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Development of a method for the simultaneous analysis of anionic and non-ionic surfactants and their carboxylated metabolites in environmental samples by mixed-mode liquid chromatography–mass spectrometry

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Abstract

A new methodology capable of performing the simultaneous analysis of the main surfactants – linear alkylbenzene sulfonates (LAS), alkyl ethoxysulfates (AES), alkyl sulfates (AS), nonylphenol polyethoxylates (NPEOs) and alcohol polyethoxylates (AEOs) – and their carboxylated metabolites – sulfophenyl carboxylic acids (SPCs) and alkylphenol ethoxycarboxylates (APECs) – in environmental samples has been developed for the first time. Extraction is carried out by solid-phase extraction (SPE) and pressurized liquid extraction (PLE) from water and sediment, respectively. Identification and quantification of the target compounds is performed using a liquid chromatography–mass spectrometry (LC–MS) system equipped with an electrospray interface (ESI) operating in mixed-mode. Optimization of parameters such as pH, ionic strength, temperature and solvents has been carried out in order to obtain recoveries in the range from 70 to 107% for most homologs, while the limits of detection are 0.05–0.5 ng mL^{-1} in water and 1–10 ng g^{-1} in sediment. The proposed methodology has been applied for the simultaneous determination of all the target compounds in samples taken from aquatic ecosystems in the SW of Spain. Values for LAS, AS, AES, NPEOs and AEOs are up to 38.7, 3.0, 2.9, 5.0 and 1.2 μg L^{-1} in waters, and in the ranges of 1.73–12.80, 0.11–0.24, 0.02–0.59, 1.94–2.70 and 0.64–3.64 mg kg^{-1} in sediments, respectively. The highest concentrations of metabolites found in water are 149.6 μg L^{-1} of SPCs and 3.9 μg L^{-1} of APECs.

Keywords: Sediments; Waters; Anionic surfactants; Non-ionic surfactants; Carboxylic acids; Pressurized liquid extraction; Mass spectrometry

1. Introduction

Since the middle of the past century, the use of synthetic surfactants in a wide variety of applications – mainly as surface-active ingredients in detergents, shampoos and other cleaning compounds – has shown a fast and continuous growth, reaching an annual worldwide production volume of 15,000 million kg [1]. The two main groups of surfactants, classified according to the charge in their hydrophilic moiety, are the anionic and non-ionic types. Data available from CESIO (Comité Européen des Agents de Surface et de leurs Intermediaries Organiques) show that these two groups account for 90% of the total production of surfactants in the EU. Among the anionics, which constitute 40% of the European market, linear alkylbenzene sulfonates (LAS) have the highest production level followed by alkyl ethoxysulfates (AES) and their non ethoxylated homologs, the alkyl sulfates (AS). In the case of the non-ionics, which account for 50% of the total production, alkylphenol polyethoxylates (APEOs) are being phased out due to the estrogenic properties of some of their metabolites [2], and have recently been displaced from first to second position. Thus, nonylphenol polyethoxylates (NPEOs) – the main type of APEOs – are being replaced by alcohol polyethoxylates (AEOs), which overall are the type of synthetic surfactant with the highest production volume in the EU today. The chemical structure of all these compounds is illustrated in Fig. 1, this shows that they are manufactured as complex mixtures containing homologs with varying length of alkyl chain and/or ethoxymers with differing degrees of ethoxylation.

Degradation of surfactants takes place with high effectiveness in wastewater treatment plants (WWTPs) [3] mainly throughout aerobic processes that generate carboxylated acids (Fig. 1)

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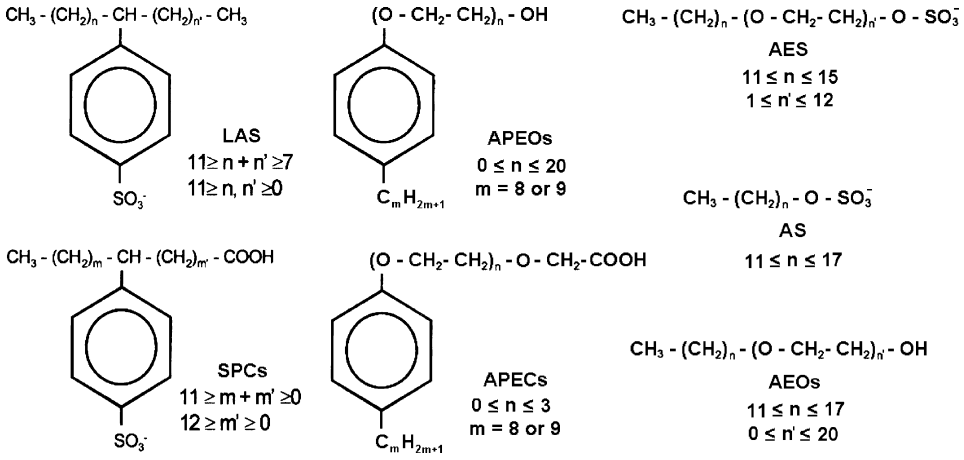

Fig. 1. Chemical structures for the main surfactants and their carboxylated metabolites.

such as sulfophenyl carboxylic acids (SPCs) and alkylphenol ethoxycarboxylates (APECs) in the cases of LAS and APEOs, respectively [4]. In spite of this, significative quantities of these chemicals reach aquatic environments in the form of discharges of both treated and untreated wastewater as well from the deposition of WWTP sludges. Numerous articles regarding methodologies for determining environmental levels of LAS and NPEOs have been published during the last two decades, and these have been reviewed by Reemtsma [5] and Lee [6]. Usually the presence of an aromatic ring indicates that these two compounds represent good candidates for their analysis by means of liquid chromatography coupled to ultraviolet and fluorescence detectors (LC–UV–FLU) [7–9]. Gas chromatography with mass spectrometry (GC–MS) has been already used but it implies further sample treatment because derivatization is needed in the case of LAS homologs and only short chain NPEOs ethoxymers can be analyzed due to volatilization issues [10,11]. Less frequently, other papers have also described the identification of SPCs and APECs using both LC–UV–FLU and GC–MS techniques [11–14]. Analysis of AES and AEOs by these previously-cited techniques is more complicated due to their lack of UV absorbance, fluorescence and volatility, and because they are constituted by several homologs each containing tens of different ethoxymers that often co-elute in chromatographic separations [15–17]. The recent development of new interfaces such as electrospray interface (ESI) has facilitated this work by allowing the determination not only of these surfactants but also of LAS and NPEOs by liquid chromatography–mass spectrometry (LC–MS), as has been reviewed recently by Di Corcia [18] and Reemtsma [19]. Unequivocal identification of metabolites such as SPCs and APECs has also been possible by the use of this technique [20,21]. Due to their charges, usually the analysis of anionics – LAS, AES, SPCs and APECs – is performed under negative ion mode (NI), separately from the non-ionic NPEOs and AEOs, detected in positive ion mode (PI) [20–26], although some attempts have been made with mixed-mode to achieve simultaneous detection of NPEOs and nonylphenol ethoxycarboxylates (NPECs) [27,28].

