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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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Table 1. Color Additives Investigated in This Study

21 CFR listing	color additive	common name	E no.	CI no.	major component	dye classifiation
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-[ethy[(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

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.

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.

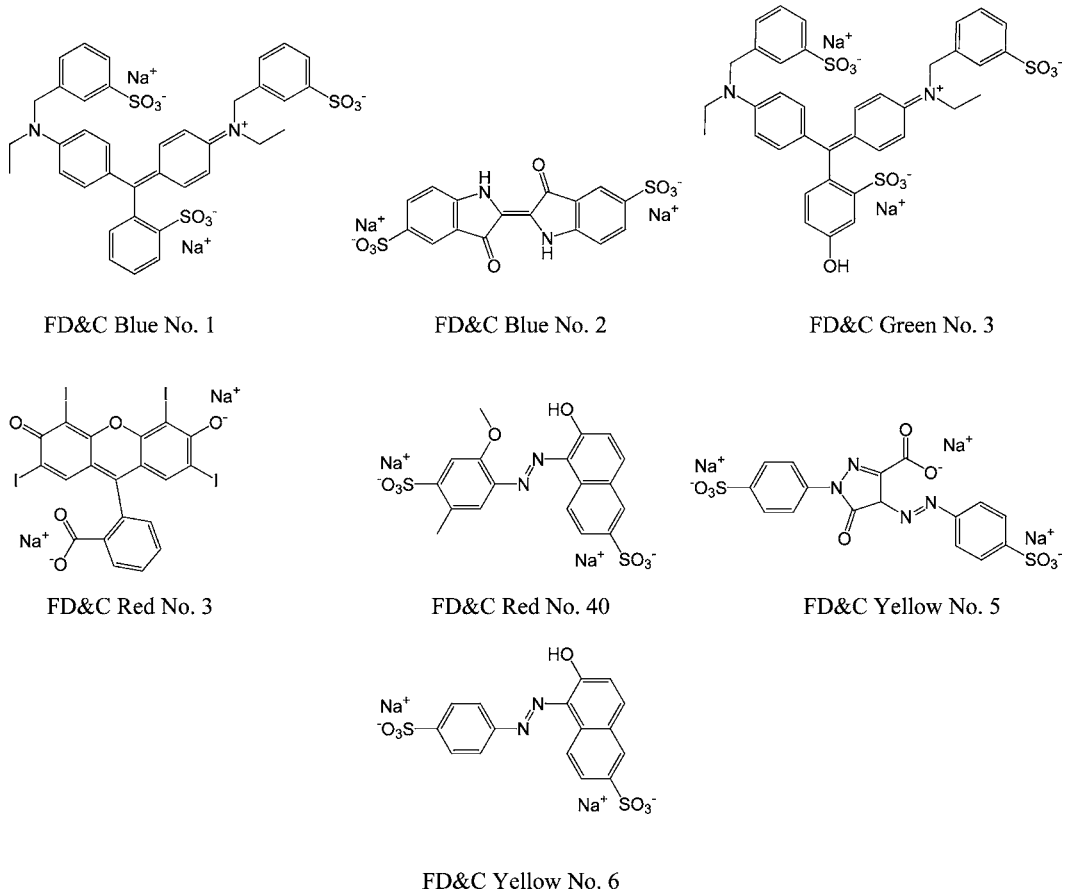

Figure 1. Structures of color additives investigated in this study.

Table 2. LC Quantitation Data for Color Additive Standards

peak	color additive	retention time (min)	λ_{max} (nm)	total dye content (%)	subsidiary colors (%)	primary dye components (%)
1	FD&C Yellow No. 5	7.3	429	82.6	0.1	82.5
2	FD&C Blue No. 2	8.6	609	85.9	<0.2	85.9
3	FD&C Yellow No. 6	13.0	487	83.6	3.5	80.1
4	FD&C Red No. 40	21.4	510	84.7	<0.2	84.7
5	FD&C Blue No. 1	39.6	628	90.1	1.8	88.3
6	FD&C Green No. 3	40.3	625	89.1	2.6	86.5
7	FD&C Red No. 3	46.3	530	87.2	3.1	84.1

Sample Preparation. *Extraction Procedure for Color Additives in Beverages, Frozen Treats, Powder Mixes, and Gelatin Products.* A 5 g sample of each beverage or frozen treat product was weighed and transferred to a 10 mL volumetric flask. (Carbonated drinks were sonicated to flatness before weighing.) Approximately 100 μL of 10% (v/v) aqueous NH_4OH was added to each flask, and methanol was added to a final volume of 10 mL. Powder mixes were prepared by weighing out a 0.1 g sample in a 50 mL volumetric flask and dissolving in a 50% methanol solution containing ~100 μL of 10% aqueous NH_4OH with sonication. A 0.5 g sample of each gelatin product was weighed and transferred to a 10 mL volumetric flask and diluted to volume with a 50% methanol solution containing ~100 μL of 10% aqueous NH_4OH. A 2 mL portion of each sample solution was filtered with a 0.45 μm filter into an LC vial for analysis.

