Determination of Parts Per Trillion Level of Carboxylic Degradation Products of Linear Alkylbenzenesulfonates in Coastal Water by Solid-Phase Extraction Followed by Liquid Chromatography / Ionspray / Mass Spectrometry Using Negative Ion Detection

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Key Words

Column liquid chromatography
Ion spray-mass spectrometry
Solid-phase extraction
Carboxylic degradation products
Alkylbenzenesulfonates in coastal water

Summary

Long-chain sulfophenyl carboxylate compounds (SPC) with more than five carbon atoms, which are degradation products of linear alkylbenzenesulfonates (LAS), have been isolated by solid-phase extraction (SPE) followed by identification with liquid chromatography / ionspray – mass spectrometry (LC/ISP-MS). The isolation procedure involved extraction in a C_{18} minicolumn and subsequently in a strong anionic exchange (SAX) column, the final determination was accomplished by Negative Ion LC/ISP/MS using a two step approach. First, a chromatographic run at a cone voltage of 80 V was performed. At this voltage the major fragment ion corresponded either to m/z 183 (C11 SPC and LAS) or to m/z 170 (C6SPC to C10SPC), and permitted the confirmation of LAS and SPC. The second step involved an extraction cone voltage of 20 V. In this case $[M-H]^-$ ion was the base peak for all the studied compounds (LAS and SPC) and was used as a quantitative ion. The linear range of the proposed method varied from 0.4 to 10 ppb with a limit of detection ranging from 2–20 ppt when 250 mL of coastal water were preconcentrated. The present method represents an important advancement for the unequivocal trace determination of the polar degradation products of LAS, SPC, since current established protocols involve either the use of derivatization/gas chromatography-mass spectrometry and/or liquid chromatography with UV detection. By using the protocol described the direct confirmation and quantitative trace determination of both LAS and their polar metabolites SPC is feasible.

Introduction

Linear alkylbenzenesulfonates (LAS) are the most commonly used anionic surfactants. From the 6 million metric tons of surfactants produced all over the world in 1994, almost 2.4 million tons correspond to LAS [1]. Commercially available LAS are mixtures of secondary isomers, with a alkyl chain lengths of 10–13 carbon atoms. After use, LAS are discharged into domestic or industrial wastewaters. Routine determination of LAS in surface waters involves the use of solid-phase extraction (SPE) followed by derivatization/gas chromatography mass spectrometry (GC-MS) [2, 3]. Such methods are tedious since they involve a derivatization step prior to GC-MS determination. A method involving continuous flow fast atom bombardment -MS has also been developed that permitts the direct determination of LAS in waste water and river samples [4].

The fate of LAS in the aquatic environment involves their biodegradation under various environmental conditions. LAS biodegradation intermediates are sulfophenyl carboxylic acids[5, 6].Quantitative determination of the polar SPC metabolites together with the parent compounds LAS can be achieved by SPE followed by LC-fluorescence detection [5]. However, in environmental analysis, the use of mass spectrometry is required for unequivocal identification of the analytes. In a previous paper from our group [6] we have used LC/ionspray-MS for the identification of LAS and some polar SPC metabolites. We showed that by using the specific diagnostic ion at m/z 183 it was feasible to identify both LAS and SPC. This specific ion at m/z 183 was previously found as a product ion of LAS by tandem MS [4].

In the present paper we have developed a quantitative method based on LC/ISP/MS for the determination of SPC and investigated the possibility of such a method as a generic approach for identifying any SPC metabolites that could be formed in the samples and for which no authentic standards are available. For this purpose it was decided to use SPE followed by LC/ISP-MS for the

