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Note

Use of a rotating disc multiwavelength detector operating in the visible region of the spectrum for monitoring ball pen inks separated by high-performance liquid chromatography

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The separation of dyes in ball pen inks using high-performance liquid chromatography (HPLC) can be of considerable value in a forensic context[1,2], with the complex chromatograms providing a powerful basis on which to make comparisons. A drawback with conventional single-wavelength monitoring, however, is that replicate analysis may often be necessary to ensure that the component dyes are adequately detected and this is particularly true when they have widely separated absorption maxima. Detectors incorporating linear diode arrays provide an obvious way of scanning a wide spectral region instantaneously, but it has been our experience that at the present time the data they produce are rarely in a convenient form for our use. A rapid sequential multiwavelength detector developed in this laboratory[3,4] is able to monitor four wavelengths in rapid succession and by coupling this to a suitable microprocessor it is possible to produce a print-out of retention time, absorbances and absorbance ratios which has been shown to be a valuable aid in characterising an eluting compound with minimal computing and data storage requirements[5]. Prior to the investigation reported here the detector had been used at wavelengths in the UV region, this paper describes its application in the visible region of the spectrum.

EXPERIMENTAL

Sample preparation

Extracts of ball pen ink were prepared by cutting, or scraping marks of not less than 5 mm in length. The paper and ink sample produced was placed in a tapered glass tube and *ca.* 50 μl of the eluent described later was added. The suspension was agitated in an ultrasonic bath until the dye was extracted, and the supernatant solution was injected on to the chromatographic column without additional clean-up.

Chromatographic conditions

Three different reversed-phase packing materials were studied: A, a laboratory-prepared 5 μm silica bonded with octadecyltrichlorosilane and trimethylchlorosilane[6]; B, Spherisorb ODS 5 μm (Phase Sep., Queensferry (U.K.); C, 5 μm ODS Hypersil (Shandon Southern Products, Runcorn (U.K.).

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These packings were slurry packed into 12.5 cm × 4.8 mm I.D. stainless-steel columns and were conditioned with eluent for several hours before being used. The eluent contained acetonitrile–tetrahydrofuran–water (924:432:644, v/v) and to each 2 l of this mixture 3.5 g of citric acid and 1.5 g of hexane sulphonic acid were added. The pH was adjusted to 4.0 by the dropwise addition of concentrated ammonium hydroxide, and was measured using a pH meter. Samples were injected via a valve fitted with a 20-μl loop (Rheodyne Model 7125).

Detection conditions

Detection was in the visible region using either a variable-wavelength detector (SpectroMonitor III from Laboratory Data Control, Stone (U.K.) or the rotating disc multiwavelength detector[3,4] fitted with four narrow bandpass interference filters. Details of these filters and other technical information about the detector are given in Table I. The output to the recorder permitted any two wavelengths to be recorded simultaneously; the selection of the best wavelengths was made on the colour of the ink being examined.

TABLE I

TECHNICAL DETAILS OF THE DETECTOR

Narrow bandpass interference filters	max. (nm)	Half bandwidth (nm)
	400	14
	500	13
	550	14
	600	14
Diameter of filters	32 mm	
Diameter of rotating disk	125 mm	
Microcomputer	PET (Commodore) Model 4032 with dual 5.25" disc drive Model 8050, Tracor printer Model 4022	
Programming language	Basic-Pseudo compiled using PET-SPEED (Oxford Computer Systems)	
Chart recorder	Dual-pen Kipp & Zonen Model BD 9	
Output channels to recorder	400 and 500 nm for yellow and red inks, 550 and 600 nm for green, blue and black inks	
Rotation speed of filter disc	2 cps	

RESULTS AND DISCUSSION

The eluent composition was arrived at by optimisation experiments[7] and gave a superior separation with the packing materials tested to that described by Lyter[1]. The eluent is only suitable for the separation of basic and neutral dyes, and acidic dyes are non-retained under the described conditions. The chromatograms shown in Fig. 1 indicate that the retention of dyes is dependent upon the packing material used. ODS Hypersil was found to provide an adequate separation with minimum retention and was selected for further study as it permitted the most rapid analysis. Pyridine has been recommended as an extractant for removing ball pen inks from

