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Note

Separation of non-dye components of Brown FK by high-performance liquid chromatography

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Considerable interest is being shown in the use of high-performance liquid chromatography (HPLC) to analyse synthetic food colours. A recent draft specification[1] proposes that HPLC should be used to characterise the composition of dyes and, in particular, to identify and quantify the presence of "organic" compounds other than dyes. This paper describes an HPLC system for the separation and quantitation of the non-dye components in Brown FK.

Brown FK is a permitted food colour which is particularly suited for colouring kippers. The specification allows for the marketing of a commercial colour preparation containing up to 50% diluent *e.g.*, sodium chloride. It is manufactured by the reaction of diazotised sulphanilic acid with a mixture of *m*-phenylenediamine and 2,4-diaminotoluene under carefully controlled conditions and has been shown to consist of six major coloured components[2]: 2,4-diamino-5-(*p*-sulphophenylazo) toluene (I), 1,3-diamino-4-(*p*-sulphophenylazo) benzene (II), 1,3-diamino-4,6-bis(*p*-sulphophenylazo) benzene (III), 2,4-diamino-3,5-bis(*p*-sulphophenylazo) toluene (IV), 1,3-diamino-2,4-bis(*p*-sulphophenylazo) benzene (V) and 1,3-diamino-2,4,6-tris(*p*-sulphophenylazo) benzene (VI) (Fig. 1).

A comparison is made between the HPLC technique and a previously reported thin-layer chromatographic (TLC) separation[2].

Application of the HPLC method to several commercial samples is also described.

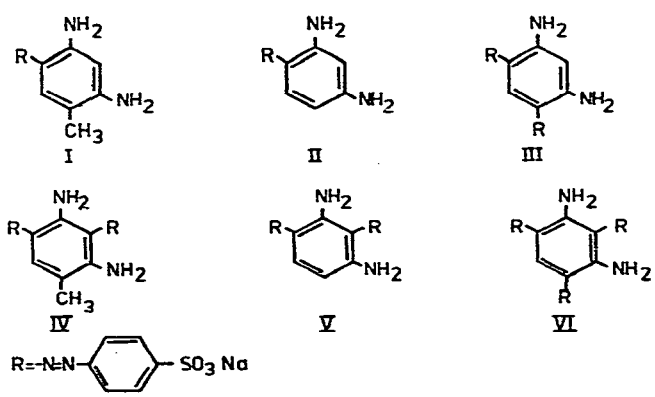

Fig. 1. Structures of Brown FK dye components.

EXPERIMENTAL

High-performance liquid chromatography

The liquid chromatograph consisted of a reciprocating pump (Applied Chromatography Systems, Luton, Great Britain) and a Cecil 212 UV monitor (Cecil, Cambridge, Great Britain) set at 254 nm. Injection was achieved using a Specac injection valve (Spectrascopic Accessories, Sidcup, Great Britain) fitted with a 20-μl loop. Gradients were formed using a deci-linear gradient programmer (Applied Chromatography Systems). Two types of bonded phase columns were used: (i) 20 cm $\times$ 0.49 cm I.D. packed with Partisil 5 (Whatman, Maidstone, Great Britain) with a 7% loading of aminopropyl phase; (ii) 20 cm $\times$ 0.49 cm I.D. packed with 5-μm Li-Chrosorb Si 100 (Merck, Darmstadt, G.F.R.) with a 21% loading of octadecyl phase. Columns were made from grade 316 seamless stainless-steel tubing (Tube Sales, Southampton, Great Britain).

The details of the aminopropyl phase preparation have been previously reported[3], and consist of shaking γ-aminopropyltriethoxysilane and moist silica in dry hexane for 5 min.

The octadecyl phase was prepared by refluxing 10 g of silica, 11.7 g of octadecyltrichlorosilane and 1 ml of pyridine in 50 ml of dry xylene for 15 min. The silica was dried overnight in an oven at 100° prior to bonding. Residual hydroxyl groups were removed by adding 2 ml of hexamethyldisilazane and refluxing the phase in xylene for a further 10 min. The phase was washed with xylene (2 $\times$ 50 ml) and hexane (2 $\times$ 50 ml) and dried in an oven at 50°. Polymeric material was removed by passing the phase through a 50-μm mesh sieve.