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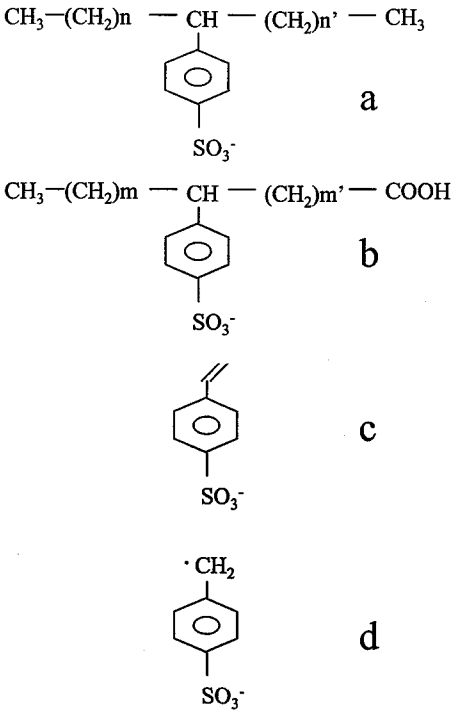

Figure 1
General chemical structures of the linear alkylbenzenesulfonates (a) and the sulfophenyl carboxylate (b) compounds and chemical structures of the specific fragment ion with m/z 183 (c) and m/z 170 (d).

Table I. Recoveries (%) and standard deviation, for LAS and their transformation products (SPC) using two stage SPE (C_{18} and SAX).

Compound	av Recovery	SD
C_{10} LAS	94	2
C_{11} LAS	96	1
C_{12} LAS	98	2
C_{13} LAS	94	1
C_6 SPC	51	2
C_8 SPC	78	1
C_{10} SPC	82	1
C_{11} SPC	92	1

quantitative determination of all the target analytes. Previous papers showed that LC/electrospray-MS could be used for the determination of nonionic polyethoxylated surfactants [7] and polyethylenglycols and related compounds [8]. Our group has used LC/ISP-MS for the determination of organophosphorous pesticides [9], phenols [10], sulfonated azo dyes [11] and acidic pesticide metabolites [12]. Since the quantification of the long-chain degradation products SPC has not been achieved up till now using ISP/MS, the purpose of this work was: (i) to develop an LC/MS method for the trace level quantification of LAS and their long-chain acidic degradation products (SPC) and (ii) to apply the method as a generic approach in several marine water samples to identify the presence of SPC metabolites with greater than five carbon atoms.

Experimental

Chemicals

HPLC water and methanol (MERCK, Darmstadt, Germany) were filtered through a 0.45 μm filter from Scharlau (Barcelona, Spain) before use. Acetic acid was purchased from MERCK (Darmstadt, Germany) and triethylamine (TEA) from Fluka Chemie (Buchs, Switzerland). The commercial LAS with a low dialkyltetralinsulfonates (DATS) content (< 0.5 %) were supplied by Petroquímica Española S. A. in a single mixture standard (i. e. not single standard solutions of each LAS). The proportional composition of the different homologues is as follows: C_{10} (3.9 %), C_{11} (37.4 %), C_{12} (35.4 %), C_{13} (23.1 %), C_{14} (0.2 %). The C_6, C_8 and C_{10} SPC standards were supplied by Jennifer A. Field (Oregon State University) and C_{11} SPC standard was prepared by sulfonation of the corresponding acid in the University of Cadiz. Chemical structures are shown in Figure 1.

Study Area and Sample Pretreatment

The study area and sample pretreatment are the same than those described in a previous paper [6, 13]. Real marine water samples from the bay of Cadiz, in the southwest of Spain, were analyzed by the method developed in this work. Water samples (250 mL) were acidified to pH 3, then filtered (1 μm) and concentrated by solid-phase extraction in a C_{18} hydrophobic-type mini-column and eluted with methanol on to a SAX strong anionic exchanger. The second elution was carried out with 3 mL of 2 M HCl in methanol. The eluate was evaporated until dry and was redissolved in 1 mL of the same mobile phase used in the liquid chromatography analysis. Recoveries for the studied compounds are shown in Table I.

