Journal of Chromatography, 445 (1988) 441–448
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 20 507

Note

High-performance liquid chromatographic system for the separation of basic dyes

R. M. E. GRIFFIN*, T. G. KEE and R. W. ADAMS
Northern Ireland Forensic Science Laboratory, 180 Newtownbreda Road, Belfast BT8 4QR (U.K.)
(Received March 21st, 1988)

Comparatively few papers are devoted to the high-performance liquid chromatographic (HPLC) analysis of basic[1] compared to acid dyes[2,3] and most of this literature is concerned with individual or a narrow range of basic dyes[1,4].

The aim of this study was to develop an HPLC system which would be capable of separating and detecting a range of synthetic basic dyes used in the dyeing of acrylic fibres.

A silica column with a buffered (pH 9.7) water–methanol eluent was found to provide an acceptable separation system for the twenty-one commercial basic dyes available. A gradient elution program was used which varied the buffer concentration while maintaining a 9:1 ratio of methanol and buffer.

EXPERIMENTAL

The equipment consisted of two Waters Assoc. Model 510 HPLC pumps, a Water Assoc. Model 680 gradient controller and a Shandon (160 × 4.5 mm I.D.) stainless-steel column. This was packed with Phase Sep Spherisorb silica (5 μm). A Rheodyne valve injector fitted with a 20-μl sample loop was used. Detection was mainly by a Waters 990 photodiode array detector coupled to a NEC-APC111 computer and Waters 990 printer/plotter. Initial experiments were carried out using a Pye-Unicam LC–UV detector and Phillips PM8251 single pen recorder.

UV–VIS spectra were determined between 200 and 800 nm on a Hewlett-Packard HP 8450 diode array spectrophotometer using a 1-cm pathlength.

Reagents

Mobile phase. A volume of 94 ml of concentrated ammonia and 21.6 ml of concentrated acetic acid were added to 884 ml of glass distilled water and the pH adjusted to pH 9.76 using either conc. ammonia or conc. acetic acid. The buffer was diluted with glass distilled water (1:3) as required for solvent B. 100 ml of buffer was mixed with 900 ml of HPLC-grade methanol (Fisons) and the eluents filtered under vacuum through a Gelman 0.2-μm FF Vericel ™ membrane filter. All solvents were degassed for 30 min in the Decon FS200 ultrasonic bath before use.

Standards. Fifteen basic dyes were obtained from Ciba Geigy and nine from Bayer U.K. (see Table I). Basic blue 7 was obtained from Aldrich. Solutions of these

0021-9673/88/$03.50

TABLE I

RRT OF THE BASIC DYES WITH RESPECT TO BLUE 45

Basic dye	*Manufacturer*	*RRT**	λ_{max} *(methanol) (nm)*
Blue 3	Ciba Geigy/Bayer	0.340	642 (596)
Blue 7	Aldrich	0.112	619
Blue 124	Ciba Geigy	0.335	612
Blue 151	Ciba Geigy	0.284	618
Blue FGRL	Bayer	0.330 (0.225)**	600
Blue GRL	Ciba Geigy	0.266 (0.298)**	616
Yellow 21	Bayer	0.261	416
Yellow 28	Ciba Geigy/Bayer	0.390	444
Yellow 40	Ciba Geigy	0.352	434
Yellow 87	Ciba Geigy	0.710	414
Yellow 91	Ciba Geigy	0.705	434
Red 14	Bayer	0.290	522
Red 46	Bayer	0.308 (0.587)**	526
Red B-LN	Ciba Geigy	0.304 (0.570)**	534
Red 18:1	Ciba Geigy/Bayer	1.01 (0.588)*	466
Red 27	Ciba Geigy	0.265 (0.194, 0.286)**	534
Red 51	Ciba Geigy	0.465 (0.263)**	524
Red 109	Ciba Geigy	0.388 (0.286)**	514
Red GRL	Ciba Geigy	0.278 (0.557)**	526
Red 46	Ciba Geigy	0.364 (0.313, 0.232)**	522
Violet 16	Bayer	0.182 (0.286)**	552

* Slight variation of RRT occurred when the column was repacked.
** Minor peak.

dyes were prepared by dissolving 100 mg of dye in 100 ml of HPLC-grade methanol and further diluting to the required concentration, *e.g.* 100 μl to 10 ml gave 100 ng in 10 μl injection.

