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Inter-laboratory comparison of liquid chromatographic techniques and enzyme-linked immunosorbent assay for the determination of surfactants in wastewaters

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Abstract

Seven laboratories participated in an inter-laboratory comparison exercise within the framework of the PRISTINE, SANDRINE and INEXsPORT European Union Projects. Solid-phase extraction (SPE) methodologies were used for the extraction of target analytes from wastewaters. The analytical strategies were based on liquid chromatography (LC) coupled to mass spectrometric (MS) or to fluorescent (FL) detection in all cases with the exception of one laboratory using a test-tube enzyme-linked immunosorbent assay kit. Samples were spiked with the surfactants nonylphenolpolyglycol ether, coconut diethanolamide, linear alkylbenzene sulfonate, nonylphenolpolyglycol ether sulfate, alkylpolyglycol ether and secondary alkane sulfonate. After enrichment on previously conditioned SPE cartridges, the SPE cartridges were distributed among the participating laboratories without the information about the amount of spiked surfactants. In addition, SPE cartridges loaded with a real-world environmental sample containing a tannery wastewater were also analyzed. The results of the programme showed that SPE followed by LC–MS techniques are reliable for the surfactants determination at submicrogram to microgram per liter levels in wastewaters. Inter-laboratory precision values were calculated as the reproducibility relative standard deviation (RSD_R) which was determined from the reproducibility standard deviation (s_R) and the average concentration at a particular concentration level. When data from all laboratories were pooled, the RSD_R values ranged from 5.1 to 28.3% for the determination of target analytes. The most accurate result corresponded to that given for linear alkylbenzene sulfonates. Taking into account that different methodologies were used (including non-chromatographic techniques) and the complexity of the samples analyzed, it can be considered that acceptable reproducibility values were obtained in this inter-laboratory study.

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1. Introduction

A number of compounds of environmental interest are polar, non-volatile and/or thermally labile. Thus they are not amenable to conventional gas chromatography (GC) analysis. To address this problem, efforts are now underway to develop suitable techniques for the determination of these compounds. In this respect, different liquid chromatography–mass spectrometry (LC–MS) methods involving particle beam and atmospheric pressure ionization (API) were developed for the analysis of highly polar and water-soluble organic pollutants [1,2].

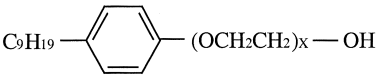
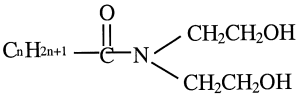
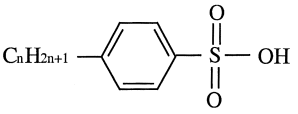
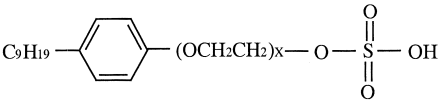
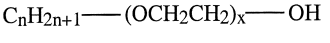
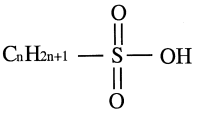
Surfactants (anionics, non-ionics, cationics and amphoterics) are used in different fields such as cosmetics, metal working, mining, agriculture, paper and leather industries and obviously they are employed in large quantities for many applications in households, institutions and industries [3]. From the group of non-ionic surfactants, nonylphenol ethoxylates ($NPEO_x$) and alcohol ethoxylates (C_nEO_x or $AEO_{n,x}$) are the most commonly used. Recent data reports an annual production of $800 \cdot 10^6$ kg of C_nEO_x in Western Europe [3] indicating that they are still the most widely used non-ionic surfactants as over 80% of all non-ionic surfactants are based on C_nEO_x [4]. Anionic surfactants [mainly linear alkylbenzene sulfonates (C_n LASs)] are also used on a large scale ($420 \cdot 10^6$ kg of LASs were produced in 1997) in the chemical industry as well as in household applications. The group of cationic and amphoteric surfactants only represented 8% of the total production in 1997. In the present inter-laboratory exercise, commonly used surfactants from different chemical groups were selected. The list included nonylphenolpolyglycol ether ($NPEO_x$), coconut fatty acid diethanolamide (C_nDEA), linear alkylbenzene sulfonate (C_n LAS), nonylphenolpolyglycol ether sulfate ($NPEO_x$-SO_4), alkylpolyglycol ether (C_nEO_x) and secondary alkane sulfonate (SAS) whose chemical structures are given in Table 1.

Analysis of surfactants is generally carried out by either chromatographic procedures that need derivatisation like GC or by LC [4–6]. Improved analytical procedures for the determination of the whole group of surfactants require the optimization of the sample pre-treatment together with the use of LC–MS. Recent works report the use of solid-phase microextraction [7] and solid-phase extraction (SPE) with different sorbents such as alkyl-bonded silica [5], graphitized carbon black (GCB) [8] and styrene–divinylbenzene resins [9]. Sequential solid-phase extraction (SSPE) combined with LC–MS has been successfully employed for the extraction of surfactants allowing to improve sensitivity and selectivity of the global analytical strategy [2,10].

SPE followed by chromatographic techniques has turned out to be the most currently used method allowing the isolation and characterization of surfactants from various environmental matrices. LC–MS with electrospray ionization (ESI) was used for the analysis of raw and treated wastewater from sewage treatment plants (STPs) with limits of detection (LODs) of 0.6 μg/l [8], whereas one of our groups [11] employed LC–MS and tandem MS (LC–MS–MS) coupled by a thermospray (TSP) interface for the detection of surfactants in STP samples. The use of LC–MS not only eliminates the need of derivatisation steps, but also provides a single-step analysis with selective mass detection. Since LC–MS methodologies are currently being used in many analytical laboratories, there is the need for inter-laboratory exercises to evaluate their performance for the determination of surfactants.

One of the objectives of the PRISTINE, SANDRINE and INEXsPORT European Union projects is to provide accurate methods for the determination of surfactants in wastewaters. One way to achieve this purpose was to organize an inter-laboratory exercise involving seven participants analyzing the same sample(s) by the analytical methods previously agreed and discussed within the participating laboratories. Among the difficulties encountered in conducting inter-laboratory studies on emerging technologies, the major problem is in constraining the participants, who frequently use instruments from a variety of manufacturers, to a rigid set of conditions. The complexity of the instrumentation leads

Table 1
Target compounds, their chemical structures and chemical names

Compound name	Chemical structure	Chemical name
Arkopal N100		Nonylphenolpolyglycol ether ($NPEO_x$)
Marlamid DF 1218		Coconut diethanolamide (C_nDEA)
Marlon A 350		Linear alkylbenzene sulfonate (C_n LAS)
Rewopol NOS 5		Nonylphenolpolyglycol ether sulfate ($NPEO_x$-SO_4)
Genapol C 050		Alkylpolyglycol ether (C_nEO_x)
Marlon PS 65		Secondary alkane sulfonate (SAS)

to many variables contributing to performance. For example, in the case of LC–MS inter-laboratory studies, the geometry of the interface, the design of the nebulizer and desolvation chamber of atmospheric pressure interfaces are generally different from each manufacturer. Therefore, specifying an exact parameter may be counter-productive to the goals of the inter-laboratory study. It has been observed that the more experienced the instrument operator, the more likely the laboratory will perform according to the method under study [12]. Consequently, a specific set of instructions was given to the laboratories to follow essential features of the method (e.g.,, calibration standards, elution procedures), but allowing other parameters to be optimized by the participant depending upon the results from their own calibration methods.

