Journal of Chromatography, 368 (1986) 450–455
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 957

Note

Separation of synthetic dyes by high-performance liquid chromatography on 3-μm columns

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(First received May 12th, 1986; revised manuscript received July 22nd, 1986)

Synthetic dyes are used widely to colour cosmetics, pharmaceutical formulations and foods. They are easily prepared stable and colour intense, in contrast to the expensive unstable and sometimes insipid natural pigments which they have largely replaced.

Several hundred dyes are known and this diversity has always posed a problem of identity for the analyst as many dyes have similar characteristics and some are even mixtures. In their turn paper chromatography[1], thin-layer chromatography[2] and more recently high-performance liquid chromatography (HPLC)[3] have aided the chemist in the examination of synthetic colours.

The introduction of HPLC transformed the analysis of dyes and has made quantitative analysis routine for the first time. Conventionally such separations employ reversed-phase ion-pair chromatography[4]. Often a silica-ODS column is used with a quaternary ammonium salt as the counter-ion[5]. Numerous workers have adopted this approach for a variety of applications[6,7]. Nevertheless the reported separations are somewhat indifferent when compared against results achieved, for other compound classes, with modern liquid chromatography[8].

Alternative separation strategies have been employed in the examination of dye intermediates. Aromatic sulphonic and carboxylic acids were successfully separated by reversed-phase chromatography in the presence of inorganic electrolytes[9]. We have extended this concept to the separation of the common synthetic food colours on reversed-phase columns. There is a considerable improvement both in resolution (as the eluted peaks are much sharper) and in reproducibility (as retention times are more stable).

The dyes may be identified not only on retention time but also by selective absorption of radiation. They can be divided into yellows, reds, blues, etc. by their differing responses in the visible region of the absorption spectrum whilst all can be detected in the UV region. We were able to separate and detect fifteen dyes in 21 min on an ODS column by this approach.

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0021-9673/86/$03.50

EXPERIMENTAL

Apparatus

A Beckman Model 344 gradient liquid chromatograph was used, fitted with an Ultrasphere-ODS, 3-μm (7.5 cm $\times$ 0.46 cm I.D.) column and coupled to a Beckman Model 165 scanning UV–visible detector. Further, a dual-pen chart recorder or dual-channel integrator and a single-pen chart recorder were used.

Materials and reagents

All solvents (water and methanol) were HPLC grade. Chemicals (orthophosphoric acid and anhydrous sodium sulphate) were AnalaR grade. Stock solutions of synthetic dyes were prepared by dissolving individual compounds in water at concentrations of approximately 300 μg/ml. Aliquots of these solutions (3–10 μl) were injected into the liquid chromatograph.

Chromatography

The colours were separated by gradient elution on an Ultrasphere-ODS, 3-μm (7.5 cm $\times$ 0.46 cm I.D.) column. Solvents were degassed prior to use.

Solvent A was 0.1 *M* aqueous sodium sulphate adjusted to pH 2.5 with orthophosphoric acid.

Solvent B was solvent A–water–methanol (1.5:7:22). Eluent flow-rate was 1.5 ml/min. The solvent composition was changed from 0 to 100% B over a period of 20 min. At 30 min the composition reverted from 100 to 0% B over 5 min.

The column eluate was monitored at two wavelengths; 254 nm for all colours and either 430 nm for yellow dyes or 520 nm for red dyes or 640 nm for the blue/green dyes. Both detector channels were set at an attenuation of 0.1 absorbance units, full scale deflection (a.u.f.s.d.). Absorption spectra were obtained "on the fly" by scanning emerging peaks (between the wavelengths shown) and adjusting detector sensitivity to obtain a satisfactory response.

