

Determination of Linear Alkylbenzene Sulphonate in Treatment Plant Wastewater Streams Using On-Line Solid-Phase Extraction Followed by HPLC with Fluorescence Detection

INTRODUCTION

Surfactants are the major active ingredients of laundry detergents. During the washing process, they lower the surface tension of water and loosen and release stains from fabric. Historically, the first surfactant used in laundry cleaning was soap (mixture of animal fat and ashes). Soap is not stable in hard or acid waters. It readily precipitates with divalent metal ions and is, thus, soluble in alkaline soft waters. Other surfactants, including anionic, cationic, non-ionic, and amphoteric surfactants have been developed to improve cleaning performance. Linear alkylbenzene sulphonate (LAS, CAS 68411-30-3) is an anionic surfactant. It was introduced in 1964 as the readily biodegradable replacement for highly-branched alkylbenzene sulphonate (ABS). The commercial product, the subject of this application note (AN), is a mixture of closely-related isomers and homologues, each containing an aromatic ring sulphonated at the para position and attached to a linear alkyl chain (Figure 1).

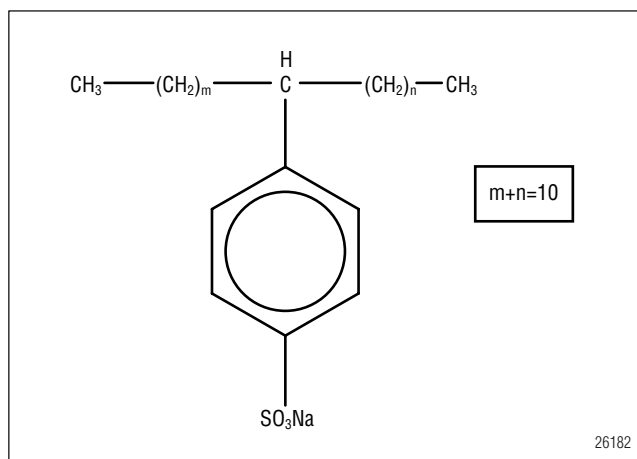


Figure 1. LAS chemical structure (C13 homologue).

The linear alkyl chain typically consists of: 10–13 carbon units, approximately in the mole ratio C10:C11:C12:C13 = 13:30:33:24; an average carbon number near 11.6; and a content of the most hydrophobic 2-phenyl isomers in the 18–29% range.¹ Thus, commercial LAS consists of more than 20 individual components. The ratio of the various homologues and isomers representing different alkyl chain lengths and aromatic ring positions along the linear alkyl chains is relatively constant across the various household applications. The global production of LAS is 2.2×10^6 t/yr¹. The present risk assessment is based on recent environmental safety data and updates the previous LAS terrestrial risk assessments in the literature.¹⁻⁹

LAS surfactants have a strong affinity for sorption to sediments. However, data regarding the fate and effects in sediments following release into the environment have not been reported in great detail.¹⁰ The concentration range of LAS in wastewater treatment plant effluent is 19,000–71,000 ng/L while the total removal in wastewater treatment plants for LAS is 95–99%.^{11,12} Approximately a 20% loss over a 4.3 min residence time in stream surface water has been observed. Hence, the half-life (DT50) of LAS in river water is conservatively set to <12 h.^{1,13} However, in some situations, continuous inputs to the environment (for example, via wastewater treatment plants effluent, other known and unknown point sources, runoff etc.) of compounds that biodegrade rapidly may replace dissipated material resulting in chronic low exposures.

Sanderson et al. (2005) demonstrated the adapted analytical method for the AS/AES homologues and low likelihood of risks.¹⁰ However, it was also concluded that additional monitoring with more sampling locations, in combination with both biota and habitat up- and downstream of wastewater treatment plants, was needed to better elucidate the potential risks. Figure 2 shows the movement of LAS in the environment.

