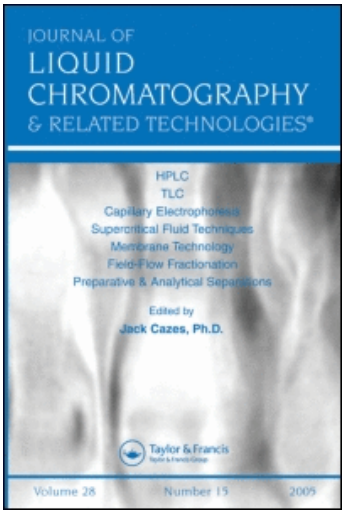


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# DETERMINATION OF A LINEAR ALKYLBENZENESULFONATE AND ITS BIODEGRADATION INTERMEDIATES IN SEA WATER USING SOLID-PHASE EXTRACTION AND RP-HPLC WITH UV DETECTION

L. Sarrazin[a]; W. Wafo[a]; P. Rebouillon[a]
[a] Laboratoire d'Hydrologie et Molysmologie aquatique, Marseille, Cedex 5, France

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# DETERMINATION OF A LINEAR ALKYLBENZENESULFONATE AND ITS BIODEGRADATION INTERMEDIATES IN SEA WATER USING SOLID-PHASE EXTRACTION AND RP-HPLC WITH UV DETECTION

L. Sarrazin,* W. Wafo, P. Rebouillon

Laboratoire d'Hydrologie et Molysmologie aquatique
Faculté de Pharmacie
13385 Marseille Cedex 5, France



## ABSTRACT

A rapid and selective method for determining a linear alkylbenzenesulfonate (LAS) and its biodegradation metabolites in sea water samples using solid-phase extraction and reversed-phase high performance liquid chromatography with UV detection is proposed. A solid-phase extraction (SPE) procedure is described for concentrating the compounds of interest on Mega Bond Elut C18 mini-column cartridges (2 g of adsorbent). Factors affecting the recovery of the investigated molecules such as conditioning of the SPE cartridge, flow-rate of the sample loaded on the cartridge, volume and pH of the sample, addition of salt to the sample, and elution of the analytes from the SPE cartridge are commented. Most of the tested compounds were extracted at recovery rates of > 70%. The obtained eluates were analyzed on a LiChrospher 100 RP-18 column and a mobile phase gradient of acetonitrile-phosphate buffer (pH=2.2) was used to separate the metabolites. The determination of LAS was made isocratically. Linear calibration curves showed good linearity for each molecule with correlation coefficients greater than 0.998. This method was applied to evaluate the LAS biodegradability in sea water during a laboratory experiment.

## INTRODUCTION

Linear alkylbenzenesulfonates (LASs) presently constitute a major group of detergent compounds in domestic and industrial usage.[1] Due to their high-volume use in consumer products, LASs have the potential for broadscale release into the aquatic environment and may be harmful towards organisms.[2-4] LAS biodegradability has been demonstrated and decreases the toxic effects of this class of surfactants.[5] Primary biodegradation is said to have occurred when the original molecule of LAS has its structure altered by bacterial action.

LAS biodegradation intermediates (Figure 1) are sulfophenylcarboxylic acids (SPCs) that are produced by ω-oxidation of the alkyl chain terminal carbon followed by successive β-oxidation.[6] Desulfonation of the benzene ring may generate hydroxyphenylcarboxylic acids, dihydroxyphenylcarboxylic acids and phenylcarboxylic acids.[7-9] Ultimately, the molecules are converted to components such as $CO_2$, water and inorganic salts (mineralization).[10,11]



The degradation pathway of LASs has been mainly studied with biodegradability tests conducted on river waters or activated sludges.[12] They are carried out with addition of bacteria from waste water at high densities, which are then particularly adapted. In the marine environment, little has been done on the biodegradation of LASs in the sea.[13] The belief in the sea's great powers of dilution is one of the reasons for this. In fact, the detection of LASs and their degradation metabolites in sea water is a complex problem due to the presence of salts and numerous organic substances that may interfere with the analysis of the samples and the development of a suitable analytical method would be necessary.

