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An improved high-performance liquid chromatography system for the analysis of basic dyes in forensic casework

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Abstract

Acrylic fibres are frequently encountered as physical evidence in casework in the Northern Ireland Forensic Science Laboratory. Separation and analysis of the basic dyes, used in the garment industry for the dyeing of acrylic fibres, was carried out by isocratic HPLC with a narrow bore column. The HPLC eluent used was a mixture of methanol and aqueous ammonium acetate solution (pH 9.76) in a ratio of 9:1. The sensitivity of the system is such that the dyes extracted from 0.2 cm of a black acrylic fibre could be detected using three injections on a single channel detector at wavelengths of 400, 500 and 600 nm (for yellow, red and blue dye components respectively).

1. Introduction

Textile fibres contain a number of dyes which may be mixed in different proportions to give varying shades and colours. Methods used by forensic scientists for the comparison of fibres are: (a) an identification of the fibre type by polarising and infrared spectroscopy and (b) examination of the dye components by comparison microscopy, microspectrophotometry and thin-layer chromatography (TLC) [1].

High-performance liquid chromatography (HPLC) systems have been described for the analysis of acid, disperse [2], direct and pigment dyes in forensic science. These include the analysis of fibres [3–7], lipstick smears [8,9], inks [10,11], illicit tablets [12], and counterfeit notes [13].

Acrylic fibres are one of the most frequently occurring fibre types encountered in casework in the Northern Ireland Forensic Science Laboratory. These are found particularly in terrorist incidents where masks and gloves have been used. Basic (cationic) dyes are commonly used in the dyeing of these acrylic fibres.

A few HPLC systems have previously been reported for the analysis of basic dyes. Individual [14,15] or a narrow range of basic dyes [16,17] can be analysed with these systems.

An HPLC method for basic dyes was described previously by this laboratory [18]. It consisted of gradient elution with mixtures of methanol and aqueous ammonium acetate solutions on a normal-phase silica column (160 × 4.5 mm I.D.) and was found to provide acceptable separation of twenty-one basic dyes. However, baseline drift was found to occur in the gradient system due to refractive index changes. This was most noticeable at visible wavelengths, when high sensitivity (0.0005 AUFS) was required for the analysis of the small quantities of extracted fibre dyes available in forensic science casework.

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An isocratic HPLC system was developed in this laboratory [5] for the analysis of basic dyes. This gave acceptable separation of seventeen of the standard dyes analysed.

Acrylic fibres were obtained from local manufacturers, dye pattern cards and casework. The dyes were extracted from the fibres and analysed on the HPLC system using a single channel detector and an autoinjector. Three injections were required to detect the dyes at 400, 500 and 600 nm. Using this system picogram levels of basic dyes were detected and dyes extracted from less than 0.2 cm of heavily dyed fibres could be detected.

The stability of the dyes was assessed during extraction, after extraction and over a six day period. Dye batch variations were examined on fibres from different areas of the garments and over the length of a single fibre.

2. Experimental

The equipment consisted of Waters Assoc. Model 510 HPLC pump, Model 680 gradient controller and a Severn Analytical 6504 absorbance detector. A Waters 990 photodiode array detector was used, coupled to a NEC personal computer. The photodiode array detector had 512 diodes and a wavelength range of 190 to 800 nm with a resolution of 1.4 nm. The cell had a volume of 8 μl and a 10 mm pathlength. The results were recorded either with a Phillips PM 8251 single-pen recorder or on a Tandon microcomputer utilising the Drew Scientific Roseate chromatography system for data capture and manipulation. The autoinjector consisted of a Waters Associates Model 712 Wisp instrument. This was electronically linked to the Severn detector and the Tandon computer.

A Shandon stainless-steel column (250×4.5 mm I.D.) was packed with Phase-Sep Spherisorb silica (5 μm). A flow-rate of 2 ml/min was used. A Jones Chromatography stainless-steel column (250×2.1 mm I.D.) or a Phase-Sep (250×2.0 mm I.D.) column packed with Spherisorb W silica (5 μm) was used at a flow-rate of 0.46 ml/min. A Rheodyne multiloop injector was used for all manual injections (10 or 20 μl loop) and a Waters Guard-Pack precolumn module (with a silica precolumn insert) was fitted.