According to the Italian official methods, the determination of surfactants in industrial discharges or surface waters must be carried out by colorimetric methods. Recently, the European Community introduced a new regulation on detergents (Regulation EC No. 648/2004 of the European Parliament and the Council of 31 March, 2004) which establishes standard methods for detergents.¹⁴ This regulation states that for those

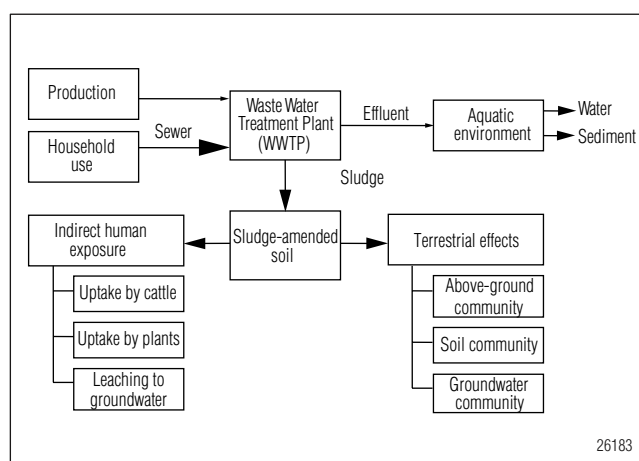


Figure 2. Fate of LAS in the environment.

surfactants not reacting with colorimetric methods (MBAS for anionic, BiAS for non-ionic, and BBAS for cation surfactants) “if it seems more appropriate for reasons of efficiency or precision, appropriate specific instrumental analyses such as high performance liquid chromatography (HPLC) or gas chromatography (GC) are to be applied.” This is the first statement in favor of the introduction of chromatographic methods for surfactant determination. The LAS surfactants are very diffuse in discharges because they replace alkylphenolethoxylates in domestic detergents which have been banned for many years.

In 2000, the European Union published the third draft of a future sludge directive entitled *Working Document on Sludge* (Third Draft, 27 April, 2000) adopted in the Italian regional legislation (*Regione Emila Romagna, Determinazione del Direttore generale ambiente, difesa del suolo e della costa n. 11046 del 29/0//2005*), where more restricted concentration limit values of some organic compounds are included for the first time.¹⁵ Some of the organic compounds included are LAS surfactants. According to the directive draft, the concentration of LAS has to be lower than 2600 mg/kg SS (Suspended Solids) for land application of sludge. The control of final concentration of LAS in sludges in the wastewater treatment plants (WWTPs) needs continuous monitoring in the effluents in each step of the depuration process. This monitoring can be accomplished by developing an automated chromatographic system for LAS analysis that should not require a manual concentration step.

The determination of LAS can be used as an indicator of the effectiveness of the depuration process. The importance of monitoring the LAS concentration is mainly due to the agricultural destination of resulting sludge, which is regulated for LAS content in some nations.¹⁶ Using sewage sludges for agricultural purposes is an economical pathway for disposal. If the LAS limit is exceeded, the sludge must be delivered to an incinerator to burn at 10 times the cost.

In the recent past, several analytical methods have been described in the literature for the determination of LAS in environmental samples, mainly in agricultural soils and sediments.¹⁷ Most of these methods are based on determination by liquid chromatography with ultraviolet diode array (UV-DAD), fluorescence (FLD), or mass spectrometric detectors.¹⁸⁻²¹ The method detection limits (MDLs) of LC techniques employing direct injection of samples are too high for the detection of the low levels allowed in natural waters. Therefore, water samples require preconcentration before analysis. Solid-phase extraction (SPE) is one of the most important techniques for sample enrichment because it overcomes many of the disadvantages of liquid-liquid extraction. Unfortunately, preparing individual samples is time consuming and a new SPE cartridge must be used for each sample. The expense of using multiple SPE cartridges and the associated manual labor can be eliminated with on-line SPE combined with HPLC. This technique delivers a simple, rapid, and accurate means for determining phenols at low concentrations in real samples.²²

The Dionex UltiMate[®] 3000 system was designed to easily execute more advanced HPLC methods, such as parallel LC, 2D-LC, and on-line SPE/HPLC. An UltiMate 3000 system, together with an autosampler capable of injecting large volumes, can be used to execute an on-line SPE method to determine LAS in water streams. This AN details an on-line SPE method, followed by HPLC with fluorescence detection, for determining LAS at the concentrations required by world regulatory agencies.

