Identification of Polar, Ionic, and Highly Water Soluble Organic Pollutants in Untreated Industrial Wastewaters

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This paper presents a generic protocol for the determination of polar, ionic, and highly water soluble organic pollutants on untreated industrial wastewaters involving the use of two different solid-phase extraction (SPE) methodologies followed by liquid chromatography—mass spectrometry (LC-MS). Untreated industrial wastewaters might contain natural and synthetic dissolved organic compounds with total organic carbon (TOC) values varying between 100 and 3000 mg/L. All polar, ionic and highly water soluble compounds comprising more than 95% of the organic content [Betowski, L. D.; Kendall, D. S.; Pace, C. M.; Donnelly, J. R. *Environ. Sci. Technol.* **1996**, *30*, 3558] and with major contribution to the total toxicity of the sample cannot be analyzed by conventional gas chromatography—mass spectrometry (GC-MS), and LC-MS is a good alternative. In this work two extraction procedures were used to obtain fractionated extracts of the nonionic polar compounds: a polymeric Isolute ENV+ SPE cartridge for the preconcentration of anionic analytes and a sequential solid-phase extraction (SSPE) method percolating the samples first in octadecylsilica cartridge in series with the polymeric Lichrolut EN cartridge. Average recoveries ranging from 72% to 103% were obtained for a variety of 23 different analytes. Determination of nonionic pollutants was accomplished by reverse-phase liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS), while anionic compounds were analyzed by ion pair chromatography-electrospray-mass spectrometry (IP-ESI-MS) and LC-ESI-MS. This protocol was applied to a pilot survey of textile and tannery wastewaters leading to the identification and quantification of 33 organic pollutants including phenolic compounds, benzothiazoles, nonionic surfactants (nonylphenol- and alcohol poly-ethoxylates and poly(ethylene glycol)), anionic surfactants (alkylbenzenesulfonates), benzene- and naphthalene sulfonates, and related industrial compounds. In the case of textile wastewaters, poly(ethylene glycol) (PEG), decyl alcohol polyethoxylate ($C10EO_5$), and 2-naphthalene-sulfonate were found as major pollutants in concentrations up to 1.2, 3.5, and 2.4 mg/L, respectively. Main contaminants in tannery wastewaters were poly(ethylene glycol), 4-chloro-*m*-cresol, diethylphthalate, and polyethoxylate tridecyl

alcohol ($C13EO_4$) with levels of 2.3, 2.3, 2.0, and 1.1 mg/L, respectively.

Introduction

Commonly, analysis of industrial wastewater constituents has to face a complex mixture of various organic substances that hamper the extraction and analysis of the compounds under investigation. This is especially true for untreated wastewater from leather and textile production that is heavily loaded with inorganic (e.g. chromium derivatives) and organic constituents. Its purification is an important task worldwide as some polar pollutants in these wastewaters are hardly or nonbiodegradable or can be biotransformed to persistent and toxic metabolites during their degradation (*1–5*). A significant fraction of this group corresponds to surfactants which are discharged with wastewater after application. Nonionic surfactants (nonylphenol ethoxylates (NPEO) and alcohol ethoxylates (AEO)) are being used increasingly to remove skin grease in the leather industry. Anionic surfactants (linear alkyl benzene sulfonates (LAS)) are also used on large scale in the chemical industry as well as in household applications. In contrast to LAS, which have been found to be readily biodegradable (*6*), other aromatic sulfonates without long alkyl side chains are reported to be persistent, especially naphthalene disulfonates and sulfonated stilbenes (*7*) mainly used as intermediates for the manufacturing of azo dyestuffs and as optical brightners in detergent formulations, respectively. Aromatic sulfonates are commonly used in textile industry as dye bath auxiliaries and rather in tannery industry as dispersants and wetting and suspending agents. Apart from surfactants and aromatic sulfonates, other pollutants may be present in industrial effluents depending on the origin of the sample. For example, the characterization of some phenols, phthalates, and phosphates among other polar pollutants in industrial effluents (petrochemical and hazardous waste) was carried out by ELISA (Enzyme Linked ImmunoSorbent Assay) followed by off-line SPE-LC-APCI-MS (*8*).

The determination of organic pollutants present in industrial effluent discharges that are mixed with the domestic sewage collecting system is a priority in Europe. In this respect, the Directive 91/271/EC indicates that all wastewater in the territories of the European Union (EU) will have to be properly collected and subjected to secondary (biological treatment with secondary settlement) or equivalent treatment before being discharged into estuaries or coastal waters. For the correct implementation of this directive, there is a need to identify the various organic pollutants present in the different wastewaters, specially those of industrial origin.

