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Determination of Linear Alkylbenzene Sulphonates and their Biodegradation Intermediates by Isocratic RP-HPLC

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An HPLC method was developed to determine linear alkylbenzene sulphonates (LASs) and their biotransformation products sulphophenyl carboxylates by isocratic chromatography using two C_{18} columns in series. Solid-phase extraction with a (C_{18}) cartridge was employed to isolate LAS from sediment and sewage effluent samples. Sodium perchlorate and ammonium perchlorate were used as ion pair agents. The recovery of the compounds was 94% ± 2 and 98% ± 2 respectively for sediment and sewage effluent. It was demonstrated that it is possible to separate LAS compounds and their isomers and homologues using this system.

Key Words: Linear alkylbenzene sulphonates, solid phase extraction, sewage effluent, water, sediment, separation, HPLC, ion pair agent.

Introduction

Linear alkylbenzene sulphonates (LASs) are highly water soluble surface active agents widely used in synthetic laundry detergent formulation and household cleaning products. LASs are synthesized by Friedel-Crafts alkylation of benzene followed by sulphonation of the aromatic ring, predominantly at the para position[1]. LAS is a mixture with chain lengths ranging from C_{10} to C_{14} with isomers having phenyl positions ranging from 2 to 7, resulting in 26 isomers[2]. They are discharged into the environment through industrial/household or launderette effluent into waterways and by sludge disposal and are significant environmental pollutants, as their biodegradation involves the consumption of bio-available oxygen resulting in an increase in chemical oxygen demand. They affect the aquatic environment and change the physiological properties of water and the exchange rate of oxygen across the gills of fish. Kimerle [3] compared reported ranges of LAS toxicity (0.1-100 mg/L) to reported ranges of LAS environmental concentrations and found

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safety margins (toxic effect concentration/water concentration) from 10 to 1000 for freshwater algae, invertebrates and fish. The lowest reported value for LAS toxicity in sediments is about 200 mg/kg. The relative amounts of LAS found in the environment reflect the amount of each LAS component entering the environment minus the amount of each that has been destroyed or removed by biological or physicochemical processes[4]. The biodegradation of LAS can be considered in two stages. Primary biodegradation is said to have occurred when the original molecule has its structure altered by bacterial action[5,6]. Secondary biodegradation is the further conversion of the LAS molecule, ultimately to carbon dioxide, water, inorganic salts and products associated with the normal metabolic processes of bacteria[7,8]. Swisher's distance principle states that biodegradation rates increase with increasing distance between the sulphonate group and the far end of the alkyl chain; thus, 2-phenyl C_{12} LAS degrades faster than 6-phenyl C_{12}LAS and C_{13} LAS degrades faster than C_{10} LAS[9]. Hence, separation of LAS homologues and isomers are important for observing the biodegradation kinetics of LASs. The varying lengths of the unsubstituted alkyl chain cause each isomer to have different characteristics relating to sorption and biodegradation. Sorption increases with increasing chain length and as the phenyl position approaches the end of the alkyl chain[10].

The main objective of this work was to develop an RP-HPLC system able to yield the best separation of a complex mixture, such as that containing sulphophenyl carboxylic acid (SPC) and respective LAS precursors. In this paper individual homologues and isomers of LASs in standards, sediments and sewage effluent were determined. A secondary objective was to evaluate the ability of an octadecyl-bonded silica (C_{18}) cartridge to extract compounds of interest from both sewage effluent and sediment. The method for analysing LAS in a variety of environmental samples such as river water, sediment, sludge, and effluents was intended to be simple, rapid, specific, sensitive, quantitative, and inexpensive. The procedure developed reduces the number of steps and total time required for sample clean-up, preparation and analysis. An evaluation of the selectivity characteristics offered by the ion-suppression and the ion-pair HPLC techniques was performed.

SPCs are metabolities formed during the biological degradation of LAS[5,11,12]. The presence of SPC in water and sediment is important as it may indicate ongoing biodegradation of LAS. Dialkyltetralin sulphonates (DATSs) are impurities resulting from the LAS manufacturing process. DATS found in the environment may imply the presence of LAS[13,14]. The structures of a typical LAS and a biotransformation product are shown in Figure 1.