The goal of this inter-laboratory exercise and consequently of the work to be carried out was (1) to help the improvement of analytical procedures for surfactant determination by investigating the analytical state-of-the-art in the determination of surfactants, (2) to compare different analytical determination methods, like LC–fluorescence detection (FL), LC–MS and/or enzyme-linked immunosorbent assay (ELISA) employed in the inter-laboratory exercise, and finally (3) to evaluate LC–MS methodology with atmospheric pressure interfaces for the

determination of surfactants. Although some inter-laboratory exercises have been reported using TSP and particle beam (PB) interfaces [12–14], inter-laboratory exercises using LC and/or LC–MS with atmospheric pressure interfaces for the determination of surfactants were not reported until now.

2. Experimental

2.1. Description of the inter-laboratory exercise

Seven European laboratories participated in an inter-laboratory exercise for the determination of surfactants. Four of them were using LC–MS techniques, three using LC–FL and one used a test-tube ELISA kit. Cartridges previously loaded with spiked municipal effluent samples and non-spiked tannery effluent samples were dried and stored at 4°C. These cartridges were given to all participants together with appropriate standards. An analytical protocol was distributed among the participants describing in detail the elution procedure and the minimum analytical requirements. The results and the description of the analytical procedures had to be reported and are included below.

2.2. Selection of test compounds

Based on their use for industrial and domestic applications and abundance in environmental samples, six surfactants were selected by the participants and are listed in Table 1. Two different standard mixture solutions (standards 1 and 2) were distributed to all participants for the preparation of the calibration graphs. Standard 1 contained Arkopal N100 (nonylphenolpolyglycol ether, $NPEO_x$), Marlamid DF 1218 (coconut fatty acid diethanolamide, C_nDEA) and Marlon A 350 (linear alkylbenzenesulfonate, C_n LAS) at 1000 mg/l for each compound, and standard 2 contained Rewopol NOS 5 (nonylphenolpolyglycol ether sulfate, $NPEO_x$-SO_4), Genapol C 050 (alkylpolyglycol ether, C_nEO_x) and Marlon PS 65 (secondary alkane sulfonate, SAS) at 1000 mg/l for each compound. These blends were also used by laboratory 3 for the preparation of the spiked wastewaters.

2.3. Participants

A total number of seven laboratories from four different European countries participated in the programme. The present study takes into account the results of these laboratories in order to evaluate the programme. Fixed codes were assigned to each participant and are presented in Table 2.

2.4. Sample preparation procedures

Sample preparation procedures were common to all laboratories in order to eliminate the error associated to this critical step and enabling to evaluate the performance of the analytical method. Thus ready-to-elute cartridges and instructions for elution were distributed to the participants. Laboratory 3 was responsible for preparation of spiked samples, preconcentration in octadecylsilica (C_{18}) cartridges and distribution of cartridges to the participants. Municipal effluent samples were spiked with standards 1 and 2 to produce samples 1 and 2, respectively. A 200-ml volume of samples 1 and 2 was loaded on a C_{18} cartridge. Blank samples were also run along with the spiked ones. Each participant received five cartridges from laboratory 3 corresponding to two replicates from the preconcentration of spiked samples 1 and 2 and a blank sample. Selective elution according to an elution protocol described elsewhere [15] was applied to the C_{18} cartridges in order to obtain four fractions.

Laboratory 1 was responsible for preconcentration of 200 ml of non-spiked tannery wastewater according to a SSPE methodology described elsewhere [16]. An octadecylsilica (C_{18}) sorbent LiChrolut RP (500 mg, 6 ml) and a styrene–divinylbenzene sorbent LiChrolut EN (200 mg, 6 ml), both from Merck, were used in series. This type of extraction allowed one to achieve clean-up and extraction of target analytes from very complex matrices in the same analytical step. Therefore, four cartridges were delivered to each participant corresponding to two replicates of the preconcentration of the tannery wastewater for the analysis of target surfactants. Differential elution according to the elution protocol of the SSPE method [2] was applied to the C_{18} cartridges in order to obtain three different extracts containing the analyzed compounds. Two portions of

Table 2
Analytical procedures and chromatographic conditions used during the inter-laboratory exercise by each participant (except laboratory 4 using test-tube ELISA)[a]

Laboratory	Analytical procedure	Chromatographic conditions				
		Mobile phase A	Mobile phase B	Gradient		Column
				t (min)	% B	
1	LC–APCI-MS LC–ESI-MS	ACN–MeOH (1:1), 0.5% HAcO	Water, 0.5% HAcO	0 10 20	60 60 0	Hypersil Green ENV (125×4.6 mm, 5 μm)
2	LC–FL	ACN	Water, 14 g/l NaClO	0 3 23 26	60 60 30 10	LiChrospher RP-18 (250×4.6 mm, 5 μm)
3	FIA–ESI-MS	Water–MeOH (1:1), 1% HAcO	–	–	–	Bypassing analytical column
	FIA–APCI-MS FIA–MS–MS	Water–MeOH (3:7), 0.05 *M* NH_4AcO	–	–	–	Bypassing analytical column
	LC–ESI/APCI-MS LC–UV	MeOH	Water–MeOH (8:2)	0 15	90 10	Spherisorb 5 ODS 2 (125×4.6 mm, 5 μm)
5	LC–ESI-MS ($NPEO_x$)	Water–MeOH (50:50), 20 m*M* NH_4AcO	MeOH–water (95:5)	1 12	60 99	Supelcosil LC-18 (250×2.1 mm, 5 μm)
	LC–ESI-MS (C_n LASs)	MeOH–water (10:90)	MeOH–water (90:10)	1 16	60 100	RPC_{18} Hypersil MOS (250×2.1 mm, 5 μm)
	LC–ESI-MS (C_nDEA)	Water–MeOH (50:50), 20 m*M* NH_4AcO	MeOH–water (95:5)	1 15	40 100	Supelcosil LC-18 (250×2.1 mm, 5 μm)
6	LC–ESI-MS (C_nDEA, $NPEO_x$)	MeOH	Water	0 5	40 5	LiChrospher RP-18 (125×2 mm, 3 μm)
	LC–ESI-MS (C_n LAS)	MeOH, % TEA, % HAcO	Water, % TEA, % HAcO	0 4	20 5	LiChrospher RP-18 (125×2 mm, 3 μm)
	LC–ESI-MS (C_nEO_x)	MeOH	Water	0 4 5	10 10 5	LiChrospher RP-18 (125×2 mm, 3 μm)
	LC–ESI-MS ($NPEO_x$-SO_4, SAS)	MeOH, % TEA, % HAcO	Water, % TEA, % HAcO	0 10 17 18	70 10 10 5	LiChrospher RP-18 (125×2 mm, 3 μm)
	LC–FL ($NPEO_x$)	MeOH	Water	0	20	LiChrospher RP-18 (125×2 mm, 3 μm)
7	LC–FL	MeOH, 10 g/l $NaClO_4$	Water, 10 g/l $NaClO_4$	0	20	LiChrosorb RP-8 (250×4.6 mm, 10 μm)

