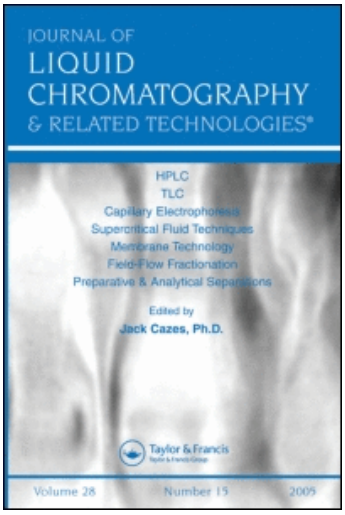

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COMPARISON OF HPLC AND MICELLAR ELECTROKINETIC CHROMATOGRAPHY IN DETERMINATION OF SULFONATED AZO DYES IN WASTE WATER

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ABSTRACT

Capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) are compared for the analytical separation of eight environmentally significant azo dyes, mono-, and di-sulfonated compounds. Optimum separation of these dyes by MEKC was achieved using a buffer of ammonium acetate 9.5 mM, Brij 35 0.1%, pH 9 and by applying electromigration injection at 12 kV for 30 s. Identification was made by the absorbance spectra of each peak. High-performance liquid chromatography separations were performed on a Discovery RP Amide C16 5 μm with a gradient from 25% to 50% acetonitrile and a buffer, made of 1 μg/L tetrabutylammonium bromide, 0.02%

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acetic acid and of 50 mg/L potassium hydroxide solution. The dyes were detected by absorbance at the wavelength of 214 nm.

The linear range of the proposed methods varied from 63 to 7600 μg/L with a limit of detection ranging from 19–230 μg/L by CE and varied from 73 to 5700 μg/L with a limit of detection ranging from 22–280 μg/L by HPLC regarding the assay of 500 mL of preconcentrated water sample.

INTRODUCTION

Azo dyes are a very important group of synthetic chemicals. They are widely used as colouring 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 excessively. There is also evidence that synthetic precursors, intermediates, and degradation products of mentioned dyes could be potential health hazards due to their toxicity and their carcinogenic effects. These compounds are quite difficult to remove by conventional water treatment procedures and can, thus, be distributed from urban wastewater via rivers because of their high solubility in water.[1]

Up to the present, analytical methods for azo dyes have been frequently based on detection using ultraviolet-visible (UV-Vis) absorbance of these compounds.[2–4] For many sample types, including wastewater and solid waste, separation of these components from complex matrices is required prior to quantitative analysis using high-performance liquid chromatography (HPLC).[5,6] Unfortunately, the potential for interferences and errors in HPLC-UV-Vis methods is high, since the resolution obtained by HPLC is not always sufficient to completely separate all components in complex mixtures, and a great number of naturally occurring compounds and industrial contaminants also absorb in the same UV-Vis region. Therefore, in order to assess the potential environmental impact of these series of compounds, definitive analytical methods are needed. Recently, capillary electrophoresis (CE) has been developed as a rapid method for their determination, showing high resolution and good analytical performance. Capillary electrophoresis (CE) is a modern analytical technique which permits rapid and efficient separations of charged components present in small sample volumes. Separations are based on differences in the electrophoretic mobilities of ions in electrophoretic media inside small capillaries.[7–13] These capillaries consist of fused silica where all properties are of importance to obtain successful separations. The studied compounds were acid blue 113, acid red 73, acid red 13, mordant yellow 8, acid red 1, acid red 14, acid red 9, and acid yellow 23. Their structures are presented in Fig. 1. The aim of the present work is to compare the performance of CE and HPLC dye separations.