SPE followed by LC-MS has turned out to be an effective analytical technique allowing the isolation and characterization of contaminants from wastewater matrixes (*9, 10*). The use of LC has been seldom reported in the characterization of polar analytes detected in industrial effluents. The U.S. EPA has published two methods for the analysis of solid waste (SW-846), involving either particle beam (method 8325) (*11*) or thermospray (TSP) (method 8321) (*12*). Both methods were applied to the characterization of many organic acids found in Superfund sites (*1*). LC-TSP-MS was used to confirm that approximately half of the unidentified total organic halo-carbon (TOX) content in leachates from a hazardous waste site was 4-chlorobenzenesulfonic acid (*13*). Few studies using LC-APCI-MS (*8–10*) or LC-ESI-MS (*14–16*) techniques have

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been performed for the analysis of wastewaters. In a previous work from our group (8), we have used APCI for characterizing a variety of organic pollutants in industrial effluents. ESI has been used for the characterization of linear alkylbenzene sulfonates (LAS) and their degradation products in coastal waters (17). However, the complete analysis of polar, ionic, and highly water soluble analytes requires not only LC-APCI-MS but also LC-ESI-MS in combination with a selective tailor-made isolation procedure for each type of analyte.

The objectives of the present work were (i) to determine nonionic and anionic surfactants in complex wastewater matrixes by applying two different analytical methods based on sequential solid-phase extraction (SSPE) followed by LC-APCI-MS (9) and SPE followed by LC-ESI-MS (18, 19), (ii) to apply IP-ESI-MS for the determination of benzene- and naphthalene sulfonates (BS and NPS) in wastewater, and (iii) to use the above-mentioned methods for the identification of other polar compounds present in this type of industrial wastewaters such as phenolic compounds and benzothiazoles among others. The final goal is to achieve a high level of knowledge about the composition and the concentration of polar, ionic, and highly water soluble organic pollutants present in textile and tannery wastewaters in order to comply with the above-mentioned directive. The control of industrial effluent discharges is a very important issue in Europe since under the terms of the Directive 91/271/EC industries that discharge waste into municipal collecting systems and into water treatment plants are required to seek prior authorization. In practice, this permission exists, and many domestic treatment plants receive a high impact of industrial effluents. The comprehensive characterization of such effluents is a priority in water treatment processes in Europe.

Experimental Section

Chemicals and Reagents. LC solvents were obtained from Merck (Darmstadt, Germany), and acetic and sulfuric acid proanalysis grade were from Panreac (Barcelona, Spain) and Merck, respectively. TEA was purchased from Merck. Referring to the standard test substances it should be pointed out the lack of standards for some of the retrieved compounds. All the polyethoxylated surfactants corresponding to a mixture with an average number of ethoxy units were from Kao Corporation (Barcelona, Spain). Phenols were purchased from Merck. Diethylphthalate (DEP) was from Aldrich (Milwaukee, WI, U.S.A.), and bis(2-ethylhexyl)phthalate (DEHP) was a gift from Mario Negri Institute (Milano, Italy). BS and NPS were a gift from AGBAR (Aigües de Barcelona, Barcelona, Spain), and commercial LAS were supplied by Petroquímica Española S.A. in a single mixture standard (not single standard solutions of each LAS). The proportional composition of the different homologues in the standard mixture is C_{10} (3.9%), C_{11} (37.4%), C_{12} (35.4%), C_{13} (23.1%), and C_{14} (0.2%).

Sample Collection. Samples were collected in Pyrex borosilicate glass containers. Each bottle was rinsed with tap water and with high-purity water prior to sample addition. Sample preservation was accomplished by storing the bottles at 4 °C immediately after sampling.

Effluent waters were collected as 24-h composite samples from a tannery and a textile industry located in the area of Porto (Portugal) and in Igualada (Barcelona, Spain). Three different samples from each factory were analyzed. Tannery samples were collected in August 1997 and two of them in October 1997, whereas textile samples were collected in January 1997, September 1997, and May 1998. The average TOC values were of 1960 mg C/L and 330 mg C/L for tannery and textile wastewaters, respectively.

Sample Preparation Procedures. The wastewater samples were filtered with a 0.45 μm membrane filter and allowed to
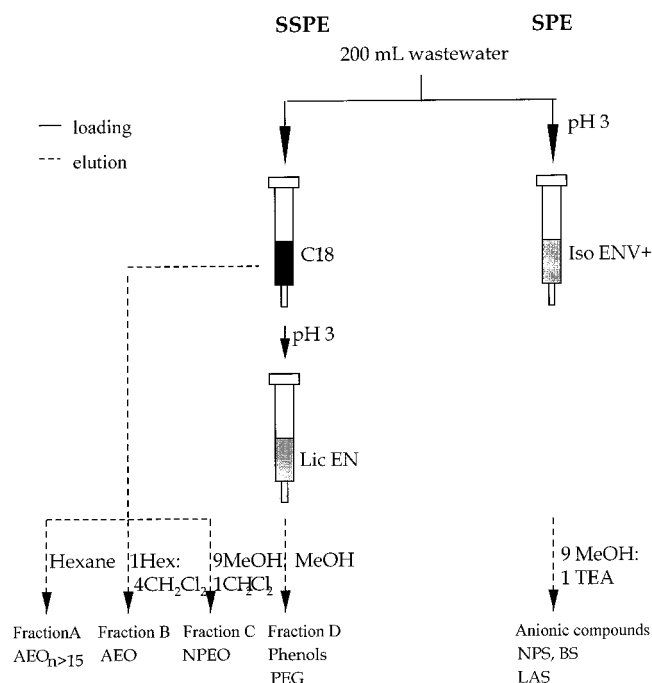

FIGURE 1. Sequence of the SPE- and the SSPE-phases, their elution and the compound classes found in the respective fraction.

equilibrate to room temperature before preconcentration, and afterward, two different extraction procedures were used. All the experiments were performed using an automated sampler processor from Gilson (Villiers-le-Bel, France). A detailed description of this system could be found elsewhere (9).

