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Short communication

High-performance liquid chromatographic analysis of a linear alkylbenzenesulfonate and its environmental biodegradation metabolites

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Abstract

A simple and fast method is described for determining a linear alkylbenzenesulfonate (LAS) and its potential sulfonated and unsulfonated metabolites in natural waters. This method includes extraction of 60 ml of water with an octadecyl-bonded silica (C_{18}) mini-column and analysis of the extract by high-performance liquid chromatography. A reversed-phase column with a 0.008 *M* potassium phosphate buffer (pH 2.2)–acetonitrile gradient as the mobile phase provides the separation. A UV detector, set at 215 nm, is employed.

Keywords: Environmental analysis; Alkylbenzenesulfonates; Surfactants

1. Introduction

Linear alkylbenzenesulfonates (LASs) are currently the most used anionic surfactants after soaps [1]. Their toxicity towards organisms has been proven [2,3] and abundant release in an aquatic environment induces severe ecological problems. LAS biodegradability nevertheless decreases its toxic effects. The first step of biodegradation is the oxidation of the terminal part of the alkyl chain and this is followed by β-oxidation [4], with formation of *p*-sulfophenylcarboxylic acids (SPCs). Desulfonation may produce hydroxyphenylcarboxylic acids or phenylcarboxylic acids [5,6]. At the end of the biodegradation process, the molecules are mineralized by bacteria [7]. The standard methylene blue method is commonly used for quantitative measurement of LASs [8,9], but this method is not suitable for the detection of metabolites. Procedures have been described with nuclear magnetic resonance or gas chromatography–mass spectrometry (NMR, GC–MS) [10,11] for identifying biodegradation products. These procedures require experience. Most frequently, derivatization of investigated molecules is needed. High-performance liquid chromatography (HPLC) coupled with UV or fluorometric detection is now employed in many laboratories. It can be used for the measurement of undegraded LASs [12,13] or for detection of SPCs [14–17]. Injection without sample pretreatment can only be used for relative clean aqueous samples [18]. Generally, an extraction step and concentration are necessary before analysis, and different methods have been developed for this purpose, based on solid-phase extraction with various sorbents, such as anion exchangers [11], graphitized carbon black [14], octadecyl-bonded silica [16]

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or XAD-4 [17]. They provide good results but are time-consuming.

The aim of this work is to propose an improved technique for rapid and sensitive HPLC identification of LASs and their biodegradation products, including unsulfonated metabolites (Fig. 1). This technique is divided into two steps: extraction (including purification and concentration) of the aqueous sample with a C_{18} mini-column cartridge and determination of investigated compounds by reversed-phase HPLC with UV detection.