Both high-performance liquid chromatography (HPLC) and gas chromatography (GC) techniques have been used to measure alkylbenzene sulphonates in environmental samples[15,16,17,18]. The determination of LAS in the aquatic environment has also been carried out by gradient analysis[19,20]. HPLC was preferred to GC for the routine determination of environmental samples containing LAS, as it does not require the derivatization or desulphonation of LAS and SPC biotransformation products prior to analysis. SPCs have been determined by ion-pair RP-HPLC with UV detection[20]. Another advantage of RP-HPLC over the GC techniques is that the simultaneous determination of both LAS and nonylphenol polyethoxylates (NPEO) using a C_{18} column is possible[20]. Ion-pair HPLC was employed by adding to the mobile phase either a tetraalkyl ammonium salt such as tetramethylammonium bromide or an inorganic salt, such as sodium perchlorate[20,21]. The addition of tetraammonium bromide to the mobile phase has been widely adopted for the fractionating of LAS[20].

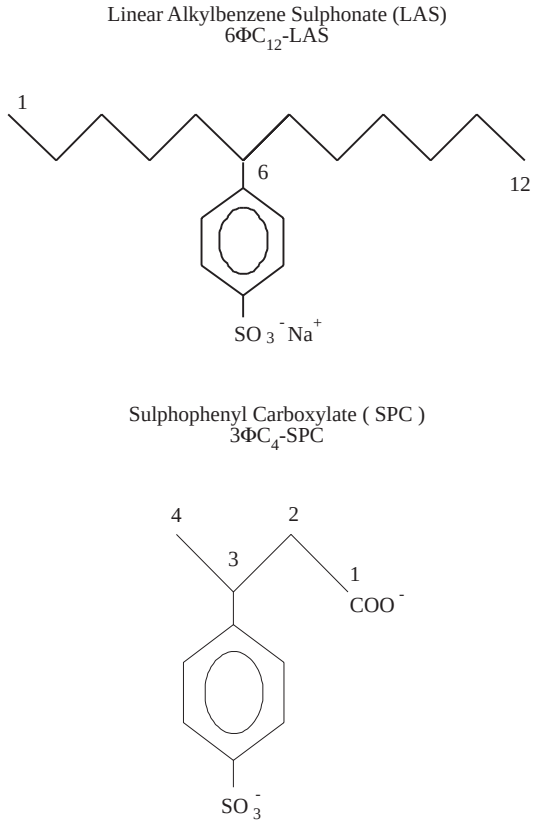

Figure 1. Structure of linear alkylbenzene sulphonate (LAS), and sulphophenyl carboxylate (SPC).

According to Comellas et al.[22] the alkaline methanol treatment of samples improves LAS extraction as LAS sorption on lipid organic matter decreases. Soxhlet extraction with solid sodium hydroxide provides these conditions only in the first few cycles. When the sample is submitted to a continuous alkaline methanolic treatment, lipids are saponified, separation of the lipid organic fraction of sediment occurs and interactions between organic matter and the mineral matrix are halted. Therefore, associations between surfactant, lipid and mineral sediment fractions are reduced and extraction is made easier. The strong basic medium resulting from saponification or the subsequent increase in salt content that a neutralization would represent makes direct injection of the extract on HPLC impossible. Therefore, it is advisable to perform a C_{18} micro-column clean-up to obtain LAS elution in a clear methanol solution. This eliminates polar compounds and inorganic salts simultaneously. Direct methanolic extract clean-up is not possible, because LASs are too soluble in methanol and would not interact with the C_{18} phase in the micro-column. However, the addition of water to the methanolic solution improves the affinity of LASs for the stationary phase. Cartridge behaviour has been studied as a function of methanol-water proportion in the solution to be cleaned[22] and the optimum was found to be 30% methanol in water.

According to this Comellas et al.[22] 30% methanol in water must be used when purifying sediment extracts and water samples, as only 30% methanol completely dissolves the sediment extracted fraction. They also reported[22] that in lower proportions methanol would form emulsions that would envelop LASs and prevent their interaction with the stationary phase.

Experimental

Reagents and Materials

Arylan SE (a commercial mixture of homologues and isomers of linear alkylbenzene sulphonic acids with C_{10-12} chain lengths) was used as the LAS standard and was supplied by Akro Chemicals, England. Marlon A, an LAS mixture supplied by Huls, Germany, has an average alkyl chain length of C_{10-13} and was also used in this work. C_{18} cartridges (1 g) were purchased from Altech (USA). The HPLC mobile phase modifiers trifluoroacetic acid (TFA), ammonium perchlorate (NH_4ClO_4) and tetramethylammonium bromide (TMAB) were supplied by Aldrich; sodium perchlorate was from BDH. Sodium dodecyl sulphate was supplied by Sigma.

