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Extraction and isolation of linear alkylbenzene sulfonates and their intermediate metabolites from various marine organisms

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Abstract

A method has been developed for the determination of linear alkylbenzene sulfonates and its degradation intermediates (sulfophenylcarboxylic acids) from different marine organisms, which includes the stages of extraction and analysis by liquid chromatography with fluorescence detection. The extraction stage (Soxhlet and solid-phase extractions) was optimised by the selection of the appropriate solvent, minicolumns and different clean-up stages. Recoveries varied in the range from 80 to 104%, with a standard deviation between 1 and 9%. Detection limits were 15 ng g^{-1} wet mass for undecylbezene sulfonate and 30 ng g^{-1} wet mass for sulfophenylundecanoic acid using HPLC–fluorescence detection. The complete analytical method was successfully applied to different marine organisms from the Bay of Cadiz (SW Spain).

Keywords: Environmental analysis; Extraction methods; *Sparus aurata*; *Diplodus vulgaris*; *Ruditapes decussatus*; Alkylbenzene sulfonates, linear; Surfactants; Sulfophenylcarboxylic acids; Carboxylic acids

1. Introduction

Synthetic surfactants are used as the principal constituents of commercial detergents. Among these, linear alkylbenzene sulfonates, LAS, are the most commonly used anionic surfactants [1]. In coastal zones, the presence of LAS and their degradation intermediates (sulfophenylcarboxylic acids, SPC), is now a fact [2], because most of the detergents consumed are discharged via urban sewer systems into the marine medium, to a large extent without prior treatment of the sewage.

Studies of LAS bioconcentration are important for the following principal reasons: (i) Due to their surfactant character, LAS tend to accumulate at interfaces, both liquid–gas and liquid–solid [3]; in fact, the concentrations found in marine sediments are up to three orders of magnitude higher than those found in water; and among these solid–liquid interfaces are the membranes of marine organisms; and (ii) biological tissues have a high lipid content and therefore LAS (lipophilic nature) should be easily dissolved in these lipids. The study of their degradation products, SPC, is also important. They are more polar products and therefore, if the accumulated LAS are transformed into SPC [4–6], this degradation pathway could be a natural mechanism of the organisms for purification.

Published bioconcentration studies of LAS are scarce [7] and were performed with radiolabelled LAS. Since LAS are biotransformed in fish [4–6] and since biotransformation products were not separated from the intact LAS, the data available from these previous studies do not provide a quantitative

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description of the bioconcentration of LAS. Recently, the only study available of quantitative bioconcentration of LAS in fresh water fish has been realized [8]. Hence, it is necessary to develop a quantification method that is accurate, sensitive and specific for LAS and SPC simultaneously in different marine biological matrices. Such a method should also be applicable to diverse organisms because disparate results are obtained depending on the organism tested and on the method employed [9]. Currently, LAS analyses in marine species are scarce [10–11] and to our knowledge, no method exists for quantitative extraction and isolation of SPC from marine species.

High-performance liquid chromatography (HPLC) with fluorescence detection (FL) has been proved to be a successful method for the determination of LAS in water and sediment marine samples [3,12]. However the isolation of LAS from biological tissues must be a complicated and laborious task because of the nature of the matrix. This paper reports a method based on Soxhlet and solid-phase extractions followed by separation and quantification by HPLC–FL.

The objectives of this study were: (i) to optimize a Soxhlet extraction method using different eluents and to develop an optimal clean-up using solid-phase extraction cartridges, for the quantification of LAS and SPC by HPLC–FL in different marine organisms and (ii) to apply the method to the analysis of LAS and SPC in samples of fish and mussels, which are commercially very important in the zone.

2. Experimental

2.1. Chemicals and materials

Hexane was purchased from Romil Chemicals, Loughborough, UK. Ethyl acetate (EtOAc) was purchased from Panreac, Barcelona, Spain. HPLC-grade water and methanol (MeOH) were purchased from Scharlau, Barcelona, Spain. Sodium perchlorate was purchased from Merck, Darmstadt, Germany. The cellulose extraction thimbles of 20 mm×80 mm size were purchased from Whatman. The C_{18} and SAX minicolumns were purchased from Supelco, Bellefonte, PA, USA. The HPLC separation was performed with a C_8 column of 25 cm×0.46 cm internal diameter and 10 μm particle size, purchased from Teknokroma, Barcelona, Spain.