Extraction of anionic compounds (LAS, BS, and NPS) was accomplished with the styrene-divinylbenzene based sorbent Isolute ENV+ from IST (Cambridge, United Kingdom) (19). The sorbent was conditioned with 7 mL of methanol and 3 mL of water at 1 mL/min and 200 mL of sample acidified to pH 3 were percolated at 15 mL/min. The elution step was performed adding 2 × 5 mL of 5 mM TEA, 5 mM acetic acid/methanol (1:9, v/v) to the cartridge at 1 mL/min, and the obtained extract was concentrated to a final volume of 1 mL with a stream of nitrogen.

The second extraction procedure was a SSPE for preconcentration of nonionic polar pollutants present in highly contaminated industrial matrices (10). Two different sorbents were used: a C18 phase (500 mg, 6 mL) in series with the styrene-divinylbenzene sorbent Lichrolut EN (200 mg, 6 mL) both from Merck. The same conditioning step as that described above was used for both cartridges. A loading volume of 200 mL of wastewater was applied to the C18 cartridge at 5 mL/min, and the residual water was acidified to pH 3 and loaded on the Lichrolut EN cartridge at 15 mL/min that was eluted with 2 × 5 mL of methanol (fraction D). Differential elution was applied to the octadecylsilica cartridge in order to obtain three fractionated extracts. The eluants system utilized for the stepwise desorption were as follows: 2 × 5 mL of hexane was allowed to obtain fraction A, fraction B was eluted from the cartridge with 2 × 5 mL of dichloromethane/hexane (4:1, v/v), and 2 × 5 mL of methanol/dichloromethane (9:1, v/v) led to fraction C. Finally, total evaporation of the extracts was carried out with a stream of nitrogen. The extracts were reconstituted to a final volume of 1 mL in the appropiate HPLC mobile phase prior to analysis.

Figure 1 illustrates the SPE and the SSPE phases, their elution, and the compound classes found in each fraction.

Chromatographic Conditions. Twenty μL of the SSPE and the SPE extracts were injected in the LC systems using the conditions described below.

Water (B) and 50% acetonitrile, 50% methanol (A) both acidified with 0.5% of acetic acid were used for the separation of nonionic pollutants using an Hypersil Green ENV column (150 mm × 4.6 mm i.d., 5 um particle size) equipped with a guard column both from Shandon HPLC (Cheshire, U.K.). The mobile phase composition was 70% B during the first 10 min, then linearly decreased to 0% B in 15 min and kept isocratic for 5 min. The flow rate was maintained at 1 mL/min. In these conditions, AEOs were separated according to the alcoholic alkyl length as a reverse-phase system was employed (20).

Anionic compounds were separated by means of a Hypersil BDS C-18 analytical column from Shandon (Cheshire, U.K.) of 250 mm length and 2 mm internal diameter, with a particle size of 5 μm using water (B) and 80% acetonitrile, 20% water (A) as mobile phase both containing 5 mM triethylamine and 5 mM acetic acid. The elution gradient for the separation of LAS started with 100% B and was linearly decreased to 0% B in 20 min. The flow rate of the mobile phase was of 0.3 mL/min. In the case of BS and NPS the mobile phase composition was 100% B during the first 5 min, then linearly decreased to 95% B in 3 min, and kept isocratic for 10 min; a decrease to 75% B was performed in 17 min, and continuous decrease to 60, 30, and 0% B were performed at min 45, 50, and 55, respectively. The flow rate of the mobile phase was of 0.8 mL/min, and a postcolumn addition of 0.2 mL/min of methanol was necessary.

Analytical Procedures. For LC-APCI-MS experiments a VG Platform from Micromass (Manchester, U.K.) equipped with a standard atmospheric pressure ionization (API) source, which can be configured for APCI or ESI, was used. Source and probe temperatures were set at 150 and 400 °C, respectively, corona discharge voltage was maintained at 3 kV, and the cone voltage was set at 30 V. The HV lens voltage was set at 0.20 kV. Nitrogen was used as nebulizing and drying gas at a flow rate of 10 and 300 L/h, respectively. In full scan mode the m/z range was from 100 to 400 in negative ion (NI) mode and from 100 to 1000 in positive ion (PI) mode of ionization. Characteristic peaks in PI mode at 133 and 177 m/z units for polyethoxylated nonionic surfactants were checked. To distinguish between the different functionalities, the presence of peaks at m/z 151 and 195 were checked for $AEO_{n,x}$, at m/z 271 and 291 for $NPEO_x$, and at m/z 101 and 145 for PEG_x. In addition to this comparison, nonionic polyethoxylated surfactants were identified by checking correspondence with the following expressions: $AEO_{n,x}$ ($C_nH_{2n+1}(OCH_2CH_2)_xOH$) should correspond with $M_W = 14n + 44x + 18$; $NPEO_x$ ($C_9H_{19}-C_6H_4(OCH_2CH_2)_xOH$) with $M_W = 220 + 44x$, PEG_x ($H(OCH_2CH_2)_xOH$) with $M_W = 18 + 44x$, and mono- ($H(OCH_2CH_2)_xOCH_2COOH$) and dicarboxylate polyethoxylated glycol ($HOOC-CH_2(OCH_2CH_2)_xOCH_2COOH$) with $M_W = 76 + 44x$ and $M_W = 134 + 44x$, respectively (10).

