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Journal of Chromatography A, 890 (2000) 225–237

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Solid-phase extraction of polar hydrophilic aromatic sulfonates followed by capillary zone electrophoresis–UV absorbance detection and ion-pair liquid chromatography–diode array UV detection and electrospray mass spectrometry

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Received 16 February 2000; received in revised form 4 May 2000; accepted 16 May 2000

Abstract

A comprehensive comparison of four different polymeric solid-phase extraction (SPE) materials for the extraction of 22 different aromatic sulfonates of environmental concern was performed. The investigated adsorbents were the polystyrene–divinylbenzene materials LiChrolut EN from Merck, Isolute ENV+ from International Sorbent Technology, HR-P from Macherey–Nagel and the new Oasis HLB poly(divinylbenzene-co-*N*-vinylpyrrolidone) copolymer from Waters. Different SPE parameters like the elution solvent and the drying step of the cartridges were optimized. Analyses were performed by capillary zone electrophoresis–UV absorbance detection (CZE–UV) and ion-pair liquid chromatography–diode array UV detection coupled in series with electrospray mass spectrometry (IP-LC–DAD-ESI-MS) in the negative ionization mode. LC–MS offers a higher separation efficiency than CZE. The best adsorbents were LiChrolut EN and HR-P followed by Isolute ENV+ and Oasis HLB. The recoveries for most of the onefold negatively charged aromatic sulfonates were >50% for the extraction from spiked ground water at 50 μg/l. Recoveries for LiChrolut EN and HR-P were approximately 20% higher than for Isolute ENV+. Very hydrophilic sulfonates containing more than one negative sulfonate group could not be extracted by any of the tested adsorbents.

Keywords: Solid-phase extraction; Polymeric adsorbents; Adsorbents; Sulfonates, aromatic

1. Introduction

Aromatic sulfonates like benzene-, naphthalene-, anthraquinone- and stilbenesulfonates are large-volume chemicals widely used in industrial and domestic processes. For example, substituted benzene- and naphthalenesulfonates are used in the chemical industry as intermediates for the manufacturing of pharmaceuticals, dyes and tanning agents. Sulfonated naphthalene–formaldehyde condensates are important commercial plasticizers for concrete, dispersants and tanning agents [1–5]. Sulfonated azo dyes are extensively applied in the textile industry to color natural fibers, inks and pigments [6]. In the paper industry stilbenesulfonates are applied as whiteners [7,8]. Alkanesulfonates and linear alkylbenzene sulfonates (LASs) are frequently used anionic surfactants in detergents and laundry [9–13].

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00616-6

Aromatic sulfonates are very acidic ($pK_a < -1$) and strongly hydrophilic. Most of the aromatic sulfonates without a hydrophobic alkyl chain (Table 1) are biodegradable compounds. There are only a few compounds which are quite persistent under aerobic conditions (such as 1,5-naphthalenedisulfonate, 1,3,6-naphthalenetrisulfonate and naphthalene–formaldehyde condensates). Despite the widespread use of aromatic sulfonates, only little is known about their toxicology, ecotoxicology and environmental behavior [14]. Because of their low octanol–water partition coefficients ($\log K_{OW} < 2.2$) [14–16], they possess high mobility within the aquatic system. Therefore, they can easily cause pollution of surface waters, they are regularly found in natural waters [1,2,5,7,12,13,17–23]. The concentrations encountered in waste waters from chemical industries and water treatment plants are much higher, values in the mg/l-range have been reported [2,15,21,24–28]. Knowledge about the presence and concentration of such compounds in the environmental compartments is therefore of great importance for the protection of our natural waters. Therefore, an effective analytical method is needed for the determination of these compounds.

Nowadays, solid-phase extraction (SPE) is the preferred extraction and enrichment procedure in biological and environmental analysis. Moreover, SPE is also very important for the clean-up of complex samples – that means matrix removal of interfering compounds – to increase the selectivity of the entire analytical method. It is generally accepted that sample preparation is the most important step in the whole analytical method.

In the past, the most frequently applied method for the enrichment of very water-soluble aromatic sulfonates was ion-pair SPE with cationic ion-pairing reagents (like tetrabutylammonium or cetyltrimethylammonium) and hydrophobic sorbents (reversed-phase C_{18}). Quite good recovery values for many aromatic sulfonates have been reported [8,15,18,19,26,27,29–32]. However, due to different problems encountered with ion-pair SPE [13,18,27,33], in recent publications ''classical'' SPE

Table 1
Aromatic sulfonates numbered in order of their LC retention times (UV-DAD) in Fig. 2[a]

No.	Name	T	M_w	M_m (m/z)
1	2-Amino-1,5-NDS	2.5	302	302
2	1,3,6-NTS	2.7	365	367
3	1,3-BDS	2.9	236	237
4	1,5-NDS	3.4	286	287
5	2,6-NDS	4.8	286	287
6	1-OH-3,6-NDS	7.3	302	303
7	1-Amino-5-NS	8.5	223	222
8	BS	8.6	157	157
9	1-Amino-4-NS	9.4	223	222
10	2-OH-3,6-NDS	11.1	302	303
11	1-OH-6-amino-3-NS	11.9	239	238
12	3-Nitro-BS	12.7	203	202
13	1-Amino-6-NS	13.5	223	222
14	4-Methyl-BS	13.9	171	171
15	1-OH-4-NS	13.9	223	223
16	4-Chloro-BS	15.6	191/193	191
17	2-Amino-1-NS	16.0	223	222
18	1-Amino-7-NS	16.6	223	222
19	4-Chloro-3-nitro-BS	17.3	237/239	236
20	1-NS	17.7	207	207
21	2-NS	18.2	207	207
22	Diphenylamine-4-sulfonate	18.7	249	248