Chromatographic conditions

Solvent A: 90% methanol, 10% ammonium acetate solution–water (1:3); solvent B: 90% methanol, 10% ammonium acetate solution pH 9.76. For gradient programme, see Table II.

TABLE II

GRADIENT PROGRAMME

Time (min)	*Flow (ml/min)*	*% A*	*% B*	*Curve**
Initial	1.00	100	0	
20.00	2.00	50	50	6
26.00	2.00	31	69	6
35.00	1.00	100	0	6

* Curve 6 denotes a linear gradient.

RESULTS AND DISCUSSION

Initial studies were carried out using the single-wavelength detector at 280 nm. This wavelength was determined from the UV–VIS spectra as suitable for the detection of all the components of the dye mixture. The relative retention times (RRT) of the dyes were calculated using basic blue 45 as the internal standard and the results are shown in Table I and Fig. 1. Seventeen peaks were obtained when the mixture of twenty-one dyes was injected onto the HPLC system, indicating that some of the dyes were not completely resolved by this system. For example, the peak at 17 min contains two components basic yellow 87 and basic yellow 91 (Fig. 2).

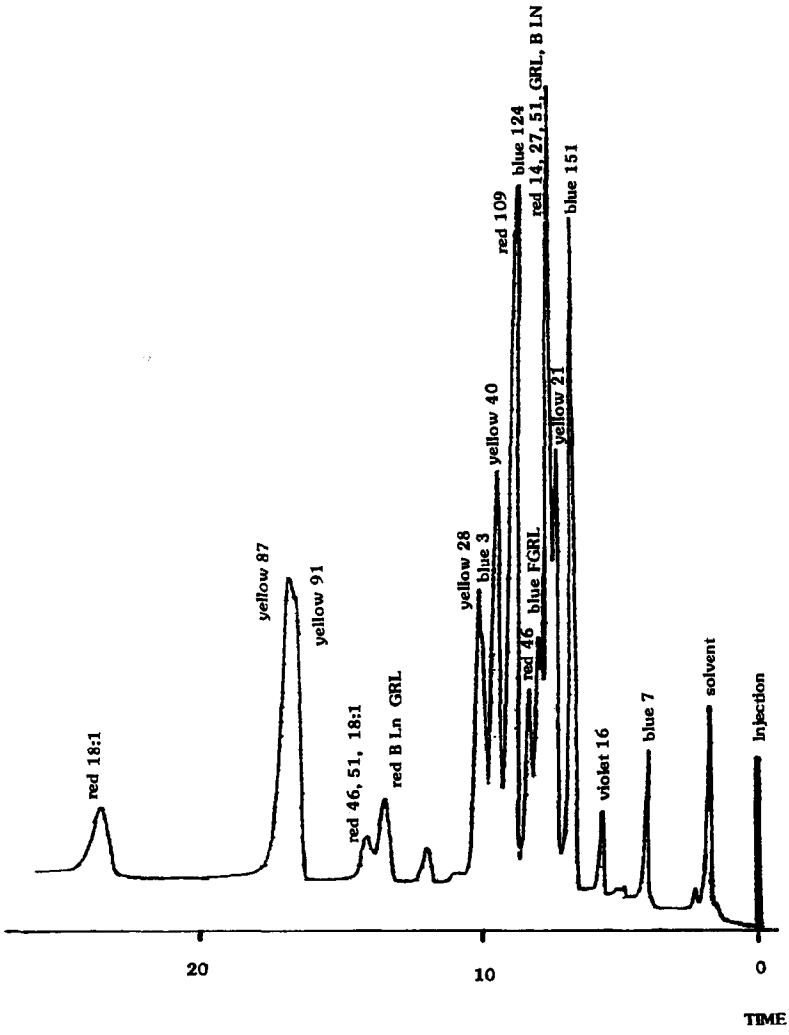

Fig. 1. Chromatogram illustrating the separation of twenty-one basic dyes.

