the animals involved, including controls; the method of housing and feeding the animals; the length and nature of the studies; procedures for evaluating the animals before, during, and at the end of the studies; and methods for recording, statistically evaluating, and reporting the data. Similar considerations must be given to stability studies, skin-painting studies, etc. In all cases, all details of the studies must be properly recorded. The kind of toxicological testing needed depends on the type of colorant being developed. Testing an FD&C color, for example, might require 2-year feeding studies in dogs and rats, repeated dermal application tests on rabbits and mice, and two-generation reproduction studies with rats. In each case, the test animals are compared with control groups with respect to survival, appearance, behavior, appetite, elimination, organ weights and ratios, tissue

structure, skeletal structure, and other variables, depending on the test involved. Where reproduction studies are concerned, the offspring are similarly evaluated.

All the data gathered should then be incorporated into a petition to the FDA for their review. Public notice of the filing of the petition and the FDA ruling on it is given in the *Federal Register.* If the petition is found to be complete and convincing, the color will then be listed for use in the kinds of products tested and petitioned for—foods, drugs, cosmetics, medical devices, or all four.

PART B

COLORANT ANALYSIS

6

IDENTIFICATION

The techniques used for the analysis of color additives differ little from those used for the examination of other commercial compounds. Because of the extent to which these colorants are examined, particularly the certified food colors, they are among the most thoroughly analyzed group of chemicals available today.

Unlike technical dyestuffs, color additives are usually analyzed on an absolute basis rather than versus a standard sample. The reason for this, of course, is the need to know the exact nature of any compound consumed by man or applied to his body.

As is the case with most branches of chemistry, the methodology used to analyze colorants is experiencing a renaissance with many of the old wet procedures being replaced or supplemented by sophisticated instrumental techniques. The majority of the procedures in use today have been developed by both industry and government (FDA), and many have been collaboratively studied for their precision and accuracy. Although few methods are designated as official, many have been used for so long that they often appear as such.

The most frequently used sounding board for the dissemination of new technology in the field of color-additive analysis is the Journal of the Association of Official Analytical Chemists. Time-tested methods can be found in a book entitled *Official Methods of Analysis of the Association of Official Analytical Chemists.*

IDENTIFICATION OF COLOR ADDITIVES

Numerous procedures have been used to identify color additives. The methods employed are usually limited only by the inventiveness of the analyst and the equipment to which he or she has access. In general, those described here presume that the chemist has a single colorant and that it is not in a food, drug, cosmetic, or medical device. The isolation of colorants from product matrices and the resolution of mixtures of colorants are separate problems and are considered in detail later.

In attempting to identify any unknown, the colorant's physical properties, including its solubility, crystal structure, melting point, if any, and color in solution as well as its color under ultraviolet (UV) light, both as is and in solution, provide important clues.

Migration rates such as a colorant's R_f in thin-layer (TLC) and paper-chromatography systems, its retention time or retention volume during column chromatography, its ionic mobility in electrophoresis experiments, and its partition coefficient during solvent-solvent extraction are all useful as methods of identification. However, one must realize that such constants are not unique and are only conclusive as means of identification when determined versus knowns and in a variety of media.

The behavior of a colorant when mixed with various reagents, including nitric acid, sulfuric acid, hydrochloric acid, sodium hydroxide, and sodium carbonate can be very informative (Table 6.1). Qualitative tests for various functional groups and metals can also be meaningful.

One of the more elegant approaches to the identification of azo colorants involves their reduction followed by the identification of the reduction products. Water-soluble colors are usually reduced in hot water with sodium hydrosulfite. Oil-soluble colors are best cleaved in alcohol and under a stream of inert gas using titanium trichloride as the reducing agent. Usually, the reduction products obtained consist of the amine originally diazotized and used to form the dye (or a reduction product of

this amine) plus an amino derivative of the compound to which the diazo component was originally coupled. Basic components obtained can be separated from neutral and acidic materials by steam distillation or by extraction from alkaline solution, whereas neutral components can be steam distilled or extracted from neutral solutions. Acidic materials such as sulfonic acids can be neither steam distilled nor extracted from water using simple liquid/liquid techniques and thus remain behind in either of the preceding schemes. Alternately, the reduction products can be separated using chromatographic procedures similar to those described for the determination of uncombined intermediates in color additives. In any event, the products obtained are best identified using UV spectrophotometry. A major advantage of this approach is the need for only a few milligrams of sample to run the test.

The most widely used and in general the most conclusive procedures for identifying color additives are instrumental in nature. Thermal techniques such as differential thermal analysis (DTA) and differential scanning calorimetry (DSC) are useful since the thermograms produced are "fingerprints" of the compounds examined. Thermal methods, though, are generally more valuable as qualitative tools for the study of purity and stability and thus far have found little application as methods of identification. The real work-horse in this area is spectrometry.

UV and visible spectrometry are usually the simplest to perform and require the least amount of sample, often as little as 0.1 mg. Where possible, spectra of the unknown should be compared with those of knowns in several solvents since, although two colorants may have almost identical spectra in any one solvent, it is rare that their spectra will be the same in several of them. The solvents chosen for such comparisons should be as different as possible—aprotic versus protic, acid versus alkaline, polar versus nonpolar, and so on—with due consideration, of course, of the solvent's spectral characteristics. The use of different modes of recording—absorbance, transmittance, first and second derivative, and so on—can often enhance spectral differences and thus improve one's chances of identifying an unknown. Spectra can usually be adequately compared by visual inspection alone; however, sophisticated electronic equipment is available, particularly for work in the visible region, that can evaluate and compare them mathematically. When working in the UV region, one must be careful that the colorant does not contain colorless UV-absorbing excipients that can clutter the spectra and

TABLE 6.1. Reactions of Some Natural Coloring Materials[a]

Coloring Matter	Concentrated Hydrochloric Acid	10% Sodium Hydroxide Solution	Sodium Hyposulfite
Annatto	Remains orange, little change		Little affected
Caramel	Little or no change	Little change or slightly browner	Slightly paler
Carotene and xanthophyll	Little change, perhaps slightly paler	Little or no change	Little affected
Cochineal	Little or no change	Violet	No marked change
Logwood	Deep red with excess of acid	Violet to violet-blue	Almost decolorized, color returning imperfectly by reoxidation
Saffron	Little or no change	Remains yellow	Little affected
Turmeric (solution in ethyl ether or ethanol characterized by pure yellow color and light green fluorescence)	Orange-red or carmine-red on addition of several volumes of concentrated acid	Orange-brown	Little affected

From: *Official Methods of Analysis,* 11th ed., The Association of Official Analytical Chemists, Washington, D.C., 1970, p. 581.

[a]Procedure: Dissolve the color in a small amount of ethanol and dilute with water. To individual portions of this solution apply reagents as follows:

Hydrochloric acid—Add one or two drops of concentrated hydrochloric acid, then dilute to three or four times the sample volume with concentrated hydrochloric acid.

Sodium hydroxide solution—Make solution slightly alkaline with one drop of 10% sodium hydroxide.

Sodium hyposulfite—Add $Na_2S_2O_4$ crystals.

mislead the analyst. UV and visible spectrometry are fast, reliable, and relatively simple procedures for identifying dyestuffs and should be used whenever possible. Their use requires only a modest amount of training, whereas the necessary equipment is moderate to expensive in price.

Infrared (IR) has also been used extensively for the identification of unknowns. IR techniques are generally a little more complicated and

0.5% Ferric Chloride Solution	10% Alum Solution	5% Uranium Acetate Solution	Sulfuric Acid on Dry Color
No marked change, perhaps somewhat browner			Blue
No change			Blue, reaction obtained with difficulty
Slightly darker		Green	
Dark shades of violet, brown, or black (first hue often fleeting)	Rose red (change rather slow)	Violet, quickly fading	Red, changing to yellow
No marked change, perhaps somewhat browner	Little change	Not affected	Blue
No marked change, perhaps somewhat browner	Little change	Somewhat browner	Red

Ferric chloride solution—Add fresh 0.5% $FeCl_3$ solution dropwise; colors are not always obtained if an excess is used.

Alum solution—Add to the test solution 20% of its volume of 10% potassium alum or ammonium alum solution.

Uranium acetate solution—Add 5% UO_2 $(OAc)_2$. $2H_2O$ solution dropwise.

Sulfuric acid on dry color—Dry a small portion of the color in an evaporating dish. Cool. Treat the residue with one or two drops of cold concentrated sulfuric acid. The color formed is sometimes extremely transitory and may be noted only when the acid wets the residue.

expensive to use than UV and visible spectrometry but usually provide a higher order of certainty when dealing with unknowns. Infrared spectra have been obtained as Nujol mulls, as KBr pellets, in solution, and as complexes in liquid ion exchange resins such as Amberlite, LA-2 (Rohm and Haas, Philadelphia, PA). The procedure to use, of course, depends on the nature of the dyestuff being examined. Organic diluents such as

dextrin and sugar and certain inorganics such as sulfates, present naturally or as deliberately added diluents, complicate IR spectra and should be removed prior to analysis or taken into account when interpreting them. When comparing spectra prepared as mulls or KBr pellets, it's also necessary to remember that differences observed can be due to purely physical reasons, including the sample's particle size, the pressure and time used in preparing the KBr pellet, and other variables and may have nothing to do with the structure of the dyestuff itself.

Recently proton nuclear magnetic resonance (NMR) has been used to identify both primary and secondary color additives. Good spectra of the certified water-soluble food colors have already been obtained and published using a mixed, deuterated solvent (water: dimethylsulfoxide; D_2O:DMSO-d_6, 2:1 v/v) at 100–105°C, and it can be presumed that more work using proton, ^{13}C, ^{14}N, and other forms of NMR will soon follow. NMR is one of the least sensitive, most complicated, and most expensive of the spectral techniques in use today, but is an excellent tool for identification purposes and for studying the structure of organic compounds. Inorganic salts such as sodium chloride and sodium sulfate do not interfere with NMR spectra, but protonated impurities, such as acetates, sugars, and other dyestuffs, do.

Not much has been reported in the field of color additives using Raman spectroscopy; however, this tool should be excellent for the identification of colorants, particularly the water-soluble ones.

A great many spectra of color additives have been published to date, some of which are referred to in the bibliography at the end of this chapter. Many of these are of high-enough quality that they can be used as standards for comparison purposes; however, comparisons are best made against knowns prepared by the same analyst at the same time and on the same equipment used to prepare the unknowns.

Selected thermograms and spectra are included here to illustrate the value of the various techniques (see Figs. 6.1*a–p*, 6.2*a–n*, and 6.3*a–h*). The reactions of some natural coloring materials with various reagents are shown on pages 202–203.

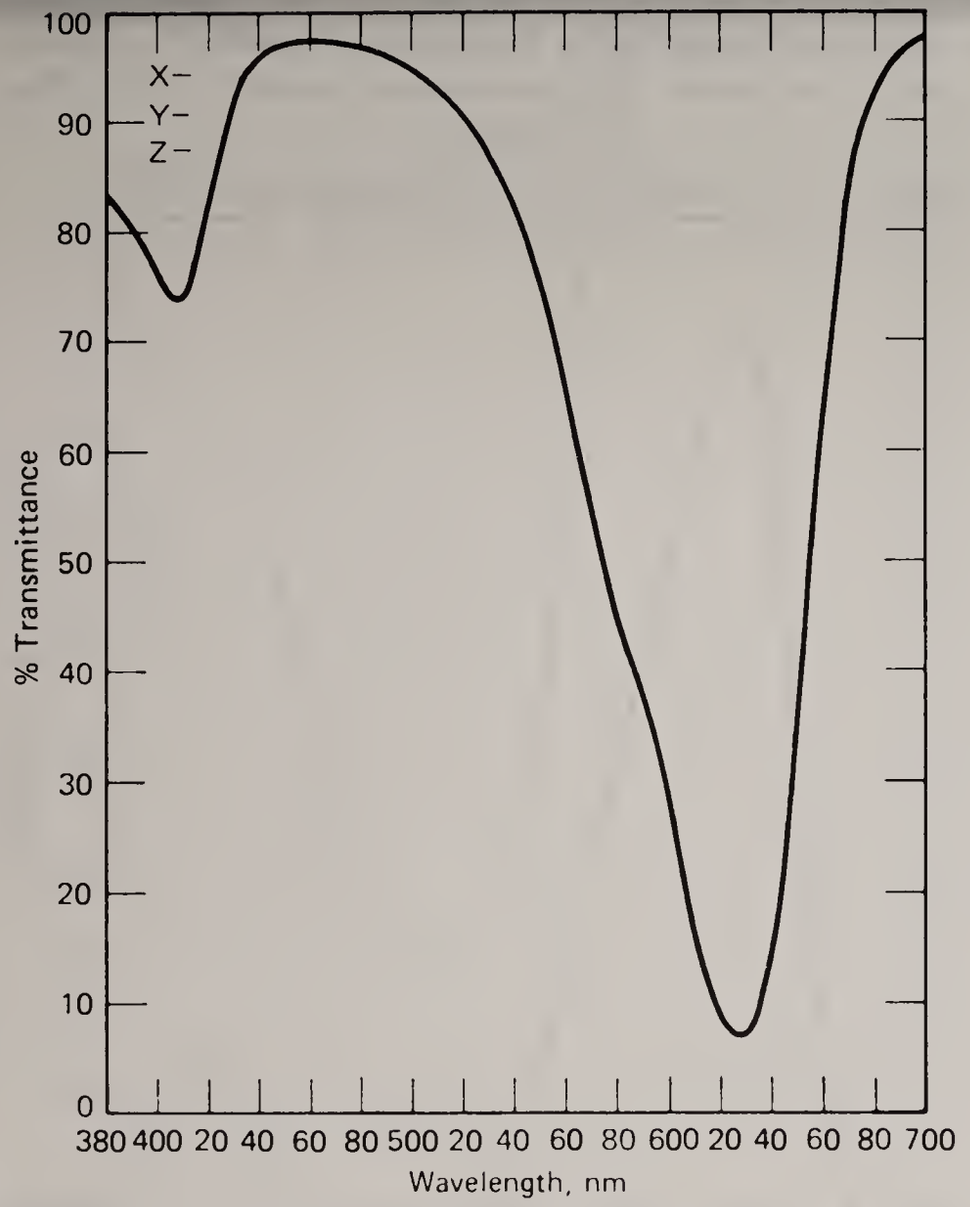

Figure 6.1*a*. Visible Spectrum of FD&C Blue No. 1: concentration, 8 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

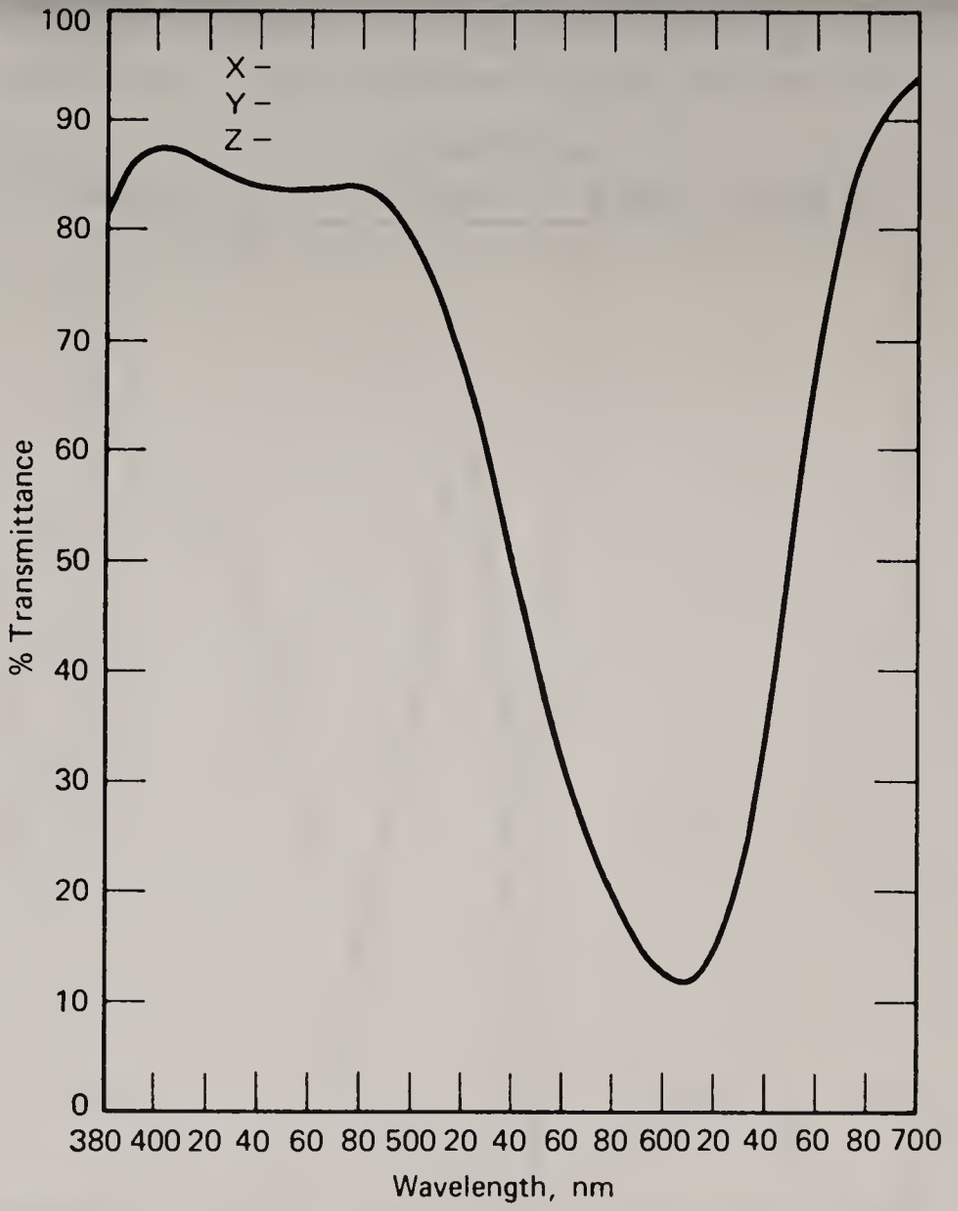

Figure 6.1*b*. Visible Spectrum of FD&C Blue No. 2: concentration, 22 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

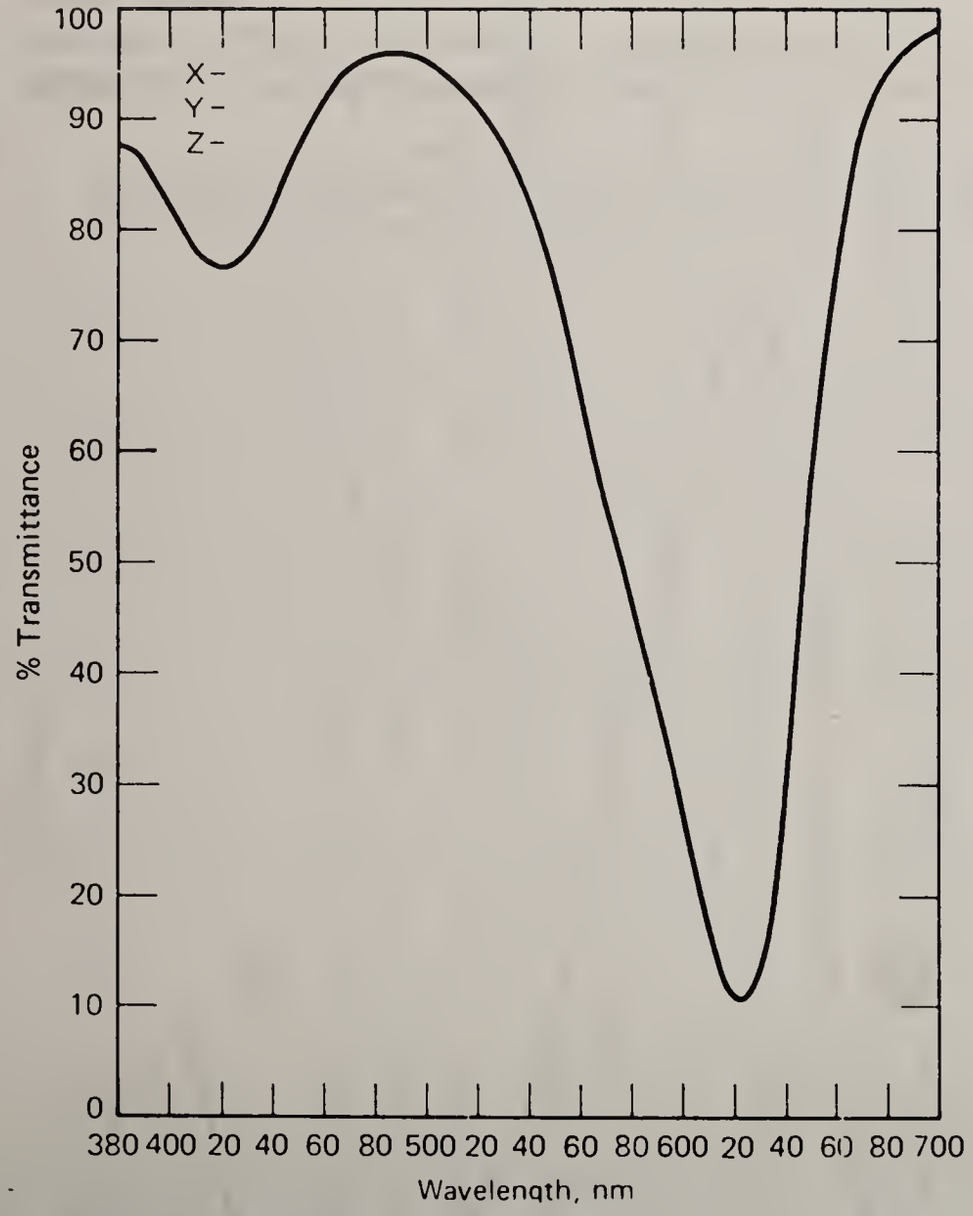

Figure 6.1*c*. Visible Spectrum of FD&C Green No. 3; concentration, 7 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

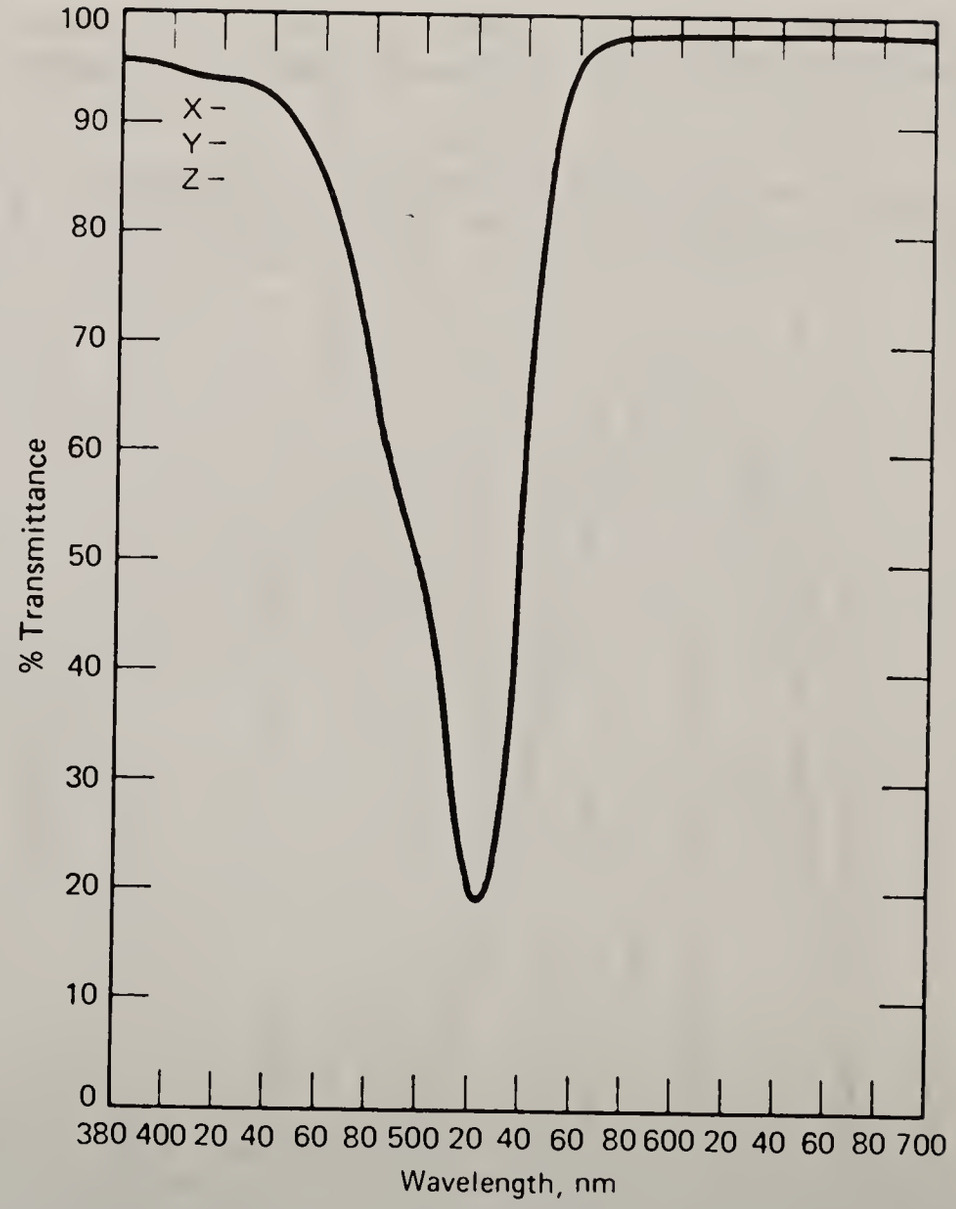

Figure 6.1*d*. Visible Spectrum of FD&C Red No. 3: concentration, 7.5 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

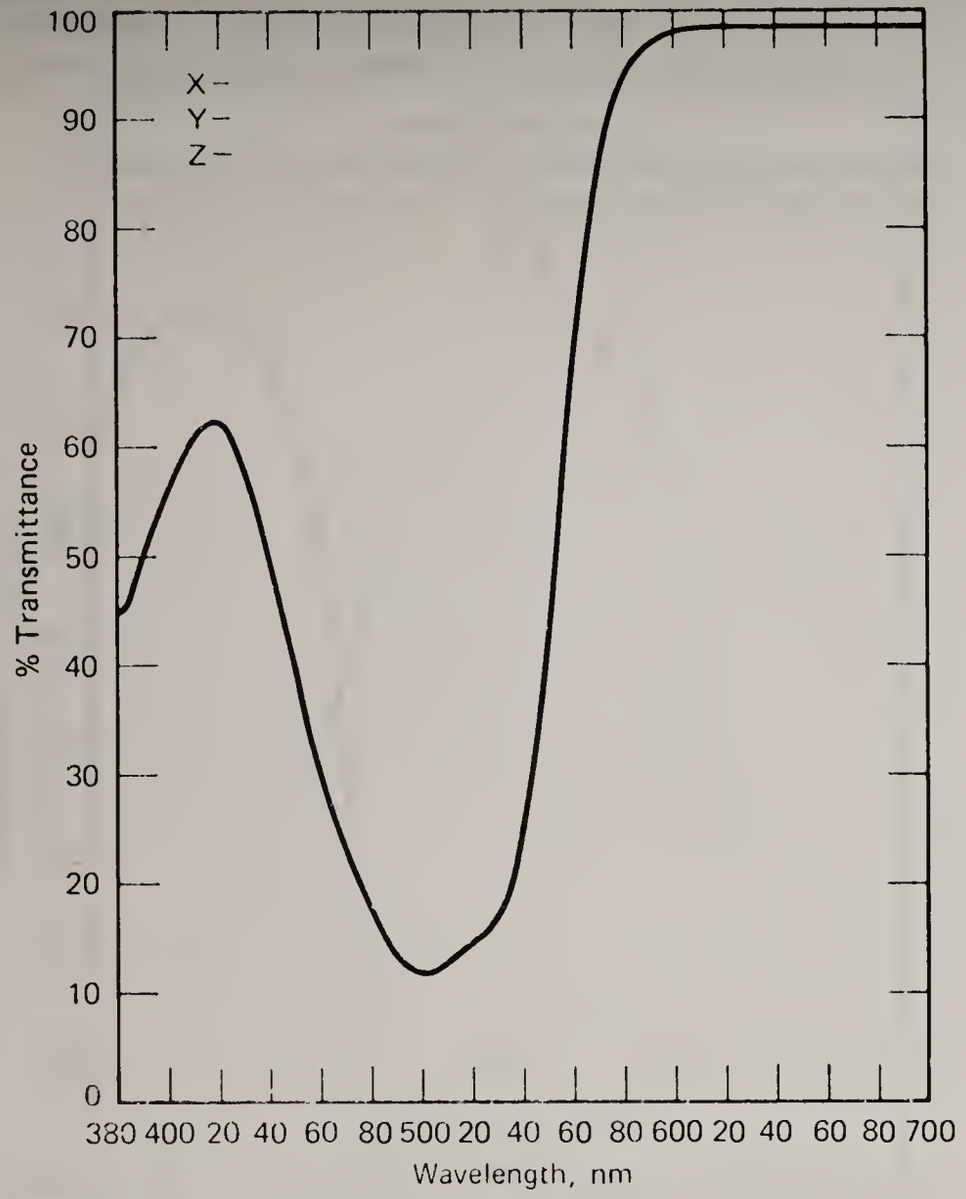

Figure 6.1*e*. Visible Spectrum of FD&C Red No. 4: concentration, 20 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

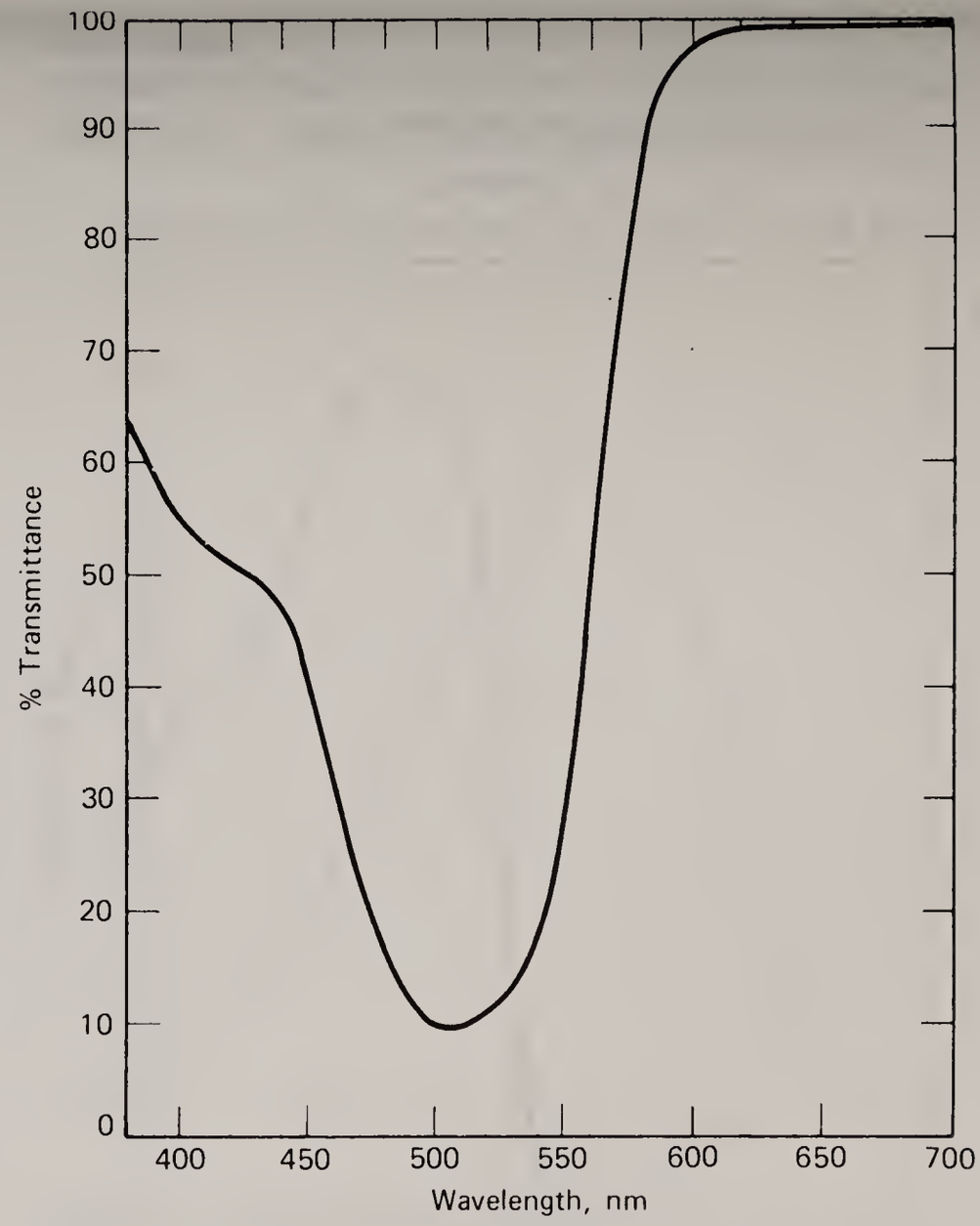

Figure 6.1*f*. Visible Spectrum of FD&C Red No. 40: concentration, 20 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

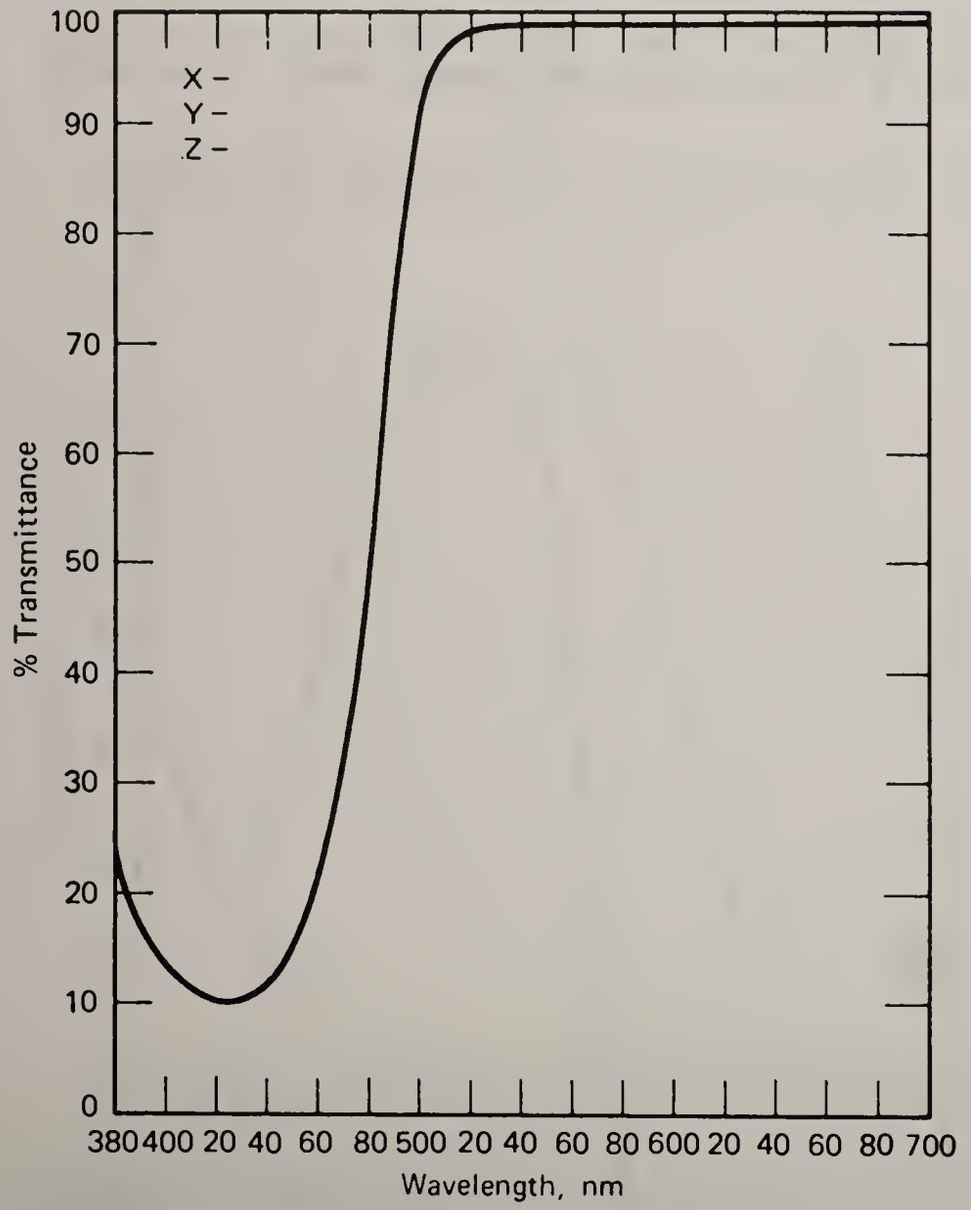

Figure 6.1*g*. Visible Spectrum of FD&C Yellow No. 5: concentration, 20 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

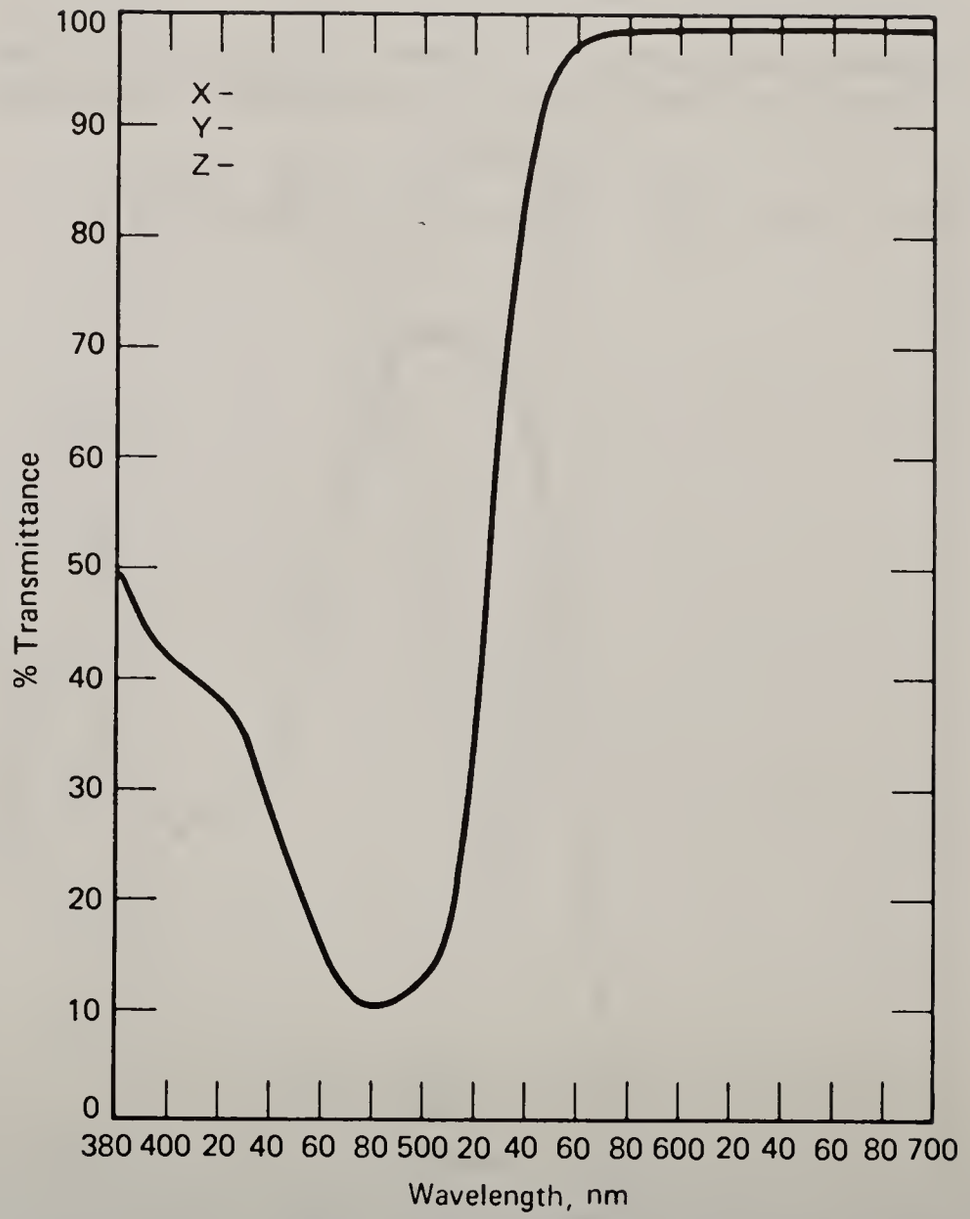

Figure 6.1*h*. Visible Spectrum of FD&C Yellow No. 6: concentration, 20 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

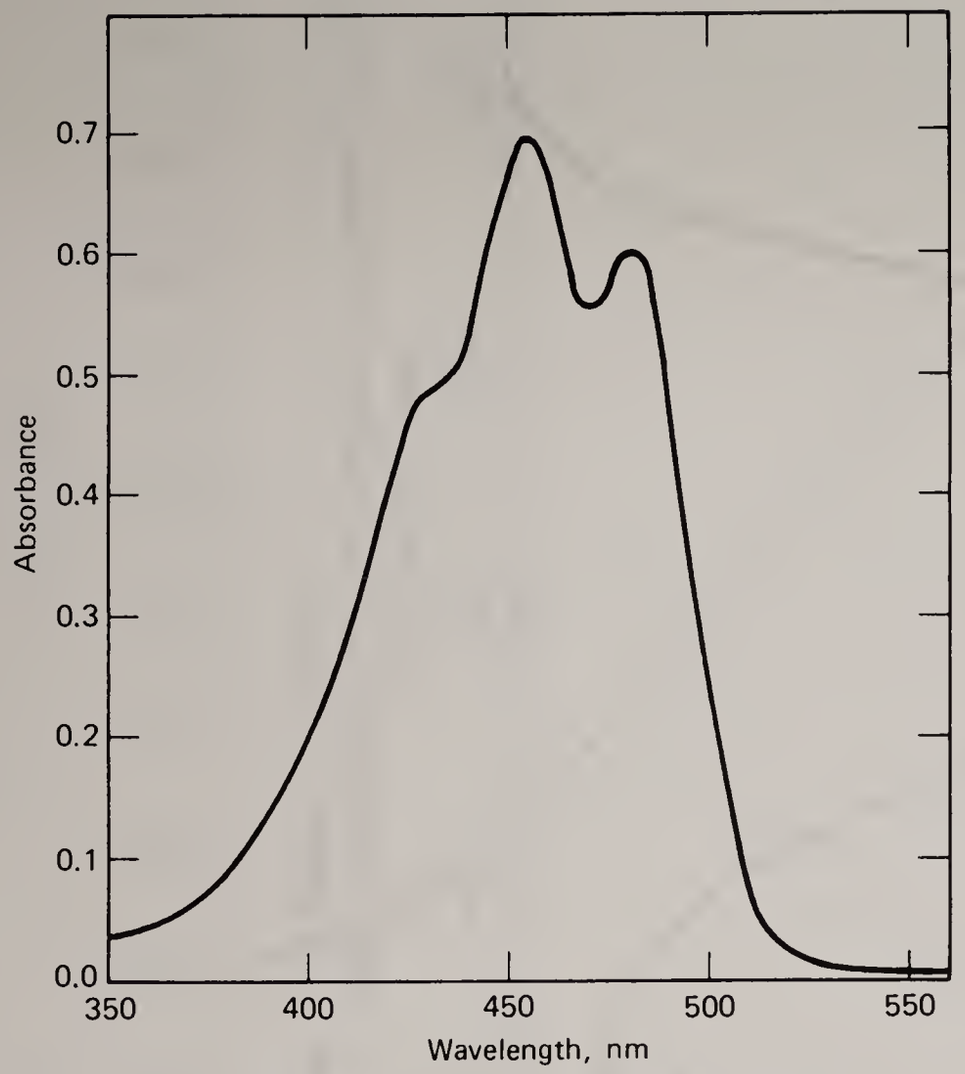

Figure 6.1*i*. Visible Spectrum of All-*trans* β-Carotene: concentration, 2.68 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

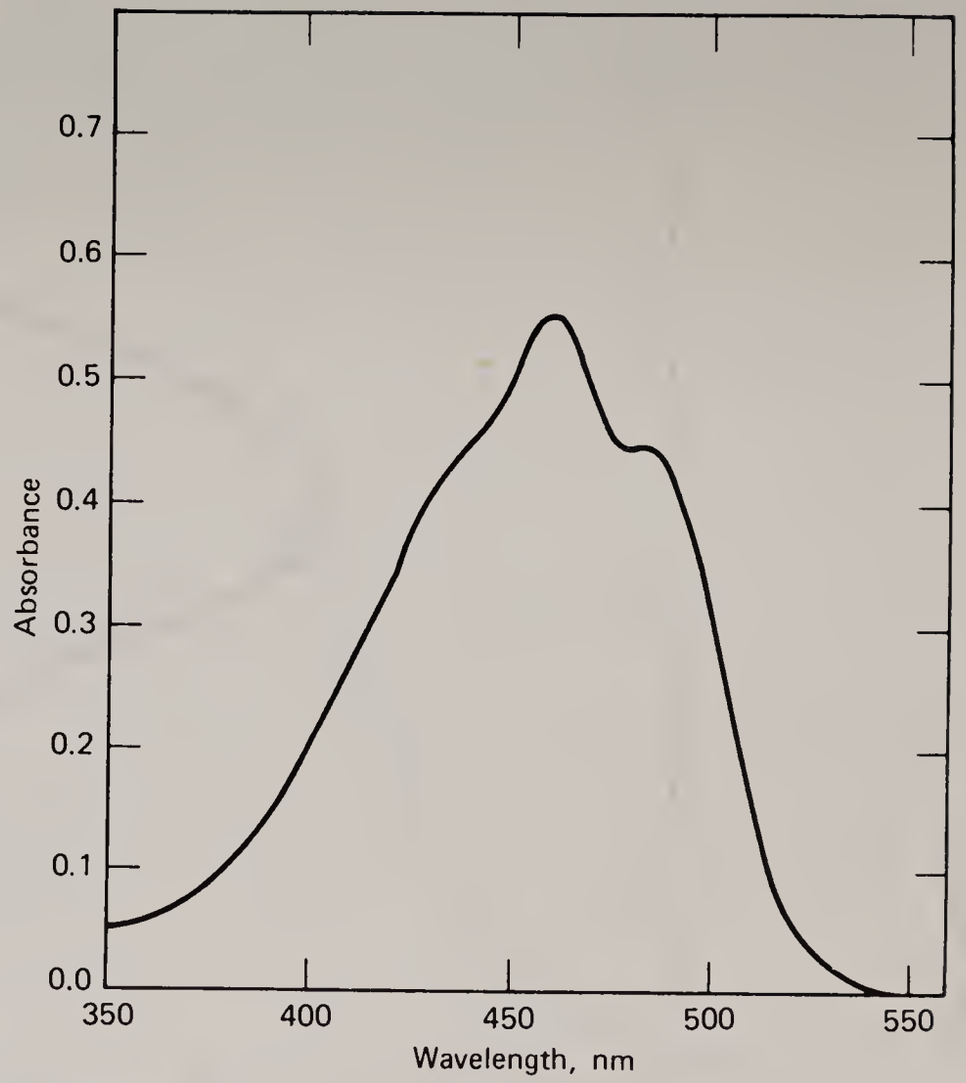

Figure 6.1*j*. Visible Spectrum of All-*trans* β-apo-8′-Carotenal: concentration, 2.11 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

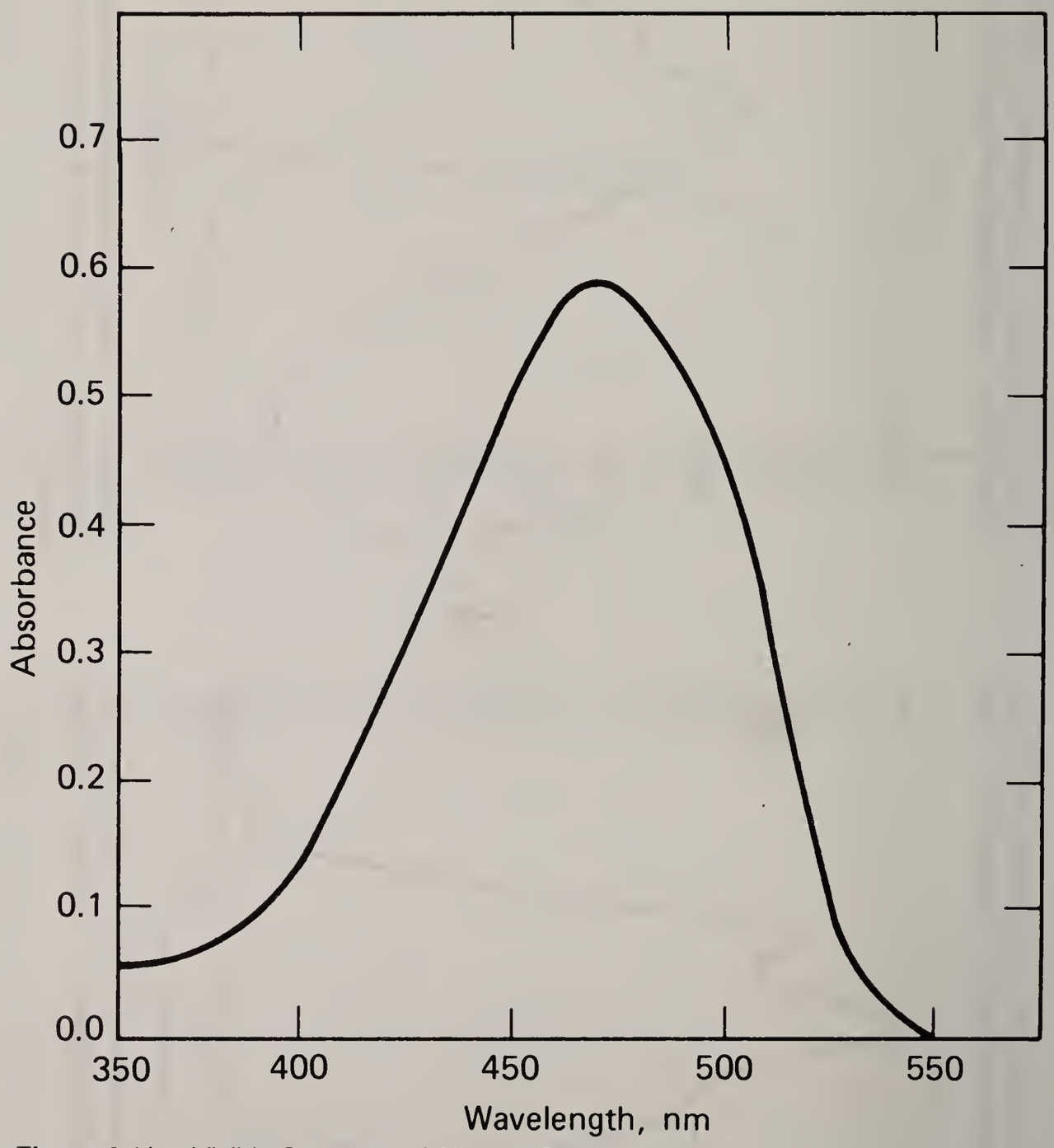

Figure 6.1*k*. Visible Spectrum of All-*trans* Canthaxanthin: concentration, 2.67 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

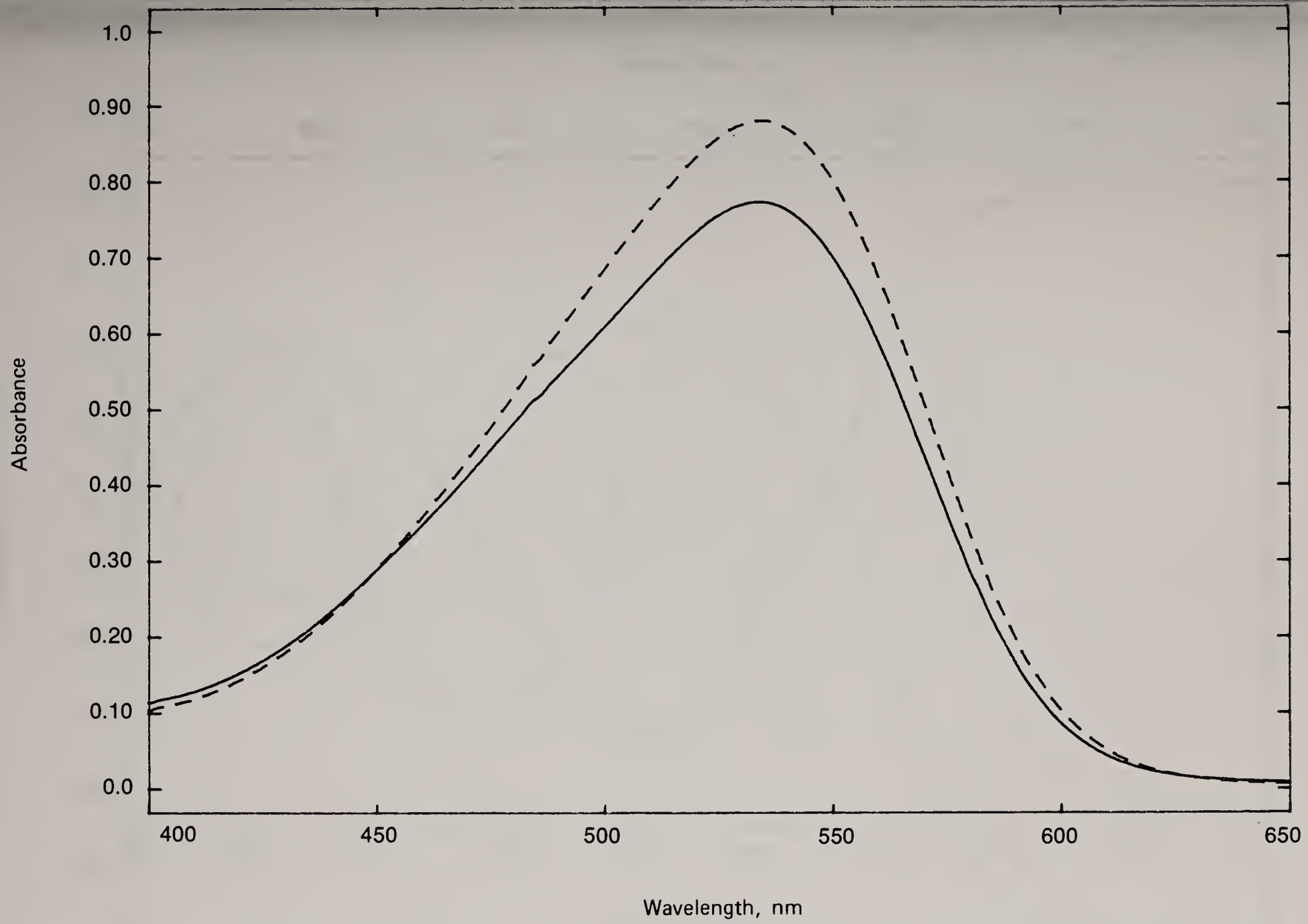

Figure 6.1*l*. Visible spectra of two liquid beet juice concentrates; concentrations, 2500 μg/mL; cell thickness, 1 cm; solvent, water; reference, water

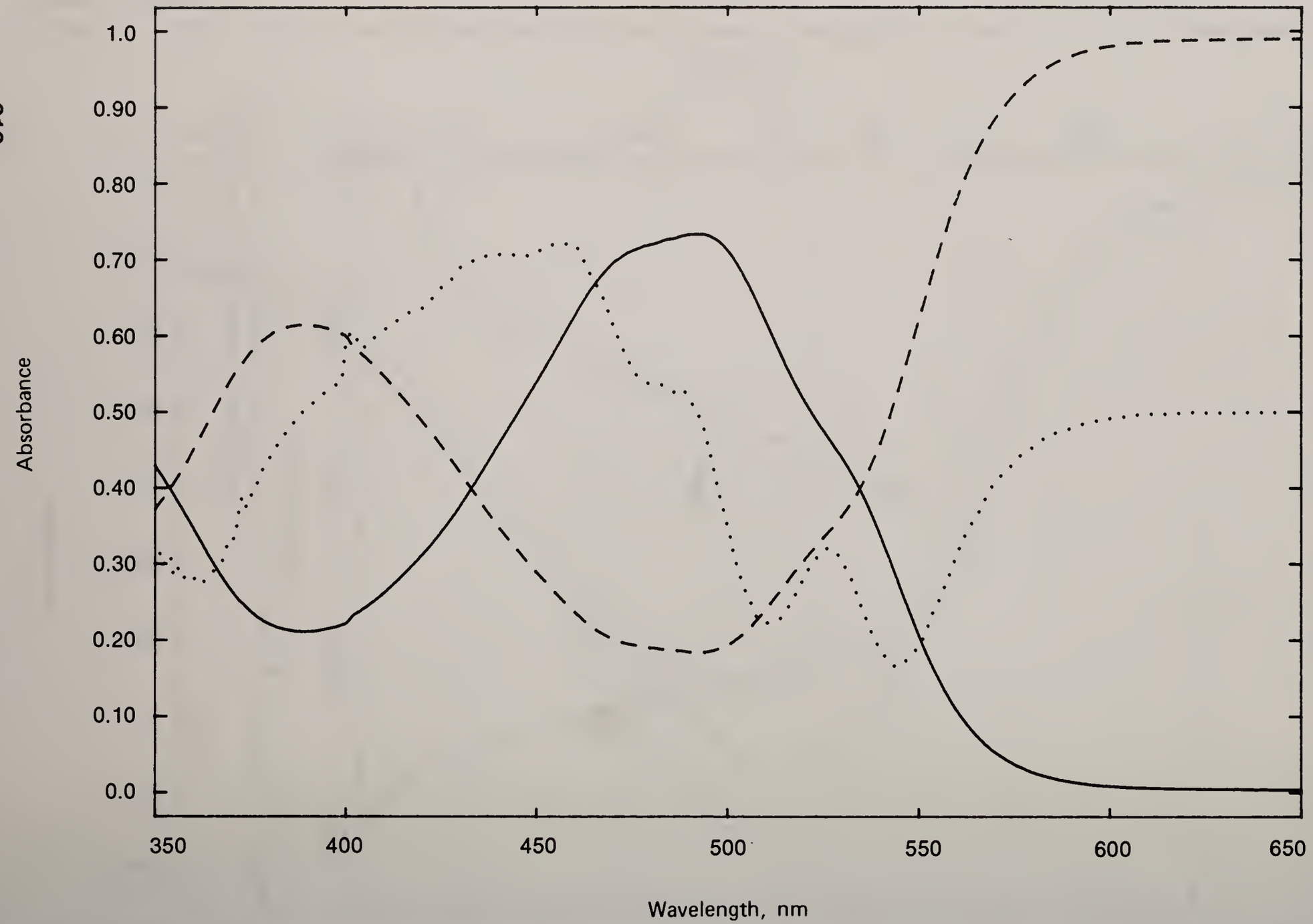

Figure 6.1*m.* Visible spectrum of a 54% carmine lake: concentration, 100 μg/mL; cell thickness, 1 cm; solvent, 0.2% v/v aq. HCl; reference, water; (——) absorbance; (- - - -) transmittance; (· · · ·) dA/dλ

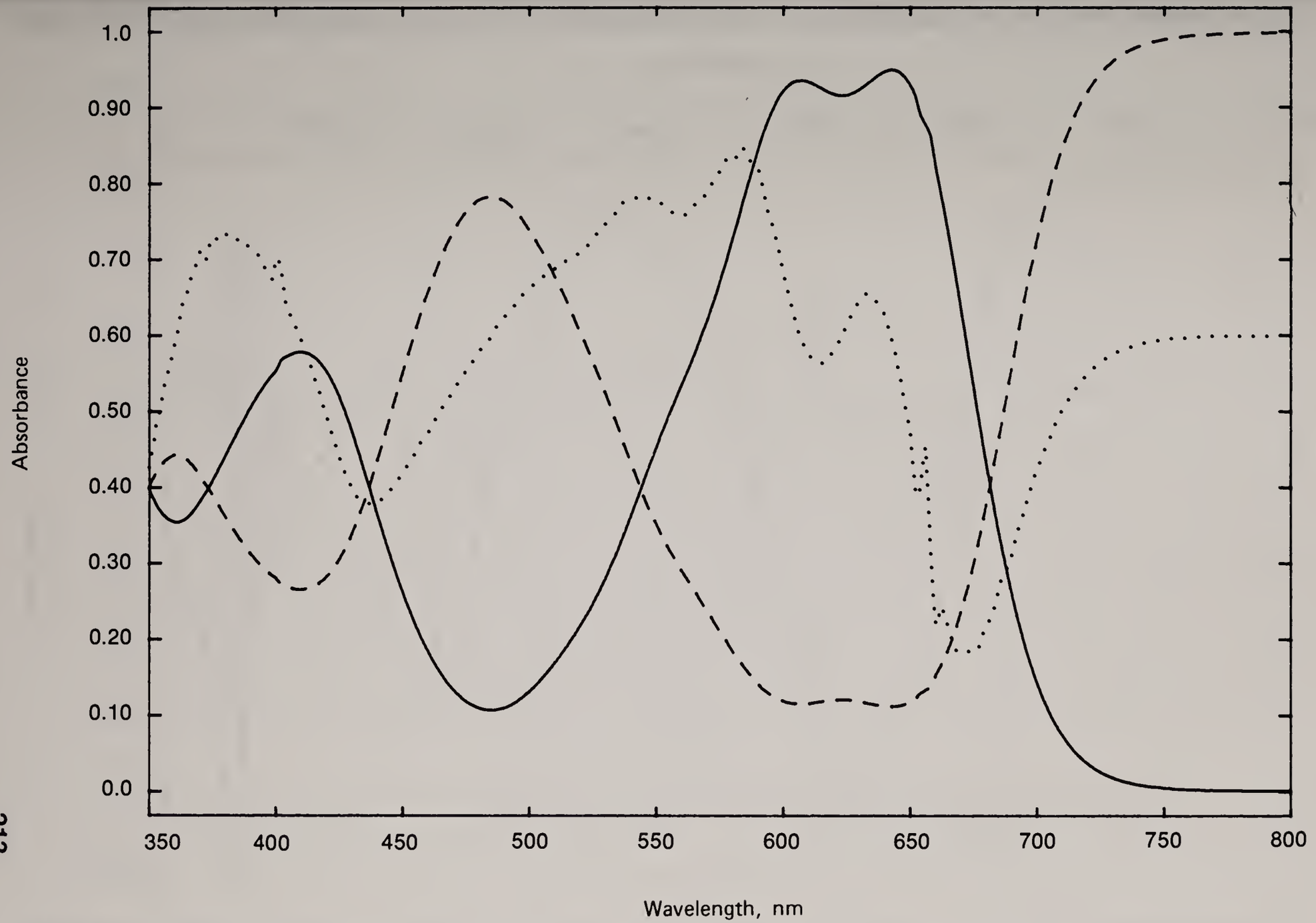

Figure 6.1*n*. Visible spectrum of D&C Green No. 5: concentration, 44 μg/mL; cell thickness, 1 cm; solvent, water; reference, water; (——) absorbance; (- - - -) transmittance; (· · · ·) dA/dλ

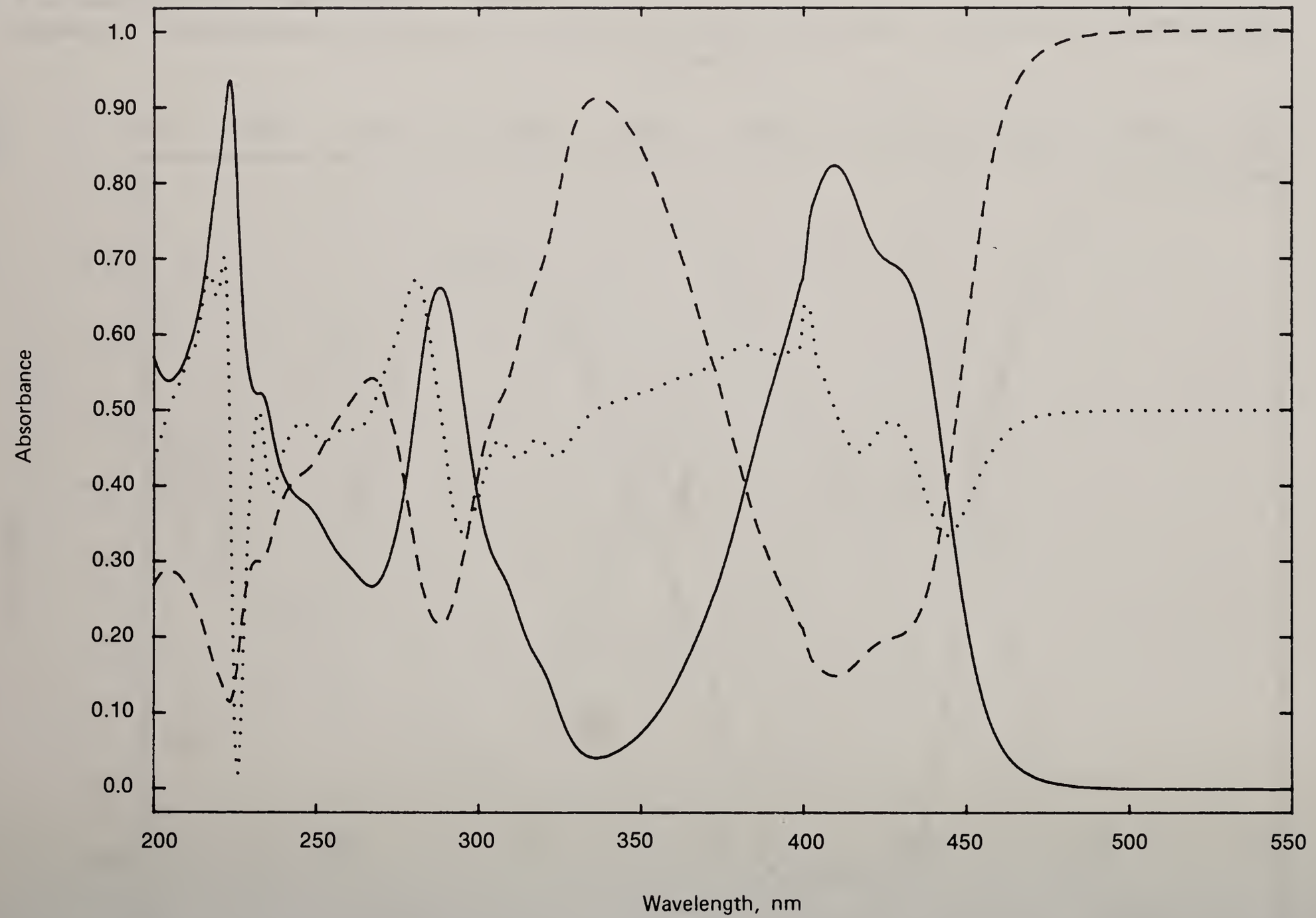

Figure 6.1*o*. Visible spectrum of D&C Yellow No. 10: concentration, 9 μg/mL; cell thickness, 1 cm; solvent, water; reference, water; (——) absorbance; (- - - -) transmittance; (· · · ·) dA/dλ

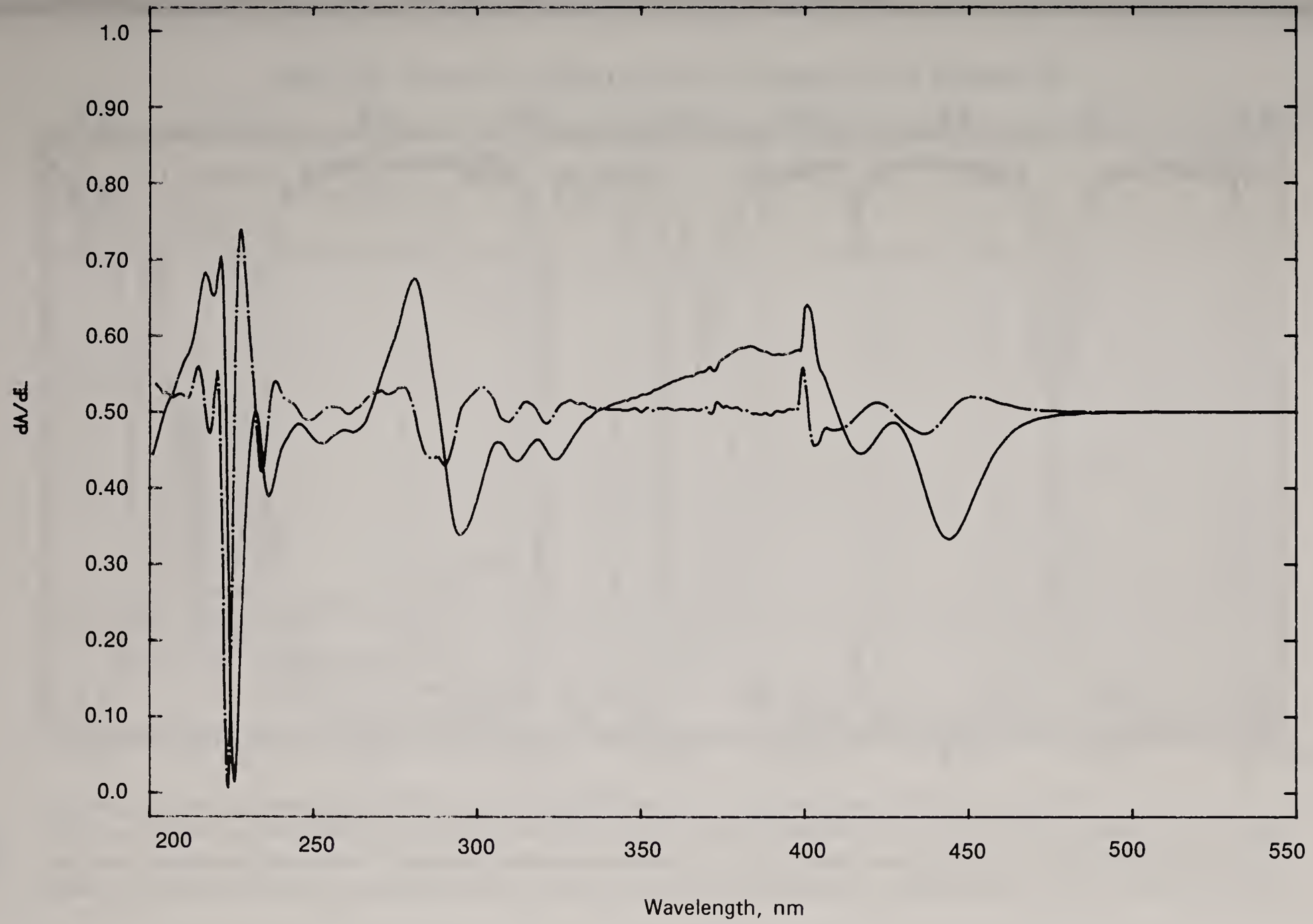

Figure 6.1*p*. Visible spectrum of D&C Yellow No. 10: concentration, 9 μg/mL; cell thickness, 1 cm; solvent, water; reference, water; (——) $dA/d\lambda$; (- · -) $d^2A/d\lambda^2$

Figure 6.2 Nuclear magnetic resonance spectra obtained on Varian A60 Spectrometer equipped with variable-temperature probe and using the following operationg conditions: probe temperature, 100–105°C; filter bandwidth, 0.4 Hz; radiofrequency field, 0.07 mG; sweep time, 500 sec; sweep offset, 0 Hz; solvent, D_2O/DMSO-d_6 (dimethylsulfoxide)(2 + 1, v/v); marker TSP (sodium 3-trimethylsilylpropionate-2,2,3,3,-d_4)

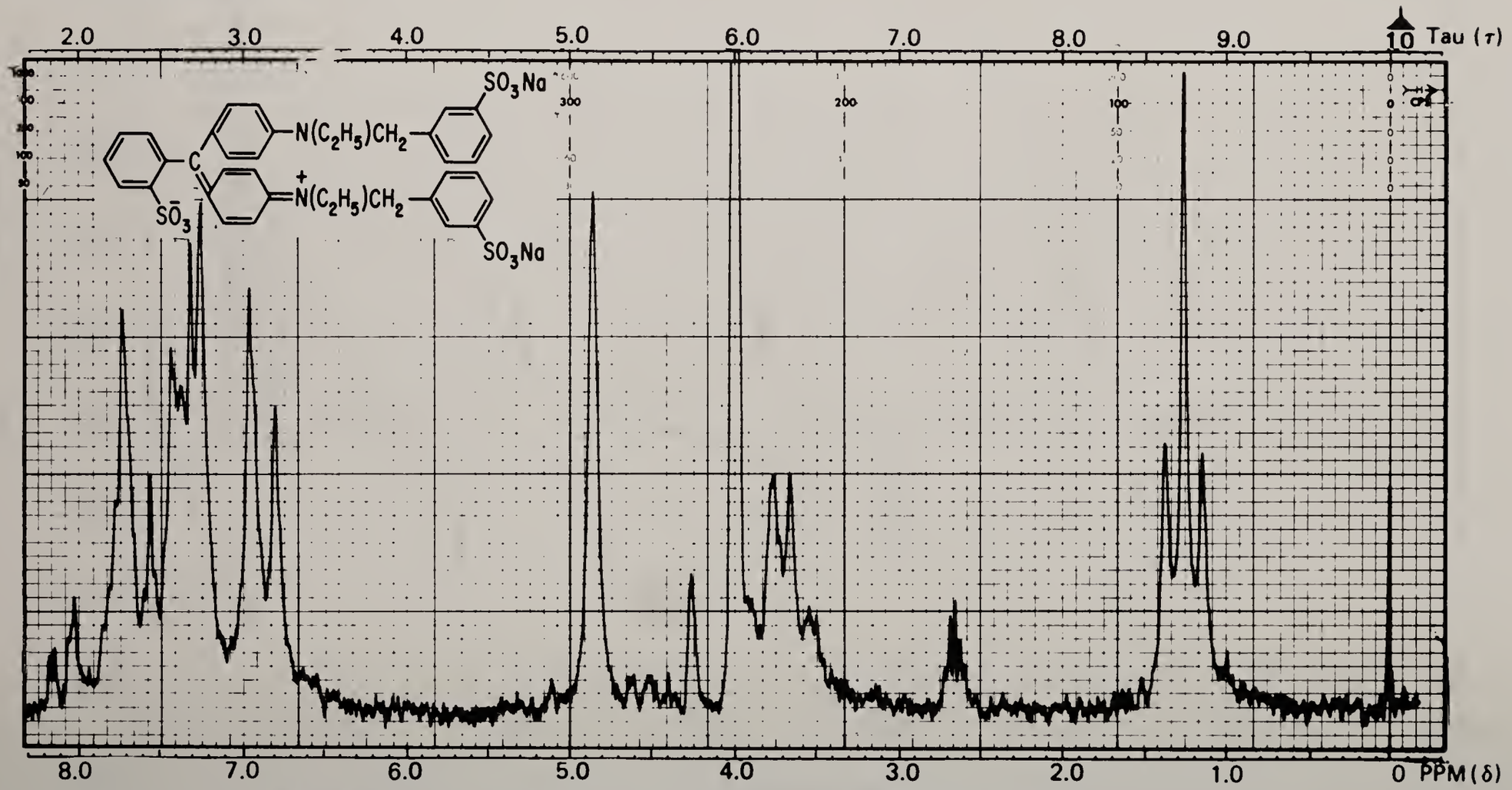

Figure 6.2*a*. Spectrum of FD&C Blue No. 1: concentration, 20%; amplitude, 80

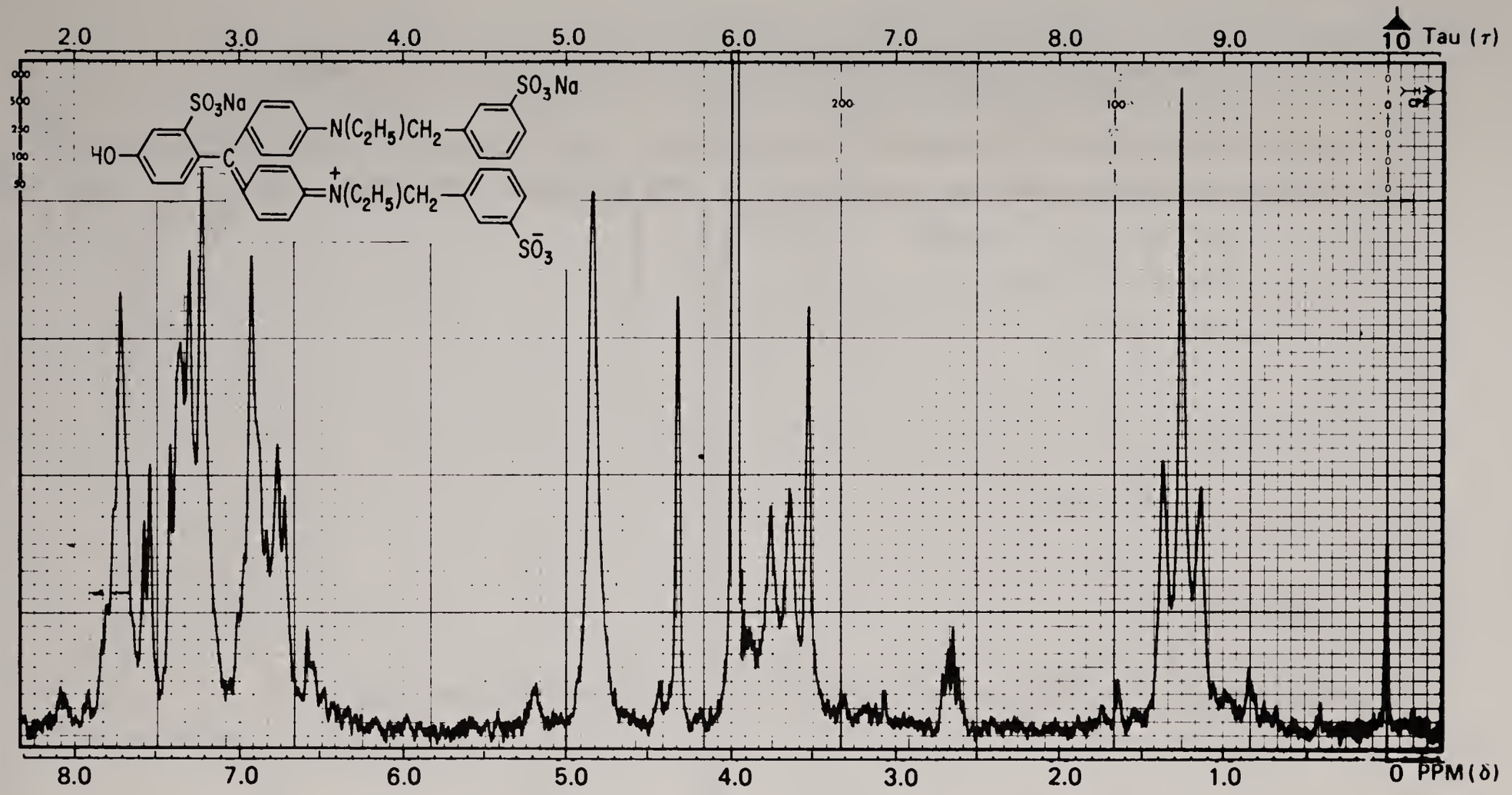

Figure 6.2*b*. Spectrum of FD&C Green No. 3: concentration, 20%; amplitude, 80

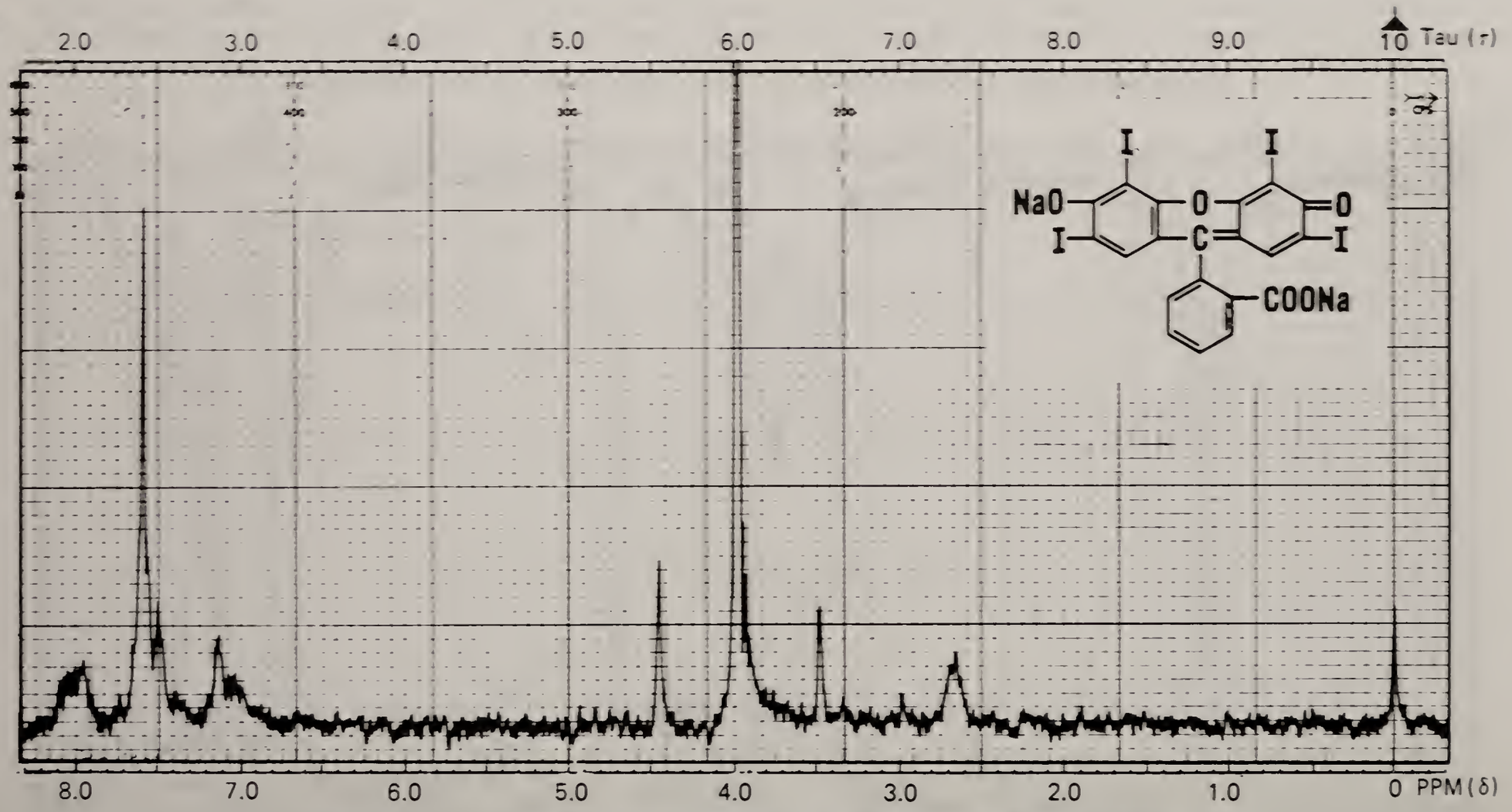

Figure 6.2*c*. Spectrum of FD&C Red No. 3: concentration, 20%; amplitude, 80

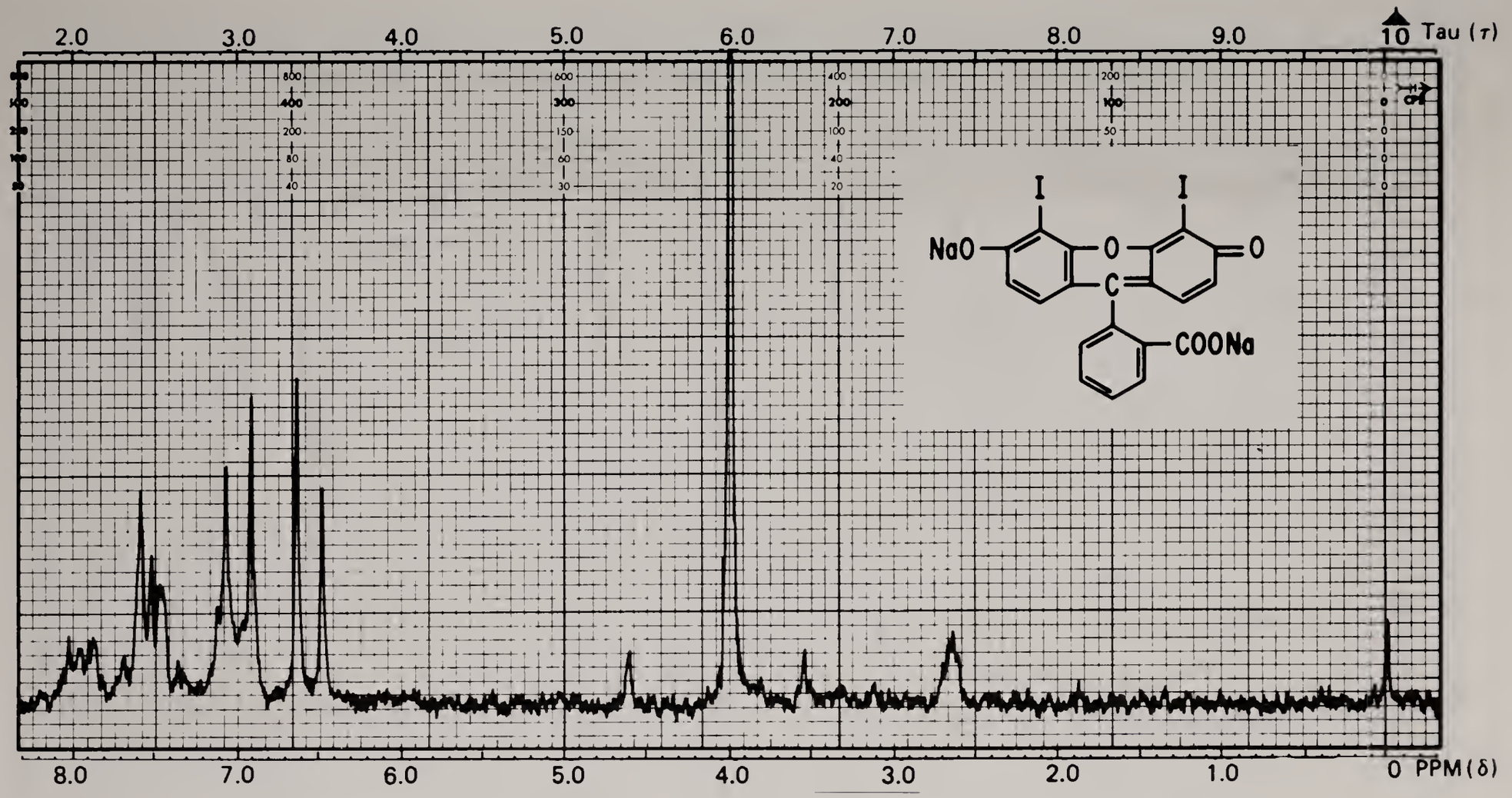

Figure 6.2*d*. Spectrum of D&C Orange No. 11: concentration, 20%; amplitude, 80

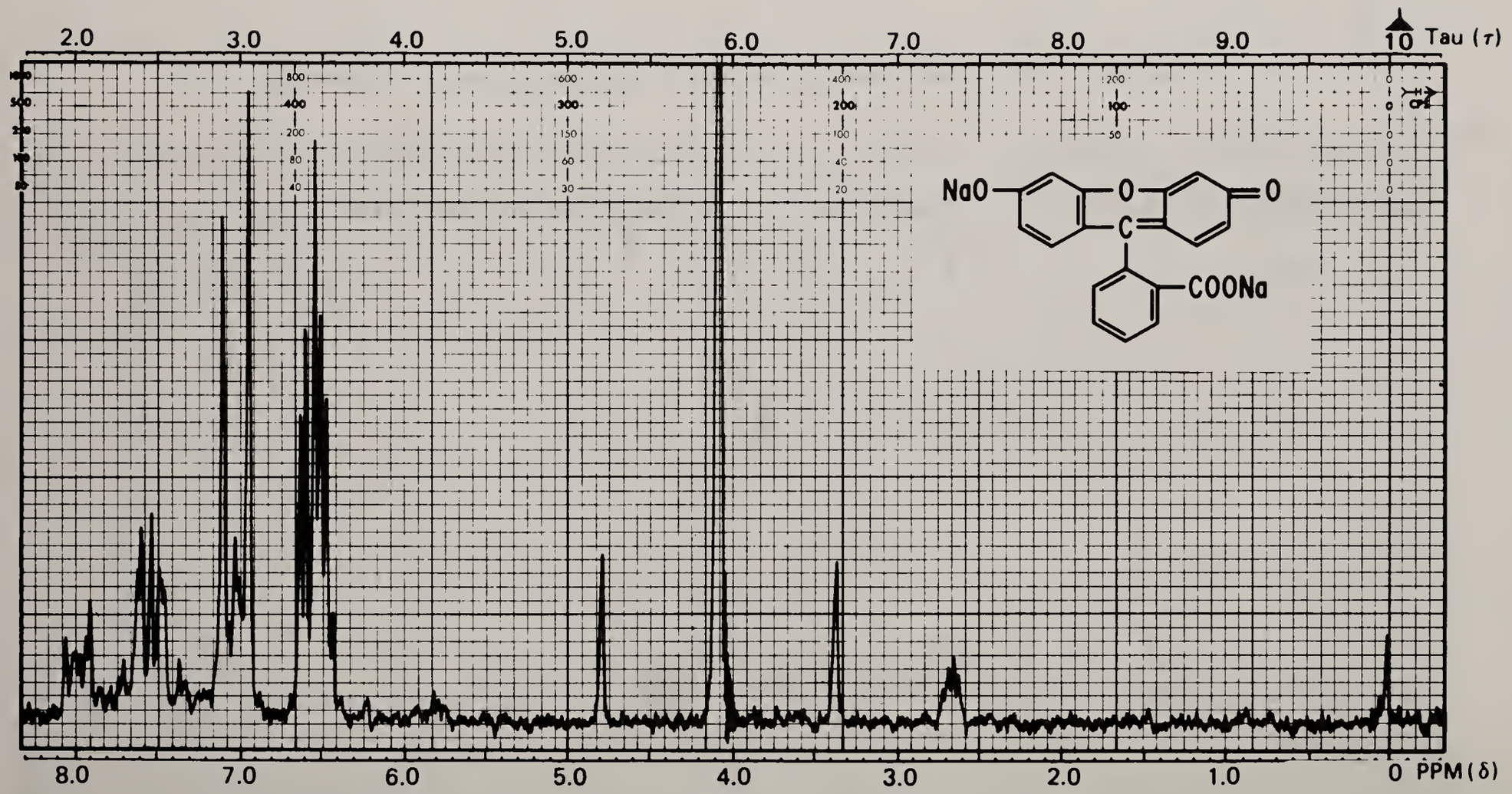

Figure 6.2*e*. Spectrum of D&C Yellow No. 8: concentration, 20%; amplitude, 63

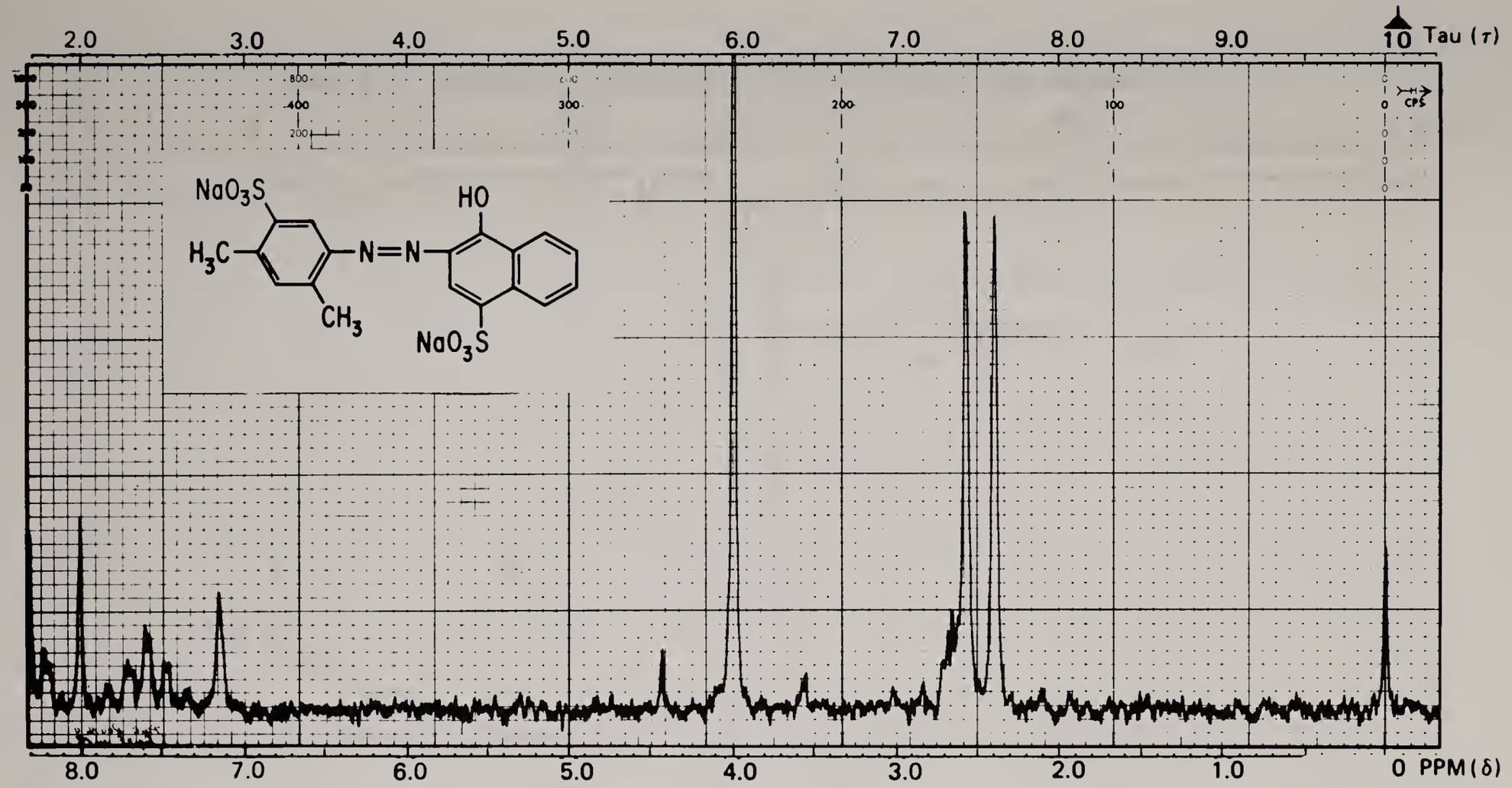

Figure 6.2*f*. Spectrum of FD& C Red No. 4: concentration, 15%; amplitude, 80

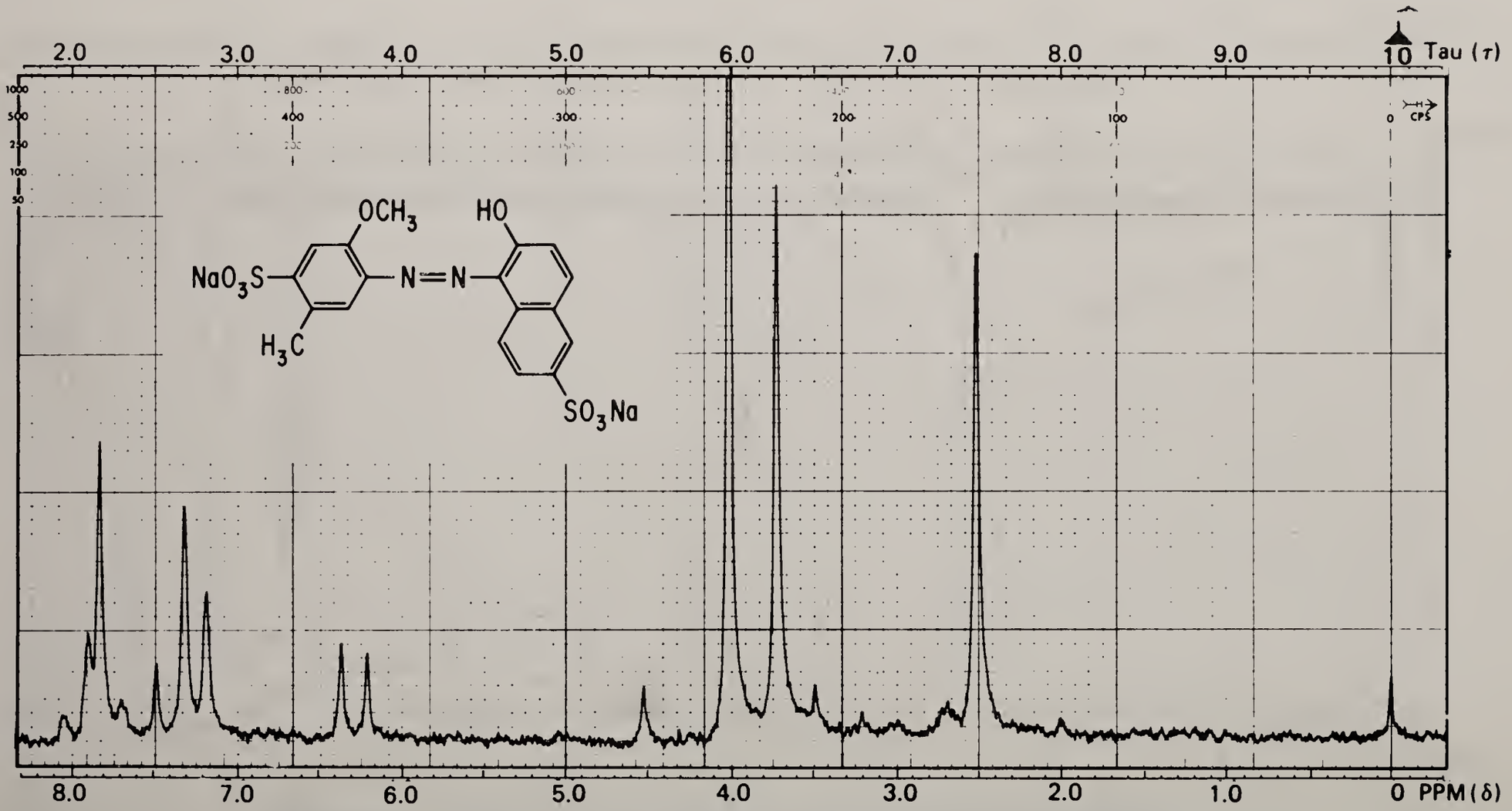

Figure 6.2*g*. Spectrum of FD&C Red No. 40: concentration, 20%; amplitude, 40

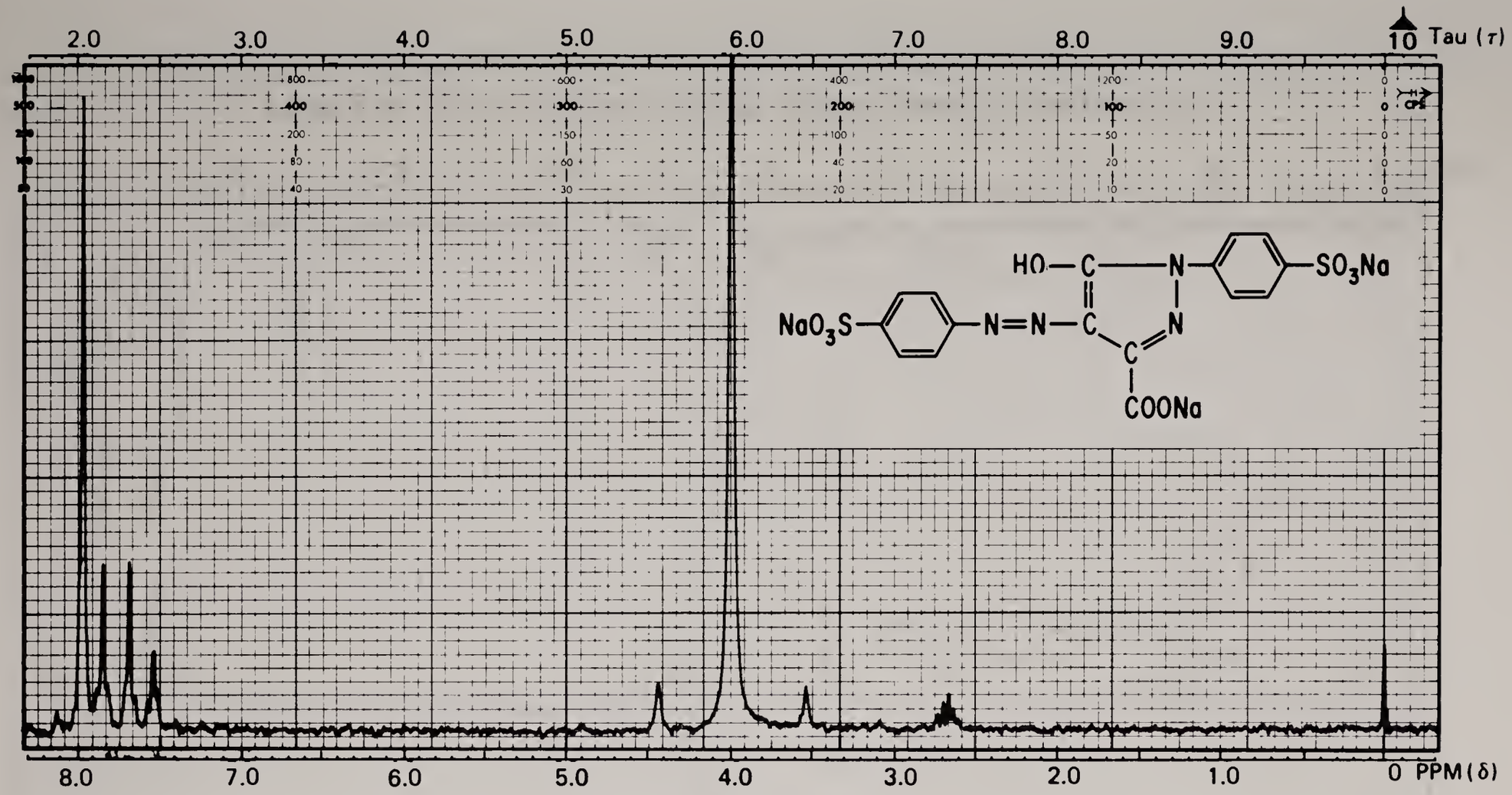

Figure 6.2*h*. Spectrum of FD&C Yellow No. 5: concentration, 20%; amplitude, 25

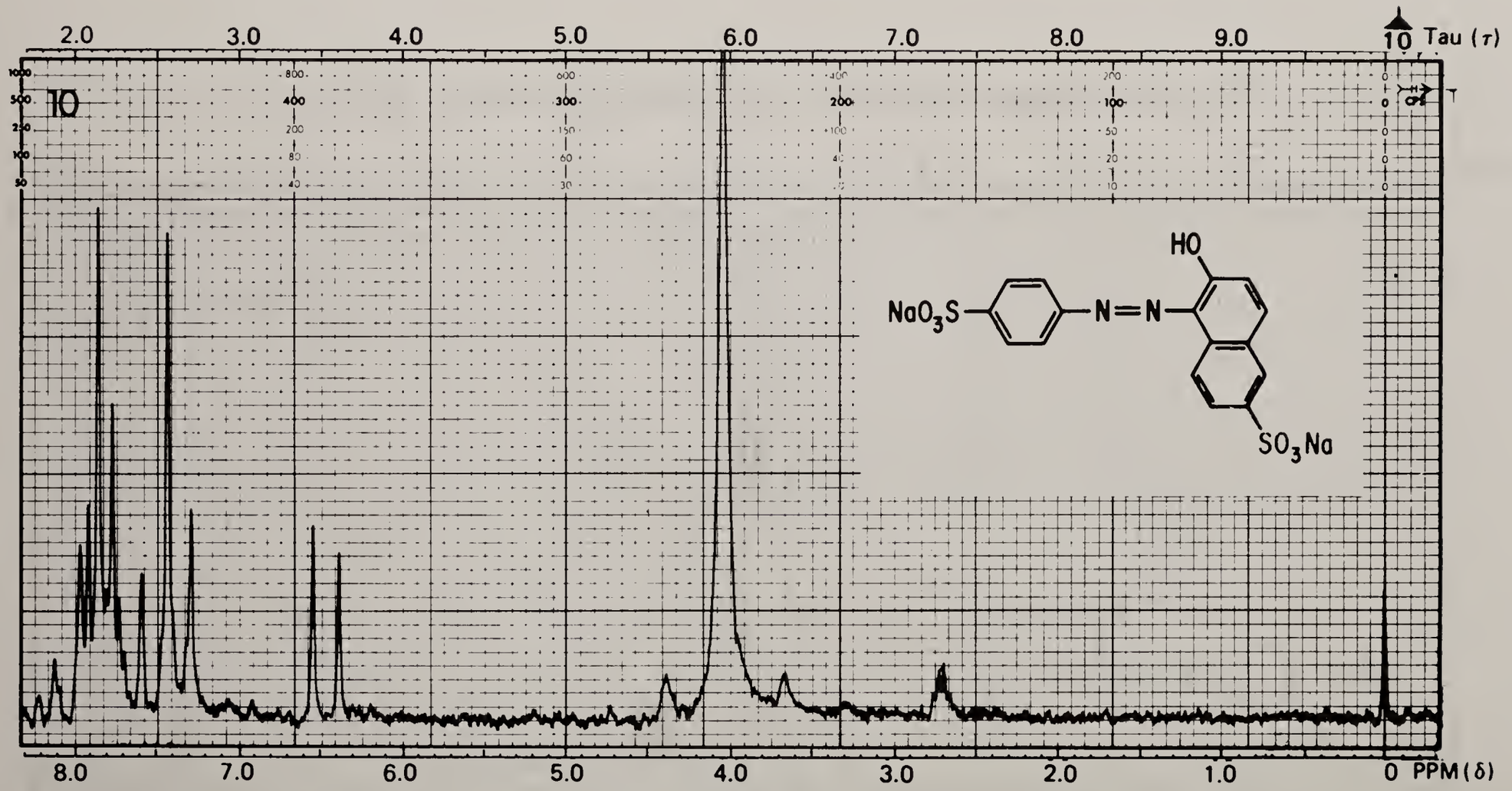

Figure 6.2*i*. Spectrum of FD&C Yellow No. 6: concentration, 20%; amplitude, 50

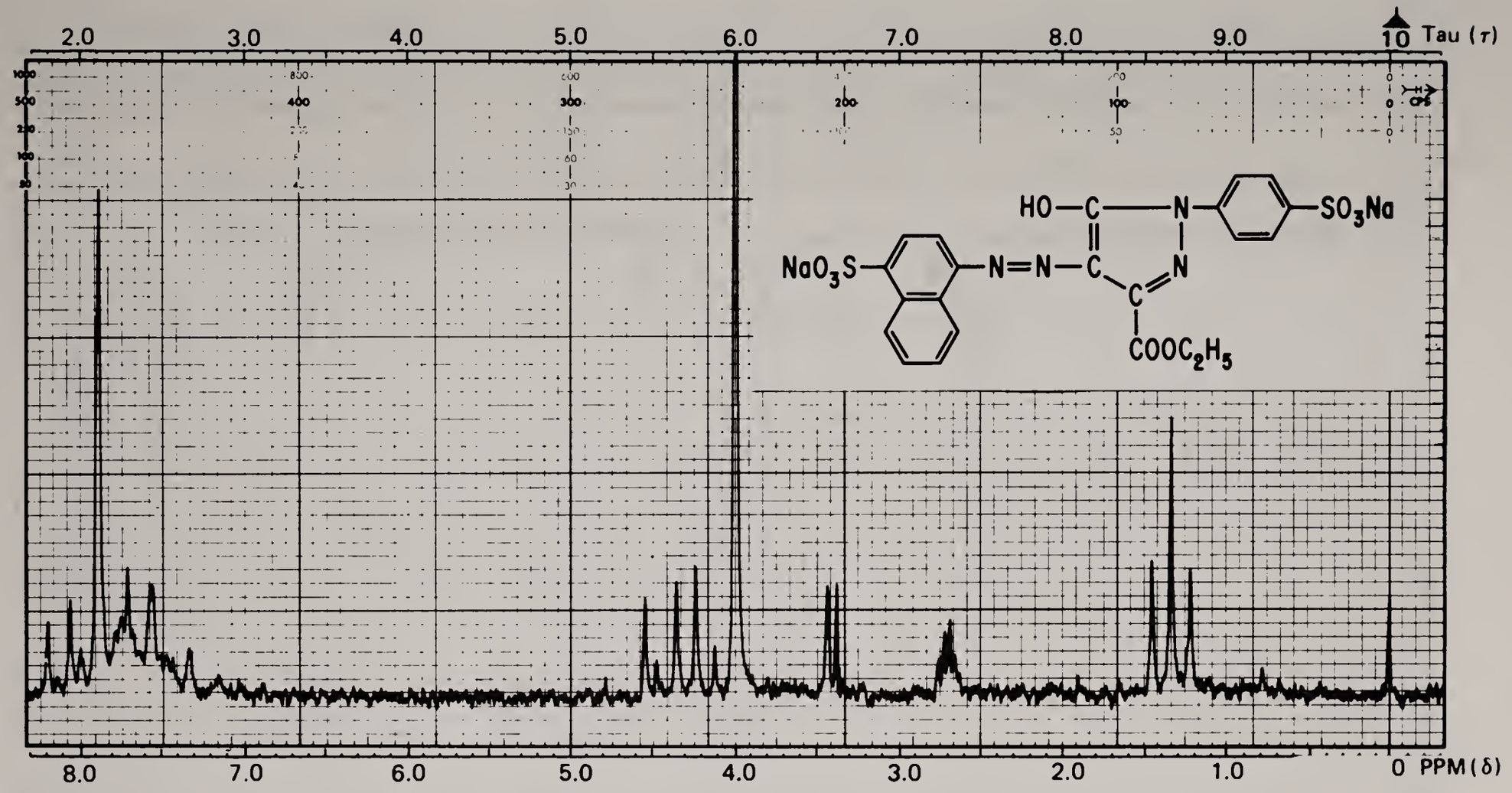

Figure 6.2*j*. Spectrum of Orange B: concentration, 10%; amplitude, 63

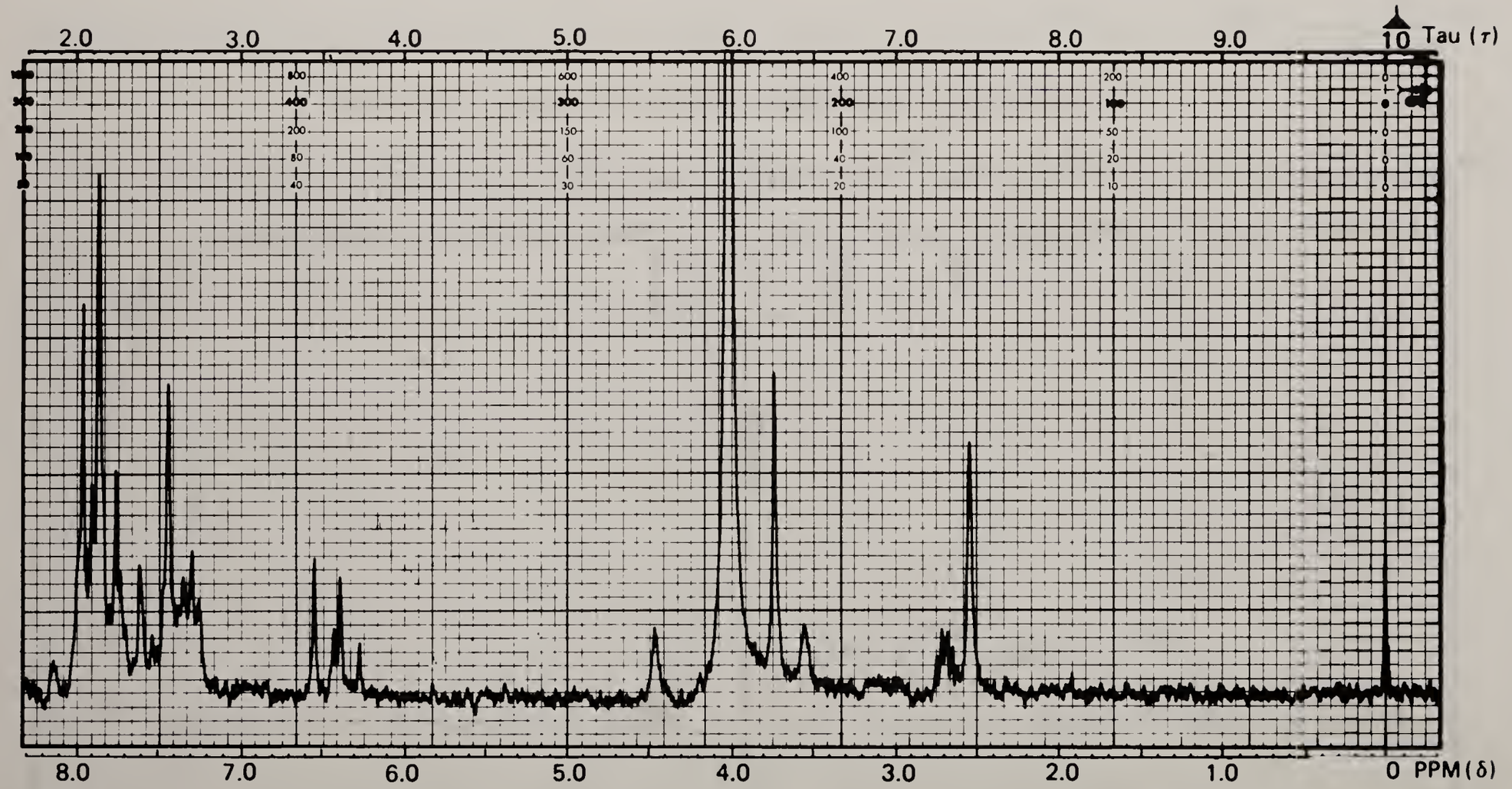

Figure 6.2*k*. Spectrum of "Orange"—a secondary colorant containing 25% FD&C Red No. 40, 20% FD&C Yellow No. 5, and 55% FD&C Yellow No. 6