[a] Numbers, names, LC retention times (T), molecular masses (M_w) and measured masses M_m (m/z) of aromatic sulfonates. Abbreviations: NS: naphthalenesulfonate, BS: benzenesulfonate, NDS: naphthalenedisulfonate, BDS: benzenedisulfonate, NTS: naphthalenetrisulfonate, OH: hydroxy.

with polymeric polystyrene–divinylbenzene (PS–DVB) adsorbents [12,13] or carbon [2,5] has been preferred.

Previously, SPE of aromatic sulfonates was studied with LiChrolut EN [13] and Isolute ENV+ [12] adsorbent materials. These publications contain a very comprehensive introduction/overview of the SPE ''state of art'' for polar sulfonates. The objective of the present work was: (i) to optimize the quite difficult SPE procedure for these compounds, (ii) compare four different SPE adsorbents and (iii) investigate different parameters for the SPE enrichment to better understand the adsorption chemistry of these polar hydrophilic compounds. The investigated SPE materials were the PS–DVB materials LiChrolut EN from Merck, Isolute ENV+ from International Sorbent Technology, HR-P from Macherey–Nagel and the new Oasis HLB poly(divinylbenzene-co-*N*-vinylpyrrolidone) copolymer from Waters.

Analyses were performed by capillary zone electrophoresis (CZE)–UV absorbance detection and ion-pair liquid chromatography–diode array UV detection coupled in series to electrospray mass spectrometry (IP-LC–DAD-ESI-MS).

2. Experimental

2.1. Chemicals and reagents

Acetone, acetonitrile, methanol, HPLC-grade water, the ion-pairing reagent triethylamine (TEA), sulfuric acid (96%), sodium hydroxide, ammonium acetate and ammonia (25% in water) all of analytical grade were obtained from Merck (Darmstadt, Germany). Sodium tetraborate ($Na_2B_4O_7$, anhydrous) was from Fluka (Buchs, Switzerland), analytical-grade acetic acid was from Panreac (Barcelona, Spain). The aromatic sulfonates (Table 1) were obtained from Fluka and Aldrich (Steinheim, Germany).

Sulfonate standard stock solutions of 1000 mg/l were prepared by dissolving 50 mg of each compound in 50 ml ultra-pure water (Merck). The working standard solutions were prepared by further diluting the stock standard solutions with water. The standard mixtures were produced from these single-compound solutions. The standard mixtures were further diluted for capillary electrophoresis (CE) and LC analysis, calibrations and preparation of fortified SPE samples. All solutions were stored at 4°C in the dark.

2.2. Solid-phase extraction

The SPE procedure for the concentration of water samples was performed off-line but automatically with an ASPEC XL apparatus (Gilson, Villiers-le-Bel, France). This system is equipped with an external 306 LC pump for sample dispensing through the SPE cartridges. The solid-phase adsorption material LiChrolut EN was obtained from Merck, Isolute ENV+ from International Sorbent Technology (IST, Cambridge, UK), HR-P from Macherey–Nagel (Düren, Germany) and the Oasis HLB from Waters (Milford, MA, USA). Disposable 6-ml SPE cartridges with 200 mg adsorbent were used in the case of LiChrolut EN, Isolute ENV+ and HR-P. The Oasis HLB cartridges only contained 60 mg adsorbent.

The adsorbents were activated and conditioned first with 7 ml methanol and after that with 3 ml water (acidified to pH 2.5 with sulfuric acid) at a flow-rate of 1 ml/min. The sorbents were not allowed to dry, and subsequently different volumes of spiked water samples were passed through the cartridges at a flow-rate of 5 ml/min. After passing the water samples, the cartridges were not dried with nitrogen (in the optimized procedure). For recovery studies, 1-l ground water samples were spiked with known volumes of a sulfonate standard mixture and were pH-adjusted with sulfuric acid (96%) to pH 2.5 (150 ml water was extracted with each cartridge). The compounds were eluted into glass vials, first with 1 ml water containing 5 m*M* TEA–acetic acid and then with 6 ml of methanol–acetone (1:1, v/v). The organic methanol–acetone solvent was evaporated under a gentle stream of nitrogen until the 1 ml water was left. If necessary, (if some water had been evaporated) the vials were filled up with water (containing 5 m*M* TEA–5 m*M* acetic acid) to a final volume of approximately 1 ml (the overall enrichment factor by the extraction of 150 ml water was 150). Absolute recoveries were determined using external calibrations.

