FULL PAPER
THE ANALYST
www.rsc.org/analyst

Determination of linear alkylbenzene sulfonates and their polar carboxylic degradation products in sewage treatment plants by automated solid-phase extraction followed by capillary electrophoresis-mass spectrometry

J. Riu and D. Barceló*

Department of Environmental Chemistry, IIQAB-CSIC, c/Jordi Girona 18-26, 08034 Barcelona, Spain. E-mail: dbcqam@cid.csic.es

Received 23rd January 2001, Accepted 2nd April 2001
First published as an Advance Article on the web 23rd May 2001

Linear alkylbenzene sulfonates (LAS) were determined by solid-phase extraction (SPE), followed by capillary electrophoresis and mass spectrometry detection (CE-MS). The linear range of the proposed method varied from 33 to 316 and from 215 to 2057 μg L^{-1}, depending on the compound, with limits of detection ranging from 4.4 to 23 μg L^{-1} when 200 ml of wastewater were preconcentrated. The analysis and confirmation of the polar carboxylic metabolites of LAS, the sulfophenyl carboxylic acids (SPC) was also achieved, and their presence was detected in both, influent and effluents of the sewage treatment plant (STP). $[M - H]^-$ ions were used for CE-MS confirmation and quantification. CE-MS diagnostic ions were the same ones used in LC-electrospray (ESI)-MS and corresponded to *m*/*z* 297, 311, 325 and 339 for C10LAS, C11LAS, C12LAS and C13LAS, respectively. For SPC identification, diagnostic ions corresponded to *m*/*z* 215 to 369 (with 14 mass unit steps) for C2 to C13SPC, respectively. LAS were determined in wastewater samples of the influent and effluent of three sewage treatment plants (STP), two of them using biological treatment with secondary settlement and receiving mainly domestic wastewater whereas one of the plants was operated with physicochemical treatment and received mainly industrial wastewater. The concentration levels of total LAS varied from 1000 to 1900 μg L^{-1} in the influents of STP, whereas in the effluents the concentrations varied from 125 to 360 μg L^{-1}.

Introduction

Routine determination of linear alkylbenzene sulfonates (LAS) in surface waters involves the use of solid-phase extraction (SPE) followed by derivatization and gas chromatography-mass spectrometry (GC-MS).[1,2] Such methods are tedious because they involve a derivatization step prior to GC-MS determination. A method involving continuous flow fast atom bombardment-MS was also developed and permitted the direct determination of LAS in wastewater and river samples.[3]

Recently various applications were published describing the determination of ionic compounds using capillary electrophoresis (CE). With its high separation efficiency and low solvents costs CE has become useful for the environmental analysis of many dyes and other compounds employed in the dye industry.[4] This efficiency of the CE technique can be additionally enhanced by an automated SPE involving a preconcentration step that also includes sample clean-up with elimination of interferences from the matrix under consideration, *i.e.*, industrial effluents and wastewater. An automated SPE method involving the ASPEC XL system, followed by CE-UV and CE-MS, was developed by our group for the determination of sulfonated azo dyes.[5] Although CE is generally coupled to UV detection, in environmental analysis MS detection is needed to avoid the large number of interferences present.

CE has been increasingly used for the determination of LAS in environmental matrices in recent years.[6–9] For the separation of LAS homologues an organic modifier, like acetonitrile or γ-cyclodextrin, is added to a phosphate or borate buffer.[6,7] Without adding organic solvents all homologues and isomers of LAS produce only one peak in the electropherogram. This method is useful for the determination of total LAS.[8] For the determination of isomeric compounds, CE has been suggested as one of the best methods available. Generally additives like SDS[6] or α-cyclodextrins[7] are used with a phosphate or borate buffer and acetonitrile as organic modifier, although some authors found a better resolution of some isomeric LAS using CZE with no SDS present in the buffer.[9] Under CE-MS conditions the use of additives like SDS is not allowed because of sensitivity problems.

