

# **Determination of Food Dye Concentrations in an Unknown Aqueous Sample Using HPLC**

## **Abstract:**

High performance liquid chromatography (HPLC) was used to determine the identity and concentrations of food dyes in an unknown sample. Individual dye concentrations in the unknown sample were determined to be 5.17 ppm Yellow No. 5 and 12.51 ppm Red No. 40.

## **Introduction:**

Recent research has been conducted concerning the degradation of synthetic dyes due to their toxic effects on the environment.<sup>1</sup> In addition it has been demonstrated that such dyes can induce serious side effects ranging from eye, skin, mucous membrane, and upper respiratory irritation, to severe headaches, nausea, and anemia.<sup>2</sup> Due to the nature of these side effects, it is desirable that a method be obtained to measure each dye's concentration. A method that has been proposed for such analysis is high performance liquid chromatography (HPLC). To test the viability of this proposed method, HPLC was used to determine the concentration of food dyes Red No. 40, Red No. 3, Blue No. 1, and Yellow No. 5 in an unknown sample.

## **Experimental Section:**

### ***Materials:***

A Hewlett Packard Series 1090 Liquid Chromatograph was used to measure the absorbance of four dyes Blue No. 1, Red No. 3, Red No. 40, and Yellow No. 5 that were obtained from Pylam products in the solid state. An Ultra C18 5 $\mu$ m column (serial number 05010164T) obtained from Restek was used for all chromatographic analysis. The polar solvent used consisted of water (resistivity 18.6  $\mu$ m) obtained from a Millipore

MilliQ A-10 system and HPLC grade methanol purchased from Fisher Scientific. All prepared solutions were made using water (resistivity 18.6  $\mu\text{m}$ ) obtained from a Millipore MilliQ A-10 system. The .20  $\mu\text{m}$  filters used were obtained from Fisher Scientific.

***Methods:***

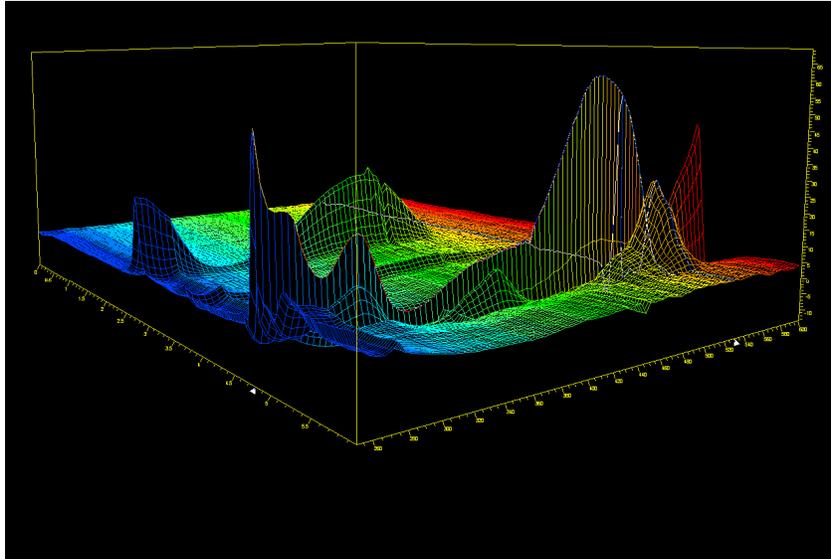
All solutions were syringe-filtered with .20  $\mu\text{m}$  filters prior to injection. 15  $\mu\text{L}$  of each solution was injected into the HPLC at an initial flow rate of .5 mL/min. The polar solvent gradient used varied the relative concentration of water to methanol from initial percentages of 90/10 to a final value (reached at 2.5 min.) of 10/90 to obtain adequate peak separation. In addition, the flow rate was increased to its final value of 1 mL/min after 2.5 min. to decrease total runtime to 6 min.. Specific wavelengths scanned by the HPLC diode array detector (DAD) corresponded to peak absorbance wavelength values of 511 nm, 531 nm, 427 nm, and 598 nm for Red No. 40, Red No. 3, Yellow No. 5, and Blue No. 1, respectively..

Chromatograms were analyzed using both two and three-dimensional plots to enhance accuracy of peak identification. Peaks obtained from the unknown sample were identified based upon peak absorbance wavelength and elution time. The concentrations of food dyes present in solution were determined using external standard calibration plots, in which integrated peak area was plotted as a function of dye concentration.

