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Characterization of Organic Pollutants in Industrial Effluents Using Liquid Chromatography–Atmospheric Pressure Chemical Ionization–Mass Spectrometry

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Contaminated industrial effluents often contain a variety of organic pollutants which are difficult to analyse by standard GC–MS methods since this technique often misses the more polar or non-volatile fraction of these organic compounds. In the present work a method for the characterization of complex mixtures of organic contaminants present in various industrial effluents is proposed. The protocol consists of setting-up a methodology based on solid phase extraction (SPE) using an Automated Sample Preparation with Extraction Columns system (ASPEC XL) and Lichrolut EN sorbent material for preconcentrating 300–500 ml of water volumes spiked with a variety of pollutants: phenolic compounds, benzophenone, isothiocyanate-cyclohexane, ethylbenzoate, 1-methyl-2-pyrrolidinone, 2-methylbenzenesulphonamide, benzidines, acridine, 1,1,3,3-tetramethyl-2-thiourea, 2,2-dimethyl-1,3-propanediol, phosphates, phthalates and non-ionic detergents characterized by LC–MS using atmospheric pressure chemical ionization in the positive and negative ion modes. The developed protocol permitted unequivocal identification of target analytes such as pentachlorophenol, tributyl phosphate, 4-nonylphenol, dibutylphthalate, dimethylphthalate, bis(2-ethylhexyl)phthalate, isothiocyanate-cyclohexane, ethylbenzoate, 2-methylbenzene-sulphonamide, tetramethyl-thiourea, 2,2-dimethyl-1,3-propanediol and 1-methyl-2-pyrrolidinone at concentration levels varying from 0.16 to 54.4 μg l^{-1}. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

A great number of synthetic organic chemicals have been released to the environment. Many of them are toxic at low concentrations or may accumulate in sediments and organisms. As a consequence, strict characterization of contaminated effluents needs to be done. In this respect the European Union (EU) promulgated several years ago a so-called 'blacklist' of 132 dangerous substances (Directive 76/464/CEE) of target analytes that should be monitored as dangerous substances discharged into the aquatic environment.[1] The blacklist includes several organohalogens such as polychlorinated biphenyls (PCBs), chlorotoluenes and chloropropanes, some organophosphorus compounds such as pesticides and tributylphosphate, chlorophenols and polycyclic aromatic hydrocarbons (PAHs). Recently a new Directive on Integrated Pollution Prevention Control (IPPC) has been promulgated by the EU.[2] This new Directive expands the range of pollutants that should be monitored in industrial effluent discharges and involves a multiannual work programme that covers many industrial sectors such as the paper and pulp industry, refineries and textiles. It is indicated in the Directive that all the substances discharged by the various industrial sectors should be monitored and the former EU blacklist expanded by adding new compounds. It is of interest to the EU to develop monitoring strategies for the characterization of a variety of pollutants. From this perspective, research in the area of characterizing new pollutants in contaminated industrial effluents will be encouraged and expanded during the coming years.

Common methods for identifying organic pollutants in contaminated industrial effluents generally involve

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the use of either dichloromethane liquid–liquid extraction (LLE) or solid phase extraction (SPE), followed by gas chromatography–mass spectrometry (GC–MS) techniques with electron impact (EI) ionization,[3–7] although a few works have also been reported using chemical ionization.[8,9] By this approach a variety of non-polar compounds are generally determined in waste waters and effluents, e.g. phthalates, phosphates, benzenes, PAHs, etc.[3,5–7,9] However, many polar, ionic, heavy and thermally unstable compounds cannot be analysed by GC techniques. A different approach must be used for these pollutants which usually comprise more than 95% of the organic content.[4]

LC techniques have several advantages over GC: less sample clean-up is required thermally labile compounds are more easily analysed, derivatization is usually not required and polar and high-molecular-weight compounds can be identified. The use of LC has rarely been reported in the characterization of polar analytes detected in industrial effluents, although a few papers have been published determining a variety of non-ionic and anionic surfactants in waste waters.[10,11] In addition, the US EPA has published two methods for the analysis of solid waste (SW-846), involving either particle beam (PB), method 8325[12] or thermospray (TSP), method 8321A.[13] These methods involve the determination of different kinds of pollutants including disperse azo dyes, phosphates, pesticides and benzidines in waste waters. Both methods were recently applied to the characterization of many organic acids found in Superfund sites.[4] LC–TSP–MS was used to confirm that approximately half of the unidentified total organic halocarbon (TOX) content in leachates from a hazardous waste site is 4-chlorobenzene sulphonic acid.[14] However, the main obstacle to routine analytical applications of LC–MS has been the unavailability of rugged and reliable LC–MS interfaces. The development of atmospheric pressure ionization (API) LC–MS interfaces provides structural information similar to that obtained by chemical ionization techniques, overcoming the limitations of other LC–MS interfacing devices, such as poor structural information or sensitivity related to TSP and PB respectively. We have recently reported the use of LC–APCI–MS for the determination of nitro- and chlorophenols detected in pulp effluents at levels ranging from 4 to 27 $\mu g\, l^{-1}$.[15]

In the present work an atmospheric pressure chemical ionization (APCI) interface has been used for the direct coupling of LC to MS. LC–APCI–MS techniques permit the analyst to obtain both structural and molecular weight information. To our knowledge this technique has scarcely been used for characterization of waste water effluents.