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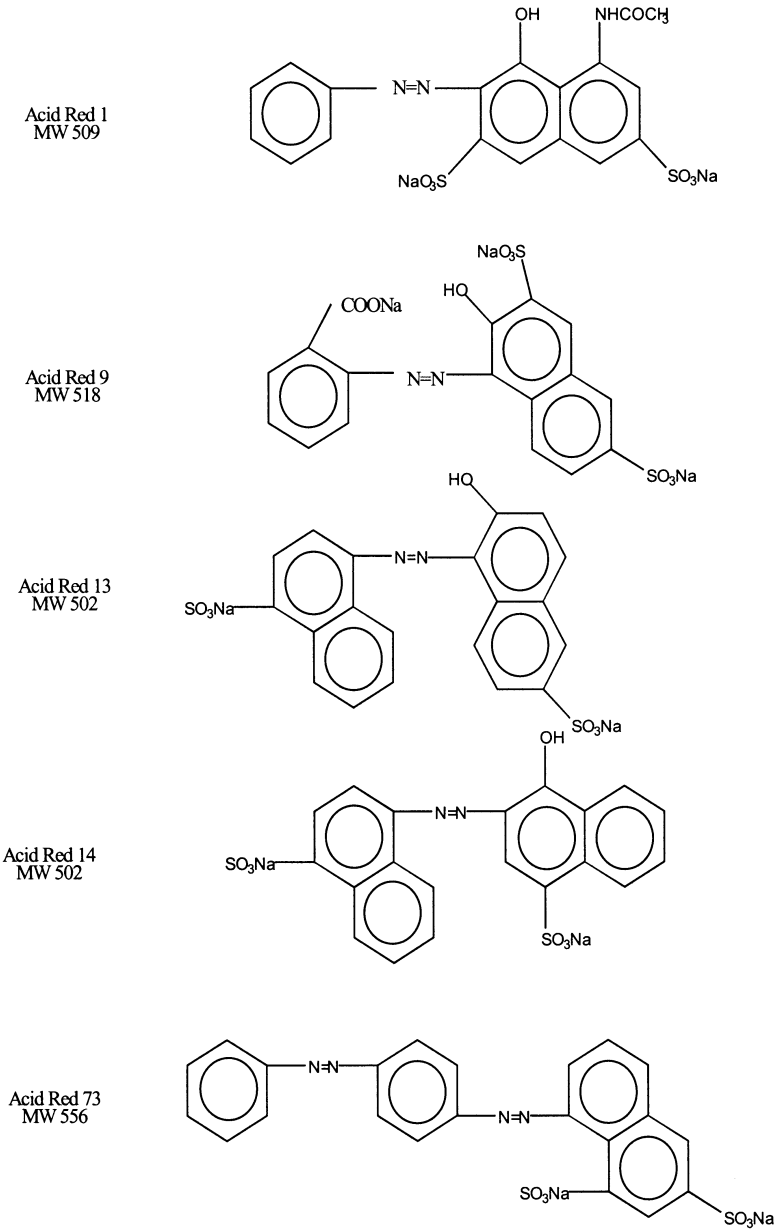
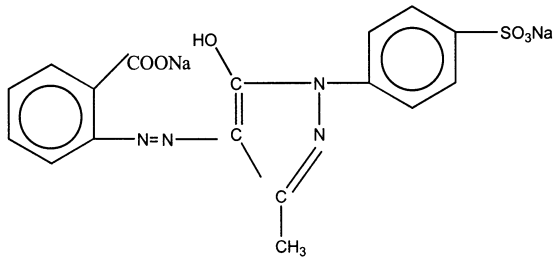


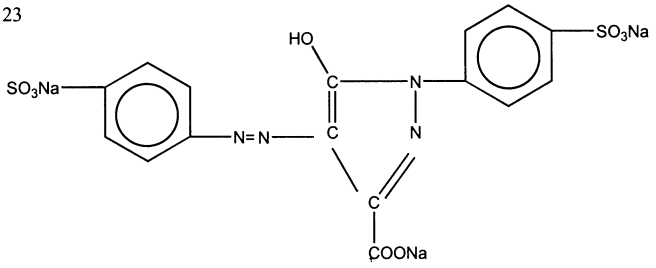
Figure 1. Chemical structures and molecular weights of the eight sulfonate azo dyes studied.

(*continued*)

Mordant Yellow 8
MW 446



Acid Yellow 23
MW 534



Acid Blue 113
MW 681

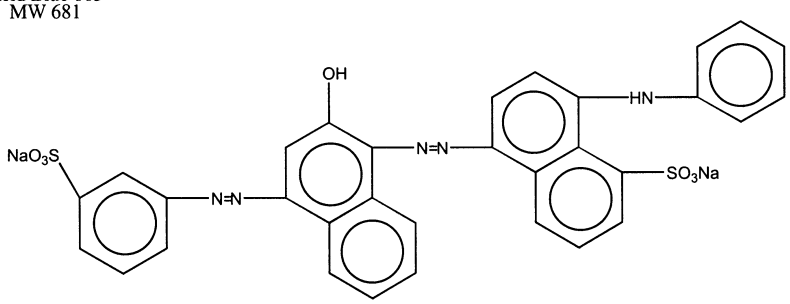


Figure 1. Continued.

EXPERIMENTAL

Reagents

Ultrapure Milli-Q water from Millipore was used for the preparation of solutions. All HPLC-grade solvents, methanol, acetonitrile, ammonia, ammonium acetate, potassium hydroxide, tetrabutylammonium bromide, acetic acid, triethylamine, and Brij 35 were obtained from Merck (Germany).

Pigment standards of sulfonated azo dyes were a loan from Ciba-Geigy and Hoechst (Portugal), ICI (Barcelona, Spain). They were, in most cases, of purity > 99%.

Disposable Isolute ENV + (International Sorbent Technology, UK) was used in the preconcentration step.

Instrumentation

The capillary electrophoresis system employed was the Quanta 4000 (Waters Chromatography Division of Millipore, Milford, MA, USA) including a positive power supply. A Zn lamp was used for UV detection at 214 nm. AccuSep polyamide fused-silica capillaries of the dimension of 60 cm × 75 μm I.D. were used throughout the series of experiments. The temperature of the capillary was set at 25°C. Data acquisition was carried out using the software facilities of Waters Millennium 2010 Chromatography Manager. Modules (Sat/in) connected the CE system with the data station.

The chromatographic determinations were carried out on a Waters HPLC system, which includes an automated gradient controller, two HPLC-pumps (W 510), a column oven with a temperature control module, a six-port manual injector (Rheodyne 7725i), and a photodiode array detector (W.996), all from Waters.

