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ORIGINAL PAPER

New sample treatment for determination of linear alkylbenzene sulfonate (LAS) in agricultural soils by liquid chromatography with fluorescence detection

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Abstract A new sample-treatment procedure has been developed for determination of total linear alkylbenzene sulfonate (LAS), i.e. homologues and isomers, in agricultural soil. The procedure involves two steps, ultrasound-assisted extraction of LAS from the samples with methanol then clean-up of the methanolic extracts and preconcentration of the LAS by solid-phase extraction on two adsorbent cartridges (SAX and C_{18}). The ultrasound-assisted procedure reduces extraction time (10 min in contrast with 6–12 h for conventional Soxhlet extraction) and requires only small volumes of organic solvent. The effect of different variables interacting in the ultrasound-assisted extraction process was studied. Finally, separation and quantification of the homologues and isomers of LAS was performed by liquid chromatography with fluorescence detection (LC−FD). 2-Octylbenzenesulfonic acid sodium salt (Na-2ØC_8-LAS) was used as internal standard. The proposed method was satisfactorily used for determination of LAS in agricultural soil samples from the fertile plain of Granada (Spain).

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Introduction

Linear alkylbenzene sulfonate (LAS), the most important class of anionic surfactant, is used mainly in domestic and industrial detergent formulations. It was introduced commercially in 1964 as a substitute for nonbiodegradable branched alkylbenzene sulfonates (ABS). Commercial LAS is a mixture of 20 different closely related isomers and homologues, each containing an aromatic ring sulfonated at the *para* position and attached to a linear alkyl chain which usually varies from 10 to 13 carbon atoms [1].

European production of LAS was 487 kton in 2004 [2]. Of this approximately 80% was used for detergents, which gives an idea of the amount of wastewater discharged into the environment with or without adequate disposal. LAS is always present in urban wastewater and is thus found in many different environmental compartments [3, 4]. This wastewater is occasionally treated, reducing thus the load of LAS in the environment, but LAS may be introduced as a contaminant in natural ecosystems by direct use of untreated wastewater as irrigation water and by use of sewage sludge as a fertilizer on agricultural soil [5]. LAS may also enter the soil as a result of the use of pesticides, because detergents are used as emulsifiers and as dispersing and spreading agents in pesticide formulations [6]. In the terrestrial environment LAS can be inhibitory to bacteria, fungi, and other inhabitants of the soil ecosystem [7–9]. Exposure of agricultural soil to LAS may therefore affect

microbial activity, which is important for soil quality and nutrient cycling [10]. For these reasons, determination of LAS in environmental samples is of great interest.

During recent decades determination of LAS in different environmental samples has been extensively studied. Liquid chromatography (LC) using UV [11–15], fluorescence [12, 16–23], or mass spectrometric [24–27] detection can be regarded as routine methods for LAS analysis. Chromatographic analysis usually involves tedious sample treatment, however, to extract the compounds from a complex matrix, for example soil. Determination of LAS in soil requires many steps that involve solvent extraction and solid phase clean-up. Isolation of LAS from soil samples is accomplished by use of a variety of extraction procedures. Almost all traditional methods, e.g. Soxhlet [11, 16, 17, 24], reflux [12, 13], and shaking [18, 28] extraction consume much solvent and time.

New methods of sample treatment have been developed to reduce the volume of organic solvent used and the time needed for total extraction from this type of matrix. They are based on CO_2–supercritical fluid extraction with methanol as organic modifier [19, 20], microwave-assisted extraction [21], and/or pressurized-liquid extraction [22, 25, 26], all of which require complex and expensive instrumentation.

Extraction assisted by ultrasound can be an advantageous alternative both to conventional extraction (Soxhlet) and, occasionally, novel extraction techniques, for example supercritical-fluid extraction (SFE) and microwave-assisted extraction [29]. Another advantage is that the equipment for ultrasonication is very simple, quickly effective, less expensive, and easy to operate [14, 15, 27, 30–33]. It is based on a sonochemical phenomenon associated with acoustic cavitation—the formation, growth, and implosive collapse of gas vacuoles in a solution. The collapse may proceed as an adiabatic compression and generate high temperature and pressure [34] and the implosion of cavities establishes an unusual environment for chemical reactions [35, 36]. As a result of the process of cavitation, ultrasonic radiation is a powerful tool for acceleration of different stages of the analytical process for solid [37] and liquid samples [38]. This type of energy can be used to facilitate and accelerate the pretreatment of solid samples, for example extraction of organic compounds [30], because sonication results in more efficient contact between solid and solvent.

The objective of this study was to develop a routine method for analysis of total LAS, i.e. homologues and isomers, in soil samples by using ultrasound-assisted extraction to reduce the total analysis time.

The proposed method has been satisfactorily applied to soil samples from twelve agricultural plots of the fertile plain of Granada (Spain).