Extraction Procedure for Color Additives in Candies, Icings, Jellies, Spices, Dressings, Sauces, Baked Goods, and Dairy Products. All products were homogenized using a blender. A 5 g sample of each product was weighed into a 50 mL centrifuge tube, followed by two ceramic homogenizers and 10 mL of 7:3 MeOH/10% aqueous NH_4OH (v/v). The mixture was vortexed for 1 min, sonicated for 1 h at 38 °C with periodic shaking, and centrifuged for 5 min at 8500 rcf. The aqueous extract was transferred to a clean 50 mL centrifuge tube. The remaining product was washed by adding 10 mL of 7:3 MeOH/10% aqueous NH_4OH, sonicating with heat for 5 min, and centrifuging for 5 min at 8500 rcf. The aqueous extract was combined with the previously collected extract. The washing step was repeated two more times. The centrifuge tube containing the complete extract was cooled in a freezer for 1 h to separate any matrix particles. The extract was then centrifuged for 10 min at 8500 rcf and carefully decanted into a 50 mL volumetric flask. (The freezing and additional centrifugation steps are not needed for dairy products.) The extract was diluted to volume with water and shaken to mix. A 5 mL aliquot was transferred to a centrifuge tube, mixed with 2 mL of *n*-hexane, and centrifuged for 5 min at 8500 rcf. A 2 mL portion of the aqueous lower layer was transferred to a beaker, mixed with ~20 μL of concentrated acetic acid, and filtered with a 0.45 μm filter into an LC vial for analysis.

Calibration Solutions. Stock solutions containing ~1.0 mg/mL of each color additive were prepared using water as the solvent. The solution flasks were covered with aluminum foil and stored in a drawer. A standard solution was prepared weekly by combining 1 mL of each stock solution without further dilution. Calibration solutions were prepared from diluting aliquots of standard solution with 1:1

methanol/water and ranged from 0.23 to 13.0 mg/L of each color additive. Calibration solutions containing 0.5 g/mL of one of each matrix were prepared similarly and ranged from ~0.25 to ~13.0 mg/L of each color additive.

Analytical Method. Separations were performed using an Alliance 2690 separation module and 996 PDA detector monitored at 420, 520, and 620 nm (Waters Corp., Milton, MA, USA). A 100 μL aliquot of the test solution was injected into an Xterra RP18 column (250 × 4.6 mm, 5 μm) (Waters Corp.). The color additives were separated using (A) 0.1 M ammonium acetate in water and (B) 0.1 M ammonium acetate in methanol with a gradient of 10−30% B in 5 min, 30−33% B in 5 min, hold at 33% B for 25 min, and 33%-100% B in 12 min. All separations were performed at a flow rate of 1 mL/min and column temperature of 25 °C. The color additives were identified and quantified in the sample solutions by comparing their LC retention times and PDA absorption spectra with those of the standards. Three wavelengths were used for identifying the color additives: 420 nm for FD&C Yellow No. 5; 520 nm for FD&C Yellow No. 6, FD&C Red No. 40, and FD&C Red No. 3; and 620 nm for FD&C Blue No. 2, FD&C Blue No. 1, and FD&C Green No. 3. Although those three wavelengths do not all correspond to the maximum absorption wavelengths for the individual color additives, they were optimal for detecting all of the color additives in one analysis. Chromatograms of the standard solution at the three wavelengths are shown in Figure 2.

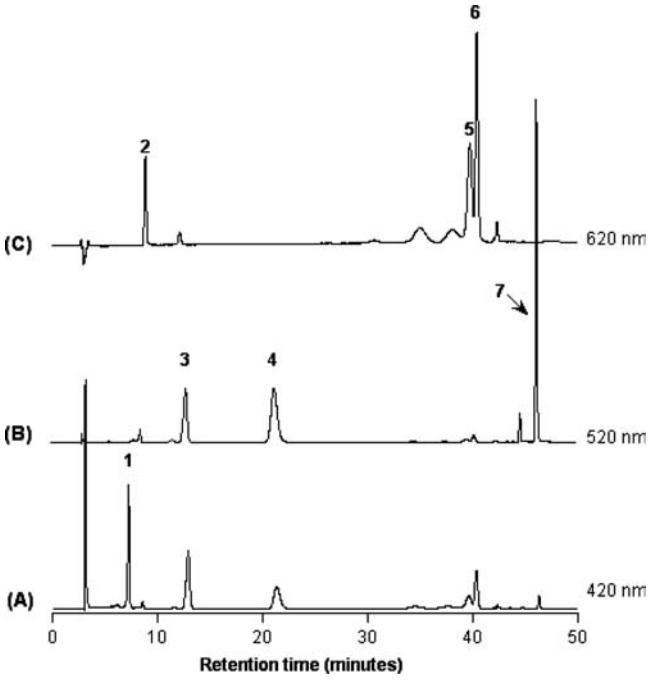

Figure 2. Chromatograms of standard solution at (A) 420 nm, (B) 520 nm, and (C) 620 nm. Peaks are identified in Table 2.