The pH of the electrolyte, spiked water samples, and wastewater was adjusted by adding ammonia and/or acetic acid by used of a Model 516 pH meter (Metrohm, Herisau, Switzerland) connected with a common pH glass electrode. The pH meter was calibrated with standard buffer solutions (pH 4.0; 7.0 and 9.0) purchased from Metrohm (Metrohm Ltd., Herisau Switzerland). Off-line SPE experiments were performed using an autosampler from Gilson (Villires-Le-Bel, France). This system consists of an automated sample preparation device with extraction columns system (Aspec XL) fitted to an external 306 LC pump for dispensing the samples through the SPE cartridges. The drying step was carried out using a VAC Master-20 (International Sorbent Technology, UK).

Analysis by Capillary Electrophoresis

Sample Preparation

The standards were first dissolved in water to provide a stock solution of 1 g/L. Then 500 mL of water was diluted starting from the stock solution to obtain a concentration of 3 mg/L for each compound and subsequently was acidified to

pH 3. The cartridges packed with 200 mg Isolute ENV + (International Sorbent Technology, UK), which had 6 mL capacity, were attached to the Aspec system (Gilson, Villers-le-Bel, France). The SPE cartridges were conditioned with 7 mL of MeOH followed by 3 mL of water (pH 3) at a flow rate of 1 mL/min. The spiked water and waste water samples were dispensed with a flow rate of 5 mL/min through the cartridge columns and, subsequently, a clean-up step rinsing with 2 mL of water (1 mL/min pH3) was performed. After this, a drying step was introduced, which took about 30 min using a vacuum system. The dyes were eluted with two aliquots of 5 mL of MeOH-H_2O-triethyl amine regarding rest of 5 min between the elution steps. The eluate was evaporated under a gentle stream of nitrogen at 60°C and, thereafter, diluted to the appropriate concentration range by adding water in a proportion of 1 : 2 prior to injection into the CE system. The blanks were, of course, not diluted.[14]

In all cases, samples were introduced by electrokinetic injection at the anode end of the capillary, for 30 seconds.

Preparation of the Electrolytes

The electrophoresis buffer solution used was prepared by dissolving 9.5 mM ammonium acetate and 0.1% Brij 35 in water and subsequently adjusted to pH 9.0. Prior to usage, the capillary was regenerated and equilibrated with 0.5 M NaOH (20 min), water (20 min) and the working buffer solution (20 min). Besides, every time between the separation runs, the capillary was washed with 0.5 M NaOH (10 min) and subsequently with electrolyte for 10 min. The buffer solution was filtered through a membrane of pore size 0.2 μm. All working electrolytes were prepared fresh daily and degassed prior to use.[14]

Analysis by HPLC

The HPLC analyses were carried out on a Discovery RP Amide C16 250 mm × 4.6 mm (i.d.), particle size 5 μm column. The operation proceeded at 30°C, a flow rate of 1 mL/min and a gradient from 25% to 50% acetonitrile in deionised water. Discovery columns are manufactured using the sol-gel process whereby billions of tiny "sol" particles (<10 nm) are aggregated and fused. This process results in highest quality and most stable silica particles.

Discovery bonded phases ensure quality due to maximal, reproducible phase density and endcapping. Phase density and endcapping affect the quality in two ways. First, as the degree of coverage increases, the peak shapes of basic compounds improve because more silanols are bonded. Secondly, keeping the degree of coverage constant from batch to batch helps to maintain reproducible

separations. Discovery's spherical, rigid silica gel particles have been size-controlled to provide a very distinct particle size distribution. This feature requires a uniformly packed column bed. All these parameters are very important to allow a very good separation of the compounds studied.

The eluent used contains 1 μg/L tetrabutylammonium bromide, 0.02% acetic acid and 50 mg/L potassium hydroxide solution. The detection was by UV absorbance at 254 nm. Samples were introduced onto the column via an injection valve fitted with a 20 μL sampling loop.

All eluents and buffer solutions were filtered and degassed. Deionized water was used throughout.

RESULTS AND DISCUSSION

Capillary electrophoresis has frequently been compared with high-performance liquid chromatography. CE has attracted considerable attention in recent years because of its potential to achieve very high efficiencies. The main reason for the extraordinary high efficiency in CE is attributable to its characteristically flat flow profile. In general, the flow of mobile phase in HPLC is maintained by a pump and, therefore, under normal operating conditions, a parabolic flow profile is observed. As a result of its contributions to peak broadening, the flow profile inherently limits the separation efficiency theoretically achievable in HPLC separations.

To preserve the high efficiency capabilities of capillary electrophoresis, the injection system must not introduce significant zone broadening. It is important to ensure that the sample injection method employed is capable of delivering small volumes of sample (typically several nanoliters) onto the column, efficiently and reproducibly. Consequently, the most commonly employed injection methods for CE are direct on-column methods, such as electromigration and hydrodynamic flow.

