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Journal of Chromatography A, 1026 (2004) 41–46
JOURNAL OF CHROMATOGRAPHY A
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Screening method for linear alkylbenzene sulfonates in sediments based on water Soxhlet extraction assisted by focused microwaves with on-line preconcentration/derivatization/detection

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Received 6 May 2003; received in revised form 17 October 2003; accepted 6 November 2003

Abstract

A screening method for linear alkylbenzene sulfonates (LAS) in sediments has been developed. Soxhlet extraction with water assisted by focused microwaves provides recoveries better (>90%) than obtained by conventional Soxhlet extraction (70–80%). Coupling of the extractor with an on-line preconcentration/derivatization/detection manifold through a flow injection (FI) interface allows a fully automated screening approach. A yes/no answer can be obtained in less than 2 h (for the whole analytical process), a short time compared with the at least 24 h of Soxhlet extraction (without final detection). Due to the use of water as leaching agent, the proposed method is environmentally friendly.

Keywords: Water Soxhlet extraction; Focused microwaves; Alkylbenzene sulfonates, linear

1. Introduction

Linear alkylbenzene sulfonates (LAS) are the anionic surfactants most widely used in both domestic and industrial detergents formulations, with an annual production rate of 1.8 million tonnes [1]. Although the environmental legislation does not permit surfactant products with less than 90% biodegradability to be marketed [2], relatively high concentration of LAS has been detected in the environment. Because of their continued presence in the environment and their properties they tend to be absorbed and accumulate in sediments, where they are found in a higher concentration as compared with water [3,4]. Therefore, LAS can be considered as a secondary contamination source for water when the sediment is re-suspended by high water flows. Moreover, LAS remaining in the water can be accumulated in aquatic organisms with toxic effects. Thus, determination of anionic surfactants in environmental samples is of great interest, after maximum priority pollutants such as pesticides, polycyclic hydrocarbons or polychlorobiphenyls.

Both Soxhlet extraction [5–7] and shaking extraction [8] methods using methanol as extraction solvent are mainly used for the extraction of LAS from sediment. Some attempts performed in order to reduce both the volume of organic solvents used and the time needed for total extraction, have been based on CO_2-supercritical fluid extraction with ion-pair formation [9,10] or methanol as organic modifier [11,12], and pressurized liquid extraction with methanol [13].

In 1997, a new technique based on Soxhlet extraction but assisted by focused microwaves was developed providing surprising results in both environmental and food analysis as compared with the conventional extraction methods [14,15]. However, one of the lacks of the first focused microwave-assisted Soxhlet (FMAS) extractor was the impossibility of using water as leaching agent due to the too long length of the glassware. To solve this problem, in 2001, a new extractor was designed and constructed showing good results for the extraction of acid herbicides from different types of soils using water as extraction agent [16]. In the present work, this extractor has been used for the extraction of linear alkylbenzene sulfonates from sediments. A modification in the distillation flask of the extractor has also allowed the coupling of the extractor to a preconcentration/derivatization/detection system, thus providing a fully automated screening approach.

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doi:10.1016/j.chroma.2003.11.047

Several methods based on titration [17], chromatography-fluorimetric detection [5,13,18], voltammetry [19] and spectrophotometry [20–22] have been reported for the determination of LAS. Between them, the spectrophotometric method is one of the most frequently used for the analysis of LAS in environmental samples, due to its high sensitivity and simplicity. This spectrophotometric method—based on the competition of an anionic chromogenic dye with an anionic surfactant in the interaction with a cationic surfactant—has been selected due to its facility to be implemented in the FI system.

2. Experimental

2.1. Instruments and apparatus

The device used for the focused microwave-assisted Soxhlet extraction (SEV, Puebla, México) is a new prototype that has been designed to enable the use of water as extractant and has been successfully applied for the extraction of acid herbicides from different types of soils [16]. In this work, the distillation flask was modified in order to allow removal of the extract directly by aspiration using a peristaltic pump (Fig. 1).

