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Separation and Quantification of 13 Synthetic Dyes in Foods Using the ACQUITY UPLC H-Class System

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief successfully develop a rapid analytical method for the simultaneous detection and quantification of 13 dyes in food products using reversed-phase chromatography with UV detection.

Benefits

The separation of all 13 dyes was achieved in less than eight minutes.

Introduction

According to regulations, a color additive is defined as "any dye, pigment, or other substance that can impart color to a food, drug, or cosmetic or to the human body." Color additives are commonly used in food products to improve visual impact, hide defects, and to enhance consumer perception of the association between color and flavor. Since appearance plays a significant role in the selection of food products, some manufacturers may make food more appealing through the use of natural or synthetic additives. Color additives of natural origin are considered safe for use and are exempt from certification by regulatory bodies. Synthetic color additives, however, can be potentially toxic for human consumption and are controlled in food products by global regulations and require certification. According to the US FDA, those synthetic dyes that are permissible in food include: brilliant blue, indigo carmine, sunset yellow, tartrazine, allura red, fast green, and erythrosine. In the United States, the FDA ensures safe and appropriate use of these dyes by certifying and monitoring their use, including correct product labeling. The legislation for synthetic dyes varies from country to country.

For example, P4R and brilliant black are banned in the United States, Belgium, Denmark, France, Germany, Sweden, Austria, Switzerland, Japan, and Finland. Though banned in some of the European Union countries, they are approved by the European Union.

Food dyes are analyzed using a variety of techniques such as ion-pairing high performance liquid chromatography (HPLC) with di or tetraalkylammonium salts, colorimetric analysis, electrophoresis, thin layer chromatography, and spectrophotometric methods. Although these techniques are useful, they are complex, tedious, time consuming, and not applicable for the simultaneous analysis of all the dyes. The ability to simultaneously analyze permitted and non-permitted food dyes using a single, fast and straightforward

UPLC method has the potential to increase sample turnover and reduce the cost of analysis. This improvement can result in higher productivity for food testing laboratories. A simple and rapid method for investigating synthetic dyes was developed in order to monitor appropriate usage and ensure consumer safety.

Results and Discussion

The separation of 13 synthetic dyes (permitted and non-permitted) was achieved using Waters ACQUITY UPLC H-Class System, shown in Figure 1, in less than eight minutes. Separation was performed on an ACQUITY UPLC BEH C_{18} 2.1 x 100 mm, 1.7 μm Column using 30 mM ammonium acetate, methanol, and acetonitrile gradient, as shown in Figure 2. The ACQUITY UPLC H-Class, a quaternary UPLC system, provides the flexibility of using up to four different solvent lines, which eliminates the need to premix the solvents. PDA detection was programmed to acquire the data at 400 nm for yellow dyes, 500 nm for orange and red dyes, and 630 nm for green and blue dyes. Individual standards of each of the dyes were also used to create a library of UV spectra. The spectra of the dyes detected in foodstuffs can be matched against the library to increase confidence in the identification of those dyes.

Figure 1. ACQUITY UPLC H-Class System with PDA Detector.