Samples

Sample Collection, Preservation and Preparation

The environmental samples collected included river water, sediment and sewage effluent. Grab samples of river water were taken from a local sewage treatment works at stage 1 and sediments were taken from Aust near the River Severn UK. River water samples (2 L) were collected in plastic containers, preserved on site with 1% formaldehyde, and stored in a refrigerator at 4°C. Sample bottles were completely filled with the sample after rinsing them twice with the sample to be analysed.

Sediment samples were taken from the top 5 cm and between 10 and 15 cm of the river bed, i.e. the sediment most exposed to river water. The sediment was filtered to remove water, air dried ground. and stored in a refrigerator until needed for analysis.

Instruments

HPLC separation was achieved on an analytical Zorbax ODS C_{18} 4.6 mm diam. X 25 cm 5 μm column from Hichrom. The chromatography system consisted of a Pye-Unicam (LC-XPD) pump with a 100 μl loop injector. A model Pye-Unicam LC-UV detector and a Model 3390 A Hewlett Packard integrator were used.

Chromatography Conditions

HPLC methodology was based on reversed-phase separation with water-acetonitrile as mobile the phase containing NH_4ClO_4 and TFA as a phase modifier.

Column:	Zorbax ODS	4.6 mm X 25 cm
Flow rate:	1 ml/min	
Pressure:	350 bars	
Detector:	UV at 225 nm	
Mobile phase:	Acetonitrile/Water	
	50:50, 0.1M NH_4ClO_4	

Sample Isolation and Concentration

Isolation of LASs from Sediment (Solid Extracts) Samples

Air-dried sediment samples (20 g) were extracted for 12 h using a soxhlet apparatus with 200 ml of methanol and 20% w/w solid NaOH mixed with the sample (Figure 2). The methanol extract was partially vacuum

evaporated to 2-4 ml on a 35°C bath and transferred into a 100 ml beaker. The round-bottom flask was rinsed twice with hot methanol in order to obtain the compounds of interest, which were then added to the beaker. The combined extract was dried under a gentle stream of nitrogen on a boiling water bath. The residue containing LAS and SPC was dissolved in about 50 ml 30:70 MeOH/H_2O mixture. All isolated samples were stored as described. The dried residue was isolated from the sediment extract, or concentrated from a liquid sample such as water or wastewater with a C_{18}SPE cartridge and dissolved by ultrasonication for 5-7 min in 5-10 ml methanol/water mixture (30:70) containing 0.005 M sodium dodecyl sulphate (SDS) to aid the resolubilization of surfactant. The extract was made up to a known volume prior to HPLC analysis. If the isolated sample was not analysed immediately then it was dried under a stream of nitrogen and kept in a refrigerator until it was analysed. The dried residue was dissolved in MeOH/H_2O (30:70) containing 0.00 5M SDS and ultrasonicated for 2 min. This solution was neutralized with HCl and ultrasonicated again for at least 1 min to dissolve sodium chloride. The C_{18} cartridge was preconditioned with 5 ml of methanol and 5 ml of deionized water before use. The column was not allowed to become dry. Half the sonicated solution was percolated through the C_{18} column at a flow rate of 1-2 ml per min. The column was washed with 5 ml of deionized water. LASs and SPCs were eluted with 15 ml of methanol. The methanol eluate was evaporated to half of its original volume under a gentle stream of nitrogen on a boiling water bath and diluted to a known volume with water containing 5 x 10^{-3}M SDS and the final ratio adjusted to 50/50 MeOH:H_2O. This solution was injected into the HPLC system. The compounds of interest were quantified by peak area. The pH levels of standards used in the procedure were adjusted to 1.0 in order and protonate the surfactant sulphonic group, to increase the affinity for the stationary reversed phase. If a pH of < 4 or > 7 is used in this clean-up procedure, or acidification of the aqueous sample with HCl (pH$\cong$ 2) is required, then *C_{18} (trifunctional) should be used because mono functional C_{18} can only be used at pH between 4 and 7. It is recommended that *C_{18} is used at pH < 3 or > 7 but can also be used at pH 4-7. A lower pH assists separation and affinity on the C_{18} column in HPLC but a pH less than 4 is not recommended as affects the stationary phase. The methanol-water proportion in the solution to be cleaned was used by suitably modifying methods previously reported by Camellas et al.[22].