The same instrument and conditions were employed for LC-ESI-MS experiments applying a voltage of 3.7 kV to the needle tip. These conditions enabled the analysis of LAS by a two-step approach: a chromatographic run at a cone voltage of −80 V was first performed in order to check the presence of the diagnostic ion $[CH_2=CH-C_6H_4-SO_3]^-$ at m/z 183 and a second run at −20 V permitted to unequivocally identify each of the four $C_{10-13}LAS$ by their $[M-H]^-$ ion with m/z values of z 297, 311, 325, and 339, respectively (18).

For the determination of BS and NPS by IP-ESI-MS, an LC system model 1090 A from Hewlet Packard (Palo Alto, U.S.A.) was used. The detection was accomplished by a model 1040M diode array detector that was coupled on-line with a mass spectrometer HP 1100 HPLC-MSD API-ES all from Hewlett-Packard. Nitrogen was used as nebulizing and drying gas, with a nebulizer pressure of 55 psi and a drying gas flow rate of 12 L/min and a temperature of 350 °C. The capillar voltage was set at 3500 V, and fragmentor voltage was set at 80 and 150 V. In full scan mode the m/z range was from 75 to 350 in negative ion (NI) mode (19). When analyzing NPS and BS by IP-ESP-MS with fragmentor voltage set at 150 V, common losses of the groups HSO_2 or HNO_2 (for nitrosulfonates) were observed; therefore, $[M-HSO_2]^-$ or $[M-HNO_2]^-$ were used as diagnostic ions. However, when applying lower voltages (80 V), the mass spectra is dominated by the presence of the $[M-H]^-$ ion for all monosulfonate compounds. The m/z values monitored for each target analyte corresponding to $[M-H]^-$ ion were m/z 222 for

TABLE 1. Mean Percent Recoveries (Rec), Limits of Detection in Full Scan Mode (LOD_{FS}), and in Selected Ion Monitoring Mode (LOD_{SIM}) and Repeatability (CV for $n = 3$) Obtained for Some Target Analytes Using the Analytical Procedures Described in the Experimental Section

compound	analytical procedure	Rec (CV)	fraction	LOD_{FS} (μg/L)	LOD_{SIM} (μg/L)
PEG_4	SSPE-LC-APCI-MS (PI)	73 (7)	D	4.1	0.8
PEG_7	SSPE-LC-APCI-MS (PI)	72 (9)	D	7.5	0.7
$NPEO_4$	SSPE-LC-APCI-MS (PI)	94 (9)	C	1.4	0.2
$NPEO_6$	SSPE-LC-APCI-MS (PI)	92 (9)	C	1.5	0.2
$C12EO_x$	SSPE-LC-APCI-MS (PI)	74 (8)	B	2.6	0.2
$C13EO_x$	SSPE-LC-APCI-MS (PI)	82 (7)	B	1.1	0.1
4-chloro-m-cresol	SSPE-LC-APCI-MS (NI)	83 (13)	D	38	7.2
2,4-dinitrophenol	SSPE-LC-APCI-MS (NI)	73 (11)	D	3.6	0.9
nitrophenol	SSPE-LC-APCI-MS (NI)	102 (13)	D	2.7	0.6
pentachlorophenol	SSPE-LC-APCI-MS (NI)	90 (9)	C	2.8	0.37
DBP	SSPE-LC-APCI-MS (PI)	69 (7)	C	1.3	0.08
DMP	SSPE-LC-APCI-MS (PI)	70 (12)	C	1.1	0.06
DEHP	SSPE-LC-APCI-MS (NI)	71 (9)	C	1.5	0.1
$C_{10}LAS$	SPE-LC-ESI-MS (NI)	78 (3)		0.47	0.003
$C_{11}LAS$	SPE-LC-ESI-MS (NI)	93 (4)		2.99	0.005
$C_{12}LAS$	SPE-LC-ESI-MS (NI)	82 (3)		3.54	0.006
$C_{13}LAS$	SPE-IP-ESI-MS (NI)	57 (3)		4.6	0.005
3-nitroBS	SPE-IP-ESI-MS (NI)	93 (1)		0.33	0.16
1-amino-6-NPS	SPE-IP-ESI-MS (NI)	91 (8)		0.07	0.07
4-chloro-BS	SPE-IP-ESI-MS (NI)	102 (11)		0.122	0.06
1-amino-7-NPS	SPE-IP-ESI-MS (NI)	85 (3)		0.18	0.18
2-NPS	SPE-IP-ESI-MS (NI)	101 (2)		0.03	0.03
1-Hydroxy-4-NPS	SPE-IP-ESI-MS (NI)	103 (8)		1.47	0.59

