Introduction

The United States Food & Drug Administration (FDA) regulates the approval of color additives for their safe use in foods, cosmetics, pharmaceuticals, and medical devices. The Federal Food, Drug, and Cosmetic Act (FDCA) and its regulations provide for which color additives are required to be approved by the agency, while the Code of Federal Regulations (CFR), the FDA’s rulebook, contains additional guidance for color additives. The regulations in 21 CFR parts 73 and 810 identify the new color certified FD&C colors approved for food use within the USA. Among the colors listed and routinely tested are FD&C Blue No. 1 and FD&C Yellow No. 5. Thorough testing for safety is required from every batch before any FD&C color can be used within the USA. Testing does not eliminate the risk of allergic reactions from consuming foods or beverages using FD&C certified colors.

In 2013, the CFR noted that FD&C Yellow No. 5, also known as tartrazine, used in foods and beverages must have a label to state that it may cause allergic reactions. Research has shown that FD&C Yellow No. 5 is absorbed in the GI tract. Reported health effects from food consumption with Blue No. 1 are allergies. The addition of FD&C colors to beverages creates a more visually appealing sport drink or juice that can impact specific flavors to match the color. Many sport and juice beverages use the FD&C Blue No. 1 and FD&C Yellow No. 5 in different concentrations to create various hues of green associated with the color green and an indication of a healthy beverage. The addition of FD&C Blue No. 1 and FD&C Yellow No. 5 in sport drinks and juices with simple solid phase extraction (SPE) automation using the SmartPrep® Extractor, with the purpose of confirmation of FD&C color presence through qualitative analysis using a portable spectrometer.

Materials

- J Jay Baker: BAKERSMITH® SPE C18, 6 mL, 100 mg
- Laboratory purified MILLI-Q® water
- Milli-Q® Millennium® 10,000 MW weight cut-off concentrate
- Watermelon-flavored juice drink
- Inspiration FRUIT-RAIN.LAKE beverages

Phenomenex® Analytical Collection Fractions of each sample were analyzed for qualitative comparison of the wavelength with the maximum absorbance. The fractions were collected in 1 mL collection tubes. The collection tubes were numbered from 1 to 4 for each batch.

SmartPrep® Extractor Process

- **LOAD - CLEANUP**
  - 380 nm – 950 nm. Collected fraction spectra were overlaid with the diluted corresponding FD&C spectra for comparison.
  - All samples and diluted FD&C were read across a range of wavelengths to generate a spectrum from 350 nm – 950 nm.

**Methanol Elute Step (FD&C Blue No. 1)**

- 2 mL vent @ 10 mL/min into 2nd collection tube
- Elute @ 2 mL/min
- Vent 1 mL
- 3 mL Methanol @ 10 mL/min into 3rd collection tube
- 4 mL at 5 mL/min and collect in 1st collection tube
- 3.5 mL Reagent Water @ 30 mL/min into 2nd collection tube
- 3 mL Methanol @ 10 mL/min into 3rd collection tube
- 1.5 mL Reagent Water @ 30 mL/min into 3rd collection tube
- Soak 2 sec.
- 3 mL Methanol @ 10 mL/min into 3rd collection tube
- 1.5 mL Methanol into 2nd collection tube
- Add 4.5 mL Reagent Water
- Mix @ 40 mL/min for 2 min
- Soak 10 sec.
- 3 mL Methanol @ 10 mL/min
- Soak: 2 sec.
- Add 4.5 mL Reagent Water
- Mix @ 40 mL/min for 2 min
- Soak: 10 sec.
- 3 mL Methanol @ 10 mL/min
- Soak: 2 sec.
- Add 4.5 mL Reagent Water
- Mix @ 40 mL/min for 2 min
- Soak: 10 sec.
- 3 mL Methanol @ 10 mL/min
- Soak: 2 sec.
- Add 4.5 mL Reagent Water
- Mix @ 40 mL/min for 2 min
- Soak: 10 sec.
- 3 mL Methanol @ 10 mL/min
- Soak: 2 sec.

**Eluate**

- The first and second fractions collected from each beverage sample were scanned and overlaid with the diluted FD&C Yellow No. 5 spectra. Frost fraction 1 and Watermelon fraction 1 had no absorbance at 589 nm corresponding to FD&C Blue No. 1. Frost fraction 3 and Watermelon fraction 2 had no absorbance at 426 nm corresponding to FD&C Blue No. 5. This indicates that these samples were the load step for both Frost and Watermelon samples. The third fractions collected from each beverage sample were scanned and overlaid with the diluted FD&C Blue No. 1 spectra. Frost fraction 1 and Watermelon fraction 2 had maximum absorbance at 589 nm corresponding to FD&C Blue No. 1. Frost fraction 3 had significantly less blue color in the beverage sample when compared to the Watermelon fraction 3 that had nearly four times more blue color. Lack of absorbance at 426 nm indicates that Frost fraction 1 and Watermelon fraction 3 are pure fractions and contain no FD&C Yellow No. 5. Overlaid spectra of the FD&C Yellow No. 5 with the second fractions collected from the Frost and Watermelon beverages show maximum absorbance at 426 nm and strong qualitative compatibility with FD&C Yellow No. 5.

Conclusion

- Automated solid phase extraction using the SmartPrep Extractor is an efficient means for isolation and purification of FD&C colors for rapid quality control analysis.
- Qualitative verification was accurately and simply performed following purification using a portable spectrometer to confirm presence of FD&C Yellow No. 5 and FD&C Blue No. 1 in Coca-cola Frost and Welch’s Watermelon beverages samples.
- The process of using SPE allowed for individual fractions to be collected for each sample, simplifying method development.
- Additional FD&C colors could be further identified using the same SPE purification principle, allowing efficient qualitative and routine analysis of banned or hazardous dyes present in foods, beverages, and spices.

References

5. Livestrong.com, Health