Parallel to this evolution in the chromatography and detection field, extraction and isolation procedures for surfactants and their metabolites have also been improved. Generally, after a prior Soxhlet or ultrasonic extraction with methanol, hexane or other solvent mixtures from sediments or sludges, LAS and NPEOs in aqueous extracts and water samples are preconcentrated and purified by means of solid phase extraction (SPE) [10,13,22,23]. Different SPE cartridges such as octadecylsilica (C_{18}) or graphitized carbon black (GCB) have been tested with satisfactory results for these surfactants but lowering of pH and/or salt addition is needed in order to enhance the recoveries for their more polar carboxylated intermediates [12,13]. SPE and Soxhlet extraction are also employed for the extraction and isolation of AEOs [23,29,31] and AES [15,30,32] although fewer protocols have been developed for these surfactants. In order to minimize the time and solvent consumption in Soxhlet and ultrasonic extractions, new extraction techniques such as pressurized liquid extraction (PLE) have been developed. Several articles have described the use of PLE in order to extract LAS and NPEOs and their metabolites from solid matrices [24,25,33–35] but its efficiency with respect to AES and AEOs has not yet been optimized [25].

In spite of the numerous methods presented above for the determination of surfactants in environmental samples, there are some current inconveniences: (a) most of the work done to date has been related only to the extraction of the compounds in water samples and mainly to the analysis of LAS and NPEOs; (b) commonly each surfactant has had to be determined by a specific protocol; and (c) methodologies regarding the determination of some surfactants and metabolites, such as AES and SPCs, respectively, are scarce and they are not optimized. These issues mean that currently, to carry out a global study of the distribution, fate and effects of surfactants in specific aquatic ecosystems is usually a tedious, expensive and time-consuming procedure. As a consequence, although the environmental behaviour of LAS and NPEOs is moderately well-known, information about other related and relevant compounds such as AES, AS and AEOs and metabolites is scarce. Therefore, in order to resolve these deficiencies we have proposed and optimized a new and rapid methodology for the simultaneous extraction, preconcentration, purification, identification and quantification of all the major surfactants (LAS,

AES, AS, NPEOs and AEOs) and some carboxylated intermediates (SPCs and APECs) in both water and sediment samples. Only one single extraction from sediment and water, by means of PLE and SPE, respectively, and one single-run by means of mixed-mode LC–MS are required. Moreover, this procedure has been validated by determining the concentrations of the target compounds at ppb level in several water and sediment samples taken from aquatic ecosystems located in the southwest of Spain.

2. Experimental

2.1. Materials and standards

Triethylamine and all solvents were of chromatography quality, purchased from Scharlau (Barcelona, Spain): hexane (Hex), dichloromethane (DCM), acetone (Ace), methanol (MeOH) and ethyl acetate. Orthophosphoric acid, sodium sulfate, sodium chloride, sodium acetate, potassium dihydrogenphosphate and formaldehyde were purchased from Panreac (Barcelona, Spain) and water was Milli-Q quality. The solid-phase extraction mini-columns used (500 mg) were supplied by Varian (Bond Elut C_{18}).

The 99% pure 2ΦC_{16} LAS internal standard and the commercial LAS mixture were supplied by Petroquimica Española (PETRESA), with the following homolog distribution for the latter: C_{10} (10.9%), C_{11} (35.3%), C_{12} (30.4%), C_{13} (21.2%) and C_{14} (1.1%). Commercial NPEOs, AEOs and AES mixtures were supplied by KAO Corporation (KAO) with the following homolog distribution: C_{12} (53.4%), C_{14} (32.6%), C_{16} (14.0%) for AEOs and C_{12} (68.5%), C_{14} (29.8%), and C_{16} (1.7%) for AES. Their ethoxylated chains have an average number of 12.2, 11.5 and 3.4, respectively. The 99% pure 5ΦC_5 to 13ΦC_{13} SPCs, octylphenol and nonylphenol monoethoxycarboxylates (OP_1EC and NP_1EC) standards were kindly supplied by F. Ventura (AGBAR, Spain) and J.A. Field (Oregon State University, USA).

2.2. Study area and sample collection

The samples were collected at three points from different aquatic environments in the southwest of Spain. The first point (P1) is located at the sea, to the north of the Bay of Cadiz (36°34′25.62″N, 6°14′43.01″E), near to the mouth of the Guadalete river. The second sampling point (P2) is in the estuary of the Guadalete river (36°38′24.63″N, 6°7′44.85″E), situated one hundred meters from the discharge outlet of a WWTP which treats the wastewaters of a town of 200,000 inhabitants and discharges into the river. The third station (P3) is located at the Bornos dam (36°48′42.18″N, 5°43′42.06″E), which has a water capacity of 200 hm^3, a protected area employed only for recreational activities. Superficial waters were taken using 2.5 L amber glass bottles and adding 4% of formaldehyde. The bottom sediment (top 10 cm) was sampled from a boat using a Van Veen grab. All samples were kept at 4 °C during their transport to the laboratory and frozen until their analysis. In the laboratory the sediment was dried in a heater at 75 °C until constant weight, and later milled and strained through a 63 μm sieve.

