Automation of AOAC 988.13 for Increased Throughput in the Identification of FD&C Color Additives in Food

Megan York and Toni R. Hofhine, Gilson, Inc., Middleton, WI, USA and Judy Hadley, Ph.D. and Rick Laurell, Agilent Technologies, Santa Clara, CA, USA

OVERVIEW

Purpose

- Color additives for food are commonly found in many places: candies, powdered drink mix, fruit skins.
- The addition of synthetic color additives is regulated closely by the FDA.
- Color additives have come under food safety scrutiny recently because of their potential adverse physical and mental health effects that may be linked to ingestion, especially in children.
- AOAC Official Method 988.13 provides a manual process for extraction, separation, and identification of color additives from various food products.
- Automation of all three steps: sample preparation, solid phase extraction, and spectrophotometric analysis through single software control allows for increased throughput and consistency of sample testing.
- Automation of common manual procedures can decrease sample backlogs by providing added efficiency and increased daily throughput for a typical laboratory.

Figure 1. Gilson GX-274 ASPEC with 406 Dual Syringe Pumps

A Gilson GX-274 ASPEC™ System in conjunction with an Agilent 8453 UV-visible Spectrophotometer with Multicell Transport was used to automate the solid phase extraction (SPE), sample preparation, and sample analysis of FD&C color additives in food. AOAC Official Method 988.13 was successfully automated and FD&C color additives were effectively separated and qualitatively identified from multiple samples.

Figure 2. Agilent 8453 UV-Visible Spectrophotometer with Multicell Transport

EXPERIMENTAL

The FD&C color additives contained within three different samples were separated via solid phase extraction and qualitatively identified against a prepared FD&C standard. The FD&C color additives examined were FD&C Yellow No. 5 (E102, Tartrazine), FD&C Red No. 40 (E129, Allura Red AC), and FD&C Blue No. 1 (E133, Erioglaucine). Spectra of each of the standards can be seen below.

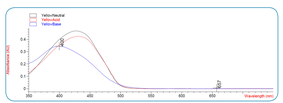


Figure 3. Standard Spectra for FD&C Yellow No. 5

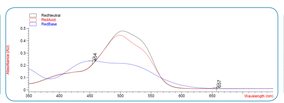


Figure 4. Standard Spectra for FD&C Red No. 40

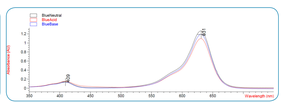
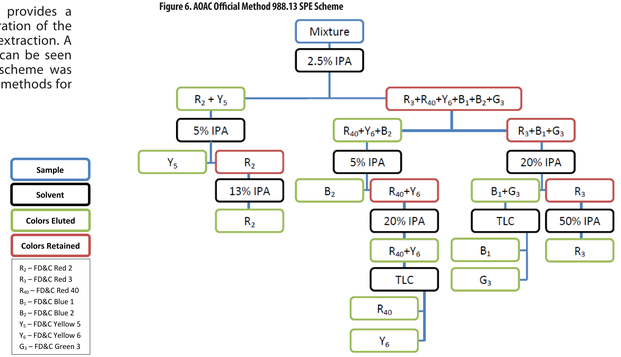


Figure 5. Standard Spectra for FD&C Blue No. 1

AOAC Official Method 988.13 provides a scheme to follow for the separation of the color additives via solid phase extraction. A representation of this scheme can be seen to the right in Figure 6. This scheme was utilized to establish proper SPE methods for the samples tested.

Figure 6. AOAC Official Method 988.13 SPE Scheme



METHOD

Samples and Solvents

Allura Red AC (Sigma, P/N 458848)
Tartrazine (Sigma, P/N T0388)
Erioglaucine (Sigma, P/N 861146)
Isopropanol (B&J, P/N 10071758)
2.5, 13, and 20% solutions were prepared with NanoPure water
Acetic Acid (Sigma, P/N 320099)
1% solution was prepared with NanoPure water
Sodium Hydroxide (EM Science, P/N SX0600-1)
50% solution was prepared with NanoPure water
Hydrochloric Acid (Sigma, P/N 258148)
NanoPure Water
Black Food Coloring (McCormick)
Kool-Aid® (Grape and Orange, powder)

