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Determination of Sulfonated Azo Dyes in Groundwater and Industrial Effluents by Automated Solid-phase Extraction Followed by Capillary Electrophoresis/Mass Spectrometry

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One monosulfonated (Mordant Yellow 8) and seven disulfonated azo dyes (Acid Red 1, Mordant Red 9, Acid Red 13, Acid Red 14, Acid Red 73, Acid Yellow 23 and Acid Blue 113) were determined in spiked groundwater samples and industrial effluents by automated solid-phase extraction followed by capillary electrophoresis with UV detection and by capillary electrophoresis/mass spectrometry (CE/MS). The MS parameters were optimized to achieve maximum sensitivity and minimum fragmentation. The main ions observed were losses of Na^+ cations replaced by hydrogen atoms. Two polymeric solid-phase extraction cartridges (Isolute ENV+ and LiChrolut EN) were compared for the preconcentration of 300 or 500 ml water samples. The recoveries varied from 64 to 80% for all dyes, with the exception of Acid Blue 113 and Acid Yellow 23, the recoveries of which varied from 15 to 34% when using Isolute ENV+. The protocol developed in this work was applied to the determination of sulfonated azo dyes in real samples from industrial effluents. The method detection limit ranged from 10 to 150 μg l^{-1} for CE/UV detection and from 100 to 800 μg l^{-1} for CE/MS under time-scheduled selected-ion monitoring conditions, with the exception of Acid Red 73 (>1700 μg l^{-1}), which exhibited a low response. © **John Wiley & Sons, Ltd.**

KEYWORDS: sulfonated azo dyes; solid-phase extraction; groundwater; industrial effluents; capillary electrophoresis/mass spectrometry

INTRODUCTION

Large quantities of dyes are produced and used in diverse applications, including textile dyes, paint pigments, printing inks and food coloring. According to recent information, nearly 20% of the world dye production, which rose more than 10% annually to 2.2 billion lb in 1994, is manufactured in Western Europe.[1] The textile industry is the largest consumer of these products, accounting for two-thirds of the dyestuff market. Sulfonated azo dyes possess acid groups and are widely used in the textile industry to color natural fibers. The sulfonic acid groups are often present as sulfonate anions and provide very good water solubility. Recent estimates indicate that ~12% of the synthetic textile dyes used each year are lost to waste streams during manufacturing and processing operations and that 20% of those losses will enter the environment through effluents from waste water treatment plants.[2] Removal of these compounds is difficult in waste water treatment procedures and they can be transported from municipal sewers through the rivers. In addition, azo dyes have been shown to undergo reduction in natural waterways and the environment and the degradation products include carcinogenic amines. Their presence in effluents and industrial wastewaters is of considerable interest because of health risks carried by the potential for contamination of groundwater and drinking water supplies.[3] For instance, the drinking water of Barcelona is supplied by the heavily polluted river Llobregat, where many factories are situated on both river banks,[4] the surfactant and textile industries being the most important. It has also been shown that synthetic precursors, intermediates and by-products of these dyes could be potential health hazards owing to both their toxicity and their carcinogenity.[5] Therefore, the detection, identification and quantification of azo dyes in wastewaters at low levels is important for the protection of natural waters.

The analysis of dyes poses special problems because these products do not belong to one group of chemical compounds, but encompass many chemical functionalities with large differences in solubility, volatility, ionization efficiency, etc. Additional complications in the analysis of sulfonated azo dyes arise because some of the manufacturing precursors to dyes are carried over to, and are not removed from, the final product. The result is a complex mixture characterized by the dye

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itself and several other compounds.[6]

Sulfonated azo dyes are non-volatile and thermally unstable, and consequently cannot be analyzed by gas chromatography (GC). For this reason, liquid chromatography/mass spectrometry (LC/MS) has been recommended. Current official analytical methods, e.g. US EPA 8321A,[7] involve the use of LC/thermospray (TSP) MS for the analysis of disperse azo dyes but are not applicable to sulfonated azo dyes. Problems were encountered with the interface[8] owing to the polarity of these compounds with difficult TSP ionization and also for their baseline separation.

With the development of LC/atmospheric pressure ionization (API) MS interfaces, good sensitivity and structural information can be achieved, as reported for many analytes such as phenols[9] and organophosphorus pesticides.[10,11] Capillary electrophoresis (CE) is a powerful alternative to classical chromatographic techniques and it can overcome the problems with LC in the separation of sulfonated azo dyes.[12]

Earlier studies by our group involved the analysis of mono- and disulfonated azo dyes by LC/MS. Although each compound could be detected with high sensitivity and reproducibility by MS using an electrospray ionization (ESI) interface, difficulties arose in their separation by LC.[13] Recently, various applications have been published describing the analysis of dyestuffs using CE, but there have been only a few publications on sulfonated azo dyes and the problems encountered in their analysis (especially with disulfonated and polysulfonated azo dyes) have always been greater than those for other dyes.[14–18] Few of these previous studies involved the determination of azo dyes in water matrices. In this respect, most of these publications used LC/MS or CE/MS of some isolated dyes but without combining solid-phase extraction (SPE) for a broad group of sulfonated dyes.

The efficiency of CE and HPLC can be additionally supported by automated solid-phase extraction (SPE), which involves a preconcentration step as well as the elimination of interferences from the matrix under consideration, i.e. industrial effluents and wastewaters. This is specially true in the case of CE, where its low injection volumes are a serious drawback to the achievement of good detection limits.

An automated SPE method was performed with an ASPEC XL system which was previously optimized in our laboratory for the determination of a variety of pollutants such as organophosphorus pesticides[10] and phenols.[19] In the work cited, it was shown that polymeric sorbents such as LiChrolut EN were appropriate for the most polar analytes. Following SPE, the use of CE/MS is the best solution for the determination of sulfonated azo dyes since it offers the possibility of unequivocal confirmation of analytes.

In view of the lack of methods for the MS determination of disulfonated azo dyes in water, and the problems found in previous work on determination by HPLC,[13] the objectives of this work were (i) the development of an automated extraction method using ASPEC by comparing the performance of the polymeric sorbents LiChrolut EN and Isolute ENV+ for the determination of sulfonated azo dyes in groundwater at the ppb level, and (ii) coupling CE with MS and testing it in terms of linearity, sensitivity and selectivity for the characterization and confirmation of the dyes in spiked and real samples of industrial effluents.