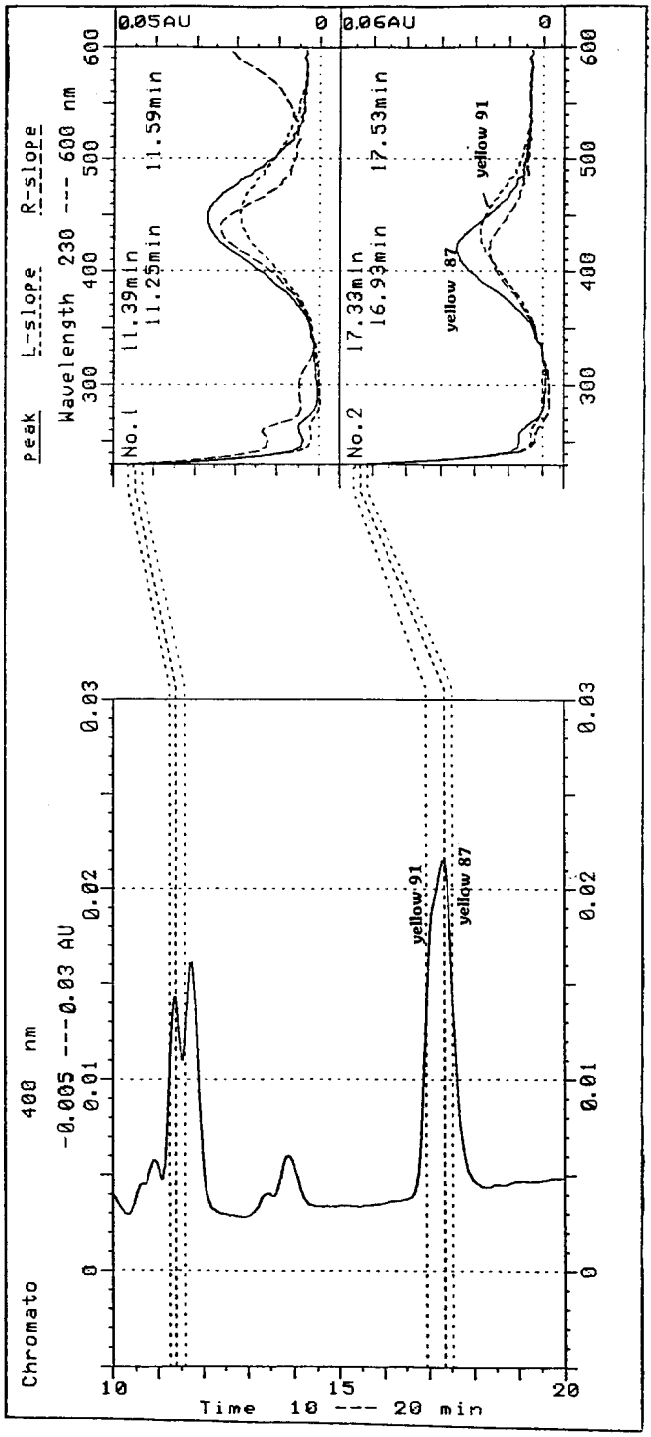

Fig. 2. Diagram showing the identification of dyes by UV-VIS spectroscopic analysis of unresolved peaks.