The most widely accepted method for the quantitative determination of anionic surfactants in aqueous sample is the standard methylene blue method (MBAS).[14] This traditional colorimetric technique is least affected by sea water, is able to record alteration of LASs[15] (primary biodegradation), but is not selective and can not detect their biotranformation products.

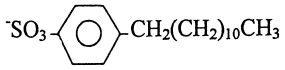
At the present time, the analytical procedures most often used for the measurement of undegraded LASs or for determination of metabolites generally include :

a) Isolation and concentration of investigated molecules from water sample by solid-phase extraction (SPE) with various sorbents such as anion exchanger,[16] graphitized carbon black[17] or octadecyl-bonded silica.[18,19]

b) Identification by gas chromatography-mass spectrometry (GC-MS)[20] which requires derivatization techniques (LASs and SPCs are not sufficiently volatile to permit direct injection by GC techniques)

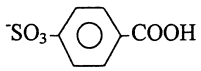
**A Linear Alkylbenzene Sulfonate (LAS)**

1-(*p*-Sulfophenyl)dodecane

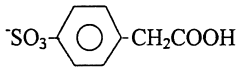


**Sulfophenylcarboxylic acids**

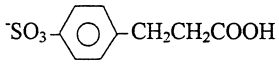
*p*-Sulfobenzoic acid



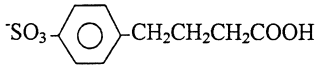
*p*-Sulfophenylacetic acid



3-(*p*-Sulfophenyl)propionic acid

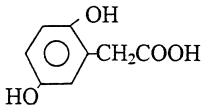


4-(*p*-Sulfophenyl)butyric acid

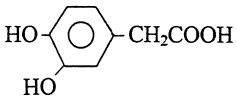


**Dihydroxyphenylcarboxylic acids**

2,5-Dihydroxyphenylacetic acid

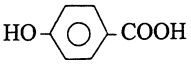


3,4-Dihydroxyphenylacetic acid

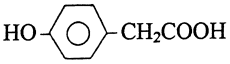


**Hydroxyphenylcarboxylic acids**

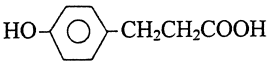
*p*-Hydroxybenzoic acid



*p*-Hydroxyphenylacetic acid

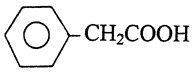


3-(*p*-Hydroxyphenyl)propionic acid



**Phenylcarboxylic acids**

Phenylacetic acid



3-Phenylpropionic acid

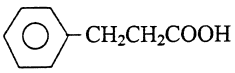


**Figure 1**. Structure of the LAS used in this study and its potential biodegradation metabolites.

or analysis by reversed-phase high performance liquid chromatography (RP-HPLC) with spectrophotometric (UV) detection.[21-23.] Because of its rapidity and its reliability the combination SPE with RP-HPLC is attractive.

In a previous paper we reported a chromatographic method for determination of a linear alkylbenzenesulfonate and its environmental biodegradation intermediates in natural aqueous samples (river and pond water).[19] This technique could be improved for the most polar metabolites which were just partially detected. In the present study, we have developed a RP-HPLC method for determining LAS and all the potential metabolites, including the most polar such as dihydroxyphenylacetic acids, in sea water using solid-phase extraction. The method is applied to follow the LAS degradation in sea water during a laboratory experiment.

## EXPERIMENTAL

### Reagents and Materials

Acetonitrile, acetone, and methanol HPLC-grade were supplied by SDS (Peypin, France). The water used was deionized and filtered through a Milli-Q™ water purification system (Millipore, New Bedford, MA, USA). HCl (2 *M*) was prepared from concentrated hydrochloric acid from Prolabo (Paris, France). Analytical-grade sodium chloride, potassium dihydrogen phosphate, and orthophosphoric acid were provided by Carlo Erba (Milan, Italy). The standard compounds, namely hydroxyphenylcarboxylic acids, dihydroxyphenyl-carboxylic acids and phenylcarboxylic acids were purchased from Acros Organic (Noisy Le Grand, France) and Interchim (Montluçon, France).