Chromatographic Conditions

The eluent was delivered by a gradient system from Waters 616 pumps coupled to a Waters 600 S controller (Waters, Milford, MA, USA). Separation of the SPC and LAS was performed with a Hypersil BDS C-18 analytical column from Shandon (Cheshire, UK) of 250 mm length and 2 mm internal diameter, with a particle size of 5 μm. Eluents used were A, 100 % water and B, 80 % acetonitrile/20 % water. Additives to A and B were 5 mM triethylamine and 5 mM acetic acid. The LC eluent conditions varied from 95 % solvent A and 5 % solvent B to 100 % of solvent B in 20 min. Isocratic conditions were maintained until complete elution of all the compounds.

LC/ISP-MS Conditions

LC-MS analysis were performed on a VG Platform from Micromass (Manchester, UK) equipped with a quadru-

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Table II. Typical fragment ions of LAS and SPC and relative abundances obtained in LC-ISP-MS under cone voltages of 20, 40, 60 and 80 V using NI mode. Relative abundance fluctuation: 10–20 %.

Mn	Compounds	m/z and tentative ions	20 V	40 V	60 V	80 V
272	C6 SPC					
		271 $[M-H]^-$	100	100	100	67
		183 $[C_8O_3SH_7]^-$	0		9	15
		170 $[SO_3C_6H_4CH_2]^{\cdot-}$	0	0	0	100
300	C8 SPC					
		299 $[M-H]^-$	100	100	100	93
		183 $[C_8O_3SH_7]^-$	0	0	3	13
		170 $[SO_3C_6H_4CH_2]^{\cdot-}$	0	0	0	100
328	C10 SPC					
		327 $[M-H]^-$	100	100	100	80
		183 $[C_8O_3SH_7]^-$	0	0	0	1
		170 $[SO_3C_6H_4CH_2]^{\cdot-}$	0	0	0	100
342	C11 SPC					
		341 $[M-H]^-$	100	100	100	61
		183 $[C_8O_3SH_7]^-$	0	0	9	100
298	C10 LAS					
		297 $[M-H]^-$	100	100	100	33
		183 $[C_8O_3SH_7]^-$	0	0	12	100
312	C11 LAS					
		311 $[M-H]^-$	100	100	100	24
		183 $[C_8O_3SH_7]^-$	0	0	50	100
326	C12 LAS					
		325 $[M-H]^-$	100	100	100	26
		183 $[C_8O_3SH_7]^-$	0	0	33	100
340	C13 LAS					
		339 $[M-H]^-$	100	100	100	36
		183 $[C_8O_3SH_7]^-$	0	0	14	100

pole mass spectrometer and a Megaflow ISP-MS interface. The interface technique and its optimization are extensively discussed elsewhere [14]. A voltage of 3.7 kV was applied to the needle tip, meanwhile the extraction cone voltage was optimized between –20 and –80 V. Nitrogen drying gas and nebulizer gas flow rates were 350 and 15 Lh^{-1}, respectively. The ion source temperature was held at 150 °C. Negative ions were monitored for both, LAS and SPC.

By variying the sample cone voltage, it is possible to control the extent of fragmentation of molecular ions, therefore adding identification capability. By increasing this voltage, ions are accelerated and may thus gain internal energy upon collision with surrounding molecules; the increased internal energy leads to more abundant fragmentation, with spectra comparable to MS/MS CID spectra [15]. However, the increase of fragmentation and identification capability is obtained at the detriment of sensitivity. For this reason the analysis for LAS and SPC was done in two ways: one with a higher cone voltage for identification of SPC and LAS in real environmental samples and another with a low voltage (20 V) in order to obtain the maximum sensitivity with no fragmentation, for quantification purposes.

