ELSEVIER

Journal of Chromatography A, 674 (1994) 263–270

JOURNAL OF CHROMATOGRAPHY A

Separation of acid, basic and dispersed dyes by a single-gradient elution reversed-phase high-performance liquid chromatography system

S.J. Speers*, B.H. Little, M. Roy

Northern Ireland Forensic Science Laboratory, 151, Belfast Road, Carrickfergus BT38 8PL, UK

Abstract

A gradient elution high-performance liquid chromatography system with an end-capped narrow-bore reversed-phase column and photodiode-array detection has been developed for the separation and characterisation of acid, basic and dispersed dyes. The system exhibits good reproducibility, efficiency and sensitivity. Using a range of commercial dyes, a database of relative retention times and spectral data has been produced. The HPLC method has been applied to the analysis of dyes extracted from single fibres.

1. Introduction

The examination of textile fibres forms a major part of casework at the Northern Ireland Forensic Science Laboratory. An important element of fibre analysis is the comparison of extracted dyes by thin-layer chromatography (TLC). While TLC is a good discriminating technique it does have the following limitations. Relatively large amounts of extracted dyes are required for the comparison of all components. Various dye classes require different eluent systems. Unless TLC conditions are strictly controlled the reproducibility of the R_F values can be poor [1]. TLC is essentially a qualitative technique.

An alternative chromatographic method is high-performance liquid chromatography (HPLC). HPLC systems have been developed for the analysis of disperse dyes extracted from polyester fibres [2,3], acid dyes from wool fibres [4], basic dyes from acrylic fibres [5,6] and natural and early synthetic dyes from archaeological textiles [7]. Comparisons of HPLC and TLC have concluded that HPLC offers better sensitivity and resolution [2–6]. Detection limits for the HPLC analysis of basic dyes [6] and disperse dyes [3] are 25 and 200 picograms respectively.

A limitation of previous HPLC systems, as with TLC is that each dye class requires different running conditions. Other workers have developed a general gradient elution system for the analysis of a range of dye classes using a reversed-phase column and ion-pairing agent but because of practical difficulties it was not recommended for use [8].

In this paper the separation and characterisation of acid, basic and dispersed dyes by gradient elution with a narrow-bore, end-capped reversed-phase column and photodiode-array (PDA) detector was investigated. The development of a database using standard dyes for the identifica-

* Corresponding author.

0021-9673/94/$07.00 © 1994 Elsevier Science B.V. All rights reserved
SSDI 0021-9673(94)00096-R

tion of dyes extracted from casework size fibres was also investigated.

2. Experimental

2.1. HPLC equipment

The equipment consisted of a Waters 600E multisolvent delivery pump, 717 Wisp autosampler and 996 photodiode-array detector (Millipore, Watford, UK). Analysis of data, gradient control and sample injection controlled by Millipore Millennium 2110 chromatography manager on a NEC 486–33 MHz personal computer with a Hewlett-Packard 550C deskjet printer. Real time display and printed chromatograms are Maxplots. This plots each dye at its maximum absorbance wavelength.

The PDA was operated over the range 380 to 600 nm, resolution 4.8 nm and data acquisition rate 1 spectrum per second. PDA flow cell 8 μl and path length 10 mm. Sample injection size 5 μl. System dead volume kept to a minimum. Analysis run time 25 min with 10 min equilibration time between injections.

2.2. HPLC column

Inertsil ODS-2, 150 × 2.1 mm I.D., 5 μm particle size and 150 Å pore size. (GL Sciences Inc., Tokyo, Japan).

2.3. HPLC eluent

Eluent A consisted of water deionised using a Milli-Q plus 185 with Milli-RO_{10} pretreatment pack. Eluent B consisted of HPLC grade acetonitrile. Both eluents were acidified to pH 2.5 by the addition of 1 ml per litre of 2.5 *M* sulphuric acid (analar grade). Gradient flow-rate 0.5 ml/min. The column was washed with 25 ml of HPLC grade methanol at the end of each batched analysis run. Eluents were continuously sparged with helium at 25 ml/min.

2.4. Standards

Samples of commercial acid, basic and dispersed dyes were obtained from Bayer UK and Ciba-Geigy UK. The dyes are listed in Table 1. Internal standard Rhodamine B (80%) C.I. 45170 was obtained from Aldrich Chemicals (Gillingham, UK). Rhodamine B is a basic dye not used in the dyeing of textile fibres.

