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Note

High-performance liquid chromatographic detection and quantitation of synthetic acid fast dyes with a diode array detector

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A previously published high-performance liquid chromatographic (HPLC) method for the analysis of synthetic dyes[1] has been re-examined. In this study, we have used tetrabutylammonium phosphate as an ion-pairing agent to produce a more acceptable separation, and a diode array detector to enhance detection sensitivity. For the nine dyes examined, detection limits ranging from 0.08 to 0.29 ppm have been realized representing a better than ten-times increase in sensitivity. Further, the method as presented more effectively resolves the dyes FDC Green 3 and FDC Blue 1 into their major constituents.

EXPERIMENTAL

Apparatus

The liquid chromatograph used was an Altex Model 322 with two, Model 110A pumps and a C_{18} column, 5 μm, 250 × 4.6 mm (Altex), fitted with a Rheodyne 20-μl syringe loading sample loop injector. Detection was by means of a Hewlett Packard 1040A diode array detector (6 mm path length, 4.5 μl volume), coupled with an HP85B computer, an HP2225 printer (for data output) and an HP7470 plotter (Hewlett Packard).

Reagents

Mobile phase. (A) Tetrabutylammonium dihydrogen phosphate (Aldrich) (1.70 g) was dissolved in 1 l HPLC-grade water (Caledon Labs., Georgetown, Canada), containing ACS-grade disodium hydrogen phosphate (1.42 g) and potassium dihydrogen phosphate (1.36 g). (B) A volume of 200 ml of mobile phase (A) was diluted to 1 l with HPLC-grade methanol (Caledon Labs.).

Standards. Acid fast dyes (see text) of known dye strength from McCormick-Stange Flavor Division (Chicago, IL, U.S.A.).

Sample preparation. Solutions of standards were prepared by dissolving 100 mg in 1 l water and further diluting 10 ml to 100 ml. Samples are prepared by methods appropriate to the matrix containing them (*e.g.* see ref. 1).

Chromatographic conditions

A solvent gradient of 45% B to 100% B in 35 min at a flow-rate of 1 ml/min was used.

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TABLE I

FDC DYES, WAVELENGTHS USED FOR QUANTITATION AND LIMITS OF DETECTION

Dye	*Wavelength (nm)*	*μg detectable per 20 μl injected*
FDC Blue 2	290	0.0035
FDC Yellow 5	428	0.0039
Amaranth	500	0.0057
FDC Yellow 6	500	0.0045
FDC Red 40	500	0.0038
FDC Red 4	500	0.0043
FDC Red 3	528	0.0016
FDC Green 3	595	0.0040
FDC Blue 1	595	0.0052

RESULTS AND DISCUSSION

The nine dyes were initially chromatographed individually using detection at 280 nm (which we had earlier used on a fixed-wavelength detector) and on-the-fly spectra were recorded. From the wavelength maxima found, five wavelengths were selected that were close to optimal for the nine dyes. This is given in Table I, together with the number of micrograms detectable (with a pre-set area reject of 10 milli-absorbance units on the detector).

The separation efficiency of the system is shown in Fig. 1. We found 280 nm to be the best single wavelength to use for mixtures of the dyes. Fig. 2 shows the effect on sensitivity with variation in detection wavelength. In Fig. 3, part of the chromatogram recorded at 595 nm is shown in expanded form, showing a reasonable

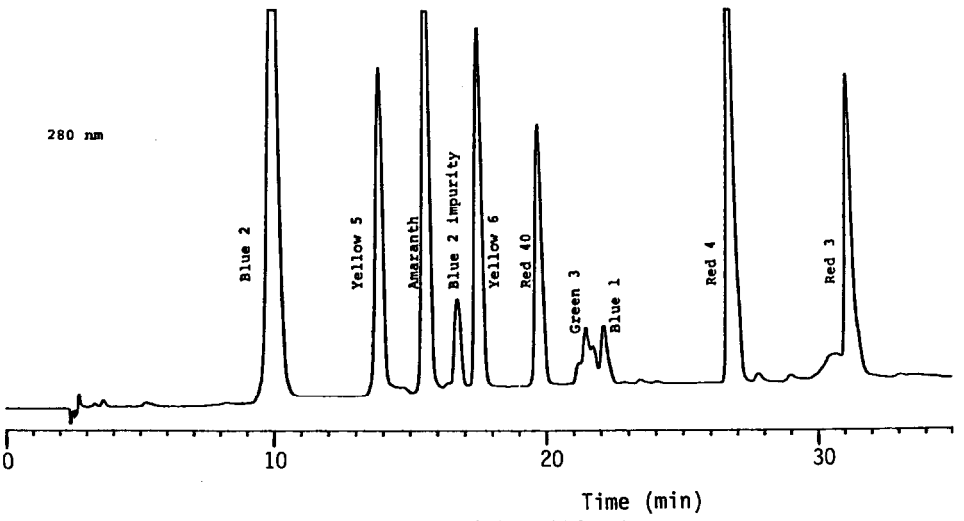

Fig. 1. Chromatogram illustrating the separation of nine acid fast dyes.