Experimental

Chemical and reagents

All reagents were of analytical grade unless specified otherwise. Water was purified using a Milli-Q system from Millipore (Bedford, MA, USA). Commercial LAS was kindly supplied by Petroquímica Española (Madrid, Spain) as an aqueous solution of the sodium salt with an LAS content of 44.05% (*w*/*w*). The average molecular weight of the Na-LAS was 342.2 g mol^{-1} and the distribution of the linear alkyl chain homologues was C_{10} 14.14%, C_{11} 37.72%, C_{12} 29.74%, and C_{13} 23.40%. 2-Octylbenzenesulfonic acid sodium salt (Na-2ØC_8-LAS; 97.6%, *w*/*w*), used as an internal standard, was also supplied by Petroquímica Española.

Stock solutions of LAS and Na-2ØC_8-LAS (1.0 mg mL^{-1}) were prepared in methanol and stored at 4 °C in the dark. Both solutions were stable for six months. These solutions were used to spike the soil samples. All glassware was cleaned with chromic acid to minimize LAS contamination. Working standards were prepared just before use, LAS being diluted with methanol and Na-2ØC_8-LAS with deionised water.

Methanol (HPLC-gradient grade), *n*-hexane and formaldehyde were supplied by Merck (Darmstadt, Germany). Sodium dodecyl sulfate (SDS) and hydrochloric acid were purchased from Fluka (Buchs, Switzerland). Acetonitrile (HPLC-gradient grade), sodium hydroxide, and phenolphthalein were supplied by Panreac (Barcelona, Spain).

Solvents and aqueous solutions used were filtered through 0.45 μm Nylon membranes (Millipore).

Isolute C_{18} (500 mg/3 mL) and Isolute SAX (500 mg/3 mL) solid-phase extraction (SPE) adsorbent cartridges were purchased from Isolute Sorbent Technologies (Mid Glamorgan, UK).

Instrumentation

Ultrasonic irradiation was applied by means of a Digital Sonifier (20 kHz, 400 W), model S-450D, from Branson (Danbury, USA), equipped with an ultrasonic generator, a model 102 converter, standard 12.70-mm diameter titanium disruptor horn, flat and replaceable 12.70 mm diameter titanium tip, and temperature probe. The horn was immersed in a distilled water bath (1,500 mL). The container used as sonication bath was a stainless steel conical cylinder of height 13.50 cm, inferior base 12.00 cm I.D., superior base 15.00 cm I.D., wall thickness 0.20 cm and 1,750 mL capacity. The container had two lateral openings designed for circulation of the water to keep the temperature of the bath constant.

These openings were located in different regions, the inlet in the lower part and outlet in the upper part of the conical cylinder. Extraction flasks were placed in this container. These flasks were stainless-steel cylinders 10.1 cm long and 4 cm I.D. and were useful either for performing the extraction or for centrifugation. The energy of ultrasonic irradiation was fixed at the desired level by using a power setting in the 10–100% range. This sonifier has a digital display which provides a continuous read-out of the power (range 105–125 W in this work) delivered to the end of the tip.

A model Universal 32 centrifuge from Hettich (Tuttlingen, Germany) and a model Frigomix U plus Thermomix 1441 thermostat from B. Braun (Melsungen, Germany) were also used.

SPE was performed on a Supelco (Madrid, Spain) vacuum manifold for twelve columns connected to a Supelco vacuum tank and to a vacuum pump. The polypropylene (3 mL) cartridges were attached to reservoirs with 60 mL capacity.

Analysis was performed with an Agilent Technologies (Palo Alto, CA, USA) 1100 series high-performance liquid chromatograph equipped with a quaternary pump, an on-line degasser, an autosampler, an automatic injector with a loop of up to 100 μL, a thermostatted column compartment, and a fluorescence detector (flow-cell volume 8 μL) connected on-line. ChemStation for LC 3D software (Agilent) was used for instrument control and for data acquisition and analysis.

Compounds were separated on a 125 mm×4 mm I.D., 5 μm particle size, LiChrospher-100 RP-8 column with a 15 mm LiChrospher-100 RP-8 safeguard column and on two 250 mm×4 mm I.D., 5 μm particle size, LiChrospher-100 RP-18 columns, purchased from Merck.

Statgraphics software package [39] was used for statistical analysis of data.

Sample collection and preparation

Soil samples were collected from twelve plots on the fertile plain of Granada. The soil had not been sprayed with pesticides and herbicides for the last five years nor had it ever received sewage sludge. All the soil collected was from the arable layer. Soils were sampled by use of a soil drill. The samples were placed in plastic containers and conserved by immediate addition of 3% (*v*/*v*) formaldehyde. Sub-samples (200 g) of this mixture were dried at room temperature for 48 h. The dried samples were then ground with a mortar and pestle and passed through a 30-mesh sieve to enhance the extractability of the analyte. Soil samples were stored in the dark at 4 °C until analysis.

Preparation of spiked soil samples

Soil samples were accurately weighed and placed in stainless steel flasks. Spiked soil samples were prepared by addition of suitable volumes of LAS standard solution to 5.0 g of each sample Methanol was then added until the solvent completely covered the soil particles. The suspension was thoroughly mixed for 1 min with a mechanical shaker to enable analyte distribution throughout the soil. The bulk of the solvent was slowly evaporated at room temperature over a period of 15–17 h and the spiked soils were then ready for the experiments.

