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Journal of Chromatography A, 871 (2000) 227–234

JOURNAL OF CHROMATOGRAPHY A

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Determination of sulphonated dyes in water by ion-interaction high-performance liquid chromatography

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Abstract

An ion-interaction high-performance liquid chromatography method for quick separation and determination of the sulphonated dye Acid Yellow 1, and the sulphonated azo dyes Acid Orange 7, Acid Orange 12, Acid Orange 52, Acid Red 2, Acid Red 26, Acid Red 27 and Acid Red 88 has been developed. An RP-ODS stationary phase is used, and the mobile phase contains an acetonitrile–phosphate buffer (27:73, v/v) mixture at pH 6.7, containing 2.4 m*M* butylamine as ion-interaction reagent. Good separations were obtained using isocratic elution and spectrophotometric detection at 460 nm. The detection limits for the eight dyes ranged from 7 to 28 μg/l for an injection volume of 100 μl. Spiked tap water samples (100 ml), containing different concentration levels (0.3–1.2 μg/l) of the dyes were analyzed after acidification (pH 3) and preconcentration in disposable solid-phase extraction C_{18} cartridges.

Keywords: Dissociation constants; Dyes; Azo dyes

1. Introduction

Azo dyes are a very important class of synthetic chemical. They are widely used as coloring agents in a variety of products, such as textiles, paper, leather, gasoline and foodstuffs. However, some synthetic dyes may be pathogenic if they are consumed in excess. It has also been shown that synthetic precursors, intermediates and degradation products of these dyes could be potential health hazards owing to both their toxicity and their carcinogenicity. These compounds are quite difficult to remove in water treatment procedures and can be transported from municipal sewers through rivers because of their high water solubility.

In the past, many methods to determine synthetic colorants have been reported, including thin layer chromatography [1,2], spectrophotometry [3], voltammetry [4], and titrimetry [5]. In recent years, several papers have described the separation of aromatic sulphonic acids and related dyes by using capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) [6–11]. However, these techniques have sensitivity problems as a result of small injection volumes. High-performance liquid chromatography (HPLC) methods, particularly ion-pair chromatography, are suitable for selective determination of synthetic dyes. An extensive work about separation of cosmetic dyes by ion-pair reversed-phase HPLC method has been described [12], but there is no data about quantification. Gennaro [13], described the separation and determination of three red dyes using an ion interaction HPLC method with good results.

The aim of the present work is to combine a preconcentration step by solid-phase extraction

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0021-9673/00/$ – see front matter
PII: S0021-9673(99)01053-5

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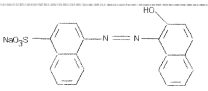
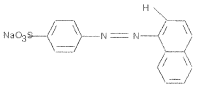
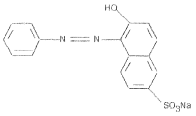
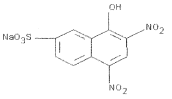
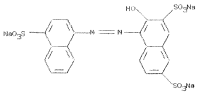
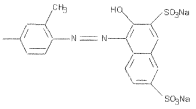
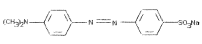
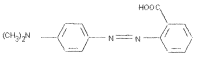
	Acid Red 88	15620 Acid Red 88		
	Acid orange 7	15510 Acid Orange 7		D&C Orange No 4
	Acid orange 12	15970 Acid Orange 12		
	Acid yellow 1	10316 Acid Yellow 1		D&C Yellow No. 7
	Acid red 27	16185 Acid Red 27 Food Red 9	E123	F D&C Red No. 2
	Xylidine Acid red 26 (2,4 Xylidine)	16150 Acid Red 26		D&C Red No 5
	Acid orange 52	13025 Acid orange 52		
	Acid red 2	13020 Acid Red 2		

[1] Colour Index

[2] European Community

[3] Food and Drug Administration official name

Fig. 1. Structure, names and numbers of the dyes used.

