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New extraction method for the analysis of linear alkylbenzene sulfonates in marine organisms☆ Pressurized liquid extraction versus Soxhlet extraction

D. Álvarez-Muñoz, M. Sáez, P.A. Lara-Martín, A. Gómez-Parra, E. González-Mazo*

Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Avd República Saharaui s/n, 11510 Puerto Real, Cádiz, Spain.

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Abstract

A new method has been developed for the determination of linear alkylbenzene sulfonates (LAS) from various marine organisms, and compared with Soxhlet extraction. The technique applied includes the use of pressurized liquid extraction (PLE) for the extraction stage, preconcentration of the samples, purification by solid-phase extraction (SPE) and analysis by liquid chromatography with fluorescence detection. The spiked concentrations were added to the samples (wet mass of the organisms: *Solea senegalensis* and *Ruditapes semidecussatus*), which were homogenized and agitated continuously for 25 h. The samples were extracted by pressurized hot solvent extraction using two different extraction temperatures (100 and 150 °C) and by traditional Soxhlet extraction. The best recoveries were obtained employing pressurized hot solvent extraction at 100 °C and varied in the range from 66.1 to 101.3% with a standard deviation of between 2 and 13. Detection limit was between 5 and 15 μg kg^{-1} wet mass using HPLC–fluorescence detection. The analytical method developed in this paper has been applied for LAS determination in samples from a Flow-through exposure system with the objective of measuring the bioconcentration of this surfactant.

Keywords: Pressurized liquid extraction (PLE); Linear alkylbenzene sulfonates; Environmental analysis; Surfactants; Extraction methods; *Ruditapes semidecussatus*; *Solea senegalensis*

1. Introduction

LAS are among the major anionic surfactants in current commercial use; and their annual consumption in laundry detergents in Europe was estimated at about 270 × 10^6 kg/year in 2000 [1]. Because the LAS used are discharged via sewage systems to the environment, the risk of aquatic exposure depends largely on the presence or absence of wastewater treatment plants. The LAS concentration found in untreated wastewater is in the range of 1–15 mg l^{-1} [2,3], but sewage is treated, 98% of the LAS present are removed [4] thus substantially decreasing its concentration in the effluent and its consequent presence in the environment. However, a small fraction of the surfactant is not removed and the aquatic biota will be exposed to this fraction.

The first method reported for LAS extraction from fish tissue [5] consisted of MeOH extraction, dilution of the extract with water, and solid-phase extraction of this solution over four C_{18} columns in series, but the recovery rate was not specified and the performance of the method cannot be evaluated. In some studies, LAS was determined by employing non-specific techniques, such as a method based on radio analysis [6]; this method was inappropriate for quantitative determination of LAS because it did not allow differentiation

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* Corresponding author. Tel.: +34 9560 16159; fax: +34 9560 16797.
E-mail address: eduardo.gonzalez@uca.es (E. González-Mazo).

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between the parent compound and its metabolites, hence, the measured concentrations overestimate the surfactant concentration. An accurate determination of LAS requires a specific quantification of the compound; this was achieved in fish exposed to LAS in the laboratory by fractionated matrix solid-phase dispersion (MSPD) [7] followed by protein precipitation and centrifugation and subsequent isolation of the parent LAS by ion-pair liquid–liquid (IP-LL) extraction of the pellet obtained after protein precipitation. The recovery from spiked fish exceeded 70% and the limit of quantification was found to be around 0.2 mg kg^{-1}. Sáez et al. [8] developed another quantitative and specific method for the determination of LAS and their degradation intermediates, sulfophenylcarboxylic acids (SPC), from marine organisms. This method was applied using Soxhlet extraction with hexane for 9 h followed by methanol for 6 h, Solid-phase extraction was used as the clean up stage, and LAS detection was performed with HPLC–fluorescence detection (FL). Although extraction using only methanol has been proved to be successful for LAS and SPCs in marine sediments [9,10], it is not suitable for marine organisms because their tissues are complex biological matrixes and contain high quantities of lipophylic substances; the first extraction using hexane decreases the amount of interference. The recoveries obtained for this method were higher, between 80 and 104% and the LAS detection limit was 15 ng g^{-1}wet mass.

