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Differentiation and quantification of linear alkyl benzenesulfonate isomers by liquid chromatography-ion-trap mass spectrometry

Loreto Lunar, Soledad Rubio, Dolores Pérez-Bendito*

Department of Analytical Chemistry, Facultad de Ciencias, Edificio Anexo Marie Curie, Campus de Rabanales, 14071 Córdoba, Spain

Abstract

Discrimination and quantitation of the 20 positional isomers of C_{10}–C_{13} linear alkyl benzenesulfonates (LASs), based on the use of reversed-phase liquid chromatography-electrospray ionisation in negative ion mode ion-trap mass spectrometry, was undertaken. Discrimination was achieved by LAS MS-MS analysis into the ion trap, by monitoring specific fragment ions resulting from the benzylic cleavage of the carbon alkyl chain on both side of the LAS phenyl group. Parameters affecting the electrospray ionisation source and the ion-trap operation were optimised. Calibration curves for the different isomers were established and this permitted their quantitation by mass spectrometry for the first time. MS-MS responses were dependent on both the position of the phenyl group on the alkyl chain and the length of this alkyl chain, these responses being higher for the external isomers and the longer alkyl chain homologues. The precision, expressed as relative standard deviation ranged between 9 and 13%. Detection limits for LAS isomers were between 0.03 and 0.07 mg/l and therefore the method is sensitive enough to be applied to environmental samples.
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1. Introduction

Linear alkyl benzenesulfonates (LASs) are the major anionic surfactants used on the market. The global consumption of LAS was estimated to be about 2.2×10^9 kg in the year 2000 [1]. The most important application products are household detergents although other minor final uses are also of interest, namely in the field of textile and fibres, chemicals, agriculture and flotation of ore minerals [2].

Commercially available LASs in Europe are specific and rather constant mixtures of homologues and isomers. A LAS molecule contains an aromatic ring sulfonated at the *para* position and attached to a linear alkyl chain at any position except the terminal carbons. The linear alkyl chain has typically 10–13 carbon units, approximately in the following mole ratio C_{10}:C_{11}:C_{12}:C_{13} = 13:30:33:24, an average carbon number near 11.6 and a content of the most hydrophobic 2-phenyl isomers (ϕ_2) in the 18–29% range [3–5]. This commercial LAS consists of at least 20 individual components.

Disposal of LASs results in high concentrations in wastewaters. For this reason, the fate and behaviour of LASs in sewage treatment plants (STPs) and the environment need to be assessed. In activated sludge STPs, LAS removal reaches 98–99.9% [6–8]. The following split can be assumed on the basis of mass balance studies [7,9,10]: 80–90% degraded, 10–20% adsorbed into sludge and about 1% released to surface waters. However, persistence of LASs under methanogenic conditions in sewage sludge digesters has been demonstrated [11,12]. On the other hand, in many countries and regions most of population are not connected to municipal STPs [13].

The determination of total LAS content is sufficient for declaration product and general assessment of the pollution of wastewater and surface water. On the other hand, homologous and isomeric separation of LASs is important in industrial and environmental samples, because detergency, biological degradation and aquatic toxicity depend on the alkyl chain length and the position of the phenyl ring. Biodegradation is faster for the higher alkyl chain LASs [10,14,15] and external (2- and 3-phenyl) isomers [16]. Hydrophobicity is also the driving force for bioconcentration with the longer chain lengths and the 2-phenyl isomers being the most toxic LASs [17].

Separation of LAS homologues is easily achieved by gas [18] (GC) and liquid [19] (LC) chromatography and capillary electrophoresis [20] (CE), LC being the best selection for product control and environmental applications.

* Corresponding author. Tel.: +34-957-218644; fax: +34-957-218644.
E-mail address: qa1pebem@uco.es (D. Pérez-Bendito).

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Isomeric separation has been undertaken by using the same techniques under a variety of conditions, however, to date, no complete resolution of internal (5-, 6- and 7-phenyl) isomers has been achieved [20–25]. For this application, capillary GC [18] and CE [20,25,26] surpass LC [21–23]. The use of mass spectrometry as a selective detection ion method does not discriminate between coeluting positional isomers since they have identical molecular masses.

Differentiation of LAS isomers has been undertaken by CG-MS-MS techniques by monitoring specific fragmentations [16,24,27]. Since LASs and not sufficiently volatile to permit direct analysis by GC techniques, their derivatization to the corresponding ester derivatives is required [28–31]. Fragmentation pathways for LASs have been studied by several authors, these pathways being similar for the different mass spectrometric techniques investigated (e.g. fast atom bombardment [32], field desorption [32], electron impact [16], etc.). Typical fragmentations of LASs consist of cleavage of the C–C bonds of the alkyl chain with concomitant hydrogen transfer (alkane losses) leading to a series of weak fragment ions. In this series the most abundant fragment is the vinyl-substituted benzenesulfonate anion $[CH_2{=}CHC_6H_4{\cdot}SO_3]^-$ at *m/z* 183, which is common for all LASs [32]. Fragment ions that depend on the position of the aromatic ring are also obtained and they have been the basis for the identification of positional isomers of LASs [16,24,27]. However, the lack of standards and the unknown mass response for the different isomers has precluded their quantification.

