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Determination of Linear Alkylbenzenesulfonates and Their Degradation Products in Soils by Liquid Chromatography-Electrospray-Ion Trap Multiple-Stage Mass Spectrometry

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Linear alkylbenzenesulfonates (LAS) (C_{10}−C_{13}) and their degradation products, sulfophenyl carboxylate compounds (SPCs) (C_2−C_6, C_8, C_{11}), have been extracted from soil samples with methanol, isolated, concentrated by solid-phase extraction, and determined by liquid chromatography/negative ion electrospray quadrupole ion-trap tandem mass spectrometry (MS^n). The ion fragmentation processes and pathways were studied in detail by MS, MS^2, and MS^3. Upon collision-induced dissociation, the deprotonated molecules of LASs render the ethylene-substituted benzenesulfonate ion (m/z 183), the fragmentation of which gave the intense signal at m/z 119, corresponding to the ethylene-substituted phenoxide ion formed by the loss of sulfur dioxide. The fragmentation pattern of SPCs shows that, for the analytes of large carbon atom chains (>5C), the neutral loss of water is favored whereas for those of short carbon atoms chain, the loss of carbon dioxide is more frequent. Multiple reaction monitoring using isolation only for MS and using isolation and fragmentation for MS^2 and MS^3 were used to identify and quantify each compound. The three MS modes have been validated in terms of sensitivity, selectivity, and precision, showing that each MS stage used reduces sensitivity 10 times. Recoveries from soil were higher than 65% at LOQ level for all the analytes tested, except for C_2−C_4 SPCs by any MS mode, with relative standard deviation lower than 19%. The utility of the method is demonstrated by successfully quantifying real samples treated with these products. Quantification limits for the methodology developed in this work ranged from 0.5 to 50 μg kg^{-1} by MS, from 2 to 400 μg kg^{-1} by MS^2, and from 20 to 4000 μg kg^{-1} by MS^3. Concentration levels of LASs and SPCs—ranging from 0.1 to 15 mg kg^{-1}—were found in soil samples amended with sludges, thus indicating their input and persistence in the soil compartment.

Linear alkylbenzenesulfonates (LASs) are the most commonly used anionic surfactants, which can pollute soil through the application of organic amendments from several sources (mainly from sewage treatment plants).[1,2] The majority of LASs are not degradable under anaerobic conditions because of the restricted metabolic pathways. However, under aerobic conditions, LASs are biodegraded to sulfophenyl carboxylic acids (SPCs).[3] Surfactants are known to enhance the apparent aqueous solubility of hydrophobic organic contaminants and to be toxic for bacteria, plants, and animals.[3−5]

As an example of the problem magnitude, the European Union (EU) produces annually more than 6 millions tons of sludges (dry matter), the principal elimination route of which is their use as soil fertilizers. The feature of this practice is the recycling of the organic matter and the fertilizing elements contained in the sludge that contributes to improve the productivity of agricultural soils. However, its use could be limited by the surfactant toxicity, since variable amounts of these substances persist in the soil surface much time after their application.[3] Observations of the ecological impact of sewage sludge treatments or applications of LAS spiked into sludge are very contradictory.[6−8] Some researchers[6,7] indicate a lower toxicity of LAS when applied in sludge, whereas others postulate that they have important adverse environmental and toxic effects.[3,8]

Methods to extract and analyze surfactants in water, sediment, soil, and biological materials are required for studies of contamination, behavior, and fate of these compounds in the environment. The determination of LAS has so far been performed using the color test by formation of an ionic pair with methylene blue, gas chromatography, liquid chromatography (LC), and capillary

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electrophoresis.[3,9−13] Nowadays, LC coupled to a mass spectrometer (MS) using an electrospray (ESI) interface, which is especially suited to analyze ionic compounds, provides clear advantages in terms of achieving the detection of these substances without tedious derivatization steps and higher sensitivity/selectivity than any of the other methods.[3,13,14] The LC/ESI-MS methodology has been successfully applied for the detection of LASs and SPCs in coastal water,[15,16] communal wastewater,[17] and sewage treatment plants.[18−21] Some features of this technique to determine ionic surfactants and their metabolites are that it provides chromatographic separation of the individual analytes, detects compounds lacking UV absorption, and identifies their molecular masses. These incentives make the approach one of the most useful tools for studying contamination by surfactants.

However, there are still several analytical deficiencies to be solved when single-stage mass spectrometry is used, derived from the characteristics of the mass spectrum and caused by known and unknown compounds that provide isobaric interferences—as the chlorinated nonylphenol ethoxylate that coelutes with C_{11}LAS and has the same molecular weight (MW = 312) and base ion m/z 311—or multiple-component spectrum that is definitively useless.[22,23]

Tandem mass spectrometry uses two stages of mass analysis, one to preselect an ion and the second to analyze the induced fragments, for instance, by collision with an inert gas such as argon or helium. This analysis can be tandem in space or tandem in time.[24,25] Tandem in space means that two mass spectrometers are in series and that the various steps of the process take place simultaneously but are separated in space. The triple quadruple has already been efficiently used for structural identification of LASs[26] and for determining them in fish.[27]

Dual analysis can also be tandem in time, as achieved on quadrupole ion trap (QIT), in which the sequence of events take place in the same space but is separated in time. Although the limited dynamic range of QIT could make quantification of analytes less reliable in difficult matrixes, its unsurpassed sensitivity, down to the subpicogram range, and its multiple fragmentation stages have allowed its application to the analysis of different organic contaminants in dirty matrixes such as biological materials and soils.[28,29] To date, no report describing the application of QIT for analyzing LASs and SPCs in environmental matrixes has been published.