Petroquímica Española supplied the undecylbenzene sulfonate (C_{11}-LAS) standard (Fig. 1a), of 94.9% purity. The sulfophenylundecanoic acid (C_{11}-SPC) standard (Fig. 1b), of more than 99% purity, was synthesized in the University of Cadiz, Spain, by sulfonation of the corresponding phenylundecanoic acid following the procedure described by Marcomini et al. [13]. C_{11}-LAS has been chosen because it is the major homologue in the commercial LAS formulation and C_{11}-SPC had been chosen because it had the same alkyl chain length as C_{11}-LAS. Both standards are isomer mixtures.

2.2. Sample preparation

The clam, *Ruditapes decussatus*, and the marine fishes, gilt head sea bream (*Sparus aurata*) and the two-banded bream (*Diplodus vulgaris*) were gifts from the Marine Culture Wet Laboratory Facilities of the Cadiz University. Also some specimens from the Cadiz Bay (SW Spain) were collected. One of the species is benthic (clam) and the other two are pelagic (fishes). This provides us with a view of the distribution of these substances in species subjected to different degrees of surfactant content in their medium. Organisms were netted out of the aquarium (or the sea), carefully blotted with paper tissue and subsequently killed by immersion in liquid N_2 and stored at −20°C until analysis. After being chopped to obtain a material easy to grind, this was then homogenized with an Ultra-turrax T25. The paste obtained was divided in samples of 10 g each

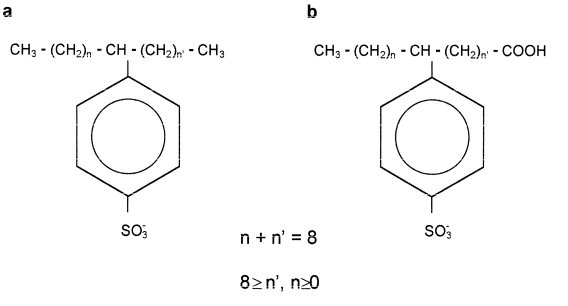

Fig. 1. General chemical structures of the C_{11}-LAS (a) and C_{11}-SPC (b) compounds.

(henceforth referred to as samples). The samples were spiked with C_{11}-LAS and C_{11}-SPC at different concentrations (1, 5, 10, 25, 50, 100 μg g^{-1} wet mass of C_{11}-LAS and 0.2, 1, 5, 10, 20, 50, 100 μg g^{-1} wet mass of C_{11}-SPC) in order to evaluate the recovery method. The LAS concentrations used are higher than for SPC because SPC is more polar and has less affinity for the biota. The frozen samples were lyophilised, weighed again and ground in a mortar with a pestle and transferred into cellulose extraction thimbles.

2.3. Sample pretreatment

Washed extraction thimbles were filled with each sample (≈10 g), which were then extracted in a Sohxlet apparatus with 60 ml of solvent at different times. Different extraction solvents were tested: hexane, ethyl acetate, and methanol. Afterwards the extract was evaporated until dry in a rotavapor, and the dry residue was redissolved with 100 ml of warm water in an ultrasonic bath. The solution was acidified to pH 3, passed either through a C_{18} cartridge only, or through the C_{18} and subsequently a SAX strong anionic exchanger for further clean-up. The C_{18} cartridge was washed with 6 ml MeOH–water (30:70). It was eluted with 10 ml of MeOH and either analyzed or passed through a SAX minicolumn, afterwards cleaned with 5 ml of MeOH (AcH 2%) and subsequently eluted with 3 ml of 2 M HCl in MeOH. The eluate was evaporated until dry and redissolved in 1 ml of the same mobile phase used in the liquid chromatography analysis.

The following extraction methods were tested:

Method 1: Soxhlet extraction with MeOH for 6 h followed by solid-phase extraction on a C_{18} minicolumn.