TABLE 2. Identified Compounds, Main Ions, and Average Concentration in Three Tannery and Textile Wastewaters Using SSPE-LC-APCI-MS, SPE-LC-ESI-MS and SPE-IP-ESI-MS

time (min)	ion mode	m/z	retrieved compound	concentration (µg/L)	
				tannery	textiles
SSPE-LC-APCI-MS					
2.9	PI	101, 106	PEG_2	nf[b]	1210
3.3	PI	133, 151 187 ± 44	$MCPEG_2$	nf[b]	830
3.5	PI	127, 139	cyclohexanecarboxylic acid	nq[a]	nf[b]
4.0	PI	133, 145, 195	PEG_4	2250	nf[b]
5.0	NI	136, 151, 166, 181, 299	MTBT	nq[a]	nf[b]
9.3	NI	92, 108, 138	nitrophenol	7	nf[b]
14.0	NI	182, 196	MsiBT	nq[a]	nf[b]
20.0	NI	141, 143	4-chloro-*m*-cresol	2340	nf[b]
19.8	PI	157, 175	indole acetic acid	nq[a]	nf[b]
21.5	PI	149, 177, 223	DEP	2020	nf[b]
22.3	PI	149, 205, 256	DBP	nf[b]	226
24.7	PI	133, 151 341 ± 44	$MCPEG_6$	750[c]	nf[b]
25.2	PI	429 ± 44	$MCPEG_8$	190[c]	nf[b]
26.2	PI	443 ± 44	$DCPEG_7$	250[c]	nf[b]
26.5	PI	531 ± 44	$DCPEG_9$	120[c]	nf[b]
26.9	PI	133, 151, 195 413 ± 44	$C3EO_8$	820[c]	nf[b]
31.5	PI	133, 177, 151 401 ± 44	$C10EO_5$	nf[b]	3513
32.3	NI	223, 299	BBT	nq[a]	nf[b]
32.3	NI	271 419 ± 44	$NPEO_4$	49	nf[b]
32.7	PI	133, 151, 195 341 ± 44	$C12EO_3$	830	
34.0	PI	133, 151, 195 399 ± 44	$C13EO_4$	1130	nf[b]
35.0	PI	133, 151 413 ± 44	$C14EO_4$	290[c]	nf[b]
36.6	PI	149, 167, 279 391	DEHP	380	173
SPE-LC-ESI-MS					
19.8	NI	183, 297	C10LAS	1	14
20.9	NI	183, 311	C11LAS	9	39
22.0	NI	183, 325	C12LAS	16	35
23.5	NI	183, 339	C13LAS	2.4	22
SPE-IP-ESI-MS					
33.4	NI	80, 156, 202	3-nitroBS	35	132
34.2	NI	80, 158, 222	1-amino-6-NPS	64	nf[b]
35.9	NI	80, 159, 223	1-hydroxy-4-NPS	nf[b]	21.4
41.4	NI	80, 127, 191	4-chloro-BS	5	nf[b]
43.4	NI	80, 158, 222	1-amino-7-NPS	4.4	2.4
48.3	NI	80, 143, 207	2-NPS	1013	2377

[a] Not quantified due to the lack of standard, nq. [b] Not found, nf. [c] Estimated concentration

1-amino-4-naphthalenesulfonate, 1-amino-6-naphthalenesulfonate (1-amino-6-NPS), and 1-amino-7-naphthalenesulfonate (1-amino-7-NPS); *m/z* 202 for 3-nitrobenzenesulfonate (3-nitro-BS); *m/z* 223 for 1-hydroxy-4-naphthalenesulfonate (1-hydroxy-4-NPS); *m/z* 171 for 4-chlorobenzenesulfonate (4-Chloro-BS), and *m/z* 207 for 2-naphthalenesulfonate (2-NPS).

Quantitation. External calibration was used for quantitation. No internal standard was used for quantification purposes as the broad range of pollutants present in the samples made difficult its selection. As reported previously (*10, 18, 19*), a series of injections of the available standards in the same of range of sample's concentration were used to obtain the calibration equations. Table 1 reports the limits of detection (LOD) achieved with the developed analytical methods either using SIM and full scan conditions.

Results and Discussion

Recoveries. Industrial wastewaters involve a complex matrix, and, therefore, it was necessary to perform a preliminary study in order to evaluate the recoveries of target nonionic and anionic compounds. In this sense the SPE and the SSPE methods described in the Experimental Section were applied for the preconcentration of 200 mL of water sample spiked with 100 µg/L of each compound listed in Table 1, among them phenolic compounds, nonionic polyethoxylated surfactants, phthalates, LAS, BS, and NPS. Wastewater sample for this experiment was provided by U.K. certification organization on water analysts Aquacheck (WRC, Medmenham, U.K.) that periodically distributes nonspiked wastewater samples and a spiking solution of target analytes for interlaboratory exercises. Therefore, the wastewater was free from the studied analytes. The main percent recoveries obtained using the different extraction methods for some target compounds are shown in Table 1.