This work describes an LC/ESI-MS^n method with an ion trap mass spectrometer that allows fast, selective, and sensitive quantification of LASs (C_{10}−C_{13}) and SPCs (C_2−C_6, C_8, C_{11}) in soil extracts by multiple-stage MS experiments (ES-MS^n). This is the first known report that applies the MS^3 product ion for quantifying LASs and SPCs in soil samples.

EXPERIMENTAL SECTION

Reagents. LAS certified standard mixture containing C_{10} (3.9%), C_{11} (37.4%), C_{12} (35.4%), C_{13} (21.1%), and C_{14} (0.2%) homologues with low content (<0.5%) in dialkyltretralinsulfonate was kindly provided by Petroquimica Española (Madrid, Spain). The SPCs were synthesized by sulfonation of the corresponding phenylalkanoic acids according to the procedure reported by Taylor and Nickless.[30] The overall yield of the procedure was estimated around 30−50% depending on the length of the alkyl chain. After synthesis, in all cases, the purity was more than 96%. Phenylacetic, 2-phenylpropionic, 4-phenylbutiric, 5-phenylvaleric, 6-phenylhexanoic, and 8-phenyloctanoic acids were obtained from Promochem (Barcelona, Spain) and phenylundecanoic acid (mixture of isomers) was from Acros (Geent, Belgium). Sulfuric acid, diethyl ether, sodium hydroxide, hydrochloric acid, and 2-propanol were from Panreac (Barcelona, Spain). Standard solutions were prepared by dissolving 20 mg of each SPC in 20 mL of methanol. Composite working standard solutions were prepared by suitable mixing of the standard solutions mentioned above. When not used, all the standard solutions were stored at 4 °C. Phenylalkyl acids are hardly disposable, because of this only C_2−C_6, C_8, and C_{11}−SPC could be synthesized, characterized, and determined.

MFE C_{18} solid phase (particle diameter in the range of 45−55 μm and pore diameter of 60 Å) was acquired from Análisis Vínicos (Tomelloso, Spain). A C_{18} column was prepared by transferring 0.5 g of solid phase to a 100 mm × 9 mm i.d. glass column, fitted with a coarse frit (No. 3) and covered with a plug of silanized glass wool.

For LC, distilled water was further deionized by passing it through the Milli-Q Plus apparatus (Millipore, Bedford, MA). Methanol of HPLC grade was obtained from Merck (Darmstadt, Germany). Acetic acid and ammonium acetate (analytical grade) were bought from PanReac (Barcelona, Spain), and tributylamine was from Fluka Chemie (Buchs, Switzerlands). All the solvents were passed through a 0.45-μm cellulose filter from Scharlau (Barcelona, Spain) before use.

Liquid Chromatography/Mass Spectrometry. Mass spectra were obtained using a Bruker Daltonic (Bruker Daltonik GmbH)

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Esquire 3000-LC ion trap mass spectrometer equipped with a Bruker data analysis system and LC/ESI-MS interface. The mass spectrometer was interfaced to an Agilent 1100 Series LC system (Agilent, Palo Alto, CA) that includes a quaternary pump, an autosampler, and a variable-wavelength detector.

Separation was performed on a Agilent Zorbax SB-Aq (50 × 4.6 mm, 5-μm particle size) with a guard column containing the same material using a binary mobile-phase gradient with 10 mM tributylamine, 10 mM ammonium acetate, and 10 mM acetic acid in methanol/water (20:80) (solution A) and 10 mM tributylamine, 10 mM ammonium acetate, and 10 mM acetic acid in methanol (solution B) at a flow rate of 0.4 mL min^{-1}. The gradient was linearly increased from 0% solution B to 90% solution B in 15 min, maintained isocratic 15 min, and then returned to initial conditions in 10 min. The sample injection volume was set at 5 μL.

The Esquire3000 was equipped with an ESI source and operated in negative polarity. The mass spectrometer was tuned for LASs and SPCs, optimizing the ionization source parameters, voltages on the lenses, and trap conditions in the ExpertTune mode of the Daltonic Esquire Control software while infusing a standard solution (10 μg mL^{-1}) by a syringe pump at a flow rate of 250 μL h^{-1}, which was mixed with the mobile phase at 0.4 mL min^{-1} by means of a T piece. Operating conditions of the source were end plate, 450 V; capillary voltage, 4500 V; nebulizer pressure, 50 psi; and drying gas flow, 9 L min^{-1} at a temperature of 350 °C. The lens and block voltages selected were skimmer, −50 V; capillary exit, −210 V; octopoles 1 and 2, −5.0 and −1.7 V, respectively; trap driver, 72.76%; octopole reference, 119.67%; and lenses 1 and 2, 10 and 70 V, respectively.

The mass spectrometer was run in full scan and multiple reaction monitoring (MRM) modes. The trap parameters were set in ion charge control using rolling averaging set at 2 with a target of 70 000 and maximum accumulation time of 100 ms at m/z range from 50 to 400 u. Negative ions were detected at unit resolution (scan speed 10 300 u/s). Fifteen scans were summarized for one spectrum, resulting in a spectral rate of 0.15 Hz. Collision-induced dissociation (CID) was performed on the ion of interest by collisions with the helium background gas present in the trap for 40 ms. In these experiments, the deprotonated molecule was subjected to CID to produce a first set of fragment ions, MS/MS or MS^2. Subsequently, one of the fragment ions from $[M - H]^-$ was isolated and fragmented to give the next set of fragment ions, MS^3. The fragmentation steps for each compound were optimized visualizing the changes in the intensities of fragments ions, whereas the fragmentation cutoff and the fragmentation amplitude were manually varied. For the MS^2, the width was set at 1.0 m/z, the cutoff at 150 m/z, and the amplitude to 2V, whereas for the MS^3, the width was set at 1.0 m/z, the cutoff at 100 m/z, and the amplitude to 2V.