The general objective of this work was to evaluate the capability of liquid chromatography-electrospray ion-trap mass spectrometry (LC-ESI-IT-MS) to identify and quantify LAS isomers. The use of LC is a more convenient technique than GC for this purpose since it avoids the need of using time consuming derivatization techniques. The investigation was developed to cover three specific aims. First, to find characteristic fragments for LAS isomers by using LC-ESI-IT-MS that permit their identification and quantification. The second aim was to determine the mass response function for individual isomers. Homologue and isomeric distribution of LASs in the mixture used as standard was found from LC-UV and CE-UV. Finally, we assessed the figures of merit of the calibration curves obtained in order to determine their usefulness for LAS isomer quantitation. To our knowledge it is the first time that LAS isomers are quantified by mass spectrometry.

2. Experimental

2.1. Chemicals

Reagents in their highest available grade were used. 4-Octylbenzenesulfonic acid (C_8-LAS), sodium dodecylsulfate (SDS), heptafluorobutyric acid (HFBA), tetrahydrofuran (THF) and triethylamine (NEt_3) were obtained from Aldrich. Acetic acid and HPLC-grade acetonitrile were purchased from Panreac (Barcelona, Spain). Trisodium phosphate was supplied by Merck (Darmstadt, Germany). The commercial LAS mixture C_{10}–C_{13} used, Petrelab P-550, was provided by Massó and Carol (Barcelona, Spain). The 2 and 5 C_{10}-phenyl and 2 C_{12}-phenyl isomers standards were kindly supplied by Petresa (Cádiz, Spain). Stock solutions of analytes were prepared in distilled water.

2.2. Apparatus

For LC-UV and LC-MS-MS experiments a liquid chromatography-UV-electrospray-ion-trap mass spectrometry system (1100 Series LC/MSD, Agilent Technologies, Waldbronn, Germany), equipped with an automatic injector, was used. The injection volume was set at 20 μl. The stationary phase was a Nova Pack C_{18} 4 μm column (300 mm × 3.9 mm; Waters P/N WATO11695). The Hypercarb column used in different experiments (200 mm × 4.6 mm, 5 μm) was supplied by Thermo. CE analysis to determine the LAS isomeric distribution of the standard mixture was performed on a P/ACE 2000 system (Beckman, Fullerton, CA, USA).

2.3. Liquid chromatography-mass spectrometry

The mobile phase used for chromatographic separations was a mixture of solvent A (acetonitrile–water, 80:20) and solvent B (water), both containing 2.5 mM acetic acid and 2.5 mM triethylamine. Elution of LAS was carried out using a linear gradient (solvent B ranged from 75 to 19% in 26 min). A column re-equilibration time of 10 min was used between runs. The flow rate was 0.6 ml/min. From 0 to 15 min the eluent flow was diverted to waste.

Mass spectrometric analysis of LASs was carried in the ESI(−) mode. The molecular ions of the target LAS homologues (C_{10}, *m/z* 297; C_{11}, *m/z* 311; C_{12}, *m/z* 325; C_{13}, *m/z* 339) and the internal standard (C_8-LAS, *m/z* 269) were isolated and fragmented into the ion trap. Excitation of the ions was accomplished through collision with helium. The isolation width was set at 1 *m/z* unit. Different time segments were established to isolation of parent ions (C_8, 15–17.7 min; C_{10}, 17.7–19.8 min; C_{11}, 19.8–21.7 min; C_{12}, 21.7–23.5; C_{13}, 23.5–26.0 min). The set of instrumental parameters used for LAS isomers MS-MS analysis is shown in Table 1. Parameters affecting LAS electrospray ionisation were optimized by directly analyzing the LAS mixture Petrelab P-550 (250 mg/l) in solvent A–solvent B (70:30, v/v) using a KD Scientific, model 100, syringe pump (New Hope, MN, USA) at 400 μl/h. Selection of the best value for each of these parameters was based on the criteria of sensitivity and precision for the molecular ion of each homologue. Parameters influencing the performance of the ion trap to LAS isomers MS-MS analysis were optimized by liquid chromatography separation of LASs and isolation and fragmentation of parent ions. Selection of the optimal value for each

Table 1
ESI-MS-MS parameters set for the analysis of LAS isomers

Parameter	Value
ESI	
Capillary voltage	3 kV
Capillary exit voltage	150 V
Skimmer	20 V
Source temperature	350 °C
Drying gas	10 min^{-1}
Nebulizer gas	60 psi
Ion-trap	
Trap drive	80
Maximum accumulation time	10 ms
Resonance excitation	1.3 V
Fragmentation time	100 ms

parameter (Table 1) was based on criteria of sensitivity and precision for the characteristic fragments of LAS isomers.

Quantitation was carried out under full scan conditions (*m/z* range 150–360) by using the extracted ion chromatograms at the *m/z* of the selected fragments for each isomer (ϕ_2, 184; ϕ_3, 198; ϕ_4, 212; ϕ_5, 226; ϕ_6, 240; ϕ_7, 254) and measuring the corresponding peak areas. The *m/z* fragment selected for the internal standard LAS-C_8 was 170. The amount of C_8 added to LAS standard solutions was 100 mg/l. Smooth chromatograms were obtained by using the Gauss function (width = 1.6 s, cycles = 2). Correlation between peak areas and LAS isomer concentrations (0.45–30 mg/l for ϕ_2 and 0.5–35 mg/l for ϕ_3–ϕ_7 isomers) were determined by linear regression and were in the range 0.98–0.998.