(SPE) with an ion-interaction HPLC separation, in order to obtain better detection and quantification limits than those reported before. The chromatographic parameters (pH, mobile phase composition and concentration of ion-pairing reagent) have been optimized to obtain the best separation.

The presented method allows the quantification of the analytes at 0.3–1.2 μg/l levels in spiked tap water samples after the preconcentration step.

Structure, names and numbers of the dyes used are shown in Fig. 1.

2. Experimental

2.1. Reagents

Ultrapure Milli-Q water from Millipore was used for the preparation of solutions. Dyes were obtained from Aldrich Stock standard solutions between 68 and 670 μg/ml were prepared in Ultrapure Milli-Q water and diluted as required. A mixed standard solution containing the seven dyes was used to examine the optimum conditions of chromatographic separation. The solvents (methanol and acetonitrile) were LiChrosolv HPLC grade. Butylamine and all other chemicals used to prepare buffer solutions were of analytical-reagent grade.

Disposable Isolute SPE end-capped cartridges C_{18} (Varian), 100 mg, were used in the preconcentration step.

Before use, the dyes were purified by recrystalization with dichloromethane to separate them from impurities such as salts and surfactants.

2.2. Equipment

Two chromatographic systems were used, first a LKB Bromma 2150 HPLC isocratic pump equipped with spectrophotometer UV–Vis Ovikon 720 LC, HPLC Autosampler 465 Kontron and integrator Merck Hitachi D-2500 Chromato-Integrator. The second system consisted of an isocratic pump Gynkotek M480 G, autosampler MSV and a Perkin-Elmer Lambda 19 spectrophotometer as detector.

Separation was carried out on a 15 cm×4.6 mm Supelcosil LC-PAH (5 μm) column and RP-18 guard pre-column at room temperature. The dead volume in the described conditions was 0.4 ml.

Sample preconcentration was done using a peristaltic pump Watson Marlow.

The dissociation constants were determined using a Beckman DU-7 single-beam spectrophotometer, Radiometer PHM 84 pH-meter, with an Orion 81-02 Ross combination electrode and thermostatic bath at 25°C.

2.2.1. Dissociation constants determination

The dissociation constants of the dyes in aqueous solution were determined spectrophotometrically, to confirm the predicted influence of pH in the chromatographic separation. The pK_a values are presented in Table 1. Absorbance data were obtained by batch experiments by measuring the spectra of several series of solutions, in which the dye concentration was kept constant and the pH varied by the addition of buffer solution (succinic acid, sodium dihydrogenphosphate trishydroxymethylaminomethane, boric acid, hydrochloric acid and sodium hydroxide), according to Perrin and Dempsey [14]. The temperature was kept constant at 25±0.2°C, and the ionic strength was 0.01 *M*. The final concentration of the dyes was about 2×10^{-5} *M*.

Absorbance data were acquired and processed by using the DUMOD and STAR programs [15] respectively.

2.2.2. Sample preparation and preconcentration

Spiked tap water samples, containing different concentration levels (see Table 2) of the dyes, were acidified with 0.1 *M* hydrochloric acid to give pH 3.

Table 1
Dissociation constant(s), detection and quantitation limits, and linear range for the chromatographic separation of studied dyes

CI name	pK_a	LOD (μg/l)	LOQ (μg/l)	Linear range (μg/l)
Acid Yellow 1 (NYS)	–	8	27	27–303
Acid Red 88 (AR88)	11.06	28	56	56–490
Acid Orange 7 (OII)	10.62	7	15	15–364
Acid Orange 12 (CO)	10.43	21	43	43–464
Acid Orange 52 (MO)	3.20	7	34	34–383
Acid Red 2 (MR)	2.88	4.74	21	28–498
Acid Red 26 (P2R)	11.59	14	44	44–326
Acid Red 27, Food Red 9 (AMR)	10.36	15	53	53–334