Two low-pressure peristaltic pumps (Gilson, Worthington, OH, USA), three low-pressure injection valves (Rheodyne, Cotati, CA, USA), a home-made mini-column (6 cm in length and 4 mm i.d.) packed with the sorbent material, and Teflon tubing of 0.5 mm i.d. were used to build the dynamic preconcentration-detection manifold (Fig. 2). A Heλios spectrophotometer (Thermo Spectronic, Cambridge) equipped with an 18 μl flow cell (Hellma, Jamaica, NY) was used to monitor the absorbance.

The individual separation of the analytes in the extract was performed by an HP1100 liquid chromatograph (Hewlett-Packard, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, a Rheodyne 7725 high-pressure manual injector valve (20 μl injection loop) and a Hitachi, model F-1050 chromatographic fluorimetric detector (Hitachi, Tokyo, Japan), equipped with a 12 μl flow cell and a D-2500 integrator (Hitachi). Kromasil C_8 (250 mm × 4.6 mm; 5 μm particle size from Análisis Vínicos, Ciudad Real, Spain) was used as the analytical column.

2.2. Reagents

The linear alkylbenzene sulfonates (sodium dodecylbenzene sulfonate (Fluka, Buchs, Switzerland), sodium tridecylbenzene sulfonate (Chem Service, Philadelphia, USA) and sodium octylbenzene sulfonate (Sigma–Aldrich, Steinheim, Germany)) were used for preparing the stock standard solutions in distilled water. Distilled water was used as extractant. The sorbent used in the preconcentration step was C_{18}-Hydra (Panreac, Barcelona, Spain). HPLC-grade methanol (Panreac) was used as both eluent in the preconcentration step and mobile phase in the chromatographic

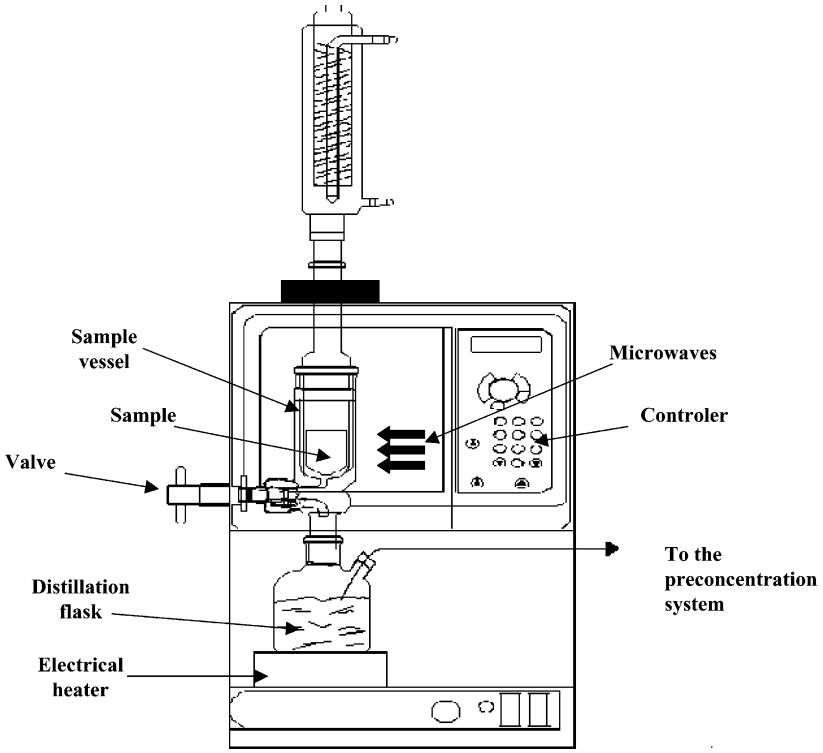

Fig. 1. Scheme of the focused microwave-assisted Soxhlet extractor.