[a] ACN denotes acetonitrile, MeOH denotes methanol, HAcO denotes acetic acid, NH_4AcO denotes ammonium acetate, TEA denotes triethylammine.

5 ml of methanol were passed through the LiChrolut EN phase in order to elute surfactant derivatives and polar related compounds. Finally, the eluates were collected and evaporated with a gentle stream of nitrogen. Prior to analysis the extracts were reconstituted to a final volume of 1 ml in the appropriate HPLC mobile phase.

2.5. Analytical procedures

Several analytical methods have been applied for the analysis of the same samples during the presented inter-laboratory exercise. Table 2 summarizes all the applied methods and the chromatographic conditions for laboratories using LC separation. In addition, laboratory 3 used flow injection analysis (FIA) in combination with MS detection (FIA–MS) and tandem MS detection (FIA–MS–MS) in order to obtain a first screening of the qualitative surfactant content [11]. Regarding the chromatographic conditions, all participants were using reversed-phase chromatography (except laboratory 4) with different C_{18} phases and appropriate mobile phases depending on the performance of the instrument (see Table 2). The flow-rate for column separation using gradient elution was 1 ml/min in all cases with the exception of laboratories 5 and 6 which used 0.2 ml/min and 0.25 ml/min, respectively, due to different LC column dimensions. Laboratory 3 added 0.5 ml/min of 0.1 *M* ammonium acetate to the mobile phase after passing the UV-diode array detector, where a control of aromatic surfactants took place, resulting in an overall flow-rate of 1.5 ml/min. The post-column split ratio was 1:2 in favor of the MS in atmospheric pressure chemical ionization (APCI) and ESI mode or waste, respectively.

The analytical parameters for LC–MS used by laboratories 1, 3, 5 and 6 are presented in Tables 3 and 4. Regarding laboratories 2 and 7 using LC–FL, the fluorescence detection was accomplished with an excitation wavelength of 225 nm and an emission wavelength of 295 nm; whereas laboratory 6 used excitation and emission wavelengths of 225 nm and 310 nm, respectively. Finally, laboratory 4 used a previously developed test-tube ELISA kit [16] for the determination of alkylphenol ethoxylates. The

Table 3
LC–MS systems and analytical parameters used by participating laboratories 1, 3, 5 and 6[a]

Parameter	Laboratory 1:		Laboratory 3:			Laboratory 5:	Laboratory 6:
	VG Platform: MS mode		TSQ 700 MS mode		MS–MS mode:	PE Sciex 150: MS mode:	Thermoquest Navigator aQa: MS mode:
	APCI	ESI	ESI	APCI	APCI	ESI (TurboIon)	ESI
Vaporizer temperature (°C)	400	400	–	400	400	200	–
Source temperature (°C)	150	150	–	–	–	–	220
Capillary temperature (°C)	–	–	200	200	200	–	–
Spray voltage (kV)	–	3.7	4.5	–	–	+4.5/−3.0	4
Cone voltage (V)	30	–	–	–	–	+44/−36	20
Corona current or discharge	3 kV	–	–	4 μA	4 μA	–	–
Capillary lens voltage (V)	–	–	10	10	10	–	–
Tube lens voltage (V)	200	200	40	40	40	–	–
Octapole voltage (V)	–	–	−3	−3	−3	–	–
Electron multiplier (V)	–	–	1200	1200	1200–1700	1800	650
Conversion dynode (kV)	–	–	15	15	15	–	–
Collision energy (eV)	–	–	–	–	−10/−50	–	–
Sheath gas pressure (p.s.i.)	–	–	40	40	40	–	–
Drying gas flow (l/h)	300	300	–	–	–	–	–
Nebulizing gas flow (l/h)	10	10	–	–	–	10	–
Ion source pressure (Torr)	–	–	0.3	0.3	0.5	–	–
Collision gas	–	–	–	–	Ar	–	–
Collision cell pressure (mTorr)	–	–	–	–	1.0	–	–

[a] 1 p.s.i. = 6894.76 Pa; 1 Torr = 133.322 Pa.

Table 4
M/z ions used for quantification of target analytes with the different interfaces used in the inter-laboratory exercise

Compound	Laboratory 1	Laboratory 3	Laboratory 5	Laboratory 6
$NPEO_x$	APCI (PI)	APCI (PI)	ESI (PI)	ESI (PI)
	m/z: 133, 177, 271, 291, 419, 573	m/z: 133, 271, 291, 419	m/z: 502, 546, 590, 634, 678, 722, 766, 810, 854	m/z: 419, 458, 502, 546, 590, 634, 678, 722, 766, 810, 854
C_nDEA	APCI (PI)	APCI (PI)	ESI (PI)	ESI (PI)
	m/z: 88, 106	m/z: 88, 106	m/z: 254, 282, 310, 338	m/z: 232, 254, 260, 282, 288, 310
C_n LAS	ESI (NI)	ESI (NI)	ESI (NI)	ESI (NI)
	m/z: 325	m/z: 183, 297, 311, 325, 339	m/z: 183, 297, 311, 325, 339	m/z: 297, 311, 325, 339
$NPEO_x$-SO_4	ESI (NI)	ESI (NI)	n.d.[a]	ESI (NI)
	m/z: 475	m/z: 475		m/z: 343, 387, 431, 475, 519, 563, 607, 651, 695
C_nEO_x	APCI (PI)	APCI (PI)	n.d.	ESI (PI)
	m/z: 133, 177, 151, 195, 465	m/z: 133, 151, 195, 465		m/z: 417, 445, 489, 533, 473, 517, 561, 457, 501, 545
SAS	ESI (NI)	ESI (NI)	n.d.	ESI (NI)
	m/z: 291	m/z: 291		m/z: 263, 277, 291, 305, 319

