FAST SCREENING FOR BANNED FOOD COLORINGS IN BEVERAGES USING ULTRA PERFORMANCE LC®

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INTRODUCTION

Artificial food colorings are often added to beverages and powdered fruit drinks for esthetic appeal, color stability and to eliminate the use of more costly natural products. These colorings, many of which are azo dye coal tar derivatives have been linked to:

- Cancer risks
- Attention deficit disorders
- Hyperactivity in children[1]

There is no international consensus on banned/permitted dyes. This makes it difficult to monitor imports from various parts of the world. Additionally, many common dyes have different trade names.

The United States permit these 3 common colors in food:

- **Yellow 5 (Artrazine, E 102)**
- **Red 40 (Allura Red, E 129)**
- **Blue 1(Brilliant Blue, E 133).** [2]

Some banned colors are:

- **Red 14 (Azorubine, E 122)**
- **Amaranth (E 123)**
- **Quinoline Yellow (E104)**
- **Patent Blue V (E 131)**.

The fast screening method illustrated here includes these dyes mentioned plus five color banned in Japan[3]:

- **Red 10B**
- **Orange II**
- **Orange 3**
- **Amido Black 10B (Naphthol Blue)**
- **Red 4 (Fast Red E)**

Figure 1. ACQUITY UPLC System with Extended Lambda PDA

METHODS

System: Waters ACQUITY ® UPLC

Column: ACQUITY BEH C18, 2.1X 100mm @ 64° C
Eluent: Water / Methanol Gradient (0.5% formic acid in each)
Flow Rate: 500 µl / min
Injection: 5 µl
Detection: ACQUITY Extended Lambda PDA, 300-700 nm
Software: Empower™ 2154

Standard Preparation:
Approximately 0.01 gram of each dye was dissolved separately in 20 ml aliquot of a citrate buffer prepared by adding Sodium Citrate to a 1M Citric acid solution to a pH of 3.1. Each standard mixture was further diluted by a factor of 1:100 (~ 5 ppm)and Injected into the UPLC. The analytes were grouped according to their maximum absorbance, red dyes at 500 nm, yellow at 440 nm and blue at 630 nm. Spectra extracted at peak maxima were incorporated into a PDA Library.

Sample Preparation:
Three commercial beverages, a soft drink containing Red 40, a health drink colored with Blue 1 and a powdered diet beverage colored with Yellow 5 were used. The powdered drink was reconstituted in HPLC grade water according to instructions; the soft drink was sonnicated to remove carbonation. Each sample was filtered through a 0.45 micron PVDF filter and injected into the UPLC along with a similar sample spiked with banned dyes of similar color.