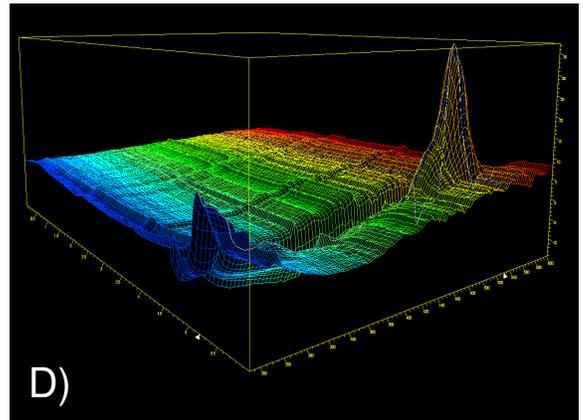
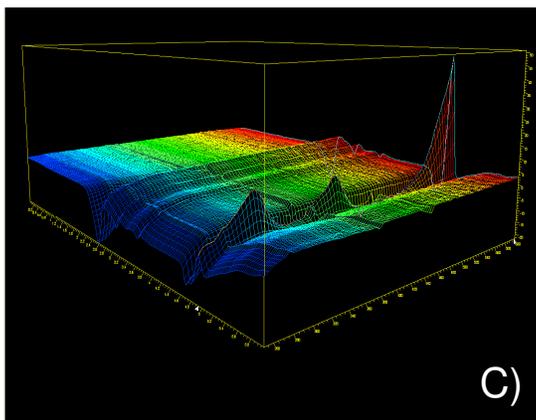
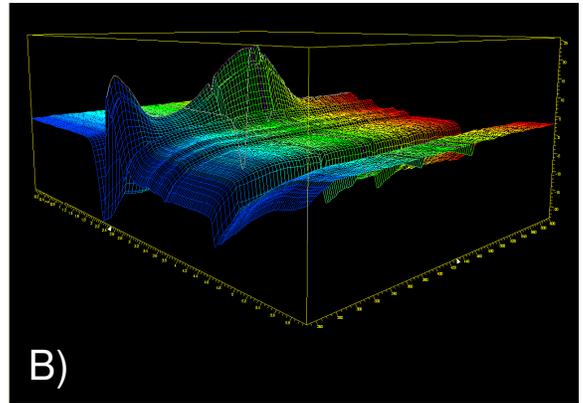
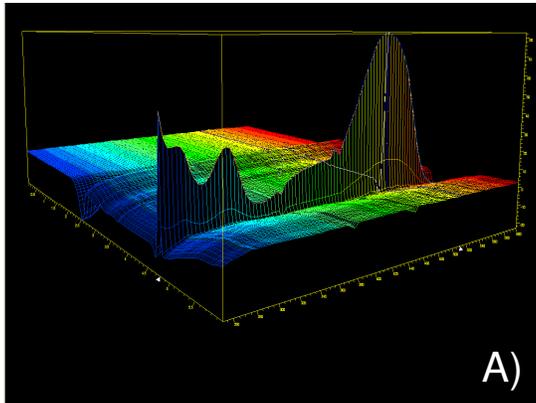
**Results and Discussions:**

As can be seen in Figure 1, the four food dyes that may be present in the unknown sample not only elude at different times but also have different peak absorbance wavelengths. In order to identify the food dye peaks in Figure 1, each dye was individually injected and the chromatograms pictured in Figure 2 were obtained. The elution times for each food dye (corresponding to peak absorbance wavelength) were 2.48 min., 4.79 min., 4.94 min., and 5.17 min. for Yellow No. 5, Red No. 40, Blue No. 1,

and Red No. 3, respectively. Individual peak absorbance wavelengths were for Yellow No. 5, Red No. 40, Blue No. 1, and Red No. 3 were 427 nm, 511 nm, 598 nm, and 531 nm, respectively.