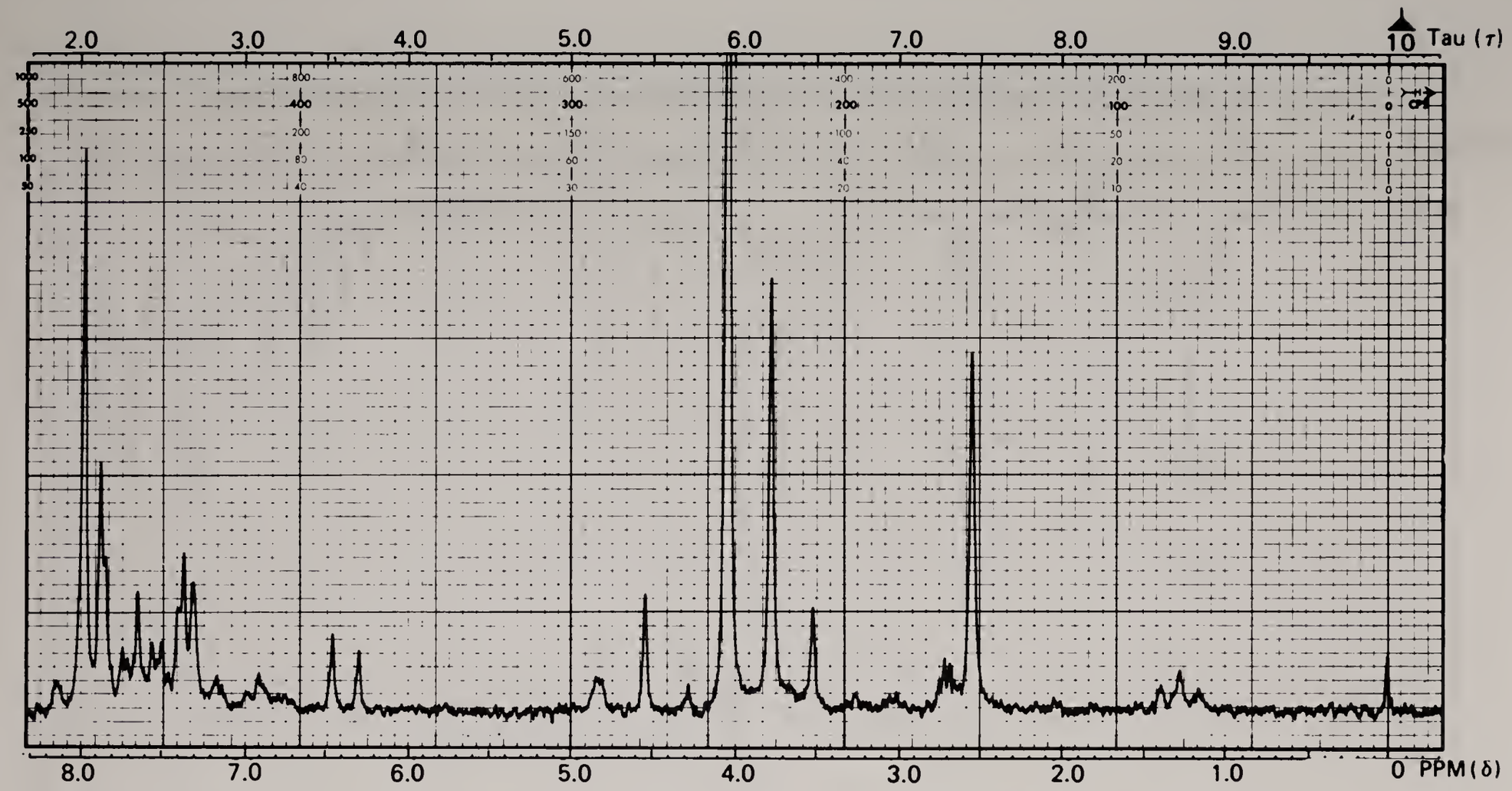

Figure 6.2*l*. Spectrum of "Chocolate"—a secondary colorant containing 10% FD&C Blue No. 1, 45% FD&C Red No. 40, and 45% FD&C Yellow No. 5

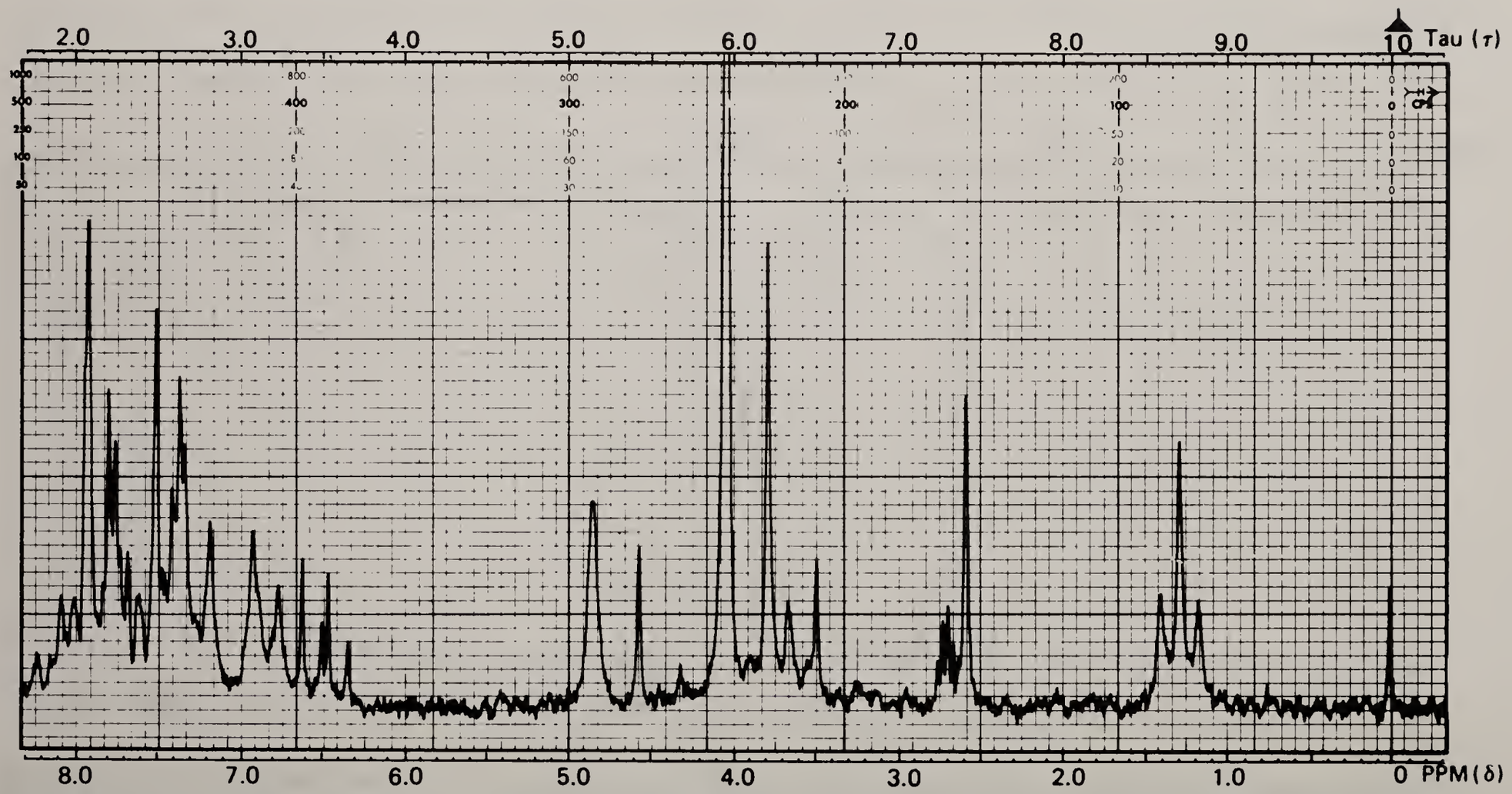

Figure 6.2*m*. Spectrum of "Black"—a secondary colorant containing 36% FD&C Blue No. 1, 22% FD&C Red No. 40, and 42% FD&C Yellow No. 6

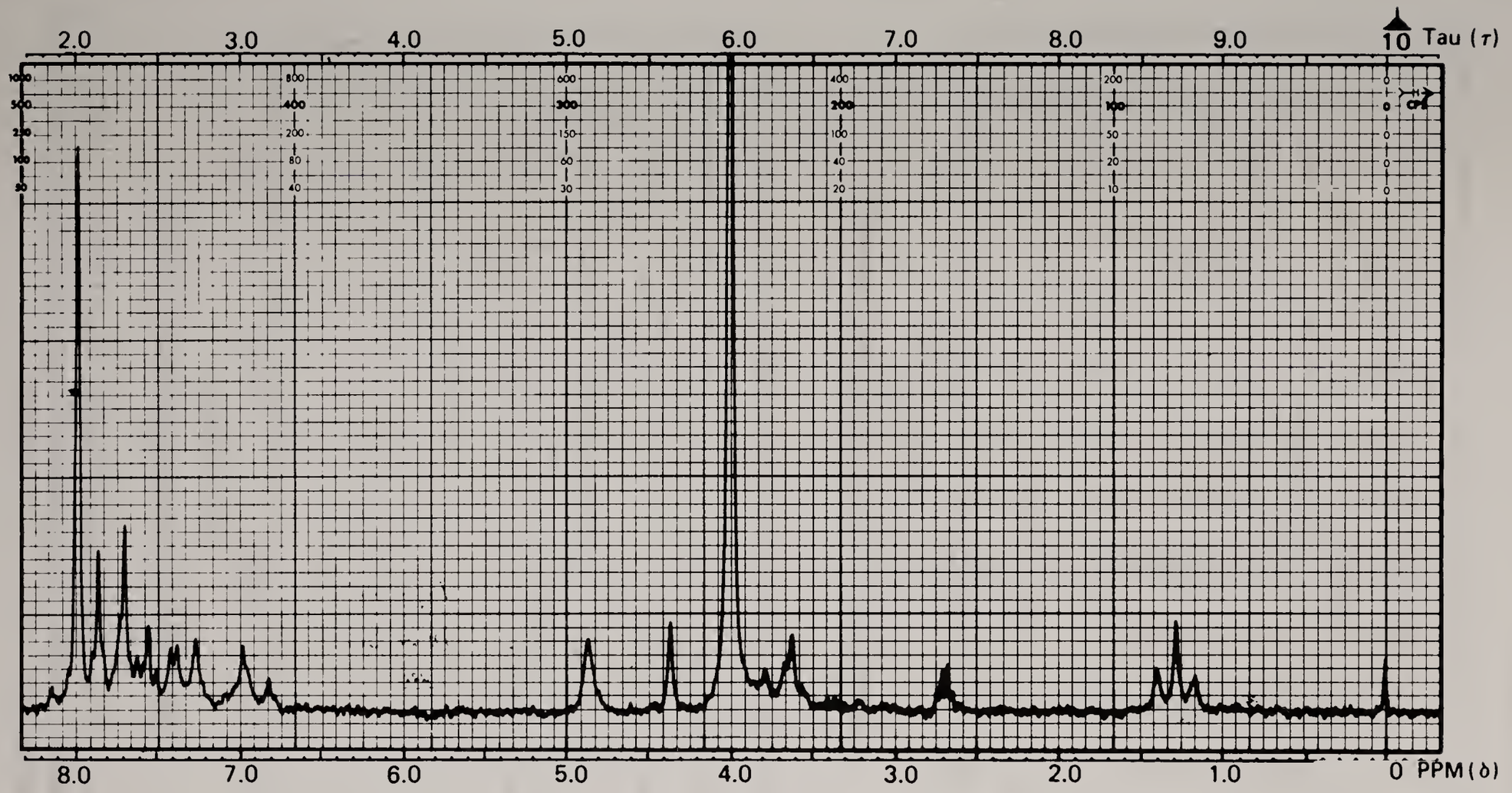

Figure 6.2*n*. Spectrum of "Mint Green"—a secondary colorant containing 25% FD&C Blue No. 1 and 75% FD&C Yellow No. 5

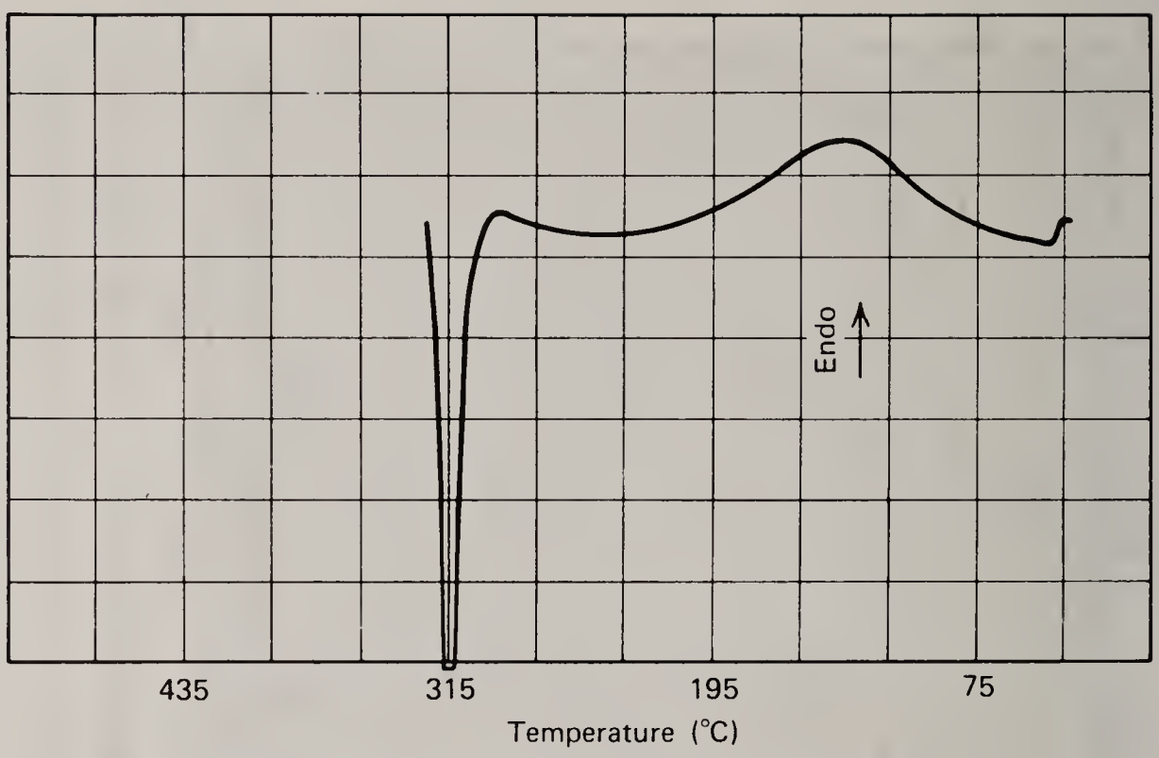

Figure 6.3*a*. Thermogram of FD&C Blue No. 1

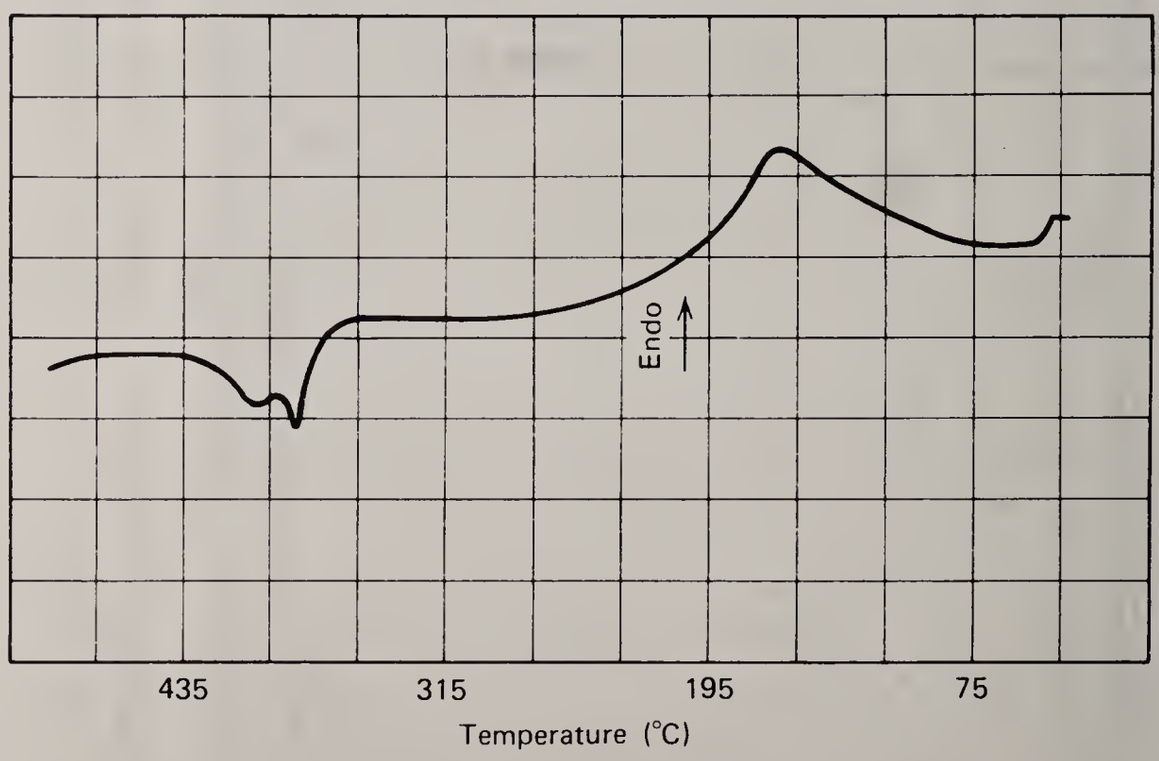

Figure 6.3*b*. Thermogram of FD&C Red No. 3

Figure 6.3*a–h*. Thermograms obtained using a Perkin-Elmer DSC-1B Calorimeter and the following conditions: sample weight, 9.8–10 mg (3.5 mg for D&C Red No. 17); scan rate, 40°/min; range, × 32; atmosphere, N_2.

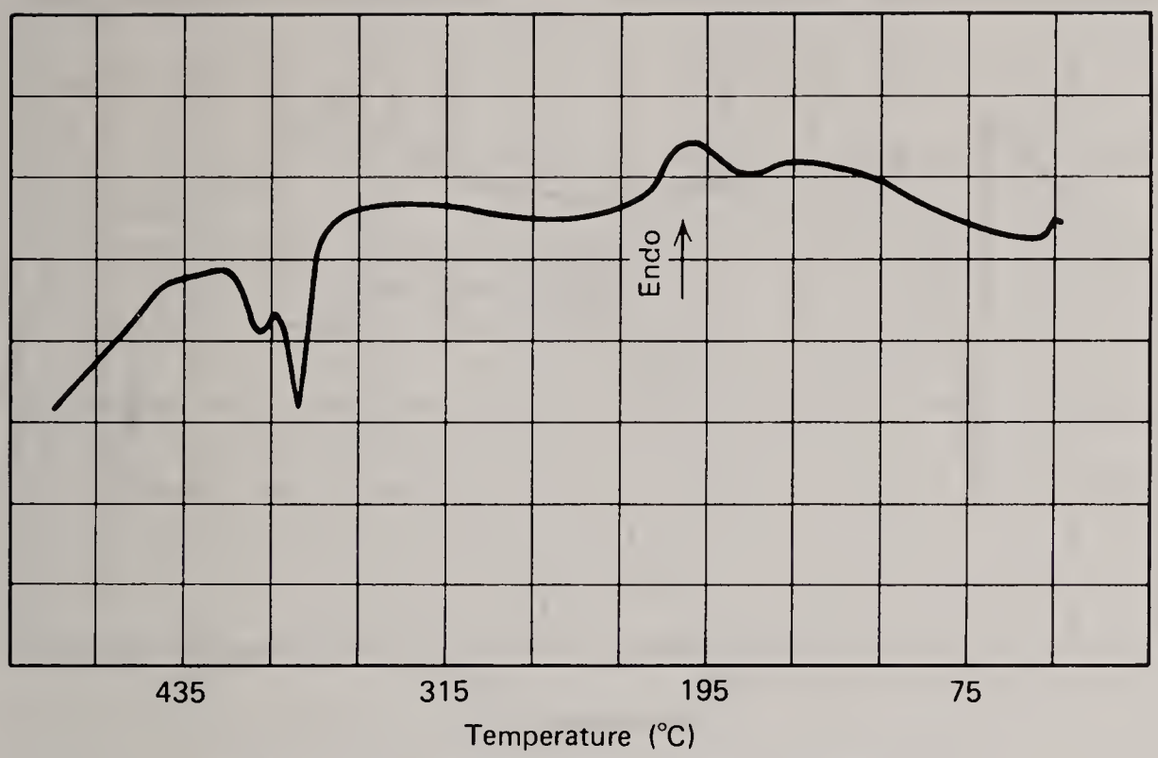

Figure 6.3*c*. Thermogram of FD&C Red No. 4

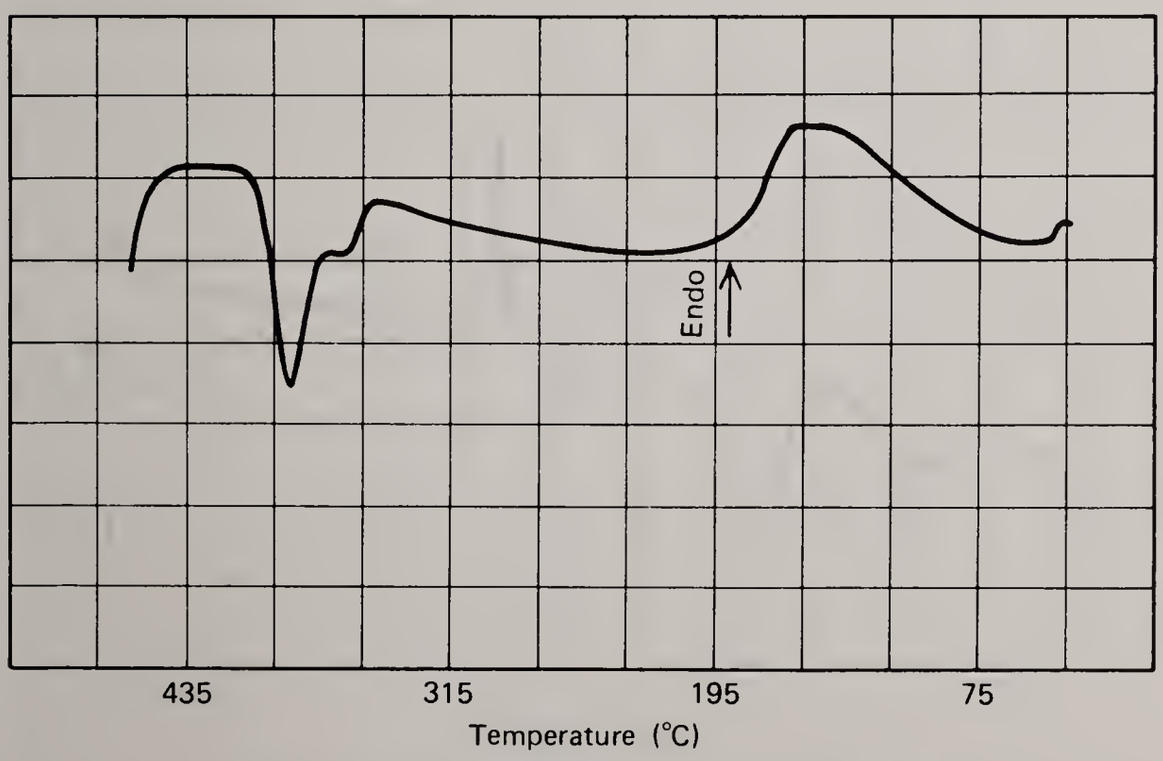

Figure 6.3*d*. Thermogram of FD&C Yellow No. 5

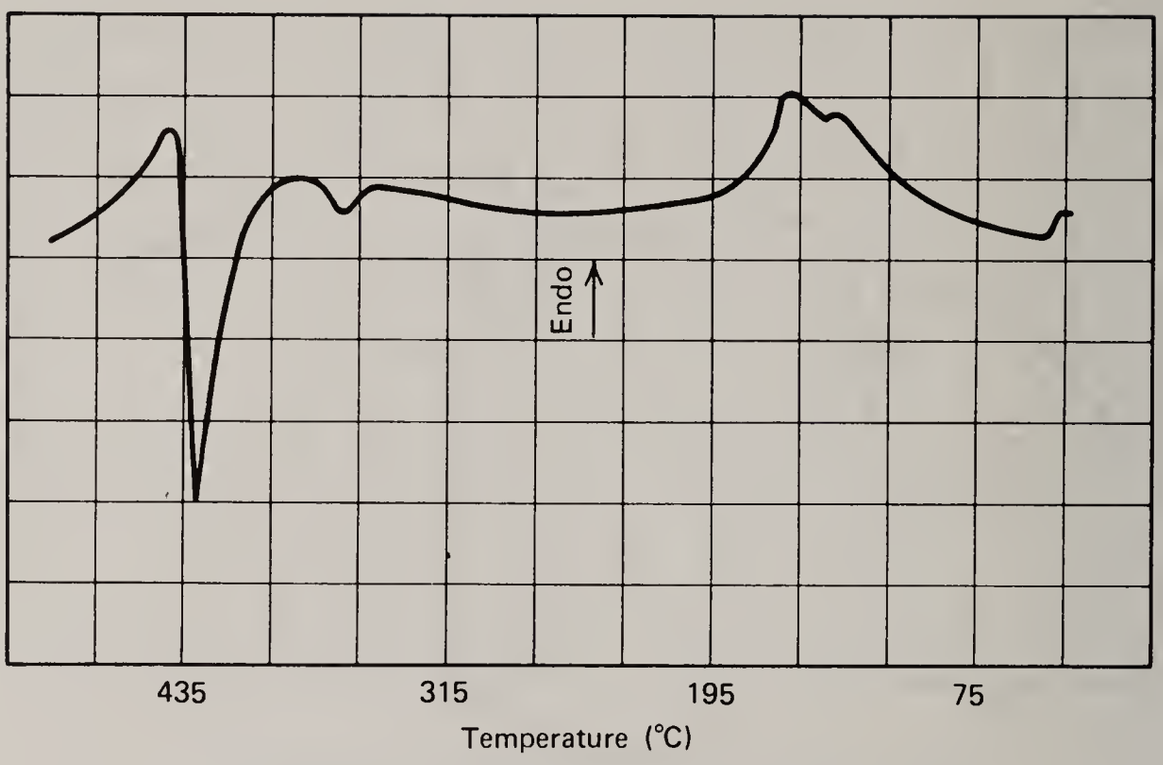

Figure 6.3*e*. Thermogram of FD&C Yellow No. 6

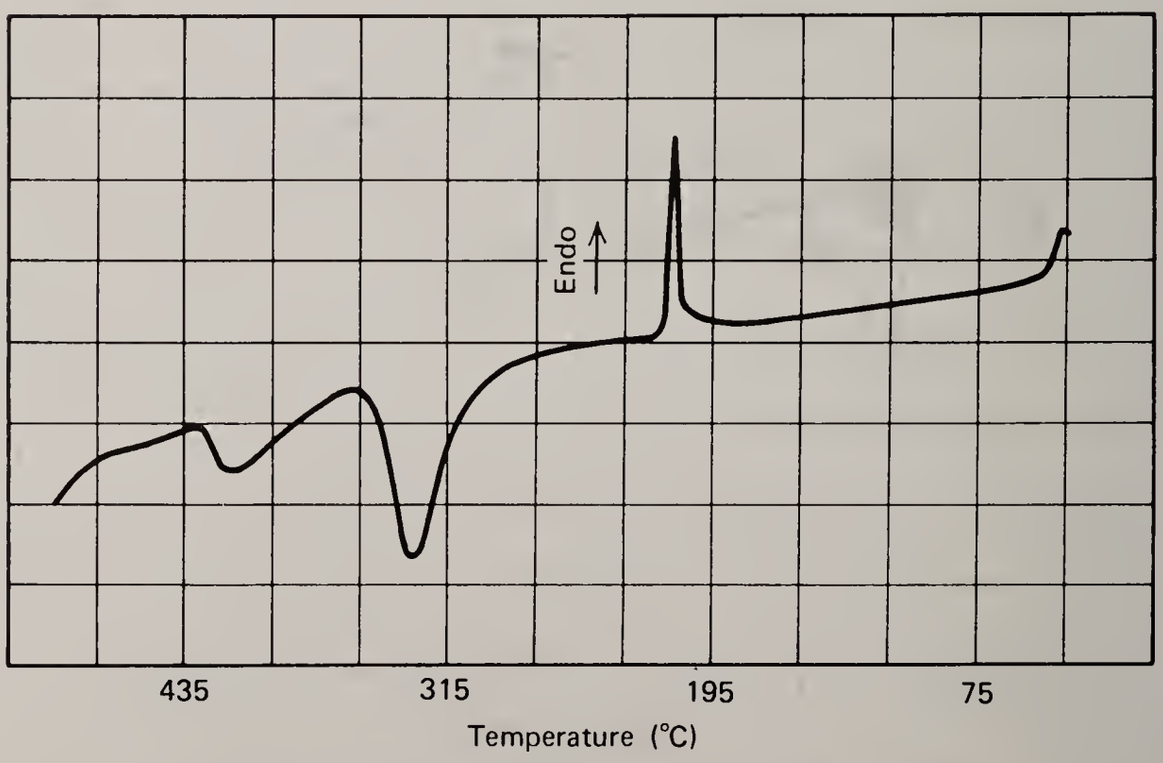

Figure 6.3*f*. Thermogram of D&C Red No. 17

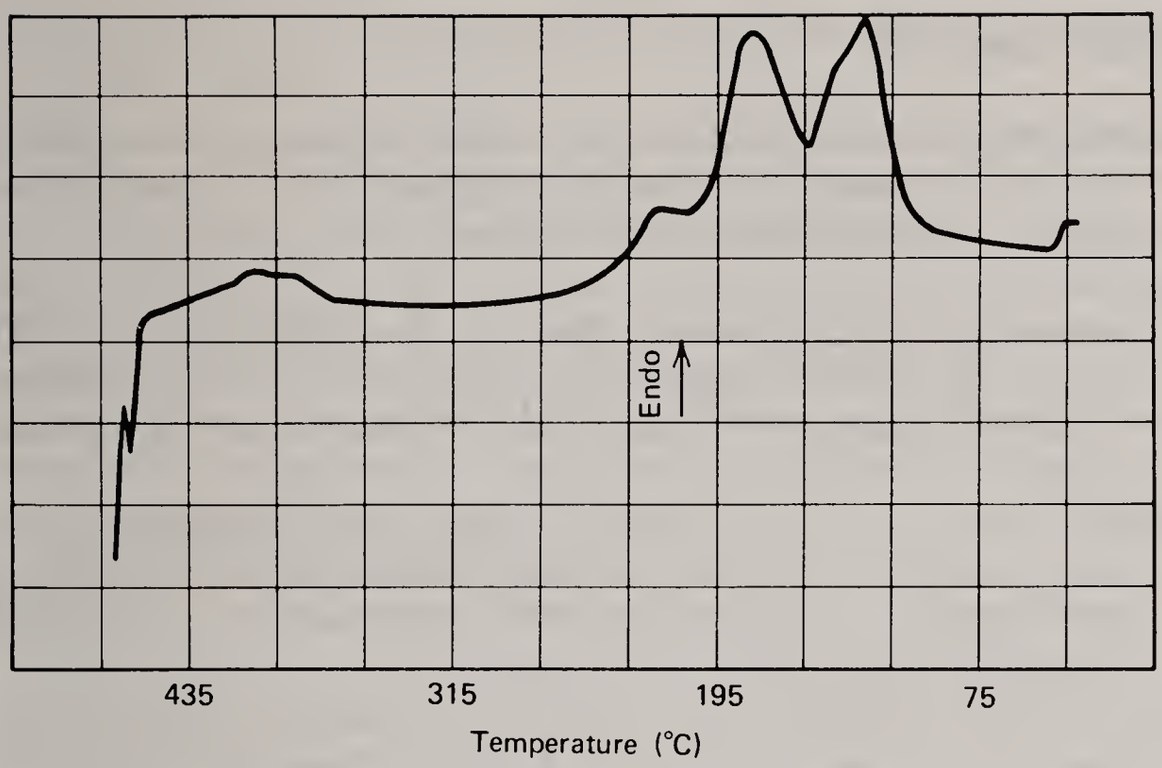

Figure 6.3*g*. Thermogram of D&C Yellow No. 8

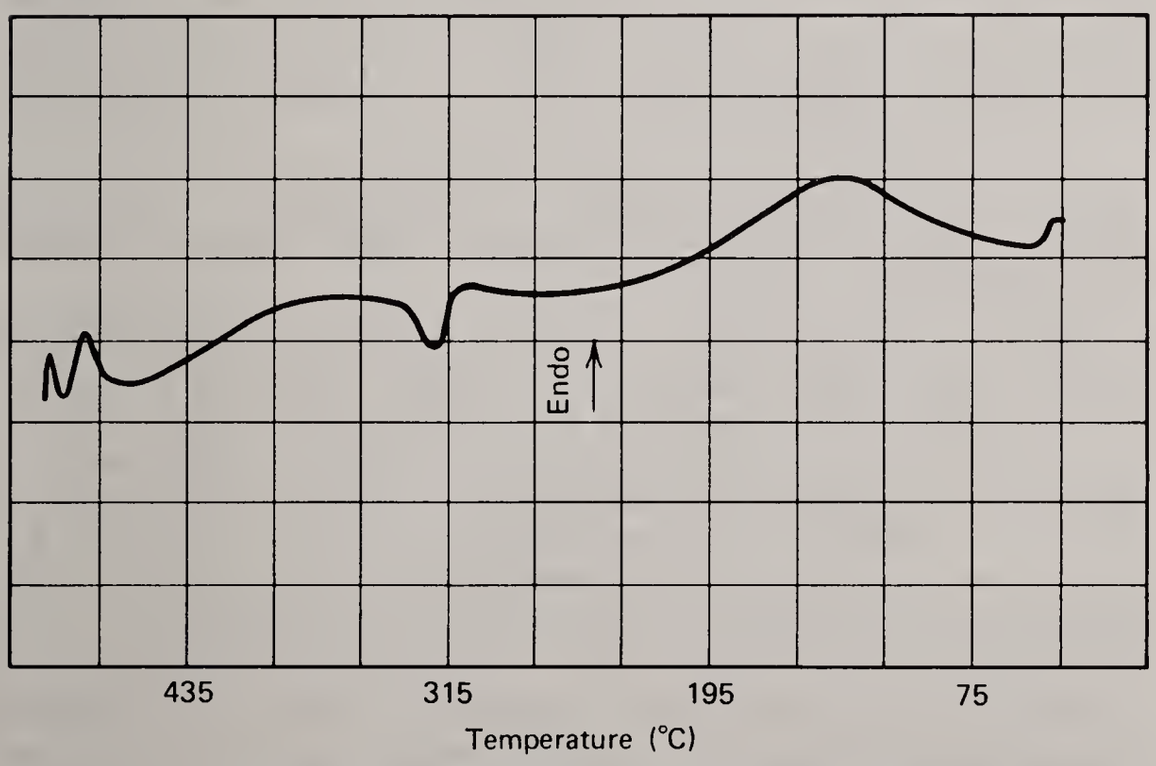

Figure 6.3*h*. Thermogram of D&C Yellow No. 10

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PUCHE, R. C. T. Publ. Inst. Invest. Microquim., Univ. Nacl. Litoral *25*, Nos. 23–24, 58–62 (1959–1960). Absorption maxima and minima for 13 food dyes are listed.

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SADTLER STANDARD SPECTRA, Sadtler Research Laboratories, 3316 Spring Garden St., Philadelphia PA. 19104. A collection of visible, UV, and IR spectra. The color additives included in this collection are listed as follows by page number.

	Page in Sadtler Collection	
Color Additive	IR	Visible/UV
FD&C Blue No. 1	X2082	U394
FD&C Blue No. 2	X2081	U393
FD&C Green No. 3	X2090	U396
FD&C Red No. 3	X2068	U391
FD&C Red No. 4	X2055	U388
FD&C Yellow No. 5	X2042	U387
FD&C Yellow No. 6	X2041	U386
D&C Brown No. 1	X21	U2
D&C Blue No. 4	X10	
D&C Blue No. 6	X12	
D&C Blue No. 9	X15	
D&C Green No. 5	X31	
D&C Green No. 6	X32	
D&C Orange No. 4	X42	U3
D&C Orange No. 5	X43	
D&C Red No. 6	X66	
D&C Red No. 7	X67	

Color Additive	Page in Sadtler Collection	
	IR	Visible/UV
D&C Red No. 17	X77	
D&C Red No. 21	X81	
D&C Red No. 22	X82	
D&C Red No. 27	X87	
D&C Red No. 28	X88	U5
D&C Red No. 31	X91	
D&C Red No. 33	X93	U6
D&C Red No. 34	X94	
D&C Red No. 36	X96	
D&C Red No. 39	X99	
D&C Violet No. 2	X106	
D&C Yellow No. 7	X118	
D&C Yellow No. 8	X119	
D&C Yellow No. 10	X121	U7
D&C Yellow No. 11	X122	
Ext. D&C Violet No. 2	X172	
Ext. D&C Yellow No. 7	X184	U9 and U10

SHELTON, J. H., GILL, J. M. T. J. Assoc. Public Analysts, *1* 88–91 (1963). Paper Chromatographic Identification of Food Dyes. The chromatographic method of Yanuka is used to identify food colors permitted in the United Kingdom.

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SZOKOLAY, A., PAGACOVA, A. Prumysl Potravin, *12*, 656–658 (1961). Identification of Food Colorants by Light-Absorption Measurement. Includes visible and ultraviolet spectra of various colorants.

TONET, N. Mitt Geb. Lebens. Hyg., *60*, 201–205 (1969). Use of High-Voltage Electrophoresis as a Supplementary Technique for the Identification of

Water-Soluble Dyes. Seventy-five synthetic dyes and several natural colorants were studied by electrophoresis at 4500 V with 20% acetic acid or 0.1 *M* Aqueous NH_3: 3.3 m*M* acetic-acid buffer adjusted to pH = 10.3.

VILLANÚA, L., CARBALLIDO, A., MUÑIZ, J. An. Bromatol. *20*, 113–136 (1968). Artificial Food Colours. XI. Ultra-Violet and Visible Spectrometry of Some Water-Soluble Colours. Spectrophotometric parameters in the range 350–700 nm are reported for 46 water-soluble dyes permitted in Spain.

YANUKA, Y., SHALON, Y., WEISSENBERG, E., NIR-GROSFELD, I. Analyst *87*, 791–796 (1962). A Paper-Chromatographic Method for the Identification of Food Dyes. Dyes are run in BuOH:EtOH:H_2O (1:1:1) adjusted to eight different pH values; R_f values are then plotted to form a characteristic curve.

7

DETERMINATION OF STRENGTH

Color additives, like most commercial products, are rarely 100% pure. Although the impurities present are usually little more than inorganic salts and water, there is a constant need to know a colorant's strength. The Food and Drug Administration uses strength or "pure dye content" as a means of monitoring an additive's overall purity and of ensuring a consistency in its batch-to-batch manufacture. The economic importance of a dyestuff's strength is obvious.

To a dye chemist, terms such as strength, pure dye, and coal-tar dye content relate to the absolute measure of the dyestuff's principal active ingredient and usually serve as the basis of sale and a criterion for purity. This is in contrast to the relative or effective strength used by the colorist to compare batches of colorant of equal chemical composition but different application properties. The latter is more of a physical phenomenon dependent on particle size and crystal structure and is most important when using colorants in the solid state, such as pigments, lakes, and plating types. Absolute dye strength is typically measured by titrimetry, gravimetry, elemental analysis, or spectrophotometry or some other pro-

cedure based on chemistry or chemical physics, whereas effective strength is generally determined visually after plating the colorant on sugar, or after making filter-paper pourouts of colorants in solution, or drawdowns of pigments pasted in oil. When determining effective or relative strength, a control or "type" is run along with the sample tested. Absolute strength and effective strength are not necessarily directly related.

TITRATION WITH TITANOUS CHLORIDE

Reduction with titanous chloride is currently the wet method most widely used for determining strength and is the titration procedure favored by both the FDA and the color manufacturers. In principle, the method is applicable to any colorant that is readily and quantitatively reduced to a colorless compound. The types of compounds that can be analyzed by this technique include azo, nitro, and nitroso.

Reduction of azo dyes: $R{-}N{=}N{-}R' + 4H^+ + 4Ti^{3+} \rightarrow R{-}NH_2 + R'{-}NH_2 + 4Ti^{4+}$

Reduction of nitro dyes: $R{-}NO_2 + 6H^+ + 6Ti^{3+} \rightarrow R{-}NH_2 + 2H_2O + 6Ti^{4+}$

Reduction of nitroso dyes: $R{-}NO + 4H^+ + 4Ti^{3+} \rightarrow R{-}NH_2 + H_2O + 4Ti^{4+}$

There are several variations of the $TiCl_3$-reduction method, each useful for analyzing certain colorants. The modification to use, the milliequivalent weight, and the volume of 0.1 *N* titanous chloride needed per gram of color titrated are shown in Table 7.1. for a number of colorants.

In general, titanous chloride titrations require a certain degree of skill if one is to obtain consistent results. Care must be taken to exclude all oxygen from the reduction flask during the titration by purging the system with carbon dioxide or nitrogen. In addition, close attention must be given to the volumes of reagents used, the reagent blank, and the end-point timing and color.

In some cases, the titration end point is indicated by a sharp decoloration of the sample. Often, though, the change in color is so gradual that it is better to add a slight excess of titrant then back-titrate the excess with a standard solution of a suitable dye such as Methylene Blue (CI No. 52015). At other times the addition of an internal indicator that is reduced after the sample has reacted with $TiCl_3$ works best. Light Green SF Yellowish (CI No. 42095) is good for this purpose.

TABLE 7.1. $TiCl_3$ Titration Factors

Color	Milliequivalent Weight	Milliliters of O.1*N* $TiCl_3$/g of Color	Appropriate Method
FD&C Blue No. 1	0.3964	25.23	3
FD&C Blue No. 2	0.2332	42.89	3
FD&C Green No. 3	0.4044	24.73	3
FD&C Red No. 4	0.1201	83.26	3
FD&C Red No. 40	0.1241	80.58	3
FD&C Yellow No. 5	0.1336	74.86	3
FD&C Yellow No. 6	0.1131	88.43	1
Citrus Red No. 2	0.07709	129.7	8
Orange B	0.1476	67.74	3
D&C Blue No. 4	0.3915	25.54	3
D&C Blue No. 6	0.1311	76.26	7
D&C Brown No. 1	0.05605	178.4	3
D&C Green No. 5	0.3113	32.12	2
D&C Green No. 6	0.2093	47.79	
D&C Orange No. 4	0.08758	114.2	3
D&C Red No. 6	0.1076	92.95	3
D&C Red No. 7	0.1061	94.24	6
D&C Red No. 17	0.04405	227.0	5
D&C Red No. 30	0.1967	50.85	7
D&C Red No. 31	0.07783	128.5	6
D&C Red No. 33	0.1168	85.58	3
D&C Red No. 34	0.1151	86.87	6
D&C Red No. 36	0.03277	305.1	
D&C Red No. 39	0.08234	121.4	
D&C Yellow No. 7	0.1662	60.18	5
D&C Yellow No. 8	0.1881	53.15	5
Ext. D&C Violet No. 2	0.2157	46.36	2
Ext. D&C Yellow No. 7	0.02985	335.0	3

Standard titanous chloride is usually prepared from commercial $TiCl_3$ solution (Lamotte Chemical, Chestertown, Maryland 21620, or similar). A procedure is given in the text that follows.

Preparation of 0.1 *N* $TiCl_3$

Add 500 mL of HCl and 500 mL of 20% $TiCl_3$ solution to a 7-L plastic bottle containing about 5 liters of distilled water. Dilute to 7 L with water

and mix well. Pass nitrogen through the solution for 1 hr, stopper, and let the solution stand for 2 days.

Weigh 3.0 g of ferrous ammonium sulfate ($FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$) into the titration flask shown in Fig. 7.1. Purge the system with nitrogen or carbon dioxide and add 50 mL of recently boiled water and 25 mL of 40% (w/w) H_2SO_4. Then, without interrupting the flow of gas, add 40 mL of 0.1 *N* potassium dichromate (4.9032 g of dry NIST $K_2Cr_2O_7$/liter of solution). Slowly add about 90% of the $TiCl_3$ solution calculated as required and then quickly add 5 g of ammonium thiocyanate (NH_4SCN) and complete the titration. Determine a reagent blank and correct for it.

$$\text{Normality} = \frac{\text{mL } K_2Cr_2O_7 \times \text{normality } K_2Cr_2O_7}{\text{net mL } TiCl_3}$$

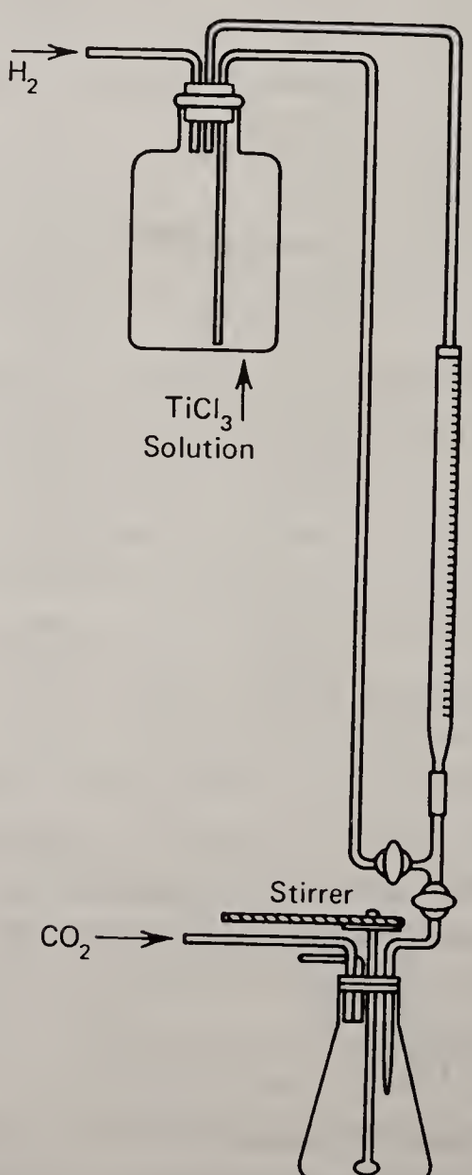

Figure 7.1. Titanous chloride titration apparatus (Reprinted with the permission of the Association of Official Analytical Chemists.)

Titanous chloride solutions should be stored under an inert gas and restandardized at least weekly.

Titration Procedures

Method 1: Prepare a 1% aqueous sample solution. Pipette an amount equivalent to about 20 mL of 0.1 *N* titanous chloride into a 500-mL wide-mouthed Erlenmeyer flask. Add 15 g of sodium citrate and adjust the volume to 150–200 mL with water. Using the apparatus shown in Fig. 7.1., blanket the sample with nitrogen or carbon dioxide and, while boiling, titrate with 0.1 *N* $TiCl_3$ to the disappearance of color.
Method 2: Prepare a 0.5% ethanolic sample solution. Proceed as in Method 1, substituting 50% ethanol for water.
Method 3: Proceed as in Method 1, substituting 15 g of sodium acid tartrate for sodium citrate.
Method 4: Proceed as in Method 3, using a freshly-prepared solution containing about 10 mg of Light Green SF Yellowish as an indicator. Determine a reagent blank and correct accordingly.
Method 5: Prepare a 0.5% ethanolic sample solution and proceed as in Method 4, substituting 50% ethanol for water.
Method 6: Dissolve 0.200 g of sample in 5 mL of sulfuric acid in a 500-mL wide-mouthed Erlenmeyer flask. Add 100 mL of ethanol and heat and stir the sample until in solution. Dissolve 20 g of sodium acid tartrate in 100 mL of boiling water and then add 20 mL of 30% sodium hydroxide solution. Stirring rapidly, add this solution to the sample. Attach the flask to the apparatus shown in Fig. 7.1, blanket the sample with nitrogen or carbon dioxide, and titrate with 0.1 *N* titanous chloride.
Method 7: Place a sample equivalent to about 20 mL of 0.1 *N* titanous chloride in a 50-mL beaker. Pour 2 mL of fuming sulfuric acid (20% free SO_3) down the side of the beaker. Mix and place on a steam bath for 30 min. Pour the solution into a 500-mL wide-mouthed Erlenmeyer flask containing 100 g of ice. Add ice to the beaker and wash any remaining color into the flask. Add 50 mL of ethanol and 20 g of sodium acid tartrate to the flask. Attach the flask to the apparatus in Fig. 7.1 and titrate the sample as in Method 1.
Method 8: Add 0.150 g of sample and 125 mL of acetone to a 500-mL Erlenmeyer flask; heat cautiously to dissolve. Dissolve 15 g of sodium acid tartrate in 75 mL of distilled water and add this to the flask. Connect

the flask to the apparatus in Fig. 7.1, blanket with nitrogen or carbon dioxide, and titrate with 0.1 *N* titanous chloride.

GRAVIMETRIC DETERMINATIONS

Because of their insolubility in dilute acid, xanthene and fluoran colorants can be assayed gravimetrically. Those that are water-soluble sodium salts are precipitated from water solution and then weighed and corrected for molecular weight differences using Method 1 (below). Colorants that are free acids and hence water insoluble are first dissolved in dilute alkali and then precipitated with acid (see Method 2).

Gravimetric procedures are slow in comparison to titrimetric and spectrophotometric methods but require little, if any, sophisticated equipment and are generally more precise and accurate.

Method 1: Transfer 0.500 g of sample to a 400-mL beaker; add 100 mL of distilled water and then heat to boiling. Add 25 mL of dilute HCl (1:49) and bring to a boil. Wash down the sides of the beaker with distilled water and then cover and keep on a steam bath for several hours or overnight. Cool to room temperature and then quantitatively transfer the precipitate to a tared Gooch crucible with dilute HCl (1:99). Wash the precipitate with two 15-mL portions of distilled water and then dry the crucible for 3 hr at 135°C. Cool in a desiccator and weigh.

$$\text{Percent pure dye} = \frac{\text{Weight of precipitate} \times \text{conversion factor} \times 100}{0.500}$$

Color	Gravimetric Conversion Factors
FD&C Red No. 3	1.074 to disodium salt, monohydrate
D&C Orange No. 11	1.075 to disodium salt
D&C Red No. 22	1.068 to disodium salt
D&C Red No. 28	1.056 to disodium salt
D&C Yellow No. 8	1.132 to disodium salt

Method 2: Transfer 0.500 g of sample to a 400-mL beaker. Add 50 mL of 0.1 *N* NaOH and swirl to dissolve. Add 50 mL of distilled water and then proceed as in Method 1, beginning with "heat to boiling."

$$\text{Percent pure dye} = \frac{\text{Weight of precipitate} \times 100}{0.500}$$

No conversion factors are needed here since the colors for which this method is useful are free acids. These include D&C Orange Nos. 5 and 10, D&C Red Nos. 21 and 27, and D&C Yellow No. 7.

SPECTROPHOTOMETRIC DETERMINATIONS

Many of today's additives can be assayed spectrophotometrically. The procedures used are, for the most part, simpler, more expedient and to some extent more specific than wet methods and have the added advantage of being able to provide qualitative information through evaluation of the spectra generated. As with most benefits, these are costly, and the price here is usually paid in the form of more expensive equipment and less precise results.

The majority of "spectro" methods in use necessitate little more than dissolution of the sample in a suitable solvent and measurement of its absorbance versus that of a standard. These are treated as a group, and the calculations given in this general procedure serve as a guide for the remaining methods. A few procedures require more care and are described in greater detail.

The absorption coefficients or absorptivities used to calibrate spectro methods are normally obtained in one of two ways—by measuring the absorption of a sample of the compound of interest that has been shown by chemical analysis to be essentially 100% pure, or by establishing a relationship between the absorbance of a large number of typical commercial samples and their strengths determined by some chemical means, usually $TiCl_3$ reduction. In most instances the numbers are the same. Published absorptivities, including those shown here, should be considered as only close approximations of the true values. For best results each laboratory must obtain its own factors using its standards and its spectrometer.

Spectrophotometric Procedures

General Procedure

Dissolve a portion of well-mixed sample in a spectrograde quality of the solvent shown in Table 7.2 and dilute this solution to obtain an absorbance at the absorption maximum within the range recommended as the

most accurate for the spectrophotometer used. Correct this absorbance for solvent blank and apply the following formula:

$$\text{Percent pure dye} = \frac{A \times 100}{a \times b \times c}$$

where: A is the blank-corrected sample absorbance, a is the color's absorptivity (in L/g-cm; see Table 7.2), b is the absorption cell's path length (in cm), c is the concentration (in g/L) of the solution presented to the spectrophotometer, and 100 is the factor for conversion to percent.

FD&C and D&C Lakes

To analyze a lake for pure dye content spectrophotometrically, the sample must first be treated to release the colorant from the substrate to which it is bound, then diluted to a volume consistent with the lake's strength. Details for various colorants follow.

FD&C Lakes (except FD&C Red No. 3)—Transfer 0.050 g of the sample, 15 g of sodium tartrate, and 100 mL of distilled water to a beaker. Heat the mixture to boiling, then cool and make to volume with water.

FD&C Red No. 3, D&C Orange Nos. 5 and 10, and D&C Red Nos. 21 and 27—Transfer 0.050 g of sample, 5 mL of 50% sodium hydroxide and 100 mL of water to a beaker. Warm and stir the mixture until in solution, cool to room temperature, then make to volume with water.

D&C Red Nos. 6, 7, 31, and 34—Add 5 mL of 96% sulfuric acid to 0.030–0.040 g of sample, then stir the mixture with a glass rod until in solution. Dilute the mix cautiously with 150 mL of ethanol-water (1 + 1) then, if need be, heat and stir the mixture until completely dissolved. Cool, then make to volume with 50% ethanol.

Insoluble Ca, Ba, and Sr Salts and Lakes of Some Colors

Reagents

10% EDTA solution—Dissolve 25 g of ethylenediaminetetraacetic acid in 165 mL of 10% aqueous NaOH. Dilute to 250 mL with distilled water.

Dilute EtOH—Mix equal volumes of 95% EtOH and distilled water.

TABLE 7.2. Absorption Coeffients

	Solvent System[a]	Wavelength of Maximum Absorbance (in mm)	Absorptivity (in L/g-cm)
FD&C Blue No. 1	Water	630	164
FD&C Blue No. 2	Water	610	47.8
FD&C Green No. 3	Water	625	156
FD&C Red No. 3	Water	527	110
FD&C Red No. 4	Water	502	54.0
FD&C Red No. 40	Water	502	54.0
FD&C Yellow No. 5	Water	428	53
FD&C Yellow No. 6	Water	484	55
Citrus Red No. 2	$CHCl_3$	515	70
Orange B	Water	437	35.5
D&C Blue No. 4	Water	630	170
D&C Blue No. 6	$CHCl_3$	603	79.0
D&C Blue No. 9	96% H_2SO_4	458	18.9
D&C Brown No. 1	Water	430	93.5
D&C Green No. 5	Water	610	21.3
D&C Green No. 6	$CHCl_3$	648	39.2
D&C Green No. 8	Water $(OH)^-$	454	51
D&C Orange No. 4	Water	484	65.3
D&C Orange No. 5	Water $(OH)^-$	503	163
D&C Orange No. 10	Water $(OH)^-$	510	122
D&C Orange No. 11	Water $(OH)^-$	510	131
D&C Red No. 6	Alcohol & water (1 + 1)	511	61.5
D&C Red No. 7	Alcohol & water (1 + 1)	516	57.2
D&C Red No. 17	$CHCl_3$	514	94
D&C Red No. 21	Water $(OH)^-$	518	150
D&C Red No. 22	Water	518	140
D&C Red No. 27	Water $(OH)^-$	537	129
D&C Red No. 28	Water	537	122
D&C Red No. 30	$CHCl_3$	537	44
D&C Red No. 30	Xylene	496[b]	24.8
D&C Red No. 31	Alcohol & water (1 + 1)	518	80
D&C Red No. 33	Water	530	66.2
D&C Red No. 34	Alcohol & water (1 + 1)	526	65.5
D&C Red No. 36	$CHCl_3$	490	84
D&C Violet No. 2	$CHCl_3$	588	36.3
D&C Violet No. 2	Toluene	580	35.7
D&C Yellow No. 7	Water $(OH)^-$	489	247
D&C Yellow No. 8	Water	489	228
D&C Yellow No. 10	Water	413	92
D&C Yellow No. 11	$CHCl_3$	420	135
Ext. D&C Violet No. 2	Water	570	24.4
Ext. D&C Yellow No. 7	Water	430	49

[a]All neutral water systems are buffered with 0.01 *N* ammonium acetate.

[b]The wavelength of maximum absorbance of D&C Red No. 30 in xylene is actually 537 nm. The isosbestic point of the *cis* and *trans* forms of D&C Red No. 30 present in xylene is 496 nm [Hobin, N. K., JAOAC *54*, 215 (1971)].

Weigh 0.1 g of sample into a 250-mL beaker. Add 7 mL of 10% EDTA solution, 3 mL of 10% aqueous NaOH, and 15 mL of distilled water. Cover the beaker and bring to a boil. Stir the sample until wetted and boil an additional 2 min longer. Remove the beaker from the hot plate and stir for 30–60 sec. Add 25 mL of 95% EtOH and 125 ml of dilute EtOH; mix. Cover the beaker and digest the sample just below the boiling point until all the color is in solution. Cool to room temperature and quantitatively transfer to a 250-mL volumetric flask with dilute EtOH. Dilute to volume with same. (If talc or TiO_2 insolubles are present, filter the sample through a fine-porosity sintered-glass filter before transferring to the volumetric flask.) Determine the pure dye content spectrophotometrically against a standard similarly prepared.

D&C Blue No. 6

Transfer 0.5 g of sample to a 50-mL beaker. Add 5 mL of 100% H_2SO_4 and blend the mixture into a smooth paste with a stirring rod. Place the beaker and rod in an oven at 100 ± 5°C for 30 ± 5 min. Cool the sample to room temperature and then drown it in about 400 mL of distilled water. Transfer the solution to a 1000-mL volumetric flask and then dilute to volume with water and mix. Pipette 10 mL of this solution into a 500-mL volumetric flask and dilute to volume with water. Determine the sample's absorbance (A) in a 1-cm cell versus distilled water at the absorption maximum near 608 nm. Correct for solvent blank.

$$\text{Percent pure dye} = \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{81.1 \times 1 \times 0.01}$$

[Phthalocyaninato (2-)] Copper

Prepare a dispersing solution by transferring 50 mL of 2-ethoxyethanol ("cellosolve", Eastman Kodak No. 1697) and 20 mL of Dispersol VL (Ahcowet VL, Arnold Hoffman Co.) to a 1000-mL volumetric flask and diluting to volume with distilled water. Place 150 mL of this solution into a 250-mL beaker and stir magnetically at a rate to give a vortex about 2/3 the depth of the solution. Pipette 50 mL of sample solution (0.04 g in 250 mL of 96% H_2SO_4) into this vortex and mix well. Transfer to a 250-mL

volumetric flask, dilute to volume with dispersing solution, and measure the solution's absorbance in a 5-cm cell at the absorption maximum near 600 nm (A_1) and the absorption minimum near 470 nm (A_2). Correct for solvent blank.

$$\text{Percent pure dye} = \frac{A \times 100}{a \times b \times c} = \frac{(A_1 - A_2) \times 100}{39.6 \times 5 \times 0.032}$$

Results for duplicate dispersions should agree within 3% relative.

Annatto, Oil Soluble

Method A. Dissolve 0.1 g of sample in chloroform and dilute with chloroform to 100 mL in a volumetric flask. Using a 1-cm cell, determine the sample's absorption spectrum between 600 nm and 400 nm. Measure the absorbance at the absorption maximum near 503 nm and at 404 nm and correct for solvent blank.

$$\text{Percent pure dye (as bixin)} = \frac{[A_{503} + A_{404} - 0.256\ (A_{503})](100)}{(282.6)(1)(1)}$$

where A represents absorbances of the sample solution at the indicated wavelengths, 1 is cell path length (in cm), 1 is sample concentration (in g/liter), 0.256 is the factor relating the absorbances of bixin in chloroform at 404 nm and 503 nm, and 282.6 is the absorptivity of bixin at 503 nm (in liters/g-cm).

Method B. Determine the blank-corrected absorbance of a chloroform solution of sample at the maximum near 467 nm. Calculate percent pure dye as bixin using 320 liters/g-cm as the absorptivity.

Annatto, Water Soluble

Dissolve the sample in 2–5% aqueous KOH and measure its absorbance at the maximum near 480 nm. Calculate percent pure dye as bixin using 287 liters/g-cm as the absorptivity.

Annatto, Emulsions

Dissolve the sample in chloroform:methanol; 1:1 v/v. Make just acid with a few drops of glacial acetic acid and measure its absorbance against

the same solvent at the absorption maximum near 500 nm. Calculate percent pure dye as bixin using 287 liters/g-cm as the absorptivity.

Canthaxanthin, All-trans Crystals

Measure the absorbance of a cyclohexane solution of sample at the absorption maximum near 470 nm. Calculate percent pure dye as *trans* canthaxanthin, using 220 L/g-cm as the absorptivity.

Canthaxanthin, Water-dispersible Beadlets

Dissolve 0.5 g of reagent-grade iodine in 50 mL of 3A alcohol. Dilute a portion of this solution 1000-fold with cyclohexane.

Carry out the following work in subdued light. Transfer 0.1 g of beadlets into a 200-mL volumetric flask. Add about 100 mL of distilled water and warm on a steam bath until well dispersed. Cool to room temperature, make to volume with distilled water, and mix well. Transfer 10 mL of this solution into a 50-mL glass-stoppered centrifuge tube. Add 2 mL of 1 *N* HCl and 25 mL of chloroform and shake for 5 min. Break any emulsion that forms with two or three drops of 3A alcohol and then centrifuge at 2500 rpm for about 3 min. If the aqueous phase is not colorless, add about 1 g of sodium chloride, shake for an additional 5 min, and then recentrifuge. Transfer about 15 mL of the chloroform extract into a second glass-stoppered centrifuge tube containing about 2 g of anhydrous sodium sulfate. Mix well then centrifuge for about 3 min. Pipette 4 mL of the clear chloroform extract into a 50-mL volumetric flask. Place the flask in a 40°C water bath and evaporate to near dryness under a stream of nitrogen. Dissolve the residue in about 30 mL of cyclohexane, add 2.5 mL of iodine solution (prepared as described in the preceding paragraph) and dilute to volume with cyclohexane. Mix and then store in the dark for 2 hr at room temperature. Measure the absorbance (A) of this solution in a 1-cm cell versus cyclohexane at the absorption maximum near 470 nm.

Percent pure dye (as *cis-trans* equilibrium mixture)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{197 \times 1 \times 0.016}$$

β-*Carotene, All-*cis *Crystals*

Prepare the sample in subdued light and use low-actinic glassware. Transfer 0.065 g of sample to a 100-mL volumetric flask. Dissolve the sample with 10 mL of acid-free chloroform and then dilute to volume with cyclohexane and mix well. Pipette 5 mL of this solution into a 50-mL volumetric flask, dilute to volume with cyclohexane and mix. Pipette 5 mL of this second solution into a 50-mL volumetric flask. Dilute to volume with cyclohexane and mix. Using cyclohexane as the reference, determine the blank-corrected sample absorbance (A) in a 1-cm cell at the absorption maximum near 340 nm.

Percent pure dye (as *cis* β-carotene)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{101 \times 1 \times 0.0065}$$

β-*Carotene, All-*trans *Crystals*

Prepare the sample in subdued light and use low-actinic glassware. Transfer 0.05 g of sample to a 100-mL volumetric flask, dissolve the sample with 10 mL of acid-free chloroform, and dilute to volume with cyclohexane. Mix well. Pipette 5 mL of this solution into a 100-mL volumetric flask and dilute to volume with cyclohexane. Pipette 5 mL of this second solution into a 50-mL volumetric flask and dilute to volume with cyclohexane. Using a 1-cm cell, determine the blank-corrected absorbance (A) of the final solution at the absorption maximum near 455 nm.

Percent pure dye (as *trans* β-carotene)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{250 \times 1 \times 0.0025}$$

β-*Carotene, Water-dispersible Beadlets*

Transfer 0.25 g of sample into a 250-mL low-actinic glass separatory funnel containing 50 mL of distilled water. Swirl to disperse the beadlets. Add 50 mL of 3A denatured alcohol and extract with 50-mL portions of petroleum ether, (shaking for 3 min each time) until the aqueous layer is colorless (ca. three extractions). Combine the extracts in a 250-mL low-

actinic glass volumetric flask and dilute to volume with petroleum ether. Add 2–3 g of granular anhydrous sodium sulfate and shake for approximately 3 min. Allow the sulfate to settle, then pipette 5 mL of the clear solution into a 50-mL low-actinic glass volumetric flask, and evaporate this to dryness with a stream of nitrogen. Use no heat. Dissolve the residue and dilute it to volume with cyclohexane. Measure the absorbance (A) of this solution in a 1-cm cell at the maximum near 452 nm, using cyclohexane as a reference.

Percent pure dye (as *cis-trans* equilibrium mixture)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{223 \times 1 \times 0.1}$$

β-Carotene, Vegetable-oil Suspension

Weigh 0.1 g of sample and assay as *trans* β-carotene using the procedure given under All-*trans* Crystals. (Make proper adjustment in calculation for differences in sample weight.)

β-Carotene, Emulsions

Weigh 0.25 g of sample and assay as *cis-trans* isomers, using the procedure reported under Water-dispersible Beadlets. (Make proper adjustment in calculations for differences in sample weight.) If an emulsion forms during the extractions with ether, add about 5 g of sodium sulfate or sodium chloride to the separator.

β-Apo-8′-Carotenal, All-trans Crystals

Weigh 0.04 g of sample and analyze using the procedure reported under β-Carotene, All-*trans* Crystals. Determine the net absorbance (A) in a 1-cm cell at the absorption maximum near 461 nm.

$$\text{Percent pure dye} = \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{264 \times 1 \times 0.002}$$

β-Apo-8′-Carotenal, Water-dispersible Beadlets

Prepare the sample in subdued light and use low-actinic glassware throughout the analysis. Transfer 0.15 g of sample to a 200-mL volu-

metric flask containing 100 mL of distilled water. Warm the sample on a steam bath and swirl to effect complete solution. If an emulsion forms, add two or three drops of 3A alcohol. Cool to room temperature and then make to volume with distilled water and mix. Pipette 5 mL of this solution into a 50-mL glass-stoppered centrifuge tube and add 1 mL of 1 *N* HCl and 20 mL of chloroform. Shake vigorously for 5 min and then centrifuge at 2500 rpm for 3 min. If the water layer is not colorless, shake again for 5 min and recentrifuge. Transfer 15 mL of the chloroform extract to a 50-mL glass-stoppered centrifuge tube containing 5 g of anhydrous sodium sulfate, shake well, then centrifuge for about 3 min. Pipette 5 mL of this dried solution into a 50-mL volumetric flask and evaporate to dryness on a water bath (40°C) under a stream of nitrogen. Dissolve the residue in cyclohexane, make to volume with cyclohexane, and mix well. Determine the blank-corrected absorbance (A) of the solution in a 1-cm cell at the absorption maximum near 457 nm.

Percent pure dye (as *cis-trans* equilibrium mixture)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{240 \times 1 \times 0.01875}$$

β-Apo-8′-Carotenal, 20% Vegetable-oil Suspension

Prepare all sample solutions in subdued light using low-actinic glassware. Wash 0.100 g of sample into a 50-mL volumetric flask with cyclohexane, swirl to dissolve, make to volume with cyclohexane, then mix well: solution = x. Pipette 2.0 mL of x into a second 50-mL volumetric flask, dilute to volume with cyclohexane, then mix well; solution = Y. Pipette 5.0 mL of Y into a third 50-mL flask, dilute to volume with cyclohexane, then mix well; solution = Z. Using 1-cm cells measure the blank-corrected absorbance (A) of Z at the absorption maximum near 460 nm.

Percent pure dye (as β-apo-8′-carotenal)

$$= \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{264 \times 1 \times 0.008}$$

Carmine (Carminic Acid)

Dissolve 0.1 g of sample in 30 mL of boiling 2 *N* HCl. Cool and dilute to 1 liter with water. Using a 1-cm cell, determine the blank-corrected absorbance (A) of this solution at the absorption maximum near 494 nm.

$$\text{Percent pure dye (as carminic acid)} = \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{13.9 \times 1 \times 0.1}$$

Cochineal Extract

Transfer 1 g of sample to a 500-mL volumetric flask containing 30 mL of boiling 2 *N* HCl. Cool and make to volume with deionized water. Filter a portion of the sample through Whatman No. 1 filter paper and then determine the blank-corrected absorbance (A) of the filtrate at the absorption maximum near 494 nm.

$$\text{Percent pure dye (as carminic acid)} = \frac{A \times 100}{a \times b \times c} = \frac{A \times 100}{13.9 \times 1 \times 2}$$

Riboflavin

Protect the sample solution from direct sunlight throughout the entire procedure. Dry the sample for 2 hr at 105°C, weigh 0.5 g, and transfer it to a 1-L volumetric flask with water. Add 5 mL of glacial acetic acid and dilute to about 800 mL with water. Heat on a steam bath until dissolved. Cool to about 25°C and dilute to 1 liter. Dilute a 10-mL aliquot with water to 1 liter and measure the fluorescence of this solution in a fluorometer at the maximum near 460 nm. Immediately add 0.01 g of sodium hydrosulfite to the sample solution, mix to dissolve, and again measure the fluorescence at the maximum near 460 nm. Similarly prepare and measure the fluorescence of a USP riboflavin reference standard.

$$\text{Percent riboflavin} = \frac{A - B}{A_s - B_s}\,(100)$$

where A is fluorescence of the sample before sodium hydrosulfite addition, B is fluorescence of the sample after sodium hydrosulfite addition, A_s is fluorescence of the standard before sodium hydrosulfite addition, and B_s is fluorescence of the standard after sodium hydrosulfite addition.

Turmeric (Curcumin)

Method A. Weigh 0.1 g of powdered sample into a 100-mL volumetric flask. Add 60 mL of glacial acetic acid and place the mixture on a water bath (90°C) for 1 hr. Add 2 g of boric acid and 2 g of oxalic acid to the flask and place it on the bath for an additional 10 min. Cool to room

temperature and dilute to volume with glacial acetic acid. Pipette 5 mL of this solution into a 50-mL volumetric flask and dilute to volume with acid. Determine the absorbance in a 1-cm cell at 540 nm against a similarly prepared standard.

Method B. Boil 0.5 g of ground turmeric (<0.5 mm diameter) with 30 mL of 96% ethanol for 2.5 hr, filter the mixture, then dilute the filtrate to 100 mL with ethanol. Dilute a portion of this solution 50-fold with ethanol, then measure its absorbance at 425 nm. Compare to a curcumin standard similarly prepared.

Method C. Prepare a 0.01–15 μg/mL sample solution in acetone–0.05M $NaHCO_3$–0.1M NaOH buffer (10:10:1, pH 11), then measure the purple color formed at 520 nm vs a buffer blank. Compare with curcumin standards similarly prepared.

Turmeric Oleoresin

Weigh 100–200 mg of a well-mixed sample into a 100-mL volumetric flask. Add approximately 75 mL of acetone and shake until the sample is completely in solution. Bring to volume and mix thoroughly. Pipette a 1-mL aliquot into a 50-mL volumetric flask and bring to volume with acetone; mix thoroughly. Using a Beckman Model B (or similar) spectrometer, a 1-cm cell, and a tungsten light source, obtain the sample absorbance at 422–425 nm, using acetone as a blank. If the absorbance is not between 0.2 and 0.4 absorbance units, adjust the concentration accordingly by varying the size of the aliquot taken from the first solution.

$$CV = \frac{\text{Absorbance}}{\text{Sample wt. @ 1/5000}}$$

$$\text{Percent Curcumin} = \frac{CV\ (1/5000)}{33.00}$$

where 33.00 is CV of Curcumin (EK No. 1179) at 1/5000 in acetone, 422–425 nm.

ASSAY BY ELEMENTAL ANALYSIS

In principle, colorants can be assayed by determining the amount of any of its elements, then relating the percentage found to that predicted by

theory. In comparison to spectroscopy, gravimetry, and titrimetry, elemental analysis is usually more time consuming, requires greater skill on the part of the analyst, and often produces less satisfying results. The advent of automated equipment for the determination of carbon, nitrogen, hydrogen, sulfur, and other elements has minimized some of the problems associated with elemental analysis, but these tools are costly and, unless one is interested in using them for structural determinations, it would very likely be wiser to spend the money on a different method of assay.

General methods are included here for the determination of sulfur and nitrogen, because these are among the most useful elements to analyze for (particularly when dealing with certified colors), and because the methods represent two different procedures for the decomposition of colorants, both of which are useful when determining other elements. In using either method it is important to realize that neither is capable of identifying the source of the element measured. Thus, it is necessary to know the nature of the sample, particularly if it is suspected to contain such materials as sodium sulfate or sodium chloride. Some specific methods are also included.

Assay Through Organic Sulfur Content

Weigh 0.2 g of sample into a Parr peroxide bomb. Mix with about 14 g of sodium peroxide, 1 g of sugar, and 0.1 g of potassium chlorate, and cautiously ignite the bomb. Place the opened bomb in a beaker, cover with water, heat at 50–60°C until the reaction ceases, and then rinse the bomb into the beaker with water. Cool the solution and neutralize it with concentrated hydrochloric acid; then add 5 mL excess. Filter the solution into a second beaker, washing the filter paper with water. Heat the filtrate to boiling and while boiling, add 25 mL of 10% barium chloride solution. Digest on a steam bath for 1–2 hr or allow the sample to stand in a warm place overnight. Filter through a tared Gooch crucible and wash the precipitate with water until it is free of chloride. Dry at 100°C for 20 min, and then at 700°C for 30 min.

$$\text{Percent total sulfur} = \frac{W(0.1373)(100)}{w}$$

$$\text{Percent organic sulfur} = \%\ \text{total sulfur} - \%\ \text{inorganic sulfur}$$

$$\text{Percent pure dye} = \frac{(\%\ \text{organic sulfur})(100)}{\text{theoretical \% organic sulfur}}$$

where W is weight of barium sulfate precipitate in g and w is sample weight in g.

Assay Through Organic Nitrogen Content

For Colors Requiring Reduction Prior to Digestion: Weigh a quantity of sample containing 2 mg of nitrogen and transfer it to a 20-mL Kjeldahl flask. Add 0.5 mL of a 9:1 mixture of 50% (w/v) hydriodic acid and 50% (w/v) hypophosphorous acid. Reflux 5–10 min, turning the flask occasionally to ensure solution and reduction. Remove most of the liquid by distillation. Remove the flask from the heater, cool, add 5 mL of 1:1 sulfuric acid, and evaporate to fumes. If considerable iodine remains, add 1–2 mL of water and again evaporate to fumes. Remove the flask from the heater, cool, and wash down the sides with 0.5 mL of water. Add 0.6 g of anhydrous sodium sulfate and 0.5 mL of 20% mercuric acetate solution. Place on a heater and digest until the solution clears and then heat for an additional hour. If the dye contains ring nitrogen, heat for 2.5 hr after clearing and add more sulfuric acid if needed. Remove the flask, cool, and add 10–12 mL of water to dissolve the salts. Transfer the sample to a micro-Kjeldahl distilling apparatus using about 10 mL of water. Set the electric controller of the steam generator to distill 20 mL in about 10 min. Add 5–6 mL of 50% sodium hydroxide solution and 3 mL of 21% sodium thiosulfate solution. Prepare an indicator solution by dissolving first 0.3 g of methyl red in 60 mL of ethanol and diluting with water to 100 mL, then 0.2 g of methylene blue in 100 mL of 50% ethanol, and mixing the two solutions. Add three drops of the indicator solution to a 50-mL Erlenmeyer flask containing 5 mL of 2% boric acid solution. Position the flask so that the outlet from the condenser dips below the level of the liquid and steam distill for 5 min. Lower the receiving flask so that the condenser outlet is above the liquid in the flask and distill for 1–2 min to flush the condenser tube.

Titrate the solution in the receiving flask with standardized 0.02 N hydrochloric acid. Make a blank determination.

$$\text{Percent nitrogen} = \frac{(A - B)N(0.014)(100)}{w}$$

where A is mL of titrant used for sample, B is mL of titrant used for blank, N is normality of titrant, and w is sample weight in g.

$$\text{Percent pure dye} = \frac{\%\ \text{nitrogen (100)}}{\text{theoretical \% nitrogen}}$$

For Colors that Do Not Require Reduction Prior to Digestion: Weigh and transfer a sample as described in the preceding paragraphs. Add 5 mL of 1:1 sulfuric acid and heat until thoroughly charred. Cool, and then add 0.6 g of sodium sulfate and 0.5 mL of 20% mercuric acetate solution. Wash down the side of the flask with a minimum amount of water and proceed as described in the preceding paragraphs, starting with "Place on a heater and digest . . ."

Organically Combined Iodine in FD&C Red No. 3

Wash 0.5 g of sample into a 100-mL volumetric flask with about 50 mL of hot distilled water. Swirl the flask to dissolve the sample and then add 6 mL of 10% aqueous NaOH. Cool the solution, make it to volume with distilled water, and mix well.

Pipette 25 mL of this solution into a 500-mL Erlenmeyer flask and add 100 mL of distilled water, 25 mL of 7% aqueous $KMnO_4$, and a few glass beads. Boil the mixture for 5 min and then remove from the hot plate. When the boiling ceases, cautiously add 10 mL of HNO_3 and boil for an additional 5 min. Remove the flask from the heat and wash down the sides of it with distilled water. While swirling, quickly add 4 mL of 12% aqueous $NaNO_2$ to the flask, then add more $NaNO_2$ dropwise until the suspension begins to clear. Continue the dropwise addition of $NaNO_2$, allowing each drop to react before the next one is added. Continue this addition until only a small amount of undissolved MnO_2 remains. Do not attempt to destroy or dissolve the last traces of MnO_2, but instead immediately add 1% $KMnO_4$ solution in 1-mL portions until the solution turns pink. (If more than 2 mL of 1% $KMnO_4$ is required or if a brown color appears, add 10 mL of $KMnO_4$ to the solution and again heat it to boiling. Repeat the dropwise addition of 12% $NaNO_2$ and 1% $KMnO_4$ to again obtain a pink color.)

Using suction, rapidly filter this solution through a Gooch crucible fitted with glass-fiber filter paper. Wash the flask and filter thoroughly with distilled water. (The filtrate must be pink at this point.) Add 12%

$NaNO_2$ solution dropwise to the filtrate with shaking until 1 drop has been added in excess of that needed to decolorize the solution.

Add 10 mL of 10% aqueous sulfamic acid, wash down the sides of the flask, and then swirl the contents. Cool the solution to room temperature, add 2–3 g of solid KI, and titrate the liberated iodine with 0.1 *N* $Na_2S_2O_3$, adding starch iodide indicator when the solution becomes lemon-yellow. Continue the titration until the blue color just disappears. Similarly determine a reagent blank.

$$\text{Percent total iodine} = \frac{(A - B)(N)(0.02115)(100)}{0.125}$$

where *A* is mL of titrant used for sample, *B* is mL of titrant used for blank, *N* is normality of titrant, 0.02115 is milliequivalent weight of I, 100 is factor for conversion to percent, and 0.125 is sample weight in g.

Organically Combined Bromine and Chlorine in D&C Red No. 28

Weigh 0.2 g of sample into a Parr oxygen bomb containing 15 mL of distilled water. Assemble and ignite the bomb. Using distilled water, quantitatively transfer the contents of the bomb to a 400-mL beaker. Adjust the sample volume to about 200 mL with distilled water, add 15 mL of HNO_3, and stir well. Add 25 mL of 0.1 *N* $AgNO_3$ to the sample and place it on a magnetic stirrer. While stirring vigorously, add 5 mL of nitrobenzene. Add 5 mL of ferric alum indicator, stir for about 3 min, and then titrate with 0.1 *N* KSCN to a faint persistent red-brown color.

$$\text{Percent total halogen as chlorine} = \frac{(AN_1 - BN_2)(0.03546)(100)}{0.200}$$

where *A* is mL of $AgNO_3$ solution = 25, N_1 is normality of $AgNO_3$, *B* is mL of KSCN solution, N_2 is normality of KSCN, 0.03546 is milliequivalent weight of chlorine, 100 is factor for conversion to percent, and 0.200 is sample weight in g.

Bomb a second 0.2 g sample as above and quantitatively wash the combustion products into a 500-mL iodination flask with distilled water. Adjust the sample volume to about 125 mL with distilled water.

While working in a fume hood, add 12 mL of 85% phosphoric acid, 3 mL of 5% potassium cyanide, and 5 mL of saturated potassium per-

manganate to the flask, wetting the sides of the flask as each reagent is added. Stopper the flask and mix the contents by gentle swirling, wetting the entire inside surface. Allow the sample to stand for at least 7 min. Add about 2 g of solid ferrous ammonium sulfate hexahydrate and then wash down the sides of the flask with distilled water and swirl the sample to mix it. (A clear, nearly colorless solution should result. If the solution is still colored, add more ferrous ammonium sulfate; a 2-g excess does no harm.)

Add 2 g of potassium iodide and immediately titrate the liberated iodine with 0.05 *N* sodium thiosulfate to a pale yellow color. Add 2–3 mL of starch iodide indicator solution and continue titrating with 0.05 *N* sodium thiosulfate to the disappearance of the blue starch iodide color. Similarly determine a reagent blank.

$$\text{Percent bromine} = \frac{(A - B)(N)(0.03996)(100)}{0.200}$$

where A is mL of $Na_2S_2O_3$ required to titrate sample, B is mL of $Na_2S_2O_3$ required to titrate blank, 0.03996 is milliequivalent weight of bromine, 100 is factor for conversion to percent, and 0.200 is sample weight in g.

$$\text{Percent chlorine} = (\%\text{ total halogen as chlorine}) - (\%\text{ bromine})\frac{35.457}{79.916}$$

where 35.457 is atomic weight of chlorine and 79.916 is atomic weight of bromine.

Organically Combined Bromine in D&C Red No. 22

Determine bromine using the method described for D&C Red No. 28

MISCELLANEOUS PROCEDURES

Calcium Carbonate

Weigh 1 g of calcium carbonate, previously dried at 200°C for 4 hr, and transfer to a 250-mL beaker. Moisten thoroughly with a few milliliters of

water and then add, dropwise, sufficient diluted hydrochloric acid to effect complete solution. Transfer the solution to a 250-mL volumetric flask, dilute to volume, and mix. Pipette 50 mL of the solution into a suitable container, add 100 mL of water, 15 mL of 4% (w/v) sodium hydroxide, and 300 mg of hydroxy naphthol blue indicator, and titrate with 0.05 *M* disodium ethylenediaminetetraacetate until the solution is a distinct blue in color. One milliliter of 0.05 *M* disodium ethylenediaminetetraacetate is equal to 5.004 mg of $CaCO_3$.

Ferrous Gluconate

Prepare an *o*-phenanthroline indicator solution as follows: Dissolve 1.48 g of ferrous sulfate, $FeSo_4 \cdot 7H_2O$, in 100 mL of water. Immediately dissolve 0.15 g of *o*-phenanthroline in 10 mL of this solution. Add 1.5 g of sample, 75 mL of water, and 15 mL of 10% (w/v) sulfuric acid to a 300-mL Erlenmeyer flask. Stir to dissolve. Add 0.25 g of zinc dust. Close the flask with a stopper containing a Bunsen valve and allow it to stand for 20 min at room temperature. Filter the sample through a thin layer of zinc dust. Wash the filter cake with 10 mL of 10% (w/v) sulfuric acid and then 10 mL of water. Immediately titrate the filtrate to an *o*-phenanthroline end point with 0.1 *N* ceric sulfate. Similarly determine a blank. One milliliter of 0.1 *N* ceric sulfate is equal to 0.04462 g of anhydrous ferrous gluconate ($C_{12}H_{22}FeO_{14}$).

Titanium Dioxide

Prepare zinc amalgamate by dissolving 10 g of granulated zinc in about 20 mL of mercury, heating and stirring at 150°C. A liquid amalgam is formed on cooling.

Mix 0.3 g of sample with 3 g of potassium hydrogen sulfate in a platinum dish and fuse. Cool the fusion and dissolve it in 150 mL of about 2 *N* sulfuric acid. Activate a freshly charged zinc amalgam reductor by passing 100 mL of 2 *N* sulfuric acid through the column, followed by 100 mL of water. Pass 200 mL of *N* sulfuric acid through the column, followed by 100 mL of water. Collect the effluent in a receiver containing 50 mL of 15% ferric ammonium sulfate in 0.5 *N* sulfuric acid. Titrate the solution with 0.1 *N* potassium permanganate; this is the blank. Reactivate

the column with 100 mL of *N* sulfuric acid. Pass the sample solution through the column followed by 100 mL of *N* sulfuric acid and 100 mL of water; collect as above. Titrate the effluent and subtract the blank titration. One milliliter of 0.1 *N* potassium permanganate is equal to 0.00799 g of titanium dioxide.

RELATIVE METHODS

Because of their indefinite composition, certain colorants are analyzed on a relative basis by comparing them with a house standard (a "type"), or a chemically nonrelated color standard such as colored glass or solutions of inorganic salts. Such measurements are made either visually or with the aid of a spectrometer. The methods are usually empirical in nature and are more a measure of color value or tinctorial strength than of purity or assay. The procedures are rarely standardized within an industry, and the numbers obtained with them are often quoted in terms that are meaningless out of context, so extreme care must be exercised in interpreting them. Colorants frequently analyzed this way include paprika, turmeric, and caramel. Paprika and its oleoresins are rated visually versus Lovibond glasses, colorimetrically versus standard solutions composed of potassium dichromate and cobaltous chloride, and spectrophotometrically by measuring the absorbance of sample solutions in acetone at 460 nm. Turmeric oleoresins also are often measured spectrophotometrically in acetone at 422–425 nm, whereas caramel is measured colorimetrically using either a Klett-Summerson color comparator equipped with No. 52 and No. 64 glasses, or a spectrophotometer operated at 610 nm or 560 nm. Typical examples of such methods are given in the paragraphs that follow.

American Spice Trade Association (ASTA) Color Value of Capsicums and Paprika Oleoresins

Prepare a standard color solution by drying $CoSO_4(NH_4)_2SO_4 \cdot 6H_2O$ for 1 week in a desiccator over Drierite. Dissolve 0.3005 g of $K_2Cr_2O_7$ and 34.960 g of dried $CoSO_4(NH_4)SO_4$ in 1.8 *M* H_2SO_4 and dilute to 1 liter with 1.8 *M* H_2SO_4; absorbance (*A*) in a 1-cm cell at 460 nm = 0.600. For a glass reference standard use a NIST SRM 2030 glass filter with an *A* specified by NIST in the range 0.4–0.6 at 465 nm.

Capsicums. Grind samples to pass a No. 40 sieve (a No. 18 if the NIST standard is used). Weigh accurately 70–100 mg of ground sample and transfer it to a 100-mL volumetric flask. Make to volume with acetone, stopper, shake well, and let stand 4 hr (16 hr if the NIST standard is used) at room temperature in the dark. Shake the flask again and let the particles settle for 2 min. Determine A of the clear solution in a 1-cm cell at 460 nm versus acetone. Determine A of the color standard at 460 nm versus 1.8 M H_2SO_4, or the NIST standard at 465 nm.

Oleoresins, using a color standard. Weigh 50–80 mg of sample to the nearest 0.1 mg on a 5-cm square of glassine paper. Transfer the paper and sample to a 100-mL volumetric flask and dilute to volume with acetone. Extract for at least 15 min with occasional shaking. Pipet 10 mL of the extract into a second 100-mL volumetric flask, dilute to volume with acetone, and mix well. Filter the diluted extract through Whatman No. 40 paper, discarding the first 10–15 mL of filtrate. Measure A of the filtrate in a 1-cm cell at 460 nm versus acetone.

Oleoresins, using a NIST glass standard. Weigh 70–100 mg of sample to the nearest 0.1 mg and transfer to a 100-mL volumetric flask. Dilute to volume with acetone, shake, and let stand 2 min. Pipet 10 mL of the extract into a second 100-mL volumetric flask, dilute to volume with acetone, and mix well. Determine A at 460 nm versus acetone.

To correct for instrument and cell variations, calculate the correction factor, $I_f = 0.600/A$ of the standard color solution at 460 nm, or $I_f =$ declared A of the NIST standard at 465 nm/actual A of the NIST standard at 465 nm. I_f should be redetermined each time the spectrophotometer is turned on.

For proper measurement, A of an extract should be 0.30–0.70. Extracts with $A > 0.70$ should be diluted with acetone as needed. Those with $A < 0.30$ should be discarded and a larger sample extracted.

$$\text{ASTA color value of capsicums} = \frac{(A_{ext} \text{ at } 460 \text{ nm}) \times (16.4\ I_f)}{\text{sample wt in g}}$$

$$\text{ASTA color value of oleoresins} = \frac{(A_{ext} \text{ at } 460 \text{ nm}) \times (164\ I_f)}{\text{sample wt in g}}$$

where 16.4 and 164 are conversion factors to ASTA color values.

EOA (Essential Oil Association) color value for oleoresin

$$= \frac{(A_{ext} \text{ at } 460 \text{ nm}) \times (61{,}000\ I_f)}{\text{sample wt in g}}$$

100 EOA color units = 2.69 ASTA units.

Colorimetric Color Value (CV) of Paprika Oleoresin

Weigh 1 g of sample into a 100-mL volumetric flask. Make to volume with acetone; mix well. Dilute the sample with acetone as needed (see table that follows).

Pipette the required amount of dilute solution (see table that follows) into a 100-mL Nessler tube. Bring the volume almost to the mark with acetone and compare it through the length of the tube against a blank. Make small additions until sample and blank match. Calculation is as follows:

$$CV = \frac{100 - (A \times B)}{A \times B} \times 100$$

where A is mL of dilute solution used and B is percent of dilute solution.

Blank preparation: Into a 100-mL Nessler tube pipette 10 mL of 0.1 *N* potassium dichromate solution (4.904 g $K_2Cr_2O_7$ per liter) and 1 mL of 0.5 *N* cobaltous chloride solution (5.948 g $CoCl_2 \cdot 6H_2O$ per 100 mL) and make to 100 mL with distilled water.

Color Value	Percent Dilution	Milliliters of Solution 1 used for dilution	Milliliters Dilute Solution to be Used
100,000	0.01	1 mL/100 mL of acetone	8–10
50,000	0.02	1 mL/50 mL of acetone	8–10
40,000	0.02	1 mL/50 mL of acetone	10–13
30,000	0.02	1 mL/50 mL of acetone	15–20
20,000	0.04	2 mL/50 mL of acetone	10–13
10,000	0.10	5 mL/50 mL of acetone	8–10

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$$\text{Tinctorial power} = \frac{A_{560}}{C}$$

$$\text{Hue index} = 10 \log (A_{510}/A_{610})$$

MARSHALL, P. N., HOROBIN, R. W., Stain Technology *49*, 19–28 (1974). A Simple Assay Procedure for Carmine and Carminic Acid Samples. A variety of samples were analyzed qualitatively by gel-filtration on Sephadex LH20 using salt-saturated dimethylformamide as the mobile phase, and by thin-layer chromatography on precoated cellulose sheets (Eastman Chromagram 6064) using either acetone–glacial acetic acid–water (1:1:1), or isopropanol–water–0.5 *M* H_2SO_4 (5:5:1). The "available carminic acid" content of samples was determined by refluxing 20–50 mg of sample for 10 min in 100 mL of 0.02 *M* aqueous HCl, diluting the cooled solution appropriately with 0.02 *M* HCl, then determining its absorbance at the maximum near 490 nm. "Total carminic acid" content was determined by dissolving 20 mg of sample in 4–5 drops of 1% v/v aqueous ammonia solution (0.880 sp. gr.), diluting the sample to 250 mL with 0.02 *M* aqueous HCl, then refluxing, cooling, and measuring the solution's absorbance as above.

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PRASAD, U. V., KUMARI, P. L., DIVAKAR, T. E., SASTRY, C. S. P. Acta Cienc. Indica (Ser.) Chem. *11c*, 115–117 (1985). Determination of Some Permitted Food Colours with *N*-Chlorosuccinimide. Amaranth, FD&C Yellow No. 6, FD&C Yellow No. 5, carmoisine and FD&C Blue No. 2 were determined titrimetrically in 1 *M* HCl with *N*-chlorosuccinimide by adding the titrant in excess and determining the unconsumed amount iodimetrically. The end-point occurs at a titrant-to-dye molar ratio of 1:1 except for FD&C Blue No. 2, were it occurs at a ratio of 1:2. Direct titrations based on the appearance of a yellow color at the end-point do not give accurate results.

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RUKMINI, N., KAVITHA, V. S. N. P. Fresenius' Z. Anal. Chem. *298*, 159 (1979). Direct Oxidimetric Determination of Indigo Carmine (FD&C Blue No. 2) With Vanadate. Treat 20–50 μmol of sample with enough 10 *M* H_2SO_4 to yield a 6–8 *M* solution when the mixture is diluted (Caution!) to 50 mL. Titrate the sample with 0.02–0.08 *M* $NaVO_3$ solution to a golden-yellow end point. Accuracy is ± 0.6%. The titration can also be monitored spectrophotometrically at 610 nm when 0.5–1.0 μg of sample is titrated with 0.5 *M* NH_4VO_3 while the sample is purged with CO_2. Accuracy here is estimated as ± 1%. Starch, D-glucose, acacia and oxalic, tartaric and citric acids do not interfere.

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TRUHAUT, R., CASTAGNOU, R., LARCEBAU, S., LASSALLAE-SAINT-JEAN, V. Bull. Soc. Pharm. Bordeaux *100*, 145–158 (1961). Photocolorimetric Method for the Measurement of the Coloring Power of Caramels and Their Acid Resistance. The coloring power of caramels was measured photocolorimetrically at 430 nm and the results compared with those obtained by the Lovibond method.

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mine B, Dichloramine B and Dibromamine B. Test solutions containing 2–60 μmol of sample made 0.01–0.1 *M* in NaOH, or 1–22 μmol of sample, or 1–22 μmol of sample in anhydrous acetic acid, was added to an excess of bromamine B, or dichloramine B or dibromamine B (0.05 *N* soln. in each instance) and, after 30 min, unconsumed oxidant was determined iodimetrically. The error in recovery of indigo carmine was <1%. The isatinsulfonate formed by oxidation of the colorant could also be determined by spectrophotometry at 410 nm.