Apparatus

Gilson GX-274 ASPEC™ with two 406 Dual Syringe Pump
(4) 10 mL syringes
GX Transfer Port Assembly (Special 1785)
Code 386 rack for 6 mL SPE cartridges
Agilent 8453 UV-visible Spectrophotometer
Multicell Transport (8-cell)
(8) Flow Cell (1 mm, 40 µL)
Phenomenex Strata® C18-E SPE Cartridge (6 mL/1000 mg), P/N 8B-S001-JCH
Grace Alltech® Extract-Clean™ Filter Columns (8.0 mL), P/N 211108

Protocols

All tests were run in quadruplicate. Figure 7 below shows the complete protocol followed for the separation and identification of black food coloring.

Sample Preparation

Reference Standard Solutions: (Stock) 100 mg of the reference material was diluted to 100 mL with NanoPure water. (Standard) 10 mL of stock solution was diluted to 100 mL with the appropriate isopropanol solution.

Black Food Coloring: A 1:200 dilution of black food coloring was prepared with NanoPure water.

Kool-Aid: 1 g powder was dissolved in 100 mL NanoPure water. Filtered on bed.

Solid Phase Extraction

The SPE scheme from AOAC 988.13 was utilized, with some volume modifications to adjust for cartridge size. This original SPE scheme can be found below in Figure 6. The volumes used for the application can be found in Tables 1, 2, and 3.

Table 1. SPE Parameters used for the Separation of Black Food Coloring

Step	Solvent	Volume (µL)	Air Push (µL)
Condition #1	IPA	2000	1250
Condition #2	1% Acetic Acid	2500	1750
Load	Black Food Coloring	1500	600
Fractionate #1	2.5% IPA	2250	750
Fractionate #2	13% IPA	1500	1000
Fractionate #3	20% IPA	3000	2000

Table 2. SPE Parameters used for the Separation of Grape Kool-Aid

Step	Solvent	Volume (µL)	Air Push (µL)
Condition #1	IPA	2000	1250
Condition #2	1% Acetic Acid	2500	1750
Load	Kool-Aid	2000	1200
Wash	2.5% IPA	3000	1500
Fractionate #1	13% IPA	2000	1000
Fractionate #2	20% IPA	3000	2000

Table 3. SPE Parameters used for the Separation of Orange Kool-Aid

Step	Solvent	Volume (µL)	Air Push (µL)
Condition #1	IPA	2000	1500
Condition #2	1% Acetic Acid	2500	2000
Load	Kool-Aid	2000	1200
Fractionate #1	2.5% IPA	4000	2500
Fractionate #2	13% IPA	3000	2000

Sample Preparation and Analysis

The fractions collected from the SPE process were prepared for identification using the GX-274 ASPEC.

(1) The fraction was transferred to a clean test tube; volume transferred was 250 µL less than the amount of solvent used for elution in the Fractionate step
(2) The fraction was diluted to 6 mL with appropriate IPA solution and volume
(3) 2 mL was transferred to each of two sets of clean tubes
(4) 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the neutral diluted fraction
(5) A drop (23 µL) of concentrated hydrochloric acid was added to the second set of test tubes and the solution was mixed
(6) 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the acidic fraction solution
(7) A drop (23 µL) of 50% sodium hydroxide solution was added to the third set of test tubes and the solution was mixed
(8) 1500 µL was transferred to the flow cells via the transfer ports, and an absorbance reading was taken on the basic fraction solution

A blank of the appropriate IPA solution was taken prior to each set of absorbance readings. The readings were taken from 190 to 1100 nm, however the spectra were only analyzed from 350 to 750 nm, as specified in AOAC 988.13. The flow cells and lines were rinsed with 5 mL NanoPure water after each reading to eliminate carryover between samples.