Ultrasonic extraction procedure

The sonication bath was prepared by addition of distilled water (1,500 mL). Each phase of the extraction was performed using two metallic capsules within the sonication bath at the same time. Capsules containing the soil samples (5.0 g) and methanol (100 mL) were arranged in the bath so they were 2 cm apart and 2 cm from the probe tip (Fig. 1). The samples were ultrasonicated for 10 min with an amplitude of 75% and then immediately centrifuged at 4,000 rpm for 10 min. The soil residues was then washed with 30.0 mL methanol and centrifuged again, and the methanolic extracts were combined.

Solid-phase extraction procedure

Each methanolic extract was subjected to SPE on an Isolute SAX cartridge previously been activated with *n*-hexane (3 mL) and methanol (5 mL). The methanolic extracts

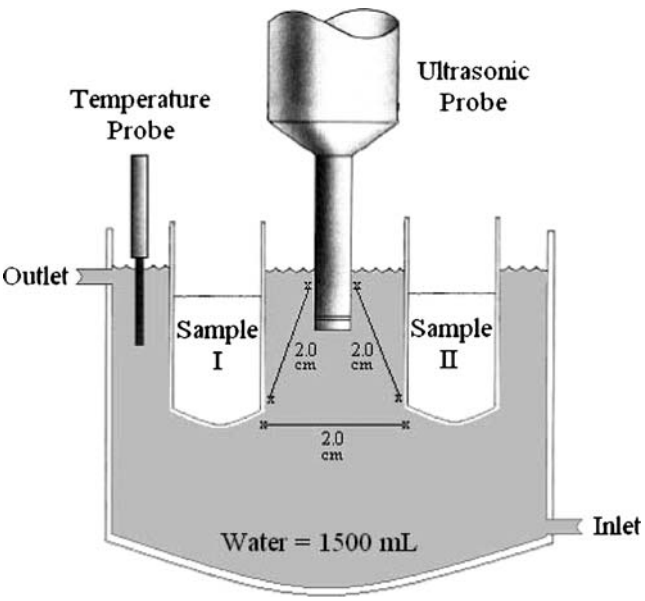

Fig. 1 Schematic diagram of ultrasound bath

(~130 mL) were passed through the cartridges at 2–3 mL min^{-1}. Clean-up was performed using methanol (5 mL) and the analytes were then eluted with 2 mL methanol–water 3:2 (*v*/*v*) containing 4 mol L^{-1} HCl into a 10-mL volumetric flask. The eluate was neutralised with 4 mol L^{-1} NaOH solution (using phenolphthalein as indicator) and, finally, the solution was diluted to volume with deionized water.

These 10-mL solutions were then passed through a Isolute C_{18} cartridge previously preconditioned with methanol (5 mL) then deionised water (5 mL). The solution was passed through the cartridge at 2–3 mL min^{-1}. Clean-up was performed with 3 mL methanol–water 35:65 (*v*/*v*) and the analytes were eluted with methanol (3 mL). The eluate was evaporated to dryness, by means of a stream of nitrogen at room temperature, and the residue was dissolved in 1 mL MeOH–H_2O 3:2 (*v*/*v*) containing 2 μg mL^{-1} Na-2ØC_8-LAS as internal standard.

Chromatographic analysis

The extract (100 μL) was injected into the chromatograph using experimental and instrumental conditions which depended on the resolution required for the analysis: total LAS, homologues, or isomers [23].

Determination of total LAS

Quantification of total LAS was achieved by assuming that total LAS was the sum of all the homologues present.

Determination of homologues

Analytical separation of the homologues was performed on a LiChrospher-100 RP-8 analytical column (125 mm× 4 mm I.D., 5 μm particle size) and a LiChrospher-100 RP-8 safeguard column (15 mm) with a mobile-phase gradient prepared from methanol (component A) and 30 mmol L^{-1} SDS (component B). The initial conditions were 55% A and 45% B and the percentage of A was then increased linearly to 70% in 15 min and then returned to 55% in 1 min. The amount of B was then set at 45% for 5 min to restore the initial conditions. The total run time was 16 min and the post-delay time was 5 min. The mobile phase flow rate was 1.0 mL min^{-1}. The column oven temperature was 40 °C and fluorescence measurements were performed with excitation and emission wavelengths of 230 and 290 nm, respectively, with a PMT gain of 13.

Determination of isomers

Resolution of the isomers was performed by use of two coupled LiChrospher-100 RP-18 columns (250 mm×4 mm I.D.; 5 μm particle size). The isomers were eluted with a flow rate gradient from 1.0 to 0.25 mL min^{-1} and a linear mobile-phase gradient prepared from acetonitrile (component A) and 5 mmol L^{-1} SDS (component B). The acetonitrile content was increased linearly from 20 to 40% in 160 min then returned to 20% A, with flow rate of 1.0 mL min^{-1} in another 2 min. The composition was then set to 20% A for 10 min to restore the initial conditions.

