Talanta 115 (2013) 606–615

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of alcohol sulfates and alcohol ethoxysulfates in marine and river sediments using liquid chromatography–tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 11 January 2013
Received in revised form
20 May 2013
Accepted 27 May 2013
Available online 17 June 2013

Keywords:
Anionic surfactants
Marine sediments
River sediments
Principal components analysis
LC–MS/MS
Pressurized liquid extraction

ABSTRACT

A novel and successful method has been developed for the identification and quantification of alcohol sulfates (AS) homologues and alcohol ethoxysulfates (AES) ethoxymers in marine and river sediment samples. The method involves the extraction of 5.00 g of dry sample with methanol using pressurized liquid extraction (PLE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). 2-Octylbenzene sulfonic acid sodium salt ($2ØC_8$-LAS) was used as internal standard. The analytical methods were applied to marine sediments collected from the coast of Almeria (South-east Spain) and river sediments collected from the Monachil river (Granada, South-east Spain). For AS homologues, the found limits of detection were 0.04–0.08 $\mu g\ g^{-1}$ for marine and river sediments. For AES ethoxymers, the found limits of detection were 0.03–0.09 $\mu g\ g^{-1}$ and 0.06–0.22 $\mu g\ g^{-1}$ for marine and river sediments, respectively. The highest concentrations of AS and AES were found in river sediment samples. Significant differences were also observed between the behavior of short-chain compounds (C_{12}) and long-chain compounds (C_{14} to C_{18}). The influence of the physic-chemical properties of water on the occurrence of these compounds was also evaluated, and differences between long- and short-chain compounds were also observed. Additionally, principal components analyses were carried out in order to study the relationship between variables and to evaluate the sources of data variability and behavior patterns. Finally, important conclusions were drawn regarding the environmental behavior of AS and AES.

1. Introduction

Surfactants are active ingredients in detergent formulations, cleaning and personal care products, emulsifiers, pesticides, adjuvants and wetting agents. These compounds are produced and consumed in large quantities. In 2010, total consumption (not including soaps) in Europe was 2.94 million tons [1]. Two of the most abundant anionic surfactants, especially in household detergents and surface cleaners, are alcohol sulfates (AS) and alcohol ethoxysulfates (AES). These products are high production volume (HPV) chemicals, and as a result many of these chemicals are ultimately released into the environment (at $ng\ L^{-1}$ to $\mu g\ L^{-1}$ levels) [2].

Over the last few years, due to the increasing public concern over environmental safety, laws regarding the use of these compounds have become stricter because of their potential to produce adverse effects on ecosystems and the wildlife that live in them [3]. Coastal ecosystems are the receptors of large amounts of surfactants from urban wastewaters that are discharged, either treated or untreated, directly into the sea or estuary, or indirectly via rivers or groundwater [4]. Surfactants are chemicals that typically contain hydrophobic and hydrophilic groups. The hydrophobic domain is usually a hydrocarbon whereas the hydrophilic group can be non-ionic, positively or negatively charged, or amphoteric. These characteristics give them specific physical and chemical properties. Because of the low solubility and great ability to associate with particles, surfactants are always present in sediments. Marine sediments act both as reservoirs and as potential sources of these chemicals and can adversely affect sediment-dwelling organisms by causing direct toxicity or altering benthic invertebrate community structure [5,6]. In addition, the aqueous ionic composition also influences the sorption of ionizable organic contaminants, since processes such as ion exchange or ion pair formation are directly influenced by the composition of the medium [7,8]. Consequently, the sorption of these pollutants is different in fresh and sea water. In general, sorption coefficients of

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http://dx.doi.org/10.1016/j.talanta.2013.05.058

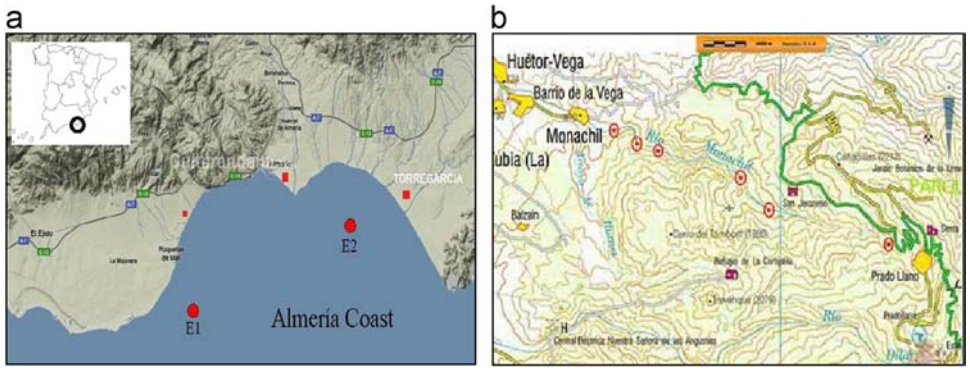

Fig. 1. Maps with sampling sites in (a) the coast of Almeria and (b) the Monachil river.

contaminants in marine environment are higher than in fresh water media [9]. Some of these contaminants are persistent in the environment, and the cumulative effects in coastal environments are expected to be considerable.

Some studies have been conducted to understand the distribution of major anionic surfactants in marine environments [10–14]; however, there are few papers on the determination of AS and AES in river [15] and marine sediments [16]. The main reason for this is the limitations of existing analytical techniques available over the last decade. The analysis of these compounds is complicated due to their structure, the complexity of the matrices and also because these compounds are generally found in very low concentrations. Therefore, it is necessary to develop new analytical methods to improve the isolation and extraction of these compounds. Different methods for the determination of AS and AES in environmental samples have been published in the scientific literature [2, 15–25]. For example, our research group has recently proposed a new procedure for the determination of AS in wastewater samples. The method includes an SPE procedure prior to a hydrolysis-derivatization procedure in one single step to directly convert AS into trimethylsilyl derivatives [18]. On the other hand, the lack of UV absorbance of AS and AES is one of the main problems when trying to detect these compounds using high-performance liquid chromatography with ultraviolet (HPLC-UV) or fluorescence detection (HPLC-FD). To overcome this problem, a derivatization reaction is required. These techniques could be an alternative to the determination of these surfactants in environmental matrices when LC–MS is not available. Beneito-Cambra et al. [19] proposed a method for the determination of fatty alcohol ethoxylates (FAE) and alkylether sulfates (AES) in industrial samples and seawater, where these compounds were extracted using a strong anionic exchanger (SAX), and esterification for FAE and transesterification of AES with a cyclic anhydride was performed. Finally, the separation of the derivatized ethoxymers was achieved using reversed phase (RP), RP-HPLC-UV and mass spectrometry (MS) detection. However, in the last decade, the use of liquid chromatography with mass spectrometry detection (LC–MS) or with tandem mass spectrometry (LC–MS/MS) has become the most powerful tool for surfactant analysis in environmental samples due to its specificity and unequivocal identification of compounds, even allowing their simultaneous determination [2,15,16,19–25].

The aim of the present work was to develop and validate accurate and sensitive analytical methods for the determination of AS homologues and AES ethoxymers in marine and river sediments based on a pressurized liquid extraction (PLE) procedure, followed by a liquid chromatography–tandem mass spectrometric (LC–MS/MS) analysis. After validation, the methods were successfully applied to the analysis of sediment samples collected from the two major wastewater outfalls (at the points of discharge into the Mediterranean Sea) of the coast of Almeria, and from the Monachil river (fed by Sierra Nevada, a mountain range in the province of Granada, Spain). Next, a monitoring and a statistical study, based on the correlation and multivariable analysis, for both AS and AES, were developed to compare the behavior of the compounds in these environmental compartments.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade unless otherwise specified. Individual standard of sodium dodecyl sulfate (AS–C_{12}) (purity 99%) was supplied by Fluka (Madrid, Spain). Sodium 1-tetradecyl sulfate (AS–C_{14}), sodium *n*-hexadecyl sulfate (AS–C_{16}) and sodium *n*-octadecyl sulfate (AS–C_{18}) (purity 95–99%) were supplied by Alfa Aesar (Barcelona, Spain). The commercial mixture of AES (COSMACOL AES 70-2-24) was supplied by Sasol Italy S.p.A. (Milan, Italy) as an aqueous solution of the sodium salt with an AES (AES–C_nE_x content of 70.0% (w/w) with the following homologue distribution: AES–C_{12} (55.0%) and AES–C_{14} (45.0%) and an average numbers of ethoxylated units (EO) of 2.0. The internal standard, 2-octylbenzene sulfonic acid sodium salt (2ØC_8-LAS; 81%, w/w) was supplied by Cepsa Química S.A. (Madrid, Spain). Stock solutions of AS and AES (100 μg mL^{-1}) were prepared in methanol. The solutions were stored at 4 °C in the dark, remaining stable for at least six months. Working standards were prepared immediately before use by dilution in methanol. Methanol and acetonitrile (both HPLC-grade) used as mobile phase were supplied by Merck (Darmstadt, Germany). LC–MS grade water, triethylamine, acetic acid and formaldehyde were supplied by Sigma-Aldrich (Madrid, Spain). Methanol (PAI grade) used in the extraction and clean up were supplied by Panreac (Barcelona, Spain). Water (18.2 MΩ cm) was purified with a Milli-Q plus system (Millipore, Bedford, MA, USA). Prior to injection into the LC system, the samples were filtered through regenerated non-sterile cellulose filters (pore size, 0.20 μm, and 4 mm in diameter) supplied by Sartorius (Goettingen, Germany).