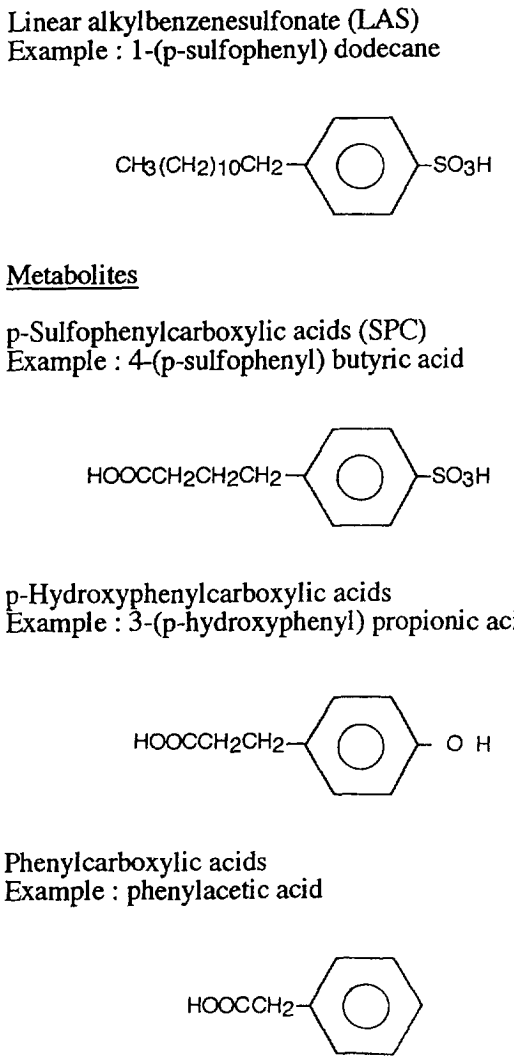

Fig. 1. Structure of LAS and its potential sulfonated and unsulfonated metabolites. Compounds studied in this work were LAS (1-(*p*-sulfophenyl)dodecane), SPCs including *p*-sulfophenylacetic acid, 3-(*p*-sulfophenyl)propionic acid, 4-(*p*-sulfophenyl)butyric acid, 5-(*p*-sulfophenyl)valeric acid, 6-(*p*-sulfophenyl)hexanoic acid, 7-(*p*-sulfophenyl)heptanoic acid, *p*-hydroxyphenylcarboxylic acids: *p*-hydroxyphenylacetic acid, 3-(*p*-hydroxyphenyl)propionic acid and the phenylcarboxylic acids: phenylacetic acid, 3-phenylpropionic acid, 4-phenylbutyric acid and 5-phenylvaleric acid.

2. Experimental

2.1. Materials

Bond Elut C_{18} mini-columns (500 mg of sorbent) were purchased from Varian (Harbor City, CA, USA). All solvents were of HPLC grade and were provided by SDS (Peypin, France). Glass double-distilled water was used throughout. The salts and acids used were of analytical-reagent grade and were purchased from Prolabo (Paris, France). The reference molecules, namely *p*-hydroxyphenylcarboxylic acids and phenylcarboxylic acids, were obtained from Acros Organics (Noisy Le Grand, France), Sigma-Aldrich (Saint Quentin Fallavier, France) and Interchim (Montluçon, France). The commercial detergents are mixtures of LASs with different alkyl homologues (C_{10}–C_{14}) and phenyl positional isomers. The LAS taken as a reference in this study was a pure isomer: 1-(*p*-sulfophenyl)dodecane. It was synthesized by sulfonation of 1-phenyldodecane (supplied by Acros Organics) using a standard procedure [19].

SPCs are unavailable from chemical product suppliers and must be prepared by sulfonation of phenylcarboxylic acids. The common technique takes a long time [15,17] and we have developed a faster technique involving the isolation of SPCs in the form of calcium salts that are soluble in water, as follows: a 1-g amount of phenylcarboxylic acid and 2 ml of H_2SO_4 (95%) were mixed at 100°C for 2 h. The mixture was diluted with 80 ml of water and washed with 3×30 ml of diethyl ether, to remove unsulfonated compounds. The aqueous phase was progressively neutralized with 6 g of $CaCO_3$ and filtered to eliminate the $CaSO_4$ that was formed. The recovered liquid was evaporated and the dry residue (SPC salt) was recrystallized in boiling water.

The structure of synthesized compounds was

confirmed by IR, UV and ^{1}H, ^{13}C NMR spectra. The purity of sulfonated molecules was checked by acidometry. Reaction yields and purities are reported in Table 1.

2.2. Sample preparation

The C_{18} mini-column cartridge was rinsed just prior to use with 10 ml of acetone followed by 10 ml of water. An aqueous sample (60 ml) was fortified with 5 *M* NaCl and 0.05 *M* KH_2PO_4 and the pH was adjusted to 1.5 with concentrated H_3PO_4. The aqueous solution was passed through the mini-column at a flow-rate of 3 ml/min. The temperature must not exceed 25°C if satisfactory retention of the investigated molecules on the C_{18} stationary phase is to be obtained. After rinsing the column bed with 1 ml of water (to remove salts and impurities), the absorbed compounds were eluted with 5 ml of acetone. The eluted solution was maintained at 0°C in an ice bath and gently evaporated to dryness using a stream of nitrogen. The dry residue was collected with 1 ml of a methanol–water (1:1, v/v) mixture, which can dissolve a large range of molecules.