Except for the *p*-sulfobenzoic acid which was provided by Acros Organics, the SPCs are not commercially available. They must be synthesized by sulfonation of phenylcarboxylic acids according to conditions reported previously.[19] The *p*-sulfophenylacetic acid, 3-(*p*-sulfophenyl)propionic acid and the 4-(*p*-sulfophenyl)butyric acid were synthesized and characterized by IR, UV, and $^{1}H$, $^{13}C$ NMR spectra. Commercial LAS materials are complex mixtures of various alkyl homologues that may vary from C9 to C13 and phenyl positional isomers. In this work, we have examined a single linear alkylbenzenesulfonate, the 1-(*p*-sulfophenyl)dodecane, which was synthesized by direct sulfonation of n-dodecylbenzene (Acros Organics) using a common procedure.[24]

The extraction of studied compounds from sea water was accomplished by employing Mega Bond Elut C18 mini-column cartridges containing 2 g of adsorbent (Harbor City, CA, USA).

## Instruments

An analytical LiChrospher 100 RP-18, 250x4 mm I.D, 5 μm column and a 25x4 mm precolumn with the same coating, both from Merck (Darmstadt, Germany), were used. HPLC analysis was performed on a Varian Model 5000 (Walnut Creek, CA, USA) equipped with a Rheodyne (Cotati, CA, USA) sample 10-μL loop injector. A variable-wavelength UV detector (Varian UV-100) set at 215 nm and a Waters 746 integrator (Waters, Milford, MA, USA) were also used.

## HPLC Analysis

HPLC was done at room temperature (*ca.* 21°C) and the filtered (0.2 μm), degassed mobile phase was maintained at a flow-rate of 0.8 mL/min. The mobile phase was acetonitrile and a 8 m*M* $KH_2PO_4$ buffer adjusted at pH=2.2 with $H_3PO_4$. Gradient elution was carried out for separating the metabolites. The initial mobile phase composition was 5% acetonitrile and 95% phosphate buffer. The acetonitrile concentration was raised linearly to 40% within 35 min. The analysis of LAS was made isocratically with 60 % acetonitrile and 40 % phosphate buffer.

## Calibration

A stock solution was prepared in methanol-water (1:1, v/v) with the concentration of each compound studied between 20 and 70 mg/L. Working standard solutions which are 8, 6, 4, and 2 times diluted were generated by dilution of the stock solution. All these solutions were stored in the dark at 4°C, to prevent an eventual photochemical degradation of hydroxyphenyl and dihydroxyphenyl acids. On the day of analysis, external calibration curves were constructed for each investigated molecule by injection of the four working standard solutions. The integrated peak areas were used to quantitate the LAS and its metabolites.

## Sample Extraction and Concentration

Before extraction, the aqueous samples were vigorously shaken to ensure adequate mixing and suspension of particulate material. For recovery determinations, samples of sea water that were not polluted by LASs, were spiked by the addition of known volumes (0.5-2 mL) of a standard solution. The cartridges were conditioned with 5 mL of acetone and 5 mL of water prior to use. Marine water samples (30-100 mL) were fortified with 30 % (w/w) NaCl and the pH value is adjusted to 1.0 by addition of 2 *M* HCl.