The LAS from river and water streams of a depuration plant are trapped on an IonPac[®] NG1, a small polymeric reversed-phase column, and then separated on a speciality polar-embedded reversed-phase column, the Acclaim[®] Surfactant column. This automated method is a cost-effective way to determine LAS in river and water stream samples and follow their fate after water treatments. The integrated on-line SPE solution proposed uses an easy instrumentation setup through Chromeleon[®] software. This combination represents a simple and economically convenient analytical solution, even for routine laboratory operators. Compared to any alternative system, a Dionex dual pump system will save money and reduce maintenance costs. Also, the elimination of the disposable cartridge in the off-line SPE procedure will save money, solvents, and workload. The same system can be easily configured to perform other common, direct, or SPE-based HPLC applications, such as the determination of phenols or polycyclic aromatic hydrocarbons (PAHs).

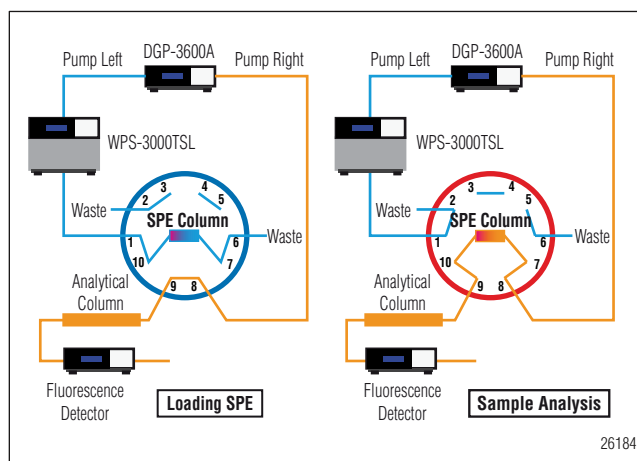


Figure 3. Schematic of devices for determination of LAS using on-line SPE followed by HPLC with fluorescence detection.

EQUIPMENT

Dionex UltiMate 3000 HPLC system consisting:
DGP-3600A dual gradient pump
SRD-3600 solvent rack with integrated vacuum degasser
WPS-3000 TSL equipped with 500 µL loop
TCC-3200 Thermostatted Column Compartment with one two-port, ten-position (2P–10P) valve
RF 2000 Fluorescence Detector
Chromleon Chromatography Data System, 6.80 SP4

REAGENTS AND STANDARDS

Use only ACS reagent grade chemicals for all reagents and standards.
Deionized (DI) water ASTM grade I (18.2 MΩ-cm) from a Barnstad or other water purification system
Acetonitrile (CH₃CN), HPLC grade (Merck, Darmstadt)
Ammonium acetate (CH₃COONH₄), analytical reagent grade
The starting standard solution contains 1463 mg/L of C₁₀₋₁₃ LAS (Sigma-Aldrich) in methanol. To this standard solution, bisphenol A, nonyphenol, nonyphenol-1-ethoxylated, and nonyphenol-2-ethoxylated were added for determination of additional information not considered in this paper. This information does not interfere with the LAS determination.

CONDITIONS

Solid-Phase Extraction

Column: IonPac NG1, 5 µm, 4 × 250 mm (P/N 039567)
Mobile Phases for SPE
(Left Pump): A. Water
Flow Rate: Rinse/Loading 2 mL/min with 100% A
Temperature: 30 °C
Inj. Volume: 0.5–5 mL
For the detailed program see Tables 1A-C.