Results and Discussion

General Remarks about Mass Spectra

Table II shows the main ions for the studied compounds obtained in LC-ISP-MS at cone voltages of 30, 40, 60 and 80 V. LAS and SPC are anionic compounds and in negative ion mode and applying a low extraction voltage of 20 V, the deprotonated molecules were mainly observed. At a cone voltage of 60 V, the fragment ion with m/z 183 with its chemical structure depicted in Figure 1 is formed for all LAS and SPC compounds and its relative abundance grows at higher voltage. The formation of this ion is produced at the expense of the deprotonated molecular ion and therefore the sensitivity is reduced. The maximum sensitivity is achieved with a cone extraction voltage of 20 V and this was used for quantification purposes. A cone voltage of 80 V was used for the identification of the studied compounds in real water samples. At this high cone voltage

Table III. Calibration data obtained with LC/ISP-MS in time-scheduled SIM-NI mode for the studied LAS and SPC

Compound	Calibration equation	r^2	Linear Range (ppb)	LOD (ppb)
C10 LAS	$\gamma = 71200\,\chi + 2010$	0.998	0.02–0.50	0.003
C11 LAS	$\gamma = 60500\,\chi + 12500$	0.999	0.2–5	0.005
C12 LAS	$\gamma = 51000\,\chi + 10200$	0.999	0.2–4.7	0.006
C13 LAS	$\gamma = 40400\,\chi + 5300$	0.999	0.1–3	0.006
C6 SPC	$\gamma = 8100\,\chi - 430$	0.997	0.8–20	0.4
C8 SPC	$\gamma = 18500\,\chi - 5300$	0.999	4–80	1.84
C10 SPC	$\gamma = 23400\,\chi - 2900$	0.995	0.4–10	0.17
C11 SPC	$\gamma = 16200\,\chi + 500$	0.999	0.4–10	0.05

Table IV. Concentration (μgL^{-1}) of LAS and SPC found in real samples form different sampling points in the Bay of Cadiz. Coefficient of variation = 15–25 % (n = 4)

Sample	C10 LAS	C11 LAS	C12 LAS	C13 LAS	C6 SPC	C8 SPC	C10 SPC	C11 SPC	C7 SPC	C9 SPC	C12 SPC
1	118	347	306	140	0.77	n.d.	7.5	3.8	n.q.	n.q.	n.q.
2	118	310	232	79	1.5	7	15	8.5	n.q.	n.q.	n.q.
3	23.21	77	62	23	3.5	64	39	7.3	n.q.	n.q.	n.q.
4	6.41	21	20	6	n.d.	8.8	5.3	0.53	n.d.	n.q.	n.d.
5	2.30	9	6.4	2.6	n.d.	6.8	5.3	0.78	n.d.	n.q.	n.d.
6	1.48	6	5	2	n.d.	12.7	7.5	0.62	n.q.	n.q.	n.d.

n.d. not detected; n.q. detected but not quantified

two ions of each analyte were used for the unequivocal identification of each analyte (see Figure 1). The m/z ion at 170, the structure of which corresponds to $[SO_3C_6H_4CH_2]^-$, was found to be up to 10 carbon atoms for SPC. This ion has been previously observed under fast atom bombardment for sulfonated azo dyes [16]. When the carbon atom chain is above 10 C, then the loss of CH from m/z 183 is not favored due to the longer carbon chain.

Finally, it should be added that due to the specific fragmentation pattern of SPC, the acquisition of the different m/z ions pointed out above allows the identification of other SPC in real samples. This can be accomplished by monitoring the specific diagnostic ions at m/z 170 or 183 and the corresponding deprotonated molecular ion of the different alkyl chain lengths.

Calibration Graphs and LOD

External standard calibration was used for quantification of the extracts after off-line SPE. Calibration was performed by plotting peak area (γ) vs amount injected (χ, μgL^{-1}) using negative ion mode and time-scheduled SIM. Calibration graphs were constructed with standard solutions that were treated in the same way as the samples. Linearity of the system was studied at four points, 0.4, 1, 4 and 10 μgL^{-1} for LAS and 0.4, 1, 2 and 10 μgL^{-1} for SPC with the exception of C_6 SPC and C_8 SPC which exhibit a lower sensitivity. This is due to the fact that these analytes are more polar than the rest of SPC and LAS and are eluted earlier in the chromatographic traces, with higher amount of water in the eluent. It has been reported [9] that at higher water percentages in the eluent the sensitivity under ISP-MS is decreased. The linear range of each LAS was determined by the different percentage of each one in the composition of the commercial mixture standard used for this work.