The primary dye peaks are identified in Table 2; the small adjacent peaks are subsidiary colors. The LC conditions were chosen to achieve optimal resolution, peak symmetry, calibration curves, and quantitation for each color additive. Five of the seven color additives are well separated and do not interfere with one another. FD&C Blue No.1 (peak 5) and FD&C Green No. 3 (peak 6) are not completely separated from each other due to the similarity of their chemical structures, which differ by a single OH− group (Figure 1). Baseline separation of the two color additives by using 1:1 methanol/acetonitrile as an elution solvent has been reported, whereas this method uses methanol.[23] This method is intended for a comprehensive survey of certified color additives in foods that are likely to be consumed by children. Because several hundred food products will be analyzed, use of methanol rather than acetonitrile will significantly reduce the cost of the survey and will provide satisfactory results. The excellent recovery results and other method validation data described below indicate that this method will provide satisfactory quantitation of all of the color additives.

RESULTS AND DISCUSSION

Color Additive Standards. The dyes certifiable as 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 all consist of primary dye components as well as smaller amounts of subsidiary colors with higher or lower numbers of substituent groups.[28,29,33−37] The subsidiary colors contribute to the total color additive content in food products but do not need to be determined in the products if the amounts of primary dye components in the color additive standards are known. The primary dye content was obtained by subtracting the subsidiary color content from the total dye content (Table 2), and the primary dye content was used for quantitating the color additives in the products.

The stability of the dyes in the standard stock solutions was monitored over a 6 month period. FD&C Blue No. 1, 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 were found to be stable in the solutions as expected. Although it is well-known that FD&C Blue No. 2 (indigotine) gradually decomposes in solution, particularly in the presence of light, the FD&C Blue No. 2 in the standard stock solution, if kept in the dark and sealed, was stable for about a week.[27,32]

Optimization of Extraction Procedures. Complete homogenization of the food products was found to be essential for reproducibility of the color additive analyses. Using both the blender and the ceramic homogenizers achieved satisfactory homogeneity. Sonication extracted all of the color additives. The freezing steps precipitated out some product matrix components.

The solvent mixture of 7:3 MeOH/10% aqueous NH_4OH was found to optimize the extraction of color additives in food products. Basic conditions facilitated the release of the color additive from the food matrix, and methanol mixed with water further improved the recoveries of the color additives, particularly for FD&C Red No. 3.

For the nonbeverage products, using ~20 μL of acetic acid to adjust the pH of the sample solution to close to neutral resulted in better peak resolution. The final solution was diluted to volume with water instead of methanol to make the ratio of water to methanol consistent with the calibration solutions. Small quantities of hexane (2 mL) were needed to collect any immiscible (fatty) components in the extract solution.

The comprehensive survey of foods will include products such as cake mixes that will be prepared as instructed prior to analysis. However, products such as Jell-O cannot be prepared as instructed due to the difficulty of analyzing gelatinized products, so the unprepared powders must be analyzed.

Method Validation. The matrices used for the validation studies were clear soda, white candy, ranch dressing, crackers, and milk, each containing no color additives. The matrices are identified as beverage, candy, dressing, baked goods, and dairy. Samples of the matrices were spiked with various concentrations of the seven color additives for the recovery and validation studies. Table 3 summarizes the method validation data.

The calibration curves in the absence and presence of the matrices were linear from ~0.25 to ~13.0 mg/L and were all within acceptable ranges. Both sets of calibration curves were