3. Results and discussion

3.1. LAS isomer discrimination by LC-ESI-IT-MS

3.1.1. Chromatographic studies

Separation of LAS isomers using reversed-phase LC-UV has involved the use of an electrolyte [22,23,33] or an ion interaction reagent [34,35] in the mobile phase. In this way, separation of LASs according to the alkyl chain length is straightforward. Also, the 2-phenyl isomers are well separated, however, the other isomers are only partially resolved.

Several studies on the separation of LASs with ESI-MS in the negative mode have been published [36–38]. The proposed methods involves the use of a LC mobile phase containing equimolar amounts of HOAc and NEt_3, which results in the formation of a volatile ion-pair agent (NEt_3H) necessary to cause sufficient retention of LASs in the C_{18} column [36,37], or conversion of carboxylic groups of LASs to their methyl esters and addition of ammonium acetate [38]. Again, homologues are well resolved but isomers coelute. The volatility of the commonly used ion-pairing reagents (i.e. tetraethylamomnium or tetrabuthylammonium) or electrolytes (i.e. $NaClO_4$, NaCl) is not compatible with the ESI interface and therefore they cannot be used for LAS separation.

Different strategies were investigated here in order to achieve the best possible resolution of LAS isomers. Initially, a porous graphitic carbon (PGC) column (Hypercarb), which has been known to provide high selectivity for the separation of positional isomers [39–41], was used. Anionic compounds such as LASs could be separated on PGC based on electronic interactions between the solutes and the delocalised electron clouds on the graphitised carbon, or they could be retained by reversed-phase interaction with the hydrophobic carbon surface in the presence of ion-pairing reagents. Electronic interaction-based LAS isomer separations were investigated using a mobile phase made up of 5–80 mM heptafluorobutyric acid in acetonitrile–water in proportions ranging from 0:100 to 100:0. HFBA is a volatile strong acid used to compete with the solutes for electronic interaction with the π-electrons of PGC. No elution of LASs was achieved under the different experimental conditions investigated and THF was required for elution of analytes. This behaviour indicated high retention of LASs on PGC by hydrophobic interactions, probably through the hydrocarbon chain. Accordingly, LAS isomer separation was attempted by using a mobile phase made up of acetonitrile–water–THF both containing 5 mM HOAc and 5 mM NEt_3. Homologues separation was easily achieved for percentages of THF above 50%. Some isomeric separation was achieved under determined experimental conditions (i.e. Fig. 1a), however, it was completely insufficient for quantitation purposes. Substitution of acetonitrile by methanol did not enhance the selectivity of the LAS isomer separation.

The second strategy investigated was based in the addition of 1–4 mM α-cyclodextrin into the mobile phase with the aim of forming inclusion complexes with LASs. Cyclodextrin additives are widely used as chiral selectors for separation of optical isomers, but they have been also used for separation of positional isomers [42]. Different mobile phases were studied including water, water–acetonitrile and water–methanol, in the presence and absence of HOAc and NEt_3 as ion-pairing reagents. Strong adsorption of LAS on the C_{18} phase from α-cyclodextrin solutions was observed under all experimental conditions investigated, the use of tetrahydrofuran being necessary for elution of the target compounds. Irreversible adsorption of analytes on the RP-18 phase in the presence of cyclodextrins has been previously described [43]. The use of THF as a mobile phase component did not improve the isomeric resolution provided by the porous graphitic carbon (Fig. 1a).

From the above results, the option of using a C_{18} column and a mobile phase made up of a volatile ion-pair agent (i.e. NEt_3H) and conventional solvents (i.e. water, acetonitrile, methanol) was selected to study the best possible resolution of LAS isomers. A wide study was undertaken using different gradient elution programs with various combinations of solvents, at various pH values and concentrations of the ion-pair agent. The selected conditions are specified