In order to reduce the time and cost of analysis, a new extraction method, an ASE-200 apparatus from Dionex was used; this technique uses conventional liquid solvents at elevated temperatures and pressures to achieve quantitative extraction from solid samples in a short time and with a small amount of solvent [11]. PLE was designed for environmental applications, for the analysis of food, pharmaceuticals and natural products, and for polymers, and its use is included in the EPA Method SW-8463545 A. Accelerated hot solvent extraction has been applied for determining compounds such as BNAs, PCBs, PAHs, and chlorinated herbicides from solid waste samples [12,13], and for PCBs and lipids from fish tissue [14,15,16]. PLE has been compared with traditional extraction methods for the analysis of herbicides in soils [17], with the best recoveries being obtained with PLE (≈100%), with modified Bligh and Dyer extraction for a lipid biomarker from pure culture and environmental samples [18]; no significant differences were found for the vegetative biomass or water and soil samples, but recoveries were significantly higher for the spores and the airborne biomass. For the analysis of environmental organic compounds in soils two conventional supercritical fluid extractor systems (SFE) have been modified to function as a PLE system [19] and have been compared with sonication and microwave-assisted extraction (MAP). The recoveries were found to be comparable to MAP and superior to sonication. All these applications show that accelerated solvent extraction is becoming recognized as an effective analytical technique; but to our knowledge, PLE has not yet been used for the determination of LAS from marine species.

The objectives of this study were to develop a new method for LAS determination in various different marine organisms, which is fast, efficient, safe and reliable, and to apply the method to the analysis of LAS in samples of fish from a flow-through exposure system.

2. Experimental

2.1. Chemicals

Hexane was purchased from Merck, Darmstadt, Germany; and HPLC grade methanol (MeOH) from Scharlau, Barcelona, Spain. Sodium perchlorate was purchased from Merk. The cellulose extraction thimbles of 10 mm × 50 mm size from Whatman, UK. The C_{18} minicolumns were supplied by Varian, Harbor City, CA, USA. The SAX minicolumns were purchased from Merck. The HPLC separation was performed with a C_8 column of 25 cm × 0.4 cm internal diameter and 5 μm particle size, purchased from Merck. Formaldehyde 37% solution, reagent grade was from Scharlau, Barcelona, Spain. Syringe driven filters 0.45 μm PTFE were purchased from Millipore. Filters D28 were purchased from Dionex. The Sodium Sulphate anhydrous was purchased from Panreac, Barcelona, Spain. Petroquímica Española supplied the commercial LAS mixture (Fig. 1) with the following homologue distribution: C_{10} (11.8%), C_{11} (34%), C_{12} (30.3%), C_{13} (22.5%).

2.2. Sample preparation

Both *Ruditapes semidecussatus* and *Solea senegalensis* were gifts from the Marine Culture Wet Laboratory Facilities of Cádiz University. The organisms were netted out of the aquarium, carefully blotted with paper tissue and subsequently killed with a lethal dose of anesthetic. The clams were removed from their shell and the fish was cut into pieces to obtain a material easy to grind; this was not necessary for the clams because of the smaller size of the organism. For each organism the samples were homogenized with an Ultraturrax T25 and the paste obtained was divided into samples of 300 g wet mass each, which were then spiked with commercial LAS at two different concentrations (10, 20 μg g^{-1}). One sample per organism was not spiked, in order to evaluate the possible original presence of LAS and establish the initial state of the organisms employed. The paste with the

