HANDBOOK OF U.S. COLORANTS FOR FOODS, DRUGS, AND COSMETICS

DANIEL M. MARMION

Second Edition

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To my wife, Annette

PREFACE

Since *Handbook of U.S. Colorants for Foods, Drugs, and Cosmetics* first appeared in 1979, numerous important changes have taken place in the field of color additives—the identity, status, and permitted areas of use of many colorants have been redefined, specifications for a large number of additives have been modernized, and analytical technology has been improved. The purpose of this revision is to document these changes while still maintaining the primary goal of the first edition, which was to provide a manual on color additives that would be useful to all who are concerned with them.

In preparing this edition, the basic structure of the book was kept the same. Modifications were made where necessary and new or supplementary information was added where it seemed beneficial to do so. Also, there was a special effort to include analytical procedures that might be useful as teaching aids because students seem to enjoy working with food, drug, and cosmetic colorants, probably because it makes the learning process more interesting and more relevant.

Much of the new material incorporated into this revision was brought to my attention by others, particularly Mr. Robert G. White of Allied Corporation. I would like to thank them as well as all those who provided me with the advice and encouragement that made this book possible.

DANIEL M. MARMION

Buffalo, New York
December 1983

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.

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HANDBOOK OF U.S. COLORANTS FOR FOODS, DRUGS, AND COSMETICS

PART A HISTORY, REGULATION, DESCRIPTION, AND USE

Chapter 1 History; Colorants in Use Today

Color is as common in our environment as the air we breathe. In fact, it is so prevalent that we are not always aware just how much we depend on it. Color is important to humans 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 color has played a prominent role in three of the things most important to humans—food, medicine, and physical appearance.

History is filled with accounts of the widespread application of color additives. Paintings in Egyptian tombs dating as far back as 1500 B.C. depict the making of colored candy. Pliny the Elder tells us that wine was artificially colored four centuries before the birth of Christ, and spices and condiments are known to have been col ored at least 500 years ago. An edict issued in Paris in 1396 that forbade the coloring of butter suggests another early and perhaps improper use of color additives.

The use of colorants in drugs undoubtedly has as long a history because 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 was probably more widespread and certainly better documented than their application in either foods or drugs. In the Old Testament (3 Kgs. 9, 30–31) we read that Jezabel "painted her face with stibic stone," probably Sb_2S_3. Archeologists have evidence that Egyptians used green ore of copper as an eye shadow as early as 5000 B.C. Egyptian women are also known to have used henna to dye their hair, carmine to redden their lips, and kohl, an antimony compound, to blacken eyebrows, lids, and lashes. Thousands of years ago it was common practice in India to tint faces yellow with saffron and to dye feet red with henna. In similar times, Chinese women used vegetable extracts to dye their feet, cheeks, and the tips of their tongues, whereas the men and women of Asia Minor painted their faces with litmus and marshmallow. Romans used white lead and chalk on their faces and blue and gold dyes on their hair and beards.

Until the middle of the nineteenth century, the colorants used in foods, drugs, and cosmetics were materials easily obtainable from

natural sources, that is, animals, vegetables, and minerals. 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.

The use of some of these in foods began in Europe almost immediately and was soon extended to drugs and cosmetics. French wines, for example, were colored 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 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 colorants in drug and cosmetic products was also on the increase.

This proliferation in the use of color additives was soon recognized as a threat to 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 and to add weight or bulk to certain items.

Some of these practices were dishonest but not inherently dangerous. For example, flour was often colored yellow to conceal dirt and to make it appear to have a high egg content; ordinary oranges were injected with red dye to give them the look of blood oranges; old meat was colored to make it appear fresh; green watermelons were dyed to make them look ripe; and jams and jellies were tinted to seem rich with fruit. 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.

Other early uses of colorants were criminal and sometimes deadly. In 1820, Fredrick Accum reported the demise of a woman who customarily ate pickles while at her hairdresser—pickles colored green with copper sulfate. A Manchester tea shop is said to have been found stocked with copper arsenite, lead chromate, and indigo for coloring used tea leaves for resale. Before the advent of synthetic organic dyes, candy was generally colored with mineral pigments such as red lead (Pb_3O_4), 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 is that of the druggist who in 1860 gave a caterer copper arsenite to use in making a green pudding for a public dinner. Two people died as a result.

As disturbing as the above abuses of colorants were, so too was the fact that often little or no control was exercised over the purity

of those added to foods, and that dyes found unsatisfactory for textiles were sometimes deliberately channeled into food products. Such misuse was exemplified in the report given 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. About 90% of them were found to contain arsenic which was traceable to the colorant used to dye them. The colorant was an otherwise unsalable mother liquor recovered during the manufacture of magenta and shown to contain 8% arsenic.

Because of increasing public concern over such practices, some measures were eventually taken by American food manufacturers to police their own industry. One example was the list published in 1899 by the National Confectioners Association of 21 colorants that they considered unfit for coloring foods. However, the effect of such actions by industry was marginal, and it was soon obvious that some form of government control was necessary if the public was to be protected.

The first effective step taken by the U.S. government to check such practices was when, under the Appropriations Act of 1900 for the Department of Agriculture, the Bureau of Chemistry was given funds to investigate the relationship of coloring matters to health and to establish principles that should be followed to govern their use. Results came quickly with the issuance by the Secretary of Agriculture of a series of Food Inspection Decisions (FID). One (FID 4[3c], issued 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 decision exempted fabricated confections from this adulteration proviso, except in those cases where 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 to declare on the label the presence of such substances as imitation chocolate (FID 29, issued September 27, 1905). A third decision (FID 39, issued May 1, 1906) contained the first direct statement by the department concerning a coal-tar dye considered unsafe in foods. In effect, it stopped importation of macaroni colored with Martius Yellow.

At about the same time a thorough study was undertaken by the Department of Agriculture to determine which dyes, if any, were safe for use in foods and what restrictions should be placed on their use. This task was monumental, to say the least, and eventually included a study of the chemistry and physiology of the then nearly 700 extant coal-tar dyes as well as the laws of various countries and states regarding their use in food products. Most of this investigation was done under the guidance of Dr. Bernard C. Hesse, a German dye expert.

Hesse believed that, in principle, any synthetic dye could be used in food provided that it could first be shown to be necessary and harmless. The problem was in determining which dyes these were. On this point, opinions among the "experts" differed widely and scientific evidence was both scarce and contradictory.

Upon examining the facts, Hesse found that very few colorants had ever been tested for their effects on health, and that most of those that had been were tested improperly. Sometimes the true chemical identity of a colorant studied was unknown. Other times a colorant's purity was uncertain. More often the procedure used for testing was naive.

Of the 80 colorants offered for use in foods in the United States in 1907, Hesse learned from the literature that 30 of them had never been tested at all and that their safety was therefore simply unknown, 26 had been tested but the results were contradictory, 8 were considered by most experts to be unsafe, and the remaining 16 were deemed more or less harmless. These 16 colorants were then tested physiologically by determining their acute short-range effects in dogs, rabbits, and human beings.

After considering all the information then available as well as the needs of industry, Hesse recommended the following seven colorants for use in food in the United States.

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

Much of what Hesse uncovered during his study was used in formulating the Food and Drugs Act of 1906. This act, plus FID No. 76 (July 13, 1907) put an end to the indiscriminate use of dangerous and impure coloring matters in foods. Among other things, this new legislation required that only colors of known composition, examined physiologically and showing no unfavorable results, could be used. The new regulations also established a system for certification of synthetic organic food colors by the Department of Agriculture. Certification was not mandatory, but dye manufacturers soon found it to their benefit to have their products certified; the first certification took place on April 1, 1908.

Because of the increased needs of industry, during the next three

decades there was a continual growth in the use and number of color additives. Using Hesse's rules, the list of colors certifiable for use in foods was expanded to include the following:

Original Name	Current Name	Year Added
Tartrazine	FD&C Yellow No. 5	1916
Sudan I	—	1918
Butter Yellow	—	1918
Yellow AB	—	1918
Yellow OB	—	1918
Guinea Green B	—	1922
Fast Green FCF	FD&C Green No. 3	1927
Ponceau SX	FD&C Red No. 4	1929
Sunset Yellow FCF	FD&C Yellow No. 6	1929
Brilliant Blue FCF	FD&C Blue No. 1	1929

In 1938 a new law was enacted, the Federal Food, Drug and Cosmetic Act of 1938, which instituted several new and important practices. First, it clearly stated that, henceforth, the use of any uncertified coal-tar color in any food, drug, or cosmetic shipped in interstate commerce was strictly forbidden. This restriction applied regardless of the inherent toxicity of the colorant. In effect, the colorants that could be used were limited, certification became mandatory, and governmental control was extended to the coloring of drugs and cosmetics. Next, it created three categories* of coal-tar colors:

FD&C colors. Those certifiable for use in coloring foods, drugs, and cosmetics.

D&C colors. Dyes and pigments considered safe in drugs and cosmetics when in contact with mucous membranes or when ingested.

Ext. D&C colors. 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.

Passage of the 1938 Act launched a new series of scientific investigations and public hearings regarding the safety of the colorants

*In surveying the colorants in use at the time, it was discovered that several manufacturers were selling the same dyes under different names. To clearly differentiate between a textile-grade colorant and a certified colorant with the same chemical structure but having a different level of purity, and to prevent giving one manufacturer an advantage over his competitors by selecting his trade name as the official designation of a colorant to be allowed under the 1938 law, the terms FD&C, D&C, and Ext. D&C were invented.

then on the market. These efforts culminated in the publication in September 1940 of Service and Regulatory Announcement, Food, Drug and Cosmetics No. 3, which listed specific colorants that could be used along with specifications and regulations relating to their manufacture, labeling, certification, and sale.

In the early 1950s, just when it appeared that the situation with regard to color additives was finally under control, new difficulties developed. The problems were precipitated by two events: a new round of pharmacological testing of food colors by the Food and Drug Administration (FDA)† and a number of cases of sickness in children who had reportedly eaten candy and popcorn colored with excessive amounts of dye. The new animal-feeding studies undertaken by the FDA were conducted at higher levels and for longer test periods than any experiments previously conducted and resulted in unfavorable findings for FD&C Orange No. 1, FD&C Orange No. 2, and FD&C Red No. 32.

The disputes that followed these events centered around the FDA interpretation of the 1938 act, which states that "The Secretary shall promulgate regulations providing for the listing of coal-tar colors which are harmless and suitable for use in food. . . ." The FDA felt that "harmless" here meant that a colorant must be safe regardless of the amount used, that is, harmless per se. On the basis of this argument, the FDA delisted the colorants in question. Meanwhile the food-color manufacturers argued that the FDA interpretation of the law was too strict, that a color additive need only be harmless when properly used, and that the FDA should establish safe limits. They also contended that the conditions used for the new animal feeding tests were too severe.

After a series of legal battles in the lower courts the problem was finally taken to the Supreme Court, which ruled that under the 1938 law, the FDA did not have the authority to establish limits of use for colorants and that they were obligated to decertify or delist a color if any quantity of it caused harm even though lesser amounts were perfectly safe. The FDA's hands were tied. A review of the remaining colors was started and soon several more were delisted, including FD&C Yellow Nos. 1–4. It was immediately and painfully obvious that the existing law on certifiable colors was unworkable and that the entire house of cards was about to collapse.

Through the efforts of the Certified Color Industry* and the FDA

†The FDA, which enforces the law governing color additives, was created by the Agricultural Appropriations Act of 1931.

*An informal, unincorporated association comprised of most of the certified food-color manufacturers in the United States. The committee was formed to deal with regulatory and legislative problems affecting the entire industry and involving the FDA.

a new law was formulated, the Color Additives Amendments of 1960 (Public Law 86-618). 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, 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. Other features eliminated any distinction under the law between "coal-tar" colors and other color additives and empowered the Secretary to decide which colors must be certified and which could be exempted from certification based on their relationship to public health.

Under provisions of the new law, the producers and consumers of the color additives were obliged to provide the necessary scientific data 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 these 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.

With the passage of the Medical Device Amendments of 1976 (Public Law 94–295) Congress created a new 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.

Colorants currently in use and their status are shown in Tables 1–4, and a chronological history of synthetic certifiable food colors is given in Table 5. These lists are accurate as of January 1984 but are subject to change by both addition and deletion. Such changes as well as any changes in the regulations discussed in Chapter 3 are routinely 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 FDA, Division of Colors and Cosmetics, 200 C St., S.W. Washington, DC 20204.

*The *Federal Register* is published by the office of the Federal Register, National Archives and Records Service, General Services Administration, Washington, D.C. 20408. It is distributed only through the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

TABLE 1 COLORANTS PERMITTED IN FOODS

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
	Subject to Certification		
FD&C Blue No. 1	42090		Listed
FD&C Blue No. 2	73015		Provisional
FD&C Green No. 3	42053		Listed
FD&C Red No. 3	45430		Listed
FD&C Red No. 40	16035		Listed
FD&C Yellow No. 5	19140		Listed
FD&C Yellow No. 6	15985		Provisional
Citrus Red No. 2	12156	Orange skins only; 2.0 ppm max., based on the weight of the whole fruit	Listed
Orange B	19235	Sausage and frankfurter casings or surfaces only; 150 ppm max., based on the weight of the finished product	Listed
	Exempt from Certification		
Annatto extract	75120		Listed
β-Apo-8′-carotenal	40820	Maximum—15 mg/lb of solid or semisolid food, or pint of liquid food	Listed
Canthaxanthin	40850	Maximum—30 mg/lb of solid or semisolid food, or pint of liquid food	Listed
Caramel			Listed
β-Carotene	75130 (Natural) 40800 (Synthetic)		Listed
Carrot oil			Listed
Cochineal extract and Carmine	75470		Listed
Corn endosperm oil		Chicken feed only	Listed
Dehydrated beets (beet powder)			Listed
Dried algae meal		Chicken feed only	Listed
Ferrous gluconate		Ripe olives only	Listed
Fruit juice			Listed
Grape color extract		Nonbeverage food only	Listed
Grape skin extract		Beverages only	Listed
Paprika			Listed
Paprika oleoresin			Listed
Riboflavin			Listed
Saffron	75100		Listed
Synthetic iron oxide	77491 77492 77499	Dog and cat food only; 0.25% (w/w) max.	Listed

TABLE 1 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
Tagetes meal and extract	75125	Chicken feed only	Listed
Titanium dioxide	77891	1% (w/w) Maximum in finished food	Listed
Toasted partially defatted cooked cottonseed flour			Listed
Turmeric	75300		Listed
Turmeric oleoresin	75300		Listed
Ultramarine blue	77007	Salt for animal feed only; 0.5% (w/w) max.	Listed
Vegetable juice			Listed

[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.

TABLE 2 COLORANTS PERMITTED IN DRUGS

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
		Subject to Certification	
FD&C Blue No. 1	42090		Listed
FD&C Blue No. 2	73015	Nylon sutures for use in general surgery only; 1% (w/w) max.	Listed
		Ingested drugs	Provisional
FD&C Green No. 3	42053		Listed
FD&C Red No. 3	45430	Ingested drugs	Listed
		Other uses	Provisional
FD&C Red No. 4	14700	Externally applied drugs only	Listed
FD&C Red No. 40	16035		Listed
FD&C Yellow No. 5	19140	Ingested drugs	Listed
		Other uses	Provisional
FD&C Yellow No. 6	15985		Provisional
D&C Blue No. 4	42090	Externally applied drugs only	Listed
D&C Blue No. 6	73000	Sutures only; 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	Listed

TABLE 2 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
		surgical use, 0.5% (w/w) max.; polypropylene surgical sutures for general surgical use, 0.5% (w/w) max.	
D&C Blue No. 9	69825	Cotton and silk sutures (including those for ophthalmic use) only; 2.5% (w/w) max.	Listed
D&C Green No. 5	61570	Nylon 66 and Nylon 6 nonabsorbable surgical sutures only; 0.6% (w/w) max.	Listed
		Other uses	Listed
D&C Green No. 6	61565	Polyethylene terephthalate sutures, 0.75% (w/w) max, and polyglycolic acid sutures, 0.1% (w/w) max. Sutures may be used for ophthalmic and general surgery.	Listed
		Externally applied drugs only	Listed
D&C Green No. 8	59040	Externally applied drugs only; 0.01% (w/w) max.	Listed
D&C Orange No. 4	15510	Externally applied drugs only	Listed
D&C Orange No. 5	45370:1	Ingested mouthwashes and dentifrices only	Listed
D&C Orange No. 10	45425:1	Externally applied drugs only	Listed
D&C Orange No. 11	45425	Externally applied drugs only	Listed
D&C Orange No. 17	12075	Externally applied drugs only	Provisional
D&C Red No. 6	15850		Listed
D&C Red No. 7	15850:1		Listed
D&C Red No. 8	15585	[b]	Provisional
D&C Red No. 9	15585:1	[b]	Provisional
D&C Red No. 17	26100	Externally applied drugs only	Listed
D&C Red No. 19	45170	Externally applied drugs only	Provisional
D&C Red No. 21	45380:2		Listed
D&C Red No. 22	45380		Listed
D&C Red No. 27	45410:1		Listed
D&C Red No. 28	45410		Listed
D&C Red No. 30	73360		Listed
D&C Red No. 31	15800:1	Externally applied drugs only	Listed
D&C Red No. 33	17200	[b]	Provisional
D&C Red No. 34	15880:1	Externally applied drugs only	Listed
D&C Red No. 36	12085	[b]	Provisional
D&C Red No. 37	45170:1	Externally applied drugs only	Provisional
D&C Red No. 39	13058	Externally applied quarternary ammonium germicides only; 0.1% (w/w) max.	Listed
D&C Violet No. 2	60725	Externally applied drugs only	Listed
D&C Yellow No. 7	45350:1	Externally applied drugs only	Listed

TABLE 2 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
D&C Yellow No. 8	45350	Externally appliied drugs only	Listed
D&C Yellow No. 10	47005	[b]	Provisional
D&C Yellow No. 11	47000	Externally applied drugs only	Listed
Ext. D&C Yellow No. 7	10316	Externally applied drugs only	Listed
		Exempt from Certification	
Alumina	77002		Listed
Aluminum powder	77000	Externally applied drugs only (1)	Listed
Annatto extract	75120	(1)	Listed
Bismuth oxychloride	77163	Externally applied drugs only (1)	Listed
Bronze powder	77440	Externally applied drugs only (1)	Listed
Calcium carbonate	77220		Listed
Canthaxanthin	40850	Ingested drugs only	Listed
Caramel			Listed
β-Carotene	75130 (Natural) 40800 (Synthetic)	(1)	Listed
Chromium-cobalt-aluminum oxide		Polyethylene sutures only; 2% (w/w) max.	Listed
Chromium hydroxide green	77289	Externally applied drugs only (1)	Listed
Chromium oxide greens	77288	Externally applied drugs only (1)	Listed
Cochineal extract and Carmine	75470		Listed
Copper powder	77400	Externally applied drugs only (1)	Listed
Dihydroxyacetone		Externally applied drugs intended solely or in part for imparting color to the human body	Listed
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	Listed
Ferric ammonium ferrocyanide		Externally applied drugs only (1)	Listed
Ferric ferrocyanide	77510 77520	Externally applied drugs only (1)	Listed
Guanine	75170	Externally applied drugs only (1)	Listed
Logwood extract	75290	Nylon 66, Nylon 6, and silk nonabsorbable sutures for use in general and ophthalmic surgery only; 1.0% (w/w) max.	Listed
Mica	77019	Externally applied drugs only (1)	Listed

TABLE 2 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
Potassium sodium copper chlorophyllin	75810	Dentifrices only; 0.1% max.	Listed
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	Listed
Pyrophyllite		Externally applied drugs only	Listed
Synthetic iron oxide	77491 77492 77499	5 mg/day (as Fe) in drugs that are ingested	Listed
Talc	77019		Listed
Titanium dioxide	77891	(1)	Listed
Zinc oxide	77947	Externally applied drugs only (1)	Listed

[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.

[b]Temporary regulations limit the quantity of this colorant that can be used in certain products.

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

TABLE 3 COLORANTS PERMITTED IN COSMETICS

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
	Subject to Certification		
FD&C Blue No. 1	42090		Listed
FD&C Green No. 3	42053		Listed
FD&C Red No. 3	45430		Provisional
FD&C Red No. 4	14700	External use only	Listed
FD&C Red No. 40	16035		Listed
FD&C Yellow No. 5	19140		Provisional
FD&C Yellow No. 6	15985		Provisional
D&C Blue No. 4	42090	External use only	Listed
D&C Brown No. 1	20170	External use only	Listed
D&C Green No. 5	61570		Listed
D&C Green No. 6	61565	External use only	Listed
D&C Green No. 8	59040	External use only; 0.01% (w/w) max.	Listed
D&C Orange No. 4	15510	External use only	Listed
D&C Orange No. 5	45370:1	5.0% (w/w) max. in lipstick and other lip cosmetics; ingested mouthwashes and dentifrices	Listed

TABLE 3 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
D&C Orange No. 10	45425:1	External use only	Listed
D&C Orange No. 11	45425	External use only	Listed
D&C Orange No. 17	12075	External use only	Provisional
D&C Red No. 6	15850		Listed
D&C Red No. 7	15850:1		Listed
D&C Red No. 8	15585	[b]	Provisional
D&C Red No. 9	15585:1	[b]	Provisional
D&C Red No. 17	26100	External use only	Listed
D&C Red No. 19	45170	External use only	Provisional
D&C Red No. 21	45380:2		Listed
D&C Red No. 22	45380		Listed
D&C Red No. 27	45410:1		Listed
D&C Red No. 28	45410		Listed
D&C Red No. 30	73360		Listed
D&C Red No. 31	15800:1	External use only	Listed
D&C Red No. 33	17200	[b]	Provisional
D&C Red No. 34	15880:1	External use only	Listed
D&C Red No. 36	12085	[b]	Provisional
D&C Red No. 37	45170:1	External use only	Provisional
D&C Violet No. 2	60725	External use only	Listed
D&C Yellow No. 7	45350:1	External use only	Listed
D&C Yellow No. 8	45350	External use only	Listed
D&C Yellow No. 10	47005	[b]	Provisional
D&C Yellow No. 11	47000	External use only	Listed
Ext. D&C Violet No. 2	60730	External use only	Listed
Ext. D&C Yellow No. 7	10316	External use only	Listed
Exempt from Certification			
Aluminum powder	77000	External use only (1)	Listed
Annatto	75120	(1)	Listed
Bismuth citrate		Hair dyes for scalp only; 0.5% (w/v) max.	Listed
Bismuth oxychloride	77163	(1)	Listed
Bronze powder	77440	(1)	Listed
Caramel		(1)	Listed
Carmine	75470	(1)	Listed
β-Carotene	75130 (Natural) 40800 (Synthetic)	(1)	Listed
Chromium hydroxide green	77289	External use only (1)	Listed
Chromium oxide greens	77288	External use only (1)	Listed
Copper powder	77400	(1)	Listed
Dihydroxyacetone		External use only	Listed
Disodium EDTA-copper		Shampoos only	Listed
Ferric ammonium ferrocyanide		External use only (1)	Listed
Ferric ferrocyanide (iron blue)	77510 77520	External use only (1)	Listed

TABLE 3 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations[a]	Current Status
Guaiazulene		External use only	Listed
Guanine	75170	(1)	Listed
Henna	75480	Hair dyes only, not near eye	Listed
Lead acetate		Hair dyes for scalp only; 0.6% (w/v) max. as Pb	Listed
Manganese violet	77742	(1)	Listed
Mica	77019	(1)	Listed
Potassium sodium copper chlorophyllin	75810	Dentifrices only; 0.1% max. (2)	Listed
Pyrophyllite		External use only	Listed
Silver		Fingernail polish only; 1% max.	Listed
Synthetic iron oxides	77491 77492 77499	(1)	Listed
Titanium dioxide	77891	(1)	Listed
Ultramarine blue	77007	External use only (1)	Listed
Ultramarine green	77013	External use only (1)	Listed
Ultramarine pink	77007	External use only (1)	Listed
Ultramarine red	77007	External use only (1)	Listed
Ultramarine violet	77007	External use only (1)	Listed
Zinc oxide	77947	(1)	Listed

[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.

[b]Temporary regulations limit the quantity of this colorant that can be used in certain products.

(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 4 COLORANTS PERMITTED IN MEDICAL DEVICES

Food and Drug Administration Official Name	Color Index Number	Limitations	Current Status
		Subject to Certification	
D&C Green No. 6	61565	Contact lenses only; 0.03% (w/w) max.	Listed
[Phthalocyaninato (2-)] Copper	74160	Polypropylene sutures for use in general and ophthalmic surgery; 0.5% (w/w) max. Contact lenses; 0.01% (w/w) max.	Listed

TABLE 4 (*Continued*)

Food and Drug Administration Official Name	Color Index Number	Limitations	Current Status
		Exempt from Certification	
2-[[2,5-Diethoxy-4-[(4-Methylphenyl)Thio] 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.	Listed

TABLE 5 CHRONOLOGICAL HISTORY OF SYNTHETIC FOOD COLORS IN THE UNITED STATES

Year Listed for Food Use	Common Name	FDA Name	Color 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	1918	No
1918	Butter Yellow	—		1918	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
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[a] Red AC	FD&C Red No. 40	16035	—	Yes

[a]Registered trademark of Buffalo Color Corporation.

Chapter 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 important foods such as meat, 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.

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 leads to 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 processing. 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 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 of the dairy farmer are equally 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.

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 as binders, vehicles, or di-

luents to give the product desirable application properties but that have little inherent cosmetic value.

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

Colorants are used in numerous medical devices, for a variety of reasons. At this time, color additives have been listed for use in only two kinds of devices—sutures and contact lenses. Sutures are usually colored to make them more visible during surgery. Contact lenses too are colored to make them more visible when handling and cleaning them, but also to enhance the color of lightly-colored eyes. Blue or green lenses, for example, can add sparkle to grey or blue eyes. Beauty is usually not a major consideration when adding color to most medical devices, and no official color scheme dictates what colorant to add to a particular product.

Chapter 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.

*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.

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. This 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. An example of such a request form is shown on page 25. In the case of straight colors, including lakes, a fee is charged for this certification service at the rate of $0.25 per pound of the batch, with a minimum charge of $160. 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 on pages 26 and 27.) 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

All 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 all colorants can be found in Appendix A.*

*Interestingly, 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%.

REQUEST FOR CERTIFICATION OF A BATCH OF STRAIGHT COLOR ADDITIVE

Date. . . . . .

Division of Color Technology
HFF-430, Bureau of Foods
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)

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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. AA-3003
DATE MARCH 21, 1980

NYK-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	MFG. BATCH NO	QUANTITY IN LBS.	CERT% PURE COLOR
FD&C YELLOW #6	3800-J	1212	91

CERTIFIED FOR USE IN;
FOODS, DRUGS AND COSMETICS

Keith S. Heine
KEITH S. HEINE

FOR THE
COMMISSIONER OF FOOD AND DRUGS

FORM FDH 3000 20-JUL-78

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

LOT NO. ZB 0000

DATE March 21, 1980

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	MFR BATCH NO.	QUANTITY IN LBS	CERT % PURE COLOR	CERTIFIED FOR USE IN
D&C Red #21	56	3300	99	Drugs & Cosmetics

cc: CIN-DO

Keith S. Heine

FOR THE
COMMISSIONER OF FOOD AND DRUGS.

FORM FDA 3000 (9/80) PREVIOUS EDITION IS OBSOLETE.

FD&C Red No. 40

Specifications: 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%.

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-naphthalenesulfonic 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

Specifications: 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

Specifications: 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 the meaning they once had.

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 the level necessary 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. Some years ago the European Economic Community prepared a list of approved colorants in the hope that this would pave the way for a greater universality of colors, but to date their attempts have met with little success.

More 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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Chapter 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 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 have also been 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 as is ("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, 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 6.

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.

Unsulfonated pigments such as D&C Orange No. 17 and D&C Red No. 36 are insoluble directly on coupling and contain no groups capable of salt formation. Each contains a chlorine or nitro group in the *ortho* position relative to the azo group, resulting in a sterically hindered molecule with low solubility and excellent light stability.

TABLE 6 REPRESENTATIVE SECONDARY MIXES

Shade[a]	Parts by Weight					
	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

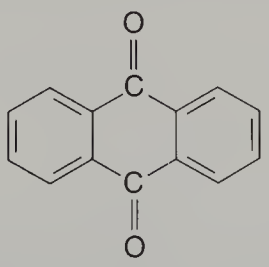
[a]Obviously, more than one combination of colorants can be used to produce a particular shade. The mixture to use depends on the effect desired and the product to be colored.

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

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, 9, and 34 are insoluble sulfonated pigments.

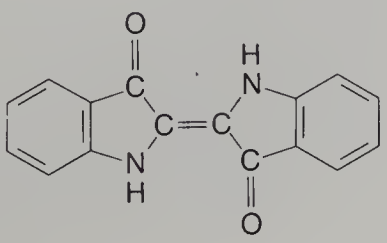
The soluble azo dyes contain one or more sulfonic acid groups. Their degree of water solubility is determined by the number of sulfonic groups present and their position in the molecule. FD&C Red No. 40 and D&C Orange No. 4 belong in this class.

Anthraquinone colorants all contain the following structure:

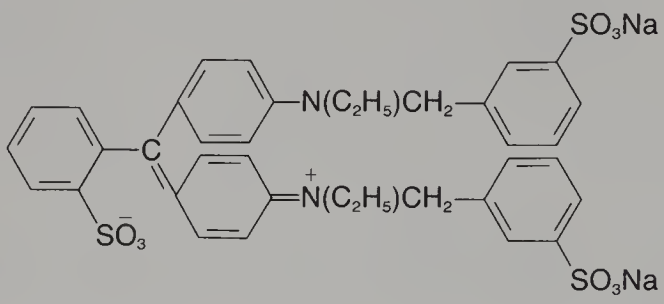


Included in this grouping are D&C Green No. 5, a water-soluble sulfonate, D&C Green No. 6, an unsulfonated water-insoluble compound, and D&C Violet No. 2, a water-insoluble hydroxyanthraquinone. Anthraquinone color additives, in general, are light stable and have good physical and chemical properties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

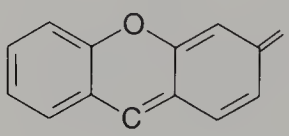
There are three color additives of the indigoid type, including D&C Blue No. 6 (indigo, an insoluble pigment), FD&C Blue No. 2 (the water-soluble disodium sulfonate derivative of indigo), and D&C Red No. 30 (an insoluble thioindigoid). All are related to the basic indigo structure. D&C Blue No. 6 has 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 systems. FD&C Blue No. 1 has the following structure:



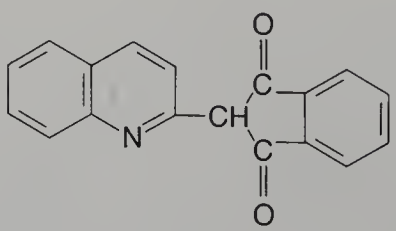
The second largest group of color additives are the xanthenes, which are characterized by the following structure:



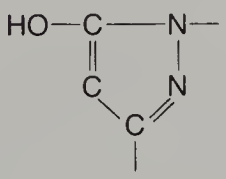
Xanthene colors can be either acidic or basic. Acid xanthenes are known to exist in two tautomeric forms. The phenolic type, or "fluorans", are free-acid structures such as D&C Orange No. 10 and D&C Red No. 21. Most have poor water solubility. In contrast to these, the quinoids or xanthenes are usually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

D&C Red No. 19 is the only certifiable basic xanthene colorant.

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. 64–90.

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 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 usually available with various pure dye contents (10–40%) and moisture levels. They 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, and their superior stability toward heat and light. Such properties have made possible the more effective and more efficient preparation of candy and pill 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, and oils, and for coloring food-packaging materials including lacquers, 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.

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 the 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 no 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 consider seriously the application properties desired 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 7–17. 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,

TABLE 7 WATER SOLUBILITY OF FD&C COLORS

Federal Name	Common Name	2°C		25°C		60°C	
		g/100 mL	oz/gal	g/100 mL	oz/gal	g/100 mL	oz/gal
FD&C Blue No. 1	Brill. 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™[a] Red AC	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

[a]Registered trademark of Buffalo Color Corporation.

TABLE 8 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 9 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 10 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 11 pH STABILITY OF FD&C COLORS

Federal Name	Common Name	pH = 3	pH = 5	pH = 7	pH = 8
FD&C Blue No. 1	Brill. 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™[a] Red AC	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

[a]Registered trademark of Buffalo Color Corporation.

TABLE 12 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	Brill. 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™[a] Red AC	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

[a]Registered trademark of Buffalo Color Corporation.

TABLE 13 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	Brill. 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™[a] Red AC	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

[a]Registered trademark of Buffalo Color Corporation.

TABLE 14 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	Brill. 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™[a] Red AC		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

[a]Registered trademark of Buffalo Color Corporation.

TABLE 15 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	Brill. 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™[a] Red AC	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

[a]Registered trademark of Buffalo Color Corporation.

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 18. 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 19 and 20. The picture Table 19 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 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 21.

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 1.1). Often, the name given is not the best, reflecting certain inconsistencies in the nomenclature 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 and represent each colorant's principal component.

FD&C Blue No. 1

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

TABLE 16 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 Orange No. 17	I	D	Ia	Ia	D
D&C Red No. 6	S	S	SS	Ia	I
D&C Red No. 7	I	D	Ia	Ia	D
D&C Red No. 8	I	D	Ia	Ia	D
D&C Red No. 9	I	D	Ia	Ia	D
D&C Red No. 17	I	SS	SS-M	SS	S
D&C Red No. 19	SF	SF	SF	SF	I
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. 37	Ia	SS	SF	SF	IE
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 16 AND 17:

a—May bleed or stain, very sparingly soluble.
B—Insoluble in water, soluble in aqueous alkaline solution.
b—Turns much bluer in hue.
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.
G—Soluble or dispersible in oils and waxes in presence of 10–25% of a fatty acid.

TABLE 16 (*Continued*)

Toluene	Stearic Acid	Oleic Acid	Mineral Oil	Mineral Wax	Et_2O	Me_2CO	AcOBu
I	C	C	C	C	I	la	I
la	D	D	D	D	I	I	I
la	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	la	la	I	I	la	la	la
I	IE	IE	IE	IE	I	la	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	D	D	D	D	la	la	I
I	I	I	I	I	I	la	I
I	D	D	D	D	I	la	I
I	D	D	D	D	I	la	I
I	D	D	D	D	I	I	I
S	S	S	S	S	SS	SS	M
I	IC	SS	IC	IC	S*	SF	I
I	D	D	D	D	M*	S	I
I	IE	IE	IE	IE	la	SS	I
I	D	D	D	D	la	SS	I
I	IE	IE	IE	IE	la	SS	I
la	D	D	D	D	la	la	la
I	I	I	I	I	I	la	la
I	I	I	I	I	I	I	I
I	D	D	D	D	I	D	D
I	D	D	D	D	I	la	D
S	S	S	IEG	IEG	S*	SF	SS
la	I	SS	I	la	S	S	SS
S	S	S	S	SW	SS	SS	S
I	D	D	D	D	SS*	S	I
I	IE	IE	IE	IE	la	kla	I
I	I	I	I	I	la	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

I—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 17 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	7l	5l	L6U
D&C Blue No. 9	7	7l	5l	6lU
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	5l	6l
D&C Green No. 8	2	l	l	5
D&C Orange No. 4	5	5	5	2m
D&C Orange No. 5	2	4al	4l	Sr
D&C Orange No. 10	2	4al	4l	Sr
D&C Orange No. 11	Similar to D&C Red No. 3			
D&C Orange No. 17	5	5l	ld	ldr
D&C Red No. 6	5	5	4	4d
D&C Red No. 7	6	5l	4l	5l
D&C Red No. 8	6	6l	4ld	4ld
D&C Red No. 9	6	6l	4l	4l
D&C Red No. 17	3	5L	4ld	5l
D&C Red No. 19	3	5	5	2p
D&C Red No. 21	2	3l	3l	5Sr
D&C Red No. 22	2	2py	1py	5
D&C Red No. 27	2	3l	3	5Sr
D&C Red No. 28	3	2p	4p	6
D&C Red No. 30	6	7l	l	6lU
D&C Red No. 31	5	5	4	5
D&C Red No. 33	5	6	3z	5
D&C Red No. 34	4	5l	4	4l
D&C Red No. 36	6	6l	4d	4d
D&C Red No. 37	3	6l	5l	la
D&C Red No. 39	2	Sv	Sv	6Sx
D&C Violet No. 2	4	5l	5l	5l
D&C Yellow No. 7	2	l	l	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	l	5l	lw
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.

TABLE 17 (*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	J-p	J-p	3	3
I	I	I	3	3
I	I	I	3	3
Similar to D&C Red No. 3				
I	I	I	3	2
6	p	p	3	1
I	4Id	4I	3	1
I	4Id	4I	3	1
I	4Id	4I	3	1
I	4Id	4I	3	1
6	6	6	3	5
I	Id	4I	4	4
6	3d	2y	4	4
I	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
Ia	I	Ia	3	5
I	4aId	I	3	3
6I	4I	4I	2	1
I	I	I	3	3
6	z-p	p	3	3
6	z	4	2	5
I	I	I	2	5
6	4z	4	3	2
6	zd	4	3	3

TABLE 18 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	7,032	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 18 (*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]	16,462.05[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	5,069,974.05
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	1,633.11
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	—

TABLE 18 (*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 Copper	524	—	—	—	154	—
	39,153	28,653	22,709	32,849	19,693	43,333

[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.

TABLE 18 (*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	133,222.91
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	7,085.41
36,731	49,811	56,348	40,206	26,293	48,925	52,016.12
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	66,243.24
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	14,395.64
18,061	3,643	3,250	8,519	2,489	2,651	1,559.17
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	9,001.05
5,866	2,460	3,520	—	4,042	—	1,653.5
7,289	4,449	22,018	25,840	38,757	66,311	55,058.11
274,692	311,972	391,040	389,989	354,554	386,150.7	423,610.84
—	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	667.99
29,045	38,909	17,788	—	—	—	—
—	—	—	—	77.3	—	—
30,797	38,909	17,788	0	77.3	881	667.99

TABLE 19 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
Meat-stamping 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 20 MAJOR CATEGORIES OF PROCESSED FOOD MANUFACTURED USING CERTIFIED COLORS AND LEVELS OF COLOR USED

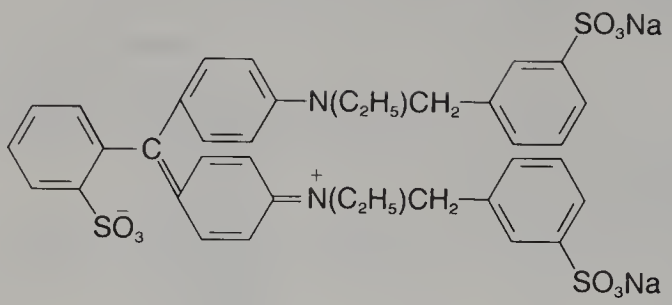
Category	Level of Color Used	
	Range (ppm)	Average (ppm)
Candy and confections	10–400	100
Beverages (liquid and powdered)	5–200	75
Dessert powders	5–600	140
Cereals	200–500	350
Maraschino cherries	100–400	200
Pet foods	100–400	200
Bakery goods	10–500	50
Ice cream and sherbets	10–200	30
Sausage (surface)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

TABLE 21 APPROXIMATE PER CAPITA CONSUMPTION OF SOME COMMON ITEMS IN THE UNITED STATES

Certified Color Additives[a]	0.024 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 1978–1981 by a population of 226.5 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.

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-sulfobenzyl)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.

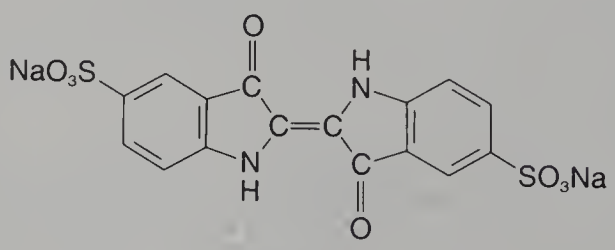
Manufacturing Process: Condense benzaldehyde-o-sulfonic acid with α-(*N*-ethylanilino)-*m*-toluenesulfonic acid ("benzylethylaniline sulfonic acid").

FD&C Blue No. 2

Synonyms: Indigotine, Indigo Carmine; CI Food Blue 1 (73015).

CAS Reg. No.: 860-22-0.

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: 466.35.

Dye Classification: Indigoid.

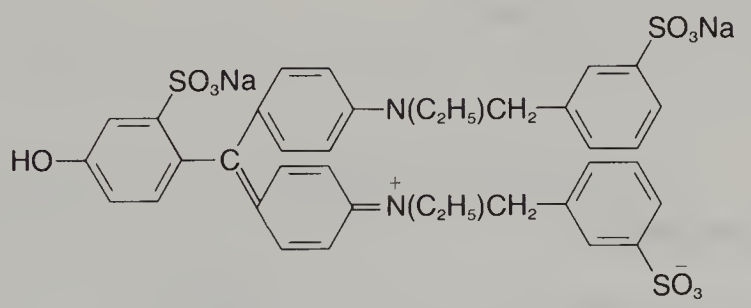
Manufacturing Process: Sulfonation of indigo.

FD&C Green No. 3

Synonyms: Fast Green FCF; CI Food Green 3 (42053).

CAS Reg. No.: 2353-45-9.

Chemical Structure:



Identity: Principally *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide, inner salt disodium salt.

Empirical Formula: $C_{37}H_{34}O_{10}N_2S_3Na_2$.

Molecular Weight: 808.84.

Dye Classification: Triphenylmethane.

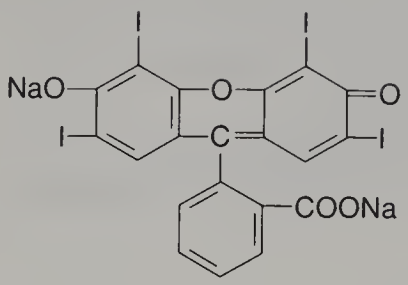
Manufacturing Process: Condense *p*-hydroxybenzaldehyde-o-sulfonic acid with α-(*N*-ethylanilino)-*m*-toluenesulfonic acid.

FD&C Red No. 3

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

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

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.