The quantification and the calculation of LOD was done using a cone voltage of 20 V in order to achieve the maximum sensitivity. The calibration data are shown in Table III. Detection limits (LOD) were obtained using spiked water solutions at low concentration and then calculating the LOD for a signal-to-noise ratio of 3. Table III shows the LOD for each compound under SIM conditions. The calibration of C_{14} LAS was not done because of the small amount (0.2 %) in the standard mixture.

Environmental Analysis

The analytical protocol developed in this work was applied to the analysis of real environmental samples. Taken from stations near the discharge outlet of the untreated urban effluents from San Fernando (Cadiz, Spain), a town of about 100,000 inhabitants. Several representative samples from this area were analyzed in order to assess the potential of the method for the determination of this kind of compounds in environmental

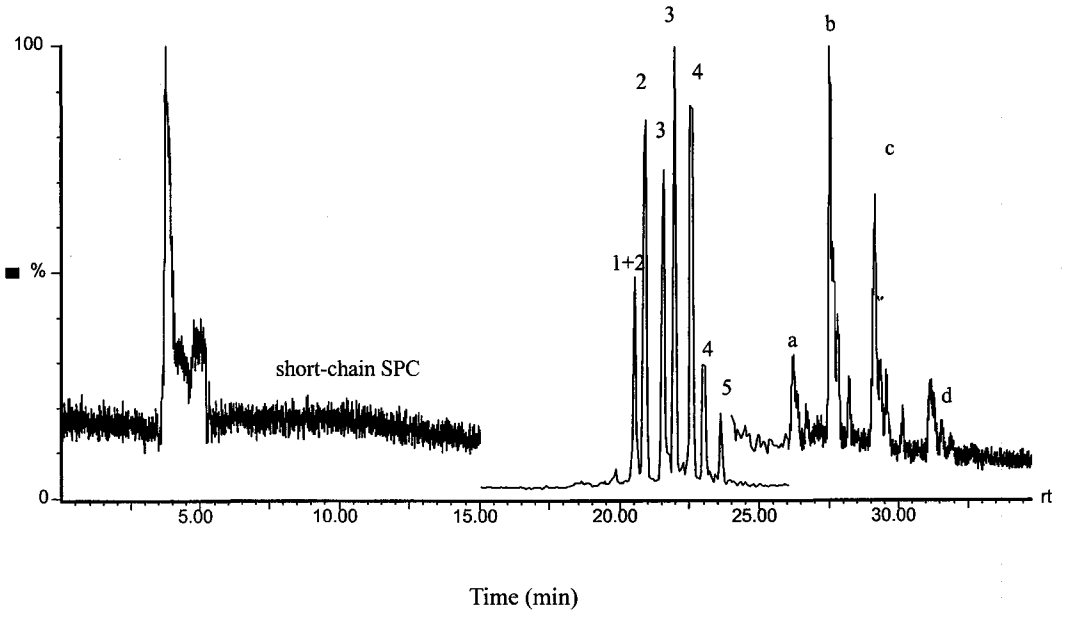

Figure 2

LC/ISP (negative ions)-MS selected ion monitoring (SIM) traces from total ion current (TIC) chromatogram corresponding to coastal water extract. Selected ions were : **1** = C_6 SPC (m/z 271), **2** = C_8 SPC (m/z 299), **3** = C_{10} SPC (m/z 327), **4** = C_{11} SPC (m/z 341), **a** = C_{10} LAS (m/z 297), **b** = C_{11} LAS (m/z 311), **c** = C_{12} LAS (m/z 325), **d** = C_{13} LAS (m/z 339).

matrices. They were collected at different distances from the coast (higher number for greater distance in Table IV).