2.3. Solid-phase extraction and pressurized liquid extraction

Surfactants were extracted from the sediment samples in triplicate using pressurized liquid extraction by means of an accelerated solvent extraction ASE 200 unit from Dionex. Quantities of dried and sieved sediment samples (4 g) were mixed together with 16 g of sodium sulfate and placed into steel cells (22 mL). Methanol was passed through the heated (120 °C) and pressurized (1500 psi) PLE cells for three cycles of 5 min each. Subsequently the extracts were evaporated until 1 mL and redissolved in 100 mL of water in an ultrasonic bath.

These extracts and the water samples, also in triplicate, were purified and preconcentrated by solid-phase extraction using mini-columns of the hydrophobic C_{18} type in an automated SPE AutoTrace unit (Zymark). These C_{18} mini-columns were rinsed with 10 mL of methanol and 5 mL of water prior to passing the 100 mL of aqueous sample previously acidified to pH 2.5 with orthophosphoric acid. They were then washed with 5 mL of water and eluted with 5 mL of methanol/acetone 1:1 and 5 mL of dichloromethane/ethyl acetate 1:1. Finally, the eluate was evaporated to dryness and redissolved in 1 mL of a methanol/water 8:2 solution containing 1 mg L^{-1} of C_{16} LAS as internal standard and 50 μM of sodium acetate.

The recoveries (percent of standard added to sample recovered during extraction) and reproducibility (relative standard deviation for quintuplicate analysis) of the methodology were determined by the analysis of spiked samples. Commercial standards of the surfactants were employed to spike 1 L of water to 100 ng mL^{-1} and 100 g of non polluted sediments to 2.5 mg kg^{-1}. Concentrations for the metabolites were 50 ng mL^{-1} and 1.25 mg kg^{-1} in the spiked water and sediment, respectively. Finally, amounts of 4 g of these spiked sediments as well as aliquots of 100 mL of spiked seawater were treated in the same way as the environmental samples.

2.4. Liquid chromatography–mass spectrometry

The HPLC system consisted of a Spectrasystem liquid chromatograph with autosampler, with the injection volume set to 100 μL. The chromatographic separation was done using a reversed-phase C-18 analytical column (LiChrospher 100 RP-18) of 250 mm × 2 mm and 3 μm particle diameter, from Merck. The detection was carried out using a LCQ ion-trap mass spectrometer (Thermo), equipped with an atmospheric pressure ionization source with electrospray interface. From 0 to 38 min ESI was used in full-scan negative ion mode in order to detect SPCs, APECs, LAS, AS and AES, setting the ion fragmentation energy to 37 V and scanning the mass/charge (*m/z*) range between 80 and 800. From 38 to 50 min the ESI was changed to full-scan positive ion mode to allow the detection of NPEOs and AEOs, setting the ion fragmentation energy to 15 V and the *m/z* range between 120 and 1000. The following mobile phase was used for an effective separation of all homologs:

Table 1
Mass/charge (*m/z*) ratios scanned to identify the target compounds

Compound	Homolog/ ethoxymer[a]	Ion 1 (*m/z*)[b]	Ion 2 (*m/z*)[c]
LAS	n_C = 10–13	297–339 (±14)	183
SPCs	n_C = 5–13	257–369 (±14)	183
AS	n_C = 12–16	265–321 (±28)	97
C_{12}AES	n_{EO} = 1–11	309–749 (±44)	97
C_{14}AES	n_{EO} = 1–11	337–777 (±44)	97
C_{16}AES	n_{EO} = 1–10	365–761 (±44)	97
NPEOs	n_{EO} = 1–17	287–991 (±44)	–
AP_1ECs	n_C = 8–9	263–277 (±14)	205–219 (±14)
C_{12}AEOs	n_{EO} = 1–17	253–957 (±44)	–
C_{14}AEOs	n_{EO} = 1–17	281–985 (±44)	–
C_{16}AEOs	n_{EO} = 1–16	309–969 (±44)	–

[a] nc and nEO represent the number of carbon atoms per homolog and number of ethoxylated groups per ethoxymer, respectively.
[b] Ion 1 represents the quasimolecular ion $[M-H]^-$ in the case of LAS, SPCs, AS, AES and AP1ECs and the sodium adduct $[M+Na]^+$ for the NPEOs and AEOs. Numbers in brackets represent the *m/z* difference between homologs/ethoxymers.
[c] Ion 2 represents a specific fragment ion for the named compound.

methanol (A) and water with 5 mM acetic acid and 5 mM triethylamine (B). The elution gradient for the first 5 min was 0% A, and was increased linearly to 70% A over 15 min, then increased linearly to 100% A over another 15 min and kept isocratic for yet another 15 min. The flow rate was 0.15 mL min^{-1}. Values of other MS parameters were needle tip voltage 4.5 kV, gas stealth flow 60 mL min^{-1}, and ion source temperature 220 °C.

Table 1 shows the ions used for the identification and quantification of the target compounds. Identification of each homolog of LAS and SPCs was carried out by monitoring their quasimolecular ions $[M-H]^-$ and their specific fragment ion at *m/z* 183. In the cases of AS and AES their specific fragment ion was *m/z* 97, and *m/z* 205 and 219 for OPECs and NPECs, respectively. Monitoring of their sodium adduct ions $[M+Na]^+$ was performed in the case of NPEOs and AEOs. Surfactant concentrations and their metabolites were determined by measuring the peak areas of the quasimolecular or adduct ions using external standard solutions (0.5–25 mg L^{-1}) prepared in methanol/water 1:1 and C_{16} LAS as internal standard (1 mg L^{-1}). In the case of AES, NPEOs and AEOs, all the ethoxymer areas corresponding to the same homolog were summed in order to obtain the overall homolog concentration. Clean water, sediment extracts and a methanol/water 1:1 solution were spiked with 1 mg L^{-1} of standards to check the influence of ion suppression (suppression of the analyte signals caused by high concentrations of matrix components) on the MS detection of the target compounds.