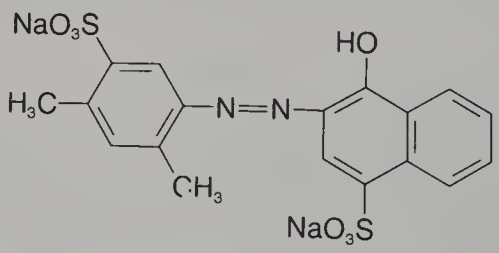
Manufacturing Process: Iodination of fluorescein (D&C Yellow No. 7).

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: 480.42.

Dye Classification: Monoazo.

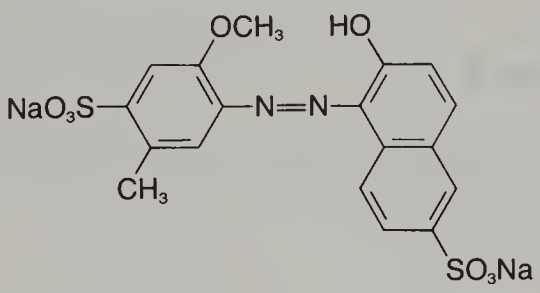
Manufacturing Process: Couple diazotized 1-amino-2,4-dimethylbenzene-5-sulfonic acid with 1-naphthol-4-sulfonic acid.

FD&C Red No. 40

Synonyms: Allura™ Red AC; 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: 496.42.

Dye Classification: Monoazo.

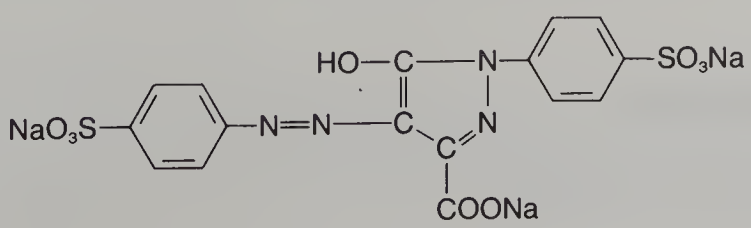
Manufacturing Process: Couple diazotized 5-amino-4-methoxy-2-toluenesulfonic acid with 6-hydroxy-2-naphthalenesulfonic acid.

FD&C Yellow No. 5

Synonyms: Tartrazine; CI Food Yellow 4 (19140).

CAS Reg. No.: 1934-21-0.

Chemical Structure:



Identity: Trisodium salt of 5-oxo-1-(*p*-sulfophenyl)-4-[(*p*-sulfophenyl) azo]-2-pyrazoline-3-carboxylic acid.

Empirical Formula: $C_{16}H_9N_4O_9S_2Na_3$.

Molecular Weight: 534.36.

Dye Classification: Pyrazolone.

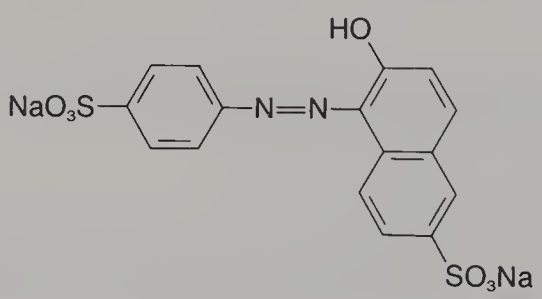
Manufacturing Processes: (a) Condense phenylhydrazine-*p*-sulfonic acid with oxalacetic ester, couple the product with diazotized sulfanilic acid, then hydrolyze the ester with sodium hydroxide or (b) condense phenylhydrazine-*p*-sulfonic acid with dihydroxytartaric acid.

FD&C Yellow No. 6

Synonyms: Sunset Yellow; CI Food Yellow 3 (15985).

CAS Reg. No.. 2783-94-0.

Chemical Structure:



Identity: Disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid.

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

Molecular Weight: 452.36.

Dye Classification: Monoazo.

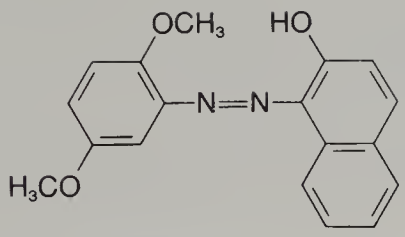
Manufacturing Process: Couple diazotized sulfanilic acid with 2-naphthol-6-sulfonic acid.

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.

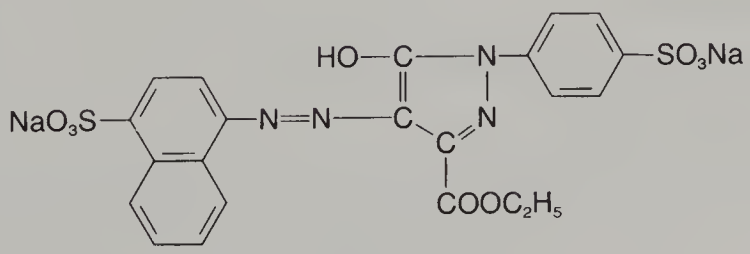
Dye Classification: Monoazo.

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-ethylcarboxy-4-(4-sulfonaphthylazo)-5-hydroxypyrazole.

Empirical Formula: $C_{22}H_{16}N_4O_9S_2Na_2$.

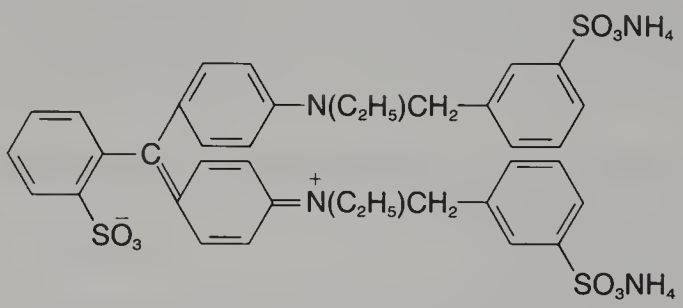
Molecular Weight: 590.49.

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

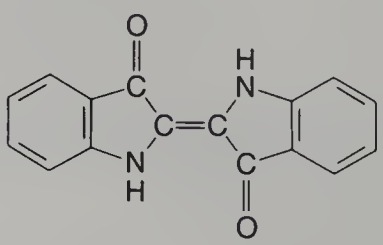
Synonyms: Alphazurine FG, Erioglaucine; CI Acid Blue 9 (42090).
CAS Reg. No.: 6371-85-3.
Chemical Structure:



Identity: NH_4 Salt corresponding to FD&C Blue No. 1.
Empirical Formula: $C_{37}H_{42}N_4O_9S_3$.
Molecular Weight: 782.94.
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.

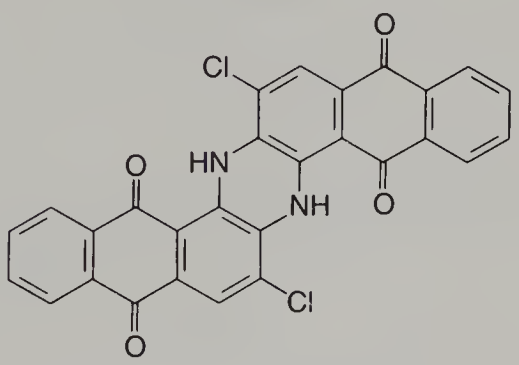
Manufacturing Processes: (a) Convert *N*-phenylglycine into pseudoindoxyl by fusion with sodium amide (or sodium and a current of ammonia) in the presence of a mixture of potassium and sodium hydroxides and sodium cyanide. Oxidize the pseudoindoxyl with air; (b) convert phenylglycine-o-carboxylic acid[*N*-(carboxymethyl)-anthranilic 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.

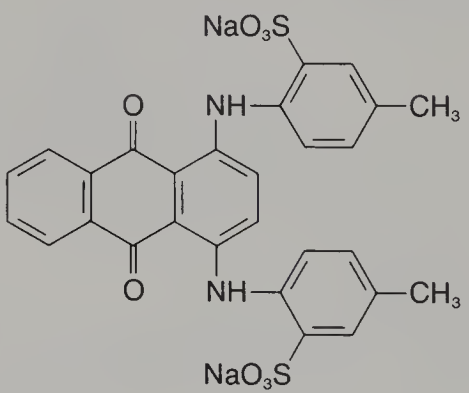
Manufacturing Process: Couple diazotized sulfanilic acid and diazotized crude xylidine with resorcinol.

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: 622.57.

Dye Classification: Anthraquinone.

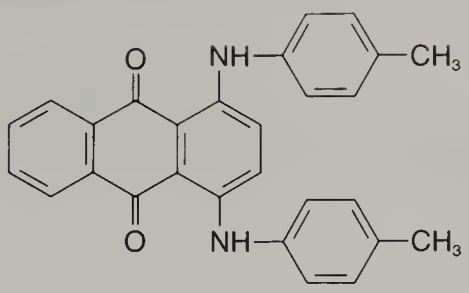
Manufacturing Process: Condense leucoquinizarin with *p*-toluidine, then sulfonate.

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.

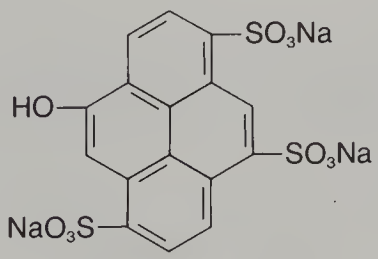
Manufacturing Process: Condense leucoquinizarin with *p*-toluidine.

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: 524.37.

Dye Classification: Pyrene.

Manufacturing Process: Sulfonate pyrene to tetrasulfonic acid, salt

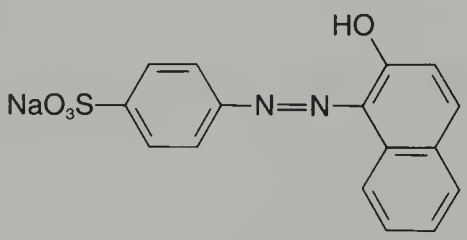
out with sodium chloride, hydrolyze in sodium hydroxide solution, add formic acid, and salt out with sodium chloride.

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: 350.32.

Dye Classification: Monoazo.

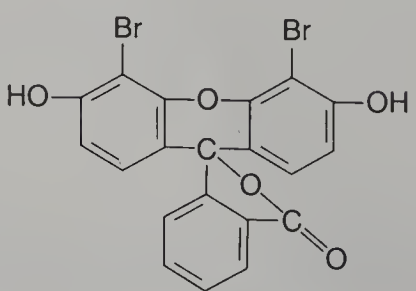
Manufacturing Process: Couple diazotized sulfanilic acid with 2-naphthol.

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 of principally 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: 490.10.

Dye Classification: Fluoran.

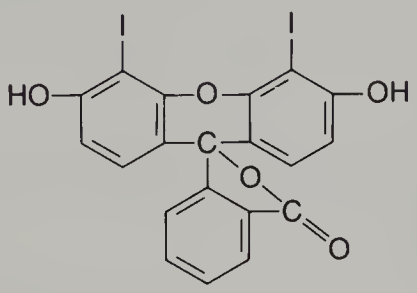
Manufacturing Process: Brominate fluorescein (D&C Yellow No. 7).

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.

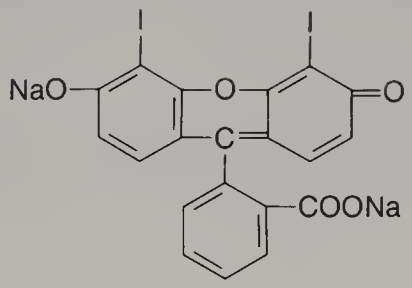
Dye Classification: Fluoran.

Manufacturing Process: Iodinate fluorescein (D&C Yellow No. 7).

D&C Orange No. 11

Synonyms: Erythrosine Yellowish Na; CI Acid Red 95 (45425).

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: $C_{20}H_8N_2O_5I_2$.

Molecular Weight: 628.07.

Dye Classification: Xanthene.

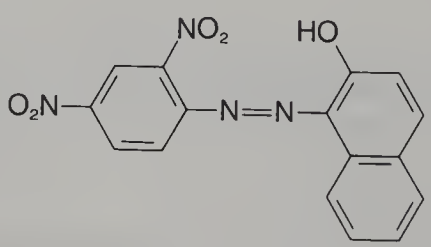
Manufacturing Process: Convert D&C Orange No. 10 to the Na salt.

D&C Orange No. 17

Synonyms: Permatone Orange; CI Pigment Orange 5 (12075).

CAS Reg. No.: 3468-63-1.

Chemical Structure:



Identity: 1-(2,4-Dinitrophenylazo)-2-naphthol.

Empirical Formula: $C_{16}H_{10}N_4O_5$.

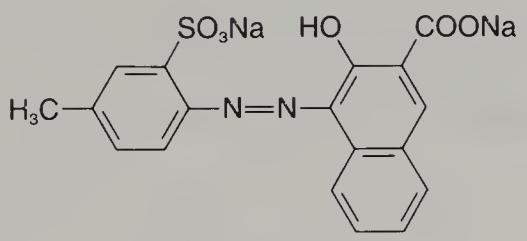
Molecular Weight: 338.28.

Dye Classification: Monoazo.

Manufacturing Process: Couple diazotized 2,4-dinitroaniline with 2-naphthol.

D&C Red No. 6

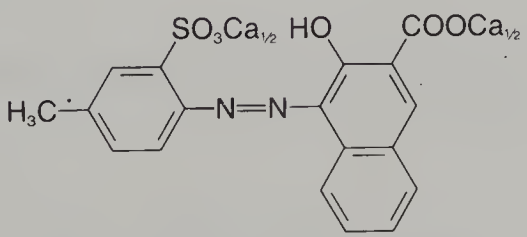
Synonyms: Lithol Rubin B; CI Pigment Red 57 (15850).
CAS Reg. No.: 5858-81-1.
Chemical Structure:



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: 430.34.
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:

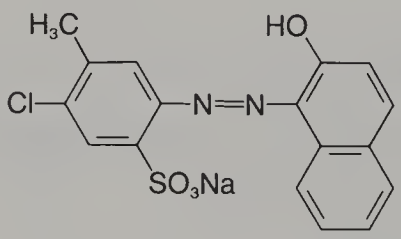


Identity: Principally the calcium salt of 3-hydroxy-4-[(4-methyl-2-sulfophenyl)azo]-2-naphthalenecarboxylic acid.
Empirical Formula: $C_{18}H_{12}N_2O_6SCa$.

Molecular Weight: 424.44.
Dye Classification: Monoazo.
Manufacturing Process: Heat D&C Red No. 6 with $CaCl_2$.

D&C Red No. 8

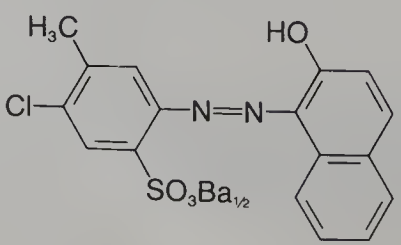
Synonyms: Lake Red C; CI Pigment Red 53 (15585).
CAS Reg. No.: 2092-56-0.
Chemical Structure:



Identity: Monosodium salt of 1-(4-chloro-o-sulfo-5-tolylazo)-2-naphthol.
Empirical Formula: $C_{17}H_{12}N_2O_4SNaCl$.
Molecular Weight: 398.80.
Dye Classification: Monoazo.
Manufacturing Process: Couple diazotized 2-amino-5-chloro-*p*-toluenesulfonic acid with 2-naphthol.

D&C Red No. 9

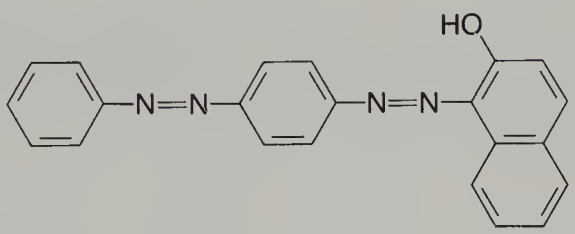
Synonyms: Lake Red C Ba; CI Pigment Red 53:1 (15585:1).
CAS Reg. No.: 5160-02-1.
Chemical Structure:



Identity: Monobarium salt of 1-(4-chloro-o-sulfo-5-tolylazo)-2-naphthol.
Empirical Formula: $C_{17}H_{12}N_2O_4SBa_{1/2}Cl$.
Molecular Weight: 444.46.
Dye Classification: Monoazo.
Manufacturing Process: Boil D&C Red No. 8 with $BaCl_2$.

D&C Red No. 17

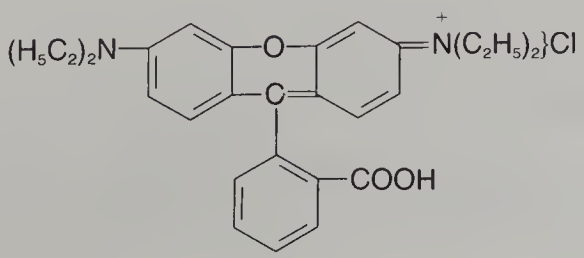
Synonyms: Toney Red, Sudan III; CI Solvent Red 23 (26100).
CAS Reg. No.: 85-86-9.
Chemical Structure:



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. 19

Synonyms: Rhodamine B; CI Basic Violet 10 (45170).
CAS Reg. No.: 81-88-9.
Chemical Structure:



Identity: 3-Ethochloride of 9-o-carboxyphenyl-6-diethylamino-3-ethylimino-3-isoxanthene.

Empirical Formula: $C_{28}H_{31}N_2O_3Cl$.

Molecular Weight: 479.02.

Dye Classification: Xanthene.

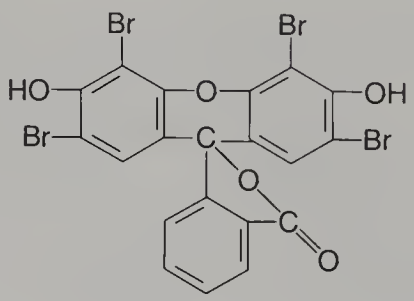
Manufacturing Processes: Fuse *m*-diethylaminophenol with phthalic anhydride, then treat the base with dilute HCl; or treat fluorescein chloride under pressure with diethylamine.

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.

Empirical Formula: $C_{20}H_8O_5Br_4$.

Molecular Weight: 647.90.

Dye Classification: Fluoran.

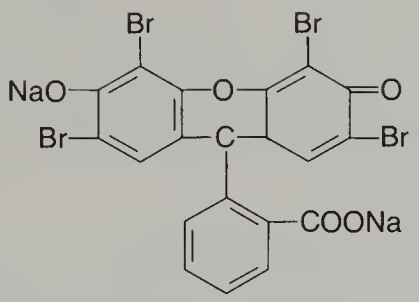
Manufacturing Process: Brominate fluorescein (D&C Yellow No. 7).

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.

Empirical Formula: $C_{20}H_6O_5Na_2Br_4$.

Molecular Weight: 691.86.

Dye Classification: Xanthene.

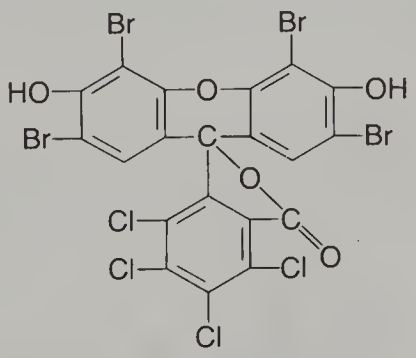
Manufacturing Process: Convert D&C Red No. 21 to the Na salt.

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: $C_{20}H_4O_5Cl_4Br_4$.

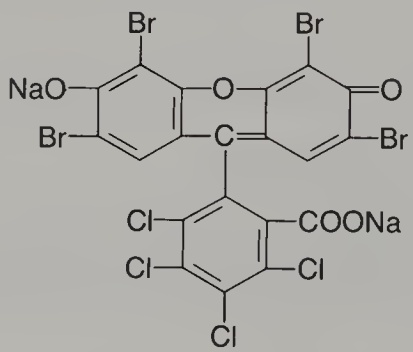
Molecular Weight: 785.68.

Dye Classification: Fluoran.

Manufacturing Process: Condense resorcinol with tetrachlorophthalic anhydride, then brominate.

D&C Red No. 28

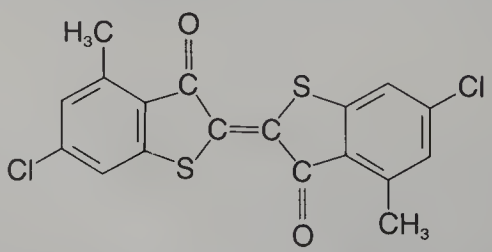
Synonyms: Phloxine B; CI Acid Red 92 (45410).
CAS Reg. No.: 18472-87-2.
Chemical Structure:



Identity: Principally the disodium salt of 2′,4′,5′,7′-tetrabromo-4,5,6,7-tetrachlorofluorescein.
Empirical Formula: $C_{20}H_2O_5Na_2Cl_4Br_4$.
Molecular Weight: 829.64.
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: 393.30.

Dye Classification: Indigoid.

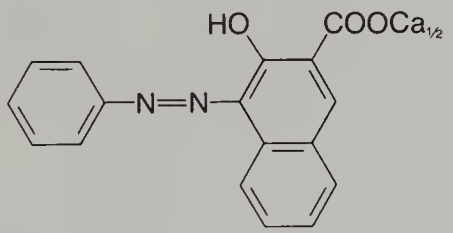
Manufacturing Process: Oxidize 6-chloro-4-methyl-thioindoxyl, or chlorinate 4,4'-dimethylthioindigo.

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.

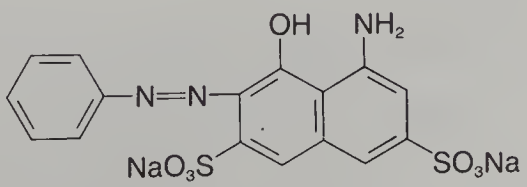
Manufacturing Process: Couple diazotized aniline with 3-hydroxy-2-naphthoic acid and then convert to the Ca salt.

D&C Red No. 33

Synonyms: Acid Fuchsine; CI Acid Red 33 (17200).

CAS Reg. No.: 3567-66-6.

Chemical Structure:



Identity: Disodium salt of 8-amino-2-phenylazo-1-naphthol-3,6-disulfonic acid.

Empirical Formula: $C_{16}H_{11}N_3O_7S_2Na_2$.

Molecular Weight: 467.38.

Dye Classification: Monoazo.

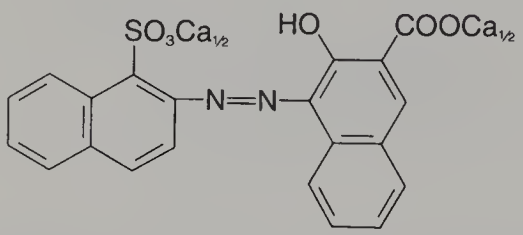
Manufacturing Process: Couple diazotized aniline with 8-amino-1-naphthol-3,6-disulfonic acid in alkaline solution.

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: 460.47.

Dye Classification: Monoazo.

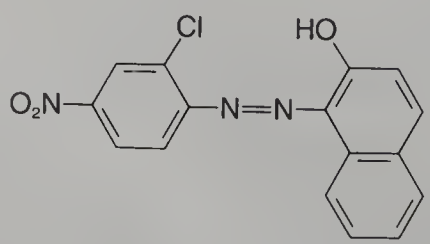
Manufacturing Process: Couple diazotized 2-naphthylamine-1-sulfonic acid with 3-hydroxy-2-naphthoic acid and then convert to the Ca salt.

D&C Red No. 36

Synonyms: Flaming Red; CI Pigment Red 4 (12085).

CAS Reg. No.: 2814-77-9.

Chemical Structure:



Identity: 1-(o-Chloro-*p*-nitrophenylazo)-2-naphthol.

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. 37

Synonyms: Rhodamine B—Stearate.

CAS Reg. No.: 6373-07-5.

Identity: 3-Ethostearate of 9-o-carboxyphenyl-6-diethylamino-3-ethylimino-3-isoxanthene.

Empirical Formula: $C_{46}H_{66}N_2O_5$.

Molecular Weight: 727.04.

Dye Classification: Xanthene.

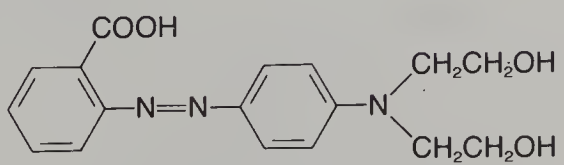
Manufacturing Process: Same as for D&C Red No. 19, except treat the base with stearic acid.

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: $C_{17}H_{19}N_3O_4$.

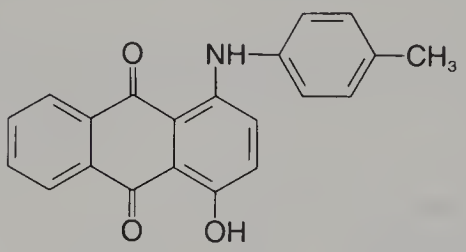
Molecular Weight: 329.36.

Dye Classification: Monoazo.

Manufacturing Process: Couple diazotized anthranilic acid with 2,2′-(phenylimino)diethanol.

D&C Violet No. 2

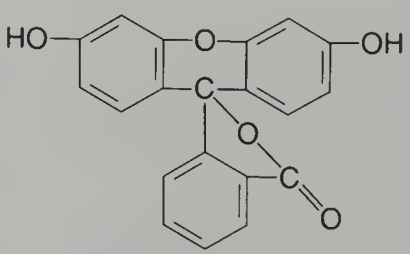
Synonyms: Alizurol Purple SS; CI Solvent Violet 13 (60725).
CAS Reg. No.: 81-48-1.
Chemical Structure:



Identity: Principally 1-hydroxy-4-[(4-methylphenyl)amino]-9,10-anthracenedione.
Empirical Formula: $C_{21}H_{15}NO_3$.
Molecular Weight: 329.35.
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:

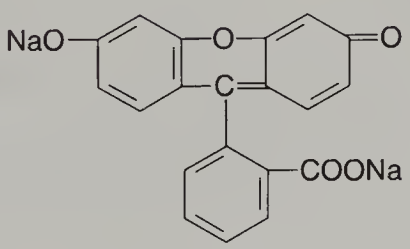


Identity: Principally fluorescein.
Empirical Formula: $C_{20}H_{12}O_5$.
Molecular Weight: 332.31.
Dye Classification: Fluoran.

Manufacturing Process: Condense resorcinol with phthalic anhydride in the presence of $ZnCl_2$ or H_2SO_4.

D&C Yellow No. 8

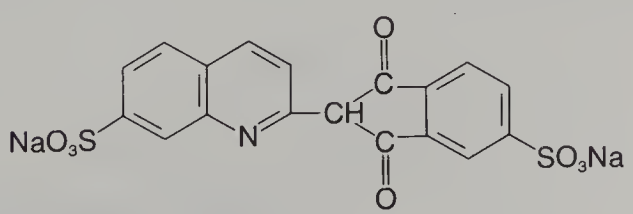
Synonyms: Uranine; CI Acid Yellow 73 (45350).
CAS Reg. No.: 518-47-8.
Chemical Structure:



Identity: Principally the disodium salt of fluorescein.
Empirical Formula: $C_{20}H_{10}O_5Na_2$.
Molecular Weight: 376.27.
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.
Chemical Structure:

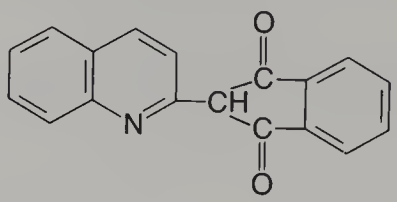


Identity: Disodium salt of the disulfonic acid of 2-(2-quinolyl)-1,3-indandione.
Empirical Formula: $C_{18}H_9NO_8S_2Na_2$.

Molecular Weight: 477.37.
Dye Classification: Quinoline.
Manufacturing Process: Sulfonate D&C Yellow No. 11.

D&C Yellow No. 11

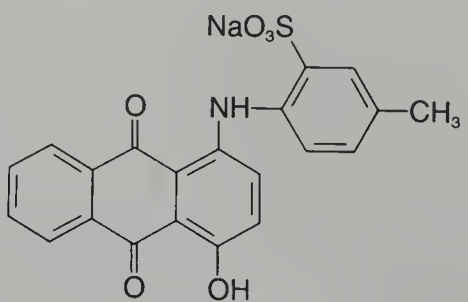
Synonyms: Quinoline Yellow SS, Quinoline Yellow Spirit Soluble; CI Solvent Yellow 33 (47000).
CAS Reg. No.: 8003-22-3.
Chemical Structure:



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: 431.39.

Dye Classification: Anthraquinone.

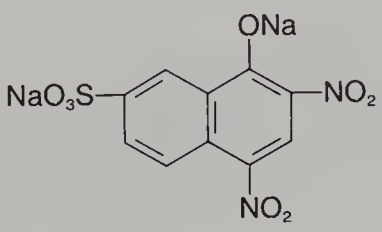
Manufacturing Process: Sulfonate D&C Violet No. 2 and then convert to the sodium salt.

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.

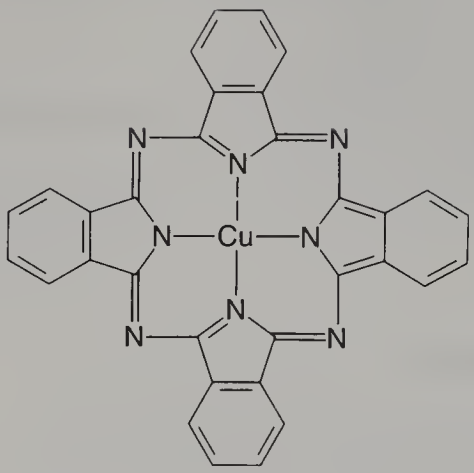
Manufacturing Process: Nitrate the di- or trisulfonic acids of 1-naphthol or the nitroso compound of the 2,7-disulfonic acid.

[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: 576.07.

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).

Chapter 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 imposed on some colorants whenever he believes that, because of their nature, certification is not needed to protect the public health (see p. 9). 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 1–4.

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 1–4 are actually unimportant as colorants per se 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 undersirable flavors and odors into the products in which they are incorporated. Also, depending on their nature and origin, exempt colorants can vary sub-

stantially 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.

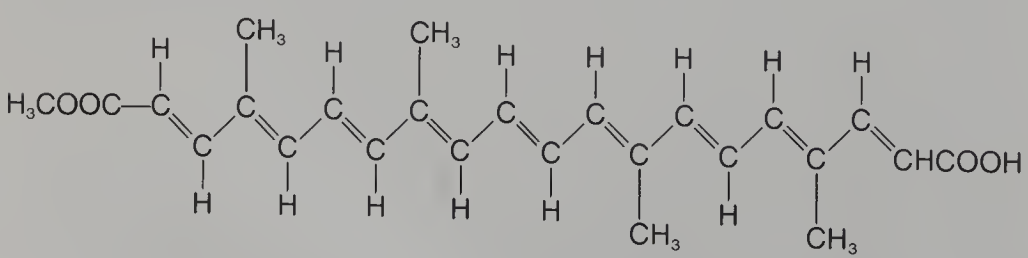
Exempt colorants are inherently neither more nor less safe than their certified cousins. However, some see them as having been obtained from nature ("natural") and, as such, imagine them as less of a health hazard than certified colorants. In fact, they, like all color additives, are fabricated products.

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 the preparation of the colorant known as annatto extract (CAS Reg. No. 8015-67-6).

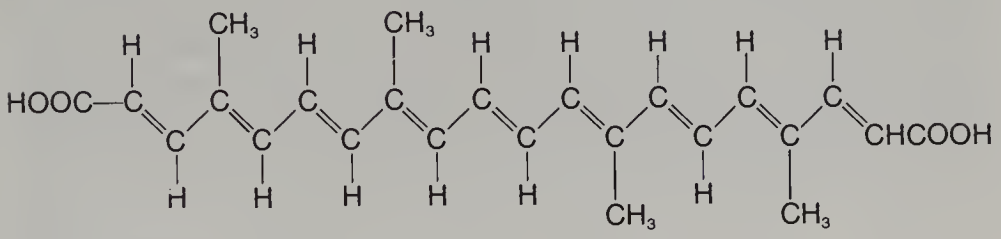
The colorant is prepared by leaching the annatto seeds with an extractant prepared from one or more approved, food-grade materials taken from a list that includes various solvents, edible vegetable oils and fats, and alkaline aqueous and alcoholic solutions. Depending on the use intended, the alkaline 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):



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)

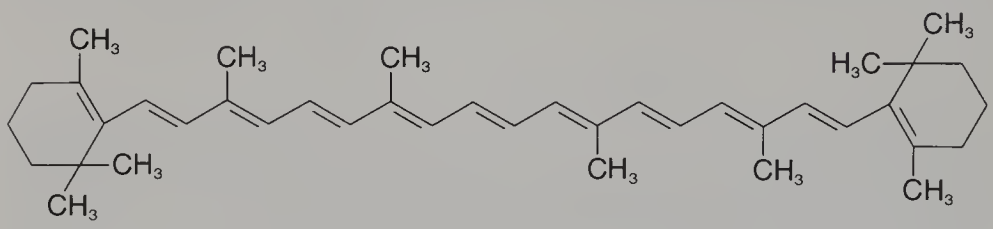
Annatto extract is sold in several physical forms, including dry powders, propylene glycol/monoglyceride emulsions, oil solutions and suspensions, and alkaline aqueous solutions, all containing 1–15% active colorant calculated as bixin. It is used in products at levels of 0.5–10 ppm as pure color, resulting 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, cooking oils, salad dressings, cereals, ice cream, ice cream cones, sausage casings, bakery goods, and spices. It is often used in combination with turmeric.

The chemistry and performance of annatto 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). 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 Hoffman-LaRoche, 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 syn-

thetically 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 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)

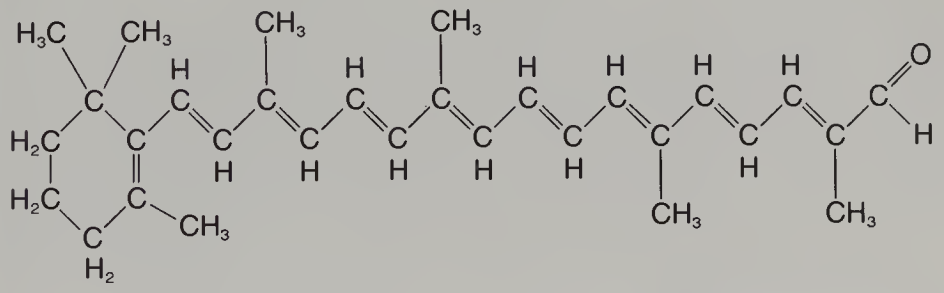
β-Carotene forms reddish-violet platelets that melt in the range 176–182°C. It is insoluble in water, ethanol, glycerine, and propylene glycol, and only slightly soluble in boiling organic solvents such as ether (0.05%), benzene (0.2%), carbon disulfide (1%), and methylene chloride (0.5%). Its solubility 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 shades.

β-APO-8′-CAROTENAL

This colorant (CI Food Orange 6, CI No. 40820, CAS Reg. No. 1107-26-2) 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: $C_{30}H_{40}O$ (mw 416.65)

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 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.

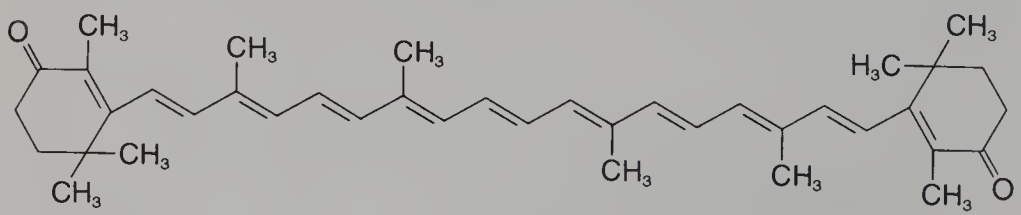
β-Apo-8′-carotenal is sold as a dry powder, as 1–1.5% vegetable oil solutions, as 20% suspensions in vegetable oil, as 2–4% solutions in a mixture of monoglycerides and *dl*-α-tocopherol, and as 10% dry beadlets. The vegetable-oil suspensions are purplish-black fluids at room temperatures that set to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

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. Conse-

quently, 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) 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 dehydrated to produce powdered colorant.

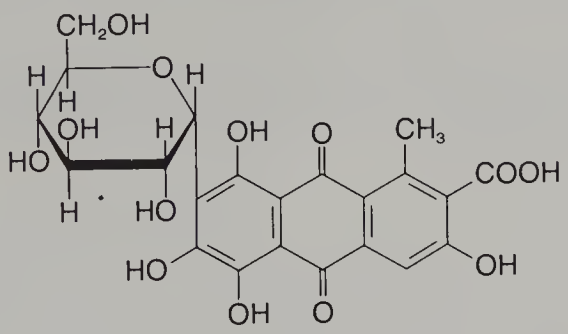
Because of the many variables in ingredients and process conditions involved in the manufacturing of caramel, its exact chemical composition is unknown. Caramel coloring is freely soluble in water and insoluble in most organic solvents. Its solubility in solutions containing 50–70% alcohol varies with 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 2.8–3.0. Most bakers' caramels, which are a less refined grade of colorant used for cookies, cakes, bread, and so on, have a slightly 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.

A major use for caramel is in soft drinks, particularly root beers and colas. It is also used extensively to standardize the hue of blended whiskeys and beer. Other uses include the coloring of baked goods, syrups, preserves, candies, pet foods, gravies, canned meat products, 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 (1000–5000 ppm), but the colorant is relatively inexpensive and shows good stability in most products.

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



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

the part 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, 75470) 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 anthraquinone comprising approximately 10% of cochineal and 2% 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 are within the range 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. Carmine is useful for producing pink shades in retorted protein products, candy, confections, rouge, eye shadow, and pill coatings.

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).

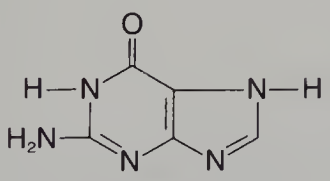
It is usually prepared by one of two methods:

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 then dry it at a high temperature.

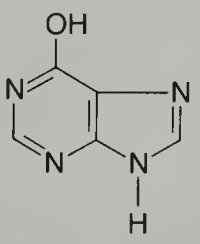
Cr_2O_3 is a yellowish (sage) green pigment. It has good strength and opacity, and excellent stability. It is used in eye makeup and soap.

GUANINE (PEARL ESSENCE)

Guanine 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.

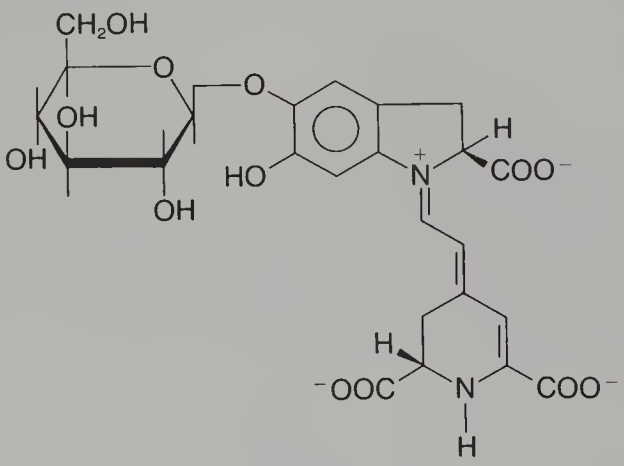
The hue of the colorant varies greatly with the amount and type of pigment found in the fish scales. Carotenoids produce reds and yellows, melanin results in blacks, and combinations of guanine and melanin produce greens and blues. Only when guanine is found alone is the product silvery or pearly white.

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

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, 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 colorant is usually sold as either concentrates prepared by evaporating beet juice under vacuum to a total solids content of 40–60%, or as powders made by spray-drying the concentrates. Both products usually contain ascorbic or citric acid as a stabilizer, and a preservative such as sodium propionate. On a dry-weight basis, beet colorant 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.

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 a color additive because of the difficulties frequently encountered in purifying them.

Iron oxide 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 oxide (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.

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 Spanish paprika (pimenton, pimiento), which has the characteristics of paprika produced in Spain.

Paprika oleoresin 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 dichloride, 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.

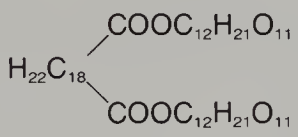
Paprika and its oleoresin are approved for use in foods in general where its application as a color additive frequently overlaps its use as a spice. Both products have good tinctorial strength and are used at levels of 0.2–100 ppm to produce orange to bright-red shades.

SAFFRON

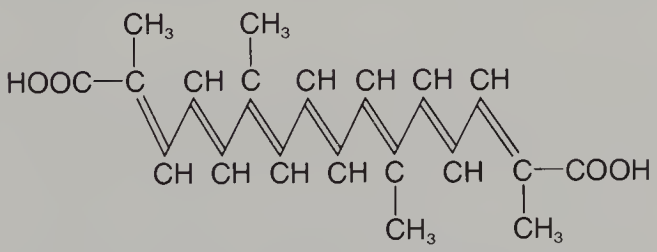
Saffron, known also as CI Natural Yellow 6 (CI No. 75100), *safran*, *crocine*, *crocétine*, and *crocus*, is the dried stigma of *Crocus sativus*,

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.

TALC

Talc, CI Pigment White 26, CI No. 77019, CAS Reg. No. 14807-96-6, is finely powdered, native, hydrous magnesium silicate (3 $MgO{\cdot}4SiO_2{\cdot}H_2O$, "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) 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 powders, eye makeup, and rouges, in amounts consistent with good manufacturing practice.

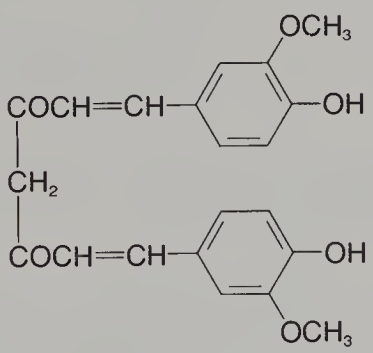
The colorant's chief disadvantages are its inability to blend well with the other ingredients usually found in powder formulations, its

tendency to produce blue undertones, and its ability to catalyze the oxidation of perfumes.

TURMERIC AND TURMERIC OLEORESIN

Turmeric (CI Natural Yellow 3, CI No. 75300) 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 com-



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

bination of the 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 as a powder and as a suspension in a variety of carriers, including edible vegetable oils and fats, and mono- and diglycerides. Turmeric oleoresin is most often sold as solutions in propylene glycol with or without added emulsifying agents. 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.