Table 2
Concentration range for the spiked tap water analyzed and achieved recoveries after preconcentration

CI name	Concentration (μg/l)	Recoveries (n=3) (%)
Acid Yellow 1	3.0–10	85±10
Acid Orange 7	3.3–11	95±4
Acid Orange 52	2.2–14	76±1
Acid Red 2	2.6–15	95±4
Acid Red 26	4.9–15	85±8
Acid Red 27, Food Red 9	3.0–20	65±8
Acid Red 88	9.8–29	100±4

The dyes were extracted from these samples by using SPE cartridges. They were previously preconditioned with 1 ml of methanol followed by 2 ml of phosphate buffer containing 2.4 m*M* of butylamine. The sample (100 ml) was passed through the cartridge using a peristaltic pump at 2 ml/min. After this the dyes were eluted with 2 ml of the mobile phase with pH adjusted to 9. The recoveries were determined as the ratio of the peak areas obtained for processed samples over the peak areas of a directly injected standard solution of the dye mixture. The results are shown in Table 2.

3. Results and discussion

3.1. Chromatographic conditions

The mobile phase organic modifier, butylamine concentration and pH were optimized to obtain the best possible separation between the different compounds.

The pH range studied (acceptable for this column) was between 4 and 8. No pH influence was observed at this range, since most of the analytes have pK_a values outside of this range, except for acid red 2 (pK_{a_1}=2.88 and pK_{a_2}=4.74). This dye has a high retention time at pH 4.75 (40 min), as indicates the ln(k') plot in Fig. 2. For this reason, a phosphate buffer (pH 6.7) was selected to study different percentages (27–50%) of organic modifier to find the best separation in a shorter time. The results obtained (Fig. 3) show that 27% of organic modifier gives good separations, and also that acid orange 12 and acid orange 7 have the same retention time regardless of organic modifier percentage. This is due to their similar molecular structure and under these conditions it was not possible to separate them. We did not consider an acetonitrile content under 27% because of the increasing in retention time for all compounds.

Butylamine was used to give butylammonium as counter-ion, and concentrations between 0 and 4 m*M* were tested. A final value of 2.4 m*M* was selected since the results did not improve over this value and the retention time for Red 88 increased drastically with the butylamine concentration. Fig. 4 shows the influence of butylamine concentration on the chromatographic separation.

The UV–Vis detector was set at 460 nm, because at this wavelength all compounds have similar absorptivity. Fig. 5 shows the spectra of the dyes in acidic medium.

The mobile phase flow rate was 1 ml/min from 0 to 6 min, and 2 ml/min from 6 min till the end of the chromatogram. This flow-gradient was used because at 1 ml/min flow rate seven analytes were eluted in 6.5 min, and the last one appeared at 28 min. By increasing the flow rate to 2.0 ml/min, the last one is eluted in 15 min.

To show the effect of mobile phase composition in the separation of the analytes, a comparison is made in Fig. 6 between two different acetonitrile percentages: Fig. 6a shows a chromatogram obtained in the best conditions, whereas Fig. 6b corresponds to a chromatogram in which the mobile phase was acetonitrile–phosphate buffer 38:62 (v/v). In both cases, the pH was equal to 6.7.

3.2. Quantification

Calibration plots with correlation coefficient $r^2 \geq$ 0.995 were obtained by reporting peak areas (relative units as given by the integrator) as a function of analyte concentrations, at values ranging between one and ten times the quantitation limit. Detection (LOD) and quantitation (LOQ) limits were calculated as the concentration corresponding to three and