For method development 0.01% (w/v) solutions of acid, basic and internal standard were prepared by dissolving 1 mg of dye in 10 ml of acidified acetonitrile–water (1:1). 0.01% solutions of dispersed dyes were prepared by first dissolving 10 mg of dye in 10 ml of methanol (0.1%) with a further dilution of 1:10 in acidified acetonitrile–water (1:1) to give a final concentration of 0.01% (w/v).

Pattern cards of known acid dyes on wool, basic dyes on acrylic and dispersed dyes on polyester fibres were obtained from Ciba-Geigy. Other fibres were obtained from the laboratory reference collection.

2.5. Extraction of fibre dyes

Basic dye extracts were obtained by placing single acrylic fibres of 10 mm or less in a capillary tube with 5 μl of formic acid–water (1:1). After sealing, the tube was heated to 100°C for 20 min. The dye extract was removed from the tube with a GC syringe and further diluted with 10 μl of eluent containing internal standard at a concentration of 0.7 ng per 5 μl injection.

Disperse dyes were extracted from polyester fibres using the procedure recommended by Wheals et al. [3]. Single fibres of 10 mm or less were placed in a capillary tube with 5 μl of chlorobenzene. The tube was sealed and heated in an oven to 130°C for 30 min. The tube was then shaken to separate the dye extract from the fibre. The end containing the fibre was removed. The dye extract was evaporated to dryness at 130°C for 30 min. The dye extract was redissolved in 15 μl of acidified acetonitrile containing internal standard at a concentration of 1 ng per 5 μl injection.

Table 1
List of commercial dyes with absorption maxima (λ_{max}), limits of detection (LOD) in picograms per 5 μl injection and relative retention times (RRT) with respect to Rhodamine B

Dye (CI name)	Manufacturer's name	Manufacturer	Dye class	RRT	λ_{max} (nm)	LOD (pg/5 μl)
Acid Red 299	Erionyl Bordeaux 5BLF	Ciba-Geigy	Disazo	1.098	520	230
Acid Red 361	Tectilon Red 2B	Ciba-Geigy		1.196	515	320
Acid Blue 225	Polar Blue RLS	Ciba-Geigy		1.244	605	640
Acid Blue 227	Tectilon Blue 4R-01	Ciba-Geigy	Anthraquinone	1.261	588	470
Acid Yellow 219	Tectilon Yellow 4R	Ciba-Geigy		1.285	396	375
Acid Red 127	Erionyl Red 2B	Ciba-Geigy	Monoazo	1.304	515	500
Acid Blue 260	Erionyl Blue RL	Ciba-Geigy	Anthraquinone	1.373	601	1250
Acid Orange 67	Erionyl Yellow RXL	Ciba-Geigy		1.401	435	235
Acid Red 260	Polar Red RLS	Ciba-Geigy	Disazo	1.492	527	1200
Basic Red 109	Maxilon Red M-4GC	Ciba-Geigy		0.107	506	110
Basic Blue 124	Maxilon Blue M2G	Ciba-Geigy		0.116	623	500
Basic Yellow 87	Maxilon Yellow M-4GL	Ciba-Geigy		0.152	412	170
Basic Yellow 91	Maxilon Yellow M-3RL	Ciba-Geigy		0.158	422	165
Basic Red 51	Maxilon Red M-RL	Ciba-Geigy	Azo	0.168	526	250
Basic Blue 151	Maxilon Blue M-G	Ciba-Geigy		0.201	625	800
Basic Red 46	Astrazon Red FBLN	Bayer	Monoazo	0.819	531	250
Basic Blue 3	Maxilon Blue 5G	Ciba-Geigy	Oxazine	0.821	650	70
Basic Yellow 28	Maxilon Golden Yellow	Ciba-Geigy	Methine	0.874	444	60
Basic Red 14	Maxilon Red 49	Ciba-Geigy	Cyanine	0.881	519	400
Basic Yellow 21	Astrazon Yellow 7GLL	Bayer	Polymethine	0.928	420	71
Basic Blue 147	Astrazon Blue F2RL	Bayer		0.942	584	75
Basic Red 27	Maxilon Pink B	Ciba-Geigy	Methine	0.943	531	40
Basic Red 18:1	Maxilon Red 2GL-N	Ciba-Geigy	Monoazo	0.978	480	120
Basic Violet 16	Astrazon Red Violet 3RN	Bayer	Methine	0.982	547	50
Basic Violet 21	Astrazon Violet F3RL	Bayer	Polymethine	1.038	558	180
Dispersed Yellow 82	Terasil Flavine 8GFF	Ciba-Geigy	Methine	0.859	475	460
Dis Violet 95	Terasil Bordeaux 2B	Ciba-Geigy	Azo	1.164	520	330
Dis Orange 45	Terasil Orange 5RL	Ciba-Geigy	Monoazo	1.211	459	380
Dis Yellow 211	Terasil Yellow 4G	Ciba-Geigy	Monoazo	1.221	445	80
Dis Blue 125	Terasil Navy SGL	Ciba-Geigy	Monoazo	1.254	584	195
Dis Red 151	Terasil Red 4G	Ciba-Geigy	Disazo	1.266	503	750
Dis Violet 57	Terasil Violet BL	Ciba-Geigy	Anthraquinone	1.285	541	1280
Dis Blue 165	Terasil Blue BG-01	Ciba-Geigy	Monoazo	1.326	610	110
Dis Red 349	Terasil Red 3GS	Ciba-Geigy	Azo	1.355	497	210

