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Note

Separation and detection of synthetic food colors by ion-pair high-performance liquid chromatography

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In recent years concern has arisen over the extensive use of synthetic coloring matter in foods. In order to prevent indiscriminate use, regulations have been developed by many countries limiting the types, purity, uses and quantities of food colors permitted in foods. As a result the need has arisen to continuously monitor foods for permitted and non-permitted food colors particularly in imported foods. In the past, methodology for both qualitative and quantitative determinations have incorporated techniques such as paper and thin-layer chromatography[1–5], titration[6,7], electrophoresis[8] and spectrophotometry[9,10].

In recent years, high-performance liquid chromatography (HPLC) has been shown to have much potential for synthetic food color analysis in terms of qualitative identification, quantitation and speed of analysis[11–17]. Although most of these used ion-exchange with gradient elution or were employed to determine impurities in perhaps a single color, they clearly illustrated the power of HPLC in the area of food colors. Reversed-phase ion-pair chromatography has also been found to be particularly useful for the separation and detection of a large number of food colors[18,19]. This technique is particularly convenient because it makes use of a reversed-phase column which, when not employed for ion-pair chromatography, can be used for usual reversed-phase analyses. We report here the use of an ion-pairing chromatographic technique for the separation and quantitation of twelve primary food colors or subsidiaries with application to the analysis of several food colors in grape beverages.

EXPERIMENTAL

Reagents

Stock solutions of the colors (except Erythrosine) were prepared at concentrations of 0.01–0.02% in water containing 0.005 *M* tetra-*n*-butylammonium phosphate (TBAP).

Erythrosine was dissolved in methanol–water (1:1) containing 0.005 *M* TBAP in order to prevent precipitation of the ion pair.

Preparation of TBAP was done by adding 52 ml of 1.11 *M* KH_2PO_4 to 25 ml of 1.54 *M* tetra-*n*-butylammonium hydroxide solution which produces a solution 0.5 *M* TBAP, pH 7.2. The solution was prepared in a brown bottle and stored in the dark

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when not in use. This was used for adjustment of all standard and sample solutions, and the mobile phases.

The colors used in the study are given in Table I. All organic solvents were distilled-in-glass grade materials. Water was distilled and deionized.

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, a Rheodyne 7125 syringe loading injection port with 20-μl loop, and a Waters Assoc. Model 450 variable-wavelength detector were employed. Separations were carried out on a Merck (Darmstadt, G.F.R.) Hibar II LiChrosorb RP-18, 10 μm (4.6 mm $\times$ 25 cm) column at ambient temperature. The mobile phases (degassed) usually consisted of methanol–water at ratios (v/v) of either (45:55) or (60:40) each containing 0.005 *M* TBAP. All mobile phases and samples were filtered through a 0.45-μm Millipore filter before use with the HPLC apparatus. At the end of each day the chromatographic system was rinsed thoroughly with 100 ml water followed by about 25 ml of methanol. This rinsing was important to protect the chromatographic system.

The Sep-Pak™ (Waters) cartridges (C_{18}) were prepared for use according to manufacturer's directions. Preparation of the beverage samples was as follows. A 100-ml volume of grape soda was made to 0.005 *M* TBAP, then 20 ml of this was passed through a Sep-Pak cartridge. The color remained on the column. The cartridge was washed with *ca.* 20 ml water then the colors eluted with *ca.* 4 ml methanol–water (1:1). The eluent was collected and evaporated to 0.5 ml, filtered through a 0.45-μm Millipore filter before HPLC analysis. The same procedure was used for a grape drink sample except only 2.0 ml of the drink was loaded onto the Sep-Pak cartridge. The synthetic colors remained on the column while most natural color was unretained as evidenced by the colored eluate which was discarded. Also, Benzyl Violet 4B required an additional 2 ml of 100% methanol for elution from the cartridge.

When carrying out the analysis of a series of food colors with the (45:55) mobile phase system, the detector wavelength and flow-rate were changed as indicated in Fig. 1 in order to best determine the compounds. The compromise wavelengths were 610 nm for the blue and green colors, and 480 nm for the reds, oranges and yellows.