The objectives of the present work were (i) to develop an analytical method based on SPE by using polymeric cartridges for the determination of medium to highly polar compounds present in contaminated industrial effluents and (ii) to characterize industrial effluents of different origin: petrochemical plant and leachate from landfill. This work is carried out in the framework of the EU Environment and Climate Program 'Pericles' (Protocol for the Evaluation of Residues in Industrial Contaminated Liquid Effluents). The final objective is to achieve a high level of knowledge about the composition and concentration of pollutants present in contaminated industrial effluents in order to comply with the recently introduced Directive.[2]

EXPERIMENTAL

Chemicals and reagents

HPLC-grade water, methanol and acetonitrile were obtained from Merck (Darmstadt, Germany) and were passed through a 0.45 μm membrane filter before use. Pentachlorophenol, 2,4-dinitrophenol, 4-nitrophenol, 1-naphthol and 2-nitrophenol were purchased from Merck. Catechol, phenol and *p*-cresol were obtained from Sigma (St. Louis, MO, USA) and acridine, 2,2′-biphenol, 3,3′-dichlorobenzidine, benzidine, 1-methylindol, benzophenone, 3,3-dimethylbenzidine, dibutylphthalate, dimethylphthalate and ethylbenzoate were from Aldrich (Milwaukee, WI, USA). Tributylphosphate was obtained from Kodak (Rochester, NY, USA). 4-Nonylphenol and ethoxylated 4-nonylphenol were from Kao Corporation (Barcelona, Spain). 1-Methyl-2-pyrrolidinone, 2-methylbenzenesulphonamide, 1,1,3,3-tetramethyl-2-thiourea, isothiocyanate-cyclohexane, bis(2-ethylhexyl)phthalate and 2,2-dimethyl-1,3-propanediol were a gift from the Mario Negri Institute (Milan, Italy). PA-grade acetic acid and sulphuric acid from Panreac (Barcelona, Spain) and Merck respectively were used.

Apparatus

Off-line SPE experiments were performed using an automated sampler processor from Gilson (Villiers-le-Bel, France). This system includes an Automated Sample Preparation with Extraction Columns system (ASPEC XL) fitted with an external 306 LC pump for the dispensing of samples through the SPE cartridges and with an 817 switching valve for the selection of samples from Gilson for the preconcentration step. The drying step was carried out using a Baker SPE 12g apparatus from J. T. Baker (Deventer, Netherlands).

An LC system, also from Gilson, consisting of two pumps, models 305 and 306, an 811c dynamic mixing chamber, an 805 manometric module and a UV detector model 117 with wavelength set at 280 and 310 nm was used for LC–UV experiments.

For LC–APCI–MS experiments a VG Platform from Micromass (Manchester, UK) equipped with a standard atmospheric pressure ionization (API) source, which can be configurated for APCI or ISP (ion spray), was used. The APCI interface consists of a heated nebulizer probe and a standard atmospheric pressure source equipped with a corona discharge needle. A detailed description of this system can be found elsewhere.[16] The solvent was delivered by a Waters 616 gradient pump system controlled by a Waters 600 S controller from Waters-Millipore (USA). The source and probe temperatures were set at 150 and 450 °C respectively, the corona discharge voltage was maintained at 3 kV and

Table 1. Mean recoveries and (in parentheses) RSD% ($n = 3$) obtained in loading different volumes of water spiked at 50 μg l^{-1} in target analytes[a] using off-line SPE with Lichrolut EN followed by LC–UV at 280 and 310 nm

Compound	V_{load} = 300 ml	V_{load} = 500 ml	V_{load} = 900 ml
Catechol	55 (13)	<5	<5
Phenol	57 (17)	<5	<5
Acridine	55 (15)	<5	<5
4-Methylphenol	63 (11)	24	<5
2,4-Dinitrophenol	59 (11)	32 (15)	23 (13)
2,2′-Biphenol	88 (10)	66 (11)	43 (12)
4-Nitrophenol	79 (13)	64 (12)	48 (13)
3,3′-Dichlorobenzidine	108 (9)	104 (9)	51 (12)
2-Nitrophenol	89 (11)	97 (12)	71 (13)
Naphthol	97 (8)	107 (9)	69 (9)
Benzidine	86 (9)	95 (8)	77 (9)
1-Methylindol	92 (8)	97 (9)	95 (10)
Benzophenone	105 (11)	104 (9)	103 (10)
3,3′-Dimethylbenzidine	103 (9)	99 (10)	104 (10)
Dibutylphthalate	54 (7)	48 (10)	41 (9)
Dimethylphthalate	69 (15)	55 (13)	46 (9)
4-Nonylphenol	34 (5)	26 (6)	13 (9)
Pentachlorophenol	87 (9)	85 (8)	79 (11)
2-Methylbenzenesulphonamide	22 (8)	9 (7)	<5
2,2-Dimethyl-1,3-propanediol	24 (9)	11 (9)	<5
Bis(2-ethylhexyl)phthalate	63 (9)	58 (10)	44 (9)

[a] Recoveries not determined for 1-methyl-2-pyrrolidinone, 1,1,3,3-tetramethyl-2-thiourea and isothiocyanate-cyclohexane owing to coelution problems. Tributylphosphate was not detected by LC–UV.

the cone voltage was between 20 and 40 V. The HV lens voltage was set at 0.20 kV. In full-scan mode the m/z range was from 80 to 400 in both negative ion (NI) mode and positive ion (PI) mode of ionization.

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.

Treated plant effluent was collected at the discharge pipe (effluent) of a petrochemical plant in Porto (Portugal) during July 1996. Two kinds of samples were supplied: A_1 from a new treatment plant in which chlorinated compounds were eliminated and A_2 from the conventional treatment plant.

Additional sampling was also conducted at an industrial leachate site in Italy (sample B). More information about this sample was reported by Benfenati *et al.* using other analytical procedures.[3]

Sample preparation

An off-line SPE method was used for preconcentration of sample A (A_1 and A_2). The styrene–divinylbenzene sorbent Lichrolut EN (200 mg, 6 ml) from Merck was used for off-line SPE purposes. The conditioning step was performed with 7 ml of methanol and 3 ml of water at 1 ml min^{-1}. The sorbent was not allowed to become dry before performing the preconcentration step. The waste water sample was filtered with a 0.45 μm membrane filter and allowed to equilibrate to room temperature if previously refrigerated. Different volumes (300, 500 and 900 ml) of water samples were loaded at 15 ml min^{-1} in the Lichrolut EN cartridges. After preconcentration the sorbent was completely dried to avoid hydrolysis of the trapped compounds. The drying step was carried out using a Baker SPE 12g apparatus connected to a vacuum system with pressure set at 15 lbf in^{-2} (negative pressure). The time used for drying was 20–30 min. The elution step was performed by adding 2 × 5 ml of acetonitrile to the cartridge at 1 ml min^{-1} and waiting 5 min between the two aliquots in order to allow sufficient contact time between the solvent and the trapped analyte. The final evaporation of the extra solvent was carried out with a stream of nitrogen. The extracts were concentrated to a final volume of 2 ml.