Method 2: Soxhlet extraction with hexane, ethyl acetate, and methanol for 6 h each followed by solid-phase extraction on a C_{18} minicolumn.

Method 3: Soxhlet extraction with hexane for 9 h and MeOH for 6 h followed by solid-phase extraction on a C_{18} minicolumn.

Method 4: Soxhlet extraction with hexane for 9 h and MeOH for 6 h followed by solid-phase extraction on a C_{18} minicolumn followed by solid-phase extraction on an SAX minicolumn.

2.4. HPLC system

C_{11}-LAS and C_{11}-SPC were analyzed in a HP1050 high-performance liquid chromatograph equipped with a fluorescence detector (λ_{ex}=225 nm, λ_{em}=295 nm). The mobile phase was MeOH–water (80:20, v/v) with 10 g l^{-1} $NaClO_4$ added. As the stationary phase a LiChrosorb RP-8 column with a particle size of 10 μm was used. C_{11}-LAS and C_{11}-SPC concentrations were determined by measuring the peak areas, using external standards (HPLC-grade water spiked with C_{11}-LAS and C_{11}-SPC). These standards were treated in the same way as the samples.

3. Results and discussion

3.1. Optimization results

A Soxhlet extraction procedure (method 1) using MeOH for 6 h (12 cycles h^{-1}) which has been proven successful for LAS and SPC analysis in marine sediments [3,12] was not suitable for spiked whole body gilt head sea bream (data not shown). Since marine organism tissues are complex biological matrices, containing high quantities of lipophilic substances, compared with sediments, a further purification of the samples is required. In order to minimize the high amount of interference, which in most cases did not allow the determination of C_{11}-LAS and C_{11}-SPC, various different extraction solvents were tested.

The average recovery values obtained for gilt head sea bream spiked with C_{11}-LAS and C_{11}-SPC (50 and 100 μg g^{-1}), using several solvents of increasing polarity in a sequential Soxhlet extraction procedure (method 2) are shown in Table 1. C_{11}-LAS and C_{11}-SPC recoveries were higher than 89% for two concentrations tested using methanol as extracting solvent and a C_{18} cartridge as the clean-up stage in the solid-phase extraction. Neither hexane nor EtOAc extracted detectable amounts of LAS or SPC; nevertheless they removed a lot of the interferences.

The elimination of the EtOAc and the lengthening of the hexane extraction time from 6 h to 9 h produced good LAS recoveries for two different

Table 1
Recoveries and standard deviation ($n=3$) for C_{11}-LAS and C_{11}-SPC in spiked gilt-head sea bream using Soxhlet extraction with various extraction solvents (method 2)[a]

Compound	Concentration ($\mu g\ g^{-1}$ wet mass)	Recovery (%)±SD		
		n-Hexane	EtOAc	MeOH
C_{11}-LAS	50	0	0	92±1
	100	0	0	89±1
C_{11}-SPC	50	0	0	101±5
	100	0	0	97±10

[a] Solvent extraction volume: 50 ml; extraction time: 6 h.

marine organisms. Table 2 shows the results obtained for C_{11}-LAS in *Sparus aurata* and *Diplodus vulgaris* using the Soxhlet extraction with methanol previously extracted with hexane (method 3). The LAS recoveries were higher than 87% for the two organisms and the various concentrations tested. Although these organisms were spiked with different C_{11}-LAS and C_{11}-SPC concentrations, the results obtained for C_{11}-SPC were not good because considerable interference appeared at the same retention time (Fig. 2a). This interference was eliminated adding a clean-up stage consisting of an SAX minicolumn after the C_{18} in the solid-phase extraction procedure (method 4). A chromatogram obtained by HPLC–FL under these conditions is shown in Fig. 2b.

In Table 3 the results obtained for the clam *Ruditapes decussatus* spiked with different concentrations of LAS and SPC, are shown. In this experiment hexane and MeOH were used as extraction solvents and C_{18}+SAX as solid-phase extraction cartridges (method 4). The recoveries are high for the two compounds and all the concentrations tested and the chromatograms obtained by HPLC–FL show high selectivity and sensitivity (Fig. 3). This indicates the capability of the method 4 to isolate C_{11}-LAS and C_{11}-SPC from complex matrices such as marine organisms.