For the analysis of environmental samples the LC/ISP-MS methodology was applied in two steps. First, a cone voltage of 20 V was applied and the acquisition of the deprotonated molecular mass ions of the studied compounds was done in order to obtain the maximum sensitivity. Figure 2 shows the LC/ISP-MS traces of a coastal water extract using time-scheduled SIM conditions under NI mode of operation. Each selected ion chromatogram contains several peaks that correspond to different positional isomers of each studied compound.

The purpose of the second step, was not only the identification and confirmation of the studied LAS and SPC previously quantified, but also to identify and confirm other SPC (which are also transformation products of the LAS studied in this work). These SPC, with no standards available, could not be quantified but their nominal mass and chemical structures are well known. This is the case of C_7, C_9 and C_{12} SPC which could not be quantified but could be identified and confirmed in several coastal water samples. This was accomplished by employing a second acquisition method with an extraction cone voltage of 80 V and acquiring the deprotonated molecular mass ions of all the SPC and LAS as well as the typical fragment ions with m/z 183 or 170 characteristic for LAS and/or SPC. In Table IV we report on the concentrations of the studied SPC and LAS found in several environmental samples, where the presence of other SPC is also indicated. The concentration of LAS and SPC that exceeded the upper limit of the linear range in the samples were obtained by diluting the extracts to reach an appropriate concentration inside the linear range. A typical chromatogram of an extracted real water sample from the bay of Cadiz is shown in Figure 3. The simultaneous separation and detection of all the LAS and SPC can be observed.

It has to be mentioned that, as can be seen in Table 4, the method reported in this paper has permitted quantification of SPC metabolites. In many real samples the concentration of these transformation products can be of the same order or even higher than the concentration of the parent LAS compounds. This leads to the conclusion that implementation of LC/ionspray-MS methods to environmental analysis brings additional information which is relevant for future environmental studies.

The protocol developed in this work can be used for the simultaneous determination and confirmation of LAS and their polar metabolites SPC, with carbon chains from 6 to 13 carbon atoms, with a high extraction cone voltage (80 V). With low cone voltages (20 V), low LODs are achieved which permit quantitative trace level determination for all the LAS and C_6, C_8, C_{10} and C_{11} SPC in environmental water samples.

The deprotonated molecular mass ions of the short chain SPC (C_2, C_3, C_4 and C_5 SPC) were also acquired from 0 to 15 min, but the presence of these compounds was not detected in any of the environmental samples. This can be attributed to the low recoveries of these highly polar