2.3. Apparatus and conditions

The concentrated extract was injected into a high-pressure chromatograph, Varian Model 5000 (Walnut Creek, CA, USA), equipped with a 10-μl loop, a variable-wavelength UV detector (Varian UV-100), which was set at 215 nm, with a 4.5-μl flow cell and a recorder (Varian Model 9176-01). The chromatographic column was a LiChrospher 100 RP-18 (250×4 mm I.D., 5 μm particle size) from Merck (Darmstadt, Germany), which was connected to a 25×4 mm guard-column containing the same coating. HPLC was performed at room temperature (21°C) with a flow-rate of 0.8 ml/min. A linear gradient program was used with acetonitrile (A) and 0.008 *M* phosphate buffer, pH 2.2 (B). The latter was prepared by dissolving 0.008 *M* KH_2PO_4 in water and adjusting the pH to 2.2 with concentrated H_3PO_4. Eluents were degassed and filtered through a 0.45-μm glass filter, prior to use.

The starting conditions were 5% A–95% B and the amount of A was increased to 65% over 60 min. Before each run, it was necessary to purge the column with water, in order to dissolve possible precipitates of KH_2PO_4 and to decrease the pressure.

Table 1
Reaction yields and the purity of synthesized molecules

Name	Yield (%)	Purity (%)
LAS		
1-(*p*-Sulfophenyl)dodecane	60	96
Sulfonated metabolites		
p-Sulfophenylacetic acid	80	62
3-(*p*-Sulfophenyl)propionic acid	75	82
4-(*p*-Sulfophenyl)butyric acid	N.D.[a]	81
5-(*p*-Sulfophenyl)valeric acid	57	76
6-(*p*-Sulfophenyl)hexanoic acid	84	83
7-(*p*-Sulfophenyl)heptanoic acid	50	75

[a]Not determined.

2.4. Quantitation

On the day of analysis, external calibration curves were made for each of the compounds studied. Working standard solutions (10–100 mg/l) of LAS and metabolites were prepared in methanol–water (1:1, v/v).

3. Results and discussion

3.1. Separation

The mobile phase modifiers used for the analysis of LASs (and SPCs) by reversed-phase HPLC are generally trifluoroacetic acid (TFA), tetrabutylammonium dihydrogenphosphate (TBA–H_2PO_4), $NaClO_4$ [14,16] or cetyltrimethylammonium ($CTMA^+$) ions [17]. Complex elution programs are then needed to obtain satisfactory separations. High $NaClO_4$ concentrations can shorten the column life.

Good results are provided when the described elution gradient is used with 0.008 *M* phosphate buffer and acetonitrile. The low molarity of the phosphate buffer increases the column's life and only requires very short re-equilibration times between each injection. The acidity of the mobile phase (pH 2.2) is sufficient to avoid ionization of analysed molecules and to efficiently increase the retention capacity of the column. An increase in the flow-rate

or the gradient decrease the analysis time but produce negative effects on peak selectivity. The choice of 254 nm as the detection wavelength is frequently made when HPLC–UV detection is used. However, LASs, SPCs and *p*-hydroxyphenylcarboxylic acids display maximum UV absorption at 222 nm, while absorption at 254 nm is weak. Phenylcarboxylic acids (with an UV maximum of 206 nm) may not be observed at 222 nm. A wavelength of 215 nm appears to be a good compromise for each family of studied compounds and the good transparency of the mobile phase permits work to be performed at this wavelength.

With the optimized chromatographic conditions, the number of theoretical plates is important (30 000 for 3-phenylpropionic acid). All of the metabolites are more polar than the LASs, which are eluted last (Table 2).

The separation of LAS and SPCs is illustrated in Fig. 2. This chromatogram clearly shows the increase in retention time due to the length of the SPC side-chain. The small peaks could correspond to the meta- and ortho-sulfonic acids. Fig. 3 shows the analysis of the LAS and its potential unsulfonated metabolites. Like SPC, the retention time increases with the number of carbon atoms in the alkyl carboxylic side-chain. For groups with side-chains of the same length, the elution order is as follows: *p*-sulfophenylcarboxylic acid<*p*-hydroxyphenylcarboxylic acid<phenylcarboxylic acid.

