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Determination of Seven Certified Color Additives in Food Products Using Liquid Chromatography

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ABSTRACT: This study describes a new method for determining FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, and FD&C Yellow No. 6 in food products. These seven color additives are water-soluble dyes that are required to be batch certified by the U.S. Food and Drug Administration (FDA) before they may be used in food and other FDA-regulated products. In the new method, the color additives are extracted from a product using one of two procedures developed for various product types, isolated from the noncolored components, and analyzed by liquid chromatography with photodiode array detection. The method was validated by determining linearity, range, precision, recovery from various matrices, limit of detection, limit of quantitation, and relative standard deviation for each color additive. A survey of 44 food products, including beverages, frozen treats, powder mixes, gelatin products, candies, icings, jellies, spices, dressings, sauces, baked goods, and dairy products, found total color additives ranging from 1.9 to 1221 mg/kg. FDA intends to use the new method for conducting a rigorous, comprehensive dietary exposure assessment of certified color additives in products likely to be consumed by children.

KEYWORDS: *certified color additives, food, liquid chromatography−photodiode array detection, synthetic organic dyes*

INTRODUCTION

The Federal Food, Drug, and Cosmetic Act requires color additives to be preapproved by the U.S. Food and Drug Administration (FDA) and listed in Title 21 of the *Code of Federal Regulations* (CFR) to be legally used in food, drugs, cosmetics, and certain medical devices marketed in the United States. FDA lists permitted color additives, which are various types of dyes and pigments, in 21 CFR parts 73, 74, and 82 depending on whether they are subject to FDA's batch certification process ("certifiable") or exempt from certification.[1]

Nine certifiable color additives are permitted for use in food. Following certification, they are called FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, FD&C Yellow No. 6, Citrus Red No. 2, and Orange B.[1] Citrus Red No. 2 and Orange B are permitted only for coloring the skins of oranges and casings of frankfurters and sausages, respectively, and were not included in this study. The other seven color additives are permitted for coloring food, drugs, and cosmetics and are water-soluble synthetic organic dyes (Table 1; Figure 1). They are required to be declared by their listed names in food product ingredient statements or, to save labeling space, may be declared by the abbreviated names Blue 1, Blue 2, Green 3, Red 3, Red 40, Yellow 5, and Yellow 6.[1]

In 2011, FDA convened a Food Advisory Committee (FAC) to explore the possible association between consumption of certified color additives and possible hyperactivity and other problematic behaviors in children.[2,3] The FAC determined that relevant scientific data did not support a causal link between consumption of certified color additives in food and children's behavior. Therefore, the committee did not recommend that information (e.g., a warning statement) in addition to the listed names be disclosed on food product labels. However, the committee did recommend that FDA conduct a robust exposure assessment to better understand children's intake of certified color additives.

The listing regulations for the seven certified color additives included in this study do not specify use levels, but rather state that these color additives may be used for coloring foods in amounts consistent with good manufacturing practice. Although FDA estimated dietary exposure to these color additives as part of its safety evaluations, the data used in the evaluations are now outdated. Furthermore, FDA does not obtain information on the use of these color additives in individual product types (food, drugs, or cosmetics) as part of the certification process. Therefore, in response to the FAC recommendation, a new method has been developed and validated for the quantitative determination of certified color additives in various food products to support the reassessment of the dietary exposure to these color additives, in particular taking into account the exposure of children.

Several types of methods have been reported for the determination of 3−40 color additives in food products.[4−25] Most of them are LC methods for water-soluble foods such as juice drinks and confectionery because the color additives can be analyzed directly with little sample preparation. The methods reported for more complex foods use procedures for extracting the color additives that are not suitable for FDA's use, because they are either very time-consuming or not applicable to a wide variety of food types or they require the use

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of large quantities of undesirable solvents. For example, defatting of sample solutions has been accomplished by using 50−150 mL quantities of dimethylsulfoxide, petroleum ether, chloroform, or *n*-hexane.[6,14,17,20]

This paper presents the development and validation of a new method for determining certified color additives in food products. The color additives are extracted from food samples by one of two procedures that have been developed for various food types. The first procedure is applicable to beverages, frozen treats, powder mixes, and gelatin products. Samples are dissolved in methanol or 1:1 methanol/water containing aqueous ammonium hydroxide. The second procedure is applicable to candies, icings, jellies, spices, dressings, sauces, baked goods, and dairy products. Samples are homogenized if needed, mixed with methanol containing aqueous ammonium hydroxide, defatted with small (2 mL) quantities of *n*-hexane, and neutralized with acetic acid. The sample extracts are analyzed by liquid chromatography (LC) with photodiode array (PDA) detection, using color additives with known dye content as standards. The new method was used in a small survey of 44 food products and is intended for use in a comprehensive survey of foods marketed in the United States that are likely to be consumed by children.