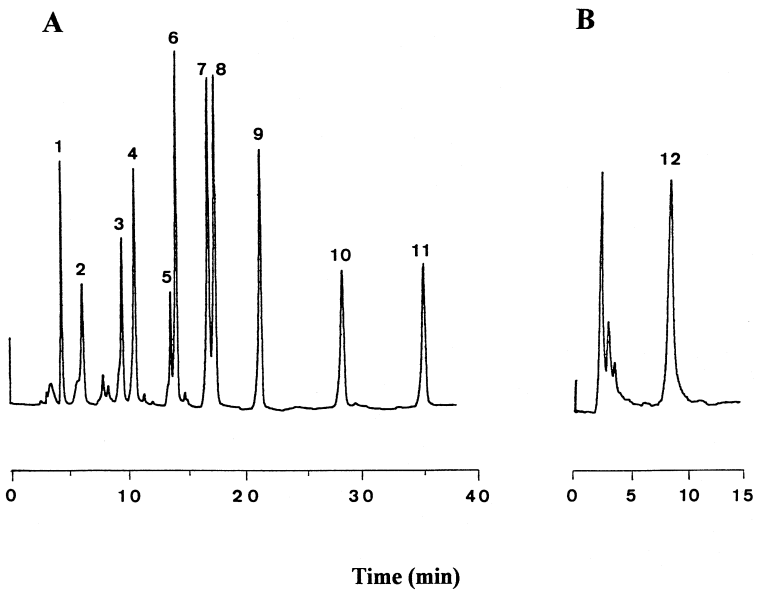



**Figure 2**. HPLC analyses of 1-(*p*-sulfophenyl)dodecane (LAS) and its potential sulfonated and unsulfonated metabolites in a spiked sample of sea water. Conditions : LiChrospher 100 RP-18 column (250 x 4 mm I.D., 5 µm). Volume injected, 10 µL. Temperature, 21°C. Detection, UV at 215 nm, 0.05 AUFS. Eluents: acetonitrile and a 8 m*M* potassium phosphate buffer adjusted at pH 2.2 with $H_3PO_4$. Flow-rate, 0.8 mL/min. Chromatogram A: gradient, linear from 5 to 40% of acetonitrile in 35 min. Chromatogram B: isocratic, 60 % of acetonitrile. Peaks: 1 = *p*-sulfobenzoic acid; 2 = *p*-sulfophenylacetic acid; 3 = 3-(*p*-sulfophenyl)propionic acid; 4 = 2,5-dihydroxyphenylacetic acid; 5 = 4-(*p*-sulfophenyl)butyric acid; 6 = 3,4-dihydroxyphenylacetic acid; 7 = *p*-hydroxybenzoic acid; 8 = *p*-hydroxyphenylacetic acid; 9 = 3-(*p*-hydroxyphenyl)-propionic acid; 10 = phenylacetic acid; 11 = 3-phenylpropionic acid; 12 = 1-(*p*-sulfophenyl)dodecane.

The obtained solutions were percolated through the octadecylsilica cartridges at flow rates below 3 mL/min. After extraction, the columns were washed with 0.5 mL of water and the analytes were desorbed from the cartridges with 3 mL of methanol.

The methanol eluates were collected in 4 mL volumetric flasks and diluted to volume with water. These solutions were filtered with Millex-LCR13 0.5 µm filters from Millipore (Bedford, MA, USA) before analysis by RP-HPLC.

## RESULTS AND DISCUSSION

### Chromatography

High-performance liquid chromatography is recognized as the method of choice for the routine determination of ionic aromatic sulfonates, but published methods enable separations of only narrow ranges of compounds.[25] Using the optimized conditions described, the detection of a large spectrum of molecules is possible and we completely separate sulfophenylcarboxylic acids, dihydroxyphenylcarboxylic acids, hydroxyphenylcarboxylic acids, phenylcarboxylic acids and the linear alkylbenzene sulfonate (Figure 2). The reversed phase column with 8 m*M* phosphate buffer and acetonitrile as eluent allows separation with good peak shapes and stable baselines. The pH value of the buffer must be accurately adjusted to 2.2. Higher values may lead to an equilibrium of the free acid and its conjugated base, inducing peak tailing or strong reduction of retention times. On the other hand, pH values below 2.2 decrease the column life.