Table 3. Validation Data for the Color Additives in Five Food Matrices

analyte	matrix	R^2	recovery (%)	LOD (mg/L)	LOQ (mg/L)	LOD (mg/kg)	LOQ (mg/kg)	intraday RSD (%)	interday RSD (%)
FD&C Blue No. 1	beverage	0.9984	93.2	0.041	0.136	0.082[a]	0.27[a]	3.1	6.1
	dairy	0.9956	114.2			0.41[b]	1.36[b]		
	candy	0.9986	103.4						
	dressing	0.9996	96.6						
	baked goods	0.9999	90.9						
FD&C Blue No. 2	beverage	0.9953	94.9	0.071	0.237	0.14[a]	0.48[a]	6.7	11.3
	dairy	0.9998	112.2			0.71[b]	2.37[b]		
	candy	0.9985	76.9						
	dressing	0.9981	112.9						
	baked goods	0.9993	93.2						
FD&C Green No. 3	beverage	0.9985	94.5	0.0065	0.022	0.013[a]	0.043[a]	0.6	3.2
	dairy	0.9989	104.3			0.065[b]	0.22[b]		
	candy	0.9974	99.7						
	dressing	0.9999	97.7						
	baked goods	0.9994	99.2						
FD&C Red No. 3	beverage	0.9997	97.1	0.035	0.118	0.071[a]	0.24[a]	3.0	2.3
	dairy	0.9988	88.3			0.35[b]	1.18[b]		
	candy	1.0000	97.3						
	dressing	1.0000	98.2						
	baked goods	1.0000	96.9						
FD&C Red No. 40	beverage	0.9998	98.4	0.014	0.047	0.028[a]	0.095[a]	1.4	0.7
	dairy	0.9991	96.3			0.14[b]	0.47[b]		
	candy	1.0000	97.9						
	dressing	0.9998	96.4						
	baked goods	0.9993	73.6						
FD&C Yellow No. 5	beverage	0.9980	110.1	0.038	0.127	0.076[a]	0.25[a]	2.1	2.4
	dairy	0.9980	82.8			0.38[b]	1.27[b]		
	candy	0.9956	96.0						
	dressing	0.9999	81.3						
	baked goods	0.9991	80.0						
FD&C Yellow No. 6	beverage	0.9995	98.7	0.015	0.050	0.030[a]	0.099[a]	1.5	2.8
	dairy	0.9990	93.0			0.15[b]	0.50[b]		
	candy	0.9998	97.1						
	dressing	0.9999	96.2						
	baked goods	0.9996	76.7						

[a]LOD and LOQ values for beverages, frozen treats, powder mixes, and gelatin products procedure. [b]LOD and LOQ values for candies, icings, jellies, spices, dressings, sauces, baked goods, and dairy products procedure.

obtained by plotting peak area versus nanograms of dye injected. Correlation coefficients (R^2) were 0.9950 or higher for all color additives in the absence of the matrices. Average R^2 values in the presence of the matrices ranged from 0.9953 for FD&C Blue No. 2 in the beverage matrix to 1.0000 for FD&C Red No. 3 in the candy, dressing, and baked good matrices and for FD&C Red No. 40 in the candy matrix.

Approximately 50% methanol in the sample solutions provided good recoveries of all of the color additives from all of the matrices. Recoveries were estimated from analyses of the matrices separately fortified at four or five different levels of each color additive. Results were calculated by dividing the slopes of the calibration curves in the presence of each matrix by the slopes of the calibration curves in the absence of each matrix. The recoveries ranged from 73.6 to 114.2%, with averages of 99.1% (beverage), 95.6% (candy), 97.0% (dressing), 87.2% (baked goods), and 98.7% (dairy). The lowest recovery was for FD&C Red No. 40 in the baked goods matrix, and the highest recovery was for FD&C Blue No. 1 in the dairy matrix. Therefore, a matrix effect was not observed. A degradation trend for FD&C Blue No. 2 also was not observed.

Limits of detection (LODs) and limits of quantitation (LOQs) for the color additives were estimated by diluting a 4 μL portion of the color additive standard solution to 2 mL with 50% methanol in water to form a solution that contained ~0.25 mg/L of all the analytes. The diluted solution was analyzed six times by LC, and the results were corrected for the standard purities. The LODs and LOQs were estimated as 3 and 10 times the standard deviation of the average value from the six replicates, respectively, and are reported in Table 3 in units of

milligrams per liter. The LODs and LOQs reported in units of milligrams per kilogram correspond to the volumes in the two extraction procedures. All of the LODs and LOQs are within previously reported ranges.[4−11,14,15,17−23] In particular, a study of auramine, malachite green, and rhodamine B that used extraction procedures very similar to those in the present study (except with larger amounts of *n*-hexane) reported very similar LODs and LOQs for sugar-based, starch-based, and fatty food matrices.[7]

To test the reproducibility of peak areas of the color additives, six individually prepared standard solutions containing ∼0.25 mg/L of all the analytes, diluted from the same concentrated standard mix, were analyzed in one day. One of these solutions was analyzed every day for 5 days. Retention times varied only minimally from day to day. The relative standard deviations (RSDs) were determined by dividing the standard deviations by the average results for the analytes and multiplying by 100%. Both the intraday and interday precisions were good, giving RSDs below 6.7% for the intraday and below 12% for the interday precisions (Table 3). In summary, very good reproducibility, precision, and ruggedness were found for the method.