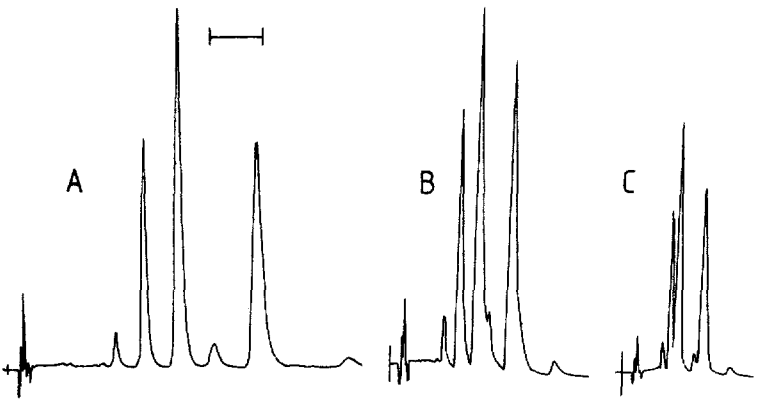

Fig. 1. Chromatograms of a blue ball pen ink on three different C_{18} modified silicas. The elution conditions were as described in the text. The monitoring wavelength was 600 nm. (A) A laboratory-prepared material; (B) Spherisorb ODS 5 μm; (C) ODS Hypersil 5 μm. The time interval marked corresponds to 5 min.

papers[1], but in our experience this solvent subsequently led to a degraded chromatogram when injected on to the column system we had used; in the work described here the eluent also functioned as the extractant.

Ball pen inks usually give chromatograms displaying considerable complexity which makes HPLC a powerful discriminatory technique. Even with single-wavelength monitoring the level of discrimination is high but ambiguity must always exist when a large number of dyes are characterised by retention characteristics alone. The advantage of multiwavelength monitoring is that every eluting component is also characterised by its light-absorbing properties at four different wavelengths. Retention and absorbance characteristics of a variety of dyes used in ball pen inks are shown in Table II. The dyes listed are either blue or red in colour and it is clear from the data that dyes of the same colour display significant differences in their absorbance ratios.

The integrity of absorbance ratios was maintained over a wide dilution range, and in tests covering the absorbance range 0.2–0.01 a.u. the major ratio (*i.e.* 600/550

TABLE II

CHROMATOGRAPHIC AND ABSORBANCE CHARACTERISTICS OF DYES USED IN BALL PEN INK FORMULATIONS

C.I. = Colour index.

Dye	*C.I. number*	*Retention time (min)*	*Absorbance ratio*		
			400/550	*500/550*	*600/550*
Methyl Violet BD*	42555	6.7	0.00	0.17	1.24
Methyl Violet B*	42535	6.7	0.00	0.17	1.24
Victoria Blue B	44045	10.1	0.09	0.14	1.94
Victoria Blue B0	42595	18.0	0.02	0.10	1.74
Rhodamine B	45170	5.5	0.04	0.16	0.04
Rhodamine 6G	45160	5.6	0.07	1.68	0.00

* Different ionic forms of the same parent dye.

in the case of blue dyes) altered by less than 2%. The stability of absorbance ratios is also brought out in the data listed in Table III. This shows the variability of retention time and absorbance ratios during a working day as determined by ten replicate injections of a standard blue ink solution. Changes in ambient temperature gave rise to substantial variation in retention times, whereas absorbance ratios remain essentially unchanged. Provided the dye produced a chromatographic peak with an absorbance in excess of 0.01 at its maximum then absorbance ratios were varying by

TABLE III

PRECISION OF RETENTION TIME AND ABSORBANCE RATIO FOR THE COMPONENTS OF A BLUE BALL PEN INK

The data were produced by injecting the same ink extract ten times during the same day.

Peak No.	*Mean retention time (min)*	*Relative standard deviation (%)*	*Mean absorbance at 600 nm*	*Absorbance ratio 600/550*	
				Mean	*Relative standard deviation (%)*
I	4.7	12	0.008	0.67	2
II	5.6	12	0.052	0.98	0.4
III	6.7	12	0.087	1.24	0.3
IV	8.0	11	0.005	1.76	4
V	10.1	12	0.061	2.01	0.3

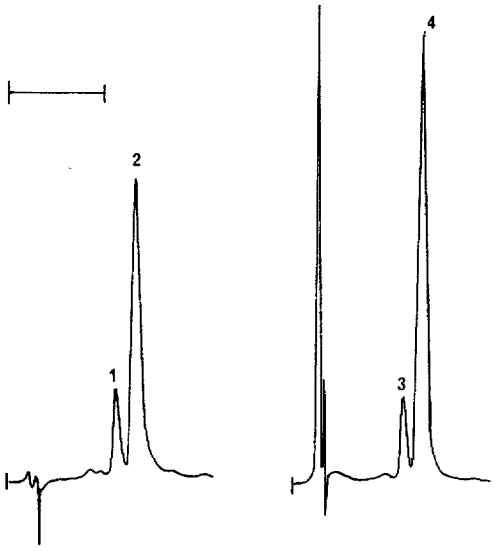

Fig. 2. Chromatograms of blue ball pen inks on ODS Hypersil illustrating how wavelength ratioing permits discrimination. The elution conditions were as described in the text. The monitoring wavelength was 600 nm. The time interval marked corresponds to 5 min.

