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Analysis of alcohol polyethoxylates and polyethylene glycols in marine sediments

Juan Manuel Traverso-Soto [a], Pablo Antonio Lara-Martín [a,*], Víctor Manuel León [b], Eduardo González-Mazo [a]

[a] Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Campus de Excelencia Internacional del Mar (CEI·MAR), Universidad de Cádiz, Campus Río San Pedro s/n, Puerto Real 11510, Cádiz, Spain
[b] Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, C/Varadero 1, San Pedro del Pinatar 30740, Murcia, Spain

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ABSTRACT

Alcohol polyethoxylates (AEOs) are the most commercially important type of nonionic surfactants, used in a wide variety of products such as household cleaning detergents, industrial cleaners, textiles, adjuvants in pesticides, wetting and dispersing agents, and emulsifiers. Our main objective in this work was to develop a methodology for the extraction, isolation and analysis of these compounds and their main degradation products and precursors (polyethylene glycols, PEGs) in solid environmental matrices. First, analytes were extracted by pressurized liquid extraction (PLE) using methanol at 120 °C as solvent and 3 cycles (5 min per cycle). Clean-up and concentration of the extracts were carried out by solid-phase extraction (SPE), using Oasis HLB cartridges and a mixture dicloromethane/methanol 1:1 as eluting solvent. Recovery percentages were usually between 54% and 106% for most compounds. Identification and quantification of analytes were performed using a liquid chromatography–mass spectrometry (LC–MS) system equipped with an electrospray interface (ESI) operating in positive ionization mode. Water content, cone voltage and adduct formation were optimized to this end. Limits of detection were usually below 50 ng g^{-1}, being higher for some shorter ethoxymers (> 100 ng g^{-1}) because of poor ionization. Finally, the protocol proposed here was applied to the determination of the concentration of AEOs and PEGs in selected surface sediment samples collected in Mar Menor Lagoon (Murcia, Spain). In this sense, this paper presents some of the first data relative to the occurrence of these analytes in coastal sediments, showing relatively high concentrations of PEGs (up to 9000 ng g^{-1}) compared to those measured for AEOs (< 100 ng g^{-1}).

1. Introduction

Nowadays, alcohol polyethoxylates (AEOs) are the main nonionic surfactants produced in Europe (e.g. 300,000 t/year just for household cleaning products [1]). This is in part due to relatively recent restrictions in the use of alkylphenol polyethoxylates (APEOs) in household detergents as a consequence of the estrogenic properties shown by their metabolites [2]. Widely used in domestic and industrial applications (e.g., detergents, emulsifiers, wetting and dispersing agents, industrial cleaners, textile, pulp and paper processing [1]), commercial AEOs consist of a mixture of several homologs of varying carbon chain length and degree of ethoxylation. Polyethylene glycols (PEGs), which have been described as the main degradation metabolites of AEOs [3], are an important group of nonionic synthetic water-soluble polymers of ethylene oxide that are also used in a wide range of applications. This includes the production of cosmetics, plastics, water-soluble lubricants, pharmaceuticals and nonionic surfactants including APEOs and AEOs [4]. In fact, the annual production of PEGs is estimated to be several millions of tons worldwide [4].

After their use, surfactant residues are discharged into aquatic ecosystems in treated or untreated wastewaters, and enter in various environmental compartments such as surface waters, sediments and biota [5]. Polyethoxylated surfactants and their metabolites are often found in wastewater effluents, receiving waters and sediments at very high concentrations compared to other targeted analytes [6], in spite of the high removal efficiencies (between 93% and 99%) in wastewater treatment plants (WWTPs). Total effluent concentrations range from 0.92 to 15.6 μg L^{-1} for NPEOs (nonylphenol polyethoxylates, which are the main type of APEOs) and AEOs [7,8]. Available studies on the presence, environmental behavior and distribution of these compounds are mainly focused on NPEOs, due to their potential adverse effects, showing concentrations between < 0.2

* Corresponding author. Tel.: +34 956 016159; fax: +34 956 016040.
E-mail address: pablo.lara@uca.es (P.A. Lara-Martín).

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and 2 μg L^{-1} in surface waters [9,10] and between <10 and 28,500 ng g^{-1} in sediments [11–13] all over the world. On the other hand, data concerning to the presence of AEOs and PEGs are rather limited. Concentrations of AEOs in sediments can be comparable to those found for NPEOs [9,12,13], showing levels ranging from 40 to 12,200 ng g^{-1}. The occurrence of PEGs has recently been reported in the U.S. East Coast [14], where concentrations in surface sediments were generally higher (up to 1490 ng g^{-1}) than those for other classes of targeted surfactants detected in the same zone. Also, some authors have found concentrations between <0.1 and 49 μg L^{-1} for AEOs [9,11,12] and PEGs [14] in surface waters. Additionally, and although they do not act as endocrine disruptor compounds, AEOs have proven to be toxic for certain aquatic species [15].

For several years now, NPEOs and AEOs have been extracted from sediments and sludges using Soxhlet or ultrasonic extraction with methanol [14,16] or mixtures containing nonpolar solvents (e.g. dichloromethane [17]). Later, extracts are often preconcentrated and purified by means of solid-phase extraction (SPE) [14,17–21]. Different SPE cartridges such as octadecylsilica (C18) [17] or various polymers (e.g. HLB [14], Porapak Rdx [22]) have been tested with satisfactory results for these compounds, while elution is performed with solvent mixtures containing methanol, dichloromethane, hexane and/or acetonitrile. More recently, new extraction methods have been developed not only to save time, but also to reduce solvent consumption without losing efficiency. Pressurized liquid extraction (PLE) is used to improve the extraction process, as the solvents remain in their liquid state at high temperatures (100–200 °C) and pressures (about 150 atm). There are some recent protocols for performing PLE of AEOs and NPEOs from sediments [7,23,24] and soils [21], using methanol, hexane or acetone as solvents in most cases. Supercritical fluid extraction (SFE) is another relatively modern extraction technique that uses water instead of organic solvents to carry out the extraction of nonionic surfactants (NPEOs and AEOs) [25] within 15 min.