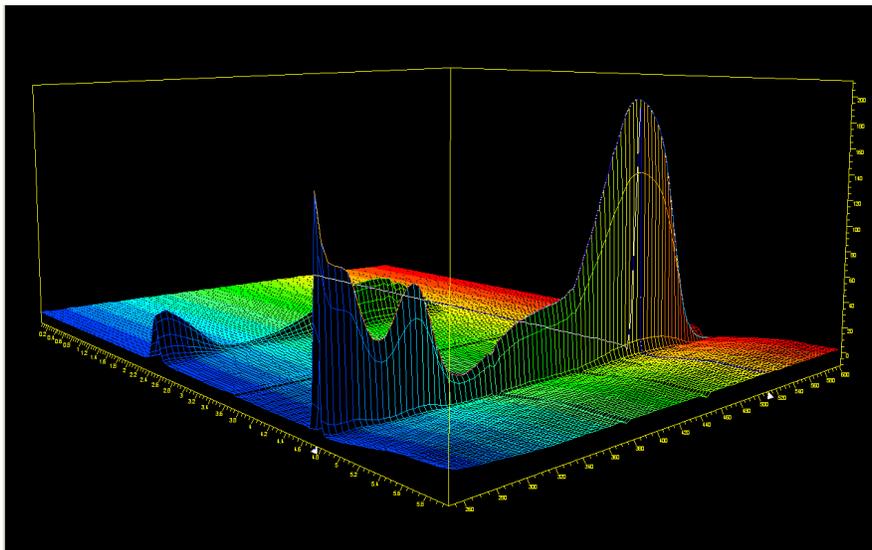


**Figure 1:** *Three-dimensional chromatogram of solution containing each food dye (Red. No. 40, Red No. 3, Blue No. 1, and Yellow No. 5) at a concentration of 5 ppm.*



**Figure 2:** *Three-dimensional chromatograms of Red No. 40 (A), Yellow No. 5 (B), Blue No. 1 (C), and Red No. 3 (D).*

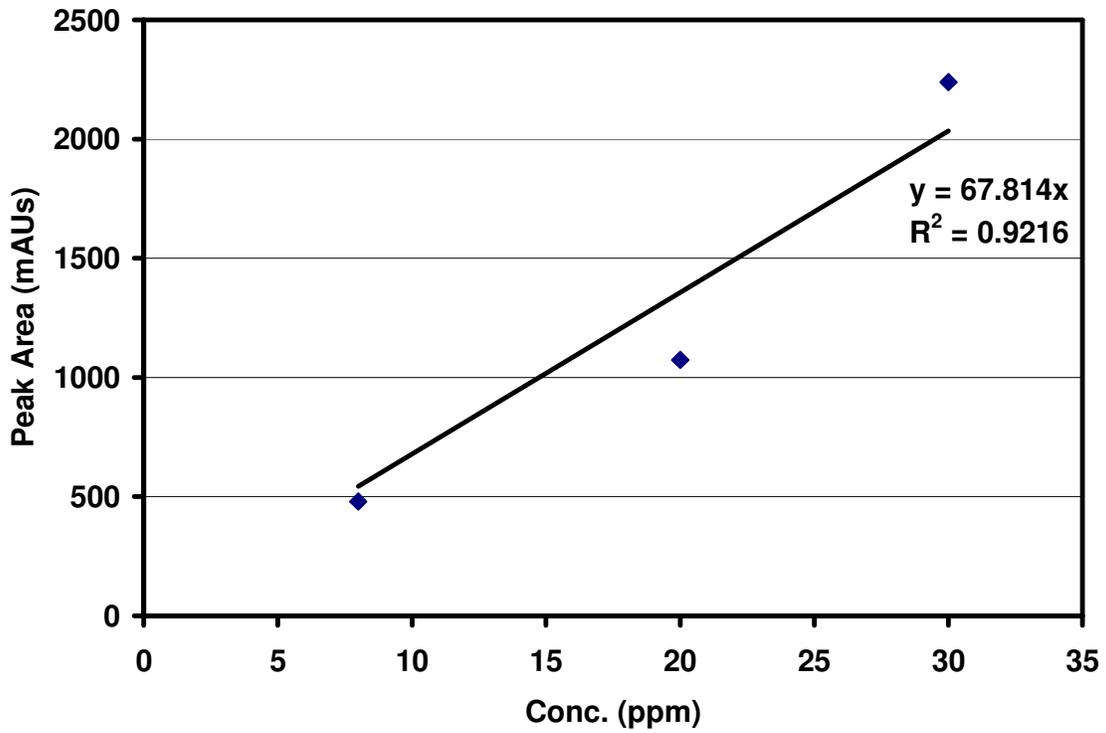
As is shown in Figure 3, the unknown sample consisted of two prominent peaks eluting at 2.51 min. and 4.60 min., respectively.



