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Determination of aromatic sulfonic acids in aqueous environmental samples by anion-exchange chromatography coupled to particle beam mass spectrometry and UV spectrophotometry

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ABSTRACT

Aromatic sulfonic acids are determined in water using anion-exchange liquid chromatography. Quantification is by UV absorption spectrophotometry (quantification limits 2–80 ng on-column) and confirmation by particle beam electron impact ionization mass spectrometry. Separations with several anion-exchange columns using acetonitrile and ammonium acetate or sodium hydroxide (requiring a membrane suppressor for desalting) mobile phases are represented. Average recoveries are 82% for six monosulfonic acids (spiked at 0.1, 1.0 and 100 ppm) and 95% for two disulfonic acids (spiked at 1.0 and 100 ppm) (S.D. 12%, UV quantification). Mass spectra (2.0 μg on-column) show molecular and major diagnostic fragment ions corresponding to losses of SO_2, HSO_2, SO_3 and HSO_3.

INTRODUCTION

Interest in the application of anion-exchange liquid chromatography (LC) for the direct separation of organic anions has grown with the appreciation of the importance of this class of compounds in agricultural, pharmaceutical and environmental chemistry. Recently more than 95% of the organic materials in aqueous samples from the Stringfellow hazardous waste site were shown to be a series of chlorinated aromatic mono- and disulfonic acid waste products from DDT manufacture [1–3]. Anion-exchange LC coupled with negative chemical ionization (NCI) particle beam mass spectrometry (MS) were used for determination of molecular weights of these previously uncharacterized compounds. Electron impact (EI, positive ion) ionization MS and UV spectrophotometry were also used to obtain essential fragmentation and structural information [3,4]. One compound in this series, 4-chlorobenzenesulfonic acid (PCBSA), has been identified as a major component of the total dissolved organic carbon at more than one hazardous waste site [1,2,5]. The polarity, non-volatility and thermal lability of these compounds had led them to be previously overlooked by gas chromatography (GC)-based methods. Similarly, the dissolved organic materials contained in other aqueous samples such as Casmalia groundwater monitoring well and Santa Clara drinking water have been shown to be optimally resolved by anion-exchange chromatography [6].

Several other studies have shown MS detection coupled with anion exchange to be a powerful combination [7–10]. Ion spray atmospheric pressure ionization MS has been applied to organic anions

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including alkyl and aromatic sulfonic acids, and collisionally activited dissociation (CAD) MS–MS experiments were used to generate sufficient characteristic fragment ions [10] or of CAD MS–MS experiments to generate fragmentation for analyte confirmation. Non-volatile tetraalkylammonium salts in the chromatographic mobile phase were made compatible with the LC–MS interface by the use of a membrane suppressor that exchanged the alkylammonium cations with the volatile hydronium ions prior to introduction into the mass spectrometer [7]. Anion-exchange chromatography of carbohydrates with thermospray (TSP) MS detection using non-volatile buffers and a membrane suppressor for desalting has also been reported [8]. Anion-exchange LC has been succesfully coupled with TSP-MS for the analysis of phenols and their corresponding glucuronides and sulfate conjugates. In that study, tandem CAD MS–MS was used to generate a much greater amount of fragmentation and structural information than was possible with TSP-MS alone [9]. The relatively soft chemical ionization available with TSP tends to generate very simple mass spectra with only molecular or solvent adduct ions. Additional structural information requires modifications such as the use of special solvents to generate sufficient diagnostic and structural information for analyte confirmation and identification.

Applications of LC–MS to the analysis of non-volatile biological molecules such as peptides, amino acids, nucleotides, steroids, lipids, phospholipids, fatty acids, carbohydrates and drugs and their metabolites have recently been reviewed [6,11]. Cation-exchange chromatography with fluorescence detection also has been reported as a sensitive method for the determination of propranolol, a β-adrenoceptor blocker, and its glucuronide and sulfate glucuronide metabolites in plasma and urine [12].