Sample B was toxicity-based fractionated according to a protocol established by Galassi and Provini[17] that follows previous work of Burkhard *et al.*[5] This method presents a hierarchic scheme for the fractionation of liquid wastes by preparative HPLC after SPE with Lichrolut EN cartridges. The procedure allows one to separate inorganic from organic species which are further split into fractions of different K_{ow} classes. Each fraction is tested for acute toxicity on *Daphnia magna* and only positive samples are analysed by the most appropriate analytical technique. LC–MS analysis is performed on the extract, whereas in the previous work of Burkhard *et al.*[5] C18 sorbent for fractionation and extraction followed by GC–MS was used for the identification of organic pollutants.

Table 2. Main ions and relative abundance obtained in LC–APCI–MS (PI and NI modes) at 20 and 40 V cone voltage for injection of 0.2 µg of all target compounds[a]

		Relative abundance[b]			
		PI mode		NI mode	
M_n[c]	Compound	20 V	40 V	20 V	40 V
184	Benzidine				
	185 $[M + H]^+$	100	100	ND	ND
	168 $[M - NH_2]^+$	8	68	ND	ND
212	3,3′-Dimethylbenzidine				
	213 $[M + H]^+$	100	97	ND	ND
	196 $[M - NH_2]^+$	16	100	ND	ND
	181 $[M - NH_2 - CH_3]^+$	ND	87	ND	ND
131	1-Methylindol				
	116 $[M - CH_3]^+$	ND	100	ND	ND
	132 $[M + H]^+$	100	89	ND	ND
179	Acridine				
	180 $[M + H]^+$	100	100	ND	ND
182	Benzophenone				
	105 $[M - C_6H_5]^+$	25	100	ND	ND
	183 $[M + H]^+$	100	14	ND	ND
110	Catechol				
	109 $[M - H]^-$	ND	ND	100	100
	169 $[M + CH_3COO]^-$	ND	ND	ND	30
139	Nitrophenol				
	138 $[M - H]^-$	ND	ND	100	30
	108 $[M - NO - H]^-$	ND	ND	ND	100
186	2,2′-Biphenol				
	185 $[M - H]^-$	ND	ND	100	100
108	4-Methylphenol				
	107 $[M - H]^-$	ND	ND	100	100
184	2,4-Dinitrophenol				
	183 $[M - H]^-$	ND	ND	100	30
	137 $[M - NO_2 - H]^-$	ND	ND	20	100
278	Dibutylphthalate				
	279 $[M + H]^+$	27	ND	ND	ND
	277 $[M - H]^-$	ND	ND	ND	64
	221 $[M - C_4H_9]^-$	ND	ND	ND	94
	167 $[C_6H_4(COOH)_2 + H]^+$	70	ND	ND	ND
	149 $[C_6H_4COOCO + H]^+$	100	ND	ND	ND
	77 $[C_6H_5]^-$	ND	ND	ND	100
194	Dimethylphthalate				
	195 $[M + H]^+$	2	ND	ND	ND
	163 $[M + H - 2CH_3]^+$	100	ND	ND	ND
220	4-Nonylphenol				
	279 $[M + CH_3COO]^-$	ND	ND	16	ND
	149 $[CH_3(CH_2)_3C_6H_4O]^-$	ND	ND	100	100
	191 $[CH_3(CH_2)_6C_6H_4O]^-$	ND	ND	62	ND
	149 $[M - (CH_2)_4CH_3]^+$	100	100	ND	ND
	280 $[M + CH_3COOH]^+$	2	ND	ND	ND
264	Pentachlorophenol				
	263 $[M - H]^-$	ND	ND	100	100
	229 $[M - Cl]^-$	ND	ND	30	25
	193 $[M - Cl - HCl]^-$	ND	ND	10	60
171	2-Methylbenzenesulphonamide				
	140 $[M - CH_3 - NH_2]^+$	100	ND	ND	ND
	172 $[M + H]^+$	17	ND	ND	ND
104	2,2-Dimethyl-1,3-propanediol				
	87 $[M - OH]^+$	99	ND	ND	ND
	105 $[M + H]^+$	100	ND	ND	ND
	149 $[M + COOH]^+$	ND	100	ND	ND
99	1-Methyl-2-pyrrolidinone				
	100 $[M + H]^+$	100	100	ND	ND
132	1,1,3,3-Tetramethyl-2-thiourea				
	133 $[M + H]^+$	34	ND	ND	ND
	88 $[M - N(CH_3)_2]^+$	100	100	ND	ND
150	Ethylbenzoate				
	149 $[M - H]^-$	ND	ND	100	ND
	105 $[C_6H_5CO]^-$	ND	ND	30	100

Table 2. Continued

		Relative abundance[b]			
		PI mode		NI mode	
M_n[c]	Compound	20 V	40 V	20 V	40 V
141	Isothiocyanate-cyclohexane				
	116 $[C_6H_{11}SH]^+$	60	41	ND	ND
266	Tributylphosphate				
	267 $[M+H]^+$100	100	18	ND	ND
	211 $[P(OH)_3(OBu)_2]^+$	25	ND	ND	ND
	155 $[P(OH)_3(OBu)]^+$	19	53	ND	ND
	99 $[M+H-3(CH_2)_4]^+$	ND	100	ND	ND

[a] Experimental conditions: acetonitrile/water (50/50) containing 0.5% acetic acid at a flow rate of 1 ml min^{-1} was used as carrier stream.
[b] ND denotes 'not detected'.
[c] M_n nominal mass.

Chromatographic conditions

One hundred microlitres of the extracts were injected in the LC system using a mobile phase of water and acetonitrile, both acidified with 0.5% acetic acid. The following solvent programming was used: from 30% acetonitrile/70% water, isocratic for 15 min, then increasing linearly to 100% acetonitrile in 15 min and back to the initial conditions in 5 min at a flow rate of 1 ml min^{-1}. A Hypersil Green ENV column (150 mm × 5 mm i.d., 5 μm particle size) equipped with a guard column, both from Shandon HPLC (Cheshire, UK), was used.