The eluent consisted of a solution of methanol–aq. ammonium acetate (pH 9.76) (9:1, v/v). This was prepared by adding 94 ml of concentrated ammonia and 22 ml of glacial acetic acid to 884 ml of deionised water. The pH was adjusted to 9.76 by addition of either a few drops of ammonia or of glacial acetic acid. This was then diluted with methanol (1:9) before use. All solvents were filtered under vacuum through a Gelman Science 0.2 μm FP Vericel TM membrane. The deionised water and methanol were degassed for 15 min using helium displacement of air.

The column was conditioned for 40 min in the eluent before use. To maintain reproducibility of retention times and separation it was necessary to clean out the column with methanol–water (9:1) for at least 1 h after use. This was mainly because of the pH of the eluent degrading the silica despite the use of an in-line filter (precolumn). For the same reason the eluent should not be left static in the column.

2.1. Standards

Chromatographic performance of the HPLC column was monitored by injecting a mixture of basic yellow 21, basic red 14, and basic blue 45 (0.002%) at the beginning of each day and at intervals throughout the day using a detecting wavelength of 280 nm.

Samples of basic dyes were obtained from Bayer UK and from Ciba Geigy UK and are: Maxilon yellow M-4GL (basic yellow 87), Astrazon Golden yellow Gl-E (basic yellow 28), Maxilon yellow M-3RL (basic yellow 91), Maxilon brilliant flavine 10GFF (basic yellow 40), Astrazon yellow 7GLL (basic yellow 21), Astrazon blue FGRL, Maxilon blue M-G (basic blue 151), Astrazon blue BG (basic blue 3), Maxilon blue M2G (basic blue 124), Astrazon red GTLN (basic red 18:1), Maxilon red M-4GL (basic red 109), Maxilon brilliant pink B (basic red 27), Maxilon red GRL Pearls (basic red 46), Maxilon Brilliant red 4G liquid (basic red 14),

Maxilon red M-RL (basic red 51), Maxilon red B-LN liquid (basic red 22), Astrazon red violet 3RN (basic violet 16).

Solutions of these dyes were prepared by dissolving 10 mg of dye in 10 ml of methanol and further diluting to the required concentration.

Fibre samples were obtained from casework and from a laboratory reference collection. Two Courtaulds fibres I and II were analysed. A Ciba Geigy pattern card which contained known dyes on acrylic fibre was used, and fibres were obtained from three local manufacturers.

2.2. Extraction of dyes

This was carried out using a 1:1 solution of HPLC-grade formic acid and deionised water. Each fibre was inserted into a capillary tube in which one end had been sealed with a bunsen burner. The fibres were pushed to the bottom of the tube. A volume of 3 μl of formic acid–water was added and the tube sealed in an oven at 100°C for 20 min. The extracts were then removed from the tubes using a 5-μl GC syringe. The solution was spotted onto a TLC plate or was diluted with 60 μl of the HPLC eluent. Aliquots of 15–20 μl were injected onto the HPLC column. TLC analysis was carried out on Kieselguhr DC Alufolien silica gel 60 F254 plates. The fibre extracts were spotted onto the TLC plate and eluted with a mixture of chloroform–methyl ethyl ketone–acetic acid (glacial)–formic acid (8:6:1:1, v/v).

3. Results and discussion

An eluent compositon was required which would separate a mixture of unknown dyes in a maximum run time of 15 min per sample injection. Initial investigations were carried out on a standard normal-phase silica HPLC column (250×4.5 mm I.D.). The eluent composition was determined by varying the methanol–aq. ammonium acetate (pH 9.76) ratio and the concentration of the ammonium acetate solution. A 9:1 ratio of methanol–aq. ammonium acetate (0.71:0.34 mol/l) solution was found to give the best separation and resolution of the commercial basic dyes.

Three dyes (basic yellow 21, basic red 14 and basic blue 45) were used to check the performance and reproducibility of the column. These dyes were injected at the start of each day and at intervals throughout the day. The wavelength chosen for the detection of these standards was 280 nm as most of the commercial dyes display a broad absorption at this wavelength.

UV–Vis spectroscopy was used to determine the wavelength maximum of the individual dyes. Wavelengths of 400, 500 and 600 nm were chosen for the detection of yellow, red and blue basic dyes, respectively. Some of the dyes were detected at two wavelengths due to the broad absorption spectrum of the dyes. Unknown samples such as in fibre extracts may need to be reanalysed at other wavelengths. The wavelengths for reanalysis can be determined from the visible spectrum of the fibres using microspectrophotometry, for example (Fig. 1) analysis at 450, 550 and 620 nm would be required to detect the dye components.