Table 2
Recoveries and standard deviation ($n=3$) for C_{11}-LAS in whole body samples of two fish species: *Sparus aurata* and *Diplodus vulgaris* (method 3)[a]

Concentration ($\mu g\ g^{-1}$ wet mass)	Recovery (%)±SD	
	S. aurata	*D. vulgaris*
1.0	89±2	104±2
5.0	94±7	104±1
10.0	91±2	n.d.[b]
25.0	88±2	87±2
50.0	93±1	97±2
100.0	90±3	n.d.

[a] Solvent extraction volume: 50 ml.
[b] n.d.=non determined.

3.2. Quantitation

Calibration was performed external standards over the concentration range from 0.2 $\mu g\ ml^{-1}$ to 20 $\mu g\ ml^{-1}$ for C_{11}-LAS and from 0.09 $\mu g\ ml^{-1}$ to 2 $\mu g\ ml^{-1}$ for C_{11}-SPC. Samples were spiked with a concentration range from 0.5 $\mu g\ g^{-1}$ to 50 $\mu g\ g^{-1}$ for C_{11}-LAS and from 0.1 $\mu g\ g^{-1}$ to 20 $\mu g\ g^{-1}$ for C_{11}-SPC. The relationship between the fluorimetric response and the concentration was found to be linear for both compounds and several organisms overall ranges tested. The linear regression equations and correlation coefficients are shown in Table 4.

The limits of detection (LOD) at a signal-to-noise ratio of 3 are 30 ng g^{-1} wet mass and 15 ng g^{-1} wet mass for C_{11}-SPC and C_{11}-LAS respectively, after the preconcentration procedure and analysis by LC.

3.3. Biological samples analysis

An example of the results obtained in the application of the method 4 to the analysis of different marine organisms collected in the Bay of Cadiz is shown in Table 5. The highest LAS concentrations correspond to the marine fish *D. vulgaris* followed by the clam *R. decussatus*, with the other fish species *S. aurata* showing the lowest concentrations. *D. vulgaris* was collected close to the shore, near the discharge outlet of untreated urban wastewater from a population of about 100 000 inhabitants. The other two species were caught at a greater distance from this outlet. Despite the species *R. decussatus* being benthic and LAS being preferentially associated with sediments, the values found for this species are less than those for *D. vulgaris* as a result of its lower degree of exposure to the surfactant at a greater distance from the coast.

No detectable quantities of C_{11}-SPC were found

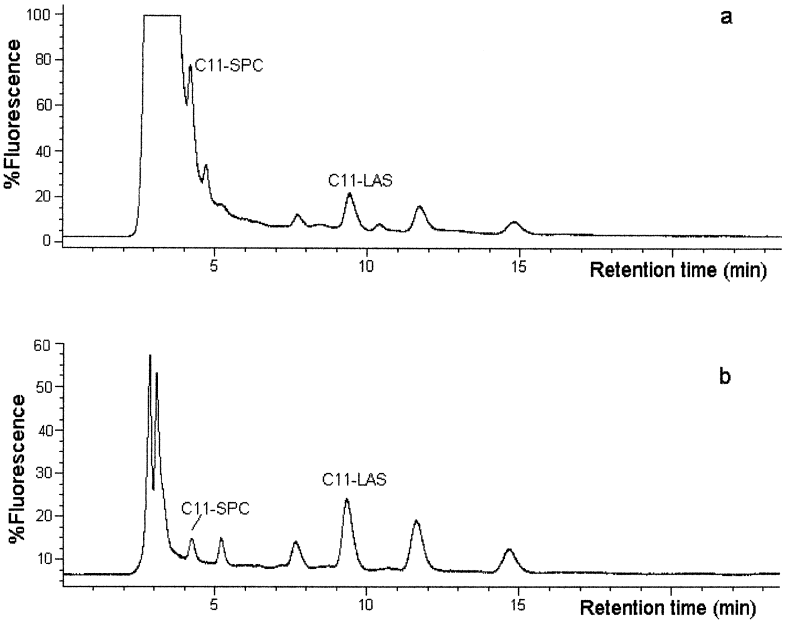

Fig. 2. LC–FL chromatogram obtained from a *Diplodus vulgaris* (two-banded bream) sample spiked with C_{11}-SPC and C_{11}-LAS using two different clean-up stages: (a) C_{18} cartridge only; (b) C_{18}+SAX cartridges. Soxhlet extraction with methanol (6 h) previously extracted with hexane (9 h) and HPLC conditions as described in section 2.4.