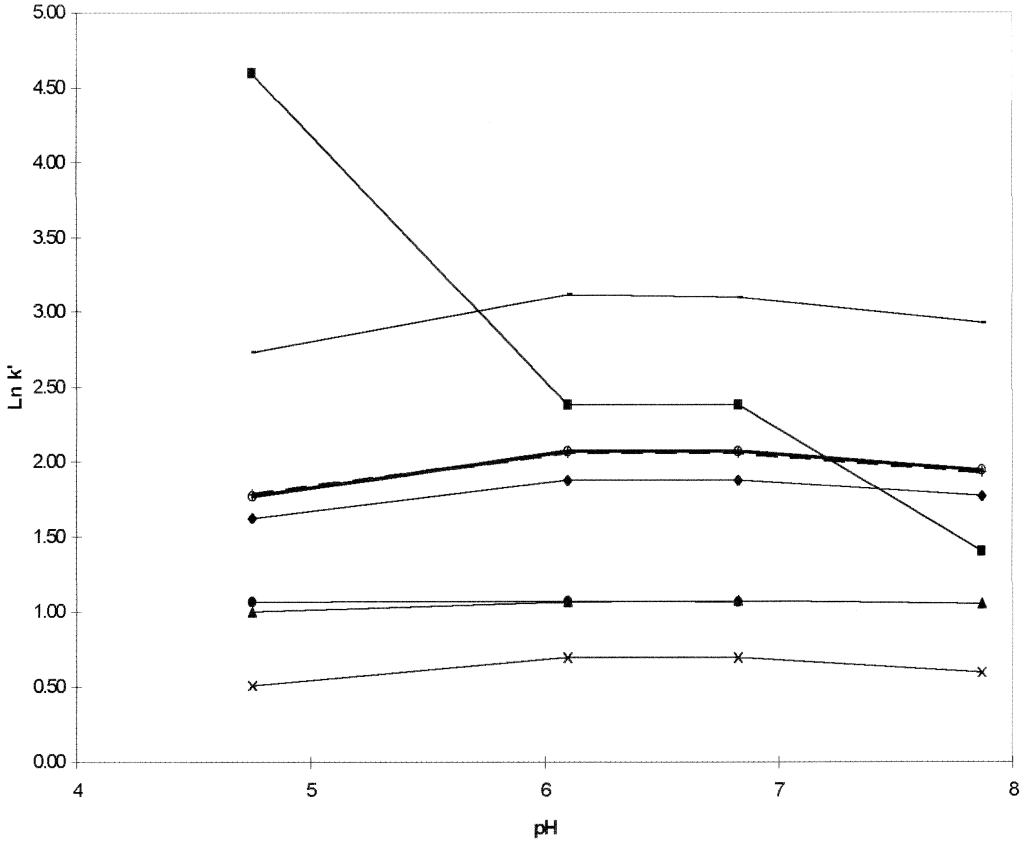


Fig. 2. Ln k' values as function of mobile phase pH. OII (+), NYS (●), MO (♦), CO (○), MR (■), P2R (▲), AMR (×) and AR88 (—).

ten times the variation in the blank response [16]. Table 1 reports LOD and LOQ for each dye.

Spiked tap water samples, prepared as described, were analyzed under the optimized conditions. Table 2 presents the concentration range for the analyzed spiked tap water, the mean values of recoveries after preconcentration and the corresponding relative standard deviations.

4. Conclusions

An ion-interaction HPLC method for the determination of one sulphonated dye and seven sulphonated azo dyes in water samples has been developed. The conditions selected were acetonitrile–phosphate buffer (27:73, v:v), 2.4 m*M* of butylamine and pH 6.7, measured in the mobile phase. The injection volume used was 100μl.

The method allows the quantification of the dyes studied in spiked tap water samples. In conjunction with solid-phase extraction it allows to analyze water samples containing between 0.3 and 1.2 μg/l of the studied compounds. This method gave reliable and reproducible results with a simple sample pretreatment operation.

Acknowledgements

M.P.U. thanks CONACYT for the scholarship. The authors also thank the Comisión Interministerial de Ciencia y Tecnología (AMB98-0327) for supporting this study.