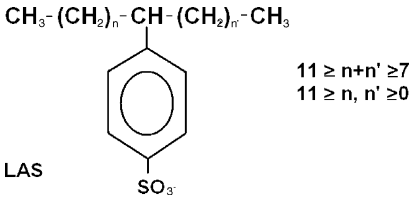

Fig. 1. General chemical structure of LAS.

respective spiked concentration was transferred to a container, and 4% of formaldehyde was added to preserve the samples. The period of contact between wet mass from organism and surfactant was 25 h under continuous agitation using a mechanical mixer (Heidolph, type: RZR 1), therefore the time necessary to achieve the equilibrium of adsorption, established at 12 h [20], was amply exceeded. The samples were frozen and stored at −20 °C until analysis; after being lyophilized and ground in a Zirconium oxide ball mill (Fristsh, Pulverisette 6), from each spiked concentration 1 g of sample was taken, in triplicate, and analyzed using the two techniques being tested.

2.3. Extraction process

For PLE the extraction cells (22 ml) were prepared by inserting a disposable cellulose filter into the cell outlet, followed by the sample (≈1 g) and using anhydrous sodium sulfate to improve cell packing. After the cells were loaded, the PLE performed the following steps automatically: (1) the cell was filled with solvent, (2) the extraction cell was heated and pressurized, (3) the sample was held at set pressure and temperature, (4) cleaned solvent was pumped through the sample, (5) sample was purged with nitrogen, (6) the filtered extract was collected in the separate collection vial and a new static cycle was performed. The solvent system was rinsed before the ASE-200 automatically loaded the next sample and the rinse was collected in the vial.

This technique was performed using a first extraction with hexane and a continuous one with methanol; the hexane extract was removed and the extraction program was repeated with methanol. The PLE conditions were: pressure 1500 psi, temperature: 100 and 150 °C (in accordance with other studies [17,18,19]), Static time: 5 min, static cycles: 3, so the total time required for the extraction was 15 min for each solvent employed. The methanolic extract volume was 30 ml; this was evaporated until dry in a rotavapor, and the dry residue was redissolved with 75 ml of warm MQ water in an ultrasonic bath. The SPE was performed using a Autotrace SPE workstation. The solution was passed through a C_{18} cartridge; it was conditioned with 10 ml of methanol and 5 ml of MQ water and eluted with 10 ml of MeOH. The eluate was evaporated until dry and redissolved in 1 ml of MeOH–water (80:20). The samples were filtered through a 0.45 μm PTFE filter before the liquid chromatography analysis.

Soxhlet extraction was performed following the methodology used by Sáez et al. [8]. Briefly, the analysis sequence was: Soxhlet extraction with hexane for 9 h and MeOH for 6 h, followed by SPE on a C_{18} minicolumn, followed by SPE on a SAX minicolumn, and finally determination by HPLC–FL.

2.4. HPLC system

The samples 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, the flow-rate was 1 ml min^{-1}. A LiChrospher 100 RP-8 column with a particle size of 5 μm was used as the stationary phase. LAS homologue concentrations were determined by measuring the peak areas, using external standards (HPLC-grade water spiked with commercial LAS).

3. Results and discussion

3.1. Separation, calibration graphs and limits of detection

The LAS separation by homologues is illustrated in Fig. 2. These three chromatograms correspond to the analysis of triplicate samples for *R. semidecussatus*. The close superimposition of peaks observed indicates the high degree of reproducibility of the method.

The chromatograms obtained for *Solea senegalensis* with the different extraction techniques employed are shown in Fig. 3. At the beginning of the chromatograms there are some peaks of unknown origin; from our research experience in LAS determination and its degradation intermediates, we believe these initial peaks could correspond to sulfophenylcarboxilyc acids (SPCs) derived from the original compound; however, formaldehyde was added to the samples to prevent the degradation of the compound and, for this reason, these peaks could also be an effect of the extraction of lipids and other coextractable materials from the organism. These interferences in the chromatograms are lower at 100 °C than at 150 °C and similar to those observed with Soxhlet extraction (Fig. 3) where two purification stages were performed (C_{18} + SAX). Thus, with PLE (at 100 °C) followed by SPE on C_{18} cartridges, SAX minicolumns are not necessary because the chromatograms obtained are easier to quantify and reproduce. This advantage has also been described for PCB extraction from fish tissues [14], thus eliminating the need for a clean up stage with sulfuric acid treatment.