Capillary electrophoresis systems are easily overloaded by large sample volumes. Sample overload can affect system efficiency by two distinct mechanisms. One mechanism relates to the volume of the sample injected relative to the total volume of the capillary. The second mechanism imposes a limit on the concentration of the sample injected and it's related to the difference in electrical conductivity of the sample and electrophoretic medium. At high sample concentrations, system efficiency can be degraded due to perturbation in the potential field gradient by the sample within the column. Several distorted peaks may result. On the other hand, if the sample injected has a slightly lower conductivity than that of the electrophoretic buffer, sample stacking can be achieved which improves peak shape and, hence, increases efficiency.

Sample stacking occurs when the conductivity of the injected sample is lower than that of the surrounding buffer and, hence, results in concentration of the analyte zone. The reason for the narrowing of the analyte zone can be attributed to the fact that the electric field depends inversely on the specific conductivity, i.e., higher field strength at lower conductivity. Therefore, the electric field strength increases in the sample zone of lower conductivity. The electrophoretic velocity increases at higher field and, hence, the analyte zone becomes narrower. This effect, described as stacking, can be utilized in both hydrodynamic and electrokinetic injections to enhance efficiency.

The advantage of MEKC is the ability to eliminate associated inherent problems of baseline shifts caused by UV changes, known to be a problem when using gradient in HPLC. Using MEKC, one can avoid gradients and, thus, the analysis of hydrophobic compounds which normally requires gradient HPLC could advantageously be run. This behaviour can be attributed to the limited operative retention window in MEKC.[15] Another advantage of MEKC is the improved peak shapes for impurities. HPLC is perhaps currently the most widely used system, but its operation and maintenance are relatively expensive, because it often requires the use of large amounts of pure organic solvents, and HPLC separation columns have to be specially prepared. Besides, method development can be labour intensive and time consuming.[16] Actually, a new line of high quality HPLC columns provides a completely different performance; this has been discussed elsewhere. Both techniques are capable of separating the compounds of interest at high efficiencies.

MEKC

All of the eight dyes studied contain carboxylic or sulfonic acid moieties in their molecules, as illustrated in Fig. 1. In acetate buffer solution, they can dissociate into multiply charged anions with a high negative electrophoretic mobility. However, when the mixture of the dyes was analysed using conventional buffer (phosphate–borate 0.025 mol/L) separation was not achieved. The use of a micellar buffer system (ammonium acetate 9.5 mM added of Brij 35 0.1%), allows a good separation of the eight sulfonated azo dyes studied. Thus, the use of MEKC improves the separation efficiency and shows that this technique has the potential to be an excellent method of analysis for the study of this class of compounds. The capillary chosen had a diameter of 75 μm since the efficiency of the MEKC depends on the longitudinal diffusion and thermal non-uniformity, which could be minimised in this way. For electromigration injection at the anode, the EOF should be large enough to facilitate the introduction of these analytes. If the EOF of the capillary is not sufficient, the mobility resulting from some, or even all, analyte species may become negative; making the separation impossible. It is also

important to recognize that the EOF's of the capillary depend on the manufacturer and can even vary from batch to batch.[18] These aspects are very important and need to be considered when performing this technique.

Since the electroosmotic migration velocity is higher than the electrophoretic migration velocity of the dyes tested, a positive potential should be applied across the capillary and the dyes should then migrate from the source to the detector at a velocity which is lower than that of the electroosmotic flow. The selectivity of separation does not depend, significantly, on the applied potential, but the migration time decrease when the potential increases. To keep the time of analysis to a minimum, the separation was run at 12 kV (Fig. 2). An increase of the potential did not bring about a significant decrease in migration times.

Precision

Repeatability and reproducibility of migration times and peak areas are presented in Table 1. The repeatability of migration times and peak areas was assessed by six replicated injections of 25 mg/L standard solution mixture. The maximum R.S.D. value of 1.59% for migration time and the maximum R.S.D. value of 14.81% for peak area obtained, is a reliable accuracy. The reproducibility was obtained by six repeated injections of 25 mg/L standard solution mixture combining, at random, the standards of all dyes on different days. We can agree that the exhibited coefficient of variation (%R.S.D.) is quite acceptable. Satisfactory results were obtained on the reproducibility of the peak areas of the dyes, with the

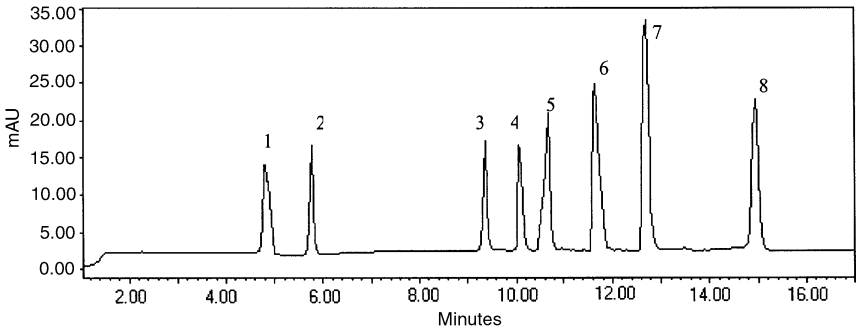


Figure 2. CE-UV (214 nm) Electropherogram of the separation of a standard mixture of eight sufonated azo dyes. Separation was carried out with a buffer solution of ammonium acetate 9.2 mM and Brij 0.05%. Peaks: (1) Acid Blue 113 (2) Acid Red 73 (3) Acid Red 13 (4) Mordant Yellow 8 (5) Acid Red 1 (6) Acid Red 14 (7) Acid Red 9 (8) Acid Yellow 23, Concentration: 50 μg/L.