Sampling. Four samples were collected in September−October 2002 from a forest area (800 Ha) located at the northern part of the Valencia province at the east of Spain. This forest area suffered a medium intensity fire—average surface temperatures ∼250 °C—in June 2001. Three months after the fire, the soils were amended with sludges from a water treatment plant to allow an easier and faster recovery of the vegetation and good evolution of some new planted trees by giving the soil an additional nutrients load. The distribution of sludges in the area affected by fire was made in two different ways depending on slope conditions, (i) distributing the sludge as uniformly as possible on the soil surface (samples 1 and 2) and (ii) applying the sludge together with surface tilling (15−20-cm depth) (samples 3 and 4). Soil samples were taken for the first 10−15-cm depth. Recovery analyses were performed on a supplementary soil sample taken from an unburned zone of the same area, with the same characteristics and type of soil, but without amendment.

The area was characterized by shallow soils of Calcic Xerochrept type according to USDA Soil Taxonomy classification[31] or Rendzic Leptosol according FAO classification,[32] developed on Jurassic limestone. These soils showed a variable depth, always less than 40 cm, good drainage, a sandy-loam texture (8.01% clay) and an alkaline pH (7.4). The cation-exchange capacity (20.08 $cmol_c$ kg^{-1}) of these soils was strongly affected by its high calcium carbonate and organic matter content that were 45.10 and 4.26%, respectively.

Before sample collection, the superficial ash layer and vegetation remainders were removed from each sampling point. After collection, soil samples were stored in polyethylene bags hermetically sealed until analysis. In the laboratory, these samples were air-dried and passed though a 2-mm sieve. A fraction of each sample was homogenized in an agate mortar before analysis.

Extraction Procedure. For recovery studies, soil samples (5 g) were spiked with volumes between 50 and 100 μL of the composite standard solution, at the appropriate concentration, letting them stand at room temperature for 3 h to achieve solvent evaporation. Soil samples were then agitated firmly for ∼1 min to allow analyte distribution in the soil and placed in a Soxhlet extraction cartridge previously washed with methanol. The soil was Soxhlet extracted over 16 h with 150 mL of methanol. A rotary evaporator set, at 40 °C and 337 mbar, was used to evaporate the extract to dryness, and then the dry residue was redissolved in 200 mL of warm water (50−55 °C) in an ultrasonic bath.

After acidification of the aqueous extract with HCl (pH 3) and preconditioning of the C_{18} with 10 mL of methanol and 10 mL of deionized water, the sample was passed under vacuum through the glass column containing the C_{18} at flow rates of ∼20 mL min^{-1}. The column was rinsed with 5 mL of deionized water. The filtrate was discarded, and the analytes retained in the solid phase were eluted by passing twice 5 mL of methanol. The eluent was collected in a graduated conical tube (10 mL) and concentrated in a thermoblock at 40 °C under a nitrogen stream to 1 mL.

RESULTS AND DISCUSSION

Mass Spectrometry. Optimization of the Experimental Conditions. Figures 1 and 2 illustrate the MS, MS^2, and MS^3 for LASs and SPCs, respectively. They are anionic compounds that lose a proton in solution and become negatively charged and determinable by ES in negative ion mode. The deprotonated molecule was the only ion observed for all the analytes, except for sulfophenylacetic acid (Figures 1A and 2A), the mass spectrum of which gave also the fragment m/z 171, corresponding to the characteristic fragment $[SO_3C_6H_4CH_3]^-$. The voltages of the lenses were adjusted to obtain the maximum response for the deproto-

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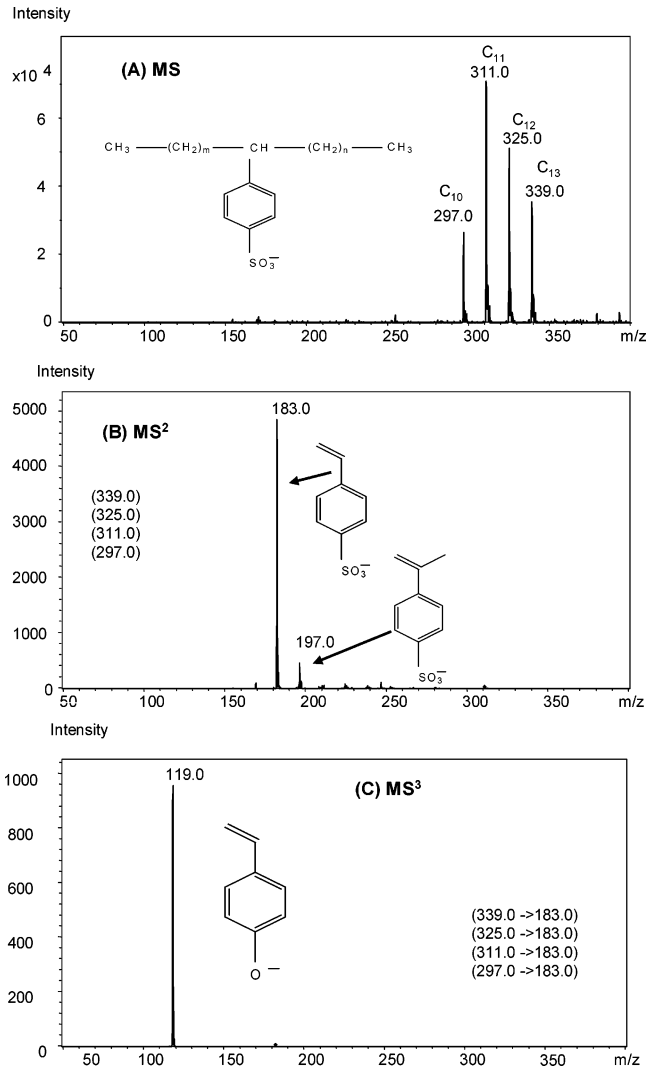

Figure 1. Multiple-stage ESI mass spectra of the linear alkylbenzenesulfonates and the proposed fragmentation scheme (A) MS, (B) MS^2, and (C) MS^3.

nated molecule $[M - H]^-$ of each studied compound, which were chosen as precursor ions in further MS^n experiments.