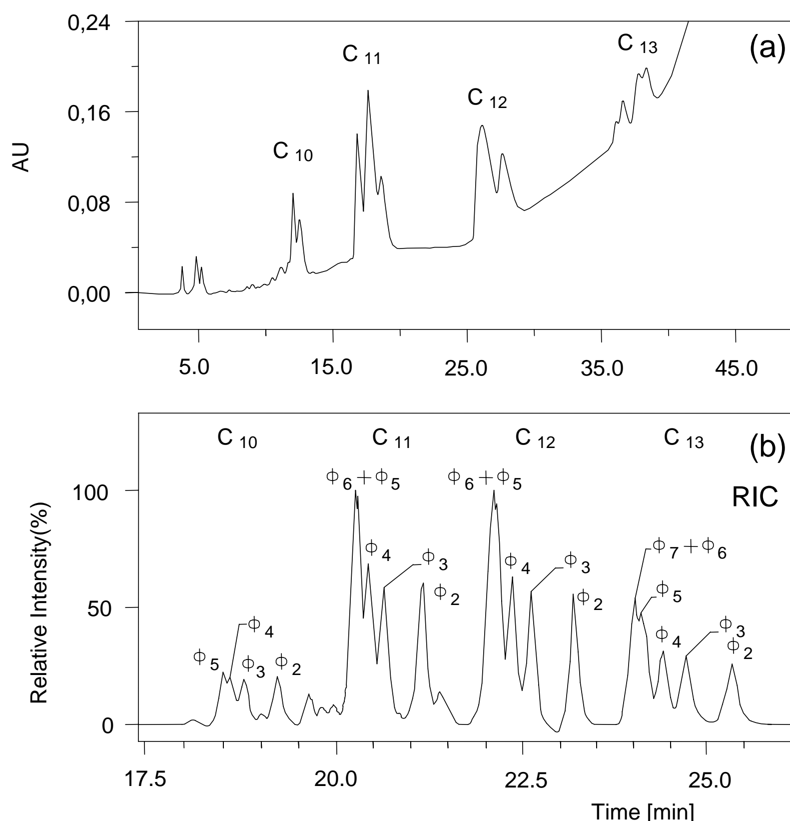

Fig. 1. (a) UV chromatogram obtained for the LASs contained in Petrelab P-550. Stationary phase: porous graphitic carbon. Mobile phase: solvent A (acetonitrile–water 80:20) and solvent B (tetrahydrofurane), both containing 5 mM acetic acid and 5 mM triethylamine. Elution gradient: linear from 100 to 90% of A in 10 min, then isocratic for 5 min, after that, linear from 90 to 70% A in 15 min and then from 70 A to 20% A in 20 min. (b) LC-ESI⁻-MS extracted ion chromatograms of LASs at the *m/z* of the homologues: C_{10}, 297; C_{11}, 311; C_{12}, 325 and C_{13}, 339. Chromatographic conditions as specified under Section 2. Petrelab P-550: 250 mg/l.

under Section 2. The temperature did not affect resolution. Fig. 1b shows the reconstructed ion chromatogram (RIC) of C_{10}–C_{13} LAS homologues acquired under negative ESI at the molecular masses 297, 311, 325 and 339, of the respective $[M–H]^-$ ions. The symbol ϕ_n indicates the phenyl position on the alkyl chain for each isomer.

Resolution of internal (4-, 5-, 6- and 7-phenyl) isomers was lesser compared to that obtained by GC [18] or CE [20,25,26], although separation between homologues was better for LC. Thus, coelution of 6-C_{13} and 2-C_{12} has been observed in CE [20], whereas in GC [34] coelution of 6-C_{12} and 2-C_{11} has been also obtained. Independently on the separation technique used, the use of fragment ions that contain relevant information to the alkyl chain is today the only potential via to quantify all individual positional LAS isomers.

3.1.2. *MS-MS LAS isomer discrimination*

Isomeric discrimination was carried out with MS-MS using the ion-trap instrument. In these studies, the molecular ion corresponding to each target compound was isolated and fragmented. Fig. 2 depicts typical full scan MS-MS spectra obtained from ϕ_4 C_{10}–C_{13} LAS isomers and Fig. 3 shows a scheme of the proposed pathways of fragmentation. These pathways were identical for all isomers and the fragment ions obtained were similar to those found by other mass techniques [32].

In addition to the molecular ion $[M–H]^-$, MS-MS spectra of LAS isomers presented common fragment ions at *m/z* 183, which are presumably generated through a six-membered ring rearrangement of the alkyl chain, and *m/z* 170 (Fig. 3). Fragment ions that depended on the position of the aromatic ring and therefore contained information relevant for discrimination of isomers were also obtained (A and B in Fig. 3). These characteristic fragments represented the benzylic cleavage of the carbon alkyl chain on both side of the phenyl group. Ion fragments corresponding to some isomers from different homologues showed the same *m/z* (i.e. fragments A for ϕ_2 from C_{10}–C_{13} homologues; fragments B for ϕ_2-C_{10}, ϕ_3-C_{11}, ϕ_4-C_{12}, ϕ_5-C_{13}), however, they could be used for identification and quantification purposes because they were no coeluting species (Fig. 1b). The results obtained from preliminary experiments indicated that higher sensitivity and precision was obtained for the quantitation of LAS isomers from fragments A compared to fragments B, and accordingly they were selected for further studies. Fig. 4 shows the extracted ion chromatograms obtained for the different LAS isomers at the *m/z* corresponding to fragments A.