Both columns were prepared using the slurry packing technique[3]. The aminopropyl phase was dispersed in water (25 ml) and packed down with acetonitrile–water (4:1) for 20 min at 5000 p.s.i. The octadecyl phase was dispersed in chloroform and packed down with acetonitrile.

Thin-layer chromatography

Precoated glass silica gel G TLC plates, layer thickness 0.25 mm (Merck, No. 5721) were used unactivated in the solvent system phenol–water (4:1). Sulphanilic acid and the aromatic amines used in the dye manufacture were detected by spraying with 1% *p*-dimethylaminobenzaldehyde (*p*-DMAB) in 50% acetic acid.

RESULTS

The separation achieved on the aminopropyl column is shown in Fig. 2. The system gave a good separation of sulphanilic acid but it proved difficult to separate *m*-phenylenediamine and 2,4-diaminotoluene which were eluted in the void volume. There was also only a partial resolution between the three disubstituted dye components, *i.e.*, III, IV and V.

However both *m*-phenylenediamine and 2,4-diaminotoluene were retained and well separated from each other on the octadecyl column (Fig. 3). Sulphanilic acid was only slightly retained eluting immediately after the void volume. This system also separated all six dyes (including the isomeric disubstituted benzene components III and V) from each other and the non-dye components.

Although the TLC system satisfactorily resolves the six dye components, it is not suitable for analysing the organic compounds other than dyes (Fig. 4 and Table

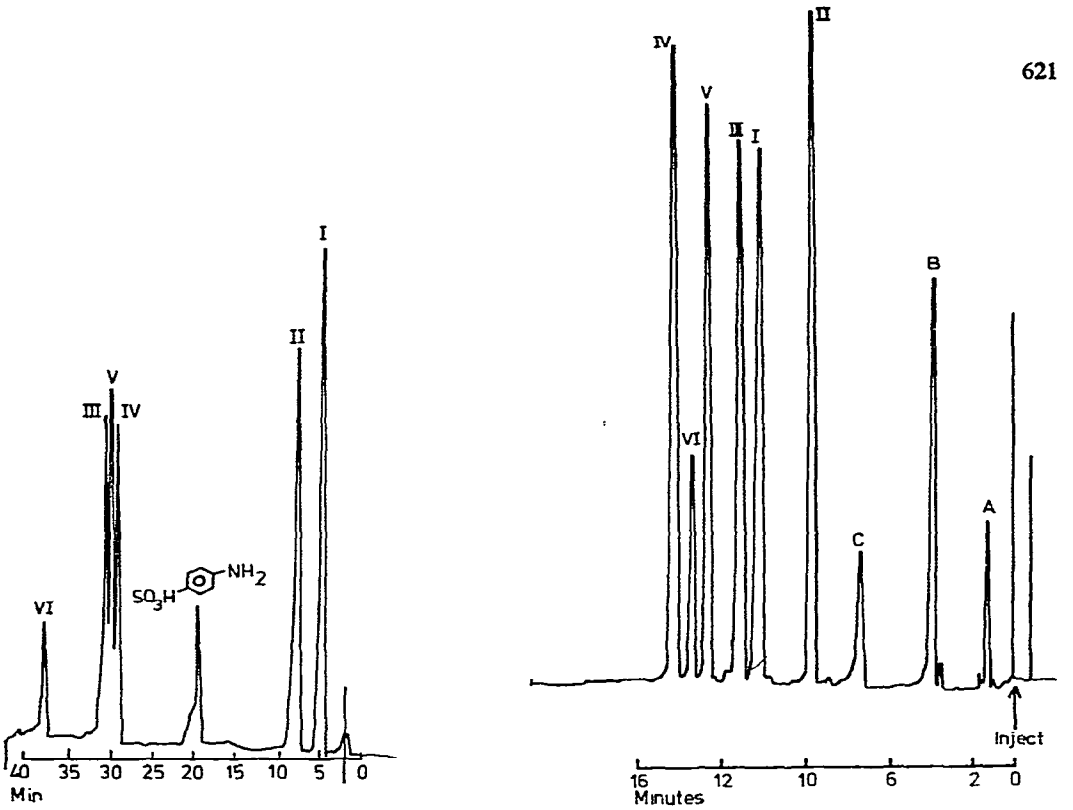

Fig. 2. Separation of Brown FK on an amino bonded-phase column. Solvent, 30-min linear gradient from acetonitrile–water (2:3) to acetonitrile–water (2:3) containing NaH_2PO_4 (2 g/l). Flow-rate, 1.5 ml/min. Detection, UV 254 nm.