8

INSOLUBLE MATTER

The amount of insolubles in a dyestuff is an indication of purity and is used as such by the colorant manufacturer, the consumer, and the Food and Drug Administration alike. In addition, insolubles represent practical problems for the user since their presence can lead to cloudy soda pop, gritty toothpaste, plugged plant filters, and so on. As a consequence, government, to protect the public health, has established limits on the insolubles content of many colorants, and industry has striven to produce products of even higher quality to meet the demands of their customers.

The methods used to determine insolubles are generally simple gravimetric procedures. Those developed and tested by the Association of Official Analytical Chemists are given here.

PREPARATION OF GOOCH CRUCIBLES

Fit a Gooch crucible with a glass fiber disk, wash it with hot water, dry at 135°C, cool in a desiccator, and weigh. Repeat the washing, heating, and drying until the weight is constant.

WATER-INSOLUBLE MATTER

Dissolve a 2-g sample in 200 mL of hot water, then let the solution cool to room temperature. Filter through a tared Gooch crucible, wash with cold water until the washings are colorless, dry for 3 hr at 135°C, cool in a desiccator, and weigh. Calculate any increase in weight as water-insoluble matter.

ALKALINE-INSOLUBLE MATTER

Proceed as described for water-insoluble matter, substituting 1% sodium hydroxide solution or 1:14 ammonium hydroxide for water.

CARBON TETRACHLORIDE INSOLUBLES

Method A. Mix 0.2–0.5 g of sample with 100 mL of CCl_4 in a 250-mL beaker, stir, and heat to the boiling point. Filter hot through a tared Gooch crucible, transfer any residue in the beaker to the filter, and then wash with 10-mL portions of CCl_4 until the washings are colorless. Dry for 3 hr at 100–105°C, weigh, and calculate any weight increase as CCl_4-insoluble matter.

Method B. Fit a Gooch crucible with a glass fiber disk and a cotton pad, then wash, dry, and tare it. Weigh 1 g of the sample into the crucible, placing the pad on top of the sample. Support the crucible in a Soxhlet extraction apparatus so that the bottom of the crucible is slightly above the top of the siphon tube. Extract with carbon tetrachloride until no more color is removed. Dry to a constant weight at 100–105°C. Cool in a desiccator and weigh.

TOLUENE, BENZENE, ACETONE, ALCOHOL, AND XYLENE INSOLUBLES

Using the appropriate solvent, proceed as described under carbon tetrachloride insolubles.

9

INORGANIC SALT CONTENT

The inorganic salts present in colors are there as a result of a step in the manufacturing process–neutralization, isolation, iodination, and so on–or because of the deliberate addition by the manufacturer to meet the strength demands of the customer. The salts most often found are sodium sulfate and sodium chloride. Others less frequently present include sodium acetate, sodium phosphate, sodium iodide, and sodium carbonate.

The procedures that follow were developed chiefly for the analysis of certifiable colors since they most often contain inorganic salts and generally have specifications controlling their salt content. The methods should, however, be applicable to many of the colorants exempt from certification, either as is or after minor modification.

Ion chromatography (IC) is probably the most useful technique currently available for determining inorganic salts. IC is faster, more specific and more easily automated than most classical methods, it can be used to determine both anions and cations, and can measure more than one ionic species in a single run. However, the equipment needed is expensive.

Ion chromatography has already been used to determine fluoride, nitrite, phosphate, bromide, sulfate, nitrate, and iodide in water-soluble colorants and in the water extracts of lakes and water-insoluble colors, and more applications are sure to follow as better equipment and columns become available.

ANIONS BY ION CHROMATOGRAPHY (4)

Colorant is trapped on a precolumn, while inorganic salts pass through for separation. The basic method can determine fluoride, chloride, nitrite, phosphate, bromide, nitrate, and sulfate in a single determination in all the water-soluble certifiable color additives. Iodide cannot be determined by the basic procedure because it elutes so slowly that its peak is too low and broad to be measured; it does not interfere with subsequent analyses. Iodide can be determined separately, though, by eliminating the 500-mm analytical column and using only the precolumn and a pump speed set at 50%.

Use a Dionex Model 10 or 14 Ion Chromatograph* equipped with a 3 × 150-mm precolumn and a 3 × 500-mm analytical column (both Dionex anion separator columns), and a 6 × 250-mm anion suppressor column, all in series.

Using 0.003 *M* sodium bicarbonate/0.0024 *M* sodium carbonate as the mobile phase, and a flow of 124 mL/hr (30% pump speed) inject 100 μL of a 0.02% sample solution in mobile phase. Monitor the effluent at 10 μmho until chloride elutes (ca. 3 min), then at 1 μmho. F^-, Cl^-, NO_2^-, PO_4^-, Br^-, NO_3^-, and SO_4^{-2} elute in about 2.1, 3.5, 4.4, 6.7, 9.0, 11.0, and 15.5 min, respectively. Using the alternate procedure I^- elutes in about 8.5 min. Compare sample peak heights or areas with those of standards similarly chromatographed.

Suppressor columns and precolumns must be regenerated about every 8 hr. Suppressor columns are regenerated by pumping 0.1 *N* H_2SO_4 through them for about 15 min, followed by a 30-min wash with distilled water. Analytical columns are reconditioned about once a month by washing with 0.1 *N* Na_2CO_3 for about 30 min, then with the standard

*An automated procedure for determining chloride, phosphate, bromide, and sulfate in color additives using a Dionex Model 12 chromatograph can be found in: Fratz, D. D. JAOAC *63*, 882–888 (1980).

mobile phase. Precolumns are regenerated outside of the instrument (to avoid attack of metal parts by HCl) by pulling 2 *N* HCl–acetone (1:1) through the column immediately followed by 15 mL of distilled water.

SODIUM PHOSPHATE (3)

Reagents

(a) Ashing reagent—Weight 392 g of cellulose powder (Whatman CF-11, Whatman LabSales, P.O. Box 1359, Hillsboro, Oregon 97124–9981) and 8.00 g of MgO into a 4-L Erlenmeyer flask, stopper the flask, then mix well for several minutes.

(b) Vanadomolybdic acid reagent—Dissolve 20.0 g of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in 200 mL of hot water. Cool, then dilute to 1 L with water. Dissolve 0.500 g of NH_4VO_3 in 100 mL of hot water, add 100 mL of HNO_3, cool, then dilute to 1 L with water. Gradually add the molybdate solution to the metavanadate solution while mixing. Store at room temperature in a polyethylene or Pyrex bottle. Discard if a precipitate forms.

(c) Standard solutions—Dry KH_2PO_4 for 2 hr at 105°C, then dilute 6.6408 g of it to 1 L with water; mix well. Dilute 0, 5, 10, 15, 20, and 25 mL of this solution to 1 L to obtain working standards equal to 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg of Na_3PO_4/10 mL, respectively.

Preparation of a Standard Curve

Place 2 g of ashing reagent into each of six 100-mL Pyrex beakers. To each beaker add 10 mL of one of the working standards, then swirl to mix. Heat the beakers at 105°C until completely dry (about 3 hr), cool them, and then mix their contents well using a small glass rod. Shake the beakers gently to level their contents, then transfer them to a cool muffle furnace (in a hood) and ash them for 3 or more hr at 500°C. After all smoking has stopped, open the muffle door, then allow the beakers to cool for one hr. Remove them from the muffle; let cool to room temperature.

Add 50.0 mL of vanadomolybdic acid reagent to each beaker, slowly at first until the ash is wet, and then rapidly. Swirl the samples until in solution, then filter them by passing each through a 65-mm diameter

Pyrex powder funnel with a 12-mm ID × 28-mm long stem that has been tightly packed with glass wool (Corning 3950, or equivalent). Collect the filtrates in 50-mL Erlenmeyer flasks with ground-glass stoppers, stopper the flasks and shake to mix. Let color develop for ≥ 10 min. (For greatest accuracy each standard should have had the same color development time at the moment that its absorbance, A, is measured.) Using 1-cm cells and vanadomolybdic acid reagent as the reference solution, determine A of each standard at 400 nm. Correct the readings by subtracting from each the absorbance of the standard to which no Na_3PO_4 was added (usually 0.005). If A for the 0.0-mg standard is abnormally high, repeat the entire determination for this standard three or more additional times, positioning the beakers at different locations in the muffle. Calculate an average blank absorbance for correcting the absorbances of the other standards, then prepare a plot of A versus mg Na_3PO_4/10 mL.

Analysis of Samples

Transfer 0.400 g of sample to a 100-mL beaker containing 2 g of ashing reagent. If the sample is granular, add ≥ 10 mL of water to dissolve completely, swirl to mix well, then dry completely at 105°C. Mix and ash the sample, develop the color, and determine its absorbance using the same technique and color development time used for preparing the standards. If the sample absorbance is greater than that for the 2.0-mg standard, repeat the analysis using an appropriately smaller sample weight.

Certain colorants require special treatment:

FD&C Red No. 3—Add 5 mL of nitric acid (1 + 4) to the dye ash and heat to dryness on a steam bath to remove iodine.

Citrus Red No. 2, D&C Red Nos. 22 and 28, D&C Yellow Nos. 8 and 11, and any other dyes whose ash contains an appreciable amount of carbon—Break up the ash with a stirring rod, add about 2 g of cellulose powder, mix and re-ash for 3 hr at 500°C.

$$\% \ Na_3PO_4 = \frac{A \times S \times 0.1}{W}$$

where A = the corrected sample absorbance at 400 nm; S = the slope of the standard curve = r/n; r = the sum of the ratios of mg of Na_3PO_4/10 mL

to the absorbance of each respective standard; n = the number of standard solutions used in the calculation; 0.1 = 100/1000, factors needed to convert to percent and grams, respectively; W = sample weight in grams.

SODIUM CHLORIDE

Volhard Method for Acid Dyes (7)

Dissolve 2 g of dye in 100 mL of water and add 10 g of activated carbon (Norit SG No. 2, American Norit Co., Inc., Jacksonville, Fla., or equivalent) that is free of chloride and sulfate. Boil gently for 2–3 min. Cool to room temperature, add 1 mL of 6 N nitric acid, and stir. Dilute with water to 200 mL in a volumetric flask, then filter through dry paper. Repeat the treatment with 2-g portions of carbon until no apparent color rises when filter paper is dipped into the filtrate.

Transfer 50 mL of filtrate to a 250-mL flask. Add 2 mL of 6 N nitric acid, 5 mL of nitrobenzene, and 10 mL or more of standardized 0.1 N silver nitrate solution, depending on the chloride content. Shake the flask until the silver chloride coagulates. Prepare a saturated solution of ferric ammonium sulfate and add just enough concentrated nitric acid to discharge the red color; add 1 mL of this solution to serve as the indicator. Titrate with 0.1 N ammonium thiocyanate solution (that has been standardized against the silver nitrate solution) until the color persists after shaking for 1 min.

$$\text{Percent sodium chloride} = \frac{(A)(N)(0.05844)(195)(100)}{(w)(50)}$$

where A is net volume of silver nitrate solution required (in mL), N is normality of silver nitrate solution, and w is sample weight (in g).

Note: Calculation is based on a 195-mL volume since 10 g of carbon occupies 5 mL.

Volhard Method for Basic Dyes (7)

Dissolve a 2-g sample in water and dilute to 200 mL in a volumetric flask. Add 10 g of carbon, stir for 1 min, and test for complete adsorption as

described previously. Repeat the carbon treatment if necessary. Filter through dry paper.

Evaporate a 50-mL aliquot to dryness and then heat to volatilize ammonium chloride. Transfer the residue to a 250-mL flask and determine sodium chloride as described previously.

$$\text{Percent sodium chloride} = \frac{(A)(N)(0.05844)(200)(100)}{(w)(50)}$$

Potentiometric Procedure for Water-Soluble Certified Colors (2,5)

Use a Fisher Scientific Co. Titralyzer or Titrimeter or any similar device designed to automatically dispense titrating solution to a predetermined millivolt setting. Equip the titrator with a Ag billet combination electrode or separate Ag indicating and Ag/AgCl reference electrodes. If it is discolored, clean the Ag electrode tip with levigated alumina (Fisher No. A-542, or equivalent) or scouring powder, then rinse thoroughly with water. Clean other electrodes as recommended by the manufacturer. It is not necessary to coat the Ag billet electrode with AgCl.

Prepare 0.01 *N* NaCl by diluting 5.844 g of NaCl (previously dried for 2 hr at 110°C) to 250 mL with halogen-free water, then diluting 25 mL of this solution to 1 liter with water. Prepare 0.01 *N* $AgNO_3$ by diluting 16.99 g of $AgNO_3$ to 250 mL with halogen-free water, then diluting 25 mL of this solution to 1 liter with water.

Standardize the system as follows: Pipette 10 mL of 0.01 *N* NaCl into the titrator beaker, dilute to 100 mL with water, and add 5 mL of HNO_3 (1 + 99). Insert the electrodes into the solution then manually titrate it with 0.01 *N* $AgNO_3$, adjusting the rate of addition of $AgNO_3$ with the rate of the voltage change so that an accurate plot of mV versus $AgNO_3$ added can be prepared. Graphically determine the end point (inflection point) and from this calculate the normality of the $AgNO_3$. Use the mV reading at this point as the end point when titrating samples. Recalibrate when any major change is made in the system.

Treat sample as follows: Transfer 1.00 g of sample (use less if NaCl content is >5% so that the titration volume is kept below 10 mL) to a 250-mL beaker, add 80 mL of H_2O, then stir into solution, heating if necessary. Quantitatively transfer the solution to a 100-mL volumetric flask,

make to volume with water, then mix well. Pipette 10 mL of this solution into the titrator beaker, dilute to 100 mL with water, then add 5 mL of HNO_3 (1 + 99). Insert the electrodes then titrate the sample automatically to the present end point (determined above) using 0.01 *N* $AgNO_3$.

$$\% \text{ NaCl} = \frac{\text{mL } AgNO_3 \times \text{normality } AgNO_3 \times 5.844}{\text{sample weight in grams}}$$

Titrations may also be done manually, using a pH meter to detect the end point. Use a direct-reading digital-type meter with scale divisions ≤10 mV and a range ≥ ± 700 mV, a magnetic stirrer operated at constant speed, 0.05 *N* $AgNO_3$, and 0.1 *N* NaCl. To prepare the sample for analysis, weight 2.0 g (less if NaCl content is > 7%) into a 250-mL beaker, add 80 mL of H_2O, then stir and heat (if necessary) until dissolved; adjust the volume to about 100 mL. Add 5 mL of HNO_3 (1 + 4) then titrate with $AgNO_3$.

Sodium Chloride in FD&C Yellow No. 5, and FD&C Yellow No. 6 Using a Selective Ion Electrode

Use a pH meter with an expanded scale (Orion Model 801 or similar) and equipped with an Orion Model 90-01 single-junction sleeve-type reference electrode and an Orion 94-17 chloride electrode.

Using the colorant of interest, prepare 5% (w/v) solutions containing 0%, 3%, 6%, and 9% NaCl. Transfer 20 mL of these solutions to 50-mL beakers and, while stirring, determine the chloride activity of each sample in millivolts. Prepare a calibration curve on semilog-arithmic paper of percent NaCl (logarithmic axis) versus millivolts (linear axis). Similarly prepare and measure the potential of the sample solutions and determine their NaCl content from the calibration curve.

SODIUM SULFATE

Titration with Barium Chloride, Acid Dyes (7)

Dissolve a 2-g sample and treat it with carbon as described under Sodium Chloride, Volhard Method for Acid Dyes, p. 281. Place 25 mL of the filtrate obtained in a 125-mL Erlenmeyer flask, and add one drop of a

solution of 0.5% phenolphthalein in 50% ethanol. Make alkaline with 0.05 *N* sodium hydroxide, and then add 0.002 *N* hydrochloric acid until the indicator is decolorized. Add 25 mL of ethanol and about 0.2 g of tetrahydroxyquinone indicator (THQ prepared sulfate indicator, Betz Laboratories, Inc., Trevose, PA). Titrate with 0.03 *N* barium chloride solution to a red end point. Make a blank determination.

$$\text{Percent sodium sulfate} = \frac{(A - B)(N)(0.07102)(195)(100)}{(w)(25)}$$

where A is volume of barium chloride solution required for sample (in mL), B is volume of barium chloride solution required for blank (in mL), N is normality of barium chloride solution, and w is sample weight (in g).

Titration with Barium Chloride, Basic Dyes (7)

Dissolve a 2-g sample and treat it with carbon as described under Sodium Chloride, Volhard Method for Basic Dyes, p. 281. Place 25 mL of the filtrate obtained in a 125-mL Erlenmeyer flask and continue as directed previously for sodium sulfate in acid dyes.

$$\text{Percent sodium sulfate} = \frac{(A - B)(N)(0.07102)(200)(100)}{(w)(25)}$$

General Gravimetric Method (10)

Weigh 5 g of sample, dissolve in 100 mL of warm water, and transfer to a 250-mL volumetric flask. Add 35 g of sulfate-free sodium chloride, stopper the flask, and let it stand for 1 hr. Swirl at frequent intervals. Dilute to volume with saturated sodium chloride solution and filter through dry filter paper. Precipitate the sulfate with barium chloride; filter, dry, and weigh the precipitate.

Turbidimetric Determination in Water-soluble Certifiable Color Additives (6)

Weigh 2 g of sulfate-free color into each of eight 400-mL beakers. Add 0 mL, 2 mL, 5 mL, 10 mL, 20 mL, 30 mL, 40 mL, and 50 mL of 0.1% w/v aqueous Na_2SO_4 stock solution to the eight beakers consecutively.

Add 10 g of activated carbon and 200 mL of water to each beaker and decolorize as described under Sodium Chloride, Volhard Method for Acid Dyes, p. 281.

Transfer the mixtures to 250-mL volumetric flasks, dilute to volume with water, mix thoroughly, and filter through dry paper. Collect filtrates when clear, and taking two 50-mL aliquots of each, add 10 mL of conditioning solution to one and 25 mL of conditioning solution to the other. Add 0.2 g of finely ground barium chloride to each sample, stir vigorously, and then immediately obtain spectra of the samples from 400-460 nm, washing the absorption cell with ethanol and water between samples. Prepare a plot on linear graph paper of sample absorbance at 440 nm against percent Na_2SO_4.

Similarly prepare and determine the absorbance of the test sample. If the sample absorbance at 440 nm is ≥0.16 when a 50-mL aliquot of the clear filtrate is treated with 10 mL of conditioning solution and 0.2 g of barium chloride, repeat the reading on a second 50-mL aliquot of sample to which 25 mL of conditioning solution has been added.

The conditioning solution is prepared by dissolving 120 g of NaCl in 400 mL of water, adding 10mL of concentrated HCl and 500 mL of glycerol, and diluting the mixture to one liter with distilled water.

Potentiometric Titration of Sodium Sulfate in Certifiable Water-soluble Sulfonated Colors (1)

(Note: This Procedure Is Not Applicable to Fluorescein-type Colors). Use a Hiranuma recording autotitrator Model RAT-11 equipped with a WB-11 double-action buret and a C-11 autocycle attachment (Rainin Instrument Co., Inc., 555 Main St., Fort Lee, NJ 07024) and a Coleman 3-571 silver-billet reference electrode and a 3-551 platinum cap indicating electrode, or similar equipment. Prior to use, clean the reference electrode with steel wool and then soak it for at least 4 hr in a solution of 252 mg $K_4Fe(CN_6)\cdot 13H_2O$ and 164 mg of $K_3Fe(CN)_6$ in 200 mL of water. Rinse the coated electrode with distilled water just prior to use. Use a freshly coated electrode each day prepared from coating solution no more than 1 week old. The indicating electrode should be cleaned after 2 days of use by electrolyzing at +22.5 V in HCl–water (1 + 3).

Pipette 10 mL of a 1–2% sample solution (depending on color and Na_2SO_4 content) to a 200-mL tall-form Berzelius beaker. Add 10 mL of

water and 100 mL of ethanol solution (360 mL of H_2O + 5640 mL of 95% EtOH) to the sample and, to ensure an end point, add 5 mL of 0.005 $M Na_2SO_4$. Similarly prepare standards using 10 mL of water in place of color solution.

Using the following control settings, titrate the sample to an end point with 0.01 M $Pb(NO_3)_2$: range, 10 MV/cm; dwell time, 90 sec; interval, 10 sec; delivery, about 0.1 mL; sensitivity, 8; stirrer, 5.

$$\text{Percent } Na_2SO_4 = \frac{\text{mL } Pb(NO_3)_2 \times 0.01 \times 0.142 \times 100}{\text{Sample weight}}$$

SODIUM ACETATE (9)

Prepare silver toluenesulfonate by dissolving silver oxide or carbonate in a solution containing 10% excess *p*-toluenesulfonic acid, evaporating to dryness, and drying at 135°C for 8 hr.

Pretreat *p*-toluenesulfonic acid by drying the monohydrate at 110°C overnight, and then cooling and powdering the material.

To a 500-mL Erlenmeyer flask add 100 mL of water, one drop of *m*-cresol purple indicator, and sufficient 0.1 *N* sodium hydroxide or 0.1 *N* hydrochloric acid to make the solution just yellow. Assemble the apparatus shown in Fig. 9.1 and place the Erlenmeyer flask under the condenser. Place 30 mL of absolute ethanol in the distillation flask, then add through a powder funnel 5 g of sample, 1 g of silver toluenesulfonate, and 5 g of *p*-toluenesulfonic acid. Wash the funnel and neck of the flask with 25 mL of absolute ethanol; add three or four boiling stones, shake the flask to mix the contents, and attach it to the condenser.

Immerse the distillation flask in a beaker of hot water and boil the water. Collect about 25 mL of distillate, remove the heat, and slowly add 25 mL of absolute ethanol to the distillation flask. Replace the heat source and collect an additional 25 mL of distillate. Make a third addition of absolute ethanol and distill as before. Finally, boil until the distillation rate is slow (ca. 30 min total distillation time from the beginning of the first distillation).

Wash down the condenser into the receiver with 50 mL of water. Add 50 mL of standardized 0.1 *N* sodium hydroxide and three or four boiling stones. Connect to a reflux condenser fitted with an absorption tube

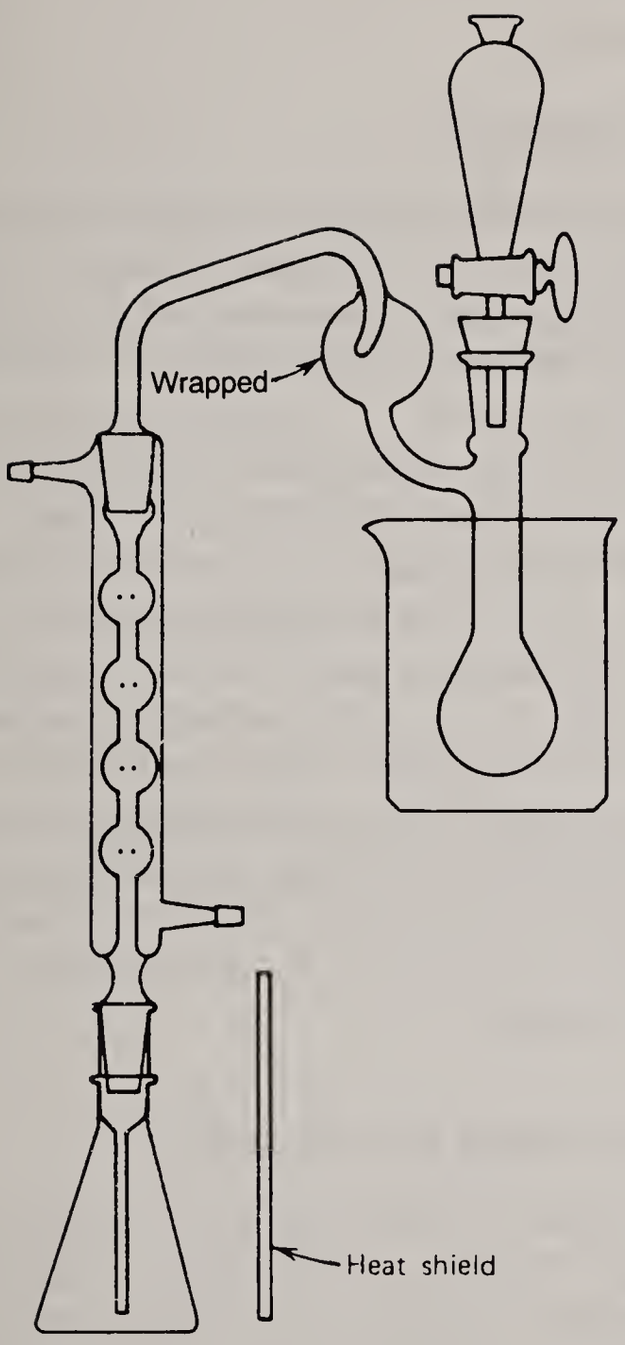

Figure 9.1 Apparatus used in sodium acetate determination. (Reprinted with the permission of the Association of Official Analytical Chemists.)

containing Ascarite; reflux for 10 min. Cool to room temperature, add a few drops of *m*-cresol purple indicator, and titrate with 0.1 *N* hydrochloric acid to a yellow-green end point.

Determine a blank by duplicating the same procedure without a sample.

Calculate the NaOAc content from the net volume of standard NaOH required. One milliliter of 0.1 *N* NaOH is equal to 0.0082 g of $C_2H_3O_2Na$.

SODIUM HALIDES

In Fluorescein Colors (7)

Place 5 g of sample in a 400-mL beaker and add about 150 mL of distilled water. If the sample is a color acid, add sufficient 10% NaOH to effect solution. Heat the mixture nearly to boiling and then add 5 mL of H_3PO_4. Digest the solution until the precipitate formed is well coagulated. Cool to room temperature, transfer to a 250-mL volumetric flask, dilute to volume with distilled water, and mix well. Filter through dry fluted paper.

Transfer a 100-mL aliquot of the filtrate to a 500-mL tall-form beaker, add 2.5 mL of 30% NaOH, and then determine iodide as described on p. 260 beginning with "25 mL of 7% aqueous $KMnO_4$ . . . ."

Take a second 100-mL aliquot and determine bromide as described on p. 261 beginning with "3 mL of 5% potassium cyanide . . . ."

Place a third 100-mL portion of filtrate in a 400-mL beaker, heat to boiling, and add enough 10% $AgNO_3$ solution to precipitate halides. Digest the solution until the precipitate is well coagulated, cool, and transfer to a weighed Gooch crucible. Wash with water and alcohol, dry at 135°C, cool, and weigh as NaCl. Correct the weight for any NaBr or NaI present in the sample.

Sodium Iodide in FD&C Red No. 3 (8)

Prepare a mobile phase by dissolving 500 g of ammonium sulfate in water and diluting to 2 liters, and then mixing the solution with 200 mL of SDH No. 30 alcohol. Weigh 0.5 g of sample, transfer it to a 150-mL beaker, and dissolve it in 15 mL of water and 10 mL of alcohol. Wash 10 g of Whatman Column Chromedia CF11 with mobile phase. (Some lots of adsorbent contain an impurity with a spectrum similar to that of sodium iodide, which must be removed.) Add the washed adsorbent and 15 mL of mobile phase to the sample and mix. Then add 50 g of ammonium sulfate and mix well. Using 25 mL of mobile phase, transfer the sample to a chromatographic column prepared and eluted as described on p. 331. Record the UV spectra of the fractions as eluted. Usually sodium iodide cannot be detected in the first two fractions and is completely eluted in the first eight fractions. It is characterized by an absorption maximum near 222 nm.

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10

METALS

For the most part, the trace metals present in color additives are there as a result of the equipment and the raw materials used in their manufacture. Their concentrations are routinely monitored to ensure both product consistency and safety. In recent years, the list of metals tested for has grown with the increased awareness of the health hazards associated with some of them, and with improvements in analytical technology that have made their determination practical. Lately, there has also been a trend toward analyses for specific elements rather than groups of them such as "heavy metals."

The battery of methods in use today for determining metals ranges from the classical to the ultramodern and includes such procedures as the Gutzeit technique for arsenic, atomic absorption spectroscopy (AAS) for chromium, copper, lead, and zinc, and X-ray fluorescence for a host of elements. In general, increased emphasis has been placed on the use of modern, automated techniques, particularly those that can determine several metals simultaneously.

Many of the newer instrumental methods have proved quite useful. Unfortunately, some, such as flame AAS, have not been the panacea they were predicted to be, since matrix interferences frequently necessitate the wet ashing of samples prior to their analyses, a step AAS was once thought capable of obviating. Flameless AAS methods, such as that for mercury, methods dependent on the generation of volatile hydrides like those for arsenic and selenium, graphite furnace methods for tin, lead, copper, etc., and inductively coupled argon plasma emission spectroscopy (ICP) should all be useful for the analysis of color additives. However, few examples of the use of these techniques for this purpose have been published to date. The growing need, though, for an ever increasing number of fast, accurate, and precise analyses for more and more metals leaves little doubt that these tools or similar ones will eventually replace most of the existing methods.

ARSENIC

Reaction with Silver Diethyldithiocarbamate (6)

Reagents:

(a) Standard arsenic solution—Dissolve 0.132 g of dry, powdered As_2O_3 in 5 mL of NaOH solution (1:5). Neutralize the solution with 10% (w/v) H_2SO_4, add 10 mL of excess, and dilute to 1000 mL with recently boiled H_2O. Pipette 10 mL of this solution into a 1000-mL volumetric flask, add 10 mL of 10% (w/v) H_2SO_4, and dilute to volume with recently boiled H_2O. Use within 3 days.

(b) Silver diethyldithiocarbamate solution—Dissolve 1 g of $(C_2H_5)_2$-NCSSAg in 200 mL of reagent-grade pyridine. Store in a light-resistant container and use within 1 month.

(c) Stannous chloride solution—Dissolve 40 g of reagent-grade $SnCl_2 \cdot 2H_2O$ in 100 mL of HCl. Store in glass and use within 3 months.

(d) Lead-acetate-impregnated cotton—Soak cotton in a saturated solution of reagent-grade lead acetate, squeeze out the excess solution, and dry in a vacuum at room temperature.

CAUTION—Some substances may react unexpectedly with explosive violence when digested with H_2O_2. Appropriate safety precautions must

be employed at all times. If halogen-containing compounds are present, use a lower temperature while heating the sample with H_2SO_4; do not boil the mixture, and add the peroxide with caution before charring begins, to prevent loss of trivalent arsenic.

Transfer 1 g of sample into the generator flask, add 5 mL of H_2SO_4 and a few glass beads, and digest on a hot plate until charring begins. (Additional H_2SO_4 may be necessary to completely wet some samples, but the total volume added should not exceed 10 mL.) After the sample has been initially decomposed by the acid, cautiously add 30% H_2O_2 dropwise, allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted material from caking on the walls or the bottom of the flask during digestion. Maintain oxidizing conditions at all times during the digestion by adding small quantities of peroxide whenever the mixture turns brown or darkens. Continue digestion until the organic matter is destroyed, fumes of H_2SO_4 are evolved, and the solution becomes colorless. Cool, cautiously add 10 mL of H_2O, evaporate to strong fuming, and cool again. Add 10 mL of H_2O, wash the sides of the flask with a few mL of H_2O, and dilute to 35 mL ± 2 mL.

Add 20 mL of dilute H_2SO_4 (1:5), 2 mL of potassium iodide solution (15:100), and 0.5 mL of stannous chloride solution; mix. Allow the mixture to stand for 30 min at room temperature. Pack the scrubber tube (Fig. 10.1c) with two plugs of lead-acetate-impregnated cotton, leaving a small air space between the plugs, lubricate joints (*b*) and (*d*) with stopcock grease, and connect the scrubber unit with the absorber tube (*e*). Transfer 3 mL of silver diethyldithiocarbamate solution to the absorber tube, add 3 g of granular Zn (20-mesh) to the mixture in the flask, and immediately insert the standard-taper joint in the flask. Allow the evolution of hydrogen and color development to proceed at 25°C ± 3° for 45 min, swirling the flask gently at 10-min intervals. Transfer the diethyldithiocarbamate solution to a 1-cm absorption cell and determine its absorbance at 525 nm, using silver diethyldithiocarbamate as the blank. The absorbance due to any red color from the sample solution should not exceed that produced by 3 mL of standard arsenic solution (3 μg of As) when treated in the same manner and under the same conditions as the

Figure 10.1 Apparatus for arsenic test. (courtesy Fisher Scientific Co)

sample. The room temperature during the generation of arsine from the standard should be held to within ±2° of that observed during the determination of the sample.

Interferences. Metals and salts of metals such as chromium, cobalt, copper, mercury, molybdenum, nickel, palladium, and silver are said to interfere with the evolution of arsine. Antimony, which forms stibine, is the only metal likely to produce a positive interference in the color development with the silver diethyldithiocarbamate. Stibine forms a red color that has a maximum absorbance at 510 nm, but at 525 nm the absorbance of the antimony complex is so diminished that the results of the determination would not be altered significantly.

Iodimetric Procedure (8)

Reagents:

(a) Potassium iodide solution—Dissolve and dilute 15 g of recrystallized KI to 100 mL with H_2O.
(b) Stannous chloride—Dissolve 40 g of $SnCl_2 \cdot 2H_2O$ in 100 mL of concentrated HCl.
(c) Absorbing solution—Dissolve and dilute 3.2 g of $HgCl_2$ (recrystallized from H_2O) and 0.1 g of powdered USP gum arabic to 200 mL with H_2O.
(d) Lead acetate solution—Dissolve 10 g of $Pb(C_2H_3O_2)_2$ in 80 mL of H_2O. Make just acid to litmus paper with acetic acid. Dilute to 100 mL with H_2O.
(e) Standard iodine solutions—Dissolve 6.35 g of I_2 and 12.7 g of KI in a little H_2O; filter; dilute the filtrate to 1 liter. Dilute 100:1000, 20:1000, and 10:1000 with H_2O. Add 25 g of KI to each liter of dilute I_2 solution.
(f) Standard arsenic solutions—Dissolve 1 g of As_2O_3 in 25 mL of 20% aqueous NaOH. Saturate the solution with CO_2 and dilute to 1 liter with freshly boiled H_2O. Dilute 100:1000, 20:1000, and 10:1000 with H_2O.
(g) Starch indicator—Make 1 g of soluble starch into a thin paste with cold H_2O. Pour into 200 mL of hot H_2O, and while still hot add two or three small crystals of HgI_2.
(h) Buffer solution—Dissolve and dilute 10 g of $Na_2HPO_4 \cdot 12H_2O$ to 100 mL with H_2O.

Assemble the apparatus shown in Figs. 10.2 and 10.3. Soak glass wool in 10% $Pb(C_2H_3O_2)_2$ solution, thoroughly dry, and insert loosely in the trap above the condenser. All connections between the condenser and the absorber should be dry. All glassware should be washed with dichromate cleaning solution and rinsed with distilled H_2O prior to use.

CAUTION—Perchloric acid is a strong oxidant. Contact with organic material may cause fire or explosion. See MCA Chemical Safety Sheet SD-11.

Weigh 10 g of sample into a 800-mL Kjeldahl digestion flask, add 10 mL of concentrated H_2SO_4 and 10 mL of concentrated HNO_3. Digest over a low flame until the mass begins to clear. Add 15 mL of H_2O and digest until clear. Add successive 5-mL portions of concentrated HNO_3 until all the organic matter is in solution. Slowly add 10 mL of a (1 + 1) mixture of concentrated HNO_3 and 60–70% $HClO_4$. Digest until the mixture in the flask is only lightly colored. Add an additional 5 mL of the $HClO_4$mixture. Heat until the initial vigorous reaction subsides. Continue the addition of 5-mL portions of $HClO_4$ mix until the digest is water-white. Heat to SO_3 fumes. Cool. Slowly add 20 mL of saturated ammonium oxalate solution and heat to SO_3 fumes. Cool and add 50 mL of H_2O. Swirl and transfer to the arsine generator (Fig. 10.2) using three 20-mL portions of H_2O. Add 5 mL of solution (a) (preceding list), 1 mL of solution (b), and dilute to 90–100 mL with H_2O. Place one mL of solution (c) in each receiving tube and place in the operating position. Add 5 g of 20–30 mesh As-free Zn to the generator. Wash down the flask neck with a few milliliters of H_2O and connect at point (*a*) (Fig. 10.2).

Heat the generator to reflux. Lower the flame and continue heating for 12–15 min, then disconnect the first receiver and the delivery tube at (*c*) (Fig. 10.3). Raise the delivery tube at (*d*) (Fig. 10.3) and add 2 mL of buffer solution (*h*) through the tube. Wash down the outside with 3–5 mL of H_2O. Place the transfer tube in position at (*d*) and suck the contents of the second receiver into the first receiver. Wash the outside of the second delivery tube with two 1-mL portions of H_2O and suck the washings into the first receiver. Remove the second receiver (*e*) and the transfer tube.

In an empty receiver tube place 2 mL of solution (*h*) and 2 mL of H_2O. Add 3 mL of solution (*e*), five drops of solution (*g*), and titrate to a colorless end point with solution (*f*). Agitate the solution during the titration by alternately sucking and blowing through the stirring tube.

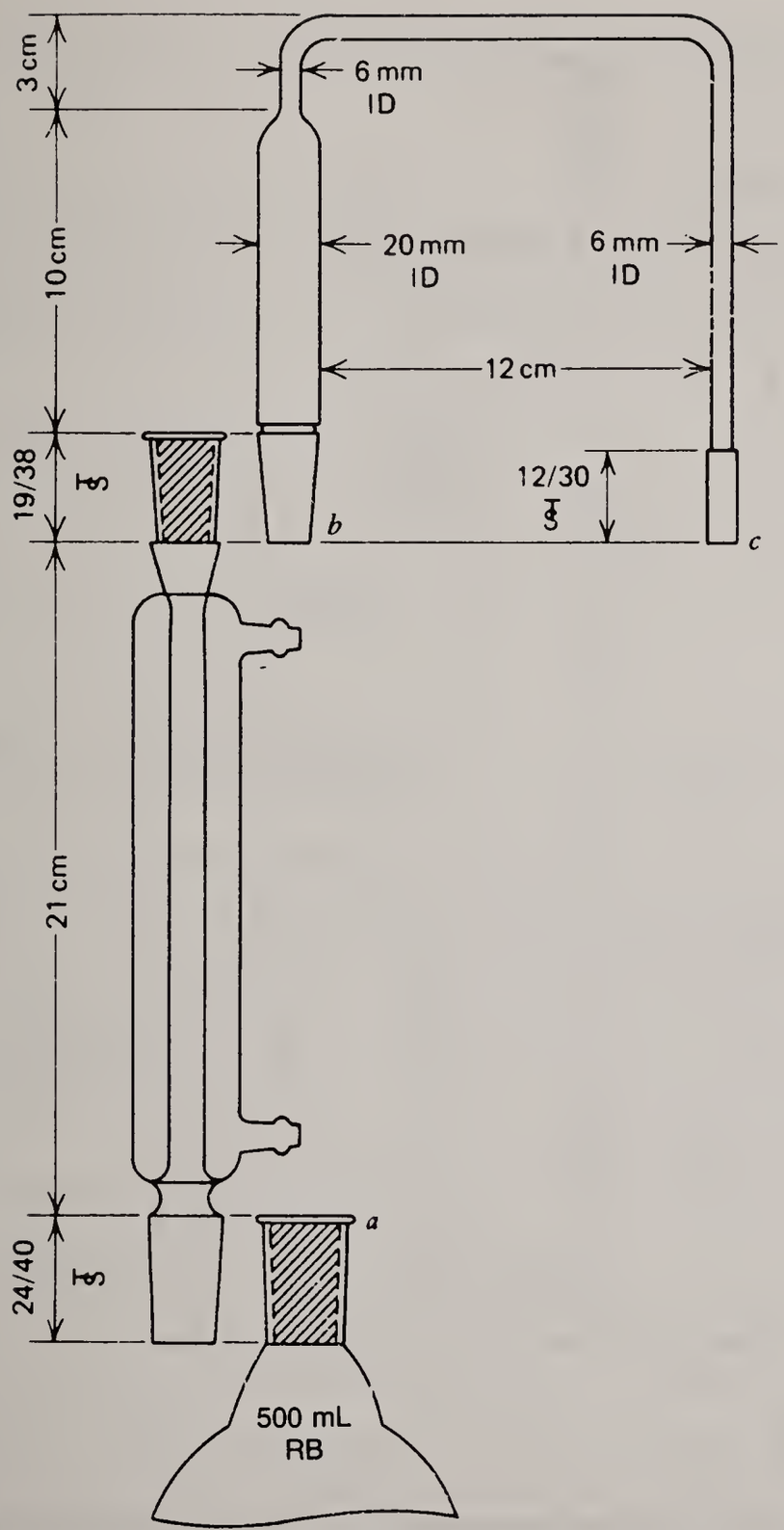


Figure 10.2 Arsine generator and distillation section: when arsenic apparatus is in operating position, absorption section is connected to distilling section at position C. (Reprinted with permission of the Association of Official Analytical Chemists.)

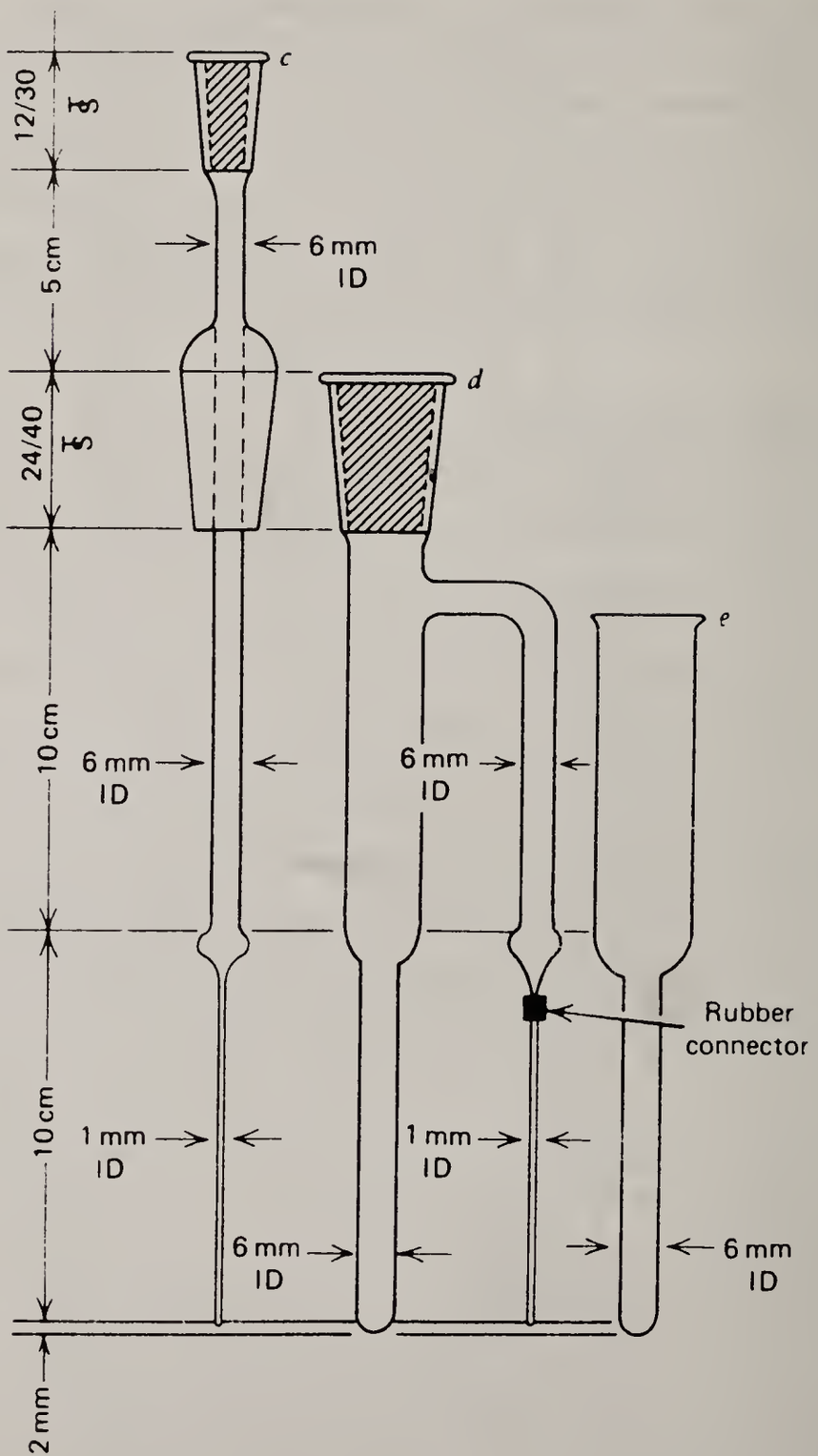


Figure 10.3 Arsine absorber and titrating tubes. (Reprinted with the permission of the Association of Official Analytical Chemists.)

$$\frac{\text{mmg } As_2O_3}{\text{mL } I_2} = \frac{\text{mmg } As_2O_3/\text{mL} \times \text{mL } As_2O_3 \text{ soln.}}{\text{mL } I_2 \text{ soln.} \times 4}$$

Place the stirring tube in the first receiver containing the sample. Add solution (e) slowly until the orange precipitate that initially forms just disappears. Add 5 drops of solution (*g*) and titrate with solution (*f*) to the disappearance of the blue color. Run a blank on all reagents.

$$\text{Percent } As_2O_3 = \frac{\text{mL } I_2 \text{ soln.} \times I_2\text{factor} - \left[\text{mL } \frac{As_2O_3}{4} \times \frac{\text{mmg } As_2O_3}{\text{mL}} \right] - \text{blank}}{\text{sample weight}}$$

CHROMIUM IN FD&C BLUE NO. 1

Weigh 5 g of sample into a platinum dish. Mix in 8 g of a fusion mix consisting of equal portions of sodium carbonate and potassium carbonate. Slowly fuse the sample over a flame, until all organic material is destroyed. Place the sample in a 800–850°C muffle furnace until it is completely ashed (ca. 2 hr). Remove the dish from the furnace, allow it to cool, and wash the contents into a 150-mL beaker with 50 mL of water. Add 25 mL of 6% hydrogen peroxide solution, stir, and boil for 10 min. Cool and then filter through Whatman No. 1 filter paper into a 100-mL volumetric flask. Rinse the beaker and filter paper into the flask and then dilute to volume. Using a 5-cm cell, determine the absorption spectrum from 450 nm to 310 nm by plotting against water. Construct a baseline connecting the absorption minima near 320 nm and 450 nm. Draw a line that is parallel to the absorbance axis and passes through the spectrum and the baseline at 370 nm. The absorbance at the intersection of this line with the sample spectrum equals A_1. The absorbance at the intersection of this line with the baseline equals A_2:

$$\text{Parts per million chromium} = (A_1 - A_2)(50.4)$$

where 50.4 is the reciprocal of the slope of a least-squares fit of the regression of A at 370 nm on ppm of chromium determined by this method.

HEAVY METALS AS LEAD (18)

Reagents:

(a) Standard $Pb(NO_3)_2$ solution—Dissolve 0.1598 g of $Pb(NO_3)_2$ in 1% (v/v) aqueous HNO_3. Dilute with H_2O to 1000 mL. Dilute 10 mL to 100 mL with H_2O. (Dilute solution must be freshly prepared.)

Weigh 1 g of sample into a porcelain crucible and ignite at low temperature until thoroughly charred. Add 2 mL of HNO_3 and five drops of H_2SO_4 to the crucible and carefully heat to SO_3 fumes. Ignite at 500-600°C to remove carbon. Cool, add 2 mL of HCl, and evaporate to dryness on a steam bath. Moisten the residue with one drop of HCl, add 10 mL of hot H_2O, and digest for 2 min. Add 6% NH_4OH dropwise until the solution is just alkaline to litmus paper. Add dilute acetic acid until the solution is slightly acid to litmus paper, and then add 2 mL excess. Filter (if necessary) into a 50-mL Nessler tube. Wash the filter and crucible into the tube with 10 mL of H_2O and dilute to 25 mL with H_2O.

Pipette 2 mL of dilute acetic acid into a second 50-mL Nessler tube. Add a volume of solution (a) containing the amount of Pb to be tested for. Add H_2O to 25 mL.

Add 10 mL of saturated H_2S solution to each tube. Mix and allow to stand 10 min. Compare the sample and standard visually. Report Pb concentration as more than or less than that of the standard.

LEAD

In Colors That Do Not Contain Calcium, Strontium, or Barium (5)

Prepare a 10% hydroxylamine hydrochloride solution by dissolving 10 g of $NH_2OH{\cdot}HCl$ in 20 mL of water and slightly alkalizing with ammonium hydroxide. Extract any lead with dithizone (diphenylthiocarbazone). Remove excess dithizone with chloroform and boil off any chloroform remaining in the aqueous phase. Acidify with hydrochloric acid and dilute to 100 mL.

Prepare a stripping reagent by adding 10 mL of glacial acetic acid to 20 mL of saturated sodium acetate solution and diluting to 100 mL.

Prepare a 0.5% starch solution by pasting 1 g of soluble starch with several mL of cold water, pouring into 200 mL of hot water, and while still hot, adding two or three small crystals of mercuric iodide as a preservative.

Prepare a standardized sodium thiosulfate solution by adding, to a 0.1 *N* sodium thiosulfate solution, 5 mL of isoamyl alcohol per liter as a preservative. On the day the analysis is performed, dilute 1:100 or 1:20 with carbon dioxide-free water (depending on the lead concentration). Standardize against a lead solution prepared by dissolving 0.3197 of lead nitrate in 100 mL of 1% nitric acid.

Transfer 5 g of sample to a 500-mL Kjeldahl flask. Add 10 mL of concentrated sulfuric acid and 10 mL of concentrated nitric acid and heat to sulfur trioxide fumes. Add 5 mL of concentrated nitric acid and again heat to sulfur trioxide fumes. Repeat the addition of nitric acid each time sulfur trioxide fumes appear, until the dye is in solution and the digest is yellow. Then add 10 mL of a 1:1 mixture of concentrated nitric acid and 60–70% perchloric acid, and continue heating until the digest is colorless or pale yellow and the bulk of the sulfuric acid is evaporated.

Cool the flask and neutralize with ammonium hydroxide. Add 20 mL of 50% citric acid solution and adjust to pH = 8.5–9 with ammonium hydroxide using thymol blue indicator. Add 5 mL of 10% potassium cyanide solution and transfer the sample to a separatory funnel. Extract the lead with 20 mL of a 0.002% solution of dithizone in chloroform. (If there is enough iron present to cause excessive oxidation of the dithizone, as indicated by a yellow color in the chloroform layer, add 10 mL of the 10% hydroxylamine hydrochloride solution to reduce the iron.) Let the chloroform layer settle; drain it into a second funnel. Repeat the extraction until the red lead dithizonate is completely removed.

Wash the combined chloroform extracts with 25 mL of water containing one drop of ammonium hydroxide. Drain the washed chloroform layer into a third funnel. Add 110 mL of 1:99 nitric acid and shake for 1 min. Drain and discard the chloroform layer and about 1 mL of the acid layer. Drain the acid layer through cotton, discarding the first 3 mL. Electrolyze a 100-mL aliquot as follows: Heat a platinum gauze cylindrical anode to red heat in the oxidizing flame of a burner. Cool the anode and place it in the sample solution. Start the electrode rotating, heat the sample solution to 60–70°C, and add 0.1 g of potassium dichromate.

Electrolyze at 70-80°C, using 100 mA. Remove the heat and siphon the sample from the beaker while playing a stream of water directly on the anode; keep the level of liquid above the deposit at all times. The acid is entirely removed when the current falls to zero.

Dissolve the deposit from the anode with 4–5 mL of stripping reagent and 1 mL of freshly prepared 2% potassium iodide solution, contained in a flat-bottom vessel of such size that the solution just covers the anode. Add a few drops of the starch solution and titrate the liberated iodine with 0.001 *N* sodium thiosulfate solution using the anode as a stirrer. Use a 0.005 *N* thiosulfate solution if the lead content is 1–5 mg. Similarly determine a blank.

In Aluminum Lakes (9)

Weigh 2 g of sample into a 500-mL Kjeldahl digestion flask, add 10 mL of concentrated H_2SO_4 and 10 mL of concentrated HNO_3, and digest on a low flame until SO_3 fumes appear. Add successive 5-mL portions of concentrated HNO_3 (waiting until SO_3 fumes appear before adding each succeeding portion until all organic matter is in solution. Slowly add 5–10 mL of a (1:1) mixture of concentrated HNO_3 and 60–70% $HClO_4$, and continue the digestion until the white precipitate formed shows the first signs of spattering. Allow the flask to cool and cautiously add 5 mL of H_2O and then a few drops of concentrated NH_4OH. Swirl the flask vigorously and cool under running water. Add 20 mL of 50% (w/v) citric acid solution and adjust the pH to 3–3.4 (Bromophenol Blue) with concentrated NH_4OH. Add 1 mL of $CuSO_4$ solution containing 1 mg of Cu per mL and transfer the solution to the precipitation tube (*b*) of the sulfiding apparatus (Fig. 10.4). Bubble H_2S through the solution at an approximate rate of two bubbles per second for 3–5 min and filter the resulting suspension through (*c*) at an approximate rate of one drop per second. When filtration is complete remove the receiver containing the filtrate and attach a suction test tube. Add 3 mL of hot concentrated HNO_3 through the separatory funnel (*a*) and draw through the filter, followed with 2 mL of hot water. Detach the filter and pass an additional 3 mL of hot concentrated HNO_3 through the filter, wetting all sides. Again follow with 2 mL of hot water. If the filter is still colored with PbS, wash again with hot concentrated HNO_3 and water. Wash the dissolved sulfides into

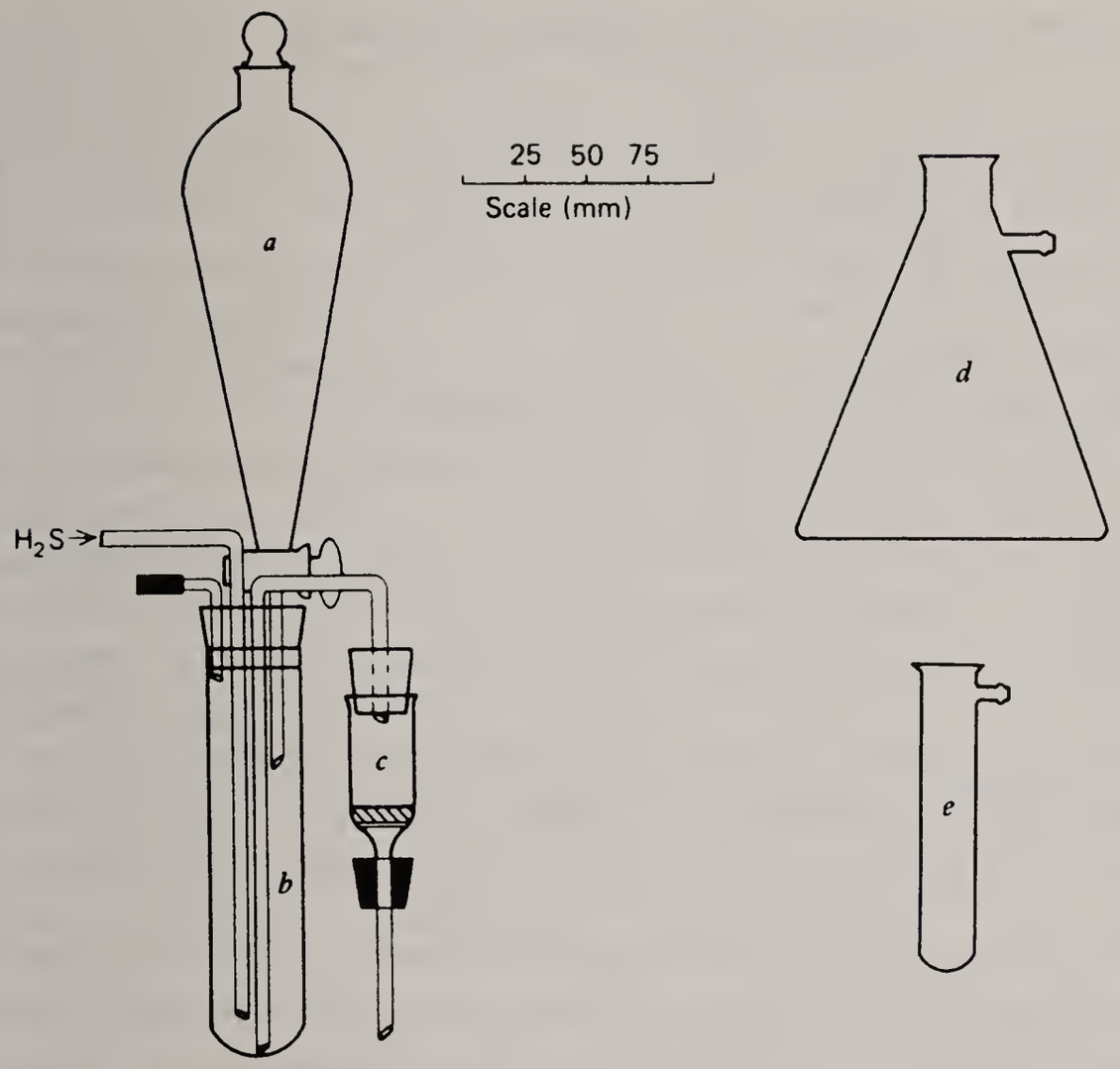


Figure 10.4 Sulfiding apparatus. (Reprinted with the permission of the Association of Official Analytical Chemists.)

the precipitation tube (*b*), wetting all sides to take up any residual lead sulfide and then into a 50–100-mL glass-stoppered conical flask. Stopper and shake for a few seconds and then remove the stopper and boil until the solution clears to remove the last traces of H_2S and to coagulate any free sulfur present.

Transfer the solution to a 250-mL separatory funnel. Wash the flask with two 5-mL portions of distilled water and add the washings to the main solution. Add 10 mL of 50% (w/v) citric acid solution, 5 mL of 10% sodium cyanide solution, and a few drops of hydroxylamine hydrochloride solution to prevent oxidation of the dithizone; adjust the pH to 8.5–9.5 (Thymol Blue) with concentrated NH_4OH.

Extract with dithizone and electrolyze as described above.

In Barium, Calcium, and Strontium Lakes (9)

Place 2 g of lake, 4 g of Na_2CO_3, 6 g of K_2CO_3, and 0.5 g of $NaNO_2$ in a platinum crucible of suitable size. Mix thoroughly. Heat carefully until the color is carbonized, then heat to about 850°C, and hold at that temperature for 15 min. If a controlled muffle furnace is available, it is only necessary to place the fusion mixture in the cold furnace and raise the temperature gradually to 850°C over a 2-hr period. Usually 15–30-min heating at 850°C is sufficient to complete the fusion.

When fusion is complete, allow the crucible and contents to cool below 100°C and then add 2–3 mL of water and heat over a low flame, using care to prevent spattering, until the contents can be separated from the crucible. Transfer the fused mixture to a 150-mL beaker with the aid of about 25 mL of hot water. Boil until the caked material is completely disintegrated, and then filter through a retentive filter paper. Wash the residue on the filter with two 15-mL portions of hot 5% Na_2CO_3 solution. Lead will be in both filtrate and residue. Transfer the filtrate to a separatory funnel and extract the lead from the filtrate as directed under aluminum lakes. Dissolve the residue on the filter in 10–20 mL of 2:5 (v/v) hydrochloric acid, wash the filter with water, and add washings to the solution. Boil the solution to expel carbon dioxide and then transfer to a separatory funnel and extract the lead as directed above. Combine with the chloroform extracts from the soluble portion of the fusion products and determine the total lead by the electrolytic method.

MANGANESE (19)

Dissolve 500 mg of sample in 5 mL of H_2SO_4, then cautiously mix in 40 mL of water, 3 mL of H_3PO_4, and 0.3 g of KIO_3. Heat this mixture for 1 hr, dilute it to 50 mL, then determine its absorbance at 525 nm versus knowns similarly prepared.

MERCURY

Ion Exchange Paper—X-ray Emission Method (12)

Place a 1.5-in. disk of ion-exchange paper (Reeve Angel, Grade SB-2 Amberlite ion resin-loaded papers, anion exchanger, strong base-type

containing Amberlite IRA-400 resin, Cl^- form) in the joint of a two-piece, 0.75-in.-ID chromatographic column, joined by a threaded aluminum coupling and having a Teflon stopcock with fine adjustment control; tighten the joint. Using light suction, draw water up through the paper. Wash the column and paper by passing 50 mL of 0.5 *N* hydrochloric acid through the column at 1 mL/min. Leave several mL of solution in the column.

Dissolve the sample in dilute hydrochloric acid and adjust the acid concentration to 0.5 *N* hydrochloric acid. Filter through fine filter paper previously washed with 0.5 *N* hydrochloric acid and rinsed with water. Dilute the filtrate to 200 mL or more with 0.5 *N* hydrochloric acid. Divide the sample in two; add a known amount (1–5 μg) of mercury to one portion. Carry a reagent blank and a standard, containing a known amount of mercury in 100 mL of 0.5 *N* hydrochloric acid, through the rest of the procedure.

Pass each solution through a column such as that described above at 1 mL/min. Follow the sample with 25 mL of 0.5 *N* hydrochloric acid, also at 1 mL/min. Drain the solution from below the resin paper, remove the paper, and dry at room temperature.

Using a standard solution containing 1.354 μg/mL of mercuric chloride, set up an X-ray emission spectrograph, equipped with a molybdenum tube, lithium fluoride crystal, scintillation counter, and pulse-height analyzer, on the mercury Lα line $2\theta = 36°$. Mount the resin-loaded disc in the instrument holder. Using at least 16,000 counts, determine the counts per second at $2\theta = 35°$, $2\theta = 36°$, and $2\theta = 37.1°$. For best results take an average of four readings, rotating the sample 90° between readings. Calculate as follows:

$$R_{36°/35°} = \frac{\text{counts/sec at } 2\theta = 36°}{\text{counts/sec at } 2\theta = 35°}$$

$$R_{37.1°/35°} = \frac{\text{counts/sec at } 2\theta = 37.1°}{\text{counts/sec at } 2\theta = 35°}$$

For blank, sample, and standards, $R_{37.1°/35°}$ should be equal if tungsten is absent. If tungsten is absent, calculate μg of mercury in the sample aliquot from $R_{36°/35°}$ as follows:

$$\mu\text{g mercury} = \frac{(R_s - R_b)Y}{R_{s+y} - R_s}$$

where R_s is calculated 36°/35° value for the sample aliquot, R_b is calculated 36°/35° value for the blank, R_{s+y} is calculated 36°/35° value for the sample aliquot to which Y μg of mercury has been added, and Y is μg of mercury added to the aliquot.

Photometric Mercury Vapor Method (21, 22)

Use the apparatus shown in Fig. 10.5. Preheat the furnace to 650°C and adjust the nitrogen flow to 1 L/min. Standardize the mercury vapor meter following the manufacturer's instructions. Adjust the attenuator so that the recorder scale is 200 mV.

Calibrate the meter by placing aliquots of mercuric chloride solution containing 0.01–0.03 μg of mercury on separate pieces of ignited asbestos (CAUTION!) in individual combustion boats. Cover the asbestos with 1–2g of anhydrous sodium carbonate. Place the boats one at a time in the tube furnace and close the inlet. After 1 min start the nitrogen flow. Prepare a plot of response versus amount of mercury.

Treat 0.025 g of organic sample or 0.25 g of inorganic sample in the same way. Iodine interferes with the determination.

Colorimetric Method (16)

Use NF-grade chloroform throughout this procedure. Prepare a 20% hydroxylamine hydrochloride solution. Remove heavy metals by shaking with 50-mL portions of 100 mg/L dithizone in chloroform. Wash with several portions of chloroform to remove excess dithizone.

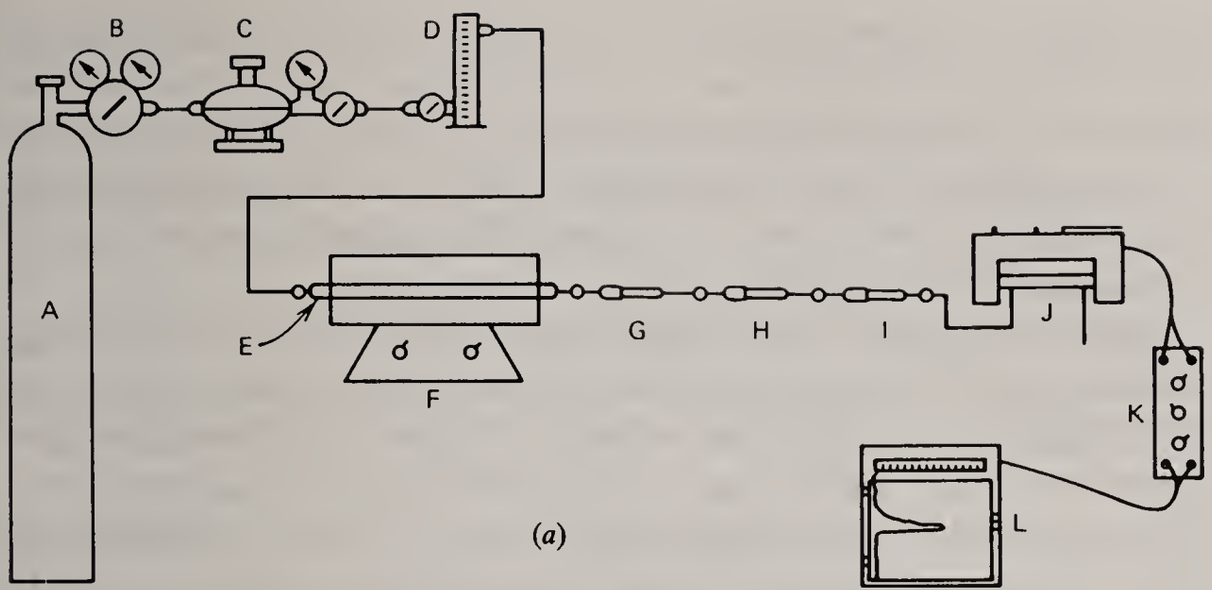
Prepare a 40% potassium bromide solution and remove heavy metals as described in the preceding paragraph. Make alkaline with one or two drops of 10% sodium hydroxide before storing.

Prepare a 50% ammonium acetate solution and remove heavy metals as described above.

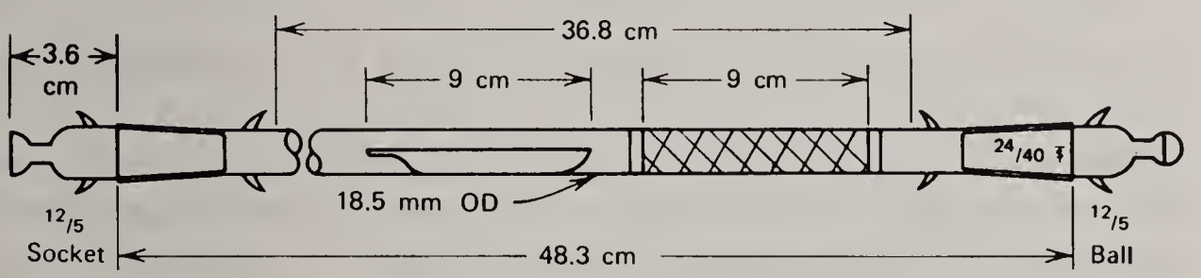
Prepare a mercury standard solution, using mercuric oxide, and containing 5 mg/L of mercury.

Prepare a dithizone solution containing 6 mg/L of dithizone in chloroform, and use this throughout the portion of the procedure that follows.

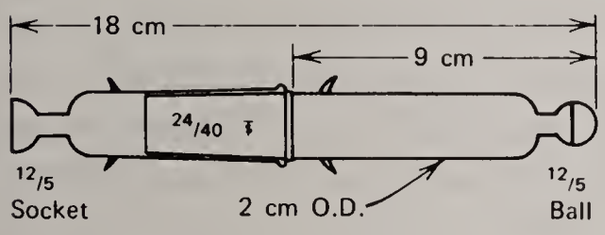
Weigh a 1-g sample and transfer it to a two-neck, 500-mL digestion flask fitted with a Freidrichs condenser and a 50-mL dropping funnel. (If



(a)



(b)



(c)

Figure 10.5 (a) Schematic diagram of apparatus for photometric mercury vapor method:

A. Tank of nitrogen
B. Two-stage pressure regulator
C. Low-pressure regulator
D. Flowmeter
E. Combustion tube
F. Combustion-tube furnace
G. Dehydrite trap
H. Ascarite trap
I. Aluminum oxide trap
J. Mercury vapor meter
K. Attenuator
L. Recorder

(b) quartz combustion tube with boat and copper oxide packing; (c) schematic diagram of trap used to contain ascarite, dehydrite, and aluminum oxide, (Reprinted with the permission of the Association of Official Analytical Chemists.)

the sample is a triphenylmethane or oil-soluble dye, use a 0.5-g sample.) Add 10 mL of 1:1:1 sulfuric acid–nitric acid–water and allow to stand for about 5 min. Heat gently for about 5 min, add 2 mL of 70% perchloric acid, and reflux for 2 hr (3 hr for triphenylmethane or oil-soluble dyes; 1 hr for lakes having a low dye content). Concurrently, run two blanks containing all the reagents.

Allow the solution to cool. Wash the condenser and the funnel, using sufficient water to bring the volume to about 100 mL. Add 10 mL of the 20% $NH_2OH \cdot HCl$ solution and reflux for 10 min. Cool. Wash the condenser and funnel with 30 mL of water and transfer the sample to a 250-mL separatory funnel, filtering if necessary. Dilute to 200 mL. Add 20 mL of the mercury standard solution to one blank; this is the standard. The second blank is the reagent blank.

Add 10 mL of the dithizone solution and shake vigorously for 1 min. Allow the chloroform layer to settle and transfer it to a second 250-mL funnel containing 50 mL of 0.25 *N* hydrochloric acid. Pass 5 mL of chloroform through the first funnel and add it to the second one. Repeat the extraction with 10-mL portions of dithizone solution until the green color of the dithizone remains unchanged. Wash the contents of the first funnel with 10 mL of chloroform and add the wash to the dithizone extracts.

Shake the second funnel vigorously for 1 min. Allow the layers to settle and transfer the chloroform layer to a third 250-mL funnel containing 50 mL of 0.25 *N* hydrochloric acid and 5 mL of 40% potassium bromide. Wash the contents of the second funnel with 10 mL of chloroform and add the wash to the third funnel.

Shake the third funnel vigorously for 1 min. Drain and discard the chloroform phase. Wash the aqueous phase with 10-mL portions of chloroform until the chloroform and aqueous phases are colorless. Discard the chloroform layer. Add 10 mL of chloroform and 20 mL of 50% ammonium acetate solution. Shake for 10 sec. Remove the funnel stopper and allow the chloroform film on the surface to evaporate. Drain the chloroform layer.

Add 10 mL of the dithizone solution; shake for 1 min. Drain the chloroform layer through absorbent cotton, discarding the first milliliter. Within 1 hr, determine the absorbances of the filtered chloroform solutions of the sample, the blank, and the standard at 490 nm.

SELENIUM (4)

Decompose 2–5 g of sample with a mixture of hot H_2SO_4, HNO_3, and $HClO_4$, cool the solution, dilute with water, heat to remove oxides of nitrogen, cool, and then make to volume with water. Treat an aliquot of this solution with hydroxylammonium chloride, formic acid, and citric acid, adjust to pH 2 with dilute aqueous NH_3, then treat with 0.5% aqueous 3,3′-diaminobenzidine hydrochloride* at 43°C (in diffuse light). Adjust the solution to pH 7, extract with $CHCl_3$, then measure the extract's absorbance at the absorption maximum near 420 nm versus a standard. For identification purposes, confirm that the extract has an absorption minimum near 372 nm and a second maximum near 340 nm.

THALLIUM (10)

Dissolve 5 g of sample in 70 mL of 3 *M* HBr and then extract the solution with three 15-mL portions of ethyl ether. (If the sample is water-insoluble, first digest it with a mixture of H_2SO_4 and HNO_3.) Combine the ether extracts, evaporate the composite to dryness in a current of warm air, dissolve the residue in 2 mL of aqueous Br, and then boil the solution to evaporate excess Br. Dilute the solution with 20 mL of water, add 0.5 mL of a 0.01% solution of methyl violet, and then extract the $TlBr_4^-$–methyl violet complex into 5 mL of isoamyl acetate. Filter the organic phase into a 1-cm absorption cell and determine its absorption at 580 nm versus a reagent blank.

URANIUM (11)

Ash 10 g of sample in a muffle at 550°C, dissolve the ash in 15 mL of 8 *N* HCl, treat this solution with 3% aq. H_2O_2, then filter it. Heat the filtrate to 50°C, then, while stirring, add an excess of 4 *N* NH_3. Filter the sample, wash the $(NH_4)_2U_2O_7$ precipitate $SO^{=}_4$ free, then dissolve it in 6 *N* HCl. Extract this solution twice with tributyl phosphate. Combine the

*CAUTION—This compound is a suspected carcinogen and should be handled with care.

organic layers, wash them with 6 *N* HCl to remove residual Fe, then dilute them with benzene. Extract the benzene solution with water. Evaporate the aqueous extract to dryness, dissolve the residue in 6 *N* HCl, then chromatograph a portion of the solution on an activated 0.25-mm Kieselgel SHR plate using H_2O-saturated ethyl ether–ethyl acetate–tributyl phosphate (25:25:1) as the mobile phase. Air dry the chromatogram, then spray it with ethanolic 0.25% 1-(2-pyridylazo)-2-naphthol. A blue-violet spot (R_f about 0.7) indicates uranium. As little as 0.1 ppm in a 10-g sample can be detected.

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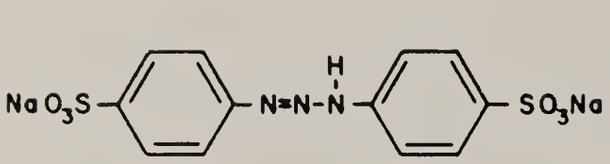
11

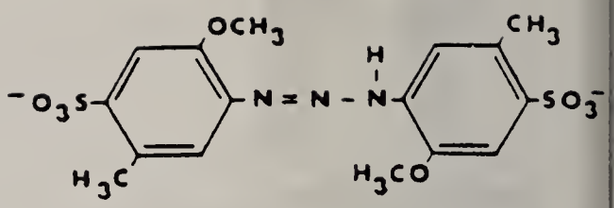
ORGANIC IMPURITIES

Several kinds of organic impurities can be present in color additives. If the colorants are factory made, they can contain traces of the reagents or "intermediates" from which they were synthesized, impurities originally present in these reagents that survive the process unchanged, isomeric colorants, subsidiary colorants, decomposition products, products formed by side reactions, and chance contaminants. Natural colorants can contain analogous impurities, and possibly pesticides and herbicides, depending on the particular colorant, its origin, its method of isolation, and so on. In general, the kinds of impurities likely can be divided into two groups—colored and colorless. It is impossible to predict all those that can be present in any one colorant, but it is useful to consider the types of contaminants that might be encountered to know what to routinely analyze for when evaluating purity, and what structures to consider when attempting to identify unknowns.

Chance contaminants, of course, are just that—impurities introduced by operator error, the use of dirty equipment, mislabeled reagents, etc.; impurities not actually a consequence of the chemistry of the process, and whose presence are not easy to predict.



DAADBSA



DMMA

Figure 11.1

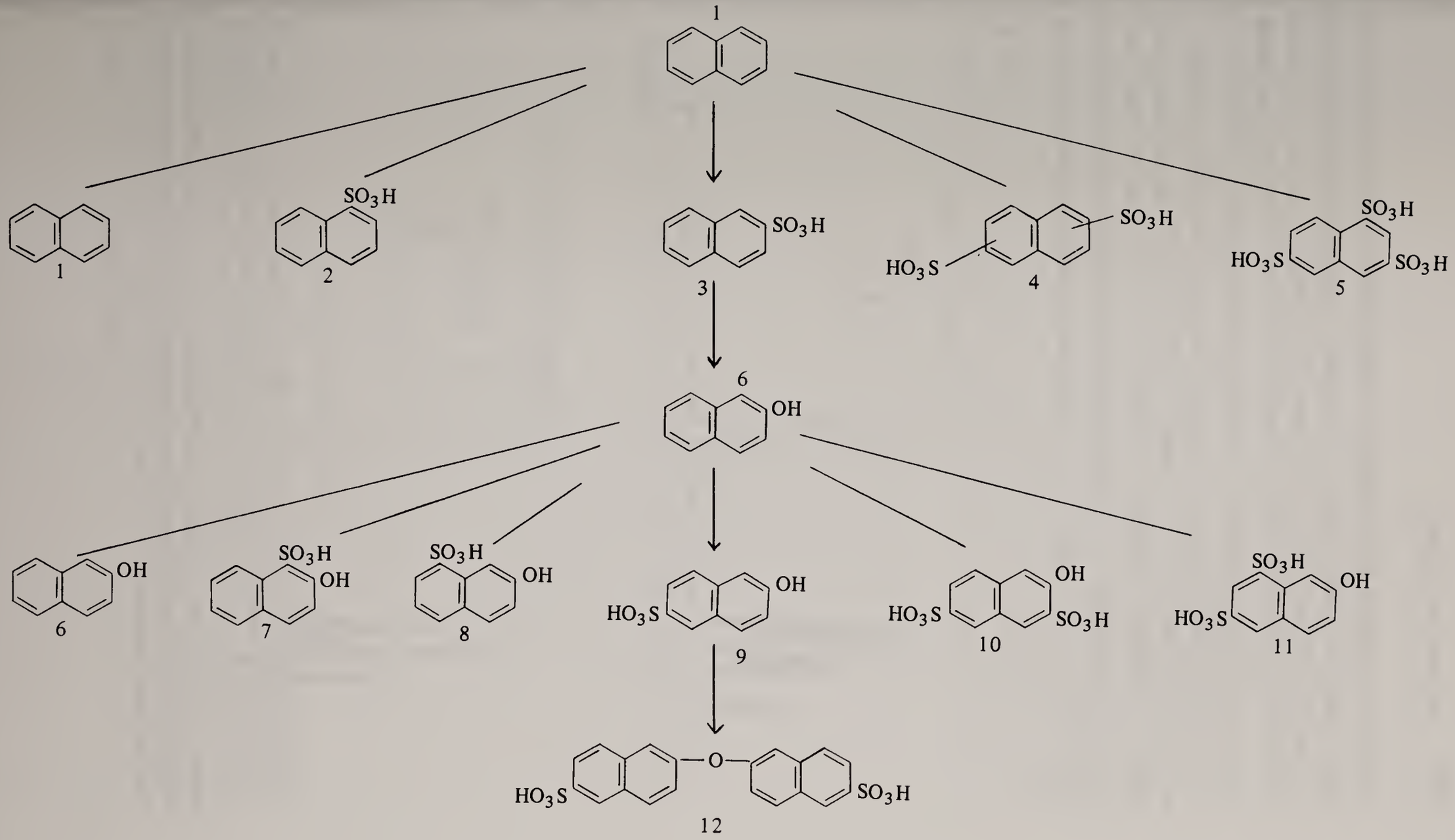
Also difficult to predict is the presence of impurities which are the result of certain side reactions. Examples are 4.4′-diazoaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA) often found in FD&C Red No. 40 and 4,4′-(diazoamino)dibenzenesulfonic acid (DAADBSA) frequently detected in FD&C Yellow No. 6. Both are the result of using insufficient amounts of nitrite or acid during the manufacture of the colorants. DMMA is produced when cresidine sulfonic acid (CSA) diazo combines with undiazotized CSA. Similarly, DAADBSA is formed when diazotized sulfanilic acid (SA) reacts with undiazotized SA. (See Fig. 11.1.)

More expected in finished colorants are small amounts of the intermediates used to synthesize them. At least one is found in each final product because of various problems associated with large-scale manufacture which make it virtually impossible to obtain perfect stoichiometric balance in the ingredients used, complete mixing of the reactants during processing, and thorough washing of the isolated product. These are the first compounds to consider when evaluating the quality of a dyestuff.

Other impurities found in color additives are a consequence of the nature and purity of the starting materials. As an example, consider Schaeffer's salt, which is used in the preparation of both FD&C Yellow No. 6 and FD&C Red No. 40. Schaeffer's salt is made by sulfonating

→

Figure 11.2. The preparation of Schaeffer's salt: 1. Naphthalene; 2. Naphthalene-1-sulfonic acid; 3. Naphthalene-2-sulfonic acid; 4. Naphthalenedisulfonic acids; 5. Naphthalene-1,3,6-trisulfonic acid; 6. 2-Naphthol; 7. 2-Naphthol-1-sulfonic acid; 8. 2-Naphthol-8-sulfonic acid (Crocein acid); 9. 2-Naphthol-6-sulfonic acid (Schaeffer's acid); 10. 2-Naphthol-3,6-disulfonic acid (R-acid); 11. 2-Naphthol-6,8-disulfonic acid (G-acid); 12. 6,6′-Oxybis (2-naphthalenesulfonic acid) (DONS).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1
SO3H
2
SO3H
3
HO3S
SO3H
4
HO3S
SO3H
SO3H
5
6
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OH
SO3H
OH
7
SO3H
OH
8
HO3S
OH
9
HO3S
OH
SO3H
10
HO3S
SO3H
OH
11
O
HO3S
SO3H
12

naphthalene, fusing a salt of the resulting sulfonic acid with alkali to form 2-naphthol, sulfonating it, then converting the product into a salt. In principle, the result is a salt of 2-naphthol-6-sulfonic acid. In practice, a mixture is produced that is mostly Schaeffer's salt but also contains small amounts of numerous related impurities. (See Fig. 11.2.) The fate of these impurities depends on just what they are. Those that can couple such as 2-naphthol, R-salt, G-salt, and Crocein acid can persist in the finished colorant unchanged, or can react to form isomeric and subsidiary dyes analogous to that produced from Schaeffer's salt itself. Those that can not couple like naphthalene-di- and trisulfonic acids and

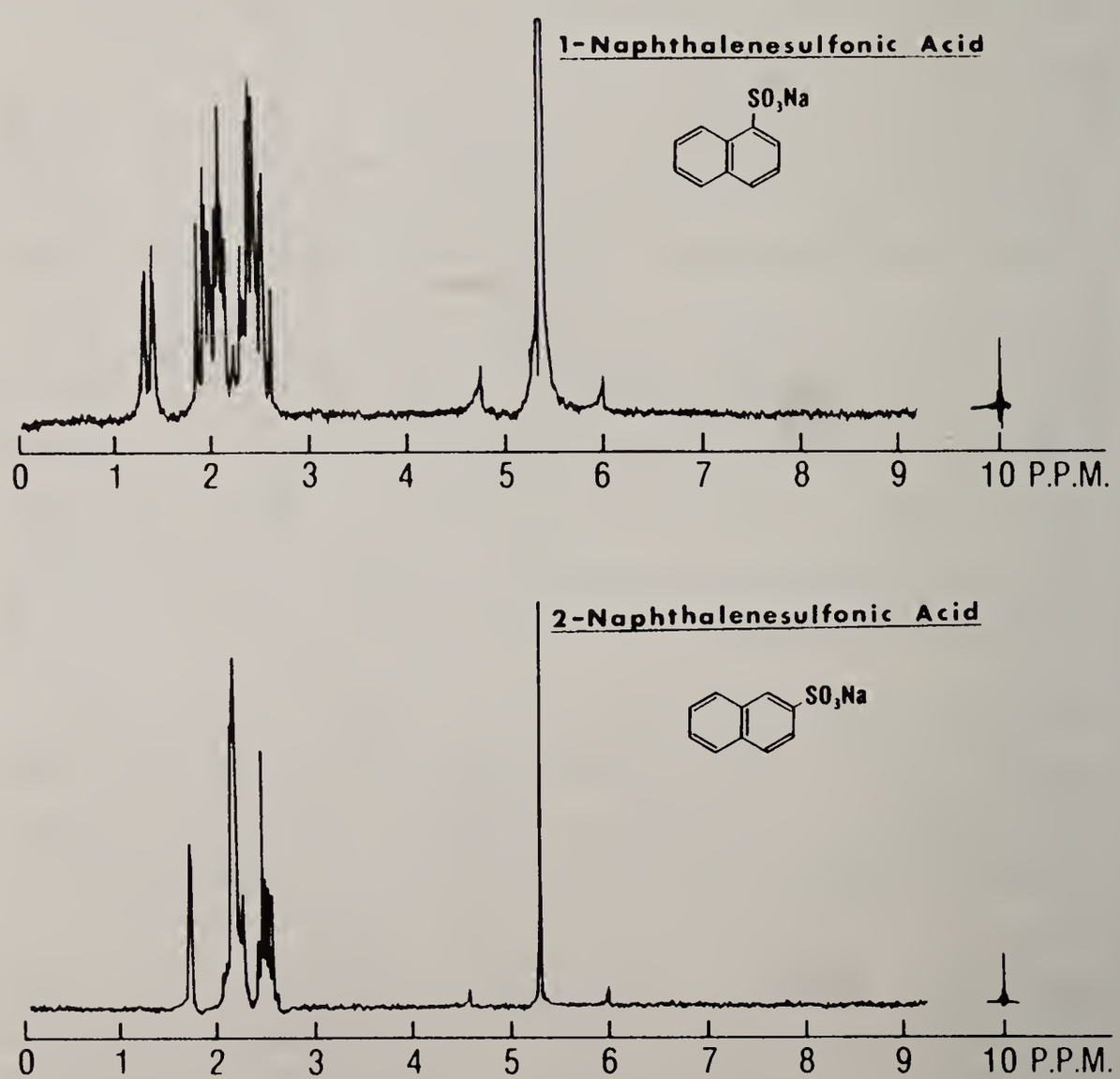

Figure 11.3. Nuclear magnetic resonance spectra of 1- and 2-naphthalenesulfonic acids obtained on a Perkin-Elmer R-32 90 MHz Spectrometer using 5% W/V D_20 sample solutions.

6,6′-oxybis (2-naphthalenesulfonic acid) (DONS) often are carried over into the finished colorant unchanged. The picture becomes more complicated when one considers the nature and purity of the other intermediate or intermediates used in synthesizing a particular colorant and the numerous combinations that can result from them.

Clearly, then, the purity of color additives depends largely on the nature and purity of the intermediates used to make them. Numerous techniques are available for evaluating these raw materials. Nuclear magnetic resonance spectroscopy (NMR) and high-performance liquid chromatography (HPLC) are excellent tools for this purpose. (See Figs. 11.3–11.6.) Thin-layer and paper chromatography are useful too. Wet chemical analyses for strength, inorganic salts, moisture and other volatiles, pH value, insolubles, sulfated ash, etc., provide invaluable in-

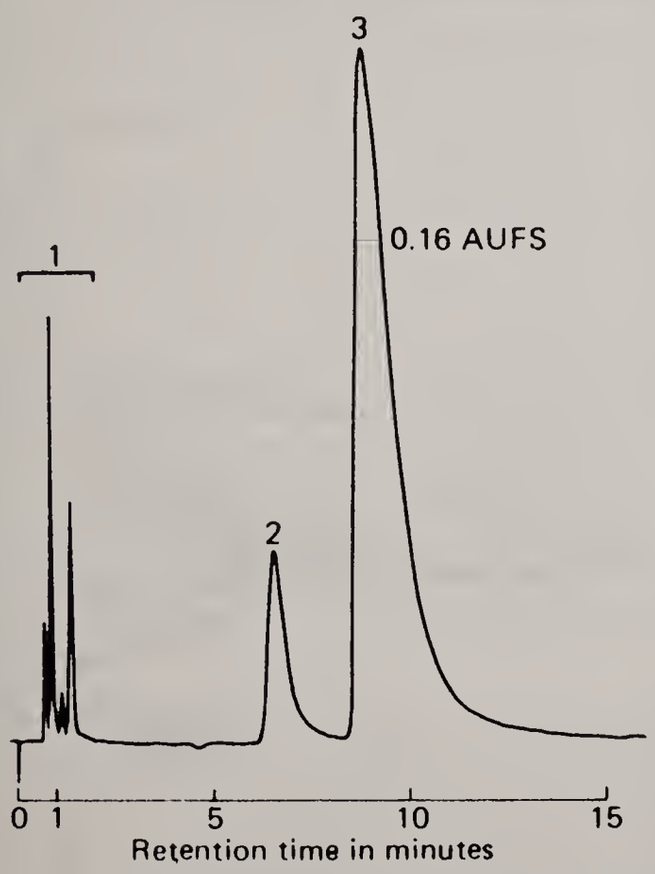

Figure 11.4. HPLC Separation of 10 μL of a 1% solution of commercial-grade 2-naphthalenesulfonic acid. Conditions: chromatograph, DuPont Model 830; column, 25-cm × 4.6-mm ID Whatman Partisil PXS10/25 ODS-2; detector, UV at 280 nm; temperature, 40°C; flow rate, 4 mL/min; mobile phase, 90 mL of isopropanol diluted to 2 liters with 0.15 *M* aqueous ammonium sulfate. Peaks: 1 = higher sulfonated homologs of 1- and 2-naphthalenesulfonic acids; 2 = 1-naphthalenesulfonic acid; 3 = 2-naphthalenesulfonic acid.

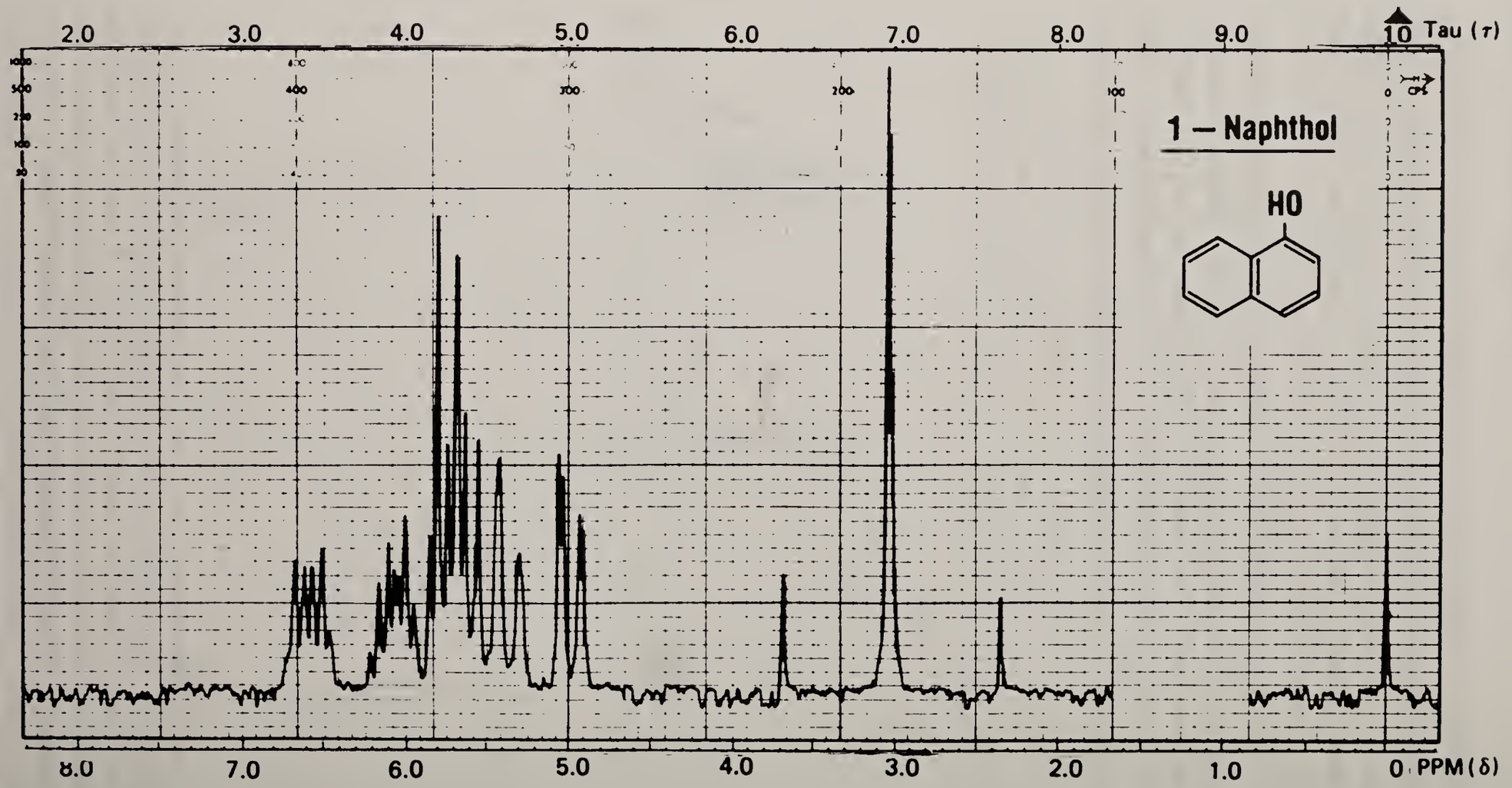

Figure 11.5. Nuclear magnetic resonance spectra of Schaeffer's salt and related compounds obtained on a Varian A60 Spectrometer, using 5% KOH in D_20 as the solvent, a sweep time of 500 sec, TSP (sodium 3-trimethylsilylpropionate-2,2,3,3,-d_4) as the marker, and a sweep offset of 100 Hz. (a). Spectrum of 1-naphthol, concentration 10%, amplitude 16.

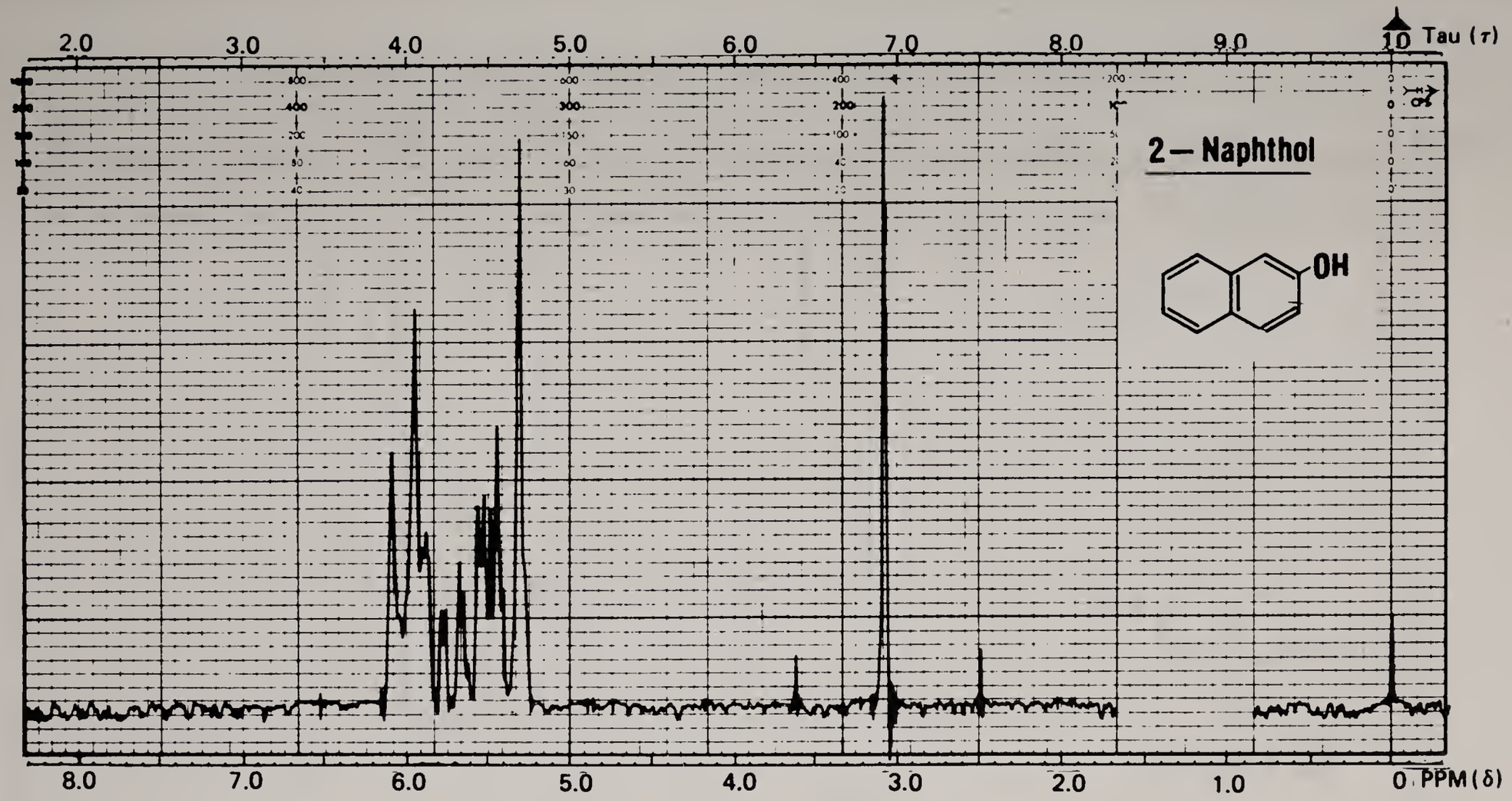

Figure 11.5b. Spectrum of 2-naphthol, concentration 10%, amplitude 16.

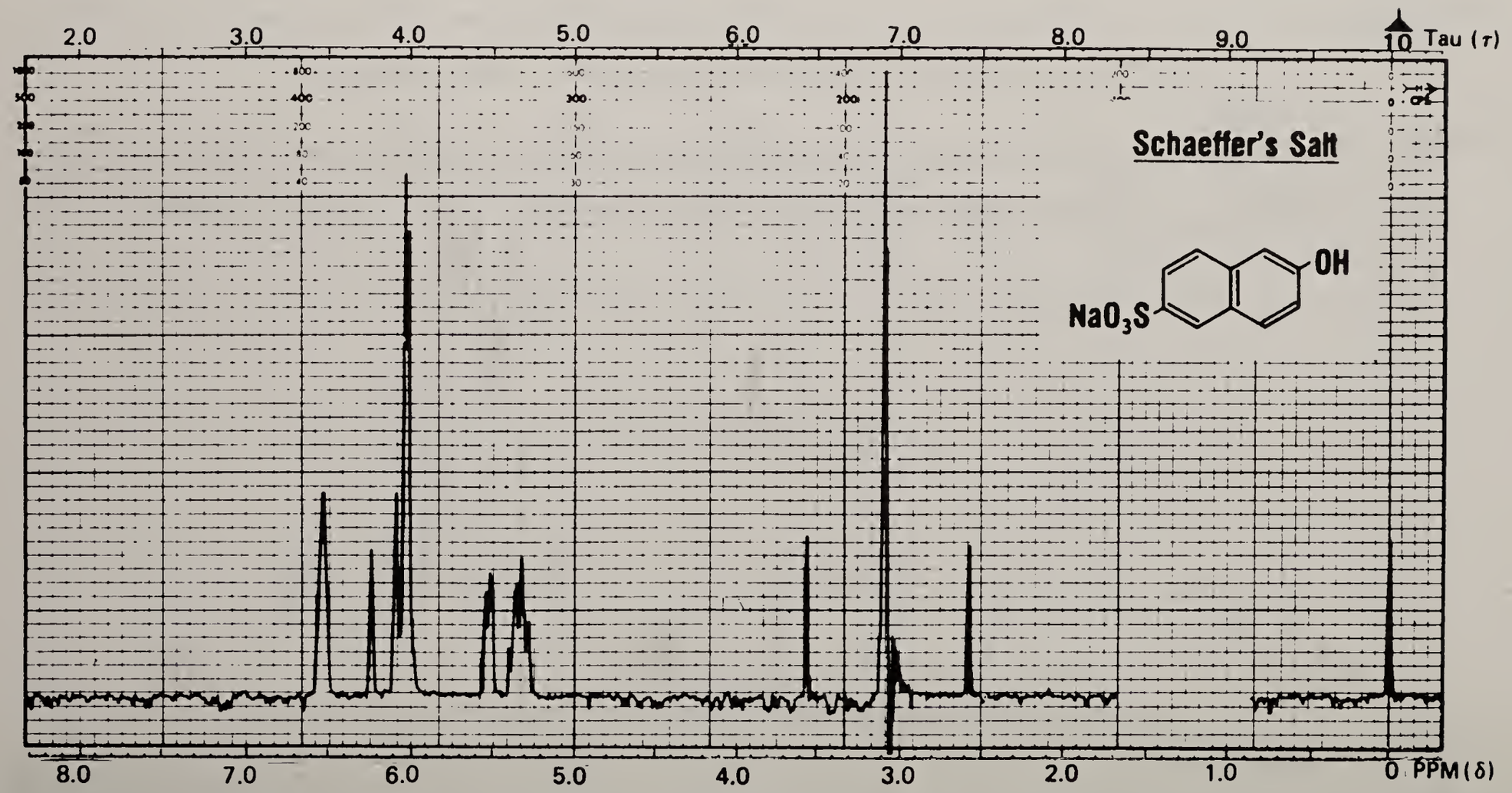

Figure 11.5c. Spectrum of Schaeffer's salt, concentration 10%, amplitude 16.

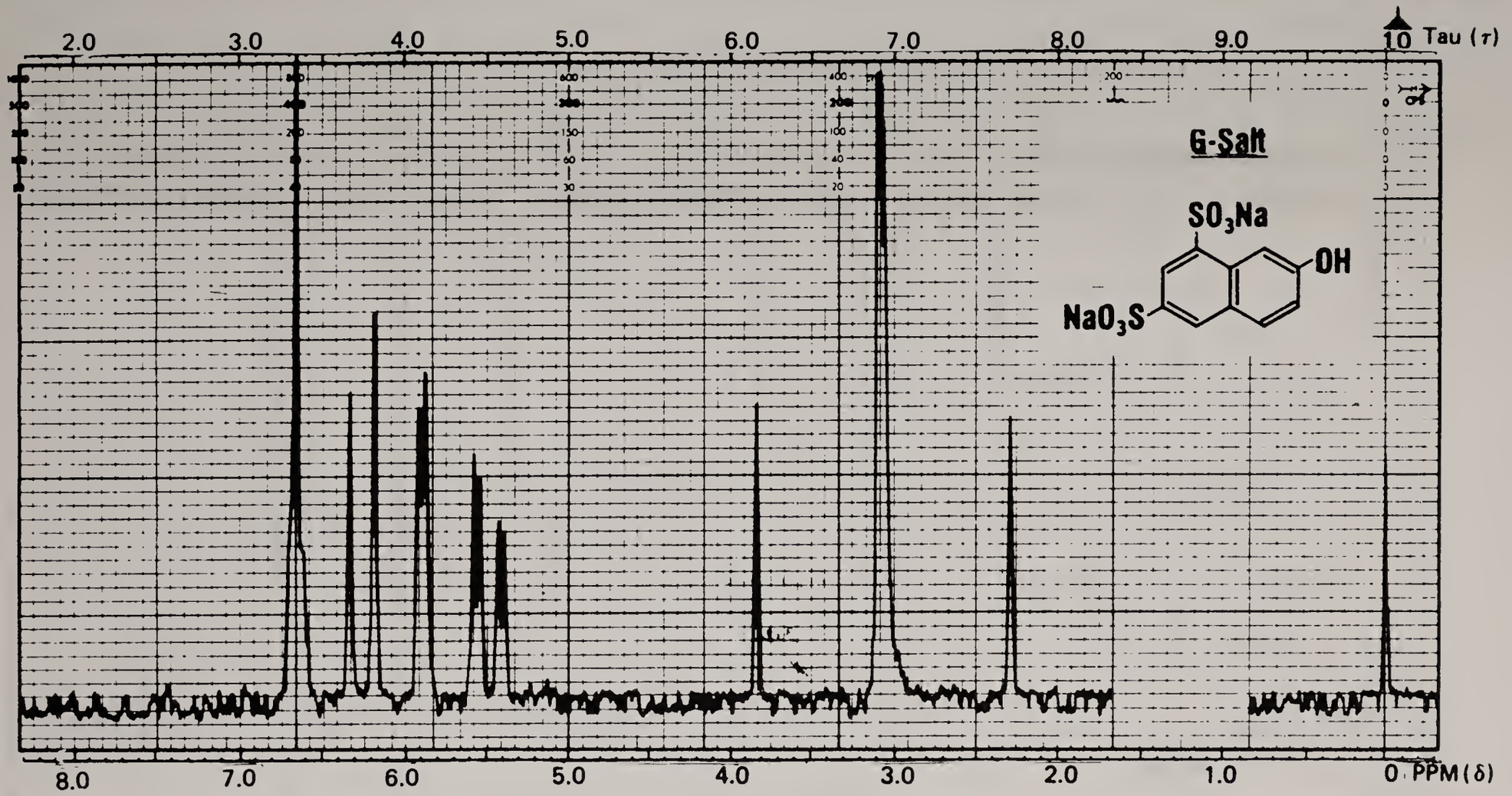

Figure 11.5d. Spectrum of G-salt, concentration 10%, amplitude 32.

Figure 11.5e. Spectrum of R-salt, concentration 10%, amplitude 32.