Table 1. Repeatability[a] and Reproducibility[b] of Migration Time and Peak Area by CE-UV (214 nm)

	Repeatability				Reproducibility			
Compounds	Retention Time (min)	R.S.D.	Peak Area UV*Sec 10^3	R.S.D.	Retention Time (min)	R.S.D.	Peak Area UV*Sec 10^3	R.S.D.
Acid Blue 113	4.86 ± 0.30	0.60	1.534 ± 0.09	6.00	4.43 ± 0.13	2.93	1.565 ± 0.07	9.94
Acid Red 73	5.76 ± 0.02	0.35	1.185 ± 0.02	1.24	5.36 ± 0.15	2.80	1.203 ± 0.02	1.52
Acid Red 13	9.49 ± 0.01	0.15	1.345 ± 0.09	6.69	9.53 ± 0.16	2.45	1.367 ± 0.10	7.30
Mordant Yellow 8	10.15 ± 0.05	0.61	1.209 ± 0.03	2.48	10.23 ± 0.35	4.84	1.234 ± 0.05	3.65
Acid Red 1	10.75 ± 0.07	0.80	1.752 ± 0.02	1.74	10.64 ± 0.47	5.43	1.774 ± 0.04	2.98
Acid Red 14	11.92 ± 0.12	1.13	1.834 ± 0.09	3.51	11.56 ± 0.22	2.08	1.823 ± 0.05	5.86
Acid Red 9	12.98 ± 0.22	1.36	2.423 ± 0.07	4.92	12.88 ± 1.34	8.70	2.639 ± 0.09	6.49
Acid Yellow 23	14.94 ± 0.27	1.59	1.995 ± 0.10	14.81	14.92 ± 1.83	11.49	1.908 ± 0.12	17.95

[a]Average of 6 injections of 25 mg/L standard mixture in the same day.
[b]Average of 6 injections of 25 mg/L standard mixture on 3 different days.

exception of the acid yellow 23. This means that a good cleaning and stabilisation of the capillary between the sample runs is very important for efficient separation.

Quantification

Calibrations were performed by preparing a standard solution mixture of all examined dyes, applying a 5 point calibration curve over the concentration range of 10–60 mg/L. The linear regression equations with slopes and correlation coefficients are shown in Table 2. The resulting values achieved for the correlation coefficients show a good relationship between the concentration of each compound and its peak area. The minimum detectable amounts (LODs) were assessed by measuring the signal-to-noise ratio of three for the eight dyes (also given in Table 2).

HPLC

In several published works,[19–22] it has been stated that the main problems appear on the separation failure with HPLC, due to the lack of thoroughly

Table 2. Calibration Data Obtained with Standard Solutions by CE-UV (214 nm)

Compounds	Calibration Equation	R^2	LOD (mg/L)	LOQ (mg/L)	Linear Range (mg/L)
Acid Blue 113	$Y = -5167.9 + 613.2X$	0.991	3	10	10–150
Acid Red 73	$Y = -1304.7 + 412.8X$	0.994	1	3	3–165
Acid Red 13	$Y = -2986.5 + 804.3X$	0.993	2	7	7–180
Mordant Yellow 8	$Y = -4035.6 + 408.1X$	0.995	7	23	23–130
Acid Red 1	$Y = -102.7 + 415.9X$	0.996	2	7	7–180
Acid Red 14	$Y = -4178.8 + 457.2X$	0.994	1	3	3–194
Acid Red 9	$Y = -3942.1 + 899.3X$	0.991	1	3	3–173
Acid Yellow 23	$Y = 79.2 + 267.9X$	0.998	8	27	27–126

conditioning the stationary phase. This completes with a good resolution of the signals. In fact, the articles examined show all the chromatograms with unresolved peaks. Looking at the chemical characteristics of the new column used in this work, we can see that, with our procedure, this handicap was overcome and the result of completely resolved peaks can be seen in the Fig. 3.

Initially, we also performed the work with a 4.6 mm × 150 mm Res Elut μm C18 column and the separation of the compounds was not satisfactory, as can be seen in Fig. 4.