In reference to nonionic pollutants, all recovery values were higher than 70% showing the good performance of the SSPE method used for its preconcentration. Furthermore, this method permitted analyte group fractionation for target analytes as polar compounds such as poly(ethylene glycol)s
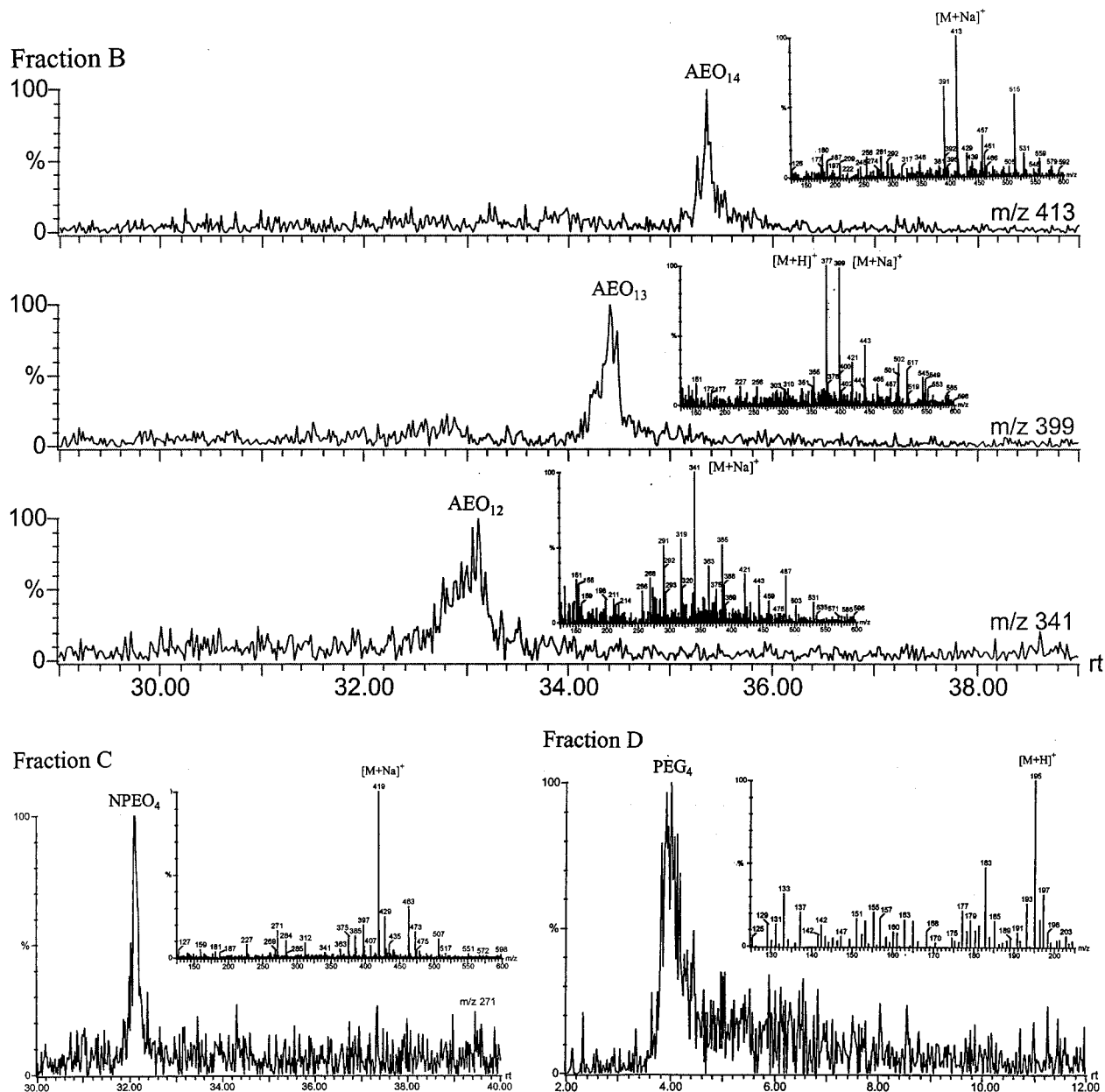
FIGURE 2. PI full-scan LC-APCI-MS chromatograms obtained with extraction voltage set at 30 V by injecting 20 μL of different fractions corresponding to the tannery wastewater. The presence of PEG_4, $NPEO_4$ and $C12EO_3$, $C13EO_4$, and $C14EO_4$ is shown in fractions D, C, and B.

and phenols were included in fraction D, aromatic compounds such as NPEOs and phthalates were mainly collected in fraction C, medium polar compounds including AEOs were found in fraction B, and mainly aliphatic analytes such as AEO with $n >15$ were recovered in fraction A. Another important advantage related to the use of SSPE is the elimination of interferents present in this complex water matrices by choosing the appropriate elution solvents in order to extract mainly target analytes.

Preconcentration of anionic target analytes using a single step extraction with the polymeric cartridge Isolute ENV+ led to recoveries from 57 to 93% for LAS, whereas NPS and BS were quantitatively enriched and desorbed with recoveries higher than 80%. Therefore, this method allowed simultaneous purification and extraction of LAS from the sample avoiding the use of two different sorbents for purification and extraction as it has been reported (17). Furthermore, the same methodology has overcome some of the difficulties described by Altenbach and Giger (6) in the SPE of NPS and

BS with graphitized carbon black. In this respect, problems associated with the irreversible adsorption of amino derivatives, reduction of sorbent capacity due to the enrichment of humics in wastewaters with high DOC values and low reproducibility of the sorbent in different lots, are solved with the use of the present methodology.