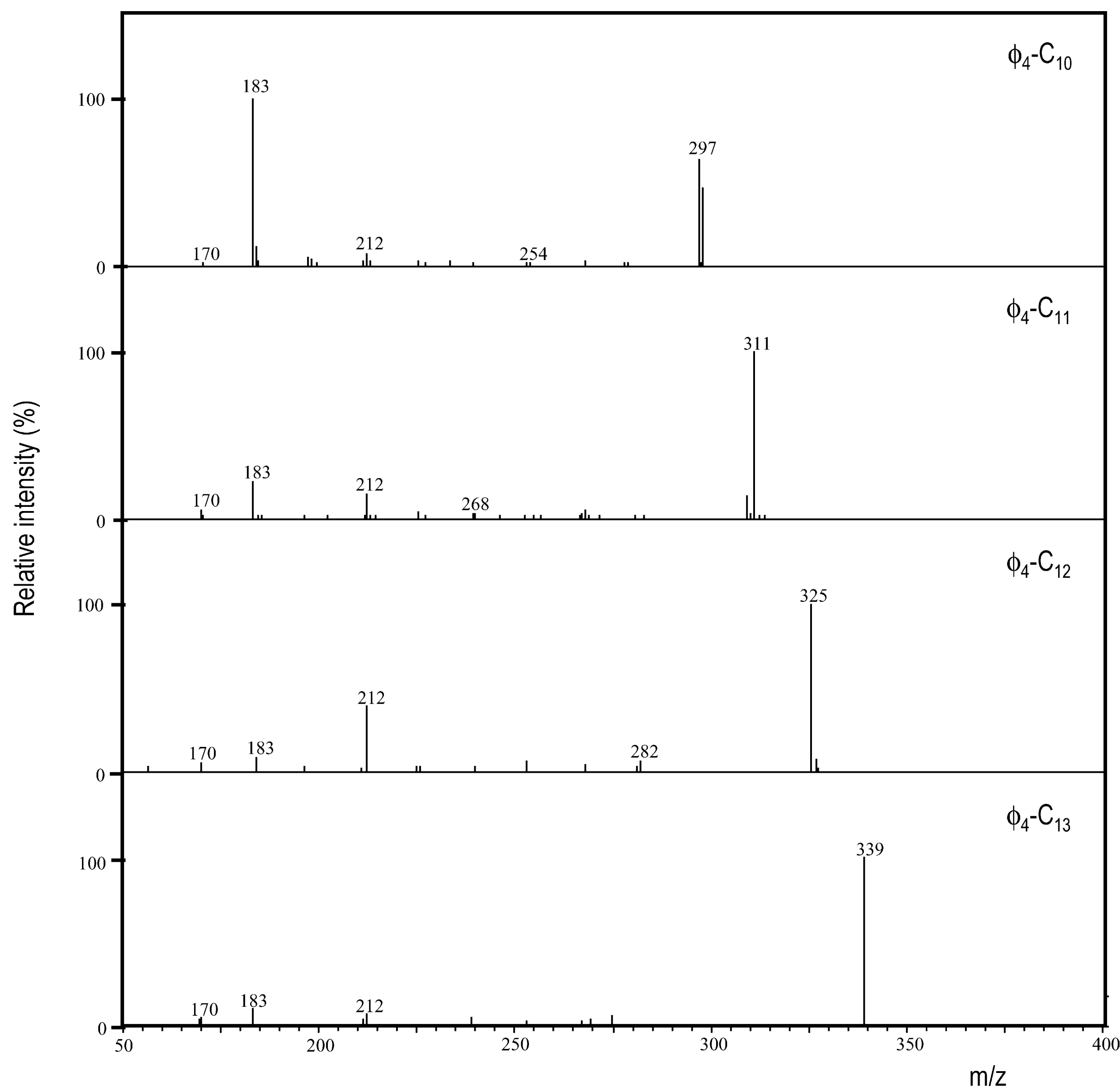

Fig. 2. MS-MS spectra of ϕ_4 isomers for C_{10}–C_{13} LAS homologues.

Different experiments were conducted to find the instrumental parameters providing the higher sensitivity and precision for the quantitation of LAS isomers. Operating conditions for the ESI source were optimised to obtain the maximum population of LAS homologue ions. Similar behaviour of the signal obtained for these homologues was found with respect to the different parameters studied; the capillary and the skimmer voltages being the most important variables affecting sensitivity. Capillary voltages above 2.5 kV were required to obtain adequate signals, although these were progressively decreasing from 3.5 kV. The optimal range found for the skimmer voltage was comprised between 15 and 25 V, the signal being decreased notably out of this interval. The optimal value selected for the different parameters studied are shown in Table 1.

Ion-trap parameters were optimised to obtain maximal accumulation of LAS homologues ions into the trap without causing space-charge effects and maximal signal for the characteristic fragments A from LAS isomers. These fragments were yield in the resonance excitation interval comprised between 0.8 and 1.6 V, fragmentation times above 60 ms being required. Table 1 shows the different values selected for LAS isomer analysis.

3.2. Mass response factors for LAS isomers

The main problem to quantify LAS isomers by mass spectrometry through their characteristic fragments is the lack of available standards. This fact has precluded their quantification by GC-MS [16,24,27]. The data available from LAS homologues quantitation by LC-ESI-MS, at the *m*/*z* corresponding to the molecular ion, indicate that mass responses vary by a decreasing factor of about two from C_{10} to C_{13} [44]. We have studied the mass responses for