In a previous paper the determination of LAS by CE using a UV detector was achieved[10] and the coupling with a mass spectrometric detector was done in order to unequivocally identify the target compounds. It also set the need for the determination of these compounds by MS detection because some overestimation is made with UV detection.

Taking into account these facts the objectives of this work were: to develop a methodology for the determination of the four commonly used LAS homologues in industrial wastewater from influent and effluent of water treatment plants from Catalonia, by SPE followed by CE-MS; to test the former methodology for the separation and determination of the polar sulfophenyl carboxylic acid (SPC) metabolites in the same environmental matrices.

Although it is known that CE-MS is less sensitive than LC-MS, in the case of LAS and SPC determination in sewage treatment plants (STP) this is not really a problem since these compounds are generally detected at higher levels than other environmental pollutants like pesticides.

Experimental

Chemicals

All HPLC-grade solvents, methanol, propan-2-ol, acetonitrile and water, also analysis-grade reagents ammonia and ammonium acetate were obtained from Merck (Darmstadt, Germany).

Acetic acid was purchased from Panreac (Barcelona, Spain). The commercial LAS with a low dialkyltetralin sulfonates (DATS) content (< 0.5%) were supplied by Petresa and C_{10} SPC standards were supplied by Jennifer A. Field (Oregon State University, USA). C_{11} SPC standard was prepared by sulfonation of the corresponding acid in the University of Cadiz.

Sewage treatment plants and sample preparation

Sample collection took place at three STP. Two of the plants carry out biological treatment (STP1 and STP2) which receive domestic effluents. The third one (STP3), consisted of primary settlement involving only physicochemical treatment, which received industrial effluents from various types of industries with major discharges (around 50%).

Water samples from the influent and effluent were collected corresponding to 24 h composite samples. The 24 h composite samples were obtained by the treatment plant operators. All water samples were collected in glass bottles. Samples were transported to the laboratory and were stored at 4 °C prior to the analysis that took place within 1–2 d. Before analysis, the pH values of the samples were adjusted to the neutral range and samples were filtered when necessary.

Disposable 6 ml cartridges packed with 200 mg Isolute ENV+ (International Sorbent Technology, UK) were attached to the ASPEC XL system (Gilson, Villers-le-Bel, France) which is fitted with an external Model 306 LC pump and connected with a Model 817 switching valve for the selection of samples. Recoveries were obtained by means of UV detection. The analytical protocol is discussed in detail elsewhere.[10]

CE analysis

CE was carried out with a Beckman P/ACE 5000 capillary electrophoresis system (Beckman Instruments, Palo Alto, CA, USA). The separation was performed in a 80–100 cm × 75 μm id fused-silica capillary (Beckman). The electrophoresis buffer solution was 10 mM ammonium acetate in water at pH 9.8 and with an organic modifier of 16% acetonitrile. The capillary was regenerated subsequently with 0.1 M NaOH, water, and working buffer solution before each analysis. The temperature of the capillary column was set at 25 °C. After a pressure injection (0.5 psi) over 20 s, a voltage of 20 kV was applied across the capillary. Data analysis was performed by System Gold software.

The pH of the electrolyte and the spiked water samples was adjusted by adding ammonia and acetic acid respectively and it was measured with a Model 691 pH meter (Metrohm, Herisau, Switzerland) connected to a pH glass electrode containing 3 M KCl and silver chloride. In order to optimize the separation several parameters were tested. Different percentages of organic modifiers (propan-2-ol and acetonitrile) were used with different buffer concentrations. Finally, several pH were tested. Because of the length of the capillary of the CE-MS interface, separation conditions were chosen in order to minimize the retention times of the target compounds. For this reason a low concentration buffer was chosen, high pH and acetonitrile instead of propan-2-ol as organic modifier. The injection time was increased to 20 s (instead of 5 s when working with CE-UV[10]) in order to gain sensitivity. Moreover, because of the length of the capillary less quantity of sample is injected when the same number of seconds is applied for the pressure injection (which is a constant), than when working in CE-UV and subsequently, with a shorter capillary. It is well-known that ESI-MS working at low flows (like CE-MS) is a mass response detector and for this reason (as CE delivers low amounts of sample into the MS system) sensitivity is a major problem, especially when working with environmental samples.[11]

CE-MS conditions

For CE-MS operation the voltage applied was 20 kV and the capillary length was 80 to 100 cm in order to extend it to the probe tip through the stainless steel sheath capillary.