EXPERIMENTAL

Chemicals

All HPLC-grade solvents, methanol, isopropyl alcohol and water, analytical-reagent grade ammonia and ammonium acetate and synthesis grade Brij 35 were obtained from Merck (Darmstadt, Germany). Purrissimum-grade triethylamine (TEA) was purchased from Fluka (Buchs, Switzerland) and acetic acid from Panreac (Barcelona, Spain). Pigment standards of sulfonated azo dyes were loaned by ICI, CIBA-Geigy and Hoechst (Barcelona, Spain).

CE and CE/MS

Micellar electrokinetic chromatography (MEKC) was carried out with a Beckman P/ACE 5000 capillary electrophoresis system (Beckman Instruments, Palo Alto, CA, USA). The UV detector was operated at a wavelength of 214 nm. Separation was performed in a 47 cm (40 cm effective length to detector) × 50 μm i.d. fused-silica capillary (Beckman Instruments). The electrophoresis buffer solution was prepared by dissolving 10 mM ammonium acetate and 0.05% Brij 35 in water and adjusting the pH to 8.6. The capillary was subsequently regenerated with 0.1 M NaOH (15 min), water (5 min) and working buffer solution (10 min) before each analysis. The temperature of the capillary column was set at 25 °C. After a pressure injection (0.5 psi) over 5 s, a voltage of 12 kV was applied across the capillary. Data analysis was performed with System Gold software.

The pH of the electrolyte and the spiked water samples was adjusted by adding ammonia and acetic acid, respectively, using a Model 691 pH meter (Metrohm, Herisau, Switzerland) connected with a glass pH electrode containing 3 M KCl and silver chloride.

For CE/MS operation, the conditions were the same but no Brij 35 was added to the buffer solution because it was found to suppress the ionization, and the separation was performed in the capillary zone electrophoresis (CZE) mode. The voltage applied was 20 instead of 12 kV and the capillary length was 80–100 cm in order to extend it to the probe tip through the stainless-steel sheath capillary.

The CE system was connected to a VG Platform ESI system from Micromass (Manchester, UK) equipped with a CE probe. The design of this probe is shown in Fig. 1 and consists of a triaxial flow arrangement whereby the CE eluent is mixed with a suitable make-up solvent at the probe tip, and then nebulized using nitrogen. The CE capillary extends fully to the probe tip through the stainless-steel sheath capillary

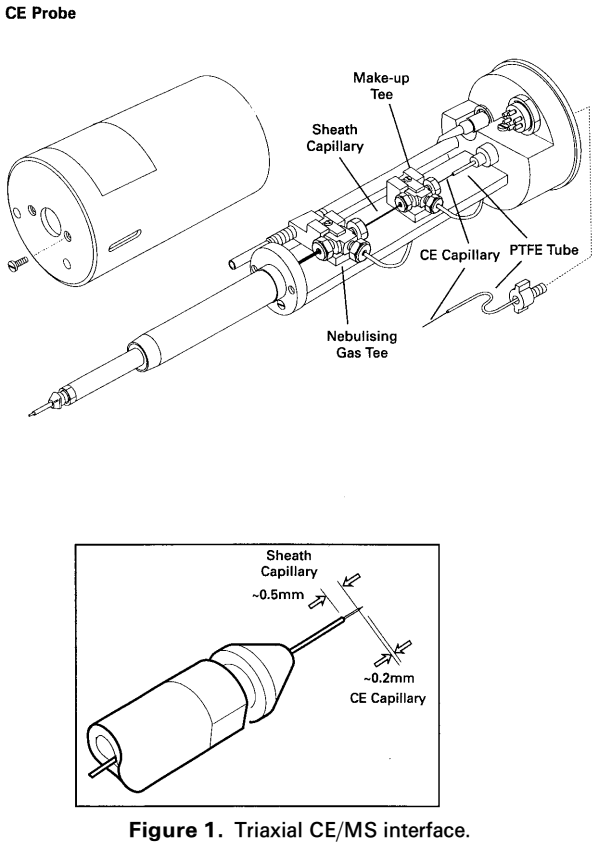

Figure 1. Triaxial CE/MS interface.

which carries the make-up solvent. Around the sheath capillary is the nebulizer capillary, through which nitrogen flows to the probe tip. The make-up solvent performs two functions: to supplement the CE flow to a level adequate for electrospray operation (the CE electroosmotic flow is in the nl min^{-1} range and as a consequence is too low for ESI operation) and to make electrical contact between the CE buffer and the probe tip. The nitrogen that flows through the probe tip maximizes the efficiency of the nebulization. The design of the source is the same as the system used in normal megaflow ESI operation by our group which has been described previously.[10]

The make-up solvent, consisting of isopropyl alcohol–water (80:20) containing 0.1% of ammonia, was delivered at a flow rate of 10 µl min^{-1} by a gradient system under isocratic conditions from Waters Model 616 pumps controlled by a Waters 600S Controller (Waters–Millipore, Millford, MA, USA).

The MS instrument was tuned by filling the capillary with compounds to be studied and monitoring the signal corresponding to the mass of the colorant ion while the voltage of the CE was applied to introduce the sample into the MS system. The operating parameters were adjusted in order to achieve maximum sensitivity (with the consequent loss of fragmentation and structural information). In this study, a nebulizer gas flow-rate of 25–30 l h^{-1} was used and the drying gas was set at a low value (of the order of 50 l h^{-1} or less). The source temperature was set at 75 °C. The cone voltage was set at 20 V in order not to produce fragmentation and to achieve the best sensitivity.

The instrument control and data processing utilities included the use of MassLynx application software installed in a Digital DEC PC 466.