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 ($\leq$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 with-

out 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—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—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—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—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.

Chromium-cobalt-aluminum oxide—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—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−).

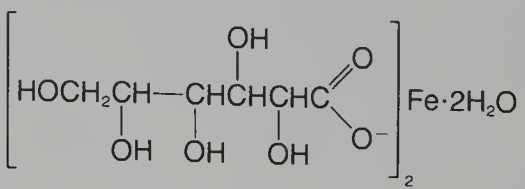
Dried algae meal—A dried mixture of algae cells (genus *Spongiococcum*, separated from its culture broth), molasses, cornsteep liquor, and a maximum of 0.3% ethoxyquin. The algae cells are produced by suitable fermentation, under controlled conditions, from a pure culture of the genus *Spongiococcum*.

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—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 482.18)

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

Grape color extract—The color additive grape color extract 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.

Grape skin extract (enocianina)—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 (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.

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

Henna—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—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—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 semiquantitatively determined by its characteristic X-ray diffraction pattern and by its optical properties.

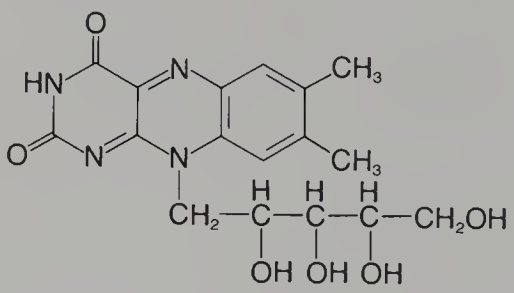
Potassium sodium copper chlorophyllin (chlorophyllin-copper complex)—A green-black powder obtained from chlorophyll by replacing the methyl and phytyl ester groups with alkali and replacing the magnesium with copper. The source of the chlorophyll is dehydrated alfalfa.

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

Pyrophyllite—A naturally occurring 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 semiquantitatively determined by its characteristic X-ray powder-diffraction pattern and by its optical properties.

Riboflavin—A yellow to 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 1 mg in 100 mL of water is pale greenish yellow by transmitted light and has an intense yellowish green fluorescence 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, 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 cited here are based on the April, 1983 edition of the Code of Federal Regulations (21 CFR 1.1) and revisions to 21 CRF 1.1 that appeared in the Federal Register through January, 1984. All are maxima unless indicated otherwise.

In addition to these requirements, color additives must be free of all impurities other than those named to the extent that such impurities can be avoided by 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; Proposed)

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; Proposed)

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%.

Lead (as Pb)—10 ppm.

Arsenic (as As)—3 ppm.

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%.

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

Volatile matter (at 135°C) and chlorides and sulfates (calculated as the sodium salts)—13.0%, total.
Water-insoluble matter—0.2%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—3 ppm.
Phenylhydrazine-*p*-sulfonic acid—0.1%.
Other uncombined intermediates—0.2% each.
Subsidiary dyes—1.0%.
Total color—87.0% min.

FD&C Yellow No. 6

Volatile matter (at 135°C)—10.0%.
Water-insoluble matter—0.5%.
Ether extracts—0.2%.
Chlorides and sulfates of sodium—5.0%.
Mixed oxides—1.0%.
Subsidiary dyes—5.0%.
Pure dye (as determined by titration with titanium trichloride)—85.0% 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.
Pure dye—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.
Pure dye—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.
Pure 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.
Pure dye—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-dihydroxyphenyl]azo]benzenesulfonic acid—29% min., 39% max.
Monosodium salt of 4[[5-[(2,5-dimethylphenyl)azo]-2,4-dihydroxyphenyl]azo]benzensulfonic acid—12% min., 17% max.
Monosodium salt of 4[[5-[(2,3-dimethylphenyl)azo]-2,4-dihydroxyphenyl]azo]benzenesulfonic acid—6% min., 13% max.

Monosodium salt of 4[[5-[(2-ethylphenyl)azo]2,4-dihydroxyphenyl]azo]benzenesulfonic acid—5% min., 12% max.

Monosodium salt of 4[[5-[(3,4-dimethylphenyl)azo]-2,4-dihydroxyphenyl]azo]benzenesulfonic acid—3% min., 9% max.

Monosodium salt of 4[[5-[(2,6-dimethylphenyl)azo]2,4-dihydroxyphenyl]azo]benzenesulfonic acid—3% min., 8% max.

Monosodium salt of 4[[5-[(4-ethylphenyl)azo]-2,4-dihydroxyphenyl]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 (for Coloring Surgical Sutures)

Volatile matter (at 135°C)—2.0%.
Water-soluble matter—0.3%.
Matter insoluble in carbon tetrachloride—1.5%.
Intermediates—0.5%.
Lead (as Pb)—10 ppm.
Arsenic (as As)—1 ppm.
Pure color—96.0% min.

D&C Green No. 6 (for Coloring Externally Applied Drugs and Cosmetics)

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°) and halides and sulfates (calcúlated 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%.
Flüorescein—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 Orange No. 17

Volatile matter (at 135°C)—5.0%.
Sulfated ash—1.0%.
Matter insoluble in toluene—1.5%.
2,4-Dinitroaniline—0.2%.
β-Naphthol—0.2%.

Pure dye (as determined by titration with titanium trichloride)—90.0% 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. 326).

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. 326).

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. 8

Volatile matter (at 135°C)—10.0%.

Ether extracts (isopropyl ether)—0.5%.

Lake Red C Amine (2-chloro-5-aminotoluene-4-sulfonic acid)—0.2%.

β-Naphthol—0.2%.

Chlorides and sulfates of sodium—5.0%.
Mixed oxides—1.0%.
Pure dye (as determined by titration with titanium trichloride)—85.0% min.

D&C Red No. 9

Volatile matter (at 135°C)—5.0%.
Ether extracts (isopropyl ether)—0.5%.
Lake Red C Amine (2-chloro-5-aminotoluene-4-sulfonic acid)—0.2%.
β-Naphthol—0.2%.
Chlorides and sulfates of sodium—6.0%.
Oxides of iron and aluminum—1.0%.
Pure dye (as determined by titration with titanium trichloride)—87.0% 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. 19

Volatile matter (at 135°C)—5.0%.
Water-insoluble matter—1.0%.
Ether extracts (from acid solution)—0.5%.

Diethyl-*m*-aminophenol—0.2%.
Chlorides and sulfates of sodium—2.0%.
Mixed oxides—1.0%.
Pure dye (as determined by titration with titanium trichloride)—92.0% 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

Volatile matter (at 135°C)—6.0%.
Water-insoluble matter—1.0%.
Ether extracts—0.5%.
Aniline—0.2%.
Chlorides and sulfates of sodium—10.0%.
Mixed oxides—1.0%.
Pure dye (as determined by titration with titanium trichloride)—82.0% 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)—5.0%.
Sulfated ash—1.0%.
Matter insoluble in toluene—1.0%.
o-Chloro-*p*-nitroaniline—0.2%.
β-Naphthol—0.2%.
Pure dye (as determined by titration with titanium trichloride)—90.0% min.

D&C Red No. 37

Volatile matter (at 80°C)—2.0%.
Sulfated ash—3.0%.
Matter insoluble in benzene—0.5%.
Diethyl-*m*-aminophenol—0.2%.
Stearic acid (not part of the dye)—50.0%.
Pure dye (as determined by titration with titanium trichloride)—50.0% 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.
Pure 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

Volatile matter (at 135°C)—10.0%.
Water-insoluble matter—1.0%.

Ether extracts—0.5%.
Quinaldine—0.2%.
Chlorides and sulfates of sodium—6.0%.
Mixed oxides—1.0%.
Pure dye (as calculated from organically combined nitrogen)—82.0% 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—0.2%.
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%.
Acid insolubles—0.2%.
Heavy metals—30 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% min.

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.

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)—5–5.5.
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: through a 100-mesh sieve—100% min.; through a 200-mesh sieve—80% min.
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.
Ratio, 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 (as Pb)—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 Ingested or Topically Applied Drugs and Cosmetics)

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%.

Acid-soluble substances as sulfate—2%.

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 15 min. 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

Colorcon, Inc., Moyer Blvd., West Point, Pa. 19846 (215-699-7733). Certified FD&C Lakes.

Color-Treme Co., 1526 S. State St., Chicago, Ill. 60605 (312-791-8250). Annatto, turmeric, and beet products.

Crompton & Knowles Corporation, 17-01 Nevins Rd., P.O. Box 415, Fair Lawn, N.J. 07410 (201-791-7100). Certified FD&C and D&C Colors, carmine lake, and beet powder.

Food Concentrates, Inc., P.O. Box 1014-A, Rahway, N.J. 07065 (201-786-1634). Caramel.

Fritzsche Dodge & Olcott, Inc., 76 Ninth Ave., New York, N.Y. 10011 (212-929-4100). Paprika and turmeric.

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

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

Hoffmann-LaRoche, Inc., Nutley, N.J. 07110 (201-235-5000). β-Carotene, β-apo-8′-carotenal, and canthaxanthin.

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

H. Kohnstamm & Company, Inc., 161 Avenue of the Americas, New York, N.Y. 10013 (212-620-4800). Certified FD&C colorants, carmine lake, annatto, caramel, turmeric, grape-skin extract, fruit juice, titanium dioxide, synthetic iron oxides, certified D&C colorants, alumina, hydrous and anhydrous chromium oxides, ultramarines, and ferric ferrocyanide.

The Mearl Corporation, 41 East 42nd St., New York, N.Y. 10017 (212-573-8500). Pearlescent pigments.

Miles Laboratories, Inc., Citro-Tech Division, P.O. Box 932, Elkhart, Ind. 46515 (219-264-8111). Annatto.

Meer Corporation, 9500 Railroad Ave., North Bergen, N.J. 07047 (201-861-9500). Beet powder, annatto, paprika, turmeric, cochineal, grape-skin extract, caramel, and saffron.

Neumann-Buslee & Wolfe, Inc., 521 Santa Rosa Dr., Des Plaines, Ill. 60018 (312-827-2153). Certified FD&C colorants.

Pfizer, Inc., 235 East 42nd St., New York, N.Y. 10017. Calcium carbonate, synthetic iron oxides and talc.

Pylam Products Company, Inc., 95-10 218th St., Queens Village, N.Y. 11429 (212-464-0860). Certified FD&C and D&C colorants and grape-skin extract.

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

Sethness Products Co., 444 N. Lake Shore Dr., Chicago, Ill. 60611 (312-527-4755). Caramels.

Smith Chemical & Color Company, 104-20 Dunkirk St., Jamaica, N.Y. 11412 (212-454-9400). Iron oxides, ultramarine blue, chromium oxide, mica, talc, zinc oxide, and calcium carbonate.

Stange Company, 342 N. Western Ave., Chicago, Ill. 60612 (312-733-6945). Certified FD&C colorants and turmeric.

Sun Chemical Corporation, 441 Tompkins Ave., Rosebank, Staten Island, N.Y. 10305 (212-981-1600). Certified D&C colorants, iron oxides, manganese violet, ultramarine blue, chromium hydroxide green, chromium oxide greens, and titanium dioxide.

Warner-Jenkinson, 2526 Baldwin St., St. Louis, Mo. 63106 (314-531-1500). Certified FD&C colorants.

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

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

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.

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.

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 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 the 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 inherently 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 chem-

ical 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.

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.

PRIMARY COLOR—A single colorant containing no diluent. See SECONDARY COLOR.

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 color additive made by mixing two or more straight colors, or one or more straight colors and one or more diluents, or both.

SHADE—Hue.

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.

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 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 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 gathered to date. 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 de-

termine 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 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 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 is then listed for use in the kinds of products tested and petitioned for—foods, drugs, cosmetics, medical devices, or all four.

PART B COLORANT ANALYSIS

Chapter 6 Identification

The techniques used for the analysis of color additives differ little from those used for the examination of other commercial compounds. However, 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 obviously 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 has access. In general, those described here presume that the chemist has a single colorant and that it is not in a food, drug, or cosmetic matrix. 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 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. 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 workhorse 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 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. These techniques are generally a little more complicated and 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

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.

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% $UO_2(OAc)_2 \cdot 2H_2O$ 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.

be presumed that more work using proton, ^{13}C, ^{14}N, and other emerging 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. 1*a-p*, 2*a-n*, and 3*a-h*). The reactions of some natural coloring materials with various reagents are shown on pages 162 to 163.

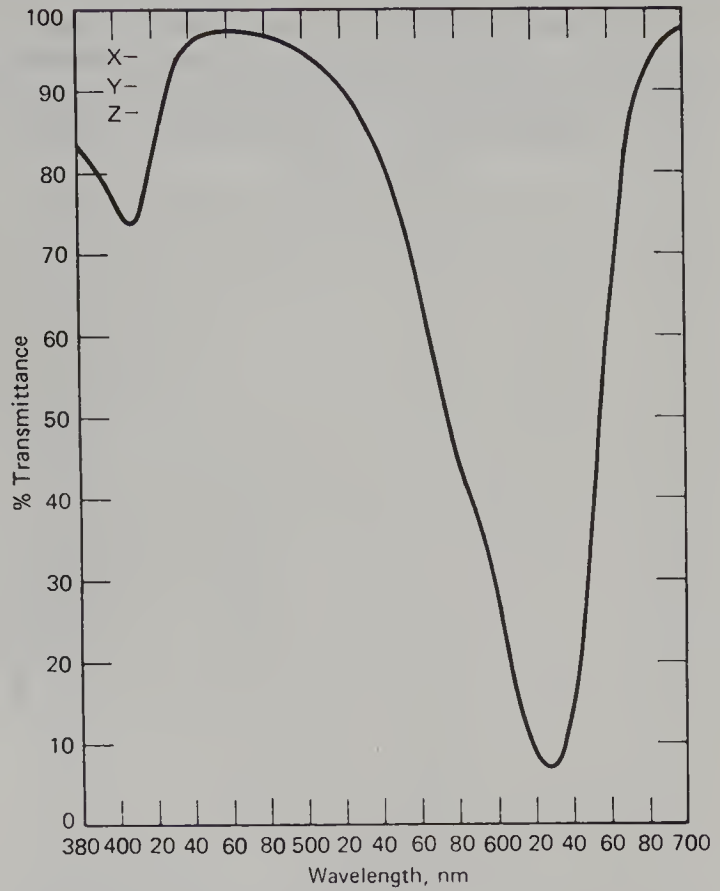

Figure 1*a* Visible Spectrum of FD&C Blue No. 1: concentration, 8 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

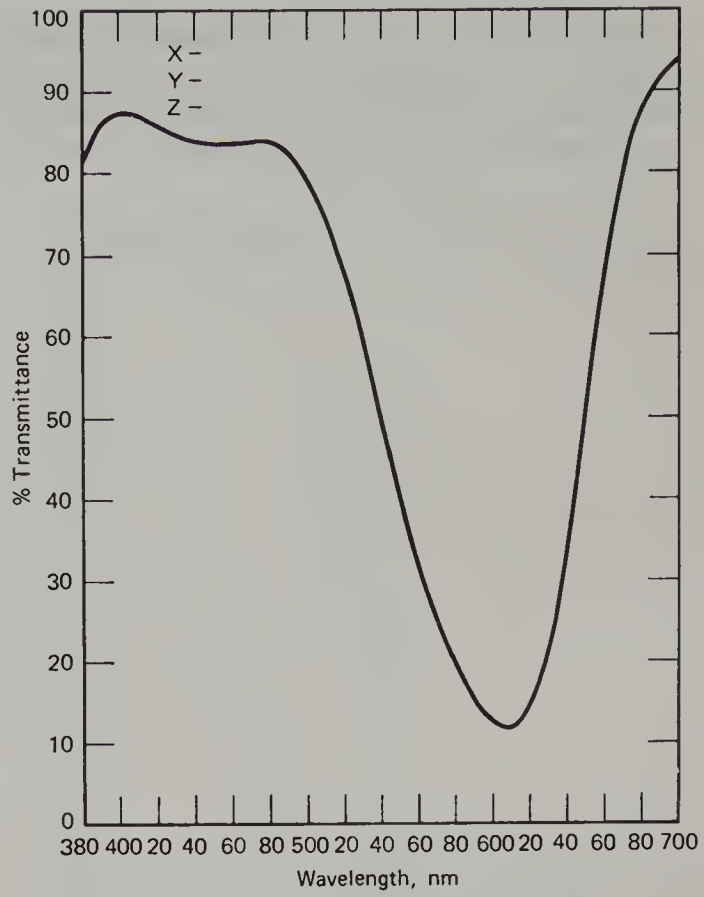

Figure 1*b* Visible Spectrum of FD&C Blue No. 2: concentration, 22 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

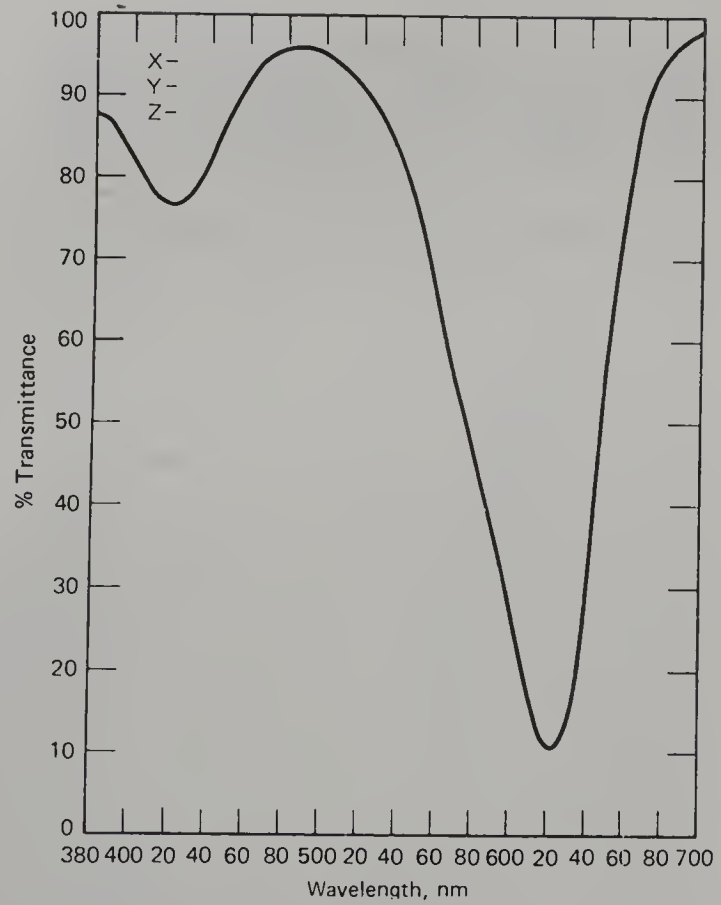

Figure 1c Visible Spectrum of FD&C Green No. 3: concentration, 7 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

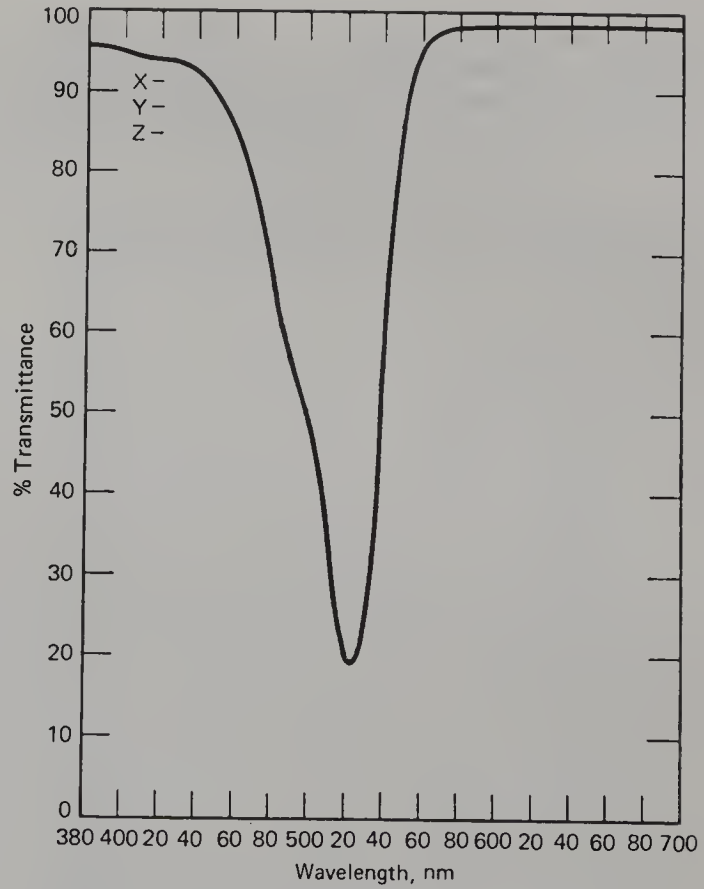

Figure 1*d* Visible Spectrum of FD&C Red No. 3: concentration, 7.5 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

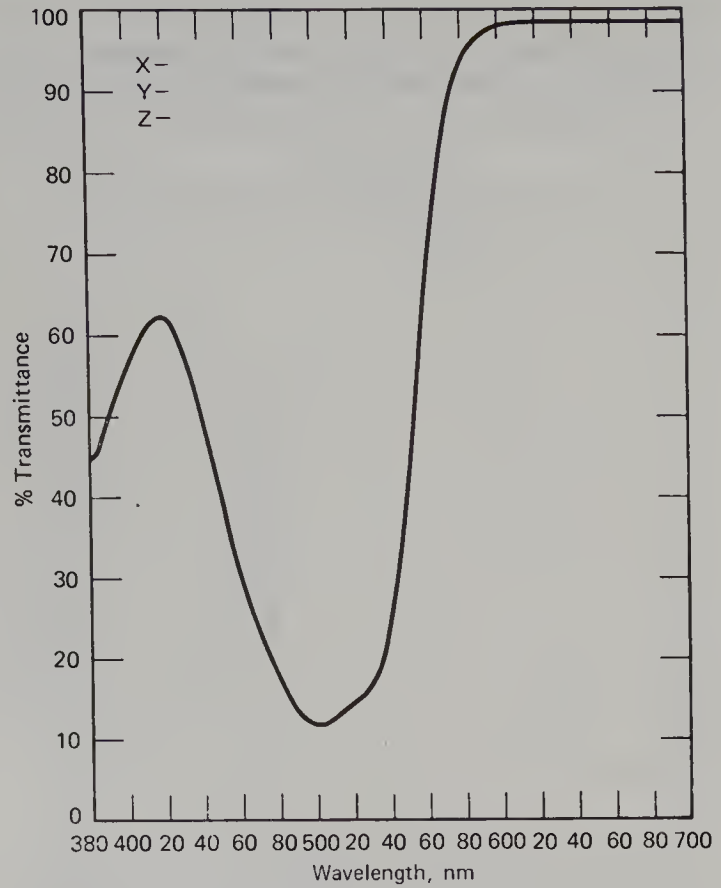

Figure 1e Visible Spectrum of FD&C Red No. 4: concentration, 20 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

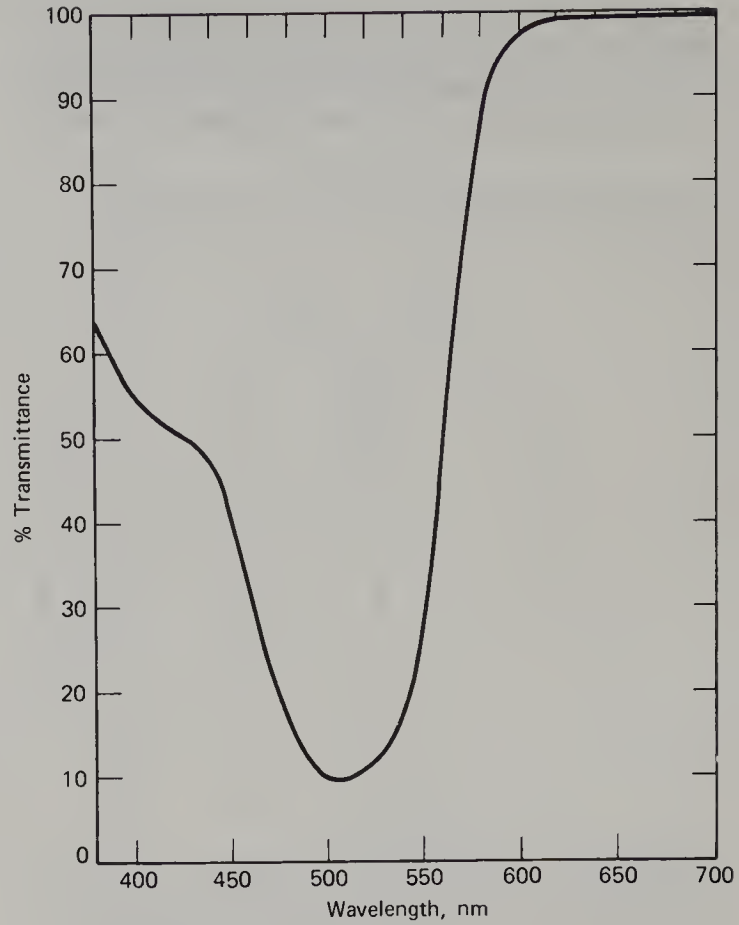

Figure 1*f* Visible Spectrum of FD&C Red No. 40: concentration, 20 ppm; cell thickness, 1 cm; solvent, water; reference, water

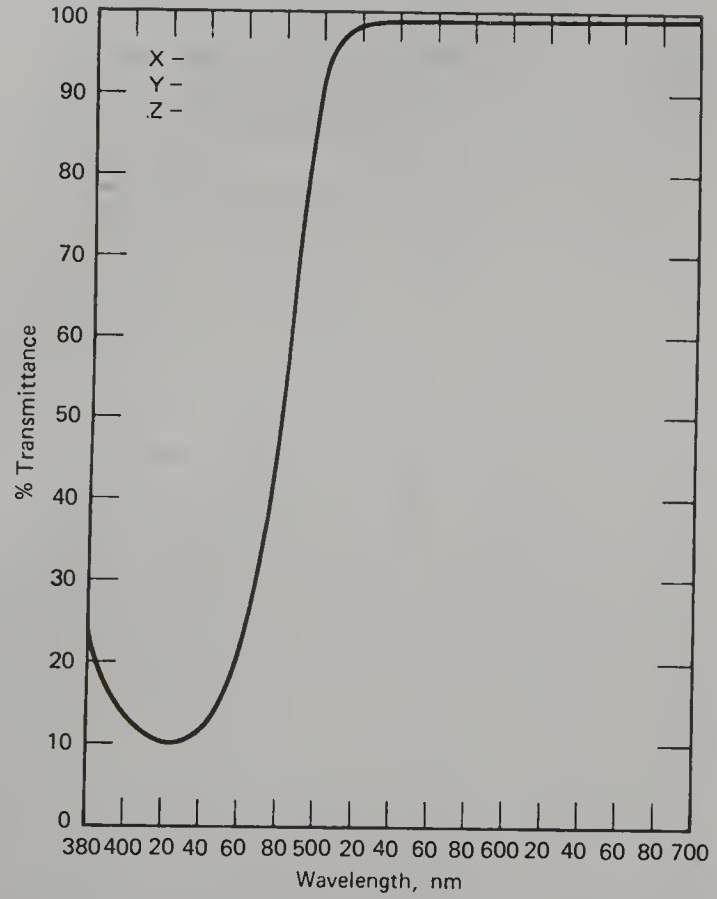

Figure 1*g* Visible Spectrum of FD&C Yellow No. 5: concentration, 20 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

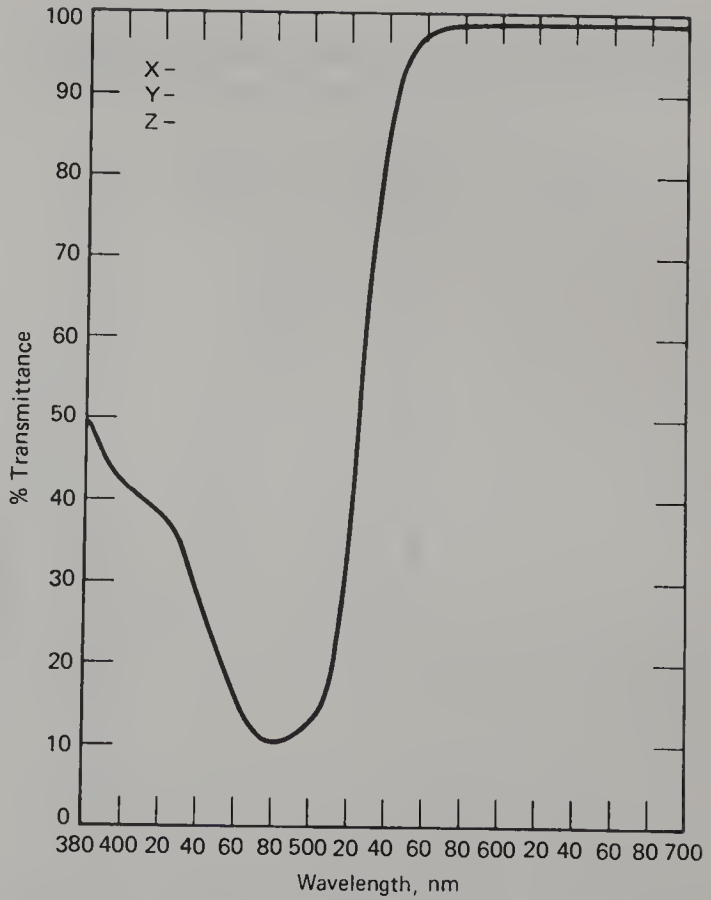

Figure 1*h* Visible Spectrum of FD&C Yellow No. 6: concentration, 20 ppm; cell thickness, 1 cm; solvent, water; reference, water (From *Encyclopedia of Industrial Chemical Analysis*, Vol. 10, Wiley, New York, 1970)

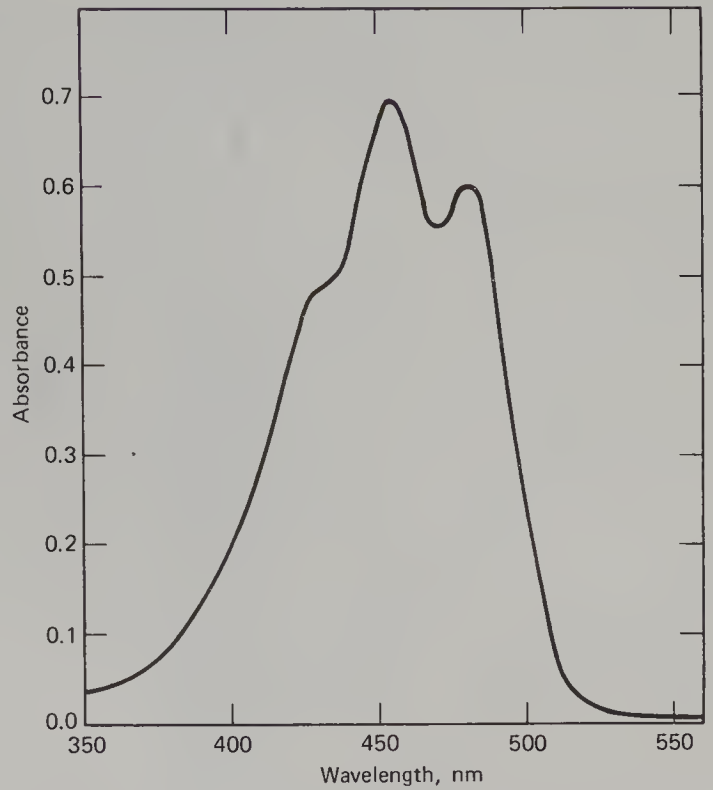

Figure 1*i* Visible Spectrum of All-*trans* β-Carotene: concentration, 2.68 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

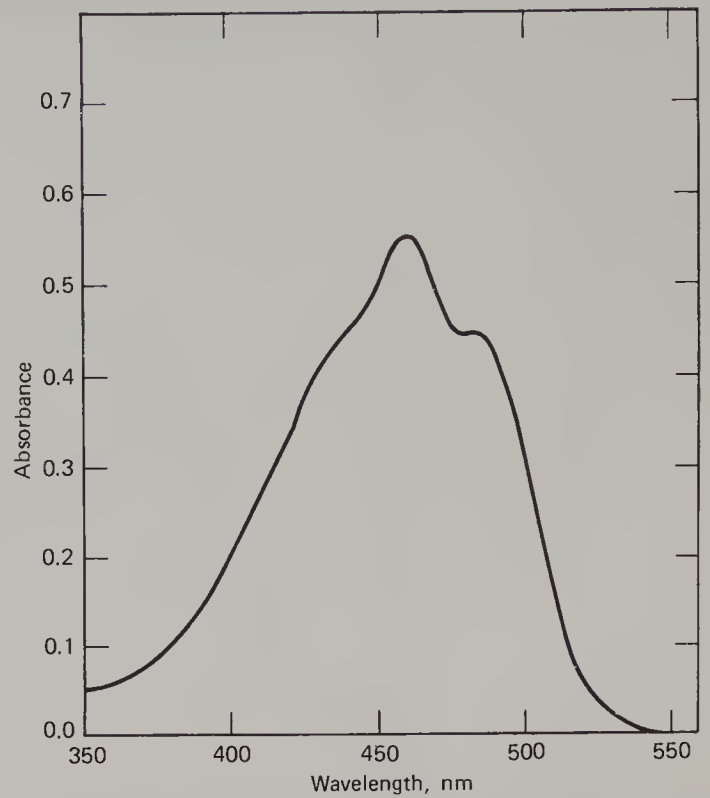

Figure 1*j* Visible Spectrum of All-*trans* β-apo-8'-Carotenal: concentration, 2.11 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

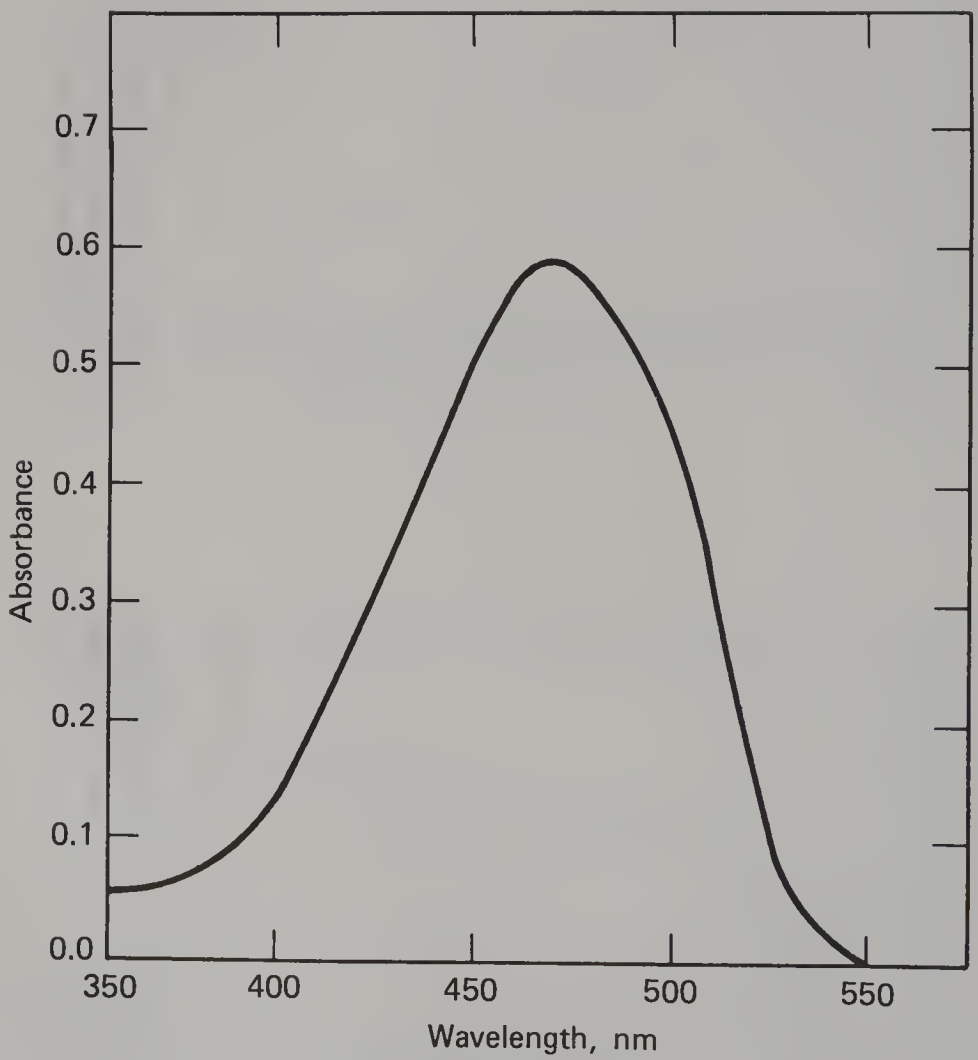

Figure 1*k* Visible Spectrum of All-*trans* Canthaxanthin: concentration, 2.67 μg/mL; cell thickness, 1 cm; solvent, cyclohexane

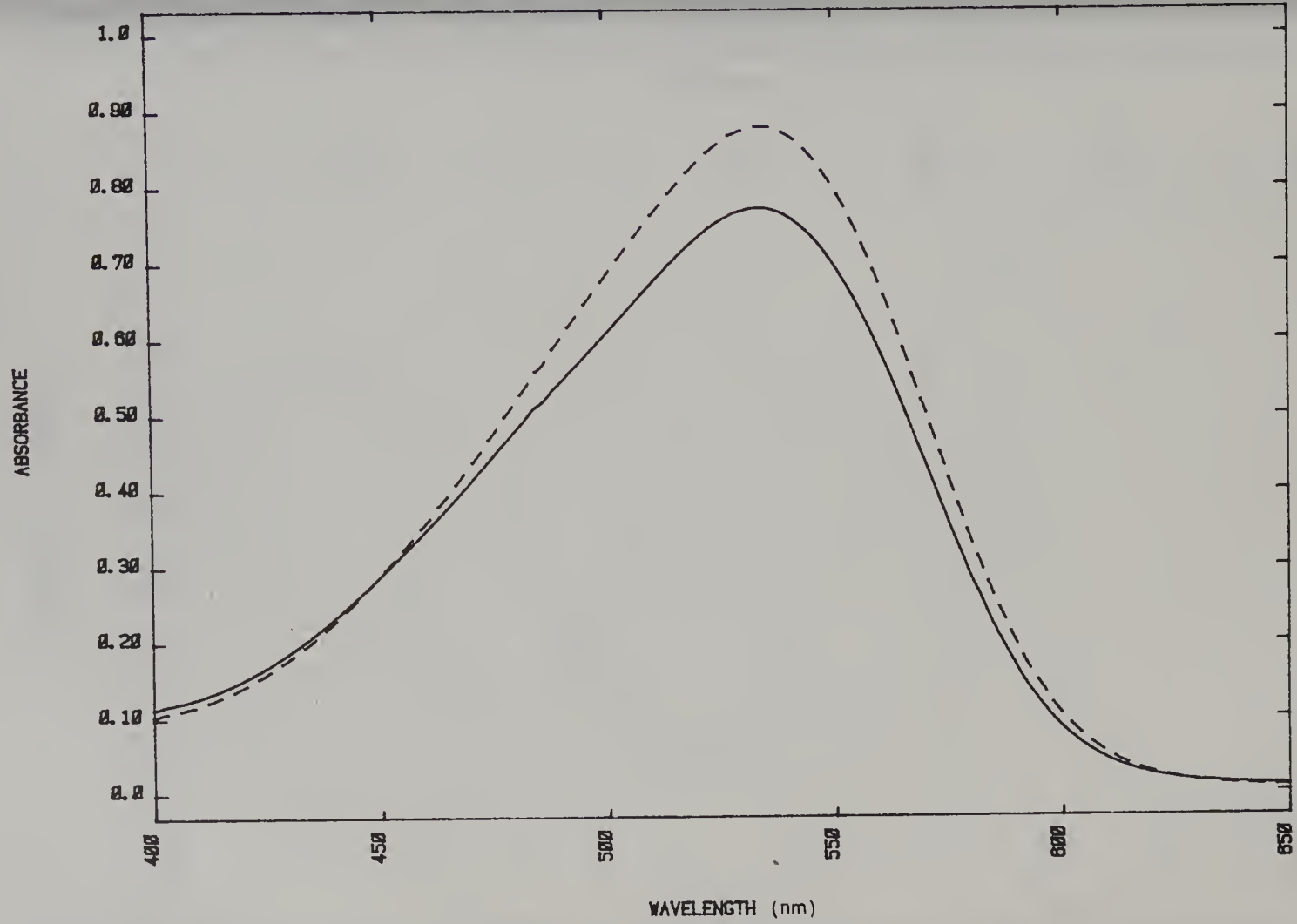

Figure 1/ Visible spectra of two liquid beet juice concentrates: concentrations, 2500 ppm; cell thickness, 1 cm; solvent, water; reference, water