RESULTS AND DISCUSSION

Table I summarizes the retention times for the various food colors examined. While six different combinations of mobile phases were evaluated, the two most useful ones were methanol–water (45:55) or (60:40), the first being the choice for most colors except Ponceau SX, Fast Red E and Benzyl Violet 4B which were analysed with latter mobile phase, and erythrosine which was analysed with methanol–water (70:30). Skyark, a subsidiary of Sunset Yellow FCF could not be completely resolved from Indigotine. However, since these two absorb at different wavelengths (Skyark, 480 nm; Indigotine, 610 nm), they could be identified by appropriate wavelength selection. Initially it was attempted to separate as many of the food colors as possible in a single run without recourse to gradient elution. Fig. 1 shows a chromatographic analysis of eight food colors using the (45:55) mobile phase. The minor peaks of

TABLE I

RETENTION TIMES OF COLOURS TESTED IN VARIOUS MOBILE PHASES

Mobile phase: methanol–water containing 0.005 *M* TBAP. Flow-rate, 1.0 ml/min. t_0 = 2.7 min.

Methanol–water ratio	*Retention times (min)*											
	Indigotine	*Tartrazine*	*Amaranth*	*Sunset yellow FCF*	*Allura Red AC*	*Fast Green FCF*	*Brilliant Blue FCF*	*Erythrosine*	*Ponceau SX*	*Fast Red E*	*Skyark*	*Benzyl Violet 4B*
40:60	8.7	18	25	32	65	>70*	>70*	–**	–	–	–	–
45:55	5.4	7.3	9.0	11	17	33	40	–	–	56	5.7	–
50:50	4.4	5.4	7.5	9.0	–	24	30	>40	–	–	–	–
55:45	4.0	4.7	5.0	5.9	7.5	9.0	9.8	–	>50	21	4.7	–
60:40	–	–	3.6	–	–	–	–	44	14.8	6.9	–	32*
70:30	–	–	2.7	–	–	–	–	9.9	4.8	–	–	–

* For Fast Green FCF, Brilliant Blue FCF and Benzyl Violet 4B, the major peak is given.

** Dashes indicate that no analyses were carried out.

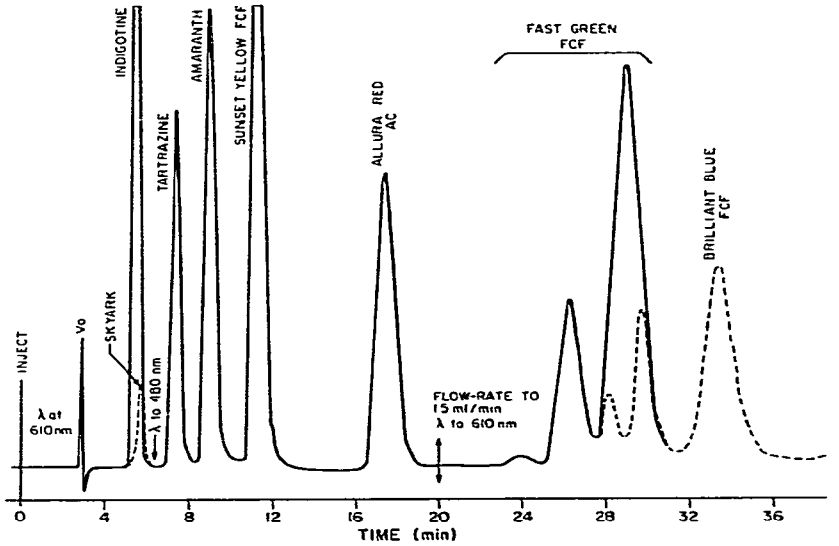

Fig. 1. Chromatogram of eight colors. Conditions as described in the text. Mobile phase methanol–water (45:55) containing 0.005 *M* TBAP; flow-rate 1.0 ml/min up to 20.0 min, then changed to 1.5 ml/min. Wavelength changes were made as indicated. Skyark and Brilliant Blue FCF peaks are in dashed lines.