Figure 2 shows the separation of all dyes using the ACQUITY UPLC H-Class System with PDA Detector. The standard curves for all compounds were prepared over the concentration range of 2 to 40 µg/mL. Excellent linearities ($r^2 > 0.999$) were achieved for all compounds. Example calibration curves for two of the compounds (P4R and brilliant black) are shown in Figure 3.
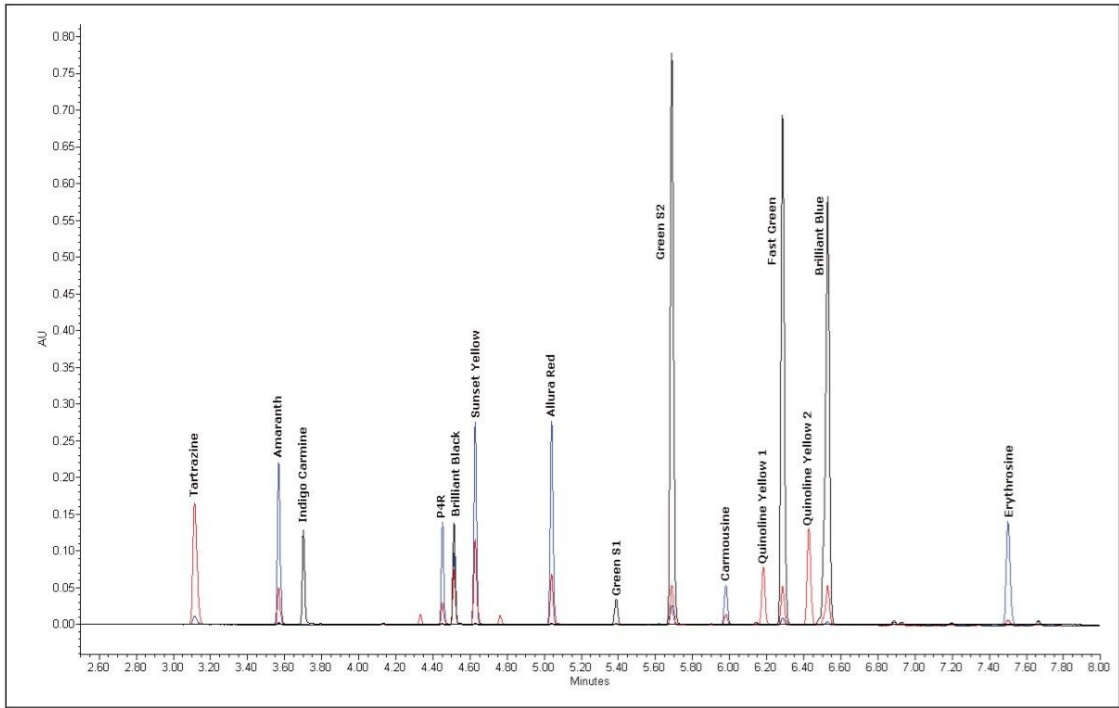
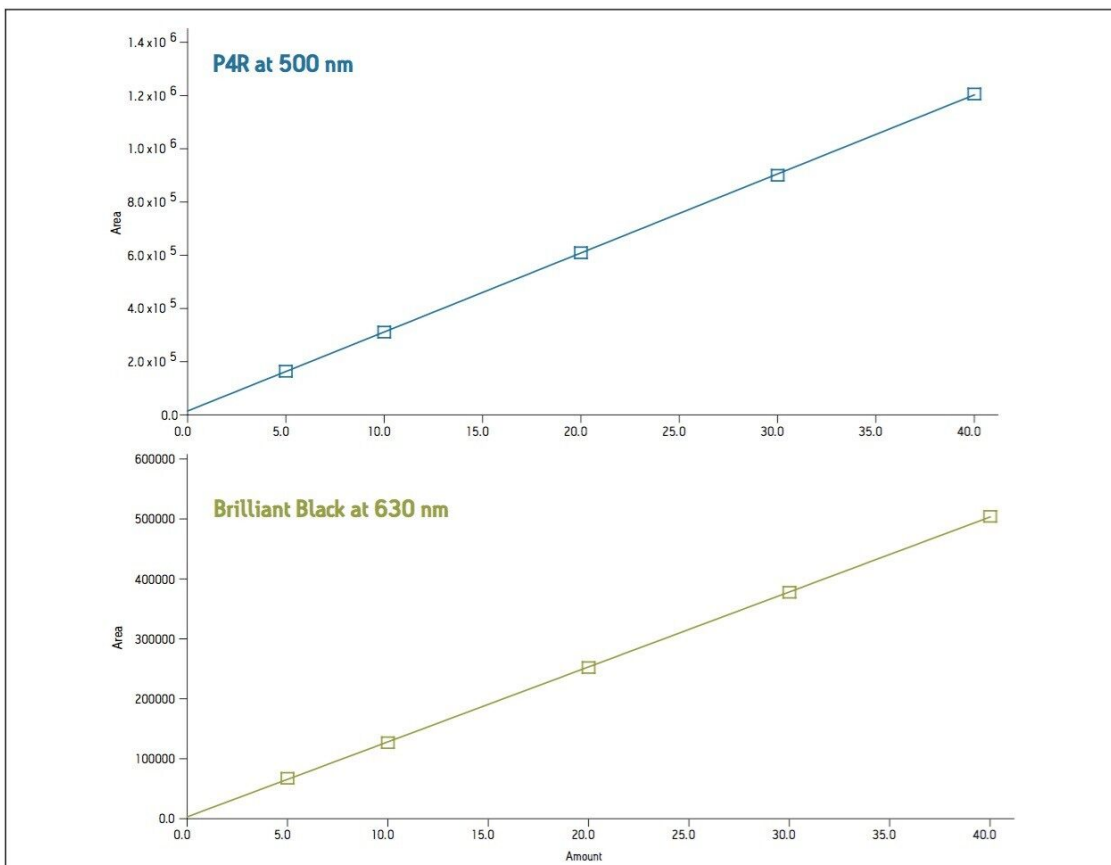*Figure 2. Overlay of the chromatograms extracted at 400 nm (Blue), 500 nm (Red) and 630 nm (Black).*

Figure 3. Calibration curve for P4R and Brilliant Black.

Conclusion

A method for the separation and analysis of color dyes in food products using reversed-phase chromatography on an ACQUITY UPLC H-Class System with PDA detectionr has been successfully developed. This method enables detection and quantification of multiple dyes in food products in a single injection. With the capability of multi-solvent blending and a flow through needle design, the ACQUITY UPLC H-Class System offers the flexibility of HPLC with UPLC quality separation.

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ACQUITY UPLC H-Class PLUS System <https://www.waters.com/10138533>

ACQUITY UPLC PDA Detector <https://www.waters.com/514225>

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