Calibration was performed by external standards, which were treated in the same way as the samples. The relation-

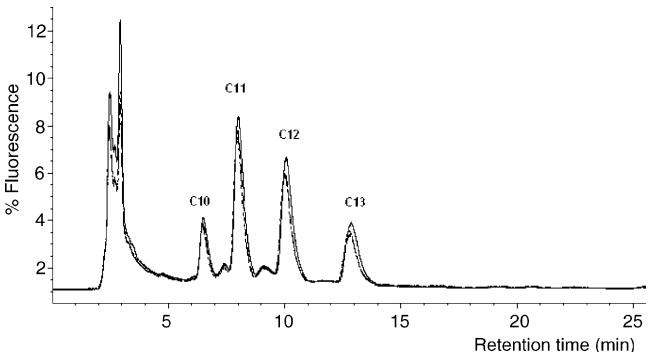

Fig. 2. LC–FL chromatograms obtained in triplicate for *R. semidecussatus* spiked with commercial LAS (10 μg g^{-1} wet mass) using PLE (at 100 °C).

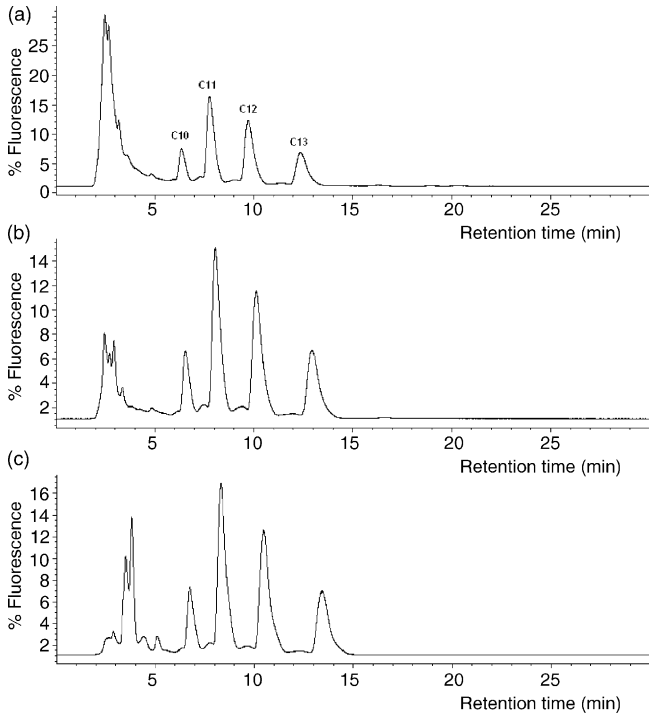

Fig. 3. LC–FL chromatograms obtained from *S. senegalensis* spiked with commercial LAS (20 μg g^{-1} wet mass) using three different techniques: (a) PLE (at 150 °C) followed by SPE on a C_{18} cartridge only; (b) PLE (at 100 °C) followed by SPE on a C_{18} cartridge; (c) Soxhlet extraction followed by SPE on a C_{18} cartridge + SAX minicolumns.

ship between the fluorometric response and the concentration was found to be linear for all the homologues and organisms tested, with excellent correlation coefficients (Table 1). The limits of detection (LODs) are at a signal-to-noise ratio of 5–15 μg kg^{-1} after the preconcentration procedure and analysis by LC.