Figure 7. Sample List for Black Food Coloring Separation and Identification

	Method Name
1	Conditions and Load
2	Fractionate
3	Fractionate
4	Fractionate
5	Sample Prep
6	2_5 Blank Injection
7	Acidify
8	2_5 Blank Injection
9	Basify
10	2_5 Blank Injection
11	Sample Prep
12	13 Blank Injection
13	Acidify
14	13 Blank Injection
15	Basify
16	13 Blank Injection
17	Sample Prep
18	20 Blank Injection
19	Acidify
20	20 Blank Injection
21	Basify
22	20 Blank Injection

RESULTS AND CONCLUSION

McCormick brand black food coloring was used as a primary test sample due to its relatively clean matrix. The product ingredient labeling for the black food coloring indicated it contained FD&C Yellow No. 5, FD&C Red No. 40, and FD&C Blue No. 1. The resultant spectra confirmed the presence of these color additives. Representative spectra from each of the fractions can be found below in Figures 8, 9, and 10. A slight carryover of FD&C Red No. 40 into the FD&C Blue No. 1 fraction can be observed in Figure 10.

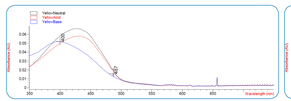


Figure 8. FD&C Yellow No. 5 from Black Food Coloring

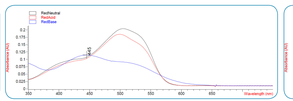


Figure 9. FD&C Red No. 40 from Black Food Coloring

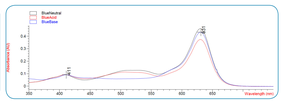


Figure 10. FD&C Blue No. 1 from Black Food Coloring

Grape and Orange Kool-Aid powder were used as secondary test samples for this application. The drink mixes provided a slightly more complex sample matrix, containing chemicals such as citric acid, calcium phosphate and natural flavorings. The Grape Kool-Aid powder was labeled as containing FD&C Red No. 40 and FD&C Blue No. 1. The Orange Kool-Aid listed both FD&C Yellow No. 5 and FD&C Red No. 40, as well as FD&C Red No. 40 Lake. Some overlapping of color bands was apparent in the spectra for both Kool-Aid flavors.

Representative spectra for the Grape Kool-Aid can be found in Figures 11 and 12. Crossover between the FD&C Red No. 40 and FD&C Blue No. 1 fractions is apparent in both spectra, but does not hinder the identification of the color additive.

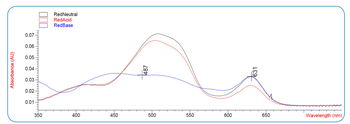


Figure 11. FD&C Red No. 40 from Grape Kool-Aid

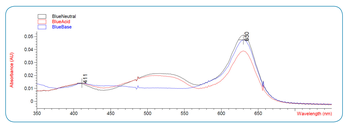


Figure 12. FD&C Blue No. 1 from Grape Kool-Aid

Representative spectra for the Orange Kool-Aid can be found in Figures 13 and 14. Despite low concentration in the Orange Kool-aid, as well as the observance of some FD&C Yellow No. 5 remaining in the fraction, the FD&C Red No. 40 in Figure 14 is qualitatively identified through the characteristic spectra displayed seen from 475 to 575 nm.

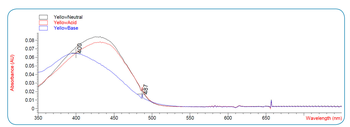


Figure 13. FD&C Yellow No. 5 from Orange Kool-Aid

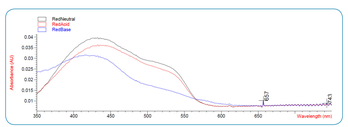


Figure 14. FD&C Red No. 40 from Orange Kool-Aid

Finding viable automation solutions to tedious and manual methods, with the added concern for worker safety in handling strong acids and bases, creates efficiency, promotes a safer work environment, and allows for day-to-day consistency. By automating standard lab practices such as spectrophotometer readings and sample preparation, a typical laboratory is able to increase sample throughput, eliminate personnel to personnel variation and use laboratory personnel for other more important laboratory work such as data analysis. The basic process of how the manual method was automated can also be applied to other manual methods requiring both qualitative, as in AOAC 988.13, and quantitative spectrophotometric sample readings, expanding its application into many different sample categories.

This application shows equivalency between established manual methodology and its automated counterpart across a variety of different sample matrices. Through additional development and optimization the overlapping of color bands could easily be eliminated. Using flow cells with a longer pathlength could also assist with obtaining higher absorbance readings for low concentration samples.

GILSON®

Agilent Technologies