Journal of Chromatography, 154 (1978) 306–312

CHROM. 10,935

Note

Separation of synthetic food dyes using high-performance liquid chromatography

J. CHUDY, N. T. CROSBY and I. PATEL

Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1 9NQ (Great Britain)

(Received February 6th, 1978)

Within a continuing review of the safety-in-use of food additives, colouring matter added to food has been the subject of particularly close scrutiny. The decision[1] of the United States Food and Drug Administration to withdraw approval for the use of Amaranth (FD and C Red 2) is just one example of the trend towards limitation. In some countries, *e.g.* Sweden[2] moves have been announced to ban the use of synthetic food dyes altogether, whilst other countries, *e.g.* South Africa[3] have imposed legislative limits on the amounts of dyes to be added to particular foods. The tendency throughout the world is to reduce the number of food dyes permitted for general use. In the United Kingdom, the process of harmonization of the relevant legislation[4] with that of the European Economic Community will be completed with the implementation of the latest amendment[5] to the Community Directive[6] on colouring matter in food. The current position is summarised in Table I.

In view of the desire to restrict the usage of synthetic food dyes, there exists a need to develop methods of analysis for these compounds, which will indicate not only the nature of the added dye but also the amount present. In the present work, the chromatographic behaviour of synthetic food dyes permitted in the United Kingdom, and also the more common ones used in other countries, has been investigated. This information is needed to establish a scheme of analysis for food dyes, involving, where necessary, extraction of dyes from the food matrix followed by qualitative and quantitative analysis of the extract by high-performance liquid chromatography (HPLC).

The analytical chemistry of synthetic dyes, including non-food applications, has been extensively reviewed in a recent monograph[7]. For food dyes, numerous schemes[8–13] have been proposed for qualitative analyses: these have depended mostly on paper (PC) and thin-layer chromatography (TLC). Quantitative analysis has received comparatively little attention. After initial treatment to extract the dyes from the food, quantitative analysis has been attempted by a number of means which have included: (a) comparison of intensities of spots on TLC plates with those of a range of standards[14], (b) spectrophotometric quantification[15,16], (c) titration with titanous chloride solution[17,18] and (d) electrophoresis on polyacrylamide gel[19]. Finally, HPLC has been used in a number of ways for dye analysis, mostly for the detection of impurities in single dyes[20–25] but also to a limited extent for the separation of dye

TABLE I

SYNTHETIC FOOD DYES USED IN THE STUDY

No.	EEC additive number	Colour index number (3rd ed., 1971)	Common name	Pure dye content of standard (%)	Retention vol. (ml)**
Red dyes (monitored at 520 nm)					
1	E122	14720	Carmoisine*	89	9.35
2	E123	16185	Amaranth (F, D and C Red 2)*	90	10.45
3	E124	16255	Ponceau 4R*	82	37.20
4	E125	14815	Scarlet GN		10.70
5	E126	16290	Ponceau 6R		18.90
6	E127	45430	Erythrosine (F, D and C Red 3)*	94	6.15
7		18050	Red 2G*	81	8.20
8		16035	Allura Red (F, D and C Red 40)	91	4.45
9		18055	Red 6B		4.85
10		17200	Red 10B		8.90
11		16045	Fast Red E		6.55
12		14780	Red FB		10.50
13		16150	Ponceau MX		9.75
14		14700	Ponceau SX (F, D and C Red 4)	91	16.35
Orange and yellow dyes (monitored at 480 and 430 nm, resp.)					
15	E102	19140	Tartrazine (F, D and C Yellow 5)*	88	9.40
16	E103	14270	Chrysoin S		2.85
17	E104	47005	Quinoline Yellow*		4.05
18	E105	13015	Fast Yellow AB		4.50
19		18965	Yellow 2G*	86	4.85
20	E110	15985	Sunset Yellow FCF (F, D and C Yellow 6)*	88	4.60
21	E111	15980	Orange GGN		5.45
22		16230	Orange G	59	11.20
23		15970	Orange RN		4.60
Green and blue dyes (monitored at 640 nm)					
24	E131	42051	Patent Blue V*	50	3.55
25	E132	73015	Indigo Carmine (F, D and C Blue 2)*	92	4.30
26		42090	Brilliant Blue FCF (F, D and C Blue 1)*	92	4.55
27	E142	44090	Green S*	88	2.40
28		42053	Fast Green FCF (F, D and C Green 3)	89	4.30
Brown and black dyes (monitored at 480 and 600 nm, resp.)					
29		—	Brown FK*		—
30		—	Chocolate Brown FB		—
31		20285	Chocolate Brown HT*		—
32	E151	28440	Black PN*	75	18.00
33	E152	27755	Black 7984*		21.10

* Permitted in United Kingdom[5].