RESULTS AND DISCUSSION

Profiles are shown in Figs. 1–3 and typical retention times given in Table I. Variations in retention are modest, such that standards would only need to be run once daily. The eluted peaks are very sharp, compared with profiles obtained by conventional reversed-phase ion-pair chromatography[10], although marked improvements in resolution have been demonstrated in recent ion-pair separations of cosmetic dyes[11]. Wandering retention indices (a problem often encountered in ion-pair work) did not occur but a gradual creep (shortening) of retention took place over a 6-month span as the columns aged. The figures quoted in this paper were obtained on a column which had reached its "steady state". No further changes were noted over a 3-year period.

Every night before shutdown the column was thoroughly washed with water followed by methanol. This was found to be necessary to maintain the column in a satisfactory condition.

Inevitably some dyes co-eluted, *e.g.*, Yellow 2G/Red 2G and Carmoisine/Brown HT but these pairs were easily distinguished by monitoring in the visible

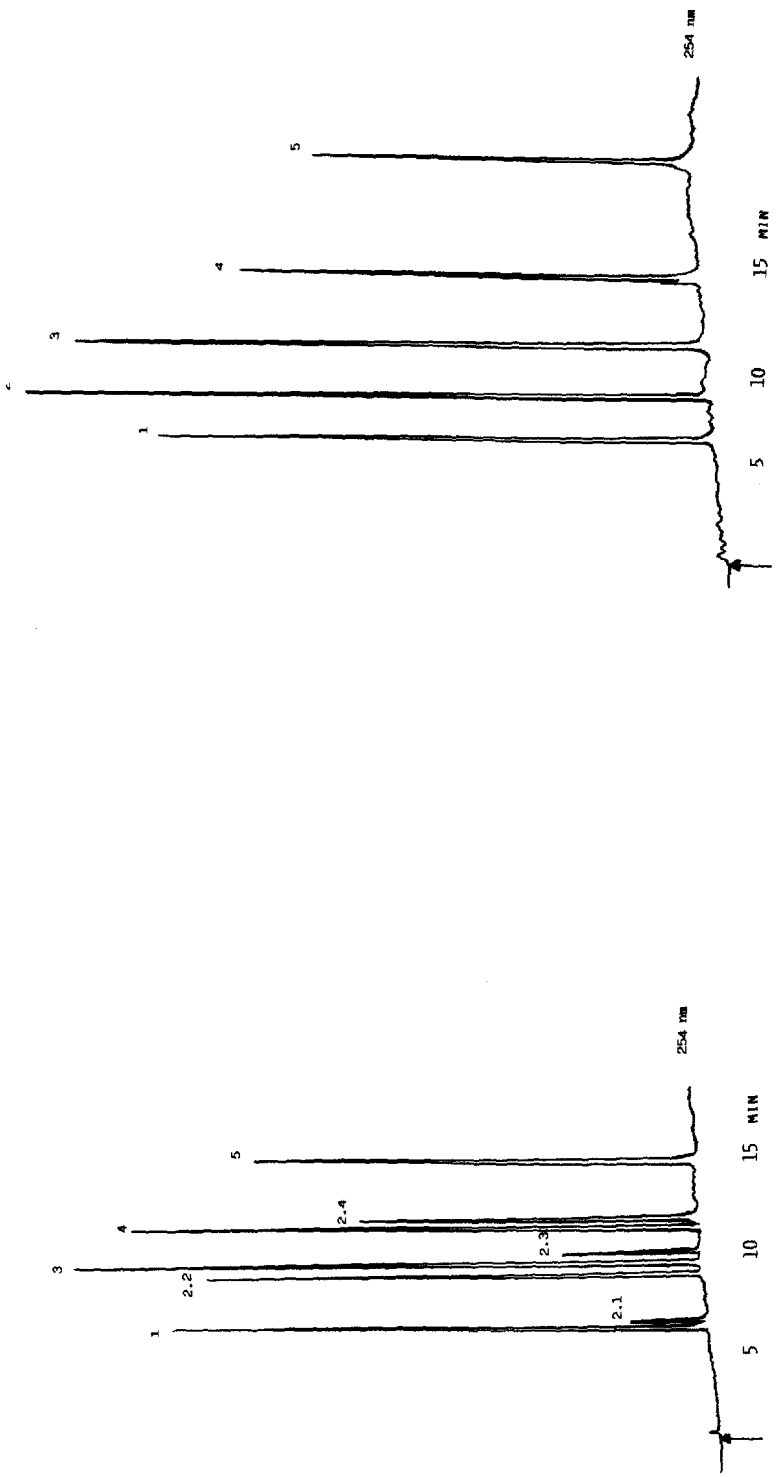

Fig. 1. Separation of yellow dyes. Peaks: 1 = Tartrazine; 2.1, 2.2, 2.3, 2.4 = Quinoline Yellow; 3 = Sunset Yellow; 4 = Yellow 2G; 5 = Brown HT.

Fig. 2. Separation of red dyes. Peaks: 1 = Amaranth; 2 = Ponceau 4R; 3 = Red 2G; 4 = Carmoisine; 5 = Erythrosine.

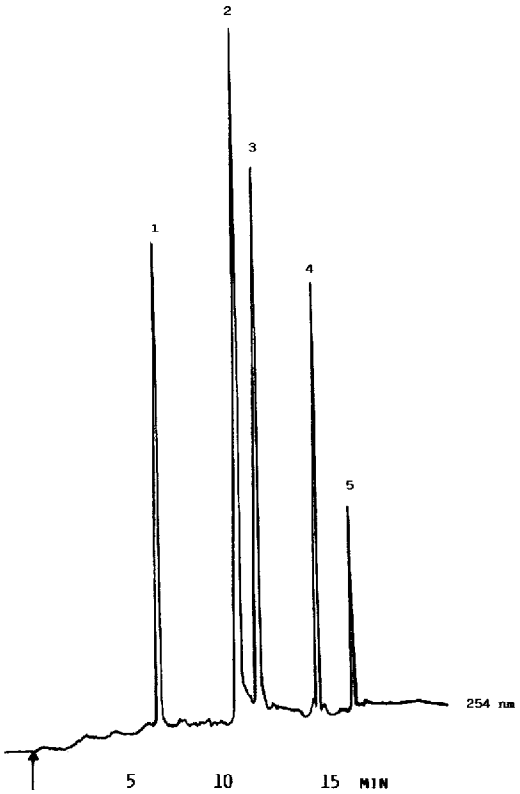

Fig. 3. Separation of blue/green dyes. Peaks: 1 = Indigo Carmine; 2 = Black PN; 3 = Green S; 4 = Brilliant Blue FCF; 5 = Patent Blue V.

TABLE I

RETENTION TIMES OF DYES

Dye	*Retention time (min)*		*Dye*	*Retention time (min)*	
	Average	*Range*		*Average*	*Range*
Yellows			*Reds*		
Tartrazine	5.8	5.7–5.9	Amaranth	6.4	6.3–6.5
Quinoline Yellow	6.1	5.9–6.2	Ponceau 4R	8.6	8.5–8.7
	8.5	8.2–8.6	Red 2G	11.2	11.1–11.3
	9.7	9.4–9.8	Carmoisine	14.7	14.6–14.8
	11.4	11.2–11.6	Erythrosine	20.8	20.7–20.9
Sunset Yellow	9.3	9.2–9.5	*Blue/greens*		
Yellow 2G	11.2	11.1–11.3	Indigo Carmine	6.7	6.5–6.8
Brown HT	14.7	14.5–14.9	Black PN	10.6	10.4–10.7
			Green S	11.7	11.5–11.8
			Brilliant Blue FCF	14.8	14.7–15.0
			Patent Blue V	16.7	16.6–16.9

region. Other dyes eluted close together but as baseline separation (Fig. 1) could be achieved between, for example, Quinoline Yellow (fourth component) and Yellow 2G where retentions differed by only 0.2 min then even here individual dyes could be determined. Effectively all fifteen dyes could be detected in the presence of each other in 21 min using multiple-wavelength monitoring.

Quinoline Yellow (Figs. 1 and 4) is a multi-component colour which is known to consist of two forms, both supposedly being disulphonated compounds. In some preparations isomeric phthalyl derivatives are also evident when impure starting materials have been used. All these possible products can be separated and monitored. "On-the-fly" scans (without stopping solvent flow) demonstrated clearly that this particular preparation contained both mono- and disulphonic acids.

The mechanism for these separations is somewhat speculative even though the behaviour of dye intermediates has been extensively investigated elsewhere[12]. There is remarkably little variation, either in response or retention, over a long period of time and eluted peaks are generally sharper than those seen in ion-pair chromatography of colours. The presence of inorganic electrolyte appears to be essential for this reproducibility. Dyes could be separated by simple gradient elution (water to methanol) but repeatability was always poor. Generally retention indices increased in the presence of electrolyte.

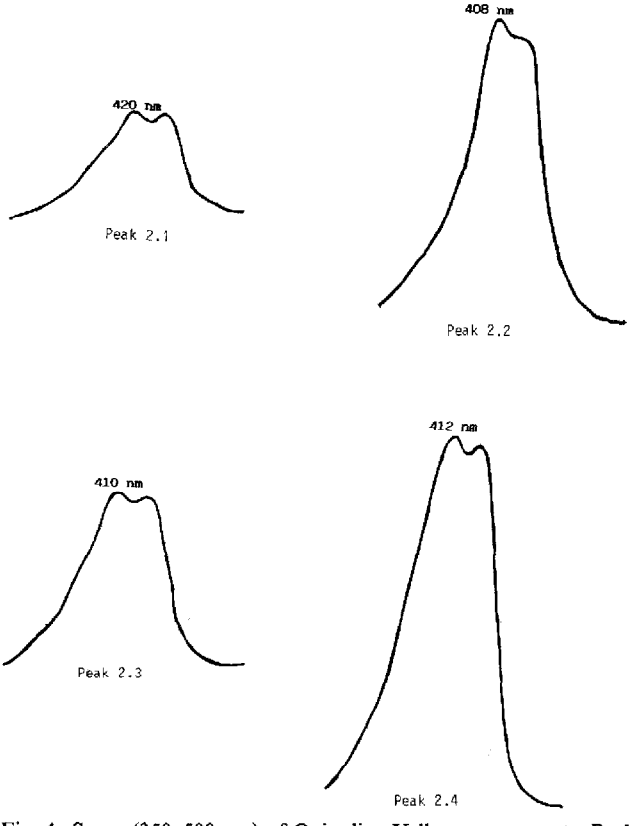

Fig. 4. Scans (350–500 nm) of Quinoline Yellow components. Peak Nos. correspond to Fig. 1.

We did not examine the effect of eluent acidity (pH) in any detail, but relatively large changes, *i.e.* greater than 0.5 pH units were needed to influence the chromatography. Some dyes were more sensitive to pH changes than others and this could be the basis for selective separations of closely eluting colours.

As retention apparently increases with increasing ionic strength then some sort of association must be taking place between the ionic groups on the dyes and the electrolyte. In essence the separation is based upon a non-polar interaction between the colours and the column packing. This is akin to hydrophobic interaction chromatography of proteins but of course these compounds are not biopolymers but merely simple amphoteric organic compounds. The detailed investigations undertaken by others[9], into the retention behaviour of sulphonic acids, also concluded that inorganic electrolyte played a central role in their resolution by this means.

CONCLUSIONS

Fifteen dyes can be separated and detected in 21 min, by gradient reversed-phase chromatography, in the presence of inorganic electrolyte. Repeatability is superior to that achieved with conventional reversed-phase ion-pair chromatography and the column can genuinely be used for other work without a deterioration in subsequent performance.

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