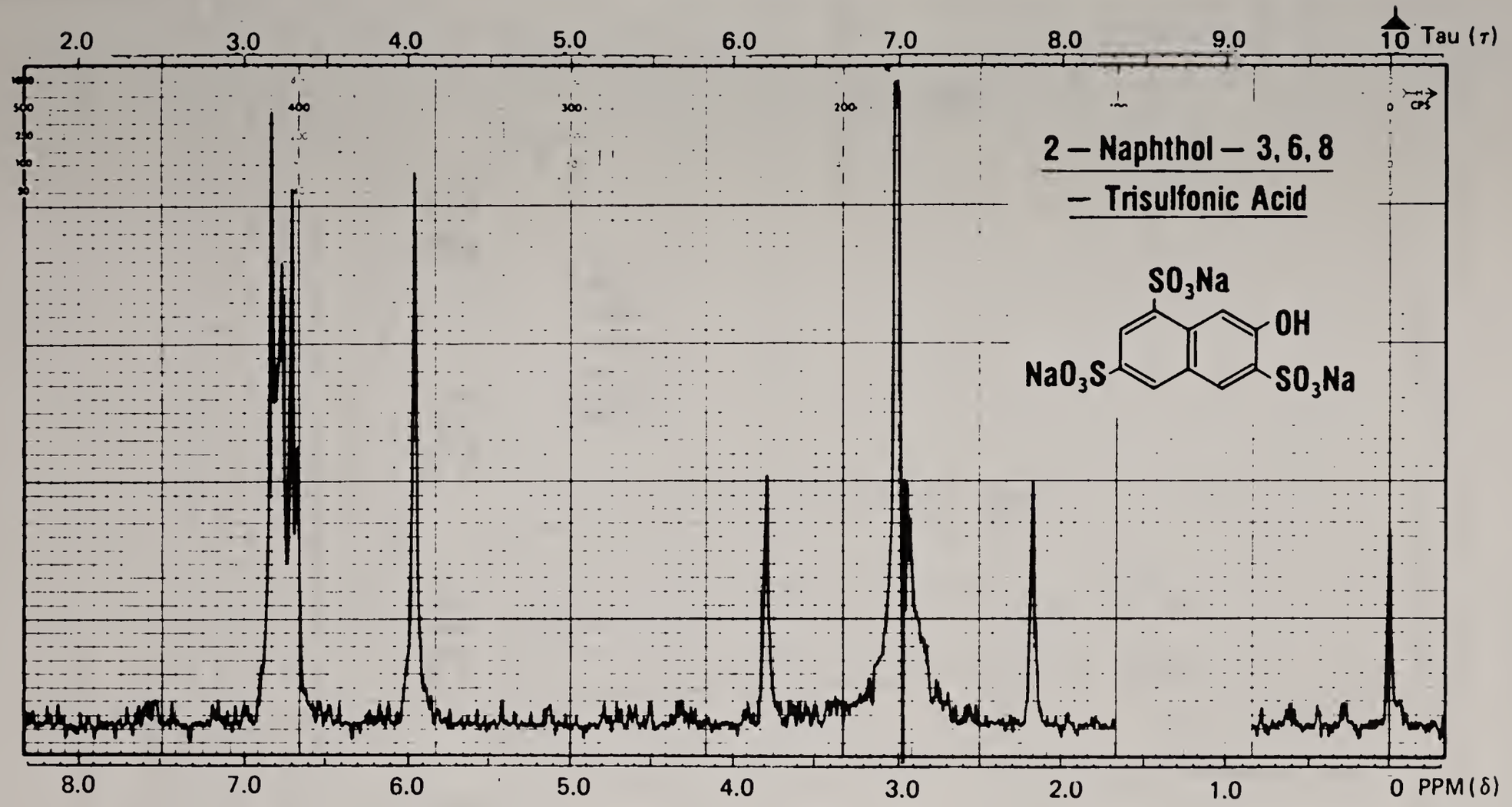

Figure 11.5f. Spectrum of 2-naphthol-3,6-8-trisulfonic acid, concentration 20%, amplitude 32.

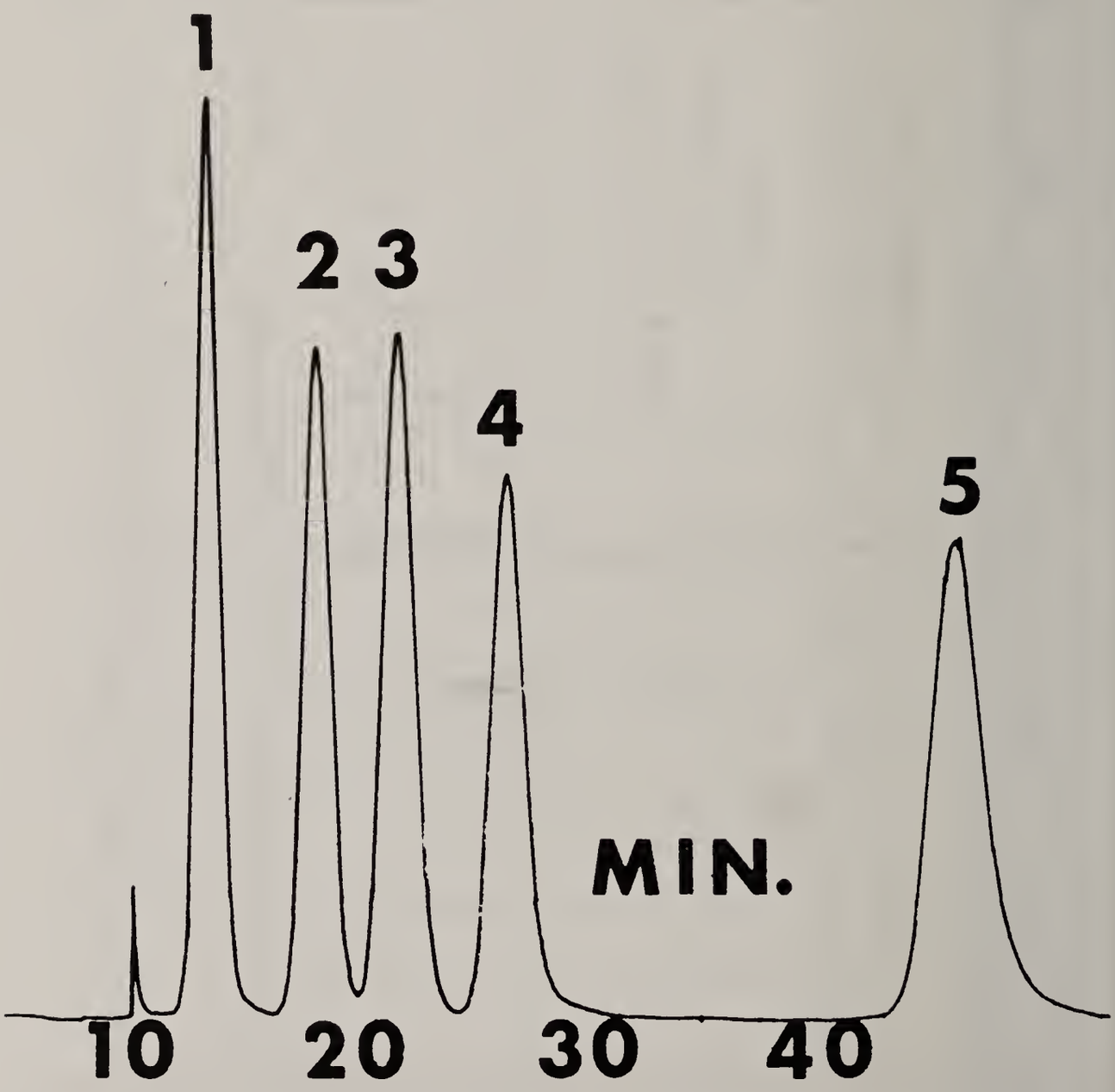

Figure 11.6. HPLC Separation of 5 μL of a solution containing about 23 mg each of Schaeffer's salt (1), *R*-salt (2), *G*-salt (3), 2-naphthol-3,6,8-trisulfonic acid (4), and DONS (5) in 200 mL of primary mobile phase. Conditions: chromatograph, Dupont Model 830; column, 1-m × 2.1mm ID DuPont Zipax SAX (strong anion exchange); detector, UV at 254 nm; flow rate, 0.75 mL/min; primary mobile phase, 0.01 *M* aqueous $Na_2B_4O_7$; secondary mobile phase, 0.01 *M* aqueous $Na_2B_4O_7$ in 0.5 *M* $NaClO_4$; gradient, nonlinear slow start function 5 clockwise; gradient rate, 1%/min for 30 min, then 3%/min.

formation. A simple comparison of a sample's strength obtained by UV spectroscopy with that obtained by one or more chemical procedures is also frequently enlightening.

Because of the special care used in selecting the starting materials for manufacturing color additives, and because of the extra efforts taken to purify the finished dyestuffs, only small amounts of a few impurities are actually ever found in color additives. The level of each depends on the contaminant and the colorant in question. In most instances, colorless impurities range from 0% to 0.3% each, whereas the concentration of colored contaminants varies from 0% to 1% each.

Methods for determining uncombined intermediates and other low-molecular-weight colorless compounds are described in Chapter 12, Procedures for determining colored impurities including homologous and isomeric colorants are given in Chapter 13.

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UNCOMBINED INTERMEDIATES AND OTHER LOW-MOLECULAR-WEIGHT IMPURITIES

GENERAL HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURES

Method A

The following procedure, which is based on ion-exchange, has been used successfully for several years for determining intermediates and other impurities in most major water-soluble food colors. The operating conditions given are those needed when using a DuPont Instruments Model 830 Liquid Chromatograph since that instrument was used to do much of the method development work, but any good chromatograph should be suitable after slight modification of the method parameters. No detector is specified in the procedure because any quality instrument should be suitable. The wavelength to monitor depends, of course, on the component or components of interest, but 254 nm is generally a good compromise. The order of elution given for the impurities in each colorant assumes that no impurity is present other than those listed.

Apparatus and Reagents

Mobile phase A—0.01 *M* aqueous $Na_2B_4O_7$.
Mobile phase B—see specific colorant.
Liquid chromatograph—DuPont Model 830 with a gradient elution accessory.
Column—DuPont strong anion exchange (SAX), l-m x 2.1-mm—ID (DuPont No. 830950405). Condition a new column before use by heating it at 50°C with 0.01 *M* $Na_2B_4O_7$ flowing through it at 1000 psi. The conditioning time needed ranges from 0–90 hr and depends on the individual column, the color being analyzed, and the resolution required. After conditioning, let the column rest 2 weeks before use.

Operating Conditions

To equilibrate the system, run a gradient of 0–100% mobile phase B at 10%/min, then pump mobile phase A through the column for 10 min. Inject the sample and chromatograph as indicated as follows.

FD&C Blue No. 1 (24).

Mobile phase B—0.25 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.
Sample size—5 μL of a 1% solution.
Flow rate—0.25 mL/min.
Gradient—linear, 0–100% secondary at 4%/min.
Order of elution—(1) *m*-sulfobenzaldehyde, (2) *o*-sulfobenzaldehyde, (3) *N*-ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid, (4) FD&C Blue No. 1, (5) ethylbenzylaniline sulfonic acid.

FD&C Blue No. 2 (70).

Mobile phase B—0.50 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.
Sample size—5 μL of a 1% solution.
Flow rate—1.00 mL/min.
Gradient—slow start exponential 3, 0–100% secondary at 1%/min.
Order of elution—(1) isatin, (2) isatin-5-sulfonic acid, (3) unknown, (4) FD&C Blue No. 2, (5) 5,7′-disulfonated indigo, (6) monosulfonated indigo.

Note: It has been reported that results obtained using this procedure can be difficult to repeat (7).

FD&C Red No. 40 (18,68,72).

Mobile phase B—0.20 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.
Sample size—20 μL of a 0.25% solution.
Flow rate—0.60 mL/min.
Gradient—linear, 0–18% in 16 min, 18–62% in 6 min more, then hold for 18 min more at 62%.
Order of elution—(1) cresidinesulfonic acid, (2) unknown, (3) Schaeffer's salt, (4) unknown, (5) 4,4′-diazoaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA), (6) unknown, (7) FD&C Red No. 40, (8) 6,6′-oxybis(2-naphthalenesulfonic acid).

Note: It has been reported (64) that DMMA is unstable in acid solution, and it is therefore necessary to keep samples alkaline by preparing them as follows: add 10 mL of 0.1 *M* $Na_2B_4O_7$ to 0.250 g of sample; add about 50 mL of water, swirl to dissolve, then dilute to 100 mL with water.

FD&C Yellow No. 5 (3,13,19).

Mobile phase B—0.10 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.
Sample size—50 μL of a 0.15% solution, prepared within 13 min of injection.
Flow rate—1.00 mL/min.
Gradient—slow start exponential 2, 0–95% secondary at 4%/min.
Order of elution—(1) phenylhydrazine-*p*-sulfonic acid, (2) sulfanilic acid, (3) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone, (4) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone, (5) 4,4′-(diazoamino)-dibenzenesulfonic acid.

FD&C Yellow No. 6 (5,17,69,72).

Mobile phase B—0.25 *M* $NaClO_4$ in aqueous 0.01 *M* $Na_2B_4O_7$.
Sample size—5 μL of a 1% solution.
Flow rate—0.50 mL/min.
Gradient—slow start exponential 3, 0–80% secondary at 3%/min.

Order of elution—(1) sulfanilic acid, (2) Schaeffer's salt, (3) 4,4′-(diazoamino)-dibenzenesulfonic acid, (4) *R*-salt dye, (5) FD&C Yellow No. 6, (6) 6,6′-oxybis(2-naphthalenesulfonic acid).

Orange B (71).

Mobile phase B—0.20 *M* $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$.
Sample size—5 μL of a 1% solution, prepared within 5 min of injection.
Flow rate—1.00 mL/min.
Gradient—slow start exponential 5, 0–100% secondary at 4%/min.
Order of elution—(1) phenylhydrazine-*p*-sulfonic acid, (2) naphthionic acid, (3) [4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, ethyl ester], (4) pyrazolone-T, (5) unknown, (6) FD&C Yellow No. 5, (7) ethyl ester of FD&C Yellow No. 5, (8) Orange K, (9) Orange B, (10) 3-[(4-sulfo-1-naphthalenyl)-azo]-4-amino-1-naphthalenesulfonic acid.

Method B (43)

Use the procedure given for determining 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazo-pyrazole, disodium salt in FD&C Yellow No. 5 (p. 393), except use methanol–water (40 + 60) containing 0.005 *M* TBAP as the mobile phase. This method has been successfully used to determine sulfanilic acid and Schaeffer's salt in FD&C Yellow No. 6 at 237 nm, sulfanilic acid and 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone in FD&C Yellow No. 5 at 249 nm, and naphthionic acid and 2-naphthol-3,6-disulfonic acid (R-salt) in Amaranth at 237 nm, and is most likely applicable to other water-soluble color additives.

Method C (61)

This procedure has been used successfully to determine organic impurities in 21 food dyes. Suspend the colorant in dilute NaOH, then apply the suspension to an Extrelut™ column. After 15 min, wash the column with ether followed by CH_2Cl_2. Collect the eluate in 0.1 *M* HCl. Evaporate the organic solvents, then dilute the remaining solution with 0.1 *M* HCl and chromatograph a portion of it using a 25-cm × 4.6-mm steel Hypersil-ODS (5 μm) column and the following mobile phases and

conditions: pH 6 NaH_2PO_4 buffer–acetonitrile (13:7) for 15 min, change to buffer–H_2O–acetonitrile (1:1:3) over 5 min, then elute with this mobile phase for 15 min more. Monitor the mobile phase at 254 nm.

GENERAL COLUMN CHROMATOGRAPHIC SCREENING PROCEDURE (24,45)

With this method, low-molecular-weight impurities usually separate from the colorants in question, but not always from each other. The analyst may have to depend on taking smaller cuts, the use of simultaneous equations, spectral shifts with changes in pH, juggling of chromatographic conditions, or some combination of these changes to make good, quantitative determinations. The modifications in chromatographic conditions usually most conducive to improved resolution include reduction of sample size, increase in the column length-to-width ratio, and changes in mobile phase strength, including the use of full or step gradients.

Scaled-up versions of this procedure have been used successfully on numerous occasions to isolate unknowns from colorants for identification.

Procedure

Affix a short length of clean rubber tubing to the tip of the glass-chromatographic column shown in Fig. 12.1. Attach a pinchcock and place a glass-wool plug in the constriction above the column tip. Slurry 60 g of Whatman Column Chromedia CF11 in 500 mL of the mobile phase given in Table 12.1. With the pinchcock open, pour the slurry into the column. Wash the column with 200 mL of mobile phase and let it drain until the liquid level is 2–3 mL above the level of the packed cellulose.

Place 0.5 g of sample in a 150-mL beaker and add the solvent indicated in Table 12.1. (The solvent indicated does not necessarily dissolve the dye sample; however, it usually extracts the impurities.) Add 10 g of Chromedia that has been previously washed with the appropriate mobile phase and mix. Add 50 g of ammonium sulfate powder to salt out the dye and mix. Using 50 mL of mobile phase, transfer the mixture to

the column. Let the column drain to the surface of the cellulose. Add mobile phase to the column at a rate equivalent to the rate of flow through the column. Collect as many 100 mL ± 1 mL fractions as can be obtained before the dye itself begins to emerge from the column. Similarly prepare and elute a blank column to which no sample has been added.

Treat the eluate fractions as directed in Table 12.1, and then record the UV spectra of the fractions versus a blank (mobile phase plus reagents) and compare them with spectra of known compounds similarly prepared. For the ideal case in which there is no interference;

$$\text{Percent intermediate} = \frac{(\Sigma A)(f + x)(100)}{(a)(b)(w)(1000)}$$

where ΣA is the sum of the absorbances (corrected for column blank) of

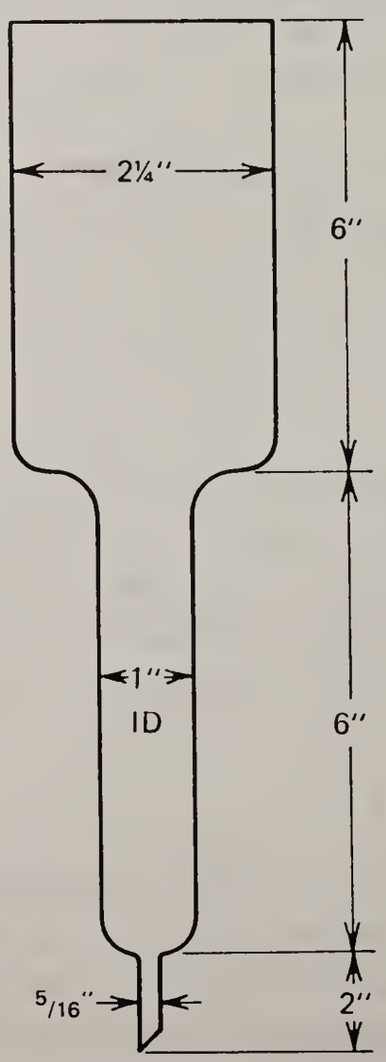

Figure 12.1 Glass-chromatographic column.

TABLE 12.1. Solvents, mobile phases, and treatment of eluate fractions for individual colors

Color	Solvent[a]	Mobile Phase (w/v)[b]	Treatment of Fractions
FD&C Blue No. 1	Water, 25 mL	Ammonium sulfate, 35%	Divide each fraction equally; add 0.5 mL of concentrated hydrochloric acid to one portion and 0.5 mL of conc. ammonium hydroxide to the other
FD&C Blue No. 2	Water, 25 mL	Ammonium sulfate, 25%	Run as eluted
FD&C Green No. 3	Water, 25 mL	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
FD&C Red No. 3	Ethanol, 5 mL; water, 25 mL	Ammonium sulfate, 25%, containing 10% ethanol (v/v)	Divide each fraction equally; add 0.5 mL of concentrated hydrochloric acid to one portion; run the other as is
FD&C Red No. 4	Water, 25 mL	Ammonium sulfate, 25%	Add 1 mL of concentrated ammonium hydroxide to each fraction
FD&C Yellow No. 5	Two drops of conc. hydrochloric acid in 25 mL of water	Ammonium sulfate, 25%, containing 0.5% hydrazine sulfate	Same as FD&C Blue No. 1
FD&C Yellow No. 6	Water, 25 mL	Ammonium sulfate, 35%	Run as eluted
Citrus Red No. 2	Ethanol, 10 mL; water, 25 mL	Ammonium sulfate, 5%, containing 5% ethanol	Run as eluted
Orange B	Two drops of conc. hydrochloric acid in 25 mL of water	Ammonium sulfate, 50%, containing 1% hydrochloric acid (v/v) for 600 mL, then 25% ammonium sulfate containing 1% hydrochloric acid (v/v) for remainder	Same as FD&C Blue No. 1

(continued)

TABLE 12.1. Solvents, mobile phases, and treatment of eluate fractions for individual colors (*Continued*)

Color	Solvent[a]	Mobile Phase (w/v)[b]	Treatment of Fractions
D&C Blue No. 4	Water, 25 mL	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
D&C Blue No. 6	Slurry with 10 mL of ethanol, then mix in 10 mL of mobile phase	Ammonium sulfate, 10%	Same as FD&C Red No. 4
D&C Blue No. 9	Water, 25 mL	Ammonium sulfate, 10%	Run as eluted
D&C Brown No. 1	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Red No. 4
D&C Green No. 5	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Green No. 6	Slurry with 10 mL of ethanol, then mix in 10 mL of mobile phase	Ammonium sulfate, 10%	Same as FD&C Red No. 4
D&C Green No. 8	Water, 25 mL	Ammonium sulfate, 40%	Run as eluted
D&C Orange No. 4	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Orange No. 5	Dissolve in a minimum of concentrated ammonium hydroxide	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Orange No. 10	Dissolve in a minimum of concentrated ammonium hydroxide	Ammonium sulfate, 25%	Same as FD&C Red No. 3
D&C Orange No. 11	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Red No. 3
D&C Red No. 6 and D&C Red No. 7	Slurry with 10 mL of ethanol, then mix in 10 mL of mobile phase	Ammonium sulfate, 10%	Divide each fraction equally; add 0.5 mL of concentrated hydrochloric acid to one portion; run the other as is
D&C Red No. 17	Slurry with 10 mL of ethanol, then mix in 10 mL of mobile phase	Ammonium sulfate, 10%	Same as D&C Red No. 6

D&C Red No. 21	Dissolve in a minimum of concentrated ammonium hydroxide, then add 5 mL of ethanol	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Red No. 22	Slurry with 5 mL of ethanol	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Red No. 27	Dissolve in a minimum of concentrated ammonium hydroxide, then add 5 mL of ethanol	Ammonium sulfate, 30%, containing 4% ammonium hydroxide (v/v)	Same as FD&C Blue No. 1
D&C Red No. 28	Slurry with 5 mL of ethanol	Ammonium sulfate, 30%, containing 4% ammonium hydroxide (v/v)	Same as FD&C Blue No. 1
D&C Red No. 30	Water, 25 mL	Ammonium sulfate, 10%	Run as eluted
D&C Red No. 31	Slurry with 10 mL of ethanol and 10 mL of mobile phase	Ammonium sulfate, 10%	Same as FD&C Blue No. 1
D&C Red No. 33	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
D&C Red No. 34	Slurry with 10 mL of ethanol, then mix in 10 mL of mobile phase	Ammonium sulfate, 25%, 1200 mL, followed by 10% ammonium sulfate	Run as eluted
D&C Red No. 36	Slurry with 10 mL of ethanol	Ammonium sulfate, 10%	Run as eluted
D&C Red No. 39	Slurry with 10 mL of ethanol	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
D&C Violet No. 2	Slurry with 10 mL of ethanol	Ammonium sulfate, 10%	Same as FD&C Red No. 4
D&C Yellow No. 7	Water, 25 mL	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
D&C Yellow No. 8	Water, 25 mL	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
D&C Yellow No. 10	Water, 25 mL	Ammonium sulfate, 40%	Same as FD&C Blue No. 1
D&C Yellow No. 11	Slurry with 5 mL of ethanol	Ammonium sulfate, 10%	Same as FD&C Blue No. 1
Ext. D&C Violet No. 2	Water, 25 mL	Ammonium sulfate, 25%	Same as FD&C Blue No. 1
Ext. D&C Yellow No. 7	Water, 25 mL	Ammonium sulfate, 35%	Run as eluted

[a]The solvent indicated may not dissolve the dye but only leach it free of impurities.

[b]The mobile phase should be essentially free of iron and other UV-absorbing impurities.

the sample fractions containing the intermediate, a is the absorptivity in L/g-cm of the intermediate at the wavelength at which A is measured, b is the absorption cell length in cm, w is the sample weight in g, f is the volume of the fraction collected in mL, and x is the volume of reagents added in mL.

Aromatic Amines in Synthetic Food Colors (37)

Adsorb the amines from an alkaline solution of dye by passing it through a column packed with Extrelut™. Wash the column with ethyl ether, then shake the eluate with 0.1 *M* HCl. Remove the ether in a rotary evaporator at ambient temperature, dilute the acid residue with 0.1 *M* HCl, then pass it through a Millipore filter. Determine the amines in the filtrate by HPLC using a 15-cm × 4.6-mm Supelcosil LC-8 column, an acetonitrile–0.15 *M* (pH 6) phosphate buffer mobile phase (1:4 or 3:7) and an electrochemical or UV detector (240 nm).

DETERMINATION OF β-NAPHTHOL

β-Naphthol is extracted with an appropriate solvent, coupled, and then determined by titanous chloride titration or spectrophotometrically.

Titration Procedure with Titanous Chloride (55)

Prepare a 0.05 *N* diazotized sulfanilic acid solution as follows. Dissolve 4.78 g of sulfanilic acid in 500 mL of 1:99 hydrochloric acid. Then dissolve 1.04 g of sodium nitrite in 300 mL of water. Place 40 mL of the sulfanilic acid solution in a 100-mL volumetric flask. Cool to 5°C, add 44 mL of the sodium nitrite solution, and allow it to diazotize. Test for excess nitrous acid with starch iodide paper; destroy any excess with a few milligrams of sulfamic acid. Dilute to volume with water. Store at 5°C.

Weigh a 10-g sample into a 2.5-cm × 8-cm extraction thimble, place the thimble in a Soxhlet apparatus, and extract for 8 hr with petroleum ether (boiling range 35–60° C).

Disconnect the extractor and add 150 mL of 1:10 hydrochloric acid to the flask. Gently boil off the ether on a hot plate and filter the solution through glass wool into a 250-mL volumetric flask. Rinse the extraction

flask several times with small quantities of 1:10 hydrochloric acid, filtering the rinses through the glass wool and collecting them in the volumetric flask. Dilute to volume with water. Mix and then divide into two equal portions. Neutralize each portion with a dilute sodium hydroxide solution, using phenolphthalein as an indicator.

Add 10 g of sodium acetate trihydrate to one portion and cool to 5°C in an ice bath. Slowly add 25 mL of the diazotized sulfanilic acid solution, stir for 5 min, and test for excess reagent with alkaline β-naphthol solution on spot paper. If necessary, add additional reagent until a positive test is obtained. Let stand for 1 hr or longer.

Heat on a water bath for 30 min to decompose any excess reagent. Test with β-naphthol solution on spot paper to determine whether decomposition is complete. To both the coupled and uncoupled portions of solution add 10 g of sodium bitartrate dissolved in 50 mL of hot water. Titrate the uncoupled blank with 0.1 *N* $TiCl_3$ to a colorless end point. Titrate the coupled portion until the dye reduces and becomes yellow. Add 1–2 mL of excess $TiCl_3$ solution and immediately back titrate with a standardized Methylene Blue solution or another suitable dye.

Subtract the volume of titrant required for the blank from the volume required for the coupled portion, and calculate percent β-naphthol. One milliliter of 0.1 *N* $TiCl_3$ is equal to 0.0036 g of β-naphthol.

Spectrophotometric Methods

General Methods (56)

Prewash all isopropyl ether once with 0.1 *N* sodium hydroxide.

Prepare a *p*-nitrobenzenediazonium chloride solution by dissolving 0.02 g of *p*-nitroaniline in 2 mL of hydrochloric acid and diluting to 200 mL with water. Then add 100 g of crushed ice and stir until the solution temperature is 5–10°C. Add 2 mL of 10% sodium nitrite solution and stir for 10–15 min. Add small portions of 10% sulfamic acid solution until the reagent gives a negative test on starch iodide paper. Store at 5°C.

For Colors Soluble in Water

Dissolve 2 g of sample in 250 mL of water. Acidify with 5 mL of 6 *N* hydrochloric acid and extract with six 30-mL portions of prewashed

isopropyl ether. Wash the combined ether extracts with 20 mL of 0.1 *N* hydrochloric acid and discard the aqueous layer. Extract with six 30-mL portions of 0.1 *N* sodium hydroxide.

Cool the combined sodium hydroxide extracts to 5–10°C with crushed ice and add *p*-nitrobenzenediazonium chloride solution slowly with constant stirring. Stir the mixture for 15 min. Heat to 90°C on a steam bath, remove from the bath, and cool to room temperature. Extract with 20-mL portions of chloroform until the extracts are colorless. Wash the combined extracts with 30 mL of 0.1 *N* sodium hydroxide. Filter the chloroform layer through cotton into a 500-mL flask. Dilute to volume with chloroform. Measure the absorbance of the sample solution at 490 nm; determine the β-naphthol content by comparison with a standard solution prepared by dissolving 0.1 g of 1-(4-nitrophenylazo)-2-hydroxynaphthalene in 200 mL of chloroform and by diluting with chloroform to obtain a concentration of 5–10 mg/liter.

$$\text{Percent } \beta\text{-napthol} = \frac{(A)(c)(144)(0.05)}{(B)(w)(293)}$$

where A is absorbance of the sample solution, B is absorbance of the standard solution, c is concentration of the standard solution in mg/L, and w is sample weight in g.

For Colors Soluble in Isopropyl Ether

Weigh a 2-g sample and add 250 mL of prewashed isopropyl ether; warm into solution on a steam bath. Extract with six 30-mL portions of 0.1 *N* sodium hydroxide. Wash the combined extracts with 30 mL of prewashed isopropyl ether and proceed as described in the preceding paragraph.

For Colors Insoluble in Water or Isopropyl Ether

Extract a 10-g sample with prewashed isopropyl ether for 8–10 hr in a Soxhlet apparatus. Transfer the extract to a 1-L separatory funnel. Rinse the Soxhlet extractor with two 20-mL portions of the isopropyl ether and add the rinses to the separatory funnel. Extract with six 30-mL portions

of 0.1 *N* sodium hydroxide and wash the combined extracts with 30 mL of the isopropyl ether. Dilute the sodium hydroxide extract to 500 mL with 0.1 *N* sodium hydroxide, place a 100-mL aliquot in a beaker, and proceed as described above.

Method for D&C Red No. 36 (42)

Prepare a *p*-nitrobenzenediazonium chloride solution by dissolving 0.040 g of *p*-nitroaniline in 2 mL of hydrochloric acid. Then chill with 35 g of ice and 15 mL of water. Diazotize with 0.04 g of sodium nitrite, stir, and let stand for 5 min. Store cold.

Stir 5 g of sample with 90 mL of ethanol and 10 mL of 10% sodium hydroxide for 30 min. Transfer the mixture to a 1-liter volumetric flask containing 600 mL of water, agitate, and dilute to volume.

Filter a 400-mL aliquot and buffer with 1 g of sodium carbonate. Cool the solution with 100 g of ice and add the *p*-nitrobenzenediazonium chloride solution. Let it stand for 10 min, stirring occasionally. Heat to 90°C, cool, and acidify with hydrochloric acid. Remove the coloring matter with 40-mL portions of chloroform continuing until the extracts are colorless. Wash the combined extracts with three 40-mL portions of water, and then with 30 mL of 0.1 *N* sodium hydroxide. Filter through absorbent cotton into a 500-mL volumetric flask. Dilute to volume with chloroform. Prepare a standard solution as described above and determine its absorbance and the absorbance of the sample at 490 nm. Calculate as under Colors Soluble in Water.

DETERMINATION OF PHTHALIC ACID DERIVATIVES (31,32,55)

Method A—in FD&C Red No. 3; D&C Orange Nos. 5, 10, and 11; D&C Red Nos. 21 and 22; D&C Yellow Nos. 7 and 8

Colors as Salts

Weigh a 2-g sample and transfer it to a 250-mL beaker with about 100 mL of water. Heat nearly to boiling and while stirring, slowly add 1:9 hydrochloric acid until precipitation seems complete. Add an additional

8.5 mL of 1:9 hydrochloric acid and dilute to about 150 mL. Digest on a steam bath for 1–2 hr. Cool and dilute with water in a 200-mL volumetric flask. Filter through dry paper.

Pipette 50 mL of filtrate into a 125-mL separatory funnel (do not grease stop-cocks) and extract with 30 mL of absolute ethyl acetate. Transfer the aqueous phase to a second funnel and extract with 25 mL of ethyl acetate. Transfer the aqueous phase to a third funnel and extract with 20 mL of ethyl acetate. Pass three successive 50-mL portions of water through the funnels in the same order that the extractions were made. Discard the ethyl acetate layers, combine the aqueous extracts, and evaporate to dryness on a steam bath.

Dissolve the residue in water and transfer to a 100-mL volumetric flask. Add 8.5 mL of 1:9 hydrochloric acid and dilute to volume. Filter through dry paper and measure the absorbance at 230 nm, 262 nm, and 276 nm against 0.1 *N* hydrochloric acid as the blank. Also measure the absorbance of a standard solution prepared by dissolving 0.13–0.135 g of potassium acid phthalate in 500 mL of water, and diluting a 10-mL aliquot to 200 mL with 0.1 *N* hydrochloric acid [phthalic acid concentration, mg/100 mL = (mg of $KHC_8H_4O_4$)(0.00813)].

Calculate *Y* for both sample and standards as follows:

$$Y = A_{230} - (A_{230} - 0.7A_{276}) - A_{262}$$

where *A* is the absorbance of the solution at the wavelengths indicated:

$$\text{Percent phthalic acid} = \frac{(Y \text{ sample}/Y \text{ standard})(c)(0.4)}{w}$$

where *c* is phthalic acid concentration of standard solution in mg/100 mL and *w* is sample weight in g.

Colors as Acids

To a 2-g sample add 6 mL of 10% sodium hydroxide and a few milliliters of water. Mix to dissolve. Dilute to about 100 mL and proceed as described above beginning with the second sentence under Method A ("Heat nearly to boiling . . . ").

Method B—in D&C Yellow No. 10

Dissolve a 1-g sample in water and transfer it to a continuous extractor. Add 1 mL of 1:100 hydrochloric acid and extract for 8 hr with 250 mL of ethyl ether. Transfer the ether extract to a separatory funnel. Rinse the flask twice with ethyl ether and add the washings to the main extract. Wash the extract with four 10-mL portions of 1:199 hydrochloric acid. Back extract the combined washings with 50 mL of ethyl ether and add the ether layer to the main ether extract. Extract the combined ether layers with four 10-mL portions of 1% sodium hydroxide. Evaporate the alkaline extracts to dryness. Transfer the residue to a 200-mL volumetric flask with water, add 2 mL of hydrochloric acid, and dilute to volume. Filter through dry paper. Measure the sample's absorbance and calculate percent phthalic acid as described in Method A.

Method C—in D&C Yellow No. 11

Transfer a 0.5-g sample to a 125-mL separatory funnel with 80 mL of chloroform. Add 20 mL of 1% sodium hydroxide and shake vigorously for 1 min. Drain the chloroform layer, wash the aqueous phase with two 30-mL portions of chloroform, and discard the chloroform layers. Add 7 mL of 1:9 hydrochloric acid and wash with two 30-mL portions of chloroform, discarding the chloroform washings. Transfer the aqueous phase to a beaker and evaporate to dryness on a steam bath. Proceed as described in Method A, beginning with "Dissolve the residue in water . . . ".

SULFOBENZALDEHYDES AND *N*-ETHYL-*N*-(3-SULFOBENZYL) SULFANILIC ACID IN FD&C BLUE NO. 1 (38,66)

Slurry 24 g of Whatman Column Chromedia CF11 in 140 mL of mobile phase (400 g of ammonium sulfate per liter of water.) Pour the slurry into the glass chromatographic tube shown in Fig. 12.2. Let the mobile phase drain at a rate of 5 mL/min or less until the liquid is 1–2 mm above the level of the packed cellulose. Close the pinch clamp.

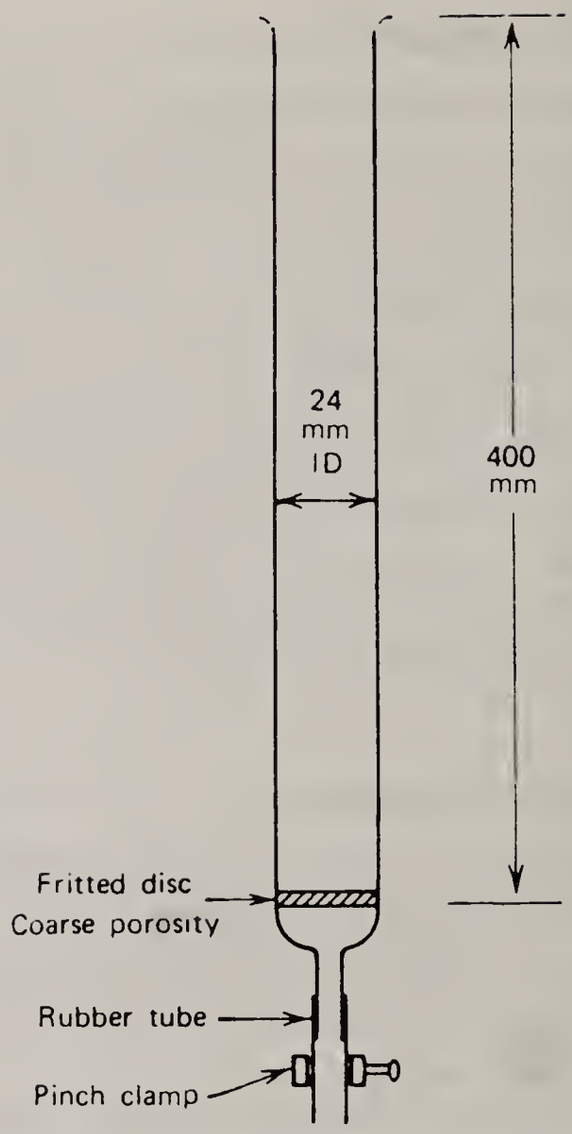

Figure 12.2 Chromatographic tube (Reprinted with permission of the Association of Official Analytical Chemists.)

Dissolve a 0.2-g sample in 10 mL of water. Add 2 g of the Chromedia and mix. Add 7 g of powdered ammonium sulfate and mix. Using 5 mL of the mobile phase, transfer the sample to the column. Drain until the flow nearly ceases. Elute the column at a rate of 5 mL/min or less, collecting as many 10 mL ± 0.05 mL fractions as necessary (ca. 30) to remove the intermediates from the column. Record the UV spectra of the fractions versus mobile phase in a 1-cm cell. Compare these against standard spectra obtained in the mobile phase.

o-Chlorobenzoic acid and *o*-sulfobenzoic acid elute just ahead of the sulfobenzaldehydes (SB) and may not be separated from them. They are identified by small maxima near 270 nm, but are not estimated by this method. To calculate a fraction as SB, the ratio A_{252}: A_{274} must be 2 or

greater. *ortho-*, *meta-*, and *para*-Sulfobenzaldehydes elute together, generally in fractions 7–15. They are calculated as total SB at 252 nm using an absorptivity of 51.6. This value is based on a mixture containing 46% *o*-sulfobenzaldehyde (*o*-SB), 46% *m*-sulfobenzaldehyde (*m*-SB), and 8% *p*-sulfobenzaldehyde (*p*-SB); 252 nm is the isoabsorptive point of *o*-SB and *m*-SB.

N-Ethyl-*N*-(3-sulfobenzyl) sulfanilic acid generally elutes between fractions 15 and 30. It is calculated at the maximum near 274 nm, using an absorptivity of 62.

Leuco Base in FD&C Blue No. 1 and FD&C Green No. 3

Oxidation with Oxygen and Cupric Chloride (21)

Dissolve 0.12 g of FD&C Blue No. 1 or 0.13 g of FD&C Green No. 3 in distilled water and dilute with water to 1000 mL in a volumetric flask. Pipette 10 mL of this solution into a 250-mL volumetric flask containing 100 mL of water, add 50 mL of *N,N*-dimethylformamide (DMF), swirl to mix, cool to room temperature, and dilute to volume with water; solution = *A*. Pipette a second 10-mL aliquot of sample solution into a 250-mL volumetric flask, add 50 mL of a solution containing 1 g of cupric chloride ($CuCl_2 \cdot 2H_2O$) in 100 mL of DMF, pass a rapid stream of air through the mixture for 30 min and then dilute to volume with water; solution = *B*. Pipette 50 mL of the 1% cupric chloride in DMF solution and 50 mL of DMF into separate 250-mL volumetric flasks containing 100 mL of water, swirl to mix, cool to room temperature, and then dilute to volume with water; solutions = *C* and *D*, respectively.

Using matched absorption cells, determine the spectra from 700 nm to 500 nm of: solution *D* versus solution *D*, solution *A* versus solution *D*, solution *B* versus solution *C*.

$$\text{Percent leuco base} = \frac{(E - E_1)100}{abcf}$$

where E is the blank corrected absorbance at the absorption maximum of solution *B*, E_1 is the blank corrected absorbance at the absorption maximum of solution *A*, b is length of cell path in cm, c is effective sample concentration in g/L, f is molecular weight of the color divided by the

molecular weight of the leuco base, and *a* is absorptivity of the color in L/g-cm.

The molecular weights of the colorants as disodium salts are: FD&C Blue No. 1 = 792.8, FD&C Green No. 3 = 808.9. The molecular weights of the leuco bases as the trisodium salts are: FD&C Blue No. 1 = 816.9, FD&C Green No. 3 = 832.9. Absorptivities in liters/g-cm are: FD&C Blue No. 1 = 164, FD&C Green No. 3 = 156.

Oxidation with Chloranil (39)

Weigh a 0.5-g sample into a 250-mL volumetric flask and dilute to volume with water. Pipette a 10-mL aliquot of the solution into each of two 100-mL volumetric flasks. To the first flask, add 15 mL of a freshly prepared solution consisting of 0.04 g of chloranil in 100 mL of *N, N*-dimethylformamide. Place the flask in a boiling water bath for 60 min, cool, and dilute to volume with water. Dilute the aliquot in the second volumetric flask to volume with water. Pipette a 10-mL aliquot from each flask into separate 500-mL volumetric flasks and dilute to volume with water. Measure the absorbance of each solution in a 1-cm cell at the absorption maximum.

$$\text{Percent leuco base} = \frac{(A - A_1)100}{(a)(b)(c)(f)}$$

where A is absorbance of the sample solution treated with chloranil, A_1 is absorbance of the untreated sample solution, b is length of cell path in cm, c is effective sample concentration in g/L = 0.004, f is molecular weight of the color divided by the molecular weight of the leuco base, and a is absorptivity of the color in L/g-cm.

Liquid Chromatographic Method (65)

System 1—Column = Waters RCM C-18, 10 μm particle size, 100 × 8 mm id, in an RCM 100 compression unit; mobil phase A = 0.1 *M* aqueous ammonium acetate; mobile phase B = 0.1 *M* methanolic ammonium acetate; flow rate = 2.0 mL/min; elution program = 57% B + 43% A for 10.0 min, to 80% B in 0.1 min, hold at 80% B for 2.9 min more. System 2—column = Waters stainless steel NOVA-PAK C-18, 4 μm particle size, 150 × 3.9 mm id; mobile phase A = 0.2 *M* aqueous ammonium

acetate; mobile phase B = methanol; flow rate = 1.2 mL/min; elution program = 55% B + 45% A for 9.9 min, to 80% B in 0.1 min, hold at 80% B for 4.0 min more. Using either system, chromatograph 20 μL of 0.5% w/v colorant in water and quantitate leuco base at 254 nm vs. solutions containing known amounts of leuco base. To prepare a stock solution of leuco base for calibration, titrate a known quantity of colorant with $TiCl_3$ using the procedure given on p. 242, then calculate the exact quantity of leuco base in the solution from the mL of titrant consumed.

Intermediates and Subsidiary Colors in FD&C Blue No. 2 (7)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 312MP equipped with two Model 110A pumps, a Model 420 microprocessor controller, a Model 155-10 variable wavelength detector set at 254 nm and 0.05 AUFS, a 20-μL Dent No. 905-42 loop injector, a Shimadzu Model C-RIA recording integrator, and an Altex Ultrasphere ODS Column No. 256-05, 4.6 mm × 250 mm, 5-μm particle size.

Mobile phase A—1.5% ammonium acetate (w/v)/0.5% acetonitrile (v/v) in water.

Mobile phase B—1.5% ammonium acetate (w/v)/50% water (v/v) in acetonitrile. To prepare, use the appropriate amount of ammonium acetate and water. Dilute this mix to volume with acetonitrile and mix, then add more acetonitrile as needed to compensate for any volume reduction on mixing.

Sample Preparation and Resolution

Dissolve 0.5 g of sample (0.1 g when measuring 5,7′-disulfoindigo) in 100 mL of water. With mobile phase A flowing at 1 mL/min, inject 20 μL of solution. Program from 0% to 60% B in A linearly in 25 min, then hold at 60% B in A for 5 min more. Possible contaminants elute at the indicated times in minutes: 5-sulfoanthranilic acid (2.61), isatin-4-sulfonic acid (4.85), isatin-5-sulfonic acid (6.53), isatin-7-sulfonic acid (8.51), anthranilic acid (9.33), FD&C Blue No. 2 (13.71), isomeric subsidiary color, 5,7′-disulfoindigo (17.98), isatin (21.45), and lower sulfonated subsidiary color, 5-sulfoindigo (27.08). Because 5-sulfoanthranilic acid elutes near t_0, it is difficult to quantitate.

PHTHALIC ACID, RESORCINOL, 2-(2′,4′-DIHYDROXY BENZOYL) BENZOIC ACID AND 2-(2′,4′-DIHYDROXY-3′,5′-DIIODOBENZOYL) BENZOIC ACID IN FD&C RED NO. 3 (29)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 420 equipped with two Model 110A pumps, a Waters 440 dual wavelength detector connected in series with an Hitachi Model 100-10 variable wavelength detector, a Micromeritics 725 autoinjector with a 20-μL loop, a Soltex Model 3314 recorder, a Columbia Scientific Industries Supergrator 3, and a DuPont Zorbax C-8 column, 25 cm × 4.6 mm ID.

Mobile phase A—0.2*M* NH_4Cl. Dissolve 10.7 g of NH_4Cl in about 970 mL of water, adjust the pH of the solution to 3.5 with 10% HCl, make to 1000 mL with water, and mix.

Mobile phase B—20% Acetonitrile in methanol; prepare fresh daily.

Sample Preparation and Resolution

Dissolve 0.250 g of sample in water and dilute to 100 mL. With 20% mobile phase B in A flowing at 1 mL/min, inject 10 μL of sample. Then program from 20% to 65% mobile phase B in 12 min, from 65% to 85% B in 18 min, then from 85% to 100% B in 5 min more. Hold at 100% B for 3 min. Measure phthalic acid at 230 nm, and the other impurities at 280 nm. Impurities elute before FD&C Red No. 3 in the order: resorcinol, phthalic acid, 2-(2′,4′-dihydroxy benzoyl) benzoic acid, 2-(2′,4′-dihydroxy-3′,5′-diiodobenzoyl) benzoic acid.

INTERMEDIATES AND SIDE REACTION PRODUCTS IN FD&C RED NO. 3 (40)

Separate a portion of sample solution using Camag DO silica gel (0.25-mm layer) as the substrate, and $CHCl_3$–anhydrous acetic acid (4:1) as the mobile phase. Visualize spots by treatment with diazotized sulphanilic acid and NH_3 vapor. Measure the amounts of each component present using densitometric scanning at 395, 415 and 420 nm, vs 700 nm as the reference wavelength. This procedure has been used successfully to determine 2,4-di-, 4,6-di-, and 2,4,6-triiodoresorcinols, and 2-(2,4-dihydroxybenzoyl)benzoic acid in FD&C Red No. 3.

5-AMINO-4-METHOXY-2-TOLUENESULFONIC ACID (CSA), SCHAEFFER'S SALT, AND 6,6′-OXYBIS (2-NAPHTHALENESULFONIC ACID) (DONS) IN FD&C RED NO. 40 (46,47,50)

Slurry 30 g of Whatman Column Chromedia CF11 in 200 mL of 40% aqueous $(NH_4)_2SO_4$ solution. Pour the slurry into a 24-mm × 400-mm glass chromatographic column (Fig. 12.2.) and let the liquid drain just to the surface of the cellulose. Wash the column with 200 mL of 40% $(NH_4)_2SO_4$, let it drain until the liquid is 1–2 mm above the level of the packed cellulose, and then close the pinch clamp.

Weigh 0.1 g of sample into a 50-mL beaker. Add 5 mL of water and stir to dissolve. Add 2 g of cellulose powder and mix. Add 7 g of $(NH_4)_2SO_4$ powder and mix. Using about 5 mL of 40% $(NH_4)_2SO_4$, transfer the mix to the column and let it drain just to the surface of the cellulose. Elute with 250 mL of 40% $(NH_4)_2SO_4$ and immediately begin collecting 50 mL ± 1 mL fractions. When the last of the mobile phase has just passed into the column elute with 20% $(NH_4)_2SO_4$ and continue collecting fractions until all the DONS elutes (ca. 1500 mL of 20% $(NH_4)_2SO_4$). A blank column to which no sample has been added should be prepared and eluted as described above.

Record the UV spectra of the fractions in a 5-cm cell from 370 nm to 220 nm. Use 40% $(NH_4)_2SO_4$ as the reference for fractions 1–8 and 20% $(NH_4)_2SO_4$ as the reference for the remaining fractions. Compare these spectra against standards obtained in the appropriate mobile phase; CSA normally elutes in fractions 4–6, Schaeffer's salt in fractions 8–10, and DONS in fractions 16–30.

$$\text{Percent CSA} = 0.186\ \Sigma(A_1 - A_2)_{252\text{ nm}}$$
(as free acid, mw 217.25)

$$\text{Percent Schaeffer's salt} = 0.396\ \Sigma(A_1 - A_2)_{282\text{ nm}}$$
(as monosodium salt, mw 246.22)

$$\text{Percent DONS} = 0.0585\ \Sigma(A_1 - A_2)_{240\text{ nm}}$$
(as disodium salt, mw 474.42)

where $(A_1–A_2)_{\times\text{ nm}}$ are the sums of the blank-corrected absorbances (also corrected for column blank where necessary) of the sample fractions containing the appropriate compound; 0.186 = 100/(53.8 × 2 × 5);

0.396 = 100/(25.3 × 2 × 5); 0.0585 = 100/(171 × 2 × 5); 100 = factor for conversion to percent; 2 = effective sample concentration in g/L; 5 = cell pathlength in cm; 53.8, 25.3, and 171 = approximate absorptivities in liters/g-cm of CSA, Schaeffer's salt, and DONS, respectively.

p-Cresidine [2-Methoxy-5-Methylaniline; 6-Methoxy-m-Toluidine] in FD&C Red No. 40 (62)

Prepare an aqueous solution of the colorant, make it alkaline with a few drops of 1 *M* NaOH, pass the solution through an Extrelut™ QE column, then elute any amines from the column using $CHCl_3$ as the mobile phase. Add 5 mM H_2SO_4 to the eluate then evaporate the $CHCl_3$. Diazotize the amines in the 5 mM H_2SO_4, couple them with R-salt (disodium 3-hydroxynaphthalene-2,7-disulphonate) then chromatograph the resultant colorants on two columns of Bio-Sil C_{18} (25 cm × 4 mm, 5 μm), previously equilibrated with a solution of 1.5 g of ammonium acetate in 0.5% acetonitrile. Elute colorants using a gradient of 0 to 40% acetonitrile in 30 min, monitoring the eluate at 546 and 254 nm. The presence of aniline as an impurity can be confirmed by using pyrazolone T as the coupling agent.

4,4′-DIAZOAMINOBIS (5-METHOXY-2-METHYLBENZENESULFONIC ACID) (DMMA) IN FD&C RED NO. 40

High-Pressure Liquid Chromatographic Procedure (6,52)

Apparatus and Reagents

Liquid chromatograph—A DuPont Model 830 equipped with a 1-m × 2.1-mm-ID DuPont SAX column (strong anion exchange, No.830950405), a gradient elution accessory, and a Model 835 multiwavelength detector fitted with a 365-nm filter (No. 835052-907).

Mobile phase A—0.01 *M* aqueous $Na_2B_4O_7$.

Mobile phase B—0.5 *M* aqueous $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$.

Instrument Parameters

Flow rate: 1.5 mL/min; temperature: ambient; gradient: 0–60% secondary at 2%/min, slow start exponential function 5.

Sample Preparation and Resolution: Weigh 0.5 g of sample into a 5-mL volumetric flask. Dissolve in mobile phase A and dilute to volume with same. Run a blank (0–60%) gradient, then pump mobile phase A through the column for 14 min. Immediately inject 5μL of sample solution into the chromatograph using a microliter syringe. Start the 0–60% gradient at once and elute until the chromatogram is complete (ca. 40 min). Pump mobile phase A through the column for 14 min and then inject the next sample. DMMA elutes in ca. 11 min.

Gravity Column Procedure (26)

Weigh 0.5 g of FD&C Red No. 40 into a 10-mL volumetric flask, add 7 mL of 0.01 *M* aqueous $Na_2B_4O_7$, swirl to dissolve, and dilute to volume with 0.01 *M* $Na_2B_4O_7$.

Slurry 3 g of Bio Rad Laboratories Cellex D (hydroxide form, standard-capacity DEAE cellulose) in 50 mL of 0.01 *M* $Na_2B_4O_7$ and pour into a 22-mm × 10-cm glass-chromatographic tube with a 200-mL reservoir top and a 22-mm fritted disk. Let the column drain to the surface of the packing and then wash with 50 mL of mobile phase (0.2 *M* aqueous $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$) and then 100 mL of 0.01 *M* $Na_2B_4O_7$.

With the column clamped off, add 1 mL of the sample solution to the top of it. Let the sample enter the column and then wash the sides of the tube twice with 10 mL of 0.01 *M* $Na_2B_4O_7$ until all the sample has entered the packing. Carefully add 10 mL of mobile phase. Allow the column to drain just to the surface of the packing and then fill the reservoir with mobile phase and elute.

Discard the first 75 mL then collect the next 150 mL or so that contain the DMMA. Measure the exact volume of the DMMA fraction then determine its absorbance at 385 nm using a 5-cm or longer cell.

$$\text{Percent DMMA} = \frac{A \times V \times 100}{W \times L \times a}$$

where A = sample absorbance at 385 nm, V = volume of sample fraction in mL, W = sample weight in g, L = cell length in cm, and a = absorptivity of standard at 385 nm in liters/g-cm.

Intermediates and Side-Reaction Products in FD&C Yellow No. 5 (41)

Apparatus and Reagents

Liquid chromatograph—A Perkin-Elmer Series 3B pump module, a Rheodyne 7125 injection port, a Perkin-Elmer LC-100 column oven, Perkin-Elmer LC-85 and Varian Varichrom detectors, a BBC Goerz Servogor 320 recorder, a Hewlett-Packard HP3353A laboratory automation system, and a 25-cm × 4.6-mm column, laboratory-packed with Lichrosorb RP-18, 7 μm (Merck).

Ion-pairing reagent—Add 1 L of 20% tetrabutylammonium hydroxide to a solution of 157.1 g of KH_2PO_4 in 540 mL of water to yield 1.54 L of 0.5 *M* tetrabutylammonium phosphate (TBAP).

Mobile phase—0.03 *M* TBAP in methanol-water. Dilute 60 mL of ion-pairing reagent to 1 L with methanol–water (40+60) and adjust the pH to 2.5 with 50% H_3PO_4.

Sample solvent—Same as mobile phase, but pH 7.9, i.e., no addition of H_3PO_4.

Sample Preparation and Resolution

Dissolve 0.250 g of sample in 100 mL of sample solvent. With the column temperature at 40°C, and the mobile phase flowing at 1.3 mL/min, inject 50 μL of sample. Elute at 1.3 mL/min for 8 min, then linear step flow gradient within 1 min to 4 mL/min. Monitor the column at 254 and 358 nm. Impurities elute in about 20 min, in the following order: phenylhydrazine-p-sulfonic acid, sulfanilic acid, pyrazolone-T, pyrazolone-T-methyl ester, 4,4′-(diazoamino)-dibenzenesulfonic acid, pyrazolone-T-ethyl ester, FD&C Yellow No. 5.

Sulfanilic Acid and 3-Carboxy-1-(4-Sulfophenyl)-5-Pyrazolone in FD&C Yellow No. 5 (78)

Transfer 1 g of sample into a 100-mL volumetric flask. Add 50 mL of water, swirl to dissolve, and then dilute to volume with methanol. Mix 1 mL of this solution with 1 mL each of *m*-chlorobenzoic acid (15 mg/mL) and 3-nitro-salicyclic acid (0.1 mg/mL), both prepared in methanol/water (50:50).

Inject 5 μL of this solution into a Waters Associates Model 202 liquid chromatograph equipped with a Model 6000 pump, a 280-nm detector, and a 30-cm × 4-mm-ID Micro Bondapack C18 column (Waters Associates). Elute at 0.9 mL/min using water-methanol-formic acid (400:400:1) containing 3×10^{-3} *M* tetrabutylammomium hydroxide and 0.6×10^{-4} *M* tridecylamine as the mobile phase.

Determine the amount of sulfanilic acid, 3-carboxy-1-(4-sulfophenyl)-5-pyrazolone, and FD&C Yellow No. 5 present using the *m*-chlorobenzoic acid and the 3-nitro-salicyclic acid as internal standards.

4,4′-(Diazoamino)-Dibenzenesulfonic Acid in FD&C Yellow No. 5 by Ion-Exchange Chromatography (27)

Apparatus and Reagents

Buffer—0.01 *M* phosphate (pH 12). Dissolve 14.2 g of Na_2HPO_4 in 1 L of water, then dilute 100 mL of this solution plus 3 mL of 50% (w/w) NaOH to 1 L with water.

Mobile phase—0.01 *M* phosphate buffer (pH 12), and 0.1 *M* sodium perchlorate. Dissolve 351.6 g of $NaClO_4 \cdot H_2O$ in 500 mL of water. Mix 20 mL of this solution, 100 mL of 0.1 *M* Na_2HPO_4, and 3 mL of 50% (w/w) NaOH, and dilute this mixture to 1 L with water.

Anion exchange resin—Whatman, Inc. DE32, (diethylaminoethyl) microgranular cellulose.

Chromatographic column—22-mm ID × 10-cm glass column with a 200-mL reservoir top, a 22-mm medium fritted disk, and a Teflon stopcock.

Transfer 50 mg of FD&C Yellow No. 5 to a 10-mL volumetric flask, add 7 mL of buffer, and swirl to dissolve. Dilute to volume with buffer and mix well.

Slurry 5.0 g of anion exchange resin with about 100 mL of buffer, then pour the mixture into the chromatographic column. Allow the resin to settle, drain the liquid just to the top of the resin, then place a 22-mm filter paper disc on top of the resin to protect it.

Wash the sides of the column with 4–5 mL more of buffer, and again drain the liquid just to the top of the resin.

Pipette 1.0 mL of sample onto the column and allow it to enter the resin. Wash the sides of the column with 10 mL of buffer and allow the

wash to enter the resin. Similarly, wash the sides of the column at least twice with 10-mL portions of mobile phase, then fill the column with mobile phase and allow it to develop.

Discard the first 50 mL of eluate, which should contain all the FD&C Yellow No. 5. Collect the next 15–25 mL, which contains the 4,4′(diazoamino)-dibenzenesulfonic acid (DAADBSA), measure the volume of this fraction, then determine its absorbance at 407 nm, using a 2.5-cm or longer cell.

$$\% \text{ DAADBSA} = \frac{A \times V \times 100}{W \times L \times a}$$

Where A = sample absorbance at 407 nm, V = volume of DAADBSA fraction in mL, W = sample weight in mg, L = cell length in cm, and a = absorptivity of a standard solution at 407 nm in A-mL/mg-cm.

Recovery of DAADBSA added to FD&C Yellow No. 5 at the 0.01–0.40% level was 90–130%. None of the at least nine subsidiary dyes that elute after DAADBSA interfere with the determination.

Intermediates, Subsidiary Colors, and Reaction By-Products in FD&C Yellow No. 6 (20)

Apparatus and Reagents

Liquid chromatograph—A Hewlett-Packard Model 1084B equipped with a variable volume injector and an automatic sampling system or a DuPont Model 850 with a Model 834 automatic sampler and a 50-µL loop, a Hewlett-Packard Model 79875A variable wavelength detector or a DuPont UV spectrophotometer, a Hewlett-Packard Model 1084B integrator, or a DuPont 850 integrator equipped with a Varian VISTA CDS 401, and a DuPont Zorbax C-8 column, 4.6-mm × 25-cm.

Mobile phase A—0.02 *M* ammonium acetate.

Mobile phase B—Methanol.

Sample Preparation and Resolution

Dissolve 0.5 g of sample in water, add 10 mL of 0.2 *M* ammonium acetate, and dilute to 100 mL with water. With mobile phase A flowing at 2

mL/min and the column temperature at 40°C, inject 50 μL of sample, program to 7% B in A in 2 min, to 12% B in 5 min more, to 60% B in 6 min more, then hold at 60% B for 5 min. Monitor the chromatogram at 232 and 358 nm. The order of elution is: 4-aminobenzenesulfonic acid (sulfanilic acid), 3-hydroxy-4-[(4-sulfophenyl)azo]-2,7-naphthalenedisulfonic acid (R-salt subsidiary color) 4,4′-(diazoamino)-dibenzenesulfonic acid (DAADBSA), 6-hydroxy-2-naphthalenesulfonic acid (Schaeffer's salt), an unknown, FD&C Yellow No. 6, 6,6′-oxybis(2-naphthalenesulfonic acid (DONS), an unknown, and 6-hydroxy-5-(phenylazo)-2-naphthalenesulfonic acid (a lower sulfonated subsidiary color).

Sulfanilic Acid, Schaeffer's Salt, and 6,6′-Oxybis(2-Naphthalenesulfonic Acid) (DONS) in FD&C Yellow No. 6 (5,47,48,49)

Slurry 30 g of Whatman Column Chromedia CF11 in 200 mL of 40% aqueous $(NH_4)_2SO_4$ solution. Pour the slurry into a 24-mm × 400-mm glass chromatographic column (Fig. 12.2.) and let the liquid drain just to the surface of the cellulose. Wash the column with 200 mL of 40% $(NH_4)_2SO_4$, let it drain until the liquid is 1–2 mm above the level of the packed cellulose, and then close the pinchcock.

Weigh 0.5 g of sample into a 100-mL volumetric flask. Dissolve in distilled water and make to volume with same; mix. Pipette 5 mL of this solution into a 50-mL beaker. Add 2 g of cellulose powder and mix. Add 7 g of $(NH_4)_2SO_4$ powder and mix. Using about 5 mL of 40% $(NH_4)_2SO_4$, transfer the mixture to the column and let it drain just to the surface of the cellulose. Open the pinchcock and elute with 250 mL of 40% $(NH_4)_2SO_4$ and immediately begin collecting 50-mL ± 1-mL fractions. When the last of the mobile phase has just passed into the column, elute with 20% $(NH_4)_2SO_4$ and collect 50-mL fractions until color elutes.

A blank column to which no sample has been added should be prepared and eluted as described above.

Record the UV spectra of the fractions in a 5-cm cell from 370 nm to 210 nm. Use 40% $(NH_4)_2SO_4$ as the reference for fractions 1–8 and 20% $(NH_4)_2SO_4$ as the reference for the remaining cuts. Compare these spectra versus standards obtained in the appropriate mobile phase. Sulfanilic acid normally elutes in fractions 3–6, Schaeffer's salt in fractions 8–10, and DONS in fractions 16–23.

$$\text{Percent sulfanilic acid} = 0.496\ \Sigma\ (A_1 - A_2)_{250\ nm}$$
(as sodium salt, mw 195.2)

$$\text{Percent Schaeffer's salt} = 0.131\ \Sigma\ (A_1 - A_2)_{232\ nm}$$
(as sodium salt, mw 246.2)

$$\text{Percent DONS} = 0.234\ \Sigma\ (A_1 - A_2)_{240\ nm}$$
(as disodium salt, mw 474.4)

where $(A_1 - A_2)_{\times\ nm}$ are the sums of the blank-corrected absorbances (also corrected for column blank were necessary) of the sample fractions containing the appropriate compound; 0.496 = 100/(80.7 × 5 × 0.5); 0.131 = 100/(305 × 5 × 0.5); 0.234 = 100/(171 × 5 × 0.5); 100 = factor for conversion to percent; 0.5 = effective sample concentration in g/liter; 5 = cell pathlength in cm; 80.7, 305, and 171 = approximate absorptivities of sulfanilic acid, Schaeffer's salt, and DONS, respectively.

2-Naphthol-6-Sulfonic Acid in FD&C Yellow No. 6 (54)

Using Schaeffer's salt-free colorant and 0.2% NaOH, prepare standard solutions containing 10 mg (100% pure dye basis) of colorant per liter of solution plus 0 μg, 5 μg, 10 μg, and 20 μg of added Schaeffer's salt. Determine the fluorescence of these solutions with a G. K. Turner Model 110 fluorometer equipped with a 7-60 primary filter and a 2A secondary filter. Prepare a calibration curve of fluorescence plotted against micrograms of Schaeffer's salt and use this to determine the Schaeffer's salt content of sample solutions similarly prepared.

4,4′-(DIAZOAMINO)-DIBENZENESULFONIC ACID (DAADBSA) IN FD&C YELLOW No. 6

High-Performance Liquid-Chromatographic Procedure (4,51,53)

Apparatus and Reagents:

Liquid chromatograph—A DuPont Model 830 equipped with a 1-m × 2.1-mm-ID DuPont SAX column (strong anion exchange, No.830950405), a gradient elution accessory, and a Model 835 multiwavelength detector fitted with a 365 nm filter (No. 835052-907).

Mobile phase A—0.01 M $Na_2B_4O_7$
Mobile phase B—0.50 M aqueous $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$.

Instrument Parameters

Flow rate: 1 mL/min; temperature: ambient; gradient: 0–100% mobile phase B at 1%/min, slow-start exponential function 4.

Sample Preparation and Resolution: Weigh 1 g of each sample into separate 10-mL volumetric flasks. Dissolve each in mobile phase A and dilute to volume with same. Before injecting the first sample run a blank (0–100%) gradient and then pump mobile phase A through the column for 14 min. Immediately inject 5 μL of sample solution into the chromatograph. Start the 0–100% gradient at once and "hold at limit" until the chromatogram is complete (ca. 50 min). Pump mobile phase A through the column for 14 min and then inject the next sample. DAADBSA elutes in ca. 17 min.

Gravity Column Procedure (25)

Weigh 0.5 g of FD&C Yellow No. 6 into a 10-mL volumetric flask. Add two drops of 50% NaOH and 7 mL of 18% aqueous Na_2SO_4 and swirl to dissolve. Dilute to volume with Na_2SO_4 solution and mix well.

Slurry 20 g of BW Solka Floc (Brown Co., Berlin, NH) in 150 mL of water and pour the slurry into a 22-mm(ID) × 20-cm glass-chromatographic column fitted with a 100–200-mL reservoir and a 22-mm fritted disk. Apply 2 psi of air pressure to the top of the column until all the liquid has entered the packing. Add 100 mL of mobile phase (150 g of Na_2SO_4, 150 g of NaCl, and 5 mL of 50% NaOH to 1 liter with water) and again apply pressure until the solution has entered the column. Add 1 mL of sample solution and wash into the column with pressure. Rinse down the walls of the column with a small amount of mobile phase, force the washings into the column with pressure, then fill the reservoir with mobile phase and elute the column under 2 psi of pressure. DAADBSA elutes just before FD&C Yellow No. 6. A narrow band of subsidiary color may elute before the DAADBSA. Collect the DAADBSA band, measure its volume, and determine its absorbance at 410 nm using a 5-cm cell.

2-Aminoanthraquinone in D&C Blue No. 9 (12)

Transfer 0.5 g of sample to a 100-mL beaker. Add 10 mL of dimethylsulfoxide, cover with a watch glass, and boil gently for 10 min. Cool the sample to room temperature and filter through a Buchner funnel with suction. With the vacuum still on, place the filtering flask on a steam bath and evaporate the solution to 0.5 mL.

Using a syringe, transfer the sample solution as a streak across a 20-cm × 20-cm TLC plate coated with 0.38 mm of silica gel G. Dry the plate, rinse the flask with a small amount of acetone, and streak the washing onto the same plate. Heat the plate 15 min at 110°C, cool, and place in a 10.5 in. × 10.5 in. × 5.5 in.-high museum jar (with a glass top) to which 150 mL of diethyl ether had been added 10–20 min earlier. Develop until the mobile phase reaches the top of the plate.

Air dry the plate, scrape off the yellow band corresponding to 2-aminoanthraquinone, extract the compound from the silica gel with 15 mL of acetic acid, filter, dilute to 25 mL, and determine the sample's absorbance in a 1-cm cell at the maximum near 428 nm. Compare against a standard.

Intermediates and Subsidiary Colors in D&C Green No. 5 (8)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 312MP equipped with two Model 110A pumps, a Model 420 microprocessor-controller, a Waters Model 440 dual wavelength UV detector set at 254 nm, an Altex 50-μL loop, a Shimadzu Model C-R1A recording integrator, and a Bio-Rad Biosil-ODS SS Column No. 125-0080, 25 cm × 4 mm ID, 5-μm particle size.

Mobile phase A—1.5% ammonium acetate (w/v)/0.5% acetonitrile (v/v) in water.

Mobile phase B—1.5% ammonium acetate (w/v)/50% water (v/v) in acetonitrile. To prepare, place the appropriate amount of ammonium acetate and water in a volumetric flask then dilute to volume with water and mix.

Sample Preparation and Resolution

Dissolve 0.250 g of sample in 100 mL of water, and mix well. Let the solution stand, with occasional mixing, for at least 1 hr before analysis

to ensure complete dissolution. With mobile phase A flowing at 1 mL/min, inject 50 µL of sample. Program from 5 to 100% B in A linearly in 25 min, then hold at 100% B for 10 min more. Contaminants elute at the indicated times in minutes: 5-amino-2-methylbenzenesulfonic acid (5.00), 2-amino-5-methylbenzenesulfonic acid (8.73), o,m-isomer of D&C Green No. 5 (20.21), o,o-isomer of D&C Green No. 5 (20.95), Ext. D&C Violet No. 2 (24.38), lower sulfonated subsidiary color of D&C Green No. 5 (31.08). To separate the m,m-, the o,o-, and the o,m-isomers of D&C Green No. 5 more completely, use isocratic elution at 60% B in A. Elutions times of the isomers in min are then 5.69, 7.79 and 8.48, respectively.

1,4-Dihydroxyanthraquinone in D&C Green No. 5 and Ext. D&C Violet No. 2 (36)

Transfer 0.1 g of sample into a 250-mL beaker. Add 100 mL of 0.1% NaOH, cover with a watch glass, and heat to boiling. Cool and transfer to a 250-mL separatory funnel, rinse the beaker with H_2O, and add the rinsings to the funnel. Add 5 mL of dilute HCl (8 + 92) and mix. Add 25 mL of isooctane and shake for 10–15 sec. Allow the layers to separate. If any quinizarin is present, the isooctane layer is yellow. Transfer the lower (aqueous) layer to a second funnel and extract with a second 25-mL portion of isooctane. Transfer the aqueous layer to a third funnel and extract with a fresh 25-mL portion of isooctane; discard the aqueous layer. Wash the isooctane in the first funnel with 25 mL of dilute HCl (1 + 199). Move this wash through the three funnels in the same manner as the initial aqueous solution. Continue to wash the isooctane layers with 25-mL portions of dilute HCl until all the colorant is removed. Discard the washes.

Draw the isooctane from the first separatory funnel into a 150-mL beaker and then pass the isooctane from the second funnel through the first funnel into the collection beaker. Similarly, pass the isooctane from the third funnel through the second and first funnels into the collection beaker.

Filter the isooctane through absorbent cotton into a 100-mL volumetric flask. Rinse the filter into the flask with isooctane, make to volume with same, and determine the sample's absorbance versus a blank in a 1-cm cell at 249 nm. Compare against standards.

Quinizarin, *p*-Toluidine, and D&C Violet No. 2 in D&C Green No. 6 (16)

Apparatus and Reagents

Liquid chromatograph—A Hewlett-Packard Model 1084A equipped with a fixed wavelength 254-nm detector and a DuPont Zorbax C-8, 4.6-mm × 25-cm column.

Mobile phase—Dilute 65 mL of 0.1 *M* aqueous sodium acetate and 222 mL of glacial acetic acid to 1 L with water, then filter through a 0.45-μm HAWPO4700 Millipore Corp. Filter. Dilute 300 mL of filtrate to 1 L with acetonitrile and mix well. Degas the mobile phase at 50°C under vacuum, then equilibrate the column with mobile phase flowing at 2 mL/min until a straight baseline is obtained (ca. 30 min).

Sample Preparation and Resolution: Dissolve 0.300 g of sample in 50 mL of $CHCl_3$, then dilute with acetonitrile to 100 mL in a volumetric flask. Chromatograph 10 μL of this solution at 50°C and a flow rate of 1 mL/min. Contaminants elute at the indicated times in minutes: quinizarin (5), *p*-toluidine (7), D&C Violet No. 2 (8), D&C Green No. 6 (16), unknown (18), unknown (47).

Note: It has been reported that the precision of *p*-toluidine results obtained using this procedure is poor.

1,4-Dihydroxyanthraquinone and 1-Hydroxyanthraquinone in D&C Green No. 6 and D&C Violet No. 2 (35,73)

Transfer 0.1 g of sample into a 250-mL beaker, add 100 mL of isopropyl ether, cover with a watch glass, and boil gently for 10 min. Cool and then transfer the solution to a 500-mL separatory funnel. If any undissolved dye is left in the beaker, add 50 mL of isopropyl ether and repeat the boiling; add the solution to the separatory funnel.

Add 50 mL of 5% NaOH solution to the funnel and shake for 10–15 sec. Transfer the aqueous phase to a clean funnel. Extract the isopropyl ether with additional 25-mL portions of 5% NaOH until an aqueous wash is colorless. (Quinizarin is intensely purple in aqueous alkaline solution.) Wash the combined NaOH extracts by shaking with successive 25-mL portions of isopropyl ether until an ether wash is colorless. Discard the ether washes.

Acidify the NaOH solution with HCl. Add 25 mL of isooctane and shake.

Transfer the aqueous phase to a clean separatory funnel and extract with 10 mL of isooctane. Repeat this process until the isooctane extractions are colorless.

Combine the isooctane extracts and wash twice with 20–25 mL portions of water. Allow the separatory funnel to stand a while after the second washing to permit as much water as possible to settle. Pass the isooctane layer through a plug of absorbent cotton (prewashed with isooctane). Rinse the separatory funnel with 20 mL of isooctane and pass this through the cotton. Transfer the isooctane to a 100-mL volumetric flask, make to volume, and determine spectrophotometrically at 249 nm.

Pyrene in D&C Green No. 8 (57)

Dissolve 2 g of sample in 200 mL of hot H_2O and let the solution cool to room temperature. Filter through a tared Gooch crucible, wash with cold water until the washings are colorless, and then dry at 135°C. Extract the isoluble residue with 50 mL of ethyl ether, filter the extract into a tared dish, evaporate to dryness at 40–50°C, dry in a desiccator over sulfuric acid for 3 hr, and then weigh.

1,3,6-Pyrenetrisulfonic Acid in D&C Green No. 8 (63)

Prepare mobile phase by dissolving 2.0 g of NaCl, 0.25 g of Na_2CO_3, and 0.25 g of $NaHCO_3$ in 110 mL of water, then mixing this solution with 15 mL of methanol. Weigh 1.000 g of sample into a beaker and dissolve it in about 60 mL of acetone-water (3+2). Transfer the sample quantitatively to a 100-mL volumetric flask and dilute to volume. Streak 0.100 mL of this solution onto a 250-μm thick, 20-cm × 20-cm TLC plate, pre-coated with 3 parts silica gel GF and 1 part MN 300 cellulose, with fluorescent indicator (Analtech, Inc.). Let the TLC plate air dry, then place it in a chromatographic tank that has been lined with Whatman Grade No. 1 chromatography paper, and equilibrated with mobile phase for about 30 min. Develop the plate until the mobile phase is 1 in. from the upper edge. Remove the plate from the tank and let it air dry. Examine the plate under a long-wavelength UV lamp. Pyrenetrisulfonic acid (PTS) appears as a violet band just above the main component. Remove the adsorbent containing PTS from the plate and extract the PTS with 0.76

N NH_4OH. Filter the adsorbent slurry through a 0.45-μm Gelman Sciences, Inc. Acrodisc, make the filtrate to volume, then measure the PTS at the absorption maximum near 281 nm. The approximate absorptivity of PTS at 281 nm is 78.5 mL/(mg × cm).

p-Toluidine in D&C Red No. 6 and D&C Red No. 7 (9)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 312MP equipped with two Model 110A pumps, a Model 420 microprocessor-controller, an Altex Model 155-10 UV/VIS variable wavelength detector set at 210 nm, a Rheodyne Model 7125 loop injector fitted with a 250-μL loop, a Shimadzu Model C-R1A recording integrator, and an Altex Ultrasphere octyl column, 25 cm × 4.6 mm ID, 5-μm particle size.

Mobile phase A—0.3 *M* ammonium sulfate-0.045 *M* sulfuric acid-1.5% (v/v) acetonitrile in water (1+1+1).

Mobile phase B—50% (v/v) acetonitrile in water.

Extraction Solution—Transfer 10.0 g of tetrabutylammonium hydrogen sulfate and 7.8 g of sulfuric acid to a 2-L volumetric flask and dilute to volume with distilled water. Mix well.

Sample Preparation and Resolution

Transfer 0.250 g of sample to a 250-mL separatory funnel. Add 100 mL of extraction solution to the funnel, then shake vigorously for 60 s. Add 50 mL of chloroform from a graduated cylinder, shake vigorously for 60 s, and let the layers separate. The lower chloroform layer should contain most of the dye and be free of suspended matter. The aqueous layer should be somewhat cloudy and light red. Drain the lower layer, being careful not to lose any insoluble material that may be at the interface. Repeat the extraction by shaking 15 s with two additional 50-mL portions of chloroform. The remaining aqueous layer should be colorless and somewhat cloudy. The aqueous layer becomes clear after standing about 30 min. With mobile phase A flowing at 1 mL/min, inject 250 μL of the aqueous layer. Program from 0–50% B in A (linearly) in 15 min, immediately change to 100% B, then hold at 100% B for 15 min more. *p*-Toluidine elutes in about 11.7 min.

Aminoazobenzene in D&C Red No. 17 (34)

Weigh a 1-g sample and transfer to an Erlenmeyer flask containing 100 mL of acetone. Heat to boiling on a steam bath. Slowly stir in 100–200 g of crushed ice and allow the mixture to stand for 15 min. Filter through a large fluted filter paper into a separatory funnel; wash the residue with 50 mL of 1:49 hydrochloric acid. Return the paper to the Erlenmeyer flask, and add 100 mL of acetone, and heat to boiling on a steam bath. Repeat the precipitation with ice and the filtration. Make the combined filtrates alkaline to litmus with 30% sodium hydroxide and extract with 50-mL portions of petroleum ether until the extracts are colorless. Wash the combined petroleum ether extracts with 20 mL of 2% sodium hydroxide and then extract with 10-mL portions of 4 *N* hydrochloric acid until the acid extracts are colorless. Heat the combined acid extracts for 15 min on a steam bath, cool, and dilute to 250 mL with 4 *N* hydrochloric acid. Determine aminoazobenzene spectrophotometrically at 500 nm.

1,3-Diphenyltriazen and Azobenzene in D&C Red No. 33 (10)

Prepare a 1% sample solution in 0.01 *M* NaOH containing 1% NaCl. Extract 100 mL of this solution with $CHCl_3$, then evaporate the $CHCl_3$ layer to dryness under vacuum. Dissolve any residue in HPLC-acetonitrile, then chromatograph a portion of this solution using a Bio-Sil ODS-5S column (25-cm × 4-mm), as follows. Equilibrate the column with aqueous 1.5% ammonium acetate containing 0.5% acetonitrile for 8 min., inject the sample, then program to 55% acetonitrile in 2 min. After 11 min. at 55%, linearly increase to 100% acetonitrile in 7 min. more. Monitor the sample at 365 nm. This procedure also separates cis-and trans-isomers of azobenzene.

Unsulfonated Aromatic Amines in D&C Red No. 33 (11)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 322MP equipped with two Altex 110A pumps, an Altex Model 420 Controller, a Waters Model 440 dual-channel UV detector set at 254 nm on both channels, an

Altex Model 155-10 variable wavelength detector set at 510 nm, a Rheodyne Model 7125 loop injector fitted with a 250-μL loop, and a 10-cm × 4.6-mm Rainin Microsorb (3 μm) C-18 column.

Mobile phase A—Dissolve 1.5 g of ammonium acetate and 0.5 mL of acetonitrile in 99.5 mL of water.

Mobile phase B—Acetonitrile.

Transfer 1.00 g of sample to a 250-mL separatory funnel containing 100 mL of a solution of 1 g of sodium chloride/100 mL of 0.01 *N* NaOH. Swirl the mixture, then extract it with 25 mL of chloroform, shaking the funnel vigorously for 60 s. Allow the layers to separate, then drain the chloroform layer through a washed (25 mL of chloroform) glass wool pledget in the constriction of a funnel into a 200-mL round-bottom flask. Extract the dye sample with two additional 25-mL portions of chloroform (30 s vigorous shaking), and drain the extracts into the flask. Add 5 mL of 0.01 *N* H_2SO_4 to the combined chloroform extracts, then remove the chloroform under vacuum using a rotary evaporator at 45°C. Take care to ensure that no chloroform droplets remain in the aqueous layer and that the flask is removed from the evaporator as soon as all of the chloroform is gone. Drive any residual chloroform vapors from the flask by using a 2-min air purge.

Chill the remaining solution in an ice bath for 5 min, add 1 mL of 1 mg/mL sodium nitrite solution, swirl to mix and wash the walls of the flask, then chill the solution for 15 min more. Add 1 mL of coupling solution containing 6 mg of sodium carbonate/mL and 5 mg of 3-hydroxy-2,7-naphthalenedisulfonic acid (R-salt)/mL. Swirl to mix and wash the walls of the flask, then chill for 15 min. Transfer the contents of the flask to a rotary evaporator and take to dryness under vacuum at 45°C. Dry the residue using a gentle air purge for 2 min. Add 5 mL of a solution containing 1.5 g of sodium dihydrogen phosphate/100 mL; swirl to dissolve.

Equilibrate the HPLC column with 100% mobile phase A for 10 min at a flow rate of 1 mL/min. Inject the sample, then program from 0 to 40% B linearly in 30 min.

The coupled amines elute in the following order: benzidine, aniline, 2-aminobiphenyl, 4-aminobiphenyl, and 4-aminoazobenzene. The method is designed to determine parts-per-billion of each.

2,4-Dinitro-1-Naphthol and 1-Naphthol in External D&C Yellow No. 7 (30)

Apparatus and Reagents

Liquid chromatograph—An Altex Model 420 equipped with two Altex Model 110-A pumps, a Waters Model 440 dual wavelength UV detector set at 280 and 436 nm, a Micromeritics Model 725 auto injector equipped with a 50-μL loop, a Soltec Model 3314 recorder, and a Whatman Partisil ODS-3 C-18 column, 25-cm × 4.6-mm ID.

Mobile phase A—0.2 *M* aqueous ammonium acetate.

Mobile phase B—Methanol

Sample Preparation and Resolution

Transfer 0.200 g of sample to a 100-mL volumetric flask, dissolve the sample in water, make to volume with water, and mix well. Adjust the mobile phase to 30% B in A, and the flow rate to 1.5 mL/min, then inject the sample. Program from 30% B to 55% B in 10 min, from 55 to 85% B in 20 min more, then from 85 to 100% B in 0.1 min more; hold at 100% B for 5 min. Ext. D&C Yellow No. 7 elutes in about 7 min, 2,4-dinitro-1-naphthol in about 14 min, and 1-naphthol in about 17 min. Monitor the eluate qualitatively at both 280 and 436 nm. Determine both impurities at 280 nm vs knowns prepared in mobile phase A containing a trace of Na_2SO_3 as a preservative.

2-Acetyl-4(5)-Tetrahydroxybutylimidazole in Caramel (44)

Dissolve 200 mg of caramel in 5 mL of water, then apply this solution to a 6-cm × 1-cm column of 100 to 200 mesh Dowex 50W-X8 resin. Wash the column with water, then elute the analyte with 0.3 *M* HCl. Evaporate the eluate to dryness at 38°C. Dissolve the residue in 5 to 10 mL of water, then analyze a portion of the resulting solution by HPLC using a 15-cm × 4.6-mm column of Supelcosil LC-18, water as the mobile phase (flow rate of 0.7 mL/min), and detection at 287 nm.

5-(Hydroxymethyl)-2-Furaldehyde in Caramel Solutions (2)

Dilute samples with water, filter them through a 0.45-μm filter, then chromatograph the filtrates using the following equipment and condi-

tions; Hewlett-Packard 1084A chromatograph equipped with a Schoeffel variable UV-visible detector set at 277 nm; Rheodyne injection valve with 20-μL loop; Whatman Inc. 25-cm × 4.6-mm ID column filled with 10 μm Partisil PXS 10/25; methanol-water gradient programmed at 2 mL/min as follows—10% methanol in water for 5 min, programmed to 30% methanol in water for 4 min, then programmed from 30% to 60% methanol in water for 4 min.

Using this procedure, the retention times for 5-(hydroxymethyl)-2-furaldehyde, 2-furaldehyde, theobromine, 5-methyl-2-furaldehyde, caffeine, vanillin, benzaldehyde, ethyl vanillin, coumarin, anethole, cinnamaldehyde, and methyl salicylate are 4.90, 6.25, 8.17, 9.91, 10.74, 11.24, 13.10, 14.39, 15.15, 15.72, 16.87, and 18.41 min, respectively.

This method can be used to monitor the quality of caramel, and to detect the presence of caramel colorant and estimate the amount of it in flavors and alcoholic beverages by measuring their 5-(hydroxymethyl)-2-furaldehyde content.

4-Methylimidazole in Caramel

Method A (14)

Dissolve 4 g of sample in 6–7 mL of water, adjust the pH to 8.5 with *N* NaOH, and add it to a column of Amberlite IRC-50 (H^+ form). Wash the column with water, then elute the 4-methylimidazole with aqueous 4 *N* NH_3. Concentrate the eluate to 0.5 mL in a rotary evaporator, dissolve the residue in saturated aqueous NaCl, make alkaline with aqueous NH_3, and extract it four times with 20 mL of chloroform. Filter the combined chloroform extracts, remove the solvent at 30° in a rotary evaporator, add 5 mL of CH_2Cl_2, and remove any residual water by azeotropic evaporation. Dissolve the residue in 0.8 mL of tetrahydrofuran containing 1 mg of imidazole as an internal standard, add 0.2 mL of acetic anhydride, heat at 60°C for 5 min, and then evaporate to 0.2 mL in a stream of nitrogen. Chromatograph 2–3 μL of sample on a 2-m × 2-mm gas chromatographic column packed with 3% OV-17 on Gas Chrom Q (80–100 mesh). Temperature program from 80–205°C at 5°/min; use nitrogen as carrier gas at 20 mL/min. Calculate the methylimidazole from the ratio of the peak area of the acetyl derivative to that of the internal standard.

Method B (76)

Add 3 *M* NaOH to the sample until it is pH 12 or higher, mix in Celite 545, then pour the mixture into a 30-cm × 2.2-cm glass chromatographic tube and allow it to settle. Fill the tube with CH_2Cl_2, and, after 5 min, drain off some of the CH_2Cl_2. Add 2-methylimidazole to the eluate as an internal standard, evaporate the mixture under vacuum at 35°C, dissolve the residue in acetone or tetrahydrofuran, then chromatograph a portion of it at 180°C using a 1-m × 4-mm glass column packed with Anakrom ABS (90–100 mesh) coated with 7.5% Carbowax 20M and 2% KOH, nitrogen (50 mL/min) as the carrier gas, and a flame ionization detector.

Method C (15)

Extract alkaline aqueous solutions of caramel with $CHCl_3$-EtOH. Caramel-containing alcohol-free beverages and dark beer should first be concentrated in a rotary evaporator at 70°C. Treat caramel-flavored yogurt with potassium oxalate to precipitate protein, extract the sample with hexane-ethyl ether (1:1) to remove fat, then concentrate the clear liquid as if a beverage. Determine 4-methylimidazole in the concentrates by gas chromatography using a 1.5-m × 3-mm glass column packed with 10% Carbowax 20M and 2% KOH on Chromosorb WAW (100–120 mesh) and operated at 190%C, using He as the carrier gas at 30 mL/min, and a Hall *N*-specific detector. The limit of detection is < 0.1 mg. 1,2,3,4-tetrahydroquinoline, 2-methylimidazole, or 2-ethyl-4-methylimidazole can be used as an internal standard.

Method D (75)

Dissolve 2.5 g of caramel color in 20 mL of 0.2 *M* potassium phosphate of pH 6.0, then adjust the pH of the mix to 6.0 with KOH. Extract 4 mL of this solution with 4 mL of 0.1 *M* bis-(2-ethylhexyl) hydrogen phosphate in $CHCl_3$, then re-extract the $CHCl_3$ layer with 3 mL of 0.1 M H_3PO_4. Chromatograph a portion of this solution using a 12-cm × 4.6 mm Nucleosil 5 C_8 column, a mobile phase composed of methanol-0.2 *M*-KH_2PO_4-H_2O (13:10:17) containing 5-m*M* Na dodecanesulfonate, and a detector set at 215 nm.

Method E (77)

Mix 10 g of sample with 6 g of 20% Na_2CO_3 solution and 10 g of Celite 545, then apply the slurry to a basic column of Celite 545. Develop the column with $CHCl_3$-ethanol (4:1), then extract the eluate with 0.05 *N* H_2SO_4. Evaporate the combined extracts to 5 mL at 55°C and 20 to 40 torr, then make the solution alkaline (pH >9) with Na_2CO_3. Chromatograph 2 μL of this solution and appropriate standards using silica gel F_{254} plates and ethyl ether-$CHCl_3$-methanol-aqueous NH_3 (20:5:5:1) as the mobile phase. Spray the plate with diazotized sulfanilic acid and compare the spots visually with the standards.

Hydroxypyridines and Hydroxypyrazines in Caramel (58)

Add 10.0 g of caramel to 10 mL of 1 *M* Na_2CO_3 and homogenize the mixture. Extract the mix with $CHCl_3$-ethanol (4:1), filter it, dry the filtrate with Na_2SO_4, then evaporate it to dryness. Dissolve any residue in $CHCl_3$, evaporate the solution to dryness under *N*, then derivatize the residue with Trisil (Pierce Chemicals) at 70°C. Chromatograph a portion of the sample using an OV-1 fused-silica column (25-m × 0.3-mm) temperature programmed from 80°C (maintained for 5 min) to 220°C at 2.5°/min. Use He as the carrier gas (50 cm/sec) and a VG7070H mass spectrometer for 70-eV electron-impact MS.

Low-Molecular-Weight Compounds in Caramel Colorants (59)

Homogenize 10 g of sample with 10 mL of 1 *M* Na_2CO_3, extract the mixture with three 100-mL portions of $CHCl_3$-ethanol (4:1), filter the combined extracts through Whatman phase-separating paper, dry the filtrate by passing it through a column of $CHCl_3$-washed Na_2SO_4, then evaporate the extract to dryness at 40°C or less. Treat the residue with 1 mL of Trisil (Pierce), allow the mixture to sit for 1 hr., then heat it at 70°C for 12 min. Using split-injection, chromatograph 1 μL of sample on an OV-101-coated silica WCOT column (25-m × 0.23-mm) operated with temperature-programming from 100°C (maintained for 5 min) to 230°C at 4°/min, a carrier gas (60 mL/min) split ratio of 1:22, and flame ionization or N-P detection.

Miscellaneous Compounds in Caramel Colorants (33)

Evaporate a portion of sample equal to 100 μg of colorant on a cylindrical probe for Curie-point pyrolysis at 600°C for 10 sec. Analyze the pyrolysis products by gas chromatography using a 30-m × 0.252-mm or × 0.329-mm fused-silica column coated with DB-210 (0.25 or 0.5 μm, respectively). Temperature program the column at 3°/min from 40°C (held for 5 min) to 210°, using He at 2 mL/min as the carrier gas, and flame ionization detection. Identify the components by GC—70eV MS, with total-ion-current monitoring.

Sugar-Degradation Products in Caramel (67)

Degradation products of sugars, including glucose and fructose, formed by heating solutions under reflux at about pH 6.5 with o-methylhydroxylamine were determined by gas-chromatography of their acetyl derivatives using a 2-m steel column packed with 2.5% SE-52 on Chromosorb G (80 to 100 mesh), temperature-programmed from 60° to 200°C at 4°/min. 1,3-Dihydroxyacetone, glyceraldehyde, erythrose, 3-deoxyhexosulose and pyruvaldehyde were detected in the caramelization mixtures.

Free Gossypol in Cottonseed Flour (22)

Pretreat all aniline used in this procedure by distilling the aniline over zinc dust (using an air condenser). Discard the first and last 10% of the distillate. Store in a glass-stoppered brown bottle in a refrigerator. Redistill when the reagent blank exceeds an absorbance of 0.022.

Grind 50 g of cottonseed through a Bauer Bros. No. 148 laboratory mill equipped with a No. 6912 plate. Grind with the plates separated so that the hulls are broken. Separate the meats from the hulls and lint by screening, then grind the meats through a Wiley mill equipped with a 1-mm screen. Do not preheat the cottonseed, and avoid heating the sample during grinding. If the sample is a cake, pellet, or meal, directly grind 50 g through a Wiley mill.

Weigh 1 g of sample into a small beaker and add 25 mL of acetone. Stir for 2 min and filter into a test tube. To one half the filtrate add a pellet of solid sodium hydroxide and heat on a steam bath for 2–3 min, swirling

frequently. The appearance of a deep orange-red to red color indicates the presence of dianilinogossypol. In this case, use Method B. If the light-yellow filtrate does not turn red on treatment with sodium hydroxide, use Method A.

Method A

Weigh 1 g of sample and transfer to a 250-mL Erlenmeyer flask. Cover the bottom of the flask with glass beads 6 mm in diameter. Pipette 50 mL of 7:3 acetone-water into the flask, stopper, and shake on a mechanical shaker for 1 hr.

Filter the solution through dry, medium-porosity filter paper into a small glass-stoppered flask, discarding the first few milliliters of filtrate. Pipette two 10-mL portions of filtrate into separate 25-mL volumetric flasks. Dilute the first portion to volume with 8:2 isopropyl alcohol-water. To the second aliquot, add 2 mL of redistilled aniline and heat in a boiling bath for 30 min, along with a reagent blank containing 2 mL of aniline and 10 mL of 7:3 acetone-water. Remove both solutions from the bath, cool to room temperature, and dilute to volume with 8:2 isopropyl alcohol-water. Measure the absorbance of the two sample solutions and the blank against isopropyl alcohol at the maximum near 440 nm. Calculate the corrected absorbance (difference between the absorbance of the samples, corrected for the blank) and compare against standards similarly prepared.

Method B

Prepare an aqueous acetone aniline solution by mixing 700 mL of acetone, 300 mL of water, and 0.5 mL of redistilled aniline. Do not use after one day.

Weigh 1 g of sample into a 250-mL Erlenmeyer flask and cover the bottom of the flask with 6-mm-diameter glass beads. Pipette 50 mL of the aqueous acetone-aniline solution into the flask. Stopper the flask and shake on a mechanical shaker for 1 hr.

Filter the solution through dry, medium-porosity filter paper into a glass-stoppered flask. Within 3 hr. pipette 10-mL aliquots into two separate 25-mL volumetric flasks. Dilute the first aliquot to volume with 8:2 isopropyl alcohol-water, mix well, and allow to stand for 25–30 min.

Treat the second aliquot and a reagent blank exactly as described in Method A. Determine the net absorbance and compare against standards similarly prepared.

Total Gossypol in Cottonseeds and Cottonseed Meals (1)

Prepare a 1.79×10^{-2} *M* iron (III) solution by dissolving hydrated ferric nitrate [$Fe(NO_3)_3 \cdot 9H_2O$, BDH AnalaR] in 40:60 (v/v) hexane–isopropyl alcohol containing a few drops of concentrated hydrochloric acid.

Prepare complexing agent solution by mixing 2 mL of 3-amino-1-propanol (E. Merck) with 10 mL of glacial acetic acid. Cool the solution to room temperature and dilute it to 100 mL with dimethylformamide in a volumetric flask.

Prepare a 4.821×10^{-3} *M* standard solution of gossypol by dissolving gossypol acetic acid (Sigma Chemical Co.) in complexing agent solution. Dilute 10-mL of this solution to 100 mL with 40:60 (v/v) hexane–isopropyl alcohol in a volumetric flask.

Grind dehulled cottonseeds or cottonseed presscake in a mill to pass through a 2-mm screen. Weigh a quantity of sample containing 2–20 mg of total gossypol, and transfer it to a 100-mL Erlenmeyer flask, then add 10 mL of complexing agent. Heat the mixture in a boiling water bath for 30 min, cool it, then dilute it to about 30 mL with 40:60 (v/v) hexane–isopropyl alcohol. Filter the solution and dilute it to 50 mL with hexane–isopropyl alcohol in a volumetric flask.

Transfer an aliquot of this solution containing 0.2–2.0 mg of gossypol to a 25-mL volumetric flask and add 2 to 4 drops of 5 *M* HCl to it. Add 5 mL of iron (III) to the flask, mix the sample well, then allow it to stand for 5 min. Add 1 mL of distilled water, then immediately dilute to volume with 40:60 (v/v) hexane–isopropyl alcohol. Measure the absorbance of the colored solution at 620 nm against the hexane–isopropyl alcohol reference mixture.

Oxalic Acid in Ferrous Gluconate (23)

Dissolve 1 g of sample in 10 mL of water, add 2 mL of hydrochloric acid, and transfer it to a separatory funnel. Extract successively with 50 mL and 20 mL of ethyl ether. Combine the extracts, add 10 mL of water, and evaporate the ether on a steam bath. Add one drop of 36% acetic acid and

1 mL of 5% calcium acetate solution. Acceptable ferrous gluconate should show no turbidity within 5 min.

Reducing Sugars in Ferrous Gluconate (23)

Prepare a cupric tartrate solution by dissolving 34.66 g of cupric sulfate, $CuSO_4 \cdot 5H_2O$, in water and diluting to 500 mL. Then dissolve 173 g of potassium sodium tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$, and 50 g of sodium hydroxide in water and dilute to 500 mL. Mix equal amounts of these two solutions when required.

Dissolve 0.5 g of sample in 10 mL of water, warm, and make the solution alkaline with 1 mL of 1:9 ammonium hydroxide. Pass hydrogen sulfide into the solution to precipitate iron and allow the mixture to stand for 30 min to coagulate the precipitate. Filter and wash the precipitate with two 5-mL portions of water. Acidify the combined filtrate and washings with 1:9 hydrochloric acid. Add 2 mL of 1:9 hydrochloric acid in excess. Boil the solution until the vapors no longer darken lead acetate paper and then concentrate to about 10 mL. Cool. Add 5 mL of 10.5% sodium carbonate solution and 20 mL of water. Filter the solution and adjust the volume of the filtrate to 100 mL. To 5 mL of this solution add 2 mL of cupric tartrate solution and boil for 1 min. Acceptable ferrous gluconate shows no red precipitate within 1 min.

Ethoxyquin (1,2-Dihydro-6-Ethoxy-2,2,4-Trimethylquinoline) in Paprika (60)

Weigh 2 g of sample into a 15-mL capped centrifuge tube, then extract it with three 10-mL portions of hexane. Centrifuge the sample at 500 rpm for 5 min. after each extraction to settle particulates. Decant the extracts into a 125-mL separatory funnel, add 15 mL of 0.3 *N* HCl to the combined extracts, then shake the funnel gently for 1 min. Allow the layers to separate completely, then transfer the aqueous (lower) layer to a 60-mL separatory funnel. Extract the hexane solution with a second 15-mL portion of 0.3 *N* HCl, then combine the aqueous layers. Add 2 mL of 4.8 *N* NaOH to the funnel, then immediately extract the solution with 10 mL of hexane. Draw off the aqueous (lower) layer and discard. Drain the hexane layer into a 100-mL round-bottom flask, rinse the separatory

funnel with 5 mL of hexane, and add the rinse to the flask. Add 5 mL of acetonitrile to the extract, then evaporate the solution to dryness on a flash evaporator, using a 35°C water bath. Dissolve the residue in 10 mL of acetonitrile, then filter the solution through a 1-μm Fluoropore filter. Chromatograph 10 μL of filtrate using a 25-cm × 4.6-mm ID DuPont Zorbax ODS column, 0.01 *M* ammonium acetate-acetonitrile (3:7) as the mobile phase at a flow rate of 1.2 mL/min, and a UV detector set at 254 nm. Ethoxyquin elutes in about 5 min.

Acid-soluble Substances in Talc (74)

Digest 1.00 g of sample with 20 mL of 3 *N* hydrochloric acid at 50°C for 15 min.

Add water to restore original volume, mix, and filter. Add 1 mL of 2 *N* sulfuric acid to 10 mL of filtrate, evaporate to dryness, and ignite to constant weight.

Reaction and Soluble Substances in Talc (74)

Boil 10 g of sample with 50 mL of water for 30 min; periodically add water to maintain approximately the original volume. Filter; the filtrate is neutral to litmus paper. Evaporate one-half the filtrate to dryness, and dry at 105°C for 1 hr.

Water-soluble Iron in Talc (74)

Using hydrochloric acid, slightly acidify the second half of the filtrate obtained in the test described above. Add 1 mL of fresh 10% potassium ferrocyanide solution. If the sample is of acceptable quality, the liquid should not turn blue.

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13

HOMOLOGOUS, ISOMERIC, AND OTHER RELATED COLORANTS

N-Ethylalanine (II) and N-Ethyl-N-Benzylalanine (III) Subsidiary Colors in FD&C Blue No. 1 (23)

Chromatograph samples by HPLC using a 15-cm × 4.6-mm Zorbax ODS column, and gradient elution from 40 to 100% methanol in 0.5% $(NH_4)_2CO_3$ solution at 5%/min. Monitor the eluate at 628, 618, and 633 nm, for FD&C Blue No. 1, 2, and 3, respectively.

Lower-Sulfonated Colors in FD&C Blue No. 1

Solvent Extraction Procedures (13, 15)

Prepare a salt-acetate solution as follows. Dissolve 125 g of sodium chloride and 13.6 g of sodium acetate in water, add 12 mL of glacial acetic acid, and dilute to 500 mL with water.

Prepare a 0.1% aqueous sample solution. To a 10-mL aliquot add 40 mL of the salt-acetate solution and extract successively in three sep-

aratory funnels, each containing 100 mL of isoamyl alcohol. Wash the alcohol extracts with 100-mL portions of the salt-acetate solution until the washings are colorless. Pass each wash successively through the funnels in the same order as described above. Dilute the alcohol layers with one or more volumes of hexane. Remove the dye from the alcohol-hexane mixtures by washing with several 10-mL portions of water, passing each washing through the funnels as described above. To the combined aqueous extracts add ammonium acetate to a concentration of about 0.04 *N*. Determine the subsidiary colors present spectrophotometrically by comparison with the spectra of a standard solution of Guinea Green B, CI No. 42085 (formerly FD&C Green No. 1).

Two other schemes have been used to separate lower-sulfonated colors in FD&C Blue No. 1. In the first, the dye mixture is dissolved in water and the solution is acidified. This solution is extracted with isoamyl alcohol, which in turn is washed with several portions of 1:99 hydrochloric acid. The trisulfonated colors plus some of the disulfonated compounds pass into the aqueous solution and the monosulfonated and disulfonated compounds remain in the amyl alcohol. After dilution with petroleum ether, the disulfonated compounds are extracted from the amyl alcohol layer with water.

If no trisulfonated compounds are present, another scheme can be applied. The dye mixture is first extracted with hot benzene; the unsulfonated compounds are dissolved and the mono- and disulfonated substances remain in the residue. The disulfonated compounds (with traces of monosulfonated substances) are extracted from the residue with hot water. The aqueous solution is acidified and extracted with isoamyl alcohol, the alcohol extract is diluted with petroleum ether, and the disulfonated compounds are extracted with water. The monosulfated colors remain in the alcohol-ether solution.