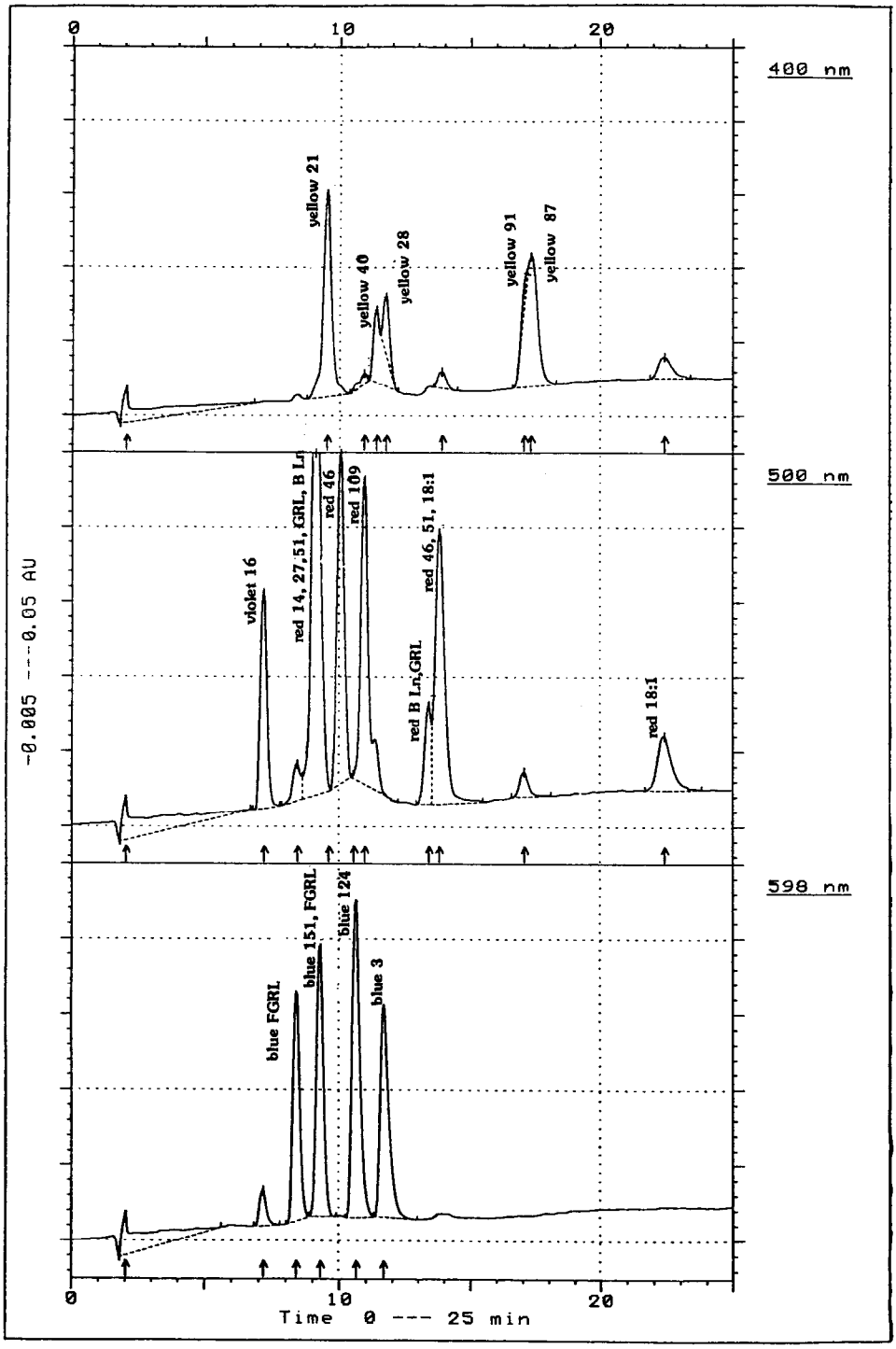

Fig. 3. Chromatograms of the dye mixture at three detecting wavelengths.

To overcome this overlap problem and provide additional information the dyes were re-analysed using the diode array detector. The chromatograms derived from three wavelengths, 400, 500 and 598 nm (yellow, red and blue dyes respectively),