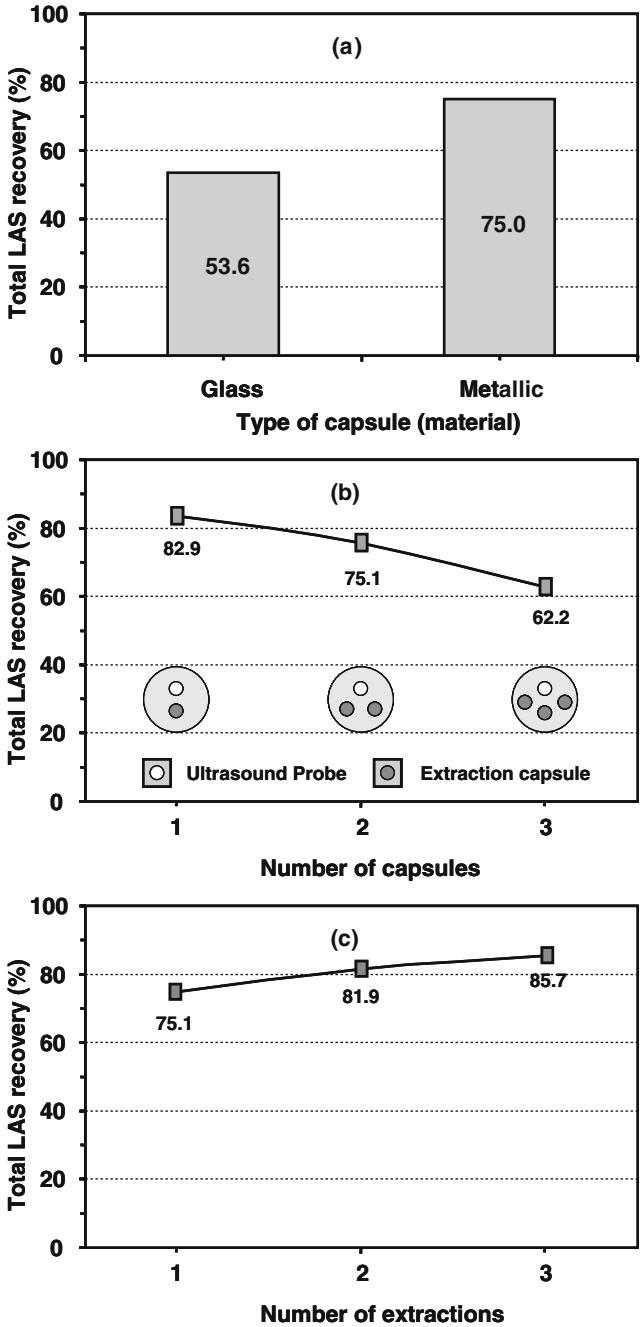

Fig. 2 Effect on the recovery of total LAS from spiked soil samples of: (**a**) use of different materials for manufacture of extraction capsules, (**b**) number of extraction capsules, and (**c**) the number of extractions

Table 1 Conditions selected for the extraction and purification steps

Condition	Values studied	Value selected
Ultrasound extraction step		
Type of capsule	Glass/Metallic	Metallic
Number of capsules	1–3	2
Number of extractions	1–3	1
Volume of methanol (mL)	20–100	100
Time (min)	2–12	10
Amplitude (%)	10–90	75
SPE step		
Flow rate (mL min^{-1})	1–4	2–3
[HCl] in methanol (mol L^{-1})	0–4	4

The total run time was 162 min and the post-delay time was 10 min. The columns were thermostatted at 40 °C and fluorescence measurements were performed with excitation and emission wavelengths of 230 and 308 nm, respectively, with PMT gain 13.

Results and discussion

Optimisation of ultrasonic extraction

In ultrasound-assisted extraction there are many possibilities of interference between the different conditions. It is, therefore, fundamental to know the most favourable conditions for effective and reproducible extraction. Conditions that can alter the process of extraction are:

1. the liquid used for propagation of the ultrasound wave;
2. the quantity of liquid in the bath;
3. the viscosity of the liquid;
4. the bath temperature;
5. the geometric form of the bath containing the ultrasonic probe;
6. the geometric shape of the extraction capsule;
7. the material used to construct the capsule used for extraction; and
8. the distance between the ultrasonic probe tip and the extraction capsule.

It was stipulated that the initial conditions used for the extractions would be:

1. use of deionized water (volume 1,500 mL) as propagation medium for the ultrasound radiation;
2. temperature of the bath to be kept constant at 25±0.5 °C by use of a cryostat, to reduce evaporation of the organic solvent; and
3. ultrasonic probe always located on the bath as described in the Instrumentation section.

Water, methanol, ethanol, and some water–alcohol mixtures were investigated as extraction solvents. The best results were obtained with methanol.

With these pre-established conditions we proceeded to study the extraction. Conditions significantly affecting the process would enlighten us about procedures resulting in more efficient extraction. In the first set of experiments the type of extraction capsule (metal capsule, 10.1 cm×4.0 cm I.D. with 100 mL capacity or glass capsule, 10.5 cm×3.5 cm I.D. with 100 mL capacity), the number of capsules in the bath (1 to 3), and the number of simultaneous extractions (1 to 3) in the bath were studied individually. For this purpose weighed soil (5.0 g) was spiked with 20 mg kg^{-1} total LAS and sonicated for 10 min (amplitude 75%) with 100 mL methanol. The first experiment was to evaluate the types of extraction capsule while performing only one extraction step by sonication. The mixtures were centri-