Particle beam MS for LC detection has been shown to be a broad-range technique for the determination of a variety of non-volatile compounds in environmental samples [2,6,13]. It has advantages over other LC–MS interfacing systems in that a single system can provide positive and negative chemical ionization with a variety of reagent gases to maximize molecular weight information, and provides conventional EI mass spectra with diagnostic fragment ions that are especially useful for analyte confirmation without the necessity for CAD MS–MS experiments. This paper described a general method for the determination of aromatic sulfonic acids in aqueous media. It uses a variety of anion-exchange LC systems with UV and particle beam MS detection.

EXPERIMENTAL

Analytical standards

Nine commercially available (Aldrich, Milwaukee, WI, USA) aromatic mono- and disulfonic acids as either the sodium salt or free acid (whichever was the purest) were used as model compounds (listed in Table I, plus 2-naphthalenesulfonic acid).

Anion-exchange chromatography

Anion-exchange chromatographic columns were obtained from Dionex (Sunnyvale, CA, USA) (OmniPac Model PAX-100 and -500, 25 cm × 2 mm I.D., polymer based) and Scientific Glass Engineering (SGE) (Ringwood, Australia) (SAX Model 250GL, 25 cm × 2 mm, silica based). Flow-rates were always 0.25 ml/min for both isocratic and gradient conditions. The following chromatographic gradient conditions were used: with the PAX-100 column with a mobile phase consisting of water–acetonitrile–ammonium acetate (250 m*M*, pH 6.0), 0 min, 88:10:2; 5 min, 70:10:20; and 10 min, 34:43:23; with the PAX-500 column were with a mobile phase consisting of water–acetonitrile–sodium hydroxide (200 m*M*), 0 min, 90:10:0; and 18 min, 0:40:60, with a membrane separator; and with the SAX column with a mobile phase consisting of water–acetonitrile–ammonium acetate (100 m*M*, pH 6.0), 0 min, 85:0:15; 10 min, 83:2:15; and 30 min, 55:30:15. For gradient elutions with the SAX column a constant aqueous ammonium acetate and variable acetonitrile mobile phase composition was used. The organic polymer-based OmniPac columns can be used with a much wider variety of mobile phases including both aqueous sodium hydroxide or ammonium acetate and acetonitrile gradients for greater chromatographic flexibility. The Dionex micromembrane suppressor, which must be used with non-volatile eluants such as sodium hydroxide, converts non-volatile sodium salts into the corresponding hydrogen form before introduction into the particle beam MS interface, *e.g.*, $NaOH \rightarrow H_2O$.

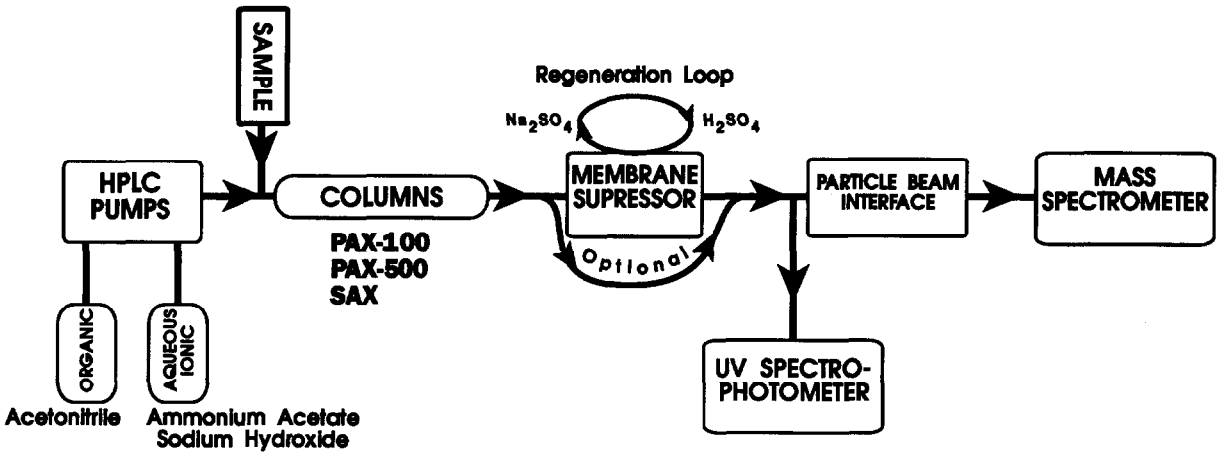

Fig. 1. Configuration of the anion-exchange LC system, the membrane suppressor (if used) and the particle beam mass spectrometer used.