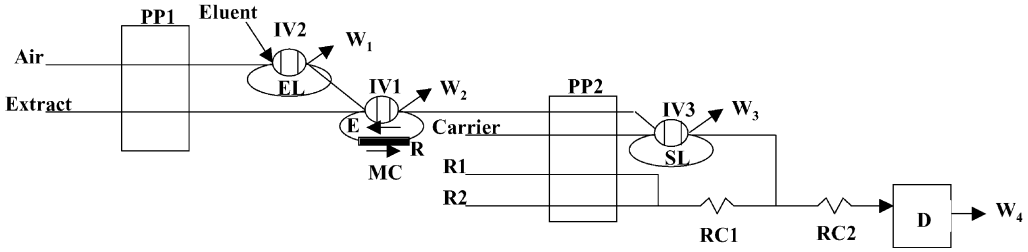

Fig. 2. Scheme of the manifold used for coupling preconcentration/derivatization/photometric detection. PP, peristaltic pump; IV, injection valve; EL, eluent loop; W, waste; E, elution direction; R, retention direction; MC, mini-column; R_1, 6.75×10^{-4} M methyl orange prepared in 0.1 M pH 5.0 acetate buffer solution; R_2, 22.25×10^{-5} M aqueous solution of cetyl pyridine chloride; SL, sample loop; RC, reaction coil; D, detector.

step. Methyl orange (MO) and cetyl pyridine (CP^+) chloride (both from Sigma–Aldrich) were dissolved in distilled water to give 1.0×10^{-3} mol/l stock solutions. Acetic acid and sodium acetate (Panreac) were used for preparing the 0.1 mol/l HAc–NaAc buffer solution at pH 5.0. Ethyl ether (Panreac) was used for sample preparation.

2.3. Sample preparation

A 300 g of dry river sediment (containing 1.9% of total organic matter) spiked with the LAS to obtain a final total concentration of 5 μg/g of each analyte was selected as matrix to carry out the optimization study. Two 50 g portions of sediment were spiked with 2.5 and 1.5 μg/g of each analyte. The spiked levels were selected in order to obtain sediments with environmentally representative concentrations. The sediment samples were aged for 3 months in order to simulate the matrix–analyte interaction in real samples.

2.4. Procedures

2.4.1. Conventional Soxhlet extraction procedure

Four grams of sample was placed in a cellulose thimble (25 mm × 88 mm, Albet, Barcelona, Spain), which was capped with cotton wool and placed into the Soxhlet chamber. The overall Soxhlet glassware was fitted to a distillation flask containing 100 ml of extractant and two to three glass-boiling regulators. Extractions with both water and methanol were carried out for 12 and 24 h.

2.4.2. FMASE procedure

Four grams of spiked sediment was weighed into a cellulose extraction cartridge, which was capped with cotton wool and placed into the sample cartridge vessel located in the microwave irradiation zone. 100 ml of distilled water was poured into the distillation flask (two or three glass-boiling regulators were also added) and the isomantle rheostat was set at 100%. The extraction program consisted of a number of cycles, each of which involved four steps: (1) filling of the sample cartridge vessel (vessel valve in load position) by water evaporation from the distillation flask, condensation in the refrigerant, and dropping on the sample; (2) microwave irradiation of the cartridge for a pre-set interval (200 s of irradiation time) at a fixed microwave power (200 W); (3) waiting for a pre-set time (120 s of delay time) in which the sample was in contact with the heated water; (4) unloading of the extraction vessel by switching the vessel valve to its unload position, thus delivering the vessel content to the distillation flask. After the last cycle, only the first step was carried out again in order to reduce the volume of the extract contained in the distillation flask to ≈50 ml.

2.4.3. On-line preconcentration/derivatization/detection procedure

After leaching, the extract (≈50 ml) was aspirated by a peristaltic pump (PP_1 in Fig. 2) from the distillation flask to the dynamic preconcentration/derivatization/detection manifold. The stream was driven (at 3.5 ml/min flow rate) to a mini-column where the analytes were retained. The mini-column was located in the loop of an injection valve (IV_1), thus allowing elution (at 0.5 ml/min flow rate, with 1:1 (v/v) water:methanol solution) in the direction opposite to retention. The eluate was driven to a 100 μl loop of the injection valve IV_3. At a pre-set time, the sample plug was injected into a carrier (water), which merged with the reagents stream in the reaction coil RC_2 (15 cm length). A previous reaction coil, RC_1 (10 cm length), was used for mixing the chromogenic reagent solution of MO, R_1 (6.75×10^{-4} mol/l in pH 5.0 HAc–NaAc buffer at 2.5 ml/min) with the cationic surfactant CP^+ chloride solution, R_2 (22.25×10^{-5} mol/l at 4.5 ml/min). Before elution of the analytes from the mini-column, the reagents stream was continuously passed through the detector in order to establish the baseline. The absorbance of the reaction product was monitored at 465 nm. Between successive samples and during the photometric determination, the sorbent in the mini-column was conditioned by circulating methanol and water through it. Deterioration of the sorbent in the mini-column was observed after around 100 samples.