RESULTS AND DISCUSSION

Matrix studies: recoveries and breakthrough volumes

Industrial waste waters contain a complex matrix characterized by the presence of different interferents and pollutants depending on the sample origin. Therefore it was necessary to perform a preliminary study of SPE before the analysis of the samples in order to know their behaviour and influence on the process of SPE. For this purpose an off-line SPE method previously developed by our group[15] was applied for the preconcentration of water samples spiked with 50 μg l^{-1} of different phenolic compounds (catechol, phenol, 4-methylphenol, 2,4-dinitrophenol, 2,2′-biphenol, 4-nitrophenol, 2-nitrophenol, pentachlorophenol, 1-methylindol and naphthol), benzidines (3,3′-dichlorobenzidine, benzidine and 3,3′-dimethylbenzidine), acridine, benzophenone, phthalates [dibutylphthalate, dimethylphthalate and bis(2-ethylhexyl)phthalate], non-ionic detergents (4-nonylphenol and ethoxylated 4-nonylphenol), tributylphosphate, ethylbenzoate, 1-methyl-2-pyrrolidinone, 1,1,3,3-tetramethyl-2-thiourea, isothiocyanate-cyclohexane, 2,2′-dimethyl-1,3-propanediol and 2-methylbenzenesulphonamide. Target compounds were chosen according to the origin of the samples and considering a compendium of contaminants commonly found in chemical disposal sites.[2,4–8] Loading volumes of 300, 500 and 900 ml were preconcentrated (in triplicate) in the Lichrolut EN cartridges to evaluate the recoveries and breakthrough volumes of target compounds. Table 1 shows the main recoveries and relative standard deviation (RSD%) obtained for the target compounds in the preconcentration of different sample volumes using off-line SPE with Lichrolut EN followed by LC–UV with wavelength set at 280 and 310 nm. Recoveries varying from 70% to 104% were obtained from the preconcentration of 900 ml for the most non-polar compounds (e.g. benzidines), although the most polar compounds such as catechol, 2-methylbenzenesulphonamide and 2,2-dimethyl-1,3-propanediol were not detected owing to breakthrough. Recoveries varying between 22% and 55% were obtained for these compounds in the preconcentration of 300 ml of waste water samples. Thus three different polarity groups were distinguished: the most polar compounds with a breakthrough volume lower than 300 ml, a medium polarity group for which breakthrough results on loading approximately 500 ml of sample and a non-polar compounds group with a breakthrough volume higher than 900 ml. Consequently, the results indicated that reliable detection of all compounds was only feasible by loading volumes of 300 ml at most. Therefore loading volumes lower than 300 ml are recommended in order to retain a maximum number of pollutants, as the main problem related to industrial waste water samples is not low concentration of contaminants but their incomplete identification mainly due to losses in the analytical process.

Low recoveries were obtained for the phthalic compounds as compared with those reported in the literature.[18,19] Nevertheless, in those reports a 500 mg C18 disc was used for preconcentration, enabling better recoveries than those obtained with polymeric sorbents owing not only to the higher amount of sorbent but mainly to the high affinity of phthalates for C8 and C18 sorbents. The matrix effect on the efficiency of the SPE process should also be noted: tap water was used for carrying out the above-mentioned work,[18] whereas industrial waste water is used in the present work. This kind of matrix is characterized by the presence of interferents and particles that can decrease the effectiveness of the SPE process by plugging the pores of the sorbent. This effect is also detected in the results obtained for phenolic compounds. When applying the same SPE procedure for the preconcentration of phenolic compounds in ground water,[17] 25% higher recoveries on average were obtained. This fact confirms the presence of a high content of interferents in industrial waste