Liquid chromatography with particle beam mass spectrometry and UV absorption spectrometry.

Fig. 1 shows the coupling of the anion-exchange liquid chromatographic system, the membrane suppressor (if used) and the particle beam mass spectrometer used in this study. The LC pumps for both UV and MS detection were a Dionex GPM-2 gradient pump (low flow-rate model) for aqueous sodium hydroxide mobile phase, or a Hewlett-Packard 1050 LC or Shimadzu LC-600 (Shimadzu Scientific Instruments, Columbia, MD, USA) equipped with a SIL-6B autoinjector for volatile mobile phases with pHs between 5 and 7.

The mass spectrometer was a Hewlett-Packard (Palo Alto, CA, USA) Model 5988A equipped with a Hewlett-Packard particle beam LC interface. The ionization modes were EI and negative- and positive-ion chemical ionization (NCI and PCI) with isobutane as a reagent gas. The MS conditions were essentially those specified by the manufacturer: source temperature, 250°C; electron energy, 70 eV (EI) or 200 eV (NCI and PCI); scan time, 2 s; source pressure, *ca.* $2 \cdot 10^{-5}$ Torr (EI) or $2 \cdot 10^{-4}$ Torr (isobutane NCL and PCI); and helium pressure for the particle beam interface, 50 P.S.I. Typically 3–6 mass spectral scans from a single peak were averaged and an equal number of scans from the previous chromatographic minimum (as a background) was averaged and subtracted.

The UV detectors were a Dionex UV–VIS fixed-wavelength detector (254 nm) and Model 4270 integrator or a Hewlett-Packard Model 1040 diode-array detector and a Model 79994A Chem Station for data acquisition. Quantification with UV absorption was at 230 nm with peak-area integration.

Spike and recovery of aromatic sulfonic acids in water

The volumes of water used for spiking experiments were determined by the sulfonic acid level required in the final concentrate to meet required quantification limits (Table II). The spiked water samples were lyophilized (Freezemobile 12 SL; Vir-Tis, Gardiner, NY, USA) over 1–72 h, treated with dry methanol (up to 10% of the original volume of the aqueous sample depending on the amount of residue after lyophilization) and the mixture was sonicated with a Branson Model 2200 sonicator (Branson Ultrasonics, Danbury, CT, USA) for 20 min at room temperature (being careful to exclude water vapor) and acetone added (6 times the methanol volume) to precipitate inorganic salts such as sodium sulfate and carbonate. After 1 h the mixture was filtered (Whatman No. 1 paper). Acetone (30 ml) was used for washing the container and the filter-paper and the combined filtrate was evaporated under reduced pressure with a rotary evaporator. This precipitation step may be repeated for samples containing large amounts of inorganic salts. The final residue was dissolved in a known volume of methanol (0.25–20 ml) for injection. The replicates referred to in Table I were complete and included individual replicate sample preparation.

Total organic carbon determination for aqueous samples

The total organic carbon in aqueous samples was measured both before and after lyophilization (and reconstitution with distilled water) with a Dohrman DC 180 total organic carbon analyzer (Rosemount Analytical Division, Santa Clara, CA, USA). It was measured initially for the whole aqueous Casmalia groundwater sample and then for a lyophilized sample reconstituted to its original volume in distilled water.