Analytical

Column: Acclaim Surfactant, 5 µm, 4.6 × 250 mm (P/N 063203)
Acclaim Surfactant Guard 4.3 × 10 mm (P/N 063215)
Mobile Phases for Analysis
(Right Pump): A. CH₃CN
B. 100 mM CH₃COONH₄ (pH 5 with HCl)
Gradient: 50 to 15% B in 20 min
Flow Rate: 1 mL/min
Temperature: 30 °C
Detection: Fluorescence, 0 min: Ex 230 nm Em 302 nm; 18 min: Ex 221 nm Em 284 nm
Total analysis time is 45 min. For the detailed program see Table 1B.

Table 1A. List of Default Conditions for SPE and Analytical Separation

Default Conditions	
	Sampler.TempCtrl = On Sampler.Temperature.Nominal = 10.0 [°C] ColumnOven.TempCtrl = On ColumnOven.Temperature.Nominal = 30.0 [°C] PumpLeft.%A.Equate = Water PumpRight.%A.Equate = ACN PumpRight.%B.Equate = NH ₄ AcOH 100 mM pH5 PumpRight.%C.Equate = "%C" InjectMode = UserProg SyncWithPump = On Emission.ExWavelength = 230 [nm] Emission.EmWavelength = 302 [nm] Emission.Sensitivity = Med PumpLeft.Flow = 2.000 [ml/min] PumpLeft.%B = 0.0 [%] PumpLeft.%C = 0.0 [%] ValveRight = 10_1 WashVolume = 1000.000 [µl] WashSpeed = 30.000 [µl/s] PrepVial= Position+1

Table 1B. List of Analytical Conditions for LAS Determination

-10.000	Sampler.TempCtrl = On Sampler.Temperature.Nominal = 10.0 [°C] ColumnOven.TempCtrl = On	Begin to equilibrate the analytical column using initial conditions for 10 min Injections at 0 min.
0.000	UV.Autozero Wait AZ_Done Emission.Autozero PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 50.0 [%] PumpRight.%C = 0.0 [%] Wait ColumnOven.Ready and Sampler.Ready Inject Emission.AcqOn PumpRight.Flow = 1.000 [mL/min] ValveRight = 1_2 PumpRight.%B = 50.0 [%] PumpRight.%C = 0.0 [%]	Starts SPE column rinse
5.200	ValveRight = 10_1	Start SPE column rinse
18.000	Emission.ExWavelength = 221 [nm] Emission.EmWavelength = 284 [nm]	Wavelengths optimized for LAS
20.000	PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 15.0 [%] PumpRight.%C = 0.0 [%]	Gradient end
30.000	PumpRight.Flow = 1.000 [mL/min] PumpRight.%B = 15.0 [%] PumpRight.%C = 0.0 [%] Emission.AcqOff ReleaseExclusiveAccess End	Isocratic step

Table 1C. Program for Multiple Sample Loading Onto SPE Column Using a WPS-3000 Autosampler Equipped with 500 µL Loop

500 µL	UdpDraw	From=SampleVial, Volume=500.000, SyringeSpeed=12.000, SampleHeight=2.000	Single step for 500 µL sample loading onto SPE column
	UdpMixWait	Duration=5	
	UdpInjectValve	Position=Inject	
	UdpMoveSyringe	Unload=500.000, SyringeSpeed=12.000	
	UdpInjectValve	Position=Load	
			To be repeated according to the final desired volume
5000 µL	UdpDraw	From=SampleVial, Volume=500.000, SyringeSpeed=12.000, SampleHeight=2.000	10th step for 5000 µL of sample loaded onto SPE column
	UdpMixWait	Duration=5	
	UdpInjectValve	Position=Inject	
	UdpMoveSyringe	Unload=500.000, SyringeSpeed=12.000	
	UdpInjectValve	Position=Load	
Final step	UdpInjectMarker		Placed at the end of any repetitive sequence last step

Table 2. Range and Determined Homologues Average Distribution of LAS in Working Standard (n=5)

	C_{10}	C_{11}	C_{12}	C_{13}
Range %	5–15	30–40	20–40	15–30
Average found %	13	33	31	23

RESULTS AND DISCUSSION

Optimization of the On-line SPE Method

The linearity of the on-line SPE procedure for the determination of C_{10-13} LAS was investigated by loading the SPE cartridge with different volumes of the C_{10-13} LAS standard prepared at the same concentration. The resulting chromatograms are shown in Figure 4. Linearity results are summarized in Table 3.