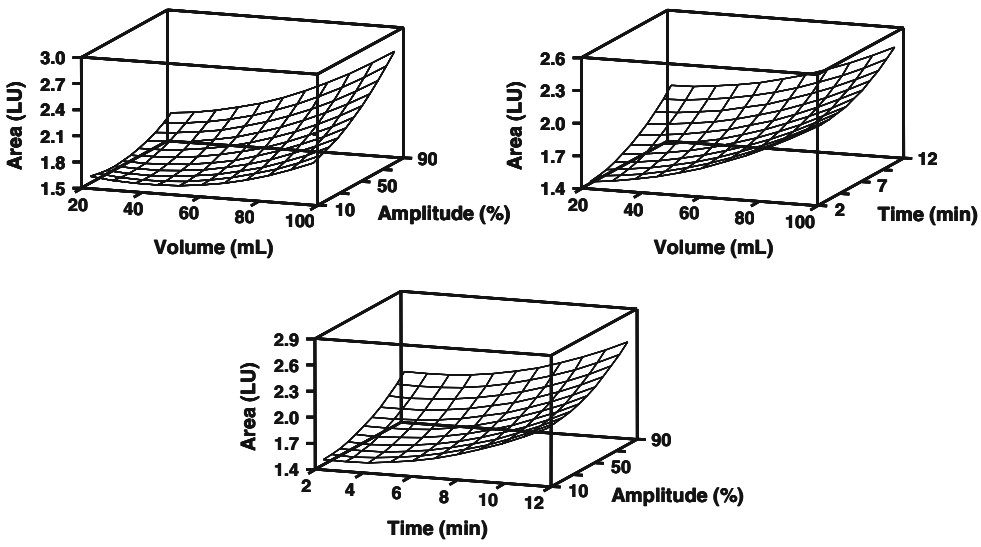

Fig. 3 Response surface of the experimental design

fuged at 4,000 rpm for 10 min, the residual soil was washed with 30 mL methanol and centrifuged again. Finally, the methanolic extracts were combined. The SPE procedure mentioned above was then applied before chromatographic separation of the homologues. The purpose of this analysis was to determine the effect on performance of diffusion and propagation of the ultrasonic wave and, therefore, which arrangement of the capsules enhanced the efficiency of the extraction. From the results obtained the capsule chosen for subsequent analysis was the metal one, because it resulted in better recoveries. These results are shown in Fig. 2a.

We then determined the best number of capsules in the sonication bath. In this experiment extraction was performed with 1 to 3 extraction capsules instead of the customary two or three; the results can be seen in Fig. 2b. Too many capsules in the bath results in reflections of the ultrasound waves, which reduced the efficiency of the sonication process. We therefore decided that extraction would be performed with two capsules.

We then determined the number of extractions necessary. Extraction was repeated up to three times, the amount of LAS extracted was determined each time, and the cumulative recovery was calculated (Fig. 2c).

A Central Composite Design (CCD) was used to study the combined effect of sonication time, extractant volume, and amplitude of ultrasound radiation on the extraction process. It involved the use of three centre points to

Table 2 Comparison of the different methods for extraction of LAS from samples

Analysis	Sample	Extraction method[a]	Time	Ref
Total LAS, homologues and isomers	River sediment	SOX (200 mL, MeOH+20% (*w*/*w*) solid NaOH)	12 h	[11]
Total LAS and homologues	Marine sediment	PLE (MeOH) SOX (MeOH)	15 min 5 h	[24]
Total LAS and homologues	Agricultural soil	SOX (80 mL, MeOH)	8 h	[16]
Total LAS and homologues	River sediment	SOX (100 mL, H_2O) SOX (100 mL, MeOH) SOX+MAE (100 mL, H_2O)	24 h 24 h 2 h	[17]
Total LAS and homologues	River sediment, sewage sludge, sludge amended soil	REF (150 mL, MeOH)	2 h	[12]
Total LAS and homologues	Sewage sludge, sludge-amended soil	REF (50 mL, 0.5 mol L^{-1} KOH in MeOH)	4 h	[13]
Total LAS, homologues and isomers	River sediment	SHE (MeOH)	24 h (×3)	[28]
Total LAS and homologues	Agricultural soil	SHE (30 mL, MeOH)	1 h	[18]
Total LAS and homologues	Sludge-amended soil, river sediment, digester sludge	SFE (MeOH modified CO_2)	30 min	[19]
Total LAS and homologues	River sediment	SFE (H_2O)	50 min	[20]
Total LAS and homologues	Preserved natural soil, Agricultural soil, Sludge amended soil, Sewage sludge	MAE (60 mL, MeOH)	50 min (×2)	[21]
Total LAS and homologues	Sludge-amended soil	PLE (MeOH+H_20, 9:1 *v*/*v*)	15 min	[25]
Total LAS and homologues	Marine sediment	PLE (ACT+MeOH, 1:1 *v*/*v*)	15 min	[26]
Total LAS and homologues	River sediment	PLE (MeOH)	45 min	[22]
Total LAS and homologues	Sludge-amended soil	USE (60 mL, MeOH+H_2O, 1:1 *v*/*v*, bath)	20 min (×3)	[29]
Total LAS and homologues	Lagoon sediment	USE (70 mL, EtOH+H_2O, 1:1 *v*/*v*, bath)	15 min	[27]
Total LAS and homologues	Agricultural soil	USE (MeOH, bath)	1 h	[14]
Total LAS, homologues and isomers	Agricultural soil	USE (100 mL, MeOH, probe)	10 min	This work