2.4.4. Chromatographic determination

After extraction, and when individual quantification of each analyte was required, a HPLC with fluorescence detection method was used. The HPLC separation of the analytes was performed using an isocratic elution regime in which a 4:1 (v/v) methanol:water mixture was used as mobile phase

at a flow rate of 0.8 ml/min. Fluorimetric detection was performed at 225 and 295 nm for the excitation and emission wavelengths, respectively. Quantification of the analytes was carried out by running three calibration curves (one for each analyte) using standard solutions between 0.5 and 35 μg/ml.

3. Results and discussion

The ranges assessed and optimum values for the variables in all steps are listed in Table 1.

3.1. Optimization of the chromatographic separation

Different mixtures of methanol/water as mobile phase and different gradients were used for separation of the LAS using the Kromasil C_8 column. The influence of the flow rate of the mobile phase was studied between 0.5 and 2 ml/min, and the best separation was obtained for 0.8 ml/min. An injection volume of 20 μl was selected in order to obtain a quantifiable fluorimetric signal. As can be seen in Fig. 3, complete separation of the analytes was achieved within 25 min with the isocratic regime commented in Section 2.

3.2. Optimization of the on-line derivatization/detection

The flow injection (FI) spectrophotometric method for the determination of LAS was based on the competitive reaction for the cationic surfactant cetyl pyridine (CP^+) chloride between the acidic dye methyl orange (MO) and the anionic surfactants. In a pH 5.0 medium the cetyl pyridine cation (CP^+) reacts with dissociated methyl orange (MO^-) to form an ion-associate complex, causing a hypsochromic shift of the absorption maximum from 465 nm for MO^- to 358 nm for the $CP^+ - MO^-$ associate. The MO^- in the ion-associate complex can be quantitatively substituted by such anionic surfactants as LAS, leading to an increase in the absorbance measured at 465 nm. This increased absorbance is proportional to the concentration of anionic surfactants.

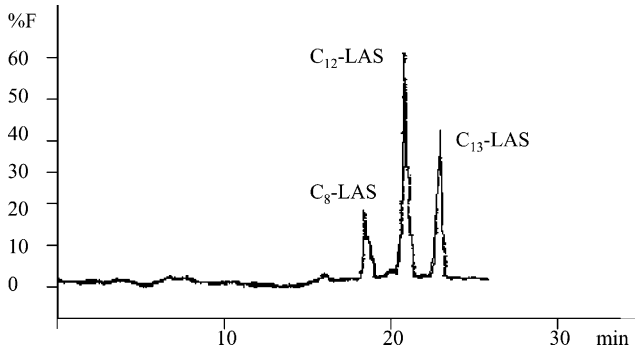

Fig. 3. Chromatogram of a spiked sample (containing 5 μg/g of each LAS) after focused microwave-assisted water extraction, under the optimum working conditions.

The variables optimized for the colorimetric reaction were the concentration of both R_1 (MO) and R_2 (CP^+ chloride), the flow rates of R_1, R_2 and carrier (water), and the volume of sample.

A half-fractioned 2^{6-1} type IV resolution design allowing three degrees of freedom and involving 32 randomized runs plus three centered points [23] was built for a screening study of these variables.