RESULTS AND DISCUSSION

Anion-exchange chromatograms of the monosulfonic acid standards with particle beam MS detection (full scan, EI) are shown in Figs. 2 and 3. Fig. 2 also shows the EI mass spectra for the six monosulfonic acids corresponding to the peaks of the chromatogram shown. Fragmentation of these aromatic sulfonic acids under EI conditions involves the loss of SO_2, HSO_2, SO_3 or HSO_3. The presence of these fragment ions in the EI spectra are invaluable for confirmation without requiring the use of more complicated MS–MS experiments. With selected ion monitoring the molecular ion and fragments corresponding to loss of SO_2, HSO_2, SO_3 and HSO_3 under these conditions, the limits of detection are less than 250 ng.

In each instance the identity of the peak is unabiguously confirmed from a molecular and corresponding diagnostic fragmentation ions. A carryover or "memory" effect can be seen in some spectra. For example, the spectrum of 4-hydroxybenzenesulfonic acid (Fig. 2) shows in addition to the M^+, $[M-SO_2H]^+$ and $[M-SO_3H]^+$ ions a much smaller cluster at m/z 192 and 194 that probably come from the earlier eluting 4-chlorobenzenesulfonic acid. Possible the low volatility of these compounds makes them remain in or near the MS ion source.

These anion-exchange chromatograms with EI

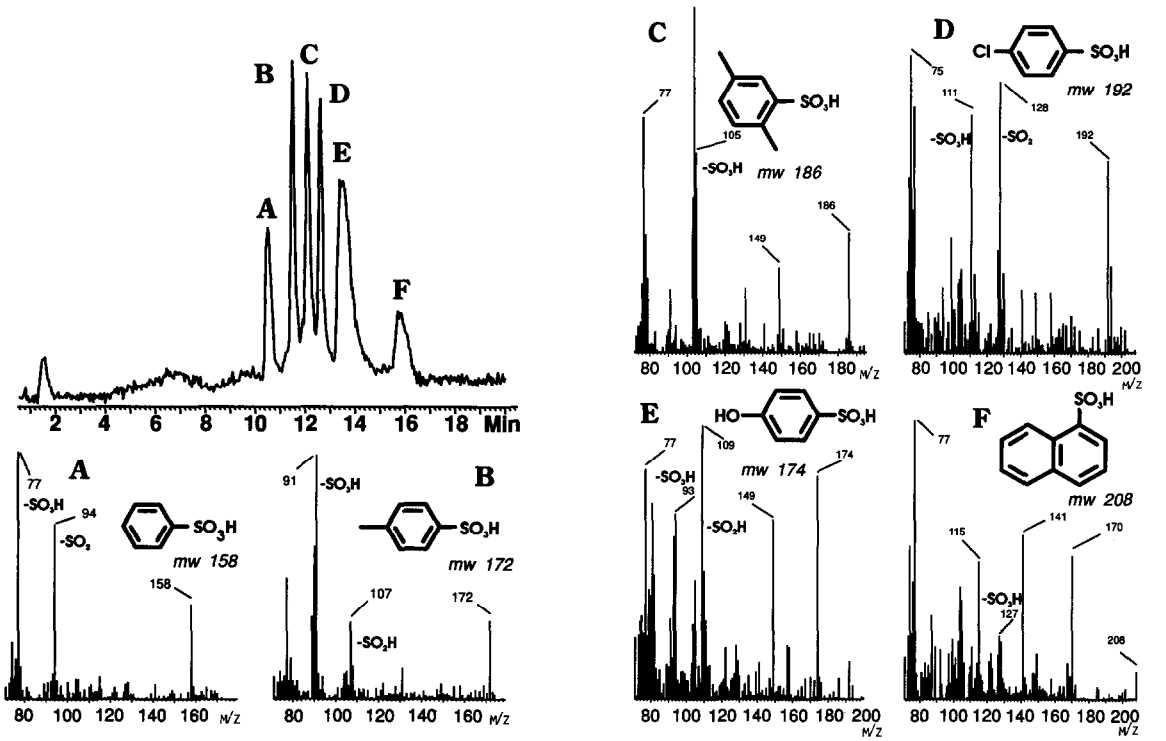

Fig. 2. Anion-exchange chromatogram (PAX-100 column with aqueous ammonium acetate–acetonitrile gradient) of six monosulfonic acid standards (2.0 μg each) with particle beam MS detection (full scan, EI) and (below) corresponding mass spectra obtained from this chromatogram. Chromatographic gradient conditions with a mobile phase consisting of water–acetonitrile–ammonium acetate (250 m*M*, pH 6.0): 0 min, 88:10:2, 5 min, 70:10:20; 10 min, 34:43:23.

