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Stability of sulfonated derivatives of benzene and naphthalene on disposable solid-phase extraction pre-columns and in an aqueous matrix

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Abstract

The stability of 14 sulfonated benzene and naphthalene compounds was investigated using polymeric solid-phase extraction cartridges, based on the styrene–divinylbenzene polymer Isolute ENV+. Several different storage conditions were tested to carry out the stability study in polymeric cartridges, which included storage at room temperature, at 4°C and at −20°C, during a period of up to 3 months. An additional stability study was carried out, not with the polymeric solid matrix, but in an aqueous matrix. This study was performed storing the samples at 4°C, during 2 months under three different conditions: acidifying the water sample to pH 2.5–3 with sulfuric acid, adding 1% of formaldehyde (additive used in waste water analyses), and storing the water sample at 4°C without any additives. The extraction of the SPE process is analyzed by ion-pair chromatography–electrospray mass spectrometry, in the negative ion mode. This study showed that the stability of polar aromatic sulfonic acids on disposable polymeric cartridges and in the water matrix is related to temperature and pH, respectively. Target aromatic sulfonated compounds stored in polymeric solid-phase extraction cartridges, are more stable at lower temperatures. The target analytes showed also good stability when stored in water at acidic pH. From the different analytes studied, substituted naphthalenesulfonates suffered more degradation than mononaphthalenesulfonates or benzenesulfonates under the experimental conditions of this work.

Keywords: Extraction methods; Stability studies; Water analysis; Benzenesulfonates; Naphthalenesulfonates; Sulfonates

1. Introduction

Sulfonated organic compounds are chemical substances that are present in many industrial processes and as consumer products. Benzene- and naphthalenesulfonates are used mainly as intermediates for the manufacturing of azo dyestuffs, pharmaceuticals and tanning agents [1–3]. A wide variety of substituted benzene- and naphthalenesulfonates are produced on a large scale in the chemical industry. Naphthalenesulfonic acids and their derivatives have been applied in the paper, textile and tanning industries as optical brighteners, dyes, azo dye coupling components and as dispersant, wetting and suspending agents. Naphthalenesulfonates are also applied as additives in cement and concrete. Benzenesulfonates are used as intermediates in the production of dyestuffs, tanning agents, catalysts, pesticides, water-soluble and ion-exchange resins, optical brighteners, pickling, wetting and finishing agents, plasticizers, pharmaceuticals and chemicals for organic synthesis.

Although the aromatic sulfonates have been produced and applied for a long time, little information is known on their toxicology, ecotoxicology [4,5],

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environmental behavior and degradation. In order to carry out more efficient and reliable environmental water monitoring it is important to know the stability of the different contaminants in water after sample collection and during transport and storage. For example, it is known that acidification prevents degradation of some volatile compounds and allows sample storage for some days or the addition of formaldehyde prevents degradation of linear alkylbenzenesulfonates and related analytes in wastewater samples. Due to their high water solubility and low biodegradability these sulfonates should be expected to be highly stable, being able to store them during some days or weeks. It is also important to know if hydrolysis and/or microbial decomposition occur during storage.

The isolation of aromatic sulfonates from untreated industrial wastewaters is difficult due to the high content of inorganic salts, total organic carbon (TOC) and to the complexity of such wastewaters. Therefore a clean-up step based on elimination of interfering chemicals with further preconcentration is required.

Due to the previous experience of our group with sulfonated compounds, Isolute ENV+ polymeric sorbent based on polystyrene–divinylbenzene was chosen to perform the preconcentration and storage of benzene- and naphthalenesulfonate compounds from water samples. First, a comparison between a SPE (solid-phase extraction) methodology, using Isolute ENV+ polymeric sorbent, and a SSPE (sequential solid-phase extraction) methodology, using two different sorbents: a C_{18} phase in series with the polymeric sorbent was carried out. The main advantage of using two sorbents in series is the elimination of interferences present in complex water matrices. Ion-pair chromatography followed by electrospray mass spectrometry (IPC–ESI-MS) in the negative ion mode was used for the quantification of benzene and naphthalenesulfonate studied compounds. ESI-MS allows one to obtain both structural and molecular mass information as well as good sensitivity. This work follows previous work from our group in the characterization of sulfonated azo dyes [6,7], and linear alkylbenzenesulfonates [8] using negative ion ESI either with LC–MS and/or capillary electrophoresis (CE)–MS.

The specific objectives of the present work were as follows: (i) to establish the storage stability of some benzene- and naphthalenesulfonates in water and in Isolute ENV+ polymeric SPE pre-columns, (ii) to evaluate the performance of the disposable pre-columns of Isolute for use in current monitoring programs by investigating their feasibility for storage and transport of samples, (iii) to carry out the stability study during a period of 3 months at three different temperatures (−20°C, 4°C and room temperature) in order to assess the ideal storage conditions for aromatic sulfonates stability in SPE pre-columns and (iv) to carry out the stability study of the water sample (aqueous matrix), storing the samples at 4°C, during a period of 2 months at three different conditions: acidifying the water sample to pH 2.5–3 with sulfuric acid, adding 1% of formaldehyde (common additive used in wastewater analyses), and storing the water sample at 4°C without any additive.

The final goal of the present study is to improve the quality assurance parameters in relation to sample handling, transport and storage of polar aromatic sulfonates in environmental water analyses and to apply the present approach to on-going monitoring programs. To our knowledge, the stability of benzene- and naphthalenesulfonates in water and also the use of disposable SPE pre-columns for storing and transport of water samples containing this group of pollutants has not been done before. The present work follows our previous research on the analyses of sulfonated aromatics in environmental water samples [9,10], and on stability studies using SPE cartridges of pesticides [11] and phenols [12].

2. Experimental

2.1. Chemicals and reagents

The compounds used in this study were purchased from Fluka (Buchs, Switzerland), Aldrich (Steinheim, Germany) or Sigma (St. Louis, MO, USA) in the form of the free acids or their sodium salts. The reference substances were: 1-amino-5-naphthalanesulfonate, 1-amino-4-naphthalanesulfonate, 1-amino-6-naphthalanesulfonate, 1-amino-7-naphthalanesulfonate, 2-amino-1-naphthalanesulfonate, 1-hydroxy-4-naphthalenesulfonate, 2-amino-

1,5-naphthalanedisulfonate, 1-hydroxy-3,6-naphthalenedisulfonate, 2-hydroxy-3,6-naphthalenedisulfonate, 1,5-naphthalenedisulfonate, 2,6-naphthalenedisulfonate, 2,7-naphthalenedisulfonate, 1,3,6-naphthalenetrisulfonate, benzenesulfonate, 4-chloro-3-nitrobenzenesulfonate, 1-hydroxy-6-amino-3-naphthalanesulfonate, 1-hydroxy-7-amino-3-naphthalanesulfonate 1,3-benzenedisulfonate 4-methylbenzenesulfonate, 3-nitrobenzenesulfonate, 4-chlorobenzenesulfonate, 1-naphthalanesulfonate, 2-naphthalanesulfonate and diphenylamine-4-sulfonate (substance chosen as internal standard for the future analyses, due to the lack of this sulfonated compound in the studied samples).