[a] n.d., Denotes not determined.

methodology employed by laboratory 4 only detected Arkopal N100 (nonylphenolpolyglycol ether NPEO). Briefly, the protocol consisted of the following steps: first, the antigen–enzyme (HRP) conjugate powder was reconstituted with 7 ml of buffer solution. The resulting solution was mixed with the sample or standard solution (1:1) and 0.5-ml aliquots of this mixture were dispensed into each antibody coated tube for incubation during 60 min at room temperature. For the washing step, six-fold wash solution was diluted with distilled water (1:5) and each tube was rinsed three times with 3 ml of this solution. In order to allow the chromogenic reaction, the chromogen solution and the substrate solution were mixed (1:100) and 0.5 ml of the mixture were dispensed into each tube. The reaction took place at room temperature and it was stopped after 30 min by adding 0.5 ml of stop solution. Finally, the absorbance at 450 nm was measured in order to quantify samples with the previously obtained standard curve.

2.6. Quantitation

External calibration was used for quantitation of target analytes. No internal standard was used for quantification purposes as the broad range of compounds made difficult its selection. A series of injections of standards 1 and 2 were used to obtain the calibration equations. The area under the selected ion currents were used for quantitation. Therefore, each participant carried out its own quantitation depending on the chosen m/z ions. As an example Figs. 1–4 include separation and spectra of target analytes allowing one to select appropriated ion currents for quantitation purposes. The quantitative results obtained for the spiked and real environmental wastewater samples are shown in Tables 5 and 6.

2.7. Statistical analysis

Summary statistics (s_R, RSD_R) were calculated for the average concentration results and for the overall method precision (see Tables 7 and 8) corresponding to the six different target analytes. The overall standard deviation (s_R, reproducibility) indicates the precision associated with measurements generated by a group of laboratories. The reproducibility relative standard deviation (RSD_R), which was determined from the reproducibility standard deviation (s_R) and the average concentration at a particular concentration level, is an indication of the inter-laboratory method precision. Summary statistic results are listed in Tables 7 and 8.

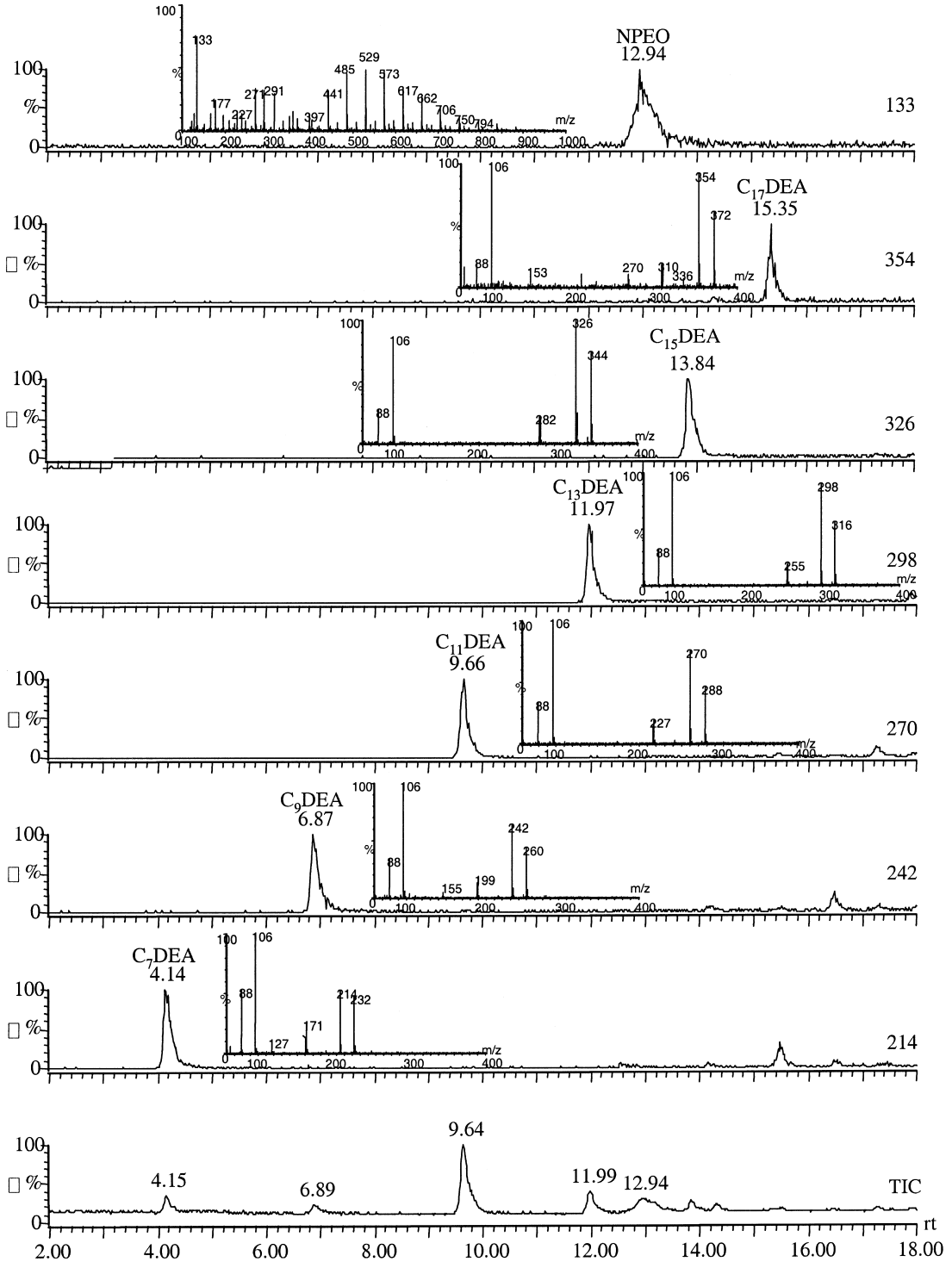


Fig. 1. Chromatogram and spectra of standard 1 obtained by laboratory 1 using LC–APCI-MS in the positive ionization mode: separation of coconut fatty acid diethanolamides (C_nDEAs) and nonylphenolpolyglycol ether ($NPEO_x$). rt = Retention time in min.