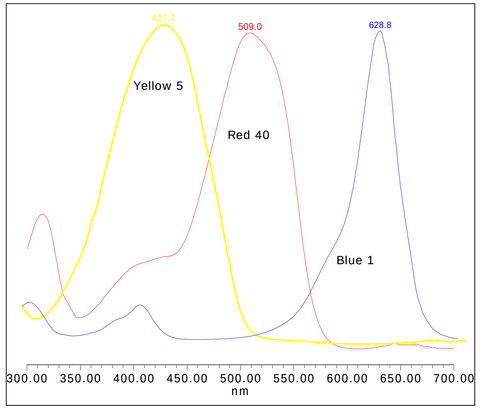

Figure 2. UV spectra of red, yellow and blue food dyes Permitted in the US

RESULTS

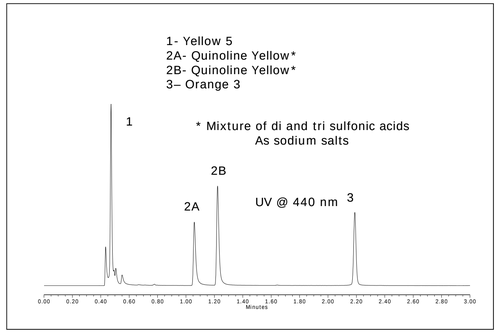

Figure 3. Separation of yellow food colorings

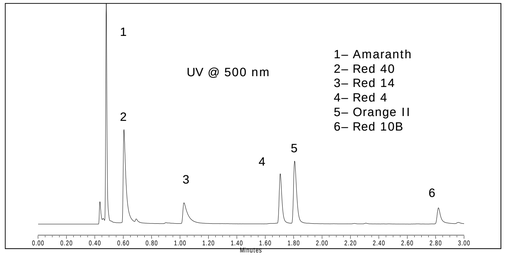

Figure 4. Separation of red food colorings

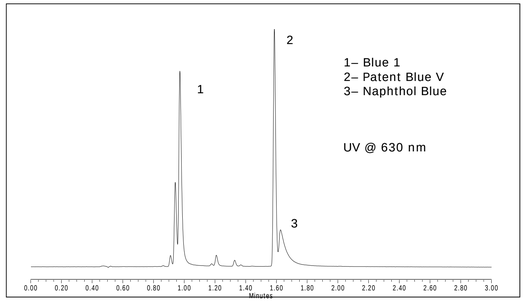

Figure 5. Separation of blue food colorings

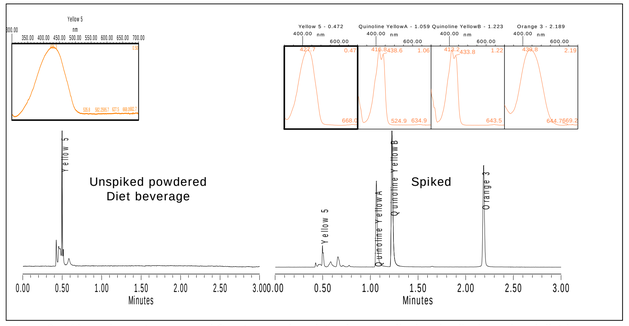

Figure 6. 440nm chromatograms and Spectrum Index plots of an unspiked and spiked powdered diet beverage

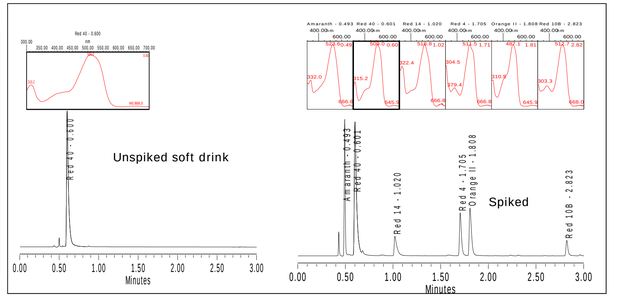

Figure 7. 500nm chromatograms and Spectrum Index plots of an unspiked and spiked soft drink

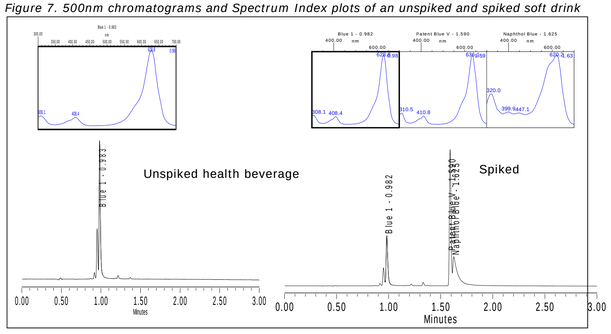

Figure 8. 630nmChromatograms and Spectrum Index plots of an unspiked and spiked health beverage

DISCUSSION

- Combinations of dyes or subtle chemical differences in dye structures can produce the same visually perceived color.
- Both chromatographic retention time and UV spectral information are important for screening if a color in a beverage product originates from a banned or permitted dye,
- ACQUITY UPLC with the extended wavelength UPLC photodiode array detection (PDA) enables separation and screening of a group of common banned and permitted food colors in 3 minutes or less.
- Screening to recognize the banned analytes in samples is facilitated by creating and using a UV spectral library in Empower software.
- The UV spectral library is expandable. Empower UV library search parameters are modifiable to help answer questions about spectrally co-eluting species or different colored analytes in the same sample.
- Empower software methods can be set to automate screening (not shown here)

CONCLUSION

- **UPLC enables food color separation: 3 minutes or less**
- **Qualitative color screening with Spectrum Index plot**
- **Minimal sample prep: dilution, sonnication, filtration**
- **Water – methanol is a preferred eluent; especially in view of the current acetonitrile shortage**

References

1. Food additives and hyperactive behavior in 3-year-old and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial", Lancet, Sept 2007
2. USFDA 21 CFR Section 74, Subpart A
3. Yoshioka N. Ichihashi K. " Determination of 40 synthetic food colors in drinks and candies by high performance liquid chromatography using a short column with photodiode array detection" Talanta 74 (2008) pp. 1409

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