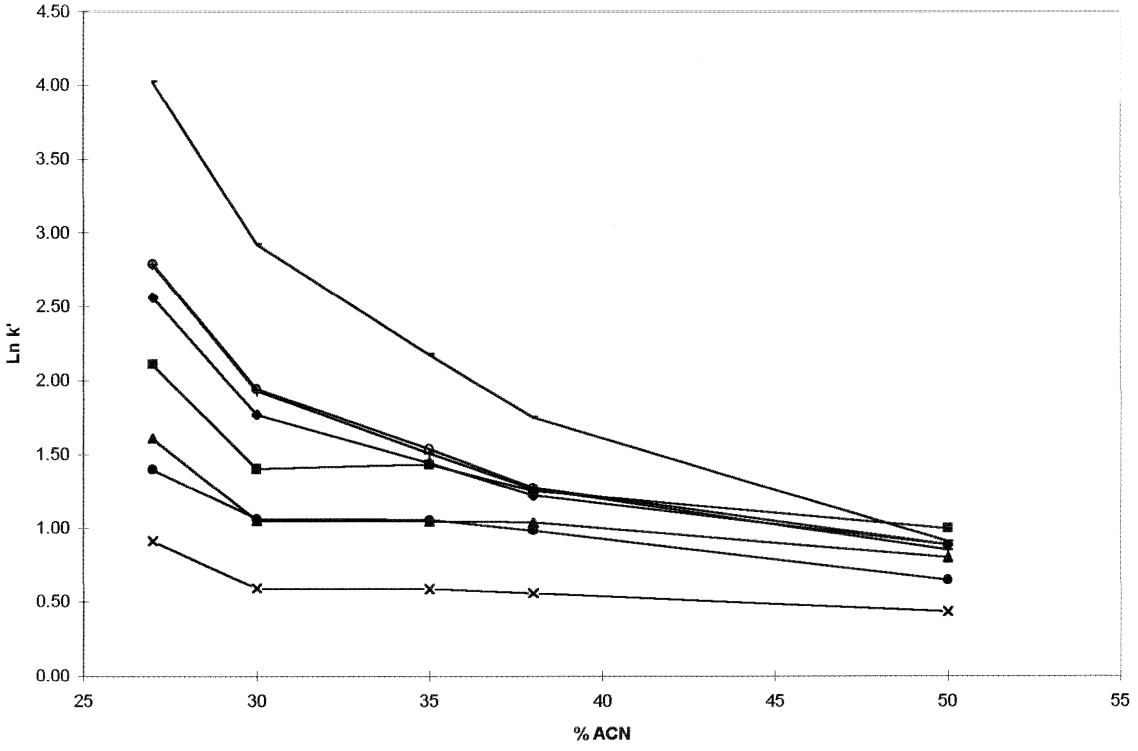


Fig. 3. Ln k' values as function of organic modifier at pH 6.7. Symbols as in Fig. 1. ACN=Acetonitrile.