A variable-wavelength detector was used but only one wavelength could be sampled at any one time. This is a lengthy process requiring a

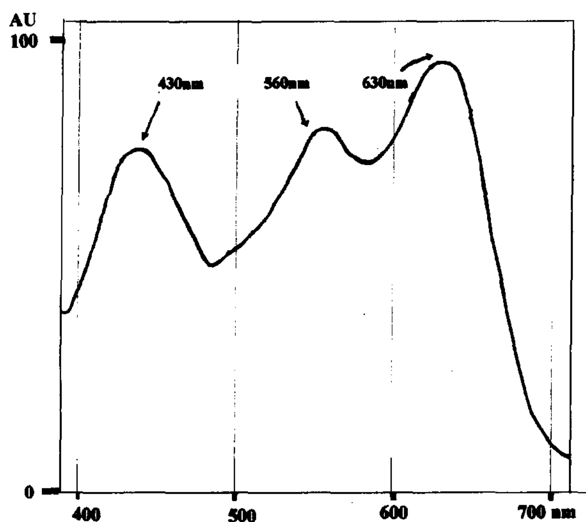

Fig. 1. Visible spectrum (400–700 nm) of a pale coloured casework fibre (Fibre 7) showing the wavelength maxima at approximately 430, 560 and 630 nm.

minimum of 45 min per fibre analysed. Attempts to change the wavelengths throughout the analysis were not feasible especially when an unknown dye mixture is present in the fibres. An autoinjector was incorporated into the HPLC system and the detector was programmed to change automatically the wavelength every 17 min. The dye extract was injected every 17 min in conjunction with the wavelength changes for the analysis of the yellow, red and blue dye components in the dye mixture. The program was repeated after every third injection.

A narrow bore HPLC column (250 × 2.1 mm I.D.) was then investigated to see if the resultant increase in sensitivity would facilitate the use of a diode array detector. The mixture of methanol–ammonium acetate (9:1) already described was used as the eluent at a flow-rate of 0.46 ml/min. The commercial basic dyes were injected onto this HPLC column individually and as a mixture of yellow, red and blue dyes. The retention times of the dyes on the HPLC column are shown in Table 1. The separation of the dye mixture is shown in Fig. 2.

The majority of the dyes were eluted between 3 and 6 min. It was found that the dyes were not all resolved by the HPLC system but if the coeluting components were of a different colour they could in most cases be identified by a combination of retention time and detecting wavelength (colour). If, however, the coeluting dyes are the same colour (basic yellow 87 and 91) then the use of a diode array detector, which has the capability of giving both retention and spectral data, would facilitate the identification of these dyes (Fig. 3). The ability of the diode array detector to detect all wavelengths at one time means that only one injection would be required and a more concentrated solution of the dye extract would be available.

A comparison of the limits of detection (LOD) of the single channel detector and the diode array detector was carried out on the narrow bore column. The lowest detectable limit (signal-to-noise, S/N, of at least 3:1) was determined for each dye.

A 0.002% stock solution of the dyes was prepared (not corrected for the pure dye content of the commercial dye) and diluted such that 4 ng to 0.02 ng of dye in 10 μl of solution was injected onto the HPLC column. The absorbances of the dyes were measured and when plotted against concentration were found to obey Beer's law.

The average LOD at 280 nm (on the single channel detector, 0.0005 AUFS) was found to be 1 ng whereas at 400, 500 and 600 nm the LOD was found to be between 12 and 25 pg using the narrow bore (2.1 mm I.D.) column and 100 pg for the wide bore (4.5 mm I.D.) column. These limits would be improved by analysis at the wavelength maxima of each dye. The LOD for the diode array detector (0.002 AUFS) was found to be 3.4 ng (yellow 21), 3.2 ng (red 14) and 1.9 ng (blue 45). To obtain a whole visible spectrum of the dyes more dye than this would be required. This quantity is not usually available in most of the fibres (less than 1 cm in length) found in forensic casework.

Fibres (5 mm in length) were removed from pattern cards. The dyes were extracted (formic

Table 1
Retention times of standard dyes on the 4.5 mm I.D. and 2.1 mm I.D. HPLC columns

Dyes	4.5 mm I.D.	2.1 mm I.D.
Yellow 21	3.5	3.9
Yellow 28	4.8	5.1
Yellow 40	4.8	[a]
Yellow 87	9.3	9.6
Yellow 91	9.2	9.4
Red 14	4.5	4.3
Red 18:1	7.4	7.0
	16.1	14.7
Red 27	3.8	4.0
Red 46	3.7	3.9
	6.5	6.6
Red 51	7.0	6.3
Red 109	5.4	4.9
Red 22	4.2	[a]
Blue 3	4.2	3.8
Blue 124	4.8	4.6
Blue 151	3.3	4.0
Blue 45	12.5	11.2

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; flow-rate, 0.46 ml/min.
[a] Not analysed.