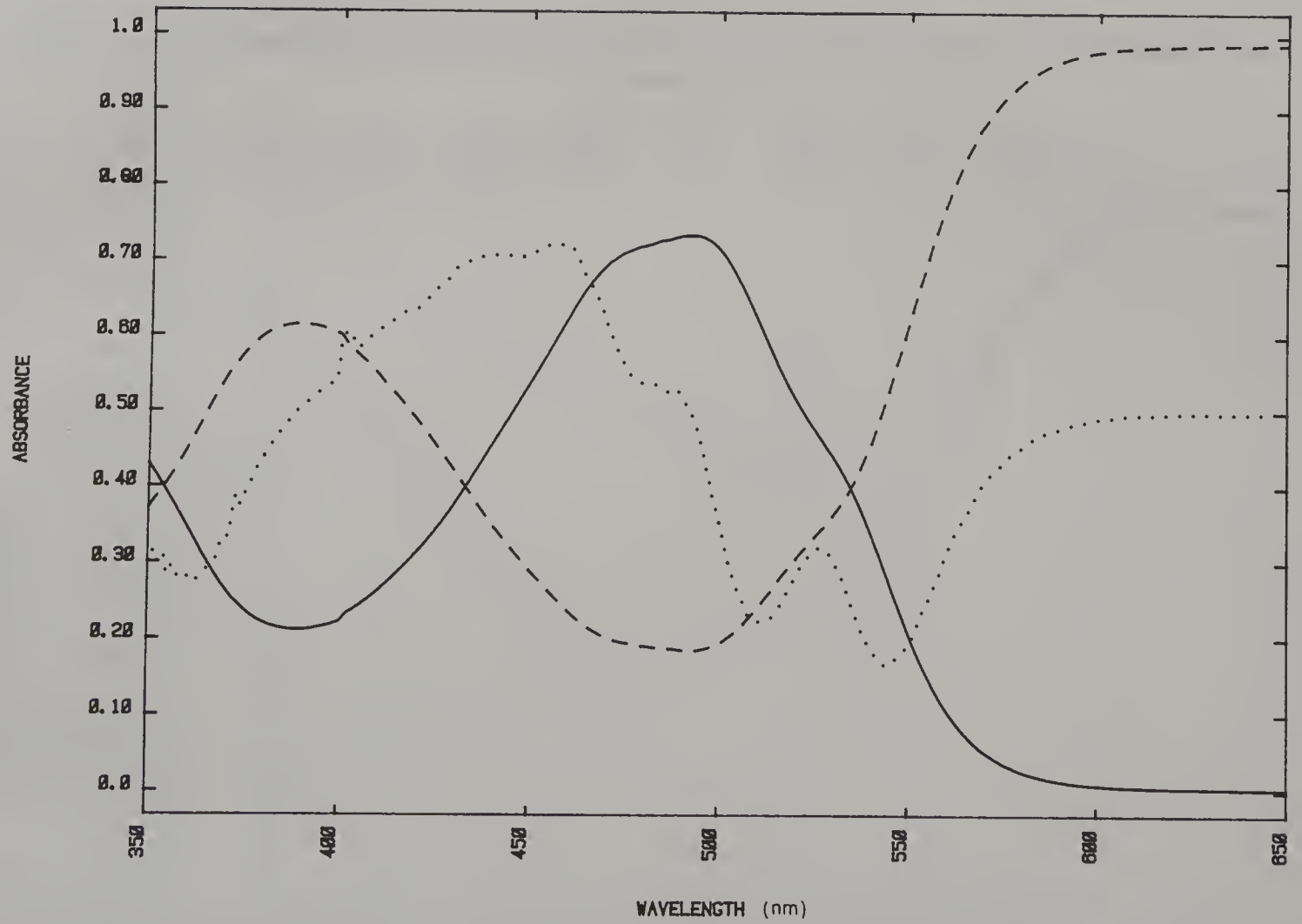

Figure 1*m* Visible spectrum of a 54% carmine lake: concentration, 100 ppm; cell thickness, 1 cm; solvent, 0.2% v/v aq. HCl; reference, water; (——) absorbance; (– – –) transmittance; (· · · ·) $dA/d\lambda$

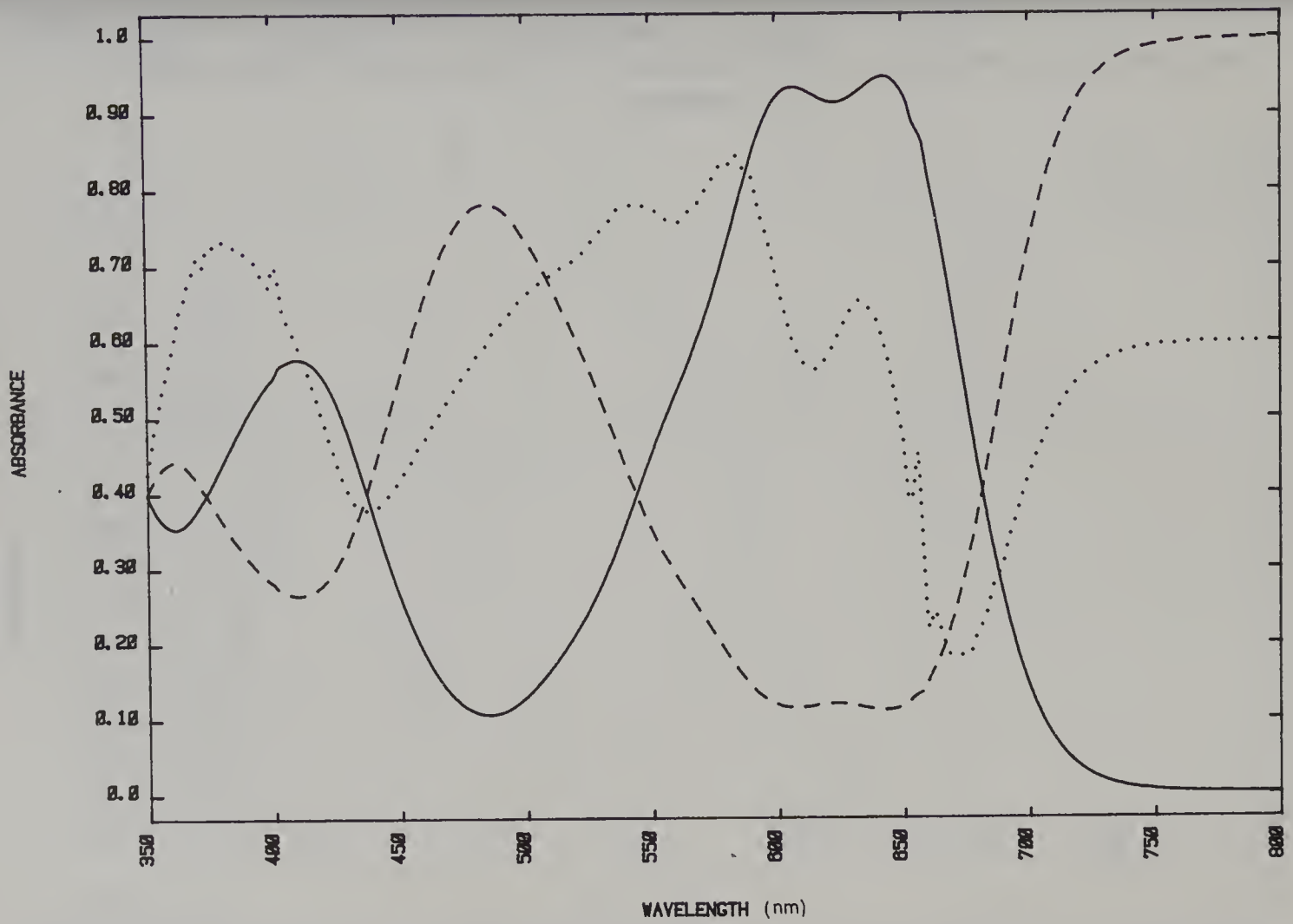

Figure 1*n* Visible spectrum of D&C Green No. 5: concentration, 44 ppm; cell thickness, 1 cm; solvent, water; reference, water; (——) absorbance; (– – –) transmittance; (· · · ·) $dA/d\lambda$

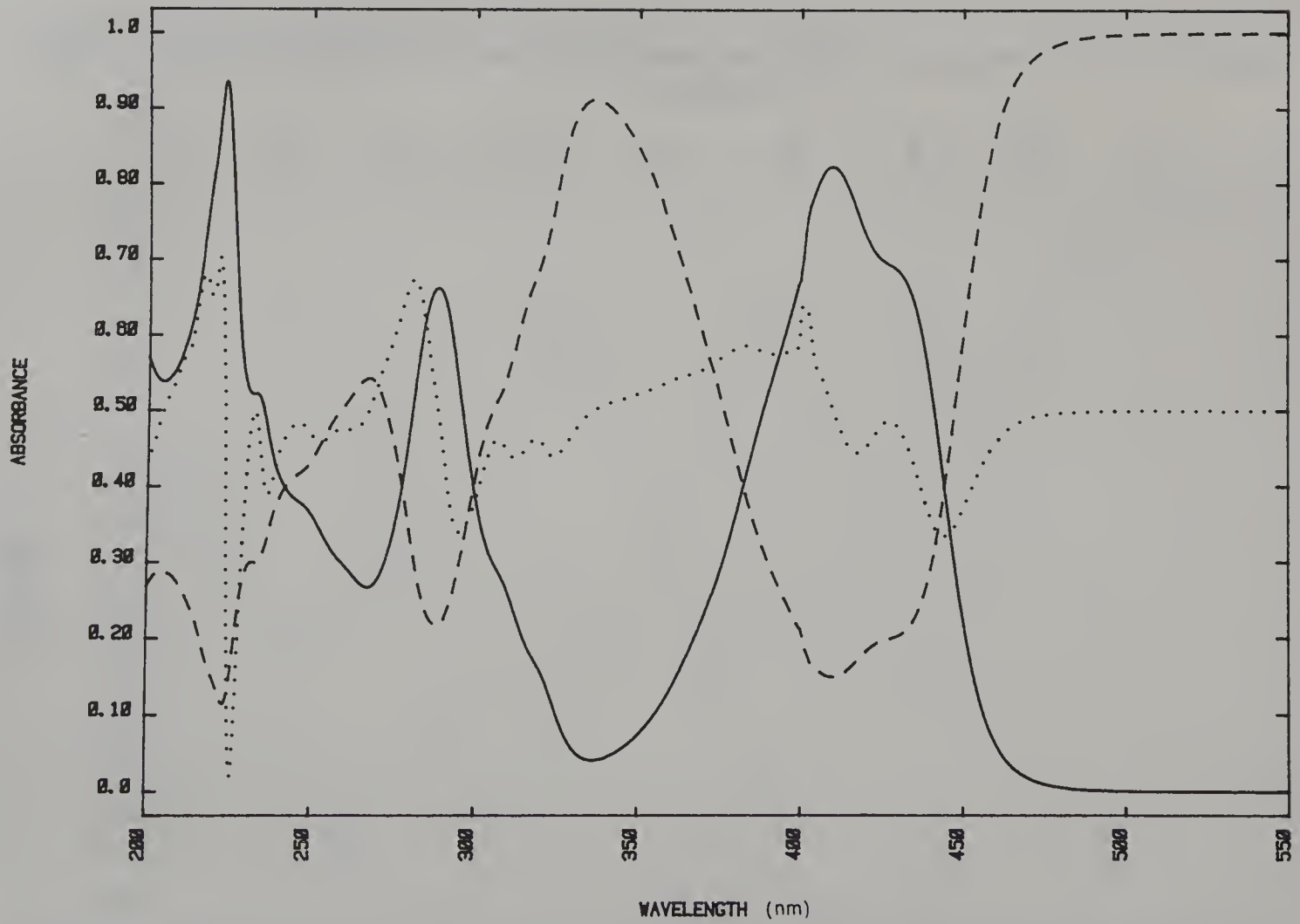

Figure 1*o* Visible spectrum of D&C Yellow No. 10: concentration, 9 ppm; cell thickness, 1 cm; solvent, water; reference, water; (——) absorbance; (– – –) transmittance; (· · · ·) $dA/d\lambda$

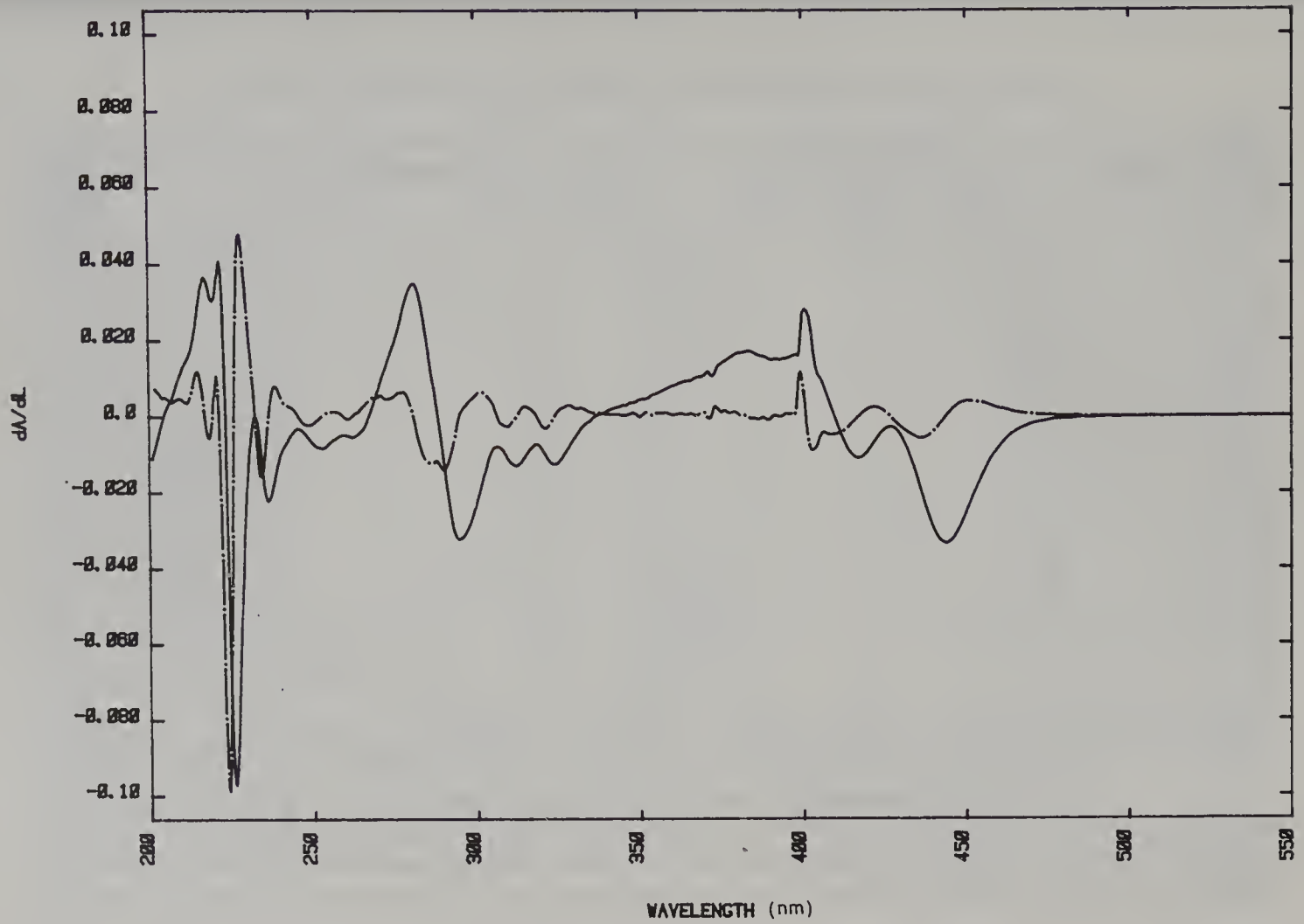

Figure 1*p* Visible spectrum of D&C Yellow No. 10: concentration, 9 ppm; cell thickness, 1 cm; solvent, water; reference, water; (——) $dA/d\lambda$; (– · –) $d^2A/d\lambda^2$

Figure 2 Nuclear magnetic resonance spectra obtained on Varian A60 Spectrometer equipped with variable-temperature probe and using the following operating 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)