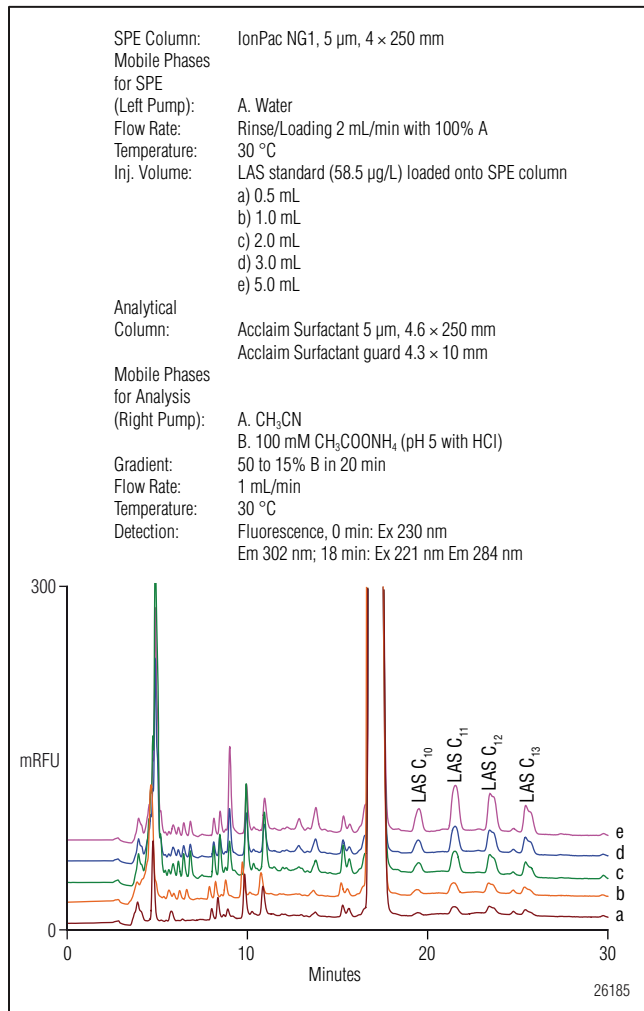


Figure 4. An overlay of chromatograms of different volumes of the same C_{10-13} .

Reproducibility, Detection Limits, and Linearity

The wide range of LAS concentrations in the analyzed samples collected in the inlet and outlet of the WWTP and the limited dynamic range of fluorescence detection, require the use of two different loading volumes for the SPE device, 0.5 and 5 mL respectively, and subsequent use of two calibration curves for 0.5 and 5 mL volumes. The reproducibility was estimated by making five replicate injections of an inlet sample and three replicates of a low concentration sample. Table 4 summarizes the retention time and peak area precision data for both samples. The method detection limits (MDLs) of the C_{10-13} LAS using the on-line SPE-HPLC are listed in Table 5. The calibration linearity for the determination of C_{10-13} LAS was investigated by making replicate injections of C_{10-13} LAS prepared at five different concentrations. The external standard method is used to calculate the calibration curve and for sample analysis. Table 3 lists the data from the calibration as reported by the Chromeleon software.

Sample Analysis

To achieve satisfactory chromatography of C_{10-13} LAS in water samples, the samples should be filtered prior to analysis, and analyzed in a short time to avoid degradation processes due to the dissolved organic matter.