Under these chromatographic conditions, there is a linear relationship between the retention time (t_R, and not log t_R) and the number of carbons (n) in the carboxylic chain within the same family of compounds: *p*-sulfophenylcarboxylic acids, $t_R = 3.78n - 1.46$, with a correlation coefficient (r) of 0.9998; phenylcarboxylic acids, $t_R = 5.58n + 18.3$, with $r = 0.9964$.

A chromatogram of a mixture of SPCs and unsulfonated metabolites is presented in Fig. 4. All of the molecules are separated and SPCs do not overlap with *p*-hydroxyphenylcarboxylic acids.

3.2. Recovery test and sample preparation

Determination of the rate of recovery was performed by spiking natural aqueous samples (river or pond water that was not polluted by detergent) with 1 ml of a standard solution containing LAS and metabolites. The spiked samples were treated by

Table 2
Identification of peaks, their retention times, concentrations in spiked aqueous samples and recoveries of LAS and metabolites

Compound	Peak number	Retention time (min)	Concentration (mg/l)	Recovery[a] (%)
LAS				
1-(*p*-Sulfophenyl)dodecane	13	57.2	0.9–1.0	81±3
Sulfonated metabolites				
p-Sulfophenylacetic acid	1	6.3	0.5–0.6	29±7[b]
3-(*p*-Sulfophenyl)propionic acid	2	9.7	0.9–1.0	92±5
4-(*p*-Sulfophenyl)butyric acid	3	13.6	0.4–0.7	96±2
5-(*p*-Sulfophenyl)valeric acid	4	17.4	0.4–0.9	96±3
6-(*p*-Sulfophenyl)hexanoic acid	6	21.2	0.5–0.8	96±3
7-(*p*-Sulfophenyl)heptanoic acid	8	25.1	0.4–0.7	96±4
Unsulfonated metabolites				
p-Hydroxyphenylacetic acid	5	17.9	0.8–0.9	88±2
3-(*p*-Hydroxyphenyl)propionic acid	7	21.9	0.8–0.9	90±1
Phenylacetic acid	9	28.9	1.0–1.1	82±2
3-Phenylpropionic acid	10	35.9	1.4–1.6	83±1
4-Phenylbutyric acid	11	40.4	0.9–1.0	87±1
5-Phenylvaleric acid	12	46.0	0.9–1.1	85±1

[a] Mean±S.D.of three determinations.
[b] The small degree of recovery is a consequence of the high polarity of this compound.

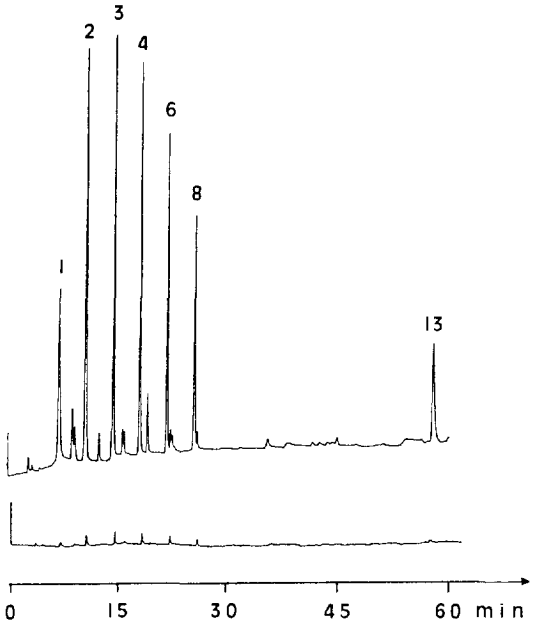

Fig. 2. Chromatogram of 1-(*p*-sulfophenyl)dodecane (LAS) and its sulfonated metabolites, namely the *p*-sulfophenylcarboxylic acids (SPCs), which were spiked in a sample of natural water. Column, LiChrospher 100 RP-18, 250×4 mm I.D. Eluent A, acetonitrile; eluent B, 0.008 *M* potassium phosphate buffer (pH 2.2). Gradient, linear from 5 to 65% A in 60 min. Flow-rate, 0.8 ml/min. Temperature, 21°C. Detection, UV at 215 nm, 0.1 AUFS. Volume injected, 10 μl, corresponding to 0.25-0.60 μg of each molecule that was investigated. Identification of peaks and their concentrations are shown in Table 2. The lower chromatogram is recorded at 254 nm.