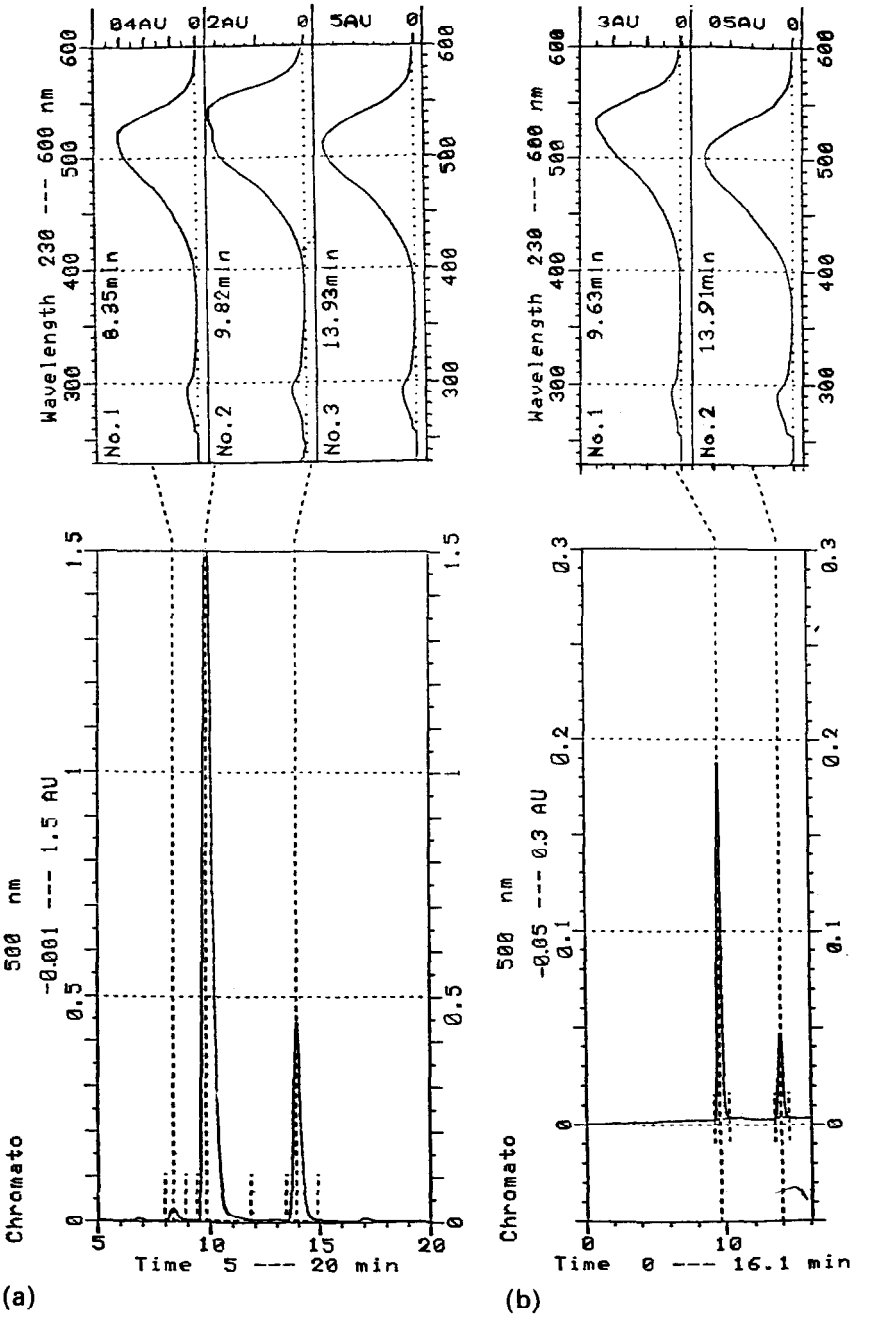

Fig. 4. Chromatograms showing components of (a) basic red B-Ln and (b) basic red GRL.

are shown in Fig. 3. It can be seen that four yellow peaks, four blue peaks and six red peaks were obtained for the dye mixture. Analysis of the UV–VIS spectra across individual peaks indicated those with more than one component. In the case of the