Sample preparation

The standards were first dissolved in water to give a mixed standard solution of 1 g l^{-1}. Figure 2 shows the structures of the compounds studied in this work. In the experiment on the determination of recoveries, 300 ml of groundwater were fortified with this solution to obtain a concentration of 3 mg l^{-1} of each compound, and subsequently acidified to pH 3. Four spiked real water samples from an industrial effluent were preconcentrated under the same conditions. Also, a blank was extracted for each type of solid phase. Disposable 6 ml cartridges packed with 200 mg of Isolute ENV+ (International Sorbent Technology, Cambridge, UK) and 3 ml cartridges packed with 200 mg of LiChrolut EN

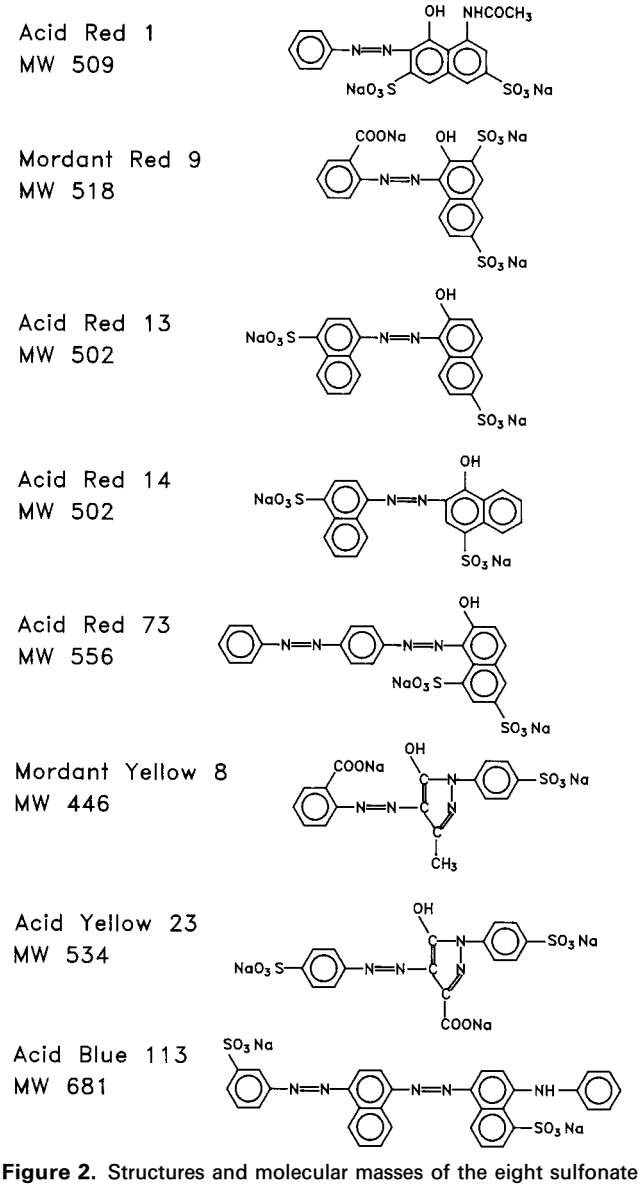

Figure 2. Structures and molecular masses of the eight sulfonate azo dyes.

Table 1. Calibration data obtained with standard solutions by CE–UV (214 nm)

Compound	Calibration equation[a]	r^2	Linear range (ppm)	LOD (ppm)
Acid Blue 113	$y = -6314.8 + 765.5x$	0.995	10–60	2
Acid Red 73	$y = -1202.5 + 389.8x$	0.995	10–60	1
Acid Red 13	$y = -3150.2 + 857.5x$	0.999	10–60	1
Mordant Yellow 8	$y = -3692.8 + 379.8x$	0.992	10–60	6
Acid Red 1	$y = -65.1 + 323.6x$	0.993	10–60	1
Acid Red 14	$y = -4103.5 + 1305.7x$	0.999	10–60	1
Mordant Red 9	$y = -3616.4 + 758.9x$	0.996	10–60	1
Acid Yellow 23	$y = 132.8 + 199.9x$	0.996	10–60	6

[a] Calibration points include 10, 20, 30, 40, 50 and 60 ppm.

(Merck) were attached to the ASPEC XL system (Gilson, Villers-le-Bel, France), which was fitted with an external Model 306 LC pump and connected with a Model 817 switching valve for the selection of samples. 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^{-1}. After the spiked groundwater samples had been dispensed at a flow-rate of 5 ml min^{-1} through the cartridge columns, a clean-up step with 2 ml of water (1 ml min^{-1}, pH 3) was performed. Subsequently, a 30 min drying step was introduced using a Baker Spe 12G combined with a vacuum system (15 psi negative pressure). The dyes were eluted with two aliquots of 5 ml of different mixtures of MeOH–H_2O in combination with TEA (see Table 5 for composition) with a waiting time of 5 min between the two elution steps. The eluate was evaporated under a gentle stream of nitrogen at 60 °C. The samples were reconstituted in 1.5 ml of water. These were diluted to the working concentration range by adding water in the ratio 1:20 just before an injection by CE, with the exception of the blank.

The protocol described above was applied to the analysis of real samples of industrial effluents. The extracted samples were analyzed by CE/MS and were also fortified with a standard mixture of the compounds under study at the level of 500 mg l^{-1} (in the extraction vial) in order to evaluate the effect of the matrix background on the determination of the dyes. Effluent water samples were collected from an effluent in the Porto region, transported in cool glass bottles and kept in a refrigerator (4 °C) before analysis. Samples were extracted and analyzed 2 weeks after sampling.

RESULTS AND DISCUSSION

Calibration graphs

The linearity of the system with UV detection was studied by external calibration carried out by quantification of spiked HPLC-gradewater in the range 10–60 mg l^{-1}. Groundwater was also spiked with different concentrations of the dyes in the range 50–250 μg l^{-1}. This was followed by SPE preconcentration.

Calibrations were performed with standard solution mixtures of the studied dyes by constituting a five-point calibration curve over the concentration range 10–60 mg l^{-1}. The linear regression equations with slopes and correlation coefficients are summarized in Table 1. The relationship between the concentration of each compound and its peak area was found to be linear, as indicated by correlation coefficients >0.996. The limits of detection (LODs), ranging between 1 and 6 mg l^{-1} at a signal-to-noise ratio of 3 for the eight dyes, are also given in Table 1.