In addition, a reliable way of obtaining structural information is to perform tandem MS experiments on specific ions of interest. CID of $[M - H]^-$ ions (MS^2) and the CID of the main fragment ion (MS^3) often yielded fragment ions formed by fragmentation of the lateral chains in the molecular structure.

The product ion of highest abundance from any of the deprotonated molecules of LAS isomers was the ethylene-substituted benzenesulfonate ion (m/z 183) (Figure 1B). The other ion present (m/z 197) corresponded to a propylene-substituted benzenesulfonate structure. Further fragmentation of the ion was verified by MS^3, showing an intense signal at m/z 119 that corresponded to the ethylene-substituted phenoxide ion formed by the loss of sulfur dioxide (Figure 1C). The ion at m/z 183 has been previously observed using other mass analyzers such as the single[18] or triple[26,27] quadrupoles and fast atom bombardment[33] and the ion at m/z 119 by fast atom bombardment.[32]

Figure 2B shows the product ion mass spectrum of the molecular anions of the different SPCs studied. C_{11}, C_8, C_6, C_5, and C_3 SPCs presented the corresponding sulfophenyl alkylaldehydes formed by the loss of a water molecule. This was the only product ion for C_8 SPCs and the most abundant one for C_5 and C_6 SPCs, which also showed as product ion a less intense fragment at m/z 171 that has been already identified[18] as $[SO_3C_6H_4CH_2]^-$. The most intense fragment for the MS^2 analysis of C_{11} SPC was the ion at m/z 183 also obtained for LASs. For the C_3 SPC, the ion at m/z 185 was obtained, which corresponds to the ethyl-substituted benzenesulfonate formed by loss of carbon dioxide. C_4 and C_2 SPCs form the products ions at m/z 199 and 171, respectively, which are the propyl- and methyl-substituted benzenesulfonates. The fragmentation pattern of SPCs showed that, for the analytes of a large carbon atom chain (>5C), the neutral loss of water is favored whereas for those of a short carbon atom chain, the loss of carbon dioxide is more frequent.

The MS^3 of the most abundant product ion from the MS^2 spectrum showed intense peaks at m/z 183 and 170 for C_4, C_5, and C_8 SPCs (Figure 2C). An additional fragment at m/z 135 is formed for C_4 by loss of sulfur dioxide. C_6 SPC presented only the ion at m/z 170. C_3 and C_{11} SPCs gave the ethylene-substituted phenoxide ion at m/z 119 and C_2 methyl-substituted phenoxide ions at m/z 107.

The selected ion transitions used for the quantification by LC/MS^2 and LC/MS^3 are indicated in Table 1. The cutoff and the amplitude were set to a value that resulted in the maximum intensity of the product ions. The selection of transitions of high intensity as well as the isolation and fragmentation of a few specific ions using time windows enhanced sensitivity; four segments were selected in the method as indicated in Table 1.

Use of MS^n conditions is especially valuable in the analysis of soil where other compounds with the same molecular ion present in the mixture can coelute with the compound of interest. To isolate and fragment this molecular ion under MS/MS or MS^3 conditions provide specific information that confirms analyte identity. In addition, complete chromatographic separation by using a C_{18} reversed phase and a volatile buffer as ion pair reagent was achieved.

The sensitivity of MS, MS^2, and MS^3 procedure was compared by calculating the detection limits (LODs) as three times the standard deviation of the noise added to the intercept. For the MS mode, LOD was 100 pg for C_2−C_4 SPCs, 40 pg for the other SPCs tested, and 4 pg for the LASs except C_{13} LAS, the LOD of which was 40 pg. With an injection volume of 5 μL, this corresponded to a LOD between 2 and 50 $\mu g\ L^{-1}$. The MS mode was 10 times more sensitive than the MS^2 mode, which was also 10 times more sensitive than the MS^3 mode. Using standards prepared in methanol, the LODs obtained were the same using full scan or isolating the ions of interest because this full scan was used in the MS operation by accumulating the ions on the trap and ejecting them from the trap to the detector to produce the spectrum. The production of MS^n spectra was performed by inclusion of two steps in the analysis cycle: isolation and fragmentation. The quantification was performed by extracting the single m/z values of interest in the data analysis.

Method Validation. Sensitivity is one of the most important parameters in the determination of environmental contaminants such as LAS and their metabolites. The comparisons of quantification limits (LOQs) obtained by LC/MS using ion isolation, LC/