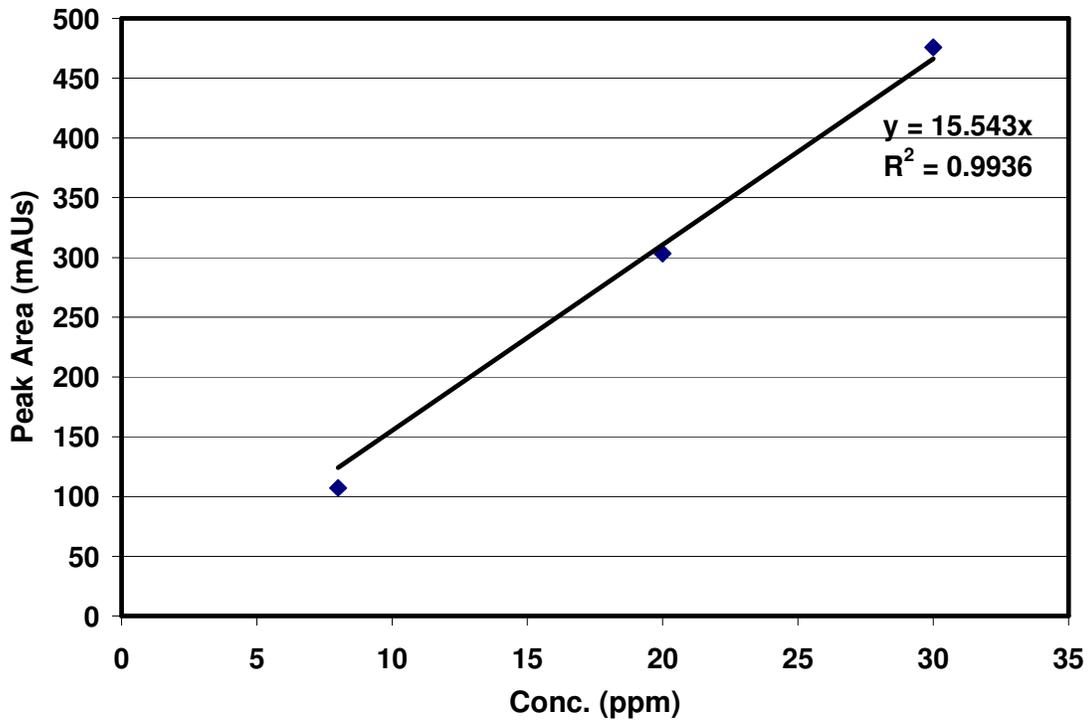
**Figure 3:** *Three-dimensional chromatogram of unknown sample.*

In addition, it is clear that the first peak has a peak absorbance wavelength of 425 nm while the second peak has a peak absorbance wavelength of 510 nm. By comparing Figure 3 with Figure 2, one can conclude that the unknown contains concentrations of Yellow No. 5 and Red No. 40.

As is depicted in Figures 4 and 5, external standard calibration plots for Yellow No. 5 and Red No. 40, respectively, were obtained to determine the concentration of each component in the unknown sample.



**Figure 4:** Calibration curve for Yellow No. 5.



**Figure 5:** Calibration curve for Red No. 40.

Average concentrations of each food dye in the unknown were calculated so as to remove random error possibly introduced by inconsistent injector volumes, flow rates, and column pressure. These average concentration values obtained for Yellow No. 5 and Red No. 40 were 5.17 ppm and 12.51 ppm, respectively.

**Conclusions:**

As has been clearly demonstrated, HPLC can be used to quantitatively determine concentrations of potentially harmful dyes when such species are intermixed. It is difficult, however, to obtain accurate results using this experimental method due to the introduction of random error. While addition of an internal standard could alleviate this problem, experimentation would be needed to determine the identity and desired concentration of such a standard. Therefore, while improvements can be made to make HPLC more applicable to dye analysis, the identity and concentration of food dyes present in an unknown sample were successfully determined to be 12.51 ppm Red No. 40 and 5.17 ppm Yellow No. 5.

### Literature Cited

1. Blackburn, Richard S., "Natural Polysaccharides and Their Interactions with Dye Molecules: Applications in Effluent Treatment." *Environ. Sci. Technol.* **2004**, 38, 4905-4909.
2. Jain, Rajeev., Meenakshi, Bhargava., and Sharma, Nidhi. "Electrochemical Studies on a Pharmaceutical Azo Dye: Tartrazine." *Ind. Eng. Chem. Res.* **2003**, 42, 243-247.

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High Performance Liquid Chromatography**

Name