** On SAS-Hypersil column.

mixtures. Anion-exchange columns have been used for this purpose and frequently gradient elution has been specified. A paper dealing with the determination of food dyes by HPLC[26] describes the separation of 7 selected permitted dyes using a strong anion-exchange column.

Initial attempts at the application of HPLC to the separation of dye mixtures were disappointing when conventional techniques were used, a fact which has already been noted in the literature[27]. Much more promise was shown by the modification in which small amounts of suitable salts are added to the mobile phase, the salts

being chosen so as to generate large ions of opposite charge to the sample molecules, thereby giving rise to reversible ion-pair complexes. This technique, known as paired-ion chromatography, has found applications in the HPLC analysis of readily ionizable compounds[27,28].

A particular kind of paired-ion chromatography uses a cationic detergent, cetrimide, to provide a large organic counter-ion in solution. Cetrimide [cetyltrimethylammonium bromide, $C_{15}H_{33}(CH_3)_3NBr$] forms reversible ion pairs with the dye molecule anions and these pairs are then separated. "Soap chromatography"[29] has been used principally for the identification of some dyes and dyestuff intermediates and has been applied in the present work to study the chromatographic behaviour of a number of food dyes.

EXPERIMENTAL

Apparatus

A Waters Assoc. 6000A constant-volume pump fitted with a stop-flow injection system and a Cecil Instr. CE 515 spectrophotometric detector together with CE 500 control record module were used. This detector can be operated in the range 200–800 nm and has facilities for recording the spectra of individual chromatographic peaks. All experiments were carried out at room temperature with columns encased in cotton wool jackets.

Ultraviolet and visible spectra were recorded in 10-mm cells on a Perkin-Elmer 402 spectrophotometer using the mobile phase as both solvent and reference.

Reagents

Where possible, samples of known pure dye content were used to make up reference standard solutions. Other dyes, not permitted for use infood in the United Kingdom, were taken from a stock held within the laboratory but no information was available as to their purity. Solutions of these dyes contained 0.1% (w/v) of the solid dissolved in water; they were kept in a shaded position to minimise fading. E132 Indigo Carmine was kept in the dark. All solvents were of analytical reagent grade and were used without further purification. Cetrimide was obtained from BDH (Poole, Great Britain). The mobile phase was freshly made up each day and filtered through a porosity 5 sinter: in addition to removing particulate matter this also served to degas the liquid.

Chromatographic conditions

Conditions were as follows. Column A: 12 cm × 4.6 mm I.D. packed with 4.6 μm SAS-Hypersil (Shandon-Southern, Runcorn, Great Britain); flow-rate: 1.0 ml/min; pressure drop, 13.8 MPa (2000 p.s.i.); detector, 0.1 a.u.f.s.; recorder, 1.0 cm/min chart speed and 1 V f.s.d.; mobile phase, iso-propanol–water–cetrimide–glacial acetic acid (41:59:0.25:0.25, v/v/w/v). Column B: 15 cm × 4.6 mm I.D. packed with 5 μm Spherisorb S5W (Phase Separations, Queensferry, Great Britain); flow-rate, 1.0 ml/min; pressure drop, 17.2 MPa (2500 p.s.i.); detector, 0.2 a.u.f.s.; recorder, as for column A; mobile phase, iso-propanol–water–cetrimide–glacial acetic acid (70:30:2.0:1.0, v/v/w/v).

Columns were packed by upward pumping of a slurry of the material at a

pressure of 34.5 MPa (5000 p.s.i.). Most of the separations could be carried out on SAS-Hypersil but in one or two cases superior resolution was achieved on Spherisorb.

Under the conditions specified 2000–2500 theoretical plates could be obtained from the SAS-Hypersil and around 1500 from the Spherisorb column. Injections onto the columns were in the range 1–5 μl.