2.3. Samples and sample pretreatment

The water used for the spiking/extraction experiments was ground water from the water supply system of the CSIC-Institute in Barcelona (quality parameters: pH 8, 75 mg/l nitrate, 387 mg/l sulfate, 254 mg/l Ca, 88 mg/l Mg, conductivity 2020 μS/cm). The samples were taken in the period between August and October 1999. No special sample pretreatment was applied for the ground water. The water sample of the waste water treatment plant (Igualada, Catalonia, Spain) influent was first filtered and then a reversed-phase C_{18} SPE clean-up (500 mg, C_{18}, Merck) was performed at the actual pH of the water (7.5) to remove interfering unpolar compounds. The percolated water was collected, pH-adjusted to 2.5 with sulfuric acid and extracted with LiChrolut EN SPE cartridges (200 mg) to adsorb the polar aromatic sulfonates.

2.4. Capillary zone electrophoresis

CZE was carried out with a Beckman P/ACE 5000 CE system (Beckman Instruments, Palo Alto, CA, USA) equipped with a fixed-wavelength UV detector which was operated at a wavelength of 214 nm and a HP^{3D}CE system (Hewlett-Packard, Waldbronn, Germany). In all cases, separations were performed with bare (uncoated) fused-silica capillaries of 75 μm I.D.×375 μm O.D. (from HP and Bio-Rad, Munich, Germany). In case of the Beckman instrument, the length of the capillary was 47 cm (40 cm effective length), and for the HP^{3D}CE instrument 64.5 cm (56 cm effective length).

A constant voltage of usually 20–25 kV was applied with the cathode end at the detector. The temperature of the capillary was maintained at 25°C by the instruments thermostatting systems. Samples were pressure injected with 0.5 p.s.i. for 5 s (1 p.s.i.=6894.76 Pa). Data acquisition was performed for the Beckman CE with the System Gold software, or with the HP software, respectively.

CZE separations were routinely performed with a 12 m*M* ammonium acetate buffer alkalinized to pH 10 with ammonia (25%) or with a 12 m*M* sodium borate buffer at pH 9.3 (no pH adjustment necessary).

The capillary was conditioned every morning before starting a sequence of runs by rinsing in the high-pressure mode for at least 20 min with 0.1 *M* NaOH, 10 min with water and 5 min with running buffer. After every third run the capillary was rinsed for 5 min with 0.1 *M* NaOH, 3 min with water and 2 min with running buffer to remove adsorbed material from the walls of the capillary. Pre-run rinsing for equilibration was performed with running buffer for 2 min.

2.5. Liquid chromatography–UV-DAD-mass spectrometry

LC separations were carried out with a Hewlett-Packard (HP) 1090 A LC-system, UV detection using a HP 1040 M diode array UV–Vis detector coupled in series with the MSD HP 1100 mass-selective detector (MSD), equipped with an orthogonal interface and a standard atmospheric-pressure ionization (API) source using electrospray ionization (ESI) in the negative mode.

Ion-pair chromatography (IPC) was used to separate the polar aromatic sulfonates with a Superspher RP-18 ''fast and short'' column (75×4 mm I.D., 4 μm particle diameter) from Merck equipped with a 10×4 mm guard column (at room temperature). This column has the advantage of shorter retention times compared to the previously used longer 250 mm column [12]. TEA, a volatile tertiary alkylamine, was used as ion-pairing reagent. Table 2 reports the gradient eluent composition during the analysis runs.

After the LC separation and the UV-DAD de-

Table 2
Gradient eluent of the IPC–ESI-MS separation[a]

Min	% B	ml/min
0	100	0.8
6	100	1.0
10	95	1.0
15	80	1.0
20	40	1.0
25	30	1.0
30	25	1.0
35	100	1.0
60	100	0.8

[a] Eluents: (A) methanol, (B) water (pH 6.5) both with 5 m*M* TEA and 5 m*M* acetic acid.

tection, the sample was introduced through the nebulizer needle into the ESI source together with a nitrogen nebulizing gas (nebulizer pressure 55 p.s.i.). Evaporation and nebulization of the LC effluent are further enhanced by concurrently adding a drying gas of heated nitrogen (350°C, 11 l/min). The molecular and cluster ions enter the first vacuum stage through an internally metal-plated fused-silica capillary, which creates a molecular leak between the API chamber and the first vacuum stage. Declustering of the solvent–analyte cluster ions takes place in this capillary and in the following collision-induced dissociation (CID) region where partial fragmentation can be achieved. The capillary voltage was set to 3500 V. The ions are focused by two skimmer systems into the high vacuum of the mass analyzer and further focused through an octapole mass filter. The fragmentation voltage was set to 80 V for quantification studies. Quantitative determination of the aromatic sulfonates was performed by time-scheduled single ion monitoring (SIM) using $[M]^-$, $[M+1]^-$, $[M+2]^-$ or $[M-1]^-$ ions in case of amino- or nitro-substituted sulfonates (see Table 1) [12].

3. Results and discussions

3.1. Capillary zone electrophoresis–UV detection

For separation and recovery studies 22 aromatic sulfonates of a wide range of different structure, substitution and polarity were chosen (Table 1).

In general, aromatic sulfonates are separated by CZE with a sodium borate–boric acid [31,34–37] or a simple sodium borate buffer [3,13,23] at pH between 8 and 10. The advantage of a simple sodium borate buffer is that no pH adjustment is necessary, the pH is about 9.3. Loos and Niessner [13] used a 25 m*M* sodium borate buffer for the separation of 14 aromatic sulfonates and explained the migration order of the compounds. In the present work we only wanted to separate 10 compounds for the recovery experiments and therefore used a 12 m*M* sodium borate buffer which shows a sufficient separation efficiency and has the advantage of shorter migration times. This separation of a 2 mg/l standard is shown in Fig. 1. Aromatic sulfonates also can be separated with a volatile ammonium acetate buffer for coupling CE to MS.