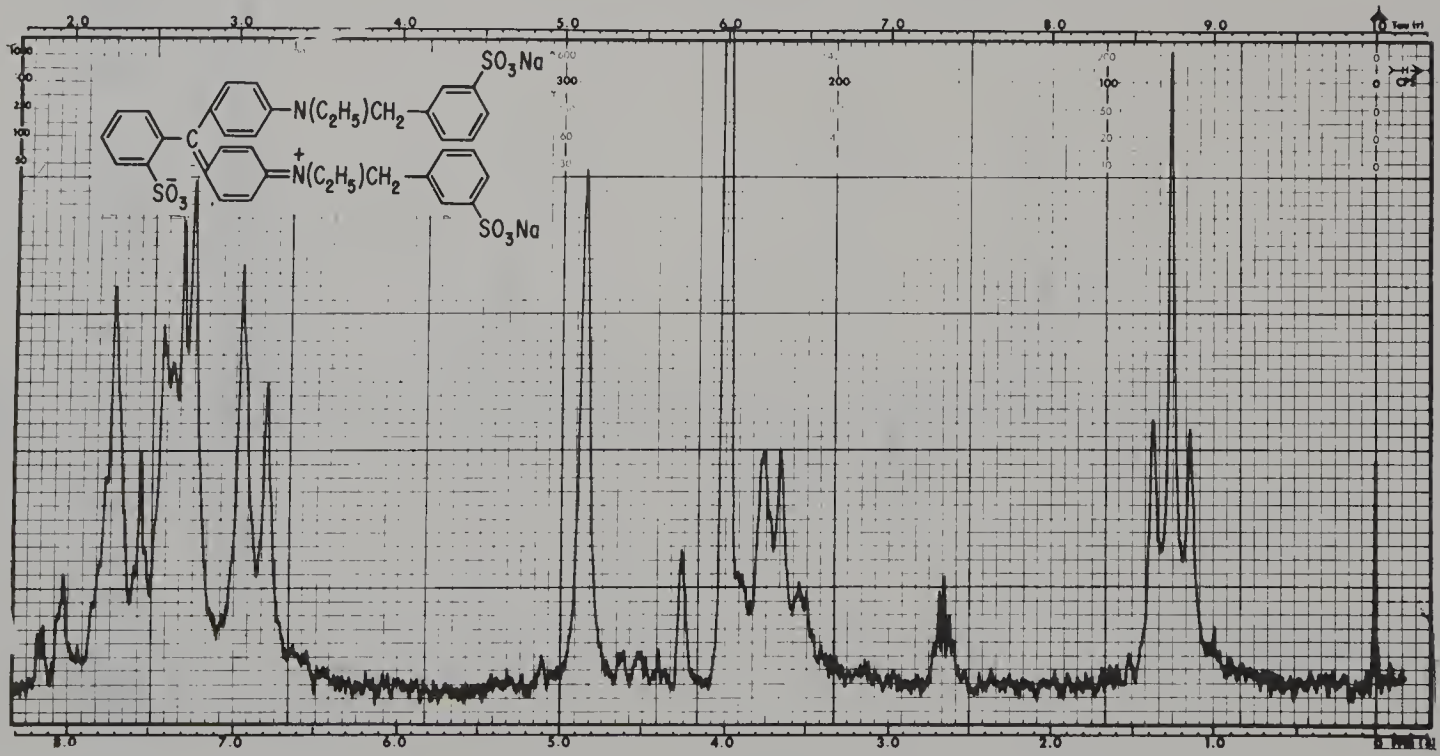

Figure 2*a* Spectrum of FD&C Blue No. 1: concentration, 20%; amplitude, 80

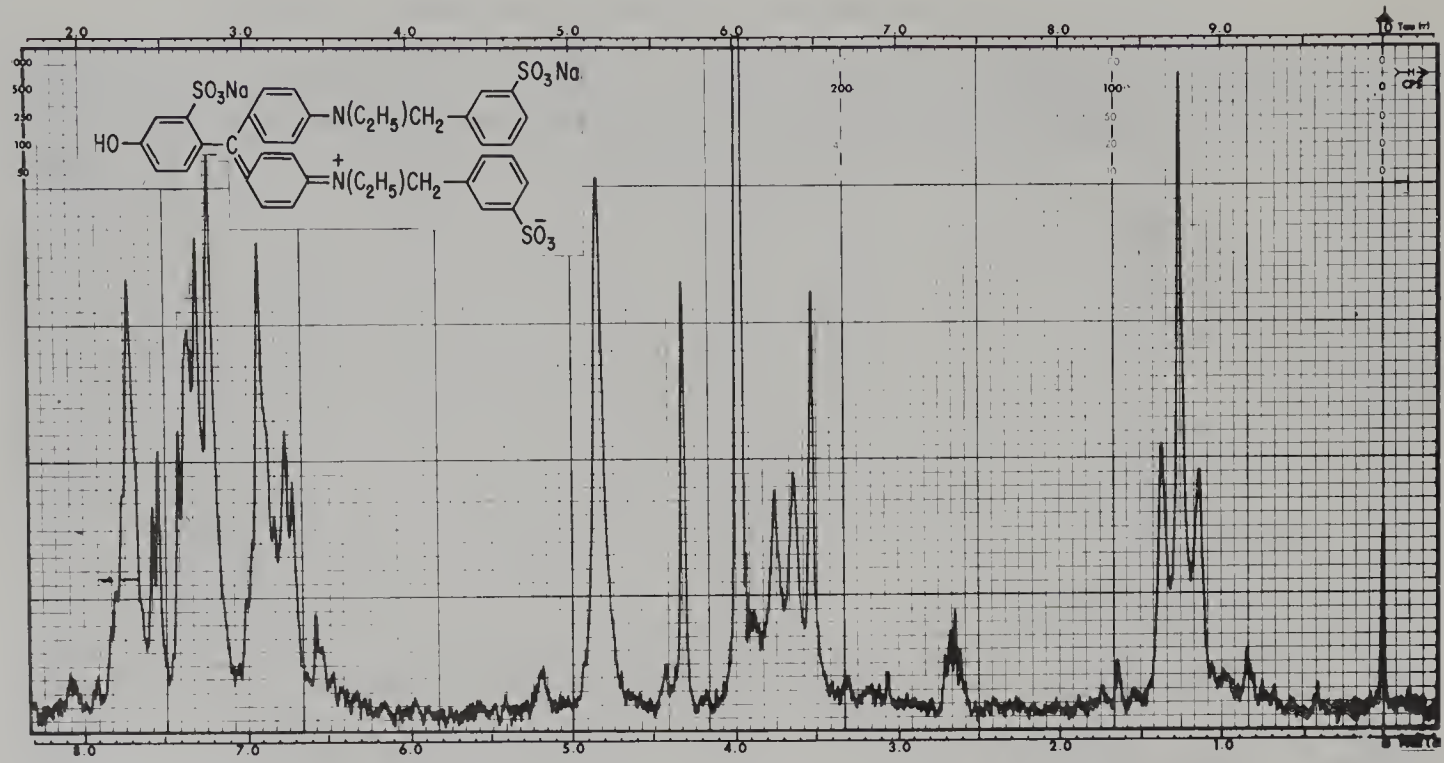

Figure 2*b* Spectrum of FD&C Green No. 3: concentration, 20%; amplitude, 80

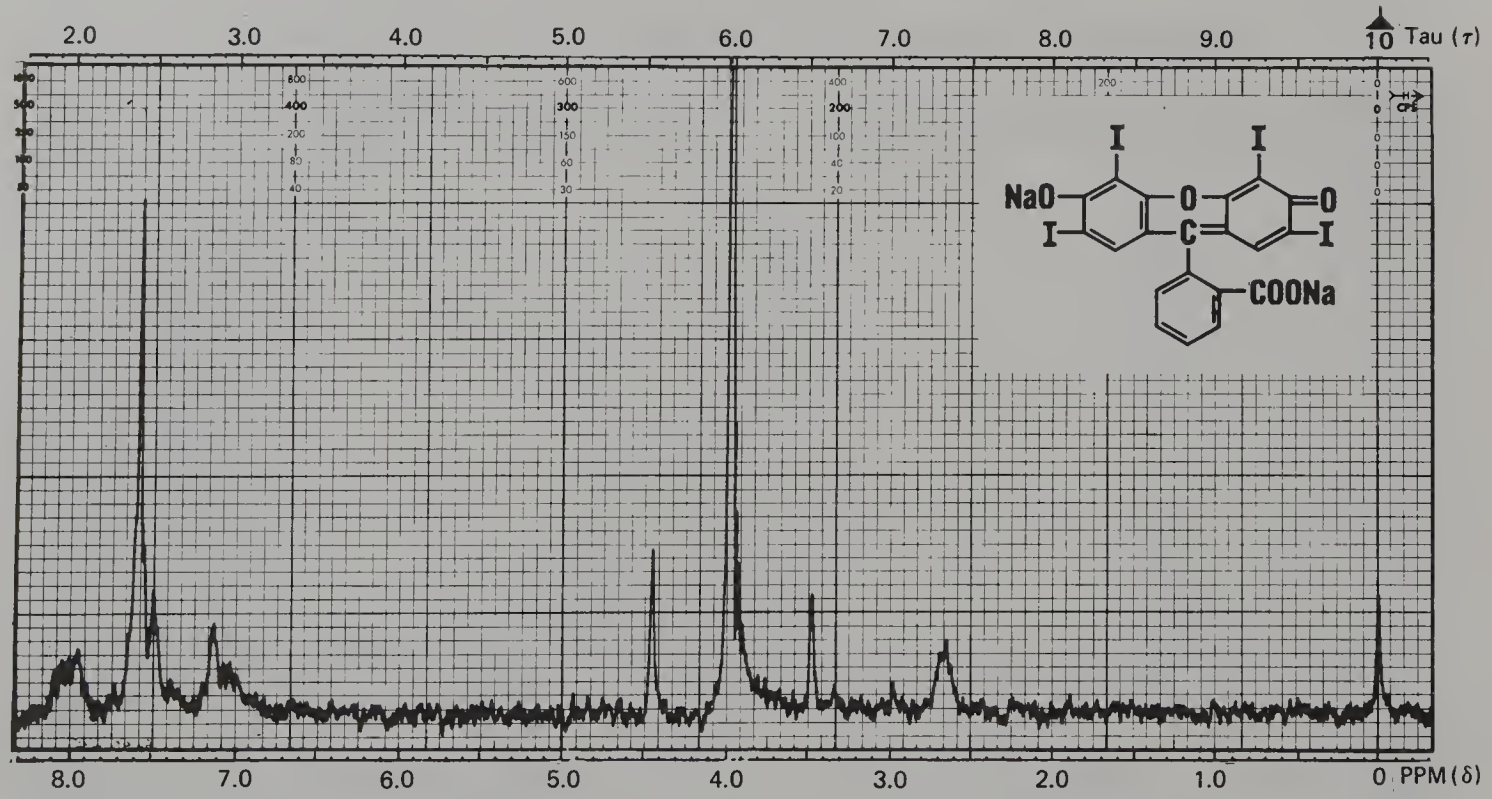

Figure 2c Spectrum of FD&C Red No. 3: concentration, 20%; amplitude, 80

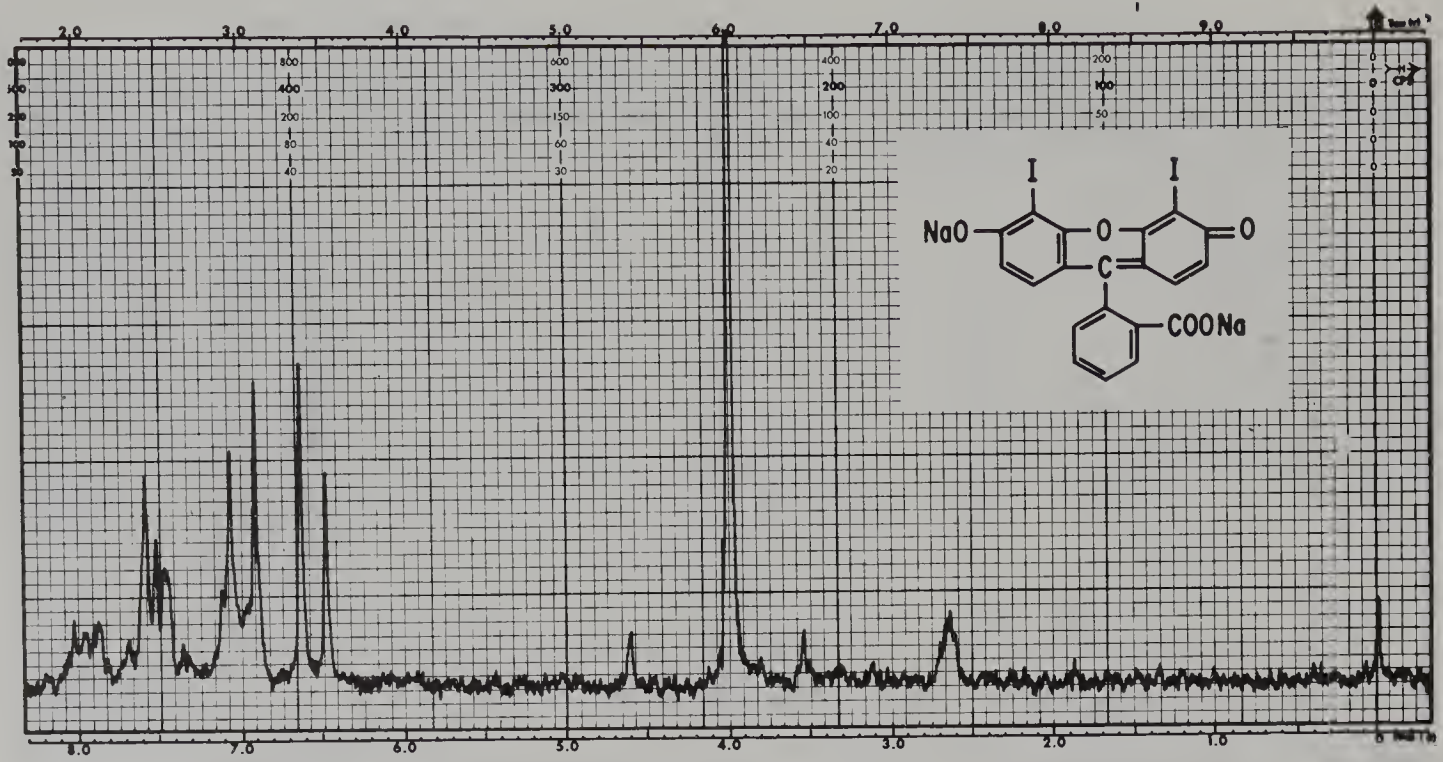

Figure 2*d* Spectrum of D&C Orange No. 11: concentration, 20%; amplitude, 80

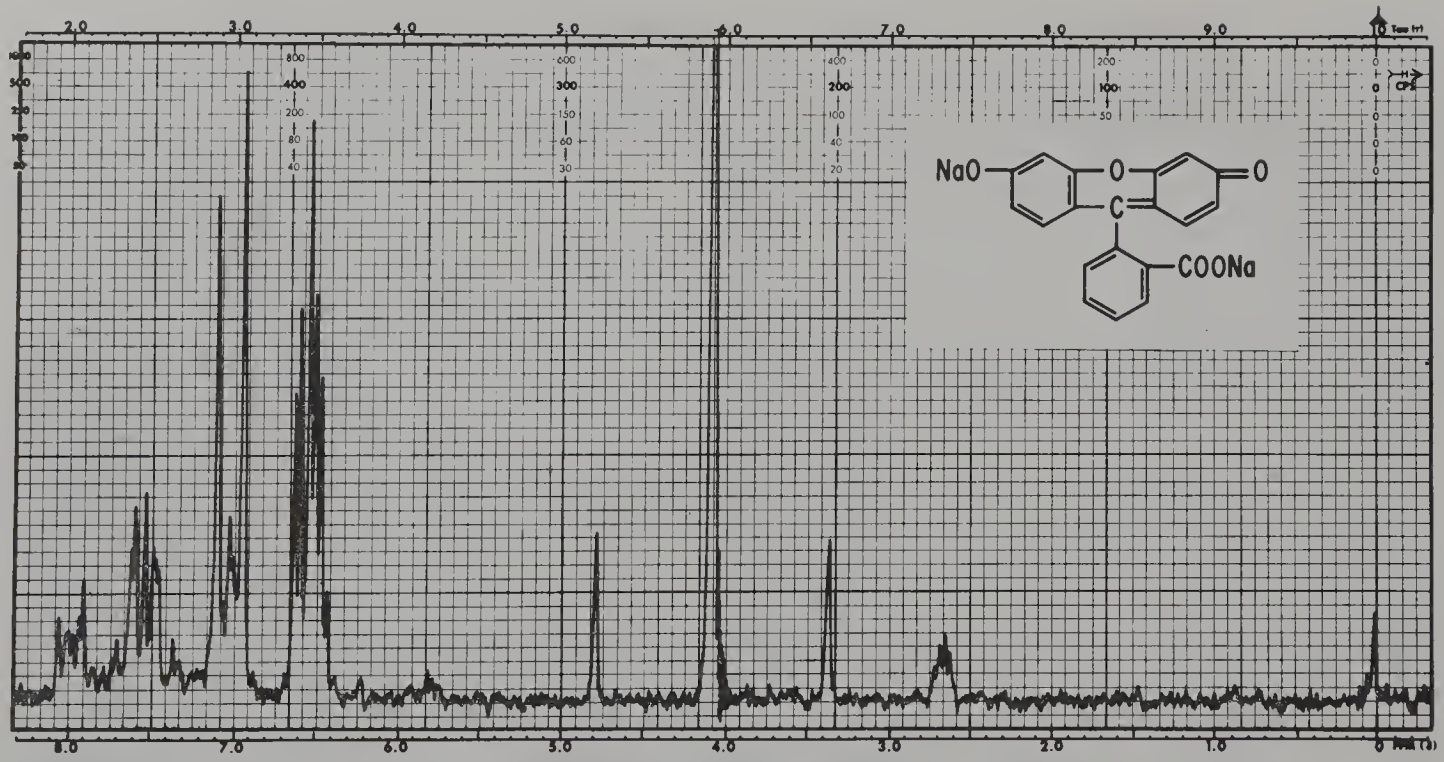

Figure 2e Spectrum of D&C Yellow No. 8: concentration, 20%; amplitude, 63

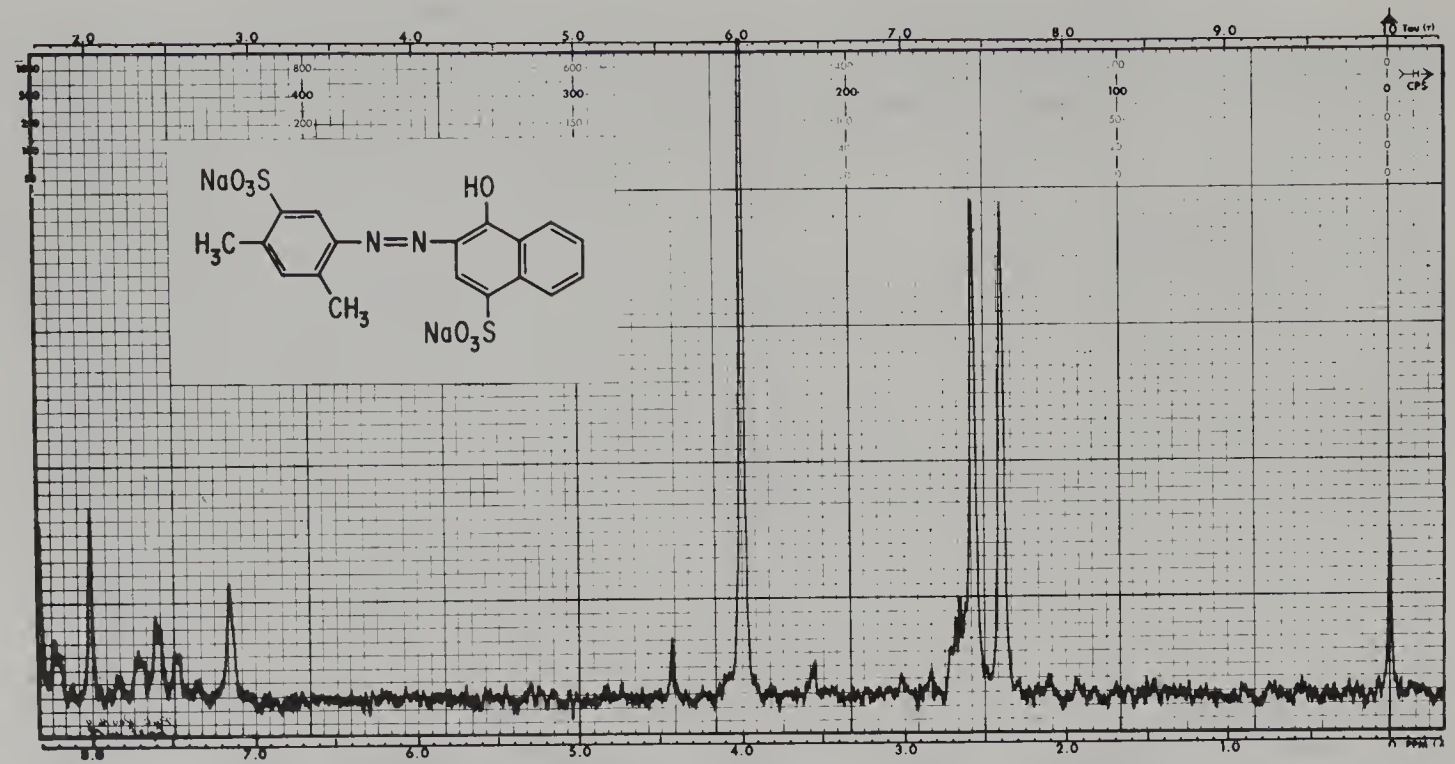

Figure 2*f* Spectrum of FD&C Red No. 4: concentration, 15%; amplitude, 80

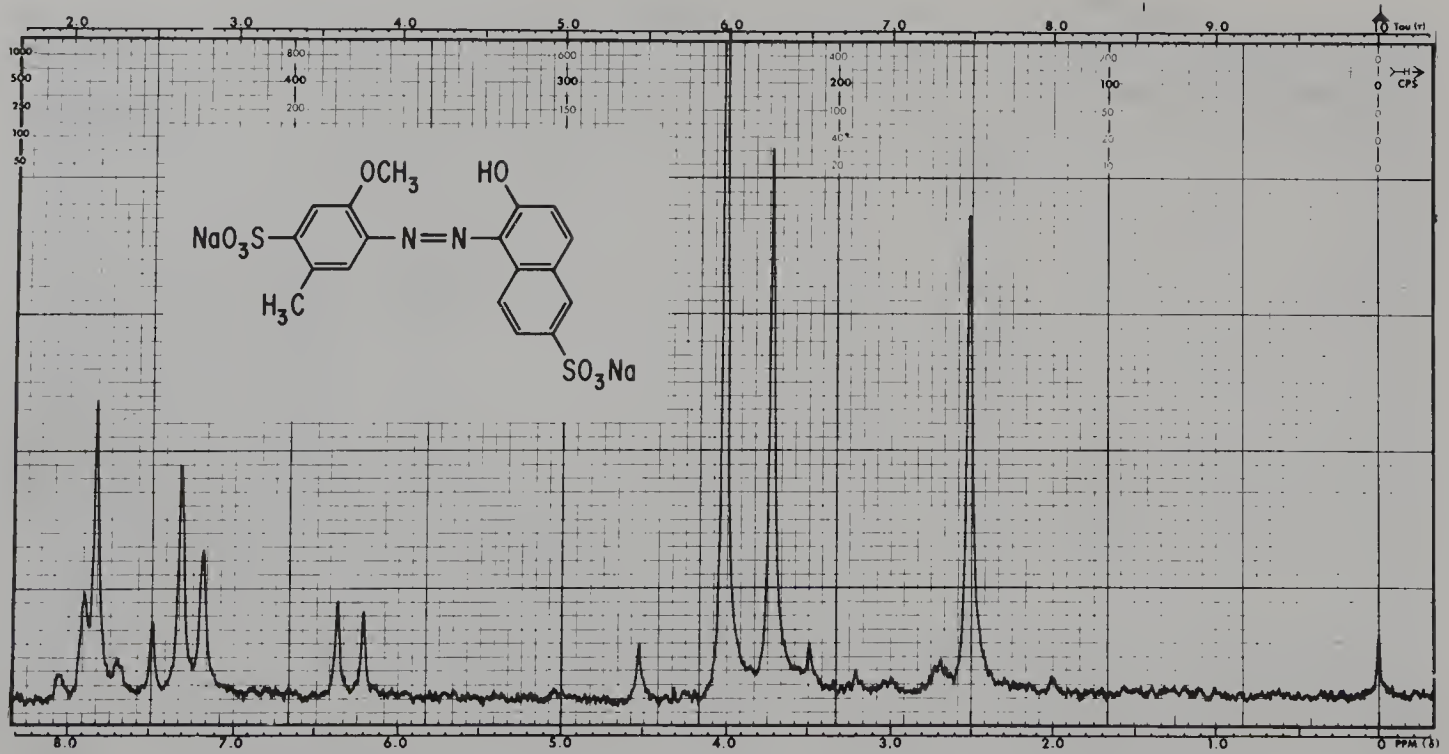

Figure 2*g* Spectrum of FD&C Red No. 40: concentration, 20%; amplitude, 40

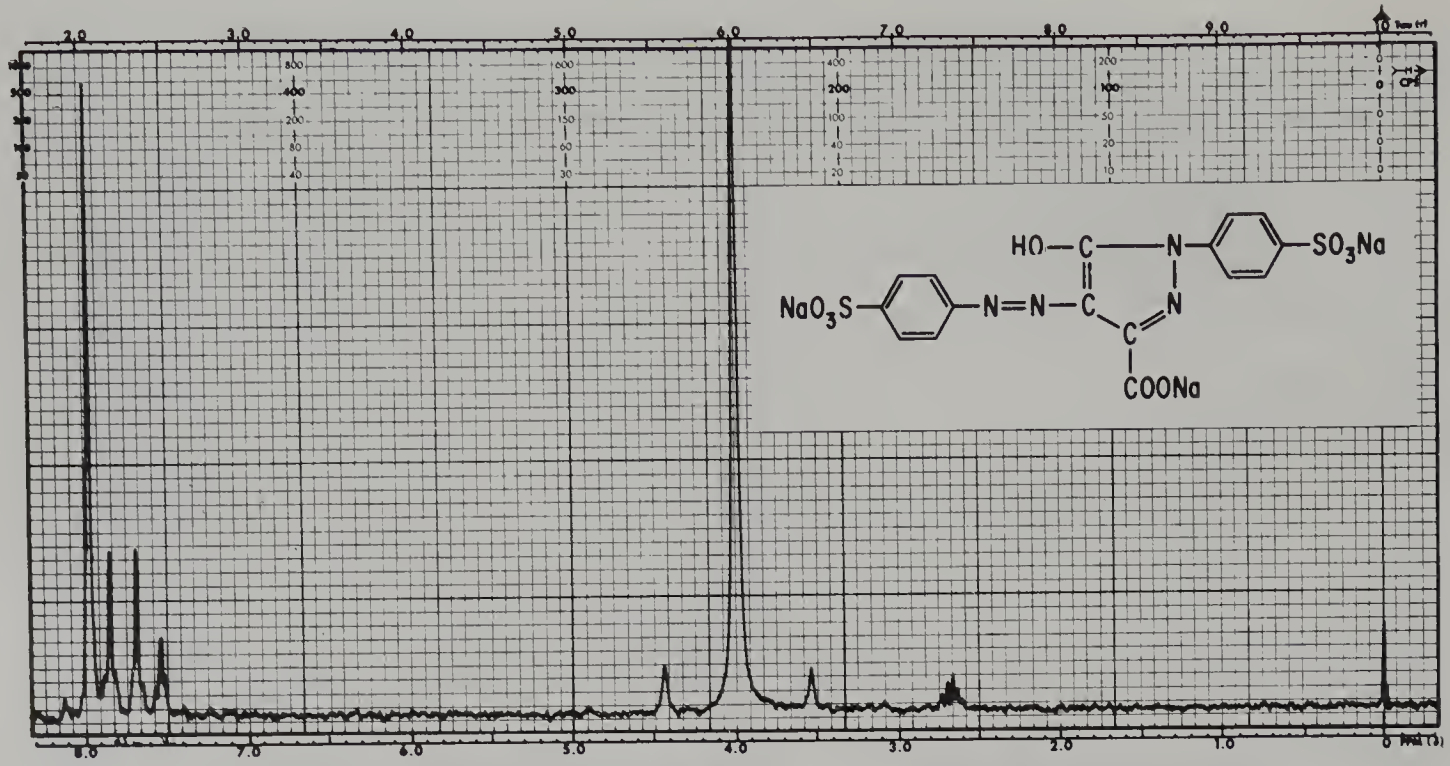

Figure 2*h* Spectrum of FD&C Yellow No. 5: concentration, 20%; amplitude, 25

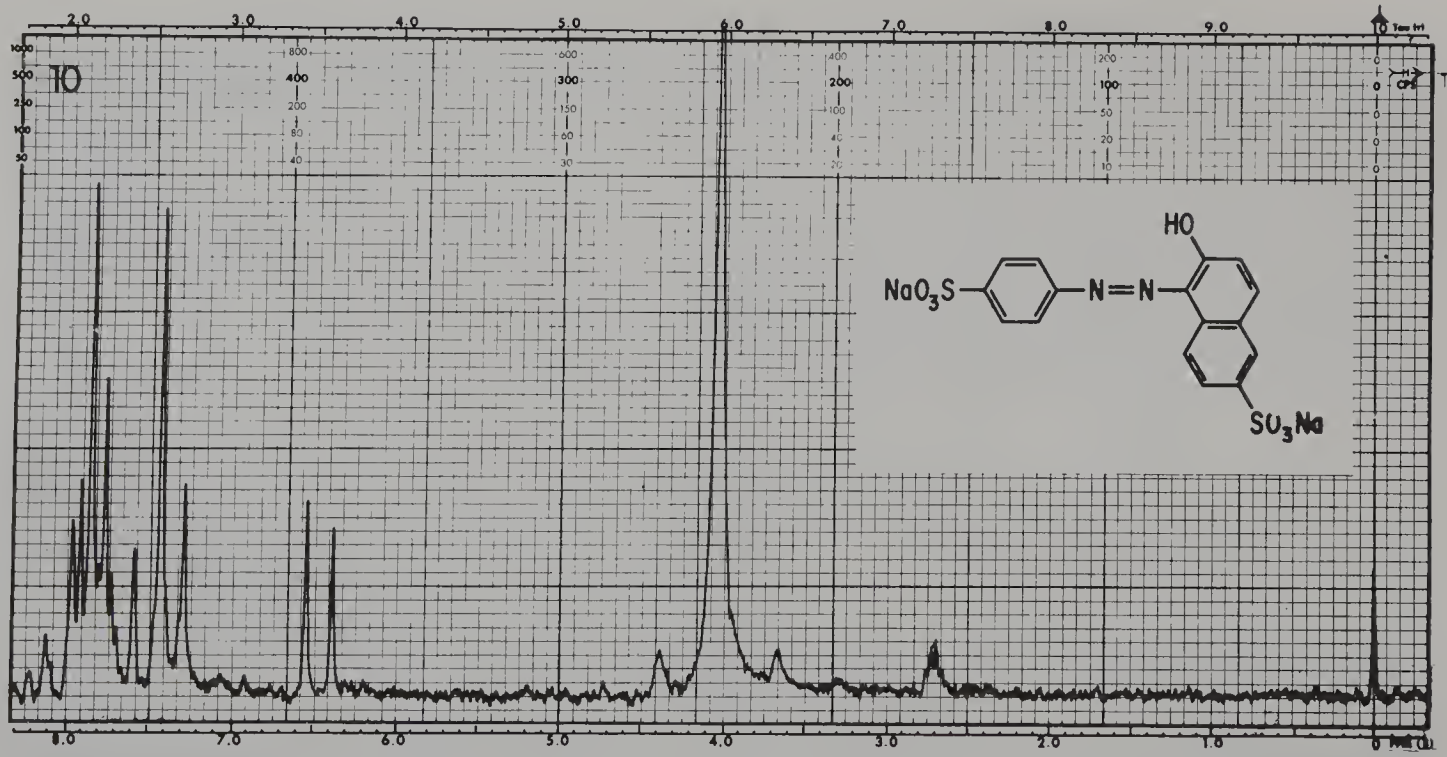

Figure 2*i* Spectrum of FD&C Yellow No. 6: concentration, 20%; amplitude, 50

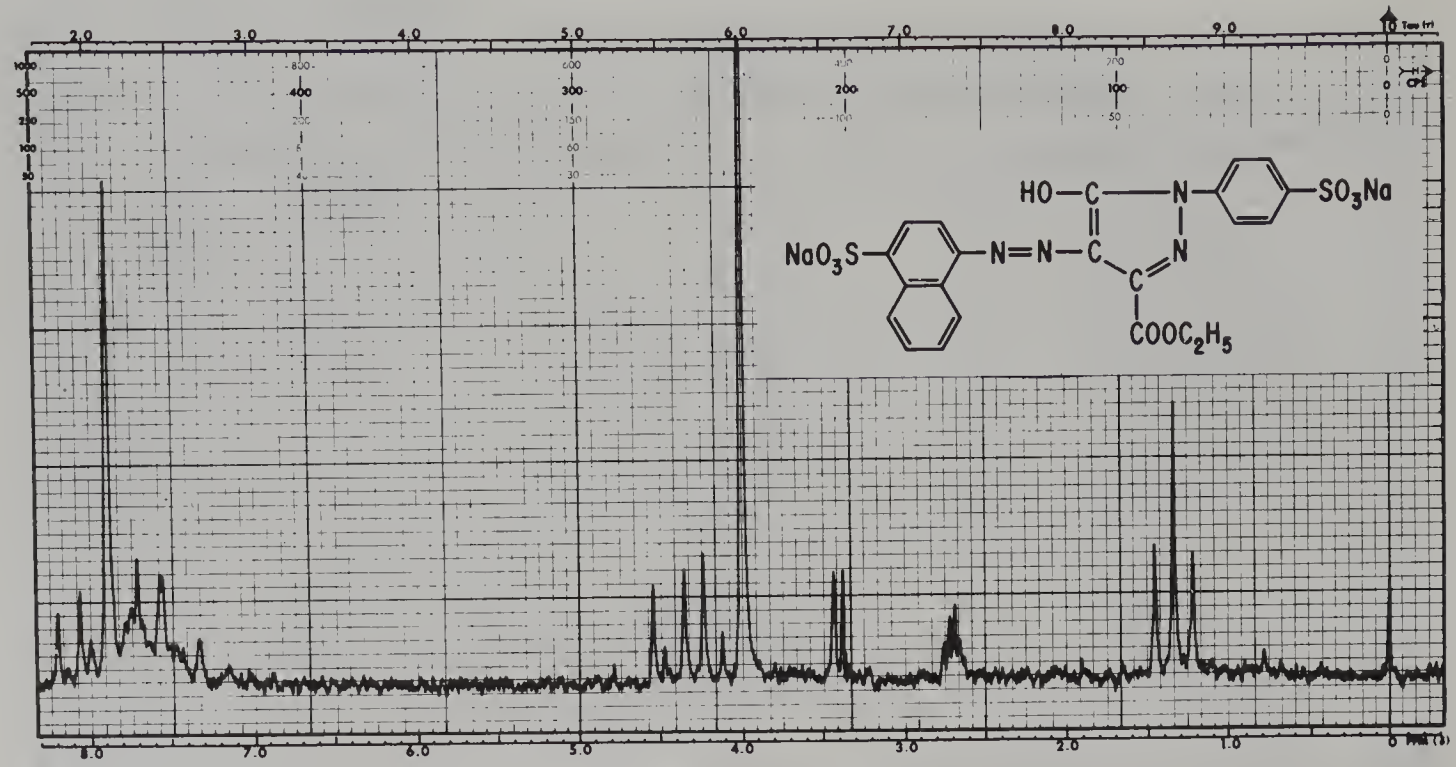

Figure 2*j* Spectrum of Orange B: concentration, 10%; amplitude, 63

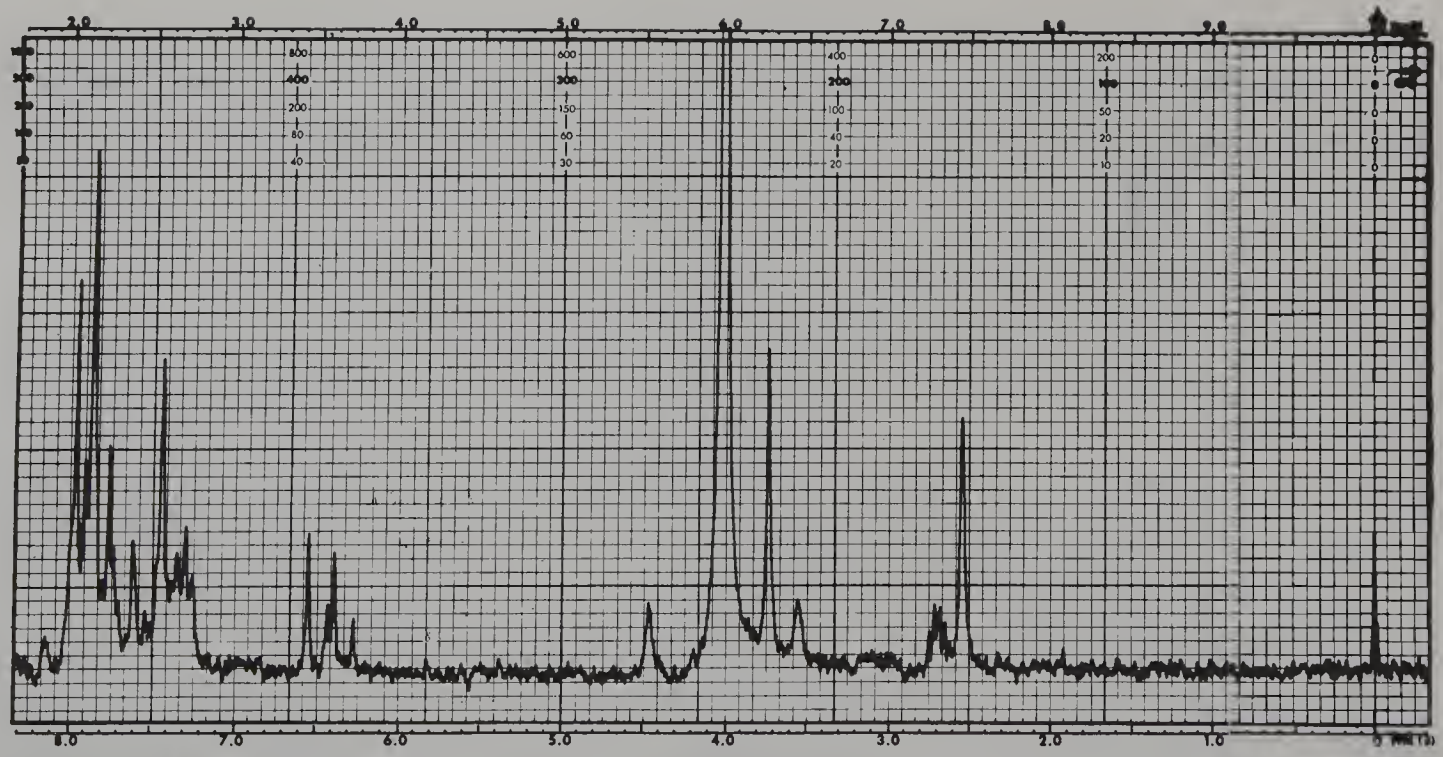

Figure 2*k* Spectrum of "Orange"—a secondary colorant containing 25% FD&C Red No. 40, 20% FD&C Yellow No. 5, and 55% FD&C Yellow No. 6

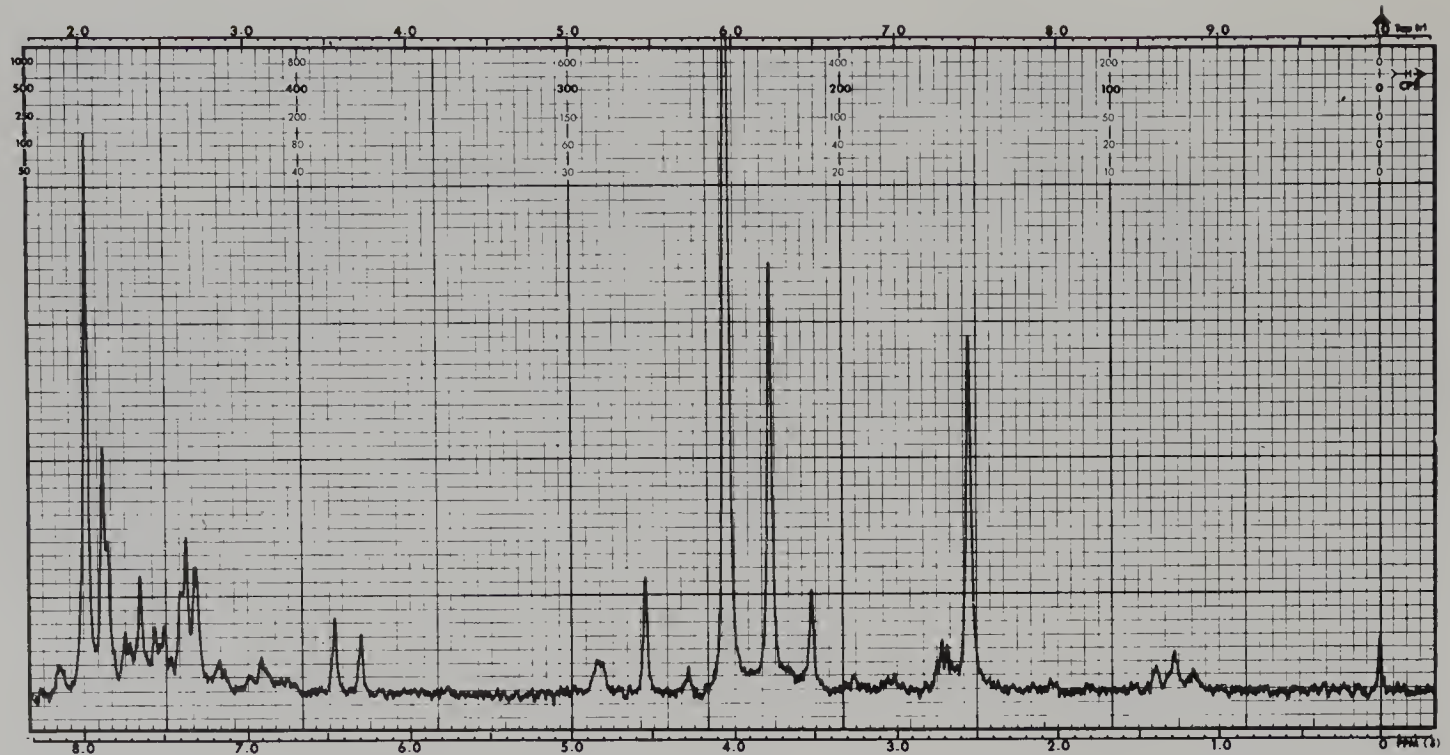

Figure 2*l* Spectrum of "Chocolate"—a secondary colorant containing 10% FD&C Blue No. 1, 45% FD&C Red No. 40, and 45% FD&C Yellow No. 5

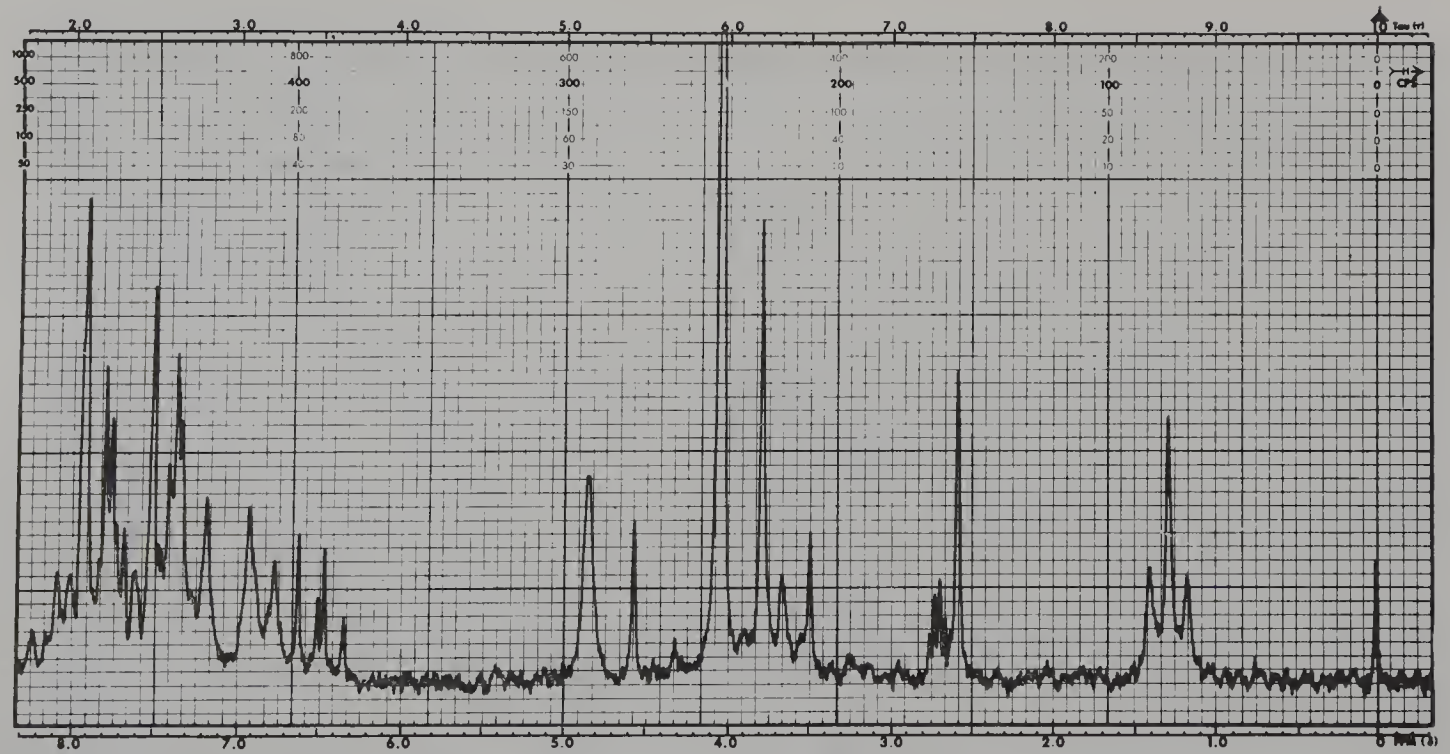

Figure 2*m* **Spectrum of "Black"—a secondary colorant containing 36% FD&C Blue No. 1, 22% FD&C Red No. 40, and 42% FD&C Yellow No. 6**

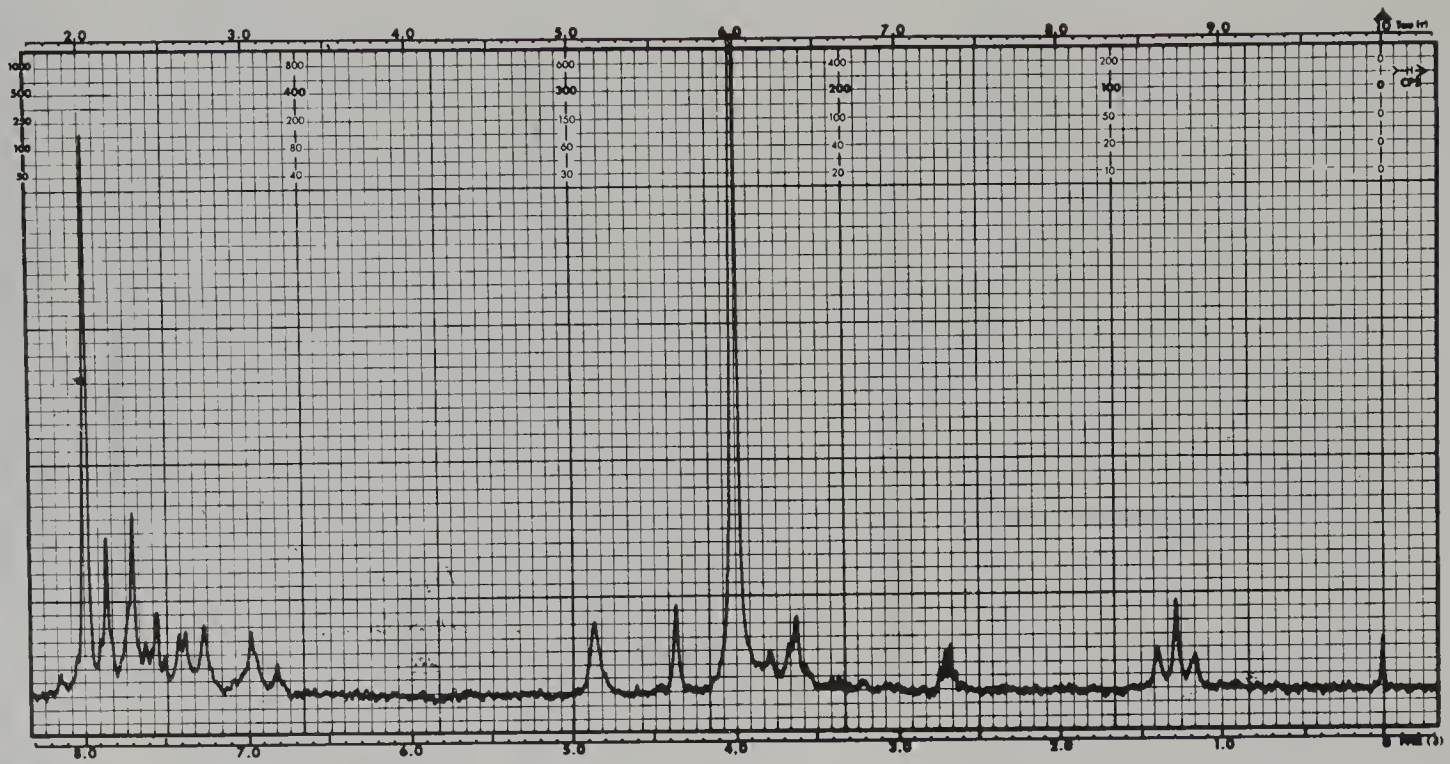

Figure 2*n* Spectrum of "Mint Green"—a secondary colorant containing 25% FD&C Blue No. 1 and 75% FD&C Yellow No. 5

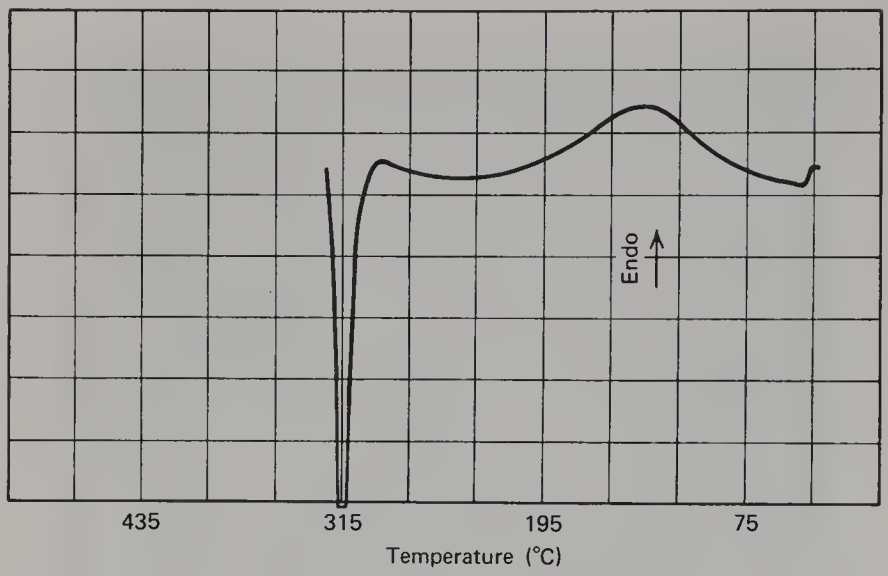

Figure 3a Thermogram of FD&C Blue No. 1

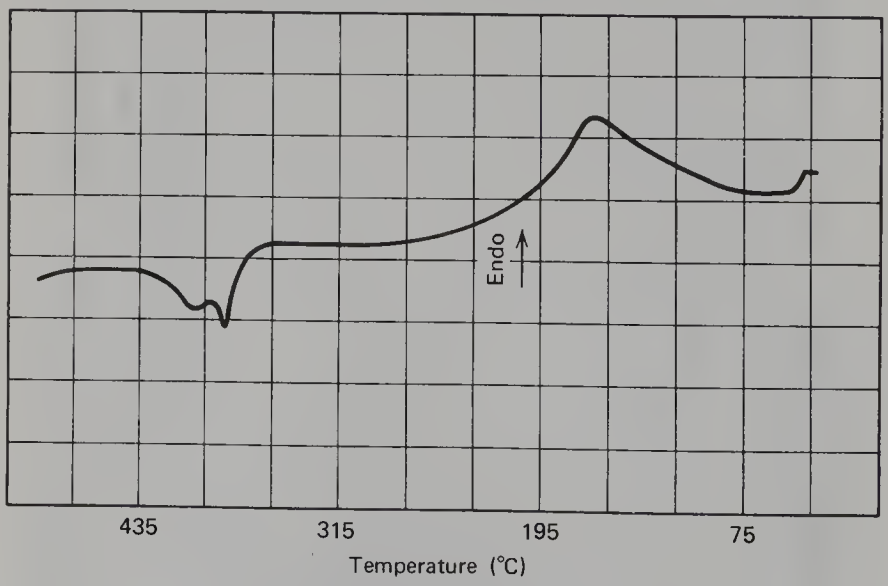

Figure 3*b* Thermogram of FD&C Red No. 3

Figure 3 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

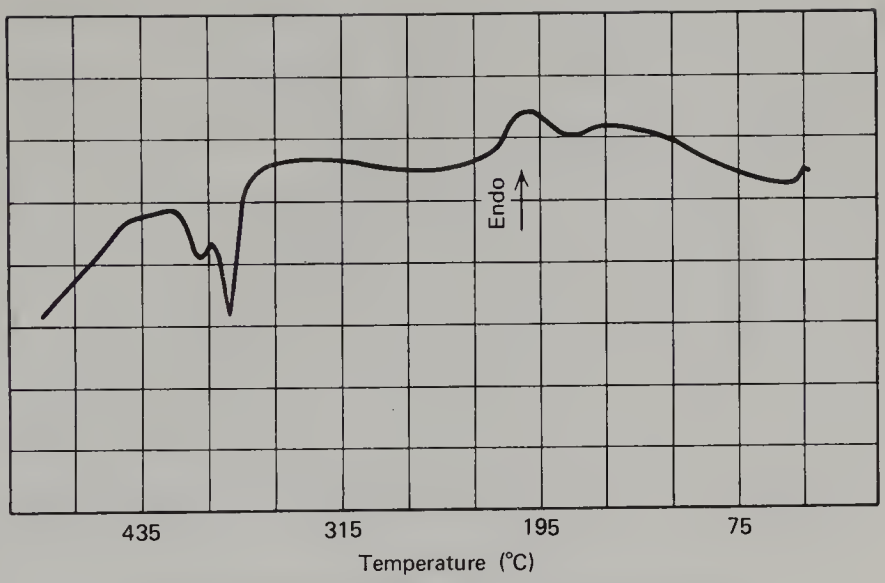

Figure 3c Thermogram of FD&C Red No. 4

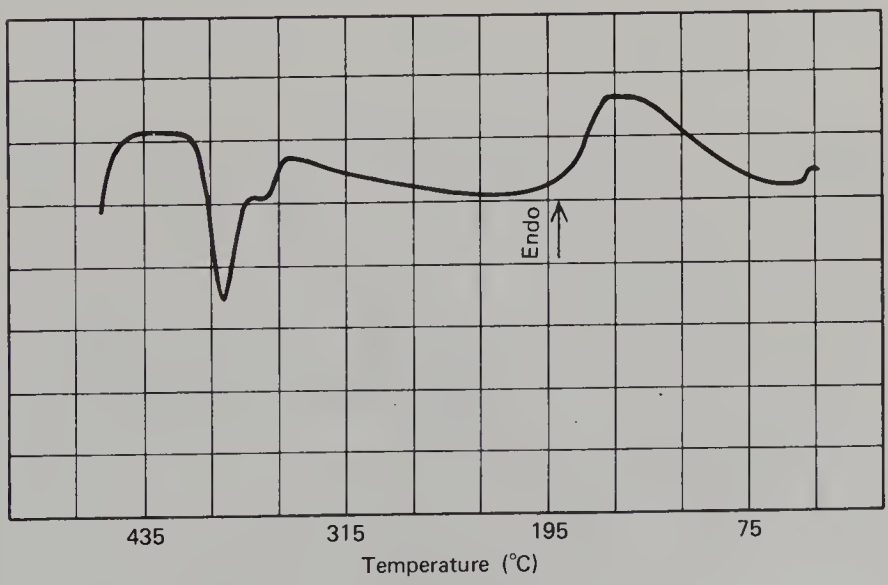

Figure 3*d* Thermogram of FD&C Yellow No. 5

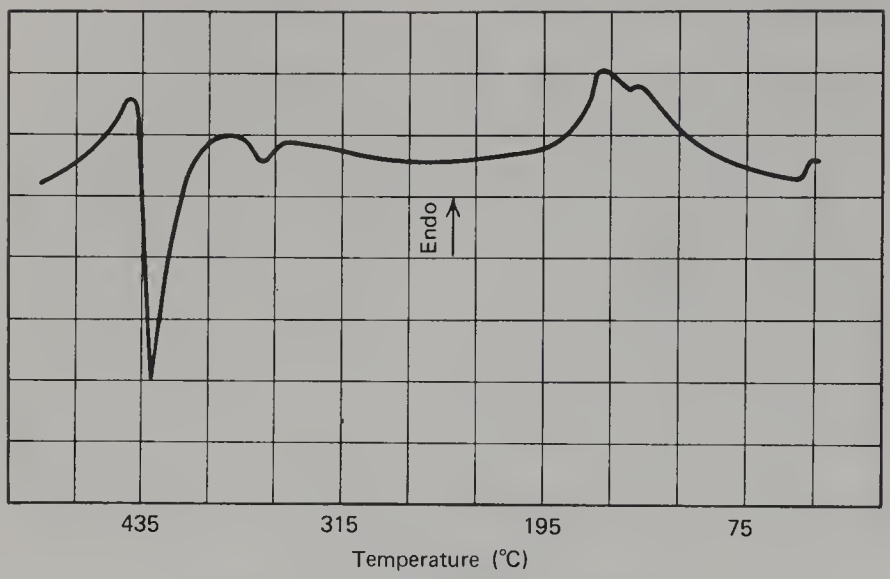

Figure 3e Thermogram of FD&C Yellow No. 6

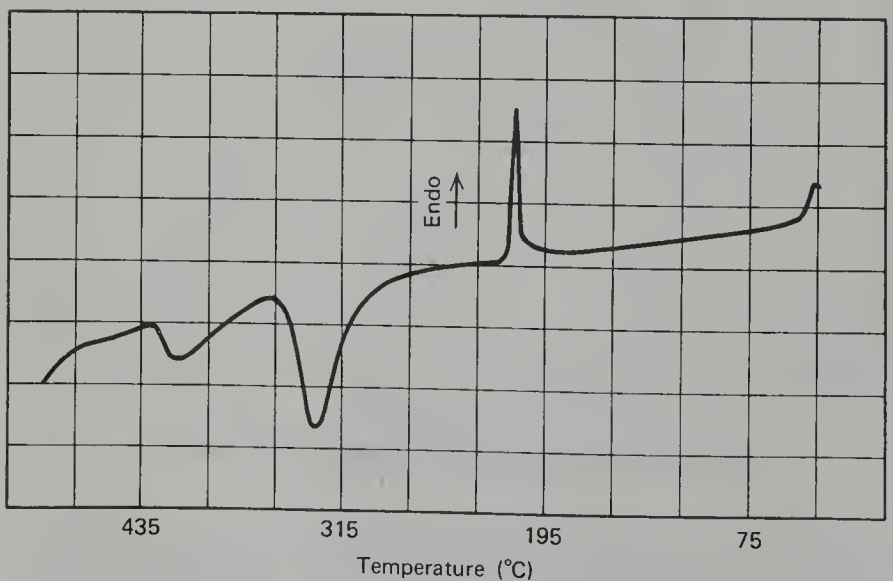

Figure 3f Thermogram of D&C Red No. 17

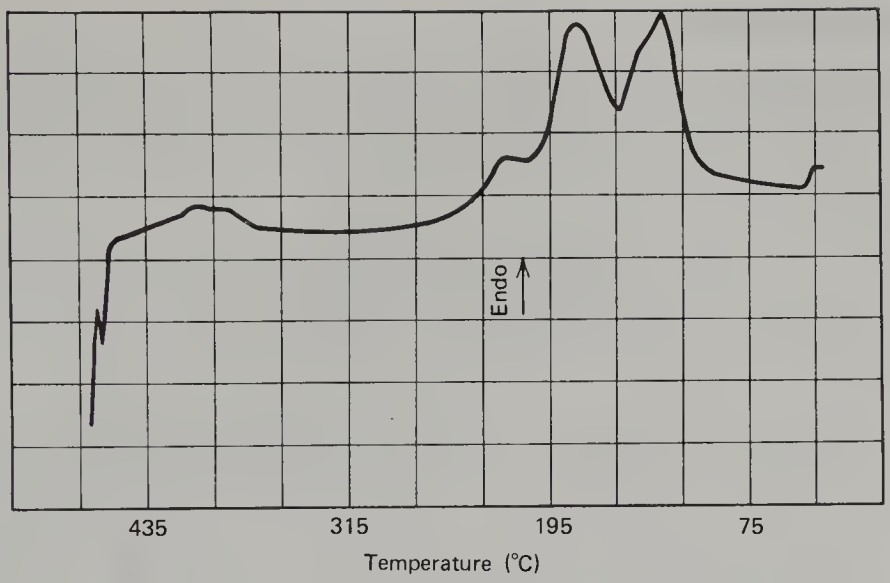

Figure 3*g* Thermogram of D&C Yellow No. 8

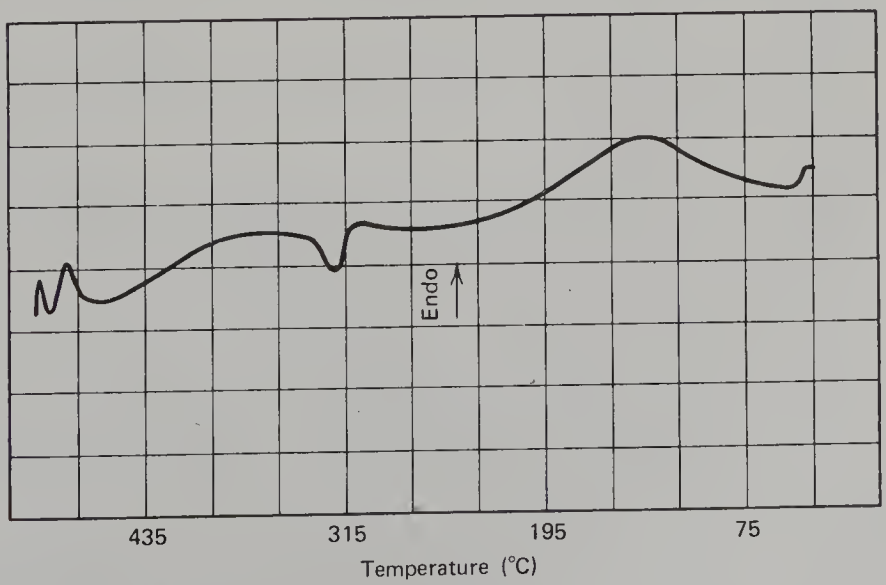

Figure 3*h* Thermogram of D&C Yellow No. 10

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sorption Spectrophotometry. Describes a procedure whereby a sample is run spectrophotometrically versus a known reference solution that is continually varied until it compensates for all the sample absorption. The method can be used to prove the identity of a sample or to analyze mixtures.

JONES, J. H., HARROW, L. S. JAOAC *34*, 831–842 (1951). The Identification of Azo Dyes By Spectrophotometric Identification of Their Reduction Products. Water-soluble colors are reduced in water with sodium hydrosulfite, and oil-soluble colors are reduced in alcohol with titanium trichloride. The reduction products are resolved by distillation, extraction, and/or chromatography and identified spectrophotometrically.

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MARMION, D. M. JAOAC *53*, 244–249 (1970). Evaluation of Color Additives Using a Differential Scanning Calorimeter. Includes thermograms of FD&C Red No. 4, FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Blue No. 1, FD&C Red No. 3, D&C Red No. 17, D&C Yellow No. 8, and D&C Yellow No. 10 and describes the use of DSC as a tool for identification.

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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.

REITH, J. F., GIELEN, J. W. J. Food Sci. *36*, 861–864 (1971). Properties of Bixin and Norbixin and the Composition of Annatto Extracts. Includes IR spectra (KBr disk) of α-bixin, β-bixin, α-norbixin, and β-norbixin.

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 Orange No. 17	X55	
D&C Red No. 6	X66	
D&C Red No. 7	X67	
D&C Red No. 8	X68	
D&C Red No. 9	X69	
D&C Red No. 17	X77	
D&C Red No. 19	X79	U4
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	

Color Additive	Page in Sadtler Collection IR	Visible/UV
D&C Red No. 36	X96	
D&C Red No. 37	X97	
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.

SUZUKI, M., NAKAMURA, E., NAGASE, Y. Analytical Studies for Dyes by Using Infrared Spectrum. I. Yellow Dyes for Food, Auramine and Butter Yellow. Yakugaku Zasshi *79*, 1116–1119 (1959); II. Green Dyes for Food and Malachite Green. Ibid. *79*, 1209–1211 (1959); III. Red Dyes for Food and Rhodamine B. Ibid. *80*, 916–919 (1960). Describes the KBr method for the identification of color additives. The discussions include Ext. D&C Yellow No. 7, FD&C Yellow Nos. 5 and 6, FD&C Green No. 3, FD&C Red Nos. 3 and 4, and D&C Red Nos. 19, 22, and 28.

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.

Chapter 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 batchs 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 procedure 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 pure dye 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: $R—NO + 4H^+ + 4Ti^{3+} \rightarrow R—NH_2 + H_2O + 4Ti^{4+}$

Several variations of the $TiCl_3$-reduction method are now in use, and the proper technique is dictated by the color analyzed. The methods to use as well as the milliequivalent weights and the volumes of 0.1 *N* titanous chloride needed per gram of color titrated are shown in Table 22 for a number of colorants.

TABLE 22 $TiCl_3$ TITRATION FACTORS

Color	Milliequivalent Weight	Milliliters of 0.1*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. 8	0.09970	100.3	6
D&C Red No. 9	0.1111	90.00	6
D&C Red No. 17	0.04405	227.0	5
D&C Red No. 19	0.2395	41.75	
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. 37	0.3635	27.51	
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

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, formerly FD&C Green No. 2) is good for this purpose.

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. 4. 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 NBS $K_2Cr_2O_7$/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.

$$\text{Normality} = \frac{\text{mL } K_2Cr_2O_7 \times \text{normality } K_2Cr_2O_7}{\text{net mL } TiCl_3}$$

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

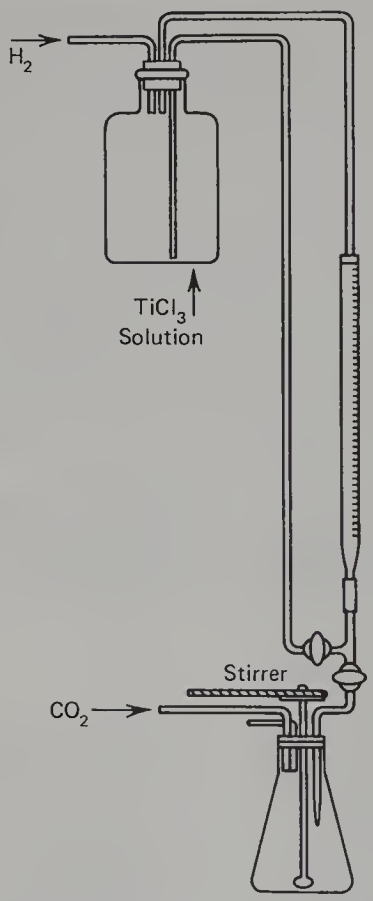

Figure 4 Titanous chloride titration apparatus (Reprinted with the permission of the Association of Official Analytical Chemists)

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. 4, 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. 4, 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. 4 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 sodium acid tartrate in 75 mL of distilled water and add this to the flask. Connect the flask to the apparatus in Fig. 4, 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 should obtain its own factors using its standards and its spectrometer.

Spectrophotometric Procedures

General Procedure for Primary Colorants: Dissolve a portion of well-mixed sample in a spectrograde quality of the solvent shown in Table 23 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 23), 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 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. 19, 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. 6–9, 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.

D&C Orange No. 17—Transfer 0.030–0.040 g of sample to a beaker, add 200 mL of chloroform, then boil and stir the mixture until dissolved. Cool; make to volume with chloroform.

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.

TABLE 23 ABSORPTION COEFFICIENTS

	Solvent System[a]	Wavelength of Maximum Absorbance (in nm)	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 Orange No. 17	$CHCl_3$	480	77
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. 8	Alcohol & water (1 + 1)	486	52.2
D&C Red No. 9	Alcohol & water (1 + 1)	486	47
D&C Red No. 17	$CHCl_3$	514	94
D&C Red No. 19	Water	554	236
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 Red No. 37	Water	545	154
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)].