Due to a technical problem with the PDA detector it was not possible to analyse extracted acid dyes.

3. Results and discussion

3.1. Choice of column

The Inertsil-ODS2 column consists of octadecyl groups bonded onto ultra-pure silica. The bonded phase has been extensively end-capped to remove silanol group interferences, enabling the separation of basic and other polar compounds without the addition of ion-pairing agents. Using an Inertsil ODS-2 column, an acetonitrile water gradient HPLC system has been developed for the analysis of acid, basic and neutral drugs [9]. As the chemical structures of drugs and dyes are similar the application of the column to the separation of acid, basic and dispersed dyes has been investigated.

3.2. Optimisation of gradient

Mixtures of standard acid, basic and dispersed dyes were initially chromatographed with a linear gradient of 2% to 98% acidified acetonitrile for 20 min and held at 98% for 5 min. This was to take into account the wide range of polarities of the dye mixtures. The gradient was repeated over a range of pH values and flow-rates. The optimum eluent pH value was 2.5 and the flow-rate was 0.5 ml/min.

All chromatograms of mixtures exhibited symmetrical peak shape and good efficiency. The gradient range was further optimised (20–98% acidified acetonitrile) to remove the initial portion of the gradient at which no peaks were eluted. The linear gradient time was reduced from 20 min to 10 min with minimal effect on resolution. Table 2 lists the optimum gradient profile.

Although the gradient run was completed in 15 min it was a further 10 min before the last peak was eluted. The cause of the lag can be explained by an inherent dead volume in the HPLC pump due to the pulse dampers (volume 2.5 ml) and the tubing between the low pressure mixing chamber and the pump (volume 2.5 ml). The system was therefore operating isocratically at the initial gradient conditions for the first 10 min of the chromatographic run. The pulse dampers can be removed, but since this would increase baseline noise and because the chromatographic runs were reproducible this was not done.

Table 2
Optimum gradient profile

Time (min)	Flow-rate (ml/min)	Eluent		
		%A	%B	%C
Initial	0.5	80	20	0
10	0.5	2	98	0
15	0.5	2	98	0
20	0.5	80	20	0
40	0.1	0	0	100

Eluent A acidified water, eluent B acidified acetonitrile, eluent C methanol.

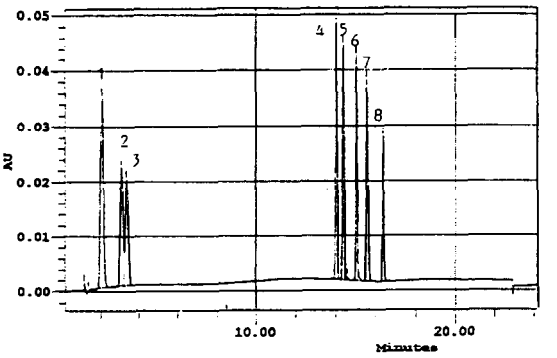


Fig. 1. Maxplot HPLC chromatogram of basic dye mix 1. Chromatographic conditions: Inertsil ODS-2 column, 150 × 2.1 mm I.D.; eluent, acidified acetonitrile–water pH 2.5; gradient 20–98% acidified acetonitrile over 10 min; flow-rate 0.5 ml/min; PDA detector operating over the range 380 to 600 nm; concentration approx. 100 ng/μl; Peaks: 1 = Basic Red 109; 2 = Basic Yellow 87; 3 = Basic Yellow 91; 4 = Basic Blue 3; 5 = Basic Yellow 28; 6 = Basic Blue 147; 7 = Basic Red 18:1; 8 = Basic Violet 21.