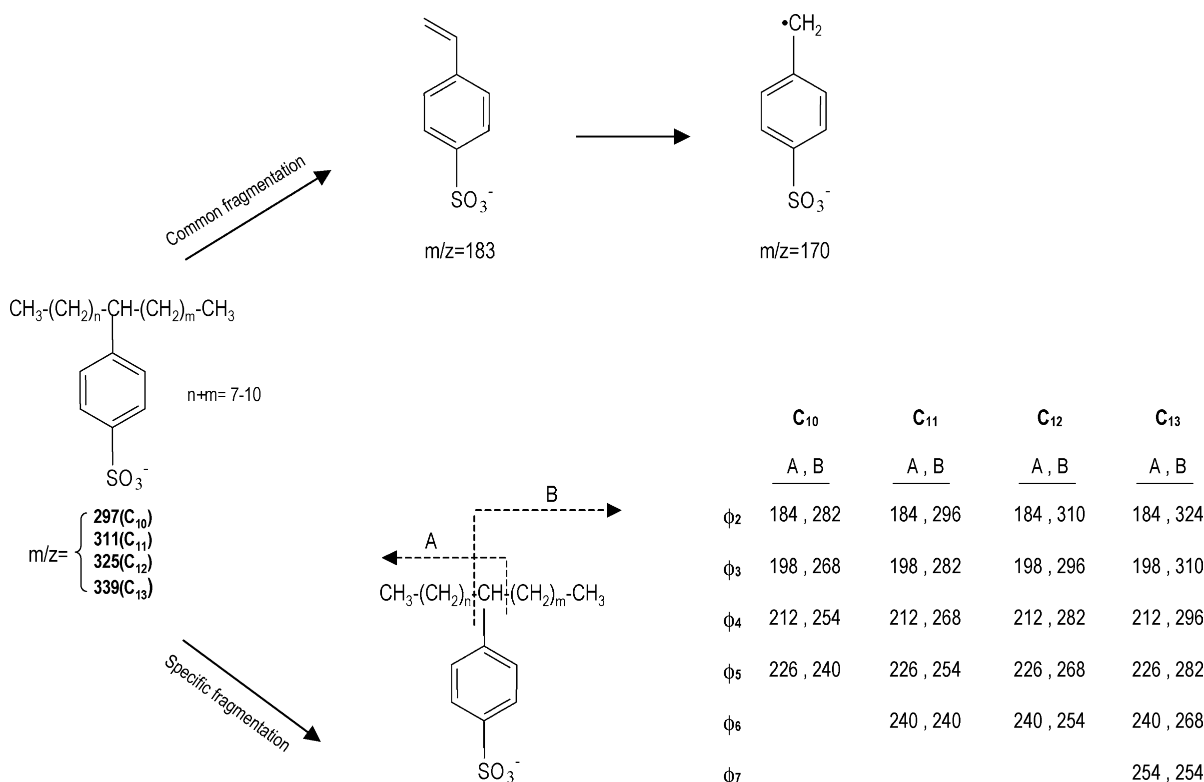

Fig. 3. Fragmentation scheme for LASs and *m/z* of the common and specific fragments obtained from the different isomers.

the same homologues from the measurement of the common fragment ion at *m/z* 183 (Fig. 3), under the conditions described in Section 2, these responses varying by an increasing factor of about 4. The slope of the calibration curves obtained were respectively: C_{10}, 1.1×10^7; C_{11}, 2.0×10^7; C_{12}, 3.0×10^7 and C_{13}, $4.2 \times 10^7\,\mathrm{l\,mg^{-1}}$. On the contrary to the behaviour observed for MS measurements [44], MS-MS responses were more sensitive for the higher alkyl chain LASs. These results indicate that different mass responses will be obtained for fragments A, and therefore the exact composition of isomers in LAS mixtures must be known for calibration prior to any quantitative analysis.

In order to use Petrelab P-550 as a standard for LAS isomers in mass spectrometry, we tried to determine its isomer distribution by combining CE-UV and LC-UV analysis on the basis that CE [20,25,26] provides the highest resolution for internal isomers, LC offers high selectivity for homologues and ϕ_2 separation, and UV response factor for isomers is identical [21–23]. Liquid chromatography analysis was carried out using the mobile phase gradient specified under Section 2. Detection was performed at 230 nm. Separation of isomers by CE [20] was carried out using a fused capillary of 47 cm length (40 cm to the detection window) $\times$ 50 μm i.d. The separation voltage was 25 kV (detection at the cathode), and the temperature of the capillary was 25 °C. Detection was performed at 230 nm. Injections (at the anodic end) were performed in the pressure mode and the injection time was set at 5 s. The buffer consisted of 10 mM phosphate (pH 6.8)–40 mM SDS–30% acetonitrile. The CE resolution used as basis of the quantitative analysis was similar to that achieved previously by Heinig et al. [20]. The quality of the determinations was verified by parallel quantitation of ϕ_2-C_{10}, ϕ_5-C_{10} and ϕ_2-C_{12} prior calibration with the standards kindly supplied by Petresa. Table 2 lists the percentages of LAS isomers found in the standard Petrelab P-550. The amount of ϕ_5 and ϕ_6 from C_{11} and ϕ_6 and ϕ_7 from C_{13} could not be determined since these isomers were not separated by CE. The LAS homologue distribution was found as follows: C_{10}, 8.3%; C_{11}, 35.6%; C_{12}, 36.1%; C_{13}, 20.2%.