RESULTS

The chromatographic behaviour of the dyes is summarised in Table I and some separations are illustrated in Figs. 1–3. Fig. 1 shows the separation of 10 out of the 14 red dyes, 5 of which are permitted in the United Kingdom. The remaining 4 non-permitted red dyes (4, 10, 12 and 13) gave peaks in the region around 7, 1 and 2 and could not be separated under these conditions but they can be separated by PC or TLC.

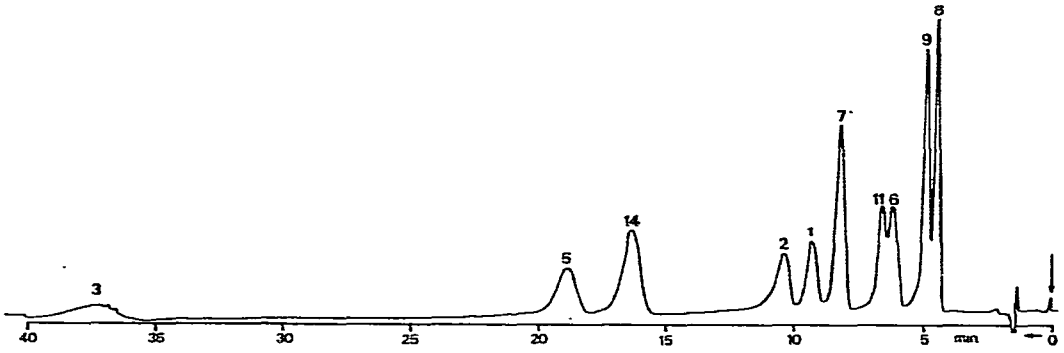


Fig. 1. Separation of 10 red dyes on column A. 5 μl of a mixture of 0.1% (w/v) solutions of each dye were injected. Chromatographic conditions were as in the text, peak numbers refer to dyes listed in Table I.

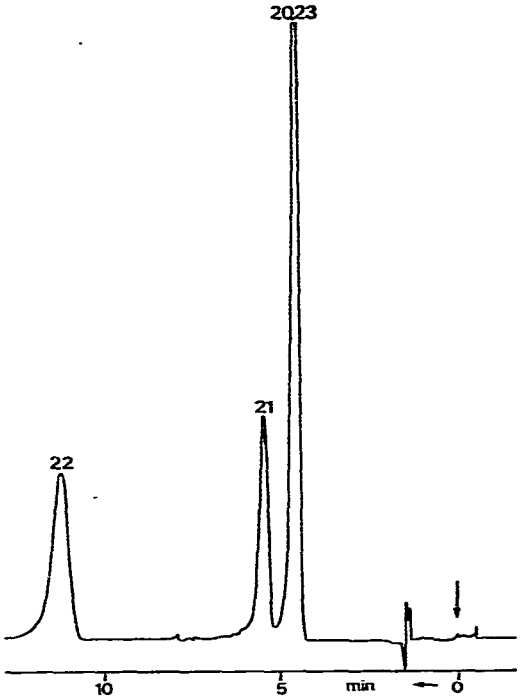
Fig. 2 shows the separation of the orange dyes of which only one is permitted. 20 and 23 can be separated on column B. Fig. 3 shows the separation of 5 yellow dyes, 3 of which are permitted. 17A is a minor component of Quinoline Yellow.

Of the brown dyes only Brown FK elutes under the conditions described, giving rise to 4 distinct peaks having retention volumes of 1.45, 2.45, 2.70 and 3.50 ml. The other two brown dyes remain on the column.

DISCUSSION

As can be seen from the chromatograms, practically all common food dyes can be separated on the SAS-Hypersil column. The three brown colours require other means of identification, *e.g.* TLC, and so do the red dyes Red 10B, Red FB, Scarlet GN and Ponceau MX. These dyes can be separated by PC[8,11,12].