Figs. 1–7 show representative chromatograms of the dye mixtures (concentration approx. 100 ng/μl). In the legends the components of the dye mixtures are listed with their corresponding peak.

3.3. Reproducibility of the HPLC system

Basic dye mixture 2 was injected 10 times with 10 min equilibration time at initial conditions at the start of each run. The relative standard

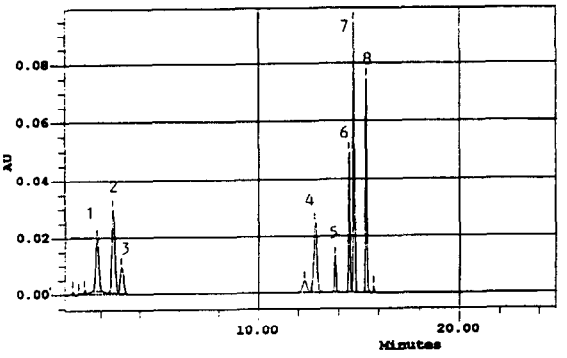


Fig. 2. Maxplot HPLC chromatogram of basic dye mix 2. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Basic Blue 124; 2 = Basic Red 51; 3 = Basic Blue 151; 4 = Basic Red 46; 5 = Basic Red 14; 6 = Basic Yellow 21; 7 = Basic Red 27; 8 = Basic Violet 16.

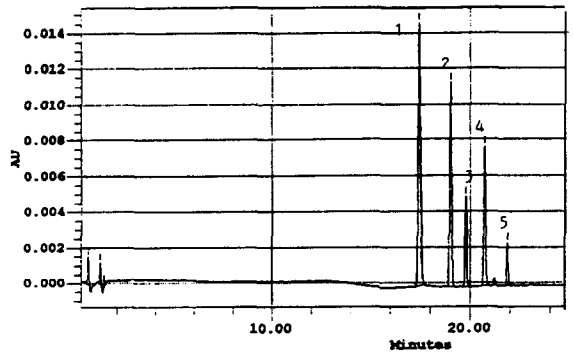


Fig. 3. Maxplot HPLC chromatogram of acid dye mix 1. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Acid Red 299; 2 = Acid Red 361; 3 = Acid Blue 225; 4 = Acid Red 127; 5 = Acid Blue 260.

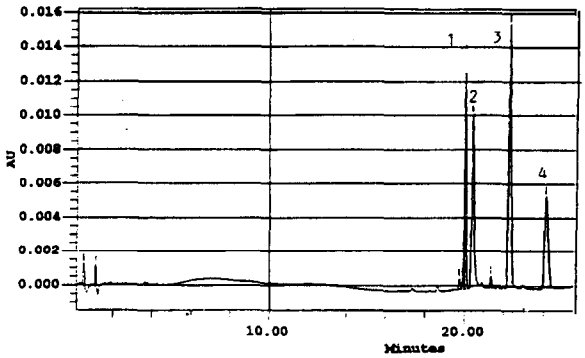


Fig. 4. Maxplot HPLC chromatogram of acid dye mix 2. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Acid Blue 227; 2 = Acid Yellow 219; 3 = acid Orange 67; 4 = Acid Red 260.

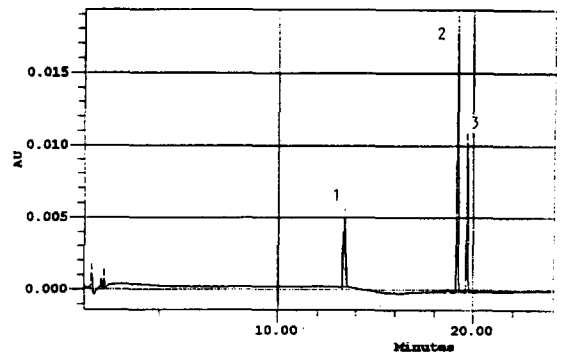


Fig. 5. Maxplot HPLC chromatogram of disperse dye mix 1. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Dispersed Yellow 82; 2 = Dispersed Yellow 211; 3 = Dispersed Blue 125.