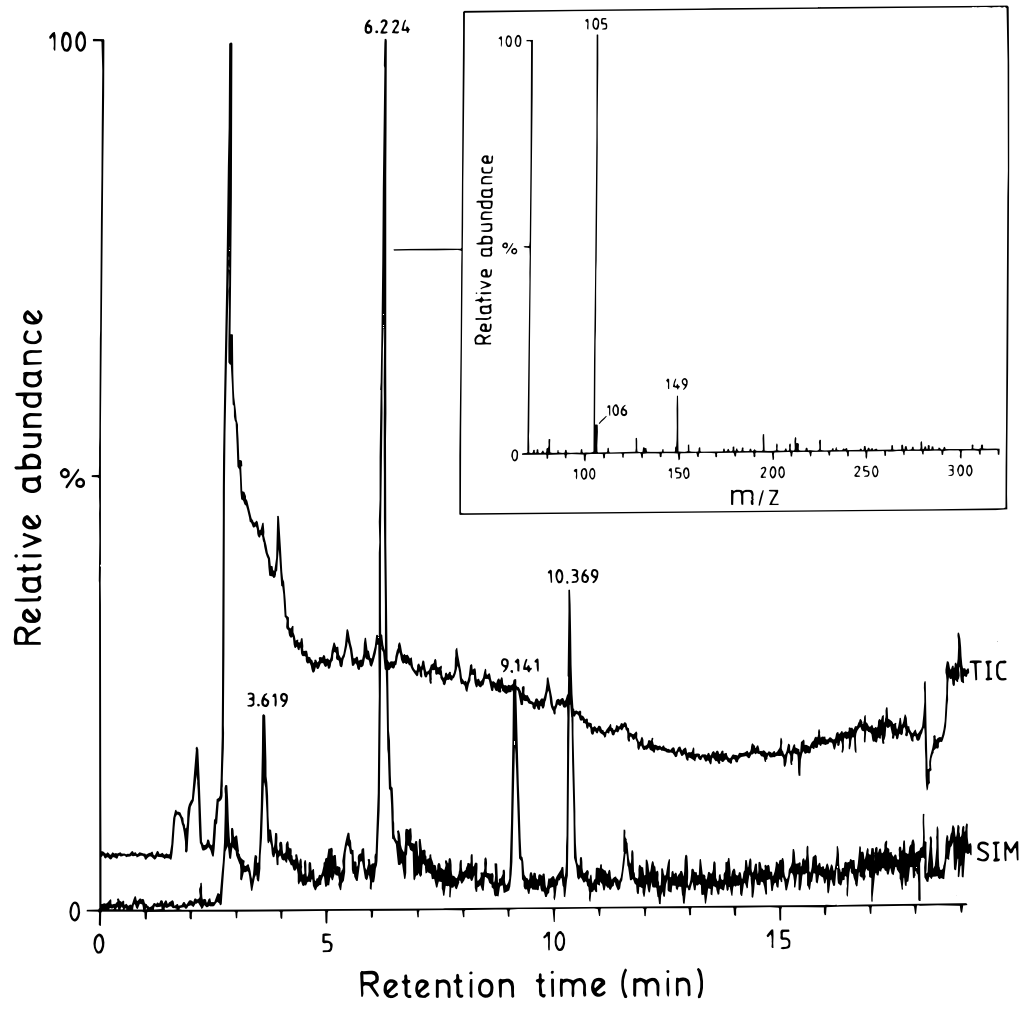

Figure 1. Full-scan LC–MS chromatogram and SIM LC–MS chromatogram for *m/z* 105 obtained with extraction voltage set at 40 V under NI conditions in injection of 100 µl of extract obtained in preconcentration of 200 ml of sample A_1 in Lichrolut EN. Inset: LC–APCI–MS spectrum for ethylbenzoate. See Experimental for chromatographic conditions.

waters that decreases the efficacy of the SPE process by diminishing the active surface area of the sorbent and therefore accelerating the breakthrough of target analytes which occurs for lower water volumes.

Further comment needs to be made about the recoveries obtained for 4-nonylphenol. Although apparently low values have been recovered for this compound, these results agree with those obtained by other authors[10] percolating water volumes no higher than 100 ml.

LC–APCI–MS optimization

Characterization of water samples was carried out by means of LC–MS techniques. This approach provides identification and characterization of the most polar, heavy and thermally unstable pollutants which cannot be analysed using GC techniques. An atmospheric pressure chemical ionization interface was used and its efficiency for detecting target compounds was checked before analysing waste water samples. Preliminary flow injection analysis (FIA) was performed in order to establish the analysis conditions. Two ionization modes (PI and NI) and different cone voltages (20 and 40 V) were tested. Table 2 shows the results obtained in this experiment. In general, more fragmentation was obtained via collision-induced dissociation (CID) by raising the cone voltage, which allows one to get structural information for the identification of unknowns. However, an extraction voltage of 20 V was preferred because it led to higher sensitivity than 40 V with enough structural information in both NI and PI modes. For instance, for 3,3′-dimethylbenzidine the mass spectrum was dominated by the $[M + H]^+$ ion (*m/z* 213) and the $[M - NH_2]^+$ ion (*m/z* 196) could be detected with a relative abundance of 16% with the cone voltage set at 20 V. An increasing fragmentation was observed at 40 V. In these conditions the base peak was the $[M - NH_2]^+$ ion (*m/z* 196) and the $[M - NH_2 - CH_3]^+$ ion (*m/z* 181) was detected with a relative abundance of 87%. However, regarding the sensitivity for 3,3′-dimethylbenzidine, the optimal cone voltage was 20 V, which gave enough structural information and the best sensitivity.

It is interesting to note that $[M + H]^+$ and $[M - H]^-$ ions were detected in PI and NI modes respectively for almost all target compounds (except