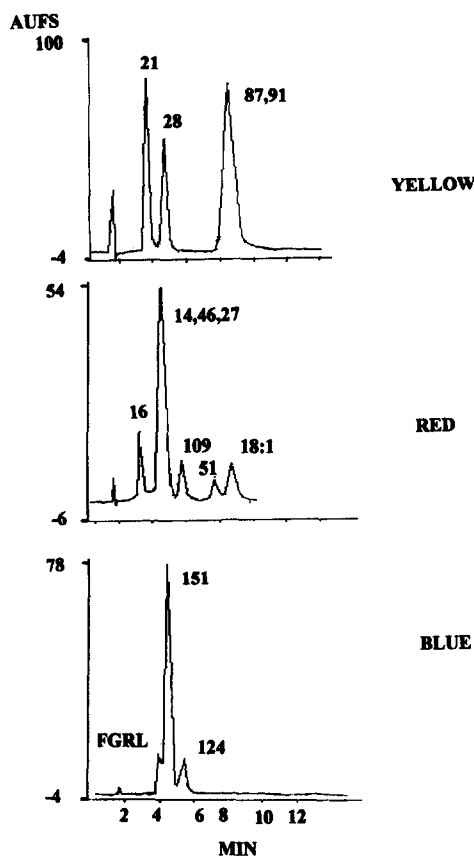

Fig. 2. HPLC analyses showing the separation of a mixture of yellow, red, and blue standard basic dyes using detecting wavelengths of 400, 500 and 600 nm respectively. Eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column, 2.1 mm I.D.; flow-rate, 0.46 ml/min.

acid–water) and analysed by HPLC. The peak heights of the dye components were compared with a known quantity (1 ng in 10 μl) of the standard dye. The results are shown in Table 2.

Problems could arise with the use of the autoinjector if the extracted dyes were to decompose prior to injection as some samples will then be analysed after 24 h.

Some of the basic blue dyes are known to degrade in the pyridine and water extracting solvent [19,20] giving a different coloured solution. This is possibly due to deprotonation of the dye. A similar mechanism may be occurring in formic acid and water. It was found that for some of the black fibres examined pink solutions

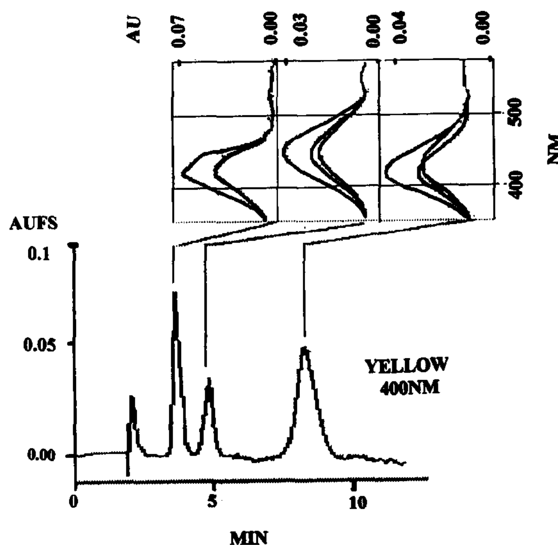

Fig. 3. HPLC analysis and visible spectra obtained using a diode array detector. Eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column 2.1 mm I.D.; flow-rate, 0.46 ml/min.

were obtained on extraction. The colour changed to blue when the extract was spotted onto a TLC plate or when diluted with the HPLC eluent. Other studies in this laboratory [18] have shown that basic blue 151 decomposed in solution to a red dye over a period of one week.