The conclusions are that R_1 and carrier flow rates were not influential factors in the ranges under study. Better signals were obtained with the highest R_1 flow rate and with the lowest carrier flow rate tested. However, when low carrier flow rates were used wide peaks were obtained. Thus, the highest value tested for both R_1 and carrier flow rates (2.5 ml/min in both cases) were selected for further experiments. The volume of sample was also a non-influential factor and the lowest value tested (100 μl) was selected for subsequent experiments. The other variables, namely R_1 and R_2 concentrations and R_2 flow rate, were influential factors. Higher values were tested using a two-level full factor design involving eight randomized runs plus three centered points. In this case, R_2 flow rate was not influential factor and the lowest value tested (4.5 ml/min) was selected. Analyzing the

Table 1
Optimization of the method

Step	Variables	Tested range	Optimum value
Derivatization	R_1 concentration (M)	0.5×10^{-4} to 8.75×10^{-4}	6.75×10^{-4}
	R_2 concentration (M)	2.5×10^{-5} to 22.5×10^{-5}	22.25×10^{-5}
	R_1 flow rate (ml/min)	0.5–2.5	2.5
	R_2 flow rate (ml/min)	0.5–4.5	4.5
	Carrier flow rate (ml/min)	0.5–2.5	2.5
	Sample volume (μl)	100–300	100
Preconcentration	Retention flow rate (ml/min)	0.5–4.5	3.5
	Elution flow rate (ml/min)	0.3–1	0.5
	Eluent volume	0.5–2.5 ml	2
	Breakthrough	15–150 ml	75
Leaching	Irradiation power (W)	200–400	200
	Irradiation time (min)	20–200	200
	Delay time (min)	0–120	120
	Number of cycles	1–9	9

Table 2
Study of the sorbent material and eluent

Recovery (%)		C_{18}	C_{si}	C_{18}-Hydra
Water:methanol	3:1 (v/v)	20	19	67
	1:1 (v/v)	36	25	89
	1:3 (v/v)	35	20	87
Water:acetonitrile	3:1 (v/v)	25	27	43
	1:1 (v/v)	46	48	66
	1:3 (v/v)	41	50	67
Water:acetone	3:1 (v/v)	22	30	55
	1:1 (v/v)	35	53	70
	1:3 (v/v)	32	45	66

design for both R_1 and R_2 concentrations, which were the key variables, a second-order polynomial equation was obtained. The optimal values, 6.75×10^{-4} for R_1 concentration and 22.25×10^{-5} for R_2 concentration, were obtained by equalizing to zero the first derivatives of the polynomial, solving the resulting equation systems, and decoding the results.

3.3. Optimization of the on-line preconcentration

The selection of the sorbent material (C_{18}-Hydra) and the eluent (1:1 (v/v) water:methanol solution) was based on the results from a study where three types of sorbent (C_{18}-Hydra, C_{18} and C_{si}) and different water mixtures of methanol, acetonitrile, and acetone were investigated (Table 2). Then, the retention and elution flow rate and the volume of eluent were optimized as in reference [16]. The ranges tested and the results obtained are summarized in Table 1. Finally, sample volumes between 15 and 150 ml, which contained 5 μg of each analyte, were passed through the mini-column. The signal remained constant up to 75 ml and decreased for higher volumes, so 75 ml was the breakthrough volume.

3.4. Optimization of the FMASE

The variables optimized were the irradiation power, the irradiation time, the number of cycles and the delay time (interval during which the sample is in contact with the hot solvent after microwave irradiation and before draining from the irradiation vessel).

A screening study of the behavior of the main variables affecting the extraction efficiency was performed using a half-fractioned 2^{4-1} type IV resolution design allowing three degrees of freedom and involving eight randomized runs plus three centered points.

The conclusions were that the irradiation power was not an influential factor; thus, the lowest value tested (200 W) was selected for subsequent experiments. The irradiation time, the number of cycles and the delay time were influential factors. Higher values were tested using a full two-level factorial design involving an overall of $2^3 = 8$ experiments, in addition to three centered points. The study showed that the irradiation time and the delay time were not influential factors in the new range studied. However, the results showed better recoveries with the highest values tested. Thus, 200 s of irradiation time and 120 s of delay time were selected. The number of cycles is the key variable of the extraction.