Thin-layer chromatographic procedure (5, 48)

Dissolve 1 g of dye in water and dilute to 50 mL in a volumetric flask. Streak 0.1 mL of this solution about 2 cm from the edge of a 20-cm × 20-cm silica gel G (200 μ thick) chromatographic plate. Dry the plate for 5 min at 100°C and then develop using isoamyl alcohol–acetonitrile–methyl ethyl ketone–water-NH_4OH (50:50:15:5:5) as the mobile phase. Leach the colors from the plate with EtOH and determine spectrophotometrically.

5.7′-Disulfo-3,3′-Dioxo-$\Delta^{2,2'}$-Biindoline and 5-Sulfo-3,3′-Dioxo-$\Delta^{2,2'}$-Biindoline in FD&C Blue No. 2 (45)

Prepare chromatographic solvents as follows. Mix 1 volume of concentrated HCl with 8 volumes of 2.5% (w/v) aqueous hydroxylamine hydrochloride. In a separatory funnel, mix 2 volumes of this reagent with 1 volume of butanol and 1 volume of chloroform. Shake for 1 min and allow the layers to separate. The lower (organic) layer is the mobile phase and the upper (aqueous) layer is the stationary phase.

Add 7 mL of stationary phase to 12 g of Johns-Manville Celite 545 and mix thoroughly. Transfer to a 40-cm × 2.5-cm-ID chromatographic tube and pack firmly with a plunger.

Dissolve 6 mg of FD&C Blue No. 2 in 5 mL of stationary phase, add 10 g of Celite, and mix thoroughly. Transfer the mixture to the chromatographic tube and pack firmly. Dry wash the beaker with about 1 g of Celite and pack the wash into the column.

Develop the chromatogram with mobile phase. The monosulfonated color elutes first, followed by the isomeric color. Collect each as a separate fraction and dilute each with an equal volume of chloroform.

Extract the solution of monosulfonated color with three 5-mL portions of water and dilute the combined extracts to 25 mL with water. Extract the solution of isomeric color with three 15-mL portions of water and dilute the combined extracts to 50 mL with water. Using 1-cm cells, obtain the visible spectra of the solutions from 700 nm to 400 nm and compare against standards.

Trisulfoindigo in FD&C Blue No. 2 (3)

Apparatus and Reagents

Liquid chromatograph—A Varian Model 5060 equipped with a Vista 401 data system, a Varian Model 5000 detector set at 254 nm, an Altex Model 155-10 UV/VIS detector set at 610 nm, a 20-μL loop injector, and a 25-cm × 4.6-mm ID, 5-μm Supelcosil LC-18 column (Supelco Inc).

Mobile phase A—1.5% Ammonium acetate (w/v)/0.5% acetonitrile (v/v) in water.

Mobile phase B—1.5% Ammonium acetate (w/v)/50% water (v/v) in acetonitrile. To prepare, use the appropriate amount of ammonium

acetate and water. Dilute this mix to volume with acetonitrile and mix, then add more acetonitrile as needed to compensate for any volume reduction on mixing.

Sample Preparation and Resolution

Dissolve 0.5g of sample in 100 mL of water. With mobile phase A flowing at 1 mL/min, inject 20 μL of solution. Program from 0% to 60% B in A linearly in 25 min, then hold at 60% B in A for 5 min more. Impurities elute in the following order: isatin-5-sulfonic acid, 5,5′,7-trisulfoindigo, unknown, 5,5′-disulfoindigo, 5,7′-disulfoindigo, 5-monosulfoindigo.

Lower-Sulfonated and Isomeric Colors in FD&C Green No. 3 (50)

These colorants can be determined using the solvent extraction and the TLC procedures described for FD&C Blue No. 1 (see pp. 379–380).

Lower-Iodinated Colorants in FD&C Red No. 3

High-performance Liquid Chromatographic Procedures (10)

Weigh 0.200 g of sample into a 100-mL volumetric flask, dilute to volume with water, and mix well.

Using an Altex Scientific Inc. Model 420 chromatograph equipped with a microprocessor and two Model 110-A pumps, a Waters Associates, Inc. Model 440 dual-wavelength detector set at 436 nm and 546 nm, and a DuPont Zorbax C-8 25-cm × 4.6-mm ID column, chromatograph 20 μL of sample at a flow rate of 1 mL/min using either of the following methods.

Method 1

Mobile phase A, 0.1 M ammonium acetate; mobile phase B, methanol. Program from 45% to 66% B in 20 min, 66% to 100% B in 0 min more, then hold at 100% B for 4 min. Components elute in the following order: fluorescein; 4-iodofluorescein plus 4,5-diiodofluorescein; 2-iodofluorescein; 2,5-diiodofluorescein; 2-7-diiodofluorescein; 2,4,5-triiodofluorescein; 2,4,7-triiodofluorescein; FD&C Red No. 3.

Method 2

Mobile phase A, 2% v/v glacial acetic acid in water; mobile phase B, acetonitrile. Elute sample at 43% B for 15 min, program to 100% B in 3 min more, then hold at 100% B for 4 min. Components elute in the following order: fluorescein; 4-iodofluorescein; 2-iodofluorescein; 4,5-diiodofluorescein; 2,5-diiodofluorescein; 2-7-diiodofluorescein plus 2,4,5-triiodofluorescein; 2,4,7-triiodofluorescein plus FD&C Red No. 3.

Paper-chromatographic procedure (56)

Prepare a mobile phase by mixing 400 mL of methyl ethyl ketone, 100 mL of acetone, 100 mL of water, and 1 mL of concentrated ammonium hydroxide. Place two 3-in.-wide blotting-paper drapes in an 18-in. × 6-in. tank, one on each side, add the mobile phase to the tank, and allow time to equilibrate. Prepare a 0.1% sample solution in 2:98 ammonium hydroxide. Apply a 0.1 mL aliquot as a 0.5-in. × 2.5-in. band to 3-in.-wide Schleicher & Schuell No. 2043 chromatographic paper, 1.5 in. from the bottom of the strip. Suspend the strip in the tank so that the lower edge dips 0.5 in. into the mobile phase. Develop for 6 hr protected from light. Remove the strip and dry in the dark. Extract each spot from the chromatogram with small amounts of 1:199 ammonium hydroxide. Dilute as needed, filter, and determine the individual colors spectrophotometrically. The order of elution, absorption maxima, and approximate absorptivities of the disodium salts are listed in the table that follows.

Order of Elution	Color	Absorption Maximum (in nm)	Absorptivity of Disodium Salts (in L/g-cm)
1	2,4,5,7-Tetraiodofluorescein	527–530	108[a]
2	2,4,7-Triiodofluorescein	517–520	140
3	2,4,5-Triiodofluorescein	516–519	116
4	2,7-Diiodofluorescein	511–513	179
	2,5-Diiodofluorescein	509–511	145
5	4,5-Diiodofluorescein	507–509	122
6	2-Iodofluorescein	501–503	193
7	4-Iodofluorescein	497–500	154
8	Fluorescein	491–493	228

[a]For monohydrate.

Column-chromatographic procedure (17)

Prepare ethanol wash solution by diluting 75 mL of 95% ethanol to 100 mL with water. Prepare each of the following mobile phases:

Mobile phase No. 1—350 mL of 25% aqueous sodium chloride solution containing 1.75 mL of concentrated ammonium hydroxide.
Mobile phase No. 2—500 mL of 2% aqueous sodium sulfate solution containing 2.5 mL of concentrated ammonium hydroxide.
Mobile phase No. 3—1000 mL of 1% aqueous sodium sulfate solution containing 5 mL of concentrated ammonium hydroxide.
Mobile phase No. 4—600 mL of 0.5% (v/v) ammonium hydroxide.
Mobile phase No. 5—400 mL of 60% ethanol containing 2 mL of ammonium hydroxide.

Use a glass chromatographic column equipped at the top with the mobile phase distribution system shown in Fig. 13.1. Place a glass-wool plug or a disc of Lectromesh screen in the constriction above the column tip. Fill the tube with 3 in. of water; add 0.5 g of dry Whatman Column Chromedia CF11 and allow to settle. Add a slurry of 16 g of Solka Floc BW-100 in 150 mL of water to the column. Open the outlet and drain the liquid nearly to the top of the packing. Add 100 mL of the ethanol wash solution and drain nearly to the top of the packing. Add 100 mL of mobile phase No. 1, washing the cellulose from the sides of the tube and keeping a level surface on the column. Drain the liquid to about 1 in. above the column packing. Wrap the 1-in.-diameter section of the column with aluminum foil to protect the sample from light.

Dissolve a 0.1-g sample in 100 mL of 1:199 ammonium hydroxide. Pipette 5 mL of the sample solution onto the column, mixing it with the mobile phase present and taking care not to disturb the surface of the packing. Drain the solution just to the packing surface. Carefully wash down the sides of the tube with mobile phase No. 1 and drain into the column. Add 20 mL of mobile phase No. 1 and about 3 g of the Chromedia to the column. When the cellulose has settled to form a protective cap over the surface, fill the column with mobile phase No. 1. Place any remaining mobile phase No. 1 in a flask and connect it to tube No. 1. Place mobile phases Nos. 2–5 in suitable flasks and connect them to the appropriate tubes. Elute the column successively with mobile phases

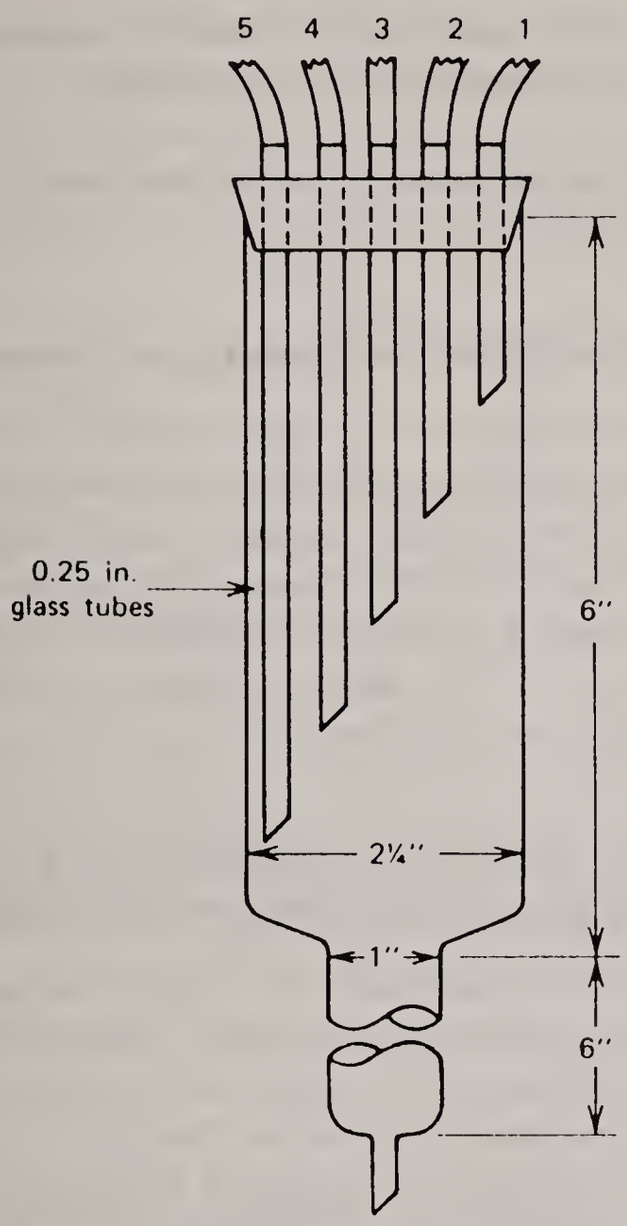

Figure 13.1. Mobile phase distribution system for column-chromatographic determination of subsidiary colors in FD&C Red No. 3 (numbers above inlet tubes refer to serial number of respective mobile phase).

Nos. 1–5. Collect the individual bands eluted (protect from light) and determine the colors spectrophotometrically. The order of elution and the identity of the subsidiary colors are as follows:

Moble phase No. 1—Band 1, unidentified color; Band 2, fluorescein.
Mobile phase No. 2—Band 3, mixture of 2- and 4-monoiodofluoresceins; Band 4, mixture of 2,4-, 2,5-, and 2,7-diidofluoresceins.
Mobile phase No. 3—Band 5, mixture of 4,5-diiodofluorescein and 2,4,7-triiodofluorescein; Band 6, 2,4,5-triiodofluorescein.

Mobile phase No. 4—Band 7, 2,4,5,7-tetraiodofluorescein.
Mobile phase No. 5—Band 8, unidentified color.

The amount of the individual colors can be estimated using the absorptivities given on p. 384.

Subsidiary Colorants in Xanthene-Group Dyes (36)

Fluorescein and its mono-, di-, tri- and tetrachloro-, bromo- and iodo-derivatives can be separated on two 50-cm × 2.1-mm columns in series, each containing Hitachi Gel 3011 (polystyrene-divinylbenzene copolymer) beads maintained at 35°C. Detection is at 254 nm. The mobile phases giving the best separations contain 0.05 *M* $HClO_4$ in H_2O–acetonitrile–methanol (3:3:14 for chloro-derivatives, 1:2:7 for bromo-derivatives, and 3:8:29 for iodo-derivatives.)

2-(2,4-Xylylazo)-1-Naphthol-4-Sulfonic Acid and 2-(5-Sulfo-2,4-Xylylazo)-1-Naphthol in FD&C Red No. 4 (17)

Extract 150 mL of *n*-butanol with three 50-mL portions of 10% sodium hydroxide and then wash the butanol with 50-mL portions of water until neutral (prepare fresh daily). Mix 100 mL of the washed butanol with 100 mL of carbon tetrachloride in a separatory funnel and add 100 mL of 2% hydrochloric acid containing 20 mg/mL of hydroxylamine hydrochloride. Shake the mixture for 3 min and then allow the layers to separate. Use the upper layer as the stationary phase and the lower layer as the mobile phase.

Mix 10 g of Celite 545 with 5 mL of the stationary phase, and firmly pack the mixture into a 45-cm × 2.5-cm chromatographic column.

Dissolve 0.05 g of sample in 25 mL of the stationary phase, mix a 5-mL aliquot of this solution with 10 g of Celite, and pack the mixture on the top of the column. Elute with mobile phase and collect the subsidiary color in the leading edge of the eluate.

If the subsidiary color is red, dilute it to 25 mL with the mobile phase and determine the color spectrophotometrically against a standard solution of 2-(2,4-xylylazo)-1-naphthol-4-sulfonic acid. If the eluate is yellow, transfer it to a separatory funnel and add an equal volume of chloroform. Wash with two 10-mL portions of 10% ammonium sulfate

solution. Add a second volume of chloroform and extract with two 10-mL portions of 10% ammonium sulfate solution containing 10mg/mL of sodium hydroxide. Adjust the aqueous extract to 25 mL with the extracting solution and determine the color spectrophotometrically against a standard solution of 2-(5-sulfo-2,4-xylylazo)-1-naphthol.

2-(4-Sulfo-1-Naphthylazo)-1-Naphthol-4-Sulfonic Acid in FD&C Red No. 4 (17)

Prepare mobile phase by dissolving 50 g of sodium chloride in water containing 0.5 mL of concentrated ammonium hydroxide and then diluting this solution to 1 liter with water. Slurry Solka Floc BW-40 in water, pack two 1-in. chromatographic columns 12 in. high with the slurry, and wash each with 50 mL of mobile phase.

Dissolve 0.025 g of sample in 10 mL of water and add it to the first column. When the color settles on the column, wash it with mobile phase until most of the FD&C Red No. 4 is eluted. Strip the remaining color from the column with water and collect. Add 20 g of solid sodium chloride to each 100 mL of this solution and transfer it to the second column. Elute the remaining FD&C Red No. 4 with mobile phase and then elute the subsidiary color with water and determine it spectrophotometrically.

2-(3-sulfo-2,6-Xylylazo)-1-Naphthol-4-Sulfonic Acid in FD&C Red No. 4 (55)

Prepare mobile phase by dissolving *800 g* of anhydrous sodium sulfate in 5 liters of 0.2 *M* hydrochloric acid. Slurry powdered cellulose (Whatman Column Chromedia CF11 or equivalent) in mobile phase, pour sufficient slurry into a 24-in. × 2-in. chromatographic column to make a 15-in. bed, and wash the column with 100 mL of mobile phase.

Prepare a 0.5% aqueous sample solution and pipette a 20-mL aliquot onto the column. Elute the column with mobile phase; collect the fraction containing the subsidiary color, which elutes before FD&C Red No. 4. Saturate this fraction with sodium sulfate and pass the resulting solution through a 1-in.-diameter column packed with a 3-in. bed of cellulose. Elute the color with 100 mL of water and determine it spectrophotometrically against a standard solution.

2-(6-Sulfo-2,4-Xylylazo)-1-Naphthol-4-Sulfonic Acid in FD&C Red No. 4 (20)

Slurry Solka Floc BW-40 in water, pack a 1-in.-diameter chromatographic column 12-in. high with the slurry, and wash it with 50 mL of a 20% sodium sulfate solution. Prepare a 0.5% aqueous sample solution, pipette a 10-mL aliquot onto the column, and elute with 20% sodium sulfate solution. FD&C Red No. 4 is quantitatively adsorbed. Collect the subsidiary color eluted and determine spectrophotometrically against a standard.

This compound can also be separated from FD&C Red No. 4 by paper chromatography using 200:88:2:40 butanol–water–ammonium hydroxide–ethanol or 300:150:5:80 butanol–water–acetic acid–ethanol as the mobile phase.

Subsidiary Colors in FD&C Red No. 4 (17)

Thin-layer Chromatography

Using a microliter syringe, spot about 4 mg of color as a band onto a 20-cm × 20-cm TLC plate coated with 250 μm of silica gel G. Elute with ethyl acetate–ethyl alcohol–diethylamine-water (55:20:10:10) until the solvent front nears the top of the plate. Leach the colorants from the plate with water or water-alcohol (1:1) and determine spectrophotometrically.

The order (top to bottom) in which the colorants appear on the plate is:

2-(5-Sulfo-2,4-xylylazo)-1-naphthol.
2-(2,4-Xylylazo)-1-naphthol-4-sulfonic acid.
2-(5-Sulfo-2,4-xylylazo)-1-naphthol.
2-(6-Sulfo-2,4-xylylazo)-1-naphthol-4-sulfonic acid.
FD&C Red No. 4.
2-(4-Sulfo-2,5-xylylazo)-1-naphthol-4-sulfonic acid.
2-(3-Sulfo-2,6-xylylazo)-1-naphthol-4-sulfonic acid.

Paper Chromatography

Prepare a mobile phase by mixing 70 volumes of methyl ethyl ketone, 30 volumes of acetone, 30 volumes of water, and 0.2 volume of concentrated ammonium hydroxide. Use a tank suitable for ascending chromatography and saturate its atmosphere with mobile phase. Prepare a 1% aqueous sample solution and apply 0.1 mL within a 18 cm × 0.7-cm rectangle, 2.5

cm from the bottom of a 20-cm × 20-cm sheet of Whatman No. 1 chromatographic paper. Allow the paper to dry at or below 50°C. Mount the sheet in the tank so that the mobile phase is 1 cm below the baseline of the sheet. Elute to a height of 17.5 cm or until the separation is satisfactory. Visually compare the chromatogram with knowns similarly prepared or extract the colors from the paper and determine spectrophotometrically.

Screening Procedures for Subsidiary Colors in FD&C Red No. 40

Paper-chromatographic Method (28)

Apply 0.01 mL of 5% aqueous sample solutions as 1.5-in. bands on Whatman No. 1 chromatographic paper (10 in. × 10 in.). Dry for 15 min in a 50°C air oven and then develop for about 2 hr in a 10-in. × 10-in. glass chromatographic tank (Arthur H. Thomas Co., 3108-B05), using 130 mL of methyl ethyl ketone-acetone-water (70:30:30) as the mobile phase. Compare with standards run simultaneously on the same sheet. Figure 13.2. is a schematic representation of the resolution of a synthetic mixture containing subsidiary colors that could be present.

Thin-layer-chromatographic Procedure (8, 17)

Spot 3 μL of a 2% sample solution and the appropriate standards on a 20-cm × 20-cm thin-layer plate coated with a 250 μm layer of silica gel G. Develop in a mobile phase composed of isoamyl alcohol–1,4-dioxane-acetonitrile–ethyl acetate–water–ammonium hydroxide (10:10:10:10:10:2). Compare sample and standards visually or extract from the plate with water and examine spectrophotometrically. Lower-sulfonated colors appear above the main band, whereas higher-sulfonated colors appear below the main band.

3-Hydroxy-4-[(2-Methoxy-5-Methyl-4-Sulfophenyl)Azo]-2,7-Naphthalenedisulfonic Acid (*R*-Salt + CSA) and 7-Hydroxy-8-[(2-Methoxy-5-Methyl-4-Sulfophenyl)Azo]-1,3-Naphthalene-disulfonic Acid (*G*-Salt + CSA) in FD&C Red No. 40 (28)

Weigh about 200 g of Celite 545 (Fisher C-212) into a Petri dish and place in a desiccator containing 25 mL of General Electric SC-77 Dri-Film. Let stand until the Celite no longer wets when mixed with water (≥3 hr).

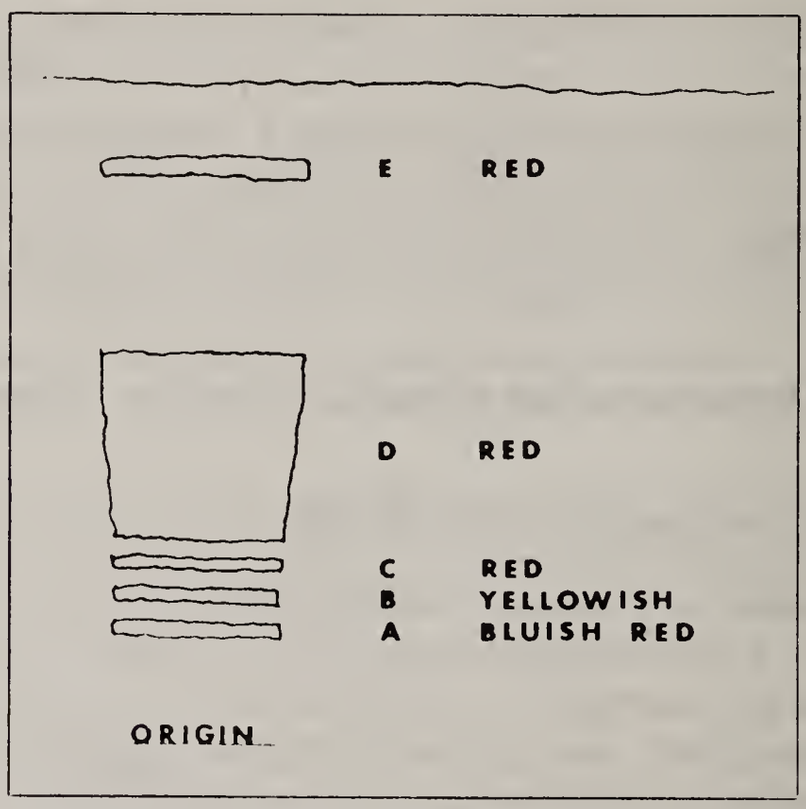

Figure 13.2. Paper-chromatographic resolution of subsidiary colors: A, *R*-salt + CSA dye; B, *G*-salt + CSA dye; C, (6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl) azo] -8- (2-methoxy-5-methyl-4-sulfophenoxy) -2-naphthalensulfonic acid); D, FD&C Red No. 40 and E, cresidine + Schaeffer's dye and CSA + 2-naphthol dye.

Place 100 mL of BuOH, 100 mL of CCl_4, and 100 mL of dilute HCl (1 + 4) into a separatory funnel, shake for 3 min, and allow the layers to separate. The lower layer is the stationary phase and the upper layer is the mobile phase.

Mix 15 g of silane-treated Celite with 7.5 mL of stationary phase. Using a rammer, pack firmly into an 8-in. × 1-in.-OD glass chromatographic tube. Wash the column with 15 mL of mobile phase. (This wash frequently flushes a yellow color from the column just ahead of the red subsidiary dye. The yellow color is leached from the packing itself and should be discarded.

Prepare a 0.2% sample solution in mobile phase. Pipette 3 mL onto the column and let drain to the surface of the packing. Elute the column with mobile phase. Collect the desired fraction in a 25-mL volumetric flask and dilute to volume with mobile phase. Similarly prepare and elute a blank column to which no color has been added.

Record the visible spectra of the sample and blank from 680 nm to 480 nm in a 1-cm absorption cell against mobile phase. The *G*-salt + CSA dye

has an absorption maximum near 503 nm. The *R*-salt + CSA dye has an absorption maximum near 512 nm.

$$\text{Percent } R\text{-salt + CSA dye (mw 599.48)} = A \times 8.41$$
$$\text{Percent } G\text{-salt + CSA dye (mw 599.48)} = A \times 9.04$$

where 8.41 is equal to 100/(49.4 × 1 × 0.24); 9.04 is equal to 100/(46.1 × 1 × 0.24); 100 is factor for conversion to percent; 1 is cell path length in cm; 0.24 is effective sample concentration in g/liter; 49.4 and 46.1 are absorptivities in liters/g-cm of *R*-salt + CSA dye and *G*-salt + CSA dye, respectively; and *A* is blank-corrected absorbance (also corrected for column blank where necessary) of the sample fraction at the appropriate absorption maximum.

6-Hydroxy-5-[(2-Methoxy-5-Methylphenyl)Azo]-2-Naphthalenesulfonic Acid (Cresidine + Schaeffer's Salt) and 4-[(2-Hydroxy-1-Naphthyl)Azo]-5-Methoxy-2-Methylbenzenesulfonic Acid (CSA + β-Naphthol) in FD&C Red No. 40 (28)

Add 100 mL of BuOH, 100 mL of CCl_4, and 100 mL of dilute HCl (2 + 98) to a 500-mL separatory funnel. Shake for 3 min and let the layers separate. The upper layer is the stationary phase and the lower layer is the mobile phase.

Pipette 5 mL of stationary phase onto 10 g of Celite 545; mix well. Pack this mixture firmly into an 8-in. × 1-in.-OD glass chromatographic column. Pipette 5 mL of a 0.2% sample solution (in stationary phase) onto a second 10-g portion of Celite, mix well, and then pack firmly into the column with a rammer. Elute the column with mobile phase. Collect the desired fraction in a 25-mL volumetric flask and dilute to volume with mobile phase.

Immediately record the visible spectrum of the sample and blank (similarly prepared) from 680 nm to 480 nm in a 1-cm absorption cell against mobile phase.

$$\text{Percent cresidine + Schaeffer's dye (mw 394.4)} = A \times 4.19$$
$$\text{Percent CSA + β-naphthol dye (mw 394.4)} = A \times 3.88$$

where 4.19 is equal to 100/(59.7 × 1 × 0.40); 3.88 is equal to 100/(64.4 × 1 × 0.40); 100 is factor for conversion to percent; 1 is cell path length

in cm; 0.40 is effective sample concentration in g/liter; 59.7 and 64.4 are absorptivities in liters/g-cm of cresidine + Schaeffer's dye and CSA + β-naphthol dye, respectively; and A is blank-corrected absorbance (also corrected for column blank where necessary) of the sample fraction at the absorption maximum near 508 nm.

Screening Procedures for Subsidiary Colors in FD&C Yellow No. 5

Paper Chromatography (9)

This procedure separates the lower-sulfonated colors as well as the ethyl ester of the parent compound from FD&C Yellow No. 5. It does not separate the lower-sulfonated colors from each other.

Prepare the mobile phase by mixing 70 volumes of methyl ethyl ketone, 30 volumes of acetone, and 30 volumes of water. Use a tank suitable for ascending chromatography and saturate its atmosphere with the mobile phase. Prepare a 1% aqueous sample solution and apply 0.1 mL within a 18-cm × 0.7-cm rectangle, 2.5 cm from the bottom of a 20-cm × 20-cm sheet of Whatman No. 1 chromatographic paper. Allow the paper to dry at or below 50°C. Mount the sheet in the tank so that the mobile phase is 1 cm below the baseline of the sheet. Elute to a height of 17.5 cm or until the separation is satisfactory. Visually compare the chromatogram with knowns similarly prepared, or extract the colors from the paper and determine spectrophotometrically.

Thin-layer Chromatography (17)

Dissolve 2 g of sample in 100 mL of water. Spot 3 μL of this solution and the appropriate standards onto a silica gel thin-layer plate. Dry the plate and elute using 1,4-dioxane-isoamyl alcohol-water-ammonium hydroxide (10:10:4:1). Leach the colorants from the plate and determine spectrophotometrically.

Lower-Sulfonated Colors in FD&C Yellow No. 5

Liquid-liquid Extraction Method (12)

Dissolve 0.2 g of sample in 100 mL of warm water. To 50 mL of this solution add 1 mL of concentrated hydrochloric acid and extract the solution successively in three separatory funnels, each containing 50 mL

of amyl alcohol. Wash the alcohol extracts by shaking successively with 50-mL portions of 0.25 *N* hydrochloric acid until the washings are practically colorless. Pass each acid portion through the funnels in the order used for the original alcohol extraction. Dilute the alcohol extracts in each funnel with 1–2 volumes of petroleum ether and extract the lower-sulfonated colors by washing with several 10–20-mL portions of water. Pass each portion through the funnels in the order reverse to that previously followed. Transfer the water solution to a 100-mL volumetric flask, add about 1 g of solid ammonium acetate, and dilute to volume with water. Determine the compounds present spectrophotometrically at 434 nm against a standard.

Column-chromatographic Method (17)

Transfer 100 mL of BuOH, 100 mL of CCl_4, and 100 mL of dilute (1 + 19) HCl to a separatory funnel. Shake the mixture for 3 min and then allow the layers to separate. Use the lower organic layer as the mobile phase and the upper aqueous layer as the stationary phase.

Mix 5 g of Celite 545 and 2.5 mL of stationary phase. Place a pledget of glass wool in the constriction of a 250-mm × 22-mm-ID glass chromatographic column then firmly pack all but a little of the mixture into the column.

Dissolve 0.04 g of color in 5 mL of stationary phase. Mix the solution with 5 g of Celite and then pack the mixture firmly into the column. Rinse the beaker with the reserved Celite and pack the rinse into the column. Elute with mobile phase and collect the lower-sulfonated colors which elute first.

Transfer the eluate containing the subsidiary colors to a separatory funnel, add an equal volume of hexane, and extract the color with several small portions of water. Make to a known volume and examine spectrophotometrically.

3-Carboxy-5-Hydroxy-1-p-Sulfophenyl-4-Phenylazo-Pyrazole, Disodium Salt in FD&C Yellow No. 5 (27)

Prepare 0.5 *M* TBAP ion-pair reagent by adding 25 mL of 1.54 *M* aqueous tetra-*n*-butylammonium hydroxide to 52 mL of 1.11 *M* aqueous KH_2PO_4, then filtering the mixture through a 0.45-μm Millipore filter.

Weigh 0.15 g of sample into a 100-mL volumetric flask, add 1.0 mL of 0.5M TBAP, then dilute to 100 mL with water and mix well.

Chromatograph 20 μL of sample using an E. Merck Hibar II LiChrosorb RP-18, 10 μm (4.6-mm × 25-cm) column at ambient temperature, and methanol-water (56.5 + 43.5) containing 0.005 *M* TBAP as the mobile phase. Use a flow rate of 1.0 mL/min and monitor the eluate at 433 nm. Approximate elution times in minutes are: FD&C Yellow No. 5 (4); 3-carboxy-5-hydroxy-1-*p*-sulfophenyl-4-phenylazopyrazole (8.3); unknown (11.5). The unknown is possibly 3-carboxy-5-hydroxy-1-phenyl-4-*p*-sulfophenylazopyrazole.

Screening Procedures for Subsidiary Colors in FD&C Yellow No. 6

Paper Chromatography

The lower- and higher-sulfonated compounds can be determined in one step by paper chromatography according to the method given above for FD&C Yellow No. 5 except that a mixture of 350 volumes of methyl ethyl ketone, 150 volumes of acetone, 150 volumes of water, and 1 volume of concentrated ammonium hydroxide is used as the mobile phase. The higher-sulfonated compounds are separated from each other, but the lower-sulfonated compounds are not.

Thin-layer Chromatography (7)

Spot 3 μL of a 2% aqueous sample solution onto an 8-in. × 8-in. chromatographic plate coated with 0.25 mm of silica gel G. Air dry the plate and then elute using isoamyl alcohol–acetone–water–ammonium hydroxide (65:50:20:5). If present, lower-sulfonated colors appear above the main band and higher-sulfonated colors appear below the main band.

Higher-Sulfonated Dyes in FD&C Yellow No. 6

Extraction Procedure (14)

Dissolve 0.1 g of sample in 100 mL of 1:25 hydrochloric acid. Dilute 10 mL of this solution with 40 mL of 1:25 hydrochloric acid. Extract by shaking the solution successively in five separatory funnels, each containing 50 mL of isoamyl alcohol. Transfer the acid layer to a 100-mL volumetric flask. Wash the alcohol extracts with two 25-mL portions of the 1:25 hydrochloric acid, passing each portion through the funnels in

the same order used for the original extraction. Add the washings to the acid layer in the flask and dilute to 100 mL with water. Determine the higher-sulfonated colors spectrophotometrically against a standard.

Chromatographic Method (17)

In a separatory funnel mix 100 mL of *n*-butanol and 100 mL of carbon tetrachloride. Add 100 mL of 1:4 hydrochloric acid, shake for 3 min, and allow to settle. Use the lower layer as the stationary phase and the upper layer as the mobile phase. Prepare silane-treated Celite by adding 25 mL of General Electric GS-77 Dri Film to the bottom of an empty desiccator, placing a dish containing 200 g of Celite 545 into the desiccator, and leaving it covered for at least 3 hr. Test completion of the silanization by mixing a small amount of the treated Celite with water; the Celite should not be wetted.

Mix 15 g of the treated Celite with 7.5 mL of the stationary phase, pack the mixture into a 45-cm × 2.5-cm chromatographic column and then wash the column with 15 mL of mobile phase. Dissolve 0.05 g of sample in 25 mL of the mobile phase. Transfer a 5-mL aliquot of this solution to the top of the column and elute with mobile phase. The higher-sulfonated colors will elute as one band before the parent compound. Determine their content in the fractions spectrophotometrically against a standard.

Lower-Sulfonated Dyes in FD&C Yellow No. 6

Reverse-phase HPLC Procedure (4)

6-Hydroxy-5-(phenylazo)naphthalene-2-sulfonic acid and 4-(2-hydroxy-1-naphthylazo)benzenesulfonic acid can be determined by reverse-phase HPLC on a Waters 15-cm × 3.9-mm Novapak C_{18} column (5 μm) using gradient elution with a H_2O–tetrahydrofuran mobile phase buffered with ammonium acetate, and detection at 254 nm and 490 nm.

Extraction Procedure (35)

Dissolve 0.2 g of sample in 20 mL of water, add 1 mL of concentrated hydrochloric acid, and dilute to 50 mL. Extract by shaking the solution successively in three separatory funnels, each containing 50 mL of amyl alcohol. Wash the alcohol extracts with 50-mL portions of 5% aqueous

sodium chloride solution until the washings are colorless. Dilute each alcohol layer with 100 mL of petroleum ether and extract the lower-sulfonated dye with several 10-mL portions of water. Pass each portion through the funnels in the order reverse to that previously followed. Combine the aqueous extracts in a 100-mL volumetric flask, add 1 mL of 2 *N* ammonium acetate solution, and dilute to volume with water. Determine the lower-sulfonated colors present spectrophotometrically at 485 nm against a standard solution of D&C Orange No. 4.

Chromatographic Method (17)

Wash 150 mL of *n*-butanol with three 50-mL portions of 10% sodium hydroxide and then wash the butanol with 50-mL portions of water until neutral; prepare fresh daily. Mix 100 mL of the washed butanol with 100 mL of carbon tetrachloride in a separatory funnel and add 100 mL of 2% (v/v) hydrochloric acid containing 20 mg/mL of hydroxylamine hydrochloride. Shake the mixture for 3 min and then allow the layers to separate. Use the upper layer as the stationary phase and the lower layer as the mobile phase.

Mix 10 g of Celite 545 with 5 mL of the stationary phase and firmly pack the mixture into a 45-cm × 2.5-cm chromatographic column.

Dissolve 0.05 g of sample in 25 mL of the stationary phase, mix a 5-mL aliquot of this solution with 10 g of Celite, and pack the mixture on the top of the column. Elute with mobile phase, and collect the desired fractions. Dilute them to 25 mL with mobile phase and determine the amount of each color spectrophotometrically.

1-*p*-Sulfophenylazo-2-Naphthol-3,6-Disulfonic Acid, Trisodium Salt in FD&C Yellow No. 6 (27)

Prepare 0.5 *M* TBAP ion-pair reagent by adding 25 mL of 1.54 *M* aqueous tetra-*n*-butylammonium hydroxide to 52 mL of 1.11 *M* aqueous KH_2PO_4, then filtering the mixture through a 0.45-μm Millipore filter.

Weigh 0.025 g of sample into a 100-mL volumetric flask, add 1.0 mL of 0.5 *M* TBAP, then dilute to 100 mL with water and mix well.

Chromatograph 20 μL of sample using an E. Merck Hiber II LiChrosorb RP-18, 10 μm (4.6-mm × 25-cm) column at ambient temperature and methanol-water (45 + 55) containing 0.005 *M* TBAP as the mobile phase. Use a flow rate of 1.0 mL/min and monitor the eluate at

490 nm. The subsidiary colorant elutes in about 7 min; FD&C Yellow No. 6 elutes in about 11 min.

Subsidiary Colors in Orange B

Column-chromatographic Procedure (17)

Insert a glass-wool pledget in the constriction above the tip of a 59-cm × 3.3-cm-ID glass chromatographic column. Slurry Solka-Floc BW-40 in water and add the mixture to the column to a height of about 45 cm. Wash the column with about 100 mL of 20% aqueous NaCl.

Transfer 0.2 g of sample into a small beaker and dissolve in 10 mL of water. Add 20 mL of 20% NaCl then, using small portions of 10% NaCl, quantitatively transfer the sample to the column. Drain the column just to the surface of the support and then cover the top of the support with a pledget of glass wool. Elute with 10% NaCl until the main band is halfway down the column and then elute with 2% NaCl.

Orange B elutes first followed by Orange K [1-(4-sulfophenyl)3-carboxy-4-(4-sulfonaphthylazo)-5-hydroxypyrazole]. When most of the Orange K has eluted, wash 2-(4-sulfonaphthylazo)naphthionic acid or any other subsidiary color present from the column with water. Determine the colors spectrophotometrically.

Thin-layer chromatographic Method (17)

Using a microliter syringe, spot about 3 mg of color as a band onto a 20-cm × 20-cm, 250-μm thick cellulose plate. Allow the plate to dry thoroughly in the dark (ca. 20 min) and then develop using 1,4-dioxane–isoamyl alcohol–acetic acid–water (45:25:1:20) as the mobile phase. The order of elution from top to bottom of the plate is:

> Orange B, 2-(4-sulfonaphthylazo)naphthionic acid, 1-(4-sulfophenyl)-3-carboxy-4-(4-sulfonaphthylazo)-5-hydroxypyrazole.

1[4-(2,5-Dimethoxyphenylazo)-2,5-Dimethoxyphenylazo]-2-Naphthol and 1,1′-(2,2′,5,5′-Tetramethoxy-4,4′-Biphenylene-bisazo)-di-2-Naphthol in Citrus Red No. 2 (49)

Transfer 2.5 mg of sample (in chloroform) as a band 1 in. from the bottom of a 20-cm × 20-cm TLC plate coated with 0.38 mm of silica gel G. Allow

the plate to dry and then develop it with chloroform until the solvent reaches the upper edge of the plate. Remove the plate from the tank, air dry it for about 10 min, and then leach the bands from the plate and determine them spectrophotometrically. The colors appear in the ascending order: (1) 1[4-(2,5-dimethoxyphenylazo)-2,5-dimethoxyphenylazo]-2-naphthol (dimethoxy color), (2) 1,1′-(2,2′,5,5′-tetramethoxy-4,4′-biphenylenebisazo)-di-2-naphthol (benzidine color), and (3) Citrus Red No. 2.

Lower-Sulfonated Colors in D&C Blue No. 4

This colorant is the ammonium salt corresponding to FD&C Blue No. 1 and can be analyzed by the methods given for that color.

Indirubin in D&C Blue No. 6 (53)

Weigh 0.3–0.4 g of sample into a 6-dram-capacity vial containing about 1 cm of Ottawa sand (MCB No. SX75). Add another 1 cm of sand, replace the cap, and shake. Transfer the sample and sand to a double-thickness cellulose extraction thimble containing 1–2 cm of sand. Further additions (ca. 3–4) of sand to the vial followed by shaking and transfer to the extraction thimble are made until sample transfer is essentially quantitative and the thimble is 3/4 full. Insert the thimble into the extraction section of a Soxhlet extractor with a 34/45 upper joint. Add 150 mL of glacial acetic acid to the flask and extract at a rate of four or more drops per second for 4 hr. Use boiling chips. After cooling, transfer the extract to a 200-mL volumetric flask. Rinse the flask with three 10-mL portions of acetic acid, adding the rinses to the extract. Make to volume with acetic acid. Dilute as needed and if a precipitate of indigo occurs filter through a 0.45-μm Millipore filter.

Determine the blank-corrected sample absorbance at 615 nm and 533 nm against acetic acid.

$$\text{Percent indirubin} = \frac{4.42 \times (A_{533} - A_{615})}{177.32 \times w \times (1000/200) \times b} \times \text{DF} \times 100$$

$$= \frac{0.1128\ (4.42 \times (A_{533} - A_{516})) \times \text{DF}}{w \times b}$$

where w is sample weight in g, 1000/200 is factor for conversion of

effective sample concentrations to g/L, b = cell path length in cm, DF = dilution factor, 100 = factor for conversion to percent and $(4.42 \times (A_{533} - A_{615}))/177.32$ is the solution of the following simultaneous equation for c_1.

$$A_{533} = 40.866\ bc_1 + 11.601\ bc_2$$

$$A_{615} = 3.310\ bc_1 + 51.276\ bc_2$$

where c_1 and c_2 are the effective sample concentrations in g/liter of indirubin and indigo, respectively; 40.866 and 3.31 are the absorptivities in L/g-cm of indirubin at 533 nm and 615 nm, respectively; and 11.601 and 51.276 are the absorptivities of indigo at the same wavelengths.

Monosulfonated Color in D&C Green No. 5 (24)

Transfer 10 g of sample to a 250-mL Erlenmeyer flask. Add 50 mL of a 10:2:1 glacial acetic acid-concentrated hydrochloric acid–water mixture and 10 mL of concentrated hydrochloric acid containing 1 g/mL of stannous chloride.

Boil gently until the volume is reduced to about 25 mL and then dilute the hot mixture with 100 mL of water. Transfer to a 500-mL volumetric flask and dilute to volume with water. Filter a 100-mL aliquot into a 500-mL extraction funnel; make it alkaline with 25 mL of 50% sodium hydroxide solution, cooling the funnel during the addition. Extract the liberated amine with two 100-mL portions of ethyl ether; combine the extracts and wash them with four 25-mL portions of water. Extract the amine with five 25-mL portions of 0.3 *N* hydrochloric acid and transfer the washings to an iodination flask.

Boil to expel the dissolved ether, concentrate to 100 mL, then cool. Add 25 mL of 0.3 *N* hydrochloric acid and about 100 g of crushed ice. Add 0.05 *N* potassium bromide-bromate to the agitating solution until it remains yellow for at least 30 sec, and then add 5 mL in excess. Stopper the flask and let it stand in an ice bath for 10 min. Add 2–3 g of potassium iodide and titrate while cold with 0.05 *N* sodium thiosulfate to a starch end point (add indicator internally near the end point.) Perform a blank determination. Calculate the result as *p*-toluidine; 1 mL of 0.05 *N* potassium bromide-bromate is equivalent to 1.34 mg of *p*-toluidine. Calculate the amount of monosulfonated dye present in the sample as follows:

$$\text{Monosulfonated dye, weight \%} = \frac{(W)(4.858)(100)}{w}$$

where W is amount of *p*-toluidine in mg and w is sample weight in mg.

Sodium Salts of 1-(*p*-Toluidino)-4-(*o*-Sulfo-*p*-Toluidino) Anthraquinone and 1-Hydroxy-4-(*o*-Sulfo-*p*-Toluidino) Anthraquinone in D&C Green No. 5 (51)

Streak 1–1.5 mg of sample as an aqueous solution onto a 20-cm × 20-cm thin-layer plate coated with a 250-μm layer of MN 300 cellulose. Allow the plate to air dry at room temperature for about 45 min and then develop with butyl acetate–dimethylformamide–water (10:5:1) in a tank that has been equilibrated with mobile phase for about 30 min. The colorants appear on the plate in descending order:

Unsulfonated D&C Green No. 5 (D&C Green No. 6),
Monsulfonated D&C Green No. 5 [1-(*p*-toluidino)-4-(*o*-sulfo-*p*-toluidino)-anthraquinone],
1-Hydroxy-4-(*o*-sulfo-*p*-toluidino)anthraquinone (Ext. D&C Violet No. 2),
D&C Green No. 5.

Alizurol Purple (D&C Violet No. 2) in D&C Green No. 6 (17)

Spot about 1 mg of color as a chloroform solution onto a 20-cm × 20-cm thin-layer plate coated with silica gel G. Allow the plate to dry and then develop it using hexane–trichloroethylene–diethylamine (6:2:1) as the mobile phase. Leach the alizurol purple from the plate with chloroform and determine it spectrophotometrically.

Lower-Sulfonated Colors in D&C Orange No. 4 (17)

Use the chromatographic method given for lower sulfonated dyes in FD&C Yellow No. 6 (p. 396) except prepare the mobile phase from 100 mL of washed butanol, 100 mL of carbon tetrachloride, and 100 mL of distilled water.

Related Bromofluoresceins in D&C Orange No. 5

Method A (17)

Use the Column-chromatographic procedure described under Lower-Iodinated Colorants in FD&C Red No. 3 (p. 383), but substitute the following mobile phases:

Mobile phase No. 1—350 mL of 25% aqueous sodium chloride solution containing 1.75 mL of concentrated ammonium hydroxide.

Mobile phase No. 2—1000 mL of 15% aqueous sodium sulfate solution containing 5 mL of concentrated ammonium hydroxide.
Mobile phase No. 3—800 mL of 5% aqueous sodium sulfate solution containing 4 mL of concentrated ammonium hydroxide.
Mobile phase No. 4—500 mL of 3% aqueous sodium sulfate solution containing 2.5 mL of concentrated ammonium hydroxide.
Mobile phase No. 5—500 mL of 0.5% (v/v) ammonium hydroxide.
Mobile phase No. 6—500 mL of 60% ethanol containing 2.5 mL of concentrated ammonium hydroxide.

The order of elution and the identity of the subsidiary colors are as follows:

Mobile phase No. 1—Band 1, unidentified color; Band 2, fluorescein.
Mobile phase No. 2—Band 3, mixture of 2- and 4-monobromofluoresceins; Band 4, mixture of 2,4- and 2,5-dibromofluoresceins.
Mobile phase No. 3—Band 5, 4,5-dibromofluorescein.
Mobile phase No. 4—Band 6, 2,4,5-tribromofluorescein.
Mobile phase No. 5—Band 7, 2,4,5,7-tetrabromofluorescein.
Mobile phase No. 6—Band 8, unidentified color.

Estimate the amount of each colorant present using the following values. Unknowns can be calculated as D&C Orange No. 5.

	Absorption Maximum (in nm)	Absorptivity (in liters/g-cm)
Fluorescein	492	247
2-Bromofluorescein	500	231
4-Bromofluorescein	498	206
2,4-Dibromofluorescein	504	190
2,5-Dibromofluorescein	507	186

(*Continued*)

	Absorption Maximum (in nm)	Absorptivity (in liters/g-cm)
2,7-Dibromofluorescein	507	214
4,5-Dibromofluorescein	504	163
2,4,5-Tribromofluorescein	512	151
2,4,7-Tribromofluorescein	513	182
2,4,5,7-Tetrabromofluorescein	518	150

Method B (17)

Spot 1–2 mg of colorant as an aqueous solution onto a 20-cm × 20-cm glass thin-layer plate coated with 0.25 mm of silica gel G, dry the plate, then develop it using acetone–chloroform–butylamine–water (19:5:2:2) as the mobile phase.

Method C (21)

Prepare a 1% ammoniacal sample solution and spot 5 μL on a 18-in. × 22-in. Whatman No. 1 chromatographic paper, about 1 in. from the edges. Immerse the 18 in. edge of the paper in a mobile phase composed of 1 g of sodium chloride and 1 mL of concentrated ammonium hydroxide dissolved in 10 mL of water (solution 1). Develop for 24 hr. Remove the sheet from the tank, air dry it in the dark, and then rotate it 90° and develop for an additional 48 hr using a mobile phase composed of 100 mL of solution 1, 300 mL of *n*-butanol, and 70 mL of ethanol (solution 2). Leach the spots from the paper with 1:99 ammonium hydroxide and determine the individual compounds spectrophotometrically. See Fig. 13.3.

4-Toluene-Azo-2-Naphthol-3-Carboxylic Acid in D&C Red Nos. 6 and 7 (26)

Place 0.25 g of sample in a 125-mL acetylation flask. Add 80 mL of methyl Cellosolve and 5 mL of concentrated hydrochloric acid, attach an air condenser, and reflux for 15–20 min. Transfer the solution to a separatory funnel. Rinse the flask into the funnel with four 10-mL portions of isopropyl ether and two 75-mL portions of water, shake, and then let the two phases separate. Drain the lower aqueous phase into a second separatory funnel, extract it with portions of isopropyl ether totaling 40 mL, combine the ether extracts, and discard the residual aqueous solution.

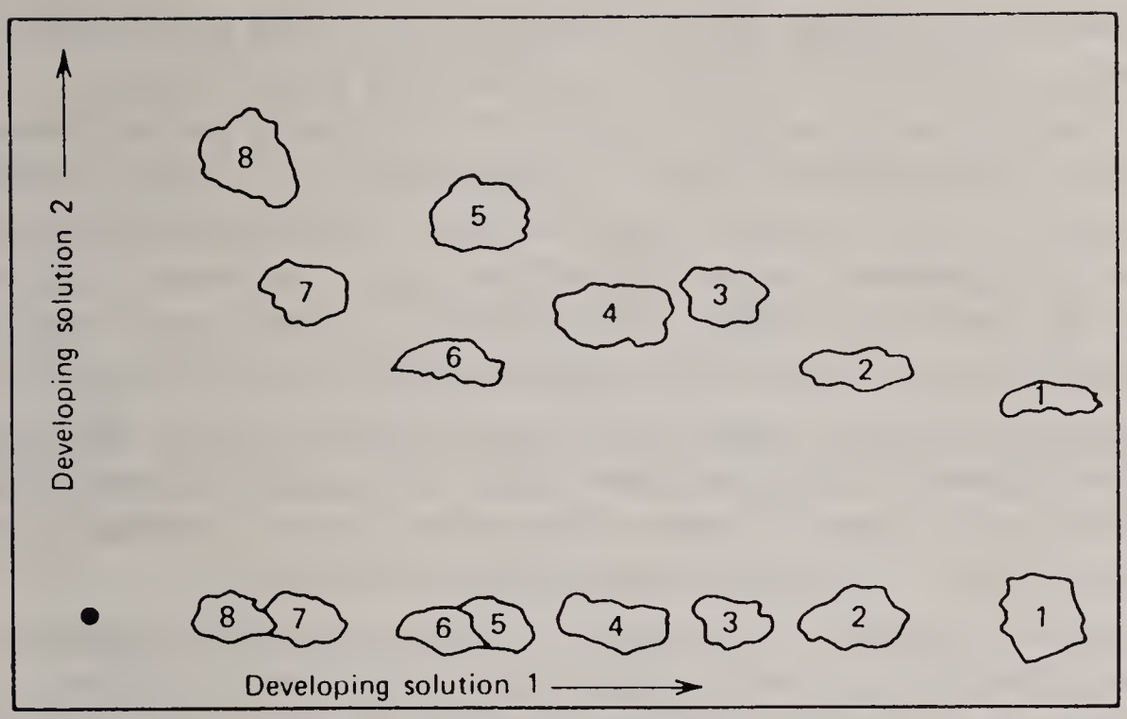

Figure 13.3. Paper-chromatographic separation of fluorescein and nine bromofluoresceins: (1) fluorescein, (2) 2-bromofluorescein and 4-bromofluorescein, (3) 2,7-dibromofluorescein, (4) 2,4-dibromofluorescein and 2,5-dibromofluorescein, (5) 2,4,7-tribromofluorescein, (6) 4,5-dibromofluorescein, (7) 2,4,5-tribromofluorescein, (8) 2,4,5,7-tetrabromofluorescein (black dot in lower left corner shows initial spot of sample containing 0.003 mg of each compound). (Reprinted with the permission of the Association of Official Analytical Chemists).

Extract the ether layer with 20-mL portions of water until the water extract is colorless. Filter the ether layer through a cotton pledget, rinse the cotton and the funnel with isopropyl ether, and dilute the filtrate to 100 mL with isopropyl ether. Compare the absorbance of the sample against a standard at the maximum near 507 nm.

Ether-Soluble Matter in D&C Red Nos. 6 and 7 (17)

Transfer 0.100 g of colorant, 75 mL of 8 *N* HCl and 100 mL of glacial acetic acid to a 250-mL beaker, then stir and heat the mixture on a hot plate until in solution. Remove the beaker from the hot plate, cover it with a watch glass, and allow it to cool to room temperature (1–2 hr).

Pour the solution into a 1000-mL separatory funnel, then rinse the beaker into the funnel using three 50-mL portions of water.

Add 150 mL of anhydrous diethyl ether to the funnel, stopper and shake for 10 sec, then invert the funnel and cautiously open the stopcock

to remove gas buildup. Shake the funnel for one minute more, venting as needed, then allow the funnel to stand until the layers separate.

Transfer the bottom (aqueous) layer to a 500-mL separatory funnel, add 100 mL of ether, then stopper and shake for 1 min, venting as needed. Allow the layers to separate. Drain the bottom layer into a waste beaker, then transfer the ether layer to the 1000-mL separatory funnel. Rinse the 500-mL funnel with 100 mL of water, adding the rinse to the 1000-mL funnel. Shake the 1000-mL funnel for 1 min, allow the layers to separate, then drain the lower aqueous layer into the waste beaker. Rinse the 500-mL funnel at least three times (total) and repeat the 100-mL water washes until no color is present in the aqueous layer. Discard the bottom aqueous layer to the waste beaker after each separation.

Wash the combined ether layers twice more with 100-mL portions of water, discarding the bottom aqueous layer after each separation.

Extract unsulfonated subsidiary color from the ether layer by shaking it for one minute with 20 mL of 2% w/w aqueous NaOH. Allow the layers to separate, then drain the lower layer into a 100-mL beaker. Repeat this extraction until no additional colorant is extracted. Combine the aqueous extracts and save for the determination of 3-hydroxy-4-[(4-methylphenyl)azo]-2-naphthalenecarboxylic acid, sodium salt.

Transfer the ether layer to a 250-mL beaker and allow it to evaporate to near dryness. Cool the beaker to room temperature. Add about 8 mL of 95% ethanol and swirl to mix the contents. Using ethanol wash the sample into a 25-mL graduate, then adjust its volume to 15 mL.

Using 1-cm matched cells, determine the spectrum of 95% ethanol versus distilled water from 400–700 nm at a scan rate of 5 nm/sec. Similarly determine the spectrum of the ethanol solution of the ether soluble material. Compare the sample spectrum with that in Figure 13.4.

To "pass test" the absorbance of the sample must not exceed that of Figure 13.4 at any wavelength.

Determination of 1-Phenylazo-2-Naphthol (PAN) in D&C Red No. 17 (33)

Prepare a 2.5% solution of the sample in chloroform and apply a 0.2-ml aliquot across a 20-cm × 20-cm glass plate coated with a silica gel G layer, 0.375 mm thick. Allow the plate to air dry. Develop with toluene in a lined chromatographic tank until the solvent front reaches the top of the plate. Remove the plate and air dry. If the orange-yellow line of PAN

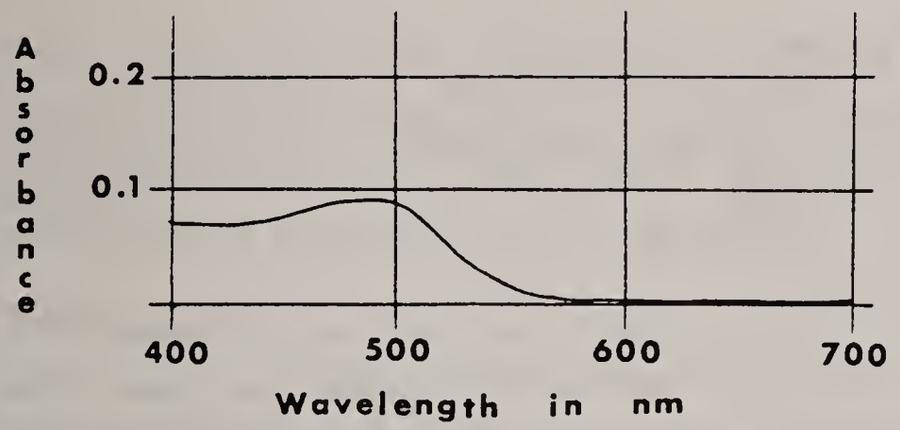

Figure 13.4. Spectrum of ether-soluble matter isolated from D&C Red No. 6 Lot AA5169, drawn to equal 150% of that actually obtained using the method on p. 403—Conditions: 1-cm cells; scan rate, 5.0 nm/sec.; reference, distilled water.

is not adequately resolved from the D&C Red No. 17, repeat the elution. If PAN is separated from the D&C Red No. 17 but mixed with another colorant, scrape the commingled colors from the plate, leach with chloroform, filter through sintered glass, evaporate the filtrate and any needed washings to 0.1–0.2 mL, and apply this solution to a 10-cm × 20-cm silica gel G plate, 0.375 mm thick. Develop the plate with methylene chloride. Leach the compounds from the plate with chloroform and determine spectrophotometrically.

Lower-Brominated Subsidiary Colors in D&C Red Nos. 21 and 22

Method A (22)

Weigh 2 g of sample and transfer it to a 100-mL volumetric flask. Add 50 mL of a solvent prepared by mixing 50 mL of S. D. No. 1 ethanol, 35 mL of water, and 15 mL of concentrated ammonium hydroxide. Shake to dissolve; warm if necessary. Dilute to volume with solvent. Apply 0.1 mL of sample solution to a 3-in. × 16-in. Schleicher & Schuell No. 2043 chromatographic paper and let it dry in the dark. Suspend the strip in a 18-in. × 6-in.-ID glass tank and develop in the dark for 24–48 hr by the ascending technique using *n*-butanol–water–ammonium hydroxide–ethanol (100:44:1:22.5). Remove the strip and dry in the dark. Wash each colored zone into a separate volumetric flask using 1:199 ammonium hydroxide, filter or centrifuge if necessary, and determine the individual substances spectrophotometrically. Their absorption maxima and approximate absorptivities are given on p. 401.

Method B (2)

Coat a 20-cm × 10-cm glass plate with a 250-μm layer of silica gel GF 254 (TLC silica gel + $CaSO_4$ binder + fluorescent indicator for use with short wave UV). Dry the plate, then activate it by placing it in a 130°C oven for 1 hr.

Dissolve 1.0 g of sample in acetone plus a minimum of water, then make to 100 mL with acetone and mix well. Spot 7.5 μL of sample and standard onto the plate, dry the spots, then develop the plate until the mobile phase travels the full distance of the surface (about 1 hr) using chloroform–benzene–methanol–formic acid (65:20:7:8) as the mobile phase. Dry the plate then examine it under UV light.

Components elute in the following order:

R_f	Identity
0.19	Fluorescein
0.32	Unknown
0.36	Unknown
0.39	Monobrominated fluorescein
0.50	Dibrominated fluorescein
0.55	Tribrominated fluorescein
0.59	The ester of D&C Red No. 21
0.63	D&C Red No. 21
0.68	Unknown

Method C (17)

Use the column-chromatographic procedure described under Related Bromofluoresceins in D&C Orange No. 5 (p. 400), but substitute the following mobile phases.

Mobile phase No. 1—450 mL of 25% aqueous sodium chloride solution containing 2.25 mL of concentrated ammonium hydroxide.

Mobile phase No. 2—2000 mL of 10% aqueous sodium sulfate solution containing 10 mL of concentrated ammonium hydroxide.

Mobile phase No. 3—600 mL of 0.5% (v/v) ammonium hydroxide.

Mobile phase No. 4—400 mL of 60% ethanol containing 2 mL of concentrated ammonium hydroxide.

Subsidiary colorants elute in the same order as given under D&C Orange No. 5, except that a ninth unknown band is sometimes detected. The colorants present can be estimated using the values given under D&C Orange No. 5.

Method D (17)

Subsidiary colorants can be separated from D&C Red Nos. 21 and 22 using the thin-layer chromatography procedure (B) described under D&C Orange No. 5.

Uranine in D&C Red No. 22 (25)

Slurry 20 g of Solka Floc SW-40-A in 800 mL of water, pack the slurry into a 120-cm × 2.2-cm chromatographic column, and wash the column with 50 mL of 5% sodium sulfate solution. Prepare a 0.3% aqueous sample solution and pipette a 5-mL aliquot into a beaker. Add 0.1 mL of 10% sodium hydroxide solution and 1 g of anhydrous sodium sulfate, dilute to 20 mL with water, and then add, with stirring, 0.5 g of Solka Floc. Transfer the mixture to the top of the column with small portions of 5% sodium sulfate solution and elute the uranine with this solution. Make the eluate alkaline with sodium hydroxide and determine the uranine spectrophotometrically at 490 nm.

Subsidiary Colorants in D&C Red Nos. 27 and 28 (17)

Spot 1–2 mg of sample as an aqueous solution onto a 20-cm × 20-cm glass plate coated with 0.25 mm of silica gel G, dry the plate, then develop it using acetone–chloroform–butylamine (88:12:9.5) as the mobile phase.

1-(Phenylazo)-2-Naphthol in D&C Red No. 31 (17)

Extract 0.1 g of sample in a Soxhlet extractor with approximately 100 mL of benzene until the leachings are colorless or have a slight persistent bleed. Transfer the extract to a separatory funnel and wash with 1% NaOH solution until the washings are colorless. Wash the benzene layer with several 50-mL portions of water to remove the excess NaOH.

Evaporate the benzene solution to dryness. Dissolve the residue in alcohol and dilute with alcohol to 100 mL or 200 mL, depending on the intensity of the color. Determine the amount of subsidiary color present spectrophotometrically.

Chromotrope 2R in D&C Red No. 33 (43)

Slurry Solka Floc BW-40 in water and pack it into a 100-cm × 2-cm chromatographic column to a settled height of 50 cm, and then wash the column with 20% sodium chloride solution. Prepare a 0.1% aqueous sample solution. To a 5-mL aliquot add 20 mL of a 20% sodium chloride solution; transfer it to the column with the sodium chloride solution. Elute the column with 10% sodium chloride solution containing 1% ammonium hydroxide. Chromotrope 2R elutes first followed by the parent compound. (An unknown blue dye frequently remains at the top of the column.) When the Chromotrope 2R band is separated by about 10 cm from the main band, change the mobile phase to a 5% sodium chloride solution containing 1% ammonium hydroxide. Neutralize the appropriate fractions with acetic acid and determine Chromotrope 2R spectrophotometrically at the absorption maximum near 508 nm.

4-Nitrophenylazo-2-Naphthol and 2,4-Dinitrophenylazo-2-Naphthol in D&C Red No. 36 (17)

Spot 0.5–1 mg of sample as a chloroform solution onto a 20-cm × 20-cm glass plate coated with 0.25 mm of silica gel G, dry the plate, then develop it using benzene as the mobile phase. Dry the plate, then develop it again using chloroform.

Quinizarin Green (D&C Green No. 6) in D&C Violet No. 2 (17)

Spot as a band about 1 mg of sample as a chloroform solution onto a 20-cm × 20-cm TLC plate coated with silica gel G. Allow the plate to air dry and then develop using hexane–trichloroethylene–diethylamine (30:10:5) until the solvent front nears the top of the plate.

The colors appear in the descending order:

Quinizarin Green.
D&C Violet No. 2

Subsidiary Dyes in D&C Yellow No. 10

Method A (41)

Weigh about 200 g of Celite 545 (Fisher C-212) into a Petri dish and place in a desiccator containing 25 mL of General Electric SC-77 Dri Film. Let stand until the Celite no longer wets when mixed with water (3–24 hr).

Prepare a 2.5% solution of triiso-octylamine in *n*-butanol and equilibrate with an equal volume of (1 + 9) HCl. Allow the layers to separate.

Thoroughly mix 18 mL of the *n*-butanol layer from the solution described above with 20 g of the silane-treated Celite and pack the mixture into a 1-in.-diameter glass column. Wash the column with mobile phase (the aqueous-acidic layer from that described above) and allow the column to drain just to the surface of the packing.

Transfer 1 mL of a 1% sample solution in mobile phase to the column and elute with mobile phase. Collect 20-mL fractions and determine the individual colorants spectrophotometrically.

When present, the subsidiary colors emerge in the following order:

2-(2-Quinolyl-6-sulfonic acid)-1,3-indandione-5-sulfonic acid.
2-(2-Quinolyl-8-sulfonic acid)-1,3-indandione-5-sulfonic acid.
2-(2-Quinolyl-6,8-disulfonic acid)-1,3-indandione.
2-(2-Quinolyl-6-sulfonic acid)-1,3-indandione.
2-(2-Quinolyl-8-sulfonic acid)-1,3-indandione.

Method B (17)

Spot 1–2 mg of sample as an ethanol–water (1:1) solution onto a 20-cm × 20-cm glass plate coated with 0.25 mm of silica gel G, dry the plate, then develop it using isoamyl alcohol–1,4-dioxane–water–ammonium hydroxide (10:10:4:1) as the mobile phase.

2-(2-Quinolinyl)-1H-Indene-1,3-[2H]-Dione and Other Organic-Soluble Matter in D&C Yellow No. 10 (19)

Apparatus and Reagents

Liquid chromatograph—With gradient elution capability, a 100-μL sample injection loop, a Waters Model 440 dual wavelength detector, a 3-cm

× 4.6-mm Brownlee RP-18 Spheri-5 cartridge guard column, a 30-cm × 4-mm Varian Micro-Pak MCH-10 C_{18} chromatographic column, and a Perkin-Elmer Lambda 5 spectrophotometer, or equivalent.

Extraction Column—Extrelut™ QE, 20-mL capacity (EM Science).
Mobile phase A—0.05 *M* ammonium acetate.
Mobile phase B—Acetonitrile.

Weigh 1.0000 g of sample into a 30-mL beaker, add 10 mL of water to the beaker, and place it on a steam bath. Stir the sample until in solution, then remove the beaker from the steam bath and allow it to cool to room temperature. Pour the solution onto an Extrelut™ QE column. Rinse the beaker with 1 mL of water and transfer the rinse to the column. Continue washing the beaker with 1-mL portions of water until the water is colorless. (Do not use more than 10 mL of water to rinse the beaker.) After the final rinse has been added to the column, let the column stand for 5 min. Extract the column with 20 ± 1 mL of chloroform. Wait 2–5 min, then extract with another 20 mL of chloroform. Continue extracting the column as above using a total of five 20-mL portions of chloroform. Combine the extracts in a 250-mL round-bottom flask then evaporate all the chloroform with a rotary evaporator. Pipet 5 mL of acetonitrile into the flask to dissolve the residue. Inject 100 µL of this solution into the chromatograph and elute as follows. Use a flow rate of 1 mL/min, ambient temperature, and detection at 436 nm. Simultaneously monitor at other wavelengths, depending upon what other organic compounds are present in the extract. Elute with 60% B for 19.9 min; program to 100% B in 0.1 min; hold at 100% B for 5 min; program to 60% B in 0.1 min; hold at 60% B for 5 min. The time needed at 100% B to wash the column of any late eluting material or the time needed at initial composition during equilibration may be adjusted to fit the needs of different LC columns or equipment.

Related Colorants in Annatto (18)

Paper-chromatographic Method (29–32)

(see McKeown, G.G. under Resolution of Mixtures, p. 436).

Thin-layer-chromatographic Methods:

Method A. (see Francis, B. J., and Ramamurthy, M K., and Bhalerao, V. R. under Resolution of Mixtures, p. 431 and 447).

Method B-Chloroform Extracts of Annatto (11). Coat 2-in. × 15-in. glass plates with silica gel containing 12% gypsum as a binder. Dry the plates for 1 hr at 100°C.

Steak 0.2 mL of a 0.2–1% chloroform solution of pigment onto the plate and elute using acetic acid–chloroform–acetone (1:50:50).

Method C-Vegetable Oil or Propylene Glycol Extracts of Annatto (39). Apply sample to a 20-cm × 20-cm cellulose thin-layer plate (Eastman Chromagram Sheet, 6065) and elute briefly with cyclohexane to separate the oil from the pigments. Air dry the plate then elute in a paper-lined, mobile phase-equilibrated tank containing cylohexane–chloroform–acetic acid (65:5:1).

Method D-Aqueous-alkaline Extract of Annatto (39). Apply sample to an untreated 10-cm × 20-cm Merk silica gel thin-layer plate. Air dry and then elute in a paper-lined, mobile phase-equilibrated tank using chloroform–absolute ethanol–acetic acid (68:2:1).

Pigments in Beet Colorants

Method A (42)

Separate samples on a Waters Associates μ-Bondapak C18 column either isocratically using CH_3OH–0.05M KH_2PO_4 (18:82, v/v) adjusted to pH 2.75 with H_3PO_4 as the mobile phase (A), or by gradient elution programming from 100% A to 80% A: 20% CH_3OH. Monitor chromatograms at 535 nm. Degradation products and the yellow pigments of beet red (vulgaxanthins) normally elute first followed by betanin, isobetanin, betanidin, and isobetanidin.

Method B (54)

Use a Waters Associates μ-Bondapak C18 column (4 mm ID × 30 cm) and 0.005 *M* PIC Reagent A (Waters Associates) in 90:10 water-metha-

nol as the mobile phase, or use the same column and program from 0.005 *M* PIC Reagent A in 90:10 water-methanol to 0.005 *M* PIC Reagent A in 70:30 water-methanol. (PIC Reagent A is tetrabutylammonium phosphate at a pH of 7.5–8). Monitor yellow pigments at 476 nm, and red pigments at 538 nm.

Method C (38)

Transfer a 5% solution of sample in 0.1% HCl to a 26-cm × 18-mm column of Dowex 50 W-X2, wash the column with 0.1% HCl, then elute the pigments with water. Concentrate the eluate, purify and fractionate it on a 20-cm × 18-mm column of Polyclar AT by elution with water, then determine betacyanin and betaxanthin pigments using the HPLC procedure given in Method A, above.

Fractionation of Caramel by Gel Filtration (34)

Caramel is separated in an aqueous medium into high- and low-molecular-weight components on Sephadex G-25 and G-50.

Separation of α- and β-Carotene (see Usher, C. D. et al. under Isolation of Colorants, Dairy Products, p. 494).

Pigments in Grape Extract (40)

Chromatograph 20 μL of sample by HPCL using a 25-cm × 4.6-mm Alltech 5-μm C_{18} column, and AcOH:H_2O (10:90) containing 0.1% H_3PO_4 (A) and MeOH:H_2O:AcOH (60:10:30) containing 0.1% H_3PO_4(B) as the mobile phase. Using a flow rate of 0.8 mL/min and a concave gradient, elute the sample with A for 4 min, then program to B at 6.7%/min. Most impurities elute within 30 min.

Carotenoids in Paprika (1)

Extract paprika (or red pepper) with benzene, concentrate the extract, then separate a portion of the concentrate by TLC, using silica gel 60 F_{254} plates and light petroleum–benzene–acetone–acetic acid (80:20:4:5) as the mobile phase. Dry the plates with N, extract the carotenoids from the adsorbent with benzene, then measure the absorbance of the solution.

Pigments in Paprika (16)

Grind paprika pods, then exhaustively extract them with acetone to produce an oleoresin. Chromatograph a portion of the resulting acetone solution using a 25-cm × 4.6-mm Zorbax C_{18} column equipped with a Vydac RP pre-column, and gradient elution at 1 mL/min using acetone–H_2O (3:1) (A) in acetone–methanol (3:1) as the mobile phase. Program from 100% A to 65% B in 10 min, to 80% B in 30 min, and to 100% B in 60 min. Monitor the system at 460 nm, and also at 510, 480, and 428 nm for some compounds. Using this procedure, four classes of compounds are separated: hypophasic xanthophylls, fatty acid carotenoid monoesters, epiphasic carotenoids and fatty acid carotenoid diesters. Oleoresin extracts can be saponified and reduced to aid identification of the mono- and diesters.

Lumiflavin in Riboflavin (46)

Shake 20 mL of chloroform with 20 mL of water for 3 min. Allow the layers to separate. Drain the chloroform and repeat the extraction twice with 20-mL portions of water. Filter the washed chloroform through dry filter paper. Shake the filtrate for 5 min with 5 g of powdered anhydrous sodium sulfate. Allow the mixture to stand for 2 hr and then decant or filter the clear chloroform.

Shake 0.025 g of riboflavin with 10 mL of washed chloroform for 5 min and filter. A color in the filtrate similar to that of potassium dichromate solution indicates the presence of lumiflavin. Acceptable riboflavin should have no more color than 0.0003 *N* potassium dichromate solution when viewed under identical circumstances.

Related Colorants in Saffron

Extraction/TLC procedure (37)

Boil 1.00 g of sample in 20 mL of water, evaporate the filtered extract to a few drops, and chromatograph the solution on Whatman No. 1 paper versus safflower and other knowns using the following mobile phases:

1 mL 0.88 Ammonia + 99 mL water
2.5% Aqueous sodium chloride

80 g Phenol + 20 g water
5 mL 0.88 Ammonia + 95 mL water + 2 g trisodium citrate

Thermomicro Separation (TAS)/TLC method (47)

Weigh the powdered sample into a glass cartridge fitted with a capillary tube, heat the tube at an appropriate temperature (ca. 200°C) and collect the distillate on a silica gel HF_{254} plate. Elute with benzene–chloroform (80:20) and examine under short-wavelength UV light.

Related Colorants in Turmeric (52)

Curcumin, dimethoxycurcumin and didemethoxycurcumin can be separated by HPLC using a 25-cm × 4.6-mm Nucleosil NH_2 column, with ethanol as the mobile phase (1.2 mL/min). Detection can be by absorption spectrometry at 254 nm, or fluorescence at 470 nm with excitation at 420 nm.

Curcuma Zedoaria and Curcuma Aromatica in Turmeric (44)

Prepare a 1% benzene solution of the essential oils steam-distilled from turmeric. Spot 10 μL of this solution onto a 10-cm × 20-cm glass plate coated with 500 μm of silica gel G (the plate should have been activated for 1 hr at 110°C), then elute in a mobile phase-saturated tank using ethyl acetate–*n*-hexane (3:17).

Spray the air-dried plate with concentrated sulfuric acid–nitric acid (50:0.5), allow it to stand 1 min, then examine visually under daylight and UV light (365 nm). Then spray with anisaldehyde–sulfuric acid–ethanol–glacial acetic acid (0.5:0.5:9.0:0.1) and similarly examine.

Compare versus standards prepared simultaneously.

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PART C

RESOLUTION OF MIXTURES AND ANALYSIS OF COMMERCIAL PRODUCTS

14

RESOLUTION OF MIXTURES

Frequently, no single dye is capable of producing a desired shade, so mixtures or "secondary colors" are used. The determination of the nature and the amount of individual colorants in such mixtures presents a special problem. If the mixtures are not too complicated and if the component colorants have sufficiently different spectra not masked or distorted by the presence of excipients, nuclear magnetic resonance (NMR), infrared (IR), or visible spectrometry (VIS) can be used to analyze them directly. Rarely, though, is the analyst blessed with such ideal conditions, and most often separation of the mixtures into their component parts is necessary for a successful analysis.

The literature is teeming with examples of the analysis of such mixtures using most every separations technique available. The method to use, of course, is dictated by the needs of the analyst and the equipment available to him. Electrophoresis, thin-layer chromatography (TLC), and paper chromatography are relatively simple methods and require a minimum of equipment, applied time, and technique but yield only semiquantitative results even after extensive calibration. These methods are

best used on a "go-no-go" basis versus an acceptable standard. Conventional gravity-column chromatography provides greater precision and accuracy but usually at the expense of longer analysis times and more attention on the part of the analyst. Solvent-solvent extraction is simple but in most cases inadequate, whereas counter-current distribution is a powerful enough tool, but its use generally requires too much sophistication and time. To date, little use has been made of gas chromatography since few colorant mixtures are amenable to separation by this method. The tool that presently offers the most promise as both a rapid and quantitative method is high-performance liquid chromatography (HPLC). Although still a relatively young technique, HPLC has proven to be a powerful weapon for the determination of impurities in color additives and for separating mixtures of colors. Unfortunately, the instrumentation needed is costly.

Procedures exist for separating groups of dyestuffs having similar properties or applications such as the carotenoids, the water-soluble food colors, lipstick dyes, etc. but no one method has yet been written that separates all the permitted color additives and most certainly none ever will. The majority of extant methods are deficient since they either deal with one or more colors no longer permitted, or fail to consider newer colorants, or both. However, these methods can frequently be modified to meet one's needs or used as the starting point for developing a better one. A number of them are summarized in the following bibliography. Others can be found under the discussion of the isolation of colorants from commercial products where they are used as a means of identification.

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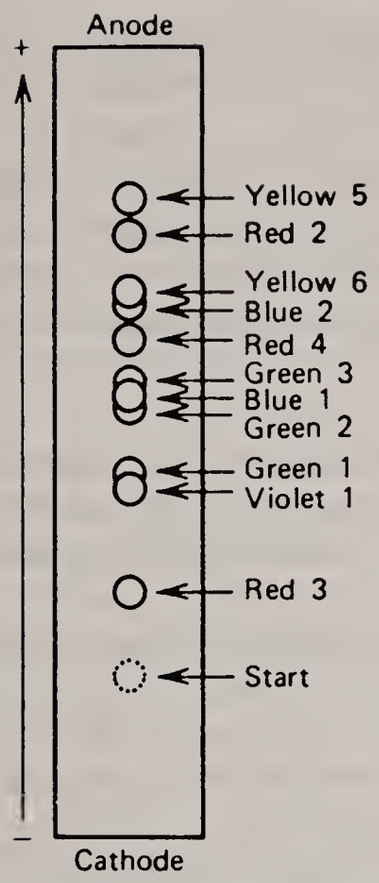

Figure 14.1. Electrophoretic separation of food colors [From J. Chem. Ed. *40,* 537 (1963)].

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BARRETT, J. F., RYAN, A. J. Nature *199,* 372–373 (1963). Thin-Layer Chromatography of Some Food Colors on Silica Gel. Describes the use of TLC on silica gel for the separation of dyestuffs including FD&C Red Nos. 3 and 4, FD&C Yellow Nos. 5 and 6, and FD&C Blue No. 2. The mobile phases studied were 9:1 EtOH–NH_4OH; 5:2:1 acetoacetic ester–MeOH–NH_4OH; 5:2:1 acetoacetic ester-C_5H_5N–NH_4OH; 10:10:1 AmOH–EtOH–NH_4OH, and 7:3:1 EtOAc–C_5H_5N–H_2O.

Bayer, J. Acta Pharm. Hung. *31B,* Suppl. 51–58 (1961). Evaluation of Paper Chromatograms of Pharmaceutical Preparations by the Densitometer. Use of

a densitometer to quantitate the components of drugs separated by paper chromatography. Includes the determination of FD&C Yellow No. 5.

BOLLINGER, H. R., KOENIG, A., SCHWIETER, U. Chimia *18,* 136 (1964). Thin-Layer Chromatography of Carotenes. Six carotenes are separated on activated MgO layers with petroleum ether (boiling range 90–110°)-benzene (50:50). For clearer separations of ε-, α-, and β-carotenes use 90:10 ether–benzene and for mixtures of δ-, γ-carotene and lycopene use 10:90 ether–benzene.

Solvents: S_1 = Light petroleum (boiling range 90–110°)–benzene (9:1).
S_2 = Light petroleum (boiling range 90–110°)–benzene (5:5).
S_3 = Light petroleum (boiling range 90–110°)–benzene (1:9).

Thin layer: "Darlington" light magnesium oxide, activated for 1 hr at 120°.

	R_f		
Compound	S_1	S_2	S_3
ε-Carotene	0.47	0.70	0.84
α-Carotene	0.26	0.66	0.80
β-Carotene	0.11	0.49	0.74
δ-Carotene	0.00	0.20	0.55
γ-Carotene	0.00	0.11	0.41
Lycopene	0.00	0.00–0.02	0.13

BROWN, J. C. JSDC *85,* 137–146 (1969). The Chromatography and Identification of Dyes. A general description of TLC, paper chromatography, and electrophoresis as tools for the separation of dyestuffs.

CALZOLARI, C., COASSINI, L., LOKAR, L. Rass. Chim. *15,* 49–60 (1963). Partition Paper Chromatography of Food Dyes.

CAMACHO, I., DUARTE, M. I. Rev. Colomb. Cienc. Quim. Farm. *1,* 5–32 (1971). Identification of Dyes Used in Lipstick in Columbia. Thin-layer and paper chromatography are used to separate and identify lipstick colorants.

CANUTI, A., MAGRASSI, B. L. Chim. Ind. (Milan) *46,* 284–286 (1964). Food Colors. I. Application of Thin-Layer Chromatography for Determining Added Artificial Food Colors. Artificial acidic food colors permitted in Italy are separated using BuOH–H_2O–EtOH–NH_4OH (50:25:25:10) as the mobile phase.

CELAP, M. B., JANJIC, T. J., JEVTIC, V. D. Mikrochim. Ichnoanal. Acta *4,* 647–651 (1965). Application of the Ring-Oven Method to the Determination

of Dyes. The Weisz ring-oven method was applied to the separation and determination of various dyes including FD&C Red No. 3, FD&C Blue No. 2 and FD&C Yellow No. 5.

CERESA, G. Ann. Sper. Agrar. (Rome) *13,* 545–571 (1959). Identification of Synthetic Dyes Used in the Food Industry. The 13 food dyes permitted by Italian legislation are separated by one-dimensional paper chromatography using EtOH–BuOH–H_2O (50:25:25) as the mobile phase. Those dyes not separated by this mixture are resolved using normal HCl or by adding 10 mL of concentrated NH_4OH to 100 mL of the mixture described above.

CHAPMAN, W. B., OAKLAND, D.J. Assoc. Publ. Analysts *6,* 124–128 (1968). Differentiation of Blue Colouring Matters in Food and Drugs With Particular Reference to Blue VRS (CI Acid Blue 1) and Patent Blue V (CI Acid Blue 3). Thin-layer chromatography and paper electrophoresis were used to differentiate 14 blue colors. Using TLC: Colors were applied as 0.1% aqueous solutions (5μL) to layers of Kieselgel G (250 μm) previously activated at 105° for 2 hr, the TLC plates were then developed with fresh isopropyl alcohol–concentrated aqueous NH_3 (4:1) for about 2 hr; or, colors were applied to layers of Cellulose CC4l previously dried overnight, then the plates were developed with isobutyl alcohol–H_2O–ethanol–concentrated aqueous NH_3 (25:25:50:2) for 1–1.5 hr. Using paper electrophoresis: Colors were applied as aqueous solutions to Whatman 3MM paper (25 cm × 10 cm) and dried. The paper was saturated with electrolyte, 0.1 *N* aqueous NH_3 or 0.25 *M* $Na_2B_4O_7$ buffer (pH = 9.2), and a 6-mA current was passed for about 2 hr.

CHAYTOR, J. P., HEAL, R. L. J. Chromatog. *368,* 450–455 (1986). Separation of Synthetic Dyes by High-Performance Liquid Chromatography on 3-μm Columns. Fifteen food dyes were separated and detected in the presence of an inorganic electrolyte by HPLC using a 7.5-cm × 4.6-mm Ultrasphere ODS (3 μm) column, and gradient elution at 1.5 mL/min. with aqueous 0.1 *M* Na_2SO_4 (pH 2.5; A), containing 0–100% of methanol–water–A (44:14:3; B) over 20 min. At 30 min, the composition reverted to 0% B in 5 min. Detection was at 254 nm for all colors, and 430 nm for yellow dyes, 520 nm for red dyes and 640 nm for blue-green dyes.

CHIANG, H. C., CHEN, C. H. J. Pharm. Sci. *59,* 266–267 (1970). Polyamide-Silica Gel Layer Chromatography of Yellow Food Dyes. Various yellow colorants including FD&C Yellow Nos. 5 and 6 were separated on mixed polyamide-silica gel plates using either MeOH–23% NH_4Cl–$CHCl_3$ (30:20:1.3) or iso-BuOH–EtOH–0.45% NaCl (3:5:1) as the mobile phase. Plates are prepared by dissolving 8 g of polyamide chip (Nylon 6, type 1022B, UBE Industrial Ltd., Osaka, Japan) in 80 mL of 90% formic acid and

then adding 20 mL of distilled water and warming and stirring the mixture to form a homogeneous solution. The mixture is then cooled to room temperature and 52 g of silica gel G (E. Merck) is added. Coated glass plates are air dried for 3 hr and then heated at 100°C for 30 min. Separations are better than those obtained on plates made from either polyamide or silica gel G alone.

CHIANG, H. C. J. Chromatog. *40,* 189–190 (1969). Polyamide-Silica Gel Thin-Layer Chromatography of Red Food Dyes. FD&C Red Nos. 3 and 4 and other red colorants are separated on plates coated with a mixture of polyamide and silica gel G. Plates are made by dissolving 7 g of polyamide (ε-polycaprolactam CM 1007S, Toyo Rayon Co., Tokyo, Japan) in 100 mL of warm 75% formic acid, adding 52 g of silica gel G and coating the mixture onto plates that are air dried for 3 hr and then heated at 100°C for 30 min. Mobile phases studied include iso-PrOH–5% NH_4Cl (8:3), ether-iso-PrOH–5% NH_4Cl (1:2:2), $CHCl_3$–iso-PrOH–5% NH_4Cl–glacial AcOH (1:5:2:1), *n*-BuOH–EtOH–5% Na citrate (6:4:3) and $CHCl_3$–iso-PrOH–5% NaCl–glacial AcOH (5:25:5:1).

CHIANG, H. C., LIN, S. L. J. Chromatog. *44,* 203–204 (1969). Polyamide-Kieselguhr Thin-Layer Chromatography of Yellow Food Dyes. Eight yellow colorants, including FD&C Yellow Nos. 5 and 6, are separated on TLC plates made from Nylon 6 and Kieselguhr G. The plates are made by dissolving 10 g of polyamide chip (Nylon 6, type 1022 B, UBE Industries Ltd., Osaka, Japan) in 80 mL of 90% HCO_2H, adding 20 mL of water and warming (< 40°C) and stirring the solution until homogenous. The mixture is then cooled and 40 g of Kieselguhr G (E. Merck) is mixed in. Glass plates are then coated, air dried for 3 hr, and heated at 100°C for 30 min. Using MeOH–$(CH_3)_2CO$–H_2O–30% AcONa–ethylenediamine (10:10:20:5:2) as the mobile phase, R_f values were 0.66, 0.11, 0.05, 0.91, 0.38, 0.31, 0.73 and 0.53, respectively for Naphthol Yellow S, Yellow AB, Yellow OB, FD&C Yellow No. 5, FD&C Yellow No. 6, Metanil Yellow, auramine, and picric acid. With EtOH–H_2O–Et_2O–5% NH_4Cl-ethylenediamine (15:15:10:5:2) the respective R_f values were 0.69, 0.35, 0.23, 0.88, 0.81, 0.53, 0.76 and 0.60.

CHUDY, J., CROSBY, N. T., PATEL, I. J. Chromatog. *154,* 306–312 (1978). Separation of Synthetic Food Dyes Using High-Performance Liquid Chromatography. Colorants were separated as ion-pairs on a 12-cm × 4.6-mm column of SAS-Hypersil (4.6 μm) using isopropyl alcohol–H_2O–cetrimide–acetic acid [164:236:1:1 (v/v/w/v)] as the mobile phase, and on a 15-cm × 4.6-mm column of Spherisorb S5W (5 μm) using the same mobile phase components but in the proportions 70:30:2:1 (v/v/w/v).

CIELESZKY, V., SOHAR, J. Koloriszt. Ertesito *6,* 358–373 (1964). The Use of Chromatographic Methods for the Separation, Identification, and Purification of Synthetic Food Dyes.

COTTER, R. L. Paper No. 41, 1975 Pittsburgh Conference. The Use of High Pressure Liquid Chromatography for the Analysis of Food, Drug and Cosmetic Colorings. Describes the use of a reverse-phase column (Micro Bondapak C-18, Waters Associates Inc., Milford, Mass.) for resolving mixtures of colorants and detecting impurities in colorants.

CRIDDLE, W. J., MOODY, G. J., THOMAS, J. D. R. J. Chromatog. *16,* 350–359 (1964). Thin Film Electrophoresis. Part I. The Electrophoretic Behavior of Coal-Tar Food Colours on Paper and Thin Films. Twenty-six colorants permitted in foods in the United Kingdom were subjected to electrophoresis for 1 hr at 200 V on thin layers of Kieselguhr, alumina G, silica gel G, and Whatman No. 1 paper. Thin films were prepared from slurries of 30 g of adsorbent in 60 mL of H_2O spread on 20-cm × 17.5-cm plates and dried at 105°C. The electrolytes used were normal HOAc, 0.1 *N* NH_4OH and buffer solutions of pH 4, 6, 8, and 9.2.

CRIDDLE, W. J., MOODY, G. J., THOMAS, J. D. R. Nature 202, 1327 (1964). Use of Thin Films for Electrophoresis of Coal-Tar Food Colours. Ten colorants, including FD&C Yellow No. 5, FD&C Red No. 3 and FD&C Blue No. 2, were separated by electrophoresis using thin layers of alumina, Kieselguhr, and silica gel. Results using Kieselguhr and a mobile phase of 0.05 *M* borax (pH = 9.18) at a potential of 200 V were compared with those similarly obtained on Whatman No. 1 paper.

CROSSLEY, J., THOMAS, J. D. R. Analyst *83,* 462–465 (1958). The Separation of Some Coal-Tar Food Colours by Paper Electrophoresis.

Apparatus: E. E. L. electrophoresis apparatus.
Substrate: Whatman No. 1 filter paper.
Electrolytes:

1. Normal acetic acid.
2. pH 4 Buffer: 6 mL of 0.1 *N* NaOH + 750 mL of 0.1 *M* monopotassium phthalate diluted to 1.5 L.
3. pH 6 Buffer: 85.5 mL of 0.1 *N* NaOH + 750 mL of 0.1 *M* potassium dihydrogen orthophosphate diluted to 1.5 L.
4. pH 8 Buffer: 702 mL of 0.1 *N* NaOH + 750 mL of 0.1 *M* potassium dihydrogen orthophosphate diluted to 1.5 L.
5. 1% Sodium tetraborate.
6. 0.1 *N* NH_4OH.

Electrolyte	Distances Moved (in mm)					
	1	2	3	4	5	6
Current density (in mA per 5 cm)	0.6	1.7	1.7	2.0	2.0	1.7
Time (in hr)	2	1.75	2	2	1.5	2
FD&C Red No. 3	0	0	0	0	9	0
FD&C Yellow No. 5	130	74	23	15	103	83
FD&C Blue No. 2	52	35	9	8	38	10
Ponceau MX			0	0	0 and 16	
Ponceau 4R			26	30	100	
Ponceau 3R			0	0	11	

CUZZONI, M. T. Farmaco (Pavia) Ed. pract. *15,* 752–758 (1960). Food Additives Permitted in Italy. Electrophoretic Determination of Synthetic Dyes.

DAMIANI, C. Ind. Aliment. *4,* 41–48 (1965). Identification of Water-Soluble Food Colors by Paper Chromatography Using a Pyridine Based Eluant. Mixtures of colorants are separated using EtOH–BuOH–Pyridine–Water (5:35:30:30) as the mobile phase.

DAVIDEK, J., JANICEK, G. Qualitas Plantarum et Materiae Vegetabiles *16,* 253–257 (1968). Thin Layer Chromatographic Separation of Fat Soluble and Water Soluble Food Dyes. Recommends a number of chromatographic systems. For fat-soluble colorants: aluminum oxide plates, petroleum ether-CCl_4; or paraffin-impregnated starch plates, MeOH–H_2O–AcOH (16.3:1). For water-soluble dyes: polyamide powder plates, NH_4OH–MeOH–H_2O (5:15:80).

DAVIDEK, J., DAVIDKOVA, E. J. Chromatog. *26,* 529–521 (1967). The Use of Polyamide in Analyses of Water Soluble Food Dyes. IV. Thin-Layer Chromatographic Separation of Water Soluble Food Dyes. Various combinations of NH_4OH–MeOH–H_2O were used to resolve colorants on polyamide powder. The best separation was achieved using NH_4OH–MeOH–H_2O (5:15:80). Plates were prepared by homogenizing 12 g of polyamide powder (Chemical Fabrics Lovosice Workshop Rudnik, Czechoslovakia) with 40 mL of MeOH, coating plates with a 0.2-mm layer of the mixture and then drying them at 40°C for 30 min.

Color	R_f
Amaranth	0.77
Azorubin	0.78

Color	R_f
Echrot	0.42
Cochenillerot	0.34
FD&C Red No. 3	0.21
FD&C Yellow No. 6	0.72
FD&C Yellow No. 5	0.88
Naftolgelb	0.62
FD&C Blue No. 2	0.70
Brillantschwarz	0.60

DAVIDEK, J., JANICEK, G. J. Chromatog. *15,* 542–545 (1964). Chromatography of Fat-Soluble Food Dyes on Thin Starch Layers With Stationary Non-Polar Phases.

DAVIDEK, J., POKORNY, J., JANICEK, G. Z. Lebensm. Forsch. *116,* 13–19 (1961). Detection and Determination of Fat Soluble Food Colors with the Aid of Thin-Layer Chromatography on Aluminum Oxide. Of the mobile phases studied, petroleum ether, CCl_4, and mixtures of these gave the best results.

DE GORI, R., CANTAGALLI, P. Boll. Lab. Chim. Provinciali *8,* 23–26 (1957). Extraction and Identification of Synthetic Coloring Added to Food. Naphthol Yellow S (Ext. D&C Yellow No. 7), FD&C Yellow No. 5, and croisidine are separated on Whatman No. 1 paper using EtOH–H_2O–BuOH–NH_4OH (1:1:2:1) as the mobile phase. The R_f values are 0.35, 0.05, and 0.89, respectively.

DE GORI, R., GRANDI, F. Boll. Lab. Chim. Provinciali *9,* 168–177 (1958). Separation and Identification of the Artificial Dyes Authorized for Alimentary Use by Decree of the High Commissioner of Hygiene and Sanitation of 22 December 1957. A discussion of the separation of a variety of colorants, including FD&C Yellow No. 5, FD&C Yellow No. 6, D&C Yellow No. 10, and FD&C Blue No. 2 on SS 2043A paper using EtOH–BuOH–H_2O (20:25:25).

PLA-DELFINA, J. M. J. Soc. Cosmet. Chemists *13,* 214–244 (1962). Systematic Identification of Food, Drug and Cosmetic Azo Dyes.

DICKES, G. J. J. Assoc. Public Analysts *3,* 49–52 (1965). Separation of Synthetic Water-Soluble Coloring Matters by Thin-Layer Chromatography. Separations were performed on Kieselgel G layers (250 μ, 20-cm × 10-cm glass plates heated at 160°C for 1.5 hr), using iso-PrOH–NH_4OH–H_2O (10:1:1) or saturated KNO_3 as the mobile phase).

DOBRECKY, J., CARNEVALE BONINO, R. C. D'A. DE. Revta Asoc. Bioquim. Argent, *32,* 12–15 (1967). Chromatographic Separation of Food Dyes

Permitted in Argentina. FD&C Red No. 3, FD&C Yellow Nos. 5 and 6, FD&C Blue No. 2, and several other colorants including Amaranth are separated by radial paper chromatography using 0.1 *M* HCl as the mobile phase.

DOBRECKY, J., CARNEVALE BONINO, R. C. D'A. DE. Revta Asoc. Bioquim. Argent. *32,* 16–19 (1967). Paper Chromatographic Separation of Dyes Permitted for Foods, Drugs and Cosmetics in the U.S.A. Food colorants are separated on paper by two-dimensional chromatography using the organic phase of a mixture of BuOH–AcOH–H_2O (4:1:5) and then a solution containing 2 g of EDTA and 5 mL of 25% aqueous NH_3 in 100 mL of H_2O.

DOBRECKY, J., CARNEVALE BONINO, R. C. D'A. DE. Revta Asoc. Bioquim. Argent. *32,* 139–143 (1967). Paper Chromatographic Separation of Dyes Permitted by the European Economic Community. Fourteen dyes permitted in foods and drugs were separated by two-dimensional paper chromatography using BuOH–AcOH–H_2O (4:1:5) and 0.1 *N* HCl as the mobile phases. Red and blue dyes in the series were separated by circular-paper chromatography using a 2% solution of EDTA in 5% aqueous NH_3 as the mobile phase.

DOBRECKY, J., CARNEVALE BONINO, R. C. D'A. DE. Revta Asoc. Bioquim. Argent. *36,* 143–145 (1971). Separation, Identification and Determination of Six Dyes Not Permitted (in Argentina) in Medicines or Foods. Ponceau 2R, Ponceau SX (FD&C Red No. 4), Rhodamine B, Naphthol Yellow S (Ext. D&C Yellow No. 7), Malachite Green, and Auramine were separated by two-dimensional paper chromatography on Whatman No. 1 paper. The first mobile phase was 2% EDTA (disodium salt) in 5% aqueous NH_3. The second was H_2O.

DOBRECKY, J., CARNEVALE BONINO, R. C. D'A. DE. Rev. Farm. *114,* 21–22 (1972). Paper Chromatography of Colouring Agents Used in Drugs and Cosmetics.

EGGER, K. Chromatographic Symposium II 1962. Société Belge des Sciences Pharmaceutiques, Bruxelles, 1963, p. 75. Eleven carotenoids, including canthaxanthin, β-apo-8′-carotenal, and β-carotene, were separated on thin layers of paraffin-impregnated Kieselguhr using various combinations of acetone and 95% ethanol.

EGGER, K., VOIGT, H. Z. Pflanzenphysiol *53,* 64–71 (1965). Carotenoid Separation on Thin Layers of Polyamide. Thirty-one carotenoids were chromatographed on thin layers of polyamide using nine mobile phases. The best were isooctane–MeOH–MeCOEt (80:10:10) and MeCOEt–MeOH–H_2O (30:30:10).

ESPADA, A. M. Inform. Quim. Anal. *24,* 63–67 (1970). Chromatographic Identification of Dyes Used in Carbonated Beverages. Eight colorants, including FD&C Red No. 4, FD&C Yellow Nos. 5 and 6, FD&C Blue No. 1, and caramel, were separated and identified by paper chromatography using Whatman No. 1 paper and a mobile phase containing 4.8 mL of 28% NH_4OH, 6 mL of BuOH, 4 g of NaCl, and 100 mL of H_2O.

FOPPEN, F. Chromatog. Rev. *14,* 133–298 (1971). Tables for the identification of Carotenoid Pigments. Includes paper, thin-layer, and column chromatography data.

FOUASIN, R. Rev. Fermentations Inds. Aliment. *7,* 195–219 (1953). A Systematic Method for Separation and Identification of Synthetic Colors Used in Foods. Based on a study of the chromatographic properties of more than 80 colorants, a scheme of qualitative analysis was devised that first separates the colorants into groups using immiscible solvents, and then subdivides them using various acid and alkaline mobile phases.

FRANCIS, B. J. Analyst *90,* 347 (1965). The Separation of Annatto Pigments by Thin-Layer Chromatography with Special Reference to the Use of Analytical-Grade Reagents. The Separation of Annatto Pigments on silica gel as reported by Ramamurthy and Bhalerao [Analyst *89,* 740–744 (1964)] was found to be dependent on the acetic acid content of the amyl acetate used as the mobile phase.

GALCZYNSKA, M., KWIATKOWSKA, M., MIKUCKA, B., SZOTOR, J. Herba pol. *20,* 352–355 (1974). Chromatographic Separation of Synthetic Dyes Permitted (in Poland) for Use in Pharmaceuticals. Eight dyes including FD&C Yellow No. 5 were investigated using TLC. The best separation was achieved on MN 300 CM carboxymethylcellulose using NaCl–H_2O–10% aqueous NH_3 (2:93:5) as the mobile phase.

GLOOR, R., JOHNSON, E. L. J. Chromatogr. Sci. *15,* 413–423 (1977). Practical Aspects of Reverse-Phase Ion-Pair Chromatography. The effects of the type, size and concentration of the counter-ion on the separation of disinfectants, amino-acids, catecholamines and food dyes are discussed. Practical guidelines for performing such separations are summarized.

GRAHAM, R. J. T., NYA, A. E. International Symposium on Chromatography and Electrophoresis, 5th Bruxelles, 1969, p. 486–490. Twenty-eight British food colors were chromatographed on silica gel thin layers using BuOH–EtCOMe–NH_4OH (d 0.88)–H_2O (5:3:1:1) as the mobile phase.

GRAHAM, R. J. T., NYA, A. E. J. Chromatog. *43,* 547–550 (1969). The Partition Chromatography of Food Dyes on Polycarbonate-Coated Foils. Twenty-eight food dyes permitted in Britain, including FD&C Blue No. 2,

FD&C Yellow Nos. 5 and 6, FD&C Red Nos. 3 and 4, and Amaranth were separated on precoated polycarbonate foils (10 cm × 10 cm) using butanol-aqueous NH_3 (*d* 0.88) (99:1).

GREENSHIELDS, R. N., HUNT, P. C., FEASEY, R., MAC GILLIVRAY, A. W. J. Inst. Brew. London *75,* 542–550 (1969). Preliminary Investigation of the Electrophoretic Properties of Caramels.

GRIFFITHS, M. H. E. J. Food Technol. *1,* 63–72 (1966). Systematic Identification of Food Dyes Using Paper Chromatography. Procedures are presented for the separation and identification of a variety of food colors permitted in the United Kingdom, the United States, and the European Economic Community. The technique of "double spotting" is recommended as a means of overcoming irregularities in R_f values caused by the impurities derived from the foodstuffs. "Double-spotting" consists in placing a spot of the unknown dye on top of the spots of knowns so that both will be equally affected by impurities present.

GROB, E. C., PFANDER, H., LEUENBERGER, U., SIGNER, R. Chimia *25,* 332–333 (1971). Separation of Carotenoid Mixtures by Counter-Current Extraction. β-Carotene, cryptoxanthin, canthaxanthin, and zeaxanthin were separated by counter-current distribution using the solvent system methanol–H_2O (19:1) and light petroleum (boiling range 50–70°C). Separations were performed under an atmosphere of nitrogen using the apparatus developed by Signer and Arm [Analyt. Abstr. *15,* 3034 (1968)].

HANSENS, M., DE RUDDER-TACK, Y. Pharm Tijdschr. Belg. *44,* 125–131 (1967). Paper Chromatographic Determination of Synthetic Water-Soluble Food Colors. Samples (0.25%) were prepared in 50% EtOH, spotted on paper, and developed with 2% tri-Na citrate in 5% NH_4OH.

HAYES, W. P., NYAKU, N. Y., BURNS, D. T. J. Chromatog. *71,* 585–587 (1972). Separation and Identification of Food Colours. III. Improved Resolution of Selected Dye Pairs. [For Parts I and II, see Hoodless et al., J. Chromatog. *54,* 393–404 (1971); 56, 332–337 (1971).] Systems were devised for dye pairs previously unresolved. For Orange GGN and FD&C Yellow No. 6, use BuOH–H_2O–AcOH (10:5:1); for Guinea Green B and Green S, use iso-BuOH–EtOH–H_2O–concentrated aqueous NH_3 (60:20:2:1); and for FD&C Blue No. 1 and Light Green Yellowish, use ethyl acetate–MeOH–concentrated aqueous NH_3 (10:3:3). All separations were on thin layers of cellulose powder (Applied Science Laboratories, microcrystalline).

HEILINGOETTER, R. Kosmet, Aerosole *44,* 970 (1971). Chromatography of Hair Dyes.