The flow-rate of the HPLC system was optimized for a good peak selectivity. Increase of flow-rate produced only a negligible gain in analysis time and diminishes the quality of the separation. The UV maxima of the LAS and its potential metabolites were obtained with a Hitachi Model U-2000 Double-Beam Spectrophotometer (Tokyo, Japan). The wavelength scanning operation was made within a range of 200 to 350 nm. Most of the investigated molecules have absorption maxima between 205 and 222 nm; the dihydroxyphenylcarboxylic acids absorb between 280 and 290 nm; *p*-sulfobenzoic acid and *p*-hydroxybenzoic acid have a maximum at 232 and 253 nm respectively (Table 1).

We chose to work at 215 nm for the routine analyses since all the studied molecules present a sufficient absorption and may be detected correctly at this wavelength. Furthermore, the calibration curves performed at 215 nm show good linearity and the correlation coefficients for each standard are over 0.998. Capacity factors are calculated for all compounds based on the retention times and the chromatograms show the elution order typical of reversed-phase systems, that is, polar components eluting before non-polar components. All of the biodegradation products are more polar than the LAS which is analyzed isocratically with a high percentage of acetonitrile in the mobile phase for decreasing the analysis time.

### Recoveries from SPE

As the content of LASs and metabolites in the samples of sea water may be low, preconcentration by solid-phase extraction is recommended. It is in the environmental field that this modern technique for preparing samples for HPLC

**Table 1**

**Capacity Factor, Linearity Data, and UV Maxima of 1-(*p*-Sulfophenyl)dodecane (LAS) and its Potential Metabolites**

| Number of Peak[a] | Capacity Factor[b] (k') | Range of Quantities Injected (μg) | Linearity Correlation Coefficient[c] (r) | UV Maxima[d] (nm) |
|---|---|---|---|---|
| 1 | 0.69 | 0.09-0.35 | 0.999 | 232 s, 260 w |
| 2 | 1.42 | 0.04-0.15 | 0.999 | 221 s, 260 w |
| 3 | 2.73 | 0.03-0.10 | 0.999 | 221 s, 260 w |
| 4 | 3.27 | 0.06-0.25 | 0.998 | 290 m |
| 5 | 4.23 | 0.03-0.10 | 0.999 | 221 s, 260 w |
| 6 | 4.54 | 0.04-0.15 | 0.998 | 280 m |
| 7 | 5.65 | 0.03-0.12 | 0.999 | 253 s |
| 8 | 5.88 | 0.05-0.20 | 0.999 | 222 s, 275 m |
| 9 | 7.42 | 0.04-0.15 | 0.999 | 222 s, 276 m |
| 10 | 10.12 | 0.07-0.27 | 0.999 | 208 s, 263 w |
| 11 | 12.80 | 0.05-0.20 | 0.999 | 206 s, 248 w |
| 12[e] | 2.23 | 0.05-0.25 | 0.999 | 222 s, 261 w |

[a] Refers to peak number in Figure 2. [b] k' = (tr-to)/to. [c] n = 4 and λ = 215 nm.
[d] s =strong absorption, m = middle, w = weak. [e] Analyzed isocratically.



analyses has revealed its potential. Indeed, aqueous samples taken from the environment and polluted by LASs are characterized by high complexity since a large variety of potential metabolites, with different polarities, may be present simultaneously, in addition to the matrix components.[25] In order to trap a maximum of these molecules C18 mini-column cartridges containing 2 g of adsorbent are necessary.

The first step of the reversed-phase SPE is the clean up of the cartridges with a water miscible solvent, e.g. acetone, to activate the octadecyl chains. Then the cartridges are conditioned with water. Sea water samples are adjusted to pH=1.0 and 30 % (w/w) of NaCl are added in order to obtain good recoveries of studied compounds. The addition of NaCl has been studied in a previous work.[19] Raising the salt concentration produced an increase of the ionic strength and improve the retention of the most polar metabolites on the mini-columns. The effects of pH on recovery rates are summarized in Table 2 using 30 mL of spiked sample. Low pH values avoid the ionization of molecules and provide quantitative recoveries for *p*-sulfobenzoic acid and *p*-sulfophenylacetic acid. Different values of flow-rate of samples can be found in the literature.[17,26]