Additionally, to evaluate the linearity of the automated off-line SPE-ASPEC XL-CE/UV method, groundwater samples were spiked with the mixtures of eight dyes in the range 50–250 μg l^{-1} and 300 ml of water were percolated and pre-concentrated to 1.5 ml. The calibration data and the LODs for the different compounds are reported in Table 2. The regression equations were characterized by correlation coefficients >0.95 and better LODs could be calculated for a signal-to-noise ratio of 3 between 10 and 150 μg l^{-1}.

Table 2. Calibration data obtained with CE–UV (214 nm) after pre-concentration of 300 ml of spiked groundwater in Isolute ENV+ cartridges

Compound	Calibration equatinon[a]	r^2	Linear range (ppb)	LOD (ppb)
Acid Blue 113	—	—	—	150
Acid Red 73	$y = -5231.1 + 532.8x$	0.991	50–250	3 5
Acid Red 13	$y = 14468.1 + 662.0x$	0.957	50–250	17
Mordant Yellow 8	$y = -10749 + 542.1x$	0.960	100–250	50
Acid Red 1	$y = -3131.9 + 317.3x$	0.970	50–250	16
Acid Red 14	$y = -9788.8 + 1686.0x$	0.983	50–250	10
Mordant Red 9	$y = -12502.7 + 954.3x$	0.984	50–250	30
Acid Yellow 23	$y = 785.3 + 244.9x$	0.968	50–250	35

[a] Calibration points include 50, 100, 150, 200 and 250 ppb in the water sample.

Table 3. Repeatability[a] and reproducibility[b] of migration time and peak area (mean ± SD/RSD) by CE/UV (214 nm)

Compound	Migration time (min)	RSD (%)	Repeatability and peak area	RSD (%)
Acid Blue 113	4.676 ± 0.009	0.20	0.128 ± 0.009	6.87
Acid Red 73	5.664 ± 0.011	0.19	0.131 ± 0.006	4.92
Acid Red 13	6.546 ± 0.027	0.41	0.251 ± 0.010	4.09
Mordant Yellow 8	8.194 ± 0.037	0.45	0.116 ± 0.010	8.27
Acid Red 1	8.966 ± 0.046	0.51	0.112 ± 0.008	6.82
Acid 14	10.295 ± 0.077	0.75	0.400 ± 0.009	2.21
Mordant Red 9	16.065 ± 0.167	1.04	0.256 ± 0.011	4.45
Acid Yellow 23	16.999 ± 0.184	1.08	0.091 ± 0.008	9.12

[a] Average of six injections of 25 mg l^{-1} standard mixture on the same day.
[b] Average of five injections of 25 mg l^{-1} standard mixture on three different days.

The repeatability and reproducibility of migration times and peak areas are shown in Table 3. The repeatability was assessed by six replicate injections of a 25 mg l^{-1} standard solution mixture. The maximum RSD of 1.1% for the migration time and 9.2% for the peak area indicate good quantitative accuracy of the method. The migration time and peak area reproducibility is a critical point in CE analysis and depends strongly on the cleaning and stabilization of the capillary with the buffer used for the separation.

Linearity of the CE/MS system was studied at six points (2, 2.5, 3, 3.5, 4 and 4.5 mg l^{-1}) using the negative ionization mode and with time-scheduled selected-ion monitoring (SIM). An internal standard (sodium naphthalene-2-sulfonate) was added in order to minimize the effects of the instability of the spray, which may cause deviations from linearity. The ions selected for quantification and the calibration graphs are shown in Table 4.

The SIM limits of detection are given in Table 4 and were calculated using a signal-to-noise ratio of 3 (the ratio between the peak intensity and the noise amplitude) by dilution of the water samples and considering the preconcentration factor. The repeatability ($n = 5$) of the compounds that gave acceptable correlation coefficients at a level of 2 mg l^{-1} varied from 5 to 15%, depending on the compound.

SPE followed by CE/UV

A typical electropherogram is shown in Fig. 3(a) for a water extract after preconcentration on a cartridge packed with 200 mg of the cross-linked polymeric sorbent Isolute ENV+ with MEKC using Brij 35 as a micellar agent. This figure shows the absence of interfering peaks in the separation of the target compounds spiked in groundwater (3 mg l^{-1}), which allows for the determination of all the dyes. The absence of interfering peaks is more evident in the electropherogram of an unspiked groundwater sample after solid-phase extraction with an Isolute cartridge (with a preconcentration factor 20 times higher to amplify the interferences) that is shown in Fig. 3(b).

Recovery studies were carried out for the eight sulfonated azo dyes spiked in groundwater at the 3 mg l^{-1} level. Cartridges (attached to the ASPEC XL system) packed with 200 mg of two polymeric SDB sorbent materials were used: Isolute ENV+ and LiChrolut EN. In addition to the assessment of the suitability of the sorbent material, the elution solvent was also optimized. For instance, to enhance the effectiveness of the elution step, an amine base such as TEA was added to the solvent mixture. This assists in the total removal of cations,[20] resulting in better elution of the compounds. Although the two sorbent materials are based on the same (organic) structure, a distinct pre-concentration behavior and effectively is obvious and it could only be traced back to the different physico-chemical characteristics of the sorbent material. Whilst the preconcentration with Isolute ENV+ using a 100% MeOH (0.01% TEA) eluent achieved recoveries up to 71%, only poor recoveries (up to 49%) relative to the LiChrolut EN pre-concentration procedure were obtained. These could not be improved even by varying the percentage water in the eluent (up to 30% of water).

Table 4. Detection limits and calibration curves for the dyes by CE/MS

Compound	Detected ion (m/z)	Calibration equation[a]	r^2	Linear range (ppm)	LOD (ppm)
Acid Blue 113	317	$y = -1.033 + 0.0071x$	0.744	2–4.5	0.80
Acid Red 73	255	—	—	—	>1.17
Acid Red 13	228	$y = -0.056 + 0.0091x$	0.987	2–4.5	0.73
Mordant Yellow 8	401	$y = -0.206 + 0.0036x$	0.935	2.–4	0.20
Acid Red 1	232	$y = -0.166 + 0.0012x$	0.915	2–4.5	0.36
Acid Red 14	228	$y = -4.897 + 0.0170x$	0.935	2–4	0.10
Mordant Red 9	225	$y = -0.300 + 0.0017x$	0.967	2–4.5	0.71
Acid Yellow 23	233	$y = -0.059 + 0.000\,27x$	0.958	2.5–4.5	0.60

[a] Calibration points include 2, 2.5, 3, 3.5, 4 and 4.5 ppm in the water sample.