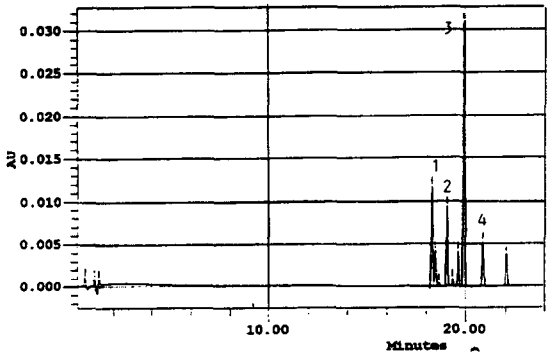


Fig. 6. Maxplot HPLC chromatogram of disperse dye mix 2. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Dispersed Violet 95; 2 = Dispersed Orange 45; 3 = Dispersed Blue 165; 4 = Dispersed Red 151.

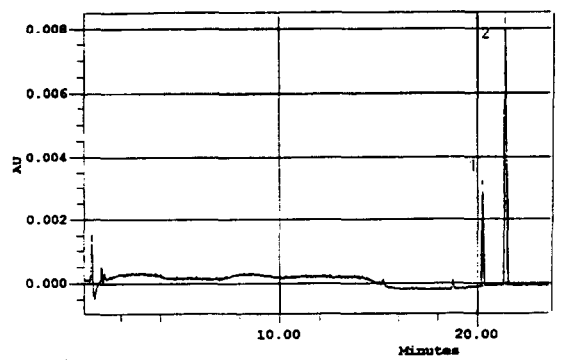


Fig. 7. Maxplot HPLC chromatogram of disperse dye mix 3. Chromatographic conditions as in Fig. 1. Concentration approx. 100 ng/μl. Peaks: 1 = Dispersed Violet 57; 2 = Dispersed Red 349.

deviation (R.S.D.) of the relative retention time (with respect to Rhodamine B) of the first eluted peak (basic blue 124) was 0.76%. The R.S.D. for the last peak basic violet 16 was 0.2%. Increasing the equilibration time between injections did not reduce the R.S.D.s further. The Inertsil ODS-2 column was used daily over a period of 10 weeks, with no deterioration in dye peak shapes.

3.4. Detection limits of PDA detector

Concentrations of the five standard dye mixtures ranging from 25 to 0.5 ng per 5 μl injection were analysed to determine the limit of detection

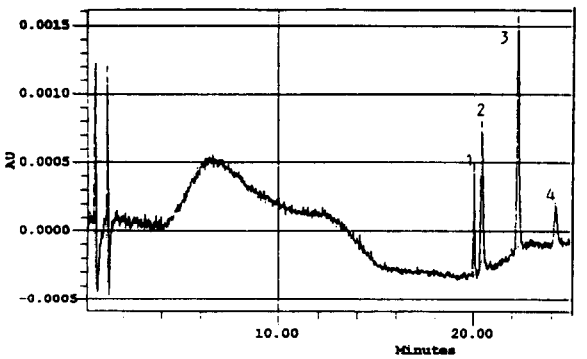


Fig. 8. Example of a Maxplot HPLC chromatogram of acid dye mix 2, used for the determination of limits of detection. Chromatographic conditions as in Fig. 1. Concentration 2000 pg/5 μl injection. Peaks: 1 = Acid Blue 227; 2 = Acid Yellow 219; 3 = Acid Orange 67; 4 = Acid Red 260.

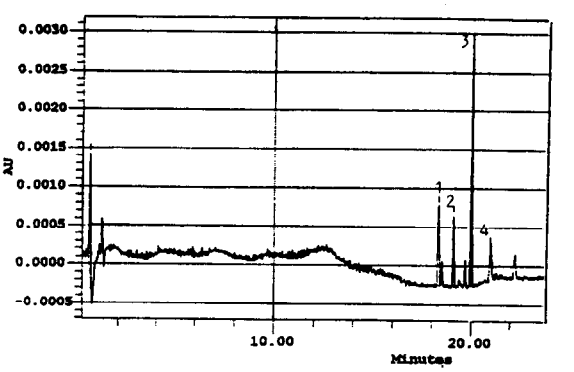


Fig. 10. Example of a Maxplot HPLC chromatogram of dispersed dye mix 2, used for the determination of limits of detection. Chromatographic conditions as in Fig. 1. Concentration 2000 pg/5 μl injection. Peaks: 1 = Dispersed Violet 95; 2 = Dispersed Orange 45; 3 = Dispersed Blue 165; 4 = Dispersed Red 151.

for each individual dye. The limit of detection (LOD) was calculated on a signal to noise ratio S/N of 3:1.