**Table 2**

**Recoveries of Sulfophenylcarboxylic Acids at Different pH Values**

| Compounds | Conc'ns. (mg/L) | Recoveries[a] (%) pH 3.2 | pH 2.0 | pH 1.0 |
|---|---|---|---|---|
| *p*-Sulfobenzoic acid | 5.6-5.7 | 11 | 85 | 83 |
| *p*-Sulfophenylacetic acid | 2.4-2.5 | 48 | 99 | 99 |
| 3-(*p*-Sulfophenyl)propionic acid | 1.6-1.7 | 90 | 90 | 94 |
| 4-(*p*-sulfophenyl)butyric acid | 1.7-1.8 | 100 | 100 | 97 |

[a] Extraction from 30 mL of spiked sea water.



The manufacturers recommend 5-15 mL/min while some users apply 10-30 mL/min. With the highest possible flow-rate a reduced sample preparation time should be obtained. Nevertheless, at high flow-rates, non-equilibrium processes can lead to a loss of retention and we recommend flow-rates through the cartridges below 3 mL/min. The recovery yields obtained for these columns versus the sample volume passed through are shown in Table 3. A sample volume of 100 mL must be selected only to detect low concentration of analytes and a volume of 30 mL is more suitable because it gives quantitative recovery yields even for the *p*-sulfobenzoic acid. By comparing our results with those obtained by utilization of C18 mini-columns containing 500 mg of adsorbent, we observe an increase in percent extractions with an increase in the total mass of the solid phase (recovery yields with 500 mg of adsorbent and 30 mL of sea water : 10 % for *p*-sulfobenzoic acid, 22 % for *p*-sulfophenylacetic acid and 39% for 2,5-dihydroxyphenylacetic acid).

All the concentrated molecules should be eluted in as small a volume as possible. A small volume of eluate is necessary to achieve a correct enrichment factor. For the elution, 3 mL of methanol are necessary. In order to increase the concentration factor, some researchers evaporate the eluate to dryness and collect the dry residue in a very small volume of an appropriate solvent. This technique is not used because it may lead to the loss of the volatile phenylcarboxylic acids. Figure 2 shows the high quality of chromatograms using the experimental conditions detailed in the study.

These results show the usefulness of the SPE method for the analysis of LASs and their biodegradation products in sea water. Indeed, this treatment of samples allows the elimination of impurities and inorganic salts simultaneously. The limit of detection of the method is approximately 0.1 mg/L for each compound (signal to noise ratio of 4:1; 30 mL of sample).

**Table 3**

**Recoveries of LAS and Metabolites with Respectively 30, 50, and 100 mL of Spiked Sea Water**

| Compounds | Conc'ns. (mg/L) | Recoveries[a](%) Sea Water Volume, mL 30 | 50 | 100 |
|---|---|---|---|---|
| **LAS** | | | | |
| 1-(p-Sulfophenyl)dodecane | 1.0 - 1.2 | 70 ± 5 | 69 ± 2 | 70 ± 5 |
| **Sulfophenylcarboxylic acids** | | | | |
| *p*-Sulfobenzoic acid | 1.7 - 5.7 | 83 ± 10 | 36 ± 2 | 31 ± 6 |
| *p*-Sulfophenylacetic acid | 0.7 - 2.5 | 99 ± 4 | 91 ± 3 | 84 ± 17 |
| 3-(*p*-Sulfophenyl)propionic acid | 0.5 - 1.7 | 94 ± 4 | 97 ± 2 | 100 ± 3 |
| 4-(*p*-Sulfophenyl)butyric acid | 0.5 - 1.8 | 97 ± 6 | 97 ± 2 | 98 ± 3 |
| **Dihydroxyphenylcarboxylic acids** | | | | |
| 2,5-Dihydroxyphenylacetic acid | 1.2 - 3.9 | 70 ± 10 | 74 ± 7 | ND[b] |
| 3,4-Dihydroxyphenylacetic acid | 0.8 - 2.5 | 94 ± 8 | 102 ± 7 | 77 ± 5 |
| **Hydroxyphenylcarboxylic acids** | | | | |
| *p*-Hydroxylbenzoic acid | 0.6 - 2.0 | 97 ± 3 | 100 ± 3 | 98 ± 10 |
| *p*-Hydroxyphenylacetic acid | 0.9 - 3.1 | 96 ± 4 | 97 ± 2 | 95 ± 9 |
| 3-(*p*-Hydroxyphenyl)propionic acid | 0.8 - 2.6 | 97 ± 2 | 100 ± 2 | 95 ± 9 |
| **Phenylcarboxylic acids** | | | | |
| Phenylacetic acid | 1.3 - 4.4 | 93 ± 3 | 93 ± 5 | 98 ± 6 |
| 3-Phenylpropionic acid | 1.0 - 3.2 | 89 ± 3 | 92 ± 2 | 95 ± 6 |