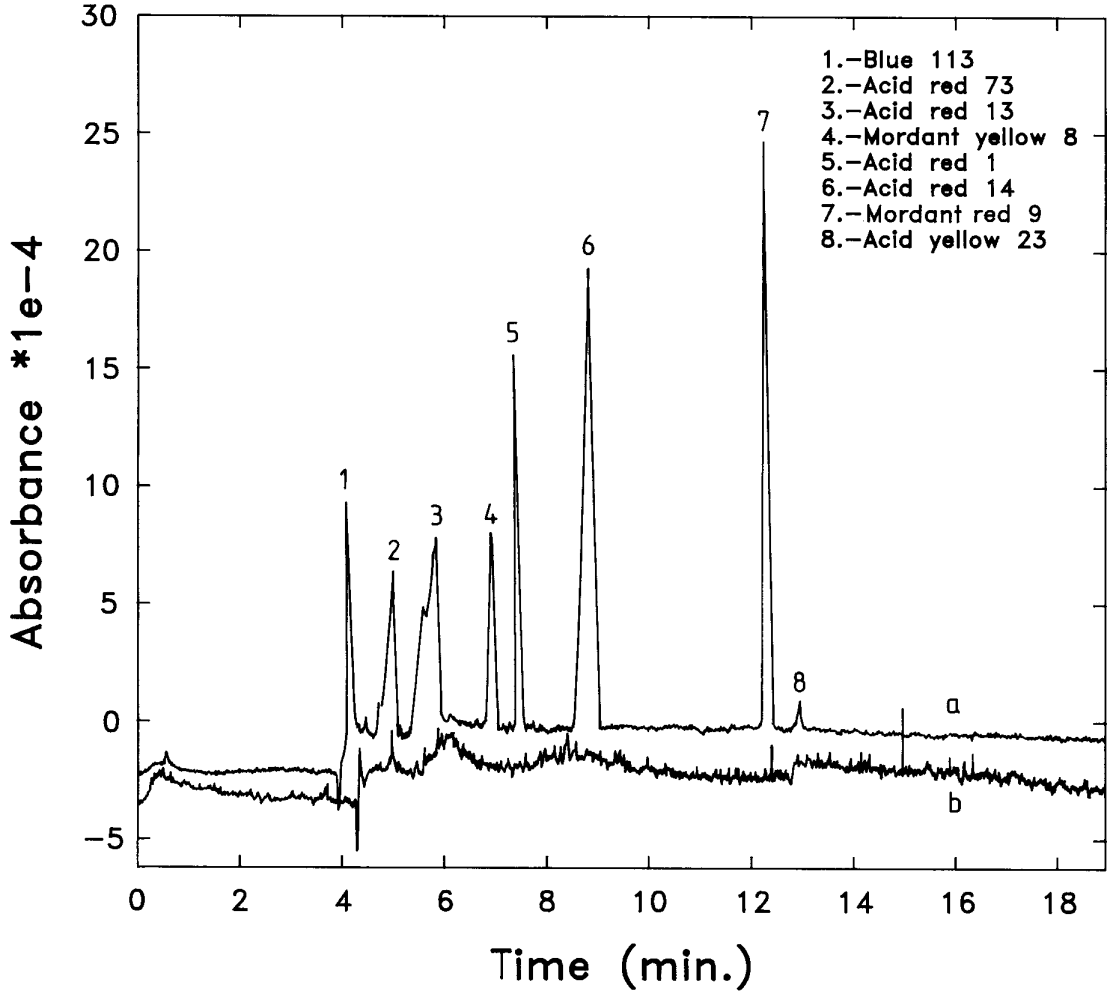

Figure 3. (a) CE/UV (214 nm) electropherogram of the separation of the eight sulfonated azo dyes spiked in groundwater (3 mg l^{-1}) after off-line solid-phase extraction with Isolute Cartridges. Separation was carried out with a buffer solution of 9.2 m M ammonium acetate and 0.05% Brij 35. Peaks: (1) Acid Blue 113; (2) Acid Red 73; (3) Acid Red 13; (4) Mordant Yellow 8; (5) Acid Red 1; (6) Acid Red 14; (7) Mordant Red 9; (8) Acid Yellow 23. Concentration: 50 μg l^{-1}. (b) CE/UV (214 nm) electropherogram of an unspiked groundwater sample after solid-phase extraction with Isolute cartridges. The injected blank was 20-fold more concentrated than sample (a).

Based on these results, the application of Isolute ENV+ for further optimization was found to be more effective. Although the recoveries of Acid Yellow 23 and Acid Blue 113 were always $<50\%$, the recoveries of the six remaining dyes could be improved to 64–83% by increasing the percentage of water and TEA in the eluent to 20% and 0.2%, respectively. In Table 5 the final conditions of the elution step and the best recoveries obtained with both stationary phases are shown.

Table 5. Recoveries obtained from 300 ml of pre-concentrated spiked groundwater samples (3 mg l^{-1}) by solid-phase extraction with LiChrolut EN and Isolute ENV+ sorbents with MeOH–H_2O (90:10) (0.1% TEA) and MeOH–H_2O (80:20) (0.2% TEA) solvents, respectively

Compound	LiChrolut EN	Isolute ENV+
Acid Blue 113	n.d.[a]	34 ± 7
Acid Red 73	39 ± 1	83 ± 14
Acid Red 13	40 ± 1	80 ± 11
Mordant Yellow 8	49 ± 1	69 ± 7
Acid Red 1	17 ± 1	69 ± 2
Acid Red 14	34 ± 3	74 ± 7
Mordant Red 9	18 ± 1	64 ± 1
Acid Yellow 23	n.d.	15 ± 2

[a] Not detected.