The time and temperature of extraction, the

Table 2
Quantities of dyes in pattern card fibres

Dye	Peak height standard	Peak height fibre/mm	Amount of dye (ng)
Blue 151	76.86	42.36	1.1
Blue 124	98.77	51.67	1.0
Blue 3	89.83	59.68	1.3
Red 109	95.77	20.92	0.5
Red 51	61.65	40.92	1.3
Red 46	73.79	81.54	1.1
Red 14	52.04	41.06	1.6
Red 18:1	17.25	12.88	1.5
Yellow 87	39.72	5.08	0.3
Yellow 91	25.87	19.99	1.6
Yellow 28	31.09	13.02	0.9

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity 0.005 AUFS; column 2.1 mm I.D.; flow-rate, 0.46 ml/min.

time in the formic acid–water after extraction, and the time in the eluent before injection were all examined by HPLC analysis. The degree of extraction (formic acid–water) of the dyes from acrylic fibres was investigated using a blue, a red and a black fibre. All fibres were found to be fully extracted within 20 min at 90°C. Generally extraction was complete after 15 min at 90°C and 10 min at 100°C. There was almost no extraction of the dyes at room temperature even after 10 days. Degradation of the dyes in the extracts was found to occur at elevated temperatures or elongated extraction times (greater than 100°C and 30 min). Fig. 4 shows the chromatograms obtained for a red casework fibre at 100 and 130°C.

The analysis of the extracts after a period of a few min up to 4 h in the formic acid–water extracting solvent showed that no degradation had occurred for seven of the eight fibres analysed. One green fibre did appear to degrade in the formic acid–water after extraction. The solution changed from green to orange after approximately 40 min. Over a 24-h period the fibre extract returned to its original green colour with an increase in the blue dye component in the chromatogram.

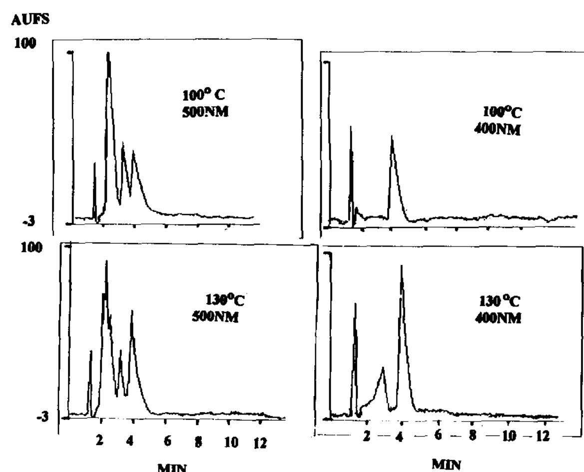

Fig. 4. HPLC analyses at 400 and 500 nm showing the degradation of a red casework fibre using an extraction temperature of 130°C compared to 100°C. Eluent, methanol–aq. ammonium acetate (9:1); sensitivity 0.005 AUFS; column, 2.1 mm I.D.; flow-rate, 0.46 ml/min.

Fibre extracts were stored at room temperature in the HPLC eluent to simulate the queue for the autoinjector. The extracts were analysed every hour for 6 h and then daily for 6 days. None of the fibres analysed showed any signs of degradation in the eluent.

Fibres from pattern cards dyed with the commercial dyes were also analysed. Slight variation in the retention times between the methanol solution of the individual dyes and the extracted or formic acid–water solutions of the dyes was found. It was postulated that ion-pair formation was occurring between the formic acid and the dye. This effect was reduced when the extract was diluted with methanol or eluent. There was also a slight variation observed in the retention times of some of the dyes when injected individually or in a mixture of dyes.

If the crime and control fibres are treated identically then a comparison can be carried out. A control fibre was therefore analysed every third or fourth sample to take account of any degradation that might have occurred.

Fibres (0.5 cm) containing mixtures of known dyes were removed from the pattern cards. Three shades each containing the same three dyes (basic yellow 91, basic red 51 and basic blue 124) were analysed. The results are shown in Table 3. It was found that between 0.1 and 0.8 ng per mm of the dyes were present in the fibres. The ratios of the peak heights indicate the relative amounts of the dyes used on each fibre.

Fibres were obtained from casework and local manufacturers to assess the HPLC system for the

Table 3
Concentration of dyes in three fibres (from the pattern card) which contain different proportions of the same dyes

Colour of fibre	Concentration of dye (ng/mm)		
	Yellow 91	Red 51	Blue124
Blue	0.3	0.1	0.2
Green	0.8	0.2	0.1
Brown	0.7	0.5	0.1

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column, 2.1 mm I.D.; flow-rate, 0.46 ml/min.