HPLC-grade water and methanol were obtained from Merck (Darmstadt, Germany). The ion-pair reagent used was triethylamine (TEA), purchased form Merck. Analytical-reagent grade acetic acid and sulfuric acid (95–97%) from Panreac (Barcelona, Spain) and Merck, respectively were used. Mobile phase was passed before use through a 0.45-μm membrane filter from Scharlau (Barcelona, Spain).

2.2. Sample preparation procedure

A mixture of all standards was prepared in water at 1000 μg/l. The stability experiment was performed using groundwater with the following characteristics: pH 7.4, conductivity: 2020 μS/cm, alkalinity: 315 mg of $CaCO_3$, chlorine: 369 mg of Cl/l, sulfates: 387 mg of SO_4/l, nitrates: 75 mg of NO_3/l and hardness 100 mg of $CaCO_3$/l. Groundwater was spiked with the standard solution to approximately 40 μg/l, a common average concentration found in wastewater samples.

An off-line SPE method with extraction columns (ASPEC XL) was used for automated preconcentration of water samples. The ASPEC XL system (Gilson, Villiers-le-Bel, France) is fitted with an external Model 306 LC pump for dispensing samples through the cartridges and is connected with a Model 817 switching valve for the selection of water samples. Disposable 6-ml cartridge columns packed with 200 mg of Isolute ENV+, from International Sorbent Technology (Cambridge, UK), were used. This sorbent is based on styrene–divinylbenzene polymers. Conditioning, washing and elution flow was 1 ml/min. The water sample was percolated through the cartridge with an optimum flow-rate of 5 ml/min. To perform the SSPE, disposable 6-ml cartridge columns packed with 500 mg of C_{18} phase, from Merck were used.

Prior to SPE, water was filtered through 0.7-μm glass microfiber filters from Scharlau to remove suspended matter and acidified to pH 2.5–3, with sulfuric acid.

Conditioning of the sorbents was accomplished by passing 7 ml of methanol and 3 ml of acidified water through the cartridges at a flow-rate of 1 ml/min. The sorbent was not allowed to dry and immediately 150 ml of water sample was loaded at 5 ml/min in the Isolute ENV+ cartridges. The washing step was carried out with 1 ml of water. The drying step applied later to the sample was previously tested and in all cases better recoveries were obtained when the cartridge was not dried, so this step was eliminated [9]. However to study the storage at −20°C this step is necessary. The drying step was carried out using a Baker LSE 12G apparatus connected to a vacuum system with pressure set at 15 p.s.i. (negative pressure; 1 p.s.i.=6894.76 Pa). Drying took 20–25 min.

The second studied extraction procedure was SSPE. The main goal of this procedure is to eliminate some interferences present in water samples. Two different sorbents were used: a C_{18} phase (500 mg, 6 ml) in series with the styrene–divinylbenzene sorbent Isolute ENV+. The same condition step as described above was used for both cartridges, with the exception that the C_{18} phase was conditioned with water at neutral pH, because the neutral water sample is loaded through this non-polar sorbent (200 ml at 5 ml/min). A 150-ml volume of the residual water was acidified to pH 2.5–3 and loaded on the Isolute ENV+, as is described above. Fig. 1 illustrates the SSPE and the SPE methodologies, their phases and the elution step.

A total of 66 pre-columns were used for preconcentrating, because the experiment design involved three replicate analyses of each of the three storage treatments and five storage periods: 1, 2, 4, 8 and 12 weeks. Six different concentrations (in triplicate) were analyzed to obtain the calibration plots. And in addition, blanks were prepared (also in triplicate) by percolating the same ground water, but they were not spiked.

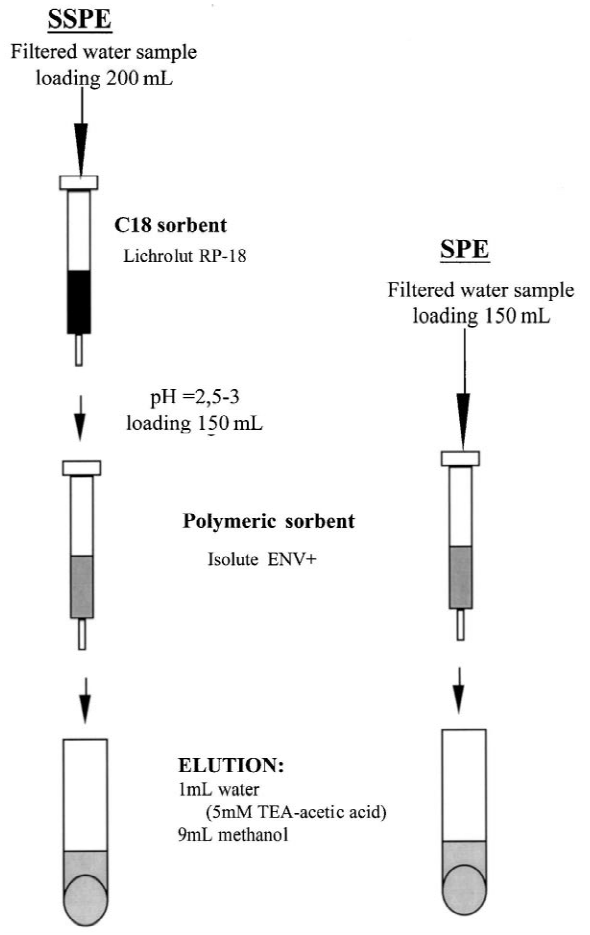

Fig. 1. Sequential solid-phase extraction (SSPE) and solid-phase extraction (SPE) methods.

In all instances, the pre-columns were stored immediately after the preconcentration step.