Environmental Samples. The developed SSPE-LC-APCI-MS method was applied to the analysis of tannery and textile wastewaters. Both NI and PI modes with extraction voltage set at 30 V were used for real samples in order to detect as many compounds as possible. Those compounds with available standards were identified by matching retention time and MS spectrum in PI and NI modes. Table 2 lists all the compounds observed in the studied samples, the main ions in their spectra, and their average concentration in the different analyzed samples.

Different nonionic surfactants were identified: PEGs were found in concentration levels ranging from 0.12 to 2.25 mg/L, AEOs concentration varied between 0.29 and 3.5 mg/L,
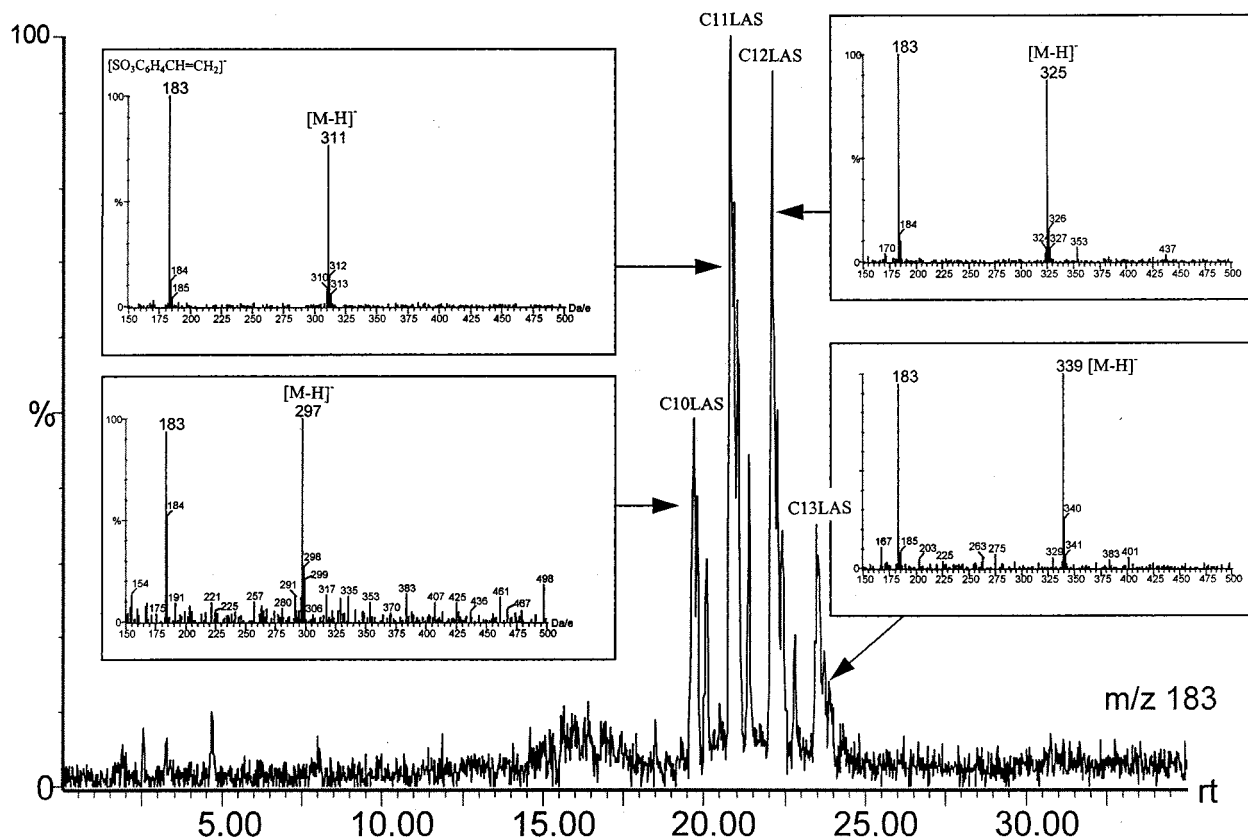
FIGURE 3. NI full-scan LC-ESI-MS chromatogram (extracted ion m/z=183) obtained with extraction voltage set at 70 V by injecting 20 μL of SPE extract from the textile wastewater. Peaks at retention time 19.8, 20.9, 22.0, and 23.5 min reveal the presence of C10LAS, C11LAS, C12LAS, and C13LAS as shown in the spectra.