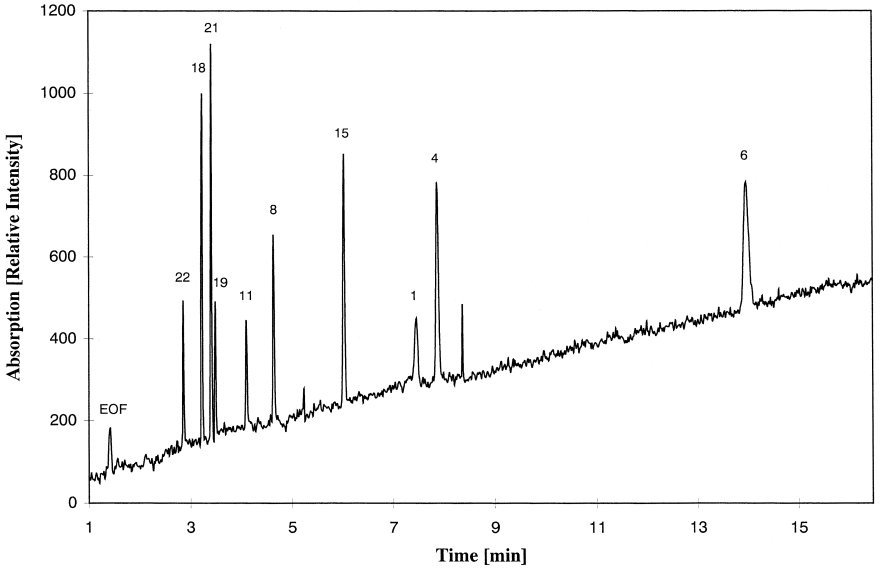

Fig. 1. Electropherogram of a 10-compound aromatic sulfonate mixture containing 2 mg/l of each compound. Conditions: running electrolyte 12 m*M* sodium borate, pH 9.3, capillary 47 cm (40 cm to detection window)×75 μm I.D., voltage 25 kV, temperature 25°C, pressure injection 0.5 p.s.i. for 5 s, UV detection at 214 nm. For peak identification see Table 1.

3.2. Liquid chromatography–UV-DAD-mass spectrometry

Aromatic sulfonates were separated by ion-pair chromatography followed by UV diode array and electrospray ionization mass spectrometric detection (IPC–UV-DAD-ESI-MS, or simple LC–MS) in the negative ion mode. The commonly used involatile ion-pairing reagents such as tetraalkylammonium salts are not suited for coupling to MS. With TEA, a volatile tertiary alkylamine, effective ion-pair formation for the aromatic sulfonates and stable MS performance were achieved [12]. In comparison to CZE–UV, LC–MS allows both structural and molecular mass information. In addition, LC usually provides due to the higher injection volume a superior detection sensitivity than CZE.

Moreover, with LC–MS a higher separation efficiency was achieved for the aromatic sulfonates compared to CE–UV. Separation of 22 compounds was performed within 20 min (Fig. 2). In comparison to conventional longer 250 mm (5 μm particle diameter) analytical HPLC columns [12], with the now used ''fast and short'' 75×4 mm I.D. (4 μm particle diameter) column the analysis time was reduced by the half. However, not all compounds are completely baseline separated by LC–DAD or LC–total ion current (TIC)-MS. But, due to the mass selective detection mode of MS, unequivocal detection of coeluting compounds is possible. The SIM masses used are reported in Table 1. Fig. 2 shows that the peaks in the UV-DAD chromatogram are more narrow than in the TIC-MS chromatogram.

Fig. 3 depicts (as example) the chromatograms of the single detected SIM masses for the well separated five isomeric aminonaphthalenesulfonates