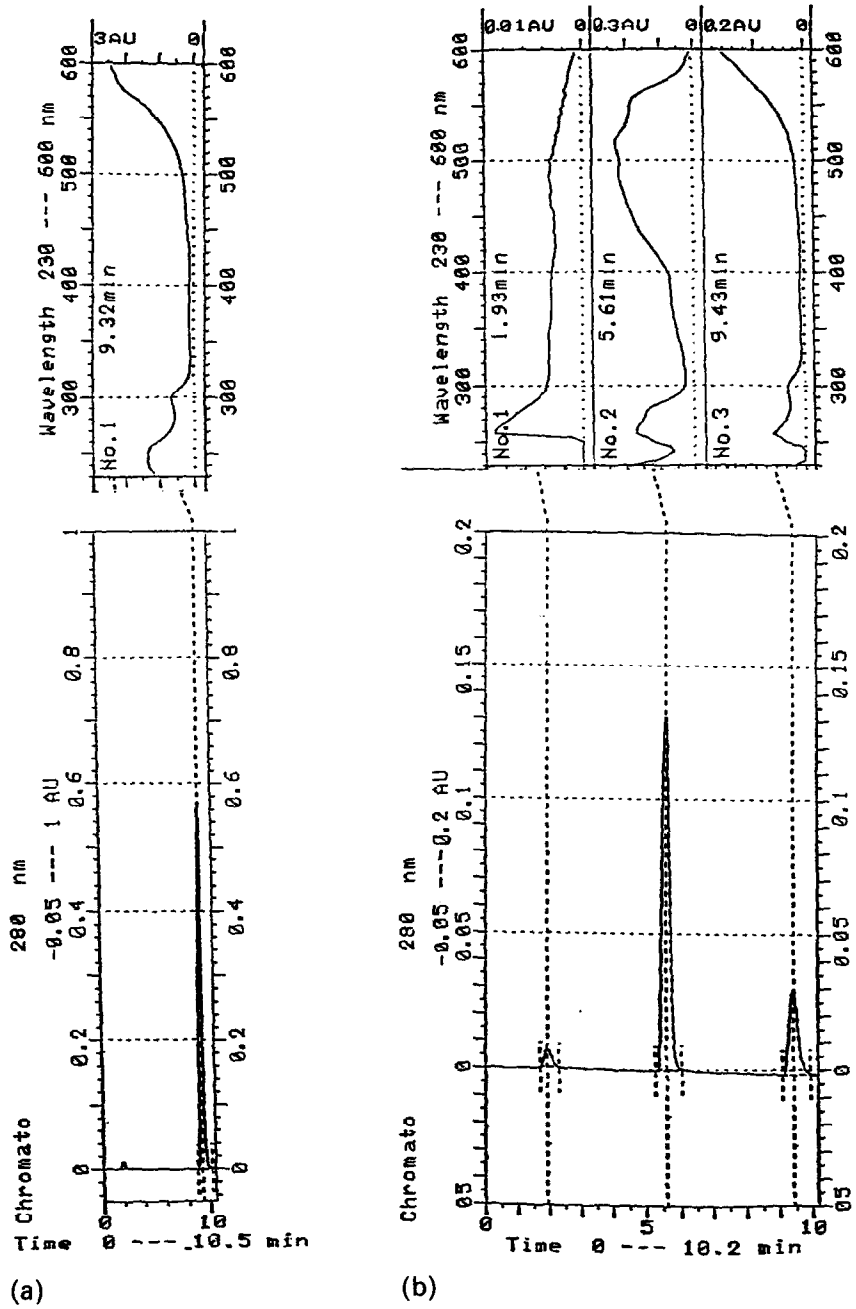

Fig. 5. Chromatograms of basic blue 151 (a) freshly prepared solution and (b) one week old solution.

yellow dyes, the peak at 17 min was shown to contain 2 dyes with λ_{max} of 420 nm (basic yellow 87) and 435 nm (basic yellow 91) (Fig. 2). Similar analysis of the chromatograms at 500 and 598 nm resulted in a total of eighteen out of twenty-one dyes being identified.

Impurities and secondary peaks were detectable by this system, *e.g.* basic red B-Ln and basic red GRL both have two distinct components but the two dyes are not resolved when co-injected. The difference in the UV–VIS spectra for the early eluting components are shown in Fig. 4.

Basic blue 151 was found to decompose in methanol solution to give a red solution over a period of about one week. This decomposition could be monitored by the HPLC system (Fig. 5). These experiments demonstrate the applicability of this HPLC system to a range of problems.

CONCLUSION

The combined use of the diode array detector with the chromatographic conditions described above provides a powerful system for the separation and detection of basic dyes used in the dyeing of acrylic fibres.

ACKNOWLEDGEMENTS

The Waters 990 photodiode array detector and data processing system were on loan from AGB Scientific Apparatus, Larne Road, Carrickfergus, U.K. The dyes were obtained from Ciba Geigy Dyestuffs and Chemicals, Glengormley, U.K. and Bayer U.K., Carryduff, U.K.

REFERENCES

1 K. Venkataraman (Editor), *The Analytical Chemistry of Synthetic Dyes*, Wiley Interscience, New York, 1976.
2 F. E. Lancaster and J. P. Lawrence, *J. Assoc. Off. Anal. Chem.*, 65 (1982) 1305.
3 J. W. M. Wegener, H. J. M. Grünbauer, R. J. Fordham and W. Karcher, *J. Liq. Chromatogr.*, 7 (1984) 809.
4 J. M. Ballard, *Anal. Chem.*, 56 (1984) 2604.