Figure 5 shows an example of an inlet stream of an urban and industrial wastewater treatment plant. The concentration of the total C_{10-13} LAS in the sample was 8689 μ g/L. The chromatogram of the effluent stream of the same plant is shown in Figure 6. The C_{10-13} LAS concentration in the sample was reduced to 740 μ g/L. Another example of an urban waste depuration plant effluent analysis is shown in Figure 7. This situation is far above the quantification limit and the C_{10-13} LAS concentration in the sample was as low as 37.1 μ g/L.

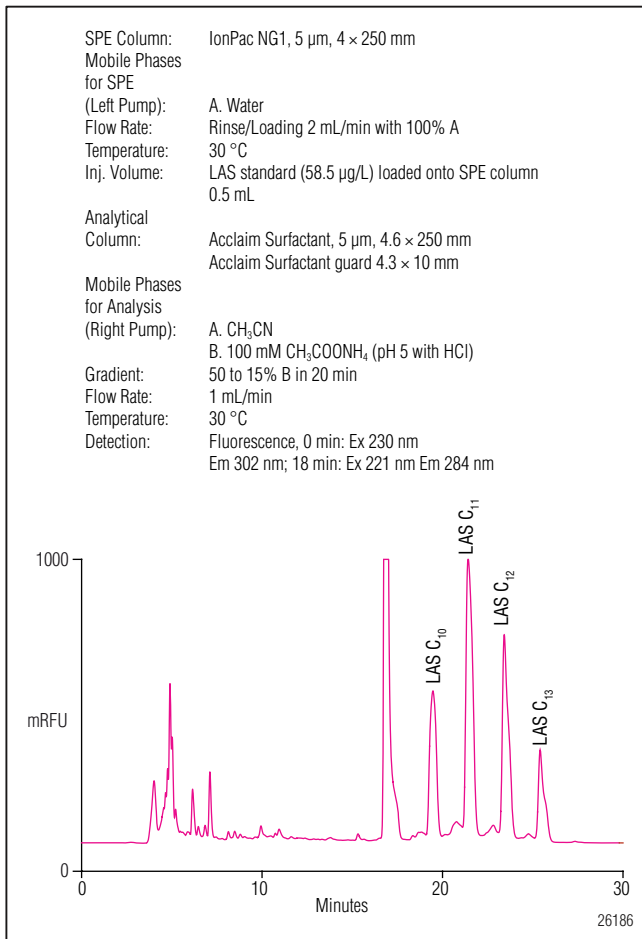


Figure 5. Chromatogram of the inlet stream of an urban and industrial wastewater treatment plant. The total LAS concentration is 8689 μ g/L.