Immediately after preconcentration, three pre-columns were eluted (time 0). The storage treatments include the following: (i) one-third of the pre-columns were stored at room temperature until the day of the analyses, at 1, 2, 4, 8 and 12 weeks; (ii) the second-third of the pre-columns were kept at 4°C (in the dark) during the previously mentioned periods; and (iii) the rest of the columns were dried during 20–25 min, stored at −20°C (in the dark) and analyzed during the same period. At the established time, the pre-columns were brought to room temperature over 8–10 h prior to analyses in order to remove the frozen water matrix enveloping the polymeric phase and to avoid precision and variability problems.

The desorption and elution step was performed by adding 1 ml of water containing ion-pair reagent (5 m*M* TEA and 5 m*M* acetic acid, pH 6.5) and 4 ml of methanol at 1 ml/min, waiting 5 min, in order to allow sufficient contact time between the solvent and the trapped compound, and then adding the second aliquot of 5 ml of methanol. The final evaporation step of the extra solvent was carried out with a stream of nitrogen. The extracts were concentrated to a final volume of 1 ml. Finally, 50 μl was injected into the LC system and analyzed by IPC–ESI-MS.

The same preconcentration and analyses procedure was carried out to do the stability study of the water sample in the aqueous matrix stored at 4°C in the dark. For this experiment, groundwater was also spiked with the standard solution to approximately 40 μg/l. Spiked water samples were stored during 2 months at 4°C, in Pyrex borosilicate brown glass containers. Three different bottles were used to study the behavior of the aromatic sulfonated compounds at three different storage treatments: acidifying the water sample to pH 2.5–3 with sulfuric acid, adding 1% of formaldehyde (additive used in the water analyses to stabilize) to the water sample and the water sample stored in the fridge without any additives. The design involved three replicate analyses of each of the three storage treatments.

2.3. Evaluation

All stability results were converted to a percentage of the initial concentration, this means that all recoveries were calculated taking into account the recoveries obtained in the extraction at time zero. The precision and variability of the off-line SPE procedure for each aromatic sulfonate compound was calculated through the relative standard deviation (RSD).

The matrix was also studied comparing the results of the stability study in water and on disposable SPE cartridges.

2.4. Liquid chromatography/mass spectrometry

2.4.1. Chromatographic conditions

In the present work, ion-pair chromatography is

used to separate polar aromatic sulfonates with a C_{18} column, at room temperature. For the chromatographic separation, a conventional column (LiChroCART-LiChrospher 100 RP-18 analytical column of 250×4 mm and 5 μm particle diameter) was compared with a fast and short column (Superspher 100 RP-18 analytical column of 75×4 mm and 4 μm particle diameter), both of them equipped with a LiChrospher 100 RP-18 10×4 mm and 5 μm particle diameter guard column; all columns were obtained from Merck.

The commonly used involatile ion-pairing reagents [13–17], such as tetrabutylammonium bromide and tetrabutylammonium hydrogensulfate are barely suitable for coupling to mass spectrometry, because they can cause contamination of the electrospray interface with additional problems of poor reproducibility and sensibility. Due to this problem, Suter et al. [18] have developed a method using ammonium acetate as buffer in the LC eluent. However, because this small cation is not a real ion-pair reagent, isomers of most polar naphthalenesulfonates, such as naphthalenedisulfonates, cannot be separated. In order to achieve optimal performance we have used an orthogonal interface and volatile reagents, enabling drainage of the waste and avoiding contamination of the MS source. The system used in this paper consisted of an orthogonal and concentric nebulizer in combination with a volatile ion-pair reagent, which permitted adequate analytical performance. TEA, a volatile tertiary alkylamine that allows ion-pair formation for aromatic sulfonates, was chosen in this work as the ion-pair reagent. Eluents were: (A) methanol (5 m*M* TEA and 5 m*M* acetic acid) and (B) water (5 m*M* TEA and 5 m*M* acetic acid, pH 6.5).

The mobile phase composition was, in the case of the conventional column, 100% B during the first 10 min, then linearly decreased to 95% B in 3 min, and kept isocratic for 10 min; a decrease to 75% B was performed in 17 min, and continuous decreases to 60, 10 and 0% B were performed at minutes 45, 50 and 55, respectively. The flow-rate of the mobile phase was 0.8 ml/min. A time of 50 min is necessary to complete the analyses.

The mobile phase composition was, in the case of short and fast column, 100% B during the first 5 min, then linearly decreased to 95% B in 5 min, a decrease to 80% B was performed in 5 min, and continuous decreases to 40, 10 and 0% B were performed at minutes 20, 25 and 30, respectively. The flow-rate of the mobile phase was 0.8 ml/min during the first 5 min, then was increased to 1 ml/min, in 1 min. A time of only 20 min is necessary to complete the analysis, when a short column with lower particle diameter is used. The difference between analysis time of a conventional column and a short column is 30 min in every injection, in every sample.

Chromatographic separation was carried out using a system from Hewlett-Packard, Model 1090 A. Detection was carried out using a HP 1040 M diode array UV–Vis detector coupled in series with the HP 1100 mass-selective detector, equipped with a standard atmospheric-pressure ionization source that was used as ESI interface. A characteristic feature of the HP 1100 is that the inlet coming from the LC system is neither directed on-axis nor off-axis, but situated perpendicular to the ion optics axis. This prevents uncharged particles from entering the low-pressure region and finally the quadrupole and thus significantly reduces the background and increases the sensitivity. When the LC effluent enters the atmospheric pressure chamber, evaporation of the solvent is enhanced by concurrently introducing a drying gas (12 l/min) of nitrogen heated up to 350°C. In the silica capillary a voltage of 3500 V is applied to initiate declustering of the solvent–analyte–cluster ions. Further declustering and partial fragmentation (the collision-induced dissociation, CID) can be achieved in the CID region, located between the end of the transfer capillary and the first skimmer at a voltage between 50 and 150 V. A distinct optimum in the abundance of the quasi-molecular ion peak was observed at 80 V. Therefore fragmentor voltage of 80 V was used for quantification of mono- and disulfonates. The m/z values monitored for each target analyte corresponded to $[M-H]^-$ ion. At a fragmentor voltage of 150 V, used for identification, significant fragmentation of the quasi-molecular ion occurs, due to loss of neutral SO_2 or SO_3 groups.

2.5. Instrument calibration

External calibration was used for quantitative determination. The IPC–ESI-MS system calibration

was performed by plotting peak area (y) vs. injected amount (x, μg/l), using the negative ion (NI) mode, time-scheduled selected ion monitoring (SIM) and 80 V as fragmentor voltage. As reported previously [9,10], the linearity range was over three orders of magnitude for target compounds. The quantitative determination of the water extracts was achieved by using time-scheduled SIM using the $[M-H]^-$ ion for each sulfonic compound (see Table 1). Calibration plots were constructed by percolating the same volume as the sample waters spiked at different levels, following the methodology described in the previous section. These points were chosen in such a way that they covered the whole concentration range in which stability study was carried out, taking into consideration the possibility of diminution of the concentration of aromatic sulfonates due to degradation. All compounds under study showed a linear behavior at this concentration range.