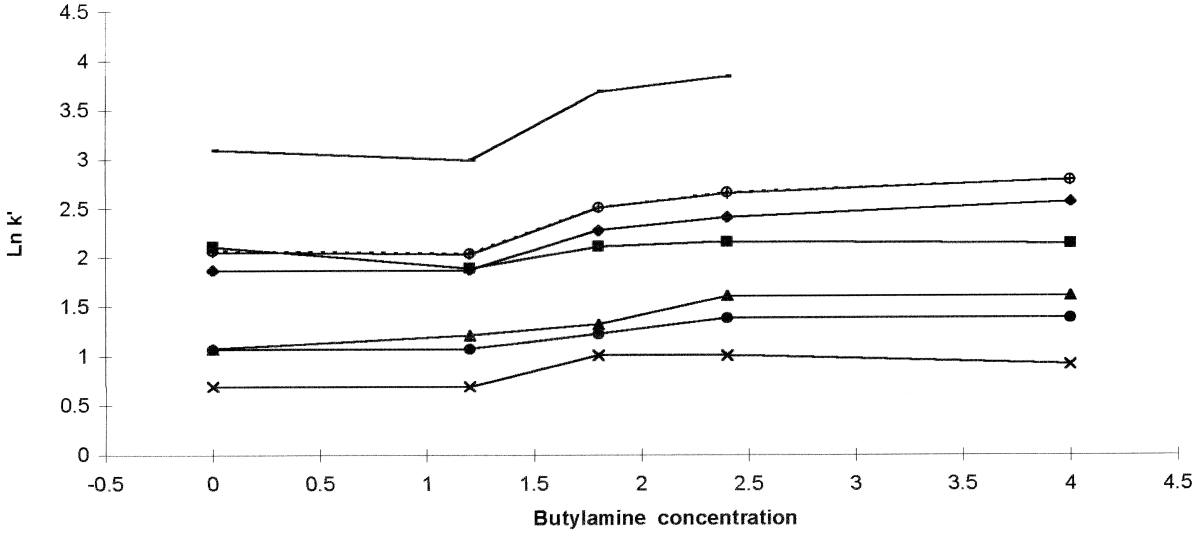


Fig. 4. Ln k' values as function of butylamine concentrations. Symbols as in Fig. 1. The value corresponding to R88 at 4 m*M* butylammonium is not reported because of the high value.