for any of the organisms. This could mean either that these organisms do not accumulate SPC or that the original compound is not biodegraded in these organisms. However, there is evidence of biotransformation of LAS in fresh water fish [4–6], and laboratory tests monitoring the evolution of the

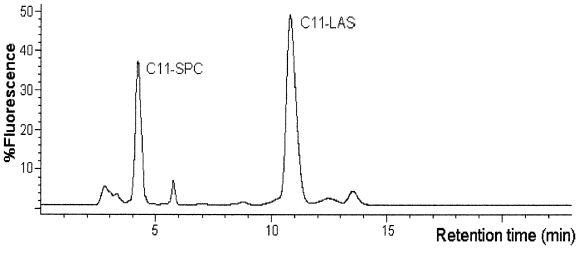

Fig. 3. HPLC–FL chromatogram of a *Ruditapes decussatus* sample spiked with C_{11}-LAS and C_{11}-SPC using method 4 and HPLC conditions as described in section 2.4.

Table 3
Recoveries and standard deviation (n=3) of C_{11}-LAS and C_{11}-SPC at several concentrations in spiked clam tissues *Ruditapes decussatus* (method 4)[a]

Compound	Concentration (μg g^{-1} wet mass)	Recovery (%)±SD
C_{11}-LAS	1	88±2
	5	81±4
	10	86±4
	25	80±2
	50	85±4
C_{11}-SPC	0.2	81±2
	1.0	75±2
	5.0	100±2
	10.0	89±2
	20.0	96±2

[a] Solvent extraction volume: 50 ml.

Table 4
Calibration data obtained for C_{11}-LAS and C_{11}-SPC spiked in seawater, *Ruditapes decussatus, Sparus aurata* and *Diplodus vulgaris*

Matrix	Compound	Calibration equation	r^2
Sea water	C_{11}-LAS	$y = 6.342x - 0.152$	0.999
	C_{11}-SPC	$y = 10.305x + 0.705$	0.999
R. decussatus	C_{11}-LAS	$y = 0.845x - 0.182$	0.999
	C_{11}-SPC	$y = 0.957x + 0.124$	0.998
Sparus aurata	C_{11}-LAS	$y = 0.903\ x + 0.111$	0.999
D. vulgaris	C_{11}-LAS	$y = 0.958\ x - 0.271$	0.996

Table 5
C_{11}-LAS and C_{11}-SPC concentration measured in different marine species collected in Cadiz Bay using the method 4[a]

Compound	Biological samples (μg g^{-1} wet mass)		
	S. aurata	*D. vulgaris*	*R. decussatus*
C_{11}-LAS	1.29	2.98	2.38
C_{11}-SPC	n.d.[b]	n.d.	n.d.

[a] Sample size: 10 g.
[b] n.d. = not detected.

concentration of SPC [14] conclude that biodegradation is very rapid in its first stage and that long-chain SPC are not detected. This may explain these negative findings.

4. Conclusion

An analytical protocol for the determination of LAS and SPC in different marine biological samples using Soxhlet and solid-phase extractions has been developed. Hexane for 9 h, followed by methanol for 6 h, was found to give the best recoveries.

Using a C_{18} minicolumn followed by an SAX minicolumn in the SPE stage produces, via HPLC–FL, chromatograms without interference and with sufficient intensity to permit an accurate quantification of LAS and SPC.

Method 4 developed in this paper was applied to the analysis of samples of marine organisms from the Bay of Cadiz (SW Spain). C_{11}LAS was detected in a concentration range varying from 1.29 μg g^{-1} to 2.98 μg g^{-1}.

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