[a] Mean ± standard deviation (n = 3). [b] Not determined.

**Table 4**

**Disappearance of 1-(*p*-Sulfophenyl)dodecane (LAS) in Sea Water Under Aerobic Conditions**

| Incubation Time (Days) | LAS[a] Concentration (mg/L) |
|---|---|
| 0 | 15 |
| 3 | 3.8 |
| 15 | 3.5 |
| 30 | 2.5 |
| 45 | 0.8 |
| 60 | 0.2 |

[a] Concentration based on the calibration curve for original LAS material.



## Example of Application

LAS biodegradation in sea water was followed during a laboratory experiment. A sample of sea water (2.5 L) as collected on the French Mediterranean Coast near Marseille. No detergents were detected in this sample and 37.5 mg of 1-(*p*-sulfophenyl)dodecane were added in order to obtain a solution containing 15 mg/L of LAS.

The sample was stored at room temperature (21°C) and was maintained under aerobic conditions in an Erlenmeyer flask. It was analyzed at appropriate intervals using the chromatographic method described above.

The concentration of undegraded LAS versus time is reported in Table 4. The disappearance of surfactant material was distinctly slower than those obtained in fresh waters[27] or by inoculation of adapted bacteria[15] and a reduction of 95% of the LAS level is observed only after 45 days. The chromatogram of the 45-day sample revealed that four peaks corresponding to potential metabolites were formed (Figure 3).

The sulfophenylcarboxylic acids were identified by comparison of UV maxima and k' with those of standards (Table 5). The content of SPCs was in the 0.63 mg/L to 3.50 mg/L range and after 45 days of experiment no significant variations in the SPCs concentrations were observed. The unsulfonated metabolites were undetected and the bacterial population in the sea water sample was not able to induce a total mineralization of 1-(*p*-sulfophenyl)dodecane.

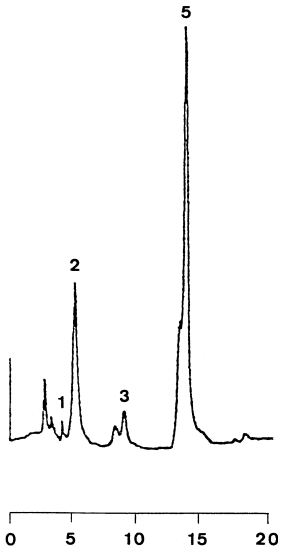



**Figure 3**. Chromatogram of a sample of sea water containing the metabolites generated by the biodegradation of LAS. Injection of 10 µL. Detection, UV at 215 nm, 0.05 AUFS. The separation conditions and the number of peaks as in Figure 2 A. The content of each metabolite is reported in Table 5.



**Table 5**

**Content of Metabolites in Sea Water After 45 Days of Experiment**

| Number of Peak | Identification | Content[a] (mg/L) |
|---|---|---|
| 1 | *p*-Sulfobenzoic acid | 0.63 ± 0.06 |
| 2 | *p*-Sulfophenylacetic acid | 1.65 ± 0.10 |
| 3 | 3-(*p*-Sulfophenyl)propionic acid | 0.35 ± 0.08 |
| 5 | 4-(*p*-Sulfophenyl)butyric acid | 3.50 ± 0.58 |

[a] Mean ± standard deviation of 3 replicates.