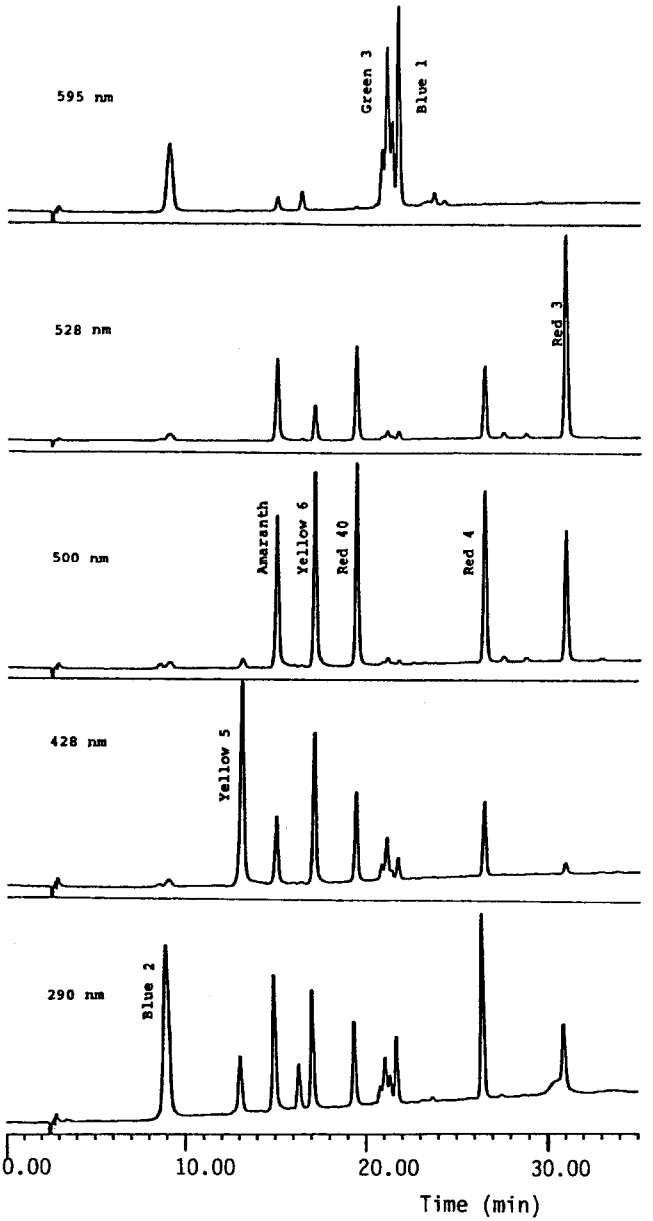

Fig. 2. Chromatograms showing effect of changes in detection wavelength on sensitivity.

separation between FDC Green 3 and FDC Blue 1, as well as between the minor components of each dye. On-the-fly spectral scan of the FDC Blue 1 peaks, and of the FDC Green 3 peaks, shows that for each dye, the UV–VIS spectra for the components are almost identical and that there is no wavelength that could be used to determine one of the components without interference from the other.

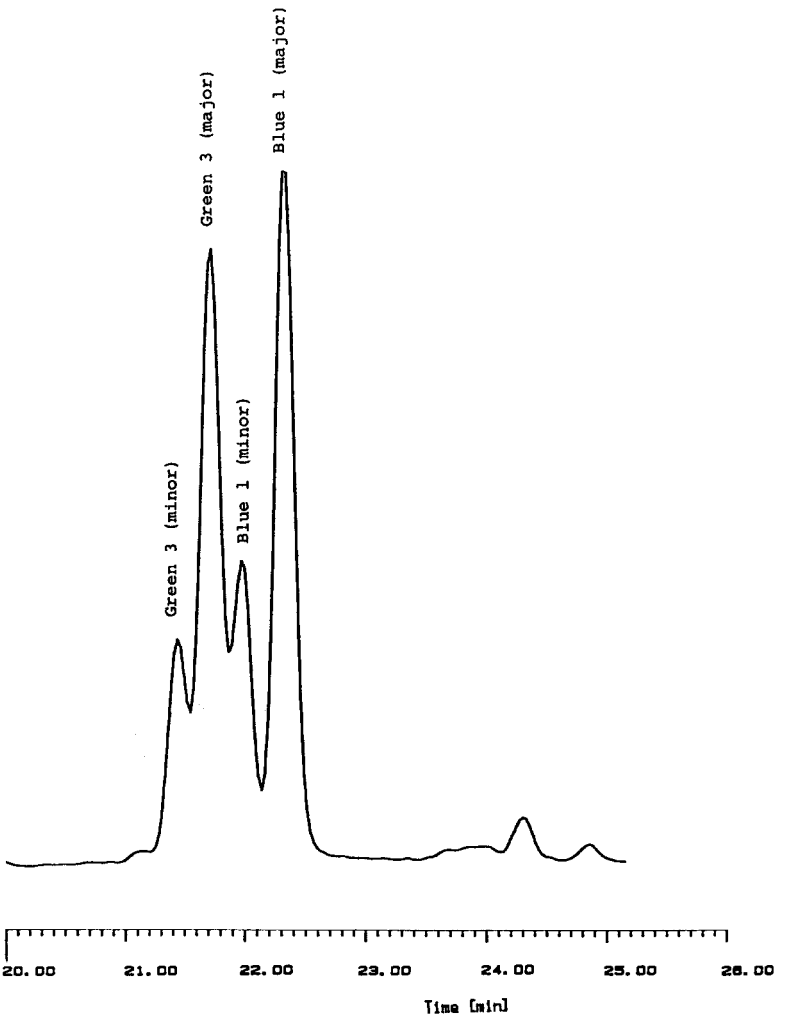

Fig. 3. Part chromatogram (595 nm) showing components of FDC Green 3 and FDC Blue 1.

REFERENCE

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