Survey Results. Forty-four food products purchased commercially were quantitatively analyzed for certified color additive content using the validated LC method. The color additives were extracted from the products using one of the two extraction procedures. Calibration in the absence of the matrices was used for quantitating the color additives. Additional dye components (subsidiary colors), matrix residues, and impurities did not interfere with any of the product analyses.

Chromatograms at 420, 520, and 620 nm are shown in Figure 3 for dry-powder iced tea (Table 4, product 2). The color additives were extracted by the procedure for powder mixes (first procedure). A peak at 7.3 min shown in Figure 3A is identified as FD&C Yellow No. 5. Peaks at 13.0 and 21.4 min shown in Figure 3B are identified as FD&C Yellow No. 6 and FD&C Red No. 40, respectively. A peak at 39.6 min shown in Figure 3C is identified as FD&C Blue No. 1. The unidentified peaks are subsidiary colors.

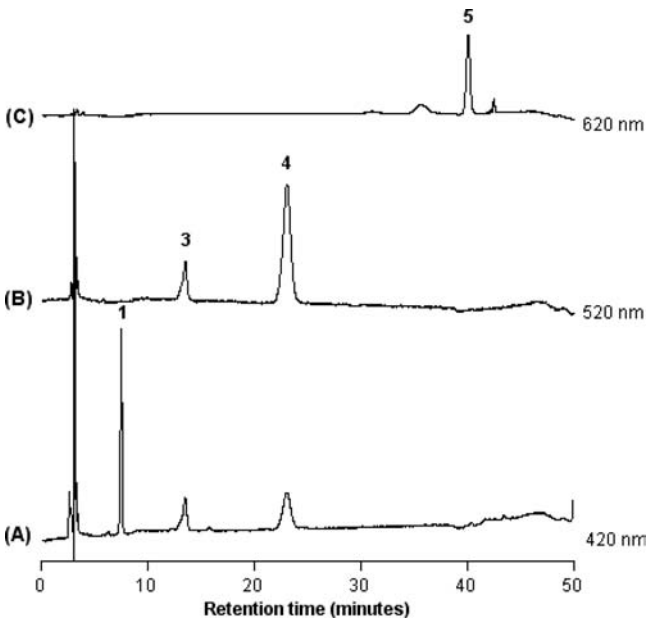

Figure 3. Chromatograms of dry-powder iced tea (Table 4, product 2) at (A) 420 nm, (B) 520 nm, and (C) 620 nm. Peaks are identified in Table 2.

Similar chromatograms are shown in Figure 4 for cereal I (Table 4, product 29). The color additives were extracted according to the procedure for baked goods (second procedure). A peak at 7.3 min shown in Figure 4A is identified as FD&C Yellow No. 5. Peaks at 13.0 and 21.4 min shown in Figure 4B are identified as FD&C Yellow No. 6 and FD&C Red No. 40, respectively. A small peak at 8.6 and a large peak at 39.6 min shown in Figure 4C are identified as FD&C Blue No. 2 and FD&C Blue No. 1, respectively. The unidentified peaks are either subsidiary colors or matrix residues.

Chromatograms are shown in Figure 5 for cereal II (Table 4, product 30). The color additives were extracted according to the procedure for baked goods. A peak at 21.4 min shown in Figure 5B is identified as FD&C Red No. 40. Peaks at 8.6 and 40.3 min shown in Figure 5C are identified as FD&C Blue No. 2 and FD&C Green No. 3, respectively. FD&C Blue No. 1 was declared on the product label and a small peak corresponding to that color additive is evident in Figure 5C as a small shoulder on the left side of the FD&C Green No. 3 peak. However, the amount found falls below the method detection limit.

The survey results are reported in Table 4. The samples were analyzed in duplicate or triplicate according to the new method and were reanalyzed when discrepancies occurred. Of the 108 eight color additives declared on the product labels, 94 were found as expected. The other 14, encompassing 10 products, either were not found or were present at levels below the detection limit of the method. Sample solutions containing color additives at levels above the upper limits of the calibration curves were diluted and reanalyzed. Two analysts performed the survey and obtained equivalent results on different days using different sample solutions, indicating the ruggedness of the method. Most of the RSDs were 10% or below, showing good reproducibility. The high RSDs in sprinkles I, sprinkles II, and raspberry walnut dressing are attributed to the inability to completely homogenize the products.

The most frequently found color additive in the survey was FD&C Red No. 40, in 30 of the 44 products. This is not surprising because FD&C Red No. 40 is certified in highest quantity.[38] The other frequently found color additives were FD&C Blue No. 1 in 19 products, FD&C Yellow No. 6 in 18 products, and FD&C Yellow No. 5 in 14 products. FD&C Blue No. 2 was found in 7 products, FD&C Green No. 3 in 2 products, and FD&C Red No. 3 in 4 products.