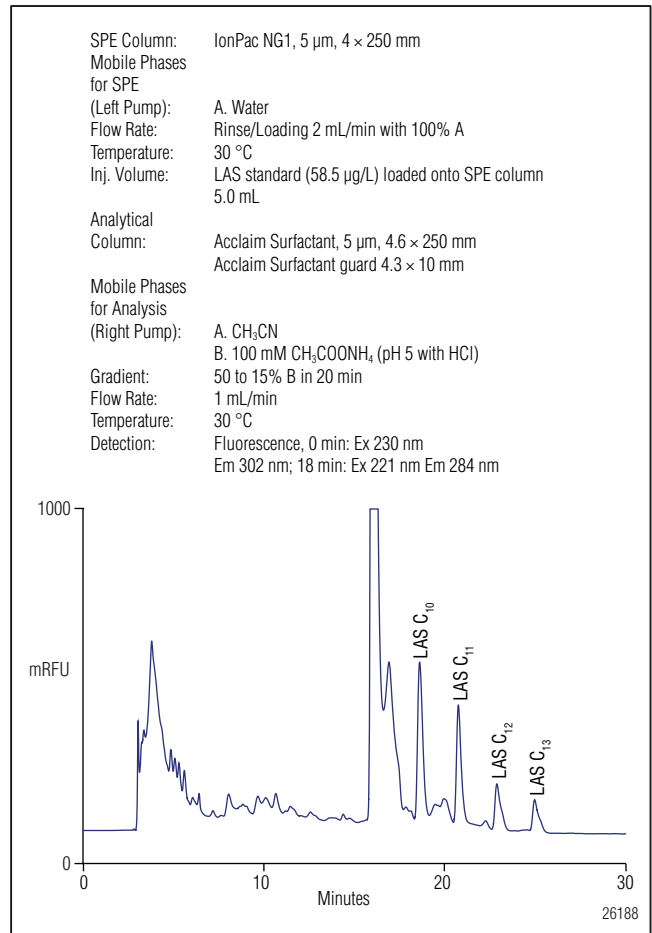


Figure 6. Chromatogram of the outlet stream of an urban and industrial wastewater treatment plant of Figure 5. The total LAS concentration is 740 μ g/L.

Table 3. LAS Calibration at Two Different SPE Volumes and Five Different Concentrations Each

SPE Loaded Volume	C ₁₀			C ₁₁			C ₁₂			C ₁₃		
	r ²	Offset	Slope	r ²	Offset	Slope	r ²	Offset	Slope	r ²	Offset	Slope
0.5 mL	0.9967	-0.3170	0.1343	0.9964	0.5495	0.1137	0.9962	0.6692	0.1117	0.9935	0.6836	0.1108
5 mL	0.9996	1.2408	1.1660	0.9988	4.2647	0.9655	0.9988	4.4579	0.8101	0.9975	3.3943	0.6974

Table 4. Retention Time and Peak Area Precisions for C₁₀₋₁₃ LAS at Two Different SPE Loading

SPE Loaded Volume	C ₁₀		C ₁₁		C ₁₂		C ₁₃	
	RT RSD	Area RSD	RT RSD	Area RSD	RT RSD	Area RSD	RT RSD	Area RSD
0.5 mL (n=5)	0.24	1.72	0.22	1.33	0.16	1.39	0.12	3.25
5 mL (n=3)	0.95	11.1	0.03	9.9	0.04	15.1	0.03	15.4

Table 5. Detection Limits for C₁₀₋₁₃ LAS at Two Different SPE Loading Volumes

SPE Loaded Volume	C ₁₀		C ₁₁		C ₁₂		C ₁₃	
	LOD (µg/L)	RSD	LOD (µg/L)	RSD	LOD (µg/L)	RSD	LOD (µg/L)	RSD
0.5 mL (n=5)	5.1	3.29	14.2	3.89	15.4	1.1	12.3	3.86
5 mL (n=3)	0.19	11.4	0.51	12.1	0.46	12.4	0.31	15.3