2.2. Instrumentation and software

Pressurized liquid extraction (PLE) was made using a model 200 accelerated solvent extractor (ASE) from Dionex (Dionex Corp, Sunnyvale, CA, USA). A centrifuge, model Universal 32, from Hettich (Tuttlingen, Germany) was used to separate solid and liquid phases. Analyses were performed using an Agilent 1200 series (Agilent Technologies Inc., Palo Alto, CA, USA) high-performance liquid chromatography system equipped with a binary pump, a vacuum membrane degasser, a thermostated

column compartment, an automatic sampler and coupled to an API 2000 (Applied Biosystems, Foster City, CA, USA) triple–quadrupole mass spectrometer system that can use either atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI) interfaces. Analyst software version 1.4.2 was used for instrument control and for data acquisition and analysis. Statgraphics 5.0 software package (Manugistics Inc, Maryland, USA) was used for statistical and regression analysis.

2.3. Sample collection

Marine sediment samples were collected from the two major wastewater outfalls of the coast of Almeria (Spain) (at the points of discharge into the Mediterranean Sea). The outfalls are located at 36°52′19.60″N, 2°08′46.70″W (Outfall 1) and 36°52′33.40″N, 2°08′42.60″W (Outfall 2). The samples were collected seasonally from May 2002 to October 2003 from four different points of each outfall (East, West, immediately below the outfall, and South). Fig. 1a shows the location of the outfalls (labelled E1 and E2) and the sampling sites. Sampling was divided into seven campaigns: campaign 1 (May 2002), campaign 2 (June 2002), campaign 3 (July 2002), campaign 4 (January 2003), campaign 5 (April 2003), campaign 6 (July 2003) and campaign 7 (October 2003). It is important to highlight that although the samples should have been collected from four different points around the outfall (South, East, West and immediately below the outfall), this was not possible due to the absence of fine sediments in some of these sampling points in the different seasons. In some points only rocks were collected. On the other hand, the distance between each of the sampling points and the outfall (central point) was 500 m and only one replicate was collected from each sampling point. According to the marine chart, the samples were collected from a depth of 150 m. In total, 44 representative samples were analyzed for the determination of AS and AES. The sampling process was performed following two published guidelines, the TBT Assessment Project [26] and the EPA Recommended Guidelines for sampling marine sediment, water column, and tissue in Puget Sound [27].

River sediment samples were collected from six sampling sites along the Monachil river bed (Fig. 1b). The temperature, conductivity, redox potential, pH and dissolved O_2 were performed in situ on a routine basis to determine the physico-chemical characteristics of the environment and to detect variations in the quality of the waters of Sierra Nevada. The sampling process was performed according to the ISO 5667-6:2005 [28] and ISO 5667-12:1995 [29]. The samples were obtained between June 2008 and June 2010. The location of the sampling sites was as follows: 37°05′43.82″N, 3°24′13.47″W, altitude 2028 m (Point 1); 37°06′12.47″N, 3°27′16.89″W, altitude 1435 m (Point 2); 37°06′ 52.60″N, 3°28′7.58″W, altitude 1301 m (Point 3); 37°07′15.95″N, 3°30′15.73″W, altitude 1074 m (Point 4); 37°07′23.56″N, 3°30′46.90″W, altitude 1008 m (Point 5) and 37°07′39.79″N, 3°31′14.34″W, altitude 930 m (Point 6). A total of 23 representative samples was analyzed for AS and AES.

After collection, the samples were placed in glass bottles previously cleaned with hydrochloric acid (1:1, v/v) and preserved by immediate addition of formaldehyde 3% (v/v). The samples were then transported to the laboratory and stored refrigerated in the darkness at 4 ± 2 °C. The usual precautions were taken to avoid contamination. The samples were lyophilized for at least 48 h or until the process was completed, and then sieved through a 2 mm mesh. Spiked samples were prepared by adding 2 mL of a methanolic solution containing the analytes AS–C_{12}, AS–C_{14}, AS–C_{16}, AS–C_{18} and commercial mixture of AES to 5.00 g of sample. The bulk of solvent was slowly evaporated at room temperature for 24 h.

2.4. Sample treatment

Aliquots of 5.00 ± 0.01 g of dried sediment samples were weighed and transferred into a cylindrical cell of the extractor (33 mL). Methanol was used as solvent. Each extraction began with a 2 min preheating phase, followed by 5 min heating and 10 min static extraction. Static extraction was performed at constant temperature and pressure (125 °C, 1000 psi). One extraction cycle was used. After the extraction process was completed, methanol was added to the extract to a final volume of 50 mL in a volumetric flask and 500 μL of the methanolic extract plus 500 μL aqueous solution of the internal standard (2ØC_8-LAS) were transferred to a chromatographic vial. Finally, the sample was shaken for 1 min and injected into the LC–MS/MS system using the instrumental conditions described in the following section.

2.5. LC–MS/MS analysis

The separation of AS homologues and AES ethoxymers was performed using a Zorbax Eclipse XDB–C_{18} analytical column (50 × 4.6 mm i.d., 1.8 μm particle size) from Agilent. The mobile phase was a mixture of triethylamine/acetic acid (50 mM) solution in water (solvent A) and acetonitrile/water (80:20, v/v) solution (solvent B). A linear gradient was used for the separation of AS homologues. The gradient conditions were the following: 0.0 to 30 min, 50 to 100% B; back to 50% B in 5.0 min to restore initial conditions. The total run time was 35 min, and the post-delay time for reconditioning the column was 5 min. Flow rate was 0.5 mL min^{-1}, injection volume 5 μL, and the column temperature was maintained at 30 °C. For AES ethoxymers, the gradient conditions were the following: 0.0 to 15 min, 30 to 100% B; back to 30% B in 5.0 min to restore initial conditions. The total run time was 20 min, and the post-delay time for reconditioning the column was 5 min. The injection volume was also 5 μL, the mobile phase flow rate 0.5 mL min^{-1}, and the column temperature was 30 °C.

For MS/MS detection, electrospray ionization (ESI) was used in the negative ion mode. The tandem mass spectrometer was operated in the selected reaction monitoring mode (SRM) and Q1 and Q3 quadrupoles were set at unit mass resolution (± 0.7 Da). The mass spectrometric conditions were optimized for each compound by infusing standard solutions of AS (8.0 μg mL^{-1} of each one) and AES (35.0 μg mL^{-1} commercial mixture). The ion source temperature was maintained at 450 °C. The IonSpray voltage was set at $^{-}$4.2 kV. Nitrogen was used as both curtain gas (45 psi) and ion source gas 1 and 2 (50 and 55 psi, respectively). Collision gas was air at 4 psi. Other adjustments like entrance potential (EP), declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) were optimized for each analyte. The dwell time for each compound was set to 50 ms. Table 1 summarizes the instrumental conditions of the mass spectrometer.