3. Results and discussion

3.1. Optimization of solid-phase extraction

The percentage recoveries for the SPE extraction procedure are shown in Tables 2 and 3. Several protocols were tested employing C_{18} mini-columns in order to obtain satisfactory results when performing a simultaneous extraction for all the target compounds. Table 2 shows the influence of the ionic strength and pH of the aqueous samples in the recoveries using the elution protocol named A1, a modification of a previous protocol reported by Petrovic and Barceló [22] which was validated for a sequential extraction of AEOs and NPEOs, and LAS and NPECs, into two respective fractions. At neutral pH this procedure is effective for the preconcentration of homologs of LAS (75–93%), NPEOs (84%) and AS, AES and AEOs (74–99%) with the shortest alkyl chain (C_{12}). In the case of the SPCs the recovery values are increased in function of the length of the alkyl chain. The explanation is that the interaction between the mini-columns and the shortest alkyl chain SPCs is too poor due to their high polarity so a part of these compounds is washed out when the sample passes through the mini-columns. An opposite trend can be observed for most homologs of the surfactants, where the extraction percentages decrease towards the longest alkyl chain homologs. The very strong interaction of these most hydrophobic homologs of AS, AES and AEOs with octadecylsilica minimizes their elution with the solvent mixture tested.

Table 2
Influence of salt addition (250 g L^{-1} of NaCl) and pH in the SPE for the elution procedure A1 (5 mL of DCM/Hex 1:4 and 5 mL of DCM/Ace 9:1)

Compound	pH = 7		pH = 2.5		Salt and pH = 2.5	
	%	SD	%	SD	%	SD
C_{10}LAS	93	5	92	5	87	6
C_{11}LAS	88	4	85	7	62	8
C_{12}LAS	83	6	82	4	60	5
C_{13}LAS	75	3	78	6	65	6
C_5SPC	1	2	51	4	54	16
C_6SPC	6	4	71	3	47	16
C_7SPC	14	5	79	1	36	6
C_8SPC	34	11	82	2	39	6
C_{10}SPC	52	5	91	7	27	1
C_{11}SPC	70	3	89	6	37	4
C_{12}SPC	72	2	93	12	37	2
C_{13}SPC	89	3	108	10	35	3
C_{12}AS	99	4	115	6	60	2
C_{14}AS	49	5	34	4	50	3
C_{16}AS	54	4	26	8	53	7
C_{12}AES	74	3	85	6	42	4
C_{14}AES	55	5	43	5	68	5
C_{16}AES	41	4	37	6	35	10
NPEOs	84	12	90	10	51	16
OP_1EC	39	5	44	4	25	8
NP_1EC	31	4	29	2	51	8
C_{12}AEOs	80	6	86	11	69	9
C_{14}AEOs	57	7	63	17	52	13
C_{16}AEOs	40	8	51	15	13	6

Recoveries in percentage (%) and standard deviations (n = 5) for the target compounds are shown.

The first issue, lower recoveries for the more polar metabolites, has been solved by several authors [12,35] by lowering the pH so the dissociation of the carboxylic groups is minimized and the interaction of these compounds with the octadecylsilica is encouraged. By lowering the pH to 2.5 this effect can be observed in Table 2, where the efficiency in the extraction of shorter alkyl chain SPCs homologs has been dramatically

Table 3
Influence of the solvent mixture in the SPE for the elution procedures A1, A2, A3 and A4 (pH = 2.5 and without salt addition)

Compound	A1[a]		A2[b]		A3[c]		A4[d]	
	%	SD	%	SD	%	SD	%	SD
C10 LAS	92	5	96	2	100	4	95	3
C11 LAS	85	7	89	2	99	4	96	0
C12 LAS	82	4	89	2	94	2	94	3
C13 LAS	78	6	86	2	91	2	78	3
C5 SPC	51	4	55	5	38	8	35	4
C6 SPC	71	3	74	7	52	6	59	3
C7 SPC	79	1	81	9	69	9	65	1
C8 SPC	82	2	84	9	67	8	69	7
C10 SPC	91	7	92	9	92	7	88	2
C11 SPC	89	6	94	6	93	8	97	4
C12 SPC	93	12	98	13	91	12	104	16
C13 SPC	108	10	104	14	103	12	106	13
C12 AS	115	6	120	8	114	5	104	9
C14 AS	34	4	36	6	50	4	89	8
C16 AS	26	8	32	5	35	5	59	8
C12 AES	85	6	93	5	96	5	91	2
C14 AES	43	5	50	2	54	1	82	4
C16 AES	37	6	42	4	24	2	67	2
NPEOs	90	10	40	7	57	8	72	4
OP_1EC	44	4	42	5	38	0	72	14
NP_2EC	29	2	21	3	35	1	71	17
C12 AEOs	86	11	34	5	87	5	86	17
C14 AEOs	63	17	27	5	68	12	61	17
C16 AEOs	51	15	30	5	49	6	59	13

Recoveries in percentage (%) and standard deviations ($n = 5$) for the target compounds are shown.
[a] Elution with 5 mL of DCM/Hex 1:4 and 5 mL of DCM/Ace 9:1.
[b] Elution with 10 mL of MeOH.
[c] Elution with 5 mL of MeOH and 5 mL of DCM.
[d] Elution with 5 mL of MeOH/Ace 1:1 and DCM/Ethyl acetate 1:1.

improved (from 6 to 71% in the case of C_6SPC as an example), while the recoveries from the rest of the target compounds remain practically unaltered. Addition of salts has been also suggested [13] in order to increase the ionic strength of the aqueous matrices and therefore, drive the organic compounds into the octadecylsilica phase (a process known as salting-out). Addition of 25 g of sodium chloride and 0.5 g of potassium dihydrogenphosphate to the 100 mL of water samples at pH 2.5 was performed in order to test this effect. As expected, the results in Table 2 show that recoveries from C_5 to C_8 SPCs are improved in comparison with the absence of additional salt and neutral pH, but there are severe losses in the case of the most hydrophobic compounds. Taking into account that the use of most hydrophobic solvents (data not shown) does not improve the recovery percentages, these losses could be attributed to sorption onto the glass of the receptacles and onto the tubing of the SPE AutoTrace unit.