The LOD for the standard basic dyes ranged from 40 pg for basic red 27 to 800 pg for basic blue 151. For standard acid dyes the range was 230 pg for acid red 299 to 1250 pg for acid blue 260. For standard dispersed dyes the range was 80 pg for dispersed yellow 211 to 1280 pg for dispersed violet 57. The majority of the dyes had an LOD between 50 and 500 pg. All figures quoted are per 5 μl injection. For examples see Figs. 8–10.

Studies in this laboratory indicate the concentration of dyed fibres to be of the order of a few nanograms or more per 10 mm depending on the percentage dyeing.

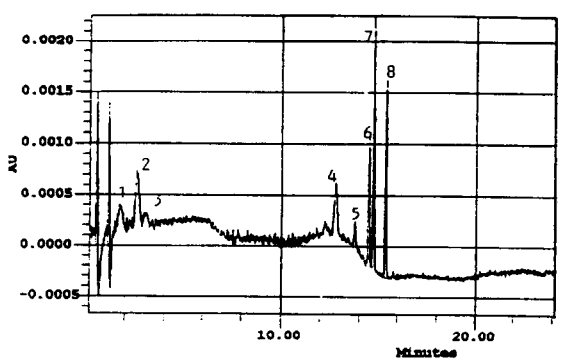


Fig. 9. Example of a Maxplot HPLC chromatogram of basic dye mix 2, used for the determination of limits of detection. Chromatographic conditions as in Fig. 1. Concentration 500 pg/5 μl injection. Peaks: 1 = Basic Blue 124; 2 = Basic Red 51; 3 = Basic Blue 151; 4 = Basic Red 46; 5 = Basic Red 14; 6 = Basic Yellow 21; 7 = Basic Red 27; 8 = Basic Violet 16.

3.5. Baseline stability

Refractive index (RI) disturbances at the PDA detector flow cell were minimised by the low pressure mixing of eluents A and B by the Waters 600E multisolvent delivery system. The baseline at high detector sensitivity (<0.002 AUFS) is acceptable, although not as smooth as an isocratic HPLC chromatogram. A previous attempt to develop a gradient system using high pressure mixing of eluents with two pumps, T-piece and a dynamic mixer produced excessive baseline drift and was unsuitable for detecting low levels of dyes.

3.6. Characterisation of dyes extracted from textile fibres

Spectral data of the standard commercial dyes separated by the HPLC system, together with their relative retention times with respect to Rhodamine B (as listed in Table 1) were stored

in a library created using the Millennium PDA software. Chromatograms of unknown fibre dye extracts can be searched against this library. A display of the best library match is overlaid onto the spectrum of the unknown dye. The system was tested using 10 fibre dye extracts.

Five known dispersed dyed fibres and five known basic dyed fibres 10 mm in length were removed from Ciba-Geigy pattern cards. A range of colours were chosen. The dyes were extracted according to the procedures described in the experimental section. The five dispersed dyed fibres contain blue 125, yellow 82, orange 45, red 151 and violet 57. The five basic dyed fibres contain blue 147, blue 124, yellow 21, yellow 87 and red 51.

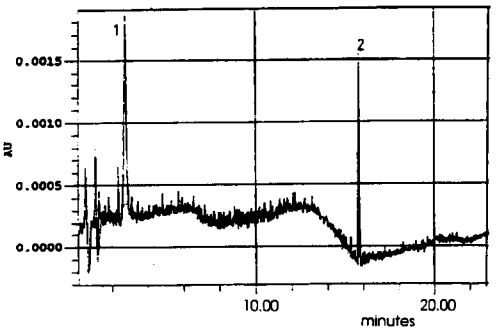
In all cases the extracted dyes gave identifiable peaks. All extracted dyes were matched with their respective standard library dye on the basis of relative retention time with respect to Rhodamine B and visible spectrum match.