Table 1. Color Additives Investigated in This Study

21 CFR listing	color additive	common name	E no.	CI no.	major component	dye classifation
74.101	FD&C Blue No. 1	Brilliant Blue FCF	E133	42090	disodium salt of ethyl [4-[*p*-[ethyl (*m*-sulfobenzyl)amino]-α-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadien-1-ylidene] (*m*-sulfobenzyl) ammonium hydroxide	triphenylmethane
74.102	FD&C Blue No. 2	Indigotine	E132	73015	disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indoline-5-sulfonic acid	indigoid
74.203	FD&C Green No. 3	Fast Green FCF	none	42053	inner salt disodium salt of *N*-ethyl-*N*-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfobenzenemethanaminium hydroxide	triphenylmethane
74.303	FD&C Red No. 3	Erythrosine	E127	45430	monohydrate of 9 (*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3*H*-xanthen-3-one, disodium salt	xanthene
74.340	FD&C Red No. 40	Allura Red AC	E129	16035	disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-naphthalenesulfonic acid	azo
74.705	FD&C Yellow No. 5	Tartrazine	E102	19140	trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[4-sulfophenylazo]-1*H*-pyrazole-3-carboxylic acid	pyrazolone
74.706	FD&C Yellow No. 6	Sunset Yellow FCF	E110	15985	disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonic acid	azo

■ MATERIALS AND METHODS

Materials. The color additives used as standards were obtained from the FDA's Office of Cosmetics and Colors and were analyzed for the CFR-specified components and impurities in FDA's certification laboratories. Total dye content, which includes the primary dye component(s) and any subsidiary colors, was obtained twice by visible spectrophotometry using referenced absorptivity values and once either by titanium trichloride titration (all except FD&C Red No. 3) or gravimetric analysis (FD&C Red No. 3).[26−32] Subsidiary color content was determined by LC.[33−37] The content of primary dye components used for quantitation was obtained by subtracting the subsidiary color content from the total dye content. The results are reported in Table 2. The standards also contain residual amounts of volatile matter (water), salts (sodium chloride and sodium sulfate), starting materials, and other impurities (not reported).

Water, methanol, ammonium acetate, glacial acetic acid (all of LC grade), and ammonium hydroxide were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). LC grade *n*-hexane (95%) was purchased from J. T. Baker (Phillipsburg, NJ, USA). Products were homogenized using a Waring commercial laboratory blender (Torrington, CT, USA) and ceramic homogenizers purchased from Agilent Technologies (Santa Clara, CA, USA). Extraction procedures were performed using a Branson 2510 sonicator with mini-vortexer and temperature control (VWR, Radnor, PA, USA) and Eppendorf centrifuge 5804 with rotor FA-45-6-30, maximum 8500 relative centrifugal force (rcf) (Hauppauge, NY, USA). Polypropylene centrifuge tubes (50 mL) were purchased from VWR. Syringeless glass microfiber filters with 0.45 μm pore size and polypropylene housing were purchased from Whatman Inc. (Piscataway, NJ, USA).

Forty-four different products, including beverages, frozen treats, powder mixes, gelatin products, candies, icings, jellies, spices, dressings, sauces, baked goods, and dairy products, were purchased from retail stores in Washington, DC, and surrounding Maryland counties. The seven certified color additives were declared a total of 108 times on the product labels. Additional products containing no color additives were purchased for use as matrices: clear soda (beverage matrix), white candy (candy matrix), ranch dressing (dressing matrix), crackers (baked goods matrix), and milk (dairy matrix). Prior to analysis, the products were refrigerated or frozen as needed or stored at room temperature. The manufacturer or distributor, food type, net quantity of contents, expiration date, and manufacturing code were recorded for all products prior to opening.