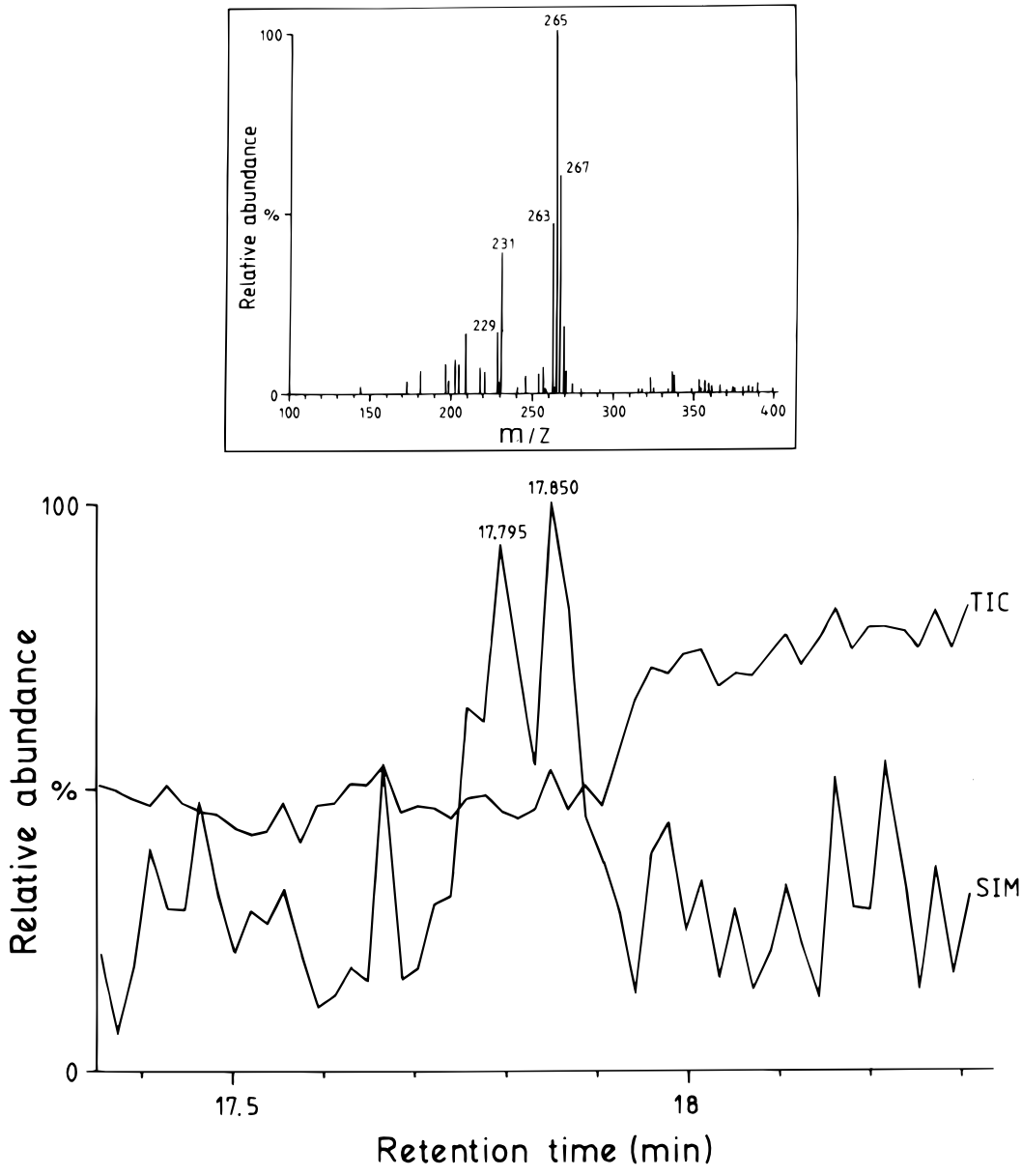

Figure 2. (a) Full-scan LC–MS chromatogram and SIM LC–MS chromatogram for (a) *m/z* 263 and (b) *m/z* 149 obtained with extraction voltage set at 20 V under NI conditions in injection of 100 µl of extract obtained in preconcentration of 200 ml of sample A_2 in Lichrolut EN. Inset: LC–APCI–MS spectrum for (a) pentachlorophenol and (b) nonylphenol. See Experimental for chromatographic conditions.

isothiocyanate-cyclohexane in PI mode and 4-nonylphenol in PI and NI modes). Therefore this technique allows one to obtain important molecular weight information. This is especially true for those compounds whose spectrum's base peak is one of the mentioned ions. In the case of detection of $[M + H]^+$ or $[M - H]^-$ ions they can be used to confirm the molecular weight of the analyte.

The results obtained for some of the target compounds agree with those reported previously by other authors. For example, in the case of tributylphosphate the characteristic ions of the phosphate compounds group at *m/z* 155 and 211 corresponding to $[P(OH)_3(OBu)]^+$ and $[P(OH)_2(OBu)_2]^+$ respectively were detected, matching with the results obtained by Barceló *et al.* using GC–MS.[9] All phenolic compounds gave similar spectra to those found by Puig *et al.*[20]

In the case of 4-nonylphenol the spectrum was characterized by the presence of the ions at *m/z* 149 and 279 corresponding to $[M - (CH_2)CH_3]^-$ and $[M + CH_3COO]^-$ respectively.

Environmental samples

The developed method of SPE followed by LC–APCI–MS was applied to the analysis of industrial effluents (A_1 and A_2) and samples from landfill B. Both NI and PI modes with extraction voltage set at 20 and 40 V were used for real samples in order to detect as many compounds as possible. All compounds were identified by matching the retention time and mass spectrum in PI and NI modes with authentic standards.

Total organic carbon was also measured in samples A_1 and A_2 with values of 13 and 25 mgC l^{-1}, which are higher as compared with surface waters used for drinking purposes (4–7 mgC l^{-1}) but are much lower than those found in highly contaminated industrial wastes.[4]

Figure 1 shows the negative ion full-scan LC–MS chromatogram (TIC) and selected ion monitoring (SIM) LC–MS chromatogram for *m/z* 105 obtained with extraction voltage set at 40 V in the injection of 100 µl of the extract from the preconcentration of 200 ml of sample A_1 in Lichrolut EN. The chromatographic profile obtained in full-scan mode showed chromatographic peaks containing unresolved components. Nevertheless, SIM chromatograms provided a more selective method avoiding the presence of interferences. This improved sensitivity allowed detection of ethylbenzoate (see Fig. 1 for spectrum) frequently used in chemical processes as a solvent, although it also has non-industrial applications, e.g. as a sunscreen agent in cosmetics.[21] This compound is not particularly toxic (its oral LD_{50} for rats is 2100 mg kg^{-1}) and therefore is not registered or included in any list from official institutions.

The full-scan LC–MS chromatogram and SIM LC–MS chromatogram for *m/z* 263 under NI conditions at 40 V, obtained by injecting 100 µl of the extract resulting from the preconcentration of 200 ml of sample A_2 in Lichrolut EN, are shown in Fig. 2(a). Unequivocal identification of pentachlorophenol was possible. This compound is frequently formed in bleaching processes and its presence in industrial effluents has been reported,[22–24] although its degradation products such as less substituted chlorophenols are more frequently detected.[6,7,22] Pentachlorophenol is on the Hazardous