Fig. 3. Separation of Brown FK and non-dye components on an octadecyl bonded-phase column. A = Sulphanilic acid, B = *m*-phenylenediamine, C = 2,4-diaminotoluene. Solvent, 17-min linear gradient from 5 to 40% acetonitrile in water. The water contained NaH_2PO_4 at the level 1.2 g/l and Na_2HPO_4 at 2.4 g/l. Flow-rate 2 ml/min. Detection UV 254 nm.

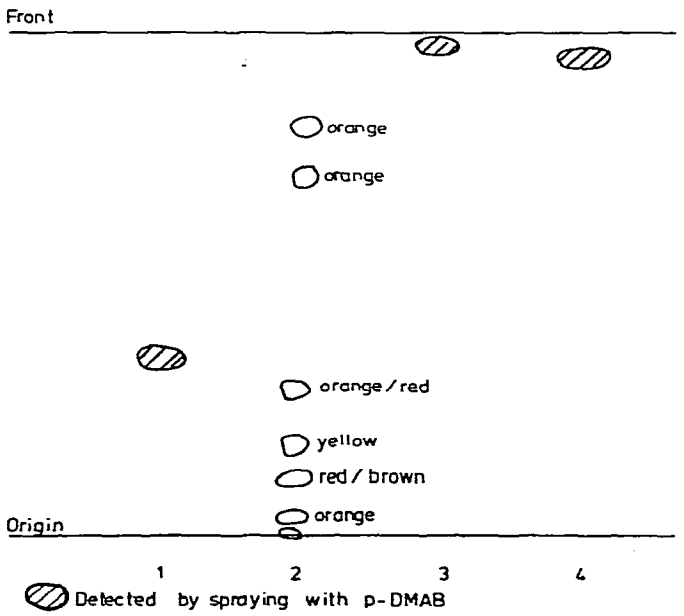

Fig. 4. TLC separation of Brown FK dye components and the compounds used in the dye manufacture. 1 = Sulphanilic acid; 2 = Brown FK; 3 = 2,4-diaminotoluene; 4 = m-phenylenediamine.

TABLE I

TLC CHARACTERISTICS OF DYE COMPONENTS AND COMPOUNDS USED IN DYE MANUFACTURE

Compound	$R_F \times 100$	*Colour*
2,4-Diaminotoluene	96	Yellow*
m-Phenylenediamine	93	Yellow-orange*
I	79	Orange
II	70	Orange
Sulphanic acid	34	Yellow*
IV	31	Orange-red
V	18	Yellow
III	11	Red-brown
VI	4	Orange

* Colour observed after spraying with *p*-DMAB.

I). Sulphanilic acid was not completely resolved from component IV whilst the two aromatic amines were not well resolved, both running close to the solvent front.

Analysis of commercial samples of Brown FK including one (sample No. 1) which had been used for toxicological testing were analyzed by HPLC using the two column systems (Table II). Quantitative data for sulphanilic acid and the aromatic amines was obtained by comparison of peak heights with those of standard solutions. All four samples exhibited very low levels of non dye components.

TABLE II

PERCENT OF ORGANICS OTHER THAN DYES IN COMMERCIAL BROWN FK SAMPLES

Sample No.	*Sulphanilic acid*	*m-Phenylenediamine*	*2,4-Diaminotoluene*
1	0.05	<0.01	0.06
2	0.02	<0.01	0.03
3	0.03	<0.01	0.03
4	0.03	<0.01	<0.01
Detection limit	0.005	0.01	0.01

CONCLUSION

Although TLC separates all six dye components, the organic compounds other than dyes are not well resolved. However, HPLC, using an octadecyl column, is not only suitable for analysing the low levels of sulphanilic acid and aromatic amines in commercial Brown FK samples but will also resolve the six dye components.

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