In order to solve the second issue, the elution of the most hydrophobic surfactants, the influence of the solvent mixture used for the elution in the SPE has been also tested. Apart from A1 (elution with 5 mL of DCM/Hex 1:4 followed by 5 mL of DCM/Ace 9:1), three other different procedures were developed at pH 2.5 and without salt addition. The first one, A2, consists of an elution with 10 mL of MeOH. Methanol is a polar solvent commonly used by most authors for an effective extraction of LAS [9] and SPCs [13,35]. Table 3 shows that recoveries are good for these compounds (86–96% for LAS and 74–104% for SPCs) as well as for C_{12} AS and C_{12} AES but the longer alkyl chain homologs of these two surfactants and the more non-polar NPEOs and AEOs suffer from a sharp decrease in the recovery percentages, in comparison with A1, where hexane and dichloromethane prove to be more effective. In the case of the procedures A3 (elution with 5 mL of MeOH and 5 mL of DCM) and A4 (elution with 5 mL of MeOH/Ace 1:1 and 5 mL of DCM/ethyl acetate 1:1) the recovery values for LAS are similar to those reported for procedures A1 and A2 although shorter alkyl chain SPC percentages show a slight decrease (Table 3). Extraction of AS, AES and AEOs, however, is generally more efficient for A3 and, especially, for the procedure A4, where the highest recovery values are obtained for the more non-polar homologs of AS (59–104%), AES (67–91%) and AEOs (59–86%) as well as for the carboxylated metabolites APECs (71–72% with respect to 21–44% obtained for the rest of the procedures). Standard deviations are low enough in all the four procedures to ensure good reproducibility of the results.

Taking into account all the data presented in Tables 2 and 3 and the considerations above, the usage of octadecylsilica minicolumns together with the acidification of the samples to pH 2.5 and the elution with a mixture of methanol/acetone 1:1 and dichloromethane/ethyl acetate 1:1 account is recommended for an effective extraction, preconcentration and purification of most homologs of the main surfactants and their carboxylated metabolites from aqueous matrices.

3.2. Optimization of pressurized liquid extraction

In order to achieve a fast and efficient extraction of the target compounds from sediments using PLE, the correct operational parameters such as temperature and extraction solvents must be selected. To check the influence of the temperature, spiked sediment samples were extracted using a mixture of MeOH/Ace 1:1 at four different temperatures (75, 100, 125 and 150 °C) with subsequent SPE clean-up. Results are shown in Table 4 and, despite the fact that this solvent mixture was not effective at all for extracting SPCs, moderate to high recovery values were obtained for the rest of the surfactants and metabolites. In general terms, there were no great differences in the extraction for the four selected temperatures, although lower recovery percentages are found for the most hydrophobic homologs of the surfactants in the case of 75 °C. The increase in their recovery is significant when temperature is raised to 100 and 125 °C, but it is not enough to justify extraction at 150 °C, which at the same time, would cause substantial losses for the shorter ethoxylated chain NPEOs due to volatilization [21]. Previous papers have reported this positive correlation between extractability and extraction temperature in the case of NPECs [21] and LAS [36]. Therefore, the optimum value should be between 100 and 125 °C, as has been suggested by most authors for the extraction of NPEOs [24,25,33] and LAS [24,25,34–36].

Selection of the proper solvent or solvent mixture is another key factor in PLE. Similar to the case of SPE in aqueous matri-

Table 4
Influence of the temperature in the PLE (solvent = MeOH/Ace 1:1, P = 1500 psi, t = 3 cycles of 5 min each)

Compound	75 °C		100 °C		125 °C		150 °C	
	%	SD	%	SD	%	SD	%	SD
C_{10}LAS	100	13	102	4	97	7	102	14
C_{11}LAS	89	15	98	3	103	12	114	8
C_{12}LAS	79	10	96	7	98	9	103	3
C_{13}LAS	67	10	82	11	84	8	78	3
C_5SPC	10	3	16	4	8	2	15	3
C_6SPC	23	3	24	6	19	1	21	4
C_7SPC	32	5	27	4	25	1	28	4
C_8SPC	26	5	24	2	22	1	25	4
C_{10}SPC	21	3	24	3	16	2	24	3
C_{11}SPC	26	6	23	2	21	2	23	3
C_{12}SPC	24	4	25	3	20	1	24	1
C_{13}SPC	26	3	24	2	21	1	25	2
C_{12}AS	107	17	100	13	108	9	104	5
C_{14}AS	91	7	106	26	100	13	97	3
C_{16}AS	70	16	73	12	75	13	65	12
C_{12}AES	82	6	71	2	82	5	72	1
C_{14}AES	78	16	96	9	109	5	87	8
C_{16}AES	66	9	69	5	73	6	70	9
NPEOs	70	10	87	8	92	7	83	11
OP_1EC	66	8	59	4	69	3	61	2
NP_1EC	74	8	75	1	68	4	74	1
C_{12}AEOs	54	11	57	10	74	8	69	12
C_{14}AEOs	55	12	66	8	72	5	66	9
C_{16}AEOs	47	9	48	12	66	11	63	10

Recoveries in percentage (%) and standard deviations (n = 3) for the target compounds are shown.

Table 5
Influence of the solvent mixture in the PLE (T = 120 °C, P = 1500 psi, t = 3 cycles of 5 min each)

Compound	MeOH/Ace 1:1		MeOH		MeOH/DCM 7:3	
	%	SD	%	SD	%	SD
C_{10}LAS	107	11	105	10	94	6
C_{11}LAS	103	6	99	6	105	6
C_{12}LAS	95	5	92	10	99	6
C_{13}LAS	85	3	78	9	89	3
C_5SPC	24	8	73	9	38	6
C_6SPC	29	9	86	10	55	6
C_7SPC	36	13	87	1	69	7
C_8SPC	44	12	90	5	70	8
C_{10}SPC	45	4	82	1	61	6
C_{11}SPC	41	12	73	1	47	4
C_{12}SPC	59	13	90	10	63	6
C_{13}SPC	44	11	70	1	44	8
C_{12}AS	92	14	89	11	94	7
C_{14}AS	72	5	73	12	77	2
C_{16}AS	65	4	64	9	75	9
C_{12}AES	92	8	88	14	84	5
C_{14}AES	82	2	75	5	105	5
C_{16}AES	71	12	63	5	86	8
NPEOs	86	2	70	3	76	3
OP_1EC	55	5	65	6	56	2
NP_1EC	63	12	74	0	62	5
C_{12}AEOs	77	1	73	4	86	3
C_{14}AEOs	64	3	60	2	74	4
C_{16}AEOs	68	8	57	3	68	7

Recoveries in percentage (%) and standard deviations (n = 5) for the target compounds are shown.