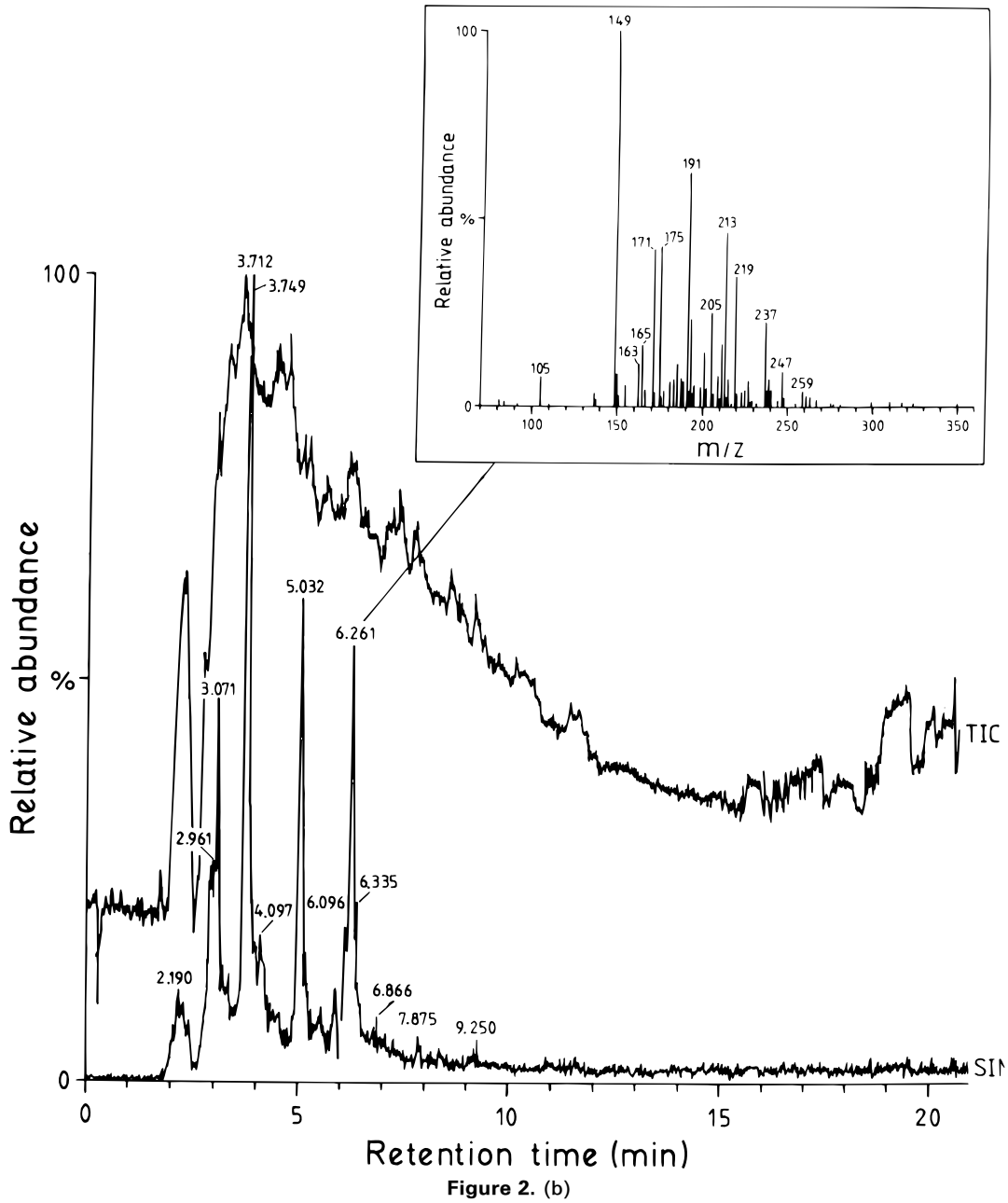

Figure 2. (b)

Substance List because it is regulated by OSHA [the legal airborne permissible exposure limit (PEL) is 0.5 mg m^{-3} averaged over an 8 h workshift] and cited by ACGIH. Its oral LD_{50} for rats is 25–200 mg kg^{-1}, so this compound is classified as toxic.[25]

LC–APCI–MS in SIM mode for *m/z* 149 [see Fig. 2(b)] showed the presence of 4-nonylphenol in sample A_2, which was confirmed by injecting an authentic standard. Nonylphenols are degradation products of nonylphenol ethoxylates (non-ionic surfactants) often added to separators to break up oil/water emulsions. They have also been used as plastic additives[26] an in Matacil (insecticide) formulations,[27] although these inputs are relatively minor. 4-Nonylphenol is persistent, lipophilic and toxic to aquatic organisms, with 96 h LC_{50} values for salmon and trout ranging from 0.13 to 0.23 mg l^{-1}.[28] It has recently been found to be oestrogenic.[26,29]

Figure 3 shows the PI full-scan LC–MS chromatogram and SIM LC–MS chromatogram (extraction voltage set at 20 V) obtained by injecting 100 µl of the most toxic extract of sample B. It was possible to detect two phthalic derivatives [dibutylphthalate and bis(2-ethylhexyl)phthalate] by monitoring the ion at *m/z* 149 that can be attributed to the phthalic acid anhydride. It