3. Results and discussion

3.1. Comparison of two sorts of octadecylsilica column

In this work the separation of aromatic sulfonic acids was improved using a C_{18} fast and short column, with 4 μm particle diameter. Fig. 2 shows the separation of 19 aromatic sulfonate compounds in a conventional C_{18} column, with 5 μm particle diameter, by IPC–ESI-MS, in the negative ionization mode, using time-scheduled SIM at 80 V as fragmentor voltage, using the $[M-H]^-$ ion for each sulfonic

Table 1
Retention times and monitored molecular ion in IPC–ESI-MS, using negative ionization and SIM mode at 80 V as fragmentor voltage, of sulfonate compounds separated in (1) a conventional C_{18} column of 250×4 mm and 5 μm particle diameter (LiChroCART-LiChrospher 100 RP-18 analytical column, from Merck) and (2) a fast and short C_{18} column of 75×4 mm and 4 μm particle diameter (Superspher 100 RP-18 analytical column, from Merck)

Retention time (1) (min)	Retention time (2) (min)	Sulfonate compound	Monitored ion (m/z)
22.7	9.6	1-Amino-5-naphthalenesulfonate	222
25.1	10.4	1-Amino-4-naphthalenesulfonate	222
36.1	14.1	1-Amino-6-naphthalenesulfonate	222
43.1	16.6	2-Amino-1-naphthalenesulfonate	222
44.3	17.1	1-Amino-7-naphthalenesulfonate	222
39.1	14.1	1-Hydroxy-4-naphthalenesulfonate	223
10.0	3.0	2-Amino-1,5-naphthalenedisulfonate	302
19.9	8.9	1-Hydroxy-3,6-naphthalenedisulfonate	303
27.2	11.8	2-Hydroxy-3,6-naphthalenedisulfonate	303
15.2	4.1	1,5-Naphthalenedisulfonate	287
17.8	5.9	2,6-Naphthalenedisulfonate	287
27.3	n.s.	2,7-Naphthalenedisulfonate	287
28.5	12.6	1-Hydroxy-6-amino-3-naphthalenesulfonate	238
37.8	14.5	4-Methylbenzenesulfonate	171
42.9	16.3	4-Chlorobenzenesulfonate	191
34.6	13.4	3-Nitrobenzenesulfonate	202
47.6	18.1	1-Naphthalenesulfonate	207
48.0	19.0	2-Naphthalenesulfonate	207
31.5	n.s.	1-Hydroxy-7-amino-3-naphthalenesulfonate	238
n.s.	3.5	1,3,6-Naphthalenetrisulfonate	367
n.s.	9.9	Benzenesulfonate	157
n.s.	17.9	4-Chloro-3-nitrobenzenesulfonate	236
n.s.	3.5	1,3-Benzenedisulfonate	237
n.s.	19.1	Diphenylamine-4-sulfonate	248

n.s.: Compound not separated.