Spectral information

The parameters of the mass spectrometer were optimized in order to achieve the maximum sensitivity. By changing the cone voltage it is possible to induce fragmentation in the source region and consequently to obtain structural information, but at the expense of sensitivity. Owing to the small amounts injected in CE (of the order of nanoliters), the sensitivity is a serious drawback of this technique. This is the reason why a low cone voltage (20 V) was employed in order not to induce fragmentation and improve the detection limits. All the compounds studied were detected as anions and the negative ionization mode in time-scheduled SIM conditions was used for all of them. Table 6 shows the main fragment ions obtained for the dyes under the conditions described in the Experimental section and the ions employed to detect the compounds in SIM conditions are shown in Table 4. In each case the mass ion which give the best signal-to-noise ratio for each

Table 6. Typical fragment ions and relative abundances (RA) in CE/MS in negative ionization mode

Compound	M_r	m/z	Fragment ions	RA (%)
Acid Red 1	509	173		(100)
		231/232	$[M-2Na]^{2-}$	100(14)
		194/195		63(9)
		311		40(6)
Mordant Red 9	518	225	$[M-3Na+H]^{2-}$	100
Acid Red 13	502	253		20
		228	$[M-2Na]^{2-}$	100
		212		43
Acid Red 14	502	479	$[M-Na]^{-}$	1
		457	$[M-2Na+H]^{-}$	1
		239	$[M-Na-H]^{2-}$	10
		228	$[M-2Na]^{2-}$	100
Acid Red 73	556	255	$[M-2Na]^{2-}$	100
		241		23
		227		40
		171		42
		281		23
		311		17
M. Yellow 8	446	423	$[M-Na]^{-}$	30
		401	$[M-2Na+H]^{-}$	60
		200	$[M-2Na]^{2-}$	62
		178	$[M-2Na\text{-}COO+H]^{-}$	100
		255		30
		213		28
Acid Yellow 23	534	211	$[M-3Na-COO+H]^{2-}$	100
		233	$[M-3Na+H]^{2-}$	10
		255		11
Acid Blue 113	681	317	$[M-2Na]^{2-}$	100

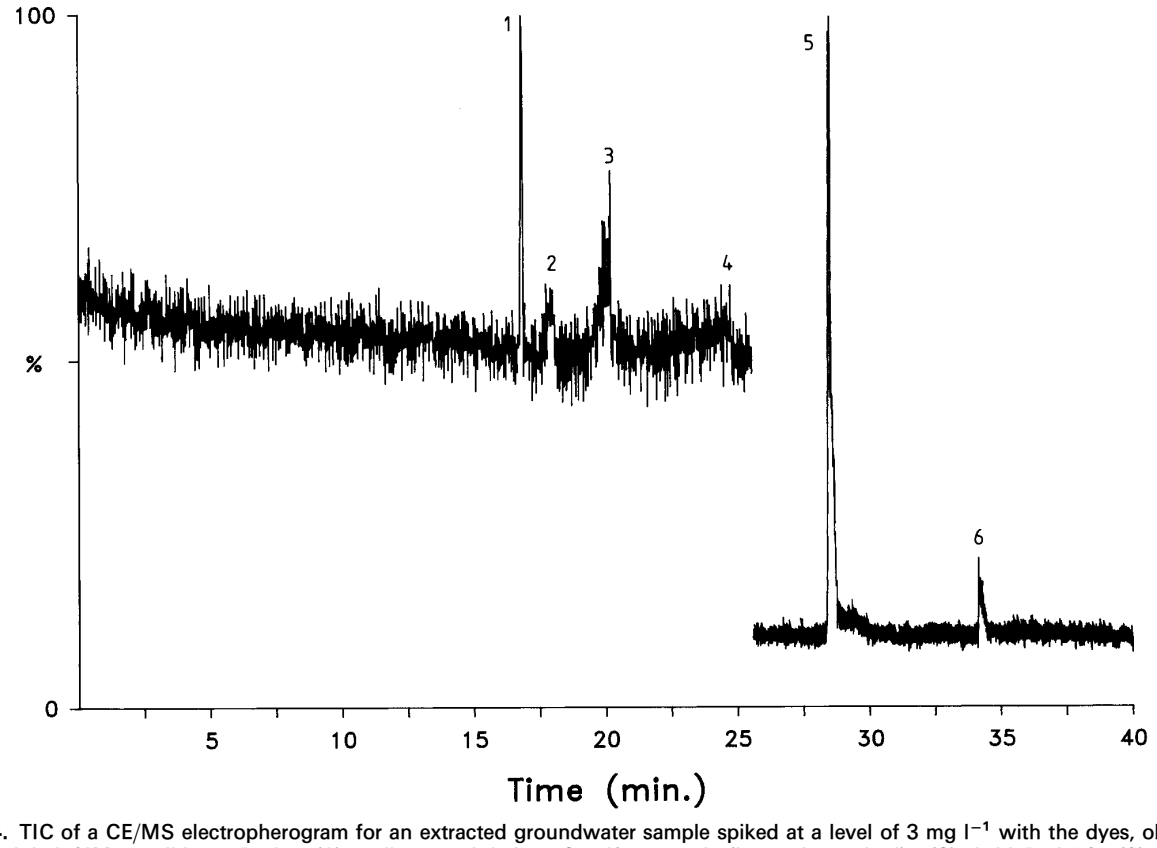

Figure 4. TIC of a CE/MS electropherogram for an extracted groundwater sample spiked at a level of 3 mg l^{-1} with the dyes, obtained in time-scheduled SIM conditions. Peaks: (1) sodium naphthalene-2-sulfonate salt (internal standard); (2) Acid Red 73; (3) Acid Red 1 + Mordant Yellow 8; (4) Acid Red 13 + Acid Blue 113; (5) Acid Red 14; (6) Mordant Red 9.

