Colorful Confections: Analyzing Artificial Food Dyes in Sports Drinks, Gelatins, and Gummies

Gordon T. Fujimoto, Lindsay E. Hatch, Sarah E. Dowd; Waters Corporation, Milford, MA 01757 USA

INTRODUCTION

Artificial food dyes are widely used in foods and beverages to enhance their appearance and entice consumers. Some dyes have been linked to a variety of health issues including carcinogenicity, allergic reactions and behavioral issues [1]. The potential for health problems has led individual states to restrict the use of artificial dyes in foods. There are upcoming restrictions in California to ban six artificial food dyes in meals, drinks and snacks served in public schools [2] and in West Virgina to ban seven artificial food dyes in all food products statewide [3]. In addition, artificial food dyes are on the list of food additives regulated in Europe [4]. To characterize the expected dyes in food products, techniques like HPLC-UV can be utilized, but is limited to higher concentrations. With the new bans, methods will be needed with new limits to certify that a food product is free of artificial dyes. In this study, an LC/MS MRM method has been developed for the analysis of artificial food dyes and tested on a selection of food products (sports drinks, gelatins, hard and soft candies).

SAMPLE PREPARATION

Food samples were obtained from local grocery stores in the Milford, MA USA area.

Food Type	Portion	Extraction Procedure
Gummies 	One serving (approx. 4-6 grams)	30 mL of 50/50 water w/2 mM ammonium acetate (pH=5) /acetonitrile, heated to 50C periodically for 10 h
Hard Candies 	One serving (approx. 4-6 grams)	30 mL of 70/30 water w/1.4 mM ammonium acetate (pH=5) /acetonitrile, heated to 50C periodically for 2 h
Gelatin 	4 grams	30 mL of 70/30 water w/1.4 mM ammonium acetate (pH=5) /acetonitrile, heated to 50C periodically for 2 h
Cake Icing 	4 grams	30 mL of 70/30 water w/1.4 mM ammonium acetate (pH=5) /acetonitrile, heated to 50C periodically for 2 h

- The extracts were filtered through 0.2 µm, 13 mm PTFE syringe filters.
- Filtered extracts were diluted 1:10 using a solvent mixture of 90/10 water w/1 mM ammonium acetate (pH=5)/methanol. When appropriate, the samples were further diluted when the concentration of the dyes in samples were outside the concentration range of this analysis.

Sports drinks were filtered through a 0.2 µm, 13 mm PTFE syringe filter and diluted 1:10 and analyzed.

METHOD DEVELOPMENT

Infusion experiments were performed to obtain the optimum MRM parameters for each of the dyes studied. Both the positive and negative ion electrospray modes were tested.

---------- Dyes Studied in this project ----------

Dye	CAS No	Dye	CAS No
FD&C Red No. 40	25956-17-6	Patent Blue V	3536-49-0
FD&C Red No. 3	16423-68-0	FD&C Green No. 3	2353-45-9
FD&C Red No. 2	915-67-3	D&C Green No. 5	4403-90-1
Acid Red 18	2611-82-7	FD&C Yellow No. 5	1934-21-0
Carmoisine	3567-69-9	FD&C Yellow No.6	2783-94-0
FD & C Blue No. 1	3844-45-9	D&C Yellow No. 10	8004-92-0
FD & C Blue No. 2	860-22-0		

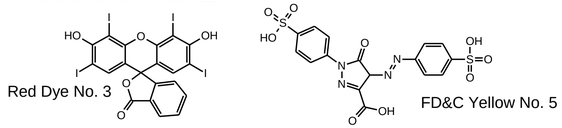


Figure 1. Molecular structures of two of the dyes studied. The majority of the dye compounds contained one or more sulfonate groups and often available commercially in their sodium salt forms.

Example of optimized MRM Parameters [5]

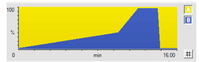
Dye	RT	Mode	Prec	Prod	CV	CE
FD&C Red No. 40	5.75	Neg	225.01	207	43	14
			225.01	79.96	43	40
FD & C Blue No. 2	3.40	Neg	420.98	342	40	24
			209.99	80	45	24
FD & C Blue No. 2	3.40	Pos	423.00	342	80	29
			423.00	341	80	23

With standard reverse phase chromatography, FD&C Yellow No. 5 and FD&C Blue No. 2 are poorly retained. To increase the chromatographic retention of these compounds, a mixed mode C18 column was selected.