2.6. Statistical analysis

To carry out the statistical analysis, the concentrations of homologues AS–C_{12}, AS–C_{14}, AS–C_{16} and AS–C_{18} and the concentration of the different ethoxymers, AES–C_nE_x ($n=12, 14$; $x=0$–6) were selected as response variables. For river sediment, the physic-chemical properties of water were also included. The following physic-chemical parameters in river water were evaluated: chloride concentration, pH, temperature, conductivity, redox potential and dissolved O_2 concentration. For marine sediments, the relationship between the response variables and other marine sediment variables including sampling site, outfall type, and sampling frequency was determined.

In order to simplify the statistical treatments, first, a preliminary data reorganization was performed. The data from each marine

Table 1
MRM transitions selected and optimized potentials: declustering potential (DP), focusing potential (FP), entrance potential (EP); collision energy (CE) and collision cell exit potential (CXP).

Compounds	Parameters					
	Transitions[a]	DP (V)	FP (V)	EP (V)	CE (V)	CXP (V)
IS	268.9→183.5	−40	−350	−10	−50	−15
Alcohol sulfates						
AS–C_{12}	265.2→96.9	−30	−350	−10	−34	−20
AS–C_{14}	293.0→97.0	−30	−350	−10	−42	−25
AS–C_{16}	321.0→97.0	−39	−350	−10	−44	−28
AS–C_{18}	349.5→96.9	−39	−351	−8	−50	−32
Alcohol ethoxysulfates						
AES–$C_{12}E_1$	309.0→97.0	−34	−350	−10	−50	−16
AES–$C_{12}E_2$	353.3→97.3	−45	−350	−10	−54	−17
AES–$C_{12}E_3$	397.0→97.1	−41	−350	−10	−57	−18
AES–$C_{12}E_4$	441.1→97.0	−73	−350	−10	−69	−23
AES–$C_{12}E_5$	485.1→97.0	−76	−350	−10	−80	−25
AES–$C_{12}E_6$	529.4→97.0	−80	−350	−10	−93	−21
AES–$C_{12}E_7$	573.3→97.1	−87	−350	−10	−100	−20
AES–$C_{12}E_8$	617.2→97.0	−95	−350	−10	−100	−20
AES–$C_{12}E_9$	661.2→97.0	−100	−350	−10	−100	−20
AES–$C_{12}E_{10}$	705.2→97.0	−113	−350	−10	−110	−15
AES–$C_{12}E_{11}$	749.2→97.0	−117	−350	−10	−100	−20
AES–$C_{12}E_{12}$	793.5→97.0	−118	−350	−10	−97	−20
AES–$C_{14}E_1$	337.0→96.9	−40	−350	−10	−50	−13
AES–$C_{14}E_2$	381.1→97.0	−50	−350	−10	−57	−15
AES–$C_{14}E_3$	425.1→97.0	−60	−350	−10	−63	−16
AES–$C_{14}E_4$	469.4→97.0	−70	−350	−10	−75	−15
AES–$C_{14}E_5$	513.5→97.1	−80	−350	−10	−86	−15
AES–$C_{14}E_6$	557.4→97.2	−90	−350	−10	−93	−16
AES–$C_{14}E_7$	601.5→97.0	−100	−350	−10	−96	−16
AES–$C_{14}E_8$	645.3→97.0	−110	−350	−10	−97	−20
AES–$C_{14}E_9$	689.5→97.2	−116	−350	−10	−97	−20
AES–$C_{14}E_{10}$	733.3→97.0	−119	−350	−10	−97	−20
AES–$C_{14}E_{11}$	777.4→97.0	−122	−350	−10	−100	−20
AES–$C_{14}E_{12}$	821.5→97.0	−125	−350	−10	−100	−20

[a] Transition used for quantification.

outfall were divided in two groups (outfall 1, labeled as 0 and outfall 2, labeled as 1). The data regarding sampling frequency were divided into seven groups (labeled 1 to 7) corresponding to the seven campaigns. Finally, the data regarding the sampling sites were classified into four groups (East, West, South and immediately below the outfall).

In order to reduce the number of response variables, principal component analysis (PCA) was developed [30]. First, a correlation study between variables was developed and the Pearson's correlation coefficients were calculated to evaluate the degree of correlation between variables and to determine whether a PCA is useful. The nature of the experimental data and the results measured regarding the physic-chemical properties of the water, did not allow us to construct a robust model to correlate these variables with the concentration of AES and AS in sediments. In order to do that, the experimental variables should be evaluated within a higher range and under controlled conditions to correlate the variation of the independent variables with the concentration of AES and AS. Therefore, the aim of apply PCA in this work was as exploratory analysis technique to reduce the number of correlated variables to a new set of non-correlated variables and to identify meaningful latent variables.

3. Results and discussion

3.1. Liquid chromatographic separation

Chromatographic separation was based on the procedure proposed by Lara-Martin et al. [16]. The main objective was to obtain an improvement in sensitivity, selectivity and peak shapes using shorter chromatographic times. We tested both a Zorbax XDB–C_{18} (100 × 2.1 mm i.d., 1.8 μm particle size) and Zorbax XDB–C_{18} (50 mm × 4.6 mm i.d., 1.8 μm particle size) liquid chromatography column from Agilent Technologies. Although the former column provided better resolution for all analytes investigated, clogging of the column occurred with sediment samples. The 50 × 4.6 mm i.d. column was therefore selected for our study.

Some modifications were also performed in the mobile phase. First, the effect of substituting methanol for acetonitrile/water (80:20, v/v) as solvent B and increasing both the concentration of triethylamine and acetic acid to 50 mM in water as solvent A were evaluated. Peak shapes improved and a good resolution of all the ethoxymers was obtained, so this mobile phase was selected for further experiments. The elution gradient was modified for both AS and AES as described in the previous section. The influence of flow rate was also analyzed and 0.5 mL min^{-1} was selected in order to reduce the analysis time. Finally, a study to improve the peak shape by increasing the injection volume was also performed. A range from 5 to 20 μL was studied and 5 μL was selected as injection volume. Fig. 2 shows a chromatogram of an AS standard and a commercial standard mixture of AES in methanol.

3.2. Mass spectrometric analysis

The MS/MS detection method was optimized by direct infusion of individual compounds in order to optimize the response of the precursor ion and product ions. ESI and APCI interfaces in positive and negative modes were evaluated for all the compounds analyzed. ESI in negative mode was selected because of its higher sensitivity for all the compounds. The selected precursor ions for AS, 2ØC_8-LAS – used as internal standard – and AES correspond to the loss of the sodium atom $[M–Na]^-$. The selected ions are shown in Table 1. Due to the low fragmentation that the precursor ion underwent, only one product ion was monitored for SRM method. The parameters optimized for the precursor ions were declustering potential (DP), focusing potential (FP) and entrance potential (EP); and for the product ions were collision energy (CE) and collision cell exit potential (CXP). Regarding sensitivity, the most influential parameters were DP and CE.

3.3. Extraction procedure

Marine and river sediments (free of contamination), spiked with 40 μg g^{-1} of compounds were used for optimization of the most influential variables affecting the PLE procedure. A sample weight of 5.00 g was used for all the experiments. The effect of solvent, temperature, pressure and number of cycles on the extraction efficiency of PLE were evaluated. First, methanol, dichloromethane and hexane were tested. The best recoveries were obtained using methanol as extraction solvent and therefore this was chosen for further optimization of the PLE procedure. Extraction temperature, extraction time and number of cycles were optimized. Temperatures from 75 to 150 °C, extraction times from 1 to 20 min, and 1 to 3 numbers of cycles were evaluated. The optimal values for all compounds were obtained at 125 °C, 10 min and 1 cycle.