Table 4
Retention times of dyes extracted from samples of fibres from local manufacturers

Manufacturer	Colour of fibre	Retention times (min)		
		400 nm	500 nm	600 nm
A	Green	8.5	6.4	4.4
A	Navy	4.7	3.4, 3.6, 4.7, 6.5	3.3
A	Black	4.7	3.3, 3.5, 4.5, 6.2	3.2
B	Black	4.4	4.3, 5.5	4.4
B	Blue	3.3	3.0, 3.5	3.0
B	Red	3.1, 4.2, 5.5	5.5	3.1
B	Light green	4.2	3.3, 4.2	3.1
B	Dark green	4.2	3.1, 4.4	3.1
C	Black	4.2	3.1, 4.4, 6.1	3.1
C	Orange		5.5	
C	Yellow	4.0, 10.2		
C	Red		4.3	
C	Blue		5.6	4.2
C	Pink		7.3	
C	Purple		4.0	

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column, 4.5 mm I.D.; flow-rate, 2 ml/min.

separation and detection of mixtures of unknown basic dyes. Fibres (1 cm in length) were extracted and analysed by both TLC and HPLC. The extract for the HPLC analysis was split into three portions for injection at the three wavelengths. The whole extract was used for TLC

Table 5
Retention times of extracted dyes from black casework fibres

Sample	Retention times (min)		
	400 nm	500 nm	600 nm
1B9	3.9, 4.6, 6.5	3.3, 3.8, 4.5, 6.5	2.1, 4.5
1B15	4.5, 6.5	4.5	
2P2	4.6	3.1, 3.6, 4.0, 4.7, 6.9	3.1, 4.6
2U9	4.4, 6.5	2.8, 3.2, 4.5, 6.6	2.9, 4.3
2B13	4.8	3.1, 3.5, 4.0, 4.8, 7.1	3.1, 4.4
3P6	4.4	3.4, 4.2	4.4
3P1	1.5, 2.4, 3.5	2.7, 4.0, 4.7, 5.3, 6.6	
4C5	4.3	3.2	4.1
4U12	4.4	3.3, 5.7	4.1
5C4	1.8, 3.6	3.7	
5C13	1.8	3.7	3.5
6C	4.0	4.2	5.0
6C12	3.9	3.6	
7U11		2.3	
7B11		1.5	3.4
8B8	3.4, 4.6, 14.4	13.7	
8B2	3.5, 4.6, 14.4	3.9, 14.5	

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity 0.005 AUFS; column, 4.5 mm I.D.; flow-rate, 2 ml/min.

analysis. Fibres from three different manufacturers were analysed. The dye composition of two of these was known; (A) basic yellow 87, basic red 51 and basic blue 124 and (B) basic yellow 28, basic red 46 and basic blue 41. The dyes in these fibres had been mixed in different proportions according to the colour required for the final product. The results are shown in Table 4.

The black fibres were found to contain numerous dyes. On discussion with the manufacturers it was found that in black or dark coloured fibres either a known mixture of dyes is used, or the dye baths from previous dyeing processes may be used. Therefore not every batch of these fibres is the same.

Black casework fibres which had previously been grouped into 8 groups by TLC analysis were extracted and injected onto the HPLC system (Table 5). It was found that more discrimination was found by HPLC and also quantitative differences were detectable (Fig. 5). The black fibres in group 2 were found to be similar in dye content to a sample of a black Courtaulds fibre (I). It is known that the black dye used in this fibre is bought from a supplier and adjusted to the correct shade by the addition of a red or a yellow dye.

Three black masks which were indistinguishable by TLC were also analysed and differences in the dye content detected at 500 nm. Black fibres from different sources (A–R) in one household were also examined. HPLC was found to differentiate between the different black acrylic fibres. Also three fibres (C, I and S) contained peaks similar to those found in a Courtaulds fibre (II). The same 6 or 7 dyes were present in all the fibres suggesting that these fibres were dyed by the Courtaulds process (Table 6).

Pale coloured case fibres (approximately 6 mm in length) which had shown no visible spots on TLC were extracted and analysed. Bulk extracts were prepared from 5 or 6 fibres and these solutions were analysed by TLC to determine the dye components present in the fibres. Some dye components were still not detectable due to the small quantities present in the 6 mm of fibre. HPLC was found to give peaks for all but one of the fibres (Table 7). Analysis of the unextracted fibre by microspectrophotometry indicated that the dye components in the fibres have sharp absorption maxima at 440, 550 and 630 nm and minimum absorption at the detecting wavelengths (Fig. 1). Reanalysis at these wavelength maxima would be necessary.