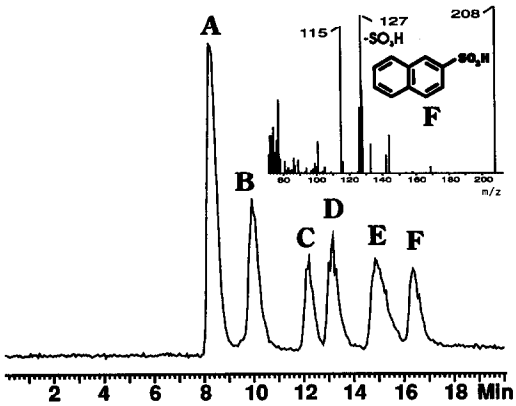

Fig. 3. Anion-exchange chromatogram (PAX-500 column with aqueous sodium hydroxide–acetonitrile gradient and micromembrane suppressor) of six monosulfonic acid standards (0.4 μg each) with particle beam MS detection (full scan, EI) and corresponding mass spectrum of 2-naphthalenesulfonic acid (mol.wt. 208; peak F) obtained from this chromatogram. Chromatographic gradient conditions with a mobile phase consisting of water–acetonitrile–sodium hydroxide (200 m*M*): 0 min, 90:10:0; 18 min, 0:40:60.

particle beam MS detection were produced with the OmniPac PAX-100 (with ammonium acetate, no suppressor, Fig. 2) and PAX-500 (with sodium hydroxide and the micromembrane suppressor, Fig. 3) columns. Because of the pressure limitations of currently available membrane suppressor desalting systems, the particle beam LC interface is ideally

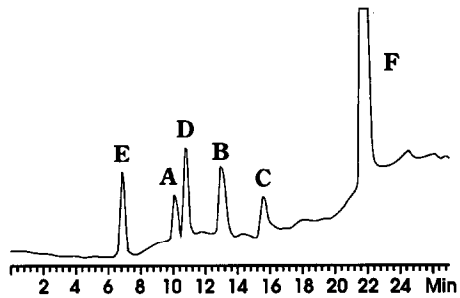

Fig. 4. Anion-exchange chromatograms (SAX column with constant aqueous ammonium acetate–gradient acetonitrile) of six monosulfonic acid standards (20 ng each) with UV detection (230 nm). Chromatographic gradient conditions with a mobile phase consisting of water–acetonitrile–ammonium acetate (100 m*M*, pH 6.0): 0 min, 85:0:15; 10 min, 83:2:15, 30 min, 55:30:15.

matched because of its inherently low back-pressure. Fig. 4 shows the same six standards as used in Fig. 2 (20 ng each), chromatographed on the SAX column using ammonium acetate and UV detection. The relative elution order of the six monosulfonic acids changes considerably between the PAX columns and the SAX column.

EI ionization gives approximately equal responses with six aromatic sulfonic acids (Figs. 2 and 3). NCI only gives a good response and spectral quality for 4-chlorobenzenesulfonic acid (base ion *m/z* 156, [M − HCl] [2]. PCI gives a poor response for all the standards used in this study.

TABLE I

SPIKE AND RECOVERY RESULTS FOR SIX MONO- AND TWO DISULFONIC ACID STANDARDS SPIKED INTO TAP WATER (TW) AND DISTILLED WATER (DW)

A = benzenesulfonic acid; B = 4-toluenesulfonic acid; C = xylenesulfonic acid; D = 4-chlorobenzenesulfonic acid; E = 4-hydroxybenzenesulfonic acid; F = 1-naphthalenesulfonic acid; G = 2,6-naphthalenesulfonic acid; H = 1,5-naphthalenesulfonic acid.