The CE system was connected to a VG Platform mass spectrometer from Micromass (Manchester, UK) equipped with a CE probe and an electrospray interface (ESI). The design of this probe consists of a triaxial flow arrangement whereby the CE eluent is mixed with a suitable make-up solvent at the probe tip, and then nebulized using nitrogen gas. The CE capillary extends fully to the probe tip through the stainless steel sheath capillary, which carries the make-up solvent. Around the sheath capillary is the nebulizer capillary through which the nitrogen gas flows to the probe tip. The make-up solvent performs two functions: to supplement the CE flow to a level adequate for electrospray operation (the CE electroosmotic flow is in the range of 10 nl min^{-1} and is too low for ESI operation without make up solvent) and to make electrical contact between the CE buffer and the probe tip. The nitrogen gas that flows through the probe tip maximizes the efficiency of the nebulization. The design of the source is not different from the system used in the normal megaflow ESI operation which has been previously described by our group.[12]

The make-up solvent, consisting of propan-2-ol–water (80 + 20) with 0.1% of ammonia, was delivered at a flow rate of 10 μL min^{-1} by a gradient system used in isocratic condition from a Waters 616 pump controlled by a Waters 600S Controller from Waters-Millipore (Millford, MA, USA).

The MS instrument was tuned by filling the capillary with the studied compounds and monitoring the signal corresponding to the mass of the tuning ion while the voltage of the CE was applied to introduce the sample into the MS. The operating parameters were adjusted in order to achieve maximum sensitivity (with the consequent loss of fragmentation and structural information). In this study a nebulizer gas of 25–30 L h^{-1} was used and the drying gas was set at a low value (in the order of 50 L h^{-1} or less). The source temperature was set at 75 °C. The cone voltage was set at 20 V in order not to produce fragmentation and achieved the best sensitivity.

The instrument control and data processing utilities included the use of the MassLynx application software installed on a Digital DEC PC 466.

Results and discussion

Calibration graphs

Linearity of the CE-MS system was studied with standard solution mixtures of the studied LAS at five points (0.85, 1.1, 1.7, 2.8 and 5.5 mg L^{-1}, total LAS concentration) using the negative mode of ionization and with time-scheduled SIM. An internal standard (1-naphthalene sulfonate) was added at a concentration level of 0.5 mg L^{-1} in order to minimize the effect of the instabilities of the spray, which may cause deviations from linearity.

The relationship between the concentration of each compound and its peak area was found to be linear, as indicated by correlation coefficients higher than 0.992. The minimum detectable amounts (LODs) ranging between 4.4 and 23 μg L^{-1}, were calculated by injecting more and more diluted standard solutions until the peak height was comparable to the noise amplitude. At this stage, the concentration for a signal-to-noise ratio of 3 (the ratio between the peak height with SIM conditions and the noise amplitude) was determined. The LODs for the studied compounds are given in Table 1. The quantification limits were calculated in the same way but for a signal-to-noise ratio of 10. The reproducibility (n = 5) of the compounds that

gave acceptable correlation coefficients at a level of 2 mg L^{-1} varied from 8 to 12% depending on the compound.

Spectral information

The spectral ions obtained from LAS and polar SPC with ESI interface (which is the one used in CE-MS) is well known and is supplied in a former work.[13] The parameters of the mass spectrometer were optimized in order to get the maximum sensitivity. By changing the cone voltage it is possible to induce fragmentation in the source region and consequently to obtain structural information but at the expense of sensitivity. Due to the low amounts injected in CE (in the order of nL) the sensitivity is a serious drawback of this technique. This is the reason that low cone voltages (20 V) were employed in order to not induce fragmentation and improve detection limits.