ces, to extract a wide variety of organic compounds from sediments or sludges, certain solvents are recommended for the more hydrophobic compounds (e.g., hexane or dichloromethane in the case of short ethoxylated chain NPEOs [8,22,33]) while more polar solvents such as methanol are required to ensure high efficiency in the extraction of the more hydrophilic analytes LAS [7,10] and SPCs [13,35]. Therefore, we have tested three different solvent mixtures in spiked sediment samples at the selected temperature of 120 °C. The extraction process by PLE is efficient for LAS (85–107%), AS (65–92%), AES (71–92%) and NPEOs (86%) employing a mixture of MeOH/Ace 1:1 (Table 5). Similar results have been obtained previously by González et al. [24] for NPEOs using the same solvents, although LAS recovery values dropped to 56–72% because a lower temperature (50 °C) was used in order to avoid volatilization of alkylphenols. In the case of AEOs, however, recoveries are only moderate (57–68%) and, as mentioned above, are low for SPCs (24–59%). Methanol as the sole extraction solvent has been employed successfully for the extraction of LAS [34,36] in sediments by PLE. Table 5 shows that this solvent improves recovery values for SPCs to 70–90% with a slight alteration for the rest of the target compounds when compared with the MeOH/Ace 1:1 mixture. Lower values for LAS and SPCs have been obtained in soils when 10% of water was added to methanol by Eichhorn et al. [35], apart from the fact that this protocol is not applicable to sediments because clays are not permeable. The last solvent mixture tested is MeOH/DCM 7:3, which has proven to be efficient for the extraction of LAS, NPEOs, NPECs and AEOs in sewage sludges by sonication [22]. In spite of the lower polarity of the mixture in comparison to pure methanol, extraction of longer alkyl chain homologs of the surfactants is only slightly improved in the case of AS, AES and AEOs whereas SPCs suffer from lower recovery percentages (Table 5). Results of this kind are expected when trying to develop a valid methodology for the simultaneous extraction of analytes with a wide range of polarity, therefore, we recommend the use of methanol alone as extraction solvent in the case of the major surfactants and their carboxylated metabolites.

The efficiency of PLE is comparable with traditional techniques such as Soxhlet and ultrasonic extractions, also the entire extraction process for all target compounds is completed in only 15 min by PLE while sonication takes 1 h [22] and Soxhlet extraction from 4 to 12 h [13,23,30]. Also the volume of solvent used is minimized to 30–40 mL and the reproducibility is good according to the low standard deviations obtained. Overall, from the data shown in Tables 4 and 5, it can be inferred that the extraction of the target compounds from sediments can be achieved rapidly by employing PLE at 120 °C and methanol as solvent, as against other more time- and solvent-consuming techniques.

3.3. Chromatographic separation, calibration and limits of detection

Fig. 2 shows the chromatograms resulting from applying the optimized methodology described above to a standard mixture

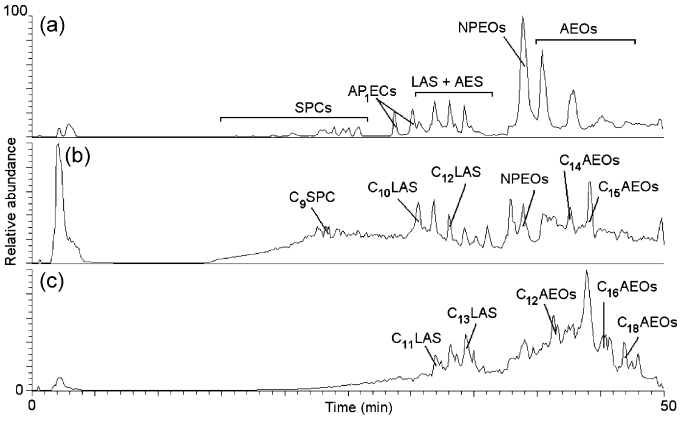

Fig. 2. Full-scan LC/ESI/MS mix-mode chromatograms corresponding to: (a) a standard mixture; (b) a water sample after SPE; and (c) a sediment sample after PLE + SPE. Chromatograms were obtained under the specific analytical conditions described in Section 2.4.

of surfactants and metabolites (Fig. 2a) and to water and sediment samples (Figs. 2b and c, respectively) collected from the study area. From 0 to 38 min the anionic compounds are detected in NI mode while the polarity of the instrument was switched to PI mode after the elution of the last LAS homolog, in order to detect NPEOs and AEOs. Full-scan mode chromatograms show that most homologs of the different surfactants and metabolites are effectively separated and analyzed in a single chromatographic run by the gradient conditions employed along the RP-18 column selected. Shorter alkyl homologs of the more polar compounds such SPCs and NPECs elute first, followed by LAS homologs (including the internal standard C_{16} LAS) and AES ethoxymers. The non-ionic surfactants are the last analytes to elute because their greater hydrophobicity increases their retention time in the column. Elution of LAS and AES is simultaneous because they have homologs with the same length of alkyl chain with similar properties [30]. In the case of NPEOs and AEOs all their ethoxymers with the same length of alkyl chain co-elute under the same chromatographic peak.

In spite of the limitations of the octadecylsilica column for achieving a complete separation of every homolog and ethoxymer, the use of mass spectrometry enables all the target compounds to be distinguished because of their specific ions, represented in Table 1. Fig. 3 shows the characteristic mass spectra of several surfactants and metabolites. It can be observed that, for the anionics, identification of every single analyte can be performed by means of its quasimolecular ion $[M-H]^-$ while their characteristic specific fragment ions can be used to distinguish between different groups: *m/z* 183 in the case of LAS and SPCs (Fig. 3a and b, respectively), *m/z* 205–219 for AP_1ECs (Fig. 3d) and *m/z* 97 for AS and AES (Fig. 3e). Information on the ethoxymer distribution in the case of NPEOs (Fig. 3c) and AEOs (Fig. 3f) homologs can be obtained by extracting the selected *m/z* ratios for the sodium adducts $[M+Na]^+$ formed by the addition of sodium acetate.