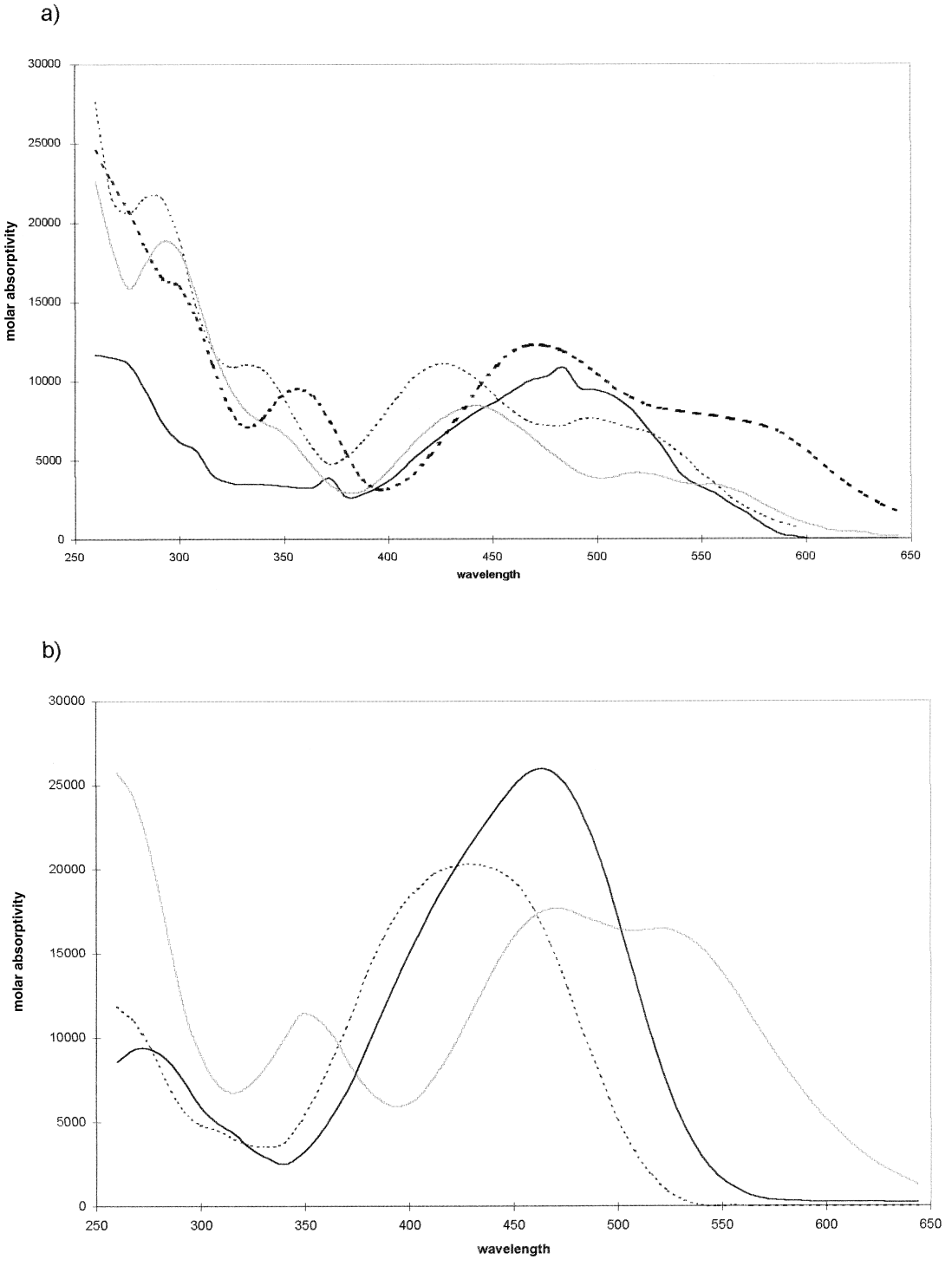


Fig. 5. UV–Vis spectra of the dyes in acid form. (a) OII (—), CO (----), P2R (–), AMR (- - -). (b) MR (----), and AR88 (—), MO (–).

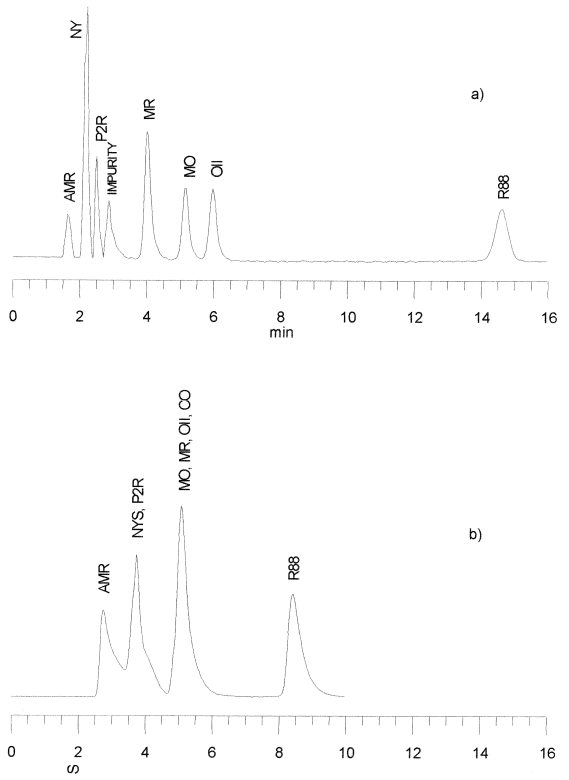


Fig. 6. (a) Chromatogram of an extracted spiked tap water sample. The concentration injected was NYS (126.1 μg/l), P2R (61.2 μg/l), AMR (167.1 μg/l), OII (90.9 μg/l), CO (116.2 μg/l), R88 (245.1 μg/l), MO (215.5 μg/l) and MR (124.5 μg/l). Chromatographic conditions: An RP-ODS stationary phase is used and the mobile phase acetonitrile–phosphate buffer (27:73, v/v), pH 6.7 containing the ion-interaction reagent butylamine. Flow rate of 1 ml/min from 0 to 6 min and after 2 ml/min to the end. (b) Chromatogram of a dye mixture. Chromatographic conditions: mobile phase acetonitrile–phosphate buffer (38:62, v/v), pH 6.7 containing the ion-interaction reagent butylamine. Flow rate of 1 ml/min.

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