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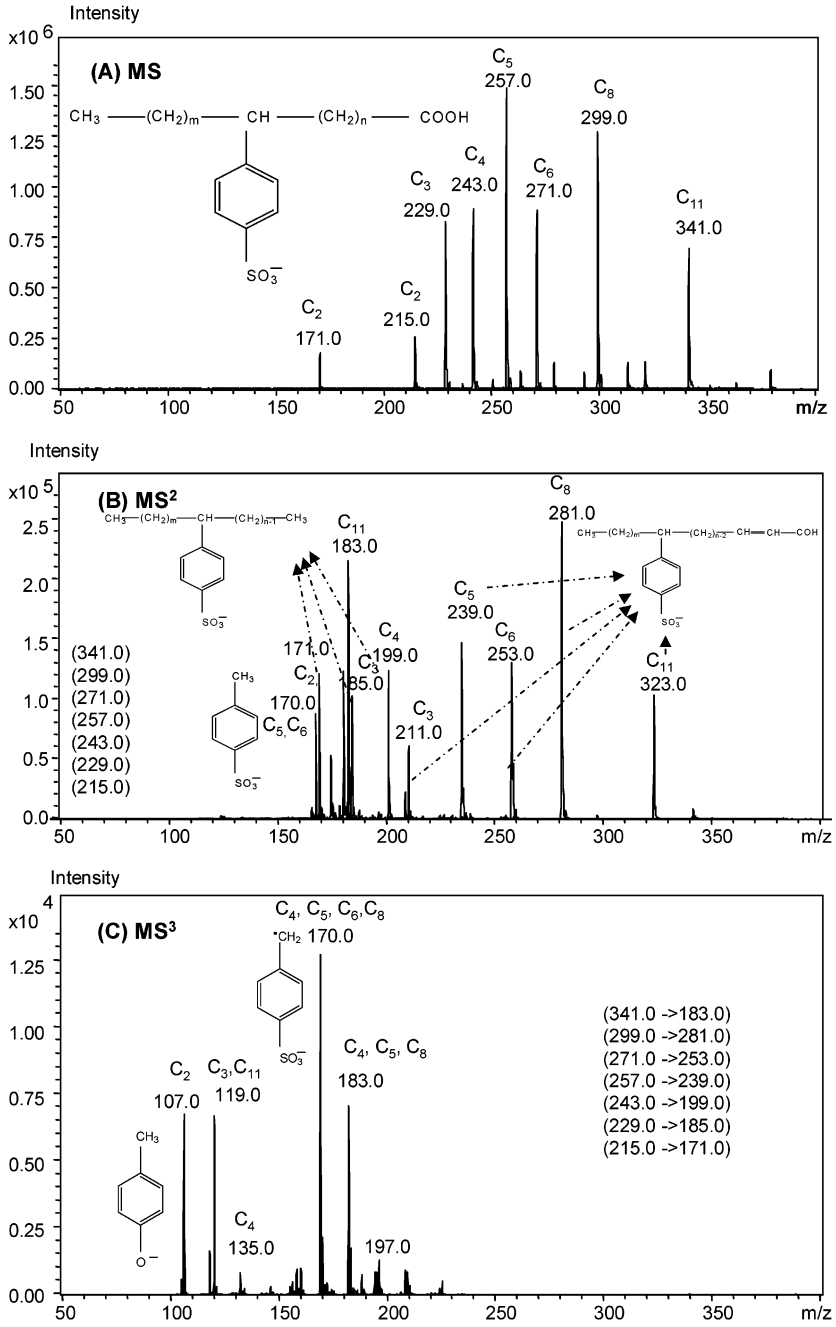

Figure 2. Multiple-stage ESI mass spectra of the sulfophenyl carboxylate compounds and the proposed fragmentation scheme (A) MS, (B) MS^2, and (C) MS^3.

MS^2, and LC/MS^3 in MRM mode are shown in Table 2. LOQs were calculated as the signal-to-noise (S/N) ratio equal to 10 in the selected conditions. The reliability of these LOQs was checked by calculating recoveries and repeatabities. The LOQs ranged from 0.5 to 80 μg kg^{-1} by MS, from 2 to 400 μg kg^{-1} by MS^2, and from 20 to 4000 μg kg^{-1} by MS^3. Differences noted in sensitivity between the three MS modes were very similar to those reported by calculating the LODs of standard solutions. This method of isolation before producing the MS spectrum is analogous to selected ion monitoring used with a quadrupole mass spectrometer. The software has 10 isolation steps available and because of that the same segment time for MS^2 and MS^3 was used. In that way, it is possible to increase the sensitivity of the measurement of the m/z values of interest by emptying the trap of background at other m/z values. This makes it possible to have the maximum number of ions per spectrum at the masses of interest and to realize benefits, such as greater sensitivity and dynamic range, because more signal is available at the masses of interest, providing a greater insensitivity to sample contamination. The subsequent isolation and fragmentation steps provided slight background noise reduction compared with that obtained in the first isolation step; in contrast, each isolation/fragmentation step reduces sensitivity, which affects the three modes applied in terms of their applicability for different purposes. The MS^2 and MS^3 chromatograms were recorded in product scan mode for studying the different product ions.

The application of sewage sludge to soil can result in surfactant levels, generally, in a range 0−3 mg kg^{-1}.[7] Although the MS mode

Table 1. Time Schedule and Transitions Used for Quantification by MS^n Operation

time (min)	ion	retentn time (min)	compds	MS^2 transition precursor → product (m/z)	MS^3 transition precursor → product → product (m/z)
0−13	1	9.32	C_2SPC	215 → 171	215 → 171 → 107
	2	10.88	C_3SPC	229 → 185	229 → 185 → 119
	3	12.01	C_4SPC	243 → 199	243 → 199 → 170
	4	12.65	C_5SPC	257 → 239	257 → 239 → 170
	5	13.72	C_6SPC	271 → 253	271 → 253 → 170
14−15	1	14.96	C_8SPC	299 → 281	299 → 281 → 170
15−18	1	16.59	C_{11}SPC	341 → 183	341 → 183 → 119
18−30	1	20.01	C_{10}LAS	297 → 183	297 → 183 → 119
	2	21.69	C_{11}LAS	311 → 183	311 → 183 → 119
	3	23.42	C_{12}LAS	325 → 183	325 → 183 → 119
	4	24.09	C_{13}LAS	339 → 183	339 → 183 → 119