Isolation and Concentration of LAS from Aqueous Samples

A 200 ml water sample was taken and methanol added so that the final MeOH/H_2O ratio was 30:70. This ratio is the optimum to concentrate water samples and isolate compounds of interest. This solution was then percolated through the pre-conditioned C_{18} column as described previously at a flow rate of 1-2 ml/min. Because of their nature, surfactants will tend to become adsorbed onto any suspended solids, as well as on the walls of the containing vessel. For this reason, centrifugation of the wastewater samples is preferable to filtration before percolation through C_{18} columns. After percolating the sample through the column; the column was rinsed with 5-10 ml of distilled water prior to elution, in order to remove interferences.

An aqueous sample was concentrated using the same procedure (Figure 2) as described previously for dissolved sediment extract residue for HPLC analysis of LAS and SPC. After elution of the compounds from the column with methanol the extract was concentrated to a known volume by evaporation under nitrogen. This solution was then analysed by HPLC.

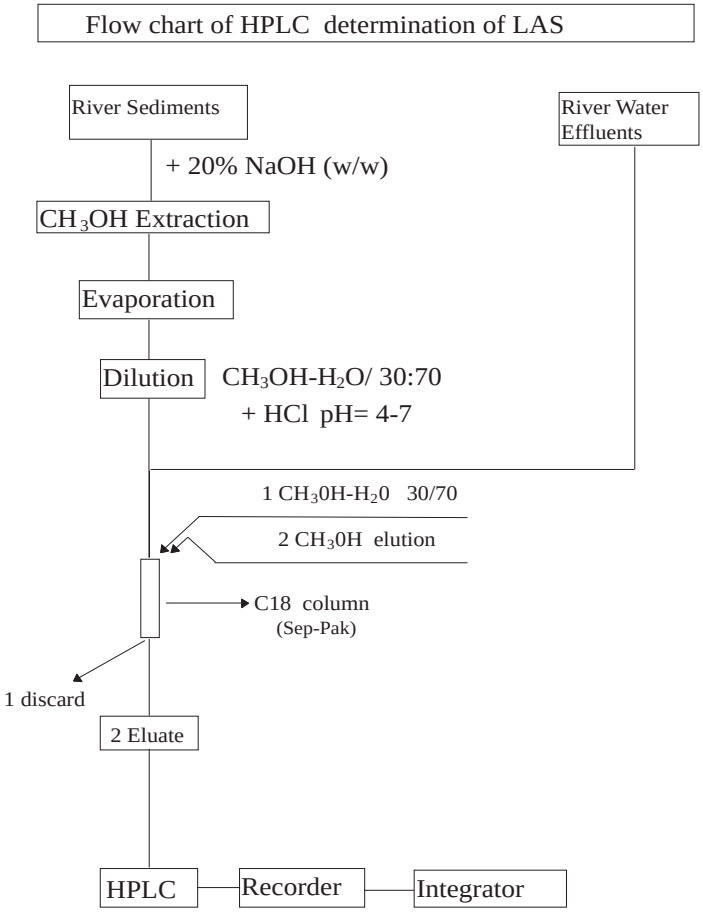

Figure 2. Flow chart of HPLC determination of LAS.

Results and Discussion

The effect of the $NaClO_4$ concentration on LAS separation using a C_{18} column was examined by Marcomini et al.[23] and it was found that the best concentration to use was 10-15 g/L of 0.1M $NaClO_4$ to aid separation of LAS homologues and isomers. This was confirmed by preliminary work for this study. It was also found that 0.1 M NH_4ClO_4 gave the same good separation. They also reported[23] that this concentration was accepted as it was the optimized concentration to be used with the newly developed method and good resolution was obtained. In this procedure, no separation was achieved without using an ion-pair agent. Figure 3 is a chromatogram of alkylbenzene sulphonic acid standard solution showing the separation of the homologues and isomers with alkyl chain lengths of 10-12 carbon atoms. The separation of these compounds was comparable to that in the literature using gradient systems.