3.4. Analytical performance and validation of the method

Calibration graphs were obtained in the selected reactions monitoring (SRM) mode for spiked sediment (marine and river) samples using the analytical procedure described above. The samples were spiked approximately 24 h before the analyses. 2ØC_8-LAS was used as internal standard. The method was applied

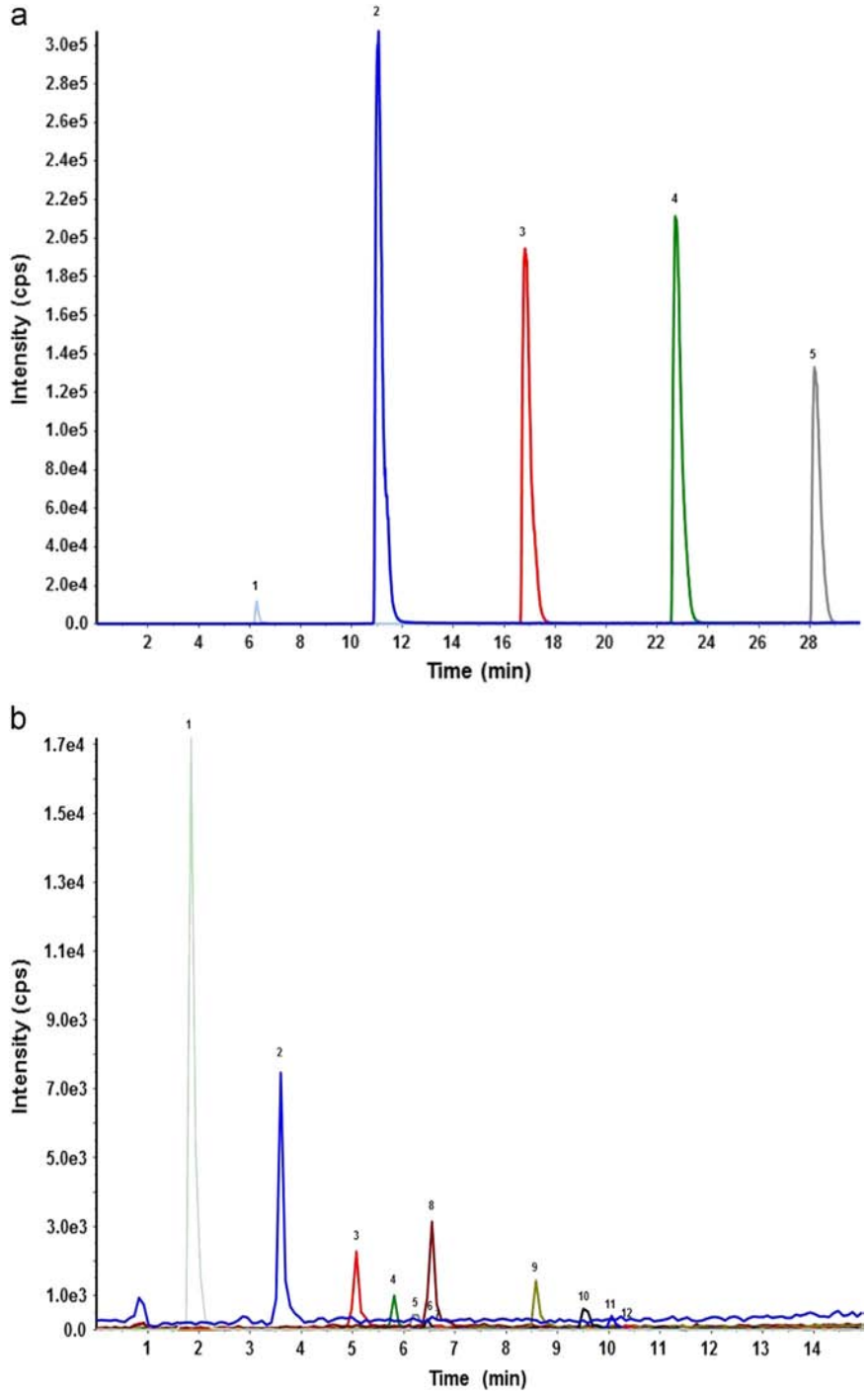

Fig. 2. SRM chromatogram of a methanolic standard of: (a) An AS mixture: **1**. IS, **2**. AS–C_{12}, **3**. AS–C_{14}, **4.** AS–C_{16}, **5**. AS–C_{18}. (b) A commercial mixture of AES: **1**. IS, **2**. $C_{12}E_0$, **3**. $C_{12}E_1$, **4**. $C_{12}E_2$, **5**. $C_{12}E_3$, **6**. $C_{12}E_4$, 7. $C_{12}E_5$, **8**. $C_{14}E_0$, **9**. $C_{14}E_1$, **10**. $C_{14}E_2$, **11**. $C_{14}E_3$, **12**. $C_{14}E_4$.

to blank sediment samples to confirm the absence of target compounds above the limits of detection of the method. Absence of analyte contamination from the containers and material used to handle the samples was accurately checked. Calibration curves were built using the analyte/internal standard peak area ratio *versus* analyte concentration. Table 2 shows the results for the slope (b) and correlation coefficient (R^2) obtained for AS and for the major components of the commercial mixture of AES ($C_{12}E_x$ and $C_{14}E_x$) respectively. The *p*-values of the *lack-of-fit* test, P_{lof} (%), were higher than 5% in all cases. These facts indicate a good linearity within the stated ranged.

The LODs (limits of detection) and LOQs (limits of quantification) were calculated from the blank standard deviation. In order to estimate the chromatographic blanks, we applied the methodology proposed by González-Casado et al. [31]. It can be assumed that the chromatographic peak shape is a Gaussian-type one, the estimation of base width (W_b) for 99.73% of the peak-area is $W_b = 6\sigma = 2.548 W_{0.5\ h}$, where $W_{0.5\ h}$ is the half-width of the peak. Extrapolation in the graph of $W_{0.5\ h}$ at different concentrations of analyte can give us a statistically significant idea of the width of the base for "zero concentration". The blank signal for each analyte can be determined by integration of the chromatograms' baseline

Table 2
Quality and statistical parameters.