Table 2. Method Quantification Limits and Recoveries by LC/MS, LC/MS^2, and LC/MS^3 for the Analysis of the Selected LASs and SPCs in Soil Samples

	LC/MS		LC/MS^2		LC/MS^3	
compd	LOQ (mg/kg)	recovery, % (RSD, $n = 5$)	LOQ (mg/kg)	recovery, % (RSD, $n = 5$)	LOQ (mg/kg)	recovery, % (RSD, $n = 5$)
C_2SPC	0.08	13 (19)	0.4	18 (15)	4	20 (14)
C_3SPC	0.05	22 (14)	0.3	27 (12)	2	29 (12)
C_4SPC	0.05	16 (17)	0.3	22 (16)	2	21 (13)
C_5SPC	0.008	65 (15)	0.08	68 (12)	1	69 (10)
C_6SPC	0.008	72 (8)	0.08	72 (9)	1	74 (7)
C_{11}LAS	0.0005	81 (10)	0.002	85 (9)	0.02	77 (6)
C_{11}LAS	0.0005	81 (10)	0.002	85 (9)	0.02	85 (10)
C_{12}LAS	0.0005	83 (10)	0.002	90 (11)	0.02	93 (8)
C_{13}LAS	0.005	86 (12)	0.02	91 (7)	0.2	92 (9)

Table 3. Matrix Effects on the Accuracy of LAS and SPC Determination at LOQ Levels in Soil Samples by LC-Ion Trap-MS/MS

	concn[a]	analytical data				
compd	(mg mL^{-1})	X[b]	SD[c]	RSD[d]	$\mu \pm$ SD[e]	t[f]
C_2SPC	2	215 203	26 251	12	544 128 ± 51 026	17.8
C_3SPC	1.5	194 377	17 188	9	331 541 ± 13 650	6.3
C_4SPC	1.5	220 058	16 353	7	288 988 ± 14 133	6.7
C_5SPC	0.4	300 435	24 382	8	314 561 ± 21 824	3.0
C_6SPC	0.4	281 965	12 669	4	277 790 ± 19 915	−0.2
C_8SPC	0.1	247 742	7 700	3	263 399 ± 2 060	2.5
C_{11}SPC	0.1	220 148	14 994	7	219 295 ± 6 777	−0.2
C_{10}LAS	0.01	268 498	4 134	2	253 042 ± 3 605	−0.6
C_{11}LAS	0.01	571 405	26 221	5	569 551 ± 32 911	−0.1
C_{12}LAS	0.01	405 016	20 762	5	375 823 ± 9 296	−0.4
C_{13}LAS	0.1	355 204	24 572	7	343 286 ± 4 555	−0.4

[a] Concentration in the injected extract. [b] X, mean values of peak areas obtained by analyzing final extracts of three samples of different types spiked with known amounts of LASs and SPCs. [c] SD, standard deviation. [d] RSD, relative standard deviation. [e] $\mu \pm$ SD, mean values and standard deviations of peak areas calculated by injecting three times known amounts of LASs and SPCs. [f] t, Student's t-test.

presents excellent sensitivity for environmental samples, it lacks adequate selectivity because it works like a single quadrupole with all the drawbacks in terms of isobaric interferences or multiple-component spectra. The LOQs obtained in this study were between 10- and 100-fold more sensitive than those reported previously by other methods developed for sludges and sediments using a single quadrupole.[13,20,21] For all these reasons, the method should not be discarded as a method of choice to determine very low concentrations of LASs and their metabolites. The MS^2 mode shows an adequate combination of sensitivity and selectivity to be used in routine determination of LASs and SPCs in soil samples, improving the characteristics and performance of the already reported procedures[13,20,21] because it offers a sensitivity of the same order of magnitude and includes detection of specific parent/product ion transitions that increase selectivity. The MS^3 mode enhanced selectivity, which is very important, but lacks enough sensitivity for quantitative environmental purposes. However, it can be an important tool to unequivocal confirmation of the identity of the compounds in highly contaminated samples.

Qualitative identification criteria in the target compounds were based on the LC retention time of the analyte compared to that of a standard (±2%), the specific transition selected, and the ratio of different product ions (when it is possible) within 10% of the ratios obtained for the standard.

Quantification was performed by monitoring the reported parent−product ion transitions by an external standard method using solutions of pure standards, as is the commonly applied method for LC quantification of the compounds. Quantitative data analysis was performed after data acquisition ("postacquisition") for both single-component and multicomponent analyses. It consists of building calibration curves for each component, with subsequent quantification of the unknown and blank samples against the regression curve. Linearity was studied over a range of spiking levels from the LOQs obtained for MS^3 to 10 mg kg^{-1} by MS, MS^2, and MS^3. For all the compounds and all the MS modes, the calibration curves were linear in this range with regression coefficients of >0.998.

However, in quantitative analysis, one of the major problems is the suppression/enhancement of the analyte signal in the presence of matrix components, which has been reported by many authors.[15−21] Response suppression caused by sample matrix components using the ES interface has been widely discussed in the literature.[15,19−21] The presence of soil constituents in charged liquid droplets could interfere with the ionization process by complex mechanisms. Matrix interferences can be established by comparing the signal intensity, obtained in a standard solution (methanol), with those obtained in matrix-matched standards. Table 3 shows the differences in response of each analyte in pure solvent standard and in matrix-matched standard at LOQ concentrations for the MS^2 mode. After measuring for each analyte, the resulting ion signal was averaged and compared with those obtained for the standard solution by applying the t-test ($n = 3$, $p = 0.05$). The calculated t-values were lower than the critical value (4.30) for all the compounds except C_2−C_4 SPCs, indicating that only for the most polar compounds, which elute at the beginning of the chromatogram, there was a significative suppression of the response. The matrix effect can be reduced, if not completely removed, by a careful sample cleanup and LC separation. In this way, the developed method included an appropriate LC separation and a purification step via C_{18} columns that provides a clean extract to inject. The matrix effect was similar in other MS modes and at other concentrations. These results can be expected since matrix interferences are related to the ionization source employed and not to the mass analyzer or MS mode.