Peak	t_R (min)	400/550	500/550	600/550
1	5.85	0.04	0.26	0.86
2	6.87	0.00	0.21	1.09
3	5.82	0.02	0.28	1.04
4	6.67	0.00	0.18	1.25

less than 2% (*i.e.* relative standard deviation). This stability was also maintained in the long term, and absorbance ratios of test dyes measured after a period of six months displayed no change in value. Thermostating or the use of relative retention values substantially reduced the variability in retention times and in experiments similar to that described for producing the data in Table III, relative standard deviations for the retention times was reduced to less than 2%.

An example of the way in which the use of absorbance ratios aids an ink comparison is shown in Fig. 2. The retained peaks are dyes extracted from paper marked with blue ball pen inks. The retention times of the dyes in both samples are similar and the two chromatograms produced when monitored at 600 nm are virtually identical. The non-identity of these samples becomes immediately apparent, however, when the absorbance ratios are compared. All four dyes are different and that marked as peak 4 has the characteristics indicative of methyl violet.

In Fig. 3 are shown chromatograms of two blue inks containing several components. There is no doubt that these two inks are different but it would be of interest to know whether any dyes are common to both inks. The two samples were run at different times and changes in ambient temperature had given rise to slight changes in retention. Despite this complication peaks 1, 2 and 3 are identical in absorbance

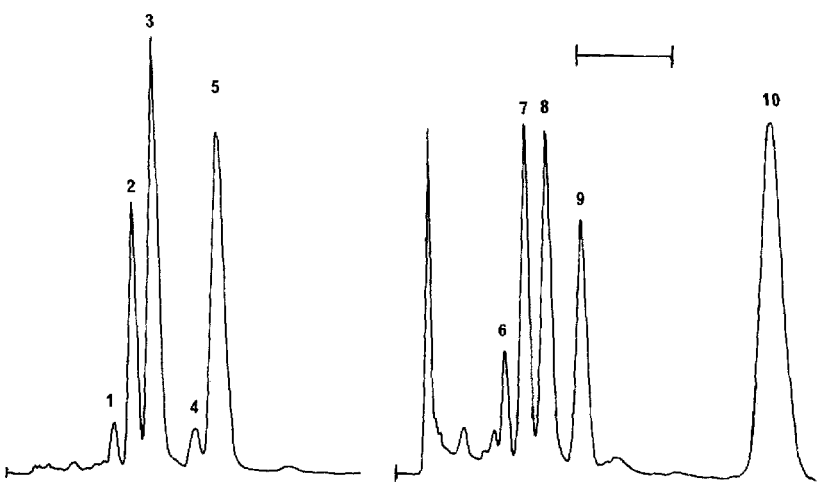

Fig. 3. Chromatograms of blue ball pen inks on ODS Hypersil illustrating how wavelength ratioing permits dyes common to both inks to be identified. The elution conditions were as described in the text. The monitoring wavelength was 600 nm. The time interval marked corresponds to 5 min.

Peak	t_R (min)	400/550	500/550	600/550
1	4.85	0.00	0.30	0.68
2	5.65	0.00	0.22	0.99
3	6.57	0.00	0.17	1.26
4	8.95	0.04	0.19	1.59
5	9.90	0.09	0.13	1.95
6	4.86	0.01	0.27	0.69
7	5.73	0.00	0.23	0.99
8	6.73	0.01	0.18	1.24
9	8.78	0.01	0.21	0.89
10	18.62	0.02	0.16	1.55

characteristics to peaks 6, 7 and 8 respectively; the latter peak in each group of three closely resembling Methyl Violet. In addition, peak 5 in the first chromatogram has characteristics of Victoria Blue B.

Absorbance ratioing in the visible region adds significantly to the discriminatory potential of HPLC in the context of ball pen ink examinations. The qualitative information it provides about dye components may also prove to be useful in helping to date inks, or to pinpoint particular formulations.

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