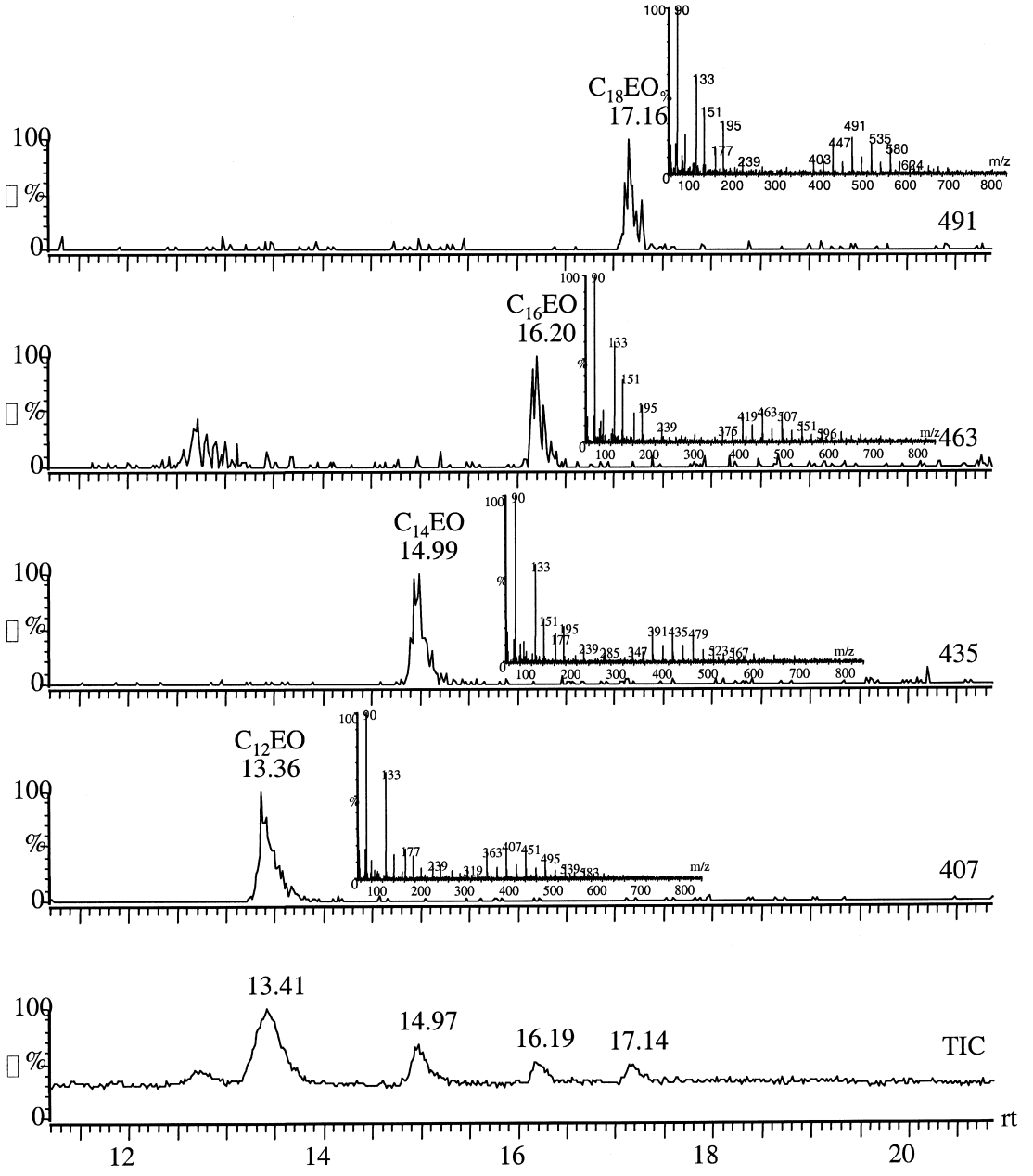


Fig. 2. Chromatogram and spectra of standard 2 obtained by laboratory 1 using LC–APCI-MS in the positive ionization mode: separation of alkylpolyglycol ether (C_nEO_x). rt = Retention time in min.

3. Results and discussion

3.1. General comments

The results obtained for the analysis of diverse classes of surfactants by different analytical methodologies are listed in Tables 5 and 6. Laboratories 1, 3 and 6 gave the concentration results for all target analytes, laboratory 5 analyzed nonylphenolpolyglycol ether, linear alkylbenzene sulfonates