[a] REF: reflux; SOX: Soxhlet; MAE: microwave-assisted extraction; PLE: pressurized liquid extraction; SFE: supercritical-fluid extraction; SHE: shaking extraction; USE: ultrasound extraction; MeOH: methanol; ACT: acetone; EtOH: ethanol.

evaluate experimental error. The experimental design and statistical analysis were performed using the Statgraphics software. The sum of the peak areas of the homologues was used as the response variable. Table 1 shows the upper and lower values used for each factor for accurately weighed soil (5 g) spiked with 20 mg kg^{-1} total LAS. By use of the standardized Pareto chart all the variables (ultrasound radiation amplitude, sonication time, and extractant volume) are found to be statistically significant factors in the ranges under study. The values selected for amplitude and sonication time were a compromise between reasonable recovery, reasonable analysis time, and smaller consumption of the horn tip rather than the optimum values found. The choice was inferred from predictions provided by the Statgraphics software. The maximum volume of extractant was selected as the optimum. The values selected, on the basis of results obtained by use of the response surface (Fig. 3), are listed in Table 1.

Table 2 show the times required for extraction of LAS from soils, as estimated by eighteen different authors, including ourselves. Our extraction times results are much shorter than those reported by other authors.

Solid- phase extraction

Because of the complex nature of soil a solid-phase extraction procedure was selected as appropriate for clean-up and preconcentration of LAS in the methanol extracts obtained previously. This procedure was performed in two stages. In the first stage, with SAX cartridges, two conditions were optimized—flow rate (which affects analyte adsorption) and the concentration of eluent used (which affects analyte desorption). The optimum concentration of HCl in the water–methanol solution used to desorb the LAS from the SAX phase was tested by use of different concentrations (0, 0.06, 0.12, 0.25, 0.50, 1.00, 2.00 and 4.00 mol L^{-1}). Maximum analyte peak area was obtained by use of 4.00 mol L^{-1}, which was therefore selected for subsequent experiments. Another factor studied was the flow rate used for both adsorption and desorption (1.0, 2.0, 4.0, and >4.0 mL min^{-1}). The results obtained showed that retention of the analytes was maximum if the flow rate was slightly less than 4.0 mL min^{-1}; subsequent experiments were performed at a flow rate of 2.0–3.0 mL min^{-1}. The second stage of SPE (with C_{18} cartridges) was used because of the recommendations by del Olmo et al. [23]. This stage was necessary to remove substances coeluted with the SAX, because the solution contained not only phenolphthalein but excess chloride. Breakthrough curves were evaluated for LAS to establish the capacity of the adsorbents used (SAX and C_{18}). Curves were recorded using a solution of 10 mg L^{-1} total LAS in Milli-Q water at a flow rate of 2.0 mL min^{-1}. Breakthrough volumes of LAS

Table 3 Analytical and statistical data

Parameter[a]	C_{10}	C_{11}	C_{12}	C_{13}	Total LAS
a	-0.12×10^{-3}	2.11×10^{-3}	7.69×10^{-3}	4.67×10^{-3}	
S_a	0.64×10^{-3}	1.89×10^{-3}	1.33×10^{-3}	1.0×10^{-3}	
b (kg mg^{-1})	0.484	0.484	0.484	0.484	
S_b	0.17×10^{-3}	0.21×10^{-3}	0.21×10^{-3}	0.19×10^{-3}	
LDR (mg kg^{-1})	0.06–7.07	0.17–16.36	0.15–14.87	0.11–11.70	
R^2 (%)	99.99	99.99	99.99	99.99	
$S_{y/x}$	2.90×10^{-3}	8.42×10^{-3}	6.29×10^{-3}	5.12×10^{-3}	
P_{lof} (%)	31.6	22.6	32.2	50.8	
LOD (mg kg^{-1})	0.02	0.05	0.04	0.03	
LOQ (mg kg^{-1})	0.06	0.17	0.13	0.10	
R (%)	75.1	75.2	75.2	75.0	
Accuracy (%)[b]					
Intra-day (n=12)	96.6–104.4	97.3–103.3	98.3–102.6	96.6–101.7	97.4–102.2
Inter-day (n=36)	99.3	99.1	100.1	99.2	99.6
Precision (RSD, %)[b]					
Intra-day (n=12)	1.1–1.9	1.6–2.3	1.5–2.0	1.6–2.2	1.0–1.7
Inter-day (n=36)	1.7	2.0	1.8	1.9	1.3

Accuracy and precision data of LAS homologues studied

[a] *a*, intercept; S_a, intercept standard deviation; *b*, slope; S_b, slope standard deviation; LDR, linear dynamic range; R^2, determination coefficient; $S_{y/x}$, regression standard deviation; P_{lof}, P-value for *lack-of-fit* test; LOD, limit of detection; LOQ, limit of quantification; *R*, average recovery obtained for 1.0, 5.0, 30.0, and 50.0 mg kg^{-1} total LAS and three determinations; RSD, relative standard deviation

[b] Average results obtained from three determinations of 1.0, 5.0, 30.0, and 50.0 mg kg^{-1} total LAS; measurements on days 1–3 (n=12 for each day)

under these conditions were >1 L for SAX and 76 mL for C_{18}. The values used in this work were within these limits.