Once extracted from solid or aqueous matrices, separation, identification and quantification of AEOs, PEGs and other polyethoxylated compounds can be carried out by means of several methods based on the use of high-performance liquid chromatography coupled to ultraviolet and fluorescence detectors (HPLC-UV-FLU) [18,26], and gas chromatography with mass spectrometry (GC–MS) [16]. However, analysis of AEOs by these techniques is more challenging than that for APEOs due to their lack of UV absorbance, fluorescence and volatility, so prior derivatization is required in most cases [26]. Considerable progress has been achieved during the last decade, mainly due to the development of new interfaces such as electrospray ionization (ESI), allowing the determination of these surfactants by high performance liquid chromatography–mass spectrometry (HPLC–MS). This combination offers many advantages in terms of greater sensitivity, selectivity and the possibility to simultaneously measure multiple classes of compounds together. The application of HPLC–MS for the determination of AEOs and PEGs [22,27] is severely limited compared with the relatively large number of protocols developed for nonylphenolic compounds analysis over the last decades [10,19,20]. NPEOs and AEOs have been simultaneously measured in different environmental compartments [17,21,23,25] by means of HPLC-single quadrupole/ion trap (IT)-MS. More recently, tandem mass spectrometry (MS–MS) has been used for the analysis of organic pollutants, increasing sensitivity and selectivity when determining AEOs in aqueous matrices [28,29], as well as in marine sediments [13]. Less commonly used than other HPLC–MS instrumental approaches in the analysis of surfactants, liquid chromatography coupled to time-of-flight mass spectrometry (LC-ToF-MS) constitutes an alternative for the identification of surfactants and many other organic pollutants, due to the capability for estimating their elemental composition by accurate mass measuring of ions for confirmation purposes. So far, there are only a couple of papers dealing with LC-ToF-MS analysis of AEOs and PEGs in environmental samples [14,30].

In spite of the numerous methods presented above for the determination of nonionic surfactants in environmental samples, most of the work done has been focused on the extraction of APEOs and their degradation intermediates in soils, sediments and sludges. The environmental behavior of these compounds is therefore relatively well-known today, whereas information for AEOs is increasing and it is still really scarce on many other compounds such as PEGs. In order to help solving these deficiencies, we have developed and optimized a new method for the simultaneous extraction, preconcentration, purification, identification and quantification of AEOs and PEGs in sediment samples using a combination of ultrasonic extraction, PLE, SPE and HPLC-IT-MS. Additionally, this procedure has been applied to the determination of concentrations of both compounds in surface sediments from a coastal lagoon in the southeast of Spain, allowing for a comparative study on the environmental levels of AEOs and PEGs for the first time.

2. Material and methods

2.1. Chemicals and standards

Ethyl acetate (Acet), methanol (MeOH), water, acetone (Ace), dichloromethane (DCM) and hexane (Hex) were of chromatography quality, purchased from Scharlau (Barcelona, Spain). Acetic acid, sodium sulfate, sodium acetate and ammonium acetate were purchased from Panreac (Barcelona, Spain). The solid-phase extraction (SPE) mini-columns used (6 mL, 500 mg) were supplied by Varian (Bond Elut C18) and Waters (Oasis HLB).

The individual $>98\%$ pure polyethylene glycols (PEGs) having 1, 2, 3, 6 and 8 EO units, AEO ethoxymers (C_{12}, C_{14}, C_{16} and C_{18} homologs having 1, 2, 3, 6 and 8 EO units), and a PEG 300 mixture were purchased from Sigma-Aldrich (Milwaukee, USA). The $\geq 98\%$ pure $C_{10}AEO_8$ internal standard used in positive ionization mode was also purchased from Sigma-Aldrich (Madrid, Spain).

Stock standard solutions containing individual species or mixtures of them were prepared by dissolving them in 100% methanol. Working standard solutions were made by further diluting with methanol.

2.2. Pressurized liquid extraction

Target compounds were extracted from the sediment samples using pressurized liquid extraction (PLE) 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). Extraction was optimized by testing several extraction solvents. Methanol was selected and passed through the heated (120 °C) and pressurized (1500 psi) PLE cells for three cycles of 5 min each. Subsequently the methanolic extracts were evaporated until 5 mL.

2.3. Ultrasound assisted-extraction

The extraction from dried sediment (0.5 g per sample) was performed for comparison with PLE using ultrasonic irradiation at 30 °C during 3 cycles (30 min each), and methanol/dicloromethane 1:1 (30 mL) after testing several extraction solvents. After extraction, solvent was separated from sediment samples by centrifugation and evaporated to 5 mL.

2.4. Extract clean up and concentration

Target compounds were isolated from sediment extracts using Oasis HLB 6 mL 500 mg minicolumns in an automated SPE AutoTrace unit (Zymark). These cartridges were conditioned with 8 mL of methanol and 5 mL of Milli-Q water prior to passing the extracts reconstituted in 100 mL of HPLC water. They were then washed with 16 mL of HPLC water before being air-dried. Elution was performed with 8 mL of a mixture of methanol/dichloromethane 1:1. Extracts were then evaporated to dryness and reconstituted in 1 mL of methanol. Finally, further dilutions were made in methanol/water (50:50) containing 250 $\mu g\,L^{-1}$ of $C_{10}AEO_8$ as internal standard and 50 μM of sodium acetate prior to HPLC–MS analysis. SPE was optimized by testing several minicolumns (not only HLB but also octadecylsilica, or C18) and solvent mixtures for elution.

2.5. Identification and quantification of the target compounds

Analysis of surfactants and their degradation metabolites was carried out by HPLC–MS. First, target compounds were separated on a Spectrasystem unit with autosampler, with the injection volume set to 20 μL. The chromatographic separation was done using a reversed-phase C-18 analytical column (Lichrospher 100-RP18) of 250 mm × 2 mm and 0.003 mm particle diameter, from Merck. LC conditions for AEOs and PEGs were as follows: mobile phase A was methanol and mobile phase B was HPLC water with 5 mM acetic acid. Flow rate was constant (0.15 mL min^{-1}) and the elution gradient started with 10% A and was increased linearly to 100% A over 15 min and kept isocratic for 20 min. The initial solvent conditions were then restored over a 2 min ramp and the column was allowed to re-equilibrate for an additional 3 min (total run time = 40 min).

The detection was carried out using a LCQ ion-trap (IT) mass spectrometer (Thermo), equipped with an atmospheric pressure ionization source with electrospray interface (ESI). All extracts were analyzed using ESI full-scan positive ionization mode (ESI+) in order to determine AEOs and PEGs, scanning the mass/charge (m/z) range between 100 and 1000. Ion fragmentation energy was optimized to 10 V to get the maximum signal of every target compound in positive mode. Values of other MS parameters were: needle tip voltage 4500 V, 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 AEOs and PEGs. Identification of the target compounds was based on monitoring their different adduct ions (such as $[M+NH_4]^+$ and $[M+Na]^+$), and confirmed by comparison of the retention time of the compounds with that of a standard. Quantification was carried out by measuring the peak area of adduct ions using external standard solutions (2–14500 $\mu g\,L^{-1}$) prepared in methanol/water 1:1 and $C_{10}AEO_8$ as internal standard (250 $\mu g\,L^{-1}$). The analyte response was normalized to that of the internal standard.