	Marine sediments						River sediments					
	b (g μg^{-1})	s_b (g μg^{-1})	LOD (μg g^{-1})	LOQ (μg g^{-1})	LDR (μg g^{-1})	% R^2	b (g μg^{-1})	s_b (g μg^{-1})	LOD (μg g^{-1})	LOQ (μg g^{-1})	LDR (μg g^{-1})	% R^2
AS–C_{12}	0.294	$7\cdot10^{-5}$	0.04	0.12	0.1–100	99.95	0.295	$3\cdot10^{-5}$	0.04	0.14	0.1–100	99.99
AS–C_{14}	0.173	$8\cdot10^{-5}$	0.04	0.13	0.1–100	99.95	0.173	$3\cdot10^{-5}$	0.07	0.23	0.2–100	99.98
AS–C_{16}	0.167	$9\cdot10^{-5}$	0.04	0.15	0.2–100	99.95	0.167	$2\cdot10^{-5}$	0.06	0.20	0.2–100	99.99
AS–C_{18}	0.091	$5\cdot10^{-5}$	0.04	0.15	0.2–100	99.95	0.091	$2\cdot10^{-5}$	0.08	0.26	0.2–100	99.98
AES–$C_{12}E_1$	0.254	$8\cdot10^{-5}$	0.04	0.13	0.1–164	99.99	0.255	$5\cdot10^{-5}$	0.09	0.29	0.3–164	99.98
AES–$C_{12}E_2$	0.224	$7\cdot10^{-5}$	0.04	0.14	0.1–187	99.99	0.225	$4\cdot10^{-5}$	0.10	0.33	0.3–187	99.98
AES–$C_{12}E_3$	0.201	$6\cdot10^{-5}$	0.05	0.16	0.2–208	99.99	0.201	$4\cdot10^{-5}$	0.11	0.37	0.4–208	99.98
AES–$C_{12}E_4$	0.182	$6\cdot10^{-5}$	0.05	0.18	0.2–230	99.99	0.182	$3\cdot10^{-5}$	0.12	0.41	0.4–230	99.98
AES–$C_{12}E_5$	0.166	$5\cdot10^{-5}$	0.06	0.19	0.2–252	99.99	0.167	$3\cdot10^{-5}$	0.13	0.45	0.4–252	99.98
AES–$C_{12}E_6$	0.153	$5\cdot10^{-5}$	0.06	0.21	0.2–274	99.99	0.153	$3\cdot10^{-5}$	0.15	0.49	0.5–274	99.98
AES–$C_{12}E_7$	0.141	$4\cdot10^{-5}$	0.07	0.23	0.2–296	99.99	0.142	$3\cdot10^{-5}$	0.16	0.52	0.5–296	99.98
AES–$C_{12}E_8$	0.132	$4\cdot10^{-5}$	0.07	0.24	0.2–318	99.99	0.132	$2\cdot10^{-5}$	0.17	0.56	0.6–318	99.98
AES–$C_{12}E_9$	0.123	$4\cdot10^{-5}$	0.08	0.26	0.3–339	99.99	0.124	$2\cdot10^{-5}$	0.18	0.60	0.6–339	99.98
AES–$C_{12}E_{10}$	0.116	$4\cdot10^{-5}$	0.08	0.28	0.3–361	99.99	0.116	$2\cdot10^{-5}$	0.19	0.64	0.6–361	99.98
AES–$C_{12}E_{11}$	0.109	$3\cdot10^{-5}$	0.09	0.29	0.3–383	99.99	0.110	$2\cdot10^{-5}$	0.20	0.68	0.7–383	99.98
AES–$C_{12}E_{12}$	0.103	$3\cdot10^{-5}$	0.09	0.31	0.3–405	99.99	0.104	$2\cdot10^{-5}$	0.22	0.72	0.7–405	99.98
AES–$C_{14}E_1$	0.150	$6\cdot10^{-5}$	0.04	0.12	0.1–117	99.99	0.148	$3\cdot10^{-5}$	0.07	0.24	0.2–117	99.98
AES–$C_{14}E_2$	0.134	$5\cdot10^{-5}$	0.04	0.13	0.1–132	99.99	0.132	$3\cdot10^{-5}$	0.08	0.27	0.3–132	99.98
AES–$C_{14}E_3$	0.120	$5\cdot10^{-5}$	0.04	0.15	0.2–146	99.99	0.119	$2\cdot10^{-5}$	0.09	0.30	0.3–146	99.98
AES–$C_{14}E_4$	0.109	$4\cdot10^{-5}$	0.05	0.16	0.2–160	99.99	0.108	$2\cdot10^{-5}$	0.10	0.32	0.3–160	99.98
AES–$C_{14}E_5$	0.101	$4\cdot10^{-5}$	0.05	0.18	0.2–175	99.99	0.099	$2\cdot10^{-5}$	0.11	0.35	0.4–175	99.98
AES–$C_{14}E_6$	0.093	$4\cdot10^{-5}$	0.06	0.19	0.2–189	99.99	0.092	$2\cdot10^{-5}$	0.11	0.38	0.4–189	99.98
AES–$C_{14}E_7$	0.086	$3\cdot10^{-5}$	0.06	0.20	0.2–203	99.99	0.086	$2\cdot10^{-5}$	0.12	0.41	0.4–203	99.98
AES–$C_{14}E_8$	0.081	$3\cdot10^{-5}$	0.07	0.22	0.2–218	99.99	0.080	$2\cdot10^{-5}$	0.13	0.44	0.4–218	99.98
AES–$C_{14}E_9$	0.076	$3\cdot10^{-5}$	0.07	0.23	0.2–232	99.99	0.075	$2\cdot10^{-5}$	0.14	0.47	0.5–232	99.98
AES–$C_{14}E_{10}$	0.071	$3\cdot10^{-5}$	0.07	0.25	0.2–246	99.99	0.070	$1\cdot10^{-5}$	0.15	0.50	0.5–246	99.98
AES–$C_{14}E_{11}$	0.067	$3\cdot10^{-5}$	0.08	0.26	0.3–261	99.99	0.067	$1\cdot10^{-5}$	0.16	0.53	0.5–261	99.98
AES–$C_{14}E_{12}$	0.064	$3\cdot10^{-5}$	0.08	0.28	0.3–275	99.99	0.063	$1\cdot10^{-5}$	0.17	0.56	0.6–275	99.98

b, Slope; s_b, Slope deviation; LOD, Limit of detection; LOQ, Limit of quantification; LDR, Linear dynamic range; R^2. Determination coefficient.

taking a width $t_R \pm 0.5W_{b0}$, where t_R is the retention time of the analyte and W_{b0} has been evaluated as explained above. The determination of the sensitivity relies on studying the blank standard deviation in a time interval corresponding to the peak width at its base, extrapolated to zero concentration. The LOD was 3 s_0 and the LOQ was 10 s_0. First, for marine sediments the LOD was 0.04 μg g^{-1} for each AS homologue, and ranged from 0.03 to 0.09 μg g^{-1} for AES–$C_{12}E_x$ and from 0.03 to 0.08 μg g^{-1} for AES–$C_{14}E_x$. In the case of river sediments, the LOD was 0.04–0.08 μg g^{-1} for AS homologues, and ranged from 0.08 to 0.22 μg g^{-1} for AES–$C_{12}E_x$ and from 0.06 to 0.17 μg g^{-1} for AES–$C_{14}E_x$.

Linearity of the calibration graphs was tested according to the Analytical Methods Committee guidelines [32]. The *lack-of-fit* test was applied to three replicates and three injections of each standard. The behavior of compounds was linear in the range from the LOQ to 100 μg g^{-1} for each AS homologue, LOQ–143 μg g^{-1} for AES–$C_{12}E_x$ and LOQ–103 μg g^{-1} for AES–$C_{14}E_x$, with R^2 values close to 100% for each compound.

In addition, the accuracy of the method in terms of trueness and precision was studied. Due to the absence of certified materials, a recovery assay was performed in order to validate the method in terms of trueness. Blank sediment (marine and river) samples were analyzed to ensure that they did not contain the analytes or they were below the LOD of the method. The trueness was evaluated by determining the recovery of known amounts of the tested compounds in river sediment at four concentration levels (1.00, 25.0, 50.0 and 100.0 μg g^{-1} for AS; 5.96, 35.8, 71.5 and 143.0 μg g^{-1} for AES–$C_{12}E_x$ and 4.30, 25.8, 51.6 and 103.1 μg g^{-1} for AES–$C_{14}E_x$, respectively). Samples were analyzed using the proposed methods and the concentration of each compound was determined by interpolation from the standard calibration curve within the linear dynamic range and compared with the amount of analytes previously added to the samples. The obtained recoveries are shown in Table 3. The recoveries were very close to 100% (97.0–103.8% for AS and 96.1–102.5% for AES).

To evaluate the overall precision of the method, intra- and inter-day precision (as relative standard deviation, RSD) were assessed at four concentration levels. The procedure was repeated three times on the same day to evaluate the repeatability and was repeated for seven consecutive days to determine inter-day reproducibility. Repeatability and inter-day reproducibility values (RSD) are shown in Table 3. RSD values were between 0.2–3.0% for AS and 0.3–2.9% for AES. Precision and recovery values demonstrate the accuracy of the proposed methodology.

3.5. Application to marine and river sediment samples

The analytical method was applied to the determination of the amount of AS homologues and AES ethoxymers in marine and river sediment samples. Concentration values for AS and AES (six replicates) in marine sediment samples from the coast of Almeria are shown in Table 4. For the sake of clarity, Outfall 1 and Outfall 2 were named E1 and E2, respectively. Similarly, sampling locations were named: South (S), East (E), West (W) and below the outfall (B). Seven sampling campaigns were carried out seasonally during 15 consecutive months.

AS were quantified in 18.2% ($n=8/44$) of the analyzed samples, in concentrations ranging from 1.1 to 29.9 μg g^{-1}. Both AES–$C_{12}E_x$ and AES–$C_{14}E_x$ were also quantified in 18.2% ($n=8/44$) of the analyzed samples, in concentrations ranging from 1.2 to 29.9 μg g^{-1} and from 1.2 to 7.1 μg g^{-1}, respectively. In both cases,

Table 3
Recovery study for marine and river sediment samples.