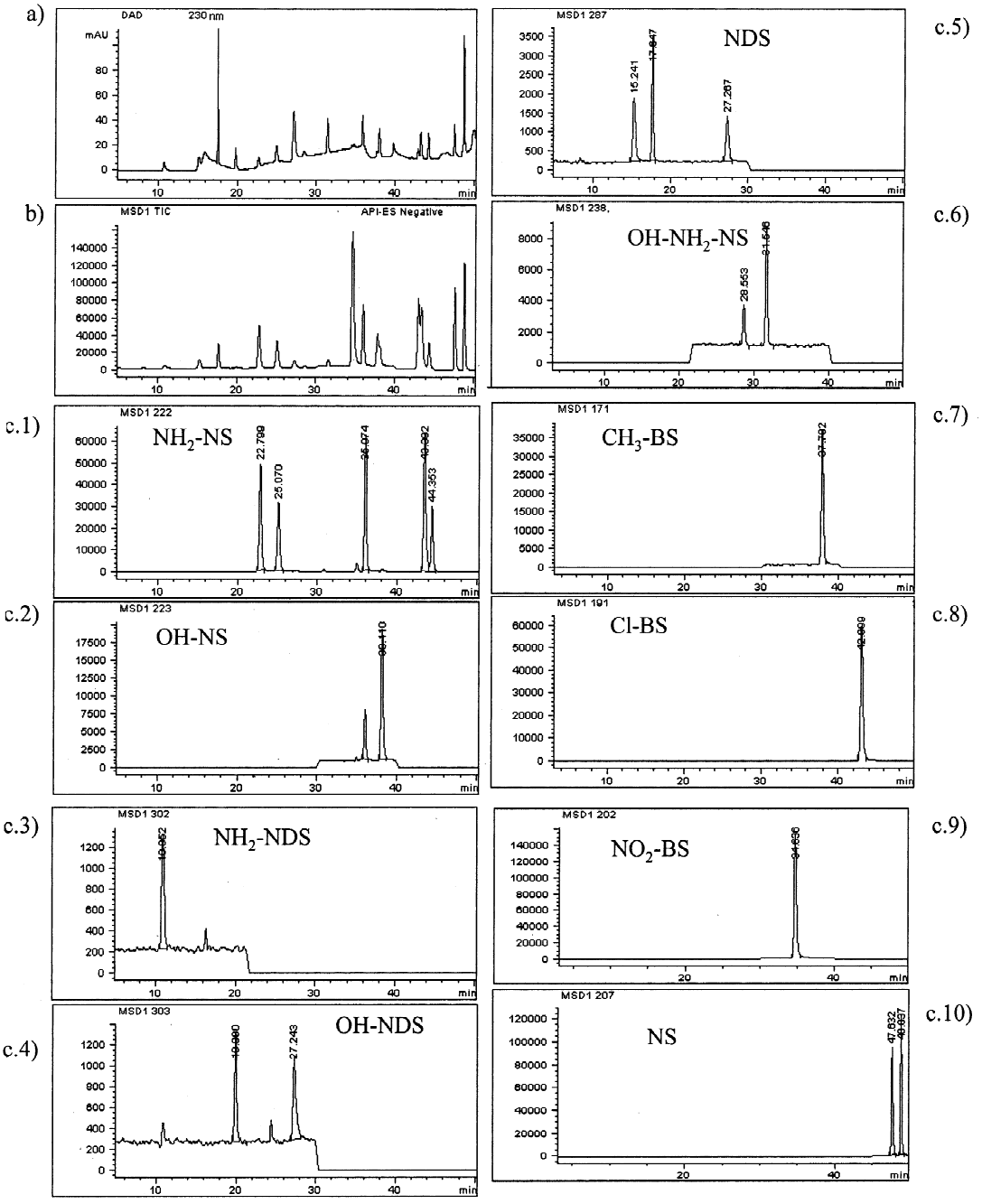

Fig. 2. Separation of 19 aromatic sulfonate compounds in a conventional C_{18} column of 250×4 mm and 5 μm particle diameter (LiChroCART-LiChrospher 100 RP-18 analytical column, from Merck): (a) IPC–UV chromatogram (230 nm), (b) total ion current (TIC) and (c) SIM mode IPC–ESI-MS chromatograms, obtained at 80 V, negative ionization, monitoring in all cases the molecular ion: $[M-H]^-$; (c.1) five aminonaphthalenesulfonate isomers, (c.2) hydroxynaphthalenesulfonate, (c.3) aminonaphthalenedisulfonate, (c.4) two hydroxynaphthalenedisulfonate isomers, (c.5) three naphthalenedisulfonate isomers, (c.6) two hydroxyaminonaphthalenesulfonate isomers, (c.7) toluenesulfonate, (c.8) chlorobenzenesulfonate, (c.9) nitrobenzenesulfonate and (c.10) two naphthalenesulfonate isomers. For more information, see Table 1 and Experimental section.

compound. Table 1 shows the retention time of each separated compound and the selected ion monitored. Using the fast and short column is possible to decrease the analysis time by half. The high efficiency and resolution of these columns allow one to analyze more compounds in a shorter time. This behavior is shown in Fig. 3, which exhibits a complete separation of 22 aromatic sulfonate compounds in a fast and short C_{18} column, with 4 μm particle diameter, by IPC–ESI-MS, in the negative ionization mode, using time-scheduled SIM at 80 V as fragmentor voltage, using the $[M-H]^-$ ion for each sulfonic compound. Table 1 shows the retention time of each separated compound and the selected ion monitored. Solvent and time saving is the greatest advantage of this sort of columns. High resolution and solvent and time saving was obtained using the ''fast and short column'' (Superspher 100 RP-18 analytical column of 75×4 mm and 4 μm particle diameter) and for this reason it was chosen for future work.

3.2. Off-line recovery studies

Recovery studies of target compounds using off-line SPE methodology were performed by spiking groundwater at a concentration of 40 ng/ml, by preconcentrating 150 ml of water ($n=3$) using polymeric cartridges based on polystyrene–divinylbenzene (Isolute ENV+). The extracts of the off-line SPE process are analyzed by IPC–ESI-MS. Quantification was done by external standard comparison. In a previous work [10], the breakthrough volumes for some benzene- and naphthalenesulfonates were already evaluated. The results showed that a volume of 150 ml of water is recommended in order to retain a maximum number of sulfonated compounds with good recoveries and without considerable losses. The results also showed that the breakthrough volumes for some naphthalenedisulfonates are lower than 150 ml.

Table 2 shows the mean recoveries of aromatic sulfonated compounds under study and RSDs. The lower recoveries observed between these data and previous data [9] is that the present recoveries are slightly lower for certain analytes. This has been attributed to a manufacturing problem of the sorbent material. A study is underway with the manufacturer to find out this variation of recoveries. The recoveries ranged from 66% to 96%, with low RSDs, with the exception of two aminonaphthalenesulfonates: 1-amino-5-naphthalenesulfonate and 1-amino-4-naphthalenesulfonate; and benzenesulfonate, whose recoveries were lower than 50%. This was attributed to breakthrough volumes of these compounds which are lower than 150 ml.

Table 2 also shows the comparison between SPE and SSPE methodologies, when 150 ml of water sample was preconcentrated as is described in the Experimental section.

The recovery difference is not very important, except for 1-amino-5-naphthalenesulfonate, which is retained in the C_{18} phase and only 2% is eluted in the polymeric sorbent. The main advantage related to the use of SSPE is the elimination of many interferences present in the complex water sample.

3.3. Storage conditions

A total of 14 compounds (eight naphthalenesulfonate and six benzenesulfonate compounds) were chosen as representatives of this group of compounds (polar aromatic sulfonated compounds) to carry out the stability study. Different isomers were also selected. A ground water matrix free of interferences was used for the preconcentration of target compounds for SPE. All results reported in this section are given in percent of recovery in relation to time=0.

3.3.1. Storage at room temperature

Storage at room temperature study was carried out to evaluate stability in polymeric cartridges without the need for cooling. This will permit an easy mailing of the samples preconcentrated on the cartridges to another laboratory without temperature control.

Percentage recoveries of target compounds and RSDs, after storing the cartridges at room temperature for up to 12 weeks are shown in Fig. 4. The results showed that at room temperature substituted naphthalenesulfonate compounds present a degradation of 50%, only in the first 2 weeks of storage. Regarding mononaphthalenesulfonates, α and β isomers presented a high stability at room temperature, even 4 weeks after the preconcentration. The stabili-