Further, advantages in the use of MS over UV and fluorescence detectors are clearly illustrated in Fig. 4, a representation of several extracted ion chromatograms from a spiked water sample. In the first chromatogram (Fig. 4a) it can be observed how effective is the separation of the different SPCs and LAS homologs by extracting their specific fragment ion *m/z* 183, which together with their $[M-H]^-$ ions, permits an unequivocal identification and easy quantification. Chromatographic separation of NPEOs from their carboxylated metabolites is also achieved (Fig. 4b) together with differentiation between OPECs and NPECs by means of the *m/z* ratios 205 and 219, respectively. Fig. 4c shows the elution sequence of

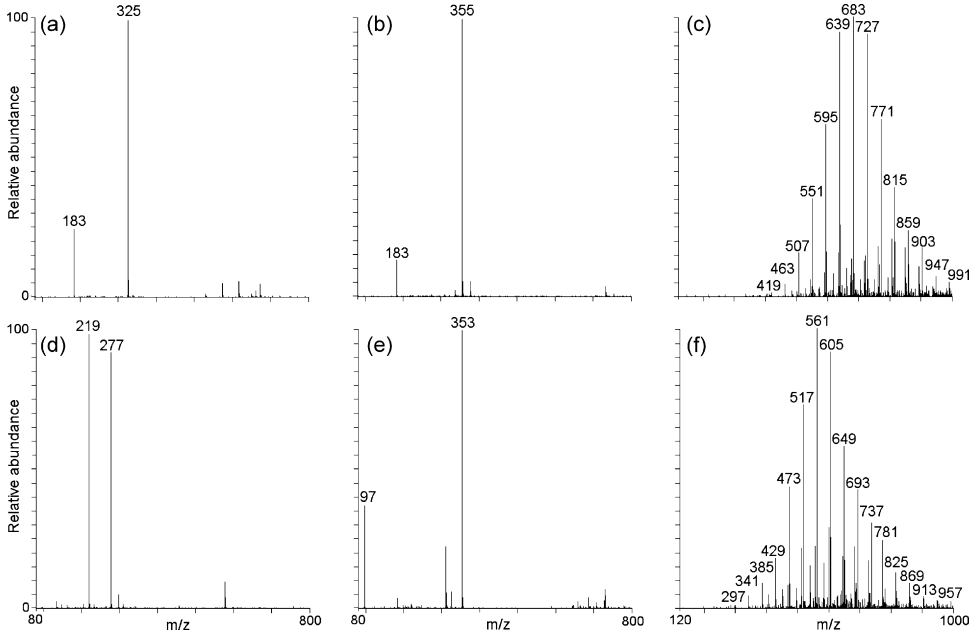

Fig. 3. Full-scan LC/ESI/MS mass spectra of: (a) C_{12} LAS; (b) C_{12} SPC; (c) NPEOs; (d) NP_1EC; (e) C_{12} AES with $n_{EO}=2$; and (f) C_{12} AEOs. Mass spectra were obtained under the specific analytical conditions described in Section 2.4.

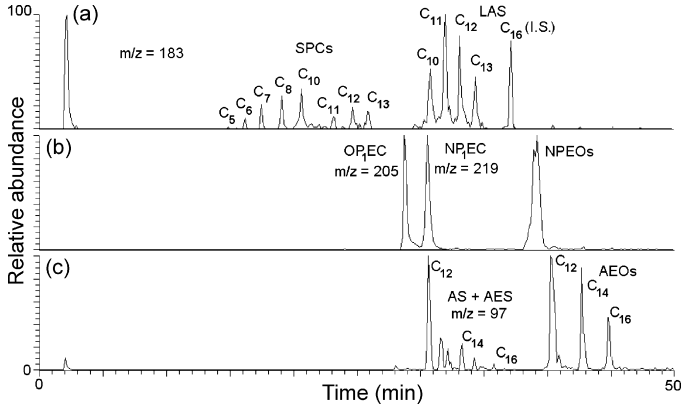

Fig. 4. Extracted LC/ESI/MS mix-mode ion chromatograms from a spiked water sample showing the identification and separation of different homologs/ethoxymers of: (a) LAS and SPCs (scanning their specific fragment ion *m/z* 183); (b) AP_1ECs (scanning their specific fragment ions *m/z* 205 and 219) and NPEOs; and (c) AS and AES (scanning their specific fragment ion *m/z* 97) and AEOs. Chromatograms were obtained under the specific analytical conditions described in Section 2.4.

the different homologs from the aliphatic surfactants. AEOs homologs appear isolated and consecutively at the end of the chromatographic run, each peak containing all the ethoxymers from the same homolog. In the case of AES, however, elution of their homologs takes place in the same time window as LAS but the specific fragment ion *m/z* 97 allows a clear identification. The lower AES ethoxymers (including AS, with zero ethoxylated groups) can be separated while the rest (n_{EO} from 3 to 11) are eluted under the same chromatographic peak.

Calibration curves were obtained for each homolog of the target compounds, assuming the same response for every ethoxymer in the case of AES, AEOs and NPEOs. Results might suffer in accuracy taking into account that intensity of the signals for the lower ethoxymers (EO1–EO3) of AEOs and NPEOs is very low so they were not considered in this study. The behavior of all compounds was linear in a range between 0.1 and 50 mg L^{-1} for the anionics and between 0.5 and 20 mg L^{-1} for the non-ionics, with r^2 values above 0.999 for each homolog. The limits of detection were calculated using a signal-to-noise ratio of 3:1, and was found to be in the range from 0.05 to 0.5 ng mL^{-1} in water and 1 to 10 ng g^{-1} in sediment. The influence of ion suppression in sediment was determined as a reduction less than 5% of the signal intensity for the anionics and less than 10% for the non-ionics. This effect was found to be negligible in aqueous samples. Calibration curves, detection limits and ion suppression were measured with and without switching from negative to positive ion detection mode and no differences were found.