	Marine sediment				River sediment			
	Spiked ($\mu g\ g^{-1}$)	Found[a] ($\mu g\ g^{-1}$)	RSD[b] (%)	Recovery (%)	Spiked ($\mu g\ g^{-1}$)	Found[a] ($\mu g\ g^{-1}$)	RSD[b] (%)	Recovery (%)
AS–C_{12}	1.00	1.01	4.0	101.0	1.00	0.97	3.8	97.0
	25.0	24.3	3.3	97.2	25.0	25.8	3.7	103.2
	50.0	49.1	3.7	98.2	50.0	49.5	3.5	99.0
	100.0	102.1	3.9	102.1	100.0	97.1	4.6	97.1
AS–C_{14}	1.00	0.96	3.9	96.0	1.00	0.98	4.0	98.0
	25.0	25.5	3.5	102.0	25.0	25.4	2.6	101.6
	50.0	49.8	3.4	99.6	50.0	51.9	3.5	103.8
	100.0	102.3	4.3	102.3	100.0	103.1	4.0	103.1
AS–C_{16}	1.00	0.97	4.2	97.0	1.00	1.03	4.8	103.0
	25.0	24.6	3.8	98.4	25.0	25.8	3.4	103.2
	50.0	50.8	3.2	101.6	50.0	49.9	4.0	99.8
	100.0	97.6	3.9	97.6	100.0	97.3	4.8	97.3
AS–C_{18}	1.00	0.97	3.9	97.0	1.00	0.99	3.2	99.0
	25.0	25.4	2.7	101.6	25.0	25.9	3.2	103.6
	50.0	49.6	3.3	99.2	50.0	51.4	2.5	102.8
	100.0	98.5	3.5	98.5	100.0	98.9	4.9	98.9
AES–$C_{12}E_x$[c]	5.96	5.75	3.6	96.5	5.96	5.70	4.3	95.6
	35.75	34.81	3.5	97.3	35.75	36.85	3.4	102.8
	71.50	73.83	3.3	103.3	71.50	70.08	2.4	98.0
	143.0	139.7	3.7	97.7	143.00	141.04	4.8	98.6
AES–$C_{14}E_x$[c]	4.30	4.48	3.3	104.2	4.30	4.18	3.4	97.2
	25.78	25.12	2.5	97.4	25.78	25.60	3.3	99.3
	51.55	52.93	3.4	102.7	51.55	52.69	4.6	102.2
	103.1	100.9	3.2	97.8	103.11	106.06	4.0	103.0

[a] Mean value of 21 determinations.
[b] Relative standard deviation.
[c] Sum of ethoxymers.

for AES, ethoxymers with three units were quantified and ethoxymers containing up to five ethoxylated units were detected.

The results revealed that AS–C_{12} concentration was significantly higher than that of the rest of compounds in most samples. Finally, it is remarkable that the maximum values were always found in samples collected immediately below the outfall, as in $(E_1.B)_3$ and $(E_1.B)_5$. The low mobility and dispersibility of the compounds could explain this fact.

On the other hand, the concentration values for AS and AES (six replicates) in river sediment from the Monachil river are shown in Table 5. Five samplings were carried out at each season from June 2008 to June 2010.

AS were quantified in 60.9% ($n=14/23$) of the analyzed samples, in concentrations ranging from 4.5 to 65.6 $\mu g\ g^{-1}$ analyzed according to the homologues. AES–$C_{12}E_x$ were quantified in 60.9% ($n=14/23$) of the analyzed samples, in concentrations ranging from 1.1 to 65.6 $\mu g\ g^{-1}$. Ethoxymers were quantified with 4 units and were detected ethoxymers containing up to 5 ethoxylated units. AES–$C_{14}E_x$ were quantified in 60.9% ($n=14/23$) of the analyzed samples, in concentrations ranging from 1.0 to 23.9 $\mu g\ g^{-1}$. Ethoxymers with 3 units were quantified and ethoxymers containing up to 4 ethoxylated units were detected.

The proposed methods present some advantages in comparison with some previously published methods found in the scientific literature [15–16]. First, a faster extraction procedure of analytes from soil samples is proposed; on the other hand, many intermediate steps such as evaporation of the methanolic extract, redissolution in water, preconcentration by SPE, evaporation of the eluate until dryness and redissolution before to be injected, have been avoided. Finally, one of the most important advantages of the proposed method is the individual quantification of each AES ethoxymer.

3.6. Statistical analysis for river sediments

Results of the correlation study are presented in Supplementary material (only significant correlations [$p < 0.05$] are shown). The study demonstrated high correlations between variables; this fact showed that the individual variables could be grouped into other variables which contain them.

Strong correlations were found between AS and AES, but it is relevant that AS–C_{12} and any of the AES–$C_{12}E_x$ did not correlate with any AS or AES with a carbon-chain longer than 12 atoms (C_{14} to C_{18}). Additionally, all compounds with similar carbon-chain length correlated with each other, being the number of ethoxylated units is not significant. This reflects that the environmental behavior, and probably the sources of AS–C_{12} and AES–$C_{12}E_x$ are different to those of AS–C_{14-18} and AES–$C_{14}E_x$. Moreover, strong correlations were found between AS/AES, dissolved O_2 and conductivity. pH only was correlated with short-chain AS (AS–C_{12}). Temperature and redox potential were not correlated with AS or AES. AS/AES were strongly correlated with dissolved O_2. AS–C_{12} and AES–$C_{12}E_x$ were positively correlated with O_2, whereas AS–C_{14-18} and AES–$C_{14}E_x$ correlated negatively.

After confirmation of correlation between variables, the PCA analysis was carried out (Table 6). The first component (PC1) had a high eigenvalue (8.76), and it explained 51.5% of data variability. Two more principal components with eigenvalues higher than 1 were obtained (3.47 and 2.38). They explained 20 and 14% of total data variability. Based on these results, it was possible to reduce the response variables to just three, called PC1, PC2 and PC3.

Positive contributions of AS–C_{12}, AES–$C_{12}E_1$ and dissolved O_2 concentrations were found. Some variables with negative contribution were obtained: AS–C_{14}, AS–C_{18}, AES–$C_{14}E_1$, AES–$C_{14}E_2$, AES–$C_{14}E_3$ and temperature. There was a negative correlation

Table 4
Values detected for AS and AES in sediments from the coast of Almería.

	AS (μg g^{-1})								AES (μg g^{-1})							
	C_{12}	C_{14}	C_{16}	C_{18}	$C_{12}E_1$	$C_{12}E_2$	$C_{12}E_3$	$C_{12}E_4$	$C_{12}E_5$	$C_{12}E_6$	$C_{14}E_1$	$C_{14}E_2$	$C_{14}E_3$	$C_{14}E_4$	$C_{14}E_5$	$C_{14}E_6$
$(E_1.B)_1$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.E)_1$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.B)_1$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_1$	2.5	D	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_1$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_1$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_2$	3.1	D	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.E)_2$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.S)_2$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.W)_2$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_2$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_2$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_3$	29.9	3.9	D	D	12.2	6.4	3.7	D	D	< LOD	3.1	2.0	D	D	< LOD	< LOD
$(E_1.E)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.S)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.W)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.B)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_3$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_4$	7.6	4.9	1.8	D	3.2	1.2	D	D	< LOD	< LOD	3.9	2.3	1.5	D	< LOD	< LOD
$(E_1.S)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.W)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.B)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_4$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_5$	12.1	7.1	3.4	3.2	5.1	D	D	< LOD	< LOD	< LOD	5.2	3.7	1.3	D	D	< LOD
$(E_1.E)_5$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.S)_5$	7.5	4.1	2.2	D	3.3	< LOD	< LOD	< LOD	< LOD	< LOD	3.5	2.2	D	D	< LOD	< LOD
$(E_1.W)_5$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_5$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_5$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_5$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_6$	5.9	4.4	1.3	D	8.7	5.6	9.2	D	D	< LOD	4.0	2.6	2.5	D	D	< LOD
$(E_1.S)_6$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.B)_6$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.E)_6$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_6$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.W)_6$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.B)_7$	8.9	6.3	2.2	1.1	3.8	D	D	< LOD	< LOD	< LOD	4.5	3.2	1.2	D	D	< LOD
$(E_1.E)_7$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_1.S)_7$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
$(E_2.S)_7$	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

LOD: Limit of detection; D: detected (between LOD and LOQ).

between concentration of dissolved O_2 and temperature ($R^2=-0.892$; $p=0$). This is in accordance with thermodynamics/gas laws which states that a decrease in temperature results in an increase in gas solubility. Therefore, PC1 is related to the solubility of compounds as a function of temperature and O_2 solubility. Concentration of AS and AES with longer chains (C_{14} to C_{16}) increases with higher water temperatures. AS and AES with C_{12} chains are predominant at lower temperatures. Concentration of AES ethoxymers with more ethoxylated units also decreases with lower temperatures. This first component represents the principal cause of data variability and the different distribution between AS homologues and AES ethoxymers, and is related to water temperature. These results are also in concordance with the results of the correlation study. The loading and score plots for PC1 and PC2 are shown in Fig. 3. This graph shows two clearly defined clusters where the variables are grouped according to their positive or negative contribution to PC1.