Identification, by comparison of retention volumes with those of standards, is aided by the fact that the dyes have absorption maxima at different wavelengths in the visible region. Even when two dyes elute very close together, monitoring the



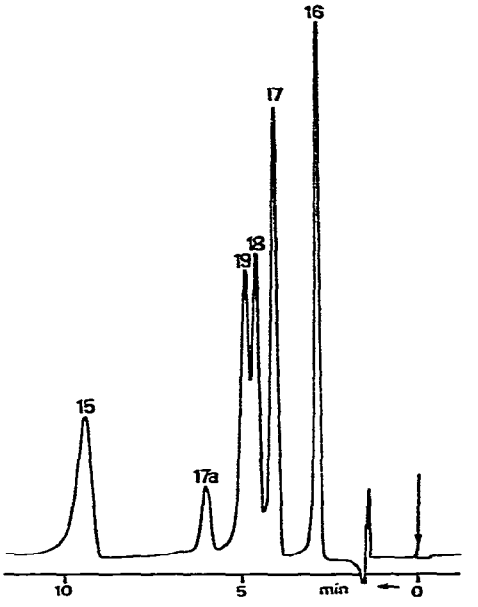


Fig. 2. Separation of orange dyes on column A. 2 μl of a mixture of 0.1% (w/v) solutions of each dye was injected. Chromatographic conditions as in text, peak numbers refer to dyes listed in Table I.

Fig. 3. Separation of yellow dyes on column A. 2.5 μl of a mixture of 0.1% (w/v) solutions of each dye was injected. Chromatographic conditions as in text, peak numbers refer to dyes listed in Table I.

column effluent at a wavelength where one absorbs weakly or not at all permits a quantitative analysis to be performed. A collection of the spectra of the dyes is required and this was recorded on the spectrophotometer but most of the spectra are available in the literature[30]. There remain, however, some cases where, in a particular sample, dyes are present whose HPLC peaks coincide or substantially overlap and where each dye absorbs significantly at the other's spectral maximum. In such cases prior separation of the dyes on *e.g.* polyamide columns[31] must be performed.

The method, as described, has potential application in the identification and quantification of food dyes, especially in routine repetitive analyses of known products. In favourable circumstances (aqueous samples such as soft drinks, fruit squashes etc.) an aliquot of the sample can be injected directly without lengthy clean-up procedures.

Non-aqueous samples must first be treated to remove the dyes from the food matrix: several methods have been suggested in the literature[13–16,31–37] and a study is in progress to determine the relative efficiencies of the procedures. Quantification of the amount of dye extracted can readily be achieved by comparison of peak heights or (preferably) areas with those of standards or with that of a single internal standard or by standard addition. From reference to spectra the wavelength of maximum absorption can be selected to monitor the column effluent thus assuring maximum sen-

sitivity. At the present time the limit of detection is of the order of 0.1 μg of pure dye in an injection volume of 5 μl which corresponds to about 10 μg dye per ml of solution used for injection. The limit of detection in foods in general cannot be precisely stated being dependent on the extent to which the dye extracts are concentrated prior to injection. In the cases where peaks are eluted late and in consequence are broad in shape, increasing the iso-propanol concentration to 45 or 50% is necessary for more rapid elution and accurate quantification, *e.g.* Ponceau 4R or the black dyes. With 50% iso-propanol, the elution volume for Ponceau 4R becomes 4.15 ml. Alternatively, gradient elution could be used.

When using the method for quantification it should be noted that most commercial food dyestuffs contain at most 80–90% of the pure dye, the rest being made up by subsidiary dyes and inorganic matter such as sodium chloride or sulphate. Standards must, therefore, be of known pure dye content.

The HPLC procedure described can also be used to monitor the behaviour of dyes during the various stages of food processing. Some dyes react with food constituents or with metal packaging to yield colourless, sometimes toxic, degradation products[38], whilst Red 2G under certain conditions gives rise to Red 10B[39], not considered suitable for food use in most countries. Since these last two dyes are resolved completely using the conditions described, the progress of the degradation can readily be followed.

ACKNOWLEDGEMENTS

We thank Williams (Hounslow) Ltd., Pointing Ltd. and D. F. Anstead Ltd. for gifts of dye samples, the Government Chemist for permission to publish and the Ministry of Agriculture, Fisheries and Food for financial support.

REFERENCES

1 *Fed. Reg.*, 41 (1976) 5823.
2 Swedish National Food Administration, *Notice M15/76*, 19 Oct. 1976.
3 *South African Food Drugs and Disinfectants Act 1929*, Regulation 6 (2) (revised); operative 6 Nov. 1977, Item R756, Government Gazette 5537 dated 6 May 1977.
4 *Statutory Instruments*, 1973 No. 1340, with amendments 1975 No. 1488 and 1976 No. 2086, London, HMSO.
5 *Off. J. Eur. Communities*, 20 (1977) C300/3.
6 *The Approximation of the Rules of Member States concerning the Colouring Matters for use in Foodstuffs intended for Human Consumption*, EEC Directive, 23 Oct. 1962.
7 K. Venkataraman (Editor), *The Analytical Chemistry of Synthetic Dyes*, Wiley, New York, 1977.
8 M. H. E. Griffiths, *J. Food Technol.*, 1 (1966) 63.
9 R. A. Hoodless, K. G. Pitman, T. E. Stewart, J. Thompson and J. E. Arnold, *J. Chromatogr.*, 54 (1971) 393.
10 D. Pearson, *J. Ass. Pub. Anal.*, 11 (1973) 127 and 133.
11 D. Pearson, *J. Ass. Pub. Anal.*, 13 (1975) 103.
12 D. Pearson and R. J. Walker, *J. Ass. Pub. Anal.*, 3 (1965) 46.
13 R. Takeshita, T. Yamashita and N. Itoh, *J. Chromatogr.*, 73 (1973) 173.
14 E. Andrzejewska, *Roczn. Panstw. Zakl. Hig.*, 26 (1975) 575.
15 C. Graichen and J. C. Molitor, *J. Ass. Off. Anal. Chem.*, 46 (1963) 1022.
16 C. Graichen, *J. Ass. Off. Anal. Chem.*, 58 (1975) 278.
17 B. Larsson, *Var. Foda*, 28 (1976) 2.
18 *Official Methods of Analysis*, Ass. Off. Anal. Chem., Washington, D.C., 12th ed., 1975, p. 636.

19 D.-B. Yeh, *J. Chromatogr.*, 132 (1977) 566.
20 J. E. Bailey and E. A. Cox, *J. Ass. Off. Anal. Chem.*, 58 (1975) 609.
21 J. E. Bailey and E. A. Cox, *J. Ass. Off. Anal. Chem.*, 59 (1976) 5.
22 J. A. Sclar and R. C. Williams, *DuPont Liquid Chromatography Applications Lab Report*, No. 73-02.
23 R. J. Passarelli and E. S. Jacobs, *J. Chromatogr. Sci.*, 13 (1975) 153.
24 D. M. Marmion, *J. Ass. Off. Anal. Chem.*, 60 (1977) 168.
25 M. Singh, *J. Ass. Off. Anal. Chem.*, 60 (1977) 173.
26 M. Attina and G. Ciranni, *Farmaco, Ed. Prat.*, 32 (1977) 186.
27 D. P. Wittner, *Anal. Chem.*, 47 (1975) 1422.
28 *Paired Ion Chromatography. An Alternative to Ion Exchange*, leaflet F61, Waters Ass., Milford, Mass.
29 J. H. Knox and G. R. Laird, *J. Chromatogr.*, 122 (1976) 17.
30 *Separation and Identification of Food Colours Permitted by the Colouring Matter in Food Regulations 1957*, The Association of Public Analysts, London, 1960.
31 R. A. Gilhooley, R. A. Hoodless, K. G. Pitman and J. Thomson, *J. Chromatogr.*, 72 (1972) 325.
32 Anonymous, *Analyst (London)*, 88 (1963) 864.
33 Y. Yanuka, Y. Shalon, E. Weissenberg and I. Nir-Grosfeld, *Analyst (London)*,88 (1963) 872.
34 G. Lehmann, P. Collet, H. G. Hahn and M. R. F. Ashworth, *J. Ass. Off. Anal. Chem.*, 53 (1970) 1182.
35 B. Drevon and J. Laur, *Ann. Fals. Fraudes*, 52 (1959) 155.
36 A. Perdih and D. Prihavec, *Z. Lebensm.-Unters.-Forsch.*, 134 (1967) 239.
37 I. Ekar and A. Perdih, *Hrana Ishrana*, 15 (1974) 263.
38 I. G. Drozdova, I. N. Putilova and L. V. Golubeva, *Izv. Vyssh. Ucheb. Zaved. Pishch. Tekhnol.* (1976) 21; also *Int. Flav. Food Additives*, 8 (1977) 154.
39 M. E. Knowles, J. Gilbert and D. J. McWeeny, *J. Sci., Food. Agr.*, 25 (1974) 1239.