Table 6
Concentration values and standard deviations ($n = 3$) measured for the target compounds in water (μg L^{-1}) and sediment (μg kg^{-1}) for the selected sampling stations

Compound	Water			Sediment		
	P1	P2	P3	P1	P2	P3
C_{10}LAS	8.5 ± 0.6	16.9 ± 1.2	5.8 ± 0.4	267 ± 47	752 ± 3	454 ± 47
C_{11}LAS	5.8 ± 0.3	12.4 ± 0.8	1.7 ± 0.0	329 ± 21	2949 ± 76	625 ± 53
C_{12}LAS	2.4 ± 0.1	6.0 ± 0.3	4.1 ± 0.4	425 ± 26	4307 ± 255	458 ± 121
C_{13}LAS	1.0 ± 0.0	3.5 ± 0.1	0.1 ± 0.0	706 ± 44	4789 ± 118	402 ± 117
Σ LAS	17.7	38.7	11.7	1727	12797	1939
C_5SPC	4.0 ± 0.3	6.4 ± 1.2	n.d.	n.d.	n.d.	n.d.
C_6SPC	2.1 ± 0.5	17.6 ± 1.3	n.d.	n.d.	n.d.	n.d.
C_7SPC	1.7 ± 0.3	22.0 ± 0.8	n.d.	n.d.	n.d.	n.d.
C_8SPC	n.d.	29.3 ± 2.3	n.d.	n.d.	n.d.	n.d.
C_9SPC	n.d.	41.2 ± 3.3	n.d.	n.d.	n.d.	n.d.
C_{10}SPC	n.d.	18.6 ± 2.0	n.d.	n.d.	585 ± 7	n.d.
C_{11}SPC	n.d.	9.7 ± 1.2	2.4 ± 0.4	n.d.	871 ± 152	n.d.
C_{12}SPC	n.d.	3.6 ± 0.5	n.d.	n.d.	377 ± 12	n.d.
C_{13}SPC	n.d.	1.2 ± 0.2	0.2 ± 0.0	n.d.	n.d.	n.d.
Σ SPCs	7.8	149.6	2.6	n.d.	1833	n.d.
C_{12}AS	0.9 ± 0.1	1.0 ± 0.2	0.1 ± 0.1	31 ± 4	90 ± 8	67 ± 19
C_{14}AS	0.5 ± 0.0	1.2 ± 0.1	n.d.	33 ± 12	88 ± 9	33 ± 12
C_{16}AS	0.1 ± 0.0	0.8 ± 0.0	n.d.	77 ± 5	64 ± 8	15 ± 9
Σ AS	1.5	3.0	0.1	141	242	115
C_{12}AES	1.4 ± 0.2	0.9 ± 0.1	n.d.	49 ± 3	224 ± 17	18 ± 2
C_{14}AES	1.0 ± 0.1	1.6 ± 0.1	n.d.	33 ± 16	301 ± 21	n.d.
C_{16}AES	0.1 ± 0.0	0.4 ± 0.1	n.d.	12 ± 3	70 ± 1	n.d.
Σ AES	2.5	2.9	n.d.	94	595	18
NPEOs	3.3 ± 0.5	5.0 ± 0.6	n.d.	1940 ± 108	2696 ± 247	2664 ± 295
OP_1EC	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
NP_1EC	n.d.	3.9 ± 0.1	n.d.	n.d.	n.d.	n.d.
C_{12}AEOs	0.9 ± 0.1	n.d.	n.d.	304 ± 19	738 ± 85	3 ± 1
C_{14}AEOs	0.3 ± 0.0	0.1 ± 0.0	n.d.	929 ± 34	1064 ± 25	65 ± 2
C_{16}AEOs	n.d.	n.d.	n.d.	656 ± 39	1839 ± 10	569 ± 54
Σ AEOs	1.2	0.1	n.d.	1889	3641	637

n.d., non-detected.

3.4. Environmental samples

Table 6 shows the concentration values for every surfactant and metabolite homolog in the samples collected at the selected sampling stations. Among the stations, the total concentration of surfactants ranges from 11.8 to 49.7 $\mu g\,L^{-1}$ in water and from 5.37 to 19.97 $mg\,kg^{-1}$ in sediment. Taking into account that the target compounds comprise more than 90% of the total volume of surfactants on the markets of European countries like The Netherlands [37], values obtained by means of the proposed methodology could be useful for estimating the overall amount of synthetic surfactants in aquatic ecosystems. The maximum values are found at the sampling point P2 due to its proximity to the discharge outlet of a WWTP. After their input into the estuary, surfactants and their metabolites are subjected to degradation, sorption and dilution processes during their transportation downstream, which explains the much lower values found at station P1. P3 shows the lowest levels due to the area having protected status, although significant levels of LAS and NPEOs are present in sediments due to occasional discharges from the surrounding populations. In general, values for LAS and NPEOs are of the same order of magnitude as those reported in previous papers in marine [13,24,33,37] and freshwater [10,21,27] environments. A similar conclusion can be inferred from the scarce data available regarding AES and AEOs concentrations in water [17,31,32] and sediments [30,38].

Environmental values up to 153.5 $\mu g\,L^{-1}$ and 1.83 $mg\,kg^{-1}$ of carboxylated metabolites were found in water and sediment, respectively. The presence of SPCs [13,14,20] and APECs [11,27] has been reported in other aquatic ecosystems at similar levels. It is notable that the greater part of these intermediates is found in water due to their higher polarity in comparison with their parent compounds. Only quantities of the most hydrophobic SPCs of C_{10}–C_{12} remain in sediments close to the WWTP discharge outlet, where higher concentrations of carboxylated metabolites are introduced in the environment after the wastewater treatment [12]. On the other hand, relatively higher percentages of the longer chain homologs of LAS, AS, AES and AEOs are found in sediment whereas the shorter, more hydrophilic homologs are more predominant in water (Table 6). This statement is also applicable when comparing overall concentrations of relatively polar surfactants like LAS, AS or AES to those showing the lowest water solubility like AEOs and NPEOs.

4. Conclusions

This paper describes the development of a novel methodology capable of the simultaneous determination of the most commonly used surfactants and their carboxylated degradation products (SPCs and APECs) in both water and sediment samples. The analysis is performed with high selectivity and reproducibility, in a simple and less time-consuming way when compared with previous specific methods for the determination of each surfactant separately. Further, it is possible to discriminate among the various homologs and ethoxymers that comprise the different surfactant mixtures. The use of modern techniques such as PLE and LC–MS has been demonstrated to be very useful for performing a faster and easier environmental monitoring of these compounds on which, in some cases like non-aromatic surfactants (AES, AS and AEOs), there is relatively little information available. Data on the environmental presence of all the target compounds in several aquatic ecosystems is also presented for the first time.

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