The amount of dye extracted from the fibres was determined from their peak heights. This value was further multiplied by 3 to account for injecting 5 μl from a total fibre extract volume of 15 μl. Table 3 lists the dyes with their relative retention times and quantity of dye in 10 mm of fibre. See Fig. 11 for an example of a chromatographed fibre dye extract.

Table 3
Identification of dyes extracted from 10-mm fibres

Dye	RRT	Standard dye RRT	Quantity of dye in fibre (ng)
Basic Blue 147	0.947	0.942	2.1
Basic Blue 124	0.118	0.116	6.0
Basic Yellow 21	0.922	0.928	1.2
Basic Yellow 87	0.155	0.152	5.1
Basic Red 51	0.161	0.168	2.6
Dis Blue 125	1.259	1.254	4.5
Dis Yellow 82	0.850	0.859	8.9
Dis Orange 45	1.203	1.211	5.8
Dis Red 151	1.269	1.266	12.2
Dis Violet 57	1.277	1.285	10.6

Relative retention time (RRT) with respect to Rhodamine B, the internal standard.



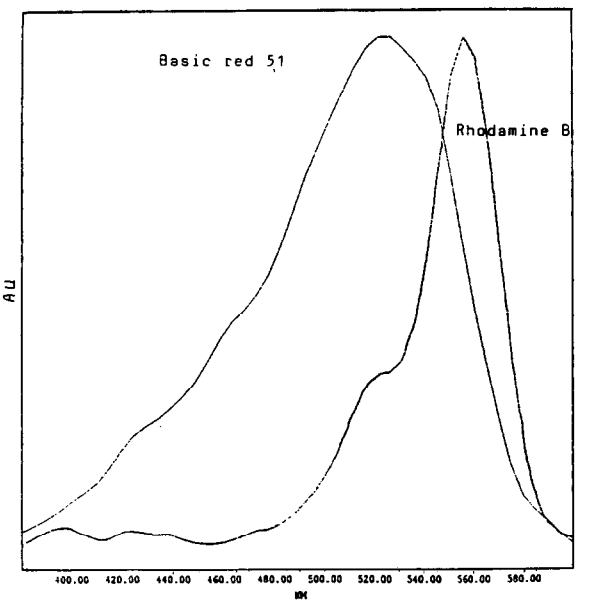


Fig. 11. Example of a Maxplot HPLC chromatogram and visible spectrum of a 10 mm fibre dye extract. Fibre dyed with basic red 51. Chromatographic conditions as in Fig. 1. Peaks: 1 = Basic Red 51; 2 = Rhodamine B (internal standard).

4. Conclusions

A single-gradient HPLC system has been developed for the separation of acid, basic and dispersed dyes. The Inertsil ODS-2 column has minimal residual silanol groups enabling the separation of a range of dye classes with good efficiency and peak shape without the need for ion-pairing agents. The HPLC system with a

PDA detector has detection limits typically less than 1 ng. Basic and dispersed dyes extracted from 10 mm fibres were qualitatively and quantitatively identified using a database of relative retention times and spectral data of standard commercial dyes. The database library will be upgraded with more commercial dyes.

Further work is in progress comparing the HPLC system with TLC and high-performance TLC in the analysis of casework size fibres. The results of this work will decide if the HPLC system is to be used in forensic casework.

A more detailed study relating the chemical structures of the dyes to their separation by the HPLC system is also ongoing.

5. References

[1] D.K. Laing and L. Boughey, unpublished results.
[2] J.C. West, *J. Chromatogr.*, 208 (1981) 47.
[3] B.B. Wheals, P.C. White and M.D. Paterson, *J. Chromatogr.*, 350 (1985) 205.
[4] D.K. Laing, R. Gill, C. Blacklaws and H.M. Bickley, *J. Chromatogr.*, 442 (1988) 187.
[5] R.M.E. Griffin, T.G. Kee and R.W. Adams, *J. Chromatogr.*, 445 (1988) 441.
[6] R.M.E. Griffin, S.J. Speers, L. Elliott, N. Todd, W. Sogomo and T.G. Kee, *J. Chromatogr. A*, 674 (1994) 271.
[7] Ch.-H. Fischer, M. Bischof and J.G. Rabe, *J. Liq. Chromatogr.*, 13(2) (1990) 319.
[8] J. Adams, N.L. Babb, K.E. Haddock and A.W. Hartshorne, unpublished results.
[9] R. Gill and S. Cosby, unpublished results.