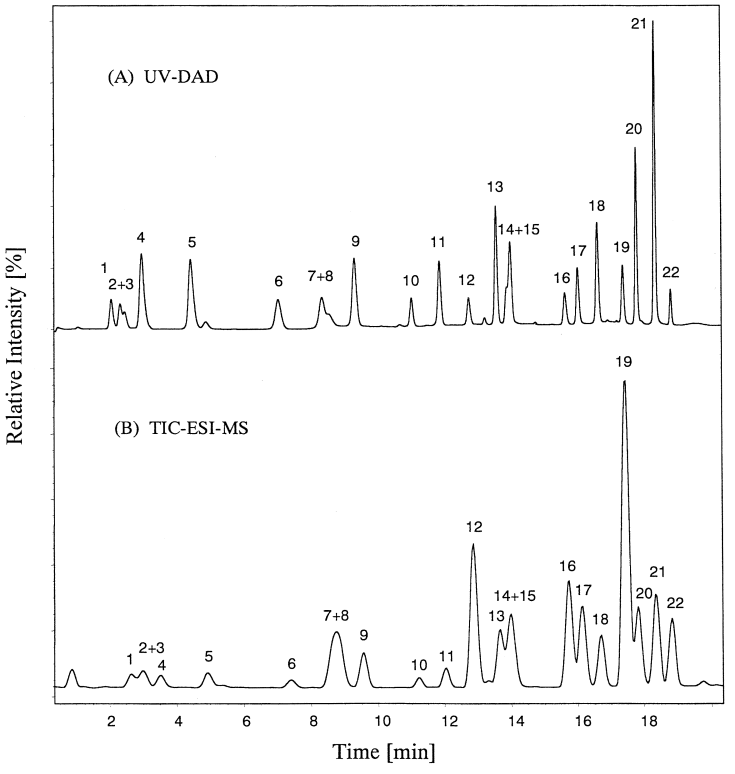

Fig. 2. IP-UV-DAD (A) and total ion current (TIC)-ESI-MS (B) chromatogram of a 22-compound aromatic sulfonate standard mixture of 4 mg/l in the SIM-NI mode. Injection volume 50 μl, fragmentation voltage 80 V. Superspher RP-18 ''fast and short'' column (75×4 mm I.D., 4 μm particle diameter). For peak identification see Table 1.

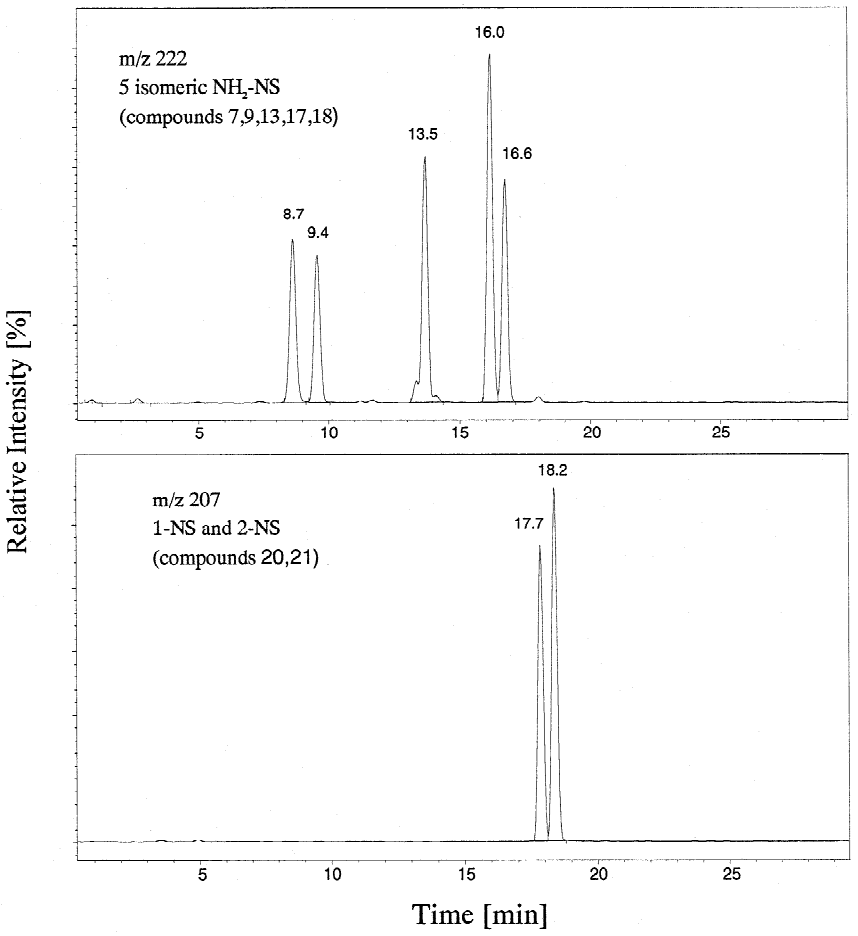

Fig. 3. IP-ESI-MS single SIM chromatograms of isomeric aminonaphthalenesulfonates and isomeric non-substituted naphthalenesulfonates (4 mg/l). Conditions as in Fig. 2. For peak identification see Table 1.

(NH_2-NS, **7**, **9**, **13**, **17**, **18**) and the two isomeric non-substituted naphthalenesulfonates 1-NS (**20**) and 2-NS (**21**).

3.3. Calibration and sensitivity

Calibration with CZE was performed for 10 compounds, with LC–MS for all 22 aromatic sulfonates listed in Table 1. Calibration with CZE was performed in the concentration range between 1 and 50 mg/l with UV detection at 214 nm, with LC–MS in the concentration range between 0.05 and 10 mg/l in the SIM-MS detection mode. Regression data are not shown. The correlation coefficients R (n=4–7) for CZE–UV were greater than 0.991 for the first nine compounds, only worse (0.969) for the last eluting compound (**6**). The correlation coefficients R (n=6) for LC–MS were slightly better, being greater than 0.994 except for two compounds (**12**, **19**).

The limit of detection (LOD) for CZE–UV is approximately 0.5 mg/l. The blank value dependent LOD of the combined SPE–LC–MS method for the extraction of 150 ml water with 200 mg LiChrolut EN was between 0.02 and 5.4 μg/l (LOD=blank value+3×RSD of the blank value), and for SPE–CE–UV in the range of 0.2 μg/l (10-times higher than LC–MS).