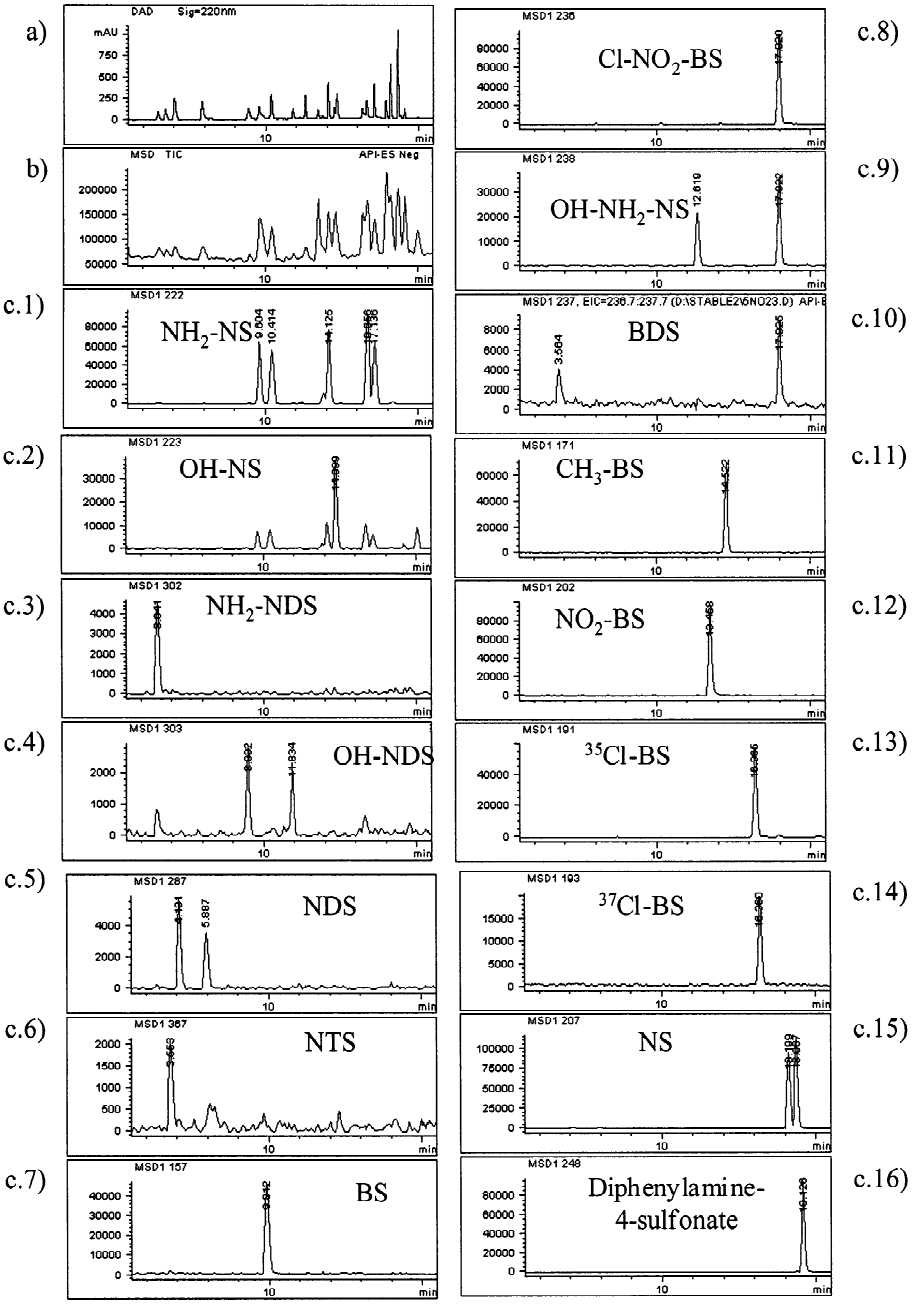

Fig. 3. Separation of 22 aromatic sulfonate compounds in a fast and short C_{18} column of 75×4 mm and 4 μm particle diameter (Superspher 100 RP-18 analytical column, from Merck): (a) IPC–UV chromatogram (230 nm), (b) total ion current (TIC) and (c) SIM mode IPC–ESI-MS chromatograms, obtained at 80 V, negative ionization, monitoring in all cases the molecular ion: $[M-H]^-$; (c.1) five aminonaphthalenesulfonate isomers, (c.2) hydroxynaphthalenesulfonate, (c.3) aminonaphthalenedisulfonate, (c.4) two hydroxynaphthalenedisulfonate isomers, (c.5) two naphthalenedisulfonate isomers, (c.6) naphthalenetrisulfonate, (c.7) benzenesulfonate, (c.8) chloronitrobenzenesulfonate, (c.9) two hydroxyaminonaphthalenesulfonate isomers, (c.10) benzenedisulfonate, (c.11) toluenesulfonate, (c.12) nitrobenzenesulfonate, (c.13) [^{35}Cl]chlorobenzenesulfonate, (c.14) [^{37}Cl]chlorobenzenesulfonate, (c.15) two naphthalenesulfonate isomers and (c.16) diphenylamine-4-sulfonate. For more information, see Table 1 and Experimental section.

Table 2
Solid-phase extraction (SPE) and sequential solid-phase extraction (SSPE) recoveries (%) and relative standard deviations (RSDs), for the extraction of 150 ml of spiked ground water at 40 μg/l, as is described in the Experimental section

Compound[a]	Monitored *m/z*	SSPE (%)	RSD (%)	SPE (%)	RSD (%)
1-NH2-5-NS	222	2.0	0.1	21.9	6.9
1-NH2-4-NS	222	42.6	1.8	34.2	2.8
1-NH2-6-NS	222	79.6	3.3	81.0	7.7
2-NH2-1-NS	222	82.1	1.7	82.6	3.1
1-NH2-7-NS	222	69.0	0.8	68.3	6.2
1-OH-4-NS	223	111.2	0.3	95.6	8.0
1 NS	207	83.3	1.6	84.6	3.3
2NS	207	79.3	2.5	83.8	0.4
BS	157	15.9	6.0	14.0	1.0
4-Cl-3NO2-BS	236	67.5	1.8	68.6	1.5
4-CH3-BS	171	57.1	4.2	66.0	5.8
3-NO2-BS	202	61.2	2.2	69.0	4.1
4-Cl-BS	191	67.8	1.9	74.7	4.0
diPh-NH-4S(*)	248	72.2	1.8	87.3	0.9

[a] NS: Naphthalenesulfonate; NDS: naphthalenedisulfonate; BS: benzenesulfonate; (*): diphenylamine-4-sulfonate.

ty of benzenesulfonate compounds at room temperature is higher than that of naphthalenesulfonate compounds. The studied benzenesulfonates showed percentage recoveries equal to or more than 70%, even when 4 weeks had passed since loading the sample. Whereas for substituted naphthalenesulfonates total degradation occurs for some compounds in the same storage period.

3.3.2. Storage at 4°C

The columns were brought to room temperature and not immediately eluted after storage to avoid precision and variability problems. Fig. 4 shows the percentage recovery of each studied compound after storing the pre-columns at 4°C (in the dark) for up to 3 months (12 weeks). The recoveries of the majority of the studied benzene- and naphthalenesulfonates were higher than those obtained at room temperature storage conditions, especially in the case of substituted naphthalenesulfonates during the first 4 weeks, with differences between recoveries greater than 10%. For benzenesulfonates and mononaphthalenesulfonates, there is no important difference between recoveries obtained at room temperature and at 4°C. Results showed that the stability of benzenesulfonate compounds is higher than naphthalenesulfonate compounds at 4°C, and is similar at room temperature. The studied benzenesulfonates and mononaphthalenesulfonate isomers showed percentage recoveries equal to or more than 60%, even after 12 weeks of storage. In contrast, total degradation occurs for the majority of aminonaphthalenesulfonates whereas for hydroxynaphthalenesulfonate 50% degradation is observed in the same storage period and at the same temperature.