HONKAWA, T. Analyt. Lett. *8,* 901–910 (1975). Two-Wavelength Spectrophotometric Determination of Food-Colour Mixtures With the Function Gen-

erator. Background absorption can be eliminated at two wavelengths by use of a function generator, which balances the signal contribution at the two wavelengths. This enables one component of a two-component mixture to be quantitatively determined without sample pretreatment.

HOODLESS, R. A., PITMAN, K. G., STEWART, T. E., THOMSON, J., ARNOLD, J. E. J. Chromatog. *54,* 393–404 (1971). Separation and Identification of Food Colours. I. Identification of Synthetic Water-Soluble Food Colours Using Thin-Layer Chromatography. A TLC method is described for the separation and identification of 49 synthetic food colors that are or have been used in food products. The R_f and R_x (with respect to Orange G) values are tabulated and a scheme for the rapid identification of the components of a mixture of dyes is proposed. Cellulose and silica gel plates were used with a variety of mobile phases.

HOODLESS, R. A., THOMSON, J., ARNOLD, J. E. J. Chromatog. *56,* 332–337 (1971). Separation and Identification of Food Colours. II. Identification of Synthetic Oil-Soluble Food Colours Using Thin-Layer Chromatography. Cellulose layers (0.25 mm) are immersed in a 10% solution of liquid paraffin in light petroleum (boiling range 80–100°C) for 1 min and then either air dried or dried in an oven at 80°C. Then 1–2 μL of dye solution is applied and the chromatogram is developed with 2-methoxyethanol–MeOH–H_2O (11:3:6). Ten oil-soluble dyes, including four that are permitted in certain countries, are separated by this procedure.

IRIMESCU, I., COCIUMIAN, L., IDU, S. M. Z. Med. Labortech. *8,* 85–93 (1967). Improved Circular Chromatographic Method. Orange GGN, FD&C Blue No. 2, Amaranth, and FD&C Yellow No. 5 are separated by paper chromatography using BuOH–C_5H_5N–H_2O (3:2:5).

JACKSON, W. P., LATER, D. W. J. High Resolut. Chromatogr. Chromatogr. Commun. *9,* 175–177 (1986). Analysis of Commercial Dyes by Capillary-Column Supercritical-Fluid Chromatography. Mixtures of commercial azo, aniline and anthraquinone dyes were separated on a 20-m × 50-μm column of fused silica coated with SE-54 (0.25 μm). Supercritical n-pentane was used as the mobile phase, at 210°C, using linear pressure programming from 38 (maintained for 5 min) to 58 atm at 0.5 atm/min, and detection at 210 or 280 nm. The technique permitted efficient separations of multifunctional, polar disperse dyes with molecular weights up to about 700.

JENSEN, A. Wiss. Veroeffentl, Deut. Ges. Ernaehrung *9,* 119–127 (1963). Paper Chromatography of Carotenes and Carotenoids. A review.

JENSEN, A., JENSEN, S. L. Acta. Chem. Scand. *13,* 1863 (1959). Separation of Twenty Five Different Carotenoids on 20% Kieselguhr Paper Using Mixtures of Petroleum Ether and Acetone.

JONES, J. H., CLARK, G. R., HARROW, L. S. JAOAC *34,* 135–148 (1951). A Variable Reference Technique for Analysis by Absorption Spectrophotometry. A solution of an unknown is placed in the sample compartment of a double-beam spectrophotometer. The sample's composition is determined by continually varying the composition of a reference solution until spectral balance is obtained. The composition of the reference solution is conveniently changed by equipping the spectrometer's reference compartment with a flow-through cell connected through a circulating pump to a titration vessel into which suspected knowns are added from burettes.

KAMIKURA, M. Shokuhin Eiseigaku Zasshi *7,* 338–342 (1966). Thin Layer Chromatography of Synthetic Dyes. IV. Separation and Identification of Water-Soluble Dyes. 1. On the Developing Solvent and Condition of Activation of Silica Gel. Silica gel chromatography plates used for separation of water-soluble dyes were prepared under three conditions of activation: no activation, 60° activation for 60 min, and 100° activation for 60 min. Using eight mobiles phases, the influence of the conditions of activation on the separation of water-soluble dyes was studied. Of the eight, MeCOEt–H_2O (20:1) and MeCOEt–Me_2CO–H_2O (10:0.1:0.4) gave clear separation for xanthene dyes, including D&C Yellow No. 7, FD&C Red No. 3, D&C Red No. 22, D&C Red No. 28, Rose Bengal, and Acid Red.

KENMOCHI, K., KATAYAMA, O. Shokuhin Sogo Kenkyusho Kenkyu Hokoku *32,* 128–132 (1977). Simple Method for Identification of Cochineal Pigment and "Lac Dye" in the Presence of Synthetic Food Dyes. Cochineal dye and "lac dye" were separated from Amaranth and identified by column chromatography using a 7-cm × 1-cm column prepared from an aqueous suspension of aminoethylcellulose–Celite (2:1). The column was washed with 1% acetic acid, the test mixture was applied, and the colorants were eluted with 0.05 *N*–NH_4Cl–NH_3 buffer of pH 7 (10 mL), pH 9 (30 mL), and pH 10 (20 mL).

KOCH, L. JAOAC *26,* 245–249 (1943). Systematic Group Separation of Mixtures of FD&C, D&C and Ext. D&C Colors by Use of Immiscible Solvents.

KRAUZE, S., PIEKARSKI, L. Acta Polon. Pharm. *16,* 395–402 (1959). Electrophoretic Separation and Determination of Dyes. Various dyes, including FD&C Yellow No. 5 and D&C Orange No. 4 were studied by paper electrophoresis using a potential of 400 V and pH = 12 phosphate buffer.

LASZLO, T. Rev. Chim. (Bucharest) *29,* 978–982 (1978). Spectrophotometric Analysis of a Binary Mixture of Carotenoids. Mixtures of β-carotene and canthaxanthin in hexane exhibit absorption maxima at 250 nm and 476 nm due to β-carotene and at 464 nm due to canthaxanthin. Using a single absorbance measurement at 520 nm and simultaneous equations the proportions of

the two components can be calculated. A nomogram correlating the absorbance with the composition of the mixture can be used to evaluate the results.

LAWRENCE, J. F., LANCASTER, F. E., CONACHER, H. B. S. J. Chromatog. *210,* 168–173 (1981). Separation and Detection of Synthetic Food Colours by Ion-Pair High-Performance Liquid Chromatography. Twelve food colors were separated by HPLC using a 25-cm × 4.6-mm LiChrosorb RP-18 (10 μm) column at room temperature and mobile phases comprised of methanol–water (9:11 or 3:2) containing 5 m*M* tetrabutylammonium phosphate. Flow rates of 1, 1.5, and 2.0 mL/min were used.

LEGRAND, P. Ann. fals. fraudes *52,* 5–14 (1959). Identification by Microelectrophoresis of Small Quantities of Synthetic Coloring Matter for Foods.

LEHMANN, G., HAHN, H. G., MARTINOD, P. Fresenius Z. Anal. Chem. *227,* 81–89 (1967). Quantitative Determination of Substances Separated on Thin Plates. After conventional thin-layer chromatography, the component of interest is scraped from the plate onto smooth parchment and then transferred quantitatively to a special microchromatographic column where it is eluted, then measured spectrophotometrically.

LIN, S. C., LIN, Y., CHIANG, H. C. T'ai-Wan Yao Hsueh Tsa Chih *19,* 45–47 (1967). Polyamide Thin-Layer Chromatography of Food Colors. Eleven colorants were separated by polyamide TLC using three mobile phases. $CHCl_3$–Me_2CO–5% NaCl (0.4:9:3), $CHCl_3$–Me_2CO–5% Na salicylate (0.4:9:3), and $CHCl_3$–Me_2CO–5% Na benzoate (0.4:9:3).

LYLE, S. J., TEHRANI, M. S. J. Chromatog. *175,* 163–168 (1979). Thin-Layer Chromatographic Separation and Subsequent Determination of Some Water-Soluble Dyestuffs. Layers (0.25 mm) of silica gel G were heated at 100°C for 30 min, purified with EtOH and heated again just before use. Colorants were applied as solutions in 80% EtOH and the chromatograms were developed for 10 cm using EtOH–BuOH–H_2O (9:2:1) as the mobile phase. Each dye zone was measured spectrophotometrically after extraction with 80% EtOH, or by densitometric scanning in situ, or by reflectance after removal of the zone containing the dye and admixture of it with clean adsorbent. Densitometry was preferred at lower levels of dye (2–5 μg). For higher loadings either of the other methods proved suitable. The extraction method did not remove any dye quantitatively, but a fixed percentage of eight FD&C colorants (77–93%, according to the dye) was recovered over the range studied (2–25 μg).

MAC DONELL, H. L. Anal. Chem. *33,* 1554–1555 (1961). Porous Glass Electrophoresis. Food colors, inks, and amino acids were separated by electrophoresis on porous glass slides (Corning, No. 7930).

MC KEOWN, G. G. JAOAC *37,* 527–529 (1954). The Separation of Amaranth and Tartrazine. Slurry alumina (Fisher Scientific adsorption alumina 80–200

mesh) in water and pack it into a 15-cm × 1.5-cm-ID column to a height of 7.5 cm. Wash the column with water until the eluate is clear. Activate the column with 1:100 hydrochloric acid. Next, dissolve a 0.01-g sample in 20 mL of 1:100 hydrochloric acid and pass it through the column. Wash the column with 100 mL of water. Colors are fixed on the top of the column as lakes. Elute FD&C Yellow No. 5 (Tartrazine) with 200–300 mL of 3% sodium acetate solution. Elute Amaranth with 100–200 mL of 0.4% sodium hydroxide. Next, adjust the eluate fractions to pH = 6 with hydrochloric acid and examine them spectrophotometrically.

MC KEOWN, G. G., THOMSON, J. L. JAOAC *37,* 917–920 (1954). A Separation of Triphenylmethane Food Colors by Column Chromatography. Slurry 80–200-mesh adsorption alumina (Fisher Scientific) in water and pack it into a 15-cm × 1.5-cm-ID column to a height of 10 cm. Wash it with water until the eluate is clear. Next, prepare an aqueous sample solution containing about 0.5 mg of each color. Adjust the solution to about 0.1 *N* with dilute acetic acid and pass it through the column. Wash the column with 50 mL of water and then develop with 350 mL of 1.5% aqueous pyridine. The basic solvent alters the color of some triphenylmethane dyes. To observe the position of the colors after development, pass 100 mL of water through the column followed by 100 mL of 1:100 acetic acid. The dyes then regain their original color.

MC KEOWN, G. G. JAOAC *44,* 347–351 (1961). Paper Chromatography of Bixin and Related Compounds. Prepare a strip of filter paper 6.5 in × 22.5 in. from Whatman 3MM paper and mark a starting line 2.5 in. from one end. Impregnate by dipping into a 50% (v/v) solution of *N,N*-dimethylformide (DMF) in acetone and let dry in air for 10 min with the paper suspended in a vertical position. After drying, rapidly spot 2-μL volumes of the solutions to be analyzed. Develop the chromatogram by descending flow, using cyclohexane–chloroform–DMF-acetic acid (85:10:3:2) for 3 hr, or until a satisfactory separation is obtained.

Compound	R_f
Stable norbixin	0.009
Labile norbixin	0.014
Stable bixin	0.09
Labile bixin	0.14
Oil Yellow AB (Reference)	0.24
Stable methylbixin	0.38
Labile methylbixin	0.56

MC MILLION, C. R., DUNNING, H. A. B., Jr. J. Am. Pharm. Assoc. *48,* 249–251 (1959). A Chromatographic Technique for the Identification of Fluorescein and Phenolphthalein Derivatives. Methods are described for the chromatographic separation and identification of fluorescein and phenol derivatives on paper strips or cellulose columns by using 0.5 *M* Na_3PO_4 as the mobile phase.

MACCIÓ, I. Anales direc. nacl. quim. *9,* 52–54 (1956). Partition Chromatography of Inverted Phase. Separation of Some Fat-Soluble Dyes. The paper strip, cut from Whatman No. 1 filter paper, was submerged for 1 min in a petroleum ether solution of Vaseline and then the petroleum ether was allowed to evaporate in the air for 3 hr. The resulting chromatogram was developed by placing the treated paper strip in a closed test tube for 18 hr. The following mixtures could be separated with a mobile phase containing 80% MeOH and 20% H_2O: (1) Sudan IV, Sudan III, and butter yellow; (2) Sudan IV, Sudan III, and Yellow OB, and (3) Sudan IV, Sudan II, and butter yellow. It is not possible to separate butter yellow from Yellow OB, or a mixture of Sudan II and Sudan III, by using this mobile phase. With mobile phases containing EtOH or MeOH and H_2O and HCl, it is possible to separate mixtures of butter yellow and Yellow OB. None of these mobile phases separate Sudan II and Sudan III.

MACCIÓ, I. Anales direc. nacl. quim. *17,* 10–12 (1956). Chromatographic Study of New Dyes Derived from Coal Tar Allowed by the Food Authority for Food Use. The following dyes were separated by using a mobile phase consisting of 80% EtOH and 5% glacial AcOH in water (R_f values after each): FD&C Red No. 3 (0.88), Rose Bengal (0.88), Orange I (0.76), Ponceau 2R (0.24), Ext. D&C Yellow No. 7 (0.34), FD&C Blue No. 2 (0.08), FD&C Yellow No. 5 (0.07), Guinea Green B (0.90), and Patent Blue (0.93). Separation required 7 hr in a glass container 10 cm in diameter by 50 cm high. Separation was done on Whatman No. 1 paper 8 cm × 30 cm in size, using a 0.1% aqueous solution of the dye.

MALKUS, Z. Chem. Prum. *28,* 83–84 (1978). Contribution to Chromatographic Separation of Xanthene Dyes. Aqueous solutions containing 0.01% each of Rhodamine B, D&C Red No. 22, D&C Yellow No. 8, FD&C Red No. 3, Rose Bengal, and Phloxine P were chromatographed on Silufol TLC sheets using chlorobenzene–ethyl acetate–acetic acid (90:10:3) as the mobile phase. Spots were detected under 254-nm radiation.

MARMION, D. M. JAOAC *57,* 495–507 (1974). Applications of Nuclear Magnetic Resonance to the Analysis of Certified Food Colors. Individual colorants in secondary mixtures are identified and quantitated by NMR. Spectra are obtained in mixed deuterated solvent (water: dimethylsulfoxide; D_2O: DMSO–d_6, 2:1 v/v) at 100–105°C.

MASIALA-TSOBO, C. Anal. Lett., Part A. *12,* 477–490 (1979). High-Pressure Liquid Chromatography of Synthetic Food Dyes. A number of colorants including FD&C Yellow No. 5 and FD&C Red No. 3 were separated on a Nucleosil 10-C8 column (25 cm × 4 mm) or a Nucleosil R5C8 column (20 cm × 4 mm) using 5 m*M* tetrabutylammonium phosphate in MeOH–H_2O (9:11, or 3:2) as the mobile phase at a flow of 1 mL/min. Most dyes tested separated well using the 9:11 mobile phase, however a few colorants, including FD&C Red No. 3, required the 3:2 mobile phase to speed elution. Detection was at 254 nm.

MASLÓWSKA, J. Chromatographia *20,* 99–101 (1985). New Chromatographic Method for the Separation of Food-Dye Mixtures on Thin Magnesia Layers. Colorants including FD&C Blue No. 2, cochineal, and FD&C Yellow No. 5 were separated on 0.25-mm layers of magnesia coated on degreased glass plates. The plates were activated for 1 hr at 130°C, samples were applied, then the plates were developed by the ascending technique at 20°C ± 0.1° for 1.5 to 2.5 hr using 15% Na citrate–methanol (4:1) as the mobile phase.

MASLOWSKA, J., MARSZAL, K. Dtsch. Lebensm.-Rundsch *77,* 275–278 (1981). High-Pressure Liquid-Chromatographic Separation of Food Dyes. Six food dyes were separated by HPLC using a LiChrosorb RP-2 column and anhydrous methanol as the mobile phase, a LiChrosorb RP-18 column and aq. 5% methanol as the mobile phase, and a μ-Bondapak C18 column, and 15% isopropyl alcohol containing Waters Associates PIC-A reagent as the mobile phase. The optimum wavelengths for detection were 313 nm and 254 nm.

MASSART, D. L., DE CLERCQ, H. Anal. Chem. *46,* 1988–1992 (1974). Applications of Numerical Taxonomy Techniques to the Choice of Optimal Sets of Solvents in Thin Layer Chromatography. The problem of making a rational selection of a restricted set from a large number of available chromatographic systems for the separation of a particular group of substances is discussed. The systems are classified according to their mutual resemblance by numerical taxonomy techniques. From the resulting groups with dissimilar separation characteristics, one system per group can be chosen according to criteria such as availability and cost. In this way, a combination of systems with desirable characteristics and yielding relatively little correlated information should be obtained. This is illustrated by the selection of a combination of three mobile phase/stationary phases from a set of ten for the separation and identification of 26 yellow, orange, and red synthetic food dyes. The selection criterion in the groups, obtained by numerical taxonomy classification, is the information content. The resulting best combination is given and is found to permit unambiguous identification of all 26 dyes.

MERLE, M. H., PUERTA, A., PUERTA, M. Ann. Falsif. Expert. Chim. *71,* 263–266 (1978). Differentiation Between Orange GGN and FD&C Yellow

No. 6. The colorants were separated by HPLC using a 30-cm Bondapak C18 column and a mobile phase of H_2O–MeOH (1:1) containing 1% of tetrabutylammonium phosphate. Detection was at 280 nm.

MIGLIETTA, E. Boll. lab. chim. provinciali *11,* 216–229 (1960). Chromatography and Spectrophotometry of Some Certified Dyes. Chromatographic values with four mobile phases and color curves are reported. A standardization of chromatographic and spectrophotometric characteristics of various certified dyes is proposed.

MITCHELL, L C. JAOAC *36,* 943–946 (1953). The Separation of Certain Anthraquinone Dyes by Paper Chromatography. Spot a dimethylformamide solution of the sample on 8-in. × 8-in. Whatman No. 1 filter paper. Uniformly impregnate the paper with 1:99 refined soybean oil–ethyl ether by rapidly spraying it from top to bottom in horizontal strips. Elute the sheet in a 12-cm × 25-cm × 25-cm glass tank with 4:1 methyl Cellosolve–water until the mobile phase approaches the top of the paper (ca. 2.5 hr). Compare against standards simultaneously prepared.

Color	R_f (approx.)
D&C Green No. 5	0.98
"Monosulfonated" D&C Green No. 5	0.86
D&C Green No. 6	0.14
Ext. D&C Violet No. 2	0.90
D&C Violet No. 2	0.24

MITRA, S. N., MATHEW, T. V., GUPTA, P. K. J. Inst. Chem. (India) *40,* 177–178 (1968). A Note on Paper Chromatography of Food Colours. Sample solutions (0.01%) were spotted on 40-cm × 10-cm Whatman No. 1 paper.

R_f Values of Food Colors Using Two Mobile Phases

	R_f	
Color	Mobile Phase A	Mobile Phase B
Ponceau 4R	0.183	0.437
FD&C Red No. 3	0.560	0.018
Carmoisine	0.10	0.085

R_f Values of Food Colors Using Two Mobile Phases

Color	R_f	
	Mobile Phase A	Mobile Phase B
Fast Red E	0.09	0.07
Amaranth	0.05	0.15
Red 6B	—	0.056
Red FB	—	0.0
Acid Magenta	—	0.89
FD&C Yellow No. 5	—	0.471
FD&C Yellow No. 6	—	0.21
FD&C Blue No. 2	0.113	—

Mobile phase A–Iso-amyl alcohol: 95% ethanol: NH_4OH : H_2O (4:4:1:2); 18 hr; descending.

Mobile phase B–2% Sodium citrate in 5% NH_4OH; 3 hr; descending.

MITRA, S. N., CHATTERJI, R. K. J. Proc. Inst. Chemists *27,* 169–176 (1955). Separation of Permitted Coal-Tar Food Colors by Paper Chromatography. Paper chromatographic methods for the separation of FD&C Red No. 3, FD&C Yellow No. 5, FD&C Blue No. 2, Amaranth, and Orange I are described. The most suitable mobile phases were 5% NaCl or 0.25-1*N* HCl. Separations can be improved by two-dimensional chromatography using iso-BuOH-5% NaCl as the second mobile phase.

MORI, I., KIMURA, M. J. Pharm. Soc. *74,* 179 (1954). Electromigration of Food Colours. A number of systems were evaluated for the separation of various color additives using Toyo filter paper No. 50 as the support.

Conditions

	Electrolyte	V	mA/cm	hr
I	30% Acetic acid	700	0.5	4
II	10% Acetic acid	700	0.6	1
III	1% Borax	500	1.0	4
IV	0.1% NH_4OH	700	0.4	1
V	5% $NaHCO_3$	200	2.5	4

Relative Separation

	I	II	III	IV	V
Amaranth	38	30(71)[a]	23[b],60[c]	80	21
D&C Red No. 22	4	0	21	22(38)	
D&C Yellow No. 7	12	−8	46	74	
FD&C Blue No. 2	54	49	48,75[d]	60	14
FD&C Yellow No. 5	72	80	85		43
Naphthol Green B	23	21	28	28	
D&C Red No. 28	0		33		

[a]Parentheses indicate fluorescence.

[b]Blue spot.

[c]Yellow spot.

[d]Small spot.

MORI, H., YOKOYAMA, T., HAMADA, K. Eisei Shikenjo Kenkyu Hokoku *81,* 57–60 (1963). Separation of Some Japanese Official Cosmetic Coal-Tar Dyes by Column Chromatography. Mixtures of red dyes were separated on a cellulose powder-alumina (1:1) column using a mixture of BuOH–EtOH–0.5*N* NH_4OH (6:2:3).

MÜLLER, K., TÄUFEL, K. Ernährungsforschung *1,* 354–361 (1956). Paper Chromatographic Separation and Identification of Food Dyes Allowed in the German Democratic Republic.

NAFF, M. B., NAFF, A. S. J. Chem. Ed. *40,* 534–535 (1963). TLC on Microscope Slides. D&C Yellow No. 7 and related fluoresceins were separated on silica gel G using toluene–acetic acid (65:35).

NETTO, I. Ann. fals fraudes *50,* No. 580 (1957). R_f Values of Food Colors. The substrate was Whatman No. 1 paper and the mobile phase was 1 *N* HCl.

Color	R_f
Ponceau 3R	0.031–0.040
Amaranth	0.063–0.077
FD&C Blue No. 2	0.099–0.131
FD&C Yellow No. 5	0.320–0.340
Ext. D&C Yellow No. 7	0.534–0.574
Guinea Green B	0.791–0.860

NEY, M., BERGNER, K. G., SPERLICH, H., MIETHKE, H. Deut. Lebensm. Rundschau *61,* 148–150 (1965). The Food Color Patent Blue. Patent Blue V (CI 42051), Patent Blue VF (CI 42045), Patent Blue AE (FD&C Blue No. 1, CI 42090), and Wool Green BS (CI 44090) were separated by paper chromatography using two mobile phases: BuOH–EtOH–25% NH_4OH–H_2O (4:4:1:3) and HOAc–pyridine–H_2O (55:25:20).

NIITSU, Y. Bunseki Kagaku *13,* 1239–1242 (1964). High-Voltage Paper Electrophoretic Analysis of Water-Soluble Coal Tar Dyes for Food. Xanthene and triphenylmethane dyes were separated on filter paper using pH = 3–11.6 buffers and an applied voltage of 3000 V. The cooling agent was hexane. Migration distance was dependent on both the pH of the buffer and the structure of the dye.

ODA, S., SAWADA, T., NOMURA, M., KAMADA, H. Anal. Chem. *51,* 686–688 (1979). Simultaneous Determination of Mixtures in Liquid by Laser-Induced Photoacoustic Spectroscopy. Laser-induced photoacoustic spectroscopy (LIPAS) was used to determine individual colorants in dilute aqueous mixtures. The combinations studied included FD&C Yellow No. 6. Many of the mixtures examined have been previously found to be extremely difficult to analyze by conventional colorimetric and dual-wavelength spectrometry.

OHNISHI, S., NISHIJIMA, Y., KIJIMA, K., KANO, S., TONOMURA, M. Bunseki Kagaku *25,* 353–357 (1976). Separation of Tar Dyes for Cosmetic Products by High-Speed Liquid Chromatography. Six water-soluble azo-dyes, and five water-soluble xanthene dyes were separated on a 60-cm × 4-mm column of TSK GEL LS-140 porous polymer operated at 25°C, using methanolic 1 to 2% triethylamine–methanolic 1 to 2% acetic acid (10:1) as the mobile phase at a flow rate of 0.5 mL/min, and UV detection. Five of the colorants were also separated on a 30-cm × 2.1-mm column of Vydac AX using a linear gradient of 0.1 *M* $Na_2B_4O_7$ and 0.01 *M* $Na_2B_4O_7$ in *M* $NaClO_4$ as the primary and secondary mobile phases, respectively. By slightly adjusting column parameters and mobile phase molarities, this method was also used to separate fluorescein and mono-, di-, tri- and tetrabromofluorescein. The xanthene dyes and the bromo-substituted fluoresceins were also separated on a 30- or 50-cm × 0.5-cm column of TSK GEL LS-212 or LS-213 using either methanol or 0.05% H_2SO_4 in methanol as the mobile phase.

OHTA, H., AKUTA, S., OKAMOTO, T., OSAJIMA, Y. Kyushu Diagaku Nogakubu Gakugei Zasshi *33,* 101–107 (1979). Column-Chromatographic Separation of the Major Anthocyanin Pigments from Grapes. Extracts of homogenized grapes were passed through a column of Dowex 50W-X4 resin, pigments were washed from the column with ethanolic 1% HCl, and any

anthocyanins present were isolated using Polyclar AT (PVP) and polyamide C-200 (PA). Malvin, malvidin 3-glucoside, peonin, and peonidin 3-glucoside were detected by TLC on Avicel SF. Adsorption of the pigments by PVP and PA and their recoveries at pH values of 1–7 were measured by spectrophotometry at 530 nm; both adsorption and recovery were highest at pH 7 for both adsorbents. Recovery from PA was 80% (compared with 50% from PVP) at all pH values tested, but adsorption on PVP was stronger than that on PA. A column (40 cm × 1 cm) of PVP loaded with 1 mL of solution from 100 g of grapes (homogenized in 500 mL of ethanolic 1% HCl) separated the four pigments cited with 0.1% HCl solution in 30% ethanol (or in 60% methanol) as the mobile phase at a flow of 0.33–0.4 mL/min. A similar column of PA gave the same separation by stepwise elution with 0.1% HCl in H_2O and in 20% ethanol. With grape juice, the PA column showed less resolution than the PVP column.

OKA, H., IKAI, Y., KAWAMURA, N., YAMADA, M., INOUE, H., OHNO, T., INAGAKI, K., KUNO, A., YAMAMOTO, N. J. Chromatog. *411,* 437–444 (1987). Simple Method for the Analysis of Food Dyes on Reversed-Phase Thin-Layer Plates. Mixtures of food dyes were separated by TLC on C_{18}-modified silica gel plates. Various mobile phases were tried, and pH was adjusted with NH_3 or acetic acid. The determination of 13 dyes was achieved by using methanol–acetonitrile–aqueous 5% Na_2SO_4 (3:3:10), then methanol–ethyl methyl ketone–aqueous 5% Na_2SO_4 (1:1:1) as mobile phase in the same direction. Separation was best at pH 6.0–7.0. After drying the chromatogram, the absorbance of each spot was measured by UV densitometry. Calibration graphs were rectilinear for 0.2–1.0 μg in each instance.

OMORI, T. BUNSEKI KAGAKU *29,* 189–193 (1980). Simultaneous Determination of Water-Soluble Food Dyes by High-Performance Thin-Layer Chromatography. Eleven water-soluble dyes were separated on Si 60 gel using ethyl methyl ketone–MeOH–aqueous 28% NH_3 (8:4:1) as the mobile phase. Dyes unstable in aqueous conc. NH_3 were separated on layers of cellulose using BuOH–ethyl methyl ketone–aqueous 1% NH_3–H_2O (4:2:1:1) as the mobile phase. Between 5 and 20 ng of each dye was determined using a dual-wavelength densitometer at 430, 550, or 630 nm.

PAN, C. Fenxi Huaxue *11,* 218–220 (1983). Determination of Food Dyes by Dual-Wavelength Spectrophotometry. The method of Sasaki (see) was applied to mixtures of citron yellow, carmine, amaranth and indophenol blue, using their maxima at 440, 510, 530, and 620 nm, respectively.

PANOPOULOS, G., MEGALDOIKONOMAS, J. Chim. Anal. *36,* 68–69 (1954). Application of Chromatography to Identify the Dyes Used in Coloring Food Products.

PARIS, R. R., ROUSSELET, R. Ann. Pharm. Franc. *16,* 747–756 (1958). Characterization of Dyes of Vegetable Origin by Paper Chromatography. The R_f values and recommended mobile phases are given for a number of natural dyestuffs, including caramel, carotene, chlorophyll, indigo carmine, and saffron.

PARKÁNYI, C. Chemie *10,* 45–47 (1958). Square Capillary Analysis on Paper. Egacide Orange G and GG, FD&C Yellow No. 5, Amaranth, and Metanil Yellow O were separated on filter paper using water, aqueous 10% NH_3, or 5% HCl.

PARRISH, J. R. J. Chromatog. *33,* 542–543 (1968). Chromatography of Food Dyes on Sephadex. Separations by TLC on Sephadex G-25 (superfine grade) were used to predict separations on columns of the same material.

R_f Values of Dyes on Sephadex G-25

DYE (Cl No.)	Mobile Phases		
	A	B	C
Blue VRS (42045)	0.48	0.41	0.31
FD&C Red No. 4 (14700)	0.47	0.27	0.20
Ponceau 4R (16255)	0.46	0.27	0.21
FD&C Yellow No. 5 (19140)	0.42	0.27	0.13
Ponceau 3R (16155)	0.40	0.12	0.06
FD&C Blue No. 2 (73015)	0.36	0.13	0.06
Amaranth (16185)	0.34	0.15	0.06
Ext. D&C Yellow No. 7 (10316)	0.33	0.29	0.16
Carmoisine (14720)	0.27	0.08	0.03
Orange G (16230)	0.25	0.07	0.04

A = Water.

B = 0.1% Sodium sulfate solution.

C = 4% Sodium sulfate solution.

PEARSON, D. J. Assoc. Public Analysts *2,* 30–34 (1964). R_f Values of Permitted Synthetic Water-Soluble Coloring Matters. Data are reported using 80 g of PhOH + 20 g of H_2O as the mobile phase.

PEARSON, D., CHAUDHRI, A. B. J. Assoc. Public Analysts *2,* 22–30 (1964). R_f Values of Some Nonpermitted Synthetic Water-Soluble Coloring Matters. R_f Values were recorded for seven mobile phases.

PEARSON, D., WALKER, R. J. Assoc. Public Analysts *3,* 45–48 (1965). R_f Values of Permitted Synthetic Water-Soluble Coloring Matters. Twelve mobile phases, including those proposed by the Association of Public Analysts, the British Standards Institute, and the British Food Manufacturing Industries Research Association, were used to study 29 dyes by ascending chromatography.

PEARSON, D. J. Assoc. Public Analysts *11,* 52–56 (1973). Identification of Oil-Soluble Food Colours. Reversed-phase paper chromatography using liquid paraffin as the stationary phase was used to study 16 colors and eight mobile phases. Spectrophotometric absorption maxima for solutions of the colors in light petroleum are also reported.

PEEREBOOM, J. W. Chem. Weekblad *57,* 625 (1961). R_f Values (Thin Layer) of Fat-Soluble Dyestuffs.

PEEREBOOM, J. W. C., BEEKES, H. W. J. Chromatog. *20,* 43–47 (1965). Thin-Layer Chromatography of Dyestuffs on Polyamide and "Silver Nitrate" Layers. A study is reported of the separation of fat-soluble dyestuffs on layers of silica gel G, Kieselguhr G, aluminum oxide G, polyamide, and silver nitrate-impregnated silica gel.

PENNER, M. H. J. Pharm. Sci. *57,* 2132–2135 (1968). Thin-Layer Chromatography of Certified Coal-Tar Colour Additives. Nineteen dyes used in pharmaceutical preparations are separated on 0.25-mm layers of microcrystalline cellulose (Avicel) using the following mobile phases: ethyl acetate–BuOH-pyridine–H_2O (5:5:6:5); ethyl acetate– BuOH–aqueous NH_3 (d = 0.88) (4:11:5); ethyl acetate–PrOH–aqueous NH_3 (d = 0.88)–H_2O (7:7:4:4); and PrOH–ethyl acetate–aqueous NH_3 (d = 0.88) (13:15:12).

PFANDER, H., SCHURTENBERGER, H., MEYER, V. R. Chimia *34,* 179–180 (1980). Separation of Carotenoids by High-Performance Liquid Chromatography. I. Separation of Carotenes and Diterpenes. The α-, β- and γ-isomers of carotene and lycopene were separated in less than 10 min on a 25-cm × 4.6-mm column of 5-μm Sperisorb ODS using acetonitrile (2 mL/min) as the mobile phase and detection at 440 nm. Four diterpenes differing only in the number of double bonds were separated in 3 min on a 25-cm × 3.2-mm column of 5-μm Sperisorb Nitrile using pentane as the mobile phase (1 mL/min) and detection at 230 nm or 375 nm, and in less than 10 min on a column of 5-μm LiChrosorb Si 60 using pentane containing 0.02% acetonitrile as the mobile phase (1 mL/min).

PIETSCH, H. P., MEYER, R. Nahrung *9,* 154 (1965). Thin-Layer Chromatographic Separation of Artificial Organic Food Dyes with Kieselgel D.

PIEKARSKI, L., KRAUZE, S. Roczniki Panstwowego Zakladu Hig. *10,* 495–500 (1959). Determination of Dye Mixtures After Their Chromatographic

Separation. Nine water-soluble dyes permitted in Poland for use in food are separated by two-dimensional paper chromatography using BuOH–EtOH–H_2O (2:1:1) and *N* NH_4OH.

PINTER, I., KRAMER, M., KLEEBERG, J. Elelmiszervizsgalati Kozlemen *14,* 169–175 (1968). Thin-Layer Chromatographic Method for Detecting Various Cosmetic Dyes in Mixtures.

POPOV, A., MITSEV, I. Izvest. Inst. Org. Khim., Bulgar. Akad. Nauk *2,* 5–11 (1965). Identification of Erythrosine in the Presence of Other Red Dyes. Mixtures of erythrosine and resorcinolphthalein food dyes are separated by paper chromatography using 10% NH_4OH–20% NaOAc-tert-BuOH (with 5% H_2O) (65:20:15).

POPOVICI, V., SCHWEIGER, A., SPITZER, A. Farmacia *13,* 569–573 (1965). Paper Chromatography of Some Dyes Used in Pharmacy.

PRASAD, C. A. K., RAO, M. V., NAGARAJA, K. V., KAPUR, O. P. Indian Food Packer *37,* 74–77 (1983). Beverages and Confectionery: Thin-Layer Chromatographic Separation and Identification of Water-Soluble Green and Blue Coal-Tar Dyes. FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, and other colorants were separated using silica gel plates impregnated with 1.25% starch, 5% $CdCl_2$ or 5% $NiSO_4$, and aqueous 80% phenol as the mobile phase.

PUCHE, R. C. T. Rev. Assoc. Bioquim. Argent. *22,* 228–236 (1957). Paper Partition Chromatography of Synthetic Dyes Authorized for Coloring Foods. Food colors were spotted on S&S No. 0859 paper and developed with BuOH saturated with 10% HCl (I) or 0.5 mL of xylidine and 5 mL of concentrated HCl in 10 mL of H_2O (II). The following dyes were chromatographed (name, R_f using I, R_f using II): FD&C Red No. 3, 0.00, 0.60; Rose Bengal, 0.00, 0.42; Bordeaux Red, 0.07, 0.21; Ponceau Red 2R, 0.18, 0.28; Orange I, 0.22, 0.41; FD&C Yellow No. 5, 0.47, 0.17; FD&C Blue No. 2, 0.94, 0.42; Guinea Green B, 0.89, 0.50.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. JAOAC *64,* 1–8 (1981). Ion-Pair High Performance Liquid Chromatography of Synthetic Water-Soluble Acid Dyes. Two ion-pair HPLC systems were studied for separating fourteen food colors. System 1 used a 25-cm × 3-mm-ID RSIL C18 HL (10 μm) column, and stepwise gradient elution using 2:3 to 3:2 methanol–5 m*M* tetrabutylammonium hydroxide (A) at 1 mL/min as the mobile phase. System 2 used a 30-cm × 2-mm-ID Micro-Pak MCH (10 μm) column coated with pentanol, and 1 m*M* A in phosphate buffer (pH = 7; I = 0.1), 50% saturated with pentanol as the mobile phase.

RAI, J. Chromatographia *5,* 211–213 (1971). Separation and Identification of Water-Soluble and Fat-Soluble Food Dyes by Thin-Layer Chromatography.

The R_f values are tabulated for 21 fat-soluble dyes in four mobile phases and 21 water-soluble dyes in five mobile phases using silica gel G as the adsorbent.

RAMAMURTHY, M. K., BHALERAO, V. R. Analyst *89,* 740–744 (1964). A Thin-Layer Chromatographic Method for Identifying Annatto and other Food Colours. A simple technique is described for separating and identifying 11 yellow food colors: fat-soluble annatto, water-soluble annatto, curcumin, Oil Orange S, ethyl bixin, Oil Orange E, Yellow OB, Yellow AB, FD&C Yellow No. 5, FD&C Yellow No. 6 and β-carotene. Annatto and curcumin can be separated from other fat-soluble and water-soluble dyes on glass slides coated with H_2SiO_3 containing plaster of Paris or on silica gel G using amyl acetate as the mobile phase. The fat- and water-soluble dyes can be separated further on glass slides coated with $CaCO_3$ containing starch treated with liquid paraffin using MeOH–H_2O–NH_3 (20:5:1) as the mobile phase. (see Francis, B. J.).

RAO, T. S. S., SASTRY, L. V. L., SIDDAPPA, G. S. Indian J. Technol. *3,* 332–334 (1965). Separation of Synthetic Food Colors by Chromatography and Electrophoresis. The R_f values (using ascending chromatography) are given for FD&C Red No. 3, Amaranth, Carmoisine, Fast Red E, Red 6B, Ponceau 4RS, FD&C Yellow No. 5, FD&C Yellow No. 6, Blue VRS, Brilliant Black, and FD&C Blue No. 2 in 15 mobile phases. Five systems were studied using circular chromatography. Electrophoresis data are given for the dyes in pH = 8.6 borate, pH = 4.5 phthalate, pH = 8.6 1% $Na_2B_4O_7$, and pH = 7 phosphate buffers.

RAO, V. K., SARMA, P. S. N. J. Sci. Ind. Research *21D,* 61–63 (1962). Paper Chromatography of Food Colors. A mobile phase consisting of 1.6% ethylenediamine hydrate and 2% iso-BuOH in H_2O is used in the paper-chromatographic separation and identification of coal-tar dyes permitted under the Indian Prevention of Food Adulteration Act of 1954.

ROY, B. R., SUNDARARAJAN, A. R., MITRA, S. N. J. Sci. Ind. Res. *18,* 38–40 (1959). Analysis of Synthetic Food Colours Prescribed in India. Various chromatographic schemes are outlined for the paper chromatographic separation of food colors.

SADINI, V. Chimica *37,* 381–394 (1961). Identification of Synthetic Dyes Permitted in Foods in the Countries Included in the European Economic Community. The chromatographic behavior of 23 dyes permitted in foods in the countries included in the European Economic Community is studied by descending chromatography using 21 mobile phases.

SADINI, V. Rass Chim. *13,* 13–18 (1961). Further Developments on Chromatographic Research on the Coloring Matter of Food. A review with 100

references of research carried out during 1959 and 1960 on the chromatography of colors and pigments, natural and synthetic, both found in and added to foods.

SADINI, V. Rass. Chim. *12,* 27–35 (1960). Partition Chromatography on Paper of Food-Additive Dyes. The 13 dyes that may be used as food additives in Italy were chromatographed on paper using 150 mobile phases.

SAENEZ, I., RUIZ, L., LAROCHE, C. Bull. Soc. Chim. France 1594–1597 (1963, 8–9). Thin-Layer Chromatography of Synthetic Dyes.

SAGUY, I., MIZRAHI, S., KOPELMAN, I. J. J. Food Sci. *43,* 121–123 (1978). Mathematical Approach for the Determination of Dye Concentrations in Mixtures. A procedure is described based on the nonlinear curve-fitting of the visible spectrum of a mixture of colorants with a predicted function for each of the individual colorants. The log-normal distribution function showed excellent fit for FD&C Yellow No. 5, Amaranth, and Yellow 2G, which were used to test the mathematical model.

SAGUY, I., KOPELMAN, I. J., MIZRAHI, S. J. Food Sci. *43,* 124–127 (1978). Computer-Aided Determination of Beet Pigments. A fast, accurate method is described for determining the major beet pigments (betanin, vulgaxanthin I, and betalamic acid) and browning substances by visible spectrophotometry. The procedure is based on a nonlinear curve-fitting method [*Ibid. 43,* 121–123 (1978)], which eliminates the need for laborious, time-consuming separations. The procedure is particularly useful for the continuous monitoring of time- and temperature-related processes such as drying and storage.

SASAKI, H., IWATA, T. Shokuhin Eiseigaku Zasshi *13,* 120–126 (1972). Analytical Studies on Food Dyes. IV. Direct Densitometry of Paper Chromatograms of Food and Other Dyes by Transparent Methods. Paper chromatograms of 12 water-soluble and four oil-soluble dyes (including Amaranth and FD&C Yellow No. 6) were directly scanned using a photoelectric densitometer. The integrated readings were proportional to the square root of dye amounts or concentration.

SASAKI, H., FUKUSHIRO, S. Shokuhin Eiseigaku Zasshi *13,* 127–132 (1972). Analytical Studies on Food Dyes VI. Determination of Monoazo Food Dye Mixtures by Direct Densitometry of Transparent Types. Paper chromatograms of two or four component mixtures of Amaranth, FD&C Yellow No. 5, New Coccine, and FD&C Yellow No. 6 were scanned with a densitometer. Recovery was 95.7–100.7% or 84.8–112.1% for the two- or four-component mixtures, respectively.

SASAKI, H. Shokuhin Eiseigaku Zasshi *19,* 1–11 (1978). Quantitative Analysis of Multi-Component Mixtures of Food Colors and Similar Dyes by Dual-Wavelength Spectrophotometry. 0.02 N Ammonium acetate solutions containing three or four different color additives were analyzed by measuring the

absorbance of the samples at two different wavelengths and comparing the results to standards. Mixtures of xanthene colorants were the most difficult to quantitate.

SASAKI, H., TANSEI, H., MIWA, M., ASAKURA, M., SHIRAI, K. Shokuhin Eiseigaku Zasshi *19,* 12–22 (1978). Quantitative Analysis of Coal-Tar Dye Mixtures With An Ordinary Spectrophotometer Using a Dual-Wavelength Method. Binary mixtures of 13 dyes were analyzed using a dual-wavelength procedure (see above). Results are reported for 15 combinations containing 5 μg/mL of each of two colorants. When the method was applied to commercial dye mixtures, 86.7–103.0% of the label claims were found.

SASAKI, H. Shokuhin Eiseigaku Zasshi *19,* 34–43 (1978). Qualitative and Quantitative Analysis of Coal-Tar Dyes for Food by Derivative Spectrometry. I. Rapid Analysis of Each Component in Some Two- and Three-Component Mixtures. A time-derivative method using a double-beam spectrophotometer was developed to facilitate the detection and determination of food colors, even in turbid solution. Absorption coefficients at characteristic wavelengths, both on absorption and first- and second-order derivative spectra, were determined for 13 colorants at concentrations up to 20 μg/mL in 0.02 N ammonium acetate. Recoveries for two-component systems containing 0–10 μg/mL of each colorant, and for three-component systems containing 33.3% of each using second-order derivative spectra ranged from 99% to 106% and 86% to 112%, respectively.

SASAKI, H. Tottori Daigaku Kogakubu Kenkyu Hokoku *9,* 106–115 (1978). Qualitative and Quantitative Analysis of Coal-Tar Dyes for Food by Derivative Spectrophotometry. IV. Analysis in Solid Foods. Instant powdered beverages were dissolved in 0.02 *N* ammonium acetate, colored candies were dissolved in water at 50–60°C, ices were melted and fish cakes were shredded and then extracted with ethyl ether. Artificial food-coloring agents in these solutions were quantitated by derivative spectrometry without further purification. The results obtained correlated well with those obtained using thin-layer chromatography.

SASTRY, L. V. L., SEBASTIAN, K., KRISHNAPRASAD, C. A. J. Food Sci. Technol. *7,* 132–134 (1970). Estimation of Total Dye Content of Food-Colour Preparations. The component dyes were separated by chromatography on Whatman No. 3 paper with various mobile phases and extracted from the chromatogram with 0.1 *M* HCl in 70% ethanol. The extinction of each extract was then measured at the appropriate wavelength of maximum absorption. The recoveries of pure dyes ranged from 97% to 101%, but that of indigo carmine was only 5–29%.

SCHNEIDER, H., HOFSTETTER, J. Deut. Apotheker-Zgt. *103,* 1423–1424 (1963). Thin Layer Chromatography of Enamel-Like Pigments Used in

Drugs. Thin-layer chromatography of H_2O-insoluble enamel-type pigments can be performed after the Al salt is converted to the free acid by stirring for 2 hr with *N* HCl. An aliquot is spotted on a 0.5-mm cellulose plate and eluted with a 4:1 solution of 2.5% Na citrate–25% NH_3. Identification is by color and position of the spot compared with standards. The technique can be applied to most substances approved as food coloring, except for lightly colored sugar coatings, where no positive determination could be made because of sugar interference.

SCLAR, R., FREEMAN, K. JAOAC *38,* 796–809 (1955). Chromatographic Procedures for the Separation of Water-Soluble Acid Dye Mixtures. General methods are given for the chromatographic separation of FD&C Colors. Paper chromatography–Place one drop of fresh 1% aqueous sample solution on a 6-cm × 22-cm strip of Whatman No. 1 filter paper. The sample should be reasonably free of salt, sugar, starches, and other substances. Allow it to dry. Suspend the strip in an air-tight tank containing 0.5–1 cm of 2:3 water–ethylene glycol monomethyl ether acetate (methyl Cellosolve acetate). Alternatively, 1:4 water–methyl Cellosolve acetate can be used. Develop for 2–3 hr. If all FD&C colors are present, 10 colored zones may be observed. From the bottom up, the zones are as follows:

1. FD&C Yellow No. 5.
2. FD&C Red No. 2.
3. FD&C Blue No. 2.
4. FD&C Red No. 1 plus FD&C Red No. 4.
5. FD&C Yellow No. 6.
6. FD&C Blue No. 1 plus FD&C Green No. 2 plus FD&C Green No. 3.
7. FD&C Yellow No. 1.
8. FD&C Violet No. 1 plus FD&C Green No. 1.
9. FD&C Orange No. 1.
10. FD&C Red No. 3.

FD&C Reds No. 1 and 2, Greens No. 1 and 2, Yellow No. 1, Orange No. 1, and Violet No. 1 are no longer certifiable as FD&C colors.

Column chromatography–Prepare 30%, 25%, and 20% solutions of sodium chloride. To prepare solutions containing both ethyl alcohol and sodium chloride, mix the required reagents with water. For example, to prepare 20% ethyl alcohol plus 15% sodium chloride, mix 12 parts of 95% ethanol, 30 parts of 30% sodium chloride solution, and 18 parts of water. Slurry Solka-Floc BW-40 (manufactured by Grefco Inc., Dicalite Division, New York) in water and pour the amount indicated into a 2.5-cm-ID glass column. Allow it to stand for 12 hr. Treat sample as directed in the following table.

General Method for FD&C Colors

Pass the following solutions through the columns in the order given, allowing as little mixing as possible between successive solutions.	Collect the following bands as they separate and emerge from the column; treat as indicated.	
Column No. 1 - 50 g of Cellulose		
		mL (approx.)
1. 50 mL of 5% ethanol plus 15% sodium chloride (prewash)	Water plus prewash (discard)	350
2. Unknown colors dissolved in 50 mL of 20% sodium chloride		
3. 250 mL of 5% ethanol plus 1% sodium chloride	FD&C Green No. 3 plus FD&C Green No. 2[a] plus FD&C Blue No. 1	45
	Tailing plus ethanol solution (discard)	16
	FD&C Yellow No. 1[a] plus FD&C Yellow No. 5 (separate on column No. 4)	55
	Tailing plus ethanol solution (discard)	22
	FD&C Yellow No. 6	91
	Ethanol solution (discard)	25
4. 250 mL of 0.5% sodium chloride	FD&C Blue No. 2 plus FD&C Red No. 2[a] (separate on column No. 5)	132
	Dilute tailing (discard)	55
5. Sufficient water to remove remaining colors	Remaining colors	112
Column No. 2 - 40 g of Cellulose		
1. 50 mL of 20% ethanol plus 15% sodium chloride (prewash)	Water plus prewash (discard)	394
2. Solution of remaining colors from column No. 1 (in 112 mL) adjusted to 20% sodium chloride		

Column No. 2 - *continued*

3. 250 mL of 20% ethanol plus 15% sodium chloride	FD&C Green No. 1[a] plus FD&C Violet No. 1[a] (separate on column No. 6)	96
	Slight tailing of FD&C Violet No. 1[a] plus trace of FD&C Orange No. 1[a] (discard)	62
	FD&C Orange No. 1[a]	
4. 50 mL of 20% sodium chloride	Ethanol solution (discard)	140
5. Sufficient water to remove remaining colors	Remaining colors	42

Column No. 3 - 30 g of Cellulose

1. 50 mL of 20% NaCl (prewash)	H_2O + prewash (discard)	—
2. Solution of remaining colors from column No. 2 (in 42 mL) made to 20% NaCl		
3. 200 mL of 1% NaCl + 1% NH_4OH	FD&C Red No. 4 (in basic form)	75
	Ammoniacal solution (discard)	—
4. 50 mL of 20% NaCl + 1% acetic acid		
5. 200 mL of 0.0625% NaCl	FD&C Red No. 1[a]	—
6. 50 mL of H_2O + one or two drops of concentrated NH_4OH	Salt solution (discard)	—
7. Sufficient H_2O to remove remaining color	FD&C Red No. 3	

Column No. 4 - 20 g of Cellulose
(Separation of FD&C Yellow No. 1 from FD&C Yellow No. 5)

1. 50 mL of 20% NaCl (prewash)	Water plus prewash (discard)	—
2. Solution of FD&C Yellow No. 1[a] + FD&C Yellow No. 5, salted and freed of alcohol[b]		
3. 200 mL of 20% NaCl	FD&C Yellow No. 1[a]	—
	Salt solution (discard)	—
4. Sufficient H_2O to remove remaining color	FD&C Yellow No. 5	—

Column No. 5 - 30 g of Cellulose
(Separation of FD&C Blue No. 2 from FD&C Red No. 2[a])

1. 50 mL of 20% NaCl (prewash)	H_2O + prewash (discard)	—
2. Solution of FD&C Blue No. 2 + FD&C Red No. 2, salted and freed of alcohol[b]		
3. 200 mL of 1% NaCl + 1% NH_4OH	FD&C Red No. 2[a] (in basic form)	—
	Salt solution (discard)	—
4. Sufficient H_2O to remove remaining color	FD&C Blue No. 2	—

Column No. 6 - 30 g of Cellulose
(Separation of FD&C Green No. 1[a] from FD&C Violet No. 1[a])

1. 50 mL of 10% alcohol + 15% NaCl (prewash)	H_2O + prewash (discard)	—
2. Solution of FD&C Green No. 1[a] + FD&C Violet No. 1[a], salted and freed of alcohol[b]		
3. 250 mL of 10% alcohol + 1% NaCl	FD&C Green No. 1[a]	140
4. 50 mL of 20% NaCl	Alcohol solution (discard)	—
5. Sufficient H_2O to remove remaining color	FD&C Violet No. 1[a]	60

[a]These dyes are no longer certifiable as FD&C colors.

[b]Add sufficient sodium chloride to the fraction to bring the concentration to 20%. Extract with an equal volume of peroxide-free ethyl ether. If the dye tends to pass into the ether, add 0.5 mL of 10% sodium hydroxide for each 100 mL of dye solution. Neutralize the aqueous phase, if necessary, and remove the ether by passing air through it.

SHELTON, J. H., GILL, J. M. T. J. Assoc. Public Analysts *1,* 88–91 (1963). Paper Chromatographic Identification of Food Dyes. The method of Yanuka and colleagues is extended to the separation and identification of those food colors permitted in the United Kingdom and not included in the original paper.

SHRIVASTAVA, P. K., PRAKASH, R. Chromatographia *21,* 655–656 (1986). Thin-Layer Chromatographic Separation of a Few Food Dyes Over Scolecite as a New Adsorbent. Food colors used in the baking industry including FD&C Blue No. 1 were separated on a 3-in × 1-in slide coated with the naturally occurring zeolite scolecite. Acetone was used as the mobile phase.

SPALDING, R. C. J. Assoc. Public Analysts *2,* 111–112 (1964). R_f Values of Certain Synthetic Coloring Matters. The R_f values of 28 dyes obtained by overnight descending chromatography are compared to those obtained with 12-cm ascending runs. The values did not necessarily correspond.

STAHL, E., BOLLINGER, H. R., LEHNERT, L. Wiss. Veroeffentl. Deut. Ges. Ernaehrung *9,* 129–134 (1963). Thin-Layer Chromatography of Carotene and Carotenoid Mixtures. Some 30 carotene derivatives are separated, identified, and classified as to functional groups using $Ca(OH)_2$, Mg phosphate, and silica gel G. Mobile phases for separating carotenes were 5% CH_2Cl_2 in mixed hydrocarbons, aldehydes, esters, and CCl_4; for highly polar carotenoids, C_6H_6 or 20% EtOAc in CH_2Cl_2 was used. Separation took place under CO_2 to prevent decomposition. Ubquinones as interfering substances were separated on paraffin-impregnated 50:50 Kieselguhr-silica gel with paraffin-saturated 9:1 MeOH–iso-PrOH and visualized with 5% phosphomolybdic acid.

STEUERLE, H. Z. Lebensm. Forsch. *169,* 429–434 (1979). Enrichment, Identification and Determination of Acid Dyes by HPLC, With Special Reference to Food Dyes. Dyes are concentrated by applying 1 mL of sample to a 25-cm × 4-mm column packed with 10-μm LiChrosorb NH_2, then washing the column with acetonitrile–H_2O–anhydrous acetic acid (10:9:1) to fix the colorants at the head of the column. A neutral mobile phase of acetonitrile–H_2O–anhydrous acetic acid–aq. 26.3% NH_3 (500:380:15:14) is then pumped across the column, and the linear gradient produced separates and elutes the colorants, which are detected photometrically.

STEWART, I., WHEATON, T. A. J. Chromatog. *55,* 325–336 (1971). Continuous Flow Separation of Carotenoids by Liquid Chromatography. A liquid chromatographic system is described for the separation of complex mixtures of carotenoids. Carotenes are separated on magnesium oxide and xanthophylls are separated on zinc carbonate. The separation of complex mixtures required gradient elution. A variety of mobile phases were tried.

SYNODINOS, E., KOTAKIS, G., KOKKOTI-KOTAKIS, E. Chim. Chronika *28,* 77–79 (1963). Separation of Synthetic Dyes by Thin-Layer Chromatography. The following synthetic dyes used in Greece for coloring food, drugs, and cosmetics were separated by TLC on $CaCO_2$ using BuOH–EtOH–H_2O (2:1:1) with 10% NH_3 as the mobile phase. The R_f values include: Ponceau BR (0.71), Amaranth (0.63), FD&C Red No. 3 (0.79), FD&C Red No. 4 (0.74), FD&C Yellow No. 5 (0.54), FD&C Blue No. 2 (0.65), and FD&C Yellow No. 6 (0.72).

SYNODINOS, E., KOTAKIS, G., KOKKOTI-KOTAKIS, E. Riv. Ital, Sostanze Grasse *40,* 674–676 (1963). Separation of Synthetic Dyes by Thin-Layer

Chromatography. Seven dyes approved by the Greek Food Regulations were studied. Plates were prepared using H_4SiO_4 (I), Celite (II), MgO (III), $CaCO_3$ (IV), rice starch (V), and gypsum, alone or in mixtures. The mobile phase was 2:1:1 BuOH–EtOH–H_2O containing 10% concentrated NH_3. The dyes studied were Ponceau 3R (1), Amaranth (2), FD&C Red No. 3 (3), FD&C Red No. 4 (4), a mixture of 1 and 4, a mixture of 1, 2, 3, and 4, FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Blue No. 2, a mixture of FD&C Yellow No. 5, FD&C Yellow No. 6 and FD&C Blue No. 2, chlorophyllin a and b, and Carmine; 1 and 4 can be separated on a plate made of IV and V. The best separation between chlorophyllin a and b is obtained using a plate made with V, I, II, and III.

SZOKOLAY, A. Z. Lebensm. Forsch. *120,* 295–299 (1963). Paper Chromatographic and Spectrophotometric Detection of Fat-Soluble Synthetic Food and Cosmetic Dyes. Twelve oil-soluble synthetic food colors are separated by two-dimensional paper chromatography using dioxane–H_2O-NH_4OH (70:20:5) as the mobile phase.

SZOKOLAY, A. Cslka Hyg. *14,* 289–292 (1969). Identification by Thin-Layer Chromatography of Food Dyes Permitted in Czechoslovakia. Mixtures of FD&C Red No. 4, FD&C Red No. 3, Amaranth, FD&C Blue No. 2, cochineal red and Ponceau 6R were readily separated on starch-bound silica gel using ethyl acetate–MeOH-4.6 *N* aqueous NH_3 (25:8:5) as the mobile phase. Mixtures of FD&C Yellow No. 5 and azorubine were separated using BuOH–EtOH–H_2O–aqueous NH_3 (10:5:4:2).

TAKESHITA, R., ITOH, N., SAKAGAMI, Y. J. Chromatog. *57,* 437–440 (1971). Separation and Detection of Basic Dyes by Polyamide Thin-Layer Chromatography. The chromatographic behavior of fifteen colorants used in foods and drugs is studied in a variety of mobile phases.

TAYLOR, K. B. Nature *185,* 243–244 (1960). Chromatography of Xanthene Dyes. Commercial halogenated fluoresceins were successfully chromatographed on 20-cm × 40-cm Whatman No. 1 paper using 0.88 ammonia–EtOH–H_2O (5:10:85).

TERASHIMA, T. Shokuhin Eiseigaku Zasshi *2,* 44–51 (1961). High-Voltage Paper-Electrophoretic Analysis of Water-Soluble Coal-Tar Dyes. I. The Migration Distance of Dyes. Sixty-nine dyes, including many that are used in foods and drugs, were studied by paper electrophoresis using 5 *N* AcOH or 0.1 *N* NaOH plus 10% propylene glycol as electrolyte at 50 V/cm for 30 min.

TERASHIMA, T. Shokuhin Eiseigaku Zasshi *8,* 46–52 (1967). High-Voltage Paper-Electrophoretic Analysis of Water-Soluble Coal-Tars Dyes. VII. Systematic Separation of Dyes. 36 water-soluble food dyes are systematically classified.

TILDEN, D. H. JAOAC *35,* 423–435 (1952); JAOAC *36,* 802–810 (1953); JAOAC *37,* 812–818 (1954). Report on Paper Chromatography of Coal-Tar Colors. A study of the usefulness of paper chromatography for the separation and identification of color additives is discussed.

TONET, N. Mitt. Geb. Lebensm. Hyg. *60,* 201–205, (1969). Use of High-Voltage Electrophoresis as a Supplementary Technique for the Identification of Water-Soluble Dyes. Seventy-five synthetic and several natural dyes were studied by electrophoresis at 4500 V using 20% acetic acid or 0.01 *M* aqueous NH_3-3.3 mM acetic acid buffer adjusted to pH = 10.3 as electrolyte.

TURNER, T. D., JONES, B. E. J. Pharm. Pharmac. *23,* 806–807 (1971). Identification of Blue Triphenylmethane Food Dyes by Thin-Layer Chromatography. To separate FD&C Blue No. 1, Blue VRS, FD&C Green No. 3, Green S, and Patent Blue V, apply 1 μL of a 0.01% sample solution to a thin layer of DEAE-cellulose pre-coated on a plastic sheet (Macherey-Nagel) and elute with *M* NH_4I or 0.05 *M* or 0.2 *M* ammonium benzoate. Azo dyes have very low mobilities in these solvents.

VERMA, M. R., DASS, R. J. Sci. Ind. Res. *15C,* 186–192 (1956). Identification of Certifiable Food Colors. I. Determination of R_f Values of Single Food Colors. The R_f values of 45 dyes used in the food industry have been determined in a number of mobile phases.

VERMA, M. R., DASS, R. J. Sci. Ind. Res. *16*B, 131 (1957). R_f Values of Fat-Soluble Dyes.

VILLANUA, L., CARBALLIDO, A., OLMEDO, R. G., VALDEHITA, M. T. Anales Bromatol. *13,* 59–106 (1961). Synthetic Food Colors. VI. Characteristics, Properties, Spectrophotometry, and Circular Paper Chromatography of Prohibited Water-Soluble Dyes.

VILLANUA, L., CARBALLIDO, A., OLMEDO, R. G., VALDEHITA, M. T. Anales Bromatol. *13,* 263–285 (1961). Synthetic Food Colors. VII. Systematic Scheme for the Identification of Water-Soluble Dyes.

VILLANUA, L., CARBALLIDO, A., OLMEDO, R. G., VALDEHITA, M. T. Anales Bromatol. *16,* 377–394 (1964). Synthetic Food Colors IX. Characteristics, Properties, Spectrophotometry, and Chromatography of Some Water-Soluble Artificial Dyes.

WALTHIER, J., JENEY, E. Olaj, Szappon, Kozmet, *17,* 85–88 (1968). Thin Layer Chromatography in the Analysis of Synthetic Food Colours.

WANG, K. T. Nature *213,* (1967). Polyamide Layer Chromatography of Some Synthetic Food Colors. Ten colorants, including FD&C Red No. 4, Amaranth and FD&C Yellow Nos. 5 and 6, were separated on polyamide layers using five mobile phases.

WEAVER, K. M., NEALE, M. E. J. Chromatog. *354,* 486–489 (1986). High-Performance Liquid-Chromatographic Detection and Quantitation of Synthetic Acid Fast Dyes With a Diode Array Detector. FD&C Blue Nos. 1 and 2, FD&C Yellow Nos. 5 and 6, FD&C Red Nos. 3, 4 and 40, and FD&C Green No. 3 were determined by HPLC using a diode array detector, and a 25-cm × 4.6-mm, 5-μm C_{18} column. The mobile phase was tetrabutylammonium dihydrogen phosphate (an ion pairing reagent) in Na_2HPO_4 (A) and A-methanol (1:4) (B), using a gradient of 45–100% of B in 35 min at a flow rate of 1 mL/min.

WEISS, L. C. JAOAC *34,* 453–459 (1951). Chromatographic Properties of Oil-Soluble Coal-Tar Colors. Systems are presented for the column-chromatographic separation of 22 oil-soluble colorants.

WILL III, O. H., RUDDAT, M. LC *2,* 610–612 (1984). C_{18} Reversed-Phase HPCL Analysis of Carotenes. Mixtures of carotenes including β-carotene were separated by HPLC using a 25-cm × 4.6-mm 5-μm Ultrasphere ODS column and a mobile phase consisting of 2-propanol and acetonitrile/water (9:1) delivered in a gradient from 30:70 to 55:45, at a flow rate of 40 mL/hr. Detection was at 283, 425, or 443 nm.

ZAKARIA, M., SIMPSON, K., BROWN, P. R., KRSTULOVIC, A. J. Chromatog. *176,* 109–117 (1979). Reverse-Phase High-Performance Liquid Chromatographic Analysis for Determination of Provitamin-A-Carotenes in Tomatoes. Homogenized tomatoes are extracted with acetone, the acetone extract is diluted with water and light petroleum, then the upper phase containing the carotenes is evaporated. The residue is hydrolyzed by adding 15% KOH and allowing the solution to stand for 14 hr in the dark. The mixture is then extracted with light petroleum, and a portion of the extract is chromatographed by HPLC on a 25-cm × 4.6-mm Partisil-PXS ODS column using 8% $CHCl_3$ in acetonitrile as the mobile phase at a flow rate of 2 mL/min. Detection is at 470 nm. β-Carotene, α-carotene, and lycopene separate well.

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ANALYSIS OF COMMERCIAL PRODUCTS

The determination of colorants in foods, drugs, cosmetics, and medical devices is probably the most challenging and certainly among the most needed analysis in the field of color additives today. The challenge arises from the inherent difficulties associated with isolating the colorants, knowing when recovery is complete, and whether low recovery reflects inadequate analytical procedures or product-related colorant decomposition. The simultaneous presence of more than one color additive as well as the presence of natural colorants in the product compound the problem. The need for the determination is manifold. Government requires that only limited amounts of specified colorants be used in products, and so it must police industry. Industry wants to know what its competition is doing, and both are interested in colorant stability after incorporation into various matrices.

A number of techniques have been used to determine color additives in manufactured goods. The simplest, of course, is to measure them spectrophotometrically in situ, an approach that is viable if the colorant or colorants present are not interfered with by the presence of natural

dyestuffs or by each other. Still and carbonated soft drinks, powdered gelatin desserts, certain hard candies, and colored films can often be measured this way after only a minimum of sample preparation such as degassing, dilution, or dissolution. The chief concern in making such measurements is in being certain that the matrix does not affect the colorant's spectrum either qualitatively or quantitatively, a point that is best established using the technique of known additions.

Unfortunately, few products can be handled so simply. Items such as chocolate pudding, lavender lipstick, fruit-stripe gum, nail polish, and multicolored cold capsules can be an analyst's nightmare since the colorants present are often a mixture of both soluble dyestuffs and insoluble pigments or lakes that are widely different in chemical nature, difficult to isolate from their matrices, and a real chore to resolve from each other.

Most colorants can be isolated from their matrices by one of three techniques: leaching, solvent-solvent extraction, or adsorption onto an active substrate. Leaching has been used successfully to remove colorants from the surface of oranges, sausages, and tablets as well as from packaging films and spices. In the simplest case, the product is merely soaked in an appropriate solvent and then filtered or centrifuged to isolate the colorant-bearing liquid. Further cleanup is done as needed. Solvent-solvent extraction using simple immiscible solvent pairs, or solvent pairs, one of which acts as a carrier for a complexing agent or an ion-exchange resin, is a widely and effectively used method of isolation. The procedure pioneered by Dolinsky and Stein and developed by Graichen and Molitor in which the liquid anion exchange resin Amerlite LA-2 (Rohm and Haas Co., Philadelphia, PA.) dissolved in butanol or hexane is the extracting medium is a typical example of this type of extraction procedure. Adsorption techniques have been developed using a variety of materials such as wool, powdered leather, cellulose, alumina, and polyamide powder. In using adsorption techniques, the pH of the sample solution is adjusted as needed, then the solution is treated with adsorbent either by adding it to the sample or by passing the solution down a column packed with adsorbent. The adsorbent is freed of sample matrix and then stripped of colorant by washing with appropriate solvents.

As might be expected, no one method is capable of analyzing all kinds of samples, and only a thorough knowledge of specific procedures will enable one to develop techniques suitable for solving individual problems. Some of these methods are summarized in the following

bibliography. Where appropriate, procedures are grouped according to the class of product to which they apply. The remainder of the procedures are listed as general.

BAKED GOODS

BENDER, A. E., MACFARLANE, A. J. Analyst *90*, 536–540 (1965). Determination of β-Carotene in a Roller-Dried Food. Three procedures involving enzyme treatment, saponification, and direct solvent extraction are compared for effectiveness in determining β-carotene in a roller-dried food. The enzyme method appears to be the best.

Enzyme Procedure: Weigh 10 g of powdered food and 0.3 g of Bacterase (Associated British Maltsters Ltd., Stockport, Cheshire) and mix in a 150-mL beaker. Add 40 mL of pH = 7 buffer (0.4 *M* disodium hydrogen orthophosphate plus 0.2 *M* critic acid) at 50°C, mix to a smooth paste, and incubate the mixture for 5 min at 50°C. Add ammonia until pH = 8.5 and incubate the mixture at 50°C for an additional 5 min. Transfer the sample to a 250-mL separatory funnel using a minimum of water and extract it with successive 50-mL portions of extraction solvent (10% v/v of 99%, 74° over proof industrial methylated spirits in peroxide-free, analytical reagent-grade diethyl ether) until all color has been removed. Combine the extracts, centrifuge if necessary, and determine the absorption at the absorption maximum near 452 nm.

CASILLO, R., POLITO, A. Selezione Tec. Molitoria *14*, 108–113 (1963). Addition of Carotene and Xanthophyll to Farinaceous Products (method for cold extraction with benzene and for extraction preceded by hydrolysis with alcoholic potash).

Total carotenoids are determined by extracting with cold benzene and then measuring spectrophotometrically at 465 nm. β-Carotene and xanthophyll are separated from the total carotenoids by passing the sample solution over activated alumina. Xanthophyll is retained at the upper part of the column and elutes after β-carotene. Xanthophyll is measured at 442 nm.

DI STEFANO, F., RENZI, D. Rend. 1st. Super. Sanita *19*, 294–297 (1956). Detection of Riboflavine in Artificially Colored Food Pastes. The sample is extracted in the cold by centrifugation with water and then exposed for 45 min to UV radiation to convert riboflavine to lumichrome. A blue fluorescence under Wood's light indicates the product originally contained riboflavine.

HAYES, W. P., NYAKU, N. Y., BURNS, D. T., HOODLESS, R. A., THOMSON, J. J. Chromatog. *84*, 195–199 (1973). Separation and identification of

Food Dyes. V. Examination of Ponceau 6R Dyes: Extraction of Dyes from Confectionary Products (cakes, cake mixtures, and pastries). Weigh 5 g of sample into a glass evaporation dish and place it in a 100°C oven for 30 min. Add sufficient light petroleum (boiling range 40–60°C) to cover the sample (ca. 30 mL); stir the mixture. Allow the solids to settle and decant off the light petroleum. Repeat this procedure twice and then allow the residual light petroleum to evaporate. Grind the sample gently so as not to form too fine a powder, add 4 g of Celite 545 to the sample, and mix.

Place a plug of glass wool in the end of a chromatographic tube (250 mm × 15 mm) and transfer the powdered sample to it. Pour 30 mL of acetone on top of the column and when the solvent has percolated through the whole length of the column, apply a slight air pressure to aid uniform packing. Discard the eluate. Carefully pour 50 mL of a mixture of methanol, water, and 25% v/v aqueous tetramethylammonium hydroxide solution (40:9:1) through the column. Adjust the pH of the eluate to approximately 6 by the addition of dilute hydrochloric acid. Add 5 mL of 1% aqueous polyoxyethylene sorbitan monooleate solution and reduce the volume of the mixture to about one-half on a steam-bath with the aid of a current air blown over the surface of the liquid. Add an equal volume of water to the solution and allow it to cool.

Place a plug of glass wool in a 15-mm × 500-mm chromatographic tube and add a suspension of equal amounts of cellulose powder (microgranular/CT, without additives, Whatman LabSales, Hillsboro, OR) and silica gel (CT, without additives, Whatman LabSales) in water to the tube to give a column about 20 mm high. Rinse the walls of the tube with a small volume of acetone to aid the settling of the column packing and then place sand on top of the packed column to form a layer about 6 mm deep. Pour the solution of extracted dye through the column and wash the column three times with 5-mL portions of acetone, five times with 5-mL portions of a mixture of chloroform, absolute ethanol, water and 90% formic acid (100:90:10:1), three times with 5-mL portions of acetone, and finally three times with 10-mL portions of water. Elute the dyes with a minimum volume of acetone-ammonia solution (40 mL of acetone, 9 mL of water, 1 mL of ammonia, specific gravity = 0.88), rejecting the eluate until the dyes are eluted. Remove the ammonia by blowing a current of air over the surface of the eluate and then reduce its volume to about half on a steam bath. Add an equal volume of water and adjust the pH to approximately 6 with hydrochloric acid. Pour the solution through a column of cellulose powder-silica gel in a second 15-mm × 500-mm chromatographic tube prepared as described above and wash the column with the same volumes of solvents in the sequence as described above for the first column. Elute the dyes with the minimum volume of acetone-ammonia solution. Remove the ammonia by blowing a current of air over the surface of the liquid and then evaporate the solution almost to dryness on a

steam bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for TLC.

HILDENBRAND, K. Deut. Lebensm. Rundschau *63*, 372–373 (1967). Identification of Riboflavine and Quinoline Yellow in Bakery and Confectionery Products. Riboflavine and Quinoline Yellow are isolated from food by fixation on wool fibers, then separated by chromatography on paper or polyamide powder.

LEHMANN, G., COLLET, P. Z. Lebensm. Forsch. *144*, 104–106 (1970). Analysis of Dyes. IX. Detection of synthetic dyes in pastries, dough products, and grain. The chopped sample is dried at 110°C, ground in a mortar with sand, Celite, and acetone, then filtered. The cake is repeatedly extracted with acetone to remove fat and water. The residue is dried, finely ground, and transferred to a chromatographic tube, where it is extracted with concentrated aqueous NH_3–methanol (1:19). The natural and synthetic fat-soluble dyes in the acetone extract and the acid dyes eluted from the chromatographic tube are separated and identified chromatographically.

SINGH, M. JAOAC *53*, 23–25 (1970). Stability of Color Additives: FD&C Red No. 2 in Baked Goods. Colorants are extracted from cookies, cakes, dog biscuits, and various other baked goods using the Amberlite LA-2 amine ion exchange resin method of Graichen and Molitor (see p. 533).

BEVERAGES

AMBADE, K. A., VAIDYA, P. V., MEGHAL, S. K. J. Food Sci. Technol. *14*, 60–63 (1977). Thin-Layer Chromatography and Identification of Dyes in Indian Country Liquors. Evaporate 10 mL of sample to dryness, dissolve the residue in 1 mL of 40% EtOH, and chromatograph 10 μL of this solution on activated silica gel G (0.25 mm) using BuOH-anhydrous acetic acid–EtOH–H_2O (20:4:1:10). Extract the colorants from the plate with EtOH and determine spectrophotometrically.

ANDREY, D. Mitt. Geb. Lebensm. Hyg. *70*, 237–245 (1979). Detection of the Colouring of Orange Juice with Red Beet Pigments and with Carminic Acid. The pigments are adsorbed from an acidified sample solution (pH 1) onto Dowex 50W-X4 resin (H^+ form), eluted with H_2O and detected by thin-layer chromatography on cellulose or by spectrophotometry. Anthocyanins from blood oranges, elderberries, grapes, cherries, and bilberries do not interfere. As little as 2 ppm betanin and carminic acid can be detected by TLC, whereas 3 ppm and 40 ppm, respectively, can be detected using spectrophotometry.

BAUERNFEIND, J. C., OSADCA, M., BUNNELL, R. H. Food Technol. *16*, 101–107 (1962). β-Carotene, Color and Nutrient for Juices and Beverages.

For manufacturing control, parallel assays are run on unfortified and β-carotene-fortified juice and the amount of added β-carotene is determined by difference (Procedure 1). When the unfortified sample is not available, added β-carotene is determined by column chromatography (Procedure 2).

Procedure 1: To a 40-mL glass-stoppered centrifuge tube add 0.5 g of $CaCO_3$ and 0.25 g of Hyflo Super Cel. Add 2 mL of a 1:1:1 mixture of water, methanol, and *n*-propanol, thoroughly wetting the adsorbent mixture. Using a 2-mL blowout volumetric pipette, transfer 2 mL of the orange-juice concentrate, pre-blended for 5 min in a Waring Blender, into the tube. Follow with 20 mL of Skellysolve B, stopper, and shake 5 min in a horizontal mechanical shaker. Centrifuge briefly. Add 2 g of anhydrous Na_2SO_4. Shake and centrifuge briefly. Add 4 g of anhydrous Na_2SO_4. Shake for 5 min and centrifuge for 5 min until the Skellysolve B supernatant is clear. Dilute as needed and determine spectrophotometrically.

Procedure 2: Extract 2 mL of orange-juice concentrate as described under Procedure 1. Transfer 10 mL of the extract to a 125-mL Erlenmeyer flask, blanket with a steady stream of nitrogen, and evaporate to dryness on a 40°C water bath. Dissolve the residue in 5 mL of Skellysolve B. Pack a 10-mm-ID chromatographic column with 8 cm of Merck No. 71707 reagent aluminum oxide using a vacuum of 15–20 in. of mercury. Just before applying the sample, adjust the vacuum to obtain a solvent flow rate of about two drops per second.

Transfer the residue dissolved in Skellysolve B onto the alumina column. Rinse the flask two times with 5 mL of Skellysolve B and transfer the rinsings into the column. Elute the column with 35 mL of 2% acetone in Skellysolve B into a glass-stoppered graduated cylinder so that the final volume is 40 mL. (The colorless initial eluate is discarded to keep the volume in this range.) The carotenes (α-, β-, and ζ-) are eluted as a red-orange band; a deep yellow band that follows should be at least 2 cm from the bottom of the column after all the carotenes have been eluted. Determine spectrophotometrically.

BECKMAN INSTRUMENTS, private communication. The Determination of Color Additives in Pasteurized Fruit Drinks. Mix 2 mL of sample with 0.4 mL of 0.1 *M* aqueous potassium dihydrogen phosphate and 0.5 mL of 1% trioctylamine in dichloromethane. Shake the mixture vigorously for 1 min, then allow the layers to separate. Discard the aqueous layer, then wash the organic layer with 2 × 0.5 mL of water, discarding the aqueous layer each time. Add 0.4 mL of 1% trifluoroacetic acid in dichloromethane, shake briefly, then add 0.4 mL of 0.1 *M* aqueous potassium bicarbonate and shake the sample vigorously for 30 sec more. Allow the layers to separate, and discard the organic phase. Wash the aqueous layer with dichloromethane (2 × 0.5 mL), discarding the organic layer each time. Chromatograph 10 μL of sample using

an 3-μm Ultrasphere-ODS column (7.5 cm × 0.46 cm) and gradient elution. Mobile phase A = 0.1 *M* sodium sulphate adjusted to pH 2.5 with phosphoric-acid. Mobile phase B = mobile phase A–water–methanol (15:70:220). Use a flow rate of 1.5 mL/min, and monitor the eluate simultaneously at 254 nm and at an appropriate wavelength in the visible region.

BENK, E. Deut. Lebensm. Rundschau *57*, 324–329 (1961). Detection of Added β-Carotene in Orange Juice and Orange Juice Products. To determine total carotenoids—Extract the sample with petroleum ether–MeOH, wash the extract repeatedly with aqueous MeOH and then with H_2O, and measure spectrophotometrically at 450–470 nm.

To determine β-carotene—Transfer the ether extract to a chromatographic column containing H_2O deactivated Al_2O_3. Elute β-carotene with 40% C_6H_6 in petroleum ether and measure spectrophotometrically.

BENK, E. Essenze Deriv. Agrum. *35*, 113–118 (1965). The Detection of β-Carotene and Carotene Compounds Added to Fruit Juices and Fruit Juice Stock. Methods are described for quantitative separation and detection of carotene compounds. Includes a chromatographic separation on partially activated Al_2O_3.

BENK, E. Rieschstoffe Aromen *12*, 205–206 (1962). Detection of Coloring of Fruit Juice, Beverages, and Lemonades by Sugar Color.

Method A: Based on the change in color produced when the 5-hydroxymethyl-2-furaldehyde present in caramel is treated with resorcinol. Mix 5–10 g of syrup or juice with washed beach sand to form a soft mass. Extract the mass with Et_2O, evaporate the extract to dryness, and add a few drops of fresh 1% resorcinol in fuming HCl to the residue. A cherry-red color indicates the presence of large amounts of 5-hydroxymethyl-2-furaldehyde, a pale red or brown color indicates low or trace amounts, and an olive or dark green color indicates that none is present.

Method B: Reactions with *p*-toluidinebarbituric acid. To 30 mL of a 0.5% aqueous solution of syrup or 30 mL of beverage add 1 mL of Carrez Solution 1 (150 g of $K_4Fe(CN)_6$/liter) and 1 mL of Carrez Solution 2 (300 g of ZnOAc/liter). Dilute to 100 mL, filter, and add *p*-toluidine to the filtrate. To one aliquot of filtrate add 1 mL of water (blank) and to a second aliquot, add 1 mL of 0.5% barbituric acid. After 4–5 min, measure the absorbance of the red color formed versus the blank.

BENK, E., WOLFF, I., TREIBER, H. Deut. Lebensm. Rundschau *59*, 39–42 (1963). Detection of Added Carotenoids in Orange Juice by Thin-Layer Chromatography. Carotenoids extracted from juice with Et_2O are separated on a column of Al_2O_3 then identified by TLC using SiO_2-coated glass plates, and petroleum ether–C_6H_6–Me_2CO–AcOH (80:20:2:1) as the mobile phase.

BRICOUT, J. Bios (Nancy) *9*, 19–22 (1978). Applications of High-Performance Liquid Chromatography to the Analysis of Beverages. Applications of HPLC for determining sugars, organic acids, flavors, and natural and synthetic colorants are reviewed.

CALABRO′, G., MICALI, G., CURRO′, P. Essenze Deriv. Agrum. *48*, 359–367 (1978). Determination of α- and β-Carotenes in Citrus-Fruit Juices by High-Pressure Liquid Chromatography. To 20 mL of juice or 100 mL of beverage add 50 mg of 2, 6-di-*t*-butyl-*p*-cresol (BHT) and extract the solution with 80 mL of isopropyl alcohol-light petroleum (3:1) in the dark. Add 100 mL of ethyl éther and 20 mL of saturated aqueous NaCl and continue the extraction. Discard the lower layer, wash the upper layer with 100 mL of 10% aqueous NaCl, then evaporate it to dryness in vacuo at 35°C. Clean up the residue as needed, add 0.01% BHT, then analyze the residue by HPLC at 35°C using a 25-cm × 2.6-mm column packed with HC-ODS/Sil X and MeOH-H_2O (50:3) as the mobile phase. Use a flow rate of 0.75 mL/min and monitor the effluent at 450 nm.

DAGHETTA, A., BRUSS, O. Ann. Sper. Agrar. *11*, 117–120 (1957). Determination of β-Carotene in Fruit Juices. The method described is that reported by Wall and Kelley (see this list) using Al_2O_3 as an adsorbent in place of magnesia.

DE GORI, R., GRANDI, F., SANTUCCI, F. Boll. Lab. Chim. Provinciali *10*, 248–255 (1959). Determination of Some Dyes for Liquors. Mixtures of colors, including FD&C Blue No. 2, FD&C Yellow No. 6 and FD&C Yellow No. 5, are determined in liquors spectrophotometrically without prior isolation or separation.

DI GIACOMO, A., RISPOLI, G. Essenze Deriv. Agrum. *36*, 167–176 (1966). Countercurrent Distribution Determination of Synthetic Carotenoids Added to Orange Juice. Samples are prefractionated by column chromatography and then resolved in a 100-tube Craig apparatus using a two-phase system of petroleum ether–MeOH. The various carotenoids are identified by TLC or spectrophotometry at 340–550 nm.

DI GIACOMO, A., RISPOLI, G. Riv. Ital. Essenze-Profumi Pianti Offic. Aromi-Saponi Cosmet.-Aerosol. *48*, 631–636 (1966). Determination of Caramel Added to Orange Juice and Beverages. Samples are chromatographed on Sephadex.

DROSS, A., HARDT, R., BALTES, W. Fresenius′ Z. Anal. Chem. *328*, 495–498 (1987). Detection and Identification of Caramel Colours in Some Liquid Foodstuffs. Low-molecular weight compounds were separated from caramel colors and foodstuffs by ultrafiltration. The remaining high-molecular weight compounds were analyzed by pyrolysis-GC-MS using a Curie-point pyrolyzer (at 600°C for 10 sec) connected to a fused-silica capillary column (3 m

× 0.329 mm) coated with 0.5 μm of DB-210, with temperature programming from 40°C–210°C, He as carrier gas (2 mL/min) and MS detection. From three types of caramel (spirit caramel, beer caramel, and soft-drink caramel) 24 main pyrolysis products were separated and identified.

ETOURNAUD, A., AUBORT, J.-D. Mitt. Geb. Lebensmittelunters. Hyg. *77*, 452–459 (1986). Analysis of Carotene-Carotenoid Colours in Beverages on Fruit Juice Basis. 5 mL of samples were extracted with 3 × 1 mL of $CHCl_3$, and the extracts were dried over Na_2SO_4, combined, then evaporated under N. The residues were dissolved in 500 μL of acetone, then 20-μL portions of these solutions were chromatographed by HPLC using a 25-cm × 4.6-mm column packed with 5-μm Supelcosil LC-18. The colorants were eluted with aqueous 90% acetone at 2 mL/min, and detected at 450 nm. The procedure separated canthaxanthine, β-apo-8′-carotenal, the ethyl ester of β-apo-8′-carotenic acid and β-carotene. When other dyes such as lutein and zeaxanthin and their esters, lycopene, and bixin, are present, a gradient of 70–95% acetone in H_2O is recommended as the mobile phase. Saponification of the extract with methanolic KOH may also be necessary to identify the ester dyes.

FOGG, A. G., YOO, K. S. Analyst *104*, 723–729 (1979). Direct Differential-Pulse Polarographic Determination of Mixtures of Tartrazine and Sunset Yellow FCF in Soft Drinks (Sparkling Orangeade and Lemonade). Prepare pH 1.9 Britton-Robinson buffer by dissolving 2.47 g of boric acid in 500 mL of distilled water containing 2.3 mL of glacial acetic acid, then adding 2.7 mL of orthophosphoric acid and diluting to 1 liter with water. Adjust the pH as required using 0.2 *M* or 4 *M* sodium hydroxide solution. Pipette 10 mL of sample into a 50-mL beaker. Add 5 mL of 0.01 *M* tetraphenyl phosphonium chloride solution and 20 mL of pH 1.9 buffer. Adjust the pH to 9 with sodium hydroxide solution and dilute to 50 mL in a volumetric flask.

Deoxygenate a portion of the solution and polarograph it between −0.3 V and −1.0 V using a Princeton Applied Research PAR 174 polarographic analyzer. For differential-pulse operation, use a forced drop time of 1 sec, a pulse height of 50 mV, and a scan rate of 2 mV/sec. Use two-electrode operation with a dropping-mercury electrode and a saturated calomel reference electrode, and a water-jacketed polarographic cell maintained at 25°C. The peak potentials for Sunset Yellow FCF and Tartrazine are −0.64 V and −0.73 V, respectively.

HANN, J. T., GILKISON, I. S. J. Chromatog. *395*, 317–322 (1987). Gradient Liquid-Chromatographic Method for the Simultaneous Determination of Sweeteners, Preservatives and Colours in Soft Drinks. The procedure studied uses a 25-cm × 4.6-mm column of Spherisorb 5 ODS, and 10% methanol in 50 mM phosphate buffer at pH 3.6 as the mobile phase. Elution was isocratic for three min, followed by a gradient to 60% methanol over the next 10 min, followed by isocratic elution with 60% methanol for 2 min more. Detection

was at 214 nm. For the analysis of a fruit drink and tonic water, recoveries of the compounds studied were 77.3–103.4% in the range 10.96–150 mg/L, and the coefficient of variation (n = 5) was 0.15–1.32%.

HIGBY, W. K. Food Technol. *17*, 95–98 (1963). Analysis of Orange Juice for Total Carotenoids, Carotenes, and Added β-Carotene.

ILLI, J. Mitt. Gebiete Lebensm. Hyg. *54*, 434–437 (1963). The Isolation of Artificial Dyestuffs From Foods With the Aid of Acidic (Anionotropic) Activated Aluminum Oxide. Dilute the sample (soft drink or cordial) with water and pour the mixture onto an acidic (anionotropic) aluminum oxide column. Wash concomitant substances such as sugars from the column with 70% ethanol. Elute the color with 10 mL of 70% ethanol, to which 1 mL of 10% ammonium hydroxide has been added. Evaporate the eluate to 1–2 mL and resolve the colors by paper chromatography.

KIRCHNAWY, F., KAINZ, G., SONTAG, G. Ernaehrung *9*, 388–391 (1985). Determination of Synthetic Dyes in Beverages and Sweets by HPLC with Electrochemical Detection. Synthetic dyes were extracted from liquid or dried beverages or sweets with H_2O, and the extracts were purified on an MN-polyamide SC-6 column using methanol–aqueous NH_3 (20:1) as the mobile phase. The eluates were chromatographed by HPLC using a 20-cm × 4.1-mm RP-18 column and a mobile phase of methanol–Britton–Robinson buffer solution–0.5M-tetrabutylammonium sulphate buffer–$NaNO_3$–H_2O (96:20:2:1:81) at pH 6, and electrochemical detection with a stationary mercury-drop electrode in the differential-pulse mode. The standard deviation of the procedure was 1.1%.

KOBAYASHI, F., OZAWA, N., HANAI, J., ISOBE, M., WATABE, T. Anal. Chem. *58*, 3048–3051 (1986). Quantitative Extraction and Concentration of Synthetic Water-Soluble Acid Dyes from Aqueous Media Using a Quinine–Chloroform Solution. To 20 mL of sample, add 0.8 g of boric acid, 5 mL of 0.12 M quinine monohydrochloride, 5 mL of chloroform, and 1 mL of 0.5 M NaOH. Shake the mixture for 10 min, centrifuge it for 10 min more at 2500 rpm, then remove the aqueous layer by aspiration. Add 0.2–0.5 mL of 0.5 N NaOH to 4 mL of the organic layer then shake the mixture for 10 min. Centrifuge the sample for 10 min at 2500 rpm, remove the aqueous layer, neutralize it with 0.5 *N* H_2SO_4, and determine any colorants present by HPLC.

LEHMANN, G., COLLET, P., MORAN, M. Z. Lebensm. Forsch. *143*, 191–195 (1970). Detection of Artificial Dyes in Wines and Fruit Juices. The sample is acidified with 98% formic acid–methanol (2:3) (solvent A) and stirred with polyamide powder, and the mixture is transferred to a prepared microcolumn, which is washed with solvent A and then with H_2O until the washings are neutral. The percolate and washings, containing anthocyanins, are rejected. The adsorbed dyes are washed from the column with concentrated aqueous

NH_3–methanol (1:19), then with methanol, and the solution is diluted with H_2O. The dyes are further purified by another treatment on a polyamide column. The final solution is evaporated to a small volume, and the dyes are identified by TLC on a 0.25-mm layer of cellulose powder using 2.5% aqueous Na citrate–concentrated aqueous NH_3–methanol (20:5:3) as the mobile phase. For the identification of basic dyes, the sample is passed through a prepared column of polyamide powder and the column is washed with H_2O. The dyes and part of the anthocyanins are eluted from the column with methanol, and the eluate is passed through an anion-exchange column of DEAE-cellulose, which retains the natural colors. The basic dyes in the percolate are then adsorbed on a cation-exchange column of carboxymethylcellulose, from which they are eluted with solvent A. The eluate is evaporated to 0.2 mL and the dyes are identified by TLC.

MAGLITTO, C., GIANOTTI, L., MATTAREI, C. Boll. Lab. Chim. Provinciali *15*, 354–359 (1964). Rapid Extraction of Pigments and Their Detection by Thin-Layer Chromatography. I. Research of cuprous chlorophyllins in preserves, of malvin from hybrid wines, and of vegetable extracts added to brandies.

For juices and preserves—To 50 g of sample add 50 g of NaCl and 0.5 mL of HCO_2H. Extract with 25 mL of 3:2:5 Me_2CO–Et_2O–iso-PrOH. Evaporate the extract to dryness, add 10 mL of solvent, add NaCl, and reextract. Concentrate the residue for chromatography.

For wines, syrups and brandies—To 50 mL of sample add 13 g of NaCl. Extract with three 25-mL portions of the mixed solvent as described above, concentrate the combined extracts under reduced pressure to 5 mL, and chromatograph.

MAROVATSANGA, L., MACRAE, R. Food Chem. *24*, 83–98 (1987). Determination of Added Azo-Dye in Soft Drinks Via Its Reduction Products. The initial concentration of amaranth in soft drinks and the stability of the colorant during storage was monitored by measuring the change with time in the naphthionic acid content of the beverage. The stored drink was analyzed at monthly intervals by HPLC using a 12.5-cm × 4.6-mm stainless-steel column packed with 5-μm Spherisorb ODS-2, a gradient of methanol in 6 m*M*-tetrabutylammonium hydroxide as mobile phase at a flow of 1 mL/min, and detection at 237 nm.

MARTIN, G. E., FIGERT, D. M. JAOAC *57*, 212–218 (1974). Qualitative Determination of Coal-Tar Dyes in Alcoholic Products by Thin-Layer Chromatography. Pipette 50 mL of flavor extract or 100 mL of alcoholic beverage into a 250-mL beaker. Add sufficient HCl to reduce the pH to about 2. Add about 12 in. of wool yarn and a few boiling chips. Place the solution on a hot plate and boil it until its volume has been reduced to 25 mL. Remove the wool

from the beaker and rinse thoroughly with cold water. If the wool is white, no synthetic aromatic dye is present. If the wool retains color, add approximately 25 mL of 10% NH_4OH to wash the dye from the wool. Let stand for 15 min, remove the wool, express the dye solution with a glass stirring rod and discard the yarn.

Place the beaker on a hot plate and boil until the solution is reduced to about 2 mL.

Depending on the dye content, transfer 0.5–2.0 μL of the solution to two 20-cm × 20-cm glass thin-layer plates coated with 0.1 mm of cellulose (EM Laboratories, Elmsford, N. Y.). Similarly transfer standard solutions (0.1 g/100 mL) to the same plates. Chromatograph in separate 10-in. × 12-in. × 4-in. glass tanks using mobile phases A and B.

Mobile phase A: Ethylacetate–*n*-butanol–pyridine–H_2O (25:25:30:25).

Mobile phase B: *n*-Propanol–H_2O–triethylamine (62:28:10).

MARTIN, G. E., TENENBAUM, M., ALFONSO, F., DYER, R. JAOAC *61* 908–910 (1978). High Pressure Liquid and Thin Layer Chromatography of Synthetic Acid Fast Dyes in Alcoholic Products. Isolate colorant from flavors and alcoholic beverages as described above (Martin and Figert, JAOAC *57*, 217–218, 1974). Spot samples and standards on cellulose plates, dry the plates in a forced air oven for 4 hr at 52°C, then chromatograph them using ethyl acetate–*n*-butanol–pyridine–water (25:25:30:25) as the mobile phase.

Or, chromatograph 20 μL of sample at room temperature by high-pressure liquid chromatography using a 250-mm × 4.6-mm stainless steel RP 8 column (Hewlett-Packard, C8 chemically bonded to 10-μm particle size silica gel).

Using a flow rate of 2.0 mL/min, elute isocratically for 3 min using 10% methanol in 0.01 *M* KH_2PO_4, then solvent program linearly to 90% methanol in 0.01 *M* KH_2PO_4 in 15 min. Monitor at 290 nm.

TLC R_f Values and HPLC Retention Times for Synthetic Acid-Fast Dyes

Dyes	R_f	Retention Time (min)
FD&C Yellow No. 5 (Tartrazine)	0.125	3.45
Amaranth	0.208	4.09
FD&C Blue No. 2 (Indigotine)	0.250	4.61
FD&C Yellow No. 6 (Sunset Yellow FCF	0.416	6.27
FD&C Blue No. 1 (Brilliant Blue)	0.416	9.05
FD&C Green No. 3 (Fast Green FCF)	0.458	8.86
Ponceau SX	0.500	9.42
FD&C Red No. 40	0.500	7.63
FD&C Red No. 3 (Erythrosine)	0.925	12.78

MATTIONI, R. Boll. Lab. Chim. Provinciali *15*, 539–545 (1964). Determination of Caramel in Beverages. To beverages containing not more than 20% EtOH add 1 g Na_2SO_4 and 2 mL BuOH. Agitate a few minutes and then add one drop of a fresh saturated solution of phloroglucinol in concentrated HCl. Shake well. A dark-pale red color in the BuOH layer indicates the presence of caramel.

PALLOTTI, G., BENCIVENGA, B., GIABBAI, M., PALMIOLI, A., ROSATELLI, I. Boll. Chim. Lab. Prov. III *28,* 217–230 (1977). Spectrophotometric Method for Simultaneous Determination of Synthetic Water-Soluble Dyes in Foods and Beverages. The concentrations of two or three colorants in mixtures were determined by making absorbance measurements at suitable wavelengths and solving simultaneous equations. The method was applied to liquers, aperitifs, carbonated beverages, syrups, caramels, and water-ices.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. JAOAC *67*, 880–885 (1984). Extraction of Organic Acids by Ion-Pair Formation With Trioctylamine. V. Simultaneous Determination of Synthetic Dyes, Benzoic Acid, Sorbic Acid, and Saccharin in Soft Drinks and Lemonade Syrups. Dilute 10 g of lemonade syrup with water to 100 mL. Degas soft drinks by ultrasonication for 15 min at room temperature. Mix 5 mL of degassed soft drink or diluted lemonade syrup with 5 mL of phosphate buffer (pH 5.5, ionic strength 0.5) in a centrifuge tube. If necessary, adjust the pH of the solution to 5.5 ± 0.1 with 2 *M* sodium hydroxide. Extract the mixture with 10 mL of 0.01 *M* tri-*n*-octylamine in $CHCl_3$. Allow the phases to separate, transfer 7 mL of the organic layer to a second tube, then extract it with 7 mL of 0.1 *M* $NaClO_4$. Identify colorants by TLC using 0.1-mm Merck cellulose plates and ethyl acetate–*n*-propanol–ammonia–water (35:35:20:20) as the mobile phase, or by HPLC using a Merck 10-μm 25-cm × 4-mm RP-18 Lichrosorb octadecyl silica column, a gradient of methanol in aqueous phosphate buffer solution of pH 3 to 7, and detection at 254 nm.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. JAOAC *66*, 670–672 (1983). High Pressure Liquid Chromatographic Determination of Tartrazine in Rice Milk Following Ion-Pair Extraction With Tri-*N*-Octylamine. Dry 2 g of rice milk by lyophilization, then transfer the sample to a 40-cm × 1-cm glass chromatography tube equipped with a Teflon stopcock. Elute any colorant from the column with ammonia–methanol (95 + 5) at an elution rate of ca 0.5 mL/min. Evaporate the eluate to dryness at ca 40°C, redissolve the residue in 20 mL of phosphate buffer (pH = 5.5, ionic strength = 0.1), then extract any colorant into 5 mL of 0.1 *M* tri-*n*-octylamine solution in chloroform. Back extract 3 mL of this solution into 3 mL of 0.1 *M* $NaClO_4$, then determine the colorant present by TLC using 20-cm × 20-cm × 0.1-mm plates of cellulose (Merck, GFR) and a mobile phase of ethyl acetate–*n*-propanol–ammonia–water (35 + 35 + 20 + 20), or by HPLC using a 30-cm × 4-mm

MCH-10 column (Micro-Pak 10-μm octadecylsilica), and a mobile phase of methanol–phosphate buffer prepared as follows. Prepare two 1-L solutions of methanol–phosphate buffer (pH = 7.00 ± 0.05, ionic strength = 0.1): (a) 30 + 70 and (b) 60 + 40, each containing 0.5% (v/v) tetrabutylammonium hydroxide solution (TBA, 25% in methanol, Fluka AG). The mobile phase consists of 85% (a) + 15% (b).

RATHER, H. Riechstoffe Aromen *12*, 33–41 (1962). The Determination of Carotene and Carotenoids as Coloring Adjuvants in Orange Juice and Concentrates. Colorants are isolated by solvent–solvent extraction using alcohol, Et_2O, petroleum ether mixtures and then chromatographed on activated Al_2O_3 using C_6H_6-petroleum ether as mobile phase.

REEDER, S. K., PARK, G. L. JAOAC *58*, 595–598 (1975). A Specific Method for the Determination of Provitamin A Carotenoids in Orange Juice. Blend equivalent of 20 mL of single-strength orange juice with 20 mL of petroleum ether, 60 mL of isopropanol, and 50 mg of butylated hydroxytoluene (BHT) for 1 min under reduced light. Transfer the mixture to a 500-mL separatory funnel. Add 100 mL of diethyl ether and 20 mL of NaCl-saturated water and shake for 2 min. Discard lower layer and wash upper layer with 4 × 100 mL of 10% aqueous NaCl. Transfer organic layer to a 250-mL Erlenmeyer flask. Add 10 mL of methanolic KOH and a stirring bar and flush with nitrogen. Stopper and stir gently magnetically for 45 min. Transfer to a 250-mL separatory funnel and wash free of alkali with 10% aqueous NaCl. Reduce extract to dryness in rotary vacuum evaporator at 35°C. Dissolve residue in 2 mL of benzene–*n*-hexane (3:5) plus 0.01% BHT (mobile phase). Filter extract through 0.2 μm of regenerated Sartorius cellulose membrane into small vial. Analyze immediately or store at −10°C.

Pack a 2.1-mm-ID × 3-ft stainless-steel column with basic alumina (Woelm B 18, 18–30 μm). Deactivate by eluting with 20 mL of 15% isopropanol in hexane followed by mobile phase until baseline is stabilized.

Using a loop injector, inject 120 μL of extract onto the column and elute under pressure with mobile phase at 2 mL/min. Monitor the eluate at 440 nm. Retention times for α- and β-carotene are 4.7 min and 6 min, respectively.

ROBINSON, J. L., JOHN, J., SAFA, A. I., KIRKES, K. A., GRIFFITH, P. E. J. CHROMATOG. *402*, 201–210 (1987). Evaluation of Disposable Cartridges for Trace Enrichment from Aqueous Solutions. Toluene, naphthalene, benzo(a)pyrene, bis-(2-ethylhexyl) and dibutyl phthalate, and FD&C Red No. 2, FD&C Yellow No. 5, and FD&C Yellow No. 6 were isolated from aqueous solution on Chrom-Prep PRP-1 and Sep-Pak C_{18} cartridges preconditioned with methanol. The Chrom-Prep cartridges showed greater adsorption capacity for the aromatic hydrocarbons and the phthalate esters than Sep-Pak C_{18} cartridges; the Sep-Pak C_{18} cartridges showed greater capacity for the

dyes. The breakthrough volume for the dyes increased with pH. Both types of cartridges showed a small amount of analyte leakage during the trace enrichment step, which was measured by monitoring the cartridge effluents for the presence of ^{14}C-labelled solute.

SCHAPER, M., KRUEGER, E. Monatsschr. Brauwiss. *37*, 456–461 (1984). Detection of Dyes in Alcoholic Drinks by HPLC. Alcoholic drinks to which colorants were added were analyzed by HPLC using a 10-cm × 2.1-mm Hypersil MOS column (5 μm; Shandon), a gradient of aqueous 0.01 *M* KH_2PO_4 in methanol, and detection at 450, 520, and 563 nm for yellow, red, and blue dyes, respectively. The method is more sensitive than isotachophoresis and is therefore preferred for the determination of dyes in alcoholic drinks.

STEELE, J. A. JAOAC *67*, 540–541 (1984). High Performance Thin Layer Chromatographic Identification of Synthetic Food Dyes in Alcoholic Products. Evaporate 200 mL of sample in a 1-L beaker, add 100 mL of water, 10 mL of 3 *N* HCl, about 12 in. of unbleached wool yarn, and a few boiling chips. Boil the sample on a hot plate until the volume is reduced to 50–100 mL, but do not char. Remove the yarn and wash it thoroughly with cold water. Extract the dye from the yarn by adding 25 mL of 10% ammonium hydroxide to it in a 100-mL beaker and allowing the sample to stand for about 30 min. Remove the yarn from the beaker, then evaporate the solution on a hot plate to about 2 mL. If FD&C Blue No. 2 is present, do not boil the solution, but rather reduce its volume by means of a rotary evaporator. Chromatograph 10 μL of the concentrate using Whatman LHP-K, Analtech HETLC, or Analtech HERPS plates, and butanol–butan-2-ol–acetonitrile–tetrahydrofuran–ethyl methyl ketone–aqueous 0.5% NaCl–aqueous NH_3 (10:10:25:15:20:18:2) or propanol–acetonitrile–tetrahydrofuran–ethyl methyl ketone–ethyl acetate–aqueous 0.5% NaCl-aqueous NH_3 (20:15:25:10:10:18:2) as the mobile phase.