3.4. Solid-phase extraction and recovery studies

The SPE materials investigated for the extraction of the very polar hydrophilic and water-soluble aromatic sulfonates were LiChrolut EN, Isolute ENV+, HR-P and the new Oasis HLB (hydrophilic–lipophilic balanced) copolymer. LiChrolut EN and Isolute ENV+ are very often customary used SPE adsorbent materials in environmental analysis. The quite unknown HR-P material is very similar to LiChrolut EN. Less et al. [38] examined seven different PS–DVB SPE materials – including LiChrolut EN, Isolute ENV+ and HR-P – for the extraction of aromatic amines, and obtained the best results for HR-P. The characteristic properties of the different adsorbents are given in Table 3.

Ground water samples were spiked with 50 μg/l for each of the chosen aromatic sulfonates (Table 1), acidified to pH 2.5 (with sulfuric acid) and were extracted by SPE with the four different sorbents. The recovery results obtained at this concentration range of 50 μg/l were very similar to the ones measured previously at a lower concentration range of 2 μg/l [13,39].

The SPE recovery results for the aromatic sulfonates show that only compounds with one sulfonate group can be extracted. The very polar two- and threefold negatively charged sulfonates (compounds **1**–**6** and **10**) are not enriched at all by any of the tested adsorbents (Tables 4 and 5).

Comparing the four different adsorbent materials, the highest recoveries for most of the sulfonates were obtained with LiChrolut EN and HR-P. These two materials showed very similar recovery results, being approximately 20% higher than for Isolute ENV+ (Table 4).

In a previous publication [12] it was reported that the Isolute ENV+ adsorbent material gives much higher recoveries for some aromatic sulfonates, even for disulfonates. These recoveries were determined in 1998 with a different batch of the Isolute ENV+ adsorbent material. By means of the recently examined recovery data we report in this present paper, we have to state that morefold negatively charged aromatic sulfonates cannot be extracted from water with conventional polymeric adsorbent materials (without ion-pairing).

This different batch behavior of Isolute ENV+ can be explained by the uncontrollable catalytic production process of this adsorbent material. Isolute ENV+ is produced by the help of a nitrogen catalyst, which can sometimes cause the introduction of additional nitrogen groups into the polymer. These additional nitrogen centers are able to adsorb very polar compounds like the aromatic sulfonates. Unfortunately, this production process is not yet controllable [40]. It would be desirable to produce more of the batch used in 1998 for the extraction of the very polar aromatic sulfonates with two or three sulfonate groups.

The better adsorption behavior of LiChrolut EN and HR-P can be explained by the lower particle and pore size diameter and the higher specific surface area in comparison to Isolute ENV+ (Table 3). The different characteristics of the adsorbents can be directly observed during the extraction of the water samples as the Isolute ENV+ material shows (after passing the water samples) a brighter color than LiChrolut EN and HR-P.

The Oasis HLB material gave for some compounds (**19**, **21** and **22**) even slightly higher recoveries than LiChrolut EN and HR-P. This is quite astonishing, considering that the Oasis HLB cartridges only contained 60 mg adsorbent material.

Table 3
Characteristic properties of the investigated adsorbent materials [41] (data available from the different manufacturers)

Property	Material (manufacturer)			
	LiChrolut EN (Merck)	Isolute ENV+ (IST)	HR-P (Macherey–Nagel)	Oasis HLB (Waters)
Structure	Polystyrene–divinylbenzene			Poly(divinylbenzene-co-*N*-vinylpyrrolidone) copolymer
Specific surface area (m^2/g)	1200	1000	1300 [38]	810
Pore size diameter (nm)	3	85	2.5	8
Particle size diameter (μm)	40–120	40–140	43–120	30–100

Table 4
SPE recoveries for the extraction of 150 ml spiked ground water at 50 μg/l for the different adsorbent materials[a]

No.	Recovery (%)			
	LiChrolut EN (n=3)	HR-P (n=3)	Isolute ENV+ (n=3)	Oasis HLB (n=4)
1	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	n.d.
3	n.d.	n.d.	n.d.	n.d.
4	n.d.	n.d.	n.d.	n.d.
5	n.d.	n.d.	n.d.	n.d.
6	n.d.	n.d.	n.d.	n.d.
7	12	21	12	n.d.
8	6	4	3	n.d.
9	50	37	27	n.d.
10	n.d.	n.d.	n.d.	n.d.
11	26	25	24	2
12	105	113	78	39
13	55	54	36	n.d.
14	71	63	36	5
15	88	85	58	30
16	113	110	83	51
17	86	85	66	64
18	69	67	52	24
19	91	94	72	97
20	88	92	77	91
21	84	85	71	86
22	63	66	54	67

[a] Elution with 1 ml water (5 m*M* TEA/acetic acid) and 6 ml methanol/acetone. LC–MS measurements. (n.d.=not detected). The RSDs were smaller than 15%, except for the compounds with low recoveries (**7**, **9**, **11**).

However, for the other compounds recoveries were worse with Oasis HLB, so it was at all the worst suited adsorbent material. Though, 200 mg Oasis HLB material would probably give better recovery results.