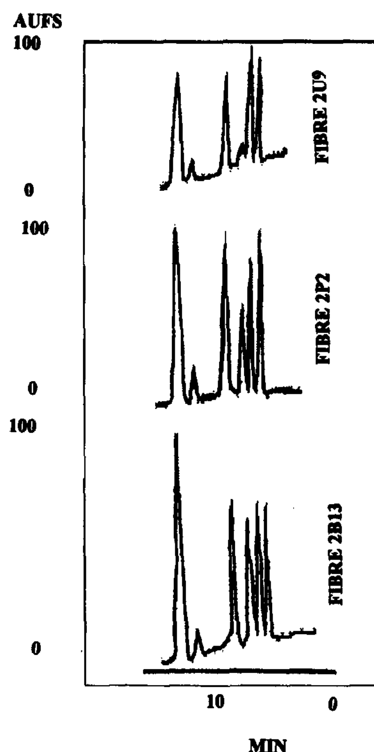

Fig. 5. HPLC analyses (500 nm) of three Group 2 TLC fibres showing the differences in dye ratios. Eluent, methanol–aq. ammonium acetate (9:1); sensitivity 0.005 AUFS; column, 4.5 mm I.D.; flow-rate, 2 ml/min.

Variation of the dyes within one fibre source was investigated. One particular acrylic fibre appears under the microscope to have variation between fibres and along the length of a single fibre. These are the producer dyed fibres made by Courtaulds UK Ltd. These fibres are known as 'Tiger Tail' fibres because of the pattern of dark and light (or coloured) bands which are visible under the microscope, even at low power. The analysis of the dye content of the Tiger Tail fibres (0.5–1 cm in length) was investigated along the length of a fibre, between individual fibres and from different areas of the garment.

Table 6
Retention times of fibres from different sources

Fibre	Retention time (min)		
	400 nm	500 nm	600 nm
Black A	5.7	5.2	
B	4.4, 5.7, 7.8, 8.4	3.0[a], 5.7, 7.6, 8.4	3.0, 6.5
C	4.5, 5.8, 7.8	4.1, 4.5, 5.9, 7.6, 8.5	2.9, 4.1, 6.4
D	4.4, 5.7	4.5, 5.7, 7.2	2.9, 4.5, 6.2
E	5.7, 7.9[a], 8.3[a]	5.7, 7.6, 8.4	2.9, 6.3
F	5.7	5.3	
G	5.8	2.7, 5.2, 5.8	
H	3.5[a]	5.3	2.9[a], 3.7
I	4.4, 5.7, 7.7	4.1, 4.5, 5.8, 7.5, 8.4	2.9, 4.1, 6.5
J	5.8	2.9, 5.8, 7.8	2.9, 6.4
L	7.5, 8.5	7.5, 8.5	4.6
O	4.4, 5.7, 7.0, 8.4[a]	5.8, 7.6, 8.4	3.0, 4.5, 6.2
P	2.9[a]	2.8, 3.4	3.4, 6.5[a]
R	5.7	4.5, 5.3, 5.7, 7.3	2.8[a]
S	4.1, 5.6, 7.8, 8.2	4.0, 4.4, 5.9, 7.9, 8.2	2.9, 4.0, 6.0
Courtaulds II	2.7, 4.0, 5.5	2.7, 2.95, 4.0, 5.5	2.7, 3.5
Blue G74	3.0	2.7, 3.1, 5.2	2.6
Red INH6/90	3.9	2.3, 3.2, 3.9	2.3[a]
Green	3.8		3.5
Blue		2.9, 3.2	2.9
Red	3.8	3.1	3.2
Yellow	3.8	4.3[a]	
Blue	3.5, 4.2	3.1, 5.5	3.6
Pale green	3.8	2.5, 3.0	2.9
Pale blue		3.0	3.8, 2.7
Blue	2.5	2.5, 3.2	2.7
Green	3.8, 12.0	3.7, 12.0	2.7, 3.3

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column, 2.1 mm I.D.; flow-rate, 0.46 ml/min.
[a] Small peak.

No differences were detected in the three wavelengths for any of the samples analysed.

4. Conclusions

It was found that degradation of the dye components could occur in formic acid–water when the extraction was carried out at temperatures greater than 100°C or for longer than 30 min and also if the extracts were not used within 40 min of removal from the oven. No degradation was noticed when the fibre extracts were diluted with the HPLC eluent and left for up to 48 h before injection onto the HPLC column thus facilitating the use of an autoinjector.