The product with the highest color additive content was sprinkles II, which was found to contain 496.8 mg/kg of FD&C Yellow No. 6, 313.7 mg/kg of FD&C Yellow No. 5, 213.7 mg/kg of FD&C Red No. 40, 174.2 mg/kg of FD&C Blue No. 1, and 22.4 mg of FD&C Blue No. 2. The candies, icings, other sweets, and spices contained from 1.9 to 1221 mg/kg (0.00019−0.12%) total color additives, the highest range for any of the product categories. The beverages, frozen treats, powder mixes, and gelatin products contained from 3.6 to 392.3 mg/kg total color additives. (Color additives in the powder drinks and gelatin products were calculated to reflect the amounts of color additives used as consumed.) The dressings, sauces, and baked goods contained from 2.9 to 514.6 mg/kg. The dairy products contained from 3.9 to 40.0 mg/kg total color additives.

The two products containing FD&C Green No. 3 were cereal II and sherbet. FD&C Blue No. 2, FD&C Red No. 40,

Table 4. Results for Color Additives in Food Products

				color additives															
				Blue 1		Blue 2		Green 3		Red 3		Red 40		Yellow 5		Yellow 6			
product category	product no.	product name	*n*	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	total dye content (mg/kg)	
beverages, frozen treats, powder mixes, and gelatin products	1	cherry popsicle	2									26.8	7.9					26.8	
	2	dry-powder iced tea	4	0.8	8.4							8.7	4.1	10.0	9.0	2.4	10.6	21.9	
	3	dry-powder orange drink	2									*a*				32.0	0.9	32.0	
	4	fruit punch drink	2	0.2	9.4							59.3	0.3					59.5	
	5	grape popsicle	2															*b*	
	6	grape-flavored electrolyte solution	2	0.7	3.1							2.9	3.8					3.6	
	7	orange popsicle	2															*b*	
	8	orange soda	2									2.1	1.8			33.4	1.2	35.5	
	9	peach-flavored gelatin	2	*a*								6.3	6.5			4.6	4.9	10.9	
	10	Popsicle, red white and blue	2	75.3	0.3					1.8	1.8	315.2	0.8					392.3	
candies, icings, other sweets, and spices	11	bubble gum	2									128.6	0.4			3.6	7.7	132.2	
	12	chocolate candy	3	34.0	9.4	*a*						60.8	0.7	32.1	4.5	152.8	1.8	279.7	
	13	chocolate syrup	2	7.5	0.2							10.6	0.1			*a*		18.1	
	14	fruit cup	2							1.9	0.3							1.9	
	15	fruit-flavored candy	2	3.9	2.3	12.0	1.0					89.8	3.7	68.6	2.2	63.9	2.9	238.2	
	16	gummy candy	2	*a*								310.1	0.3	*a*				310.1	
	17	children's vitamins	2			53.9	11.2					191.5	2.0			48.7	10.7	294.1	
	18	licorice-flavored candy	2	139.2	2.2							203.1	2.0					342.3	
	19	purple gel	2	19.4	1.0							187.3	1.5					206.7	
	20	seasoning	2											109.0	2.2			109.0	
	21	sprinkles I	3	59.7	14.4					34.3	4.6	265.1	6.3	61.5	17.0	52.7	9.4	473.3	
	22	sprinkles II	3	174.2	6.8	22.4	12.8					213.7	13.5	313.7	4.4	496.8	10.4	1220.8	
	23	vanilla pudding	2											9.4	1.0	2.1	8.7	11.5	
dressings, sauces, and baked goods	24	apple pie	2											*a*		*a*			
	25	blueberry preserves	2	3.0	4.9							150.4	3.6					153.4	
	26	bacon product	2									185.0	1.1					185.0	
	27	barbecue sauce	2	2.7	0.7							89.7	0.1			97.8	2.2	190.2	
	28	blueberry bagel	2	2.8	10.4	1.7	2.5					15.5	4.7					20.0	

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Table 4. continued

				color additives													
				Blue 1		Blue 2		Green 3		Red 3		Red 40		Yellow 5		Yellow 6		
product category	product no.	product name	*n*	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	dye content (mg/kg)	RSD (%)	total dye content (mg/kg)
	29	cereal I	2	37.5	0.6	6.7	2.1					310.4	4.5	33.9	1.1	126.1	0.0	514.6
	30	cereal II	2	*a*		3.5	9.5	1.9	5.1			2.8	3.6					8.2
	31	cheese curls	3											99.3	3.4	204.6	0.2	303.9
	32	cheese pretzels	2	*a*										12.1	4.4	19.2	8.0	31.3
	33	cherries	2									232.2	8.8					232.2
	34	chips	2	*a*								*a*		2.9	1.5			2.9
	35	chocolate wafers	2	18.5	0.1							212.4	0.5	138.3	3.4			369.2
	36	raspberry walnut dressing	2	3.6	17.4							301.0	18.5			13.2	2.7	317.8
	37	sweet relish	2											27.5	12.1			27.5
	38	thousand island dressing	2	*a*								*a*				13.2	2.7	13.2
	39	toaster pastry	2	5.6	11.7	1.6	0.7					67.5	8.9					74.7
dairy	40	blueberry yogurt	2	8.7	2.2							4.8	3.9					13.5
	41	sherbet	2					2.9	2.1			6.3	7.8	27.1	0.3	3.7	2.0	40.0
	42	strawberry cream cheese	2									9.6	1.9					9.6
	43	strawberry milk	2							3.9	2.3							3.9
	44	strawberry yogurt	2															*b*