Spike (ppm)	Matrix	Volume (ml)	Recovery ± S.D.[a] (%)							
			A	B	C	D	E	F	G	H
0.01	DW	300	NT[b]	94 ± 7.1	85 ± 10	78 ± 5.3	NT	83 ± 5.0	NT	NT
0.01	TW	300	NT	73 ± 9.4	75 ± 10	78 ± 8.0	NT	78 ± 9.4	NT	NT
1.00	DW	10	NT	81 ± 5.2	92 ± 7.6	85 ± 4.4	NT	87 ± 4.6	96 ± 3.2	142 ± 8.8[c]
1.00	TW	10	NT	75 ± 9.8	88 ± 12	74 ± 7.0	NT	79 ± 11	62 ± 4.9	96 ± 2.6
100.0	DW	5	76 ± 6.5	90 ± 3.9	82 ± 5.3	91 ± 6.6	71 ± 5.3	90 ± 6.3	77 ± 6.1	94 ± 8.8
100.0	TW	5	78 ± 3.3	82 ± 6.1	83 ± 10	89 ± 4.6	65 ± 6.9	87 ± 4.9	75 ± 11.7	116 ± 3.6

[a] Standard deviation calculated from 6–12 replicate sample extracts.

[b] NT = specific analyte not tested at this concentration.

[c] Recovery of 1,5-naphthalenesulfonic acid was consistantly high in distilled water even with freshly prepared standards, suggesting that a positive interference may be present in this blank.

Recoveries of aromatic sulfonic acid standards added into tap water and distilled, deionized water are shown in Table I. With UV detection, the quantification limits range from 2 to 80 ng injected on-column (Table II). Quantification limits for the disulfonic acids are greater than those for the monosulfonic acids. The disulfonic acids were not used in the chromatographic experiments reported here, but were included in the Table I spike and recovery experiments to demonstrate that this recovery method can be generalized to disulfonic in addition to monosulfonic acids in aqueous samples.

Application to environmental samples

The total organic carbon (TOC) concentration in the Casmalia groundwater monitoring well sample was 7400 ± 270 ppm (± standard deviation, $n = 3$). After lyophilization of this sample and reconstitution with distilled water to the original volume, the TOC became 1500 ± 32 ppm. Hence in the Casmalia sample 21% of the TOC is non-volatile and is retained after the lyophilization process. This compares with more than 95% of the TOC in Stringfellow waste site samples being non-volatile [2]. Fig. 5 shows an anion-exchange chromatogram (SAX column) of the Casmalia groundwater monitoring well sample both before (top) and after (bottom) lyophilization with UV detection (265 nm). The early-eluting peaks appear to be selectively lost during the lyophilization process.

Fig. 6a shows the corresponding anion-exchange chromatogram of the Casmalia lyophilizate with EI particle beam detection. The chromatographic conditions were slightly modified compared with Fig. 5. The presence of toluenesulfonic acid in this sample is qualitatively indicated by comparison of the extracted ion profile (Fig. 6a) with the full EI mass spectra of this compound (Fig. 2). The major ions of the toluenesulfonic acid standard, M^+, $[M - HSO_2]^+$ and $[M - HSO_3]^+$, all coincide in Fig. 6a. Spiking the sample with 10 μg of authentic standard shows co-elution with the unknown peak (Fig. 6b). A 10-μg sample of the toluenesulfonic acid alone shows a slightly shorter retention time (38.4 *versus* 37.0 min) under these conditions (Fig. 6c). We have observed that retention times with anion-exchange chromatography are at least as sensitive to both sample loading and the presence of other analytes as is typically seen for reversed-phase chromatography. The identification of toluenesulfonic acid is purily qualitative in this experiment. This is

TABLE II

CORRELATION COEFFICIENTS (R) AND QUANTIFICATION LIMITS FOR FIVE-POINT CALIBRATION WITH SIX MONO- AND TWO DIAROMATIC SULFONIC ACIDS WITH ANION-EXCHANGE LC (SGE SAX COLUMN WITH CONSTANT AQUEOUS AMMONIUM ACETATE–GRADIENT ACETONITRILE MOBILE PHASE) AND UV DETECTION (230 nm)

The calibration concentration range is 1–2000 ng injected.