3.3.3. Storage at −20°C

Before analyzing the pre-columns kept at −20°C, it is necessary to keep the pre-columns around 6–8 h before elution, in order to defrost the water matrix. Fig. 4 shows the percentage recovery of each studied compound after storing the pre-columns frozen at −20°C (in the dark) for up to 3 months (12 weeks). Recoveries of the target analytes were higher when the cartridges were stored at −20°C than those obtained at room temperature or at 4°C.

3.3.4. Storage in aqueous matrix

Table 3 compares the results obtained after storing spiked groundwater (approximately 40 μg/l) during 2 months in the fridge (at 4°C in the dark), in Pyrex borosilicate brown glass containers, at three different storage treatments: acidifying the water sample to pH 2.5–3 with sulfuric acid, adding 1% of formaldehyde to the water sample, and the water sample stored in the fridge without any additives.

Regarding the stability in aqueous matrix, the results obtained showed that addition of formalde-

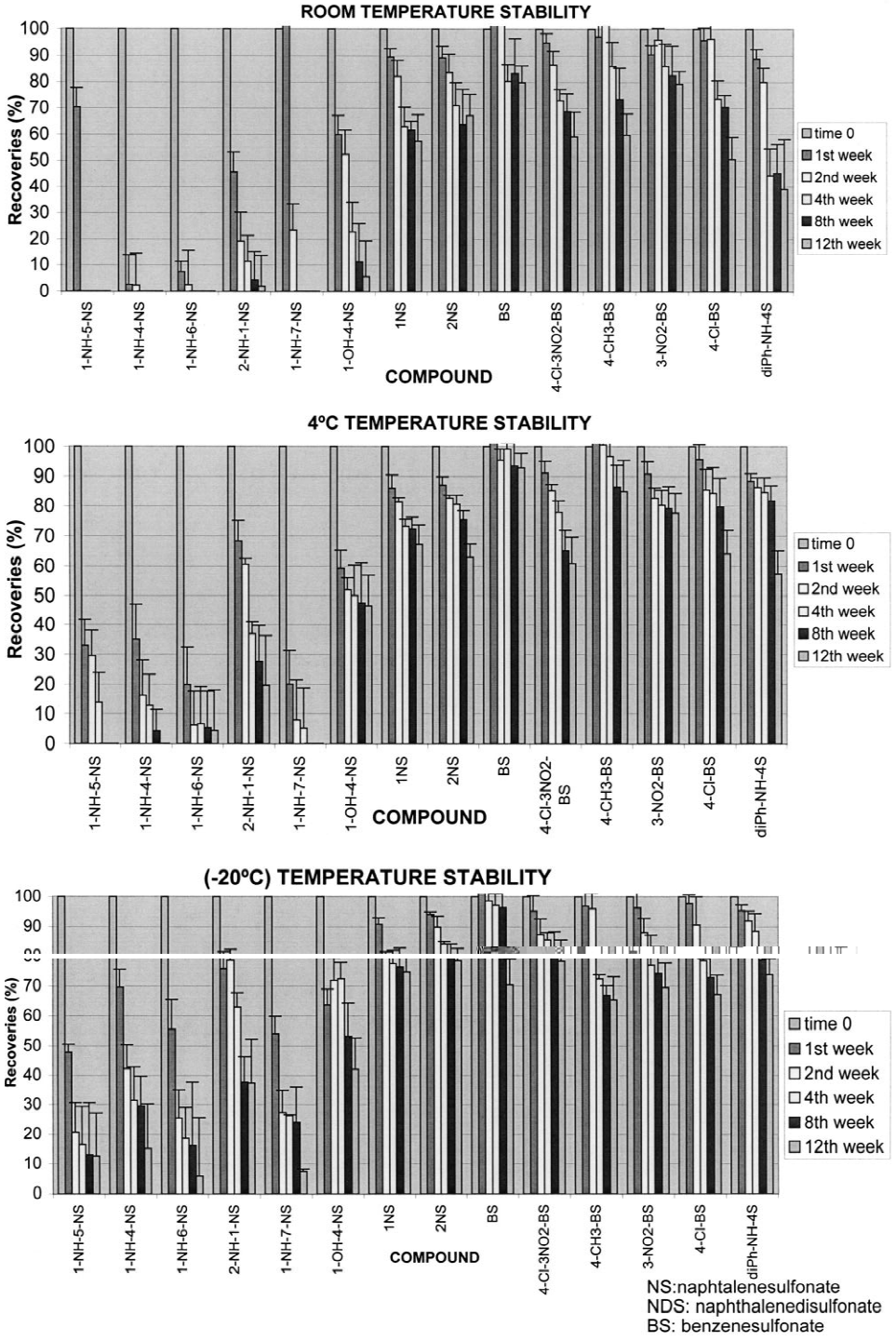

Fig. 4. Mean recoveries (%) and relative standard deviations (RSDs) of target analytes after storing the polymeric Isolute ENV+ cartridges at: (a) room temperature, (b) 4°C and (c) −20°C, for up to 12 weeks.

Table 3
Water matrix stability study recoveries (%) at three different conditions: (A) acidic pH, (B) with addition of formaldehyde and (C) without any addition

Compound[a]	Ion monitored (*m*/*z*)	Recovery (A) (%)	Recovery (B) (%)	Recovery (C) (%)
1-NH-5-NS	222	21.9	15.7	4.4
1-NH-4-NS	222	34.2	24.2	5.7
1-NH-6-NS	222	81.0	56.3	0.0
2-NH-1-NS	222	82.6	60.6	48.4
1-NH-7-NS	222	68.3	44.5	0.0
1-OH-4-NS	223	95.6	73.3	4.4
2,6-NDS	287	84.6	75.5	72.1
1-OH-6-NH-3-NS	238	55.0	0.0	0.0
1-OH-7-NH-3-NS	238	53.0	65.0	41.4
4-CH3-BS	171	66.0	43.8	37.1
3-NO2-BS	202	69.0	53.7	52.4
4-Cl-BS	191	74.7	60.6	40.9
1NS	207	84.6	62.4	65.4
2NS	207	83.8	64.3	69.6

[a] NS: Naphthalenesulfonate; NDS: naphthalenedisulfonate; BS: benzenesulfonate; (*): diphenylamine-4-sulfonate.

hyde at the 1% level is not a good treatment to store the aromatic sulfonated compounds. In some cases, the concentration of the rest of the aromatic sulfonated compounds is even lower than the limit of detection. The best treatment to store the target compounds is by acidifying the sample to pH 2.5–3.