The recoveries for most of the sulfonates containing only one sulfonate group are quite good, ranging from 50% for 1-amino-4-NS (**9**) to 113% for 4-chloro-BS (**16**) for LiChrolut EN. Only three compounds (**7**, **8**, **11**) gave recoveries below 50%. Recoveries for HR-P were very similar to LiChrolut EN (Table 4). Recoveries of the same compounds for Isolute ENV+ were in the range between 27% (compound **9**) and 83% for **16**, usually 20% lower than for LiChrolut EN and HR-P.

Different SPE parameters like the eluting solvent and drying of the cartridges were tested for the optimization of the sulfonate extraction with the different adsorbents. In summary, recoveries could be slightly increased for some compounds eluting the cartridges first with 1 ml water (containing TEA–acetic acid; ammonium acetate showed no improvement effect) and then with 6 ml methanol (in contrast to the conventional organic solvent elution) and omitting the drying step. In addition, the use of a methanol–acetone solvent mixture is preferred to pure methanol because of the better volatility of acetone. The single results of this optimization experiments which were measured by CE–UV and LC–MS are not shown.

Table 5 clearly shows the positive elution effect of TEA–acetic acid for nearly all compounds, especially 4-chloro-3-nitro-BS (**19**), which can be explained by the ion-pair characteristics of TEA. Probably, TEA forms an ion-pairing complex with the aromatic sulfonates on the SPE cartridges which supports elution of the compounds.

Moreover, the breakthrough behavior of the aromatic sulfonates was studied by extracting lower water volumes (75 and 40 ml). In conclusion, breakthrough of the compounds occurs very fast as recoveries only could be slightly increased for most

Table 5
SPE recoveries for the extraction of 150 ml spiked ground water at 50 μg/l[a]

No.	Recovery (%)			
	Isolute ENV+		LiChrolut EN	
	TEA–acetic acid, methanol–acetone (n=3)	Water, methanol–acetone (n=2)	TEA–acetic acid, methanol–acetone (n=3)	Water, methanol–acetone (n=2)
1	n.d.	n.d.	n.d.	n.d.
2	n.d.	n.d.	n.d.	n.d.
3	n.d.	n.d.	n.d.	n.d.
4	n.d.	n.d.	n.d.	n.d.
5	n.d.	n.d.	n.d.	n.d.
6	n.d.	n.d.	n.d.	n.d.
7	12	17	12	9
8	3	n.d.	6	n.d.
9	27	23	50	17
10	n.d.	n.d.	n.d.	n.d.
11	24	20	26	11
12	78	51	105	102
13	36	36	55	42
14	36	25	71	62
15	58	54	88	80
16	83	53	114	83
17	66	53	86	72
18	52	44	69	33
19	72	26	91	48
20	77	58	88	79
21	71	54	84	74
22	54	53	63	68

[a] Different elution conditions, TEA–acetic acid effect, LC–MS measurements. The RSDs were smaller than 15%, except for the compounds with low recoveries (**7**, **8**, **9**, **11**).

of the compounds at lower water volumes (single results are not shown).

3.5. Analysis of real samples

Aromatic sulfonates are often detected in waste waters from chemical industries and water treatment plants [2,15,21,24–28]. It already has been shown that conventional SPE followed by CE–UV (and fluorescence) [13,39] as well as by LC–MS analysis [12] are well suited methods for the analysis of aromatic sulfonates in real environmental water samples. However, with UV detection no unambiguous identification is possible. LC–MS is due to its higher sensitivity and selectivity better suited for the analysis of real samples.

To prove the applicability of the presented SPE–LC–MS method for the analysis of real samples, Fig. 4 shows the different chromatograms (UV-DAD, TIC-MS and SIM) determined for a waste water treatment plant SPE extract. In this influent of the plant, five aromatic sulfonates were identified: BS, 3-nitro-BS, 4-methyl-BS, 1-NS and 2-NS, however the concentrations for BS and 3-nitro-BS were near the detection limit. Table 6 reports the corresponding concentrations corrected by the SPE recoveries of the compounds.

4. Conclusions

Polar ionized aromatic sulfonates can be determined by CZE–UV detection and by IP-LC–UV-DAD-ESI-MS with a volatile ion-pairing reagent. CZE offers a very fast, simple and cheap analysis procedure. LC–MS is a more robust, selective and sensitive determination method and possesses a higher separation efficiency for aromatic sulfonates.