The HPLC system described here has been successfully used to separate the commercial basic dyes known to be used by the garment industry for the dyeing of acrylic fibres. The use of the narrow bore HPLC column has enabled the dyes from 2 mm of coloured acrylic fibres and from 5 mm of pale coloured fibres to be detected and the dye components resolved. The relative quantities of the dye components could be compared. In many fibres the small quantities of minor dye components are not visible on TLC plates and also slight variation between dye batches cannot be detected. The HPLC system

Table 7
Retention times of dyes from pale coloured fibres (6 mm)

Fibre	Colour of dye present[a]	Retention time (min)		
		400 nm	500 nm	600 nm
1	Blue, red, yellow	5.6	3.8[b], 5.0	
2	Blue, red, yellow	5.7	3.8[b], 5.0	4.4[b]
3	Purple, blue, yellow		4.4	5.7
4	Blue, red, yellow	5.7	3.9, 5.1	4.3
5	Blue, red, yellow	5.8	3.8[b], 5.1	4.3
6	Blue, turquoise		6.1[b]	4.0, 6.1
7	Turquoise, pink, orange			
8	Purple, blue		4.4	5.4
9	Blue, pink, blue		4.3	4.2
10	Pink, turquoise, blue		3.1	3.1, 4.1

Conditions: eluent, methanol–aq. ammonium acetate (9:1); sensitivity, 0.005 AUFS; column, 2.1 mm I.D.; flow-rate, 0.46 ml/min.
[a] From bulk extracts of fibres.
Small peaks.

described here has been found to be more discriminating than TLC providing greater sensitivity and resolution for the detection of basic dyes from fibre extracts. Picogram levels of detection were recorded for the dyes analysed.

The diode array detectors have not yet been proven to be sufficiently sensitive for detection of the quantities of dyes present in acrylic fibres. The single channel variable-wavelength detector was found to be more sensitive than the diode array detector although injecting the samples at three different wavelengths is time consuming.

The analysis of different sources of black fibres demonstrated the discriminating power of the HPLC system. It was found that some fibres contained the same dye components. These fibres could be from the same manufacturer and potentially the source of individual fibres could be assessed by this system.

5. References

[1] B. Beattie, R.J. Dudley and K.W. Smalldon, *J. Soc. Dyers Colour.*, (1979) 295.
[2] P.C. White and T. Catterick, *J. Chromatogr.*, 402 (1987) 135.
[3] D.K. Laing, R. Gill, C. Blacklaws, and H.M. Bickley, *J. Chromatogr.*, 442 (1988) 187.
[4] J. Yinon and J. Saar, *J. Chromatogr.*, 586 (1991) 73.
[5] R.M.E. Grifffin, T.G. Kee and L. Elliott, (1989) personal communication.
[6] Ch.-H. Fischer, M. Bischof and J.G. Rabe, *J. Liq. Chromatogr.*, 13(2) (1990) 319.
[7] J.C. West, *J. Chromatogr.*, 208 (1981) 47.
[8] D.J. Reuland and W.A. Trinter, *J. Forensic Sci.*, 20 (1980) 111.
[9] L. Gagliardi, G. Cavazzutti, A. Ameto, A. Basili and D. Toneli, *J. Chromatogr.*, 394 (1987) 345.
[10] A.H. Lyter, *J. Forensic Sci.*, 27 (1982) 154.
[11] I.R. Tebbett, *Forensic Sci. Rev.*, 3(2) (1991) 72.
[12] J.R. Joyce and D.G. Sanger, *J. Forensic Sci.*, 19 (1979) 203.
[13] R.O. Keto, *J. Forensic Sci.*, 29 (1984) 198.
[14] L.G. Rushing and M.C. Bowman, *J. Forensic Chromatogr. Sci.*, 18 (1980) 224.
[15] J.W. Wegener, J.C. Klamer, H. Govers and U.A.Th. Brinkman, *Chromatogaphia*, 24 (1987) 865.
[16] K. Venkataraman (Editor), *The Analytical Chemistry of Synthetic Dyes*, Wiley Interscience, New York, 1976.
[17] J.M. Ballard, *Anal. Chem.*, 56 (1984) 2604.
[18] R.M.E. Griffin, T.G. Kee and R.W. Adams, *J. Chromatogr.*, 445 (1988) 441.
[19] J. Home and R.J. Dudley, (1980) personal communication.
[20] J. Home and R.J. Dudley, *Forensic Sci. Int.*, 17 (1981) 71.