In the graph corresponding to loading plots, it can be observed how the variables in PC1 are clearly distributed into two groups, one group composed of AS and AES with an alkyl chain of 12 carbon atoms, and the other group composed of long chain AS and AES. In this graph the opposite behavior of AS/AES–C_{12} and AES–C_{14}/AS–C_{14-16} is more conspicuous. The score plot shows that the sampling sites are also distributed into two groups, each group including all the samples from the same campaign. This means that the distribution and behavior of AS and AES are independent of the sampling site, and it is related only to the physic-chemical properties of water and sampling date. For PC2 (vertical axis), an opposite contribution can observed between pH, redox potential and AES–$C_{12}E_2$ and AES–$C_{12}E_3$.

PC2 explained 20.4% of the total variance. A positive contribution of pH and redox potential was observed. Since the redox potential is dependent on the pH, a similar contribution is expected from both variables. No other significant positive contributions were found, but negative contributions of AES–$C_{12}E_1$, AES–$C_{12}E_2$, AES–$C_{12}E_3$, and AES–$C_{14}E_3$ were found. It can be observed that the contribution of AES to PC2 increases with the number of ethoxylated units. AS did not have a significant contribution to PC2. An explanation could be the relationship between water pH and solubility of most ethoxylated AES–C_{12}. Higher pH and higher redox potential lead to lower

Table 5
Values detected for AS and AES in sediments from the Monachil river.

Sample	AS (μg g^{-1})								AES (μg g^{-1})							
	C_{12}	C_{14}	C_{16}	C_{18}	$C_{12}E_1$	$C_{12}E_2$	$C_{12}E_3$	$C_{12}E_4$	$C_{12}E_5$	$C_{12}E_6$	$C_{14}E_1$	$C_{14}E_2$	$C_{14}E_3$	$C_{14}E_4$	$C_{14}E_5$	$C_{14}E_6$
June 2008																
2	65.6	9.4	D	< LOD	47.4	26.4	12.9	5.2	D	D	6.8	6.2	3.1	D	< LOD	< LOD
3	56.8	7.8	D	< LOD	36.3	24.8	10.9	8.6	3.5	D	4.8	2.2	D	< LOD	< LOD	< LOD
March 2009																
1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
2	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
3	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
May 2009																
1	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
2	D	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
3	D	D	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
4	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
5	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
6	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
November 2009																
1	25.3	13.5	11.1	18.6	17.9	9.5	7.6	2.3	D	< LOD	6.8	3.1	1.0	D	< LOD	< LOD
2	25.6	4.6	12.8	17.8	21.3	16.9	13.0	3.5	D	< LOD	3.0	D	< LOD	< LOD	< LOD	< LOD
3	31.6	8.9	13.4	12.4	20.5	10.5	8.3	2.7	D	< LOD	4.6	1.1	D	< LOD	< LOD	< LOD
4	31.9	4.5	14.2	13.5	26.0	12.0	8.6	2.1	D	< LOD	2.3	D	< LOD	< LOD	< LOD	< LOD
5	29.4	7.2	10.3	16.8	21.2	7.5	2.3	D	< LOD	< LOD	4.1	1.6	D	< LOD	< LOD	< LOD
6	22.5	4.7	10.8	18.2	19.8	11.5	6.3	2.0	D	< LOD	1.5	D	< LOD	< LOD	< LOD	< LOD
June 2010																
1	18.8	22.2	25.2	22.8	16.6	11.5	8.8	2.3	D	< LOD	13.7	9.1	9.0	2.8	D	< LOD
2	19.0	21.6	25.7	23.1	20.3	14.3	14.0	8.5	2.1	D	18.6	14.6	6.3	D	< LOD	< LOD
3	19.1	21.8	25.2	23.1	11.1	6.5	1.3	D	< LOD	< LOD	20.8	11.0	8.2	3.1	D	< LOD
4	19.4	21.8	25.3	22.8	7.3	3.1	D	< LOD	< LOD	< LOD	12.5	5.4	1.2	D	< LOD	< LOD
5	19.7	22.2	25.2	22.9	15.2	8.9	4.3	1.1	D	< LOD	23.9	21.2	7.8	3.3	D	< LOD
6	19.3	21.9	25.2	23.0	12.1	7.6	4.5	2.0	D	< LOD	14.2	8.7	4.3	D	< LOD	< LOD

LOD: Limit of detection; D: detected (between LOD and LOQ).

Table 6
Principal components analysis.

	PCA results for AS and AES			
	Component 1	**Component 2**	**Component 3**	**Component 4**
Eigenvalue	8.763	3.468	2.383	0.840
Proportion	0.515	0.204	0.140	0.049
Cumulative	51.5%	71.9%	86.0%	90.9%
Variable	**Loading values**			
ASC_{12}	0.305	−0.066	0.088	−0.201
ASC_{14}	−0.324	0.088	0.057	−0.109
ASC_{16}	−0.322	0.059	0.080	−0.195
ASC_{18}	−0.313	0.058	−0.087	0.214
AES–$C_{12}E_1$	0.236	−0.241	0.268	0.140
AES–$C_{12}E_2$	0.073	−0.439	0.290	−0.159
AES–$C_{12}E_3$	0.051	−0.437	0.282	−0.146
AES–$C_{12}E_4$	−0.110	−0.164	0.419	0.523
AES–$C_{14}E_1$	−0.324	0.070	0.095	0.014
AES–$C_{14}E_2$	−0.300	0.011	0.154	0.073
AES–$C_{14}E_3$	−0.268	−0.158	0.069	0.090
Cl^-	0.008	−0.384	−0.446	0.058
pH	0.169	0.333	0.156	−0.463
Temperature	−0.292	−0.184	−0.111	−0.158
Conductivity	0.046	−0.243	−0.538	0.117
Redox potential	0.19	0.363	0.035	0.468
O_2 dissolved	0.327	−0.006	−0.015	0.188

solubility of ethoxylated AES–C_{12} in sediments, or maybe to their rapid degradation in sediments. Only AES–$C_{14}E_3$ is slightly affected by this component.

Finally, PC3 explained only a 14.0% of the variance, making its interpretation more difficult. In this component, there is a strong negative contribution of conductivity (−0.538). All AS and AES, except AS–C_{18}, had and opposite contribution to conductivity. This means that, in a minor manner, a decrease in conductivity lead to a higher presence of AS and AES in sediments. This can be explained by the presence of some ions like Na^+, which play an important role in AS and AES solubility and which are related to water conductivity.

3.7. Statistical results for marine sediments

Since in almost all sampling sites the obtained results for AS and AES were below the LOQ, it was impossible to make an extensive statistical evaluation of the results and only a correlation study between AS and AES was carried out. Analytes found only in one sample or not found were not included in the study. The results of the correlation study are shown in Supplementary material. Only correlations between compounds with similar alkyl chain length were found. Although it was impossible to perform the correlation study including AS–C_{16} and AS–C_{18}, it was observed that the sample with the highest concentrations of AS–C_{14} and AES–$C_{14}E_x$ also had the highest concentrations of AS–C_{16} and AS–C_{18}; in fact, this was the only sample where these long-chain AS were detected. These results are in accordance with those obtained in river sediments, showing that different behavior is expected between AS and AES depending on the alkyl chain length.