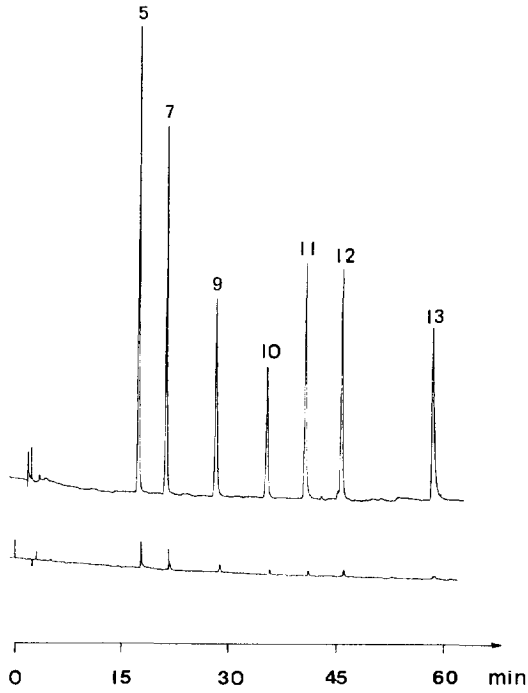

Fig. 3. Chromatogram of the separation of 1-(*p*-sulfophenyl)dodecane (LAS) and unsulfonated metabolites in a spiked aqueous sample. Detection, UV at 215 nm, 0.1 AUFS. Volume injected, 10 μl, corresponding to 0.45–0.95 μg of each compound. Identification of peaks and their concentrations are shown in Table 2. The separation conditions are the same as in Fig. 2. The lower chromatogram is recorded at 254 nm.

extraction with a C_{18} mini-column and were analysed by HPLC. The results are summarized in Table 2.

The sample preparation technique is derived from existing methods [16], but some improvements have been made to obtain better yields. The aqueous samples were not filtered because surfactants are strongly attached by particulate matters and their elimination diminishes the recovery of LASs. In the absence of filtration, a pump is necessary for percolation through the mini-column. Because the most polar metabolites are difficult to retain on a C_{18} stationary phase, the ionic strength of the aqueous phase must be increased by the addition of NaCl. This is illustrated in Fig. 5. The increase in the concentration of NaCl has a very significant effect on *p*-sulfophenylacetic acid and 3-(*p*-sulfophenyl)propionic acid recovery, while 4-(*p*-sulfophenyl)butyric acid recovery is less improved. The concentration of NaCl has no influence on the other metabolites or on the LAS. To prevent molecular ionization, the pH value is adjusted to 1.5. At temperatures higher than 25°C, the C_{18} cartridges fail to retain the most polar compounds and it is necessary to cool the aqueous phase in an ice bath to obtain suitable yields. However, at a normal temperature (20°C), no problems are observed and recovery yields are satisfactory. The eluate is evaporated to dryness at 0°C in order to avoid the loss of volatile phenylcarboxylic acids.

3.3. Sensitivity

The limit of detection was calculated using a

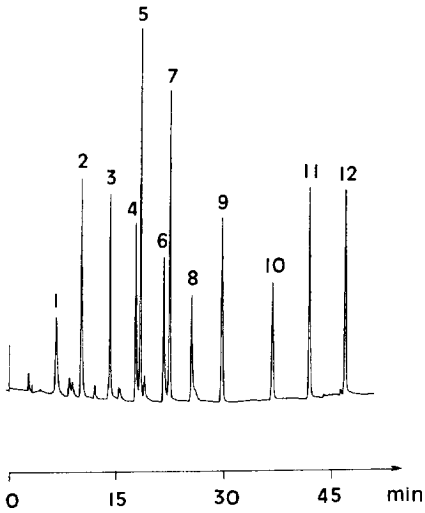

Fig. 4. Chromatogram of a mixture of sulfonated (SPC) and unsulfonated metabolites of LAS. Detection, UV at 215 nm, 0.1 AUFS. Volume injected, 10 μl, corresponding to 0.10–0.40 μg of each metabolite. Identification of peaks and their concentrations are shown in Table 2. The separation conditions are the same as in Fig. 2.

signal-to-noise ratio of three. In the case of spiked waters, the sensitivity was found to be around 0.02 mg/l for *p*-sulfophenylcarboxylic acids and for *p*-hydroxyphenylcarboxylic acids, around 0.07 mg/l

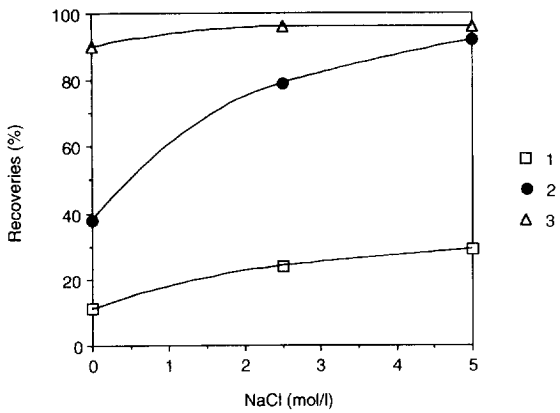

Fig. 5. Influence of the concentration of NaCl on the recovery of *p*-sulfophenylacetic acid (1), 3-(*p*-sulfophenyl)propionic acid (2) and 4-(*p*-sulfophenyl)butyric acid (3).

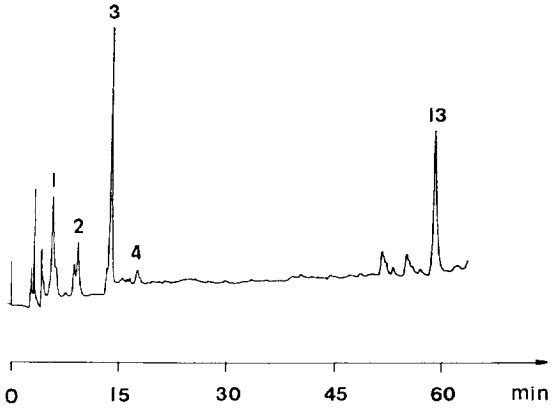

Fig. 6. Analysis of a sample of pond water containing LAS and metabolites produced by its biodegradation. Detection, UV at 215 nm, 0.05 AUFS. Volume injected, 10 μl, corresponding to 1.4 μg of LAS and 0.6 μg of SPCs. The identity of the peaks is given in Table 2. The separation conditions are the same as in Fig. 2.

for phenylcarboxylic acids and around 0.05 mg/l for LAS.

3.4. Application to a laboratory experiment

A sample of water was collected from a pond (étang de Bolmon, France). No metabolites were detected and the concentration of LAS was below 0.2 mg/l. 1-(*p*-Sulfophenyl)dodecane (15 mg) was added and the sample was maintained under aerobic conditions in a batch reactor (21°C). After four days, samples of the pond water were analysed in duplicate using the above procedure. Fig. 6 shows remaining LAS (2.3±0.2 mg/l) and SPCs formed by its biodegradation (1.0±0.2 mg/l). 4-(*p*-Sulfophenyl)butyric acid was shown to be the most important metabolite. No unsulfonated metabolites were observed in this experiment.

4. Conclusion

This chromatographic technique is simple, sensitive and reliable for rapid quantitative estimations of LAS and its metabolites in aqueous samples and therefore, it seems suitable for use in most lab-

oratories for studying the fate of LASs in polluted environments.

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