Dilute EtOH—Mix equal volumes of 95% EtOH and distilled water.

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 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 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 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 solutions in subdued light and use low-actinic glassware. Transfer 0.05 g of sample to a 100-mL volumetric flask, dissolve it 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 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 of this solution in a 1-cm cell at the maximum near 452 nm, using cyclohexane as the 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 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 volumetric 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 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 = *A*. Pipette 2.0 mL of *A* into a second 50-mL volumetric flask, dilute to volume with cyclohexane, then mix well; solution = *B*. Pipette 5.0 mL of *B* into a third 50-mL flask, dilute to volume with cyclohexane, then mix well; solution = *C*. Using 1-cm cells measure the blank-corrected absorbance of *C* 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 of this solution at the absorption maximum near 494 nm.

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 of this solution at the absorption maximum near 494 nm.

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*): 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.

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 since these are probably 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,

and 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.

$$\text{Percent total sulfur} = \frac{W(0.1373)(100)}{w}$$

$$\text{Percent organic sulfur} = \%\ \text{total sulfur} - \%\ \text{inorganic sulfur}$$

$$\text{Percent pure dye} = \frac{(\%\ \text{organic sulfur})(100)}{\text{theoretical}\ \%\ \text{sulfur}}$$

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 volume of titrant used for sample (in mL), *B* is volume of titrant used for blank (in mL), *N* is normality of titrant, and *w* is sample weight (in g).

$$\text{Percent pure dye} = \frac{\%\ \text{nitrogen}\ (100)}{\text{theoretical}\ \%\ \text{nitrogen}}$$

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 permanganate 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 Gooch crucible fitted with an asbestos mat coated with a thin layer of zinc dust. Wash the crucible 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 $CoSO_4(NH_4)_2SO_4$ 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 an NBS SRM 2030 glass filter with an *A* specified by NBS in the range 0.4–0.6 at 465 nm.

Capsicums: Grind samples to pass a No. 40 sieve (a No. 18 if the NBS 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 NBS 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 NBS 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 an NBS 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 NBS standard at 465 nm/actual *A* of the NBS

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. Extract 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

$$= \frac{(A \text{ at } 460 \text{ nm}) \times (61{,}000\ I_f)}{\text{sample wt in g}}$$

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: 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

Spectrophotometric Color Value of Turmeric Oleoresin: Weigh 100–200 mg of a well-mixed sample into 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.

$$CV = \frac{\text{Absorbance}}{\text{Sample wt. @ 1/5000}}$$

$$\text{Percent } \textit{Curcumin} = \frac{CV\ (1/5000)}{33.00}$$

where 33.00 is CV of *Curcumin* (EK No. 1179) at 1/5000 in acetone, 422–425 nm.

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$$\text{Tinctorial power} = \frac{A_{560}}{C}$$

$$\text{Hue index} = 10 \log (A_{510}/A_{610})$$

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Chapter 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 needs 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

Digest a good grade of retentive asbestos (CAUTION!) with HCl (1 + 3), wash it free of acid, and decant the supernatant liquid to remove any fine particles. Prepare a well-packed asbestos mat of suitable thickness in a Gooch crucible, wash it with hot water, dry, ignite, rewash, 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 an asbestos mat (CAUTION!) 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.

Chapter 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, whereas 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 except D&C Red No. 19, which is not retained by the precolumn. Iodide can not 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 eluant, and a flow of 124 mL/hr (30% pump speed) inject 100 μL of a 0.02% sample solution in eluant. 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 with those of standards similarly chromatographed.

Suppressor columns and precolumns must be regerated about every 8 hr. Suppressors 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 eluant. 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.

PHOSPHORUS IN CERTIFIABLE STRAIGHT COLORS (3)

Reagents:

(a) Ashing reagent—Weight 392 g of cellulose powder (Whatman CF-11, Whatman, Inc., 9 Bridewell Pl., Clifton, N.J. 07014) 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.

*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).

(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 Orange No. 17, D&C Red Nos. 22 and 28, D&C Yellow Nos. 8 and 11, and any other dyes whose ash con-

tains 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., 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, Except D&C Red No. 19 (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 preset 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, weigh 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 Specific 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 semilogarithmic 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. 230. 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. 230. 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 it 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, 2mL, 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. 230.

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 10 mL 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, N.J. 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. 5 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 containing Ascarite; reflux for 10 min. Cool to room

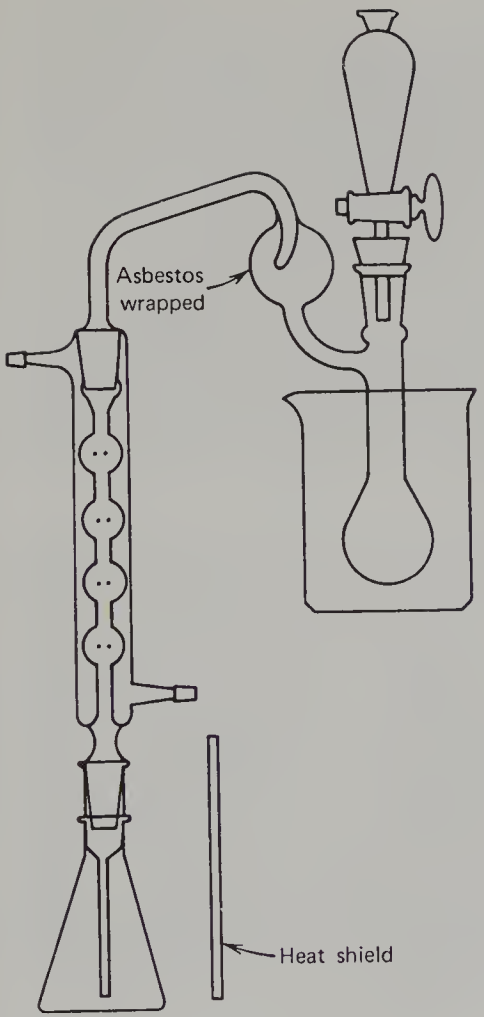

Figure 5 Apparatus used in sodium acetate determination (Reprinted with the permission of the Association of Official Analytical Chemists)

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. 214 beginning with "25 mL of 7% aqueous $KMnO_4$. . . ."

Take a second 100-mL aliquot and determine bromide as described on p. 216 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 an eluant solution 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 the eluant solution. (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 the eluant to the sample and mix. Then add 50 g of ammonium sulfate and mix well. Using 25 mL of eluant, transfer the sample to a chromatographic column prepared and eluted as described on p. 272. 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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Chapter 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 whole 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 proven 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 argon plasma emission spectroscopy should all be useful for the analysis of color additives. However, few applications of these techniques in this area have been published as yet. 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 $(C_2H_5)_2NCSSAg$ 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. 6c) 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 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.

Figure 6 Apparatus for arsenic test (Courtesy Fisher Scientific Co.)

(e) Standard iodine solution—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. 7 and 8. 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. 7a) 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. 7).

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. 7). Raise the delivery tube at (*d*) (Fig. 8) 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

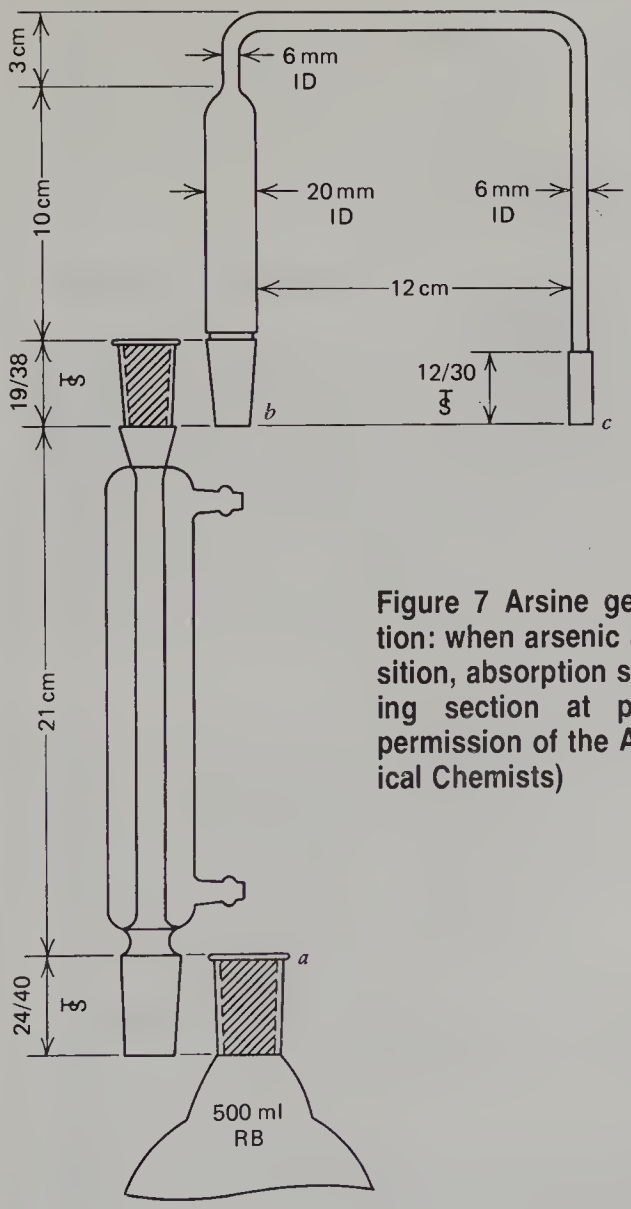

Figure 7 Arsine generator and distillation section: when arsenic apparatus is in operating position, absorption section is connected to distilling section at position C (Reprinted with permission of the Association of Official Analytical Chemists)

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 soln during the titration by alternately sucking and blowing through the stirring tube.

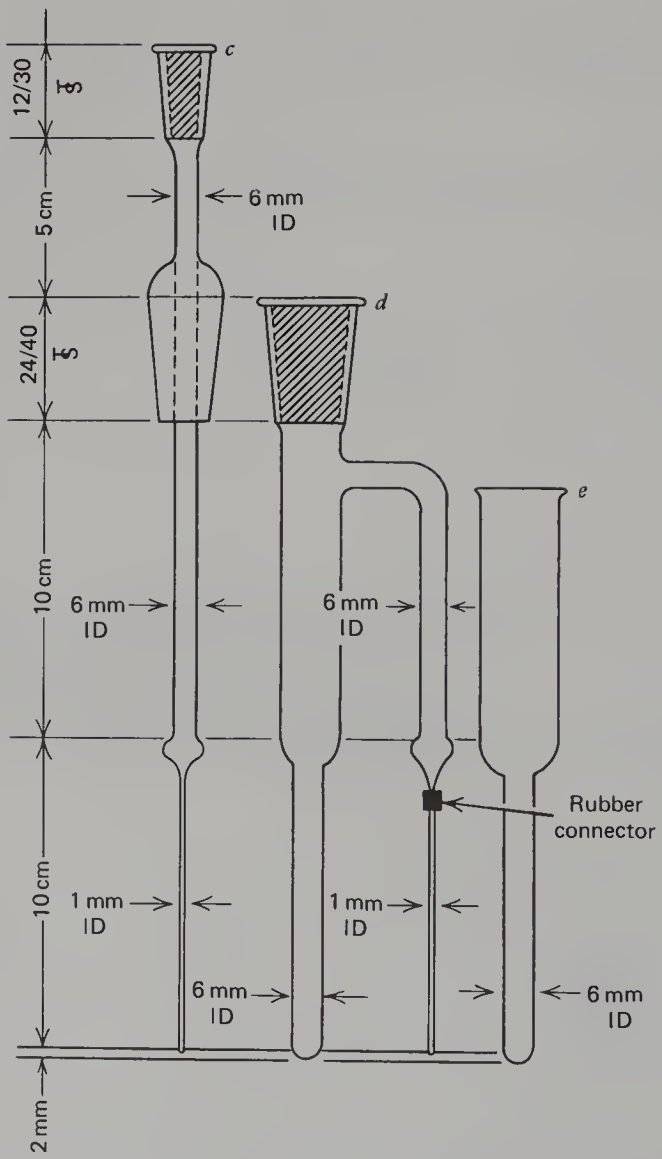

Figure 8 Arsine absorber and titrating tubes (Reprinted with the permission of the Association of Official Analytical Chemists)

$$\frac{\text{mmg } As_2O_3}{\text{mL } I_2} = \frac{\text{mmg } As_2O_3/\text{mL} \times \text{mL } As_2O_3 \text{ soln.}}{\text{mL } I_2 \text{ soln.} \times 4}$$

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.

$$\text{Percent } As_2O_3 = \frac{\text{mL } I_2 \text{ soln.} \times I_2 \text{ factor} - [\text{ml} \frac{As_2O_3}{4} \times \text{mmg } As_2O_3/\text{mL}] - \text{blank}}{\text{sample weight}}$$

BARIUM (SOLUBLE) IN D&C RED NO. 9

Rub 10 g of color into a paste with a little water and four to eight drops of alcohol. Add 150 mL of water and 40 mL of (1 + 249) HCl. (On a separate aliquot test the pH of the suspension with meta cresol purple indicator. It should be pH 2 ± 0.1). Warm on a water bath for 30 min, stirring frequently. Cool, transfer to a 250-mL volumetric flask, make to volume, mix, and filter on a dry filter. To 200 mL of filtrate, add 20 mL of concentrated HCl and heat to boiling. Add 5 mL of (1 + 3) H_2SO_4, again heat to boiling, and allow to settle for 30 min on a water bath. Filter, wash the precipitate with hot (1 + 199) H_2SO_4, ignite, and weigh as $BaSO_4$. Report as $BaCl_2$.

$$BaSO_4 \times 0.8923 = BaCl_2$$

If strontium salts are present, Ba and Sr may be separated as follows. Prepare a paste, dilute with HCl, treat on a water bath, make to volume, and filter as described above. Make the filtrate alkaline to methyl red with NH_4OH and acidify with acetic acid. Add 5 mL of a 5% solution of $K_2Cr_2O_7$. Yellow $BaCrO_4$ precipitates, which may be filtered, washed with dilute acetic acid and water, redissolved in HCl, and reprecipitated as $BaSO_4$.

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 minimum 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 (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 as more than or less than 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 g 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 by heating 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.

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. 9). Bubble H_2S through the solution

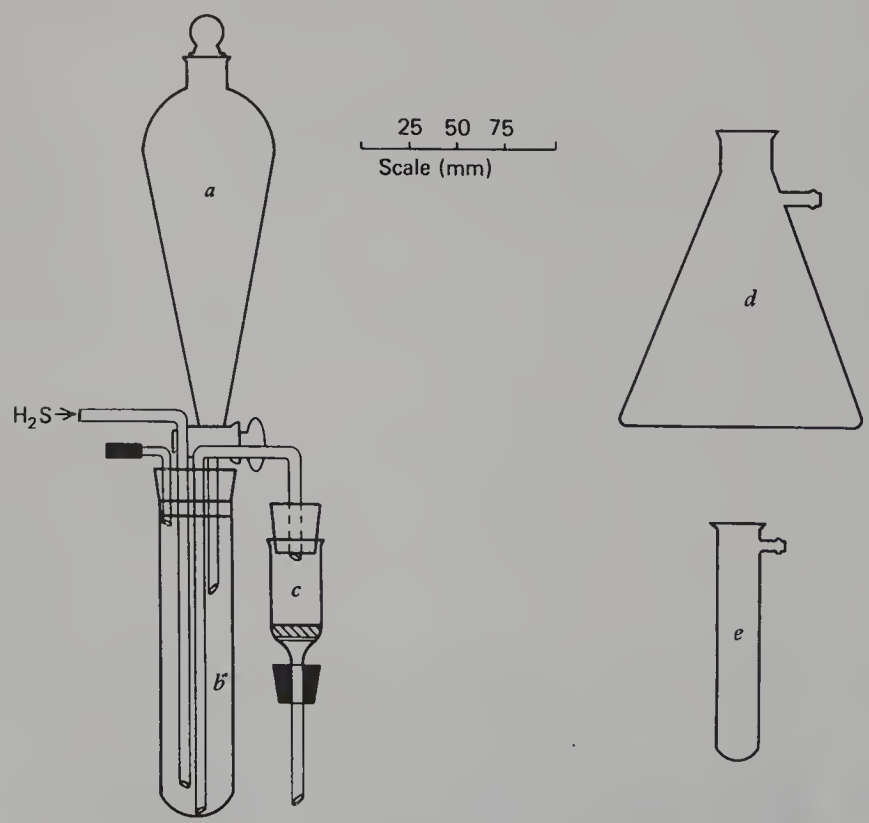

Figure 9 Sulfiding apparatus (Reprinted with the permission of the Association of Official Analytical Chemists)

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 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 the hydrochloric acid solution, 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 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 tung-

sten is absent. If tungsten is absent, calculate μg of mercury in the sample aliquot from $R_{36°/35°}$ as follows:

$$\mu g \text{ mercury} = \frac{(R_S - R_b)Y}{R_{s+y} - R_s}$$

where R 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. 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 attenator 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 in individual combustion boats. Cover the asbestos with 1–2 g 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 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.

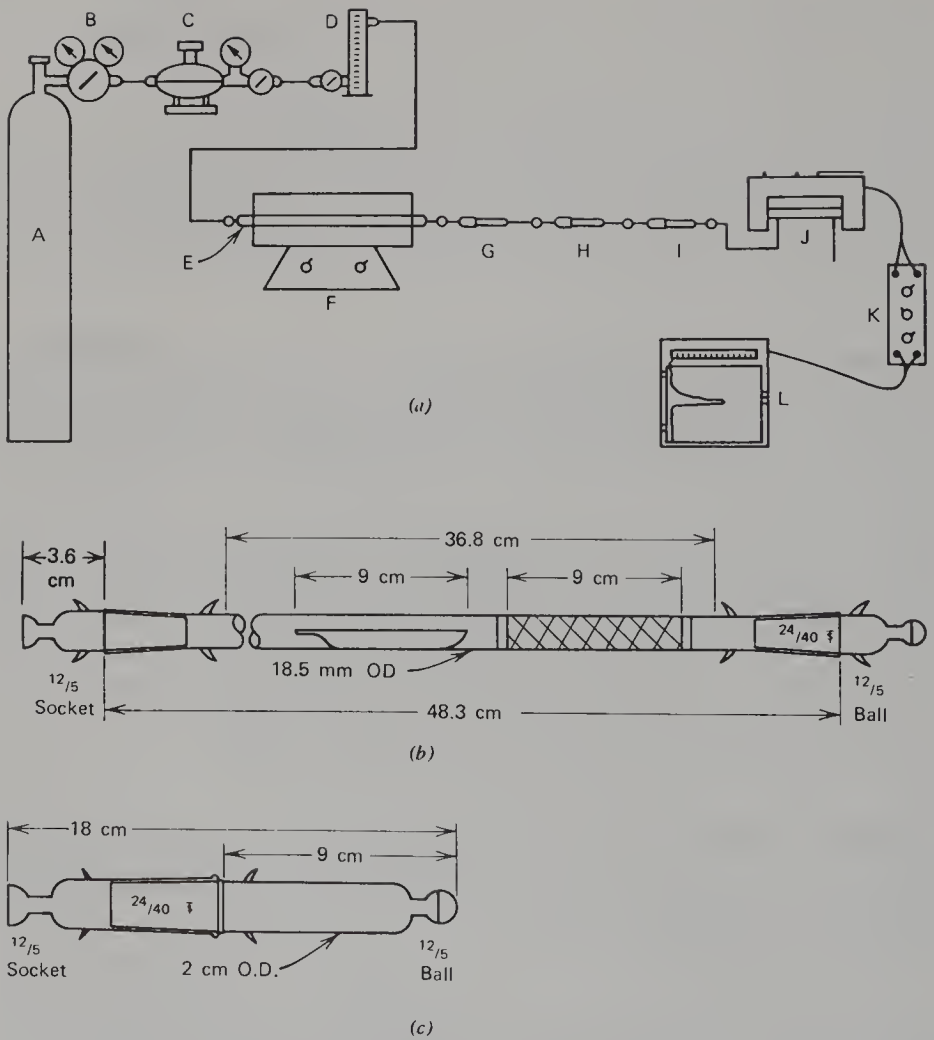

Figure 10 (a) Schematic diagram of apparatus for photometric mercury vapor method:

A. Tank of nitrogen
B. Two-stage pressure regulator
C. Low-pressure regulator
D. Flowmeter
E. Combustion tube
F. Combustion-tube furnance
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)

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

*CAUTION—This compound is a suspected carcinogen and should be handled with care.

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 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 aq. 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 eluant. 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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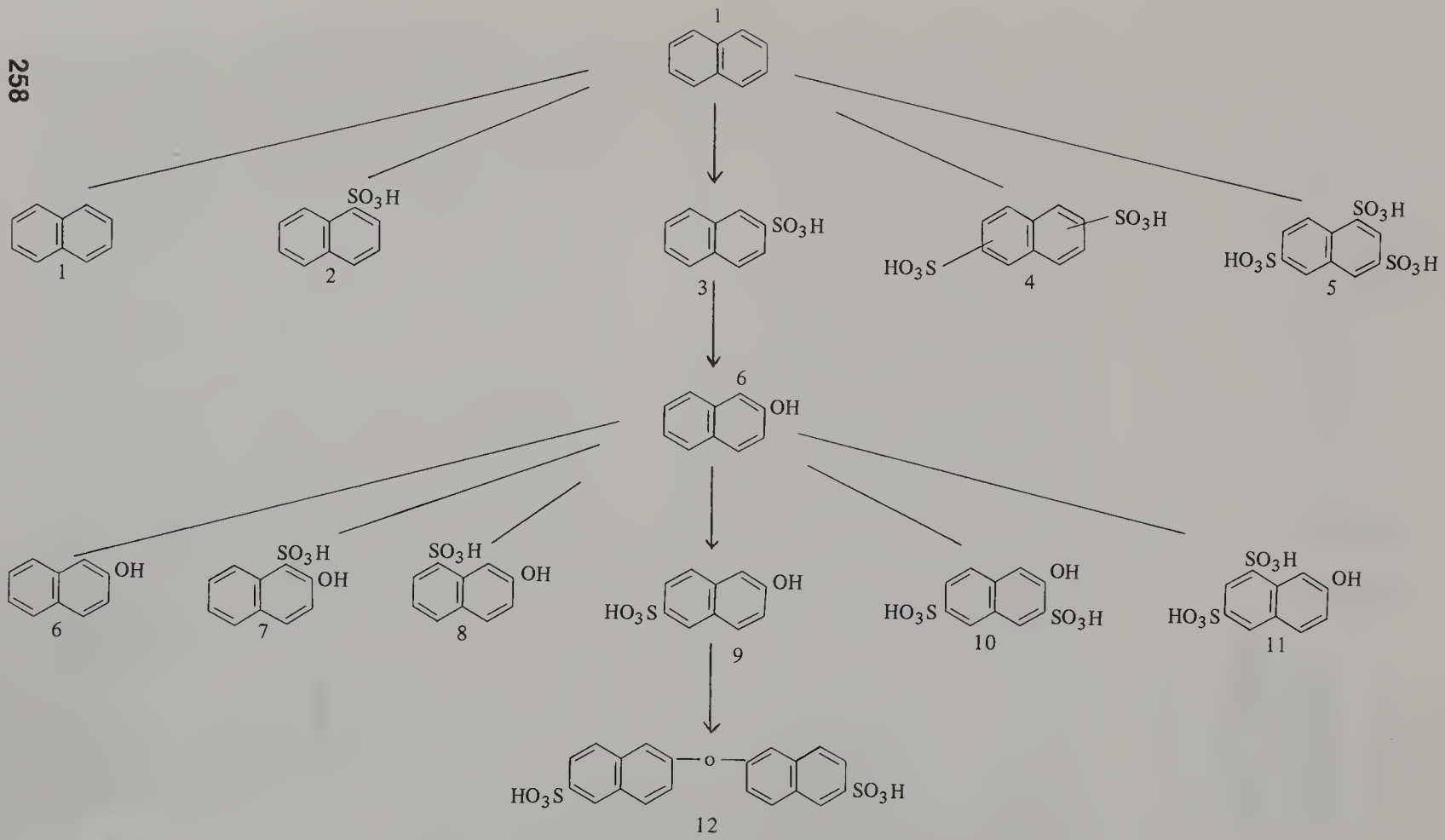
Chapter 11 Organic Impurities

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 colors, of course, can contain analogous impurities, 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, of course, 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 analyze routinely 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.

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.)

More expected in the finished colorant 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


1
SO3H
1
2
SO3H
3
HO3S
SO3H
4
HO3S
SO3H
SO3H
5
6
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6
OH
SO3H
OH
7
SO3H
OH
8
HO3S
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HO3S
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11
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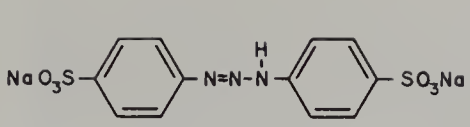
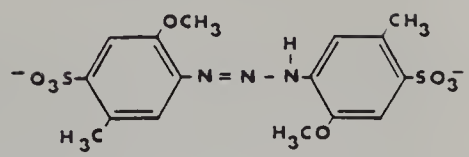
12

Figure 12



DAADBSA



DMMA

Figure 11

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 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. 12.) 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 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.

Figure 12 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)**

Clearly, then, the purity of color additives depends largely on the identity 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. 13–16.) 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 information. 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.

Figure 13 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_2O sample solutions

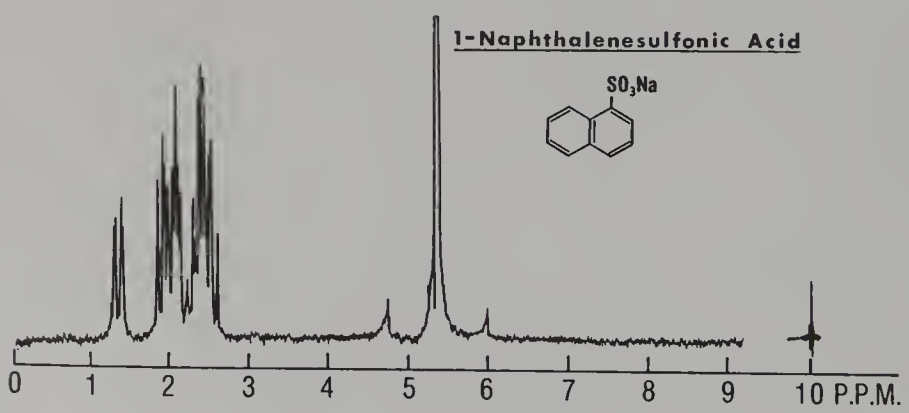


Figure 13*a* Spectrum of 1-naphthalenesulfonic acid

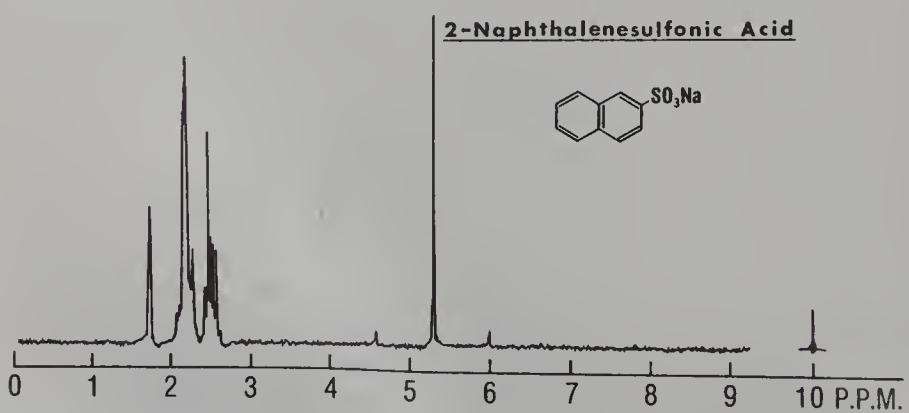


Figure 13*b* Spectrum of 2-naphthalenesulfonic acid

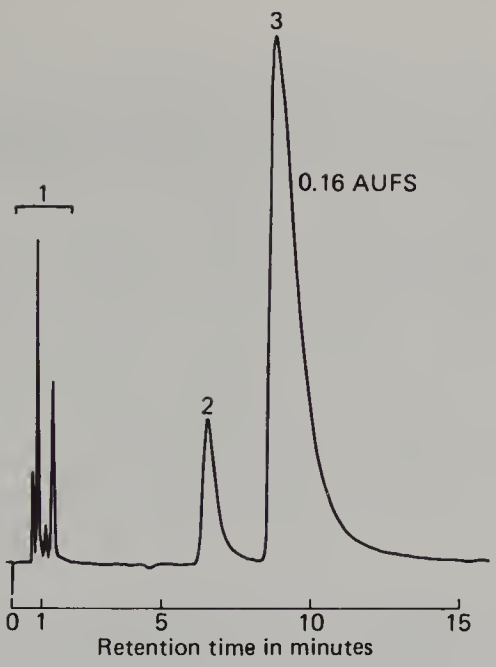


Figure 14 HPLC Separation of 10 μL of a 1% solution of commercial-grade 2-naphthalenesulfonic acid. Conditions: chromatograph, DuPont Model 830; column, 25-cm × 4.6-mm ID Whatman Partisil PXS10/25 ODS-2; detector, UV at 280 nm; temperature, 40°C; flow rate, 4 mL/min; eluant, 90 mL of isopropanol diluted 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

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.

Figure 15 Nuclear magnetic resonance spectra of Schaeffer's salt and related compounds obtained on a Varian A60 Spectrometer, using 5% KOH in D_2O 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