Precision

Repeatability and reproducibility of migration times and peak areas are presented in Table 3. The repeatability of migration times and peak areas was assessed using six replicated injections of a 25 mg/L standard solution mixture. The maximum R.S.D. value of 3.56% for migration time and the maximum R.S.D. value of 13.87% for peak area indicate a very satisfactory accuracy of the method. The reproducibility was obtained by six repeated injections of 25 mg/L standard solution mixture combining the standards of all dyes on different days. We can consider that the coefficient of variation (%R.S.D.) exhibited by almost migration times and peak areas are quite good. Satisfactory results were obtained

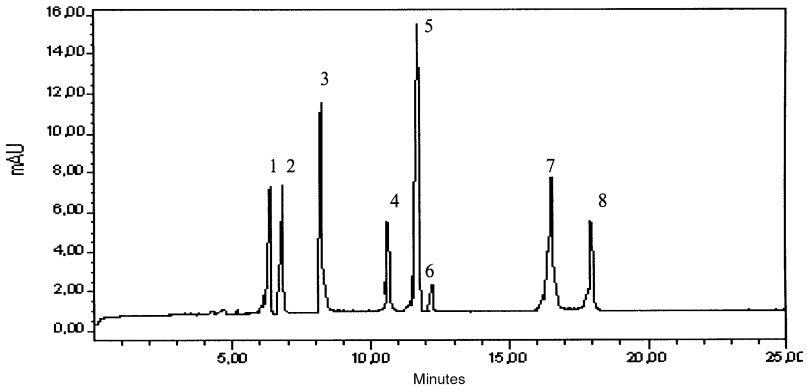


Figure 3. Separation of a standard mixture of sulfonated azo dyes by HPLC: UV detection at 214 nm; 4.6 mm × 250 mm Discovery C16 column; flow rate 1 mL/min; gradient from 25% to 50% acetonitrile in deionised water containing 1×10^{-3} mL/L tetrabutylammonium bromide, 1 mL/L 20% acetic acid and 1 mL/L of 50 g/L potassium hydroxide solution. Peaks: (1) Acid Blue 113 (2) Acid Red 73 (3) Acid Red 13 (4) Mordant Yellow 8 (5) Acid Red 1 (6) Acid Red 14 (7) Acid Red 9 (8) Acid Yellow 23, Concentration: 100 μg/L.

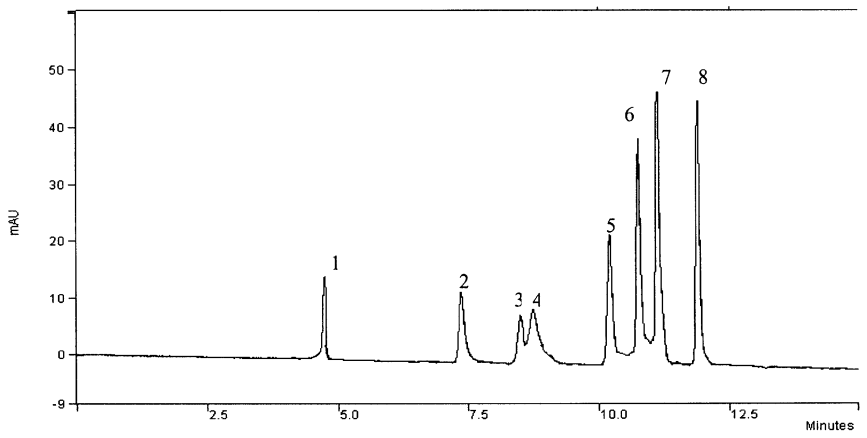


Figure 4. Separation of a standard mixture of sulfonated azo dyes by HPLC: UV detection at 214 nm; 4.6 mm × 150 mm Res Elut μm C18 column; flow rate 1 mL/min; gradient from 25% to 50% acetonitrile in deionised water containing 1×10^{-3} mL/L tetrabutylammonium bromide, 1 mL/L 20% acetic acid and 1 mL/L of 50 g/L potassium hydroxide solution. Peaks: (1) Acid Blue 113 (2) Acid Red 73 (3) Acid Red 13 (4) Mordant Yellow 8 (5) Acid Red 1 (6) Acid Red 14 (7) Acid Red 9 (8) Acid Yellow 23, Concentration: 100 μg/L.

for the reproducibilities in the peak area measurements of the dyes, with exception of the acid yellow 23.

Quantification

Calibration was performed with a standard solution mixture of all studied dyes by a five point calibration curve over the concentration range of 20–50 mg/L. The linear regression equations with slopes and correlation coefficient are shown in the Table 4. The values found for the correlation coefficient show a good relationship between the concentration of each compound and its peak area. The minimum detectable amounts (LODs), assessed by signal-to-noise ratio of three for the eight dyes, are also given in Table 4.

Extraction and Recoveries

In order to evaluate the entire performance of the automated off-line SPE-Aspec XL–CE/UV and HPLC/DAD methods, synthetic samples and textile

Table 3. Repeatability and Reproducibility of Migration Time and Peak Area Obtained with Standard Solution by HPLC (Discovery Column)