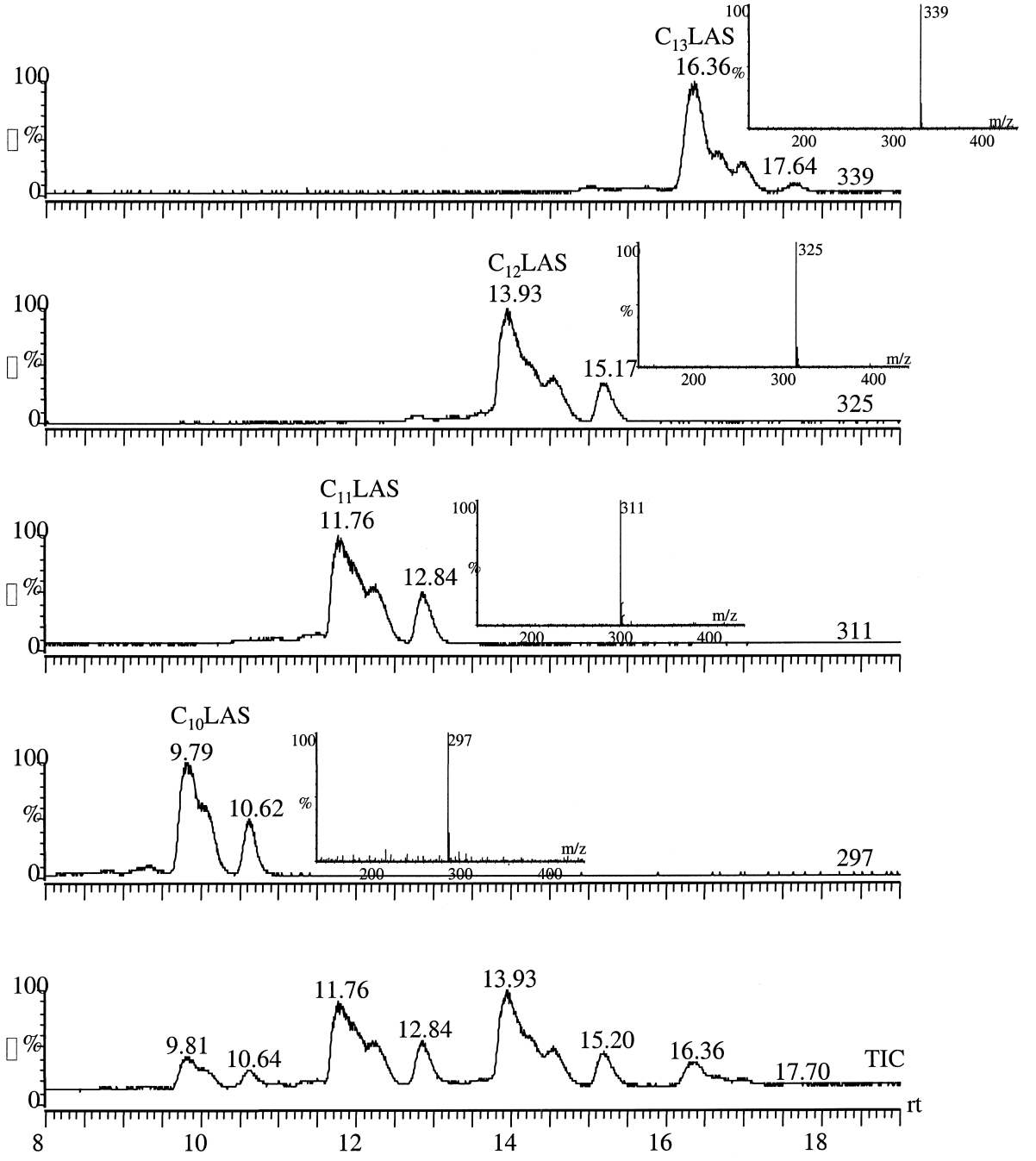


Fig. 3. Chromatogram and spectra of standard 1 obtained by laboratory 1 using LC–ESI-MS in the negative ionization mode: separation of linear alkylbenzene sulfonates (C_n LASs). rt = Retention time in min.

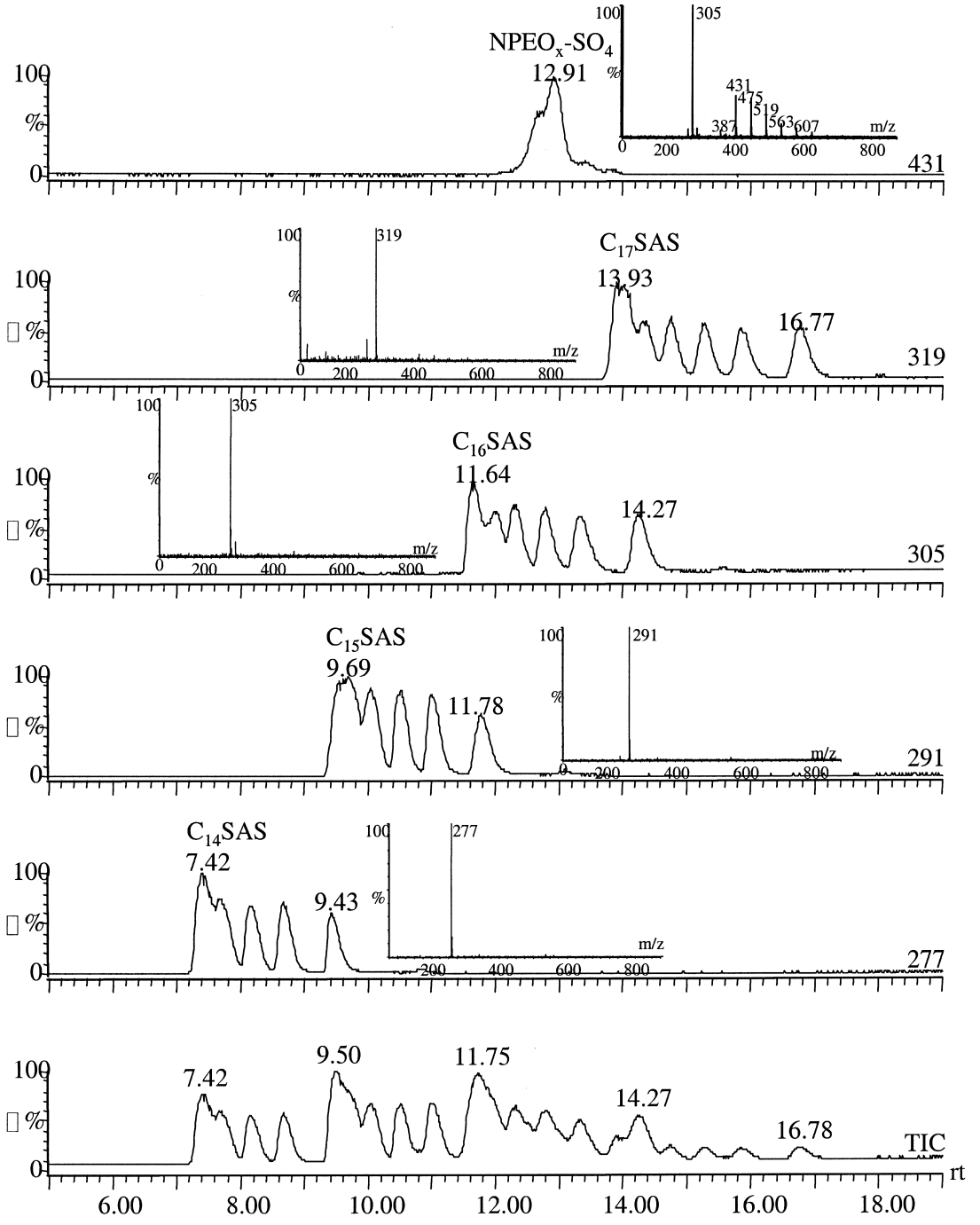


Fig. 4. Chromatogram and spectra of standard 2 obtained by laboratory 1 using LC–ESI-MS in the negative ionization mode: separation of secondary alkane sulfonates and nonylphenolpolyglycol ether sulfate (NPEO$_x$-SO$_4$). rt = Retention time in min.

Table 5
Results obtained for the analysis of spiked wastewaters

Compound	Quantified values (mg/l)							
	Spiked level	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6	Laboratory 7
$NPEO_x$	1.8	1.61	1.22[a]	1.70	1.99	1.89	1.75 1.53[a]	n.d.
C_nDEA	1.2	0.56	n.d.[b]	1.20	n.d.	0.55	1.00	n.d.
C_n LASs	1.2	0.88	0.95[a]	1.11	n.d.	1.11	1.52	1.02[a]
$NPEO_x$-SO_4	2.0	2.58	1.66[a]	1.82	n.d.	n.d.	2.09	n.d.
C_nEO_x	1.8	1.32	n.d.	1.77	n.d.	n.d.	2.38	n.d.
SAS	1.6	2.51	n.d.	1.70	n.d.	n.d.	1.72	n.d.

[a] Result obtained by LC–FL.
[b] n.d., Denotes not determined.