All the compounds studied were detected as anions and the negative mode of ionization in time-scheduled SIM conditions was used for all of them. Table 1 shows the ions used for the identification and quantification of the target compounds.

Determination of LAS in environmental samples

CE-MS in SIM conditions was used for the confirmation and determination of the studied compounds in real environmental samples. A typical electropherogram of a water extract corresponding to a STP effluent after preconcentration on Isolute ENV+ cartridges is shown in Fig. 1. The main problem in the separation of the studied compounds when working with CE-MS arises from the length of the capillary, which increases the analysis time. In order to make it shorter, pH was increased and acetonitrile instead of propan-2-ol (used for CE-UV analysis) was used as organic modifier. With these conditions, all LAS homologues were analyzed and detected in less than 20 min. This is comparable to the analysis times obtained with liquid chromatography. Although separation is achieved, resolution of LAS is not perfect and the determination of the target compounds had to be achieved by monitoring traces with different *m*/*z* ratios. Total resolution can be achieved with a low pH, but at the expense of the analysis time. In this case shorter analysis times were preferred than a better resolution because of the length of the capillary and the fact that determination is possible because of the MS capacity to acquire each compound separately (see Fig. 1).

Finally, the analytical protocol developed in this work was applied to the analysis of real environmental samples. Table 2 shows the levels of LAS found in influent and effluent waters of three STPs. The levels found are in concordance with the results of previous work[14] with removal efficiencies of LAS near 90%. Looking at Table 2, it is obvious that the lack of sensitivity is the main problem of CE-MS, especially paying attention to the effluent levels of LAS. Although there is only one sample where some LAS homologues are not detected, the levels of the studied compounds in the effluents are found below the quantification limit generally. The levels given, in these cases, are calculated making an extrapolation in the calibration graph, and for this reason these levels are only an approximation and are set between parentheses in Table 2.

Determination of polar SPC

As the determination of LAS in wastewaters was achieved, the possibility to determine their metabolites, the SPC, was investigated. Fig. 2 shows an electropherogram of the simultaneous separation of LAS and some SPC. The elution of SPC at higher retention times than the LAS is due to the fact that SPC possess two moieties with anionic properties and for this reason they are double charged at the working pH. The determination of LAS and all the SPC is achieved in 1 h approximately. This is a drawback for the simultaneous determination of these compounds, taking into account that these analysis times are higher than the time employed in HPLC to determine the same compounds. Furthermore, this is true taking into account than one of the reasons to investigate the possibility of determination of LAS, was that CE is a 'fast' technique of analysis. The loss of this advantage is due to the design of the interface that

Table 1 Linear range, limits of detection and *m*/*z* ions used for quantification of LAS

Compound	Monitored ion *m*/*z*	Linear range/ μg L^{-1}	r^2	LOD/μg L^{-1}
C10LAS	297	30–220	0.995	4.4
C11LAS	311	320–2060	0.998	23
C12LAS	325	300–1950	0.998	22
C13LAS	339	190–1270	0.993	20

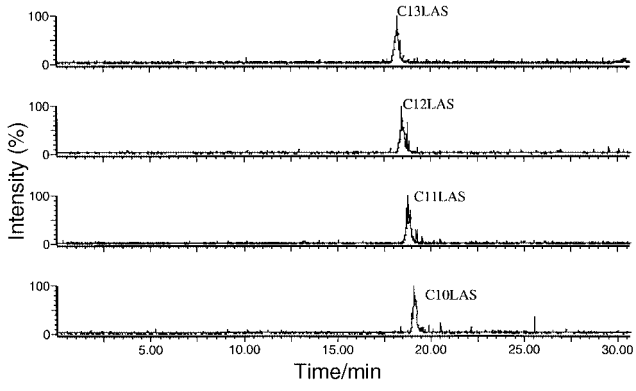

Fig. 1 Selected ion electropherograms of the determination of LAS of an extracted sample from the influent of a STP. Ions monitored corresponded to *m*/*z* 297 for C10LAS, 311 for C11LAS, 325 for C12LAS and 339 for C13LAS.