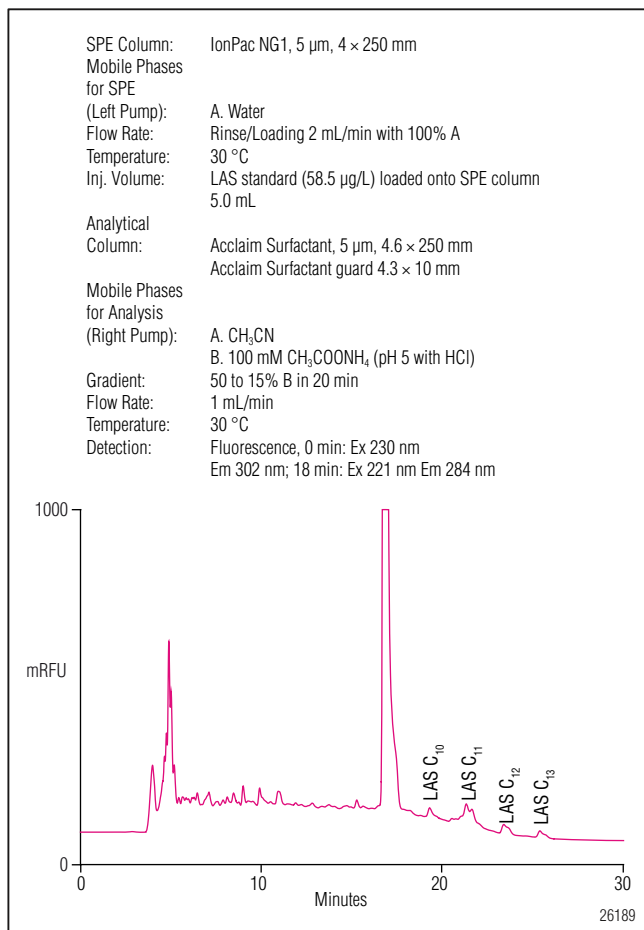


Figure 7. Chromatogram of the outlet stream of an urban wastewater treatment plant. The total LAS concentration is 37.1 µg/L.

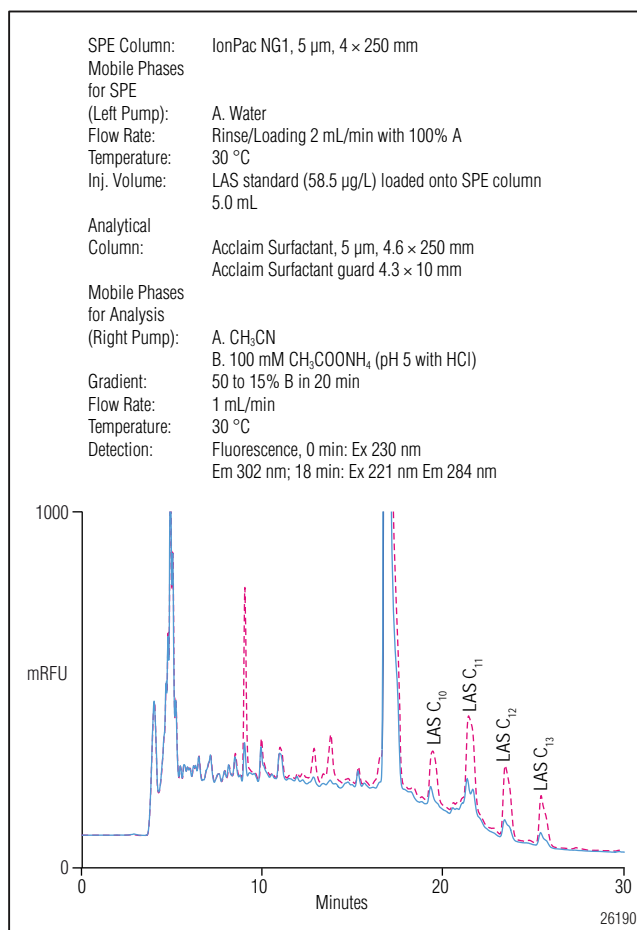


Figure 8. Chromatograms of the outlet stream of an urban wastewater treatment plant at low C₁₀₋₁₃ LAS concentration and same sample spiked with 117 µg/L of C₁₀₋₁₃ LAS.

Recovery

A recovery study was performed by spiking a low concentration sample with 117 µg/L of C₁₀₋₁₃ LAS and doing replicate analyses. Figure 8 shows the comparison between a low concentration sample (Figure 7) and the same sample spiked with 117 µg/L of C₁₀₋₁₃ LAS. The average recovery was better than 104% with a RSD of 14% (n=3).

CONCLUSION

The successful analysis of all the water samples above demonstrates that on-line SPE with a dual UltiMate system can determine the LAS without laborious off-line sample preparation. The on-line SPE method with fluorescence detection has very good reproducibility and selectivity with detection limits of 0.9 µg/L for total C₁₀₋₁₃ LAS using a 5 mL sample loaded onto the SPE column.

PRECAUTIONS

Method interferences may be caused by surfactants in solvents, reagents, glassware, and other sample processing hardware. Clean and rinse all glassware scrupulously with high-purity water and use high-purity reagents and solvents to minimize interference problems. Samples must be filtered and analyzed in a short time to avoid degradation due to dissolved organic matter.

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