	Repeatability				Reproducibility			
Compounds	Retention Time (min)	R.S.D.	Peak Area UV*Sec 10^3	R.S.D.	Retention Time (min)	R.S.D.	Peak Area UV*Sec 10^3	R.S.D.
Acid Blue 113	6.56 ± 0.10	1.12	1.923 ± 0.10	8.67	6.59 ± 0.22	4.89	2.012 ± 0.03	11.23
Acid Red 73	6.92 ± 0.08	0.89	2.044 ± 0.10	4.20	6.99 ± 0.46	6.31	2.324 ± 0.08	6.21
Acid Red 13	8.22 ± 0.15	0.93	3.425 ± 0.06	5.84	8.21 ± 0.51	7.11	3.450 ± 0.21	6.11
Mordant Yellow 8	10.72 ± 0.20	0.99	1.712 ± 0.08	6.34	10.83 ± 0.12	1.56	1.610 ± 0.11	7.21
Acid Red 1	11.80 ± 0.09	0.74	4.487 ± 0.01	2.56	11.83 ± 0.10	2.11	4.022 ± 0.09	3.12
Acid Red 14	12.11 ± 0.11	2.71	1.246 ± 0.02	3.89	12.19 ± 0.32	3.13	1.053 ± 0.20	4.16
Acid Red 9	16.82 ± 0.16	2.93	2.478 ± 0.04	4.61	16.94 ± 1.18	9.21	2.751 ± 0.21	7.90
Acid Yellow 23	18.08 ± 0.40	3.56	1.456 ± 0.30	13.87	18.11 ± 1.24	9.39	1.163 ± 0.40	18.87

Table 4. Calibration Data Obtained with Standard Solution by HPLC (Discovery Column)

Compounds	Calibration Equation	R^2	LOD (mg/L)	LOQ (mg/L)	Linear Range (mg/L)
Acid Blue 113	Y = 3.4461e + 2X + 4.7790e + 1	0.996	8	27	27–121
Acid Red 73	Y = 2.9945e + 3X + 6.2113e + 2	0.997	11	37	37–115
Acid Red 13	Y = 5.7532e + 3X + 1.4457e + 3	0.998	7	23	23–160
Mordant Yellow 8	Y = 9.7133e + 2X + 6.1134e	0.998	9	30	30–124
Acid Red 1	Y = 4.1889e + 3X + 7.2255e + 2	0.998	10	33	33–127
Acid Red 14	Y = 1.1145e + 3X − 3.2755e	0.992	13	43	43–154
Acid Red 9	Y = 1.3245e + 3X − 1.0045e + 3	0.998	6	20	20–123
Acid Yellow 23	Y = 4.867e + 2X + 1.0967e + 3	0.999	12	40	40–179

samples were spiked with the standard mixture of the eight dyes in the range of 1–4 mg/L (Table 5). The preconcentration procedure was carried out with a volume of 500 mL. These calibration data and LODs for different compounds are presented in Tables 6 and 7, respectively. The equations for regression CE method were characterised by a coefficient in the range of 0.94–0.99 and LODs could be calculated for signal-to-noise of three between 19–230 μg/L. For HPLC, the regression equations were characterised by a coefficient in the range of 0.92–0.99 and LODs could be calculated for signal-to-noise of three between 22–280 μg/L. Some authors recommend the application of copolymers, such as styrene-divinilbenzene, as the most reliable materials for SPE of very polar compounds. Licrolut ENV + was the chosen system as a consequence of higher recoveries of these compounds.[18] The effectiveness of the elution step was enhanced by adding an amine base (TEA) which allowed the total removal of cations and, consequently, yielded better recoveries.[23–26] Table 3 shows the recoveries and the standard deviation of the eight dyes from spiked water determined using MEKC or HPLC, respectively. For the acid yellow 23, no satisfying results were obtained. It is not surprising that the results presented show almost the same

Table 5. Recoveries Obtained from 500 mL Preconcentrated Spiked Water (3 mg/L) by Solid Phase Extraction with Isolute ENV + Sorbent

	Solvents (80% MeOH, 20% H_2O, 0.18% TEA)	
Compounds	MEKC	HPLC
Acid Blue 113	68 ± 4	65 ± 5
Acid Red 73	73 ± 2	78 ± 3
Acid Red 13	81 ± 1	79 ± 2
Mordant Yellow 8	72 ± 4	70 ± 4
Acid Red 1	79 ± 3	83 ± 5
Acid Red 14	80 ± 2	84 ± 4
Acid Red 9	54 ± 6	59 ± 8
Acid Yellow 23	39 ± 3	43 ± 6

Table 6. Calibration Data Obtained with CE-UV (214 nm) After Preconcentration of 500 mL of Spiked Wastewater in Isolute ENV + Cartridges

Compounds	Calibration Equation	R^2	LOD (μg/L)	LOQ (μg/L)	Linear Range (μg/L)
Acid Blue 113	Y = −20239.2 + 749.2X	0.935	230	766	766–3950
Acid Red 73	Y = −6072.9 + 671.4X	0.995	45	150	150–5670
Acid Red 13	Y = −11328.0 + 427.3X	0.965	25	83	83–6230
Mordant Yellow 8	Y = −8433.7 + 723.6X	0.980	180	599	599–7000
Acid Red 1	Y = −4052.3 + 276.4X	0.963	24	80	80–6500
Acid Red 14	Y = −10117.8 + 824.1X	0.951	19	63	63–7600
Acid Red 9	Y = −9726.1 + 856.1X	0.944	42	140	140–5100
Acid Yellow 23	Y = 987.0 + 118.5X	0.973	40	133	133–4800

Table 7. Calibration Data Obtained with HPLC After Preconcentration of 500 mL of Spiked Wastewater (3 mg/L) in Isolute ENV + Cartridges