Brilliant Blue FCF elute in the region of the major peak of Fast Green FCF. In order to monitor all food colors it was necessary to make detector wavelength changes during the run as indicated in Fig. 1. The flow-rate was increased at 20 min to 1.5 ml/min to speed up the elution of Fast Green FCF and Brilliant Blue FCF. Fig. 2

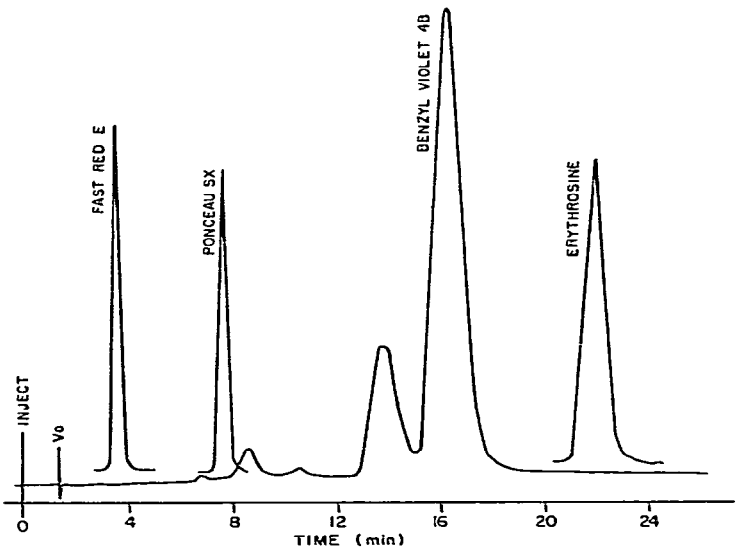

Fig. 2. Composite chromatogram of four food colors. Conditions as described in the next. Mobile phase, methanol–water (60:40) containing 0.005 *M* TBAP; flow-rate 2.0 ml/min throughout. Fast Red E, Ponceau SX and Erythrosine detected at 500 nm; Benzyl Violet 4B detected at 545 nm.

shows typical results for four other colors studied employing the (60:40) mobile phase. All four are easily separated from each other, however, for Erythrosine analysis we found that a methanol–water (70:30) mobile phase was superior in terms of analysis time and peak shape.

Application of the technique to beverage analysis is shown in Fig. 3 for a commercial grape soda drink. The results indicate the presence of both the permitted food colors, Amaranth and Brilliant Blue FCF. Results from a grape drink sample spiked with a mixture of Tartrazine, Allura Red AC, Brilliant Blue FCT and Benzyl Violet 4B showed no interferences from coextractives or natural food colors while all color peaks were quantitatively recovered when spiked in the concentration range of 4–44 μg/g.

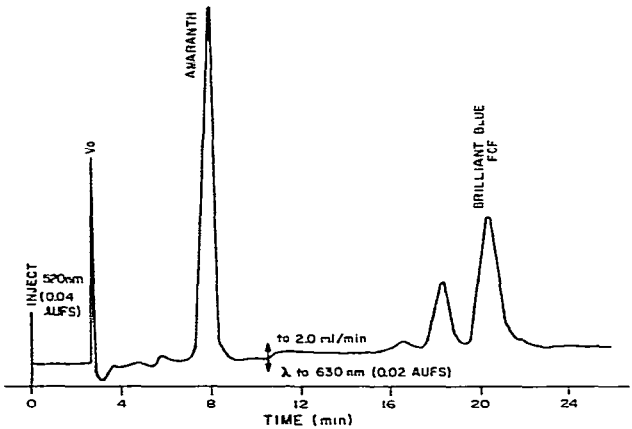

Fig. 3. Analysis of a grape soda beverage. Conditions as described in the text. Mobile phase methanol–water (50:50) containing 0.005 *M* TBAP. Wavelength and sensitivity settings (absorbance units full scale, AUFS) were as indicated.

CONCLUSION

The described HPLC method is capable of separating and quantitating twelve major food colors or subsidiary dyes. Application to grape beverages indicates the potential of the method for rapid screening of samples for non-permitted colors as well as for quantitating permitted food colors by comparison to known standards to ensure that they do not exceed the legal limit. The method is faster and less tedious than the thin-layer chromatographic–spectrophotometric techniques currently in use.

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