Recovery of this step (SAX and C_{18}) was tested and the result was 97% for 1.0–50.0 mg kg^{-1} total LAS.

The variables studied and their values selected are listed in Table 1.

Analytical data and validation of the method

Calibration plots were established by use of standard soil samples spiked at eight concentrations (1.0, 3.0, 5.0, 10.0, 15.0, 20.0, 35.0, and 50.0 mg kg^{-1} total LAS) and treated in accordance with the sample-treatment procedure described above. Each level was prepared by triplicate, and each calibration sample was analysed twice). Analyte-to-internal standard peak-area ratio was plotted against analyte concentration. The linearity of the calibration plots was tested by use of the Analytical Methods Committee [40] *lack-of-fit* test. The limits of detection (LOD) and quantification (LOQ) for the different homologues of LAS were calculated by use of the IUPAC criterion [41].

Analytical and statistical data for each homologue studied are summarized in Table 3.

To evaluate the precision of the assay, laboratory reproducibility and repeatability were estimated at four different concentrations, 1.0, 5.0, 30.0, and 50.0 mg kg^{-1} total LAS. The soil samples were spiked, extracted, and analysed in triplicate. The procedure was repeated three times on the same day to evaluate intra-day variability and was repeated on three consecutive days to determine inter-day variability. The repeatability and within-laboratory reproducibility, expressed as relative standard deviation (RSD), are summarized in Table 3.

Accuracy was evaluated by determining the recovery of known amounts of LAS in soil samples. Soil samples were spiked at 1.0, 5.0, 30.0, and 50.0 mg kg^{-1} total LAS. Recovery was evaluated by comparing the result from the spiked soil with a soil submitted to the same process of extraction and fortification before being analyzed by the chromatographic method for the homologues. The experiment was performed in triplicate. Average recovery of the LAS homologues, with the standard deviations, are listed in Table 3.

Application of the method

The method was used for determination of total LAS, homologues, and isomers in agricultural soil samples. Samples were collected from twelve plots at different locations on the fertile plain of Granada (commonly known as "Vega de Granada"): "Belicena" (four samples); "Churriana de la Vega" (two samples), "Granada" (one sample) and

Table 4 Total concentrations LAS found in the soils samples analyzed, and the proportion of each isomer as a percentage

Agricultural soil samples	Total (mg kg^{-1})	Alkyl chain distribution (%)																
		C_{10}				C_{11}				C_{12}				C_{13}				
		2Ø	3Ø	4Ø	5Ø	2Ø	3Ø	4Ø	5+6Ø	2Ø	3Ø	4Ø	5+6Ø	2Ø	3Ø	4Ø	5+6+7Ø
Plot 1 - Belicena	0.352	1.57	1.79	2.03	2.35	3.08	4.20	8.30	11.69	3.66	4.72	5.79	13.75	4.15	4.89	6.86	21.18
Plot 2 - Belicena	0.602	0.77	1.03	1.10	1.42	3.11	4.79	8.25	12.78	3.00	5.48	9.45	15.78	2.91	5.30	5.92	18.91
Plot 3 - Belicena	0.405	1.87	2.29	2.46	3.00	3.08	5.27	7.81	15.29	3.86	5.51	6.51	16.49	2.94	4.34	7.77	11.56
Plot 4 - Belicena	0.302	1.49	1.58	1.97	2.28	3.21	4.90	7.25	12.65	3.65	4.59	8.94	16.77	3.51	4.86	7.47	14.87
Plot 5 - Granada	0.599	2.18	2.37	2.50	2.63	4.84	5.98	7.30	10.74	2.62	5.22	7.66	15.43	3.37	5.17	6.89	15.10
Plot 6 - Churriana de la Vega	0.297	2.29	2.46	2.84	3.11	3.12	5.07	7.56	14.65	3.88	4.72	9.39	14.80	3.27	5.15	8.55	9.16
Plot 7 - Churriana de la Vega	0.326	1.48	1.59	1.71	2.17	2.91	4.38	5.48	15.09	2.27	6.86	11.52	14.57	4.86	5.87	7.77	11.46
Plot 8 - Las Gabias	0.266	2.02	2.19	2.51	2.87	4.99	6.81	8.26	9.20	5.51	7.68	9.21	11.68	4.08	5.01	7.15	10.86
Plot 9 - Las Gabias	0.325	1.97	2.68	2.64	2.77	3.04	4.77	9.76	14.73	3.26	7.01	10.29	13.47	3.21	4.27	5.81	10.33
Plot 10 - Las Gabias	0.356	1.90	2.24	2.49	2.58	3.47	6.53	8.51	11.26	3.08	6.94	10.37	14.02	4.12	5.02	6.61	10.86
Plot 11 - Las Gabias	0.331	1.82	2.13	2.26	2.50	4.53	5.50	7.83	9.57	4.30	6.41	8.12	11.46	4.60	6.15	10.23	12.61
Plot 12 - Las Gabias	0.351	1.73	1.92	2.12	2.59	3.67	5.01	8.25	11.18	1.99	5.58	9.22	15.85	4.35	5.82	8.83	11.89
Alkyl chain distribution of commercial product		2.66	2.94	3.63	4.91	5.04	5.67	7.23	14.78	3.92	4.65	5.79	15.38	2.77	3.19	4.06	13.38

"Las Gabias" (five samples). To determine LAS in these soils, the samples were processed by triplicate and each experimental point was analyzed twice.