A calculation of the relative percentage of alkylbenzene sulphonic acid homologues and isomers Figure 3 is shown in Table 1. The total composition of these compounds is approximately 98%.

A sample of water from the sewage treatment works was diluted and a separation of LASs and SPCs was achieved as shown in Figure 4.

The chromatogram shows the separation of the LAS homologues and isomers from sewage water effluent. It indicates that C_{10}-C_{13} LAS homologues and isomers are present. Total LASs and SPCs in water sample from the local sewage treatment works were determined to be 13,043 ppm and 2,062 ppm,

respectively. The early eluting cluster of peaks are believed to be sulphophenyl carboxylates and the first large peak may also be degradation products.

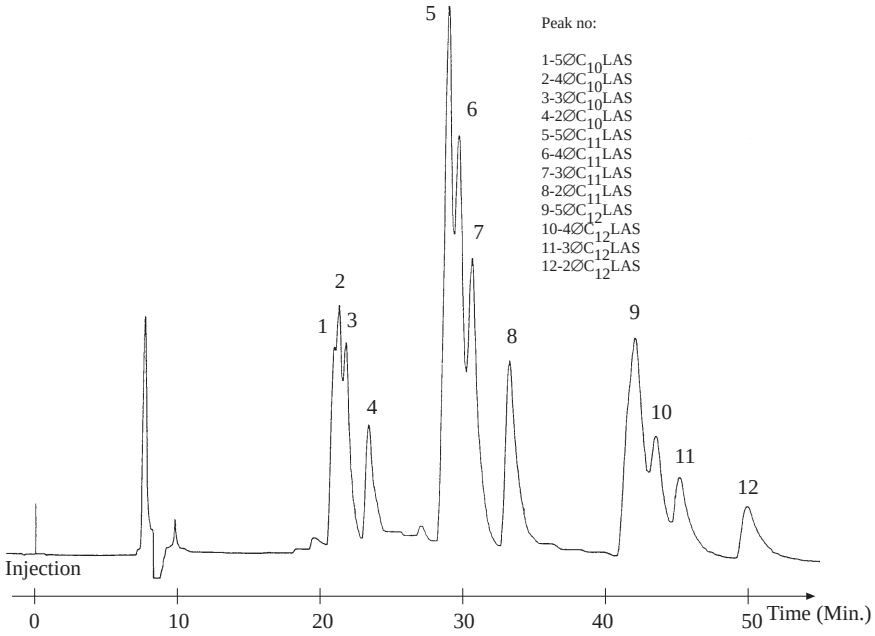

Figure 3. HPLC chromatogram of linear alkylbenzene sulphonic acid standards.

Table 1. Relative percentage of each alkylbenzene sulphonic acid homologue and isomer shown in Figure 3.

Composition of Arylan SE linear alkyl benzene sulphonic acid standard shown in Figure 3			
	LAS Homologues		
	C_{10}	C_{11}	C_{12}
Ø– position of isomers	%	%	%
2	3.742	8.435	3.852
3	5.809	12.184	5.604
4	5.222	13.723	6.674
5	2.608	(5+6Ø)	(5+6Ø)
6	-	16.226	14.010
7	-	-	-
Total: (98.086%)	17.381	50.568	30.140

A calculation of the relative percentage of each homologue and isomer detected from the sewage effluent is given in Table 2. The total of recovered homologues and isomers is approximately 78.5%. Other unidentified peaks make up the remaining 21.5%.

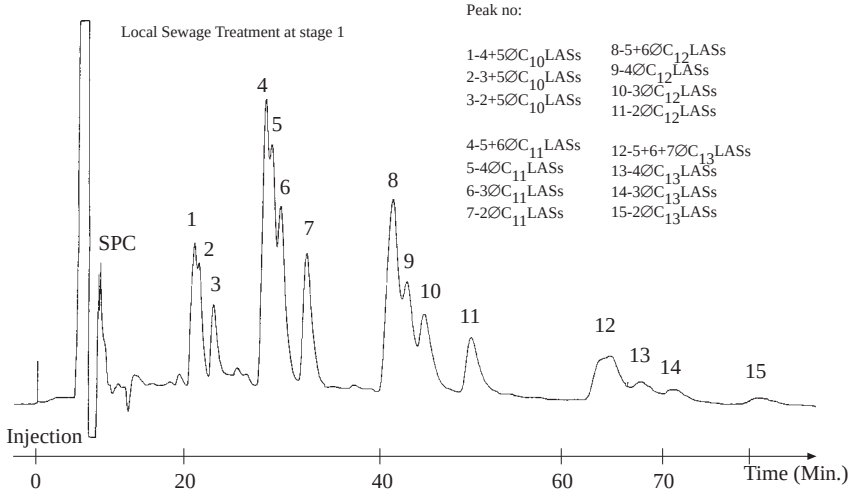

Figure 4. HPLC chromatogram of the LASs and SPCs isolated from local sewage treatment at stage 1.