Table 2 Levels (μg L^{-1}) of the concentration of LAS found in influent and effluent waters of three STPs[a]

	C10LAS	C11LAS	C12LAS	C13LAS	Total LAS
STP1 inf.	180	970	560	200	1900
STP1 eff.	50	200	bql (70)[b]	bql (50)	360
STP2 inf.	120	420	350	bql (130)	1030
STP2 eff.	50	bql (170)	bql (80)	bql (40)	330
STP3 inf.	130	830	420	210	1590
STP3 eff.	bql (40)	bql (90)	nd	nd	130

[a] nd: not detected; bql: below quantification limit. [b] See text.

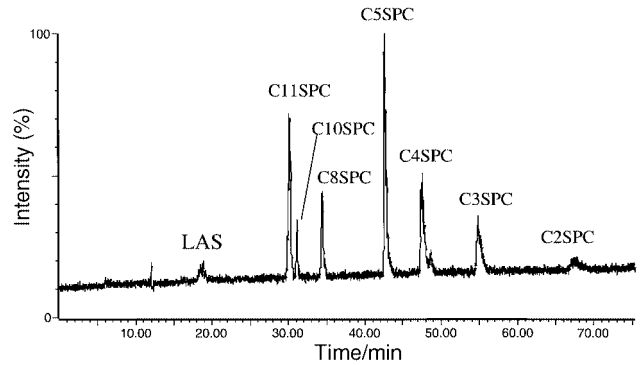

Fig. 2 Total ion current (TIC) CE-ES-MS electropherogram of the simultaneous separation of LAS and SPC of a water extract from the influent of a STP.

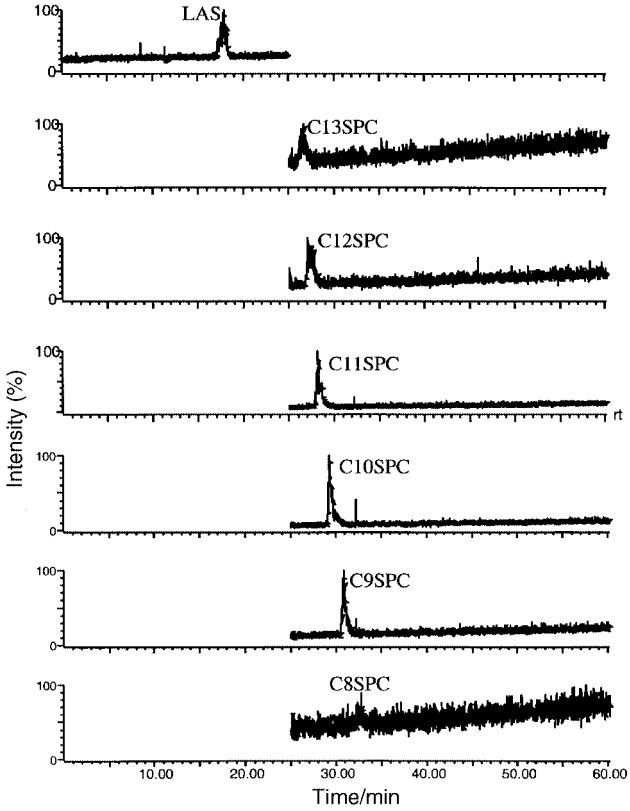

Fig. 3 Time-scheduled selected ion electropherograms of an extracted sample from the influent of a STP. Ions monitored corresponded to *m*/z 299 for C8SPC, 313 for C9SPC, 327 for C10SPC, 341 for C11SPC, 357 for C12SPC and 3372 for C13SPC. TIC of an electropherogram of LAS is also shown.

requires a very long capillary, which increases analysis time. For this reason, diffusion is increased and efficiency of separation is lost. Future designs of CE-MS interfaces have already developed shorter capillaries, thus avoiding many of these problems.