Table 1
Mass/charge (m/z) ratios (expressed as the sodium adduct $[M+Na]^+$) scanned for the determination of AEO and PEG ethoxymers.

Compound	Ethoxymer[a]	m/z[b]
$C_{12}AEOs$	n_{EO} = 1–17	253–957 (±44)
$C_{13}AEOs$	n_{EO} = 1–17	267–971 (±44)
$C_{14}AEOs$	n_{EO} = 1–17	281–985 (±44)
$C_{15}AEOs$	n_{EO} = 1–17	295–999 (±44)
$C_{16}AEOs$	n_{EO} = 1–16	309–969 (±44)
$C_{18}AEOs$	n_{EO} = 1–16	337–997 (±44)
PEGs	n_{EO} = 2–19	129–877 (±44)

[a] n_{EO} represents the number of ethoxylated groups per ethoxymer.
[b] m/z ranges are shown for AEO and PEG ethoxymers, with a difference of 44 m/z units between each consecutive ethoxymer.

2.6. Application of the analytical procedure to real samples

Surface sediment samples were collected from Mar Menor Lagoon (Fig. 1), a coastal lagoon located on the southeastern shore of Murcia (Spain), subjected to intensive agriculture, seasonal tourism and recreational nautical activities. Three WWTPs treat wastewater from a population of 50,000 inhabitants, generating of 4×10^9 L $year^{-1}$ of biologically treated sewage. A significant part of this effluent is used for irrigation and the rest is discharged into the lagoon, and the other WWTPs effluents of this area are discharged to Mediterranean Sea. Water exchange between this lagoon and the Mediterranean Sea is limited, taking place mainly through El Estacio channel. Surface sediment (0–0.05 m) samples were collected from station MM3 to station MM31 by means of a Van Veen grab during spring of 2009. Additionally, non-polluted sediment was taken from a single sampling point (36°26′26.36″N, 6°13′47.18″W) located in the salt-marsh environment of the Bay of Cadiz (in the southwest of Spain). This sample was collected from between 0.5 and 1 m depth in order to ensure the lowest level of pollution (confirmed by later analysis of PEGs and AEOs). All sediment samples were kept at 4 °C during their transport to the laboratory, where they were freeze-dried, milled and stored until their analysis. The sediments that were used in this study exhibited a wide range of physical and chemical properties, as shown in Table 3. Total organic carbon content in sediment grabs was determined by a CHS analyzer (Perkin Elmer Series II CHNS/O Analyzer 2400). In addition, fine fraction (< 63 μm) analysis was performed by wet sieving.

Extraction recoveries of target compounds were determined for non-polluted sediment samples spiked at different concentration levels ranging from 500 to 12,200 ng g^{-1}. Recoveries were determined by comparing the concentrations obtained with the initial spiking levels. The precision of the method was expressed as the standard deviation (SD) of replicate measurement (n = 3 for each experiment). Sediments collected from Mar Menor Lagoon were extracted and analyzed in duplicate. Furthermore, we also performed three successive injections of the same sample and re-analyzed a batch of samples one month after their first analysis. Limits of detection were determined from spiked sediment samples as the minimum detectable amount of analyte with a signal-to-noise ratio of 3, and taking into account the amount of sample extracted and extraction recovery percentages. Comparison of the signal intensity of spiked internal standards in methanol/water 1:1 and in sediment sample extracts was carried out to evaluate ionization suppression due to matrix effects.

3. Results and discussion

3.1. Extraction of target compounds from sediments

Several experiments were carried out to evaluate the extraction efficiency of PLE and sonication, followed by sample purification by SPE. Advantages of PLE for extraction of contaminants from solid matrices include acceptable recoveries, low SDs (typically below 20%), automation, and a faster although sequential extraction process (typical extraction time is 15–20 min per sample) [21,31]. Regarding the extraction conditions for PLE, AEOs and PEGs are fairly stable even at temperatures up to 150 °C, and the extraction pressure has no influence once samples have been dried and it is high enough to prevent the solvents from boiling [24]. Extraction pressure (1500 psi), temperature (120 °C), static time (5 min) and the number of extraction cycles

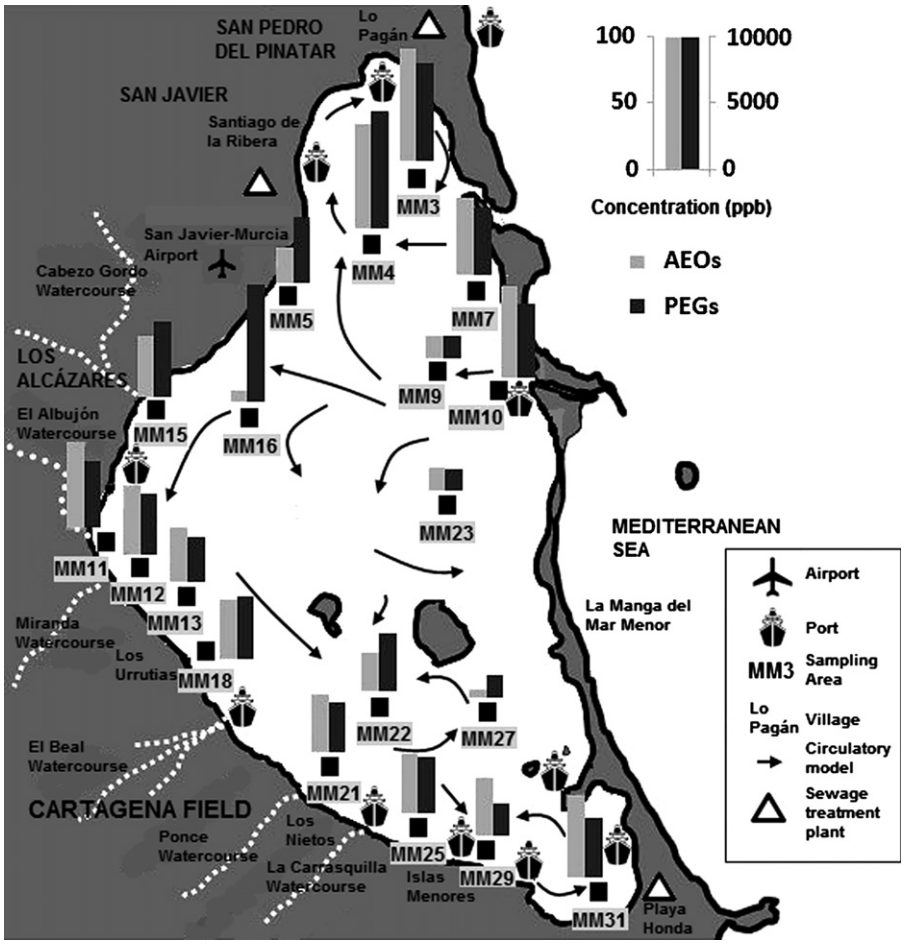

Fig. 1. Map of the Mar Menor coastal lagoon (Murcia, Spain) showing concentration values measured for the target compounds (ppb = ng g^{-1}) in surface sediment for selected sampling stations.

(3 cycles) were chosen according to previous works with surfactants [23]. We focused our attention on testing several solvent mixtures and choosing the most appropriate for extraction of target compounds. Thus, spiked sediment samples were extracted using five different solvents (methanol, acetone, and mixtures of MeOH/Acet, MeOH/DCM or Hex/Ace 1:1) to determinate extraction recoveries of target compounds. Further clean-up, concentration of PLE extracts by SPE, and LC–MS analysis were carried following conditions described in Sections 2.4 and 2.5. Results are shown in Table 2. The extraction process by PLE was efficient employing a mixture of MeOH/DCM 1:1 for AEOs (81–164%), and methanol (54–79%) or a mixture of MeOH/Acet 1:1 (63–119%) for PEGs. Significantly lower values (data not shown) were observed for PEGs using MeOH/DCM 1:1 as extraction solvent, probably due to the hydrophilic character of these compounds and the low polarity of this solvent mixture in comparison to other solvents (e.g. methanol and acetone). In order to extract both AEOs and PEGs simultaneously we considered methanol as the better option since good recoveries were also achieved for most AEO homologs/ethoxymers (see Table 2) and reproducibility was higher (lower SD). Overall, PLE extraction efficiency was also comparable to that of the other methods based on this [14,17,23] and other extraction techniques for the same analytes, such as AEOs [17] and PEGs [14] extracted by ultrasonic extraction.

Compared with PLE, ultrasonic extraction allows processing a large number of samples within the same batch in a shorter time (typically, but not limited to 24 samples/batch within less than 3 h)

Table 2
Recovery percentages ± SD ($n=3$) for the target compounds using different solvent mixtures in PLE ($T=120$ °C, $P=1500$ psi, $t=3$ cycles of 5 min each).

Compound	MeOH/Acet 1:1	Hex/Ace 1:1	Ace	MeOH	MeOH/DCM 1:1
$C_{12}AEO_2$	180 ± 13	145 ± 16	100 ± 44	164 ± 17	81 ± 14
$C_{12}AEO_3$	89 ± 16	88 ± 16	64 ± 26	96 ± 15	82 ± 15
$C_{12}AEO_6$	158 ± 29	156 ± 19	122 ± 44	176 ± 36	132 ± 20
$C_{12}AEO_8$	124 ± 22	94 ± 9	105 ± 34	126 ± 25	122 ± 22
$C_{14}AEO_2$	98 ± 12	73 ± 12	54 ± 19	84 ± 19	99 ± 18
$C_{14}AEO_3$	117 ± 25	82 ± 8	71 ± 24	106 ± 19	99 ± 14
$C_{14}AEO_6$	32 ± 6	23 ± 3	24 ± 11	27 ± 4	135 ± 20
$C_{14}AEO_8$	32 ± 6	24 ± 3	23 ± 10	27 ± 2	126 ± 19
$C_{16}AEO_2$	99 ± 1	77 ± 18	72 ± 25	99 ± 21	110 ± 14
$C_{16}AEO_3$	92 ± 21	69 ± 19	62 ± 18	80 ± 14	105 ± 14
$C_{16}AEO_6$	77 ± 20	67 ± 13	53 ± 14	65 ± 12	115 ± 20
$C_{16}AEO_8$	79 ± 22	71 ± 13	62 ± 22	69 ± 10	139 ± 23
$C_{18}AEO_2$	77 ± 7	57 ± 16	59 ± 22	85 ± 17	114 ± 8
$C_{18}AEO_3$	80 ± 20	60 ± 18	59 ± 19	77 ± 18	137 ± 10
$C_{18}AEO_6$	82 ± 20	72 ± 17	62 ± 20	73 ± 13	153 ± 21
$C_{18}AEO_8$	44 ± 8	42 ± 9	37 ± 12	40 ± 7	164 ± 23
PEG_6EO	119 ± 13	8 ± 1	29 ± 9	79 ± 6	–
PEG_8EO	63 ± 17	10 ± 3	13 ± 5	54 ± 1	–

[13,14]. One drawback is that extraction is not automated as it requires centrifugation after each cycle, and SDs are usually higher than when using PLE. In our case, sediment samples were sonicated testing three different solvents (methanol, dichloromethane and a mixture of MeOH/DCM 1:1) at 30 °C during 3 cycles (30 min

per cycle). Fig. 2 shows the recovery percentages for AEOs after sonication and later purification by SPE. It can be observed that the use of nonpolar solvents such as dichloromethane decreased the extraction efficiency of AEOs from sediment samples ($<20\%$ for AEO homologs having 6 and 8 EO units). Better results were reached using a more polar mixture MeOH/DCM 1:1 (52–83%), these recovery percentage values being higher than those obtained when methanol was used alone (28–51%) as an extraction solvent. In any case, significantly lower values (from 31% to 61%) were observed for those AEO components having 8 EO units. Other authors have reported the same trend during extraction of NPEO and AEO larger ethoxymers from solid matrixes [7,13]. This could be explained due to the affinity of these compounds for materials such as glass and the SPE cartridges, or for the sediment particles as hydrophilic interactions between the ethoxylated chain and mineral surfaces may occur [32–35]. Overall, from the data shown in Table 2 and Fig. 2, it can be inferred that ultrasonic extraction efficiency of AEOs from sediments was lower by 29–81%, depending on the homolog/ethoxymer considered, than using PLE, evaluated as the difference between better recovery percentages of both extraction techniques. Therefore, we decided using PLE over sonication for further experiments.

Optimization of clean-up and concentration of sample extracts using solid-phase extraction was also investigated employing octadecylsilica (C18) and Oasis HLB cartridges, which have been widely used for the isolation of different pharmaceuticals [6] and surfactants [22]. The extraction of sediment samples was performed following the PLE methodology previously optimized (see Section 2.2 for details). HLB cartridges were finally selected for further experiments as they showed appreciably better recoveries for PEGs than C18. This was especially significant for the more hydrophilic PEG_6EO, for which recovery was 57% lower for C18 than for HLB. This may be explained by the poor interaction between hydrophobic C18 mini-columns and the shortest ethoxy chain PEGs, so a significant fraction of them is washed out when the sample passes through the cartridges. Differences among recovery percentages for AEOs, however, were less than 10% when both types of sorbents were compared. In any case, we observed that the most lipophilic target compounds (most AEO ethoxymers) became tightly bound to the packing material and were therefore hard to elute. In this sense, Krogh and co-workers [22] tested different types of SPE cartridges and many different combinations of elution solvents going from the more polar range to the more non-polar to improve extraction recoveries for AEOs and alkylamine ethoxylates from aqueous samples. We also checked the influence of the solvent mixture used for the elution of AEOs and PEGs from HLB SPE cartridges. Different solvents were used. The first one was 8 mL of MeOH followed by 4 mL of DCM, giving low recoveries for most AEOs (21–90%) and the best results for PEGs (57–103%). In this case, the extraction percentage values decreased toward the shortest AEO ethoxymers, which may be attributed to the higher sorption of these analytes in the SPE sorbent compared with more polar compounds. Extraction of AEOs, however, was more efficient (generally over 60%) using a mixture of MeOH/DCM 1:1 instead, although low recoveries were still obtained for some ethoxymers such as $C_{14}AEO_6$ and $C_{14}AEO_8$ (27 ± 4 and 27 ± 2, respectively), which may be related to matrix-induced ionization suppression of these compounds during analysis, and/or also due to the sorption of these ethoxymers onto surfaces. Besides, extraction efficiencies of most AEO ethoxymers followed an opposite trend, with those AEOs having the shorter ethoxy chains showing the highest recovery values, to that described when 8 mL of MeOH followed by 4 mL of DCM were used as elution solvents. Acceptable recoveries (54–79%) were also obtained eluting PEGs with MeOH/DCM 1:1, despite this solvent mixture being less polar than the previous one. Results from these recovery experiments were comparable to those previously reported by other authors using both types of sorbents and different mixtures of the same elution solvents for isolating AEOs [13,16,21] and PEGs [14,17] from aqueous samples.

Finally, and considering all the data shown above and also presented in Table 2 and Fig. 2, we decided to use PLE and MeOH as extraction solvent followed by SPE clean-up using HLB cartridges, where a mixture of MeOH/DCM 1:1 was used for elution. This way, an effective and simultaneous extraction, preconcentration and purification for most AEOs and PEGs from environmental sediment samples could be achieved. If PEGs are the only analytes that need to be determined, extraction is more efficient (57–103%) using 8 mL of MeOH and 4 mL of DCM as elution solvent during the clean-up step.

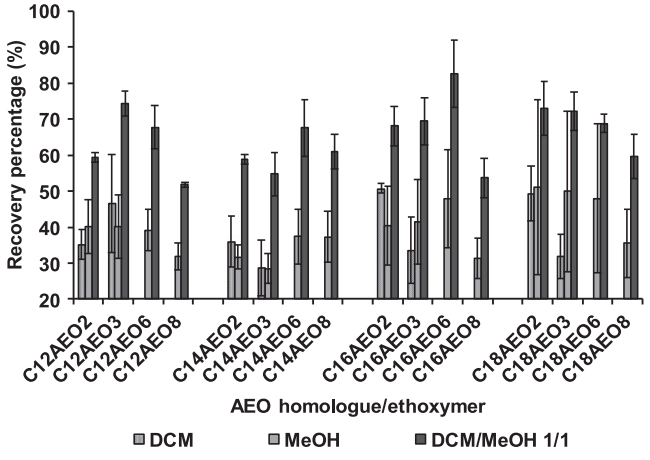

Fig. 2. Average recovery percentages (%) obtained for sediment spiked with AEOs after ultrasound assisted-extraction followed by later clean-up using SPE.

3.2. Determination by LC–MS

Fig. 3 shows ESI+ extracted ion chromatograms resulting from the analysis of a spiked sediment sample and a standard mixture of PEG 300. Individual AEO components were completely separated by their alkyl chains (Fig. 3a) using a C18 reversed-phase LC column, as retention is strongly based on the interaction between the hydrocarbon chain and the stationary phase. The more polar PEGs eluted first, followed by the AEO homologs (including the internal standard $C_{10}AEO_8$) because their greater hydrophobicity increases their retention time in the column (Fig. 3a and b). On the other hand, AEO ethoxymers with the same length of alkyl chain co-eluted under the same chromatographic peak (Fig. 3a), and PEGs were only partially separated (Fig. 3b). Although a complete separation of all components is impossible with conventional LC columns, the use of MS enables all the target compounds to be distinguished because of their specific ions, represented in Table 1. Fragmentation voltages, electrolytes added to the sample to form different ions, and water content of the methanolic extract were optimized for each analyte. First, in spite of the absence of added electrolyte to the sample during the optimization of the fragmentation voltage, AEO and PEG sodium and ammonium adducts were detected as a result of the ubiquity of these ions in solvents and samples. At relatively low source fragmentation voltage settings (0–20 V), maximum response of $[M+H]^+$ ions was obtained at 10 V for PEG shorter ethoxymers (4–10 EO), whereas $[M+NH_4]^+$ ions showed the highest intensity for those PEGs having more than 11 EO units (Fig. 4). This trend was also observed applying the same voltage for AEOs (optimum signal of $[M+H]^+$ ions for 2–3 EO, and $[M+NH_4]^+$ when $EO>6$). Additionally, target compounds were

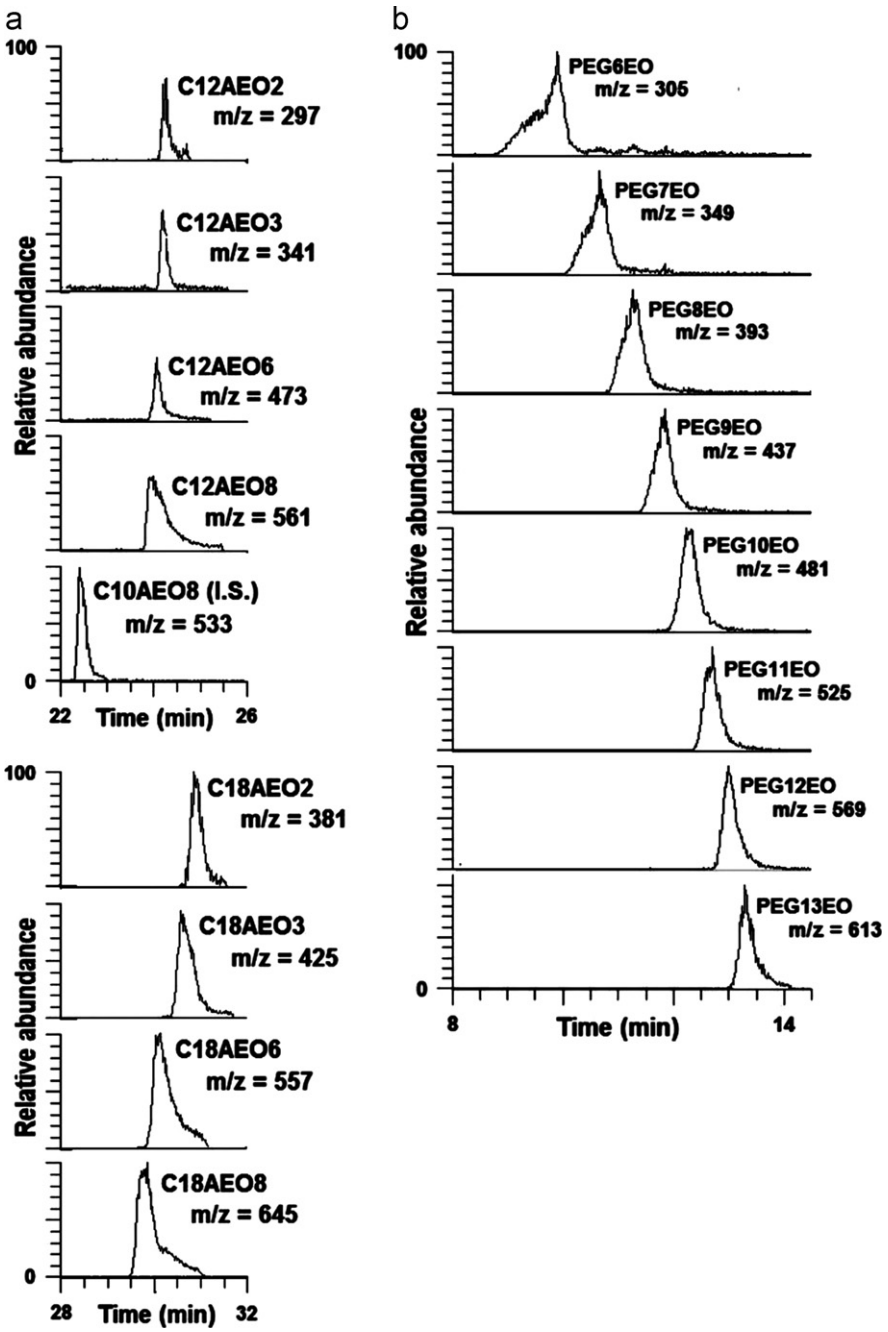

Fig. 3. Extracted LC/ESI/MS ion chromatograms showing the identification and separation of different homologs/ethoxymers (a) AEOs from a spiked sediment sample after PLE+SPE and (b) a standard solution of a PEG 300 mixture. Chromatograms were obtained under the specific analytical conditions described in Section 2.5.

spiked in pure methanol and in methanol/water (50:50) to evaluate the effect of water in the ionization efficiency. We found out that there was an average signal enhancement of 47% for PEG ethoxymers when the water content of the sample was increased, whereas variations were almost negligible for AEOs (<5–10%). Thus, ionization efficiency improved starting the elution gradient with higher aqueous content of the mobile phase, which in fact is necessary for better separation of PEG ethoxymers, as well as making further dilutions of samples in methanol/water (50:50) before LC–MS analysis.

AEOs and PEGs lack charge or acid/base functional groups, but they have high affinity for alkali metal ions with which they form several types of adducts. Many authors have exploited this for APEO analysis and fortify sample extracts or the mobile phase with sodium [10,23] or ammonium acetate [13,21,25] to provide an ion source for target compounds, so $[M+Na]^+$ or $[M+NH_4]^+$ adducts can be used for their quantification. To this end, both salts were tested to form several ions for every single compound. We could observe that signal intensity increased for longer AEO and PEG ethoxymers (Figs. 5 and 6), whereas changes in the concentration of ammonium and sodium acetate were much less important if we only consider the length of the alkyl chain of AEO homologs (Fig. 6). This is due to the interaction existing between the ethoxylated chain and the ammonium, sodium or other alkali metals [13,14,36]. These ions form AEO and PEG complexes that are more stable as the number of EO units increases. Therefore, signal intensities are enhanced toward longer chain ethoxylates until the number of EO units is high enough ($n_{EO}>7$), remaining fairly stable after this. The use of ammonium acetate improves the signal of $[M+NH_4]^+$ ions, whereas reduces the formation