According to the composition found for Petrelab P-550, calibrations were run for the different LAS isomers by triplicate. Also calibrations from the isomers standards available (ϕ_2-C_{10}, ϕ_5-C_{10}, ϕ_2-C_{12}) were obtained and the results compared. Quantifications were carried out from the extracted

Table 2
Percentage distribution of LAS isomers in Petrelab P-550

	C_{10}	C_{11}	C_{12}	C_{13}
ϕ_2	2.01	7.27	6.53	3.14
ϕ_3	1.90	6.83	5.89	2.96
ϕ_4	1.78	7.18	6.52	3.32
ϕ_5	2.62	14.3	8.30	3.81
ϕ_6	–		8.84	6.96
ϕ_7	–	–	–	

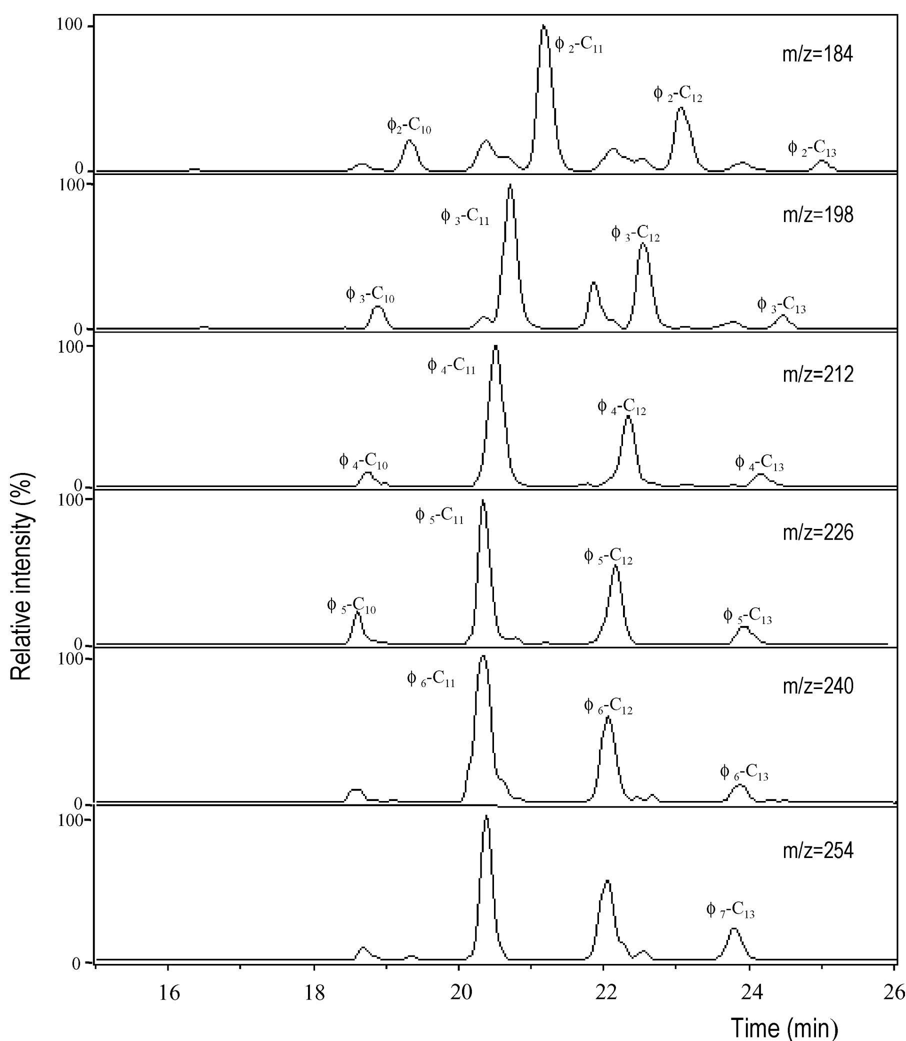

Fig. 4. LC-ESI-MS-MS extracted ion chromatograms at the *m/z* values selected for analysis of the different isomers. Petrelab P-550: 500 mg/l.

ion chromatogram for each isomer in order to improve *S/N* ratios and selectivity. 4-Octylbenzenesulfonic acid was chosen as internal standard because it does not occur in commercial LAS mixtures.

Table 3 shows the figures of merit for the calibration curves obtained for each target compound. Linearity was observed in the range of concentrations of 0.45–30 mg/l for ϕ_2 and 0.5–35 mg/l for ϕ_3–ϕ_5. The correlation coefficients indicated good fits. The instrumental detection limits were calculated using a signal-to-noise ratio of 3. Detection limits were in the range from 0.03 to 0.07 mg/l. The intra-day precision (repeatibility) of the method was evaluated by analysing 11 samples each containing 250 mg/l. The relative standard deviation ranged from 9 and 13% for the different isomers. No differences were observed between calibrations run from isomer standards and Petrelab P-550 for ϕ_2-C_{10}, ϕ_5-C_{10} and ϕ_2-C_{12}, which validated the LAS isomer composition established by LC-CE-UV.

The results obtained indicated that MS-MS responses for isomers increased linearly with the LAS alkyl chain length, similarly to the responses found for homologues by measuring the common fragment at *m/z* 183, as specified above. Thus, the plot of the calibration slopes obtained for the same positional isomers versus the alkyl chain length of LAS homologues (Table 3) was linear ($r > 0.997$) with slope values of 4.91, 2.64, 1.50 and 0.77 mg l^{-1} for ϕ_2, ϕ_3, ϕ_4 and ϕ_5 isomers, respectively. This behaviour was agreed with the high preference in mass fragmentation for the loss of the largest alkyl radical at a reactive site [45]. For this reason, the loss of the alkyl chain was most favoured for C_{13}, which resulted in higher probability for forming the fragment ion.