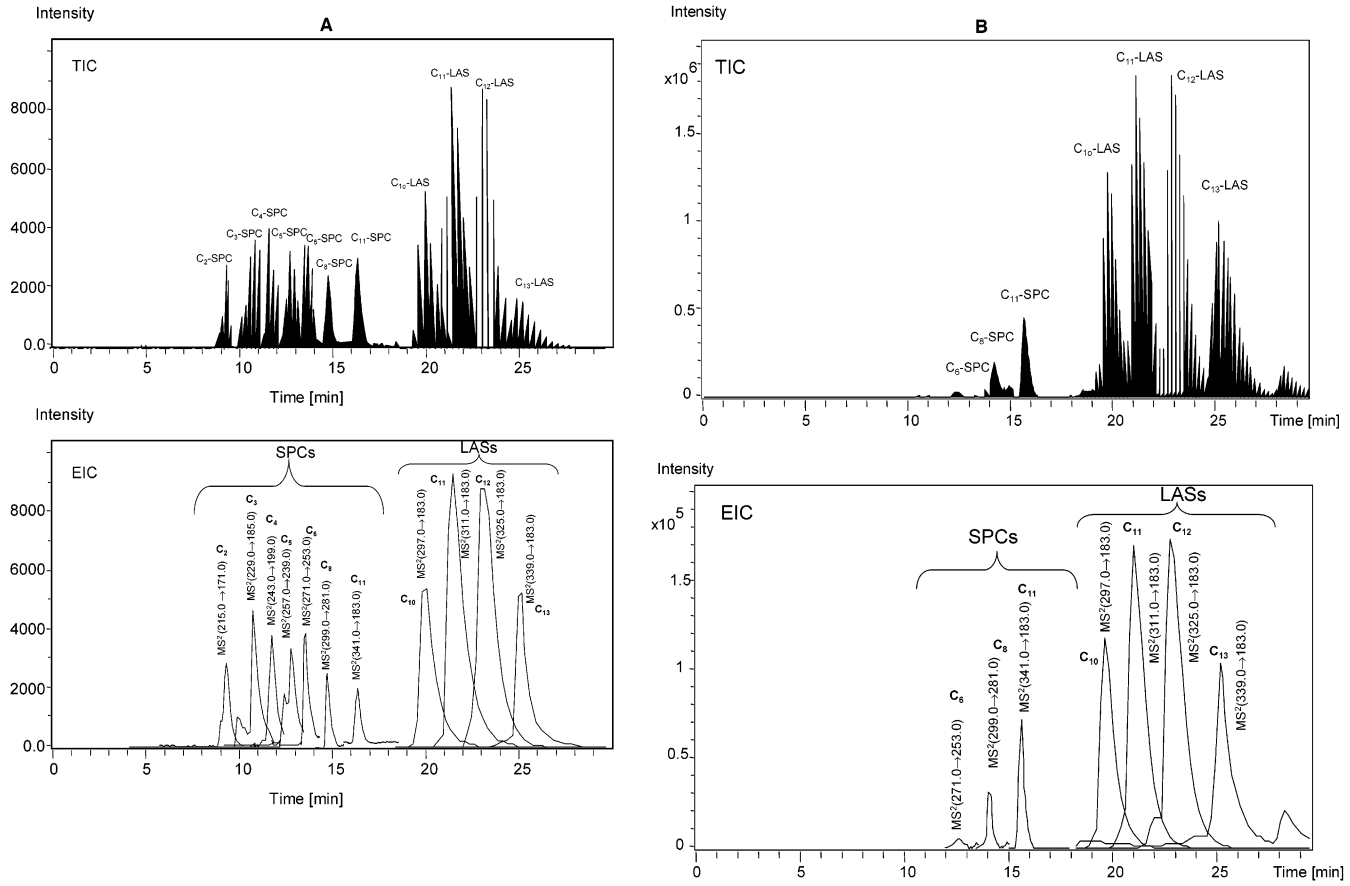

Figure 3. MRM TIC and EIC LC/ES-MS^2 chromatograms of (A) spiked soil with SPCs at LOQ and LASs at 4 μg/kg C_{10}, 35 μg/kg C_{11}, 33 μg/kg C_{12}, and 20 μg/kg C_{12}, (B) soil sample 3.

Examples of typical chromatograms are shown in Figure 3 for MRM MS^2 mode. The chromatographic resolution and peak performance were satisfactory for the LASs and SPCs in spiked samples (Figure 3A). Good chromatograms were obtained: the baseline was stable and good peak shapes were observed even for the most polar analytes. Figure 3B shows a chromatogram of a real soil sample where the $C_{10}-C_{13}$ LAS and C_6, C_8, and C_{11} SPCs were found. Resolution, peak shape, and baseline noise were comparable to those obtained for the spiked sample, proving that this method works for environmental samples.

Table 2 summarizes recoveries and repeatabilities of the three MS procedures described. They were almost equal between the MS modes. Extraction with methanol of the target analytes followed by SPE cleanup and combined with LC-ion trap-MS detection yielded recoveries from 13 to 92%. The lower recoveries were obtained for the most polar SPCs (C_2-C_4 SPCs), which are difficult to retain in the solid phase, as has already been reported by other authors.[18] The recoveries for the other analytes tested are in an acceptable range from 65 to 93%. The relative standard deviation ($n = 5$) for MS determination was below 19%, for MS^2 below 16%, and for MS^3 below 14%, showing that MS^3 is the most precise procedure followed by MS^2.