Compound	R	Quantitation Limit (ng injected)[a]
A	0.999	12
B	0.999	12
C	0.999	12
D	0.998	5
E	0.999	13
F	0.995	2
G	0.998	30
H	0.990	80

[a] Quantitation limit is the concentration of the linear five-point calibration line corresponding to the lower 95% prediction limit abscissa intercept, *i.e.*, the minimum concentration at which the predicted peak area includes zero at the 95% prediction confidence limit, or the lowest calibration concentration, whichever is the larger number.

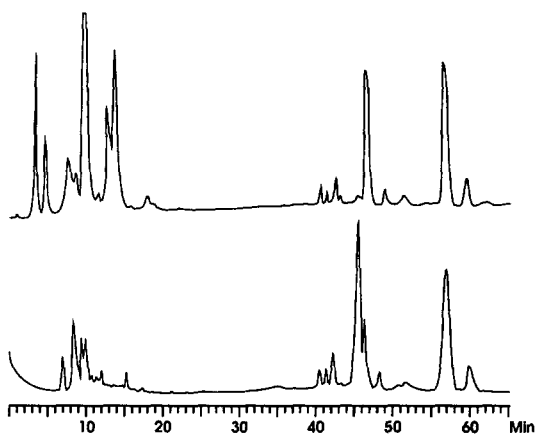

Fig. 5. Anion-exchange chromatogram (SAX column) of the Casmalia groundwater monitoring well sample both before (top) and after (bottom) lyophilization with UV detection (265 nm). The early-eluting peaks are selectively lost during the lyophilization process.

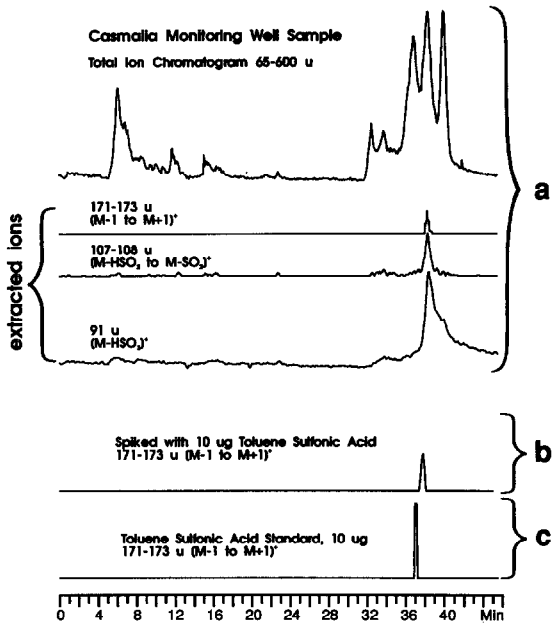

Fig. 6. (a) Anion-exchange chromatogram (chromatographic conditions slightly different to those in Fig. 5) of the Casmalia lyophilizate with EI particle beam MS detection showing both full scan (top), and extracted ion chromatograms (u = atomic mass units) (M^+, $[M-HSO_2]^+$ and $[M-HSO_3]^+$) (vertical scale $\times 10$) of the major ions of toluenesulfonic acid standard. (b) Same conditions but with the sample spiked with 10 μg of toluenesulfonic acid. (c) Same conditions but with only a 10-μg sample of the toluenesulfonic acid.

because quantification, relying on UV absorption, is effectively blocked by the large amounts of interfering material present in this real sample.

Aromatic sulfonic acids have been shown to occur as organic pollutants in aqueous samples at several hazardous waste sites. These materials are not well determined by conventional GC-based methods. The application of anion-exchange LC–particle beam MS will help to increase our understanding of this non-volatile and water-soluble organic fraction of environmental aqueous samples.

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