To determine the number of cycles necessary for quantitative recovery of the target compounds from sediment spiked with 5, 2.5 and 1.5 μg/g of each analyte, a study of the extraction kinetics was performed. In all cases, five extractions with different number of cycles ranging from 4 to 13 were performed. The other variables (power of irradiation, irradiation time and delay time) were fixed at their optimum values (200 W, 200 s and 120 s, respectively). Recovery of the analytes higher than 90% was obtained in nine cycles that required a total time of 90 min (10 min per cycle).

3.5. Performance data

After checking that the absorption maximum for all three analytes was at the same wavelength (465 nm) and their similar molar absorptivity (≈0.2 l/mol cm), a calibration graph was run for calculation of the extraction recovery. The linear dynamic range was 0.01–2.5 μg/ml (for each individual compound).

The relative detection limit ($x_{L(k=3)} = 0.35$ μg/ml), was calculated by the equation $x_L = ks_{bl}/S$, where k is a constant, S the sensitivity of the analytical method corresponding to the slope of the calibration line, and s_{bl} the standard deviation of the blank responses obtained from the analyses of 10 sediment blanks. Taking into account the amount of sample (4 g) and the volume of the extract (50 ml), this corresponds to a detection limit in the sediment of 4.4 μg/g.

The precision of the proposed screening approach was evaluated with two measurements of each analyte per day during 7 days [24]. In all experiments 4 g of sediment containing 5 μg/g of each analyte were used under the optimum working conditions. The repeatability and within-laboratory reproducibility, expressed as relative standard deviation, were 4.3 and 5.8%, respectively.

3.6. FMASE versus conventional Soxhlet extraction

As certified reference material was not commercially available, the optimized proposed approach was validated by comparing with conventional Soxhlet extraction.

Water and methanol were used in the conventional 12 and 24 h Soxhlet extractions. Water was used in order to compare its feasibility in conventional Soxhlet extraction compared with the good results obtained in FMASE. Methanol was also used, as it is the solvent commonly employed for LAS extraction. The recoveries, expressed as an average of three extractions, were 73.5 and 80.6% for water and 78.3 and 85.4% for methanol. As can be seen, the recoveries obtained were lower than that provided by the proposed approach with a within-laboratory reproducibility expressed as percentage

Table 3
Chromatographic results from the extracts obtained by the proposed approach and conventional Soxhlet

Analyte	FMASE	Conventional Soxhlet (water, 12 h)	Conventional Soxhlet (water, 24 h)	Conventional Soxhlet (methanol, 12 h)	Conventional Soxhlet (methanol, 24 h)
SDS	95	74	86	76	89
STS	98	70	86	79	87
SOS	102	72	79	70	85

Recoveries are expressed as percent.

of relative standard deviation (R.S.D.) of 4.8 and 5.4 and 5.8 and 4.2 for the extractions performed using water and methanol, respectively.

HPLC-fluorimetric quantification of extracts obtained using the proposed approach and also the conventional Soxhlet extractions was performed in order to quantify individually the target analytes. As can been seen in Table 3, the results obtained using the chromatographic analysis are similar to those obtained by the proposed approach showing its suitability as semi-quantitative method. It can also confirm that better recoveries are obtained by the FMASE method as compared with the conventional one.

4. Conclusions

A fully automated screening method for LAS in sediment samples has been developed. The proposed approach is based on the used of a focused microwave-assisted Soxhlet extractor coupled to a preconcentration/derivatization/detection FI system. The use of water as extractant has provided better efficiencies than conventional Soxhlet (with both water and methanol) but with drastic reduction of time ($\approx$2 h versus >24 h). Methyl orange, a widely available and cheap acid–base indicator, has been used as the chromogenic reagent jointly with the pairing cationic surfactant cetyl pyridine chloride, for the FI spectrophotometric determination of the target compounds. The advantages of the proposed detection method are enough sensitivity for the screening of the target analytes, fast sample-throughput rate, low analytical cost, simplicity for operation and exclusion of any toxic organic solvent.

Acknowledgements

Spain's Comisión Interministerial de Ciencia y Tecnología (CICyT) is gratefully acknowledged for financial support (project BQU-2003-01333).

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