and NPEOs were present at 0.049 mg/L level in the tannery sample. In general, the levels of nonionic polyethoxylated surfactants were higher in the tannery wastewaters than in the textile effluents due to the fact that they are extensively used in tanneries (Figure 2). The use of NPEO is being restricted in some countries due to the toxicity of its biodegradation products (16). However, these surfactants are still very much employed in the leather industry due to their effectiveness to remove grease from sheep skin. This is a worrying issue because some NPEO's metabolites such as 4-nonylphenol, 4-nonylphenol-diethoxylate, and 4-nonylphenoxycarboxylic acid have been recently found to be oestrogenic. These compounds induce the production of vitellogenin, a fish egg protein produced by female fish, in male rainbow trout (21). They have been found in paper mill effluents, municipal sewage treatment plant effluents, and river waters (22). Related acidic forms of poly(ethylene glycols) such as different mono- (MCPEG) and dicarboxylated PEGs (DCPEG) were present in the studied samples. Their concentration level has been estimated considering a similar response to that obtained for PEGs. In general, lower concentrations than that found for nonionic polyethoxylated surfactants were detected suggesting that these acidic forms derived among others from (bio)degradation process(es) of AEOs and PEGs. Actually, there is abundant evidence (15) that the initial biodegradation of AEOs occurs by cleavage at the ether bridge between the alkyl group and the polyethoxylene moiety following successive depolymerization of the ethoxy chain and leading to formation of shorter chain neutral and acidic PEGs.

Some phenolic compounds were detected in the most polar fraction of the tannery sample at 0.006 and 2.34 mg/L concentration level for nitrophenol and 4-chloro-m-cresol, respectively. Phenols are regularly found in tannery wastewaters as degradation products of unreacted monomers (23);

this is the case of the nitrophenol retrieved in the analyzed sample. On the contrary, 4-chloro-m-cresol is present due to its use as a preserving agent. Some tanneries have ceased in their use owing to the well-known influence of phenols on the taste and odor of water and to their toxic effects to aquatic biota (24).

The presence of bis(2-ethylhexyl)phthalate (DEHP) has been detected in both samples by SSPE-LC-APCI-MS in PI mode. Other phthalic compounds such as diethylphthalate (DEP) and dibutylphthalate have also been found at 2.02 mg/L in the tannery sample and at 0.23 mg/L in the textile sample, respectively. Phthalates are frequently found in industrial wastewaters (12, 25).

Different benzothiazoles derivatives were tentatively identified in the tannery sample by means of SSPE-LC-APCI-MS in NI mode. The retrieved compounds were 2-(methylthio)benzothiazole (MTBT), 2-(methylsulfinyl)benzothiazole (MSiBT), and 3-(benzothiazolyl)-benzothiazole-2-thione (BBT), and their quantitation was not feasible due to the lack of standards. These compounds are widely employed in leather production industry as fungicides instead of chlorophenols.

The analysis of LAS was accomplished by LC-ESI-MS in the NI mode as can be observed in Figure 3. The application of this methodology revealed the presence of some aromatic sulfonates in both samples although its concentration was higher in the textile one due to their extensive use in this type of industry. For example, linear alkylbenzene sulfonates (LAS) were present at concentration levels ranging from 14.2 ug/L for C10LAS to 39.1 ug/L for C11LAS. Low levels of these compounds were found in the studied industrial effluents because they are mainly used in the South of Europe as laundry and household detergents and not in industrial applications. In other European countries, like Sweden and Denmark, LAS are substituting NPEO in industrial application

due to the increased restrictions imposed in Europe to the use of nonionic surfactants.

Higher concentration levels were found for some benzene- (BS) and naphthalenesulfonates (NPS) which are used in the textile industry as dye bath auxiliaries. Among them 3-nitrobenzenesulfonate (3-NitroNBS), frequently added to most processes with reactive dyes as a mild oxidizing agent, was present at 0.132, and 2.38 mg/L of 2-naphthalenesulfonate used as hydrotropic agent was detected (7). The important amounts of this type of pollutants indicate that their presence in industrial discharges should be controlled. The widespread use of BS and NPS has led to their occurrence in surface waters and in groundwater (5).

In summary, we have shown in this paper that polar, ionic, and highly water soluble organic compounds were identified in untreated industrial wastewaters. From the data reported in Table 2 it can be noticed that the contribution to the TOC is in the range of 2−4 mg C/l. This contribution is still low for such type of waters but opens a new window to different groups of analytes not commonly monitored in untreated industrial wastewaters. The identification of nonionic and anionic surfactants and aromatic sulfonates in wastewaters was feasible by applying the combination of LC-APCI-MS, LC-ESI-MS, and IP-ESI-MS with prior tailor-made SPE procedures. Concentration levels varied from 0.9 μg/L for C10LAS to 3.5 mg/L for C10EO$_5$. Nonionic surfactants were present in higher concentration in the tannery wastewaters, whereas anionic ones were more frequently found in textile wastewaters. These differences can be related to different industrial processes. Few reports on the occurrence of some of these compounds are reported. For example, although high amounts of naphthalene- and benzenesulfonates are being used for some years, their presence in industrial effluents has been recently reported due to the lack of previous analytical techniques for their determination. As a consequence, not many studies on the toxic effect of NPS and BS to aquatic environment have been performed (26). Therefore, it is worthwhile to study in detail the organic content of wastewaters in order to identify new groups of polar pollutants whose toxicity should be studied. An additional problem occurs because many untreated wastewaters may not be totally cleaned when directed to the appropriate water treatment plants causing, therefore, additional problems to receiving waters and drinking waters. In this sense, this paper contributes to expand the range of new analytes to be monitored by the establishment of improved analytical methodologies and by their immediate application to real-world environmental samples.

Acknowledgments

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