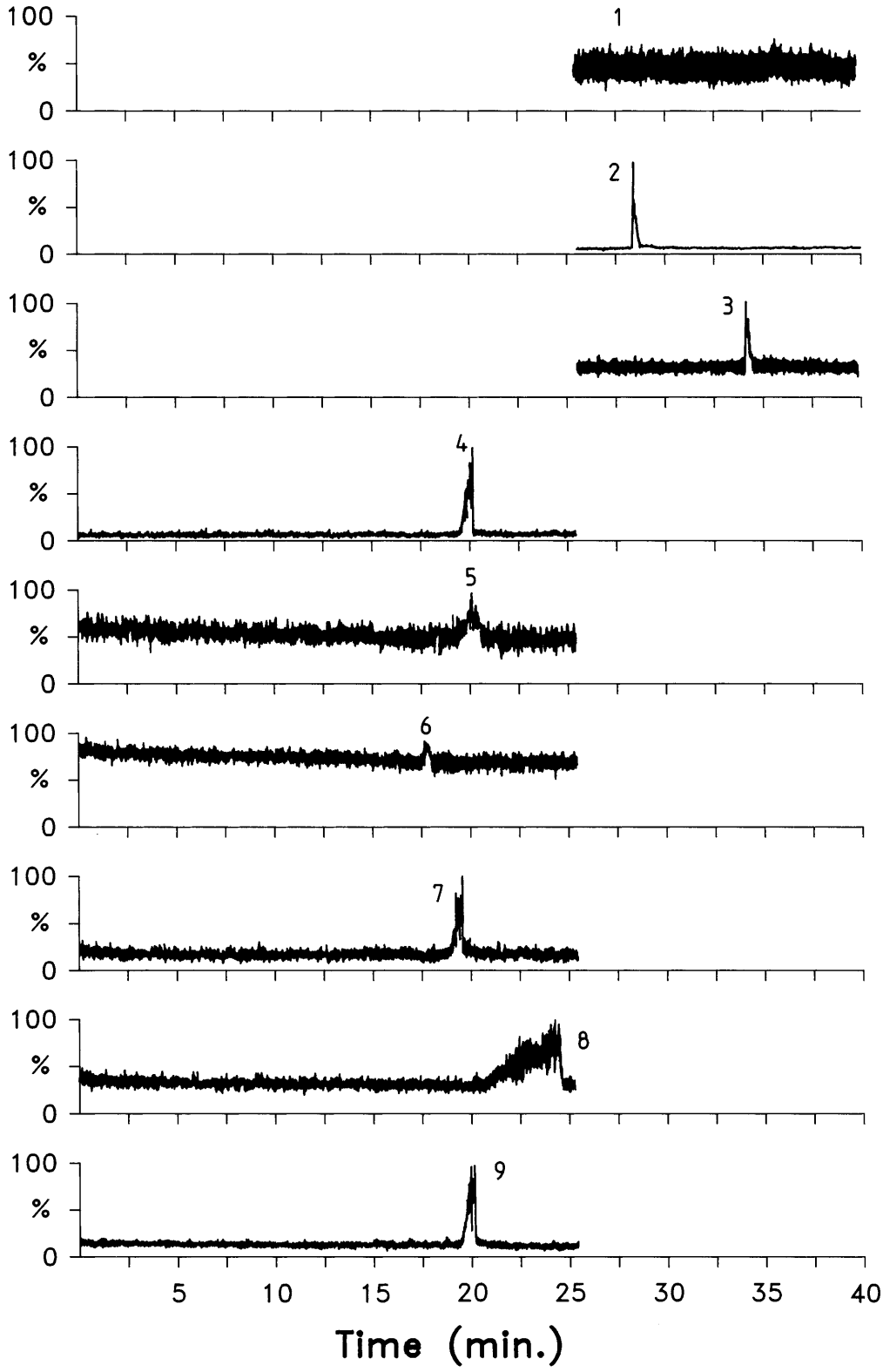

Figure 5. Selected-ion electropherogram of the extracted groundwater sample spiked at a level of 3 mg l^{-1} with the dyes, obtained in time-scheduled SIM conditions. Compounds: (1) Acid Yellow 23; (2) Acid Red 14; (3) Mordant Red 9; (4) Mordant Yellow 8; (5) Acid Blue 113; (6) Acid Red 73; (7) Acid Red 1; (8) Acid Red 13; (9) Mordant Yellow 8.

compound was chosen for CE/MS analysis. It should be pointed that this ion does not always correspond to the ion with the maximum abundance because at higher masses the noise is lower than at lower masses. This is the case with Mordant Yellow 8, which gave a base peak at *m/z* 178 but the ions selected for the analysis were at *m/z* 200 and 401. It is preferable to acquire two mass ions of a compound when problems of instability in the spectrum may occur and, consequently, the relative abundances of the ions may change. This also helps in the confirmation of the study compounds in the analysis of real samples.

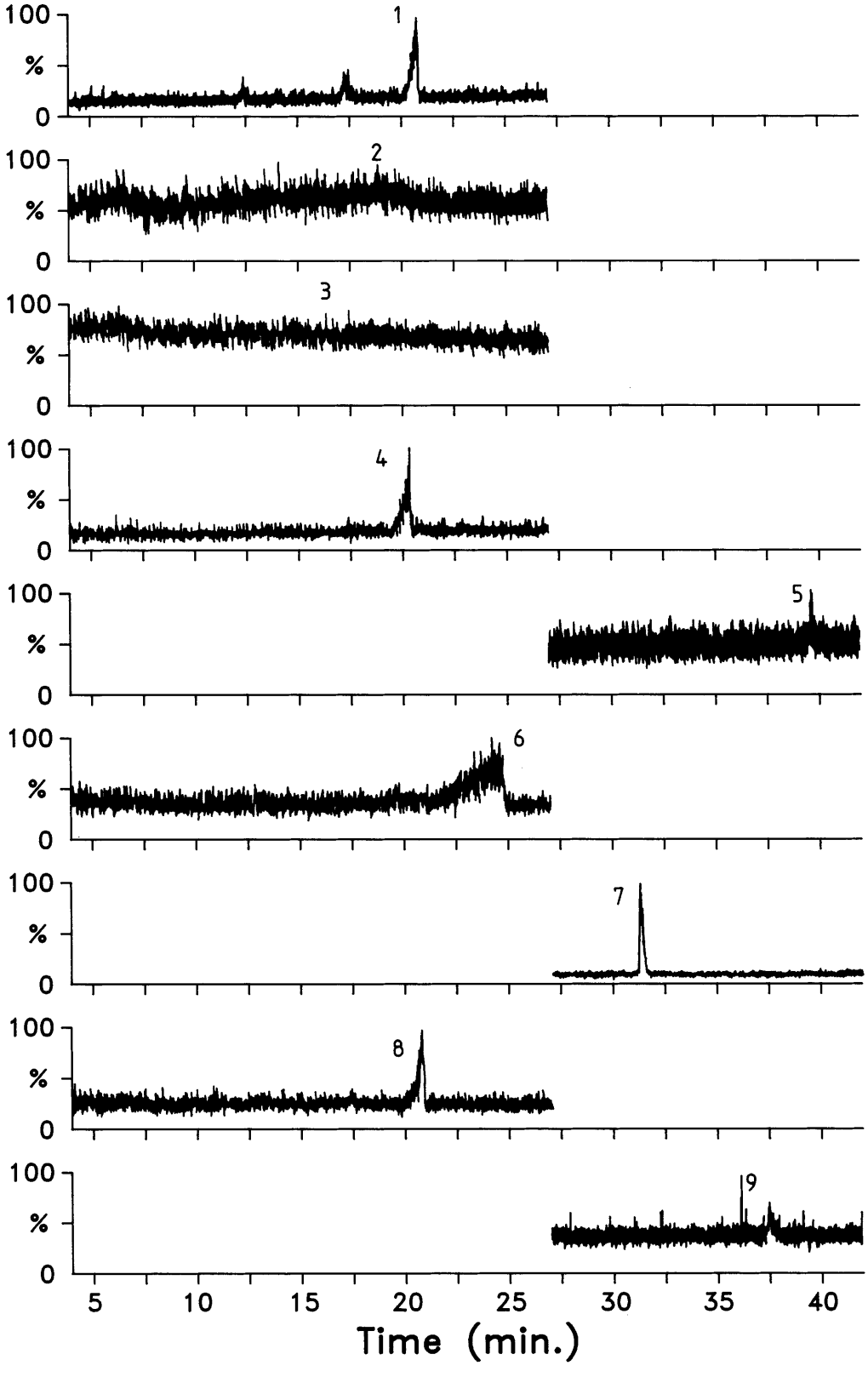

Figure 6. Selected-ion electropherograms of a real sample extracted from an industrial effluent fortified with the eight dyes, obtained in time-scheduled SIM conditions. Compounds: (1) Mordant Yellow 8; (2) Acid Blue 113; (3) Acid Red 73; (4) Acid Red 1; (5) Acid Yellow 23; (6) Acid Red 13; (7) Acid Red 14; (8) Mordant Yellow 8; (9) Mordant Red 9.

The fragmentation obtained is very similar to that obtained previously with the LC/ESI-MS interface,[13] which is to be expected because the interface used for CE/MS is based in the same principle as the ESI type. In general, the ions arising from losses of one or two Na^+ cations from the sulfonated groups and their replacement with hydrogen atoms are the main mass ions detected, but fragment ions resulting from losses of

CO_2 from the carboxylic group are also detected, as observed in LC/ESI-MS.[13]

Analysis of water samples by CE/MS

CE/MS in time-scheduled SIM conditions was used for the confirmation and determination of the dyes in spiked water samples. A typical electropherogram of a water extract spiked with the dyes after preconcentration on Isolute ENV+ cartridges is shown in Fig. 4. A micellar agent cannot be used in the separation by CE/MS as in the separation by CE/UV because it suppresses the ionization of the analyte compounds. Therefore, the separation is based only on the m/z ratio of each compound and, as can be seen in Fig. 5, there are some peaks that are not completely separated, but their determination is feasible by the acquisition of their corresponding mass ion. Acid Red 13 and 14 are isomers and consequently have the same molecular mass, but they exhibit a complete separation. This could be explained because, at the working pH of the buffer used in the separation, the two compounds do not have the same degree of protonation.

Sensitivity is another problem with this technique that has been mentioned, as can be seen in the selected ion electropherograms in Fig. 5. This is especially true for some compounds such as Acid Red 73, which could not be determined in the SPE extracts because of its poor LOD. Compounds such as Yellow 8 and Acid Blue 113 have a low response because of the low extraction recovery. Acid Blue 113 exhibited poor linearity owing to the low purity of the standard. This is a problem commonly encountered in the case of azo dyes used as standards in industrial applications.

Analysis of industrial effluents

Typical electropherograms are shown in Fig. 6 for a real sample extracted from an industrial effluent from Portugal, obtained in time-scheduled SIM conditions and fortified with the study compounds. The selectivity of CE/MS is better than that of CE/UV detection. Interferences present in the matrix from the industrial effluents are not detected in the chromatogram (see Fig. 6) as interfering peaks, and only an increase in the baseline noise is observed in comparison with the spiked groundwater. Exceptions are Acid Red 73 and Acid Blue 113 because of the low response and bad peak shape, respectively, that have already been mentioned. Overall, the use of CE/MS has advantages over LC-based methods in the analysis of industrial effluents. CE exhibits a higher separation power and matrix interferences are avoided compared with LC methods. In this respect, as an example, a slight increase in the background noise can be observed in Fig. 6 compared with Fig. 5, although the industrial effluent extract in Fig. 6 has more interferences compared with the groundwater extract in Fig. 5.

CONCLUSIONS

The determination of sulfonated azo dyes in water samples was feasible by combining SPE with CE/UV. CE/MS was used for the confirmation of the dyes in the extracted water samples. Detection limits between 10 and 150 $\mu g\ l^{-1}$ and between 0.1 and 0.8 $mg\ l^{-1}$ in the water sample were obtained with CE/UV and CE/MS in time-scheduled SIM conditions, respectively.

The structural information obtained by CE/MS was similar to that from conventional LC/ESI-MS. The main ions obtained were formed by losses of one or two Na^+ cations from the sulfonated groups and their replacement with hydrogen atoms, but also fragment ions resulting from losses of CO_2 from the carboxylic group were detected.

Of the two solid extraction phases (Isolute ENV+ and LiChrolut EN) compared for the pre-concentration of 300 or 500 ml water samples, the Isolute cartridges gave the best recoveries, ranging from 64 to 83%.

SPE followed by CE/MS was employed for the analysis of real samples extracted from industrials effluents spiked with the compounds studied. The technique demonstrated good selectivity, especially in the analysis of industrial effluents, where the number of interferences is high. In these samples the only matrix effect compared with a cleaner groundwater sample was a slight increase in the baseline noise and no interfering peaks were found.

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