3.4. Statistical evaluation

From the graphs represented in Fig. 4, it is possible formulate a hypothesis that the degradation reaction follows a first-order kinetic. So that determined values should follow the equation:

$$\ln (C/C_0) = -Kt$$

where C: final concentration (μg/l), C_0: initial concentration (μg/l), K: first-order kinetics constant (weeks^{-1}) and t: time (week).

For most of the studied analytes the linear regression coefficient obtained from the application of the above equation to the analytical data was greater than 0.8, thus indicating the assumption of a first-order degradation reaction is applicable.

Fig. 5 shows first-order kinetic constant versus each target analyte for different storage conditions: room temperature, 4°C and −20°C. This graph shows the different behavior between the studied compounds. For the first three aminonaphthalenesulfonates, at room temperature, degradation reaction is so fast that it was not possible to estimate K values, since only two concentration values are available. From this figure, is possible to see the difference between the two groups of compounds, aminonaphthalenesulfonates and hydroxynaphthalenesulfonates, that present a high kinetic constant, and benzenesulfonates and mononaphthalenesulfonates, whose constant is lower. Greater kinetic constant means faster degradation reaction, and vice versa. The obtained results show that the degradation reaction is faster at room temperature. This is especially true for substituted naphthalenesulfonates, since only in the first 2 weeks the percentage recovery of these compounds decreased by more than half. Most cases, an inhibition of the degradation reactions of the target compounds is observed with decreasing temperature.

4. Conclusion

The main advantages of the use of disposable SPE pre-columns for stabilizing aromatic sulfonic acids in water samples is the storage space, since usually bottles of 500 ml or 1 l are needed for sampling and storage. The easy shipping of the disposable SPE pre-columns containing aromatic sulfonated compounds to the central laboratory for the final analyses

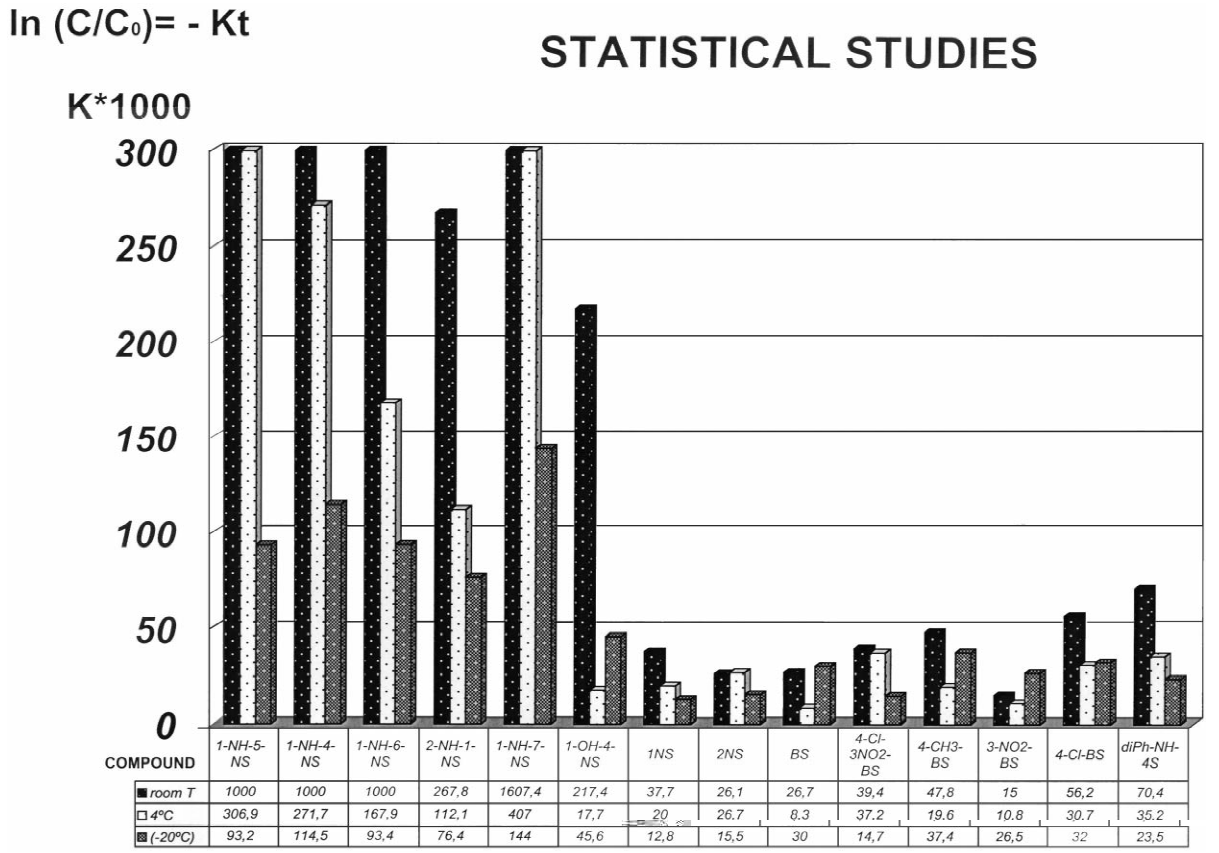

Fig. 5. Statistical analyses results: first-order kinetic constant versus each target analyte for different storage conditions: room temperature, 4°C and −20°C. (*) For the first three aminonaphthalenesulfonates, at room temperature, the degradation reaction is so fast that it was not possible to estimate *K* values, since only two concentration values are available.

is another advantage, making it unnecessary to perform the analysis immediately after sampling.

From the results reported in this work, it can be concluded that the stability of polar aromatic sulfonic acids on disposable polymeric cartridges and in water matrix is related to temperature and pH, respectively. Storage using polymeric SPE cartridges showed that target aromatic sulfonated compounds are more stable at lower temperatures. For storage in water matrix, the use of acid pH values offered more stability. An additional comment to be made is that more degradation was observed for substituted naphthalenesulfonates than for mononaphthalenesulfonates or benzenesulfonates.

The results reported in this work are of importance in the monitoring of wastewaters. These compounds are generally present in different wastewaters and water treatment plants, so by immediately storing the samples in SPE cartridges and at −20°C it is possible to analyze the samples after several weeks of storage. By storing the samples at 4°C some analytes may already suffer degradation after 1 week of storage, so 4°C it is only recommended for transportation of samples and with the maximum storage period of 1 week.

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