STRUNK, D. H., TIMMEL, B. M., HAMMAN, J. W., ANDREASEN, A. A. JAOAC *64*, 541–546 (1981). Determination of Color Intensity of Whiskey and Other Alcoholic Products. The absorbances of aged and caramel-colored alcoholic products obtained at 430, 525, and 610 nm using a narrow (≤1 nm) and an 8-nm band-width spectrophotometer are compared. Values obtained using a Klett-Summerson colorimeter equipped with Nos. 42, 54, and 60 regulator filters and 520, 540, and 560-nm narrow bandpass filters, and a Coleman Nepho-Colorimeter equipped with a 525-nm filter are also tabulated. As a result of this study, the authors recommended that measurement on such solutions be made at 525 nm using an instrument with a bandwidth of ≤10 nm. This would allow the use of simpler, less expensive spectrophotometers, and should produce values more closely related to results obtained visually.

TEWARI, S. N., SHARMA, S. C., SHARMA, V. K. Chromatographia *7*, 36–37 (1974). Paper-Chromatographic Technique for the Detection of Colouring Matter in Liquors and Wines. The sample (10 mL) is evaporated to dryness and the residue is dissolved in 50% aqueous ethanol (0.5 mL). An aliquot of this solution is spotted on Whatman No. 1 paper, together with appropriate standards, and a chromatogram is developed with butanol–acetic acid–H_2O (4:1:5).

TEWARI, S. N., SHARMA, S. C., SHARMA, V. K. J. Indian Acad. Forensic Sci. *16*, 35–43 (1977). Thin-Layer Electrophoretic Technique for Separation and Identification of Synthetic Dyestuffs Present in Liquors and Beverages. Ten mL of sample were evaporated to dryness, the residue was dissolved in 50% EtOH and then applied to a 20-cm × 20-cm silica gel G plate and separated at 300 V for 60 min using one of five electrolytes in the range pH 2–10. Comparisons were made with knowns.

VALCHER, S. Boll. Lab. Chim. Provinciali *13*, 530–542 (1962). Identification of Artificial Colors in Wines and Other Liquids.

WALL, M. E., KELLEY, E. G. Ind. Eng. Chem., Anal. Ed. *15* 18–20 (1943). Determination of Pure Carotene in Plant Tissue, Rapid Chromatographic Method. Extract 1 g of ground, dehydrated sample for 1 hr in a Soxhlet apparatus using 200 mL of acetone–Skellysolve B (30:70). Evaporate to 25–50 mL on a steam bath. Pack a 23-mm × 200-mm glass column ¾ full with a mixture of 3 parts Hyflo Super-Cel and 1 part Micron Brand No. 2641 activated MgO. Using vacuum, wash the column with 50 mL of Skellysolve B, pass the sample through the column, and then elute α- and β-carotenes with 3–5% acetone in Skellysolve B. Most noncarotene pigments remain at the top of the column.

YUFERA, E. P., MALLENT, D. Rev. Agroquim. Technol. Alimentos *4*, 499–500 (1964). Detection of Orange Juice Adultered by Addition of β-Carotene and Synthetic Carotenoids. Carotenoids are separated on silica gel G using petroleum ether–iso–PrOH (95:5). The R_f values are:

β-Apo-8′-carotenal = 0.22
Canthaxanthin = 0.12
β-Carotene = 0.75
Me ester of β-apo-8′-carotenic acid = 0.35

YUFERA, E. P., MALLENT, D. Rev. Agroquim. Technol. Alimentos *6*, 215–220 (1966). Detection of Adulterants in Citric Juices. VIII. Methods for the Characterization of Natural and Synthetic Carotenoids. Mixtures are separated on Kieselgel G using petroleum ether-iso-PrOH (95:5) for one-dimen-

sional chromatography and petroleum ether–iso–PrOH-EtOAc (80:40:5) and petroleum ether–iso-PrOH–acetone (95:5:10) for two-dimensional chromatography.

WANG, Z., ZHENG, Z., JIANG, S. Fenxi Huaxue *15*, 383 (1987). Derivative Spectrophotometric Determination of Pigments in Orange-Drink Powder. Carminic acid and Citron Yellow were determined in aqueous 20 to 40% solutions of commercial orange-drink powders by measurement of their absorbances at 468 and 548 nm. The first derivative spectrum was obtained at $\Delta\lambda = 4$ nm at a scanning rate of 1200 nm/min. At 548 nm Citron Yellow did not interfere with the determination of carminic acid, and therefore the contribution of, and correction factor for, carminic acid at the 468-nm peak could be calculated, and Citron Yellow could be quantified. Recovery was 97.3%, and the coefficient of variation was 1.0%.

WILD, R., DOBROVOLNY, H. Brauwissenschaft *29*, 93–100 (1976). Detection of Tagetes Extracts in Orange Products by High-Pressure Liquid Chromatography. Total carotenoids are extracted from juices, concentrates, or oils and are then fractionated by column chromatography on alumina. The xanthophyll ester fraction is analyzed by HPLC using a 10-μm Bondapack C18 column and methanol as the mobile phase.

CANDY AND CONFECTIONS

ANDRZEJEWSKA, E. Rocz. Panstw. Zakl. Hig. *31*, 277–281 (1980). Determination of Synthetic Organic Dyes in Chewing Gum. Extract 3 g of sample at 75°C with 15 mL of water, then with five 15-mL portions of water each mixed with 0.05 mL of aqueous 25% NH_3. Combine the extracts, acidify them with anhydrous acetic acid, then pass the solution through a column containing 1.25 g of polyamide. Wash the column with ten 10-mL portions of hot (65°C) water, three 5-mL portions of acetone, then five 10-mL portions of water. Elute the dyes with aqueous 70% MeOH–aqueous 25% NH_3 (49:1) at 55°C. Concentrate the eluate to 5 mL at 75°C, add 20 mL of water and 0.1 mL of anhydrous acetic acid, then chromatograph on a second polyamide column as above. Concentrate the eluate to 1 mL at 75°C, then chromatograph a portion of it by thin-layer chromatography.

BOLEY, N. P., CROSBY, N. T., ROPER, P., SOMERS, L. Analyst *106*, 710–713 (1981). Determination of Indigo Carmine in Boiled Sweets and Similar Confectionery Products. Grind 5–10 g of sample into a fine powder, then dissolve it in 25 mL of water on a water-bath at 55–60°C, with nitrogen constantly bubbling through the solution. Pass 5 mL of fresh HPLC mobile

phase (methanol–water–cetrimide; 78 mL + 22 mL + 0.25 g) through a Waters Associates SEP-PAK C18 reverse-phase cartridge to prime it, then remove the sample from the water bath and immediately pass it through the cartridge also. Wash the SEP-PAK with 10 mL of water to remove sugars, flavorings, etc., then elute the Indigo Carmine into a 10-mL volumetric flask using 2–3 mL of methanol. Dilute the sample to volume with water, mix well, then chromatograph 10 μL of it at room temperature on a 12-cm × 4.6-mm-ID stainless steel column packed with 5-μm SAS-Hypersil (Shandon Southern Instruments Ltd.) Use the above mobile phase at a flow of 1.2 mL/min and a detector set at 610 nm.

FREDE, W. Dtsch. Lebensm.-Rundsch *74*, 263–264 (1978). High-Pressure Liquid Chromatographic Separation (from Confectionery Products) of the Azo-Dyes E110 [FD&C Yellow No. 6, CI Food Yellow 3] and E111 [Orange GGN]. The colorants are extracted from the samples, purified by thin-layer chromatography when needed, then chromatographed on a 25-cm × 3-mm column packed with LiChrosorb RP-2, RP-8, or RP-18 using 0.021 *M* phosphate buffer (pH 5.63, 6.85, or 8.08)–methanol (10:3) as the mobile phase and detection at 480 nm.

HURST, W. J., KREISER, W. R., MARTIN, R. A. Manuf. Confect. *60*, 39–41 (1980). Use of HPLC in the Chocolate and Confectionery Industry. Methods are described for the determination of various ingredients, including FD&C Yellow No. 5.

LEHMANN, G., HAHN, H. G. Gordian *69*, 310–322 (1969). Isolation of Food Dyes from Predominantly Sugar Containing Preparations by the Polyamide Chromatography Method. Colorants are isolated from foods by polyamide-column chromatography and identified by TLC.

LEHMANN, G., ARACKAL, T., MORAN, M. Z. Lebensm. Forsch. *153*, 155–157 (1973). Analysis of Dyes, XIV. Detection of Fat Soluble Dyes in Fats and Chocolate. Oil or fat is dissolved in light petroleum (boiling range 40–60°C); chocolate is extracted with warm light petroleum and insoluble matter is removed by filtration. The light petroleum solution is shaken with dimethylformamide (I) and the I phase is separated, washed with light petroleum (to remove residual fat), and mixed with an equal volume of H_2O. Residual light petroleum is removed by distillation under reduced pressure. A portion of the I solution is applied to a column (25 cm × 15 mm) packed with polyamide powder MN SC6. The column is washed with H_2O to remove I, auramine, and riboflavine. Artificial and natural dyes are then eluted with suitable solvents, namely, methanol–$CHCl_3$ (for chocolate dyes), or methanol–aqueous NH_3 (19:1) and identified by TLC [e.g., on Kieselgel G with $CHCl_3$–methanol (1:4) as the mobile phase].

LEHMANN, G., COLLECT P. Z. Lebensm. Forsch. *143*, 418–420 (1970). Analysis of Dyes. VI. Detection of Synthetic Dyes in Marzipan and Persipan. The sample (0.5–2 g) is treated with hot H_2O (25 mL), polyamide powder (1 g) is added, the mixture is transferred to a microcolumn, and the liquid allowed to run through. Fat, and basic and fat-soluble dyes are eluted with acetone, and sugar is eluted with hot H_2O. Acid dyes are then washed from the column with 0.1% NaOH solution in 70% aqueous methanol.

MARMION, D. M. JAOAC *54*, 131–136 (1971). Analysis of Allura Red AC Dye (A Potential New Color Additive); Hard Candy (Orange Sour Balls) Containing Allura Red AC Dye and FD&C Yellow No. 6. Prepare the following mobile phases: No. 1 = 200 g NaCl and 50 mL SD No. 30 alcohol diluted to 1 liter with water; mobile phase No. 2 = 10 g of NaCl and 50 mL of SD No. 30 alcohol diluted to 1 liter with water. Slurry 30 g of Whatman Column Chromedia CF11 in 200 mL of mobile phase No. 1 and pour into a 40-cm × 2.5-cm glass column (Corning No. 38450). Wash with 100 mL of mobile phase No. 1.

Dissolve 150-g sample in 500 mL of water. Pipette 20 mL into a 50-mL beaker. Add 4 g of NaCl and 2 mL of SD No. 30 alcohol; stir to dissolve. Wash sample onto column with two 10-mL portions of mobile phase No. 1. Elute FD&C Yellow No. 6 from the column with mobile phase No. 2. Elute Allura Red AC dye from the column with water. Determine both colors spectrophotometrically.

MATHEW, T. V., MITRA, S. N., ROY, A. K. J. Proc. Inst. Chemists *36*, 301–304 (1964). Isolation and Identification of Coal-Tar Colors in Sweetmeat (Halwa) by Thin-Layer Chromatography. Colorants were isolated by leaching 50 g of sample with 100 mL of 90% EtOH followed by 100 mL of 1% aqueous NH_4OH. The combined extracts are filtered, acidified with acetic acid, and then boiled for 15 min with three white defatted wool strands to adsorb colorant. The colorant is removed from the wool by heating for 15 min with 1% aqueous ammonia, concentrated, and then chromatographed on Al_2O_3 thin-layer plates containing 5% $CaSO_4$. The mobile phase was iso-AmOH–EtOH–NH_4OH–H_2O (4:4:1:2).

PIEKARSKI, L., KRAUZE, S. Acta Polon. Pharm. *18,* 103–109 (1961). Dyes Used for Coloring Dragées. Triturate 1–2 g of sample with 5–10 mL of water; filter. Heat the filtrate on a steam bath for 10 min with 0.5 mL of 10% $KHSO_4$ and a few threads of degreased (petroleum ether) wool. Wash the wool with cold water and then heat it for 10 min in 5 mL of 1% NH_4OH. Centrifuge and then evaporate the supernatant liquid to dryness. Dissolve in a few drops of water and chromatograph using BuOH–EtOH–H_2O (2:1:1).

Alternately, extract a solution of dragées with 10 mL of pH = 3 buffer and 10 mL of quinoline. Extract the organic layer with Et_2O and 1–2 mL of H_2O, then evaporate the aqueous layer to dryness, dissolve in a minimum amount of water, and chromatograph as described above.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. JAOAC *66*, 1039–1044 (1983). High Performance Liquid Chromatographic and Colorimetric Determination of Synthetic Dyes in Gelatin-Containing Sweets, Following Polyamide Adsorption and Ion-Pair Extraction With Tri-*n*-octylamine. Dissolve 3–10 g of sweets in 20 mL of warm pH 4.0 phosphate buffer, shake the solution for 15 min with 1 g of polyamide powder, centrifuge the mixture, then remove the supernatant liquid with a pipet. Wash the polyamide powder by shaking it with 10 mL of hot water for 5 min, then centrifuging the mixture and removing the water layer with a pipet. Repeat this procedure at least three times, then extract any dyes adsorbed on the polyamide by shaking it three times with 15-mL portions of methanol–aqueous NH_3 (19:1). Evaporate the combined extracts to dryness at 40°C, dissolve the residue in 10 mL of pH 5.5 phosphate buffer then extract any colorants into 5 mL of 0.1 *M* Tri-*n*-octylamine (TnOA) in $CHCl_3$. To separate mixtures by TLC, chromatograph 10 μL of the TnOA–$CHCl_3$ extract, using precoated 20-cm × 20-cm × 0.1-mm cellulose plates (Merck, GFR) and ethyl acetate–*n*-propanol–ammonia–water (35:35:20:20) as the mobile phase. To separate mixtures by HPLC, extract 3 mL of the TnOA–$CHCl_3$ layer with 3 mL of 0.1 *M* sodium perchlorate, then chromatograph a portion of the extract using a 25-cm × 4.6-mm Merck RP18 Lichrosorb column (10 μm, octadecylsilane) and a mobile phase prepared as follows. Dilute 5 mL of 25% methanolic tetrabutylammonium hydroxide to 1 L with mixtures of methanol–phosphate buffer (pH 7.00 ± 0.05, ionic strength 0.1): phase A = 30 + 70, phase B = 60 + 40. Mix appropriate amounts of A and B, and use a flow rate of 1–2 mL/min. For samples containing only one dye, determine it by colorimetry after diluting the above methanol–ammonia phase to 50 mL.

STINSON, E. E., WILLITS, C. O. JAOAC *46*, 329–330 (1963). Separation of Caramel Color from Salts and Sugar by Gel Filtration. Slurry 475 g of 50–270-mesh Sephadex G-25 (Pharmacia, Uppsala, Sweden) with water and transfer to a 120-cm × 5-cm-ID chromatographic column. Allow excess water to drain to the top of the Sephadex. Dilute 150 mL of syrup to 200 mL, apply it to the column, and wash the column with 3 liters of distilled water at approximately 12 mL/min. The first 680 mL of eluate is colorless. The colorant appears in the next 560 mL followed by organic salts, sucrose, and sodium chloride.

WISKER, E., KOENIG, R., FELDHEIM, W. Z. Lebensm. Forsch. *170,* 267–271 (1980). Quantitative Determination of Tartrazine in Candies and Pudding Mixes. Colorants were isolated from samples by adsorption on wool or

polyamide, then extracted from the adsorbent with aqueous or methanolic NH_3, respectively. The extract was then separated on a silica gel 60 thin-layer plate using PrOH–aqueous 33% NH_3–MeOH (150:31:30) as the mobile phase and the colorants were determined by densitometry at 420 nm. R_f values are reported for Tartrazine and 10 other colorants.

COSMETICS

ALBORNOZ, A. L. Rev. Fac. Farm. Univ. Central Venezuela *5*, 57–66 (1964). Paper Chromatography of Dyes in Lipsticks Made in Venezuela. Extract 20 mg of sample with 1 mL of 10% aqueous NH_3. Evaporate the extract to near dryness and chromatograph the residue on Whatman No. 1 paper using EtOH–H_2O–AcOEt–NH_4OH (25:60:12:3).

BARKER, A. M. L., CLARKE, P. D. B. J. Forens. Sci. Soc. *12*, 449–551 (1972). Examination of Small Quantities of Lipsticks. Extract a 3-mm × 3-mm area of cloth with a few drops of acetone–trichloroethylene (1:1). Chromatograph the extract against standards on a thin-layer plate of Alumina F_{254} using isoamyl alcohol–acetone–H_2O–NH_4OH (50:50:30:0.04). Examine visually and under UV light.

COAS, V., MANCINI, P., MAGINI, N. Riv. Merceol *18*, 49–61 (1979). Thin-Layer Chromatographic Identification of Synthetic Dyes in Cosmetic Products: Lipstick. Red color additives in lipsticks were identified by TLC using AG 50W-X4 (H^+ and Na^+ forms), silylated silica, and surfactant-treated silica. Acid and basic dyes were separated from each other on surfactant-treated silica using 0.5 *M* acetic acid in 50% MeOH as the mobile phase. Basic dyes resolved best on AG 50W-X4 (Na^+ form) using 0.25 *M* ammonium acetate–aqueous NH_3 buffer containing 10% dimethylformamide as the mobile phase.

COTSIS, T. P., GAREY, J. C. Toilet Goods Assoc. *41*, 3–11 (1964). Determination of Lipstick Dyes by Thin-Layer Chromatography. Transfer about 1 g of lipstick to a flask containing 50 mL of benzene–acetone (3:1). Cover the flask with aluminum foil and reflux on a steam bath. Shake the lipstick suspension vigorously and then immediately apply 100 μL of it as a ¼-in. × 1½-in. band on a 2-in. × 8-in. glass plate coated with Adsorbosil-1 (Applied Science, P. O. Box 440, State College, PA 16801). Most colorants present can be resolved using benzene–MeOH–NH_4OH (65:30:4). For those that can't, use benzene–*n*–amyl alcohol–HCl (65:30:5) or benzene–PrOH–NH_4OH (60:30:10).

DESHUSSES, J., DESBAUMES, P. Mitt Geb. Lebensm. Hyg. *57*, 373–376 (1966). Thin-Layer Chromatographic Identification of Lipstick Dyes. Extract

0.1–0.2 g of lipstick three times with petroleum ether contrifuging and decanting the supernatant liquid each time. Dissolve the sample residue in 96% EtOH, centrifuge, and chromatograph the supernatant liquid on silica gel G (0.2 mm, according to Stahl) using PrOH–NH_4OH (90:10) as the mobile phase.

ETOURNAUD, A., AUBORT, J.-D. Mitt. Geb. Lebensmittelunters. Hyg. *74*, 372–382 (1983). Analysis of Artificial Colours in Cosmetics. I. Lipsticks and Make-Up. Extract fat and fat-soluble colors from the sample with hexane, then back-extract the organic dyes into dimethylformamide in the presence of H_3PO_4. Dilute the extract with H_2O, then centrifuge the sample to separate nonionic azo dyes. The dyes in the supernatant liquid, and acetone solutions of the precipitated colors are chromatographed on polyamide columns, then the individual dyes are identified by chromatographing on thin-layer plates of silica gel or cellulose versus standards, using various mobile phases. The technique is suitable for the analysis of greasy and nongreasy makeup as well as mascaras.

GAGLIARDI, L., CAVAZZUTTE, G., AMATO, A., BASILI, A., TONELLI, D. J. Chromatog. *394*, 345–352 (1987). Identification of Cosmetic Dyes by Ion-Pair Reversed-Phase High-Performance Liquid Chromatography. The retention times and relative absorbances at 400, 475, 525, and 600 nm (in DMF solution containing 5% H_3PO_4) are reported for 75 organic colors commonly used in the cosmetic industry. The data were obtained on a 30-cm × 5-mm silica gel 60 HPLC C_8 column (10 μm, Riedel De Haen) using a gradient of 10–95% acetonitrile in aqueous 0.10 *M*–$NaClO_4$ (pH 4) over 60 min, at a flow rate of 2.5 mL/min. Good separations were obtained for at least 50 of the dyes, with detection limits of 20–100 ng injected. The method was applied to the analysis of commercial lipsticks.

JORK, H., LEHMANN, G., RECKTENWALD, U. J. Chromatog. *107*, 173–179 (1975). Quantitative Determination of Eosin in Cosmetics. Dissolve 0.1–0.4 g of lipstick in 10 mL of dimethylformamide and extract fats with 15 mL of light petroleum (40–60° boiling range). Dilute the remaining solution with 10 mL of water and absorb the eosin on 10 g of a sand–polyamide (5:1) mixture packed in a 20-cm × 17-mm glass column. Elute the eosin with 100 mL of methanol–25% aqueous NH_3 (20:1). Spot 1 μL of eluate onto a Kieselgel thin-layer plate and develop for about 1 hr with ethyl acetate–methanol–25% aqueous NH_3 (5:2:1). Detect spots under 366-nm radiation.

KALINOWSKI, D. Roczn. panst. Zakl. Hig. *27*, 403–409 (1976). Thin-Layer Chromatographic Separation and Identification of Triphenylmethane Dyes in Cosmetics. Standard mixtures of 18 dyes, including FD&C Blue No. 1, were isolated from cosmetics by column chromatography on alumina and then

resolved by TLC. Procedures are given for removing surface-active constituents of the cosmetics and for separating alkaline and acids dyes.

KAMATA, K., KAN, T., HARADA, H. Eisei Kagaku *30*, 144–148 (1984). Determination of Guaiazulene in Cosmetics by High-Performance Liquid Chromatography. Mix the sample with Celite, place the mixture in a column, then elute guaiazulene with acetonitrile. Dilute the eluate with H_2O and extract the colorant into hexane. Concentrate the extract, then determine guaiazulene by HPLC using a 25-cm × 4.6-mm column of Finepak SIL C_{18}, aqueous 90% methanol as the mobile phase, and detection by absorbance at 284 nm, or fluorescence at 390 nm (excitation at 285 nm). Recovery of 50 ppm of added guaiazulene using this procedure was 93.8–97.2% from a variety of cosmetics. Other components of commercial cosmetics did not interfere.

LEGATOWA, B. Roczn. Panst. Zakl. Hig. *16*, 453–459 (1965). Separation and Identification of Dyes from Cosmetics. Fluorescein dyes are separated by column chromatography using Celite as the column packing and EtOH–H_2O (1:1) as the mobile phase. The eluates are evaporated to dryness, made up in 1% NH_4OH, and resolved by two-dimensional paper chromatography using 1% aqueous NH_4OH saturated with isoamyl alcohol as the first mobile phase and BuOH–EtOH–H_2O–NH_4OH (100:20:44:1) as the second.

LEHMANN, G., BINKLE, B. Seifen, Oele, Fette, Wachse *110*, 125–128 (1984). Identification of Pigments in Cosmetic Products. Methods are described for the identification of 20 inorganic pigments and 20 organic dyes. Pigments are separated from other cosmetics constituents, then determined by standard chemical reactions; organic dyes are determined using spectrophotometry and thin-layer chromatography.

LEHMANN, G., BINKLE, B., BELL, V. Fette, Seifen, Anstrichm. *86*, 208–210 (1984). Isolation and Identification of Dyes in Nail Lacquers. Digest the sample in ethyl acetate, then extract it with aqueous 50% dimethylformamide. Separate the lower dimethylformamide phase, then extract it with light petroleum to remove fat. Mix the solution with polyamide powder to adsorb the colorants, then pack the mixture into a microcolumn and wash it with methanol. Elute any colorants with concentrated aqueous NH_3–methanol (1:19) then identify them spectrophotometrically, or by separation on thin-layers of silica gel (S&S F 1500) using light petroleum, or ethyl acetate–methanol–concentrated aqueous NH_3 (5:2:1), or butanol–anhydrous acetic acid–H_2O (4:3:1) as the mobile phase. Identify carbon black by extracting the sample with ethyl acetate, oxidizing the dried insoluble residue by heating with CuO, then detecting evolved CO_2 with $Ba(OH)_2$ solution. Identify inorganic pigments by extracting the sample with ethyl acetate, igniting the

insoluble residue, ashing it at 700°C, then detecting any metals present by specific tests.

LEHMANN, G., BINKLE, B., FALLER, H. Fette, Seifen, Anstrichm. *86*, 286–288 (1984). Isolation and Identification of Dyes and Pigments in Soaps. Samples were dissolved in methanol–CH_2Cl_2 then chromatographed on thin-layers of Kieselgel using ethyl acetate–pyridine–H_2O (11:5:4) as the mobile phase. Identification of organic and inorganic pigments required preseparation of lipophilic substances and soluble dyes; the insoluble inorganic pigments were then analyzed by classical methods. Identification of organic pigments, alone or in the presence of inorganic pigments, was accomplished by (1) dissolution of the sample with acid and removal of the dyes by extraction into amyl alcohol, or (2) dissolution of the sample with dimethylformamide and removal of the dyes by adsorption on polyamide powder and subsequent elution with dilute aqueous NH_3 (for thin-layer analysis). The solutions from (1) and (2) containing the pigments were then treated with concentrated H_2SO_4, and their absorbances measured.

LEHMANN, G., EINSCHUTZ, H., COLLET, P. Z. Lebensm. Forsch. *143*, 187–191 (1970). The Concentration and Separation of Synthetic Dyes in Lipstick and Facepowder.

NEWBURGER, S. H. *Manual of Cosmetic Analysis*, 2nd ed. Association of Official Analytical Chemists, Arlington, Virginia, 1977.

OHNISHI, S., NISHIJIMA, Y., KIJIMA, K., KANO, S. Bunseki Kagaku *26*, 814–818 (1977). High-Speed Liquid-Chromatographic Analysis of Fat-Soluble Coal-Tar Dyes. Twelve fat-soluble cosmetic dyes were separated by HPLC on a 25-cm × 4-mm column packed with LiChrosorb SI 100 (5 μm) by isocratic elution using $CHCl_3$–hexane mixtures, ethyl ether–hexane (1:24) or acetone–hexane (9:91) as the mobile phase and spectrophotometric detection at 420–500 nm. Recoveries of colorants from wax-based lipsticks ranged from 91.9% to 97.0%

PERDIH, A. Z. Analyt. Chem. *260*, 278–283 (1972). Analysis of Cosmetic Dyes. III. Identification of Synthetic Organic Dyes in Lipsticks by Thin-Layer Chromatography. Schemes are presented for the separation of dye stuffs either directly by TLC using a variety of substrates and mobile phases, or by solvent extraction with dimethylformamide followed by TLC.

RUDT, U. Riechstoffe-Kosmetika—Seifen *71, 22* (1969). Fluorometric Determination of Xanthene Coloring Materials in Lipsticks. A method is described for TLC of lipstick dyes on silica with *n*-PrOH–NH_4OH (9:1).

RUSSELL, L. W., WELCH, A. E. Forensic Sci. Int. *25*, 105–116 (1984). Analysis of Lipsticks. Dissolve a portion of lipstick, or lipstick smears, in ethanol–

2 *M* HCl (10:1), then chromatograph a portion of the solution by TLC using silica gel 60 plates, and benzene–(or toluene)–methanol–anhydrous acetic acid (35:30:4), isoamyl alcohol–acetone–H_2O–concentrated aqueous NH_3(30:30:18:0.025, or 15:15:5:1), or butanol–2 *M* HCl (11:1) as the mobile phase. Examine the plates under visible light or under 254- or 366-nm UV light. Dissolve untreated lipsticks in $CHCl_3$, or extract lipstick smears on paper with $CHCl_3$, then chromatograph portions of the extracts by GLC using a 5-ft × 0.125-in stainless steel column packed with 5% PMPE on 90–100 mesh Diatomite MQ, N as carrier gas (25 mL/min), temperature programming from 160–360°C at 8°/min, and flame-ionization detection.

SHANSKY, A., CARRUBBA, P. P. Am. Perfumer Cosmet. *78*, 13–14 (1963). Qualitative Determination of Coal-Tar Dyes in Commercial Cosmetic Products. Solvent extraction and spectrophotometry are used to determine colorants in commercial cosmetic preparations.

SILK, R. S. JAOAC *48*, 838–843 (1965). Separation of Synthetic Organic Colors in Lipsticks by Thin-Layer Chromatography for Quantitative Determination. Prepare the following reagents. For buffer solution, prepare a 0.1 *M* K_2HPO_4 solution and add a few drops of toluene as a preservative. Prepare a 0.1 *M* KH_2PO_4 solution and add a few drops of toluene. Mix 5.3 mL of the first solution with 94.7 mL of the second solution. Dilute to 200 mL with water.

Mobile phase A: Mix 20 mL of 1-butanol, 4 mL of ethanol, and 3 mL of concentrated ammonium hydroxide.

Mobile phase B: Mix 15 mL of ethyl acetate, 3 mL of methanol, and 3 mL of 3:7 ammonium hydroxide:water. Prepare fresh.

Apply 0.2 mL of buffer solution as a ¼-in. band 2 cm from the bottom of a 4-in. × 8-in. glass plate coated with a 375 μm-layer of silica gel G. Air dry for about 20 min. Remove the shiny surface from the rounded end of the lipstick sample with tissue and streak 5–8 mg of it just below the buffered zone of the warmed plate.

Line a No. 11 museum jar with paper and saturate it with dichloromethane. Allow the tank to equilibrate for a few minutes. Place the warm plate in the tank and develop it in the dark until the mobile phase reaches the top of the plate. Remove and dry the plate. Redevelop two to four more times to remove oils and waxes to the top of the plate. Scrape colored zones from the plate, leach with chloroform, and determine visible spectra against standards.

Place the same plate in a covered, unlined Desaga tank (No. 25-10-20) containing 189 mL of Mobile phase A. Develop to a height of 5 cm. Dry the plate with heat and air. Repeat development once or twice until a ¼-in. zone appears above the D&C Red No. 7. The D&C Red No. 7 remains close to the

baseline while other colors present move through the buffer. Scrape the zone containing Red No. 7 from the plate, leach with 30% acetic acid, and determine the visible spectrum vs a standard.

Line three sides of a Desaga tank with paper. Pour 315 mL of Mobile phase B over the lining and equilibrate for 10 min. Place the plate in the tank with the adsorbent layer facing the liner. Add glass beads to the tank until the mobile phase reaches the edge of the continuous coated portion of the plate. Develop to a height of 15–17 cm. If necessary, dry the plate and redevelop.

If the sample contains no D&C Red No. 7, it is not necessary to treat the plate with buffer or to develop it in Mobile phase A. To detect D&C Red No. 7, develop a 2-in. × 4-in. plate in a covered 500-mL tall-form beaker. Develop twice in dichloromethane and then once in 42 mL of Mobile phase B. The appearance of multiple bands that darken on drying to a dull, nonfluorescent, deep-red color and overlap other colors suggest the presence of D&C Red No. 7. A dark-red zone at the baseline also indicates its presence. This color is frequently found in dark red or purple lipsticks.

SILK, R. S. JAOAC *46*, 1013–1017 (1963). Column Chromatographic Determination of Certifiable Colors in Lipstick. Line a chromatographic tank with filter paper and equilibrate with mobile phase for 1 hr.

Streak 8–10 mg of lipstick across a 20-cm × 22-cm sheet of Whatman No. 3MM chromatographic paper. Develop (ascending) 1½ hr using methylethyl ketone–acetone–H_2O–NH_4OH (700:200:200:2). Remove the sheet and air dry it in semidarkness. Examine it in visible and UV light against standards similarly chromatographed.

Analyze samples by column chromatography using Procedure 1 when no D&C Red No. 7 is present or Procedure 2 when D&C Red No. 7 is present.

Reagents:

(a) Immobile phase—EtOH–aqueous (1 + 9) NH_4OH (1:1).

(b) Dilute NH_4OH—NH_4OH–H_2O (1:19).

(c) 30% Acetic acid—Dilute 30 mL of glacial acetic acid to 100 mL with H_2O.

(d) Alkaline heptane–benzene—Dilute one volume of heptane with one volume of benzene. Saturate with 10 mL of (a) per 100 mL of mixture. Discard the lower phase.

(e) 1,1,1-Trichloroethane—Shake each 100 mL with 20 mL of (a). Discard the upper phase.

(f) Alkaline 1,2-dichloroethane—Saturate each 100 mL of 1,2-dichloroethane (DCE) with 20 mL of (b). Discard the upper phase.

(g) 30% *n*-Butanol in DCE—Dilute 70 mL of (f) with 30 mL of *n*-butanol. Shake with enough additional (b) to saturate the solution at room temp.

(h) 40% *n*-Butanol in DCE—Use appropriate volume. Prepared as described above.

(i) 50% *n*-Butanol in DCE—Use appropriate volume. Prepared as described above.

(j) 60% *n*-Butanol in DCE—Use appropriate volume. Prepared as described above.

(k) 80% *n*-Butanol in DCE—Use appropriate volume. Prepare as described above.

(l) Acid heptane–benzene—Dilute one volume of heptane with one volume of benzene. Saturate each 100 mL of mixture with 20 mL of (c).

(m) Acid DCE—Saturate each 100 mL of DCE with 20 mL of (c).

(n) 60% Acetic acid—Dilute 60 mL of glacial acetic acid to 100 mL with H_2O.

(o) Celite 545—Wash with chloroform and then with alcohol. Dry at 130°C and then air dry.

Procedure 1: Weigh 10 g of (o) into a 250-mL beaker. Mix thoroughly with 4 mL of (a) and pack into a 1.8-cm-ID × 45-cm glass column using a plunger.

Remove the shiny surface from the tip of the lipstick with a tissue. Smear a known weight (0.025–0.3 g) over the inner surface of a 4-oz mortar.

Thoroughly mix 3 g of (o) and 1.2 mL of (a). Transfer ⅓ of the mixture to the mortar; grind thoroughly. Then mix and grind in the remaining Celite, about 1 g at a time. Transfer the sample Celite mixture to the top of the column. Flush the mortar with an additional 1 g of (o) plus 0.4 mL of (a). Pack down the column to 15–15.5 cm high. Transfer any remaining sample to the column with a little of the first mobile phase. Elute under 4–5 lb pressure with the appropriate mobile phases. Use a flow rate of about 10 mL/min (see table that follows for the mobile phases to use). The volumes listed are approximate, the exact volume will depend on the concentration of the components to be eluted.

Evaporate fluorescein colors to dryness. Prepare a 10-g column as in Procedure 1. Mix 3 g of (o) with 1.2 mL of (a). Transfer the fluorescein colors to the column as in Procedure 1. Separate the colors as in Procedure 1 starting with step 3, using 50 mL of (f).

Determine the visible spectrum of D&C Red No. 7 in 30% acetic acid. Evaporate the other solution to dryness.

Mobile phases for Procedure 1	Comments
1. 100–150 mL of (d). Allow the column to wet before applying pressure	D&C Red No. 36 elutes.
2. 150–200 mL of (e)	
3. 50 mL of (f)	A small fraction, apparently esterified halogenated fluorescein colors, elutes
4. 100–150 mL of (g)	D&C Red No. 27 elutes; D&C Red No. 27 subsidiary dye does not elute
5. 100 mL of (h)	D&C Red No. 27 subsidiary dye elutes followed closely by D&C Red No. 21
6. 100 mL of (i)	Tribromofluorescein elutes
7. 100–200 mL of (j)[a]	D&C Orange No. 5 and/or D&C Orange No. 10 elute
8. 100 mL of (k)[b]	Monobromofluorescein plus fluorescein elute

[a]If FD&C Yellow No. 5 is present, elute the column through step No. 7 and then elute with 100 mL of (m). This procedure removes any remaining fluorescein colors. FD&C Yellow No. 5 can be removed with dilute NH_4OH.

[b]70% *n*-BuOH in DCE saturated with (b) elutes monobromofluorescein before fluorescein.

*Procedure 2:*Prepare a sample and column as in Procedure 1, except use 7 g of (o) and 2.8 mL of (a) for the column. Elutes as follows.

Mobile phases for Procedure 2	Comments
1. 100–150 mL of (d)	
2. 100 mL of (l)	Solvents (l) and (m) elute fluorescein colors
3. 50 mL of (m)	See (2)
4. 50 mL of (l)	Removes (m) from the column
5. 50 mL of (n)	Elutes D&C Red No. 7

Examine the water soluble dyes at a neutral pH. Dissolve D&C Red No. 36 in $CHCl_3$. Dissolve halogenated fluoresceins in dilute NH_4OH.

TEWARI, S. N. Arch. Kriminol. *126*, 26–32 (1960). Paper-Chromatographic Investigation of Inks, Dyes and Lipsticks. Lipstick is dissolved in warm 40% AcOH and the mixture is filtered then extracted with petroleum ether. The other extract is evaporated to dryness then taken up in 50% EtOH and chromatographed.

TONNET, N. Mitt. Geb Lebensm. Hyg. *66*, 443–472 (1975). Extraction and Identification of Colours Used in Lipsticks. After a review of the bibliography, a scheme based on liquid–liquid extraction is described for the separation of colorants into chemically defined groups.

UNTERHALT, B. Z. Lebensm. Forsch. *144*, 109–112 (1970). Determination of Lipstick Dyes. Extract the sample three times with light petroleum, centrifuging each time. Extract the residue with EtOH. Chromatograph the EtOH extract on Kieselgel G or H (0.25-mm layer) with ethyl acetate–BuOH–NH_4OH (4:11:5) or PrOH-NH_4OH (1:1) as the mobile phase.

WEGENER, J. W., KLAMER, J. C., GOVERS, H., BRINKMAN, U. A. T. Chromatographia *24*, 865–875 (1987). Determination of Organic Colorants in Cosmetic Products by High-Performance Liquid Chromatography. Heat samples at 60°C for 1 hr with DMF containing 5% H_3PO_4. Filter the mixture, mix it with aqueous 0.1 *M* tetrabutylammonium hydroxide and extract it twice with $CHCl_3$. Concentrate the combined extracts, then chromatograph a portion of the concentrate by HPLC at 30°C using a 10-cm × 4.6-mm (3 μm) Microspher C_{18} column, equipped with a guard column of the same material. Use a flow rate of 1 mL/min, and program from 25–100%, then back to 25%, of 0.5 *M* tetrabutylammoniom hydroxide (pH 7.0, adjusted with H_3PO_4) in H_2O. Use a diode-array detector to monitor the eluate between 200 and 600 nm. Using this procedure, detection limits for many synthetic colorants were 0.3–3 ng. Sensitivity was poorer for most natural colorants. The method was applied to 45 samples, including lipsticks, nail lacquers, facial makeup, shampoos, soaps, and after-sun creams. Special sample treatments were required for certain products including lipsticks. Aqueous and alcoholic liquid samples could sometimes be analyzed directly.

WEGENER, J. W. M., GRUENBAUER, H. J. M., FORDHAM, R. J., KARCHER, W. J. Liq. Chromatog. *7*, 809–821 (1984). Combined HPLC–Visible Spectrophotometric Method for Identification of Cosmetic Dyes. Dissolve the cosmetic in 5% H_3PO_4 solution in dimethylformamide, and extract any fatty material present into hexane. Dilute the acid solution with methanolic tris(tetrabutylammonium) phosphate (Waters PIC A reagent) until a suitable absorbance is reached, then chromatograph a portion of the solution on a Chrompack CP Spher C_{18} column using a gradient changing in 45 min from 50–100% methanol containing PIC A, at 1 mL/min. Monitor the eluate at four wavelengths between 350 and 700 nm. Good separation of 20 representative colorants was obtained using this method. The results suggest that a combination of HPLC with rapid-scanning visible-region spectrophotometry should be useful for determinating colorants in cosmetics, particularly if linked to on-line computing facilities.

DAIRY PRODUCTS

BENK, E., WOLFF, I. Alkohol. Ind. *77*, 16–20 (1964). Detection in Egg Liquors of Carotenoids Foreign to Eggs. Carotenoids are separated on Al_2O_3 columns using mixtures of petroleum ether, benzene and ether, and are then identified by TLC using silica gel G plates as the substrate and petroleum ether–benzene–AcOH–Me_2CO (80:20:1:2) as the mobile phase.

DALGAARD-MIKKELSEN, S., RASMUSSEN, F. Intern. Dairy Congress Proceedings 16th, Copenhagen, 1962, Section C, pp 465–473. Tracer Dyes for Rapid Detection of Antibiotics in Milk. As little as 0.03 ppm of some triphenylmethane dyes were detected in milk by the colored zone formed when 10 mL of sample were passed through a column packed with resin.

D'ALMEIDA, A. J. M. Rev. form. Bahia *2*, 6–8 (1958). Micromethod for the Determination of Annatto in Cheese. Shake 10 g of grated cheese with 30 mL of EtOH, evaporate 10 mL of the extract to dryness, dissolve the residue in 10 mL of benzene, and centrifuge. Pass the solution through a microcolumn of Al_2O_3 and identify the adsorbed dye by the blue color produced with concentrated H_2SO_4.

DHAR, A. K., GUHA, K. C., ROY, B. R., MITRA, S. N. Ind. J. Dairy Sci. *24*, 202–207 (1971). Detection of Added Colour in Milk and Milk Products. Two procedures are given for separating fat-soluble and acidic and basic water-soluble coal-tar dyes and natural dyes before identification by conventional methods.

Procedure 1: Repeatedly shake the milk with the same volume (or 3 volumes if formaldehyde is present) of ethanol–ethyl ether (1:1) until the lower phase is colorless, filter the organic extracts, and evaporate almost to dryness. Extract the residue with hot H_2O to test for water-soluble dyes, or with ether to test for fat-soluble dyes, annatto, and turmeric. Alternatively, make the milk alkaline with aqueous NH_3 and extract with ether before adding the ethanol–ether mixture; an extra volume of ethanol must then be added. This allows separate extraction of the basic coal-tar dyes, oil-soluble dyes, and annatto before extraction of the acidic dyes and turmeric.

Procedure 2: Acidify the warmed milk with acetic acid (1:3) and boil the mixture for a few minutes. Collect the casein in a fine cloth, wash it with hot H_2O, and leave it in ether overnight to extract fat-soluble dyes, annatto, turmeric, and basic dyes. For water-soluble acidic dyes, dry the extracted precipitate, heat it with 80% ethanol containing 1% aqueous NH_3, filter, and evaporate the solution almost to dryness. Caramel is left on the casein precipitate.

ESPOY, H. M., BARNETT, H. M. Food Technol., *357* (August 1955). The detection of annatto, β-carotene, Yellow AB, and Yellow OB in butter and margarine.

FEAGAN, J. T., GRIFFIN, A. T., BRAY, R. Aust. J. Dairy Technol. *20*, 22–23 (1965). An Improved Test for the Detection of Marker Dyes in Milk. Dilute 100 mL of milk with 100 mL of hot deionized water. Vacuum filter through two 1.25-in. sediment pads with 0.2 g of Dowex AC (Cl^- form) ion exchange resin between them. View the resin for color. As little as 0.0025 mg of FD&C Blue No. 1 per liter of milk can be detected using this procedure.

HAMMOND, E. G., CHANG, J., REINBOLD, G. W. J. Dairy Sci. *58*, 1365–1366 (1975). Colorimetric Method for Residual Annatto in Dry Whey. Mix 1 g of sample with 2 mL of 30% aqueous NH_3–H_2O (1:4) in a 15-mL stoppered tube for 1 min. Add 10 mL of anhydrous EtOH, shake the tube well, and then centrifuge for 3 min at 2500 rpm. Transfer the supernatant solution to a similar tube, add two drops of phosphate solution (17.1 g of $NaH_2PO_4 \cdot H_2O$ plus 10.8 g of Na_2HPO_4 in 100 mL of H_2O), shake the mixture, and then set it aside for 30 min. Centrifuge for 3 min and then measure the absorbance of the upper phase at 450 nm against a blank containing no whey.

HARTMAN, C. P., PICHAMUTHU, S. J. Inst. Chem. Calcutta *42*, 114–117 (1970). Paper Chromatographic Method for the Detection of Metanil Yellow and Vanaspati in Butter.

HORWITZ, W., (Ed.) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12 ed., Washington, D.C., 1975, pp. 268, 275, 276, 291. Color Additives in Ice Cream, Cream, Milk and Evaporated Milk. Ice Cream—Curdle 150–200 g of melted sample by adding an equal volume of water and 10–20 mL of HOAc. Heat to 70–80° with stirring and then allow to cool. Continue as below beginning with "Gather curd, when possible . . . "

Milk, cream, and evaporated milk—Warm about 150 mL of milk in a casserole over a flame, add approximately 5 mL of HOAc (1 + 3), and continue to heat slowly nearly to boiling point while stirring. Gather curd, when possible, into one mass with stirring rod and pour off whey. If curd breaks up into small flecks, separate from whey by straining through sieve or colander. Press curd free from adhering liquid, transfer to small flask, macerate with about 50 mL of ether, keeping flask tightly corked and shaking at intervals, and let stand for several hours, preferably overnight. Decant ether extract into evaporating dish, remove ether by evaporation, and test fatty residue for annatto as follows.

Pour on moistened filter paper an alkaline solution of color obtained by shaking out oil or melted and filtered fat with warm 2% NaOH solution. If

annatto is present, paper absorbs color, so that when washed with gentle stream of H_2O it remains dyed straw color. Dry paper, add drop of 40% $SnCl_2$ solution, and again dry carefully. If color turns purple, presence of annatto is confirmed. Curd of uncolored milk and milk colored with annatto is perfectly white after complete extraction with ether. If extracted fat-free curd is distinctly orange or yellowish, synthetic dye is indicated. In many cases if lump of fat-free curd in test tube is treated with a little HCl, color changes to pink, indicating presence of dye similar to aniline yellow or butter yellow or perhaps one of the acid azo yellows or oranges.

In some cases presence of synthetic dyes can be detected by directly treating about 100 mL of milk with equal volumes of HCl in porcelain casserole, giving dish slight rotary motion. In the presence of some dyes separated curd becomes pink.

JAX, P., AUST, H. Milchwiss. Ber. 145–189 (1953). The Chromatography of Butter and Cheese Dyes and the Dyes of Other Dairy Products. Procedures are described for separating and identifying mixtures of fat- and water-soluble dyes alone or in butter or cheese.

KANEMATSU, H., NIIYA, I., IMAMURA, M., KAWAKITA, H. Bitamin *33*, 52–56 (1966). The Quantitative Determination of β-Carotene and Vitamin A in Margarine. β-Carotene is eluted from a column of activated alumina using acetone–petroleum ether (1:49). Vitamin A and other pigments remain on the column.

LEHMANN, G., EINSCHUETZ, H., COLLET, P. A. Lebensm. Forsch. *143*, 187–191 (1970). Analysis of Dyes. III. Enrichment and Isolation of Artificial Dyes in Cheese-Coating Materials and Lipsticks. Cheese-coating materials—The waxy coating material is dissolved in light petroleum and the solution is extracted with H_2O–98% formic acid (2:1). The aqueous extract is passed down a microcolumn of polyamide powder, and the column is washed with H_2O until the washings are neutral. The adsorbed dyes are eluted with 5 mL of mobile phase [0.1% NaOH solution in 70% methanol, or concentrated aqueous NH_3–methanol (1:19)] and then with 5 mL of methanol. The combined eluates are acidified with methanol–acetic acid (1:1) and evaporated under reduced pressure to ≃ 1 mL. The dyes are then identified by TLC on cellulose powder, with 2.5% aqueous Na citrate–concentrated aqueous NH_3–methanol (20:5:3) as mobile phase, by comparison of R_f values with those of standards.

Lipsticks—The sample is triturated with methanol–formic acid–acetone (3:2:1) and Celite in a porcelain mortar. After evaporation of the solvent the powder is transferred to a microtube. Lipophilic dyes are eluted from the column with light petroleum, the solvent is evaporated in a rotary evaporator, the residue is dissolved in a little warm methanol, and the solution is filtered.

Other dyes are then eluted from the column with methanol followed, if necessary, by methanol–formic acid (3:2). The combined solutions are purified on a microcolumn as in the procedure described above, and the dyes are identified by TLC on Kieselgel GF_{254} with ethyl acetate–methanol–concentrated aqueous NH_3 (5:2:1) as the mobile phase.

LEHMANN, G., COLLET, P. Z. Lebensm. Forsch. *143*, 348–350 (1970). Contribution to the Analysis of Dyes. V. Detection of Synthetic Dyes in Liquid Eggs. The sample is treated with acetone, and the dyes adsorbed on the fat- and H_2O-free precipitate are desorbed with concentrated aqueous NH_3–methanol (1:19). The alkaline extract is acidified with acetic acid to pH ≃ 5, then the dyes are purified on a microcolumn of polyamide powder and reextracted with aqueous NH_3–methanol; the extract is acidified and evaporated to a small volume, and the dyes are identified by paper or thin-layer chromatography. The acetone extract, containing fat, fat-soluble dyes, and, in part, the acid dyes, is diluted with H_2O, the acetone is removed by distillation under reduced pressure, and the fat-soluble dyes are extracted with light petroleum and identified by paper or thin-layer chromatography. The aqueous phase is acidified to pH ≃ 6 and purified on a microcolumn of polyamide powder. The adsorbed basic dyes are eluted with acetone and identified.

LEHMANN, G., COLLET, P. Z. Lebensm. Forsch. *144*, 32–34 (1970). Detection of Synthetic Dyes in Milk Products. Procedures essentially the same as those used for liquid eggs (see) were applied to yogurt, cream, ice cream, and milk shakes.

LEONE, J. L. JAOAC *56*, 535–537 (1973). Collaborative Study of the Quantitative Determination of Titanium Dioxide in Cheese. Weigh 10 g of sample into a 100-mL Pt dish and char under an IR lamp. Place in a cold furnace and ignite at 850°C to a white ash.

Cool, add about 1.5 g of anhydrous Na_2SO_4 and 10 mL of H_2SO_4, cover with a watch glass, and bring to a boil on a hot plate to dissolve. Turn heat off and let cool on the hot plate. Cautiously rinse cover, add 30 mL of H_2O, and mix with a stirring rod to disperse insoluble salts. Heat on a steam bath if insoluble material forms a cake on the bottom of the dish. Transfer quantitatively to a 100-mL volumetric flask using about 40mL of H_2O. If the solution is cloudy heat on a steam bath or in a boiling H_2O bath. Cool; dilute to volume with H_2O. Pipette 3 mL of sample solution into a 5-mL volumetric flask and then dilute to volume with H_2SO_4 (1 + 9). Add 0.2 mL of 30% H_2O_2, mix well, then determine the sample's absorbance at the maximum near 408 nm. Compare against standards similarly prepared.

LUF, W., BRANDL, E. Z. Lebensm. -Unters. -Forsch. *186*, 327–332 (1988). Detection of Annatto Dyestuffs, Norbixin and Bixin, in Cheese by Means of

Derivative Spectroscopy and High-Performance Liquid Chromatography. Samples were extracted with acetone in the presence of HCl and sand (or Celite), filtered, then evaporated with ethanol to remove water. The residue was dissolved in $CHCl_3$–acetic acid (199:1) for derivative spectroscopy or in acetone for HPLC. Derivative spectra were recorded from 550–400 nm. Quantitation was by the peak-to-peak method. Extracts were resolved by HPLC using a 10-cm × 4.6-nm RP-18 Spheri 3 (3 μm) column with a 1.5-cm pre-column. The mobile phase was a gradient (1 mL/min) of acetone (65–90–65%) in H_2O adjusted to pH 2.8 with H_3PO_4. Detection was at 440 nm.

MARMION, D. M. JAOAC *54*, 131–136 (1971). Analysis of Allura Red AC Dye (A Potential New Color Additive). FD&C Red No. 40 in Ice Cream: Weigh 10 g of well-mixed melted ice cream into a 1-in. × 4.5-in. centrifuge tube. Add 35 mL of SD No. 30 alcohol and stir well. Centrifuge until clear and decant supernatant liquid into a 150-mL beaker. Repeat extraction and centrifuging, and combine supernatant liquids. Add 1 mL of glacial acetic acid to combined extracts and boil for 1 min. Let cool for 10 min and then place in ice bath for 30 min; stir occasionally. Filter through thick pad of alcohol-washed cotton into a 100-mL volumetric flask. Using chilled alcohol, wash all color from pad into flask (filtrate must be clear). Dilute to volume with SD No. 30 alcohol; mix. Similarly extract sample of ice cream containing no color. Using a suitable spectrophotometer, immediately determine absorbance of each solution in 5-cm cell (vs. SD No. 30 alcohol) at maximum near 505 nm and at 680 nm. Sample absorbance at maximum near 505 nm = A_1; sample absorbance at 680 nm = A_2; blank absorbance at 505 nm = A_3; blank absorbance at 680 nm = A_4.

Percent Allura Red AC dye

$$= \frac{(A_1 - A_2 - A_3 + A_4) \times 100}{100 \times 5 \times 52.9} = A \times 0.00378$$

where 52.9 = absorptivity of Allura Red AC dye at 505 nm in liters/g-cm; 100 = factor for conversion to percent; 100 = effective sample concentration in g/liter; and 5 = cell path length in cm.

PERDIH, A., PRIHAVEC, D. Z. Lebensm. Forsch. *134*, 239–242 (1967). Isolation of Water-Soluble Food Dyes. A method designed for isolating food colors from protein materials, including eggs, meat, fish, and milk products. Strongly polar lipids are first defatted with $CHCl_3$–EtOH (2:1); viscous liquids or liquids containing greater than 50% alcohol are diluted with water; water-soluble samples are dissolved in water; and water-insoluble samples are ground with water to form an easily extractable suspension.

Transfer 10 g of sample into a centrifuge tube and then add 2–5 mL of H_2CO and 10 mL $CHCl_3$. Shake well and then centrifuge for 2 min at 2500 rpm. Repeat this extraction four times, and then treat the residue with pH 9–9.5 NaOH or NH_4OH and reextract with $CHCl_3$. Test extracts for fat soluble and basic colorants. Then add 0.5–2 mL of 10% alkyldimethylbenzylammonium chloride (or other similar quaternary ammonium salt) to the sample residue, mix well, add 10 mL $CHCl_3$, and shake and centrifuge as described above. Repeat the $CHCl_3$ extractions as needed. Wash the combined $CHCl_3$ extracts with water, concentrate, and then chromatograph on Na alkyl sulfate or Na alkylarenesulfonate-impregnated paper.

PUTTEMANS, M., DE VOOGT, M., DRYON, L., MASSART, D. JAOAC *68*, 143–145 (1985). Extraction of Organic Acids by Ion-Pair Formation with Tri-*n*-Octylamine. Part 7. Comparison of Methods for Extraction of Synthetic Dyes from Yogurt.

Method A. Transfer 0.5 g of yogurt to a glass screw-cap centrifuge tube, add 7 mL of pH 5.5 phosphate buffer, shake the tube for 20 min, then centrifuge it for 10 min at 2000 rpm. Transfer the supernatant liquid to a 25-mL volumetric flask, and repeat the extraction with phosphate buffer twice more. Dilute the combined extracts to 25 mL with phosphate buffer. Extract 10 mL of this solution with 10 mL of 0.1 *M* tri-*n*-octlyamine in $CHCl_3$, back-extract 5 mL of the $CHCl_3$ layer with 5 mL of 0.1 *M* aqueous $NaClO_4$, then determine its dye content by photometry.

Method B. Transfer 5 g of yogurt to a glass centrifuge tube, add 10 mL of 5% ammonia, and shake the tube vigorously for 2 min. Add 10 mL of acetone, shake the tube 10 min more, then centrifuge it for 10 min at 2000 rpm. Store the tube at 4°C for 2 hr. Transfer the supernatant solution to a distillation flask and evaporate the acetone at about 60°C in a rotary evaporator. Adjust the pH of the residual solution to 4.0 ± 0.1 with 30% phosphoric acid and transfer the solution to a glass centrifuge tube. Add 0.6 g of polyamide powder and shake the tube for 15 min. Centrifuge the tube for 10 min at 2000 rpm and discard the supernatant liquid. Wash the polyamide powder 3 times with about 15 mL of double-distilled water. Shake the polyamide for 15 min with 10 mL of methanol–ammonia (95 + 5), and then centrifuge the mixture for 10 min at 2000 rpm. Transfer the methanol–ammonia to a 25-mL volumetric flask, then repeat the desorption with 10 mL of the same mixture. Dilute the combined extracts to 25 mL with methanol–ammonia and measure the dye content of the resulting solution by photometry. Method A was found useful for a number of colorants, but not for FD&C Red No. 3 or azorubine. For these colorants, Method B must be used.

RAMAMURTHY, M. K., BHALERAO, V. R. Analyst *89*, 740–744 (1964). A Thin Layer Chromatographic Method for Identifying Annatto and Other Food

Colours. Extraction of color from butter: Dissolve 10 g of sample in 50 mL of diethyl ether. Pass the solution through a 7.5-cm × 1.5-cm-diameter glass column packed with aluminum oxide (E. Merck & Co., Inc.) prepared according to Brockmann. Annatto and curcumin are adsorbed on the column, whereas other fat-soluble dyes pass through. Concentrate the eluate by evaporating the ether and then saponify the residue with alcoholic potassium hydroxide. Extract the color with three portions of diethyl ether. Wash the combined extracts with water, dry over anhydrous sodium sulfate, evaporate to concentrate, and examine by TLC. Elute annatto and curcumin from the column with 25 mL of ethanol-ammonia (2:1). Acidify the eluate with *2 N* HCl, dilute it with water, and extract it three times with diethyl ether. Wash the extract with water, dry over anhydrous sodium sulfate, evaporate to concentrate, and examine by TLC.

SADINI, V. Intern. Dairy Congr. Proc. (16th, Copenhagen) *3*, 474–486 (1962). Detection of Food Dyes in Dairy Products.

SCHWARZ, G., MUMM, H., WOERNER, F. Molkerei-u. Käserei-Ztg. *9*, 1430–1433 (1958). Coloring Cheeses with Annatto and Carotene Dyes and Their Detection. Extract 25–50 g of minced cheese for 20 hr with acetone. Evaporate the extract to dryness and then extract the residue with 15 mL of benzene. Dry the extract with Na_2SO_4 and transfer to a column packed with Al_2O_3. Elute carotene from the column with benzene and then elute annatto with chloroform.

USHER, C. D., FAVELL, D. J., LAVERY, H. Analyst *93*, 107–110 (1968). A Method for the Determination of Vitamin A, α- and β-Carotene in Margarine, Including the Results of a Collaborative Test. Weight 10 g of margarine into a 250-mL flat-bottomed flask. Add 20 mg of quinol, 60 mL of ethanol, 10 mL of 60% w/v potassium hydroxide solution, and 10 mL of light petroleum. Boil under reflux for 30 min, protecting the flask from light. (Use flasks covered with a shield of aluminum foil.) Cool, and add 80 mL of distilled water. Transfer the solution into a 500-mL separatory funnel; rinse the flask into the funnel with an additional 80 mL of water. Extract the unsaponified material with 100 mL and three 50-mL portions of diethyl ether. Combine the ether extracts and wash with four 50-mL portions of distilled water; carry out the first washing by swirling and the following three by gentle shaking. Using a stream of inert gas, evaporate the unsaponifiable extract to dryness on a water bath at 50°C. The last stages of the evaporation require full attention, because the residue in the flask must not be allowed to remain dry longer than is absolutely necessary. Immediately after all of the diethyl ether has been removed, add 2 mL of absolute ethanol and again evaporate to dryness in a current of inert gas; if the residue appears wet, repeat the addition of absolute ethanol and evaporation to dryness. Immediately dissolve the residue in 5 mL

of light petroleum and again evaporate to dryness in a current of inert gas. Repeat the dissolution in light petroleum and the evaporation to dryness twice more. Finally, dissolve the residue in 2–3 mL of light petroleum for chromatography.

Pretreat magnesia by heating magnesium oxide (heavy) at 100°C for 2 hr. Cool in a desiccator and set aside for 3–4 days in an airtight bottle.

Place a pledget of cotton wool in the tip of the chromatographic tube shown in Fig. 15-1. Add petroleum ether (boiling range, 40–60°C) to a level half-way up the center section and add 3 g of magnesia. Drain the ether just to the surface of the packing.

Using 2 mL of petroleum ether, transfer the sample solution to the column. Develop the chromatogram, under pressure if necessary, with light petroleum ether containing 4–12% ethyl ether. The exact amount of ethyl ether necessary varies with different batches of magnesia and must be determined by experience. α-Carotene elutes first as a pale yellow band. β-Carotene elutes

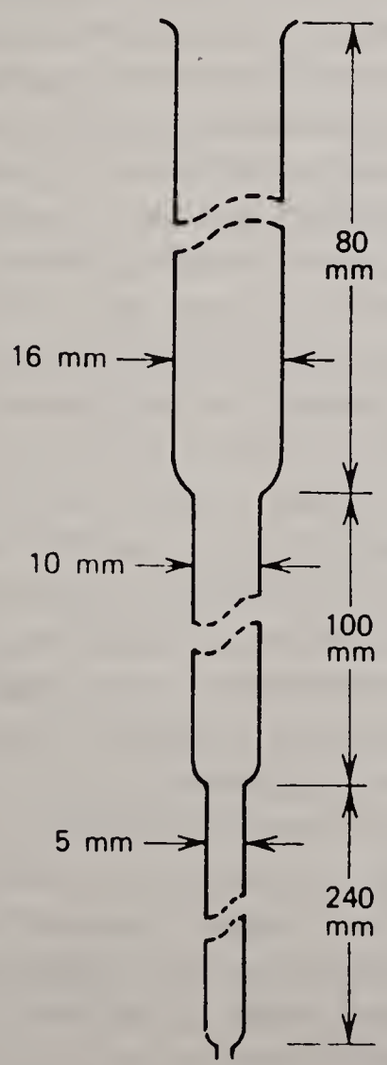

Figure 15-1. Chromatographic tube

next as a deeper orange-colored zone. After the α-carotene elutes, 1:1 ethyl ether–light petroleum may be used to speed up the eluiton of β-carotene. Determine both materials spectrophotometrically against standards.

VERMA, M. R., RAI, J., GANGOPADHYAYA, N. Ind. J. Technol. *1*, 358–360 (1963). Chromatographic Method for the Separation of Dyes from Butter and their Identification. The sample is dissolved in benzene, adsorbed on a column of alumina, and eluted with benzene then EtOH. Annatto that remains adsorbed on top of the column is removed with alcoholic ammonia.

DRUGS

AKADA, Y., KAWANO, S., TANASE, Y. Yakugaku Zasshi, *98*, 1300–1304 (1978). High-Speed Liquid-Chromatographic Determination of Colouring Matters in Gelatin Capsules. Dissolve an empty capsule in 5 mL of 0.1 *M* NaOH at 50–60°C. Pass the resulting suspension through a 5-cm × 1-cm column packed with activated charcoal. Wash gelatin from the column with 100 mL of water, then elute any dyes with aqueous 50% pyridine. Evaporate the colored eluate to dryness under reduced pressure, then dissolve the residue in 1 mL of H_2O. Chromatograph 1–5 μL of this solution on a 50-cm × 2.1-mm column of Permaphase ODS at room temperature. Elute colorants at 1 mL/min using 0.05% hexadecyltrimethylammonium bromide in aqueous methanol. Use a gradient increasing from 20% MeOH to 50% MeOH at 5%/min, then at 2%/min to 70% MeOH. Monitor at 254 nm. This procedure separates a number of colorants including Amaranth, FD&C Yellow Nos. 5 and 6, FD&C Red No. 3, and D&C Red No. 28.

ALARY, J., DUC, C. L., COEUR, A. Bull. Trav. Soc. Pharm. Lyon. *10*, 78–86 (1966). Identification of Synthetic Colorants in Drugs. Colorants in drugs are identified by ascending paper chromatography using BuOH–EtOH–NH_4OH–H_2O (50:25:10:25) or by TLC on Kieselgel using BuOH–MeOH–C_6H_6–H_2O. Both separations are performed in subdued light.

BALATRE, P., TRAISNEL, M. Bull. Soc. Pharm. Lille *1*, 41–47 (1965). Identification of Pharmaceutical Dyes by Thin-Layer Chromatography of their Complexes with a Quaternary Ammonium Compound. Colorants were extracted from the drugs, complexed with a quaternary ammonium derivative, and separated by TLC on Kieselgel G both with and without Na_2CO_3 binder, on alumina with Na_2CO_3 binder, or on cellulose MN 300. The best separations were obtained using BuOH–EtOH–H_2O (2:1:1) and EtOAc–pyridine–H_2O (7:3:1) as the mobile phases.

BALATRE, P., MULLEMAN-MARSY, D., TRAISNEL, M. Ann. Pharm. Fr., *25*, 649–653 (1967). Identification and Determination of Natural Dyes of

Vegetable Origin in Drugs. Transfer 1 mL of aqueous sample solution to a separatory funnel. Add 2 mL of 10% aqueous Na_2CO_3. Shake well. Add 20 mL of surfactant solution [0.1 g of hexadecyl-(2-hydroxycyclohexyl) dimethylammonium bromide, 0.8 g of benzyl-lauryldimethyl-ammonium bromide, and water to 100 mL] and 20 mL of $CHCl_3$. Shake the mixture for 10 min, centrifuge, and filter the $CHCl_3$ layer through absorbent cotton. Fat-soluble dyes, such as carotenes, xanthophylls, and chlorophyll, are not readily extracted. Indigotines and caramel are extracted from neutral solution, and the anthocyanins of bilberry, from acid solution containing $(NH_4)_2SO_4$. The dyes are identified and determined from the colors of the extracts under daylight and UV radiation, the absorption maximum, and the $E^{1\%}_{1cm}$. The $CHCl_3$ solution can be subjected to TLC on Kiesegel G (applied to the plates as a suspension in 1% aqueous Na_2CO_3) using butanol–ethanol–H_2O (2:1:1) or ethyl acetate–pyridine–H_2O (7:3:1) as the mobile phase.

BARROS, A. A., CABRAL, J. O., FOGG, A. G. Analyst *113*, 853–858 (1988). Use of Gelatin in the Differential-Pulse Polarographic Determination and Identification of Synthetic Colouring Matters in Drugs and Cosmetics. A systematic study was made of the effect of gelatin on the polarographic behavior of 16 food and three cosmetic synthetic-coloring additives. Gelatin was shown to have a pronounced effect on the peak currents, and a lesser effect on the peak potentials, of some of the colorants, and these effects could be used to partially identify and determine the colorants. Applications of the technique to the analysis of a colored gelatin capsule, a lipstick, and a blusher are described.

BARROS, A. A. Analyst *112*, 1359–1364 (1987). Differential Pulse Polarographic Determination of Synthetic Colouring Matters in Drugs and Cosmetics. Differential-pulse polarographic peak potentials of food and cosmetic colorants including FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Red No. 40, FD&C Red No. 3, FD&C Blue No. 2, and D&C Red No. 33 were determined at several pH values in 40 m*M* buffer solution, using a dropping mercury electrode vs silver–AgCl. The peak potentials obtained for all the colorants were similar, making their identification difficult. Addition of tetraphenylphosphonium chloride modified the peak potentials and peak currents of the colorants enough to make identification of the colorants possible. Procedures are reported for the determination and partial identification of dyes in a tablet and a lipstick.

CHEN, Y., ZHUO, Z. Fenxi Huaxue *16*, 9–12 (1988). Determination of Synthetic Food Colours in Their Binary Mixtures Using Second-Derivative Spectrophotometry. Amaranth, new coccine, FD&C Yellow No. 5, FD&C Yellow No. 6, and D&C Blue No. 4 were determined simultaneously in admixture using second-derivative spectrophotometry. Standard solutions of the colorants were prepared in acetic acid–ammonium acetate at pH 4.90–4.92, and

measurements were made between 350 and 700 nm. The procedures were applied to cold capsules and liquers.

FADIL, F., MC SHARRY, W. O. J. Pharm. Sci. *68*, 97–98 (1979). Extraction and TLC Separation of Food, Drug and Cosmetic Dyes from Tablet-Coating Formulations. Add 10 drops of 85% phosphoric acid to an appropriate amount (up to 1 mL) of tablet-coating liquid in a 50-mL centrifuge tube, and mix well by swirling intermittently for 5 min. Add 10 mL of MeOH then shake the tube for 1 min more. Add 1 mL of concentrated NH_4OH (29%) and again shake for 1 min. Centrifuge the sample, then spot 10 μL of the supernatant liquid on a 20-cm × 20-cm silica gel G plate (0.25 mm thick) that has been activated by drying at 40°C, then heating for 15 min at 105°C just prior to use. Develop the plate for 50–60 min in a 30 × 9 × 27-cm paper-lined glass tank saturated with mobile phase just prior to use, using ethyl acetate–MeOH–H_2O-conc. NH_4OH (150:40:35:5) as the mobile phase.

Colorant	R_f
FD&C Yellow No. 5	0.06
FD&C Green No. 3	0.07
FD&C Red No. 2[a]	0.07
FD&C Blue No. 1	0.16
D&C Blue No. 4	0.17
FD&C Yellow No. 6	0.22
FD&C Red No. 40	0.22
D&C Red No. 33	0.23
FD&C Red No. 4	0.24
FD&C Violet No. 1[a]	0.26
D&C Red No. 7	0.27
D&C Green No. 5	0.31
FD&C Red No. 3	0.31
D&C Red No. 28	0.33
D&C Orange No. 4	0.36
D&C Red No. 19[a]	0.36
D&C Yellow No. 10	0.37
D&C Yellow No. 11	0.77
D&C Red No. 36	0.81
D&C Green No. 6	0.85

[a]Use no longer permitted in the United States.

FELL, A. F., ALLAN, J. G. Anal. Proc. July, 291–296 (1981). Analysis of Colouring Agents in Pharmaceuticals by Derivative Ultraviolet-Visible Spectroscopy. Samples of syrups were diluted, and the colorants in them were identified and quantified based on the second derivative of their ultraviolet and visible spectra. The technique was applied to products containing amaranth, carmoisine, FD&C Yellow No. 5, and FD&C Yellow No. 6.

FOGG, A. G., BARROS, A. A., CABRAL, J. O. Analyst *111*, 831–835 (1986). Differential Pulse Adsorptive Stripping Voltammetry of Food and Cosmetic Synthetic Colouring Matters and Their Determination and Partial Identification in Tablet Coatings and Cosmetics. The method takes advantage of the strong adsorption properties of some synthetic dyes on a hanging-mercury-drop electrode. Using an accumulation time of 2 min, the sensitivity increase over differential pulse polarography at a dropping-mercury electrode was 8–100-fold. The addition of tetraphenylphosphonium chloride shifted the reduction potentials of some dyes to more negative values and either decreased or increased the peak size obtained. Increased values were obtained with carmoisine, FD&C Yellow No. 5, and quinoline yellow. Procedures for applying the method to the determination and partial identification of dyes in tablet coatings and lipsticks are described.

GALCZYNSKA, M., KWIATKOWSKA, M., MIKUCKA, B., SZOTOR, J. Farm. Pol. *33*, 645–647 (1977). Identification of Synthetic Dyes in Coloured Coated Tablets, Uncoated Tablets and Syrups. The sample of tablets or syrup is extracted with water, the extract is shaken with alumina, any adsorbed dyes are stripped from the alumina with 0.5% aqueous NH_3, and the extract is evaporated to dryness. The residue is dissolved in water and a portion of it is chromatographed on a MN 300 carboxymethylcellulose thin-layer plate using 2% NaCl solution in H_2O–10% aqueous NH_3 (93:5) as the mobile phase.

JEKABSONS, E. JAOAC *52*, 110–112 (1969). Fluorometric Analysis of Sodium Fluorescein in Ophthalmic Solutions. Dilute the sample with water so that it contains about 1 μg of sodium fluorescein per milliliter. Transfer 3 mL of sample solution and 20 mL of borate buffer (0.05 *M* boric acid in 0.05 *M* KCl adjusted to pH = 9 with 0.2 *M* NaOH) to a 100-mL volumetric flask and dilute to volume with water. Mix well and then measure the sample's fluorescence at 515 nm with excitation at 460 nm and compare with that of standards.

JENTZSCH, K., SPIEGL, P., KAMITZ, R. Scientia pharm. *38*, 50–58 (1970). Qualitative and Quantitative Investigations on *Curcuma* (Turmeric) Colouring Matters in Zingiberaceae Drugs. II. Quantitative Investigation. Transfer 0.1 g of finely powdered sample to a Soxhlet apparatus and extract for 30 min with 10 mL of 96% ethanol. Evaporate the extract to 2 mL and then dilute to

5 mL with 96% ethanol. Chromatograph 220 μL of solution on Kieselgel H (0.25 mm) using $CHCl_3$–benzene–ethanol (1:8:1). Dry the chromatogram for 30 min at 90–100°C. Examine under UV light, remove the appropriate areas from the plate, extract the colorants with 96% ethanol, and determine spectrophotometrically.

JERNAS, B., LUTOMSKI, J. Herba Pol. *24*, 125–134 (1978); *24*, 135–142 (1978); *24*, 207–213 (1978); *25*, 15–19 (1979). Determination of Dyes in Selected Colour Lakes, Parts I–IV. In Part I, the water-soluble dye in an Amaranth lake was determined densitometrically at 525 nm after chromatography at 30°C for 70 min on Whatman No. 3 paper using a 2% solution of NaCl in H_2O–aqueous 25% NH_3 (9:1) as the mobile phase. Procedures are described for determining Amaranth in suppositories and coated tablets. In Part II, Amaranth and Orange Yellow S were extracted from tablets with HCl and then with ethanol, the combined extracts were mixed with polyamide powder, and then the powder was washed with H_2O at 50°C and then with aqueous 70% MeOH. The adsorbed dyes were eluted with MeOH–H_2O–aqueous 25% NH_3 (7:2:2), the eluate was evaporated to dryness, and the residue was dissolved in water and evaluated by paper chromatography. Part III describes the determination of FD&C Yellow No. 5 densitometrically (See Part I) after separation from its lake by paper chromatography on Whatman No. 3 paper using NaCl–H_2O–25% aqueous NH_3 (1:45:5) as mobile phase. Part IV describes a similar application to Ponceau 4R and its lake.

JOYCE, J. R., HUMPHREYS, I. J. J. Forensic Sci. Soc. *22*, 253–256 (1982). Detection of Trace Impurities in Soluble Food Dyes. A method to identify the manufacturer of colorants in dye-containing illicit tablets is described. The procedure is based on the assumption that each manufacturer produces colorants with specific kinds and amounts of impurities. Aqueous solutions of the dyes, or extracts from tablets containing the dyes, were applied to a 10-cm × 5-mm SAS Hypersil column and eluted using H_2O–propanol (5:2) containing 0.25% cetrimide as the mobile phase, a flow rate of 2 mL/min and spectrophotometric detection. For some colorants, analysis time was reduced by using the detergent in H_2O-propanol (7:3) as the mobile phase. Numerous colorants including FD&C Blue No. 1, carmoisine, FD&C Red No. 3, FD&C Yellow No. 5, and FD&C Yellow No. 6 were studied using this procedure.

KOENIG, H., WALLDORF, E. Fette, Seifen, Austrichm. *83*, 281–287 (1981). Outline of a Scheme for Analysis of Toothpastes. An outline is given of methods (without details) available for the complete analysis of toothpastes. Includes procedures for the determination of color additives.

KOENIG, H., WALLDORF, E. Fresenius´ Z. Anal. Chem. *289*, 177–197 (1978). Analysis of Toothpastes. A comprehensive, systematic scheme of analysis is

proposed, with particular reference to the detection and determination of polishing agents, the separation of detergents, and the determination of moisturizers, sweetening agents, flavors, preservatives, and color additives.

LEHMANN, G., BINKLE, B., NIEDER, M. Seifen, Oele, Fette, Wachse *111*, 167–169 (1985). Identification of Dyes in Mouth- and Tooth-Care Agents. Lipophilic dyes were extracted from mouthwashes and tooth paste into light petroleum or CH_2Cl_2, and the extracts were chromatographed by thin-layer chromatography using silica Gel 60, and ethyl acetate–pyridine–H_2O (11:5:4) or ethyl acetate–pyridine–H_2O–1% $HClO_4$ (in methanol)–aqueous 25% NH_3 (11:5:4:4:2) as the mobile phase. The dyes were identified using 0.1 *M* hexadecylpyridinium chloride in methanol, 0.1 *M* pentanesulfonic acid and dimethylformamide–H_3PO_4 (17:3) as reagents.

LEHMANN, G., COLLET, P. Arch. Pharm. Berl. *303*, 855–860 (1970). Analysis of Dyes. VIII. Identification of Synthetic Dyes in Drugs. Dyes are adsorbed on Polyamide MN SC6 powder. The powder is transferred to a 150-mm × 15-mm microchromatographic column, eluted, and then identified by TLC on cellulose layers using 2.5% aqueous ammonium citrate–aqueous NH_3–methanol (20:5:3) as the mobile phase.

MARES, V., STEJSKAL, Z. Cslka. Farm. *16*, 474–479 (1967). Identification of Dyes Used for Coloring Drugs. Dyes are extracted from drugs with quinoline and then separated on Whatman No. 1 paper (descending) using 2.5% aqueous Na citrate–25% aqueous NH_3 (4:1) plus 3% triethanolamine, or BuOH–acetic acid–H_2O (1:1:1).

MERKUS, F. W. H. M., SAGEL, J. Pharm. Weekblad *99*, 1098–1116 (1964). The Use and Analysis of Synthetic Dyes in Pharmaceutical Products. Colorants are extracted with quinoline, amyl alcohol, or BuOH and the extracts are chromatographed on paper using BuOH–EtOH–H_2O (1:1:1) or 2% Na citrate in 5% NH_4OH.

MOROZOVA, G. I., SOBOLEVA, E. Ya., SHENFEL'D, I. L. Gig. Sanit. *10*, 115–116 (1978). Extraction and Identification of Some Water-Soluble Synthetic Dyes from Lip-Salve. A 0.1–0.2-g sample was mixed with 15 mL of water and the mixture was filtered; 3 mL of BuOH and 2 g of NaCl were added to the filtrate, and the mixture was shaken. The BuOH layer was washed with 10–15 mL of H_2O, then evaporated to dryness. The residue was dissolved in H_2O and chromatographed on a Silufol TLC plate using a mixture of 3 mL of toluene, 5 drops of *o*-cresol, 3 mL of alcohol and 0.5 mL of aqueous 20% NH_3.

PATEL, R. B., PATEL, M. R., PATEL, A. A., SHAH, A. K., PATEL, A. G. Analyst *111*, 577–578 (1986). Separation and Determination of Food Colours in Pharmaceutical Preparations by Column Chromatography. Fit a 600-mm ×

10-mm glass chromatographic column with a glass-wool plug, then add 30 mL of 20–50 mesh Fluka Amberlite XAD-2 resin suspended in methanol. Allow the column to drain, then wash it methanol-free with water. Transfer sample containing about 100 μg of food color to the column, then wash the column with water to remove other unretained pharmaceutical excipients. Elute disulfonated colorants from the column with 0.1% sulfuric acid in methanol, then elute trisulfonated colorants with 4.0% sulfuric acid in methanol. Determine any colorants present spectrophotometrically.

PELLERIN, F., GAUTIER, J. A., CONRARD, A. M. Ann. Pharm. Franc. *22*, 621–627 (1964) Identification of Authorized Synthetic Organic Dyes in Pharmaceuticals. The sample is extracted with 10 mL of water and filtered. Then 1 g of Na_2SO_4 and 1.5 mL of 1:5 H_2SO_4 is added to the filtrate (except for alizarin-erythrosine, which is extracted at neutral pH), a 1-cm-wide piece of polyfiber (Colcombet) is added, and the solution is heated for 30 min in boiling water. The ribbon is washed thoroughly with H_2O at 40–45°C and then dried below 50°C. Colorant is stripped from the ribbon with 2–5 mL of 10% aqueous NH_4OH, the extract is evaporated to dryness on a water bath, and the residue is dissolved in 0.5 mL of water and then resolved by paper or thin-layer chromatography.

PELLERIN, F., KIGER, J. L., CAPORAL-GAUTER, J. Ann Pharm. Fr. *32*, 427–431 (1974). Synthetic Organic Colours in Plastic Packaging Materials for Pharmaceutical Use. II. Identification in Plastics and Detection of Their Release into Drugs. The plastic, cut into fine slivers, is dissolved in 10 mL of benzene, toluene, or acetone for polyalkenes, in 1,4-dioxan or tetrahydrofuran for poly (vinyl chloride), in formic acid or cyclohexane for polyamides, and in dichloroethane or acetone for cellulose acetate. The dyestuffs are identified in the solution by: (1) TLC on silica gel, with $CHCl_3$–xylene (3:1), benzene–$CHCl_3$ (4:1) or $CHCl_3$–acetic acid (200:1) as the mobile phase, and development for 12–15 cm; (2) spectrophotometric measurements; (3) precipitation of the plastic by adding another solvent (e.g., $CHCl_3$, ethanol, or H_2O) and then TLC of the filtrate; and (4) precipitation of the plastic with H_2O, after dissolution in acetone or H_2SO_4, with the dyestuff examined for ion-pair formation in $CHCl_3$ by reaction with dodecyl sulfate or cetylpyridinium salt. The method is also applied to storage tests on semisynthetic glycerides in the presence of dyed plastic packaging materials.

PLA DELFINA, J. M., MACIAN, R. S. Galenica Acta *9*, 243–286 (1956). Chromatography of Synthetic Colors in Pharmaceutical Preparations Used Internally. Samples (10 g) were extracted with water at 60°C. Any insoluble residue was reextracted with 5% tartaric acid at 60–80°C, adsorbed on wool, eluted with 0.02 *N* NH_3, concentrated, and then chromatographed on Schleicher & Schull 2043A paper using water-saturated BuOH or

$(ClCH_2)_2CHOH$. The water extract was split in two. One portion was acidified with 5% tartaric acid, adsorbed on and stripped from wool as described above, and then extracted with AmOH. Both layers were examined for colorants. The second portion of the water extract was treated with 5% NaCl and the colors were adsorbed on wool, eluted with normal tartaric acid, and extracted with $CHCl_3$. The $CHCl_3$ extract was examined for colorant.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. J. Pharm. Boimed. Anal. *3*, 503–510 (1985). Extraction of Organic Acids by Ion-Pair Extraction With Tri-*n*-Octylamine. VIII. Identification of Synthetic Dyes in Pharmaceutical Preparations. Synthetic dyes were extracted from syrups, oral suspensions, tablets, gelatin capsules, suppositories, and granules by ion-pair formation at pH 5.5 using tri-*n*-octylamine in $CHCl_3$, then back-extracted into 0.1 *M* $NaClO_4$. Colorants were identified by TLC using cellulose ready-coated plates, and by reversed-phase HPLC using 10-μm LiChrosorb RP-18. The R_f values of dyes extracted from 47 different pharmaceuticals and the retention times of some standard dyes and dyes extracted from 13 pharmaceuticals are tabulated.

SERINI, G. Chimica *34*, 95–96, 144–145, 197–200 (1958). Separation and Identification by Paper Chromatography of Dyes Added to Aliments.

SITZIUS, F., RENTSCH, H. Pharm. Ind. Berl. *35*, 148–150 (1973). Detection of Colouring Matter in Capsules and Sugar-Coated Tablets. A suitable number of empty gelatin capsules is dissolved in 5 mL of 10% acetic acid and the mixture is passed through a 1-cm column containing 1.5 g of alumina (Brockmann). The gelatin is removed by passing 10 mL of H_2O thru the column using gentle suction. Colorant is eluted with 0.1% aqueous NH_3, the eluate is evaporated to dryness on a steam bath, and the residue is dissolved in a few drops of methanol and examined by TLC on G1440 cellulose plates.

STORCK, J. Ann. Pharm. Franc., *23*, 113–115 (1965). Detection of Dyes in Pharmaceutical Gelatin Capsules. Five gelatin capsules are dissolved in 25% HOAc and placed on an alumina column. The colorant is eluted with NH_4OH–H_2O (1:100), concentrated to 5–10 mL, and then chromatographed (descending) on Whatman No. 1 paper using tri-Na citrate dihydrate–NH_4OH–H_2O (2 g, 20 mL, dilute to 100 mL), or on 0.25 mm of Kieselgel G using Et_2NH–MeOH–EtOH (10:35:55).

SYKULSKI, J., JASKULSKA, T. Farm. Pol. *40*, 91–93 (1984). Chromatographic Identification of Synthetic Dyes in Some Coated Tablets Produced by "Polfa" Works. FD&C Yellow No. 5, Orange Yellow S, Cochineal Red, Amaranth, and FD&C Blue No. 2 are separated by thin-layer chromatography using Macherey Nagel 300 CM cellulose layers and NaCl–H_2O–aqueous 25% NH_3 (2:93:5) as the mobile phase. R_f values for standard dyes are given. To separate the dyes present in tablets, coatings are scraped from the tablets and

dissolved in H_2O, the resulting solution is centrifuged, then the supernatant liquid is acidified with 1 mL of 10% HCl and passed through a sintered-glass filter packed with a slurry of Al_2O_3 (4 g) in H_2O. The slurry is washed with 20 mL of H_2O and the dyes are eluted with 10 mL of aqueous 0.5% NH_3. This extract is then evaporated, the dry residue is dissolved in a few drops of H_2O then chromatographed as described above.

UNTERHALT, B., KREUTZIG, L. Dt. Apoth Ztg. *112*, 449–450 (1972). Detection of Dyestuffs in Cough Linctuses. Dilute 10 mL of sample with 40 mL of H_2O and acidify with $KHSO_4$ or HOAc. Adsorb colorant onto wool fibers or onto a column of polyamide powder (0.5 g). Elute colorant with methanolic NH_3, evaporate eluate to dryness, dissolve in two drops of H_2O, and chromatograph on a layer of cellulose MN 300 using aqueous NH_3–2.5% aqueous monosodium citrate (1:4), propanol–ethyl acetate–H_2O (6:1:3), or ethyl acetate–pyridine–H_2O (3:1:1).

VARSANYI, E., VIDA, L., PAAI, T. L. Acta Pharm. Hung. *54*, 146–153 (1984). Determination of Auxiliary Substances in Pharmaceutical Preparations. I. Simultaneous Determination of Talc and Aerosil in Tablets. A portion of sample is calcined to destroy any organics present, then the ash is boiled with 0.5 *M* acetic acid to remove any magnesium silicate in the ash due to the initial presence of magnesium stearate in the sample. 1 mg of dried ash is then mixed with 200 mg of KBr, and a pellet is made and examined in the infrared. Aerosil (silica) is measured at 810 cm^{-1}, and talc is measured at 670 cm^{-1}. The coefficient of variation of the method is 12.3% for Aerosil and 3.8% for talc in tablets containing 0.33–1.67% Aerosil and 1.0–5.0% talc. The accuracy of the method and experimental data for tablets of five different types are presented.

WOJCIK, Z. Farmacja pol. *25*, 419–425 (1969). Chromatographic Identification of Synthetic Dyes in Pharmaceutical Preparations. Scrape the colored coating from 5–10 tablets, dissolve the scrappings in 10–20 mL of H_2O, add 1–2 mL of 10% HCl, and mix in 2 g of alumina. Filter the mixture on a sintered-glass filter, wash with 100 mL of H_2O, and then extract colorant with 5 mL of 0.5% aqueous NH_3. Evaporate the extract to dryness, dissolve the residue in a few drops of water, and chromatograph on Whatman No. 1 paper using 2.5% aqueous Na citrate–25% aqueous NH_3–triethylamine (80:20:3) and then BuOH–HOAc–H_2O (1:1:1). Examine under daylight and under UV light.

WOJCIK, Z. Farmacja Pol. *26*, 723–729 (1970). Thin-Layer Identification of Azo Dyes Permitted in Poland for Use in Pharmaceutical Preparations. Colorants are extracted as described in the preceding paragraph, applied to a plate of MN 300 cellulose powder, activated at 100°C for 1 hr, and then developed with 2.5% aqueous Na citrate–aqueous NH_3 (7:3), PrOH–ethyl acetate–H_2O (5:2:3), or BuOH–HOAc–H_2O (25:5:12).

FATS AND OILS

BLAZQUEZ SOLANA, J. Grasas Aceites *36*, 349–352 (1985). Azo-Dyes in Edible Oils. I. Detection and Determination of Aminoazo-Compounds. Aminoazo-dyes were extracted from edible oils into 4 *M* HCl. The colorants were determined by HPLC using a 10-μm μBondapak C_{18} Radial-PAK A column, and aqueous 80% methanol containing 1 g/L of trimethylammonium chloride as the mobile phase. Flow rate was 1 mL/min, and detection was at 254, 280, 340, and 360 nm. The limit of detection was 0.1 ppm.

BLAZQUEZ SOLANA, J. Grasas Aceites *36*, 353–356 (1985). Azo-Dyes in Edible Oils. II. Detection of Hydroxyazo-Compounds. Hydroxyazo dyes were extracted from edible oils into dimethylformamide then cleaned up by TLC using silica gel G and $CHCl_3$ as the mobile phase. The colorants were determined by HPLC using a 5-μm Resolve-C_{18} column, and aqueous 90% methanol, methanol, or methanol–$CHCl_3$ (2:1) as the mobile phase, at a flow of 1 mL/min. Detection was at 254, 280, 313, 340, and 360 nm.

BOSE, P. K., ROY, B. R., MITRA, S. N. J. Food Sci. Technol. *7*, 112–113 (1970). Analysis of Oil-Soluble Dyes from Foods Using Clean-Up by Adsorption. Oil containing natural or synthetic dyes is diluted with light petroleum (boiling range 60–66°C) and then sufficient chromatographic-grade silica gel is added to adsorb the colorants. The solvent is removed by decantation and then the silica is washed with light petroleum. The adsorbed dyes are extracted from the silica gel with methanol and identified by reverse-phase chromatography.

DAVIDEK, J., JANICEK, G. Qual. Plant. Mater. Veg. *16*, 253–257 (1968). Thin-Layer Chromatographic Separation of Fat-Soluble and Water-Soluble Food Dyes. The colored fat is saponified and the dyes are extracted with light petroleum ether and then separated by chromatography.

HORWITZ, W., Ed. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12th ed. 1975, p. 279. Color Additives in Fat. Pour about 2 g of filtered fat, dissolved in ether, into each of two test tubes. To one tube add 1–2 mL of HCl (1 + 1) and to other about the same volume of 10% NaOH solution. Shake the tubes well and let stand. In the presence of some azo dyes the acid solution turns pink to wine-red, whereas the alkaline solution in the other tube shows no color. However, if annatto or some other vegetable color is present, the alkaline solution is yellow, whereas no color is apparent in the acid solution. (Red changing to yellow, especially on warming, in alkaline solution may be due to presence of gallate antioxidants.)

LINDBERG, W. Z. Lebensm. Forsch. *103*, 1–14 (1956). Detection and Identification of Fat-Soluble Coal-Tar Dyes in Food Products. The fat or oil is dissolved in petroleum ether and the colorant is extracted with acid solution

(20 mL of HCl, 10 mL of H_2O made to 100 mL with HOAc, or 40 mL of H_2SO_4, 10 mL of H_2O, and 90 mL of HOAc). The acid extract is extracted with ether, the ether is evaporated, and the residue is saponified. The unsaponified material is isolated with EtOAc and the colorant therein identified by chromatography.

MARK, E., MC KEOWN, G. G. JAOAC *41*, 817–818 (1958). Isolation of Oil-Soluble Coal-Tar Colors from Foods. Dissolve 10 g of sample in 50 mL of petroleum ether. Filter, if necessary, into a separatory funnel. Extract with three 20-mL portions of *N,N*-dimethylformamide (DMF); discard the ether layer. Combine the DMF solutions and extract with four 25-mL portions of petroleum ether, back extracting each time with 5 mL of DMF. Discard the ether extracts. Dilute the combined DMF solutions with an equal volume of water and extract with 30 mL, and then 10 mL of chloroform. Discard the aqueous DMF layer. Combine the chloroform extracts and wash them with water to remove any dissolved DMF. Evaporate the chloroform solution to dryness under vacuum at room temperature. Dissolve the residue in 25 mL of DMF and transfer it to a separatory funnel. Add 25 mL of water and extract the solution with three to five 25-mL portions of petroleum ether. Discard the aqueous DMF layer and wash the combined ether layers with water. Evaporate the ether solution under vacuum at room temperature. Examine the residue chromatographically or spectrophotometrically.

FRUITS

ADAMS, J. B., BUTLER, R. Analyst *101*, 140–142 (1976). A Rapid Method for Detecting Erythrosine in Canned Red Fruits. Weigh 20 g of macerated sample. Add 5% aqueous sodium sulfite heptahydrate to decolorize any anthocyanins present and then increase the pH of the sample to 4–6 to ensure the solubilization of the FD&C Red No. 3 (Erythrosine). Shake the mixture vigorously with 5 mL of 3-methylbutan-1-ol and centrifuge. Determine the visible spectrum of the upper (alcohol) layer from 700 nm to 300 nm. A sharp peak at 545 nm indicates the presence of Erythrosine. Identification can be confirmed by noticing the almost complete loss of absorbance at 545 nm after one or two drops of HCl are added to the sample in the absorption cell.

ANONYMOUS Chemistry *43*, 29–30 (1970). Identifying Artificial Color on Oranges. Rinse the colorant from the surface of the orange with 25 mL of $CHCl_3$, evaporate the solution to dryness on a steam bath, and then dissolve the residue in 3 mL of $CHCl_3$. Chromatograph the solution for 1 hr on paper impregnated with a solution of 5 g of mineral oil in 95 mL of Et_2O using 65% Me_2CO.

DRAPER, R. E. JAOAC *56*, 703–705 (1973). Separation and Determination of FD&C Red No. 4 and FD&C Red No. 40 in Maraschino Cherries by Column Chromatography.

Reagents:

(a) Mobile phases A and B—Add 500 mL of 5% Amberlite LA-2 resin (Rohm & Haas Co., Philadelphia, PA.) in *n*-butanol, 200 mL of water containing 7.5 mL of acetic acid, and 12.5 mL of saturated $(NH_4)_2SO_4$ solution to a separatory funnel. Shake vigorously for 1 min and let phases separate. Lower layer is mobile phase A; upper layer is mobile phase B.

(b) Hydrochloric acid—0.75% (1 + 49).

(c) Buffer solution—pH 1.5. Mix 50 mL 0.2 *M* KCl (14.911 g of KCl/liter of water) and 41.4 mL of 0.2 *N* HCl in a 200-mL volumetric flask and dilute to volume with water. Check to ensure a pH of 1.5 ± 0.02.

(d) Mobile phases C and D—Add 400 mL of *n*-butanol–CCl_4 (1:1) and 200 mL of buffer solution (c) to a separatory funnel. Shake vigorously for 2 min and let phases separate. Lower layer is mobile phase C; upper layer is mobile phase D. Prepare fresh daily.

(e) Resin-*n*-hexane—Add 500 mL of 5% Amberlite LA-2 resin in *n*-hexane and 100 mL of HCl (1 + 49) to a separatory funnel. Shake for 1 min. Discard lower phase.

(f) Adsorbent—Celite 545, acid-washed, rinsed to neutrality, and dried.

Procedure: Drain packing liquid as completely as possible from cherries and chop cherries for 15 min in a Hobart 84141 food cutter or the equivalent. Mix thoroughly while chopping. Transfer to Mason jar with tight-fitting lid.

Weigh 5-g sample into 8-oz glass mortar, add 3 mL of mobile phase A, and carefully grind with pestle for 2 min. Add 15 g of adsorbent and carefully grind for an additional 2 min. Scrape off pestle and thoroughly mix sample with spatula. Transfer mixture to a 300-mm × 23-mm-ID glass chromatographic column containing small plug of glass wool (silanized, Applied Science Laboratories, State College, PA) and firmly pack with tamping rod. Wipe off mortar, pestle, and spatula with piece of glass wool and add wipe to column. Rinse mortar with 10 mL of mobile phase B and add rinse to column. After rinse has entered column, elute column with 90 mL of mobile phase B, collecting eluate in a 125-mL separatory funnel containing 1 mL of water. Add 30 mL of hexane, shake, and let separate. Discard lower layer. Add 10 mL water (carefully rinsing around stopper and neck of separatory funnel) and 2 mL of NH_4OH. Extract color by shaking for 2 min. Allow to separate and drain lower layer into second 125-mL separatory funnel, rinsing stem with a small portion of water. Completely extract color from first separatory funnel with an additional 10 mL of water and 1 mL of

NH_4OH and add lower layer to second separatory funnel. Rinse first funnel with 5 mL of water and add rinse to second separatory funnel. Wash combined aqueous extracts with two 25-mL portions of $CHCl_3$, discarding $CHCl_3$ completely each time. Render acidic with 2 mL of HOAc and extract color with 50 mL of *n*-butanol. Continue extraction with 10-mL portions of *n*-butanol until color is visually completely extracted (3–6 extractions are usually sufficient). Combine extracts in a 150-mL beaker, rinsing each separator with 2 mL of butanol. Add 15–25 mL of ethanol, mix with stirring rod, and evaporate just to dryness on steam bath under current of air.

Mix 5 g of adsorbent and 3 mL of mobile phase D in a 100-mL beaker and transfer to chromatographic column containing small plug of glass wool. Pack with tamping rod. Dissolve color residue in 1 mL of HCl (1 + 49), being sure to dissolve color on sides of beaker. Add 2 g of adsorbent, thoroughly mix, and transfer to prepared column. Pack with tamping rod. Dry-wash beaker with 0.5 g of adsorbent and add wash to column. Wipe beaker with a piece of glass wool and add wipe to column. Rinse beaker with three 5-mL portions of mobile phase C and add rinses to column, allowing each to enter column before next one is added. Completely elute FD&C Red No. 4 with an additional 180–235 mL of mobile phase C, depending on amount of color in sample, but not exceeding 250 mL total. Collect in either a 200-mL or 250-mL volumetric flask. Dilute to volume with mobile phase C and determine spectrophotometrically at the maximum near 502 nm. After complete elution of FD&C Red No. 4, pass 20 mL of *n*-hexane through column and discard. Elute FD&C Red No. 40 with 50 mL of resin-*n*-hexane, collecting eluate in a 100-mL volumetric flask. Dilute to volume with resin-*n*-hexane, filter through glass wool if cloudy, and determine spectrophotometrically at the maximum near 500 nm.

DRAPER, R. E. JAOAC *58*, 614–616 (1975). Effect of FD&C Red No. 3 (Erythrosine) on the Determination of FD&C Azo Color Additives. When using the above procedure to analyze Maraschino cherries containing both FD&C Red No. 3 and FD&C Red No. 4, recoveries for Red No. 4 were consistently low. Further study showed that this was due to an interaction between Red No. 3 and Red No. 4, and that Red No. 3 reacted in a similar way with other azo colorants including Amaranth, FD&C Red No. 40, and FD&C Yellow Nos. 5 and 6. This interaction was found to be dependent upon the concentration of FD&C Red No. 3 and to occur only in alkaline solution and in the presence of light.

LEHMANN, G., JEKAT, F., BINKLE, B., OTTERBEIN, W. Fresenius' Z. Anal. Chem. *328*, 596–597 (1987). Contribution to the Analysis of Citrus Red 2. Citrus Red No. 2 was characterized by IR, NMR, and TLC. To detect Citrus Red No. 2 on citrus fruits, the surface of the fruit was washed with CH_2Cl_2, the washings were concentrated, then analyzed by TLC using Kieselgel 60

(250 μm), and light petroleum (40–60°)–benzene–ethyl acetate–ethyl methyl ketone (10:5:4:1) as the mobile phase. Citrus Red No. 2 was detected as a dark-red spot with an R_f of 0.65.

PRZYBYLSKI, W., SMYTH, R. B., MC KEOWN, G. G. JAOAC *43*, 274–278 (1960). Determination of Coal-Tar Colors on Oranges. Using 250 mL of chloroform, wash the color from 10 oranges. (Surface waxes, oils, and some natural pigments also wash off.) Combine the washings and dilute to 250 mL. Evaporate a 50-mL aliquot on a steam bath and dissolve the residue in about 25 mL of petroleum ether.

Fill a 2.5-cm × 10-cm glass column with petroleum ether. Sift adsorbent alumina (Fisher A-540) into the column to a height of about 4 cm.

Pass the petroleum ether solution of the sample into the column. Wash with 50 mL of petroleum ether followed by 200 mL of carbon tetrachloride. Discard the washings. Elute the coloring matter with ethanol. Evaporate the eluate to dryness on a steam bath.

Dip a 7-in. × 22½-in. strip of Whatman 3MM paper into 5% (w/v) light mineral oil in ethyl ether. Air dry. Dissolve the eluted sample in a few drops of chloroform and spot as a 6-in. band 2½-in. from the bottom of the paper. Develop for 3 hr by descending chromatography using 6:4 acetone–water as the mobile phase. Examine the chromatogram for coal-tar colors.

Dry the chromatogram and extract the individual colors from the paper with ethanol. Examine each spectrophotometrically against standards.

THIELEMANN, H. Pharmazie *32*, 729 (1977). Thin-Layer Chromatographic Indentification of 8′-Apo-β-Carotenal. Orange rind or orange juice is extracted with light petroleum, the extract is concentrated in vacuo, then chromatographed on a Silufol UV 254 sheet using benzene–methanol–ethyl ether (17:1:2) as the mobile phase. 8′-Apo-β-carotenal gives a red-violet spot ($R_f \simeq$ 0.8), and can be distinguished from the 10′- and 2′-analogues and from its 3-hydroxy derivative.

GRAIN AND GRAIN PRODUCTS

ANDRZEJEWSKI, H. Pr. Zakresu Towarozn. Chem., Wyzsza. Szk. Ekon. Poznaniu, Zesz. Nauk Ser. I. No. 25, 5–39 (1966). Determination of Riboflavin in Cereal Products. The powdered sample is heated and stirred for 1 hr at 160°C in 50% aqueous LiCl. The solution is placed on a column of K-28 cation-exchange resin (H^+ form), the column is washed with a mobile phase containing Li salts, and then the riboflavin is eluted with Me_2CO–water (1:1) and determined fluorometrically as lumiflavine.

CIRILLI, G., SANDRI, M. Tec. Molitoria *22*, 42–48 (1971). Chromatographic and Colorimetric Method for the Determination of β-Carotene. Mix 2 g of powdered corn or lucerne with 20–30 mL of benzene (or hexane)–acetone (7:3) and 0.5 mL of water. Allow the sample to stand in the dark for 15–16 hr. Then, protecting the sample as much as possible from light, dilute it to 100 mL with benzene and mix well.

Chromatograph 10 mL of the supernatant liquid on a column packed from bottom to top with 5 cm of alumina, 8 cm of Celite–magnesium oxide (1:1), and 8 cm of Na_2SO_4. Elute the β-carotene with benzene (or hexane)–acetone (9:1), dilute the eluate to 100 mL with mobile phase, and determine β-carotene spectrophotometrically at 450 nm.

HORWITZ, W., Ed. *Official Methods of Analysis of the Association of Official Analytical Chemists*, 12 ed., 1975, p. 242. Extraction, Separation, and Identification of Coloring Matter in Macaroni Products. Transfer 0.5 g of coarsely grounded sample to a 1-liter Erlenmeyer flask, add 700 mL of 80% alcohol, and shake at intervals for 24 hr or until no more color is extracted. Place the sample in a refrigerator overnight to permit dissolved protein to precipitate, filter, and then evaporate the filtrate to 100 mL. Add 25 mL of 25% NaCl solution and a slight excess of NH_4OH to the filtrate, cool, and transfer the sample to a separatory funnel. Extract the sample with equal volumes of petroleum ether (boiling point <60°C) until no more color is extracted. If colored, reserve the lower layer for further treatment.

Combine the ether extracts and wash them with several small portions of NH_4OH (1 + 50). The ether solution contains fats and oil-soluble dyes that may be identified as in (a), below. If colored, immediately acidify the aqueous alkaline solution with acetic acid and extract it with ether. Any color remaining in the ether solution may be turmeric, annatto or saffron. These may be identified as in (b).

If the original aqueous solution, freed from the ether-soluble colors, is still colored and water-soluble dyes are suspected, extract the aqueous solution with 50-mL portions of isoamyl alcohol to remove any residual saffron as well as various orange dyes and Martius Yellow; to separate these, proceed as in (c). Drain the lower aqueous layer, which, if colored, may contain Naphthol Yellow S, FD&C Yellow No. 5, and FD&C Yellow No. 6. Extract these dyes with isoamyl alcohol after acidifying the solution with HCl to about 1 *N*. Remove the FD&C Yellow No. 5 from the solvent with 0.25 *N* HCl. FD&C Yellow No. 6 is removed with slightly lower acid concentrations. Naphthol Yellow S is removed from nearly neutral solution.

(a) Extract the original petroleum ether extracts with two or three 10-mL portions of HCl–HOAc (1:5).

If yellow AB or yellow OB are present, the solution will be pink or red. A few drops of 40% $SnCl_2$ added to a small portion of the acid extract should cause either decolorization or a decided fading of such colors. These colorants can be removed from the acid extract by diluting it with water, rendering it slightly alkaline, and extracting it with petroleum ether. Any remaining colors in the petroleum ether extract may be due to natural coloring matter of wheat or eggs. The coloring principle of egg yolk, lutein, when heated with alcoholic $FeCl_3$, produces a green solution. This test is not specific, however, since carotene and xanthophyll produce similar reactions.