Table 2. Relative percentage of each alkylbenzene sulphonic acid homologue and isomer shown in Figure 4.

Composition of linear alkyl benzene sulphonates shown in Figure 4					
		LAS Homologues			
Total concent.: 13.043 ppm	SPCs	C_{10}	C_{11}	C_{12}	C_{13}
Ø– position of isomers	Total	%	%	%	%
2	2.062	6.926	7.485	3.114	0.223
3	ppm	4.286	7.076	4.964	0.264
4		(4+5Ø)	4.397	3.616	0.775
5		4.402	(5+6Ø)	(5+6Ø)	(5+6Ø)
6		-	5.890	7.191	2.124
7		-	-	-	-
Total: (78.54%)	15.807	15.614	24.848	18.885	3.386

In order to test LAS recovery, a solution was prepared and injected into the HPLC. Peak areas were calculated with a standard solution. A known volume of this solution was spiked into the sediment that had previously been shown to contain no LAS. This sediment was then air dried and extracted with methanol. The final volume of extract was made up to the same volume as the quantity initially used to spike the sample. This solution was injected into the HPLC and the peak areas and retention times were recorded electronically with an integrator as shown in Figure 5. The concentrations of LAS isomers and homologues were calculated by peak areas. The peak areas of these two solutions were compared, and the recovery was calculated as a percentage. The recovery of the compounds was 94% with relative standard deviations of 2%. The concentrations of LAS homologues and isomers were calculated using the response factor of synthesized 1 Ø C_{12}LAS standard spiked into the sample. LASs and their respective compounds were identified by co-injection with a standard mixture of alkylphenyl sulphonic acid and synthesized individual standards by comparison of retention times with those of synthesized individual LAS standards.

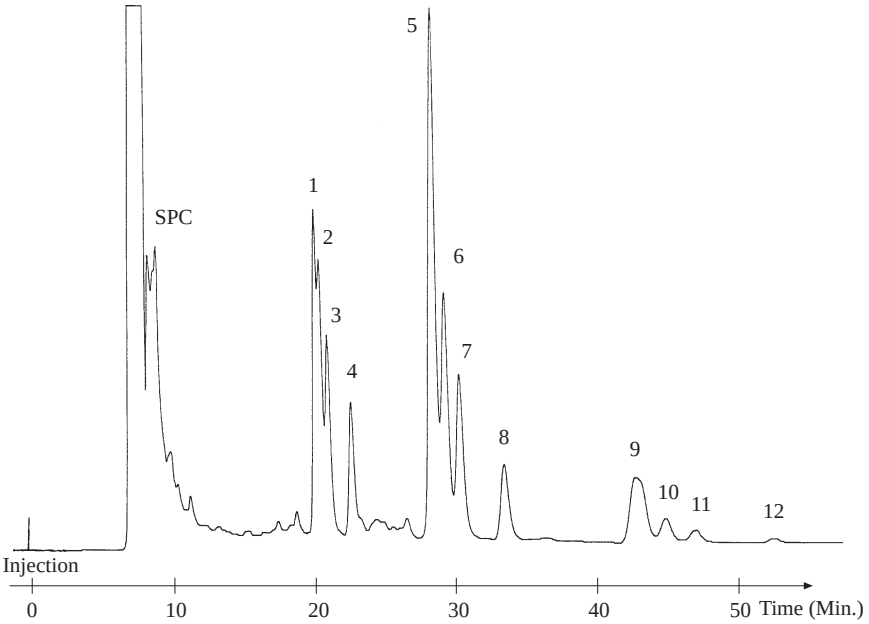

Figure 5. HPLC chromatogram of standard LASs and SPCs isolated from sediment.

The sediment sample collected from the River Severn was determined and a pattern similar to that which appeared in the water sample emerged for one set of compounds, the phenyl compounds based on the C_{11} alkyl chain. The difference in peak height reflects their rate of degradation.