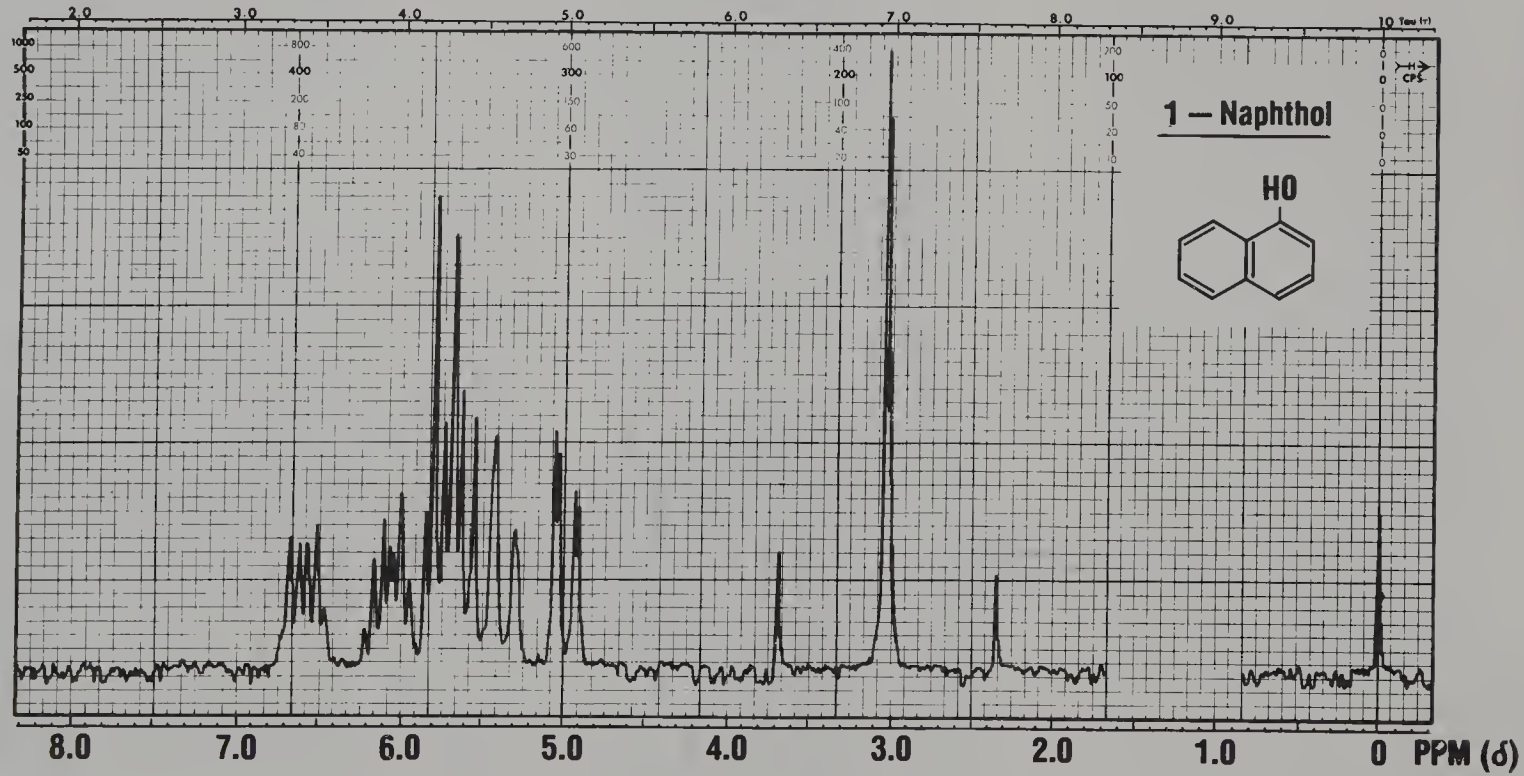


Figure 15*a* Spectrum of 1-naphthol, concentration 10%, amplitude 16

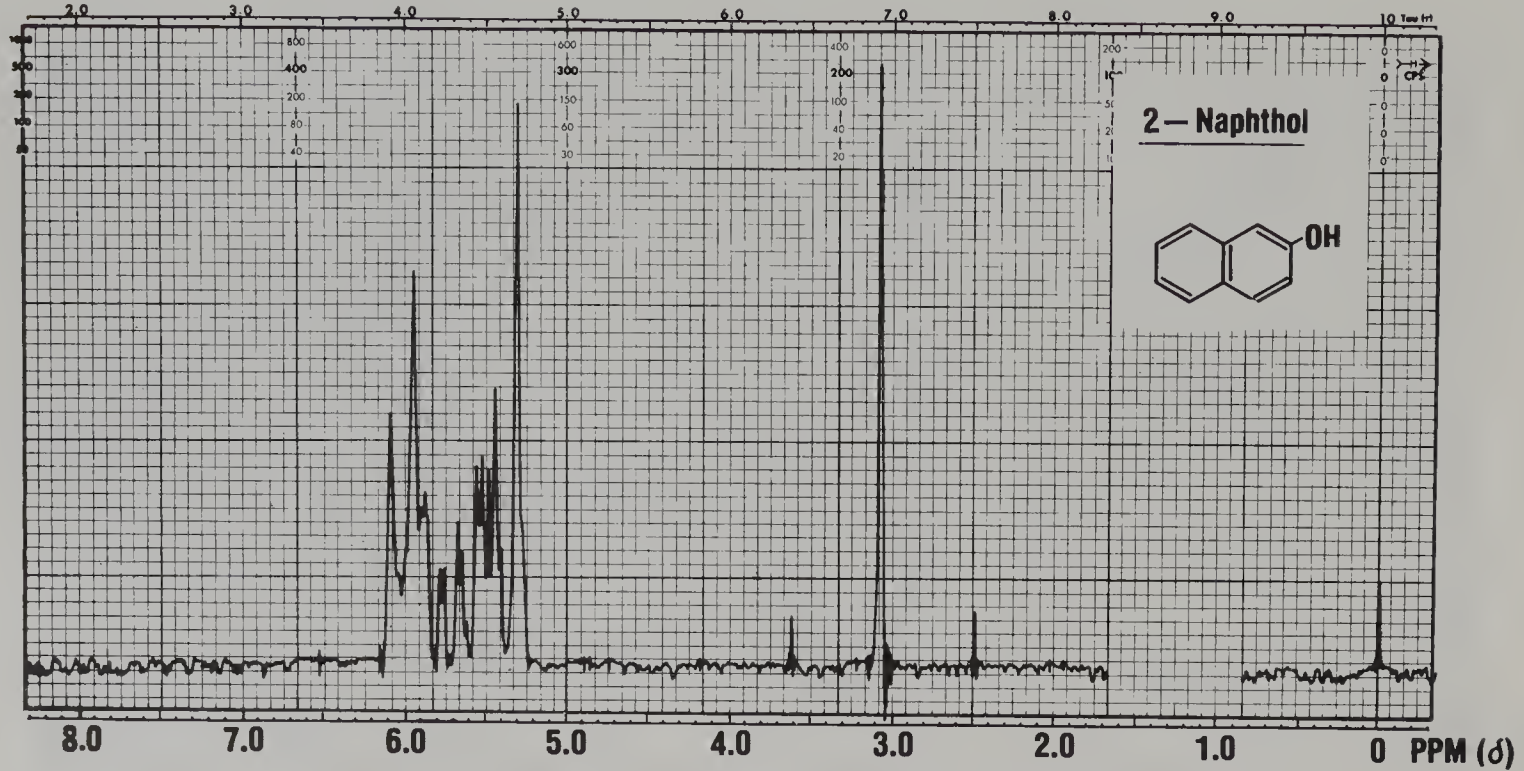


Figure 15*b* Spectrum of 2-naphthol, concentration 10%, amplitude 16

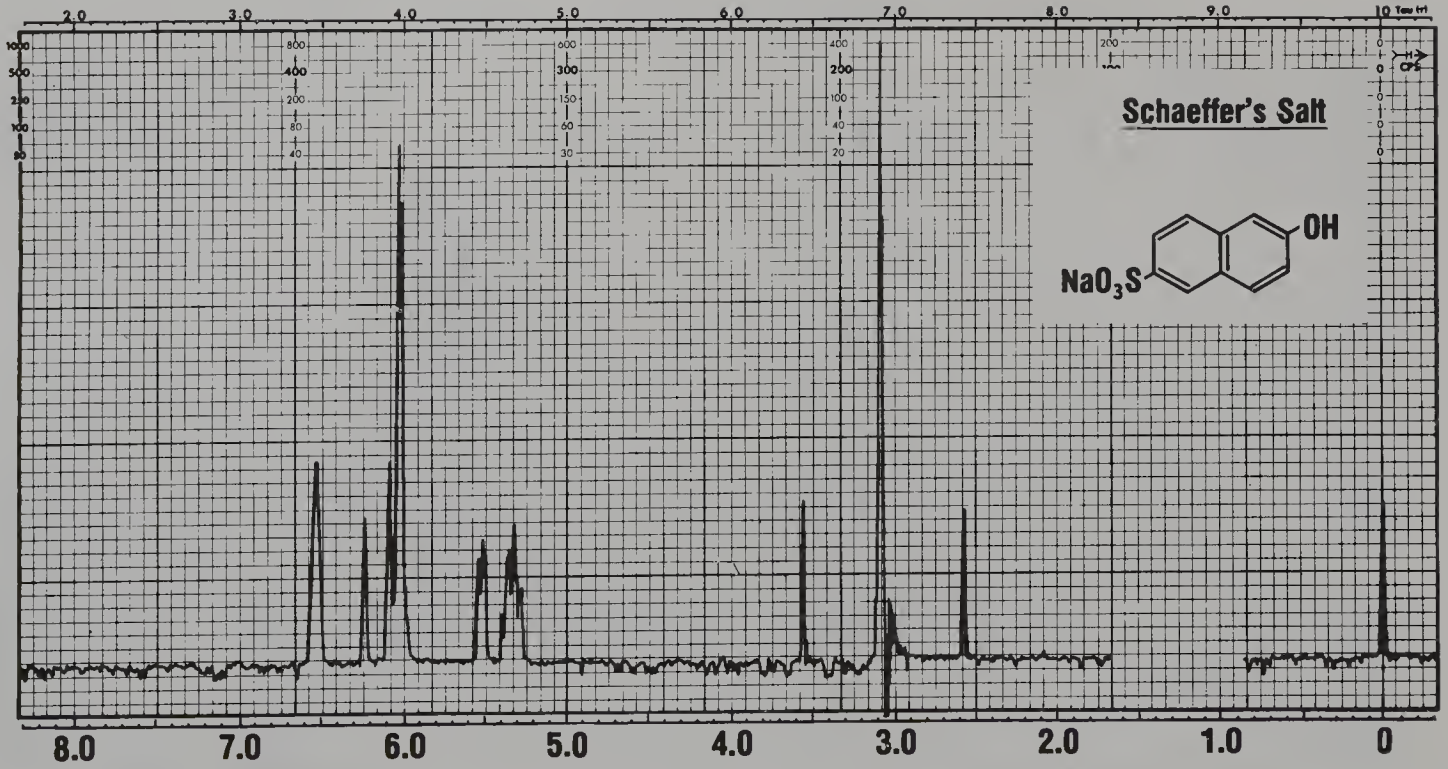


Figure 15c Spectrum of Schaeffer's salt, concentration 10%, amplitude 16

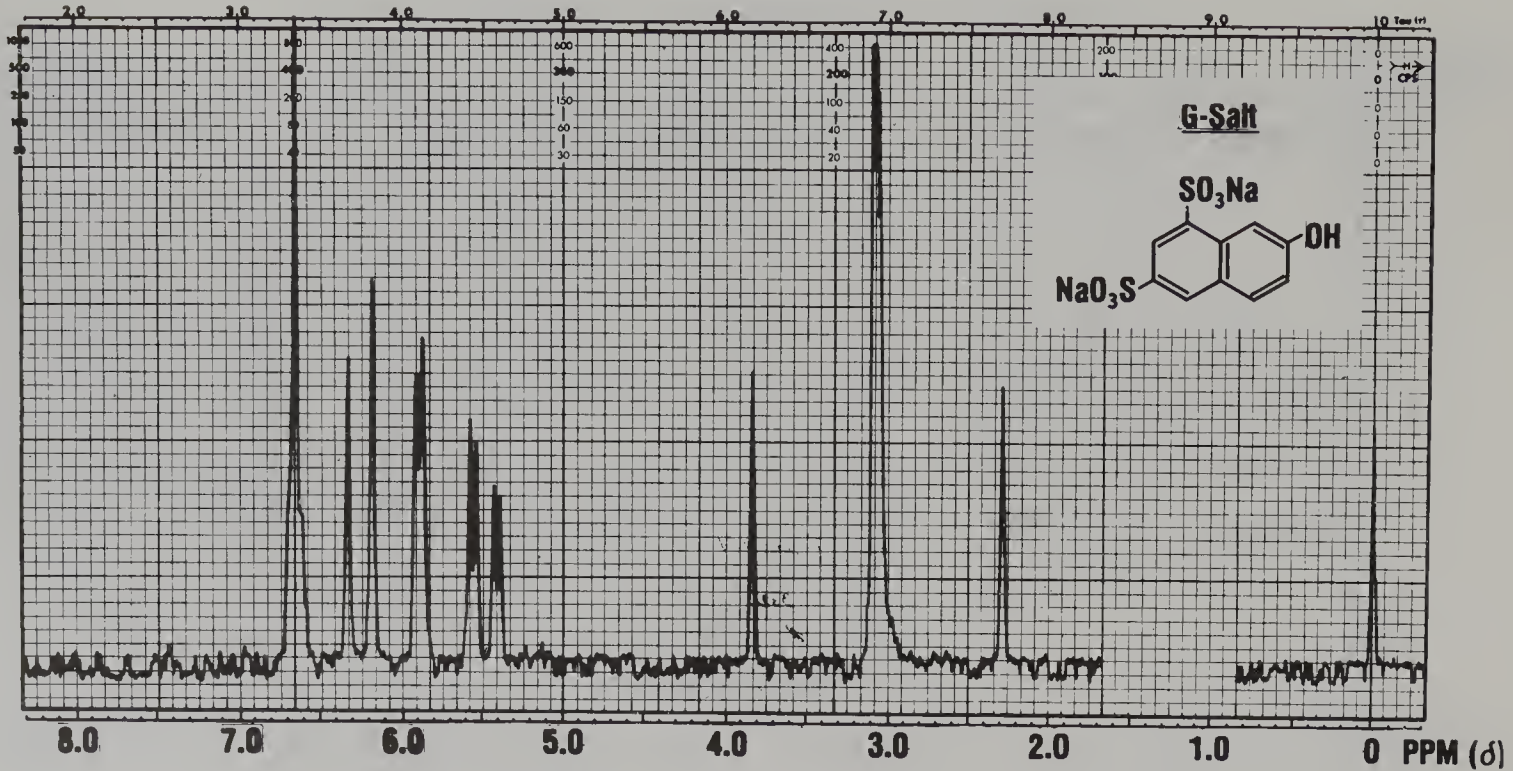


Figure 15*d* Spectrum of G-salt, concentration 10%, amplitude 32

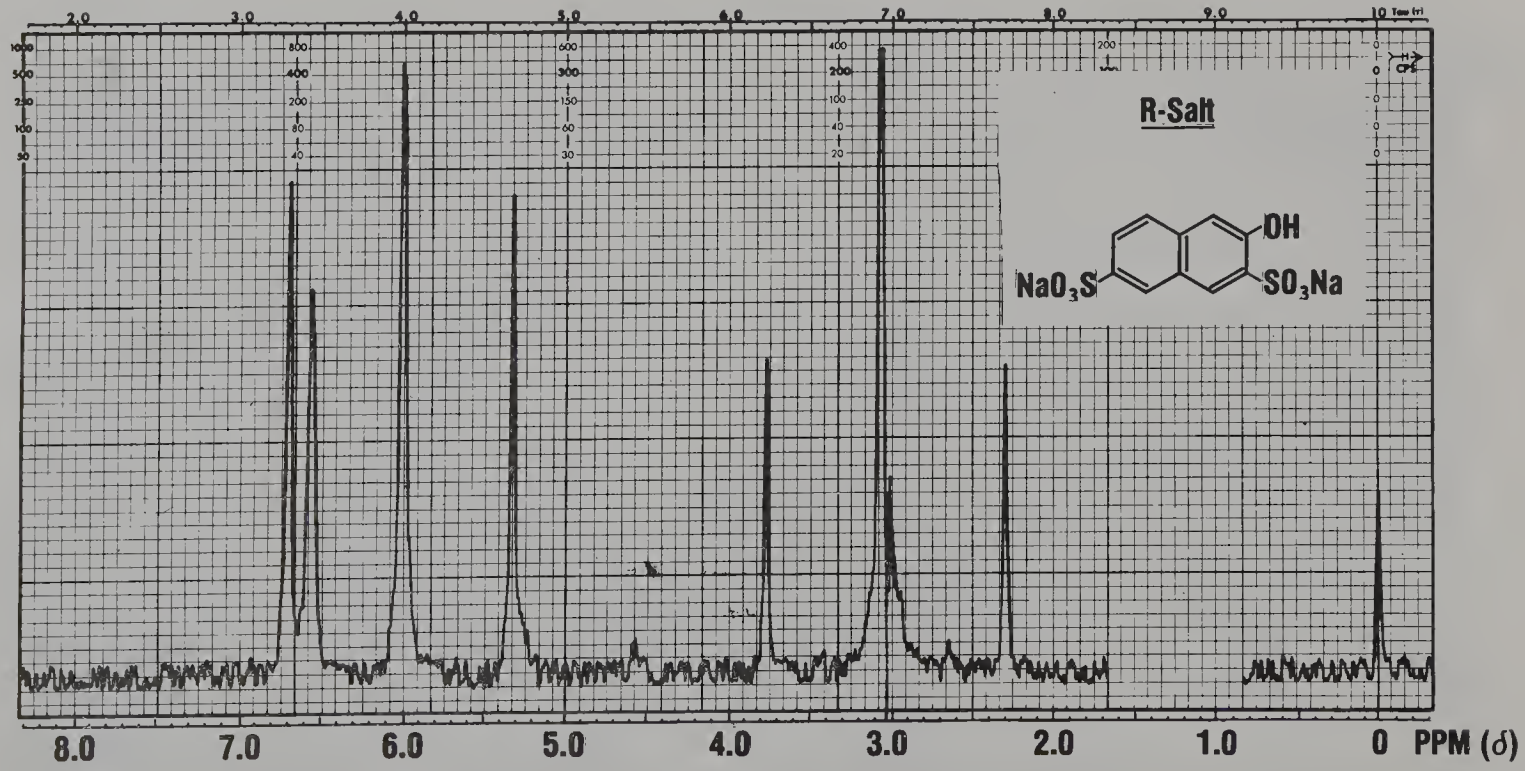


Figure 15e Spectrum of *R*-salt, concentration 10%, amplitude 32

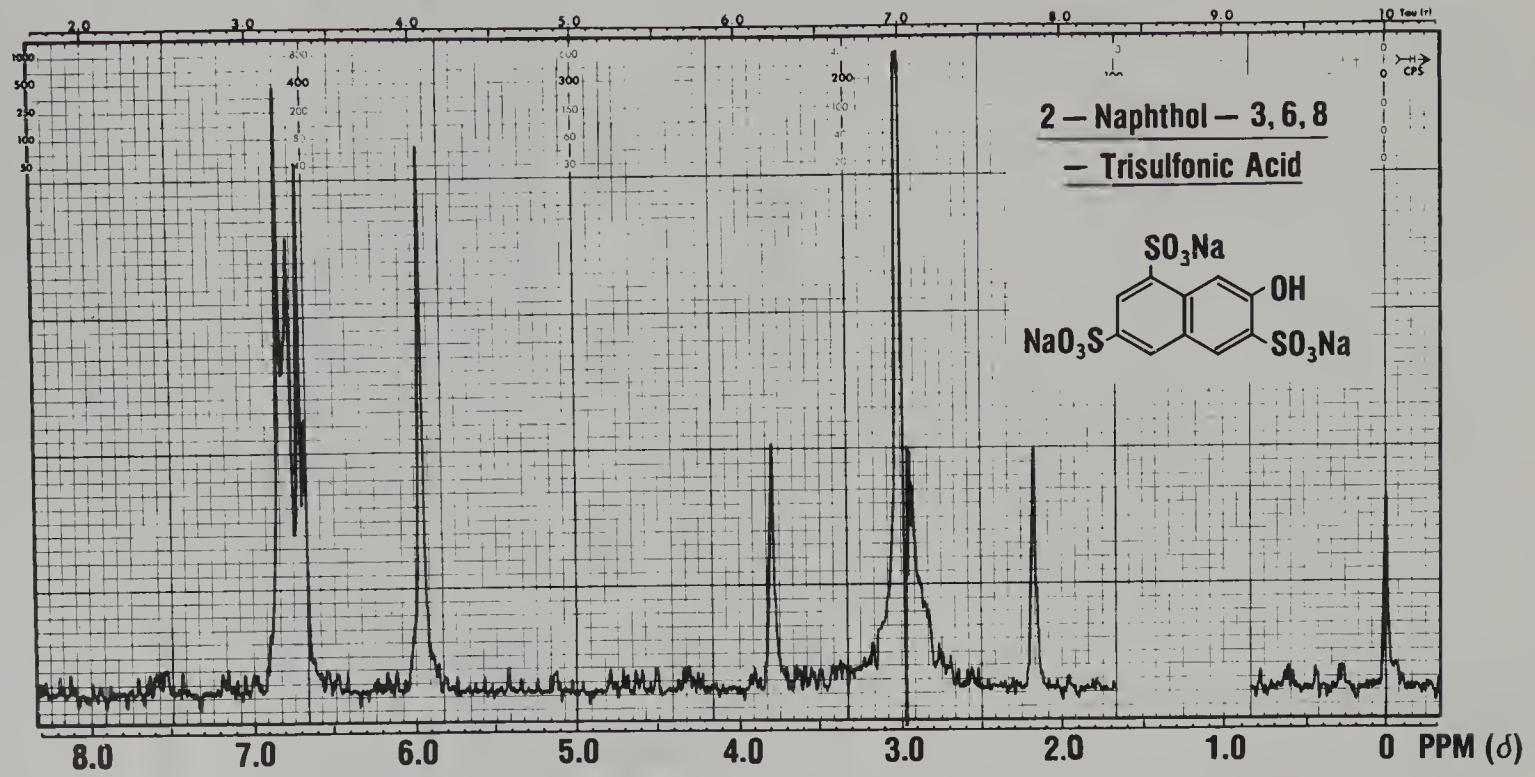


Figure 15*f* Spectrum of 2-naphthol-3,6-8-trisulfonic acid, concentration 20%, amplitude 32

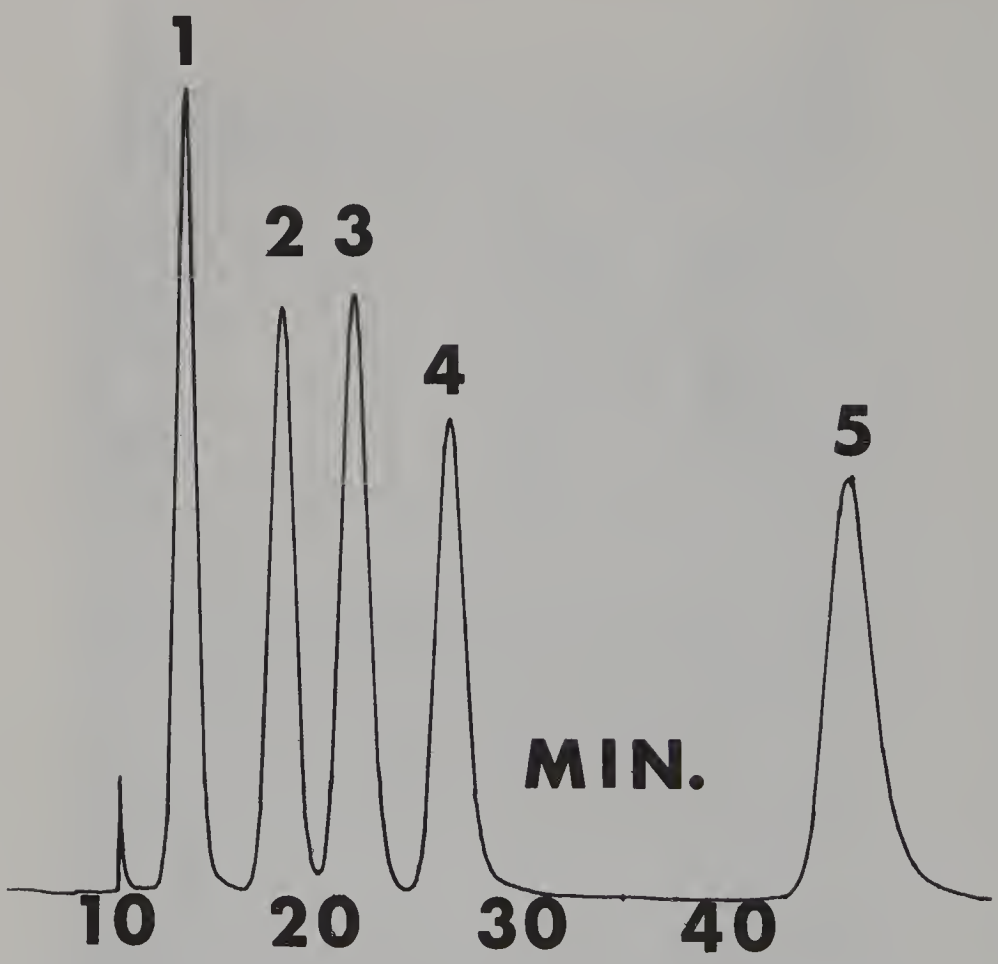


Figure 16 HPLC Separation of 5 μL of a solution containing about 25 mg each of Schaeffer's salt (1), *R*-salt (2), *G*-salt (3), 2-naphthol-3,6,8-trisulfonic acid (4), and DONS (5) in 200 mL of primary eluant. Conditions: chromatograph, Dupont Model 830; column, 1-m × 2.1-mm ID Dupont Zipax SAX (strong anion exchange); detector, UV at 254 nm; flow rate, 0.75 mL/min; primary eluant, 0.01 *M* aqueous $Na_2B_4O_7$; secondary eluant, 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

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Chapter 12 Uncombined Intermediates and Other Low-Molecular-Weight Impurities

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURE

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.

Reagents and Apparatus

Primary eluant—0.01 *M* aqueous $Na_2B_4O_7$.

Secondary eluant—see specific colorant.

Liquid chromatograph—DuPont Model 830 with a gradient elution accessory.

Column—DuPont strong anion exchange (SAX), 1 m × 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% secondary eluant at 10%/min, then pump primary eluant through the column

for 10 min. Inject the sample and chromatograph as indicated in the list that follows.

FD&C Blue No. 1 (19)

Secondary eluant—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 (53)

Secondary eluant—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 (6).

FD&C Red No. 40 (13,51,55)

Secondary eluant—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), (6) unknown, (7) FD&C Red No. 40, (8) 6,6′-oxybis(2-naphthalenesulfonic acid).

FD&C Yellow No. 5 (2,8,14)

Secondary eluant—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 (4,12,52,55)

Secondary eluant—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 (54)

Secondary eluant—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.

GENERAL COLUMN CHROMATOGRAPHIC SCREENING PROCEDURE (19,36)

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: width ratio, and changes in eluant 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. 17. 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 eluant given in Table 24. With the pinchcock open, pour the slurry into the column. Wash the column with 200 mL of eluant 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 24. (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

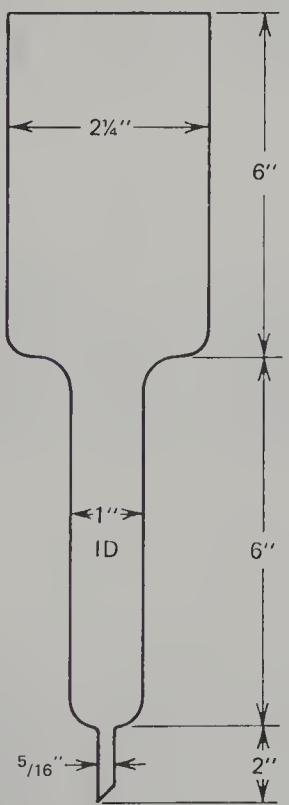

Figure 17 Glass-chromatographic column

TABLE 24 SOLVENTS, ELUANTS, AND TREATMENT OF ELUATE FRACTIONS FOR INDIVIDUAL COLORS

Color	Solvent[a]	Eluant (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

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 eluant	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 eluant	Ammonium sulfate, 10%	Same as FD&C Red No. 4
D&C Green No. 8	Water, 25 mL	Ammonium sulfate, 40%	Run as elute
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 Orange No. 17	Slurry with 10 mL of ethanol	Ammonium sulfate, 10%, containing 10% ethanol (v/v)	Run as eluted
D&C Red No. 6 D&C Red No. 7	Slurry with 10 mL of ethanol, then mix in 10 mL of eluant	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. 8 D&C Red No. 9	Slurry with 10 mL of ethanol, then mix in 10 mL of eluant	Ammonium sulfate, 10%	Same as D&C Red No.6

TABLE 24 Continued

Color	Solvent[a]	Eluant (w/v)[b]	Treatment of Fractions
D&C Red No. 17	Slurry with 10 mL of ethanol, then mix in 10 mL of eluant	Ammonium sulfate, 10%	Same as D&C Red No. 6
D&C Red No. 19	Slurry with 5 mL of ethanol	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
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 eluant	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 eluant	25% ammonium sulfate, 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. 37	Slurry with 5 mL of ethanol	Ammonium sulfate, 35%	Same as FD&C Blue No. 1
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 eluant should be essentially free of iron and other UV-absorbing impurities.

appropriate eluant and mix. Add 50 g of ammonium sulfate powder to salt out the dye and mix. Using 50 mL of eluant, transfer the mixture to the column. Let the column drain to the surface of the cellulose. Add eluant 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 24, and then record the UV spectra of the fractions versus a blank (eluant 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 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 (31)

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 eluant (1:4 or 3:7) and an electrochemical or UV detector (240 nm).

DETERMINATION OF β-NAPHTHOL

β-Napthol is extracted with an appropriate solvent, coupled, and then determined by titanous chloride titration or spectrophotometrically.

Titration Procedure

With Titanous Chloride (46): 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.

Weight a 10-g sample into a 2.5 cm × 8-cm extraction thimble, place it 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 (47): 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.

$$\text{Percent } \beta\text{-naphthol} = \frac{(A)(c)(144)(0.05)}{(B)(w)(293)}$$

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: Weight 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 (35): 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 (25,26,46)

Method A—in FD&C Red No. 3; D&C Orange Nos. 5, 10, 11, and 17; 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 Red No. 19: Dissolve a 0.5-g sample in 20 mL of hot water. Cool and transfer to a 125-mL separatory funnel. Add 80 mL of chloroform and 2 mL of 10% sodium hydroxide and shake vigorously for 1 min. Drain the chloroform layer and wash the aqueous phase with two 30-mL portions of chloroform, discarding the chloroform layers. Add 7 mL of 1:9 hydrochloric acid to the aqueous phase and then wash with two 30-mL portions of chloroform, discarding the chloroform. Transfer the aqueous solution to a beaker with water and evaporate to dryness on a steam bath. Proceed as in Method A, (beginning with "Dissolve the residue in water. . .").

Method C—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 D—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 (32, 50)

Slurry 24 g of Whatman Column Chromedia CF11 in 140 mL of eluant containing 400 g of ammonium sulfate per liter of water. Pour the slurry into the glass chromatographic tube shown in Fig. 18. Let the

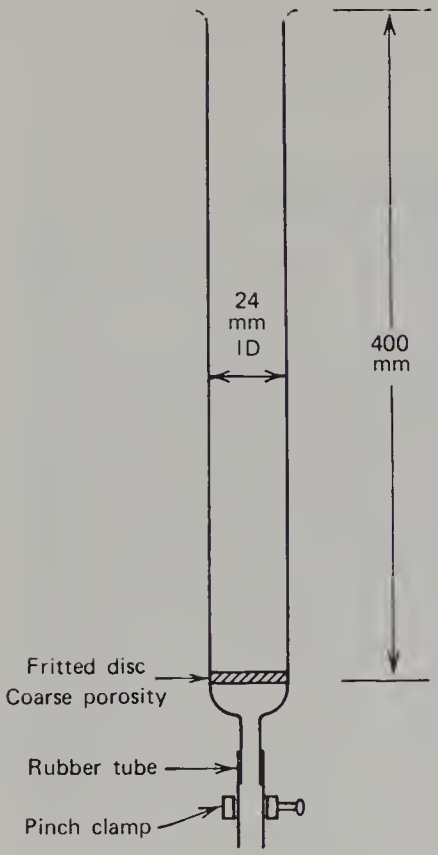

Figure 18 Chromatographic tube (Reprinted with permission of the Association of Official Analytical Chemists)

eluant 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.

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 eluant, 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 eluant in a 1-cm cell. Compare these against standard spectra obtained in the eluant.

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 (15): 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 = 792.9, 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 (33): 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).

Intermediates and Subsidiary Colors in FD&C Blue No. 2 (6)

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-R1A recording integrator, and an Altex Ultrasphere ODS Column No. 256-05, 4.6 mm × 250 mm, 5-μm particle size.

Eluant A—1.5% ammonium acetate (w/v)/0.5% acetonitrile (v/v) in water.

Eluant 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 (0.1 g when measuring 5,7′-disulfoindigo) in 100 mL of water. With eluant 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 (24)

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 Industires Supergrator 3, and a DuPont Zorbax C-8 column, 25 cm × 4.6 mm ID.

Eluant 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.

Eluant 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% eluant B in A flowing at 1 mL/min, inject 10 μL of sample. Then program from 20% to 65% eluant 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.

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 (37,38,41)

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. 18) and let the eluant 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 eluant 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 eluant; CSA normally elutes in fractions 4–6, Schaeffer's salt in fractions 8–10, and DONS in fractions 16–30.

$$\text{Percent CSA} = 0.186\ \Sigma\ (A_1 - A_2)_{252\ nm}$$

(as free acid, mw 217.25)

$$\text{Percent Schaeffer's salt} = 0.396\ \Sigma\ (A_1 - A_2)_{282\ nm}$$

(as monosodium salt, mw 246.22)

$$\text{Percent DONS} = 0.0585\ \Sigma\ (A_1 - A_2)_{240\ nm}$$

(as disodium salt, mw 474.42)

where $(A_1 - A_2)_{x\ nm}$ 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.

4,4'-Diazoaminobis (5-Methoxy-2-Methylbenzenesulfonic Acid), (DMMA) in FD&C Red No. 40

High-Pressure Liquid Chromatographic Procedure (5,43)

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).

Primary eluant—0.01 *M* aqueous $Na_2B_4O_7$.

Secondary eluant—0.5 *M* aqueous $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$.

Instrument Parameters:

Eluant 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 primary eluant and dilute to volume with same. Run a blank (0–60%) gradient, then pump primary eluant 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 primary eluant through the column for 14 min and then inject the next sample. DMMA elutes in ca. 11 min.

Gravity Column Procedure (21): 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 resevoir top and a 22-mm fritted disk. Let the column drain to the surface of the packing and then wash with 50 mL of eluant (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 eluant. Allow the column to drain just to the surface of the packing and then fill the reservoir with eluant 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 cell or longer.

$$\text{Percent DMMA} = \frac{A \times V \times 100}{W \times L \times a}$$

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).

Sulfanilic Acid and 3-Carboxy-1-(4-Sulfophenyl)-5-Pyrazolone in FD&C Yellow No. 5 (60)

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* tetrabutylammonium 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 (22)

Reagents and Apparatus:

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.

Eluant—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 eluant, then fill the column with eluant 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 interfer with the determination.

Sulfanilic Acid, Schaeffer's Salt, and 6,6′-Oxybis(2-Naphthalenesulfonic Acid) (DONS) in FD&C Yellow No. 6 (4,38,39,40)

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. 18) and let the eluant 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 eluant 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 eluant. Sulfanilic acid normally elutes in fractions 3–6, Schaeffer's salt in fractions 8–10, and DONS in fractions 16–23.

$$\text{Percent sulfanilic acid} = 0.496\ \Sigma\ (A_1 - A_2)_{250\ \text{nm}}$$

(as sodium salt, mw 195.2)

$$\text{Percent Schaeffer's salt} = 0.131\ \Sigma\ (A_1 - A_2)_{232\ \text{nm}}$$

(as sodium salt, mw 246.2)

$$\text{Percent DONS} = 0.234\ \Sigma\ (A_1 - A_2)_{240\ \text{nm}}$$

(as disodium salt, mw 474.4)

where $(A_1 - A_2)_{x\ \text{nm}}$ 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 (45)

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, 5μg, 10μg, and 20μg 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 (3,42,44)

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).

Primary eluant—0.01 M $Na_2B_4O_7$.

Secondary eluant—0.50 M aqueous $NaClO_4$ in 0.01 *M* $Na_2B_4O_7$.

Instrument Parameters:

Eluant flow rate: 1 mL/min; temperature: ambient; gradient: 0–100% secondary 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 primary eluant and dilute to volume with same. Before injecting the first sample run a blank (0–100%) gradient and then pump primary eluant 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 primary eluant through the column for 14 min and then inject the next sample. DAADBSA elutes in ca. 17 min.

Gravity Column Procedure (20): 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 eluant (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 eluant, force the washings into the column with pressure, then fill the reservoir with eluant 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 (7)

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 solvent 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.

1,4-Dihydroxyanthraquinone in D&C Green No. 5 and Ext. D&C Violet No. 2 (30)

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 (11)

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.

Eluant—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 eluant at 50°C under vacuum, then equilibrate the column with eluant flowing at 2 mL/min until a straight baseline is obtained (ca. 30 min).

Sample Preparation and Resolution: Dissolve 0.300g 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 (29,56)

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 (48)

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.

2,4-Dinitroaniline in D&C Orange No. 17 (27)

Paste a 1-g sample with 10 mL of concentrated sulfuric acid in a 500-mL beaker. Very slowly stir in 100 mL of methanol. Add 150 mL of water, mix, and evaporate to about 100 mL on a steam bath. Transfer to a 250-mL volumetric flask, cool, and dilute to volume with water. Filter 100 mL into a 500-mL separatory funnel. Add 30% sodium hydroxide until the sample solution is alkaline to litmus, cool, and extract with three 50-mL portions of ethyl ether. Wash the combined extracts with 20-mL portions of water. Transfer the ether solution into a beaker, using 10 mL of ethyl ether, and evaporate to dryness on a steam bath. Dissolve the residue in warm 0.1 *N* ethanolic hydrochloric acid and transfer it to a 100-mL volumetric flask. Rinse the beaker into the flask with two 20-mL portions of ethanolic hydrochloric acid. Dilute to volume with ethanolic acid and measure its absorbance at 290 nm, 355 nm, and 380 nm against a standard solution of 0.01 g/liter concentration.

$$\text{Percent 2,4-dinitroaniline} = \left[\frac{A_{335} - \dfrac{(A_{380} + A_{290})}{2}}{B_{335} - \dfrac{(B_{380} + B_{290})}{2}}\right] 0.25$$

where A is the absorbance of the sample at the wavelength specified, and B is the absorbance of the standard at the wavelength specified.

Lake Red C Amine (2-Chloro-5-Aminotoluene-5-Sulfonic Acid) in D&C Red Nos. 8 and 9 (16)

Weigh a 1-g sample and transfer to a 500-mL beaker with 5 mL of acetone. Add 100 mL of 2% barium chloride solution. Boil for 10 min and filter the hot mixture through Whatman No. 12 paper into a separatory funnel. Return the filter paper and the dye to the original beaker and repeat the extraction and filtering twice more.

Cool the combined filtrates, acidify with 5 mL of 1:1 hydrochloric acid, and extract with three 20-mL portions of benzene. Wash the combined benzene extracts with 20 mL of water and add the wash to the aqueous layer. Filter the aqueous layer through cotton into a beaker. Boil for 15–20 min to remove benzene. Cool. Adjust the pH to about 8 with ammonium hydroxide and dilute with water to 500 mL in a volumetric flask.

Measure the absorbance of sample against a standard solution at 247 nm.

Aminoazobenzene in D&C Red No. 17 (27)

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.

m-Diethylaminophenol in D&C Red No. 19 (28)

Prepare a *m*-diethylaminophenol standard solution by dissolving 0.2 *g* of purified *m*-diethylaminophenol in 100 mL of 50% ethanol and diluting to 200 mL with water. Then transfer a 20-mL aliquot to

a 1-liter flask, add 100 mL of *N* sodium hydroxide, and dilute to volume with water.

Place 20 *g* of potassium dihydrogen orthophosphate and 35 *g* of sodium chloride in a 500-mL round-bottomed flask, Weigh a 2.5-g sample into a 200-mL beaker and paste with 50 mL of water. Add 50 mL of water and transfer the mixture to the round-bottomed flask with several 10-mL portions of water. Dilute to 250 mL and adjust the pH to 6.6 ± 0.2 with dilute sodium hydroxide. Fit the flask with a steam distillation trap and condenser and collect 175 mL of distillate. Transfer the distillate to a 250-mL volumetric flask, add 10 mL of 10% sodium hydroxide, and dilute to volume with water. Determine the absorbance of the sample solution and the standard solution at 295 nm.

o-(2-Hydroxy-4-Diethylaminobenzoyl) Benzoic Acid in D&C Red No. 19

Follow the procedure given for determination of subsidiary dyes in D&C Red No. 19 (p. 328). The aroylbenzoic acid elutes in the first few fractions and can be measured at 365 nm.

m-Diethylaminophenol in D&C Red No. 37 (28)

Proceed as described for D&C Red No. 19, up to and including ". . . collect 175 mL of distillate." Transfer the distillate to a 500-mL round-bottom flask, rinse the receiver with several 10-mL potions of water, and add the washings to the flask. Add 30 g of sodium chloride and 15 g of potassium dihydrogen orthophosphate and adjust the pH to 6.5–6.7 with 30% aqueous sodium hydroxide. Distill, collect about 175 mL of distillate, and filter it into a 250-mL volumetric flask containing 10 mL of 10% sodium hydroxide. Wash the receiver with several 10-mL portions of water, filtering each wash into the flask. Dilute to volume with water and measure the absorbance as described above.

Phthalic Acid and *m*-Diethylaminophenol in D&C Red No. 37 (34)

Prepare a phthalic acid-diethylaminophenol standard by dissolving 50 mg of phthalic acid and 20 mg of *m*-diethylaminophenol in anhydrous methanol. Dilute to 100 mL with alcohol and store in a cool dark place.

Prepare silylating reagent by mixing equal volumes of *N*,O-bis (trimethylsilyl) acetamide and Tri-Sil/BSA (Pierce Chemical Co., Rockford, Ill.). Store under refrigeration in a rubber-capped serum vial.

Pipette 1 mL of the phthalic acid-diethylaminophenol standard into a silylation vial and evaporate to dryness in a stream of nitrogen. Weigh 0.1 g of sample into the vial. Into a second vial weigh another 0.1-g portion of sample. Cap both vials with septums, inject 1 mL of silylating reagent into each, and then heat for 10 min at 60°C.

Compare the sample against the standard by injecting 4 μL of each into a Hewlett-Packard Model 5750 (or similar) gas chromatograph equipped with a flame ionization detector and a 10 ft × 0.25 in. copper column containing 5% OV II (Supelco, Bellefonte, Pa.) on 60–80-mesh Chromosorb WAW (Johns-Manville, Celite Division, New York, N.Y.). Observe the following conditions: helium flow rate, 50 mL/min; injection port and flame ionization detector temperature, 230°C; column temperature, 150–250°C at 6°/min, then hold at 250°C until the chromatogram is complete (ca. 15 min).

o-(2-Hydroxy-4-Diethylaminobenzoyl) Benzoic Acid (HDBA) in D&C Red No. 37 (34)

Dissolve 2 g of sample in 100 mL of methanol. Apply 0.5 mL of sample solution as a streak 0.5 cm (or less) wide, 2 cm from the bottom of a 20-cm × 20-cm LQIF thin-layer plate (Quantagram LQIF, Quantum Industries, Fairfield, N. J.). Develop using 10% methanol in chloroform until the solvent front travels 12 cm. When viewed under UV light the HDBA is detected as a stripe about 1 cm above the D&C Red No. 37. Extract from the plate with methanol and determine spectrophotometrically at 350 nm.

5-(Hydroxymethyl)-2-Furaldehyde in Caramel Solutions (1)

Dilute samples with water, filter them through a 0.45-μm filter, then chromatograph them using the following equipment and conditions: 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.

The 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 (9): 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 (59): 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 (10): Extract alkaline aq. 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-methyl-imidazole can be used as an internal standard.

Method D (58): 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, an eluant 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.

Free Gossypol in Cottonseed Flour (17)

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 betweeen 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.

Oxalic Acid in Ferrous Gluconate (18)

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 (18)

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 precipate 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 (49)

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 (57)

Digest 1 g of sample with 20 mL of 1:3 hydrochloric acid at 50°C for 15 min.

Add water to restore original volume, mix, and filter. Add 1 mL of 10% (w/v) sulfuric acid to 10 mL of filtrate, evaporate to dryness, and ignite to constant weight.

Reaction and Soluble Substances in Talc (57)

Boil 10 g of sample with 50 mL of water for 30 min; periodically add water to maintain approximately the original volume. Filter. Evaporate one-half the filtrate to dryness, and dry at 105°C for 1 hr.

Water-soluble Iron in Talc (57)

Using hydrochloric acid, slightly acidify the second half of the filtrate obtained in the test described above. Add 1 mL of fresh 10% potas-

sium ferrocyanide solution. If the sample is of acceptable quality, the liquid should not turn blue.

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Chapter 13 Homologous, Isomeric, and Other Related Colorants

Lower-Sulfonated Colors in FD&C Blue No. 1

Solvent Extraction Procedures (10, 12): 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 separatory 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 alochol-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 (2, 41): 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 eluant. 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 (37)

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 separate fractions and dilute each with equal volumes 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.

Lower-Sulfonated and Isomeric Colors in FD&C Green No. 3 (43)

These colorants can be determined using the solvent extraction and the TLC prodedures described for FD&C Blue No. 1 (see pp. 306–307).

Lower-Iodinated Colorants in FD&C Red No. 3

High-performance liquid chromatographic Procedure (7): 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—Primary eluant A, 0.1 M ammonium acetate; secondary eluant 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—Primary eluant A, 2% v/v glacial acetic acid in water; secondary eluant 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 Prodedure (48): Prepare an eluant 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 a 18 in. × 6-in. tank, one on each side, add the eluant 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 eluant. 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 (13): Prepare ethanol wash solution by diluting 75 ml of 95% ethanol to 100 mL with water. Prepare each of the following eluants:

Eluant No. 1—350 mL of 25% aqueous sodium chloride solution containing 1.75 mL of concentrated ammonium hydroxide.

Eluant No. 2—500 mL of 2% aqueous sodium sulfate solution containing 2.5 mL of concentrated ammonium hydroxide.

Eluant No. 3—1000 mL of 1% aqueous sodium sulfate solution containing 5 mL of concentrated ammonium hydroxide.

Eluant No. 4—600 mL of 0.5% (v/v) ammonium hydroxide.

Eluant No. 5—400 mL of 60% ethanol containing 2 mL of ammonium hydroxide.

Use a glass chromatographic column equipped at the top with the eluant distribution system shown in Fig. 19. 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 eluant 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 eluant present and taking care not to disturb the surface of the

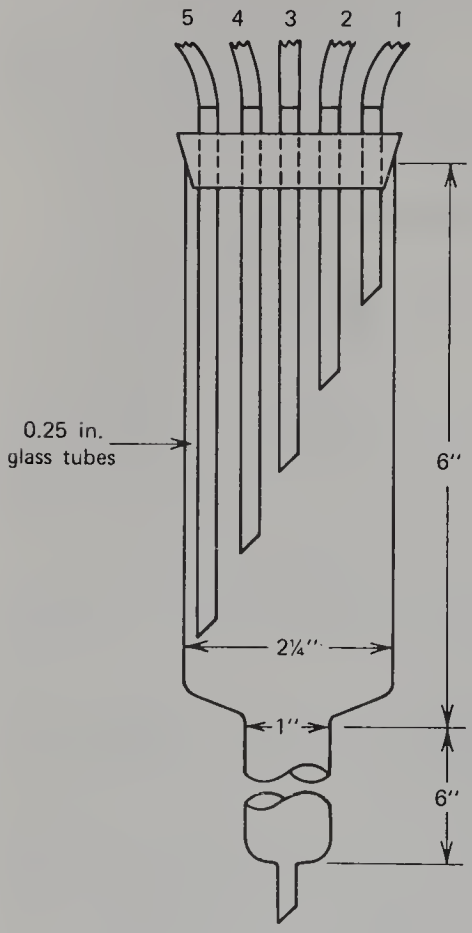

Figure 19 Eluant distribution system for column-chromatographic determination of subsidiary colors in FD&C Red No. 3 (numbers above inlet tubes refer to serial number of respective eluant)

packing. Drain the solution just to the packing surface. Carefully wash down the sides of the tube with eluant No. 1 and drain into the column. Add 20 mL of eluant 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 eluant No. 1. Place any remaining eluant No. 1 in a flask and connect it to tube No. 1. Place eluants Nos. 2–5 in suitable flasks and connect them to the appropriate tubes. Elute the column successively with eluants 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:

Eluant No. 1—Band 1, unidentified color; Band 2, fluorescein.

Eluant No. 2—Band 3, mixture of 2- and 4-monoiodofluoresceins; Band 4, mixture of 2,4-, 2,5-, and 2,7-diidofluoresceins.

Eluant No. 3—Band 5, mixture of 4,5-diiodofluorescein and 2,4,7-triiodofluorescein; Band 6, 2,4,5-triiodofluorescein.

Eluant No. 4—Band 7, 2,4,5,7-tetraiodofluorescein.

Eluant No. 5—Band 8, unidentified color.

The amount of the individual colors can be estimated using the absorptivities given on p. 309.

2-(2,4-Xylylazo)-1-Naphthol-4-Sulfonic Acid and 2-(5-Sulfo-2,4-Xylylazo)-1-Naphthol in FD&C Red No. 4 (13)

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 10 mg/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 (13)

Prepare eluant 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 eluant.

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 eluant 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 eluant 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 (47)

Prepare eluant 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 eluant, 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 eluant.

Prepare a 0.5% aqueous sample solution and pipette a 20-mL aliquot onto the column. Elute the column with eluant; 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 (15)

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 eluant.

Subsidiary Colors in FD&C Red No. 4 (13)

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 an eluant 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 eluant. 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 eluant 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 (22): 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 eluant. Compare with standards run simultaneously on the same sheet. Figure 20 is a schematic representation of the resolution of a synthetic mixture containing subsidiary colors that could be present.

Thin-layer-chromatographic Procedure (5,13): 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 an eluant 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

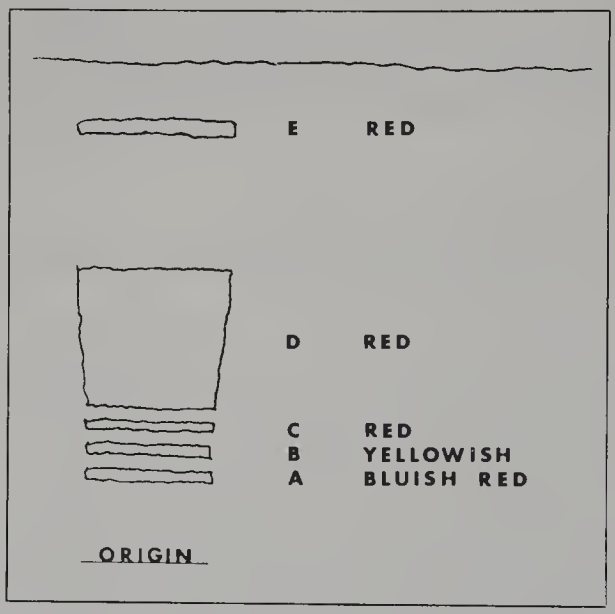

Figure 20 Paper-chromatographic resolution of dyes: A, *R*-salt + CSA dye; B, *G*-salt + CSA dye; C, (6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfonphenyl) azo] -8- (2-methoxy-5-methyl-4-sulfophenoxy) -2-naphthalensulfonic acid); D, FD&C Red No. 40 and E, cresidine + Schaeffer's dye and CSA + 2-naphthol dye

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-Naphthalenedisulfonic Acid (*G*-Salt + CSA) in FD&C Red No. 40 (22)

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).

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 eluant.

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 eluant. (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 eluant. Pipette 3 mL onto the column and let drain to the surface of the packing. Elute the column with eluant. Collect the desired fraction in a 25-mL volumetric flask and dilute to volume with eluant. 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 eluant. 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 (22)

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 eluant.

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 eluant. Collect the desired fraction in a 25-mL volumetric flask and dilute to volume with eluant.

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 eluant.

$$\text{Percent cresidine + Schaeffer's dye (mw 394.4)} = A \times 4.19$$

$$\text{Percent CSA + } \beta\text{-naphthol dye (mw 394.4)} = A \times 3.88$$

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 absorptivites 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 (6): 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-sufonated colors from each other.

Prepare the eluant 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 eluant. 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 eluant 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 make a spectrophotometric determination.

Thin-layer Chromatography (13): 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 1,4dioxane–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 (9): 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 (13): 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 eluant 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 (21)

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 eluant. Use a flow rate of 1.0 mL/min and monitor the mobile phase 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 eluant. The higher-sulfonated compounds are separated from each other, but the lower-sulfonated compounds are not.

Thin-layer Chromatography (4): 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 (11): 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 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 (13): 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

Extraction Procedure (29): 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 (13): 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 color spectrophotometrically.

1-*p*-Sulfophenylazo-2-Naphthol-3,6-Disulfonic Acid, Trisodium Salt in FD&C Yellow No. 6 (21)

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 Hibar 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 eluant. Use a flow rate of 1.0 mL/min and monitor the mobile phase 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 (13): 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 (13): Using a microliter syringe, spot as a band about 3 mg of color 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 eluant. 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′-Biphenylenebisazo)-di-2-Naphthol in Citrus Red No. 2 (42)

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 (45)

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 (18)

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 (44)

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 eluant for about 30 min. The colorants appear on the plate in descending order:

Unsulfonated D&C Green No. 5 (D&C Green No. 6),

Monosulfonated 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 (13)

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 eluant. Leach the alizurol purple from the plate with chloroform and determine it spectrophotometrically.

Lower-Sulfonated Colors in D&C Orange No. 4 (13)

Use the chromatographic method given for lower sulfonated dyes in FD&C Yellow No. 6 (p. 319) 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 (13): Use the Column-chromatographic procedure described under Lower-Iodinated Colorants in FD&C Red No. 3 (p. 309), but substitute the following eluants:

Eluant No. 1—350 mL of 25% aqueous sodium chloride solution containing 1.75 mL of concentrated ammonium hydroxide.