Finally, the determination of SPC and LAS was done for a real sample. Fig. 3 shows a typical electropherogram of the determination of the studied compounds for a waste water extract of the influent of a STP. Although LAS and long SPC (C5 to C13SPC) were detected no short SPC (C2 to C4SPC) were found in this kind of sample. In this case, the reason may be the longer retention times for these compounds. As the retention time increases, the diffusion in the capillary becomes more important and the peak width grows. This fact leads to a decrease in sensitivity.

Although no calibration graphs were produced, some SPC (C5, C8, C10 and C11SPC) were quantified in the influent of a STP approximately, by comparison with the areas found in a standard. The values found ranged from 20 to 130 μg L^{-1}, with higher levels for the long-chain SPC (C10 and C11SPC).

Conclusions

From the results reported in this paper it can be concluded that CE-MS is a feasible technique for determining LAS in complex influent and effluent of STP samples. Furthermore, the determination of SPC, the metabolites of LAS, is also possible. The use of an internal standard is recommended to minimize the effect of the instabilities of the spray and to achieve good linearity. The lack of sensitivity can also be partially overcome by the combination of automated SPE using the ASPEC XL, but is still the major problem of this technique.

The levels of LAS found in influent and effluents of STP are similar to the results given in other work using LC-ESI-MS, with elimination rates between 80 and 90%.

Acknowledgements

This work has been supported by the Environment and Climate Program of the European Union WASTE WATER CLUSTER Project PRISTINE (ENV4-CT97-0494) and by CICYT (AMB1999-0167-CE). We thank IST for providing the SPE cartridges.

References

1 J. A. Field, Th. M. Field, Th. Ploguer, Th. Giger and W. Giger, *Environ. Sci. Technol.*, 1994, **28**, 497.
2 J. A. Field, D. J. Miller, Th. M. Field, S. B. Hawthorne and W. Giger, *Anal. Chem.*, 1992, **64**, 3161.
3 A. J. Borgerding and R. Hites, *Anal. Chem.*, 1992, **64**, 1449.
4 H. Sirén and R. Sulkava, *J. Chromatogr.*, 1995, **717**, 149.
5 J. Riu, I. Schönsee and D. Barceló, *J. Mass Spectrom.*, 1998, **33**, 653.
6 K. Heining, C. Vogt and G. Werner, *Analyst*, 1998, **123**, 349.
7 K. Heining, C. Vogt and G. Werner, *J. Chromatogr. A*, 1996, **745**, 281.
8 C. Vogt, K. Heining, B. Langer, J. Mattusch and G. Werner, *Fresenius' J. Anal. Chem.*, 1995, **352**, 508.
9 P. L. Desbène, C. Rony, B. Desmazières and J. C. Jacquier, *J. Chromatogr.*, 1992, **608**, 375.
10 J. Riu, P. Eichhorn, J. A. Guerrero, Th. P. Knepper and D. Barceló, *J. Chromatogr. A.*, 200, **889**, 221.
11 J. Riu and D. Barcelò, in *Sample Handling and Trace Analysis of Pollutants: Techniques, Applications and Quality Assurance*, ed. D. Barceló, Elsevier, New York, 2000, ch. 17.
12 C. Molina, M. Honing and D. Barceló, *Anal. Chem.*, 1994, **66**, 4444.
13 J. Riu, E. Gonzalez-Mazo, A. Gomez-Parra and D. Barceló, *Chromatographia*, 1999, **50**, 275.
14 P. Eichhorn, M. Petrovic, D. Barceló and Th. P. Knepper, *Vom Wasser*, 2000, **95**, 245.