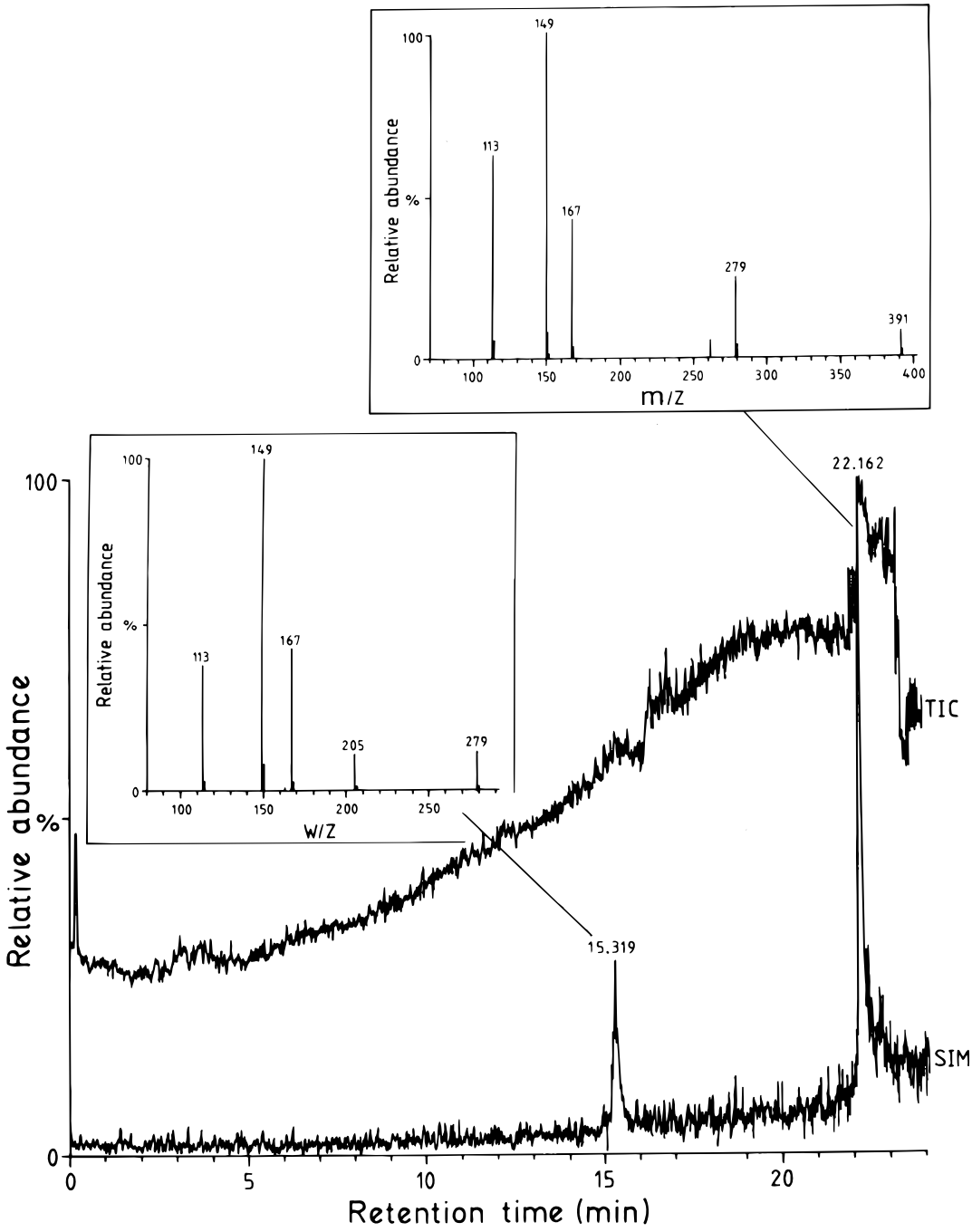

Figure 3. Full-scan LC–MS chromatogram and SIM LC–MS chromatogram for *m/z* 149 obtained with extraction voltage set at 20 V under PI conditions in injection of 100 µl of most toxic extract of sample B. Inset: LC–APCI–MS spectrum for dimethylphthalate and bis(2-ethylhexyl)phthalate. See Experimental for chromatographic conditions.

could be assured that these compounds were not laboratory contaminants, as blanks were run along with the samples. Phthalates are frequently found in industrial waste waters.[2,4,7] They have been used as plasticizers in many plastics since the 1930s, with a quarter of the total plasticizer ever produced being diethylhexylphthalate.[29] The ink used to print on plastic, board and foil-packed products frequently contains phthalates, as do some of the adhesives used in packaging.[30] They are found in food products such as baby milk formula, cheese, margarine and crisps and also in vinyl flooring and emulsion paint. Some of the most frequently used phthalates are low in toxicity (LD_{50} from 10.3 up to 33 g kg^{-1},[31] although they are hormone-disrupting chemicals. Particularly, diethylhexylphthalate is a testicular toxicant[32] and dibutylphthalate is oestrogenic with a cumulative action.[33] Owing to their persistence in the environment, phthalates are also commonly found in ground water, rivers and drinking water.[33] MACs (maximum admissible concentrations) for the most common dialkylphthalates are recommended on the level of 0.1–0.5 mg l^{-1} according to the general toxic and organoleptic indices of harmfulness.[31] Dibutylphthalate is on the Hazardous Substance List because it is regulated by OSHA (8 h PEL is 5 mg m^{-3}) and cited by ACGIH, DOT, DEP and EPA.

Tributylphosphate has also been found in sample B. Organophosphorus (OP) compounds have been widely used not only as pesticides but also in industrial and domestic products such as fire-retardant plasticizers and high-temperature functional fluids. They have been previously detected in rivers located in industrial areas.[9] These compounds are of concern because they are considerably strong inhibitors of acetylcholinesterase activity.[34]

Table 3 lists all the compounds observed in samples from sites A and B and their estimated concentration and limit of detection (LOD) for the developed off-line SPE coupled to LC–APCI–MS method. Identification was performed by comparison with the retention time and LC–APCI–MS spectrum of standards. External calibration was used for approximate quantification. No internal standard was used for quantification purposes, as the broad range of pollutants detected in the samples made difficult its selection. A single injection of 10 mg l^{-1} of each standard was done and the abundances obtained were compared with those values found in environmental samples in order to know the concentration range of the pollutants. An estimated value of concentration (see Table 3) was calculated considering the recoveries obtained for the identified compounds. The LOD was estimated as the concentration equivalent to a signal-to-noise ratio of three. The highest concentration level was obtained for ethylbenzoate, but this is not of concern owing to the low toxicity of this contaminant and the absence of legislation. On the contrary, it is remarkable the content of 1,1,3,3-tetramethyl-2-thiourea which is on the US EPA list of toxic substances. Thiourea compounds are mainly used as accelerators in the rubber industry and are frequently found in PVC plastic or adhesive, diazo paper and paint or glue remover. The rest of the pollutants are present in a lower concentration. Nevertheless, all of them (except ethylbenzoate) are included in some list regulated by the US EPA, OSHA or EC and therefore it is necessary to control pollution in order to prevent an alarming level.

Table 3. Identified compounds and their estimated LOD and concentration (results corrected for recovery) in industrial waste waters using LC–APCI–MS

Compound	Sample	LOD (μg l^{-1})	Conc. (μg l^{-1})
Tributylphosphate	B	0.17	0.66
Dibutylphthalate	B	0.08	0.78
Dimethylphthalate	A_2	0.06	0.60
Ethylbenzoate	A_1	3.82	51.0
	B		54.5
4-Nonylphenol	A_2	2.91	12.0
Pentachlorophenol	A_2	0.37	0.4
1-Methyl-2-pyrrolidinone	B	0.16	1.12
2-Methylbenzenesulphonamide	B	0.17	1.88
1,1,3,3-Tetramethyl-2-thiourea	A_2	1.97	39.3
Isothiocyanate-cyclohexane	A_1	0.96	11.2
2,2-Dimethyl-1,3-propanediol	B	1.00	14.7
Bis(2-ethylhexyl)phthalate	B	0.10	3.0

CONCLUSIONS

The application of an LC–APCI–MS technique to the characterization of industrial waste waters has been presented. LC–APCI–MS combined with SPE permits the analyst to achieve a good sensitivity of the method with only 200–300 ml of waste water samples. Although APCI techniques do not offer the complete EI spectrum, the combination of PI and NI modes at different cone voltages allows one to enhance structural information. Problems responsible for incomplete characterization of these samples included the lack of standards necessary to identify and quantify all the peaks in the chromatogram.

Even with the use of advanced techniques, up to or more than 50% of the chromatographic peaks may still be unidentified. Future efforts to characterize unknowns in complex environmental samples will include fractionation of the sample by means of SPE with different sorbents. This will lead to different polarity fractions and to a decrease in presence of interferents. The use of more selective sorbents, e.g. immunosorbents, will also improve the present results.

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