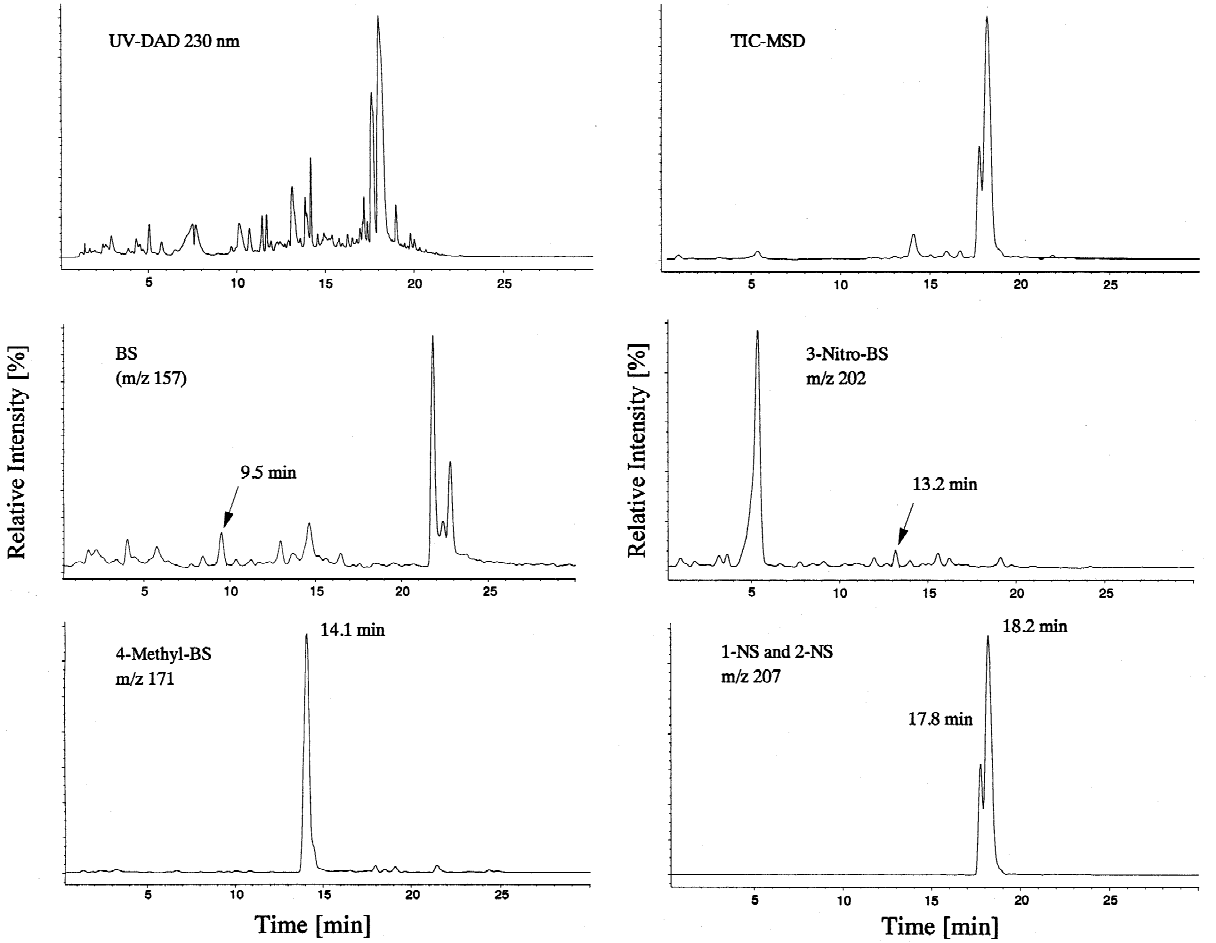

Fig. 4. IP-UV-DAD (230 nm), total ion current (TIC)-ESI-MS and SIM chromatograms of a waste water treatment plant influent sample extract. Conditions as in Fig. 2.

Very fast separations could be performed with a short HPLC column having a low particle size diameter. The comparison of four different polymeric SPE materials for the extraction of aromatic sulfonates from water revealed the best recovery results for LiChrolut EN and HR-P, resulting 20% higher than for Isolute ENV+. LiChrolut EN and HR-P adsorb these very polar, low-molecular-mass compounds slightly better than Isolute ENV+ due to their lower particle and pore size diameter and a higher specific surface area. Thus, Isolute ENV+ has a more macroporous structure compared to LiChrolut EN and HR-P with their more open structure allowing greater $\pi-\pi$ interactions. Optimization of the SPE

Table 6
Aromatic sulfonate concentrations found in an influent of a waste water treatment plant (SPE–LC–MS analysis)

Aromatic sulfonate	BS	3-Nitro-BS	4-Methyl-BS	1-NS	2-NS
Concentration (μg/l)	7.7	0.08	44.8	75.2	196.6

procedure showed that the recoveries could be increased by omitting the drying step of the cartridges and eluting the compounds first with water – containing TEA–acetic acid – and then with methanol–acetone. However, only aromatic sulfonates with one negative sulfonate group could be extracted from water without ion-pairing. Very hydrophilic sulfonates containing more than one negative sulfonate group could not be extracted by any of the tested adsorbents. Breakthrough of this compounds occurs very fast. Therefore, ion-pair SPE should be used in the future for the determination of these very polar aromatic sulfonates. The limits of detection of the combined method of SPE enrichment and CZE or LC analysis are in the low μg/l range. Both analysis methods are sufficient for real-world applications, however LC–MS provides a higher selectivity and sensitivity. Aromatic sulfonates were determined in an influent water sample of a waste water treatment plant. In the future, methods based on CE–MS or capillary electrochromatography (CEC) might be interesting for the analysis of these polar aromatic sulfonates. In addition, avoiding interferences in single MS detection, selectivity can be improved by using MS–MS or fluorescence methods.

Acknowledgements

The German Research Council (DFG) is gratefully acknowledged for the research fellowship for R.L. M.C.A. acknowledges grants from CICYT (AMB98-0913-CO3-O1), the Waste Water Cluster (Project OWWA ENV4-CT97-0608) and from CICYT (AMB98-1808-CE). We thank Merck, IST and Macherey–Nagel for supplying the SPE cartridges.

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