[a]Color additive declared on product label but not detected by LC or present below the LOD. [b]Color additives not declared and not found.

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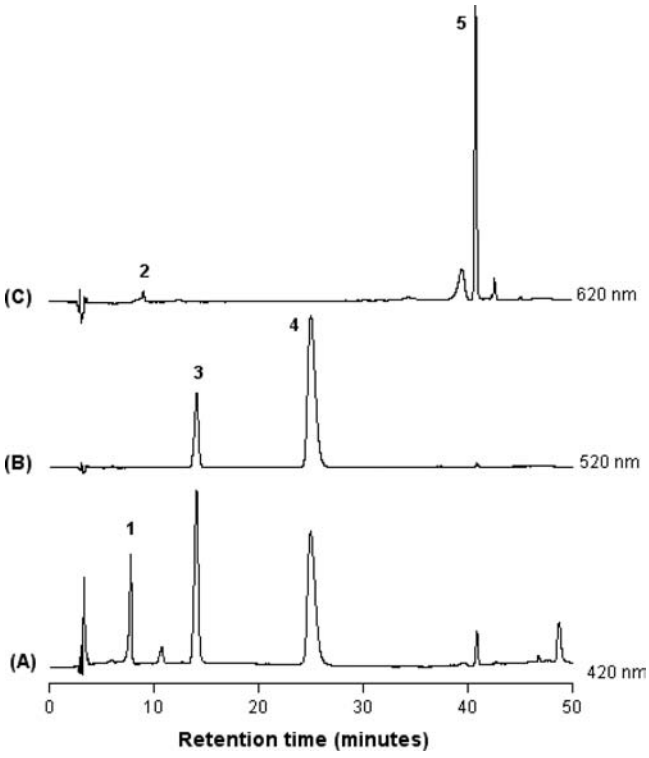

Figure 4. Chromatograms of cereal I (Table 4, product 29) at (A) 420 nm, (B) 520 nm, and (C) 620 nm. Peaks are identified in Table 2.

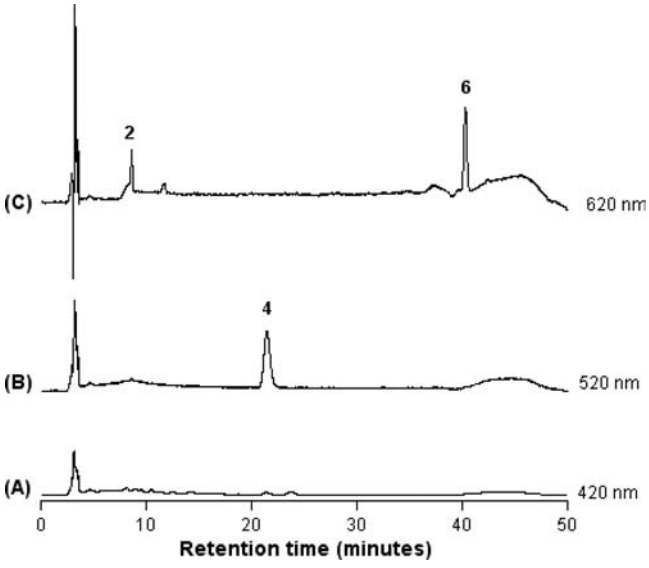

Figure 5. Chromatograms of cereal II (Table 4, product 30) at (A) 420 nm, (B) 520 nm, and (C) 620 nm. Peaks are identified in Table 2.

FD&C Yellow No. 5, and FD&C Yellow No. 6 also were found in the products. As stated above, FD&C Blue No. 1 was declared on the cereal II product label, and a small amount that was below the detection limit was found in the product. In general, the need for this method to determine FD&C Blue No. 1 and FD&C Green No. 3 simultaneously is minimal because FD&C Green No. 3 is rarely used in food products; extra effort was needed to find any food products containing FD&C Green No. 3 for the survey. Combinations of FD&C Blue No. 1 and FD&C Yellow No. 5 are used instead, as supported by FDA certification data for 2012 showing that the quantity of FD&C Green No. 3 certified was 2% of the quantity of FD&C Blue No. 1.[38] Nevertheless, the results for cereal II indicate that FD&C Blue No. 1 and FD&C Green No. 3 can be determined in a food product in the presence of each other. Confirmatory results can be obtained by reanalyzing the product with standard solutions containing FD&C Blue No. 1 or FD&C Green No. 3 alone.