METHODS

Chromatographic System: ACQUITY™ UPLC™ I-Class Plus w/FTN SM
Column: Atlantis™ Premier BEH C18 AX Column, 1.7 µm, 2.1 x 50 mm @35 °C
Mobile Phase A: Water w/1 mM NH4OAc (pH=5)
Mobile Phase B: Methanol w/15 mM NH4OH (*)
(*) = 0.1% of 28-30% solution of ammonia in water

Flow rate: 0.45 mL/min
Injection Volume: 10 µL
Total Gradient Time: 16 min



Mass Spectrometer: Xevo™ TQ-S micro
Ionization: ESI+/ESI-
Acquisition: MRM
Software: MassLynx™ 4.2

Xevo TQ-S micro Triple Quadrupole MS with a UPLC system

RESULTS

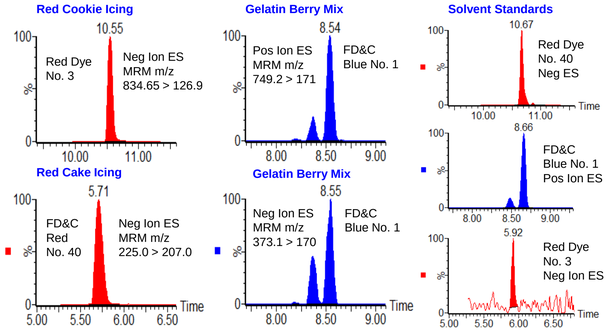


Figure 2. Example MRM chromatograms from two food products analyzed (Extracts Diluted 1:100) and solvent standards (1 ng/mL concentration)

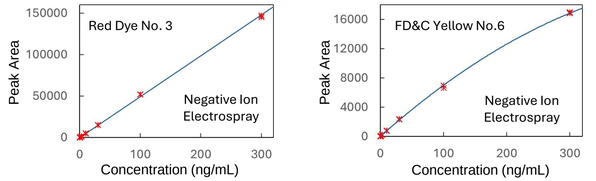


Figure 3. Calibration curves generated for two artificial dyes in solvent for 1-300 ng/mL

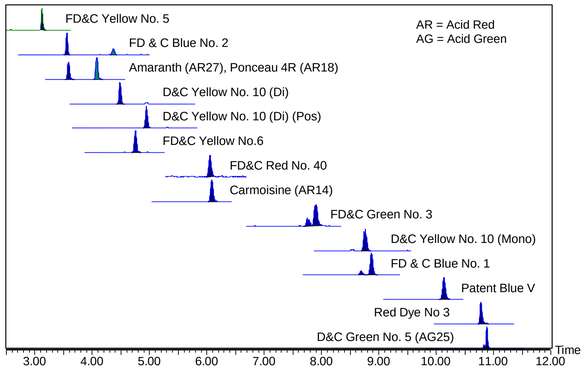


Figure 4. Representative MRM chromatograms of the dye compounds.

RESULTS AND DISCUSSION

LOQ's of 1 ng/mL were achievable in solvent standards for all of the dyes studied. The food products analyzed included gummies of various flavors, hard candies of various flavors, sports drinks, gelatin mixes, cake decorations and icings. Some of the gummies and hard candies were advertised as having no artificial colorings. None of the food products that were advertised as being 'free of artificial dyes' had detectable levels of the dyes analyzed for in this project. The products that had artificial colorings listed in their ingredients contained only those artificial colorings listed and no other artificial colorings were detected. FD&C Red No. 40 was the prominent red dye in the gummies, candies and sports drinks (20-30 mg/serving) that had artificial dyes listed in their labels.

Some food products studied produced extracts that exhibited high levels of ion suppression for some of artificial food dyes (data not shown). To potentially alleviate this, a WAX SPE clean up procedure was tested. 10 mL aliquots of the extracts and a 10 mL aliquot of a sports drink were spiked with artificial dyes and cleaned up using OASIS™ WAX SPE cartridges.

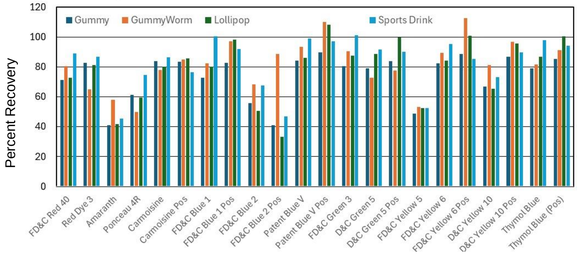


Figure 5. Recovery from spikes of standards into extracts of matrix samples that would represent artificial dyes at the level of 2 ppm in three food products and 0.2 ppm in a sports drink. ('Pos' = Positive Ion ES, all others Negative Ion ES.)

CONCLUSIONS

- A LC/MS MRM method was developed to test for a selection of artificial dyes in food products
- Further refinement of the sample preparation is needed to minimize matrix interferences
- There is no established limit for a food product to be certified as 'free of artificial dyes' but the method presented was able to detect dyes in food products at ~ 2ppm

References

[1] Environ Health Perspect. 2010 Oct;118(10) DIET AND NUTRITION: The Artificial Food Dye Blues; Carol Potera
[2] https://www.ewg.org/news-insights/news-release/2024/09/california-leads-nation-first-ban-six-harmful-food-dyes-school
[3] https://governor.wv.gov/article/governor-patrick-morrisey-signs-food-dye-legislation-law
[4] https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02008R1333-20241216
[5] Full list of MRM parameters available upon request (gordon_fujimoto@waters.com)

CONFLICT OF INTEREST DISCLOSURE: Lindsay Hatch, Sarah Dowd, and Gordon Fujimoto are employees of Waters Corporation and presenting the poster on behalf of the company.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS