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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD UTILISING SINGLE OR MULTI-WAVELENGTH DETECTION FOR THE COMPARISON OF DISPERSE DYES EXTRACTED FROM POLYESTER FIBRES

B. B. WHEALS*, P. C. WHITE and M. D. PATERSON
Metropolitan Police Forensic Science Laboratory, 109 Lambeth Road, London S.E. 1 (U.K.)
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SUMMARY

The potential of high-performance liquid chromatography for the forensic examination of disperse dyes extracted from polyester fibres is assessed. The separated dyes were monitored either by a single wavelength filter photometer or a multi-wavelength linear diode array detector. Detection limits of 200 pg of injected dye were obtained with both systems and retention data for 57 disperse dyes are reported. The method permitted dye extracts from 2–5 mm of single fibres to be compared, and provided discrimination of all fibres examined. A comparison of dye extraction methods together with the advantages and disadvantages of the two detection systems are discussed.

INTRODUCTION

Fibre comparison is forensically important and the key steps in this type of examination are chemical classification of the fibre polymer and comparative analysis of their dye content. Microscopy and IR spectroscopy[1] are of value in the former context, and microspectrophotometry[2] on the whole fibre, followed by thin-layer chromatography (TLC) of dye extracts are important in the latter. The study reported here was undertaken to assess the role that high-performance liquid chromatography (HPLC) might play in dye comparison as an adjunct or alternative to TLC.

Fibres based on polyesters constitute the most common type of synthetic fibre encountered in U.K. forensic laboratories[3], and hence this class of material was selected for study. Disperse dyes based on anthraquinone and diazo compounds are the major colouring agents in use with polyester fibres, and West[4] has shown that a normal phase gradient elution HPLC method can be used to separate such materials after extraction from fibres. West[4] concluded that HPLC offered greater sensitivity and resolution than TLC, although the data presented originated from dye extracts from several strands of fibre, and as such was not directly applicable to most forensic casework. To be of wide utility the HPLC method must be applicable to single fibres, and work on transfer[5] and persistence[6] of single fibres suggests that 2 mm lengths need to come within the scope of the procedure.

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In addition to developing a suitable separation scheme it is important to have a monitoring system of adequate sensitivity and in this study a single wavelength and multiple wavelength [based on a linear diode array (LDA)] detector have been compared. Both monitored absorbance changes in the visible region of the spectrum.

EXPERIMENTAL

Dye extraction procedures

Two alternative extraction methods were used:

With chlorobenzene. A single fibre 2–5 mm long was taken from a sample chart containing polyester fibres dyed with Dispersol disperse dyes (ICI trade name). The fibre was pushed to the bottom of a 5 cm length of glass capillary tubing of 2 mm O.D. and 1.3 mm I.D. which had been sealed at one end by heating. A volume of 5 μl of chlorobenzene was added; the tube was sealed and heated at 100°C for 15 min. After cooling the tube was shaken to separate the fibre from the dye extract and the end containing the fibre was removed. The extract was evaporated to dryness by heating in an oven at 100°C for 30 min, and the residue was dissolved in 5 μl of eluent for injection on to the HPLC column.

With dimethylformamide. A single fibre was placed in a glass tube as described above and 4 μl of a dimethylformamide–acetonitrile (1:1, v/v) mixture was added. The dimethylformamide had previously been modified by adding 1.25 g of 2,6-di-*tert.*-butyl-4-methylphenol and 1 g of citric acid to each 250 ml of the solvent. The unsealed tube and its contents were agitated by hand for 1–2 min in a silicone oil bath maintained at 120–130°C. After cooling, 6 μl of the chromatographic eluent described later were added to provide a dye solution ready for injection.

Chromatographic conditions

A Constametric HPLC pump (Laboratory Data Control, Riviera Beach, FL, U.S.A.) delivered eluent at 0.4 ml min^{-1} to a 12.5 cm, 3 mm I.D., 6.3 mm (*i.e.* 1/4″) O.D. stainless-steel column packed with 5-μm Hypersil ODS (Shandon Southern, Cheshire, U.K.). The eluent contained acetonitrile–water (4:1, v/v) and was buffered to *ca.* pH 3.2 by the addition of 1 g of citric acid per litre. Samples were introduced via an injection valve (Model No. 7125, Rheodyne, CA, U.S.A.) fitted with a 5-μl loop.

The column was packed by upward displacement of a trichloroethane–methanol (1:1, v/v) slurry of the packing material using a pneumatic amplifier pump (Stansted Fluid Power, Stansted, U.K.) delivering methanol at 6000 p.s.i.

Detection conditions

Two alternative monitoring systems were investigated:

Single wavelength detection. The eluate from the column was passed into a UV–VIS filter photometer (Knauer, Berlin, F.R.G.) fitted with a 20-μl flow cell. The monitoring wavelength in the visible region was selected by inserting one of three narrow bandpass filters transmitting at 400, 500 and 600 nm respectively. Selection of the most appropriate monitoring wavelength was based either on the absorbance spectrum of the fibre as measured by microspectrophotometry or by using the guidelines summarised in Table I.

TABLE I

GUIDELINES FOR SELECTING WAVELENGTH FOR SINGLE WAVELENGTH DETECTION

Fibre colour	*Monitoring wavelength (nm)*
Yellow–pale orange	400
Red, brown, orange or black	500
Blue–violet	600
Green	400 or 600

The detector output was coupled to both channels of a dual-pen recorder (Model BD-9, Kipp & Zonen, The Netherlands) so that signals at $\times 1$ and $\times 0.25$ of the absorbance range selected could be simultaneously recorded. Typical absorbance settings ranged from 0.016 to 0.004 aufs.

Multiple wavelength detection. The eluate was passed into a LDA detector programmed to monitor the wavelength range 250–600 nm. The detector and ancillary equipment were products of Hewlett-Packard (Palo Alto, CA, U.S.A.) and consisted of the following items: detector HP 1040A, computer HP 85B, dual floppy-disc drive for multiple signal storage HP 9121 and an *X–Y* plotter HP 7470A. A bandwidth setting of 20 nm was used throughout this study.

RESULTS AND DISCUSSION

The isocratic reversed-phase conditions described above permitted the dyes from every fibre examined to elute with retention times in the range 2.5–15 min and a summary of the data appear in Table II. It was found that retention was not influenced by the pH of the eluent, although a buffered eluent was used as a precaution. Of the fibres studied 22 (*i.e.* 38%) gave chromatograms with two or more major peaks (arbitrarily defined as any peak with a height greater than 10% of the major peak when monitored in the visible region of the spectrum), and most of the extracts characterised by one major peak also had minor peaks in the chromatogram. The long term stability of the system was found to be good and the shorter analysis times, lower instrumental costs, and proven application to single fibre extracts suggest that this method has several advantages over West's procedure[4].

The fibres examined in this study typically weighed 3–3.5 μg per 5 mm length, and had diameters of about 0.02 mm. The mass of dye extracted from such samples can be as little as a few ng and in order to generate the maximum detector signal from such extracts three factors require special attention: the dye must be extracted efficiently without chemical change; there must be minimal dilution of the sample during the chromatographic process; the detector and monitoring wavelength should be optimised. These factors will now be discussed in more detail, for a successful method must pay attention to all three.

Extraction. It has been claimed by Kissa[7], that some disperse dyes undergo oxidation when extracted with hot solvents, and he recommended the use of dimethylformamide containing an antioxidant and an organic acid as a means of minimising such decomposition. The extractant of this type worked well, but to minimise the solvent blank during HPLC it was found advantageous to dilute with acetonitrile

TABLE II

RETENTION TIME DATA FOR DISPERSOL DISPERSE DYES EXTRACTED FROM POLYESTER FIBRES

Retention times are expressed relative to that of 2,6-di-*tert.*-butyl-4-methylphenol. This compound had a retention time of 7.0 min under the chromatographic conditions described in the text. Samples marked with an asterisk gave chromatograms with two or more major peaks.

Dye (dispersol name)	*C.I. name (i.e. disperse...)*	*Chemical type*	*Monitoring wavelength (nm)*	*Relative retention time*
Violet C-BR	Violet 8	Anthraquinone	600	0.27
Yellow B-A	Yellow 1	Nitro	400	0.29
Red B-3B	Red 11	Anthraquinone	500	0.32
Yellow D-7G	Yellow 126	Anthraquinone	400	0.34
Blue B-2G	Blue 26:1	–	600	0.35*
Black D-2G	–	–	500	0.35*
Brown D-3R	Brown 4	Monoazo	500	0.35
Blue D-2R	Blue 83	Anthraquinone	600	0.36*
Yellow C-T	Yellow 42	Monoazo	400	0.37
Yellow C-5G	Yellow 119	–	400	0.37
Navy B-T	Blue 35	Anthraquinone	600	0.37*
Red C-3B	Red 82	Monoazo	500	0.40
Yellow B-GR	Yellow 39	–	400	0.44*
Red C-B	Red 91	Anthraquinone	500	0.44
Scarlet C-2R	Red 54:1	–	500	0.45*
Red D-2G	Red 282	Anthraquinone	500	0.45
Rubine C-B	Violet 33	Monoazo	500	0.46
Black D-R	–	–	500	0.46*
Black D-B	–	–	500	0.46*
Navy D-4G	–	–	600	0.46*
Scarlet C-G	Red 54	Monoazo	500	0.46
Orange C-R	Orange 30	Monoazo	400	0.46
Navy C-R	–	–	600	0.46
Black D-2B	–	–	500	0.47*
Orange B-2R	Orange 25	Monoazo	500	0.47
Navy C-2R	–	–	600	0.47*
Navy D-2G	Blue 122	Monoazo	600	0.47*
Blue C-3G	Blue 295	Anthraquinone	600	0.48
Navy D-3GR	Blue 79:1	–	600	0.49
Red C-G	–	–	500	0.53*
Red D-B	Red 131	Monoazo	500	0.53
Scarlet B-RN	Red 158	Disazo	500	0.53
Blue D-6G	Blue 296	Anthraquinone	600	0.57
Turquoise C-G	Blue 87	Anthraquinone	600	0.59
Turquoise C-R	Blue 185	Anthraquinone	600	0.60
Blue B-R	Blue 56	Anthraquinone	600	0.61*
Brown C-3G	Brown 19	–	500	0.62*
Brown C-CM	–	–	500	0.63*
Orange D-G	Orange 32	Aminoketone	400	0.64
Yellow C-4R	Orange 54	Disazo	400	0.64
Blue D-4G	Blue 200	Monoazo	600	0.68
Black B-T	–	–	500	0.72*
Yellow B-6G	–	–	400	0.75
Green C-6B	Green 9	–	600	0.78
Red B-2B	Red 60	Monoazo	500	0.78
Violet C-3R	Violet 63	Monoazo	600	0.80

TABLE II *(continued)*

Dye (dispersol name)	*C.I. name (i.e. disperse...)*	*Chemical type*	*Monitoring wavelength (nm)*	*Relative retention time*
Navy C-4R	–	–	600	0.81*
Black C-3G	–	–	500	0.81*
Black C-MD	–	–	500	0.81*
Navy C-2G	–	–	500	0.81*
Yellow D-8G	Yellow 183	–	400	0.97
Yellow C-3G	–	–	400	1.06*
Orange C-2G	–	–	400	1.19
Orange C-B	Orange 13	Disazo	400	1.35
Yellow D-3R	Orange 60	Disazo	400	1.38
Rubine B-B	–	–	500	1.54
Violet B-G	Blue 72	Anthraquinone	600	1.59

prior to extraction, and with eluent after extraction. The extract can be directly injected. The antioxidant in the extractant produces two effects, firstly it precludes monitoring in the UV region due to its substantial UV absorbance, and secondly it is retained under the described chromatographic conditions and when monitored in the VIS region gives rise to a slight baseline inflection providing an internal standard for comparing retention time data.

Chlorobenzene extraction was recommended by West[4] and has been used for many years in this laboratory to prepare extracts for TLC purposes. Injection of chlorobenzene on to the chromatographic system utilised in this study caused difficulties, and was avoided by evaporating the extracts and then redissolving in eluent. This has the advantage of permitting UV as well as VIS region monitoring and judging from the comparisons we have made is not causing additional dye decomposition.

Chromatographic optimisation. All chromatographic processes cause the analyte to be diluted and one of the most effective ways to minimise this effect in HPLC is to reduce the column volume. Columns of six different dimensions were studied

TABLE III

DIMENSIONS, INTERNAL VOLUMES, AND RELATIVE RESPONSES OF COLUMNS PACKED WITH 5-μm ODS-HYPERSIL WHEN USED TO CHROMATOGRAPH A STANDARD SOLUTION OF DISPERSOL NAVY B-T

The chromatographic conditions are described in the text. All columns were run at the same linear velocity of 8.3 cm min^{-1} and 5-μl injections of the dye solution were made.

Column	*Dimensions (length I.D. in mm)*	*Column volume (ml)*	*Relative response*	*Relative volume*
I	250 × 4.9	4.71	1.0	1.0
II	125 × 4.9	2.36	2.0	2.0
III	250 × 3.2	2.01	2.4	2.3
IV	125 × 3.2	1.00	4.2	4.7
V	250 × 2.2	0.95	4.4	4.9
VI	125 × 2.2	0.47	2.4	10.0

and the data generated are shown in Table III. Each column was packed with Hypersil ODS (5 μm) and tested with a standard dye solution using the eluent described above. For comparative purposes the detector sensitivity was held constant and the same linear velocity through each column was maintained.

It is apparent from Table III that with the exception of column VI a gain in sensitivity was achieved as the column volume decreased and this gain was roughly what would be predicted from the ratio of the column volumes. In practice, however, it becomes more difficult to pack efficient columns as the bore decreases and the slightly lower responses from columns IV and V are probably attributable to this effect. In the case of column VI the chromatogram indicated overloading and without changing to a smaller injection volume it would not be possible to exploit this column. From these experiments column IV was selected for routine use as it offered the best response per unit time, had a lower pressure drop than column V and provided adequate resolution of the dye components.

The detectors. The two detectors investigated in this work differed substantially in their cost. The single wavelength system, priced at £ 1800, was some 8–9 times cheaper than the LDA multi-wavelength detector. Does the additional information provided by the latter justify the extra expenditure? In attempting to make an impartial judgement the detectors were alternately attached to the same chromatographic column and samples were injected to assess the sensitivity and spectral information provided. Pure samples of single and multi-component dyes exhibiting absorbance maxima in the range 350–620 nm were injected. Very similar results were obtained with both detectors as is shown in Fig. 1, and minimum detection limits of about 200 pg (injected) were typical. It was noted that the bandwidth setting on the LDA system influenced the sensitivity and was optimal at about 20 nm. Dyes with maxima above 590 nm were detected with slightly less sensitivity by this detector. When applied to extracts from 2-mm fibres the absorbances of eluting dyes were noted to be

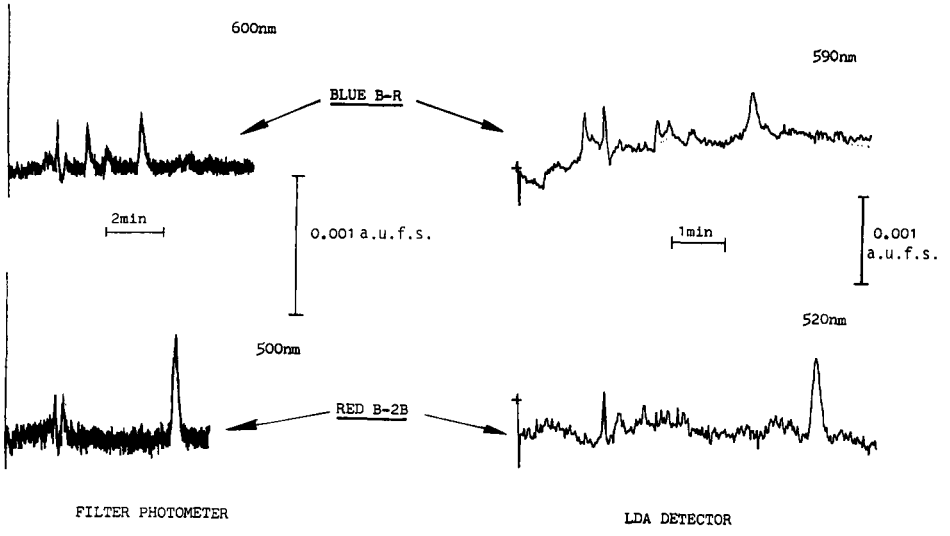

Fig. 1. Comparison of detector sensitivities. Amount of dye injected, Blue B-R; 210 pg, Red B-2B; 150 pg. Chromatographic conditions as described in the text.

more than 5 times higher than the minimum detection limit and hence both detectors have adequate sensitivity to cope with fibre analysis.

The spectral information provided by the two detectors is the area in which the LDA system proves superior. It can monitor at eight wavelengths simultaneously and hence the spectral region between 350 and 600 nm can be scanned in 50-nm increments and one channel still remains free for tuning in to a lower UV wavelength (*e.g.* 254 nm). This multi-channel facility ensures that with extracts containing dyes of different absorbance maxima no eluting compound goes undetected because the incorrect monitoring wavelength was selected, a process that cannot be guaranteed with a single wavelength instrument. Fig. 2 shows typical chromatograms from the LDA detector and it is apparent that UV absorbing species in addition to those absorbing in the VIS region of the spectrum provide chromatograms. Only the chlorobenzene extraction procedure can be applied if UV monitoring is contemplated, but no assessment of the reproducibility of extraction was made. The LDA system can in principle provide spectral information about co-eluting compounds which might permit discrimination of two fibre extracts which display no chromatographic differences. Despite the advantages just mentioned single wavelength monitoring should not be discounted; all of the dyed fibres examined could be discriminated by this mode of detection.

The way in which the LDA data are currently presented is in our view less than satisfactory. Printing out eight chromatograms is a time consuming process and a more efficient use of the information could come from presenting the eight absor-

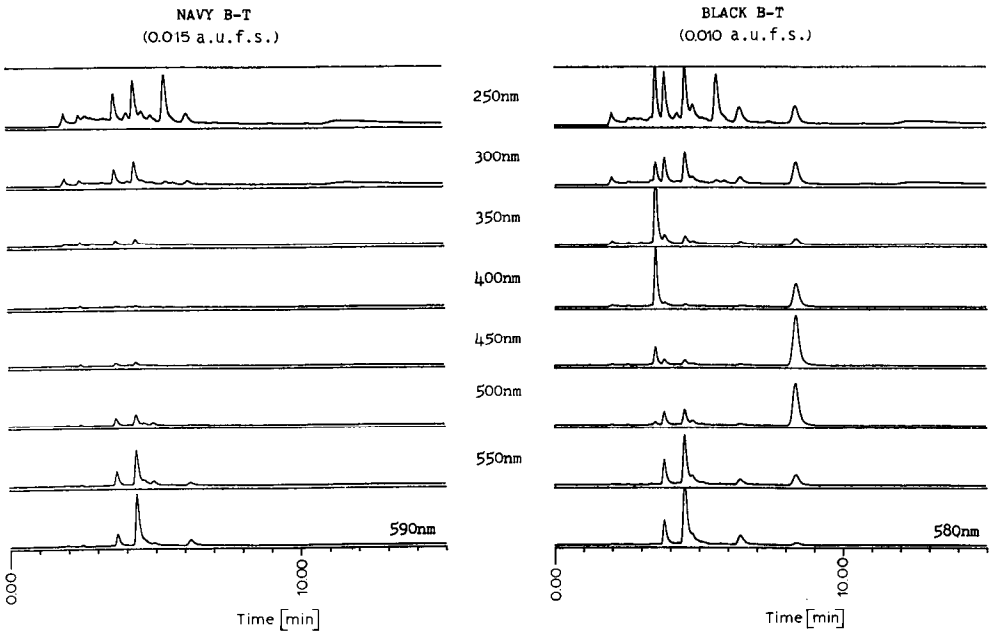

Fig. 2. Multi-wavelength monitoring of dyes extracted from 5 mm lengths of a single fibre. Chromatograms illustrate the additional information that can be gained by monitoring at wavelengths other than those related to the absorbance maxima of the dye extracts. Chlorobenzene was used as the extractant, and chromatographic conditions are described in the text.

TABLE IV

REPRODUCIBILITY DATA FOR EXTRACTS FROM 5-mm FIBRES DYED WITH THREE DIFFERENT DYES

The chromatographic conditions are described in the text and the extraction procedure used was that based on DMF. Each fibre was extracted separately and a 5-μl aliquot from each extract was then analysed using the single wavelength monitor at 600, 500 and 400 nm for Navy C-4R, Black D-B, and Yellow C-3G respectively. Peak numbering as in Fig. 3.

	Navy C-4R 5% shade Peak height (mm)			*Black D-B 9.5% shade Peak height (mm)*							*Yellow C-3G 1.5% shade Peak height (mm)*				
	I	*II*	*II/I*	*I*	*II*	*III*	*IV*	*I/III*	*II/III*	*IV/III*	*I*	*II*	*III*	*I/III*	*II/III*
	176	55	0.31	39	18	147	106	0.26	0.12	0.72	12	30	94	0.13	0.32
	169	53	0.31	45	22	174	129	0.26	0.13	0.74	12	30	86	0.14	0.35
	139	44	0.32	35	17	132	95	0.26	0.13	0.72	11	34	88	0.12	0.38
	155	48	0.31	32	15	123	89	0.26	0.12	0.72	13	32	93	0.14	0.34
	175	55	0.31	29	14	110	79	0.26	0.13	0.72	17	42	132	0.13	0.32
	159	50	0.31	36	17	135	98	0.27	0.13	0.73	14	38	102	0.14	0.37
	156	49	0.31	49	23	187	132	0.26	0.12	0.71	17	41	117	0.14	0.35
	183	57	0.31	42	20	166	118	0.25	0.12	0.71	14	34	102	0.14	0.33
	178	55	0.31								14	32	102	0.14	0.31
$\bar{x}$	165	51.8	0.31	38.4	18.2	146.7	105.7	0.26	0.12	0.72	13.8	34.8	101.8	0.13	0.34
σ	14.2	4.3	0.003	6.7	3.2	26.7	19.1	0.005	0.005	0.009	2.1	4.5	14.7	0.007	0.02
R.S.D. (%)	8.6	8.2	1.1	17.5	17.5	18.2	18.0	2.0	4.3	1.4	15.3	13.0	14.4	5.3	6.9

bance values of each peak maximum, together with just one or two chromatograms. This format would permit absorbance ratioing to be undertaken and we have shown that this treatment provides a highly reproducible way of recording the spectral characteristics of coloured compounds[8], which can also be highly discriminatory in some circumstances. The LDA system can also produce spectra as an alternative to chromatograms, but again it is our view that subtle variations in the broad spectra of most dyes are more readily compared by converting the data to absorbance ratios rather than overlaying spectra. Variation in an absorbance ratio can be recorded by a simple statistical parameter such as a standard deviation which permits greater objectivity in making comparisons than by mentally assimilating spectra.

Discrimination with single wavelength detection. The LDA detector used in our studies was only available on short-term loan, but longer term investigations have been carried out with the single wavelength instrument, and as was mentioned above this detector was able to discriminate all the fibres examined. It is apparent from Table II that dyes of a specific colour do not group closely together when arranged in elution sequence and hence the chromatographic and spectroscopic characteristics are not closely correlated. It would be interesting to relate retention to the chemical structure of the dyes but unfortunately most of the compounds studied were of undisclosed formulae. Despite the sparsity of structural information the range of retention times generated was sufficiently wide to offer considerable discrimination.

Fibre extracts containing two or more major components are relatively easy to compare as the peak height ratios provide parameters for evaluating similarity. Table IV summarises the results of replicate analyses of 5-mm fibres dyed with three different mixtures, and typical chromatograms are shown in Fig. 3. Although the

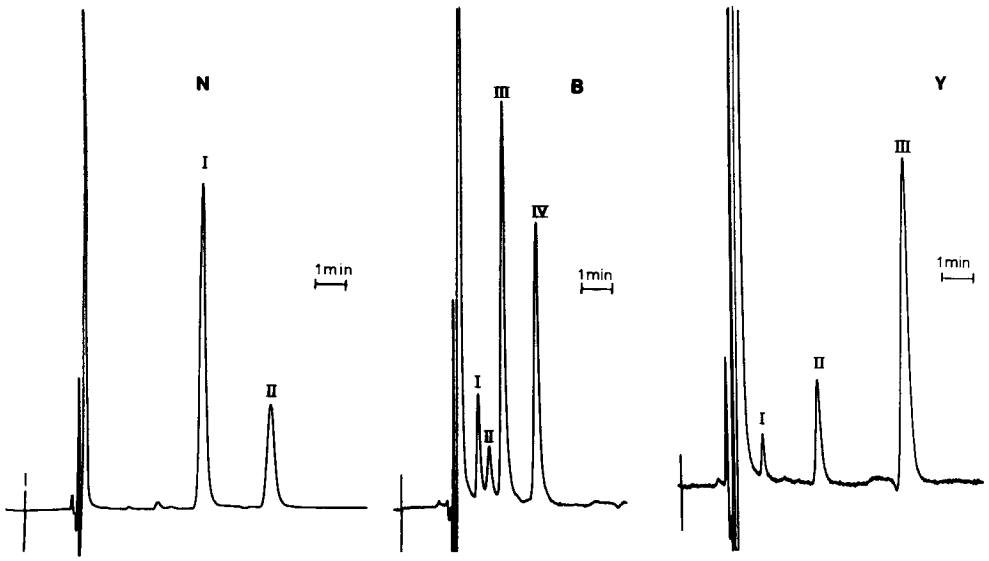

Fig. 3. Chromatograms of disperse dyes extracted from 5 mm lengths of a single fibre. The chromatographic conditions were as described in the text and the DMF extraction procedure was used. N = Navy C-4R, 5% shade, 600 nm monitoring wavelength, 0.02 a.u.f.s.; B = Black D-B, 9.5% shade, 500 nm monitoring wavelength, 0.01 a.u.f.s.; Y = Yellow C-3G, 1.5% shade, 400 nm monitoring wavelength, 0.005 a.u.f.s. The peak numbering on these chromatograms is identical with that used in Table IV.

relative standard deviation (R.S.D.) of the peak heights of specific components ranged from 8.2 to 18.2% in these extracts, when peak heights were compared the R.S.D. range fell to 1.1–6.9%. This implies that the method of extraction [N,N-dimethylformamide (DMF) in this instance] was not preferentially dissolving one component from a fibre dyed with a mixture.

Although 62% of the fibre extracts examined only contained one major dye, discrimination was possible because of differences in colour or retention characteristics. Even when dyes of the same colour yielded a major chromatographic peak indistinguishable by retention time alone the presence of minor peaks in the chromatogram invariably permitted samples to be distinguished. Whilst it cannot be disputed that the additional spectral information provided by an LDA detector would be of value in making a decision about dyes of similar chromatographic characteristics the experiments we have conducted suggest that it is not a necessity. Some typical chromatograms illustrating minor peak variation are shown in Fig. 4.