3.2. Recovery study

LAS recoveries obtained with PLE (100 °C) and Soxhlet extraction are shown in Table 2. For both techniques the results obtained are higher for *S. senegalensis* than for *R. semidecussatus* with recoveries of 100% with PLE and higher than 100% with Soxhlet. For *R. Semidecussatus* the recoveries obtained with PLE are better than with Soxhlet but lower than 100%; this difference in the results appears because these organisms are different biological matrixes with several fatty contents and therefore the intensity of the interaction of the surfactant could be different in each organism.

Table 1
Calibration data obtained for homologues spiked in Milli-Q water

Compound	Calibration equation	r^2
C10LAS	$y = 27.365x + 0.022$	0.999
C11LAS	$y = 31.528x + 2.159$	0.999
C12LAS	$y = 32.303x + 1.005$	0.999
C13LAS	$y = 29.204x - 0.565$	0.999

Table 2
Recoveries and standard deviation (n = 3) for total LAS in spiked marine organisms using accelerated solvent extraction (at 100 °C) and Soxhlet extraction

Technique	Concentration (μg g^{-1} wet mass)	Recovery (%) ± S.D.	
		R. semidecussatus	*S. senegalensis*
PLE (100 °C)	10	66.1 ± 13	101.3 ± 1
	20	81.6 ± 12	100.6 ± 2
Soxhlet	10	46.1 ± 7	142.9 ± 10
	20	73.3 ± 5	132.0 ± 14

For the purpose of obtaining better results for *R. Semidecussatus*, an increase of the extraction temperature was tested. The results obtained with PLE (150 °C) are shown and compared with PLE (100 °C) in Table 3. For all the concentrations tested the recoveries were increased, surpassing the 100% level except for one *R. semidecussatus* sample. These results show that for LAS extraction from marine organisms using PLE at 100 °C good recoveries are obtained in most cases, and at 150° C the results tend to exceed 100% LAS recovery, a finding which has been detected in PAHs extraction from different soils employing PLE at 150 °C [19] (chrysene: 126%, benzo[*b*,*k*]fluoranthene: 116%, benzo[*a*]pyrene: 116%, benzo[*ghi*]perylene: 132%). In an application to determine PCBs from fish tissues [14] (at 100 °C) the results obtained were acceptable but some of the values were outside the 95% confidence interval, which does not happen in this study.

The recoveries by LAS homologues at the two concentrations tested are shown in Table 4, for *R. semidecussatus* and for *S. senegalensis*. The efficiency of the extraction decreases with the length of the alkyl chain with PLE (100 °C) and Soxhlet for the clam. The loss recovery for total LAS is explained by the low recoveries obtained for weight homologues. This is not found at 150 °C, where the variations of the percentage recovery are minimal and there is a higher efficiency for the high-molecular-mass homologues when the extraction temperature is increased The same result was obtained for *S. senegalensis*. This behavior has also been described for PAHs of high molecular weight by Ken Li et al. [19], though the recoveries obtained at 150 °C did not result in significantly higher recovery as would be expected from the thermodynamic considerations. In *S. senegalensis* the recoveries obtained by homologues with PLE at 100 °C are practically stable with small standard deviations, which indicate the good extraction results for all the LAS homologues in this marine organism.

Table 3
Comparison between recoveries for total LAS in spiked marine organisms using pressurized liquid extraction at 100 and 150 °C extraction temperatures

Technique	Concentration (μg g^{-1} wet mass)	Recovery (%) ± S.D.	
		R. semidecussatus	*S. senegalensis*
PLE (100 °C)	10	66.1 ± 13	101.3 ± 1
	20	81.6 ± 12	100.6 ± 2
PLE (150 °C)	10	119.1 ± 10	132.5 ± 13
	20	74.4 ± 4	164.5 ± 20

Table 4
Recoveries of LAS by homologues from *R. semidecussatus* and *S. senegalensis* using the different extraction techniques, spiked at 10 and 20 $\mu g\,g^{-1}$ (wet mass)