In this study, FD&C Blue No. 1 ranged from 0.2 to 75.3 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; from 3.9 to 174.2 mg/kg in candies, icings, other sweets, and spices; and from 2.7 to 37.5 mg/kg in dressings, sauces, and baked goods; it was found at 8.7 mg/kg in one dairy product. Previous studies found ranges of 0.1−919.7 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; 0.1−4673 mg/kg in candies, icings, other sweets, and spices; 0.02−661.7 mg/kg in dressings, sauces, and baked goods; and 0.1−66.6 mg/kg in ice cream.[4−6,14−16,18−22] (Some studies reported results in units of mg/L.)

In this study, FD&C Blue No. 2 ranged from 12.0 to 53.9 mg/kg in candies, icings, other sweets, and spices and from 1.6 to 6.7 mg/kg in dressings, sauces, and baked goods. Previous studies found a range of 1.4−151 mg/kg in candies and other sweets.[14,16,18,20]

In this study, FD&C Green No. 3 was found at levels of 1.9 mg/kg in one baked good and 2.9 mg/kg in one dairy product. Previous studies found levels of 7.5 mg/kg in one sweet and 0.8 mg/kg in ice cream.[20,21]

In this study, FD&C Red No. 3 was found at levels of 1.8 mg/kg in one frozen treat; 1.9 and 34.3 mg/kg in one candy and one other sweet; and 3.9 mg/kg in one dairy product. Previous studies found ranges of 0.2−329.4 mg/kg in candies, icings, other sweets, and spices; 1.9−9.6 mg/kg in baked goods; and 0−25.1 mg/kg in ice cream.[6,16,19,20]

In this study, FD&C Red No. 40 ranged from 2.1 to 315.2 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; from 10.6 to 310.1 mg/kg in candies, icings, other sweets, and spices; from 2.8 to 310.4 mg/kg in dressings, sauces, and baked goods; and from 4.8 to 9.6 mg/kg in dairy products. Previous studies found ranges of 0.1−2335 mg/kg in beverages; 0.1−1313 mg/kg in candies, icings, other sweets, and spices; 0.04−703 mg/kg in sauces and baked goods; and 0.04−179 mg/kg in cakes and ice cream.[4,14−16,18,20]

In this study, FD&C Yellow No. 5 was found at levels of 10.0 mg/kg in one powder mix product; 9.4−313.7 mg/kg in candies, icings, other sweets, and spices; 2.9−138.3 mg/kg in dressings, sauces, and baked goods; and 27.1 mg/kg in one dairy product. Previous studies found ranges of 0.2−9450 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; 0.1−15157.5 mg/kg in candies, icings, other sweets, and spices; 0.1−4875 mg/kg in sauces and baked goods; and 0.1−66 mg/kg in cakes and ice cream.[4−6,10,14,16−22]

In this study, FD&C Yellow No. 6 ranged from 2.4 to 33.4 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; from 2.1 to 496.8 mg/kg in candies, icings, other sweets, and spices; and from 13.2 to 204.6 mg/kg in dressings, sauces, and baked goods; and it was found at 3.7 mg/kg in one dairy product. Previous studies found ranges of 0.1−4567.3 mg/kg in beverages, frozen treats, powder mixes, and gelatin products; 0.1−5815.0 mg/kg in candies, icings, other sweets, and spices; 0.1−1132.9 mg/kg in sauces and baked goods; and 0.2−59 mg/kg in cakes and ice cream.[4−6,10,14,16−22]

The highest results from this study are considerably lower than results from a few of the previous studies, none of which

were conducted in the United States. The highest reported result was 15157.5 mg/kg (~15 g/kg or 1.5%) for FD&C Yellow No. 5 in a sweetmeat (a type of candied fruit).[19] Many other color additives were found at 1000 mg/kg levels in a variety of product types. In contrast, the highest result from this study was 496.8 mg/kg (~0.5 g/kg or 0.05%) for FD&C Yellow No. 6 in a candy. The least frequently found color additives were FD&C Blue No. 2, FD&C Green No. 3, and FD&C Red No. 3 in any study. Dairy products were least frequently analyzed.

In summary, this new LC method is appropriate for the determination of certified color additives in a wide variety of food products. The lack of interference from matrix effects demonstrates the broad applicability of the method. The extraction procedures do not require large amounts of undesirable solvents, and the LC analyses of the extracts provided consistent results. The survey found excellent correlations between the color additives declared on the product labels and those found in samples of the products.

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Notes

The authors declare no competing financial interest.

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