The ease with which HPLC can be quantitated also permits fibres of different shades to be compared. Provided fibres of similar length are examined the peak heights on the chromatograms are directly related to the dye levels present. By carrying out replication experiments on the control fibre it is possible to decide whether the observed levels are significantly different or not. The lightest shades on the Dispersol chart were about 0.1% (*i.e.* 100 g of the dye had been used to colour 100 kg

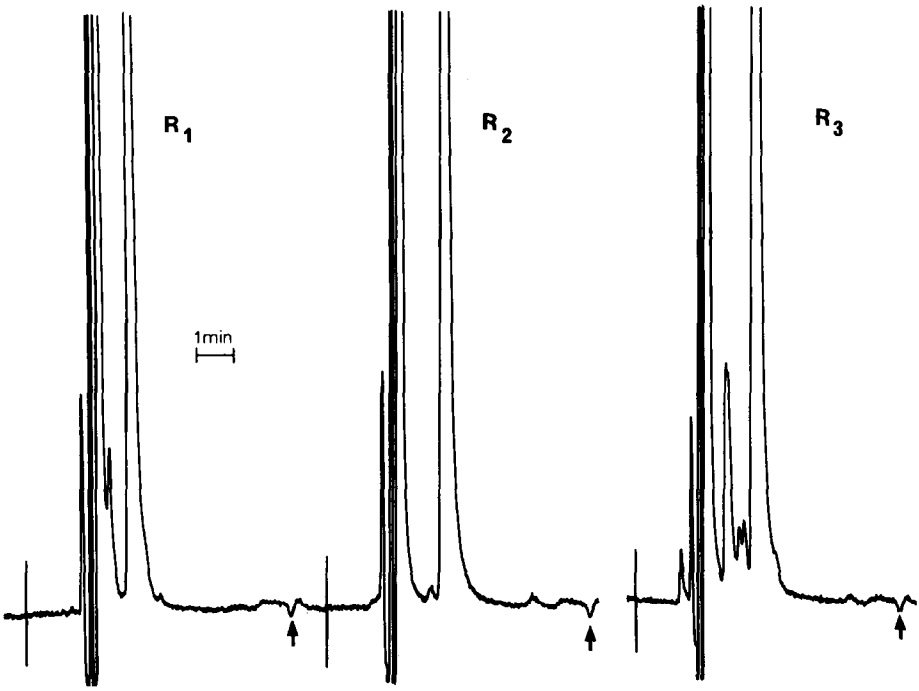

Fig. 4. Chromatograms of disperse dye extracts distinguishable by the presence of minor components. The dye extracts were from 5-mm lengths of single fibres, and the chromatographic conditions used were as described in the text. R_1, R_2 and R_3 refer to dyes Red C-3B, Red C-B, and Red D-2G respectively. The extracts were prepared using the DMF extractant and were monitored at 500 nm at 0.005 aufs. The base-line inflection marked as ▲ corresponds to the elution of the antioxidant in the extractant.

of fibre) and the highest about 10–20%. Differentiating such extremes poses no problems and fibres differing in % shade by a factor of two were amenable to chromatographic discrimination. Some of the very light shades were found to yield insufficient dye to permit very short lengths of fibre to be examined.

CONCLUSIONS

The described HPLC method was sufficiently sensitive to permit disperse dye chromatograms to be produced from single fibre extracts. Minimum detection levels of 200 pg of injected dyes were obtained with both a single wavelength filter photometer and a LDA multi-wavelength detector. These levels are 10–20 times those obtained with dye extracts originating from fibres of 2–5 mm in length, although for very light shades sensitivity was only just adequate. With a filter photometer the method permits discrimination on the basis of retention time, peak height ratios, the presence of trace impurities and on differences in shade. Multi-wavelength monitoring can provide additional discrimination by making both UV and VIS spectral data available for each eluting compound, but this detection mode is more expensive, requires a considerable amount of data processing time and in our opinion would benefit considerably if the programs offered absorbance ratios.

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