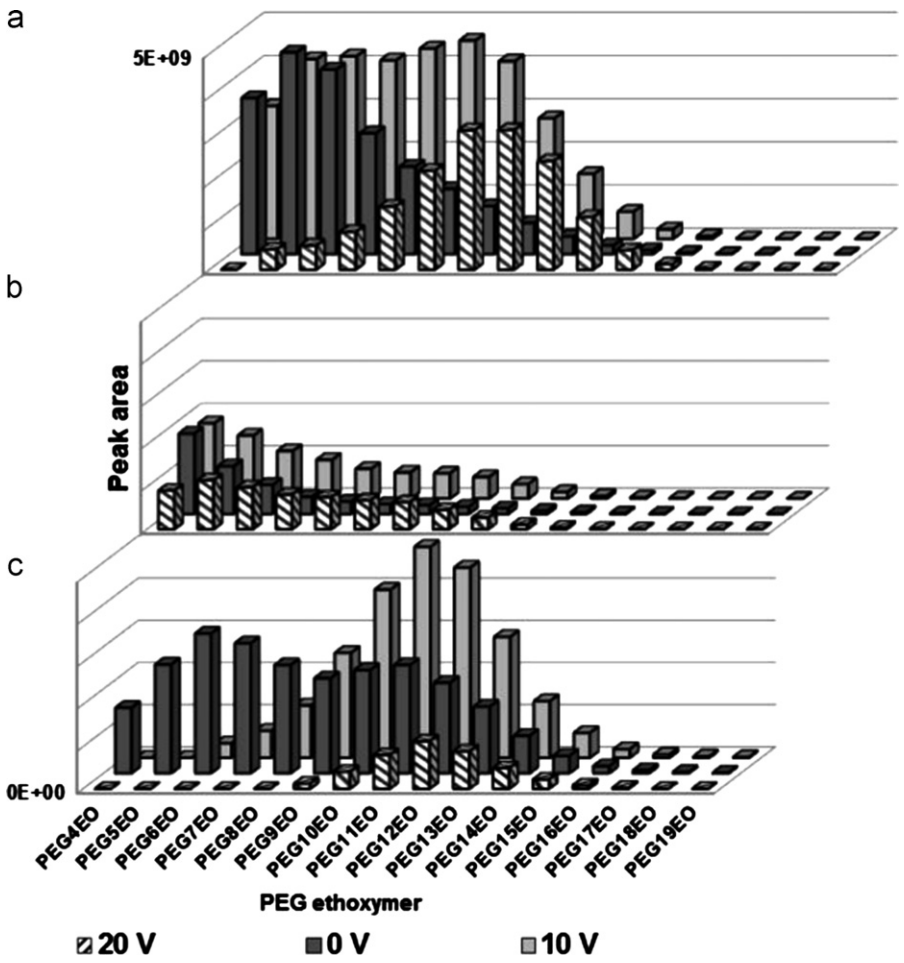

Fig. 4. Evaluation of the influence of the fragmentation voltage (*V*) in the signal intensity of PEG ethoxymers by measuring the peak area of extracted LC/ESI/MS ion chromatograms corresponding to: (a) $[M+H]^+$, (b) $[M+Na]^+$ and (c) $[M+NH4]^+$ ions.

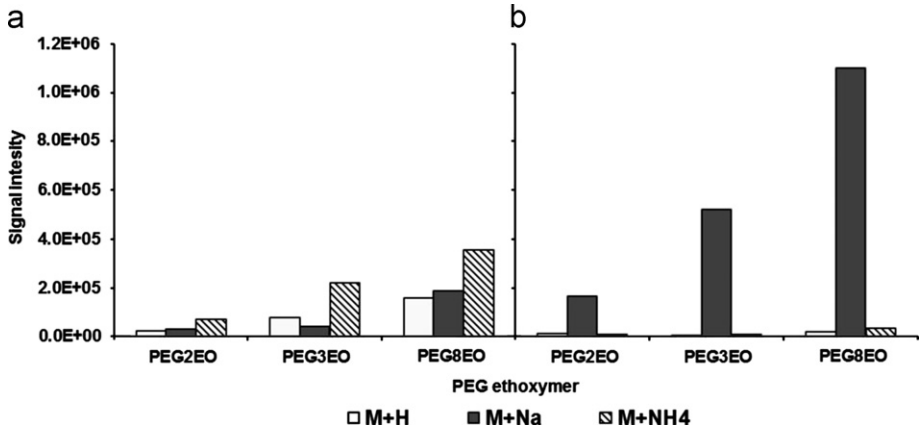

Fig. 5. Signal intensity of different adduct ions obtained by direct injection mass spectrometry of a standard solution of PEG ethoxymers containing: (a) 50 μM of ammonium acetate and (b) 50 μM of sodium acetate.

of sodium adducts and $[M+H]^+$ of AEOs and PEGs (Fig. 5a). As a consequence, identification and quantification can be carried out using these three adducts for obtaining multiple confirmation measures and, therefore, a more reliable identification of the analytes. On the other hand, the addition of sodium acetate gave the highest intensity to $[M+Na]^+$ ions and minimized the signal of $[M+H]^+$ and $[M+NH_4]^+$ for all ethoxymers of AEOs and PEGs (Figs. 5b and 6). In this case, these nonionic compounds can be only quantified using one adduct ($[M+Na]^+$) as the signal of the other two ions sharply decreases. Despite this, the usage of sodium for the analysis of AEOs and PEGs at low environmental levels of target analytes is recommended due to the higher signal intensity observed in comparison with the addition of ammonium to the samples.

The behavior of all compounds was linear in a range between 2 $\mu g\,L^{-1}$ and 14500 $\mu g\,L^{-1}$ for AEOs and between 65 and 9000 $\mu g\,L^{-1}$ for PEGs, with r^2 values above 0.999 for each homolog and ethoxymer. Limits of detection (LOD) were calculated for sediment (4 g) using a signal-to-noise ratio of 3:1, and were found to be in the range from 4 to 118 $ng\,g^{-1}$ for PEGs and

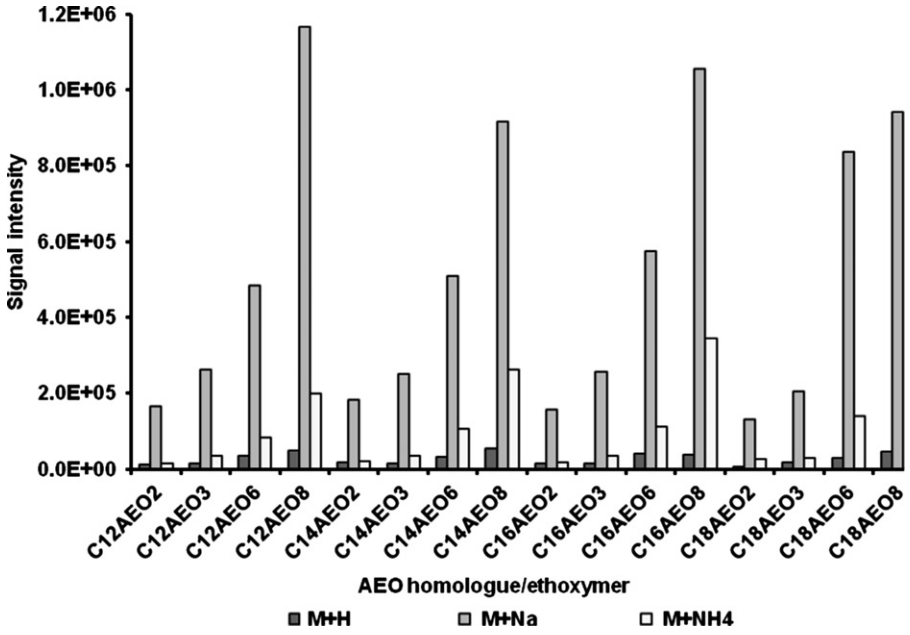

Fig. 6. Signal intensity of different adduct ions obtained by direct injection mass spectrometry of a standard solution of AEO homologs and ethoxymers containing 50 μM of sodium acetate.