(b) Wash the ether extracts with 5-mL portions of water to remove excess acid. To remove annatto and traces of saffron, wash successively with 20-mL portions of 5% $NaHCO_3$ solution. Divide the alkaline solution into two portions. Heat one to 60°C on a steam bath, dye the color on unmordanted cotton, and compare spot tests with a standard. Acidify the remaining portion of the alkaline annatto solution with HOAc and reextract with ether. Divide the ether extract into two small casseroles and evaporate to dryness. Dissolve the contents of one casserole in 10 mL of NH_4OH (1 + 9) and impregnate a strip of cotton or filter paper with the solution. An orange-yellow to orange-red stain is obtained, depending on the amount of dye present. Dry the filter paper or cotton, add a drop of 40% $SnCl_2$ solution, and dry again. If annatto is present, a purple stain is produced. Spot the contents of the other casserole with H_2SO_4 and HNO_3, which yield blue and greenish-blue colors, respectively.

Transfer two 10-mL portions of the original ether extract (from which annatto has been removed) into separate test tubes. Treat one with an equal volume of 10% NaOH and the other with an equal volume of HCl (1 + 1). In the presence of turmeric (*Curcuma*), the alkaline solution is reddish brown; the acid solution is red.

Turmeric can be further confirmed by its behavior with H_3BO_3. Test by shaking a portion of the original ether extract with an equal volume of 70% alcohol; add 1/10 the volume of HCl, mix, and divide equally into two test tubes. Then to one tube add a few crystals of H_3BO_3 and shake. Use the other tube as a control. In the presence of turmeric the solution turns red after a short time.

(c) To separate and identify saffron and the orange synthetic dyes, dilute the isoamyl alcohol extract with two volumes of petroleum ether and extract the mixed dyes with several 10-mL portions of water. To a small portion of this aqueous extract add 1/10 its volume of HOAc and a few milligrams of dry sodium hyposulfite to reduce the azo dyes. Extract the saffron with isoamyl alcohol, wash the extract with several small portions of water, evap-

orate the alcohol to dryness, and confirm the presence of saffron with spot tests.

HORWITZ, W. *Ibid.*, p. 243. FD&C Yellow No. 5 in Macaroni Products. Place 800 mL of cold water and 5 mL of NH_4OH in a 1-L Erlenmeyer flask and add 200 g of ungrounded sample. Stopper the flask and shake at intervals over a 3–4 hr period. Use a glass rod to dislodge material caking on the bottom. Centrifuge and decant the clear supernatant liquid into a 1-L flask. Add a solution of 50 g of $MgSO_4 \cdot 7H_2O$ dissolved in 100 mL of water, 10 mL of 12% silicotungstic acid solution, and 10 mL of HCl. Shake well and let stand for 1 hr to allow protein to precipitate. Then centrifuge the solution and examine the supernatant liquid spectrophotometrically.

HORWITZ, W. *Ibid.*, p. 243. Total Carotenoids and Carotene in Flour, Semolina, Macaroni, Egg Noodles and Egg Yolk. Grind macaroni and noodles to as near the fineness of flour as possible.

Weigh 20 g of flour, semolina, or macaroni, or 10 g of egg noodles, or 2 g of egg yolk into a 125-mL Erlenmeyer flask. Add 50 mL of 10% (w/v) alcoholic KOH and boil on a steam bath for 30 min under a reflux condenser. Occasionally rotate the flask (as carefully as possible) to keep the sample from collecting on the sides of the flask. Remove the flask and cool to room temperature. Filter through a Buchner medium-porosity fritted glass filter into a 250-mL suction flask, using suction, transferring most of the material with a few milliliters of alcohol from a wash bottle. Turn off the suction, rinse the flask with 25 mL of ether, pour the rinsing onto the glass filter, and stir the material with a rod to allow the ether to contact the entire sample. Filter and then repeat this operation twice.

Transfer the filtrate to a 250-mL separatory funnel and rinse with about 25 mL of ether, disregarding any soapy material in the flask. Add 175 mL of water and carefully invert and rotate the flask several times. When the layers separate remove the lower aqueous-alcoholic layer and extract this layer again with 25 mL of ether. Discard the lower layer and add the ether layer to the original ether solution. Wash the ether layer by pouring 50 mL of water through it. After the layers separate, withdraw and discard the aqueous layer. Add 50 mL of petroleum ether to the ether solution and wash with five 50-mL portions of water, carefully inverting and rotating the separator. Discard all the aqueous layers (slight emulsions usually clear in a few minutes but may be discarded, especially if there is no significant yellow tinge).

Transfer the ether–petroleum ether mixture to a 250-mL distillation flask, rinsing the separator with petroleum ether; place the flask in a beaker of water at 45–50°C. Stopper the flask, connect the side arm with vacuum, and concentrate to about 5 mL to remove ether. Filter through an Allihn-type adsorp-

tion tube with a coarse fritted glass plate containing about a 3-mm layer of anhydrous powdered Na_2SO_4, or through a 5.5–7-cm filter paper half filled with Na_2SO_4 (use a small, long-stemmed funnel reaching through the neck of the flask) into a 25-mL volumetric flask. Dilute to volume with petroleum ether that has been used to rinse the distillation flask and then passed portionwise through the filter containing Na_2SO_4. Mix the sample well and determine the carotenoid spectrophotometrically against a standard.

MITRA, S.N., ROY, S.C. Current Sci. (India) *26*, 89 (1957). Detection of Metanil Yellow in Pulses Dal. Treat a small amount of whole pulse with a little concentrated hydrochloric acid. If Metanil Yellow is present, the acid will turn purple.

To 20 g of broken (not powdered) sample add 150 mL of water and a few drops of NH_4OH and boil the mixture for a few minutes. Decant the colored solution from the pulse, render the solution just acid by the dropwise addition of 3 *N* HCl, add a few strands of white wool, and heat the mixture on a boiling water bath for 30–40 min. Stir occasionally. Wash the wool well with tap water then boil for a few minutes in 100 mL of water containing two drops of 3 *N* HCl. Wash the wool again under tap water and then strip the color from it using weak, hot ammonia.

Acidify a portion of this solution with 3 *N* HCl, add fresh strands of wool to it, and boil to adsorb the dye. If Metanil Yellow is present, the wool will turn violet when treated with concentrated hydrochloric or sulfuric acid.

Concentrate a second portion of the above-described ammoniacal solution on a water bath and chromatograph it for 18 hr against a standard on Whatman No. 1 paper using iso-butyl alcohol–ethanol–water (4:1:4) as the mobile phase. Dry the paper and test for Metanil Yellow using hydrochloric acid.

MITRA, S. N., ROY, B. R. Sci. Culture *25*, 539–550 (1960). Further Studies on the Detection of Metanil Yellow in Pulses Dal. To eliminate interference from large amounts of starch, the extraction described in the previous paragraph is done using several portions of 80% alcohol instead of aqueous NH_4OH. A new chromatographic procedure using phenol–water (80:20) as the mobile phase is also described.

MUTONI, F., TASSI-MICCO, C. Rend. Inst. Super. Sanita *25*, 567–573 (1962). Chromatographic Identification of Dyes in Macaroni. II. Mix 10 g of finely ground sample for 10 min with 25 mL of 50% ethanol. Centrifuge the mixture for 10 min at 5000 rpm. Acidify the clear solution with 8–10 drops of 2% tartaric acid. Pour onto a column 1 cm in diameter, containing a 1-cm layer of dry, ground gluten. Add 10 drops of 1.5% (v/v) ammonium hydroxide and elute. Transfer the eluate to chromatographic paper and elute with 2:1:1 butanol–ethanol–water.

OSADCA, M., ARAUJO, M., DE RITTER, E. JAOAC *55*, 110–113 (1972). Determination of Canthaxanthin in Concentrates and Feeds. Weigh 45 g of feed into a 250-mL Erlenmeyer flask, add 100 mL of warm 7% ammonium hydroxide solution containing 0.5% propyl gallate, and mix well with a glass rod. Place the flask for 15 min in a 65°C water bath. Using 150 mL of ethanol, rinse the contents of the flask into a 1-liter blender jar, cap tightly, and blend for 5 min at a speed adjusted to keep the mixture well below the cap. Add 450 mL of extracting solution (30–60°C petroleum ether–peroxide-free diethyl-ether (2:1) containing 1 g each of butylated hydroxyanisole (BHA, United Oil Products) and butylated hydroxytoluene (BHT, Shell)/liter of mixture) to the blender, cap tightly, and blend for about 5 min with stops of about 10 sec after 1.5 min and 3 min. Vent blender occasionally. Stop the blending, allow the blender contents to settle, decant the supernatant liquid into a 1-liter separatory funnel, allow the phases to separate, and drain and discard the lower aqueous phase.

For samples containing 1 g of canthaxanthin per ton of feed, transfer 100 mL of clear upper phase (50 mL for 2 g/ton feed, 25 mL for 4 g/ton feed) into a 125-mL amber, round-bottomed flask. If the upper phase is not clear, filter rapidly through a funnel containing a glass-wool plug. Evaporate in a water bath at 45°C under a stream of N_2 until no odor of ether or petroleum ether is detectable; about 2–5 mL of liquid will remain. Add 10 mL of petroleum ether, 10 mL of 50% KOH, and 0.5 g of propyl gallate to the flask and swirl carefully. Keep 15 min at room temperature; swirl occasionally.

Quantitatively transfer the contents of the flask to a 125-mL separatory funnel, rinsing with two 5-mL portions of water and two 5-mL portions of alcohol. Add the rinsings to the funnel. Finally, rinse the flask with two 10-mL portions of petroleum ether and add these rinsings to the separatory funnel. Add 25 mL of water to the funnel (do not shake) and let the phases separate. If necessary, use small amounts of alcohol to break any emulsion. Discard the aqueous phase and retain the entire ether phase in the funnel. Wash the petroleum ether extract three times with 50-mL portions of water, swirling gently each time and discarding the aqueous phase as completely as possible without losing any ether phase. Add about 3 g of anhydrous granular Na_2SO_4 to the washed extract and mix carefully. Filter the extract through glass wool into a 100-mL Erlenmeyer flask. Rinse the Na_2SO_4 into the separatory funnel with three successive 10-mL portions of petroleum ether; filter each extract through the glass wool into the flask.

Pack an 18-mm × 200-mm glass chromatographic column with 8 cm of 100–200-mesh Florisil (Fisher Scientific Co.) and then top the column with a 1-cm layer of Na_2SO_4. Prepare the column immediately prior to use. Wash the column with 10 mL of petroleum ether and then add the ether extracts and

washings to the column. Rinse the flask with two 10-mL portions of ether and add the washings to the column. Elute the column with diethyl ether (40–50 mL) until a broad yellow band appears. The brown-red band of canthaxanthin should remain close to the top of the column. Elute the canthaxanthin with 30% acetone in petroleum ether and collect the colored fraction in a 40-mL or 50-mL conical centrifuge tube. Evaporate the eluate containing the canthaxanthin (and other smaller components) almost to dryness on a 45°C water bath under a stream of N_2. Dissolve the residue in 0.5 mL of benzene.

Coat a 20-cm × 20-cm glass thin-layer plate with a 0.75-mm layer of silica gel G. (To prepare five plates, blend 120 mL of water with 60 g of silica gel G for 2 min. Air dry at room temperature for at least 4 hr and then oven dry for at least 4 hr more. The plates should be used within minutes after removal from the oven.) Streak the above benzene extract across the TLC plate as a band of 0.5 cm or less wide. Dry under a stream of nitrogen. Rinse the beaker with several 0.2-mL portions of benzene adding the washings to the TLC plate. Chromatograph in a 27-cm × 7-cm × 27-cm covered chromatographic tank using 191 mL of benzene–diethyl ether–methanol-pyridine (160:20:10:1) containing 0.25 g each of butylated hydroxyanisole (BHA, United Oil Products) and butylated hydroxytoluene (BHT, Shell). Let the plate develop (ascending) until the canthaxanthin appears separated by 0.25–0.5 cm from any interfering bands when viewed for a few seconds under white light. Scrape the canthaxanthin band from the plate through a small funnel into a 50-mL glass-stoppered centrifuge tube containing 5 mL of alcohol. Swirl for 10 sec and then pipette 20 mL of benzene through the funnel into the tube. Cap the centrifuge tube and shake for 7 min on a mechanical shaker. Add 1 g of Celite filter aid to the tube, shake for 2–3 min, and then centrifuge for 3 min at 2000 rpm. Remove the tube from the centrifuge, swirl to wash any particles adhering to the walls, and then centrifuge for an additional 10 min at 2000 rpm. Decant this solution into a second glass-stoppered tube.

Pipette 20 mL of the solution into a 25-mL amber volumetric flask. Add 0.1 mL of freshly prepared 0.3% w/v methanolic iodine solution and mix. Stopper the flask loosely and immerse the tube for 15 min in a 65°C water bath. Cool to room temperature and mix well.

Determine the sample's absorbance (A) in a 5-cm absorption cell at the absorption maximum near 480 nm against a reagent blank of alcohol–benzene (1:4).

$$\text{Grams of canthaxanthin/ton} = \frac{A \times D \times 9070}{1840 \times 5 \times 0.85}$$

where D is dilution factor $[(450 \times 25)/(45 \times V) = 250/V]$, V is mL of original extraction solution taken for evaporation, 9070 is factor for converting result to g/ton, and 0.85 is recovery factor.

JAMS AND JELLIES

ANDRZEJEWSKA, E. Roczn. Panst. Zakl. Hig. *26*, 575–580 (1975). Identification of Organic Dyes in Foodstuffs in the Presence of Natural Pigments and Some Other Components. Extract the sample with (or dissolve it in) H_2O, acidify to pH 3–4 with anhydrous acetic acid, and pass the resulting solution through a column of polyamide. Wash the column with H_2O (at 80°C) and acetone, then elute the dyes with MeOH–H_2O–25% aqueous NH_3 (35:14:1), concentrate the eluate, and chromatograph by TLC on cellulose plates. Using this technique a number of colorants including FD&C Yellow Nos. 5 and 6 and D&C Blue No. 6 were extracted from jams, juices, jellies, and honey, then identified. The procedure does not work well on foods containing proteins or fats.

DEL BIANCO, F. N., TRABACCHI, G. Chem. e Ind. (Milan) *41*, 896–898 (1959). Extraction and Identification of Synthetic Colors in Sweet Foods. Extract the dye with a neutral or highly acid aqueous medium. Condense the extract and chromatograph it on paper using Na citrate–NH_4OH–phenol (10:10:80), or BuOH–EtOH–H_2O (2:2:1).

DAVIDEK, J., DAVIDKOVA, E. Z. Lebensm. Forsch. *131*, 99–101 (1966). Application of a Polyamide in the Investigation of Water-Soluble Food Dyes. II. Isolation of Dyes From Food by Paper Chromatography. The sample solution is acidified with 10% tartaric acid or 10% $KHSO_4$, polyamide powder is added to adsorb the dye, and the sample is filtered. The colorant is stripped from the powder with 25% NH_4OH–MeOH (5:95), concentrated on a steam bath, and resolved chromatographically.

GILHOOLEY, R. A., HOODLESS, R. A., PITMAN, K. G., THOMSON, J. J. Chromatog. *72*, 325–331 (1972). Separation and Identification of Food Colours. Weigh about 5 g of sample into a beaker, add 50 mL of water, and warm into solution on a water bath. Acidify the mixture with acetic acid. Plug a 15-mm × 250-mm glass-chromatographic column with polyamide staple fiber (Nylon 66, 3.3 g per 10,000 m of fiber) and then pour enough water suspension of polyamide powder (MN CC6, Macherey, Nagel and Co.) into the column to obtain a settled height of about 20 mm. Rinse the column wall with a small amount of acetone, and then cap the column with about a 6-mm layer of acid-washed sand.

Pour the hot sample solution through the column and then wash the column with six 10-mL portions of hot water and three 5-mL portions of acetone. Elute the colors from the column with a minimum volume of fresh acetone–ammonia–water (40:1:9), rejecting the eluate until the colors elute. Remove the ammonia by blowing a stream of air over the surface of the liquid and then reduce the volume by about one-half on a steam bath. Add an equal volume of water then adjust the pH to 5–6 with hydrochloric acid.

Pour the solution through a column of polyamide powder packed in a 10-mm × 100-mm chromatographic tube packed as described above and then wash the column with five 5-mL portions of hot water. Elute the dyes with a minimum of acetone–ammonia–water solution. Remove the ammonia as before and evaporate the solution to near dryness on a steam bath. Dissolve the residue in a few drops of 0.1 *N* HCl and use this solution for TLC. (If FD&C Red No. 3 is present, dissolve the residue in water).

MEAT AND FISH

AITZETMUELLER, K., ARZBERGER, E. Z. Lebensm. Forsch. *169*, 335–338 (1979). Analysis of Food Dyes E 110 (FD&C Yellow No. 6, Cl Food Yellow 3), E 111 (CI Food Orange 2) and E 124 (CI Food Red 7) in Fish Samples by Ion-Pair High-Performance Liquid Chromatography. Wash canned saithe with $CHCl_3$ to remove residual oil, homogenize, boil briefly, cool, and filter. Wash the cake with 1:49 aqueous NH_3 and concentrate the combined filtrate and washings in a rotary evaporator. Chromatograph a portion of the concentrate on a column of Sephadex LH-20 using water as the mobile phase. Collect the colored fractions and concentrate as needed. Dilute the concentrates with water or the following mobile phase and chromatograph on a 25-cm × 4-mm-ID steel column packed with Nucleosil 10 C18 (10 μm) or LiChrosorb RP-8. Elute with H_2O–acetone (4:1) containing 0.2 g/L tetrabutylammonium chloride. Monitor at 505 nm.

ANDRZEJEWSKA, E. Rocz. Panstw. Zakl. Hig. *32*, 315–318 (1981). Detection of Cochineal, a Natural Organic Dye, in Meat Products. Extract 10 g of defatted sample with methanol–H_2O–aqueous 25% NH_3 (35:14:1) at 35°C, concentrate the combined extracts, acidify them with anhydrous acetic acid, than pass the solution through a column containing 1.25 g of polyamide powder. Wash the column with ten 10-mL portions of hot (65°C) water, three 5-mL portions of acetone, then five 10-mL portions of water. Elute any dyes with aqueous 70% MeOH–aqueous 25% NH_3 (49:1) at 55°C. Concentrate the eluate to 5 mL at 75°C, add 20 mL of water and 0.1 mL of anhydrous acetic

acid, then chromatograph on a second polyamide column as above. Concentrate the eluate to 1 mL at 75°C, then chromatograph portions of it by thin-layer chromatography on cellulose MN 300, silica gel G, and silica gel 60 PF_{254}. As little as 15 μg of cochineal can be detected on cellulose and silica gel G, and as little as 6 μg on silica gel 60 PF_{254}. Recovery by this technique was about 90%.

EGGINGER, R. Fleischwirtschaft 65, *917*, 920–921 (1985). Determination of "Beetroot" Colour Additive in Frankfurter-Type Sausage and Dry Sausage. A Quick and Simple Qualitative Test. Place a 20-mm × 1-mm diameter sample in melted kerosine (51–53°C). If the kerosine turns colored, naphthols are present. If it does not, extract a second sample with acetic acid; if this does not turn color, then acidic dyes and/or pigments are present. Extract a third sample with pyridine; if this extract turns color, then natural pigments, including betanin and anthocyanins are present. As little as 0.05% of betanin can be detected using this procedure.

GILHOOLEY, R. A., HOODLESS, R. A., PITMAN, K. G., THOMPSON, J. J. Chromatog. *72*, 325–331 (1972). Separation and Identification of Food Colours. Chop about 25 g of sample on a glass plate, add 5 g of acid-washed sand, and grind the mixture to a paste. Add 10 g of Celite 545 and mix with a palette knife into a homogeneous mixture.

Transfer the mixture to a Soxhlet thimble and extract it with chloroform for 2 hr. Remove the sample from the thimble and place it in an evaporating dish to allow residual chloroform to evaporate.

Place a plug of polyamide staple filter (Nylon 66, 3.3 g per 10,000 m of fiber) in the end of a 22-mm × 300-mm glass-chromatographic tube and add the powdered sample to the tube, tapping the column gently to aid in packing. Pass methanol–ammonia–water solution (90:5:5) through the column until all the dyes are eluted.

Add 5 mL of 1% aqueous polyoxyethylene sorbitan monooleate solution to the eluate and evaporate the solution on a steam bath, blowing a stream of air over the surface of the liquid until all the ammonia and methanol are removed. Add an equal volume of water and adjust the pH of the solution to 6 with hydrochloric acid.

Place a plug of polyamide staple fiber in the end of a 15-mm × 250-mm chromatographic tube and add a suspension of polyamide powder (MN CC6, Macherey, Nagel and Co.) in water to the tube to give a height of about 22 mm. Rinse the walls of the tube with a small volume of acetone to aid the settling of the polyamide and then add a 6-mm layer of sand on top of the polyamide.

Pour the solution of dyes through the column and then wash the column with three 10-mL portions of water, two 5-mL volumes of acetone, two 5-mL portions of chloroform–absolute ethanol–water–formic acid (100:90:10:1),

and two 5-mL portions of acetone. Elute the dyes from the column with a minimum of acetone–ammonia–water (40:1:9), rejecting the eluate until the dyes are eluted. Remove the ammonia by blowing a current of air over the surface of the liquid and then reduce the volume by about half on a steam bath. Add an equal volume of water and adjust the pH to approximately 6 with hydrochloric acid. Pour the solution through a column of polyamide in a 10-mm × 200-mm chromatographic column prepared and washed as previously described. Elute the dyes with a minimum volume of acetone–ammonia–water solution. Remove the ammonia by blowing a current of air over the surface of the liquid and then evaporate the solution to near dryness on a steam bath. Dissolve the residue in a few drops of 0.1 *N* hydrochloric acid and use this solution for TLC. (If FD&C Red No. 3 is present, dissolve the residue in water.)

LEHMANN, G., COLLET, P. Z. Lebensm. Forsch. *144*, 107–109 (1970). Detection of Synthetic Dyes in Meat and Meat Products. Grind meat paste or homogenized minced meat, sausage, or salami in a mortar with sand, Celite, and acetone. Remove the acetone by filtration and repeat the extraction until no more color is removed. Grind the residue, dry it to remove solvent, and transfer it to a small chromatographic column packed with polyamide powder. Elute the column with NH_3–methanol (1:19). Acidify the eluate, evaporate it to a small volume, and separate the dyes present by paper or thin-layer chromatography. Dilute the acetone extract with water, remove the acetone by distillation under reduced pressure, extract fat-soluble dyes with light petroleum, concentrate the extract, and identify the dyes present by paper or thin-layer chromatography. Acidify the aqueous phase to pH = 6 and purify it on a micro column of polyamide powder. Elute adsorbed basic dyes with acetone and identify.

MARMION, D. M. JAOAC *54*, 131–136 (1971). Analysis of Allura Red AC Dye (A Potential New Color Additive). Determination in wieners. Slice a 10-g length from the middle of a dyed wiener. Blend well in a small Waring blender with 100 mL of $CHCl_3$. Filter; discard the $CHCl_3$ extract.

Return the cake to the blender and blend well with 100 mL of warm mixed solvent (SD No. 30 alcohol–water–NH_4OH, 80:20:1); filter. Return the cake to the blender and extract again with 100 mL of fresh mixed solvent. Wash the blender and filter cake with two 50-mL portions of warm mixed solvent. Combine mixed solvent extracts and washings in a 500-mL volumetric flask, add 5 mL of acetic acid, and heat solution to incipient boil. Dilute to volume with SD No. 30 alcohol, mix, and let stand overnight.

Adjust the volume with SD No. 30 alcohol, mix, and filter by gravity through Whatman No. 42 paper (filtrate must be clear). Similarly extract sample containing no color.

Using a suitable spectrophotometer, determine absorbance of each solution in a 5-cm cell (vs SD No. 30 alcohol) at the maximum near 505 nm and at 680 nm. Sample absorbance at maximum near 505 nm = A_1, sample absorbance at 680 nm = A_2, blank absorbance at 505 nm = A_3, blank absorbance at 680 nm = A_4.

Parts per million Allura Red AC dye

$$= \frac{(A_1 - A_2 - A_3 + A_4) \times 1{,}000{,}000}{20 \times 5 \times 52.9} = A \times 189.0$$

where 52.9 = approximate absorptivity of Allura Red AC dye at 505 nm (in L/g-cm), 1,000,000 = factor for conversion to ppm; 20 = effective sample concentration (in g/L), and 5 = cell path length (in cm).

Mc NEAL, J. JAOAC *59*, 570–577 (1976). Qualitative Tests for Added Coloring Matter in Meat Products. Slurry the meat with a minimum amount of warm water or 80% ethanol, let the mixture stand for 5 min, and then filter. Divide the filtrate into three equal portions and evaporate each just to dryness on a steam bath; do not boil. Dissolve the residue from one portion in water and dissolve the second in 0.2 *N* HCl, and the third in 0.2 *N* NaOH. Filter if necessary and determine the spectra of the solutions against those of knowns. Alternately, the extract from above can be filtered, concentrated, and chromatographed on Whatman No. 1 paper using the mobile phases shown below.

Test for natural coloring agents as follows. Run against appropriate standards and blanks.

R_f Values of Selected Colorants

Dye	Color Index No.	Inorganic Mobile Phase[a]	Organic Mobile Phase[b]
FD&C Red No. 1[c]	16155	0.15	0.32
FD&C Red No. 2[c]	16185	0.55	0.20
FD&C Red No. 3	45430	0.05	0.70
FD&C Red No. 4[d]	14700	0.42	0.50
FD&C Red No. 40	16035	0.35	0.45
FD&C Yellow No. 1[c]	10316	0.70	0.50
FD&C Yellow No. 4[c]	11390	0.20	0.96
FD&C Yellow No. 5	19140	0.85	0.21
FD&C Yellow No. 6	15985	0.77	0.35

Dye	Color Index No.	Inorganic Mobile Phase[a]	Organic Mobile Phase[b]
FD&C Blue No. 1	42090	0.95	0.46
FD&C Blue No. 2	73015	0.18	0.21
FD&C Green No. 2[c]	42095	1.00	0.39
FD&C Green No. 3	42053	1.00	0.46
FD&C Violet No. 1[c]	42640	0.80	0.65
Methyl violet	42535	0.03	1.00
Orange B	19235	0.57	0.45
Orange No. 1	14600	0.36	0.61
Orange No. 2	15510	0.36	0.64

[a] NH_4OH-2.5% sodium citrate-water (45 + 10 + 45).

[b] *n*-Propanol-ethyl acetate-water (6 + 1 + 3).

[c] These colors are no longer permitted for use in foods, drugs, and cosmetics.

[d] Permitted in externally applied drugs and cosmetics only.

Cochineal (carminic acid, carmine red)—Weigh about 25 g of meat into a beaker. Add 100 mL of hot (80°C) 5% aqueous borax solution, mix on a steam bath for 30 min, and filter. A purple filtrate indicates the presence of cochineal; yellow is negative. The addition of borax will give a positive test if > 0.1% cochineal is present.

Beet powder—Slurry the sample with 1 *N* H_2SO_4. A purple color indicates the presence of beet powder. To confirm this, filter the slurry and divide the filtrate into three portions. Adjust these to pH = 2, 5, and 9, respectively, with dilute H_2SO_4 and NaOH and determine the spectrum of each solution from 700–400 nm. Peak maxima should be at 535 nm, 537 nm, and 544 nm at pH = 2, 5, and 9, respectively.

Annatto and saffron—Mix 25–50 g of sample with 200 mL of ethyl ether and 2 mL of concentrated HCl and filter the slurry through anhydrous Na_2SO_4 in a funnel with a glass-wool pledget. Extract 10 mL of the dried ether extract with about 3 mL of 2% NaOH. Absorb any color present on a strip of filter paper and air dry. Dip the dried paper in concentrated H_2SO_4. A blue color indicates the presence of annatto or saffron. To differentiate between the two, add 40% $SnCl_2$ to another strip on which color has been absorbed, and let air dry. If annatto is present, the paper will turn pink to purple. If annatto is absent, or if the previous test was positive due to saffron only, there will be no change in color.

Paprika and turmeric—Pack a 10-mm-ID glass chromatographic column with 10 cm of Florisil (Fisher Scientific Co., No. 100) topped with about 2 cm

of anhydrous Na_2SO_4. Prewet the column with ethyl ether. Mix 50 g of sample with 200 mL of ethyl ether and 2 mL of concentrated HCl. Let the mixture stand for 5 min and then filter the extract onto the chromatographic column through a funnel containing 10 g of anhydrous Na_2SO_4. Allow the extract to percolate through the column at 3 mL/min and then wash the column at the same rate with 50 mL of petroleum ether. If paprika is present, a red band will appear at the interface of Na_2SO_4 and Florisil. This will turn yellow and elute from the column with ethyl ether. If turmeric is present, a yellow band will appear at the interface. Elute this band with 150 mL of acetone, mix the acetone eluate with 300 mL of water, and add three or four drops of concentrated HCl and a few crystals of boric acid. A red color confirms the presence of turmeric. If both coloring agents are suspected, prepare two columns and run each of the above procedures separately.

Alkanet—Extract 25–50 g of sample with 100 mL of ethanol and filter. Add 10 mL of 10% NaOH solution. A blue color indicates the presence of alkanet.

Carotene—Blend 30 g of sample for 4 min with 40 mL of water, 40 mL of methanol, and 80 mL of $CHCl_3$. Let the blend stand for 5 min and then filter through glass wool. Dilute 5 mL of the lower ($CHCl_3$) layer to 100 mL with $CHCl_3$ and compare spectrophotometrically against knowns.

SPELL, E. Fleischwirtschaft *52*, 75–77 (1972). Detection of the Beetroot Pigment Betanin in Jellied Meats Containing Red Wine. Suspend the sample in H_2O at 30°C, strain and centrifuge the suspension, and then place it in a refrigerator and allow the gelatin to set. Remove the fat layer and then separate the betanin and the red-wine color in the gelatin by ion-exchange chromatography. Resolve the isolated colorants by thin-layer electrophoresis using cellulose as the support and pH = 4.5 citrate buffer as the electrolyte.

TONOGAI, Y., KINGKATE, A., HALILAMIAN, C. J. Food Prot. *46*, 592–594 (1983). Quantitative Determination of Colorants in Dried Shrimp Paste Using Ion-Exchange Extraction and High-Performance Liquid Chromatography. Heat 30 g of sample for 10 min with 100 mL of hexane. Decant and discard the hexane layer, then heat the residue with 100 mL of ethanol; decant and discard the ethanol layer. Heat the residue with 50 mL of 70% ethanol containing 1% aqueous NH_3, then separate the liquid, and extract it with 2 mL of acetic acid plus 10 mL of 5% Amberlite LA-2. Extract the solution with another 10 mL of 5% Amberlite in ethyl acetate, then wash the combined acetate layers with saturated NaCl then aqueous 5% NH_3. Chromatograph the various fractions by HPLC on Zorbax C_8 using a 0–100% gradient, and 1% ammonium acetate: acetonitrile–methanol (1:3) as the mobile phase.

VENTURINI, A., NOVI, M. Boll. Lab. Chim. Provinciali *16*, 175–180 (1965). Identification of Synthetic Water-Soluble Coloring Compounds from Cochi-

neal in Meat and Sausages. Place 20 g of finely ground meat in a mortar, add 30 g of quartz sand and 30 mL of Cl_3CCOOH, and grind well for 7–8 min. Filter and collect 20–30 mL of filtrate. If the filtrate is clear or slightly yellow, cochineal, enocianin, and acid azoic dyes are absent. If the filtrate is red, place 3–4 mL of it in a test tube and add NH_4OH. A greenish color indicates the presence of enocianin. Cochineal gives a purple color, whereas acid azoic dyes give no color change at all. The presence of cochineal can be confirmed by the green color formed with 5% uranium acetate. The acid azoic dyes can be separated on Al_2O_3 and identified by paper or thin-layer chromatography. If all three types of colorants are present, the azoic dyes must first be separated on Al_2O_3.

WELLNITZ, M., BENTLER, W. Fleischwirtschaft *66*, 1001–1003 (1986). Detection of Cochineal in Dry Sausages. Cochineal was recovered from defatted samples by decomposing them with 10% HNO_3, filtering the resulting solutions, making the filtrates alkaline with NH_3, evaporating the filtrates to dryness, then washing the residues with H_2O.

SPICES AND CONDIMENTS

BENK, E., PHILIPP, W. R. Gordian *69*, 537–540 (1969). Detection of Permitted Natural Coloring Matter in Mayonnaise. Extract 20 g of sample with a mixture of 100 mL of petroleum ether and 100 mL of MeOH, saponify the extract, remove any lipids, and chromatograph the extract on a column of highly activated Al_2O_3. Chromatograph the eluate on a thin-layer plate coated with Kieselgel G using light petroleum–benzene–acetone–acetic acid (80:20:2:1) or benezene–ethyl acetate–methanol–H_2O (2:5:2:1) as the mobile phase. Extract the colorants from the plate and examine spectrophotometrically.

CORRADI, C., MICHAELI, G. Boll. Chim. Unione Ital. Lab Prov., Parte Sci. *5*, 651–661 (1979). Rapid Method for Detecting and Identifying Artificial Dyes and Curcuma in Table Mustard and Related Products. Samples were treated with NH_3, then the dyes were extracted with light petroleum and cleaned up by passing the extracts through a column of polyamide. Curcumins were eluted with acetone–H_2O-acetic acid (40:9:1) and acidic artificial colorants with acetone–H_2O–aqueous NH_3 (40:9:1). Basic colorants were purified on a second column. Cucumins (curcumin, demethoxycurcumin, and didemethoxycurcumin) were separated on high-performance silica gel 60 F_{254} plates using $CHCl_3$–acetic acid (9:1), water-soluble colorants (FD&C Yellow No. 5, Chrysoin S, Quinoline Yellow, Naphthol Yellow S, and Auramine O) were resolved on cellulose plates using ethanol–butanol–pyridine–

H_2O (1:7:6:6), and fat-soluble dyes (Dimethyl Yellow and Sudan Yellow) were separated on silica gel 60 F_{254} plates using benzene as the mobile phase.

JANSSEN, A., GOLE, T. Chromatographia *18*, 546–549 (1984). Thin-Layer Chromatographic Determination of Curcumin (Turmeric) in Spices. Extract 0.1 g of spice with 1 mL of methanol, then apply 10 μL of extract to an air-dried silica gel 60 thin-layer plate. Evaporate the methanol with warm air, then develop the plate for 15 min using $CHCl_3$-anhydrous acetic acid (4:1) as the mobile phase. Dry the plate in warm air, spray it with 2.25% H_3BO_3–2.5% oxalic acid solution, heat the plate for 10 min at 100°C, then scan the plate fluorometrically (excitation at 366 nm, emission at >560 nm).

LEHMANN, G., GERHARDT, U., COLLET, P., GUTER, J. Fleischwirtschaft *50*, 946–948 (1970). Detection of Foreign Pigments in Spice Extracts Used in the Manufacture of Meat Products. Suspend the sample in water and extract fat-soluble synthetic and natural dye with light petroleum ether and identify the isolated colorants by TLC. Isolate the water-soluble colorants by adsorption on polyamide powder, DEAE-cellulose, or carboxymethycellulose and, after desorption, identify them by TLC.

LEHMANN, G., GERHARDT, U., COLLET, P. Z. Lebensm. Forsch. *144*, 345–348 (1971). Analysis of Dyes. XII. Detection of Synthetic and *Curcuma* Dyes in Mustard. Mix the sample with Celite and sand and extract it with acetone to remove fat and water- and acetone-soluble dyes. Extract the residue with methanol–NH_4OH (19:1), adjust the extract to pH = 5.6 with acetic acid, and transfer it to a microcolumn packed with polyamide powder. Elute the acid dyes with hot H_2O and identify the eluted colors by paper or thin-layer chromatography. Concentrate the acetone filtrate and chromatograph it by TLC on Kieselgel using $CHCl_3$–methanol (18:1) as the mobile phase. Examine the TLC plate for basic fat-soluble and *Curcuma* dyes.

MITRA, S. N., ROY, S. C., CHATTERJI, R. K. J. Ind. Chem. Soc., Ind. & News Ed. *19*, 155–158 (1956). Detection of Coal Tar Dyes in Turmeric. Synthetic and natural coloring matters in turmeric are distinguished by an acid-wash technique and subsequent paper chromatography. Strip 3–5 g of powdered sample by boiling with 100 mL of dilute NH_4OH, filter, acidify the filtrate with HCl, and boil the filtrate with four or five strands of pure white wool. Wash the wool with water and boil with very dilute HCl. Boil the wool for 15 min with dilute NH_4OH and divide the solution. Acidify one portion and use it to dye fresh strands of wool and for spot tests with HCl, H_2SO_4, 10% NaOH, and 12% NH_4OH; characteristic colors are produced with Orange AG, Sunset Yellow, Naphthol Yellow, Tartrazine, and Metanil Yellow. Chromatograph a portion of the concentrated extract against knowns on Whatman No. 1 paper using the organic phase from a mixture of iso-BuOH–H_2O–EtOH (4:4:1).

MITRA, S. N., ROY, S. C. J. Proc. Inst. Chemists *29*, 155–157 (1957). Detection of the Presence of Small Amounts of Turmeric in Other Spices. Triturate 20 g of sample several times with petroleum ether to remove as much oil as possible. Mix the residue with Et_2O and allow the mixture to stand for 15 min; swirl occasionally. Filter the ether extract and concentrate the filtrate to near dryness. Spot a few drops of the filtrate on filter paper, allow it to dry, treat it with aqueous boric acid solution, and heat in an air oven for 10 min. A characteristic rose-red color indicates the presence of turmeric. This may be confirmed by the greenish blue color formed when a drop of ammonia is added to the red spot.

To further confirm the presence of turmeric, condense a portion of the ether extract, chromatograph it on Whatman No. 1 paper using the organic phase prepared by mixing iso-BuOH–EtOH–H_2O (4:1:2), and spot the resolved bands with boric acid and NH_4OH as described above.

MITRA, S. N., SEN GUPTA, P. N., ROY, B. R. J. Proc. Inst. Chemists *33*, 69–73 (1961). The Detection of Oil-Soluble Coal-Tar Dyes in Chili (Capsicum). Separate portions of powdered sample are shaken with Et_2O, petroleum ether, and 90% alcohol, the extracts are treated with various concentrations of HCl and H_2SO_4, and the resultant color reactions are observed. As a confirmatory test, fresh extracts are filtered and concentrated and chromatographed on Whatman No. 1 paper that has been soaked in 5% liquid paraffin in 60–80° petroleum ether, air dried, and then dried at 100°C for 30 min. The mobile phase is 80% alcohol. With uncolored chili, only dull brown spots are resolved.

SACCHETTA, R. A. Rev. Asoc. Bioquim. Agric. *25*, 187–194 (1960). Paper Chromatography of Red Paprika Powders. Powdered samples are extracted with EtO_2 and the extracts are concentrated and chromatographed on Whatman No. 1 paper using EtOH as the mobile phase.

SARDAR, P. K., HUSSAIN, M. H., SRIVASTAVA, D., JACOB, S., DEOTALE, M. Y., MATHEW, T. V. Res. Ind. *31*, 74–76 (1986). Isolation and Identification of Some Coal Tar Colours in Spices by Thin-Layer Chromatography. Slurry 60 g of neutral alumina in H_2O, apply the slurry to 20-cm × 20-cm glass plates, then dry the plates in an air oven at 102°C for 1 hr. Shake 10 g of powdered spice sample in 50 mL of H_2O for 30 min, filter the extract, then concentrate it to 1 mL. Dilute the concentrate with methanol or ethanol, and apply a portion of the solution to the TLC plate. Develop the plate using methanol–aqueous NH_3 (4:1) as mobile phase. The method separates Rhodamine B, Metanil Yellow, FD&C Red No. 3, Fast Red E, FD&C Yellow No. 6, FD&C Yellow No. 5, and Amaranth. The method can also be used to determine synthetic colors in other foods using methanol–aqueous NH_3 (9:1) as the mobile phase.

STELZER, H. Nutr. Bromatol. Toxicol. *2*, 177–179 (1963). Identification of Synthetic Coloring in Paprika. Extracts of paprika are chromatographed on thin-layer plates using EtOH–AcOH (95:5) as the mobile phase. The plates are prepared by coating glass with a suspension of talc–wheat starch–water (7:0.04:30) and then drying the plates for 24 hr at ambient temperature.

UNTERHALT, B. Z. Lebensm. Forsch. *170*, 425–428 (1980). Turmeric and Its Application in Mustard. (Determination of Turmeric.) Mustard is ground with sand and Celite, then extracted with acetone. The extract is filtered, the filtrate is evaporated to dryness in a rotary evaporator, and the residue is dissolved in anhydrous acetic acid. Total curcumin (curcumin plus didemethoxycurcumin) is then determined by ^{1}H and ^{13}C NMR spectrometry, after formation of rubrocurcumin by complexing with H_3BO_3.

GENERAL

AMAKAWA, E., HIRATA, K., OGIWARE, T., OHNISHI, K. Bunseki Kagaku *33*, 586–590 (1984). Analytical Methods for Natural Dyes in Foods. III. Determination of Oil-Soluble Natural Dyes in Foods by High-Performance Liquid Chromatography. Homogenize 5 g of sample with 25 mL of aqueous 50% ethanol and 2 mL of sesame oil, then extract the homogenate with ether. Add one volume of hexane to the extract, then extract the solution with 5 mL of 0.5 *M* NH_3. To determine curcumin, norbixin, and bixin, treat the aqueous phase with 5 mL of 1 *M* acetic acid and extract the dyes into ether (solution A). To determine β-carotene, evaporate the organic phase to dryness then dissolve the residue in hexane (solution B). To determine paprika extracts, apply a portion of the hexane solution from B to a column of activated alumina. Wash the column with 100 mL of hexane and 30 mL of hexane–acetone (30:1), then elute any dyes with 20 mL of acetone (solution C). Analyze solutions A, B, and C by HPLC using Zorbax ODS, LiChrosorb Si-60, and Zorbax ODS columns, respectively, and methanol–H_2O–acetic acid (100:10:0.5), hexane, and CH_2Cl_2–methanol (4:1) mobile phases, respectively. Recoveries are ≥70%. Samples high in protein and oils or fats require additional preparation.

ANDRZEJEWSKA, E. Rocz. Panstw. Zakl. Hig. *31*, 587–592 (1980). Food Dying from the Viewpoint of Methods Used for Detection of Synthetic Dyes and for their Determination in Food Products. Several dyes including Tartrazine, Orange Yellow S, Cochineal Red, Amaranth, and Brilliant Black BN were determined by spectrometry after the thin-layer-chromatographic separation of their mixtures on cellulose. For the determination of dyes in food-

stuffs, food extracts were first purified on polyamide columns. The results of several determinations are tabulated, and their statistical evaluation is described. Limits of detection (in ppm) for the dyes were 0.09–0.39, 0.09–0.56, 0.09–0.80, 0.09–0.86, and 0.09–0.74, respectively. For soft drinks, the recoveries of dyes were 55–100%, and the coefficients of variation were 0.57–2.3%.

ASHWORTH, C. M., CASTLEDEN, S. L., KIRKBRIGHT, G. F., SPILLANE, D. E. M. J. Photoacoust. *1*, 151–160 (1982). Examination of Synthetic Food Dyestuffs Using Thin-Layer Chromatography and Photoacoustic Spectroscopy. Samples were dissolved in hot water, then incubated at 50°C for 3 hr with amylo-1,6-glucosidase solution in acetate buffer (pH = 4.5). The mixture was then applied to a polyamide column, and dyes were eluted with acetone–water–concentrated aqueous NH_3 (40:9:1). The eluate was evaporated to dryness, the residue was dissolved in 1 mL of water, than 1 μL of this solution was chromatographed on a silica gel 60 plate using multiple developments with propan–2-ol-concentrated aqueous NH_3–ethanol (77:13:10). Spots were examined by photoacoustic spectroscopy using the method of Adams et al., Analyst (London) *102*, 569 (1977).

BANERJEE, T. S., MAZUMDER, D., HALDER, R. C., ROY, B. R. J. Food Sci. Technol. *16*, 34 (1979). Detection of Food Colours by Gel Electrophoresis. Dyes extracted from samples (e.g., spices) with water or 80% EtOH, or present in the supernatant liquid after the precipitation of proteins in milk with EtOH, are separated by electrophoresis for 45 min in polyacrylamide gel (10 mA per gel), using citrate buffer-solution of pH 2.4 as the electrolyte. Detection limits are about 10 ppm.

BECKER, R. R. Oesterr. Chem. Z. *88*, 99–102 (1987). Artificial Food Colourings. Chromatographic methods for the determination of dyes in food are discussed.

BOLEY, N. P., BUNTON, N. G., CROSBY, N. T., JOHNSON, A. E., ROPER, P., SOMERS, L. Analyst *105*, 589–599 (1980). Determination of Synthetic Colours in Food Using High-Performance Liquid Chromatography.

Reagents

Polyamide Powder—Camlab Ltd. Grade MN polyamide SC6/CC6 for column chromatography.

Sand—Acid washed 40–100 mesh.

Acetone–Water–Ammonia Solution—Mix 40 mL of acetone, 9 mL of water and 1 mL of ammonia solution (sp. gr., 0.88). Prepare fresh daily.

Resin-in-Butanol, 5% v/v–Prepare a 5% v/v solution of Amberlite LA-2 resin in *n*-butanol. Equilibrate the solution in a 2-liter separatory funnel with 400 mL of water containing 19 mL of HCl. Discard the lower layer.

Hydrochloric Acid—0.1 *N*.

Celite 545 Filter Aid.

Sodium Chloride—1% m/v.

Ammonia Solution, 10% v/v—Dilute 100 mL of ammonia solution (sp. gr., 0.88) to 1000 mL with distilled water.

Sodium Chloride–Ammonia Solution—Dissolve 10 g of NaCl in 300 mL of water, add 10 mL of ammonia solution (sp. gr., 0.88), and make to 1L with water.

Phosphate buffer, pH 7.0—Dissolve 2.84 g of disodium hydrogen phosphate and 1.36 g of potassium dihydrogen phosphate in water and dilute to 1 L.

Acetate buffer, pH 4.6—Add 6.0 mL of glacial acetic acid and 8.2 g of anhydrous sodium acetate to 100 mL of water and dilute to 1 L.

Enzymes—Papain, lipase, phospholipase C, amyloglucosidase, pectinase, and cellulase (Sigma Chemical Co., Poole, Dorset).

Apparatus

Chromatographic Column—Place a plug of glass-wool in the bottom of a 18-mm × 280-mm column. Pack the column with 20 g of polyamide powder slurried in 80 mL of water. Allow the water to drain just to the level of the packing, wash the sides of the column down with a few milliliters of acetone, then place sand on top of the polyamide to form a layer about 6 mm deep.

Sample Preparation

Aqueous Samples and Water-Soluble Foods—Dissolve 10 g of sample in 100 mL of water, warming if necessary. Pass the solution through the polyamide column, then wash the column with 50 mL of warm water and then 15 mL of acetone. Elute the colorants with a minimum amount of acetone–water–ammonia solution, rejecting the eluate until it is colored. Evaporate the colored eluate to dryness on a water bath under a stream of air, but do not bake the residue. Dissolve the residue in 1 mL of water (or 1 mL of the 77:23:0.25 mobile phase if Erythrosine BS is present) and examine by HPLC.

Foods Insoluble in Water—Grind 10 g of sample in a mortar with 10 g of Celite and 10 mL of 0.1 *N* HCl. Quantitatively transfer the mixture to a 200-mL sintered-glass Buchner funnel (No. 3 porosity, 65-mm diameter), add 125 mL of chloroform, stir, and let stand for 5 min. Using vacuum, filter the slurry and discard the filtrate. Similarly, stir the sample with 80 mL of resin-in-butanol, allow the slurry to stand for 10 min, then vacuum filter. Repeat this extraction with two additional potions of resin-in-butanol solution. Transfer the combined extracts to a 500-mL separatory funnel and wash them with two 120-mL portions of NaCl solution, discarding the aqueous layers. Then add 240 mL of heptane, 100 mL of ammonia solution, and 50

mL of sodium chloride–ammonia solution. Shake the solution vigorously, allow the layers to separate, and collect the aqueous layer.

Repeat this extraction with two additional portions of sodium chloride–ammonia solution. Wash the combined aqueous extracts with 50 mL of diethyl ether and discard the ether layer. Transfer the aqueous layer to a 600-mL beaker and warm it on a water-bath under a stream of air for 30 min to remove ammonia and residual solvent. Neutralize the solution to pH 6–7 with glacial acetic acid (if Erythrosine BS is present, this neutralization should be carried out before heating the beaker on the water bath), then pass through a polyamide column and treat as described above for Aqueous Samples and Water-Soluble Foods.

Samples Where Incomplete Extraction of Color is Observed—Transfer 10 g of food to a 250-mL beaker and add 25 mL of buffer solution, together with the appropriate enzymes needed to hydrolyze the main structural ingredients (protein, starch, fat, etc.) of the food. For single enzymes use the conditions shown below.

For mixtures of papain and lipase use pH 7.0 buffer and for mixtures of amyloglucosidase, pectinase and cellulase use pH 4.6 buffer. Incubate the mixture for 2 hr at 35–50°C (see below), then grind the digested sample with 15 g of Celite and proceed as described above for Foods Insoluble in Water.

Conditions for the Enzyme Digestion of Major Food Constituents

Substrate	Enzyme	Amt of Enzyme/mg	Optimum pH	Temp/°C	Example of Use
Protein	Papain	100	7.0	30	Cake, fish, and meat products
Fat	Lipase	50	7.7	30	Cake, fish, and meat products
Phospholipid	Phospholipase	10	7.3	30	Sponge cake and egg products
Starch	Amyloglucosidase	100	4.5	50	Cereals, luncheon meat, jams, fruit, and modified starches
Pectin	Pectinase	50	4.0	50	Jams and fruit
Cellulose	Cellulase	50	5.3	50	Jams and fruit

See: Boley, N.P., Crosby, N.T., Roper, P. Analyst *104*, 472–473 (1979)

Determination of Extracted Colorants by HPLC—Use a Waters Associates 6000A constant-volume pump, fitted with a stop-flow injection system and a 12-cm × 4.6-mm ID stainless-steel column packed with 5-μm SAS-Hypersil (Shandon Southern Instruments Ltd.), operated at room temperature. Inject 1-5 μL of sample solution and elute using freshly prepared methanol–water–cetrimide
(77 mL:23 mL:0.25 g;
or 80 mL:20 mL:0.25 g;
or 75 mL:25 mL:0.25 g,
depending on the colorants present) at a flow of 1 mL/min.

CORRADI, C., MICHELI, G. Boll. Chim. Unione Ital. Lab. Prov. *6*, 319–326 (1981). Research and Identification of the Colouring and Flavouring Principles of Turmeric in Foods. A brief description of turmeric and its principal components is given, together with the chemical formula of its coloring and flavoring principle, curcumin. TLC and HPLC methods are described for identifying curcumin in foods, even in the presence of other yellow colorants.

CORRADI, C., MICHELI, G., SPROCATI, G. Ind. Aliment. *20*, 624–629 (1981). Investigation of Saffron Used in Compound Foods by Identification of Its Colouring, Bitter and Odorous Principles. The sample is extracted with hot water; ethanol, if present, is removed by evaporation, and crocin is hydrolyzed to aglycone crocetin by boiling with concentrated HCl. The resulting red precipitate is dissolved in acetone, and total crocetin is determined by TLC on Kieselgel 60 F_{254} plates, using $CHCl_3$–acetic acid (9:1) as the mobile phase or, if bixin and/or norbixin is present, using ethyl ether–propan-2-ol (10:1) as mobile phase. A Saturated solution of $SbCl_3$ in $CHCl_3$ (Carr–Price reagent) is used for locating crocetin. To determine "odorous and bitter" principles (free and combined safranal), the sample is steam-distilled in 3% $Ba(OH)_2$ solution to hydrolyze the bitter picrocrocin. The distillate is extracted with ethyl ether, the extract is evaporated and the residue is dissolved in methanol. Safranal is determined in the solution by TLC using Kieselgel plates (as above) using benzene–methanol (97:3) as the mobile phase, and 2,4-dinitrophenylhydrazine as the locating reagent. Using this procedure, as little as 0.05% of saffron in solid and liquid foods can be detected.

CORRADI, C., MICHELI, G. Ind. Aliment. (Pinerolo, Italy) *18*, 797–802 (1979). Rapid Method for Investigation and Identification of the Natural Dye E162 (Beetroot Red; Betanin) in Food Products. The sample, plus 10–20 mL of water, is defatted with light petroleum (2 × 10 mL). The aq. phase is acidified with 2 *N* acetic acid and introduced into a column (25-cm × 20-mm) containing ≃ 1.5 g of polyamide covered with sand. The column is repeatedly washed with water and finally with methanol then the adsorbed betanin is eluted with 3–4 mL of acetic acid–methanol (2:3). The eluate is evaporated

to dryness in an inert atmosphere. Or, the dye is purified by treating the aq. extract with ≃ 0.2 g of DEAE-Sephadex A-25; the mixture is centrifuged, the resin is washed several times with water, and the dye is desorbed from the resin by 0.5–1 mL of 2 *N* HCl–isopropyl alcohol (1:1). The resulting solution is evaporated to dryness as described above. The residue in either instance is dissolved in acetic acid–methanol (2:3) and the resulting solution is analyzed by TLC on silica gel, using acetic acid–methanol (2:3), ethanol–butanol–pyridine–water (1:7:6:6) or propanol–acetic acid–water (3:1:1) as the mobile phase.

CORRADI, C., MICHELI, G. Boll. Chim. Unione Ital. Lab. Prov. *5*, 188–200 (1979). Rapid Method for the Study and Identification of Water-Soluble Artificial Acidic Dyes in Foods. Water-soluble sample are heated with water (20–50 mL/0.5–5g) on a water bath, and the solution is diluted and acidified with acetic acid, if necessary. Partially soluble samples are treated with aqueous ethanol, sand, diatomaceous earth, and aqueous NH_3, then homogenized, heated, and centrifuged; the supernatant solution is then treated with acetic acid. Fat-containing samples are dried at 100°C and triturated with sand, diatomaceous earth, and light petroleum. The fat and solvent are removed, and the residue is heated and then extracted with aqueous ethanol and aqueous NH_3 as before. The extracts are cleaned-up on a polyamide column using acetone–water–aqueous NH_3 (40:9:1) as mobile phase, and then chromatographed on two cellulose TLC plates using ethanol–butanol–pyridine–water (1:7:6:6) and aqueous 2.5% Na citrate-aqueous NH_3-methanol (20:5:3) as mobile phases.

DEL BIANCO, F. M., TRABACCHI, G. Rass. Chim. *13* (2), 17–19 (1961). Method for Extraction of Colorants from Food Products. The procedure used by the authors for analyzing jams and jellies (see) was modified by using powdered leather treated with HCHO to adsorb colorant from weak acid solutions (pH = 5).

DEVON, B., LAUR, J. Ann Fals. Fraudes, *52*, 155–161 (1959). Determination of Coloring Matter in Food With Quaternary Ammonium Compounds. Basic colorants may be extracted directly with $CHCl_3$. For acid colorants, a sample containing 5–10 μg of colorant is adjusted to pH = 9 with Na_2CO_3 and shaken for 10 min with 10 mL of $CHCl_3$. The sample is centrifuged, and the $CHCl_3$ layer containing any basic colorants is removed. The aqueous layer including any solids that have formed at the interface of the liquid layers is mixed with a large excess of 0.1% cethylcyclohexyldimethylammonium bromide, shaken with $CHCl_3$, and centrifuged. The $CHCl_3$ solution is drawn off and evaporated at low temperature. The residue is dissolved in 0.5 mL of $CHCl_3$, and a known amount is chromatographed for 24 hr by descending chromatography using 95% EtOH–H_2O–NH_4OH (50:25:25).

DIXON, E. A., RENYK, G. J. Chem. Ed. *59*, 67–69 (1982). Isolation, Separation and Identification of Synthetic Food Colors.

Treat samples as follows:

Nonalcoholic liquids (e.g., soft drinks)—If noncarbonated, acidify slightly by adding 2–3 drops of glacial acetic acid to 30 mL of sample.

Soluble foods (jams, powdered drinks, candies, etc.)—Dissolve in 30 mL of water, then acidify with 2–3 drops of glacial acetic acid.

Starch-based foods (cakes, custard powder)—Grind 10 g of sample thoroughly with 50 mL of 2% ammonia in 70% ethanol, allow the sample to stand for 2–3 hr, then centrifuge. Evaporate the supernatant liquid to 30 mL, then acidify with 2–3 drops of glacial acetic acid.

Products high in fat (sausage, meat, fish pastes)—Defat with light petroleum ether (30–60°) and treat with hot water (30 mL) and 2–3 drops of glacial acetic acid. If oil-soluble colors are present, the organic phase will also be colored.

Cut pure, natural, untreated wool, or unbleached white knitting wool into 20-cm strips and boil in dilute NH_4OH (8–10 drops 0.880 ammonia in 50 mL of water). Rinse and boil again in water.

Add one strip of wool to about 30 mL of acidified sample solution and boil for 10 min. Wash the wool with cold water, transfer it to a small beaker, and boil it gently in dilute NH_4OH (2–3 drops 0.880 ammonia in 20 mL of water). Evaporate the colored solution to near dryness then chromatograph a portion of it on a silica gel plate using isopropanol–0.880 ammonia (4:1) as the mobile phase.

FISHER SCIENTIFIC, private communication. Extraction of FD&C Dyes From Food Products. Dissolve and filter solid foods. Adjust filtered sample solutions or liquid samples to pH 1 with 1 *N* sulfuric acid. Condition a Fisher PrepSep C_{18} column with two 2-mL portions of 2-propanol, then with 5 mL of 1 *N* sulfuric acid. Add 1 to 3 mL of acidified sample to the column, wash the column with 2 mL of 1 *N* sulfuric acid, then elute any colorant present with 0.5-mL portions of 20% 2-propanol. Chromatograph a portion of the eluate using a Waters μBondapak C_{18} chromatographic column, and a mobile phase composed of 80% 0.005 *M* tetrabutylammonium hydroxide adjusted to pH 6.5 with H_3PO_4, and 20% 2-propanol. Use a flow rate of 1.5 mL/min and detection at 254 nm.

FOGG, A. G., BHANOT, D. Analyst *112*, 1319–1321 (1987). Effect of Phosphonium and Arsonium Salts on the Differential Pulse Polarograms of Three Permitted Synthetic Food Colouring Matters. Changes in peak potential and current were compared upon adding 750 μg/mL of a phosphonium or arsonium halide to solutions of FD&C Blue No. 1, FD&C Yellow No. 5, and Yellow 2G. Polarography was at room temperature using a dropping-mercury

electrode, a platinum counter-electrode and a reference SCE electrode. For FD&C Blue No. 1, large negative shifts were obtained in acid media, which decreased in neutral media and became positive in alkaline solution. For FD&C Yellow No. 5, the shifts were small in acid and alkaline media, but larger at intermediate pH values. For Yellow 2G, the shifts were large and negative in acid and alkali. The largest changes in potential were observed with tetraphenylphosphonium chloride. The technique enables determination of each colorant when present together in foods.

GRAICHEN, C., MOLITOR, J. C. JAOAC *46*, 1022–1029 (1963). Determination of Certified FD&C Color Additives in Foods and Drugs.

Reagents

Dilute Acetic Acid—Mix one volume of glacial acetic acid with four volumes of water.

Resin-Hexane—Dissolve 50 mL of Rohm and Haas Amberlite LA-2 resin in 950 mL of *n*-hexane. Shake the solution with 200 mL of 1:4 acetic acid. Discard the lower phase.

Resin-Butanol—Dissolve 100 mL of Amberlite LA-2 resin in 900 mL of butanol. Shake the solution with 400 mL of 1:4 acetic acid and 15 mL of water saturated with ammonium sulfate. Discard the lower phase.

pH = 7.5 Buffer—Mix 75 mL of 0.1 *M* citric acid with 925 mL of 0.2 *M* Na_2HPO_4.

Resin-Butanol, pH = 7.5—Dissolve 50 mL of Amberlite LA-2 resin in 950 mL of butanol and 3 mL of glacial acetic acid. Shake the solution with 400 mL of the pH = 7.5 buffer. The pH of the lower phase should be 7.3–7.7. If it is not, repeat the preparation adjusting the amount of acetic acid. Discard the lower phase.

pH = 3 Buffer—Mix 101.5 mL of 0.2 *N* hydrochloric acid and 250 mL of 0.2 *M* potassium acid phthalate solution. Dilute to 1 L.

Resin-Butanol, pH = 3—Mix 50 mL of Amberlite LA-2 resin, 950 mL of butanol, and 8 mL of concentrated hydrochloric acid. Shake the mixture with three successive 200-mL portions of the pH = 3 buffer. The pH of the aqueous phase should be 2.8–3.2. If it is not, repeat the preparation, adjusting the amount of hydrochloric acid used.

Sample Preparation

Weigh 5 g of sample into a tissue blender. The sample should contain at least 0.2 mg of each color but no more than 5 mg of total color. Add 25 mL of 1:4 acetic acid and blend into a fine mixture. Transfer the suspension to a mortar and grind in about 5 g of Celite. Add more Celite as necessary to give the proper texture for the particular sample. The mixture should be wet enough to pack under pressure but dry enough to crumble when disturbed. With many samples the entire sample preparation can be done in a mortar.

Chromatographic Separation

Mix 20 g of Celite and 8 mL of 1:4 acetic acid. Pack about 15 g of the mixture into a 5.2-cm-ID × 20-cm glass chromatographic column. Transfer the sample onto the column and pack using the weight only of a 1300 g aluminum plunger (see Fig. 15-2). Flush the mortar with the remaining Celite–acid mixture, transfer it to the column, and pack as described above. Cover the surface with a porous disc. Elute fats and chloroform-soluble colors from the column with 100 mL of chloroform followed by 50 mL of hexane. Elute

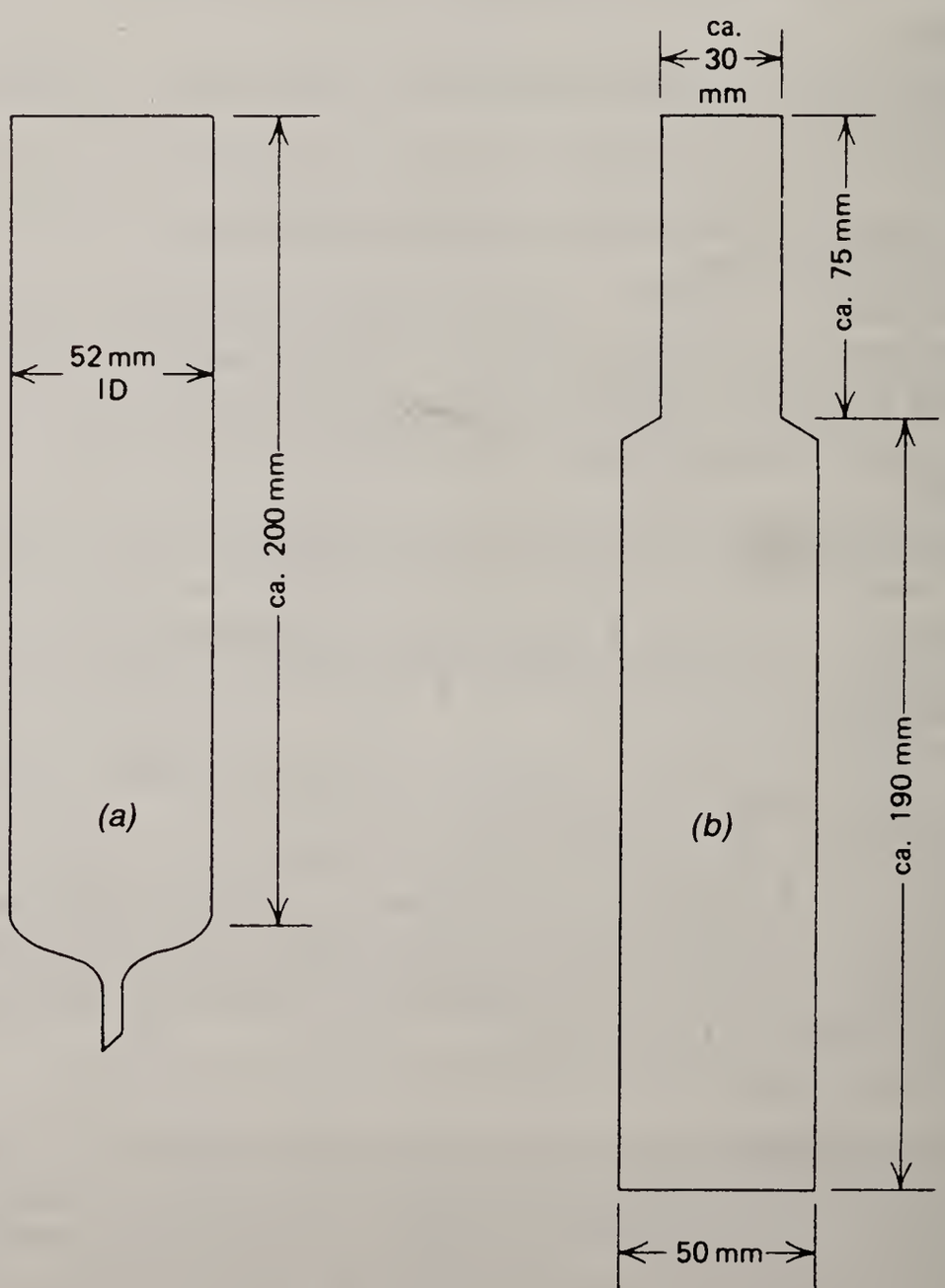

Figure 15.2. (*a*) Chromatographic tube and (*b*) aluminum plunger.

FD&C Red No. 2*, FD&C Red No. 4†, FD&C Yellow No. 5, and FD&C Yellow No. 6 from the column with 200 mL of the resin–hexane solution. Next, elute FD&C Blue No. 1, FD&C Green No. 2*, FD&C Green No. 3, and FD&C Violet No. 1* from the column with the first resin–butanol solution.

Alternate Method A—This method is preferred when FD&C Blue No. 2 or FD&C Red No. 3 is present. All colors elute. Grind the sample and pack the column as described above except use pH = 7.5 buffer in place of 1:4 acetic acid. Elute fats and chloroform soluble colors with chloroform. Elute FD&C Blue No. 2 and FD&C Red No. 3 with pH = 7.5 resin–butanol.

Alternate Method B—This method is best when aluminum lakes of colors are present. Allowing the column to stand for several hours or overnight in contact with the pH = 3 resin–butanol mobile phase improves the extraction of lakes. Grind the sample and pack the column as described above except use pH = 3 buffer in place of 1:4 acetic acid. Elute fats and chloroform-soluble colors with chloroform. Eliminate the chloroform wash if FD&C Red No. 3 is to be determined. Elute colors with the pH = 3.0 resin–butanol solution.

Isolation of Colors from the Resin Solutions

From Resin–Hexane—Wash 200 mL of extract with three 100-mL portions of water. Add 30 mL of water. Add concentrated ammonium hydroxide dropwise until the sample is alkaline, as indicated by the extraction of color into the aqueous phase. Extract all the color with 10-mL portions of dilute ammonium hydroxide. Quickly wash the combined aqueous extracts with 20 mL of chloroform, discard the chloroform, and acidify the aqueous layer with acetic acid.

From Resin–Butanol and Resin–Butanol at pH = 3—Dilute 100 mL of extract with 200 mL of hexane. Discard the aqueous layer which separates, then extract as described above.

From Resin–Butanol at pH = 7.5—Wash the organic layer with several portions of dilute ammonium hydroxide. Acidify the aqueous layer and extract with ethyl ether. Extract the ether solution with dilute ammonium hydroxide to isolate FD&C Red No. 3 from other FD&C colors.

GRAICHEN, C. JAOAC *58*, 278–282 (1975). Quantitative Determination of FD&C Colors in Foods. Describes further studies of the above procedure. [JAOAC *46*, 1022–1029 (1963)].

HENNING, W. Dtsch. Lebensm.-Rundsch. *79*, 407–410 (1983). Detection and Evaluation of the Addition of Betanin to Foodstuffs. Betanin is extracted from

*These colorants are no longer permitted in foods, drugs or cosmetics in the U.S.
†Permitted in externally applied drugs and cosmetics, only.

the sample by homogenization with H_2O acidified with a few drops of concentrated acetic acid, then the homogenate is warmed to about 40°C and filtered through paper and then through a Sep-Pak C_{18} cartridge. Betanin is eluted from the cartridge with methanol–H_2O (1:3) then the eluate is concentrated to a few drops at 35–40°C in a rotary evaporator. The colored concentrate is chromatographed on a cellulose thin-layer plate using butanol–formic acid–water (10:3:3) as the mobile phase. The pigment is identified on the TLC plate by comparison with pure betanin. It is also identified spectrophotometrically by comparison of an aqueous solution of the isolated pigment with pure betanin, in the range 400–600 nm; betanin shows a characteristic maximum at 532 nm.

HJORTH TOENNESEN, H., KARLSEN, J. Z. Lebensm. - Unters. -Forsch. *177*, 348–349 (1983). Quantitative Determination of Curcuma Dyes in Food. Shake 1 g of sample with 5 mL of methanol for 2 min, then centrifuge the extract. Chromatograph 20 μL of the supernatant liquid on a 25-mm × 4.6-mm column of Nucleosil NH_2, using ethanol as the mobile phase, and fluorometric detection at 470 nm (excitation at 420 nm). The reproducibility of the method is good, and the limits of detection for bisdemethoxycurcumin, demethoxycurcumin and curcumin are 1 pg, 0.167 ng, and 0.2 ng, respectively.

HURST, W. J., McKIM, J. M., MARTIN, R. A. J. Food Sci. *46*, 419–420, 424 (1981). Determination of Tartrazine in Food Products by HPLC. A Zipax SAX pre-column, a Zorbax NH_2 analytical column and a 0.7 *M* sodium acetate buffer mobile phase were used to determine Tartrazine in various food products. Detection was at 254 nm. This method separates Tartrazine from Amaranth and FD&C Red No. 40.

KAMIKURA, MIEKO Eisei Shikensho Hokoku *99*, 147–151 (1981). Determination of Synthetic β-carotene Added to Food. It was shown that commercial alumina could be used for column chromatography of β-carotene without the moisture conditioning with 10% Na ascorbate solution previously regarded as necessary. Recovery of β-carotene from a 10-cm × 1-cm column of alumina was 95.42% at the 97-μg level when the column was extracted in the dark or under UV light. Recovery of 97 μg of β-carotene liberated from food by solvent extraction or saponification and subsequent column chromatography on alumina was about 92%. Recovery of β-carotene from margarine, butter, sour-milk beverage, and orange juice ranged from 80–88% when the colorant was present in the food at the 5–100 μg level. Degradation of the colorant during TLC was reduced by the addition of 2,6-di-*t*-butyl-*p*-cresol to the sample before analysis.

KANI, T., DOBASHI, K., KATO, F., YUASA, Y., MIYAZAWA, F. Eisei Kagaku *32*, 54–56 (1986). Comparison of Platinum Crucible Ashing and

Porcelain Crucible Ashing for the Colorimetric Determination of Titanium Dioxide in Food and the Real Situation of Titanium Dioxide Content in Market Foods. Platinum and porcelain crucibles were compared for the ashing of samples prior to the determination of their titanium dioxide content. Foods studied included chocolate, cheese, chewing gum, and jelly beans. Porcelain crucibles proved adequate for the analysis.

KARASZ, A. B., DE COCCO, F., BOKUS, L. JAOAC *56*, 626–628 (1973). Detection of Turmeric in Foods by Rapid Fluorometric Method and Improved Spot Test. Mix 2 g of salad dressing or mashed pickle in a beaker with 3 g of Hyflo Super-Cel filter aid to a uniform mix. Add 50 mL of water-saturated *n*-butanol and stir thoroughly. Let stand for 15 min with occasional stirring and then filter through Whatman No. 42 paper. If the sample is a bread, pulverize 10 g and transfer it to a flask containing 50 mL of water-saturated *n*-butanol. Stopper the flask, shake well, and let stand for 15 min. Shake again and filter as described above.

Transfer 20 mL of filtrate to a separatory funnel, add 10 mL of NaOH solution (150 g of NaCl + 4 g of NaOH/liter), and shake vigorously for 1 min. Draw the aqueous layer and any red droplets at the interface into a second separatory funnel. Add 1 mL of glacial acetic acid and 200 mg of $Na_2S_2O_4$ and swirl to dissolve the salt. Add 20 mL of water-saturated *n*-butanol and shake vigorously for 1 min. Filter the butanol extract and determine its spectrum in a spectrophotofluorometer within 15 min as follows.

Set the fluorometer excitation scale at 435 nm and the emission scale at 520 nm. Fill the cuvette with reference solution prepared by diluting 5 mL of 0.03% curcumin in ethanol to 500 mL with water saturated *n*-butanol and then adjust slits, meter multiplier, and sensitivity to obtain 100% full-scale deflection on the recorder. Replace the reference solution with sample extract and, keeping the excitation scale at 435 nm, record its emission spectrum. The emission maximum for turmeric appears at 520 nm.

To confirm the presence of turmeric, evaporate a portion of the butanol extract to dryness, dissolve the residue in a minimum of ethanol, and spot a sufficient amount on Whatman No. 1 paper to produce a distinct yellow spot. Dry the paper in an oven at 100°C for 2 min and then add 3–4-μL portions of boric acid reagent to the yellow area. A red color that develops within 2 min at room temperature indicates the presence of turmeric.

To prepare the boric acid reagent, dissolve 1 g of H_3BO_3 and 5 mL of HCl in 95 mL of ethanol. Dry over anhydrous Na_2SO_4 and filter.

KING, R.D. Dev. Food Anal. Tech. *2*, 79–106 (1980). Determination of Food Colours. A review of methods for determining natural and synthetic colorants in food using chromatography and spectrometry. Includes 106 references.

LEHMANN, G., BINKLE, B. Lebensmittelchem. Gerichtl. Chem. *41*, 9–10 (1987). Detection of Caramel in Food Preparations. The addition of caramel to foods can be detected from the presence of 4-methylimidazole, which is formed when sugar is caramelized by treatment with ammonium compounds. Grind the sample, if necessary, and extract it with methanol. Evaporate the extract to dryness, then dissolve the residue in H_2O. Transfer the residue to a column of aromatic sulphonic acid cation exchanger. Elute the 4-methylimidazole with 0.05 *M* HCl, neutralize the eluate, then evaporate it to dryness. Dissolve the residue in $CHCl_3$, then chromatograph a portion of the residue by TLC using silica gel G plates, ethyl ether–$CHCl_3$–methanol–aqueous 25% NH_3 (20:5:5:1) as the mobile phase, and diazotized sulphanilic acid for detection. The R_f of 4-methylimidazole is 0.73.

LEHMANN, G., BINKLE, B., SCHELLER, A. Fresenius´ Z. Anal. Chem. *323*, 355–358 (1986). Identification of Dyes in Foodstuffs by Ion-Pair Extraction. Mix 5–10 g of food sample with 2–5 mL of methanolic 0.1 *M* hexadecylpyridinium chloride, then extract any ion-pairs formed into 10 mL of CH_2Cl_2. Back-extract the colorants into dilute $HClO_4$, then separate and identify them using TLC. The optimum pH for extraction is 5–9. The system can be used in the presence of egg white, which has a high affinity for anionic dyes.

LEHMANN, G., COLLET, P., HAHN, H. -G., ASHWORTH, M. R. F. JAOAC *53*, 1182–1189 (1970). Rapid Method for Detection and Identification of Synthetic Water-Soluble Coloring Matters in Foods and Drugs. Acid dyes are leached from foods with ammoniacal alcohol, acidified, and adsorbed onto polyamide powder. Protein-containing foods are treated with acetone to remove fat and water and to coagulate soluble protein. The residue is packed into a special chromatographic tube (see Fig. 15-3), and the colorants are eluted with ammoniacal alcohol, whereas the protein remains on the column. Water-soluble forms of natural colorants such as chlorophyll, carmine, annatto, alkanna red, betanin, and grape-juice red pigment can also be adsorbed on polyamide powder.

Basic dyes are adsorbed on carboxymethyl cellulose.

LEHMANN, G., HAHN, H. -G., COLLET, P., SEIFFERT-EISTERT, B., MORAN, M. Z. Lebensm. Forsch. *143*, 256–263 (1970). Analysis of Dyes. II. Rapid Determination of Water-Soluble Dyes in Foods. Dyes are Extracted from samples by methods that depend on whether the sample is soluble in or miscible with water or contains natural coloring matter, starch, pectin, or protein. The extracts are purified on microcolumns of polyamide powder, ion-exchange resin, carboxymethylcellulose, bentonite, and fuller's earth.

LEHMANN, G., MORAN, M., NEUMANN, B. Z. Lebensm. Forsch. *155*, 85–87 (1974). Analysis of Dyes, XV. Detection of Beetroot Dye (Betanin) in

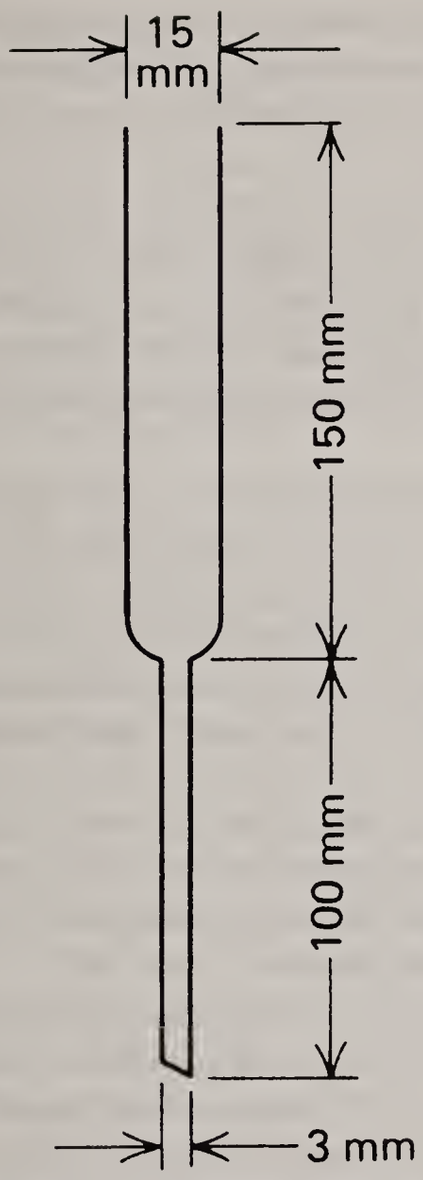

Figure 15-3. Microchromatographic tube

Foods. Betanin is isolated from H_2O-soluble samples by chromatography on a microcolumn of polyamide powder using formic acid–methanol (2:3) as the mobile phase. Protein-containing samples are treated with Celite and sand in the presence of acetone to precipitate protein and extract fat, water, and lactoflavine. The dried solids are then transferred to a polyamide column and the betanin is eluted with methanol–concentrated aqueous NH_3 (19:1). The eluate is neutralized and treated with DEAE-cellulose and the adsorbed betanin is eluted with formic acid–methanol (1:4). The concentrated eluate is chromatographed on Kieselgel using propanol–acetic acid–H_2O (3:1:1) as the mobile phase.

LEHMANN, G., HAHN, H. -G. Z. Analyt. Chem. *238*, 445–456 (1968). Detection and Determination of Water-Soluble Synthetic Food Dyes with Poly-

amide Powder. Polyamide powder is used to quantitatively adsorb dyes from aqueous or aqueous-alcoholic solutions. The dyes are washed from the polyamide with a solution of 0.5 g of NaOH in 1 L of 70% MeOH and identified spectrophotometrically.

LI, M., HU, Y., SHAO, B. Sepu *6*, 44–46 (1988). Determination of Synthetic Colours in Food by High-Performance Liquid Chromatography. Seven colorants were determined in foods by HPLC using a μBondapak C_{18} Radial-Pak column (10-cm × 8-mm), methanol–0.02 *M* ammonium acetate as the mobile phase, and detection at 254 nm. Procedures for extraction of the colors from foods are described.

LOVE, J. L. Food Technol. N. Z. *20*, 47–49 (1985). Method to Detect Synthetic Carotene Derivatives in Food and Drink and to Distinguish these from Natural Carotenoids and Xanthophylls. Synthetic carotene derivatives such as β-carotene, β-apocarotenal and canthaxanthin, natural coloring agents such as annatto, and Citrus Red No. 2, were detected in foods and beverages with thin-layer chromatography on precoated silica gel plates using light petroleum–methyl ether –acetic acid (78:19:3) as the mobile phase. Spots were visualized with HCl fumes; the lifetime of the spots could be extended to three weeks by exposure of the plates to NH_3 vapor and storage in the dark. Synthetic carotene derivatives were distinguished from natural carotenoids and xanthophylls by comparison with standards.

LOVE, J. L. N. Z. J. Sci. *27*, 113–116 (1984). A Simple Method to Identify Added Synthetic Colours in Foods. Acidify drinks (filter or centrifuge first, if cloudy) and apply to a C_{18} Sep-Pak cartridge, then elute any color from the column with ethanol made alkaline with aqueous NH_3. Extract solid foods by macerating with aqueous 50% acetone (or ethanol) made alkaline with $Na_2B_4O_7$ solution, add Celite 545, then separate solids by filtering or centrifuging. Evaporate the solvent in a rotary evaporator at 40°, acidify the residue with HCl and add it to a Sep-Pak cartridge as above. (First precipitate the protein in high-protein foods, e.g., yogurt, with ethanol before the extraction step.) Identify the colors present by thin-layer chromatography using precoated Kieselgel 60 F_{254} sheets and the following mobile phases: (a) concentrated aqueous NH_3–propan-2-ol (1:3), for red, orange, and blue colors, (b) propanol–H_2O–acetic acid (3:1:1), for red, orange, and yellow colors, (c) 2-methylpropanol–H_2O–ethanol (3:2:2), for brown colors, and (d) propan-2-ol–ethanol–H_2O–ammonium acetate (12:6:2:1 by weight), for carmoisine and Red FB. Identify the colors by comparison with standards. The best separation of permitted and nonpermitted colors is achieved using system (b), but this system does not separate all permitted colorants.

MACCHIAVELLI, L., ANDREOTTI, R. Ind. Conserve *59*, 18–21 (1984). Spectrophotometric Determination of Erythrosine in Food Products. Treat fruit

(such as apricots) with cellulase in pH 5.0 citrate buffer at 50°C, or pastry with amyloglucosidase in pH 4.5 citrate buffer at 55°C, for 2 hr to liberate Erythrosine chemically bound to the sample matrix. Cool the mixture, treat it with Celite 545 for 5 min, then filter it on a Gooch crucible (porosity 3). Extract the Erythrosine from the filter with an ethanolic solution of Amberlite LA-2, extract the color into hexane, then back-extract it into aqueous ammoniacal NaCl. Centrifuge the solution at 5000 g for 3 min, then measure the absorbance of the supernatant liquid at 520 nm against aqueous ammoniacal NaCl. The average recovery of Erythrosine is 97%, and the coefficient of variation is 0.4–11.7% (average 2.6%).

MACRAE, R., Ed. *HPLC in Food Analysis*, 2nd edition. Academic Press, San Diego, California, 1988. Includes procedures for the determination of synthetic colorants and natural pigments in foods.

MACRAE, R. J. Food Technol. *16*, 1–11 (1981). Recent Applications of High-Pressure Liquid Chromatography to Food Analysis. A review of the uses of HPLC for determining various components of foods, including color additives.

MATHEW, T. V., BANERJEE, S. K., MUKHERJEE, A. K., MITRA, S. N. Res. Indust. (New Delhi) *14*, 140–142 (1969). Isolation and Estimation of Synthetic Foods Colours by Alumina Adsorption and Paper Chromatography. The sample is diluted with water and stirred with neutral alumina powder. The alumina is removed by filtration and then extracted with isoamyl alcohol–95% ethanol–5% aqueous NH_3–H_2O (4:4:1:2) or, if indigo carmine is present, with butanol–anhydrous acetic acid–H_2O (20:5:12).

McKONE, H. T., NELSON, G. J. J. Chem. Ed. *53, 722* (1976). Separation and Identification of some FD&C Dyes by TLC. Colorant is extracted from soft drinks, juices, and Jello® products by boiling acetic acid solutions of the samples with white wool (first purified by boiling in dilute NaOH, then in water), washing the wool with cold water, then stripping the dye from it by boiling the wool with dilute ammonia. The extract is evaporated to near dryness, then a portion of the residue is chromatographed on silica gel G using *n*-BuOH–EtOH–H_2O–concentrated NH_4OH (50:25:25:10) as the mobile phase.

Colorant	R_f
Amaranth	0.63
FD&C Red No. 3	0.92
FD&C Red No. 4	0.80
FD&C Yellow No. 5	0.58
FD&C Yellow No. 6	0.73

McKONE, H. T. J. Chem. Ed. *54*, 376–377 (1977). Identification of FD&C Dyes by Visible Spectroscopy. Describes an undergraduate experiment for isolating colorants from various foods and separating them by thin-layer chromatography (see above reference), then identifying the colorants by visible spectroscopy.

McKONE, H. T., IVIE, K. J. of Chem. Ed. *57*, 321–322 (1980). An Introduction to High Performance Liquid Chromatography: Separation of Some FD&C Dyes. Colorants were isolated from liquid foods and drugs such as soft drinks, pickle and olive brines, the juice from maraschino cherries, cough medicines, mouthwashes, and liquified gelatin desserts, and from the extracts of solid foods, then resolved by high performance liquid chromatography as follows: Using a syringe pre-wet a SEP-PAK™ C18 Cartridge (Waters Associates, Milford, MA 01757) with 2 mL of 2-propanol, then flush 5 mL of 1% acetic acid through it, followed by 3 mL of sample. Discard the eluate containing sugars, flavors, etc. Elute FD&C Blue No. 2, FD&C Yellow No. 5, Amaranth, FD&C Yellow No. 6 and FD&C Red No. 40 from the SEP-PAK™ with 1 mL of 18% 2-propanol, then chromatograph 5–10 μL of the dye mixture using a Waters Associates μBondapak C18 column, a mobile phase consisting of a 1:6 mixture of 2-propanol and aqueous PIC Reagent A (0.005 *M* tetrabutylammonium hydrogen sulfate acidified to pH 6.5 with phosphoric acid) at a flow rate of 1.0 mL/min, and a detector set at 254 nm. Colorants elute in the above order. Flush FD&C Red No. 3, FD&C Blue No. 1, and FD&C Green No. 3 from the SEP-PAK™ using 1–2 mL of 50% 2-propanol, then chromatograph as above.

NISHIJIMA, M., KAMIMURA, H., KANMURI., TAKAHASHI, S., NAKAZATO, M., WATARI, Y., KIMURA, Y., NAOI, Y. Shokuhin Eiseigaku Zasshi *18*, 463–469 (1977). Determination of Dyes Permitted in Foods. Food colorants were extracted from aqueous or aqueous ethanolic solutions of food samples with Amberlite LA-2 resin in benzene–butanol (7:3), the extracts were concentrated, chromatographed on layers of cellulose or silica gel using BuOH–ethyl methyl ketone–aqueous 25% (or 1%) NH_3-H_2O (4:2:1:1) or ethyl acetate–MeOH–aqueous 25% NH_3 (45:10:7), respectively, and measured densitometrically.

ONRUST, H., HOEKE, F. Chem. Weekblad *54*, 465–470 (1958). Identification of Synthetic, Water-Soluble Food Colors. The following procedure is recommended for the analysis of foods with high sugar contents, alcoholic beverages, and milk products.

Mix one part of solid food with 4 parts of NaOAc–HOAc buffer (pH = 3), or mix 10 g of liquid sample with 20 mL of buffer. Extract the mixture with 10 mL of quinoline and centrifuge to remove the water layer. Wash the quinoline layer twice with water and then shake with 30 mL of ether, 1 mL

of H_2O, and 2 mL of 10% aqueous NH_3. Centrifuge to remove the quinoline–ether layer and then wash the colored aqueous layer with ether and analyze it chromatographically.

OSADCA, M., DERITTER, E., BUNNEL, R. H. JAOAC *49*, 1078–1083 (1966). Assay of Apocarotenal and Canthaxanthin in Foods. Carotenoids are extracted by blending or shaking the sample with an appropriate solvent and then separated from naturally occurring pigments and other added coloring agents by selective solvent extraction and/or column chromatography.

PRASAD, U. V., SASTRY, C. S. P. J. Food Sci. Technol. *20*, 263–264 (1983). Spectrophotometric Determination of Some Permitted Food Colours Using Aliquat 336. A number of food colors including carmoisine, FD&C Yellow No. 5, FD&C Yellow No. 6, and FD&C Red No. 3 have been determined by spectral measurement of their $CHCl_3$-extractable 1:1 complexes with Aliquat 336. Recoveries of FD&C Yellow No. 5, FD&C Yellow No. 6 and FD&C Red No. 3 from lemon and orange juices were between 99.08 and 100.70%. Glucose, sucrose, SO_4^{2-}, PO_4^{3-}, citric, tartaric and ascorbic acids and SO_2(except with FD&C Red No. 3) do not interfere. Benzoic acid (except with FD&C Yellow No. 6 and FD&C Red No. 3) and saccharin do not interfere in excesses below 2–6 and 1–2.5, respectively, over the dyes; NO_2^-, NO_3^- and salicylic acid interfere.

PUTTEMANS, M., DRYON, L., MASSART, D. L. Anal. Chim. Acta *113*, 307–314 (1980). Extraction of Water-Soluble Acid Food Dyes by Ion-Pair Formation with Trioctylamine. A number of colorants including FD&C Yellow No. 5 and FD&C Red No. 3 were quantitatively extracted from pH 5 phosphate buffer (I = 0.1) using 0.1 *M* tioctylamine in $CHCl_3$. (FD&C Blue No. 2 could also be extracted, but incompletely.) The colorants were back-extracted from the $CHCl_3$ solution using ClO_4^- solution.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L., JAOAC *65*, 730–736 (1982). Evaluation of Thin Layer, Paper, and High Performance Liquid Chromatography for Identification of Dyes Extracted as Ion-Pairs with Tri-*n*-octylamine. Samples are macerated with 0.1% NH_3 solution at 50°C, then filtered. The filtrates are adjusted to pH 5.5, then the dyes are extracted into $CHCl_3$ containing 0.1 *M* trioctylamine. The $CHCl_3$ extracts are examined as is by thin-layer or paper chromatography, or evaporated to dryness, redissolved in ethanol, and examined by high-performance liquid chromatography using a 30-cm × 2-mm octadecyl–silica column and various proportions of methanol–phosphate buffer (pH = 7) containing tetrabutylammonium hydroxide as the mobile phase.

PUTTEMANS, M. L., DRYON, L., MASSART, D. L. JAOAC *65*, 737–744 (1982). Isolation, Identification, and Determination of Food Dyes Following Ion-Pair Extraction. The above method was used for detecting various dyes

in grenadine, pickles, milk desserts, and alcoholic beverages. For determination by high-performance liquid chromatography, it is best to first back-extract the colorants from $CHCl_3$ into 0.1 *M* $NaClO_4$.

SALAGOITY-AUGUSTE, M. H., BERTRAND, A., SUDRAUD, P. Sci. Aliments *3*, 127–136 (1983). Determination of Several Synthetic Dyes in Various Drinks and Foods by High-Pressure Liquid Chromatography. Synthetic dyes were determined in beverages and foods by ion-pair HPLC on LiChrosorb RP-8, using aqueous 80% methanol containing hexadecyltrimethylammonium bromide as the mobile phase, and UV-visible spectrometry for detection. Liquid samples were injected directly; dyes were extracted from solid foods with water, then the extracts were chromatographed. The method showed high reproducibility and precision.

SINGH, M., GRAICHEN, C. JAOAC *56*, 1458–1459 (1973). Determination of FD&C Red No. 3 in Rat-Blood Serum. The Dye is extracted from the acidified sample with acetone–ethyl ether, extracted into dilute aqueous NH_3, and then measured spectrophotometrically.

SOHAR, J. Z. Lebensm. Forsch. *132*, 359–362 (1967). Extraction of Dyes from Food with Quaternary Ammonium Compounds. Colorants are complexed with cetyltrimethylammonium bromide and then extracted with organic solvent. The complexes are decomposed with cupferron and the free dyes are determined by the usual procedures.

SPEARS, K., MARSHALL, J. J. Assoc. Public Anal. *25*(2), 47–54 (1987). Qualitative Analysis of Synthetic Colourings in Food. To extract colorants from liquid or readily soluble foods, dissolve 10 g of sample in 90 mL of H_2O and adjust the solution to pH 2.5. Apply the solution to a Sep-Pak C_{18}cartridge, wash the cartridge with H_2O, then elute any colorant present with methanol containing a few drops of aqueous 10% NH_3. To extract colorants from insoluble and nonhomogeneous foods, homogenize 10 g of sample and extract it with 30 mL of butanolic Amberlite LA-2 anion-exchange resin. Back-extract any colorants into 25 mL of aqueous 10% NH_3. Remove excess NH_3 by warming the solution, adjust the extract to pH 3 with HCl, and pass it through a Sep-Pak cartridge as above. Analyze eluates from the Sep-Pak cartridges by TLC using cellulose plates and a mobile phase of trisodium citrate–H_2O–aqueous 10% NH_3. When blue or green colorants are present, use silica plates and a mobile phase of propan-2-ol-aqueous 10% NH_3–H_2O (7:2:1).

TSUNODA, K., INOUE, N., AOYAMA, M., HASEBE, A. Shokuhin Eiseigaku Zasshi *28*, 473–479 (1987). Rapid Analysis of the Food Colours in Foodstuffs Using Polyamide. Colorants were extracted from food samples with aqueous 1% NH_3–EtOH (2:3), the extract was filtered, the filtrate was diluted (× 2 or

3) with H_2O, acetic acid was added to pH 4–5, then 10–30 mg of polyamide powder was added to the mixture. After 10 min, the polyamide was filtered off, rinsed with hot H_2O and suspended in 100 μL of aqueous 28% NH_3–EtOH (2:3). After 5 min more, the supernatant solution was analysed by paper chromatography using BuOH–EtOH–aqueous 1% NH_3 (6:2:3) as mobile phase, or by TLC on a silica gel plate or polyamide sheet using ethyl acetate–EtOH–aqueous 28% NH_3 (33:10:10 or 45:10:7) or MeOH–EtOH–iso-amyl alcohol–aqueous 28% NH_3 (15:10:5:3:) as the corresponding mobile phase.

UEMATSU, T., KURITA, T., HAMADA, A. J. Chromatog. *172*, 327–334 (1979). Use of Amberlite XAD-2 for Isolation and Detection of Water-Soluble Acid Dyes. XAD-2 was used in a column or as a thin layer to separate eleven such dyes. In the column procedure, the XAD-2 was first milled to a particle size of 100–200 mesh. The colorants were adsorbed from a solution 0.5 *M* in triethylammonium hydrogen carbonate, then eluted with MeOH. In the thin-layer method, plates were made from a mixture of 200–400 mesh XAD-2 and silica gel G (1:2) and the mobile phase was acetone–NH_3–H_2O (3:1:6, or 6:1:3). Eluates from the column method were either analyzed spectrophotometrically or examined further by the TLC method. The methods were applied to samples of carbonated beverages, jams, candy, pickled radish, and seasoned fish.

VAN PETEGHEM, C., BIJL, J. J. Chromatog. *210*, 113–120 (1981). Ion-Pair Extraction and Ion-Pair Adsorption Thin-Layer Chromatography for Rapid Identification of Ionic Food Dyes. Samples (sweets, fruits, jellies, and caviar) were dissolved in 50% aqueous methanol containing 0.012 *M* hexadecyltrimethylammonium ion (I); the solutions were adjusted to pH 2.5 and then extracted with CH_2Cl_2. The extracts were chromatographed on silica gel impregnated with I using either methanol–acetone (9:1) plus 1% anhydrous acetic acid and 0.1 *M* I, or methanol–acetone (1:1) containing 0.1 *M* I, depending on the colorants present.

WARD, S. Shokuhin Eisei Kenkyu *37*, 61–65 (1987). Simple and Rapid Analysis of Synthetic Food Dyes. Colorants were extracted from foods with 1% NH_3–ethanol (2:3), the extract was filtered, and the filtrate was adjusted to pH 4–5, mixed with polyamide powder (0.01–0.2 g) and filtered again. A small portion of the polyamide powder was applied to a silica gel or polyamide gel plate for TLC using ethyl acetate–methanol–aqueous 28% NH_3 (33:10:10) or methanol–ethanol–isoamyl alcohol–aqueous 28% NH_3 (15:10:5:3) as mobile phase for the separation and determination of the colorants present.

WOJCIK, Z., SZYSZKO, E. Acta Pol. Pharm. *33*, 205–209 (1976). Polarographic Determination of Synthetic Dyes Used Pharmaceutically in Poland.

FD&C Yellow No. 5, FD&C Yellow No. 6, and Amaranth were determined polarograpically in pH 4.5 acetate buffer, and cochineal was determined in pH 3.3 Britton-Robinson buffer, using a dropping-mercury cathode versus a mercury-pool anode. Results agreed well with those obtained by a titanimetric method. The polarographic method is faster and can be used to determine colorants in concentrations of 10–200 μM.

YOUNG, M. L. JAOAC *67*, 1022–1024 (1984). Rapid Determination of Color Additives, Using the C_{18} Cartridge. A method is reported for isolating, separating, and identifying colorants in various products. See next reference.

YOUNG, M. L. JAOAC *71*, 458–461 (1988). Rapid Identification of Color Additives, Using the C_{18} Cartridge: Collaborative Study. Prepare a Waters Associates, Inc. Sep-Pak C_{18} cartridge (or any similar cartridge) as follows. Using a 10-mL syringe, force 3 mL of isopropanol through the cartridge, and discard the eluate. Force 5 mL of 1% acetic acid through the cartridge, and again discard the eluate. Prepare samples as follows, then transfer an appropriate amount to the cartridge.

Oriental noodles—Place 5 g of product and 20 mL of H_2O in a beaker over low heat and swirl gently until the colorant is leached from the noodles. Filter the solution, then transfer 2 mL of the filtrate to the C_{18} cartridge.

Candy—Transfer an appropriate quantity to a beaker, add 10 mL of H_2O, and proceed as for noodles.

Carbonated soda and similar drinks—Apply 2 mL of as-is sample, or an appropriate dilution of the sample directly to the cartridge.

Gelatin dessert—Place 3 g of product and 90 mL of H_2O in a beaker over low heat and swirl gently until the sample is dissolved. Filter the solution and transfer 5 mL of the filtrate to the cartridge.

Powdered drink mix—Place 1 g of sample in a beaker, add 200 mL of H_2O, and swirl the sample gently until it dissolves. Filter the solution, then apply 2 mL of the filtrate to the cartridge. Elute any colorant present using the elution scheme shown below. Elute at a flow rate that results in droplets rather than a stream of mobile phase. Monitor the separation closely and discard fraction that contain overlapping bands. Identify individual colorants by determining their neutral, acid (HCl) and basic (NaOH) spectra in the visible region. Separate mixtures of colorants by TLC using silica gel G plates and a mobile phase composed of *n*-butanol–methyl ethyl ketone–NH_4OH–H_2O (5+3+1+1). See Figure 15-4.

ZONTA, F., STANCHER, B. Riv. Soc. Ital. Sci. Aliment. *15*, 17–22 (1986). High-Performance Liquid Chromatography (HPLC) of Carotenes on Alumina Columns. Applications in Food Analysis. Homogenize low-fat foods, e.g.,

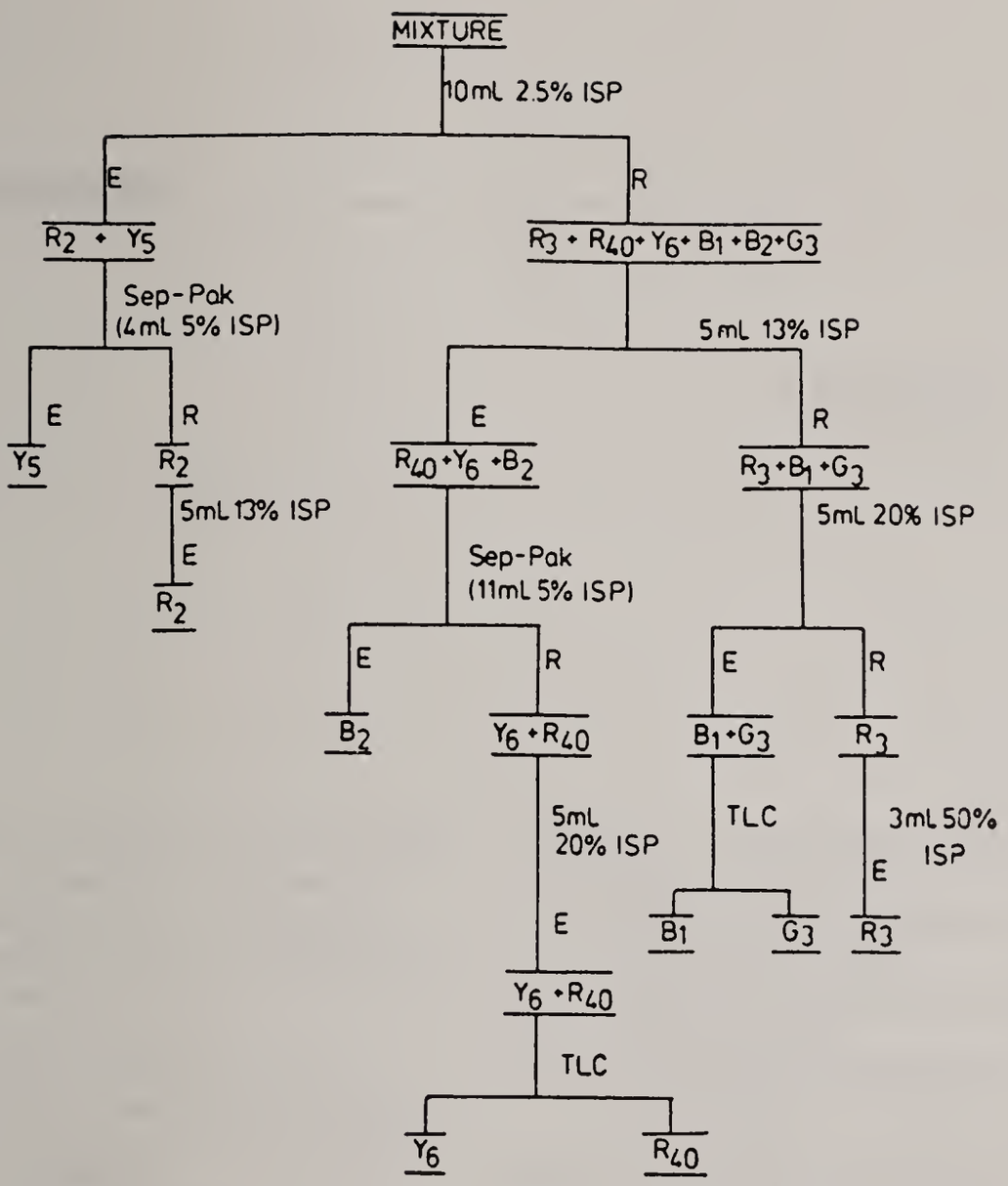

Figure 15.4. Scheme for identification of FD&C color additives: isopropanol (ISP); E = elutes and R = retains. R_2, Red No. 2; R_3, Red No. 3; R_{40}, Red No. 40; Y_5, Yellow No. 5; Y_6, Yellow No. 6; B_1, Blue No. 1; B_2, Blue No. 2; G_3, Green No. 3

carrots, in hexane–acetone–CH_2Cl_2 (2:1:1). Treat high-fat foods, e.g., cheese, with KOH solution then extract the pigments into ethyl ether. Evaporate the extracts to dryness, dissolve any residue in hexane and purify the solution by passing it through a column of neutral alumina using acetone–hexane (1:99) as the mobile phase. Analyze the percolates by HPLC using a column of LiChrosorb Alox T (5 μm), 5% CH_2Cl_2 as the mobile phase and spectrophotometric detection. The method separates α-, β-, and cis-β-carotene.

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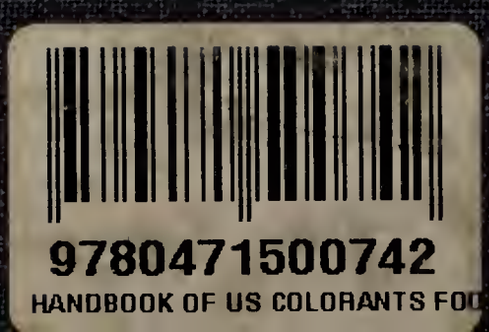

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