Eluant No. 2—1000 mL of 15% aqueous sodium sulfate solution containing 5 mL of concentrated ammonium hydroxide.

Eluant No. 3—800 mL of 5% aqueous sodium sulfate solution containing 4 mL of concentrated ammonium hydroxide.

Eluant No. 4—500 mL of 3% aqueous sodium sulfate solution containing 2.5 mL of concentrated ammonium hydroxide.

Eluant No. 5—500 mL of 0.5% (v/v) ammonium hydroxide.

Eluant 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:

Eluant No. 1—Band 1, unidentified color; Band 2, fluorescein.

Eluant No. 2—Band 3, mixture of 2- and 4-monobromofluoresceins; Band 4, mixture of 2,4- and 2,5-dibromofluroesceins.

Eluant No. 3—Band 5, 4,5-dibromofluorescein.

Eluant No. 4—Band 6, 2,4,5-tribromofluorescein.

Eluant No. 5—Band 7, 2,4,5,7-tetrabromofluorescein.

Eluant 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
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 (13): 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 eluant.

Method C (16): 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 an eluant 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 in an eluant composed of 100 mL of solution 1, 300 mL of *n*-butanol, and 70 mL of ethanol. Leach the spots from the paper with 1:99 ammonium hydroxide and determine the individual compounds spectrophotometricaly. See Fig. 21.

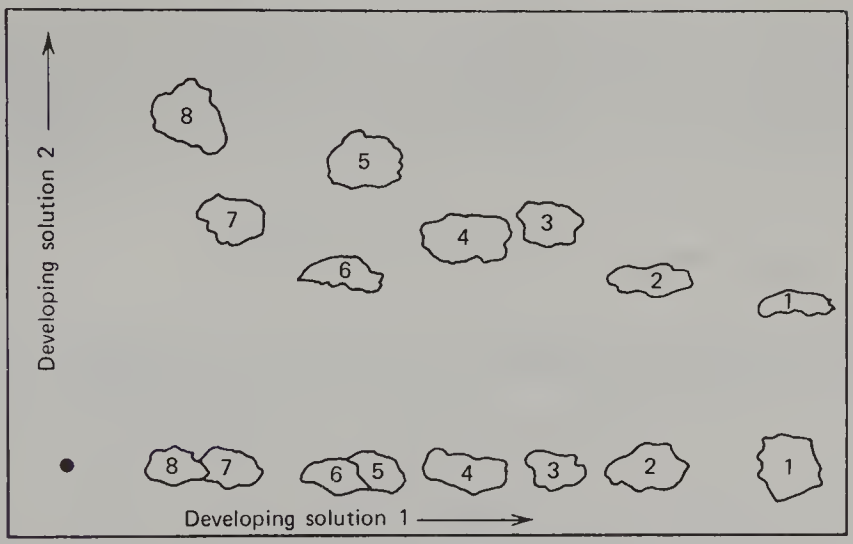

Figure 21 Paper-chromatographic separation of fluorescein and nine bromofluoresceins: (1) fluorescein, (2) 2-bromofluorescein and 4-bromofluorescein, (3) 2,7-dibromofluorescein, (4) 2,4-dibromofluorescein and 2,5-dibromofluorescein, (5) 2,4,7-tribromofluorescein, (6) 4,5-dibromofluorescein, (7) 2,4,5-tribromofluorescein, (8) 2,4,5,7-tetrabromoflourescein (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)

1-(4-Nitrophenylazo)-2-Naphthol in D&C Orange No. 17 (13)

Spot about 0.25 mg of sample as a chloroform 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 chloroform as the eluant.

4-Toluene-Azo-2-Naphthol-3-Carboxylic Acid in D&C Red Nos. 6 and 7 (20)

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.

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 (13)

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 12 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 22. Figure 22 is 150% of the absorbance obtained at each wavelength when D&C Red No. 6 Lot AA 5169 was analyzed by this procedure.

To "pass test" the absorbance of the sample must not exceed that of Figure 22 at any wavelength.

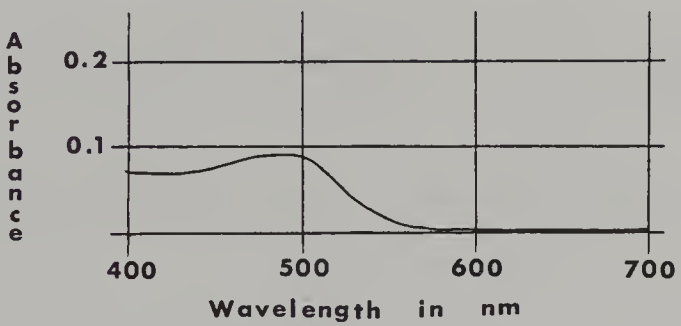

Figure 22 Spectrum of ether-soluble matter isolated from D&C Red No. 6 Lot AA5169, drawn to equal 150% of that actually obtained using the method on p. 326—Conditions: 1-cm cells; scan rate, 50 mm/sec.; reference, distilled water

4(4-Chloro-2-Sulfo-5-Tolylazo)-1-Naphthol in D&C Red Nos. 8 and 9

Method A (40): Transfer a 0.1-g sample to a 600-mL beaker. Add 100 mL of ethanol, 20 mL of 10% sodium hydroxide solution, and 80 mL of water. Cover and then boil gently for 5 min. Remove from the heat and add 4 mL of concentrated HCl, and then add hydrochloric acid dropwise until the solution is acidic. Make basic by the dropwise addition of a 2% ethanolamine solution, then add 2 mL in excess. Evaporate to 100 mL on a hot plate and then add 100 mL of a 2% ethanolamine solution, bring to a boil, cool, and filter.

Return the filter paper with the precipitate to the beaker and leach with a stream of 5:1 ethanol–10% sodium hydroxide solution. Add 80 mL of water then dilute to 200 mL with the 5:1 ethanol–10% sodium hydroxide solution. Boil, adjust the pH, and filter as described above. Combine the filtrates.

Transfer the filtrates to a 500-mL separatory funnel and acidify with about 15 mL of hydrochloric acid. Extract the dye with 75 mL of isoamyl alcohol and discard the aqueous layer. Wash the alcohol layer twice by shaking it with 50-mL portions of a 5% sodium chloride solution. Add 50 mL of isooctane to the alcohol layer and extract it with 50 mL of an aqueous solution containing 1 g of ethanolamine and 2.5 g of sodium chloride. Repeat with 25-mL portions of the same solution until no more color is extracted. Combine the extracts

in a 250-mL volumetric flask and dilute to volume with a solution of 2% ethanolamine in 5% sodium chloride. Determine the amount of dye present spectrophotometrically by comparison with a standard.

Method B (13): Spot 1–2 mg of sample as an *N,N*-dimethylformamide solution onto a 20-cm × 20-cm glass thin-layer plate coated with 0.25 mm of cellulose, dry the plate, then develop it using 0.02 *N* KOH as the eluant.

Determination of 1-Phenylazo-2-Naphthol (PAN) in D&C Red No. 17 (27)

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 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.

Triethylrhodamine and Other Subsidiary Colors in D&C Red No. 19

Column-chromatographic Method (32): Prepare silane-treated Celite as described under Chromatographic Method for FD&C Yellow No. 6 (p. 318). Slurry 20 g of the treated Celite with 18 mL of *N*-decanol that has been equilibrated with 1:99 ammonium hydroxide and then pack the slurry tightly into a 1-in.-diameter chromatographic column.

Dissolve 0.05 g of sample in 5 mL of water, add one drop of concentrated ammonium hydroxide, and add the solution to the column. Elute with 1:99 ammonium hydroxide and collect 20-mL fractions. Determine the triethylrhodamine spectrophotometrically at 540 nm. Frequently, as many as nine distinct bands are separated; triethylrhodamine elutes just before the parent compound.

Thin-layer chromatographic Method (3): Spot 0.2 mL of a 0.5% (w/v) ethanol sample solution as a band 3 cm from the bottom of a 20-cm × 20-cm TLC plate coated with 0.375 mm of silica gel G. (The plate should have been dried at room temperature and heated for 15 min at 125°C immediately prior to use.) Allow the plate to air dry and then place in an unlined tank that has been equilibrated for 10 min

with acetone–chloroform–triethylamine–water (30:45:5:1). Develop the plate until the solvent front reaches the top of the plate. Remove the plate and allow it to air dry.

The top band is D&C Red No. 19 followed by the major subsidiary, triethylrhodamine. Minor subsidiaries follow in order of decreasing ethylation. Scrape the subsidiaries of interest from the plate, slurry with ethanol, filter, and determine spectrophotometrically.

Lower-Brominated Subsidiary Colors in D&C Red Nos. 21 and 22

Method A (17): 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. 324.

Method B (1): 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 eluant travels the full distance of the surface (about 1 hr) using chloroform–benzene–methanol–formic acid (65:20:7:8) as the eluant. 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 (13): Use the column-chromatographic procedure described under Related Bromofluoresceins in D&C Orange No. 5 (p. 323), but substitute the following eluants.

Eluant No. 1—450 mL of 25% aqueous sodium chloride solution containing 2.25 mL of concentrated ammonium hydroxide.

Eluant No. 2—2000 mL of 10% aqueous sodium sulfate solution containing 10 mL of concentrated ammonium hydroxide.

Eluant No. 3—600 mL of 0.5% (v/v) ammonium hydroxide.

Eluant No. 4—400 mL of 60% ethanol containing 2 mL of concentrate 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 (13): Subsidiary colorants can be separated from D&C Red No. 21 and 22 using the thin-layer chromatography procedure (B) described under D&C Orange No. 5.

Uranine in D&C Red No. 22 (19)

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 (13)

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 eluant.

1-(Phenylazo)-2-Naphthol in D&C Red No. 31 (13)

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 (35)

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 eluant 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 (13)

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 eluant. Dry the plate, then develop it again using chloroform.

Triethylrhodamine and Other Subsidiary Colors in D&C Red No. 37

Use the TLC method described for D&C Red No. 19 (p. 328).

Quinizarin Green (D&C Green No. 6) in D&C Violet No. 2 (13)

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–trichloro-

ethylene–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 (33): 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 (13): 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 eluant.

Related Colorants in Annatto (14)

Paper-chromatographic Method (23–26) (see McKeown, G.G. under Resolution of Mixtures, p. 355).

Thin-layer-chromatographic Methods:

Method A-(see Francis, B. J., and Ramamurthy, M K., and Bhalerao, V. R. under Resolution of Mixtures, p. 350 and 363).

Method B-Chloroform extracts of annatto (8): 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.

Streak 0.2 mL of a 0.2–1% chloroform solution of pigment onto the plate and elute using an eluant of acetic acid–chloroform–acetone (1:50:50).

Method C-Vegetable oil or propylene glycol extracts of annatto (31): 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, eluant-equilibrated tank containing cylohexane–chloroform–acetic acid (65:5:1).

Method D-Aqueous-alkaline extract of annatto (31): Apply sample to an untreated 10-cm × 20-cm Merk silica gel thin-layer plate. Air dry and then elute in a paper-lined, eluant-equilibrated tank using chloroform–absolute ethanol–acetic acid (68:2:1).

Pigments in Beet Colorants

Method A (34): 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 eluant (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 (46): 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-methanol as the eluant, 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.

Fractionation of Caramel by Gel Filtration (28)

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. 402).

Lumiflavin in Riboflavin (38)

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 (30)

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 eluants:

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 (39)

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.

Curcuma Zedoaria and Curcuma Aromatica in Tumeric (36)

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 solvent-saturated tank using ethyl acetate–*n*-hexane (3:17).

Spray the air-dried plate with concentrated sulfuic 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

Chapter 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 young technique, HPLC has already 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 quite costly and much remains to be learned about its use.

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 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 dicussion of the isolation of colorants from commercial products where they are used as a means of identification.

BIBLIOGRAPHY

ALDRED, J. B. J. Assoc. Public Analysts 3, 79–82 (1965). The Identification of Violet BNP and Its Distinction from Other Violet Colors. Thin-layer chromatography is used to separate various violet colors. The method uses Kieselgel G plates and an eluant composed of iso-BuOH–EtOH–H_2O (2:2:1, v/v). The R_f values given are: Violet BNP (CI 42580), 0.38; Violet 5BN (CI 42650), 0.46; FD&C Violet No. 1 (CI 42640), 0.46; Methyl Violet (CI 42535), 0.58; and Acid Violet 4BN (CI 42561), 0.42.

ANWAR, M. H., NORMAN, S., ANWAR, B., LAPLACA, P. J. Chem. Ed. 40, 537–538 (1963). Electrophoretic Study of Synthetic Food Dyes. Thoroughly wet a cellulose acetate strip with a buffer solution consisting of equal parts of 0.1 *M* sodium acetate and isopropyl alcohol adjusted to pH = 4.6 with acetic acid. Blot between absorbent paper and spot a solution of the dye mixture on the strip. Separate by electrophoresis in the buffer solution using an applied voltage of 270 V. Fading and oxidation of triphenylmethane dyes can be minimized by conducting the separation in the dark under an inert atmosphere. See Fig. 23 for order of separation.

ATTINA, M., CIRANNI, G. Farmaco, Ed. Prat. *32*,186–191 (1977). Use of High-Pressure Liquid Chromatography for Analysis of Coloring Materials. A number of colorants including FD&C Yellow No. 5, D&C Yellow No. 10, Amaranth and Carmoisine were separated on Permaphase AAX using HCl-citrate buffers at pH values of 4–5.1.

BAINBRIDGE, W. C. JAOAC *29*, 240–242 (1946). Report on the Analysis of Mixtures of D&C Red No. 7 and D&C Red No. 10. Transfer a 0.25-g sample to a 250-mL Erlenmeyer flask. Add 100 mL of methyl Cellosolve and 5 mL of hydrochloric acid. Connect the flask to an air condenser and gently reflux the mix until it is in solution. Buffer the solution with 10 g of sodium bitartrate dissolved in 75 mL of boiling water and titrate with 0.1 *N* titanous

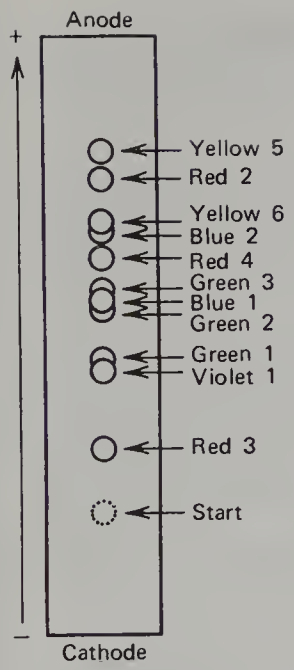

Figure 23 Electrophoretic separation of food colors [From J. Chem. Ed. *40*, 537 (1963)]

chloride solution. Record the results (in mL) as the combined titer for the two dyes. Dissolve a second 0.25-g sample in 100 mL of methyl Cellosolve and transfer the solution to a 500-mL separatory funnel. Transfer any residual dye with 10-mL portions of ethyl ether. Bring the ether volume to 150 mL and add 250 mL of 10% sodium chloride solution. Withdraw the lower layer and extract it with a second 75-mL portion of ethyl ether. Withdraw and discard the lower layer. Combine the ether extracts and wash them with 30-mL portions of water until the washings are colorless; discard the washings. Transfer the ether layer to a 250-mL Erlenmeyer flask with two 10-mL portions of ethyl ether. Evaporate the solution to dryness, add 100 mL of methyl Cellosolve, and heat into solution. Buffer with 10 g of sodium bitartrate in 75 mL of boiling water, heat to boiling, and titrate with 0.1 *N* titanous chloride solution. Calculate as D&C Red No. 10. Subtract the milliliters of titer for the D&C Red No. 10 from the milliliters of titer for the two dyes and calculate the difference as D&C Red No. 7.

BANDELIN, F. J., TUSCHHOFF, J. V. J. Am. Pharm. Assoc. *49*, 302–304 (1960). Paper Chromatography of Some Certified Dyes. Common certified dyes are separated on paper using 2% aqueous NH_4OH containing 2% iso-BuOH.

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 sulfonated dyestuffs including FD&C Red Nos. 3 and 4, FD&C Yellow Nos.

5 and 6, and FD&C Blue No. 2. The solvents 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°.

Compound	R_f		
	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.

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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 eluant. 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 solution (5 μL) to layers of Kieselgel G (250 μm) activated at 105° for 2 hr, which were then developed with fresh isopropyl alcohol–concentrated aqueous NH_3 (4:1) for about 2 hr, or to layers of Cellulose CC41 dried overnight, which 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.

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 eluant. 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, D&C Red No. 19 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. Eluants 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 Industires 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 eluant, 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 eluant components but in the proportions 70:30:2:1 (v/v/w/v).

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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 an eluant 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.

	Distances Moved (in mm)					
Electrolyte	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 eluant.

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*, 529–531 (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
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 solvents 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 eluant. 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.

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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 Nos. 2 and 3, FD&C Yellow Nos. 5 and 6, FD&C Blue No. 2, and several other colorants are separted by radial paper chromatography using 0.1 *M* HCl as the eluant.

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 solvents. 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 solvent.

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 (D&C Red No. 19), 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 developing solvent 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 solvent systems. The best eluants 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 an eluant 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 eluants.

FRANCIS, B. J. Analyst *90*, 374 (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 eluant.

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, catechol-

amines 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 eluant.

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, and FD&C Red Nos. 2, 3, and 4, 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 FD&C Green No. 1 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 Generator. 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 solvents.

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).

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 Petroelum Ether and Acetone.

JONES, J. H., CLARK, G. R., HARROW, L. S. JAOAC *34*, 135–148 (1951). A Variable Reference Technique for Analysis by Ab-

sorption 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 developing solvents, the influence of the conditions of activation on the separation of water-soluble dyes was studied. Of the eight developing solvents, 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 and identified from Amaranth 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, D&C Orange No. 4, and D&C Red No. 19, 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 cor-

relating 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 eluants 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 solvent mixtures: $CHCl_3$–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 eluant. 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 tbe 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 solvent 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 solvent mixture. With solvent mixtures containing EtOH or MeOH and H_2O and HCl, it is possible to separate mixtures of butter yellow and Yellow OB. None of these solvent mixtures 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 solvent 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 D&C Red No. 19, 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 eluant. 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 tbe 9:11 eluant, however a few colorants, including FD&C Red No. 3, required the 3:2 eluant to speed elution. Detection was at 254 nm.

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 eluant, a LiChrosorb RP-18 column and aq. 5% methanol as the eluant, and a μ Bondapak C18 column, and 15% isopropyl alcohol containing Waters Associates PIC-A reagent as the eluant. 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 solvent/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 eluants 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 Cer-

tain 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 solvent front 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 Solvents

Color	R_f	
	Solvent A	Solvent B
Ponceau 4R	0.183	0.437
FD&C Red No. 3	0.560	0.018
Carmoisine	0.10	0.085
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	—

Solvent A–Iso-amyl alcohol: 95% ethanol: NH_4OH : H_2O (4:4:1:2); 18 hr; descending.
Solvent 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 sep-

aration 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 systems found 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 system.

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. Substrate was Whatman No. 1 paper and solvent 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 solvents: 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.

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 eluant 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.

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–aq. 28% NH_3 (8:4:1) as the eluant. Dyes unstable in aq. Conc. NH_3 were separated on layers of cellulose using BuOH–ethyl methyl ketone–aq. 1% NH_3–H_2O (4:2:1:1) as the eluant. Between 5 and 20 ng of each dye was determined using a dual-wavelength densitometer at 430, 550, or 630 nm.

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 solvents 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 (CI No.)	Eluants		
	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 a solvent composed of 80 g of PhOH + 20 g of H_2O.

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 solvent systems.

PEARSON, D., WALKER, R. J. Assoc. Public Analysts *3*, 45–48 (1965). R_f Values of Permitted Synthetic Water-Soluble Coloring Matters. Twelve solvent mixtures, 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 solvent systems. 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 solvent systems; 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.

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 with solvent I, R_f with solvent 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 eluant. System 2 used a 30-cm × 2-mm ID Micro-Pak MCH (10 μm) column coated with petanol, and 1 m*M* A in phosphate buffer (pH = 7; I = 0.1), 50% saturated with pentanol as eluant.

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 solvents and 21 water-soluble dyes in five solvent systems 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 techique 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 developing solvent. 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 eluant. (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 solvent systems. 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. An eluant 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 solvent mixtures.

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 solvent systems.

SAENEZ, I., RUIZ, L., LAROCHE, C. Bull. Soc. Chim. France 1594–1597 (1963). 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 Coar-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 ammoniun 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. Artifical 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 solvent systems 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% of 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 tubes; 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		
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	—
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 + FD&C Yellow No. 5, salted and freed of alcohol[b]		
3. 200 mL of 20% NaCl	FD&C Yellow No. 1	—
	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)

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 (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 from FD&C Violet No. 1)

1. 50 mL of 10% alcohol + 15% NaCl (prewash)	H_2O + prewash (discard)	—
2. Solution of FD&C Green No. 1 + FD&C Violet No. 1, salted and freed of alcohol[b]		
3. 250 mL of 10% alcohol + 1% NaCl	FD&C Green No. 1	140
4. 50 mL of 20% NaCl	Alcohol solution (discard)	—
5. Sufficient H_2O to remove remaining color	FD&C Violet No. 1	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.

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. Solvent mixtures 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 eluant 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 solvent combinations 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 solvent system. 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 eluant 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 eluant.

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 eluant. Mixtures of FD&C Yellow No. 5 and azorubine were separated using BuOH–EtOH–H_2O–aqueous NH_3 (10:5:4:2) as the eluant.

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 solvents.

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 precoated 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 eluants.

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 solvent systems.

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.

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 eluant at a flow rate of 2 mL/min. Detection is at 470 nm. β-Carotene, α-carotene, and lycopene separate well.

Chapter 15 Analysis Of Commercial Products

The determination of colorants in foods, drugs, and cosmetics 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 cases, 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 among the most general and most useful of the methods employed today. 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* citric 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. Ist. Super. Sanita *19*, 294–297 (1956). Detection of Riboflavine in Artifically 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 sampel (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 of 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 Inc., Clifton, N. J.) and silica gel (CT, without additives, Whatman Inc.) 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 chromoatographic 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. 434).

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, preblended 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 nitro-

gen, 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.

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: Reaction 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 eluant.

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 ether and 20 mL of saturated aq. NaCl and continue the extraction. Discard the lower layer, wash the upper layer with 100 mL of 10% aq. 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 eluant. 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.

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.

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.

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 with 2.5% aqueous Na citrate–concentrated aqueous NH_3–methanol (20:5:3) as solvent. For the identification of basic dyes, the sample is passed through a prepared column of po-

lyamide 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.

MARTIN, G. E., FIGERT, D. M. JAOAC *57*, 217–218 (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: Ethyl acetate–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 eluant (Mobile phase A).

Or, chromatograph 20 μL of sample at room temperature by high-pressure liquid chromatography using a 250-mm × 4.6-mm stailess 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 liqueurs, aperitifs, carbonated beverages, syrups, caramels, and water-ices.

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 eluant.

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 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 (eluant No. 1). 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 eluant No. 1 until baseline is stabilized.

Using a loop injector, inject 120 μL of extract onto the column and elute under pressure with eluant No. 1 at 2 mL/min. Monitor eluant at 440 nm. Retention times for α- and β-carotene are 4.7 min and 6 min, respectively.

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 bandwidth spectrophotometer are compared. Values obtained using a Klett-Summerson colorimeter equipped with Nos. 42, 54, and 60 regular 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 recommend 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, I. 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. Tecnol. Alimentos *6*, 215–220 (1966). Detección 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-dimensional chromatography and petroleum ether–iso-PrOH–EtOAc (80:40:5) and petroleum ether–iso-PrOH–acetone (95:5:10) for two-dimensional chromatography.

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 eluant.

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 aq. 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 aq. 70% MeOH–aq. 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.

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 petroeum (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 solvent].

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 eluting mixtures: eluant No. 1 = 200 g NaCl and 50 mL SD No. 30 alcohol diluted to 1 liter with water; eluant 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 eluant No. 1 and pour into 40-cm × 2.5-cm glass column (Corning No. 38450). Wash with 100 mL eluant No. 1.

Dissolve 150-g sample in 500 mL of water. Pipette 20 mL into a

*Registered trademark of Buffalo Color Corporation.

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 eluant No. 1. Elute FD&C Yellow No. 6 from column with eluant No. 2. Elute Allura* Red AC dye from 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 eluant 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.

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 eluant 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 aq. or methanolic NH_3, respectively. The extract was then separated on a silica gel 60 thin-layer plate using PrOH–aq. 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) as solvent. 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 eluant. Basic dyes resolved best on AG 50W-X4 (Na^+ form) using 0.25 *M* ammonium acetate–aq. NH_3 buffer containing 10% dimethylformamide as the eluant.

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 chro-

matograph the supernatant liquid on silica gel G (0.2 mm, according to Stahl) using PrOH–NH_4OH (90:10) as eluant.

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.

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 eluant. 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 solvent and BuOH–EtOH–H_2O–NH_4OH (100:20:44:1) as the second solvent.

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 solvent mixtures, 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).

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.

Solvent A: Mix 20 mL of 1-butanol, 4 mL of ethanol, and 3 mL of concentrated ammonium hydroxide.

Solvent 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 solvent 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. Unsulfonated pigment colors separate in zones in the following descending order: D&C Red No. 36, D&C Orange No. 17, and D&C Red No. 35. 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 Solvent 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 Solvent 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 solvent 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. Remaining colors separate in the following descending order: D&C Red No. 19, D&C Red No. 8 plus D&C Red No. 10, D&C Orange No. 4, D&C Red No. 27, D&C Red No. 3, D&C Red No. 21, tribromofluorescein, D&C Orange No. 5, monobromofluorescein, fluorescein, and FD&C Blue No. 1. Scrape colored zones from the plates and leach as follows: D&C Red No. 8 plus D&C Red No. 10 in ethanol, D&C Red No. 19 in 30% (v/v) acetic acid and halogenated fluoresceins in 1:9 ammonium hydroxide. Determine the visible spectra against known standards.

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 Solvent 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 Solvent 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 eluant 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. Colors that can be identified in this manner include:

D&C Orange No. 17 or D&C Red No. 36
FD&C Yellow No. 5
D&C Orange No. 5 or D&C Orange No. 10
D&C Red No. 21
FD&C Red No. 3
D&C Red No. 27
D&C Orange No. 4

D&C Red No. 8 or D&C Red No. 10
D&C Red No. 19

Subsidiary dyes of halogenated fluoresceins also separate. Based on information obtained above, 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 (0.025–0.03 g) over the inner surface of a 4-oz mortar.

Thoroughly mix 3 g of (o) and 1.2 mL of (a). Transfer 1/3 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 eluant. Elute under 4–5 lb pressure with the appropriate eluants. Use a flow rate of about 10 mL/min (see table that follows for eluants 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 spectra of D&C Red No. 19 and D&C Red No. 7 in 30% acetic acid. Evaporate the other solution to dryness.

Eluants for Procedure 1	Comments
1. 100–150 mL of (d). Allow the column to wet before applying pressure	D&C Red No. 36 or D&C Orange No. 17 and D&C Red No. 19 elute together. Dilute the solution containing the mixture with an equal volume of heptane and extract the D&C Red No. 19 with 30% acetic acid
2. 150–200 mL of (e)	D&C Red No. 8 elutes with about 150 mL of (e); D&C Red No. 10 requires a larger volume
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. Elute as follows.

Eluants for Procedure 2	Comments
1. 100–150 mL of (d)	Pigment color and D&C Red No. 19 elute. Separate by extraction as in Procedure 1
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 the D&C Orange No. 17 and the D&C Red No. 36 in $CHCl_3$. Dissolve the D&C Red No. 8 and the D&C Red No. 10 in 95% EtOH. 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 ether 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 solvent.

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 eluant.

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 ppt, heat it with 80% ethanol containing 1% aqueous NH_3, filter, and evaporate the solution almost to dryness. Caramel is left on the casein ppt.

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 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 presence of some dyes separated curd becomes pink.

JAX, P., AUST, H. Milchwiss. Ber. 145–189 (1953). The Chromatog-

raphy 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 Artifical 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 eluant [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 solvent, 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 rotatory 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 eluant.

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 ppt 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, con-

taining 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.

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

*Registered trademark of Buffalo Color Corporation.

at 680 nm = A_2; blank absorbance at 505 nm = A_3; blank absorbance at 680 nm = A_4.

$$\text{Percent Allura* Red AC dye} = \frac{(A_1 - A_2 - A_3 + A_4) \times 100}{100 \times 5 \times 52.9} = A \times 0.00378$$

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.

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. 24. Add petroleum ether (boiling range, 40–60C°) 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

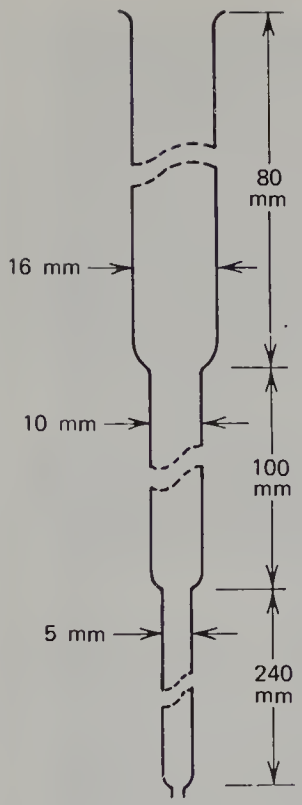

Figure 24 Chromatographic tube

batches of magnesia and must be determined by experience. α-Carotene elutes first as a pale yellow band. β-Carotene elutes 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 elution 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 aq. 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 with BuOH–ETOH–H_2O (2:1:1) and EtOAc–pyridine–H_2O (7:3:1) as eluants.

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 eluant.

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 concentratated 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 eluant just prior to use, using ethyl acetate–MeOH–H_2O–conc. NH_4OH (150:40:35:5) as the eluant.

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	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.

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% aq. 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 soln. in H_2O–10% aq. NH_3 (93:5) as eluant.

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–aq. 25% NH_3 (9:1) as the eluant. 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 aq. 70% MeOH. The adsorbed dyes were eluted with MeOH–H_2O–aq. 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% aq NH_3 (1:45:5) as mobile phase. Part IV describes a similar application to Ponceau 4R and its lake.

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 sep-

aration of detergents, and the determination of moisturisers, sweetening agents, flavors, preservatives, and color additives.

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 eluant.

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 aq. 20% NH_3.

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 eluant, and development for 12–15 cm; (2) spectrophotometric measurements; (3) ppt of the plastic by adding another solvent (e.g., $CHCl_3$, ethanol, or H_2O) and then TLC of the filtrate; and (4) ppt 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.

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 eluant 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).

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).

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 scrapings 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

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.

JONES, F. B. JAOAC *49*, 674–678 (1966). Synthetic Organic Colors in Oils. Prepare the following columns using 20-mm-ID × 300-mm glass tubes.

(a) Florisil column—Activate 60–100-mesh Florisil (Floridin Co., Englewood Cliffs, N. J.) at 650°C. Store at 130°C. For use, add 1.5 mL of H_2O to 100 g in a stoppered bottle, shake to break up lumps, mix thoroughly, and let stand overnight. Pack the column 4 in. high and wash with petroleum ether.

(b) Alumina column—Heat 80–200-mesh alumina for 1 hr at 400°C. Add 50 mL of petroleum ether to a closed chromatographic tube. Add 18 g of alumina, mix, and drain the ether to the top of the column.

(c) Magnesia column—Mix equal weights of Sea Sorb-43 and Celite 545. Pack 9 g of the mixture as described above for the alumina column. Compress with slight air pressure.

(d) Silicic acid column—Add 4 in. of a mixture of equal weights of 100-mesh silicic acid and Celite 545. Wash with *n*-hexane, using pressure.

Dilute 10 mL of sample with 10 mL of petroleum ether and pass through the Florisil column. Elute with petroleum ether, collecting the colored zone (eluate No. 1). Elute with ether, collecting the colored zone (eluate No. 2). Elute with 1:3 ethanol–ethyl ether, collecting each resolved color separately; usually the natural base-oil color, which is discarded, is followed by D&C Violet No. 2, and then D&C Yellow No. 11. Elute with acetonitrile and collect any D&C Red No. 35.

Evaporate the individual ethanol–ethyl ether and acetonitrile eluates to dryness, dissolve the residues in chloroform, and determine their visible spectra. Evaporate eluate No. 2 to dryness. Add eluate No. 1 to the residue and evaporate to about 15 mL. Transfer the solution to the alumina column and wash with 50 mL of petroleum ether; discard the eluate. Add two 10-mL portions of chloroform. If the chloroform eluate is green or blue, add it to the following alcohol–$CHCl_3$ eluate; if the chloroform eluate is colorless, discard it. Elute the column with 1:3 ethanol–chloroform until the eluate is colorless. Evaporate the eluate; dissolve any residue in petroleum ether.

Add this solution to the magnesia column. Wash with 25 mL of petroleum ether, discarding the eluate. Elute with chloroform collecting the individual colored zones. The first fraction contains D&C Green No. 6 and Ext. D&C Blue No. 5. The second fraction contains Ext. D&C Orange No. 4 and Ext. D&C Red No. 14. Elute with 1:3 ethanol–chloroform, collecting the individual colored zones. They are D&C Red No. 18, Ext. D&C Yellow Nos. 9 and 10, and D&C Red No. 17. Evaporate the individual fractions to dryness and compare the visible spectra of the residues in chloroform with standards.

If the spectrum of the blue-green portion does not conform to a known color, evaporate the chloroform and dissolve the residue in *n*-hexane. Transfer it to the silicic acid column and elute with 1:1 *n*-hexane–benzene. Collect the eluate until it is colorless. Evaporate the individual fractions to dryness and compare the visible spectra of the residues in chloroform with standards. If the spectrum for D&C Red No. 17 has a minimum at 385 nm, Ext. D&C Yellow Nos. 9 and 10 may be present. To resolve, evaporate the chloroform, dissolve the residue in a minimum volume of petroleum ether, and transfer it to the magnesia column. Elute with 1:3 ethanol–chloroform and collect the individual colored fractions. Evaporate each fraction to dryness and compare the visible spectra of the residues in chloroform with standards.

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 absoption 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) Solvents 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 solvent A; upper layer is solvent 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) Solvents 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 solvent C; upper layer is solvent 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 solvent 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 solvent B and add rinse to column. After rinse has entered column, elute column with 90 mL of solvent 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 solvent 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 solvent C and add rinses to column, allowing each to enter column before next one is added. Com-

pletely elute FD&C Red No. 4 with an additional 180–235 mL of solvent 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 solvent 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.

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 eluant. 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 eluant. 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 an eluant 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 eluant, 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 ground 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 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 (*Cur-*

cuma), 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 isoamy alcohol, wash the extract with several small portions of water, evaporate 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-liter Erlenmeyer flask and add 200 g of unground 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 adsorption 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 (4:14) as the eluant. Dry the paper and test for metanil yellow using hydrochloric acid.

MITRA, S. N., ROY, B. R. Sci. Culture 25, 539–554 (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 eluant 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 diethylether (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 minutes in a 65°C water bath. Cool to room temperature and mix well.

Determine the sample's absorbance 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 to 4 with anhyd. 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% aq. 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 and 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 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, CI 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 aq. 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 eluant. Collect the colored fractions and concentrate as needed. Dilute the concentrates with water or mobile phase and chromatograph on a 25-cm × 4-mm ID steel column packed with Nucleosil 10 C18 (10 μg) or LiChrosorb RP-

8. Elute with H_2O–acetone (4:1) containing 0.2 g/L tetrabutylammonium chloride. Monitor at 505 nm.

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 homogeous 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 fiber (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 petroluem, 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 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.

$$\text{Parts per million Allura* Red AC dye} = \frac{(A_1 - A_2 - A_3 + A_4) \times 1{,}000{,}000}{20 \times 5 \times 52.9} = A \times 189.0$$

where 52.9 = approximate absorptivity of Allura* Red AC dye

*Registered trademark of Buffalo Color Corporation.

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 inorganic or organic solvent systems.

Test for natural coloring agents as follows. Run against appropriate standards and blanks.

R_f Values of Selected Colorants

Dye	Color Index No.	In Inorganic Solvent[a]	In Organic Solvent[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
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* N_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 nm to 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.

VENTURINI, A., NOVI, M. Boll. Lab. Chim. Provinciali *16*, 175–180 (1965). Identification of Synthetic Water-Soluble Coloring Compounds from Cochineal 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 through a layer of 3–4 mm of asbestos (use suction), 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.

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 eluant. 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 eluant.

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 carboxymethycellylose 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 eluant. 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 eluant 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 eluant.

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 eluant. 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 ^1H and ^{13}C NMR spectrometry, after formation of rubrocurcumin by complexing with H_3BO_3.

GENERAL

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–conc. 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-conc. 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 soln. of pH 2.4 as the electrolyte. Detection limits are about 10 ppm.

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 phophate 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 ingregients (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 prod.
Fat	Lipase	50	7.7	30	Cake, fish, and meat prod.
Phospholipid	Phospholipase	10	7.3	30	Sponge cake and egg prod.
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. 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 soln. is evaporated to dryness as described above. The residue in either instance is dissolved in acetic acid–methanol (2:3) and the resulting soln. 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 eluant.

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 aq. ethanol, sand, diatomaceous earth, and aq. 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 aq. ethanol and aq. NH_3 as before. The extracts are cleaned-up on a polyamide column using acetone–water–aq. NH_3 (40:9:1) as eluant, and then chromatographed on two cellulose TLC plates using ethanol–butanol–pyridine–water (1:7:6:6) and aq. 2.5% Na citrate–aq. NH_3–methanol (20:5:3) as eluants.

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 hrs, 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 eluant.

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. 25). 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 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.

*These colorants are no longer permitted in foods, drugs or cosmetics in the U.S.
†Permitted in externally applied drugs and cosmetics, only.

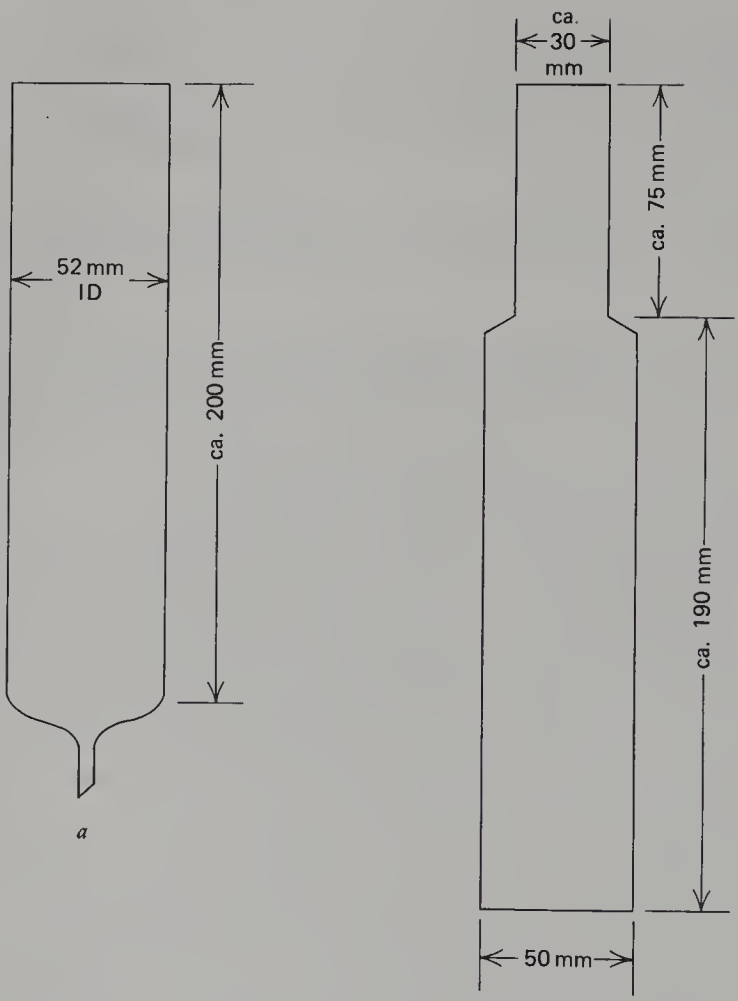

Figure 25 Chromatographic tube (a) and aluminum plunger (b)

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 eluant 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 extration 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).]

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 spectrophotoflurometer 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.

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. 26), 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, al-

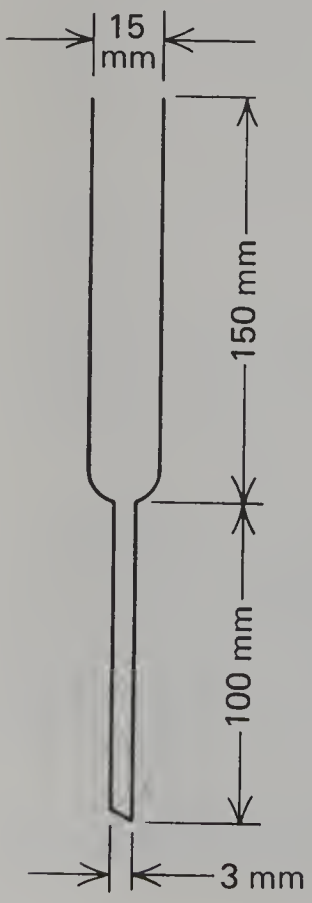

Figure 26 Microchromatographic tube

kanna 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 Fullers'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 Foods. Betanin is isolated from H_2O-soluble samples by chromatography on a microcolumn of polyamide powder using formic acid-methanol (2:3) as eluant. 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 propano–acetic acid–H_2O (3:1:1) as the eluant.

LEHMANN, G., HAHN, H.-G. Z. Analyt. Chem. *238*, 445–456 (1968). Detection and Determination of Water-Soluble Synthetic Food Dyes with Polyamide 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.

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–conc. NH_4OH (50:25:25:10) as the eluant.

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 μ Bondapak C18 column, an eluant 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 using similar conditions.

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 aq. or aq. 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–aq. 25% (or 1%) NH_3–H_2O (4:2:1:1) or ethyl acetate–MeOH–aq. 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.

PUTTEMANS, M., DRYON, L., MASSART, D. L. Anal. Chim. Acta *113*, 307–314 (1980). Etraction of Water-Soluble Acid Food Dyes by Ion-Pair Formation with Trioctlylamine 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* trioctlylamine 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 eluant.

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* $NaClO_4$.

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.

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 silic 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.

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.

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