Organism	Concentration ($\mu g\,g^{-1}$ wet mass)	LAS Homologues	Recovery (%) ± S.D.		
			PLE (100° C)	PLE (150 °C)	Soxhlet
R. semidecussatus	10	C_{10}	79.9 ± 19	147.3 ± 11	60.9 ± 9
		C_{11}	79.5 ± 17	135.6 ± 18	58.8 ± 7
		C_{12}	61.2 ± 9	125.6 ± 17	41.9 ± 4
		C_{13}	49.3 ± 11	134.2 ± 2	27.3 ± 5
	20	C_{10}	99.9 ± 18	94.1 ± 3	97.4 ± 9
		C_{11}	95.3 ± 12	83.4 ± 3	88.7 ± 6
		C_{12}	77.3 ± 13	81.3 ± 17	68.3 ± 1
		C_{13}	61.9 ± 8	67.3 ± 14	48.3 ± 1
S. senegalensis	10	C_{10}	83.1 ± 1	158.2 ± 5	152.8 ± 4
		C_{11}	98.3 ± 11	135.2 ± 1	147.8 ± 13
		C_{12}	99.8 ± 12.9	110.3 ± 1	147.7 ± 9
		C_{13}	103.2 ± 1	120.1 ± 5	136.4 ± 9
	20	C_{10}	93.9 ± 6	190.4 ± 23	123.6 ± 16
		C_{11}	103.9 ± 3	181.9 ± 27.3	147.7 ± 8
		C_{12}	102.9 ± 2	156.8 ± 17	148.1 ± 2
		C_{13}	101.8 ± 1	145.1 ± 14	144.2 ± 2

3.3. Application

This method has been applied to samples of *S. senegalensis* exposed to LAS in a flow-through system with the objective of measuring the bioconcentration of this surfactant. This was performed with a pure homologue $2\phi C_{12}$LAS with an exposure concentration of 200 $\mu g\,l^{-1}$; the concentration of the compound studied was analyzed in quadruplicate and was found to be 14.670 $\mu g\,g^{-1}$ with a standard deviation of 4. The extraction of these samples was carried out using PLE at 100 °C, followed by SPE with C_{18} minicolumns, and subsequent HPLC–FL detection. Fig. 4 show two chromatograms corresponding to duplicate samples, and, as expected, the interferences obtained were really low.

4. Conclusion

An analytical protocol for the determination of LAS in different biological marine samples using accelerated solvent extraction has been developed. Two extractions have been employed: hexane (15 min) and methanol (15 min), with the best recoveries being obtained at an extraction temperature of 100 °C.

Using C_{18} minicolumns as the clean up stage, via HPLC–FL, chromatograms with similar interference to Soxhlet followed by C_{18} and SAX, have been obtained. In addition, the intensity of the chromatograms is sufficient to permit an accurate quantification of LAS homologues.

This method has been applied to the analysis of samples of fish from a flow-through exposure system, with the $2\phi C_{12}$LAS concentration being determined with a standard deviation of 4.

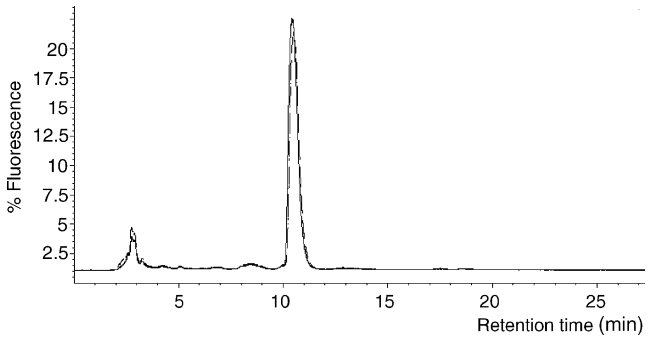

Fig. 4. LC–FL chromatograms in duplicate obtained from *S. senegalensis* in a flow-through exposure system.

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