## CONCLUSION

The utility of the described technique to detect and characterize, not only a LAS, but also its biotranformation products, has been proved during the laboratory biodegradation study. The proposed solid-phase extraction procedure (SPE) of sea water samples is fast and gives quantitative recovery yields due to the amount of packing material used in the cartridges. When coupled to RP-HPLC and UV detection, the technique is highly specific and has been shown to be suitable for the separation and quantification of the compounds of interest in sea water.

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## REFERENCES

1. G. Thoumelin, Environ. Technol., **12**, 1037-1045 (1981).

2. M. Bressan, R. Brunetti, S. Casellato, G. C. Fava, P. Giro, M. Marin, P. Negrisolo, L. Tallandini, S. Thomann, L. Tosoni, M. Turchetto, Tenside Surf. Deter., **26**, 148-158 (1989).

3. E. Argese, A. Marcomini, P. Miana, C. Bettiol, G. Perrin, Environ. Toxicol. Chem., **13**, 737-742 (1994).

4. R. A. Kimerle, Tenside Surf. Deter., **26**, 169-176 (1989).

5. C. Divo, G. Cardini, Tenside Deterg., **17**, 30-36 (1980).

6. R. D. Swisher, Soap Chem. Spec., Feb, 58-62 (1963).

7. P. Schöberl, Tenside Surf. Deter., **26**, 86-94 (1989).

8. W. E. Gledhill, Adv. Appl. Microbiol., **17**, 265-293 (1974).

9. A. J. Willetts, A. G. Cain, Biochem. J., **129**, 389-402 (1972).

10. R. J. Larson, A. G. Payne, Appl. Environ. Microbiol., **41**, 621-627 (1981).

11. R. J. Larson, Environ. Sci. Technol., **24**, 1241-1246 (1990).

12. K. J. Bock, H. Stache, "Surfactants," in **Handbook of Environmental Chemistry**, O. Hutzinger, ed., Springer, Berlin, 1982, pp.169-199.

13. D. Sales, J. M. Quiroga, A. Gomez-Parra, Bull. Environ. Contam. Toxicol., **39**, 385-392 (1987).

14. D. C. Abbot, Analyst, **87**, 286-293 (1962).

15. J. M. Quiroga, D. Sales, Tenside Surf. Deter., **28**, 200-203 (1991).

16. J. A. Field, J. A. Leeheer, K. A. Thorn, L. B. Barber, C. Rostad, D. L. Macalady, S. R. Daniel, J. Contam. Hydrol., **9**, 55-79 (1992).

17. A. Di Cortia, R. Samperi, A. Marcomini, Environ. Sci. Technol., **28**, 850-858 (1994).

18. A. Marcomini, A. Di Cortia, R. Samperi, S. Capri, J. Chromatogr. A., **644**, 59-71, (1993).

19. L. Sarrazin, A. Arnoux, P. Rebouillon, J. Chromatogr. A., **760**, 285-291 (1997).

20. M. L. Trehy, W. E. Gledhill, R. G. Orth, Anal. Chem., **62**, 2581-2586 (1990).

21. W. P. Taylor, G. Nickless, J. Chromatogr. **178**, 259-269 (1979).

22. L. Sarrazin, J. Chromatographic. Sci., **35**, 91, 237 (1997).

23. L. Sarrazin, A. Arnoux, R. Rebouillon, J. L. Monod, Toxicol. Environ. Chem. **58**, 209-216 (1997).

24. M. Doe, P. H. El-Emary, W. H. Wade, J. Am. Oil Chem. Soc., **54**, 570-577 (1977).

25. E. Gonzalez-Mazo, A. Gomez-Parra, Trends Anal. Chem., **15**, 375-380 (1996).

26. A. Marcomini, S. Capri, W. Giger, J. Chromatogr., **403**, 243-252 (1987).

27. R. D. Swisher, J. Water Pollut. Control Fed., **35**, 877-892 (1963).

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