Table 6
Results obtained for the analysis of wastewaters

Compound	Results (μg/l)						
	Laboratory 1	Laboratory 2	Laboratory 3	Laboratory 4	Laboratory 5	Laboratory 6	Laboratory 7
$C12EO_x + C13EO_x$	129	n.d.[a]	175	n.d.	n.d.	n.d.	n.d.
C_n LASs	<0.2	n.m.[b]	<0.2	n.d.	<0.2	n.d.	14

[a] n.d., Denotes not determined.
[b] n.m., Denotes not measured due to coelution problems.

Table 7
Inter-laboratory method precision for the determination of six target analytes by different analytical strategies

Compound	Spiked level (mg/l)	Average result (mg/l)	s_R	RSD_R (%)
$NPEO_x$	1.8	1.67	0.25	6.4
C_nDEA	1.2	0.83	0.33	10.6
C_n LASs	1.2	1.10	0.23	5.1
$NPEO_x$-SO_4	2.0	2.04	0.40	16.2
C_nEO_x	1.8	1.82	0.53	28.3
SAS	1.6	1.97	0.46	21.3

Table 8
Inter-laboratory method precision for the determination of six target analytes by LC–MS techniques

Compound	Spiked level (mg/l)	Average result[a] (mg/l)	s_R[a]	RSD_R[a] (%)
$NPEO_x$	1.8	1.79	0.15	2.3
C_nDEA	1.2	0.83	0.32	10.6
C_n LASs	1.2	1.16	0.26	7.1
$NPEO_x$-SO_4	2.0	2.2	0.38	14.8
C_nEO_x	1.8	1.82	0.53	28.3
SAS	1.6	1.97	0.46	21.3

[a] Values calculated removing non-LC–MS produced results.

and coconut fatty acid diethanolamide, laboratory 2 used LC–FL enabling aromatic compound analysis, laboratory 7 analyzed C_n LASs and laboratory 4 analyzed $NPEO_x$.

Comparison of the different employed techniques was feasible by considering results provided for $NPEO_x$ which was the only compound determined by all the laboratories. Although laboratory 4 did not use chromatographic techniques, acceptable results for the determination of nonylphenolpolyglycol ether were given as compared to the other laboratories (see Tables 5 and 7). This ELISA determination (laboratory 4) gave the highest value for $NPEO_x$ concentration. This fact is common when applying ELISA methods for environmental analysis since cross reacting substances may interfere and give additional signals in the biological assay [17]. The cross-reactivity (CR) pattern of APE ELISA kit (Takeda Chemical Industries) used in the present work is shown in Table 9 and indicates the high selectivity for the analysis of $NPEO_x$ related to this technique. The results obtained by LC–FL (laboratories 2 and 6) highlight the fact that they were the most uncertain ones and the lowest for the determination of $NPEO_x$, indicating an underestima-

Table 9
Cross-reactivity (CR) pattern of APE ELISA kit (Takeda Chemical Industries)

Compound	Ethoxy chain length	CR (%)
Nonylphenol ethoxylate	10	100
Nonylphenol ethoxylate	7.5	107
Nonylphenol ethoxylate	5	136
Nonylphenol ethoxylate	2	87
Nonylphenol	–	7
Octylphenol ethoxylate	10	125
Linear alkylbenzene sulfonate	–	<0.2
Sodium laurate	–	<0.2
Sodium lauryl sulfate	–	<0.2
Alkylether sulfate	–	<0.2
Phenol	–	<0.2
Polyethylene glycol	–	<0.2

tion of this compound's concentration. This is due to the fact that no specific wavelengths for the determination of $NPEO_x$ were used in the case of laboratory 2. More accurate LC–FL results were obtained in the case of laboratory 6 using optimal wavelengths for the determination of $NPEO_x$ and also by laboratories 2 and 7 for C_n LAS analysis (see Table 5). Comparison of the three techniques (LC–FL, LC–MS and ELISA) for the determination of $NPEO_x$ leads to the fact that LC–MS is the most accurate and precise technique enabling, in addition, unequivocal identification by its LC–MS spectra.

The values for s_R and RSD_R (see Tables 7 and 8) used to calculate the inter-laboratory precision indicated that the most accurate result corresponded to that given for linear alkylbenzene sulfonates (C_n LASs). These types of surfactants are widely used and routinely determined demonstrating that the more experienced the analyst is, the lower the deviation from the target value. The accuracy of the results obtained by LC–MS determination of C_n LASs was similar to that obtained by the well-established technique of LC–FL. These results demonstrated that LC–MS technology is almost as mature as LC–FL techniques.

Overall, we should comment that although very different methodologies and analytical conditions were used for the analysis of a complex mixture of compounds, acceptable reproducibility values were obtained in this inter-laboratory study. Considering the principles established by Aquacheck [18], a well-known organization distributing inter-laboratory exercises, all the obtained results were within an acceptable range except those corresponding to alkylpolyglycol ether for which a double flagged error was obtained (28.3%).

3.2. LC–MS evaluation

Regarding LC–MS techniques, they led to lower or equal deviations from target values as compared to the well-established LC–FL techniques, as has been mentioned before (see results for $NPEO_x$, $NPEO_x$-SO_4 and C_n LASs in Table 5). The inter-laboratory precision for LC–MS results varied between 2.3 and 28.3% (in terms of RSD_R) indicating the good reproducibility of LC–MS considering that four different instrumentations were used. For this reason, LC–MS techniques with atmospheric pressure interfaces are gaining acceptance as a reliable analytical technique allowing not only determination but also identification of polar common industrial contaminants and related compounds [2]. Figs. 1–4 show the LC–APCI-MS and LC–ESI-MS traces of the different standard mixtures used for the inter-laboratory studies and analyzed by laboratory 1.

The most inaccurate LC–MS result corresponded to that obtained for alkylpolyglycol ether (C_nEO_x). This is due to the fact that this product corresponds to a mixture of different homologues (from C_{12} to C_{18}) and ethoxymers (average $x=5$) whose spectrum is the most complex one (compared to the other analyzed surfactants) as can be seen in Fig. 2, including full scan (FS) spectra of target compounds. Relative abundances of high m/z ions in the spectra corresponding to ethoxylated species can suffer some variations from analysis-to-analysis due to different ionizations promoted by small differences in mobile phase, nebulization temperature, nitrogen flow, etc. Consequently, quantification based on high m/z ions leads to the most variable results in the present study. As an example, laboratory 6 which was the only group using high m/z ions for the quantification of C_nEO_x, produced the following variable results: 1.5, 2.3 mg/l; next day results: 3.1, 2.5, 2.5 and 2.4 mg/l (spiked value: 1.8 mg/l).