4. Conclusions

New analytical methods for the characterization and quantification of the homologues of AS C_{12}–C_{18} and individual ethoxymers of AES in river and marine sediment samples at μg g^{-1} levels have been developed. The methods are based on the use of PLE followed by LC–MS/MS analysis. The accuracy, reproducibility, and

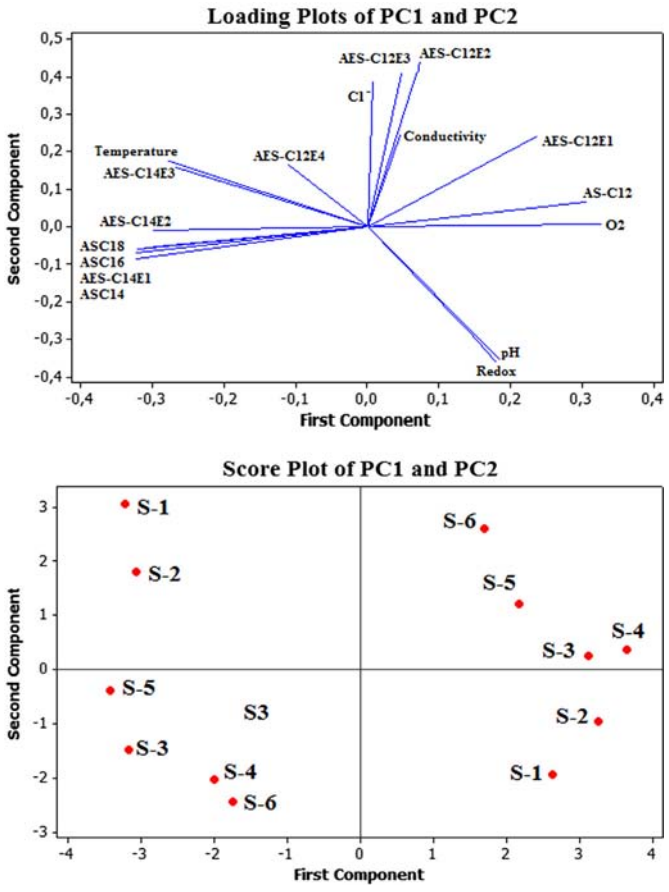

Fig. 3. Loading and score plots for Principal Component 1 and 2.

robustness as well as the viability for use in routine anionic surfactant analysis were evaluated. Good linearity was obtained from all analytes. Recovery from the PLE extraction process was acceptable taking into account the different polarities of all compounds analyzed. Finally, monitoring studies were performed in the coast of Almeria and in the Monachil river bed. Several conclusions can be drawn from the results:

(a) AS and AES concentrations in river sediments were higher than concentrations in marine sediments.
(b) The maximum value (for AS–C_{12}) was 29.9 μg g^{-1} in marine sediment, and 65.6 μg g^{-1} in river sediments.
(c) No relationship between sampling site and concentration was found, because the amount of compound depends on uncontrolled variables.
(d) In river sediments, ethoxymers with 4 units for AES–C_{12} were quantified, and ethoxymers with up to 5 ethoxylated units for AES–C_{12} were detected. For AES–C_{14}, ethoxymers with 3 units were quantified and ethoxymers with up to 4 ethoxylated units were detected.
(e) The behavior of AS homologues and AES ethoxymers varies depending on their alkyl chain length. AS and AES with similar alkyl chain length are always correlated and exhibited similar behavior in marine and river sediments.
(f) Dissolved O_2, pH and conductivity were the principal physicchemical variables affecting the presence of AS and AES in sediments.

These results could be used in the future by National or International Agencies to establish the limited values of anionic surfactants in sediment ecosystems.

Acknowledgements

The present work was supported by the Junta de Andalucía (Project of Excellence No. P06-FQM-01529) and by the Spanish Ministry of Education (Project No. CTQ2011-24210/PPQ). The authors are grateful to Cepsa Química S.A. and to the University of Granada for their valuable contribution to this study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.05.058.

References

[1] European Committee of Surfactants and their Organic Intermediates (CESIO) Statistics (2010) Available at: ⟨http://www.cefic.be/cesio⟩.
[2] H. Sanderson, B.B. Price, S.D. Dyer, A.J. Decarvalho, D. Robaugh, S.W. Waite, S.W. Morrall, A.M. Nielsen, M.L. Cano, A.K. Evans, Sci. Total Environ. 368 (2006) 695–712.
[3] J.A. Field, C.A. Johnson, J.B. Rose, Environ. Sci. Technol. 40 (2006) 7105–7105.
[4] HERA (Human & Environmental Risk Assessment on Ingredients of European Household Cleaning Products) (2009). Available at: ⟨http://www.heraproject.com⟩.
[5] X. Martínez-Lladó, O. Gibert, V. Martí, S. Díez, J. Romo, J.M. Bayona, J. de Pablo, Environ. Pollut. 149 (2007) 104–113.
[6] V. Roussiez, W. Ludwig, A. Mónaco, J.L. Probst, I. Bouloubassi, R. Buscail, G. Saragoni, Cont. Shelf Res. 26 (2006) 1843–1857.
[7] K. Schellenberg, C. Leuenberger, R.P. Schwarzenbach, Environ. Sci. Technol. 18 (1984) 652–657.
[8] C.T. Jafvert, J.C. Westall, E. Grieder, R.P. Schwarzenbach, Environ. Sci. Technol. 24 (1990) 1795–1803.
[9] T.P. Knepper, D. Barceló, P. De Voogt, Analysis and Fate of Surfactants in the Aquatic Environment, Ed, Elsevier Science BV, Amsterdam, The Netherlands, 2003.
[10] H. Takada, R. Ishiwatari, N. Ogura, Estuar. Coast. Shelf Sci. 35 (1992) 141–156.
[11] E. González-Mazo, J.M. Forja, A. Gómez–Parra, Environ. Sci. Technol. 32 (1998) 1636–1641.
[12] V.M. León, M. Sáez, E. González-Mazo, A. Gómez-Parra, Sci. Total Environ. 288 (2002) 215–226.
[13] A. Marcomini, G. Pojana, A. Sfriso, J.M. Quiroga-Alonso, Environ. Toxicol. Chem. 19 (2000) 2000–2007.
[14] T. Isobe, H. Nishiyama, A. Nakashima, H. Takada, Environ. Sci. Technol. 35 (2001) 1041–1049.
[15] P.A. Lara-Martín, A. Gómez-Parra, E. González-Mazo, J. Chromatogr. A 1114 (2006) 205–210.
[16] P.A. Lara-Martín, A. Gómez-Parra, E. González-Mazo, J. Chromatogr. A 1137 (2006) 188–197.
[17] D.D. Popenoe, S.J. Morris, P.S. Horn, K.T. Norwood, Anal. Chem. 66 (1994) 1620–1629.
[18] C. Fernández-Ramos, O. Ballesteros, R. Blanc, A. Zafra-Gómez, I. Jiménez-Díaz, A. Navalón, J.L. Vílchez, Talanta 98 (2012) 166–171.
[19] M. Beneito-Cambra, L. Ripoll-Seguer, J.M. Herrero-Martínez, E.F. Simó-Alfonso, G. Ramis-Ramos, J. Chromatogr. A 1218 (2011) 8511–8518.
[20] H. Sanderson, S.D. Dyer, B.B. Price, A.M. Nielsen, R. Compernolle, M. Selby, K. Stanton, A. Evans, M. Ciarlo, R. Sedlak, Sci. Total Environ. 368 (2006) 695–712.
[21] S. González, M. Petrovic, M. Radetic, P. Jovancic, V. Ilic, D. Barceló, Rapid Commun. Mass Spectrom. 22 (2008) 1445–1454.
[22] T. Reemtsma, J. Chromatogr. A 1000 (2003) 477–501.
[23] P.A. Lara-Martín, A. Gómez-Parra, E. González-Mazo, Trends Anal. Chem. 27 (2008) 684–695.
[24] S. Gómez, L. Ferreres, E. Pocurull, F. Borrull, Talanta 84 (2011) 859–866.
[25] M. Beneito-Cambra, L. Ripoll-Seguer, J.M. Herrero-Martínez, E.F. Simó-Alfonso, G. Ramis-Ramos, J. Chromatogr. A 1218 (2011) 8511–8518.
[26] M. Mortimer, J. Keys, TBT Assessment Project Protocol for Divers Collecting Sediment Samples Queensland Environmental Protection Agency, 2001.
[27] US-EPA, Recommended Guidelines for Sampling Marine Sediment, Water Column, and Tissue in Puget Sound Water Quality Action Team, USA, 1997.
[28] ISO 5667-6:2005. Water Quality. Sampling. Guidance on Sampling of Rivers and Streams. International Organization for Standardization. 16–Jan–2006. 28 p.
[29] ISO 5667-12: 1995. Water Quality. Sampling. Guidance on Sampling of Bottom Sediments. International Organization for Standardization. 15 June 1996. 42 p.
[30] S. Shrestha, F. Kazama, Environ. Model. Software 22 (2007) 464–475.
[31] A. González-Casado, L. Cuadros-Rodríguez, E. Alonso-Hernández, J.L. Vílchez, J. Chromatogr. A 726 (1996) 133–139.
[32] Analytical Methods Committee Analyst 119 1994 pp. 2363–2369.