Compounds	Calibration Equation	R^2	LOD (μg/L)	LOQ (μg/L)	Linear Range (μg/L)
Acid Blue 113	Y = −32173.9 + 731.2X	0.922	280	932	932–3800
Acid Red 73	Y = −7432.8 + 312.4X	0.969	49	163	163–4000
Acid Red 13	Y = −18543 + 245.4X	0.992	22	73	73–4500
Mordant Yellow 8	Y = −11234 + 567.9X	0.987	253	842	842–4900
Acid Red 1	Y = −2145.9 + 213.8X	0.983	36	120	120–5000
Acid Red 14	Y = −12123.2 + 109.1X	0.947	29	97	97–4650
Acid Red 9	Y = −11765.7 + 321.1X	0.956	49	163	163–4720
Acid Yellow 23	Y = 532.3 + 321.5X	0.972	31	103	103–5700

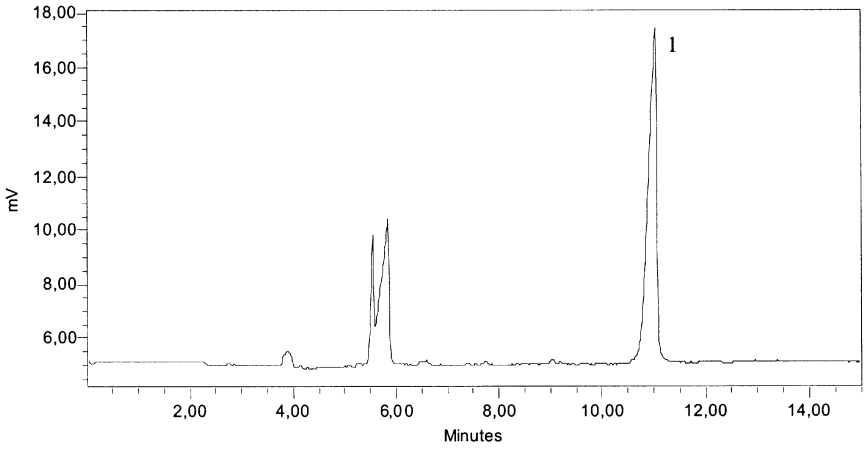


Figure 5. Electropherogram of a waste water sample from a textile industry site with a concentration of 1.1 mg/L of Acid Red 1. The sample was diluted 1 : 100 with HPLC grade water.

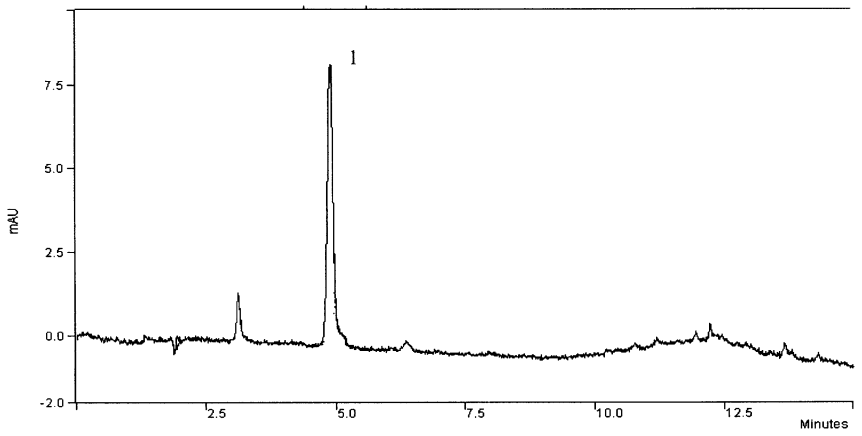


Figure 6. Chromatogram of a waste water sample from a textile industry site with a concentration of 0.9 mg/L of Acid Red 1. The sample was diluted 1 : 100 with HPLC grade water.

figures, which indicates a good reproducibility for recoveries of the dyes from water polluted samples extracted by an off-line system.

Figures 5 and 6 show the results of real samples with MEKC and HPLC, respectively.

CONCLUSIONS

Two analytical methods (MEKC and HPLC) were optimised for the determination of a mixture of eight selected azo dyes in wastewater collected from a textile industry site. In the sample preparation step, with an automated off-line SPE system, the recoveries obtained were similar for both techniques, which show a good reproducibility. A good linearity was shown in the two analytical methods, for the quantification in the range and scope of the study, as can be seen in Tables 2 and 6. Detection limits in the range between 1–8 mg/L for standard solutions and between 24–230 μg/L in spiked wastewater were achieved by MEKC. In the case of HPLC, the values are, respectively, 6–13 mg/L and 22–280 μg/L. The great selectivity exhibited by the "Discovery" column yields a better resolution and results in chromatographic peaks without tailing. Comparing MEKC and HPLC techniques, we can conclude that the first analytical technique is less expensive because it uses inexpensive capillaries and has low running

costs. From these results, we can conclude that separation of these compounds by CE is complementary with those obtained using conventional HPLC methods. The choice depends on the financial investment that the laboratory can afford.

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