On the other hand, the most accurate result corresponded to that obtained for C_n LASs (see separation and spectra in Fig. 3). The lowest C_n LAS concentration was obtained by laboratory 1 who used

the quantitation m/z ion corresponding to C_{12} LAS. Laboratories 3, 5 and 6 used all the m/z ions common to C_n LAS and laboratories 2 and 7, using fluorescence detection, also used all four chromatographic peaks for C_n LAS quantitation, explaining why laboratory 1 results are somewhat lower compared to the others.

In general terms, the variation from laboratory to laboratory (inter-laboratory) was greater than that attributed to the analytical error displayed within laboratories (intra-laboratory). There are many reasons for the inter-laboratory variation that can be attributed to operational parameters such as mobile phase flow-rate, mobile phase and buffer composition, vaporizer temperature, tip temperature and source temperature, among others.

Regarding the mass spectra, the mass fragmentation patterns were quite similar and the most intensive m/z ions were the same in all cases (corresponding to the molecular ion) whereas some differences where observed for the relative abundance of high m/z ions as mentioned before for C_nEO_x. These differences were mainly attributed to main operational parameters that control fragmentation such as cone voltage and probe temperature for APCI interfaces and capillary voltage (spray voltage) in case of ESI interfaces. It should also be taken into account that different instrumentation and analytical conditions were used as reported in Tables 3 and 4.

3.3. Real samples

Three (laboratories 1, 3 and 7) out of the seven laboratories participating in the inter-laboratory study analyzed real tannery effluents using their own LC–MS methodology.

In spite of being a very complex matrix, the SSPE protocol used for the simultaneous sample clean-up and extraction permitted the efficient concentration of target surfactants and the elimination of interferences [2]. Among the group of target surfactants, only non-ionic ones were present in the sample belonging to two series of homologues with C_{12}-(dodecanol polyethoxylate) and C_{13}-alkyl chains (tridecanol polyethoxylate). The non-ionic polyethoxylated surfactant mixture was determined by APCI-MS in the positive ionization (PI) mode and with the operational parameters established in Table 3. External calibration with the industrial blend Marlipal 013/90 containing the alcohol polyethoxylated surfactants (C_nEO_x; $n=12$ and 13; $x=2–15$) present in the tannery wastewater was performed. The results obtained by the participants are listed in Table 6 and they are in agreement for those using LC–MS considering that an inter-laboratory precision of 28.3% was obtained in this exercise for the determination of C_nEO_x. Attempt to quantify C_n LASs by LC–FL (see laboratory 7 in Table 6) led to the quantification of an interference as LC–MS methodologies showed the absence of this compounds above the limit of detection (0.2 μg/l, average limit of detection for all laboratories). Therefore, application of LC–FL for the analysis of complex industrial effluents should be performed with further confirmation, such as the used of LC columns of different polarity and/or MS detection.

4. Conclusions

The inability to impose strict guidelines was a source of statistical difficulties for the interpretation of the results obtained in this inter-laboratory study, but interesting and useful data, were still obtained. Reproducibility values (represented by the RSD_R) ranged between 5.1 to 28.3% indicating that the results were in the acceptable range of precision for an inter-laboratory exercise. Therefore, the robustness of the employed methodologies has been demonstrated. Three different analytical methods were evaluated: ELISA, LC–FL and LC–MS.

The test tube ELISA used in the present work has shown a very good performance for the determination of nonylphenolpolyglycol ether in spiked municipal wastewaters. This technique is a very valuable tool for screening purposes of target analytes in wastewaters. Further development should be centered on new devices for the determination of other common surfactants.

Regarding LC–FL methodologies, although it is a suitable tool for the determination of aromatic surfactants, there is still the need for improvement in order to obtain more accurate results. The use of acquisition windows with optimal emission and excitation wavelengths should improve the precision of this methodology. Application of LC–FL to the

analysis of surfactants in complex industrial effluents needs confirmation, either MS or LC columns of different polarity.

In spite of LC–MS performance depending on numerous instrumental parameters (e.g., mobile phase flow-rate and composition, vaporizer temperature, cone, probe and capillary voltages), the state-of-the-art LC–MS methodologies are comparable to other classical techniques such as LC–FL as shown by the results. Improvements could be reached in inter-laboratory exercises by imposing adequate m/z ions for quantification to all participants. The chosen m/z ions should include common ions for all the homologues present in target compound as well as high m/z ions frequently corresponding to the most abundant oligomers. Method performance in LC–MS was also addressed by the application of LC–MS strategies to the analysis of a real-world tannery wastewater. The results led to the determination of alcohol polyethoxylates at a concentration ranging from 0.129 to 0.175 mg/l.

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References

[1] L.D. Betwoski, D.S. Kendall, C.M. Pace, J.R. Donnelly, Environ. Sci. Technol. 30 (1996) 3558.
[2] M. Castillo, M.C. Alonso, J. Riu, D. Barceló, Environ. Sci. Technol. 33 (1999) 1300.
[3] Aaerobic Degradation of Surfactants, Scientific Review 1999, Commissioned by ERASM (Environmental Risk Assessment Steering Committee).
[4] A.T. Kiewiet, J.M.D. Van der Steen, J.R. Parsons, Anal. Chem. 67 (1995) 4409.
[5] N.J. Fendinger, W.M. Begley, D.C. McAvoy, W.F. Eckhoff, Environ. Sci. Technol. 29 (1995) 856.
[6] T.M. Schmitt, M.C. Allen, D.K. Brain, K.F. Guin, D.E. Lemmel, Q.W. Osburn, J. Am. Oil Chem. Soc. 67 (1990) 103.
[7] A.A. Boyd-Boland, J.B. Pawliszyn, Anal. Chem. 68 (1996) 1521.
[8] C. Crescenzi, A. Di Corcia, A. Marcomini, R. Samperi, Anal. Chem. 67 (1995) 1797.
[9] F. Ventura, D. Fraisse, J. Caixach, J. Rivera, J. Anal. Chem. 63 (1991) 2095.
[10] M. Castillo, F. Ventura, D. Barceló, Waste Management 19 (1999) 101.
[11] H.Fr. Schröder, J. Chromatogr. A 647 (1993) 219.
[12] T.L. Jones, L.D. Betowski, L. Lopez-Avila, Trends Anal. Chem. 13 (1994) 333.
[13] V. Lopez-Avila, T.L. Jones, J. AOAC Int. 76 (1993) 1329.
[14] T.L. Jones, L.D. Betowski, B. Lesnik, T.C. Chiang, J.E. Teberg, Environ. Sci. Technol. 25 (1991) 1880.
[15] H.Fr. Schröder, Waste Management 19 (1999) 111.
[16] J. Emnéus, personal communication.
[17] M. Castillo, A. Oubiña, D. Barceló, Environ. Sci. Technol. 32 (1998) 2180.
[18] Aquacheck, Water Research Center (WRC), Medmenham, UK.