The total amounts of LAS found in the soil samples and the proportion of each isomer as a percentage are summarised in Table 4. Figure 4 shows typical chromatograms obtained using the optimised chromatographic conditions for determination of the LAS homologues (A) and isomers (B).

Changes in distribution of the components of LAS have been demonstrated in previous work [42, 43]. This shift was explained in terms of different adsorption of the different components of the LAS—those with longer alkyl chain would be more strongly adsorbed by the organic fraction of the soil [44].

LAS was detected at low levels, approximately 0.5 mg kg^{-1}, in all the soils analyzed. The presence of the LAS could be because these agricultural soils were in contact with the analyte because of the possible exposure to wastewater, pesticides, or sludge. According to a European Commission Technical Guidance Document [45] these low levels of LAS are not believed to be a ecotoxicological hazard.

Conclusions

A simple, rapid, and practical method has been developed for determination of total LAS, homologues and isomers in agricultural soil. The approach is based on ultrasound-assisted extraction in conjunction with solid-phase extraction and then analysis by liquid chromatography with fluorescence detection. The effect of different ultrasound extraction conditions on recovery of the LAS was studied. The main advantages of the method are the low extraction times (10 min), low volumes of organic solvent used, and good reproducibility. A field study conducted on the fertile plain of Granada (Spain) detected the presence of small amounts of LAS in a series of soil samples. The findings suggest agricultural practices in the area may need to be checked.

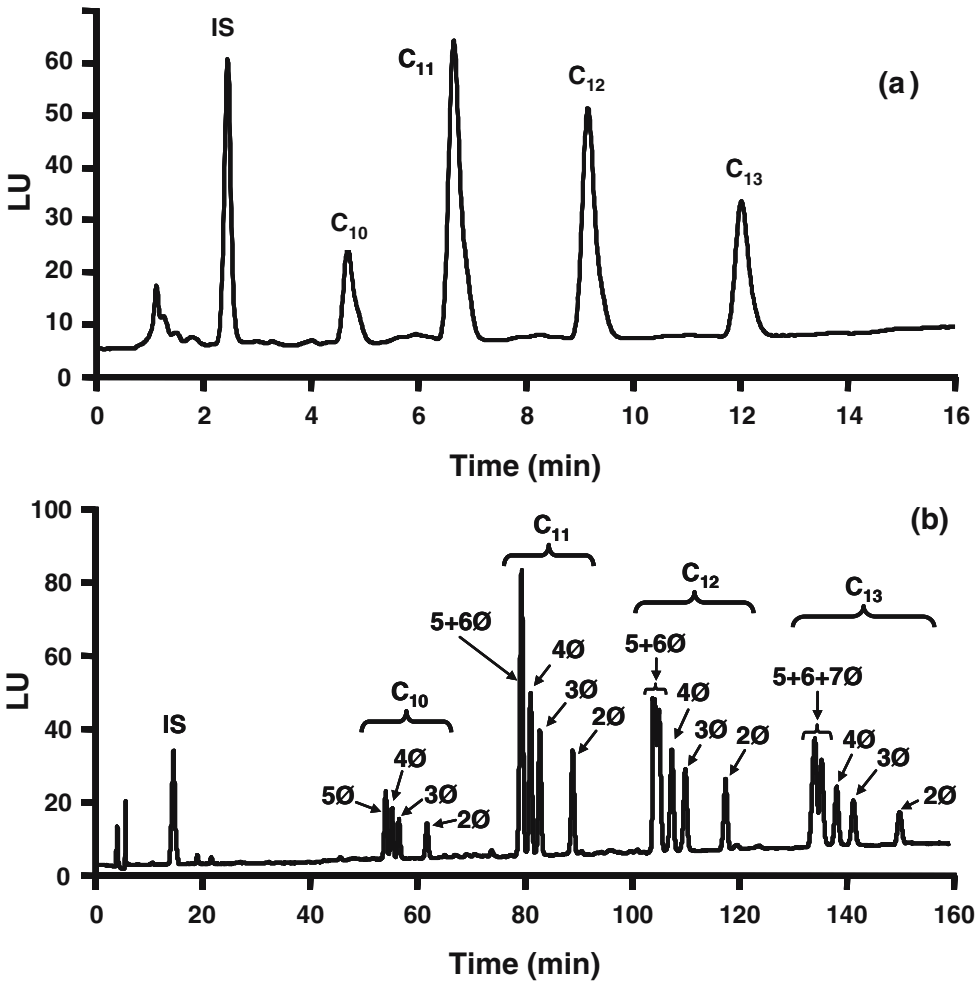

Fig. 4 Chromatographic separation of homologues (**a**) and isomers (**b**) of LAS at 5.0 mg kg^{-1}

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