A similar pattern is seen for the sediment samples as for the LAS homologues and isomers found in standards and effluent (Figure 6). Total LASs and SPCs in Severn River sediment were determined to be 0.7704 mg/kg and 0.044 mg/kg, respectively. LAS homologues in Severn River sediment were determined to be 0.282 mg/kg C_{10} – LASs, 0.27 mg/kg C_{11} – LASs, 0.1344 mg/kg C_{12} – LASs and 0.084 mg/kg C_{13} – LASs. The rising baseline and early eluting cluster of peaks may be due to sulphophenyl carboxylates. The results are in good agreement with the literature[13,20,24,25].

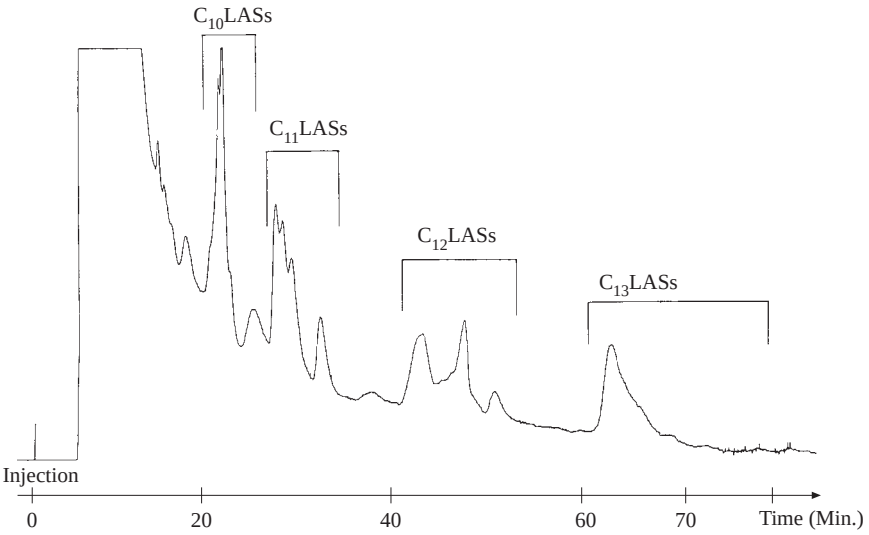

Figure 6. HPLC chromatogram of the LASs and SPCs isolated from Severn River sediment.

As $NaClO_4$ affects the thermospray interface of the mass spectrometer by precipitating the sodium and causing blockages it is important to use a more volatile salt, e.g. ammonium acetate or ammonium perchlorate. NH_4ClO_4 is more volatile than $NaClO_4$ although it also precipitates, causing problems in the interface. The same good separation was obtained with freshly prepared NH_4ClO_4 (0.1 M, pH $\cong$ 6.3) in 50/50 ACN:H_2O mobile phase. When TFA was used together with NH_4ClO_4 at pH 4 no improvement in separation was observed and retention times of peaks increased. This result therefore indicates that 0.1 M of NH_4ClO_4 was adequate to fully resolve the LAS peaks. TFA was used to improve the separation of SPCs from LASs although TFA does not improve the resolution of LAS. TFA was added and the pH was maintained at 3-4. No further improvement was observed using TFA and NH_4ClO_4 with concentrations of NH_4ClO_4 up to 0.15 M. It was found that the mobile phase (0.1 M of NH_4ClO_4) had to be freshly prepared prior to use as the NH_4ClO_4 precipitated out of the solution with time. After allowing the precipitate to dissolve in the mobile phase the separation was found to have decreased. When this new solution was used the same good separation was observed as for freshly prepared mobile phase. As can be seen, an increase in salt concentration up to 0.1 M NH_4ClO_4 allowed separation of each LAS homologue with increasing retention times. Addition of SDS to the sample to be analysed assisted the separation of compounds (LAS, SPC) on the HPLC and also prevented adsorption losses on the glassware in this procedure.

This new method was applied to sediment, river water and sewage effluent samples. This method is suitable for analysing LAS from a variety of environmental samples, either aqueous or solid. Solid-hase extraction using C_{18} was preferred to evaporation to concentrate samples and isolate LASs and their respective biodegradation intermediates for analysing without destroying any compounds of interest.

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