On the other hand, the MS-MS responses obtained decreased from the external (2- and 3-phenyl) to internal (4- and 5-phenyl) isomers (i.e. see the slope values specified for ϕ_2–ϕ_5 isomers in the above paragraph), which is probably caused by both the loss of larger alkyl radicals at the reactive site for the external isomers and the higher alkyl chain length of internal isomers compared to the external ones. It is well known that mass responses linearly decrease in proportion to the alkyl chain length increases [45]. The slope of the linear

Table 3
Calibration data obtained for LAS isomers

Isomer	*m/z* selected	Slope (±S.D.) (mg^{-1} l)	Intercept (±S.D.)	SEE[a]	r[b]
ϕ_2-C_{10}	184	9.7 (±0.6)	0.001 (±0.003)	0.003	0.995
ϕ_2-C_{11}	184	14.6 (±0.9)	0.02 (±0.01)	0.02	0.990
ϕ_2-C_{12}	184	19.9 (±0.5)	0.04 (±0.02)	0.04	0.98
ϕ_2-C_{13}	184	24.3 (±0.6)	0.002 (±0.002)	0.003	0.991
ϕ_3-C_{10}	198	2.1 (±0.1)	0.00007 (±0.001)	0.0004	0.998
ϕ_3-C_{11}	198	4.9 (±0.09)	0.0008 (±0.002)	0.001	0.997
ϕ_3-C_{12}	198	7.3 (±0.5)	0.006 (±0.005)	0.007	0.992
ϕ_3-C_{13}	198	10.1 (±0.7)	0.004 (±0.004)	0.006	0.98
ϕ_4-C_{10}	212	1.2 (±0.3)	0.001 (±0.001)	0.002	0.995
ϕ_4-C_{11}	212	2.8 (±0.4)	0.007 (±0.006)	0.07	0.98
ϕ_4-C_{12}	212	4.3 (±0.4)	0.003 (±0.005)	0.007	0.990
ϕ_4-C_{13}	212	5.7 (±0.8)	0.004 (±0.01)	0.01	0.990
ϕ_5-C_{10}	226	1.1 (±0.2)	0.001 (±0.001)	0.002	0.993
ϕ_5-C_{12}	226	2.8 (±0.3)	0.008 (±0.007)	0.006	0.991
ϕ_5-C_{13}	226	3.4 (±0.5)	0.007 (±0.005)	0.005	0.993
ϕ_6-C_{12}	240	2.4 (±04)	0.008 (±0.006)	0.002	0.996

[a] Standard error of the estimate.
[b] Correlation coefficient, $n = 8$.

relationship found for the different isomers varied by a constant factor of about 1.8 (i.e. $\phi_2/\phi_3 = 4.91/2.64 = 1.86$) and fit to the following exponential equation: ln (slope value) = $-0.608\phi_n + 2.8093$ ($r^2 = 0.9993$).

A rough estimation of the calibration slopes and concentrations in Petrelab P-550 of ϕ_5 and ϕ_6 from C_{11} and ϕ_6 and ϕ_7 from C_{13} could be performed from the data obtained for the other isomers. Thus, the calibration slope for ϕ_5-C_{11}, obtained by interpolation on the straight line for ϕ_5 isomers (0.77 mg/l, see above), was found to be 1.9 l/mg. The percentage of ϕ_5-C_{11} in Petrelab P-550 was calculated from this slope. A value of 7.27% was found. In this way, the percentage of ϕ_6-C_{11} in the Petrelab P-550 could be calculated by difference ($14.3 - 7.27 = 7.03$, see Table 2) and the calibration slope established, from this percentage and the corresponding extracted ion chromatogram at $m/z = 240$. The calibration slope was found to be 1.8 mg^{-1} l. Since the calibration for ϕ_6-C_{12} was previously established (Table 3), the calibration slope for ϕ_6-C_{13} was obtained by interpolation (2.71 mg^{-1} l). The slope obtained for ϕ_6 isomers fit to the exponential equation found with an error of ±0.01. The percentage of ϕ_6-C_{13} in Petrelab P-550 was calculated from this slope. A value of 3.91% was found. Finally, the calibration slope for ϕ_7-C_{13} was found to be 2.5 mg^{-1} l on the basis of a calculated percentage of 3.05 ($6.96 - 3.91$, see Table 2) for ϕ_7, in Petrelab P-550.

4. Conclusions

Complete discrimination and quantitation of LAS positional isomers can be achieved by LC-ESI-IT-MS in a convenient and rapid way. The use of fragment characteristics obtained from MS-MS is the only method available today for LAS isomer analysis since LC, GC, or CE do not provide enough selectivity for this purpose.

In this research the dependence of MS-MS responses as a function of both the position of the phenyl group on the alkyl chain and the length of this alkyl chain has been established for the first time. These responses indicate that the use of standards with a known isomer composition should be available to make mass techniques widely used for LAS isomer analysis. Anyway, laboratories interested in this analysis can determine the isomeric composition of commercial LAS mixtures through combination of LC-UV and CE-UV.

As previously commented, LAS isomer analysis is important in industrial and environmental samples. The detection limits achieved by MS-MS analysis are lower that those obtained by UV detection and permit its direct application to industrial and untreated wastewaters [46]. However, analysis of wastewater effluents or river samples will require preconcentration factors in the range 10–100, which can be easily achieved with the solid-phase-extraction methods used for LAS homologues determination.

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