from 0.1 to 17 ng g^{-1} for AEOs, depending on the homolog/ethoxymer. In any case, even if shorter AEO and PEG ethoxymers (<6 EO for PEGs and <3 EO for AEOs) were detected with lower sensitivity (LODs >100 ng g^{-1}) than those ethoxymers having higher number of EO units (LODs <50 ng g^{-1}), LOD values were still low enough to ensure quantification of these analytes in sewage polluted sediments. Results might suffer in accuracy taking into account that the lower ethoxymers of AEOs (1 EO) and PEGs (<3 EO) were practically undetectable within the concentration range used in this study (from 2 to 14500 μg L^{-1}) because of their extremely poor ionization. This has been previously described in some recent works, being also true for APEOs [13,14,37]. In any case, LOD values determined here were also comparable [14,21,23,36] to those determined in prior methods that do not rely on derivatization. $C_{10}AEO_8$ (Fig. 3a) was used as internal standard to account for matrix effects in real samples. The influence of ion suppression in sediment was determined as a reduction from 3% to 69% of the signal intensity, depending on the sample and compound. On the other hand, recoveries higher than 100% were observed for some analytes for both PLE and ultrasonic extraction, which was attributed to matrix-induced ionization enhancement of AEOs and PEGs during analysis. Other authors have previously observed this enhancement for different nonionic surfactants and their metabolites [14,19].

Finally, overall precision of the analytical technique was evaluated by extraction and analysis of duplicate samples of marine sediment collected from Mar Menor. In general, the resulting standard deviations (SD) were excellent, being below 10% for all analytes. The reproducibility and repeatability of the method were also evaluated by realizing three successive injections of the same sample and re-analyzing the same batch of samples one month after their first analysis, respectively. Resulting SDs were also below 10% for all analytes.

3.3. Application of the method for the analysis of environmental samples

This section shows the results from a survey in Mar Menor Lagoon (Murcia, Spain) using the methodology proposed above for measuring concentrations of AEOs and PEGs in surface sediments. Fig. 1 shows total concentration values for target compounds in samples collected at the selected sampling stations, ranging between 7 and 85 ng g^{-1} for AEOs, and 1600 and 8800 ng g^{-1} for PEGs. Levels for both the compounds were significantly higher in sediments collected near the shore than those measured inside the lagoon itself (Fig. 1). No correlation was observed between the concentrations of both analytes and the physicochemical properties of the sediments (Table 3). Hence, distribution of AEOs and PEGs could be better explained due to a combination of multiple sources: discharges from nearby WWTPs, the surrounding populations, small creeks which flow directly into the Mar Menor after collecting treated and untreated urban wastewaters, the input of surfactants used such as adjuvants in pesticides through surface and groundwaters [38], and/or from cleaning products used in ports. In general, values for AEOs were comparable to those reported in other aquatic environments [11–14,23]. Data on PEGs, however, are the first ever known in this area and it is remarkable that concentration of these chemicals, which are relatively polar metabolites, exceeded by two orders of magnitude

Table 3
Physicochemical properties of sediment samples.

Sediment	Organic carbon (%)	Fine fraction (%)	Porosity
Non-polluted	2.66	86	0.63
MM3	3.40	71.70	0.54
MM4	4.90	88.73	0.66
MM5	2.92	9.52	0.435
MM7	0.09	25.80	0.214
MM9	21.32	73.59	0.71
MM10	5.59	86.48	0.69
MM11	0.14	6.51	0.19
MM12	0.13	9.91	0.24
MM13	1.35	88.07	0.425
MM15	2.99	72.48	0.415
MM16	6.05	72.24	0.77
MM18	3.34	72.54	0.595
MM21	2.07	81.73	0.525
MM22	5.03	70.16	0.69
MM23	6.31	78.73	0.72
MM25	0.69	6.51	0.325
MM27	5.80	58.46	0.75
MM29	1.47	69.04	0.496
MM31	2.55	83.58	0.60

to that found for AEOs. A similar trend has been recently described by Lara-Martín et al. [14] in estuarine sediments from Long Island Sound (NY), where values up to 1491 and 49 ng g^{-1} were found for PEGs and AEOs, respectively. Low levels of AEOs in Mar Menor Lagoon may be related to their very effective removal in WWTPs, typically above 97% [8], where they undergo degradation by cleavage of the ether bond (which generates PEGs) and sorption on sludge. On the other hand, occurrence of PEGs in sorbed phases at concentrations above 1000 ng g^{-1} could be explained by a combination of in situ degradation of AEOs, slow degradation rate of PEGs in seawater [27] and sorption enhanced by hydrophilic interactions of the ethoxylated chain with clays [32–35]. Additionally, we have to consider that PEGs are not only AEO metabolites, but also that millions of tons of these chemicals are produced every year worldwide and most of them reach conventional sewage disposal systems after industrial utilization [4]. In this sense, the highest concentrations were detected close to the San Javier-Murcia airport, where PEGs are commonly used in aircraft cleaning products [39]. More information will available as current studies monitoring the occurrence and fate of AEOs and their possible metabolites in Mar Menor Lagoon and other aquatic systems are completed.

4. Conclusions

The present methodology permits the simultaneous determination of the most commonly used nonionic surfactants (AEOs) and their main degradation products (PEGs) in sediment samples with high selectivity and reproducibility, in a simple and less time consuming way when compared with older specific methods for the determination of each surfactant separately. Extraction efficiency depends on the nature of the target compound, ranging between 54% and 106% for most analytes, although recovery values of certain AEO larger ethoxymers dropped to 27–40% because of their high affinity for certain materials (e.g., glass, SPE cartridges) and sediment particles. This issue is also shared with many other multi-residue methods for the analysis of compounds of different chemical nature and polarity. Further it is possible to differentiate among the various AEO homologs and PEG ethoxymers. For better detection of all target compounds, electrospray ionization conditions were optimized for AEOs and PEGs on an ion-trap mass spectrometer. Limits of detection were usually below 50 ng g^{-1} per analyte when sodium adducts were formed. This methodology allowed us to establish a direct comparison between concentrations of AEOs and PEGs in the marine environment for the first time, showing concentrations in surface sediments ranging between 7 and 85 ng g^{-1}, and 1600 and 8800 ng g^{-1}, respectively. The abundance of PEGs was remarkable so further monitoring of these chemicals in aquatic systems is recommended.

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