All the experiments performed with the ion trap took about three months. Within this period, the system was continuously switched with 6−8 analyses/day. Hence, a total of over 100 samples were analyzed. The weakness attributed to this mass analyzer on the limited dynamic range, when particularly dirty matrixes are analyzed, has not been observed using the proposed procedure.

Analysis of Soil Samples. Table 4 summarizes the results of the soil samples analyzed and reveals the presence of LASs and SPCs in soils treated with sludges, at concentrations usually in the milligram per kilogram range. The untreated sample has not been included in the table because none of the studied compounds was detected on it. It is interesting to note the good agreement between the results obtained by MS, MS^2, and MS^3. Differences in repeatability between the three MS methods can be clearly observed in this table.

Disparities in LAS contents have been observed (Table 4), which could be caused by the different ways of sludge application. The higher LAS levels were found in those samples (samples 3 and 4) for which the sludge had been mixed by tilling with the soil. In addition, some SPCs metabolites (from C_6 to C_{11}) were also detected in these samples. A study on LAS and SPCs concentrations in sewage sludges[21] demonstrated that large amounts of LASs and no SPC were present in sludge samples, which could be explained by the higher polarity of SPC that diminishes their affinity for particulate matter. However, in the present study, samples subjected to tillage contain some of the studied SPCs. A probable reason could be that the aerobic degradation of LAS by soil microorganisms is favored by the oxygenation of soil produced by tillage. There were no SPC metabolites, and the content of LAS was lower in the samples corresponding to the zones in which the sludges were simply

Table 4. Concentration of the Studied Compounds in Soil Samples

sample	compd	concn (mg kg^{-1}) (RSD %, $n = 3$) MS	MS^2	MS^3
1	C_{10}-LAS	0.05 (17)	0.06 (15)	
	C_{11}-LAS	0.95 (13)	1.10 (11)	1.05 (12)
	C_{12}-LAS	0.71 (15)	0.78 (12)	0.75 (11)
	C_{13}-LAS	0.70 (17)	0.68 (15)	0.72 (14)
2	C_{10}-LAS	0.1 (16)	0.09 (12)	0.1 (10)
	C_{11}-LAS	1.6 (12)	1.8 (13)	1.9 (12)
	C_{12}-LAS	1.9 (14)	2.0 (10)	2.2 (14)
	C_{13}-LAS	2.2 (10)	2.5 (12)	2.4 (10)
3	C_6-SPC	0.1 (19)	0.1 (19)	
	C_8-SPC	1 (13)	1 (12)	4 (10)
	C_{11}-SPC	1.6 (14)	2 (11)	2.4 (12)
	C_{10}-LAS	4.4 (16)	4.6 (14)	5 (11)
	C_{11}-LAS	5.8 (11)	6.0 (9)	6.4 (8)
	C_{12}-LAS	6.0 (9)	6.0 (9)	6.4 (7)
	C_{13}-LAS	6.4 (10)	6.2 (12)	6.6 (9)
4	C_{11}-SPC	6 (15)	5 (13)	6 (10)
	C_{10}-LAS	12 (14)	13 (12)	11 (9)
	C_{11}-LAS	15 (17)	14 (15)	16 (13)
	C_{12}-LAS	14 (12)	12 (10)	13 (11)
	C_{13}-LAS	10 (11)	11 (10)	13 (7)

added only on surface (samples 1 and 2) because their incorporation to soil was probably slower and dependent on the incidence of rains and water erosion processes. By the other hand, it has to be taken in to account that the distribution of the sludges on forest soils by any method is far from being uniform, depending in many cases on the work of the brigades and the difficulty of the terrain morphology.

As an example, the chromatograms obtained for soil sample 3 by MS^2 is presented in Figure 3 (see chromatograms B). The results obtained in the samples treated with sludges demonstrated that LOQs achieved by MS^2 were excellent to determine the levels of surfactants present in real samples. On the contrary, the LOQs obtained by the MS^3 mode (see Table 2) were not always appropriate for the SPC metabolites.

The effectiveness of the method in determining these compounds can presume an important advance for the study of their distribution and fate in a matrix as complex as soil. It has to be emphasized that surfactants are widely used as components of some soil chemical restoration strategies, and sludges are increasingly used as input of organic matter and nutrients to degraded and agricultural soils. The scarcity of studies focused on LAS and SPC toxicity, tolerance, and effects on soil and its biota show the necessity of developing precise and useful methodologies, like that presented in this paper, for their detection and quantification.

It is needed to remark that Mediterranean soils are in a fragile equilibrium that allows maintaining the sustainability of resources, mainly agricultural, for subsistence in many developing countries. The alteration and degradation of soil biological properties because of contamination by these or other products could cause, in this case, the breakdown of this equilibrium with important socioeconomic consequences.

CONCLUSION

The proposed operating procedure represents a marked improvement in LC/MS applications, since it achieves high sensitivity with an unequivocal detection and confirmation of different compound structures by isolating the corresponding deprotonated molecule and segmenting it to obtain specific m/z fragments. In this way, this methodology validates its effectiveness, showing the reliability of this new approach to determine LAS and SPC homologues in complex environmental matrixes.

The low detection levels achieved, its selectivity, and its capability to analyze very complex matrixes such as soils offer a valuable tool in trace-level monitoring, providing better knowledge on the behavior of organic contaminants in soils. As it has been demonstrated, the use of LC-ESI quadrupole ion trap ES-MS^n in negative ion mode can be used for routine control of LASs and SPCs in soil samples.

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