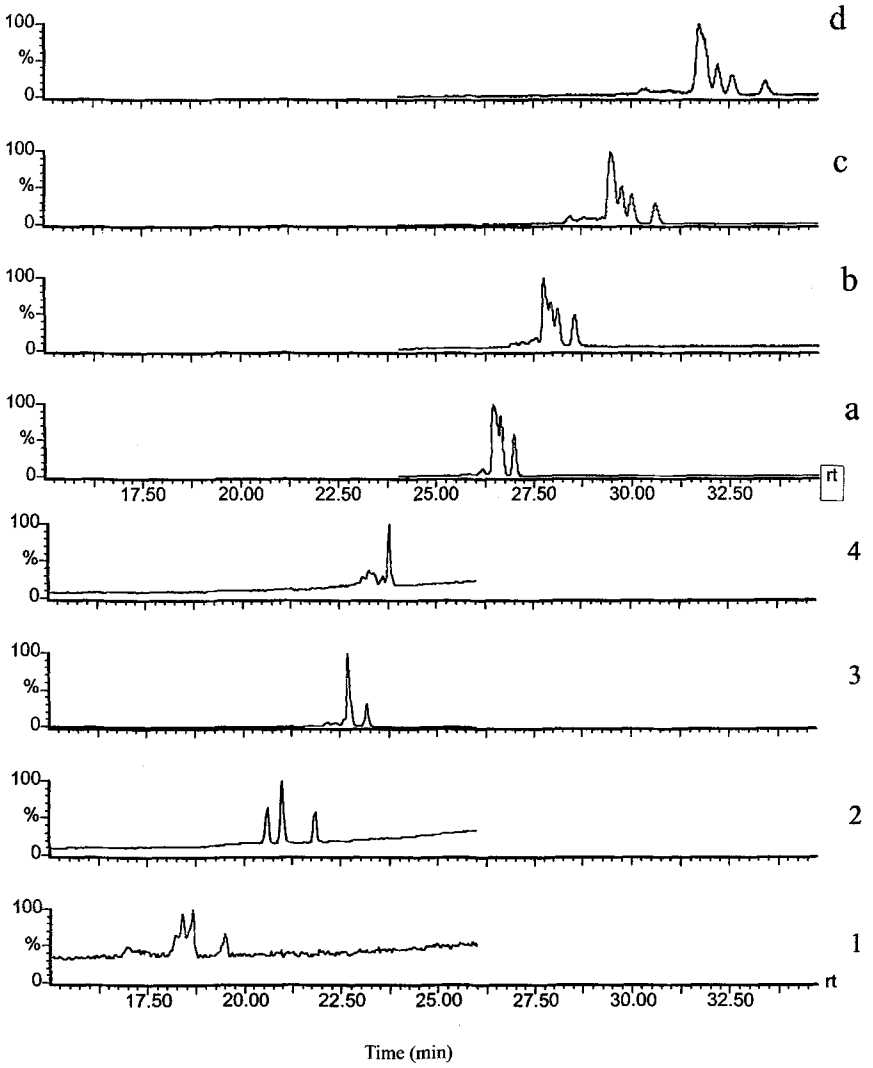

Figure 3

LC/ISP (negative ion)-MS ion chromatogram of m/z 183 of a water sample extract. Analytical column, 250 × 2 mm packed with C_{18}. Compounds identified were: **1** = C_7 SPC, **2** = C_8 SPC, **3** = C_9 SPC, **4** = C_{10} SPC, **5** = C_{11} SPC, **a** = C_{10} LAS, **b** = C_{11} LAS, **c** = C_{12} LAS, **d** = C_{13} LAS

compounds (< 20 %) using the C_{18} solid-phase extraction minicolumn.

Conclusions

By the use of a two step extraction with a C_{18} hydrophobic-type minicolumn and subsequently in a strong anionic exchanger (SAX), followed by LC/ISP-MS, the determination of metabolites (long-chain sulfophenyl carboxylate compounds) from linear alkylbenzene sulfonates in coastal waters is feasible. With an extraction cone voltage of 80 V typical diagnostic ions at m/z 183 (characteristic of all the LAS and SPC compounds) and at m/z 170 (characteristic of SPC from 6 to 10 carbon atoms) are formed. Confirmation of the presence of the studied compounds in real samples is possible with selective ion monitoring of the typical fragment ion as well as the deprotonated molecular $[M\text{-}H]^-$ ion. With low extraction voltages (20 V) and using SIM acquisition of the deprotonated ion (base ion at this cone voltage) the best sensitivity is achieved and LOD ranged from 2–20 ppt when 250 mL of marine waste water samples were obtained. The proposed method can be used as a generic approach for identifying currently unknown SPC metabolites.

Acknowledgments

Thanks to Jennifer A. Field for supplying the SPC standards without which this work could not have been done.

This work has been supported by the Environmental and Climate Program of the European Commission PRISTINE (Contract No.ENV4-CT97-494) from WASTE WATER CLUSTER and CICYT (AMB98-913-CO3)

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Received: Jan 26, 1999
Revised manuscript received: Apr 14, 1999
Accepted: Jun 14, 1999