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Determination of carboxylic acids in water by gas chromatography using several detectors after flow preconcentration

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ABSTRACT

A novel analytical method is reported that combines continuous solid-phase extraction and gas chromatography for the determination of 22 carboxylic acids in water. The highly polar and hydrophilic analytes were preferentially sorbed on a mixture of LiChrolut EN–Supelclean ENVI-18 (1:1) sorbent column and eluted with methanol; this extraction process did not require derivatisation. The extract was analysed by gas chromatography coupled to a flame ionisation detector as well as a mass spectrometer with electron impact (EI) or positive chemical ionisation modes. The highest sensitivity was achieved when using MS-EI, with good linearity in calibration curves and low detection limits (2–40 ng L^{-1}) for 50 mL of sample. The entire procedure from raw aqueous sample to a ready-to-inject methanol solution of the acids requires less than 15 min. Another benefit of this method is the good accuracy (recoveries between 93 and 102%) and precision (relative standard deviation, 3.4–6.2%), which allows the determination of carboxylic acids in environmental water and in real chlorinated and ozonated drinking water.

1. Introduction

The presence of carboxylic acids in all environmental compartments has increased in recent decades and, hence, matrices including air, environmental water, drinking water and landfill leachates have been analysed for their determination from a few μg L^{-1} to several hundreds of mg L^{-1} [1]. Short-chain carboxylic acids (containing up to six C-atoms), such as formic, acetic, oxalic, glyoxylic, pyruvic, and ketomalonic acids as well as long chain organic acids have been detected in partially treated water and also in finished drinking water where they can be formed as disinfection by-products (DBPs) during ozonation from natural organic matter present in the source water [2,3]. Approximately 25% of the DBPs formed during water ozonation are carboxylic acids [4] and taking into account that these compounds are suspected to contribute to bacterial regrowth in drinking water distribution systems, there is considerable interest in their quantification at low levels. Short-chain carboxylic acids (volatile fatty acids) are also formed during anaerobic fermentation of organic material in engineered systems or in natural environments [5]. Aromatic acids such as vanillic, p-coumaric, ferulic and salicylic acids are important compounds in the aquatic environment as degradation products of plant matter such as lignins (polyphenolic substances) that are present in vascular plants [6]. The monitoring of carboxylic acids is of growing interest since they are, together with sulphur compounds and volatile amines, responsible for odour formation in wastewater treatment.

A large number of methods are available to determine carboxylic acids in environmental waters. Factors such as the type of carboxylic acid, its concentrations, and sample matrix largely determine which analytical methods are suitable for a given sample. Although some samples are directly analysed after a simple pre-treatment such as filtration [7], the potential interference of matrix compounds requires the employment of clean-up and preconcentration steps (mainly liquid–liquid extraction and solid-phase extraction, SPE) [1]. Various analytical techniques, e.g. gas chromatography (GC) [2,3,5], capillary electrophoresis [6,7], ion-exchange chromatography [7,8], ion-exclusion chromatography [9,10] and liquid chromatography [11–15] are suitable for the environmental determination of carboxylic acids. GC is the preferred technique for the determination of short and medium chain carboxylic acids, dicarboxylic acids, and also hydroxyl- and ketoacids due to its simplicity, separation efficiency and excellent sensitivity and selectivity [1]. The more volatile carboxylic acids may be determined directly by GC but other acids should be derivatised to increase their volatility, to decrease their polarity [2,16] and/or increase the sensitivity of the method when using halogenated derivatives in conjunction with an electron capture detector [3,17]. Other authors have employed capillary

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GC columns coated with poly-ethylene glycol as the stationary phase for the direct determination of short chain carboxylic acids without derivatisation [18]. Other detectors such as a flame ionisation detector (FID) [5] and especially mass spectrometry (MS) [2,3,16,19–22] have also been employed for the GC determination of carboxylic acids in water samples.

The present study introduces a novel SPE-GC method for the simultaneous determination of aliphatic (with short, medium and long carbon chains) and aromatic carboxylic acids without previous derivatisation in a wide range of water samples in order to reduce analysis time through simplification of the sample treatment. Another purpose has been orientated to make a comparative study of several SPE sorbent materials (polymeric materials, graphitised carbon black, C_{60} and C_{70} fullerenes and nanotubes, silica-reverse phases, among others) in order to establish, for the first time, the existing similarities or differences between these materials with regard to their application as sorbents for carboxylic acids. The paper was further completed by the use of several GC detectors [FID and MS in the electron impact (EI) and positive chemical ionisation (PCI) modes] to select the best option for the determination of carboxylic acids.

2. Experimental

2.1. Chemicals and standards

Carboxylic acids (C_2–C_{18}, benzoic, o-toluic, m-toluic, p-toluic, phenylacetic, and phthalic) were purchased from Sigma–Aldrich (Madrid, Spain) at the highest purity available. Chromatographic grade solvents (methanol, ethanol, *n*-hexane, acetonitrile and ethyl acetate), 2-*tert*-butyl-4-methylphenol (2TB4MP, internal standard), benzalkonium chloride and LiChrolut EN were supplied by Merck (Darmstadt, Germany). Oasis HLB sorbent was obtained from Waters (Madrid, Spain); silica-reverse phase sorbents with octadecyl and cyanopropyl functional groups (Supelclean ENVI-18 and Discovery DSC-CN, respectively), graphitised carbon black (GCB), Florisil, silica gel and Amberlites (XAD-2, XAD-4, XAD-7 and XAD-16) were provided by Supelco (Madrid, Spain). C_{60} fullerene, C_{70} fullerene and multiwall carbon nanotubes (MWCNTs) were purchased from MER Corp. (Tucson, AZ, USA). The surface area and particle sizes of these sorbent materials are listed in Table 1.

Stock standard solutions with a concentration of 10 g L^{-1} were prepared in ethanol or methanol and stored at 4 °C until use. Standard working solutions were prepared daily by diluting the individual stock standard solutions in water purified with a Milli-Q system (Millipore, Bedford, MA, USA). Methanol, containing 100 (FID), 10 (MS-PCI) or 1 mg L^{-1} (MS-EI) of 2TB4MP (internal standard) was used as the eluent, being freshly prepared on a daily basis.

2.2. Equipment

GC–FID analyses were performed in a Clarus 500 gas chromatograph controlled by a computer running TotalChrom software (Perkin Elmer, Madrid, Spain). The GC column was a 30 m × 0.25 mm I.D., 0.25 μm HP-INNOWax (poly-ethylene glycol) capillary column from J & W (Folsom, CA, USA). The oven was maintained at 60 °C for 1 min, raised up to 200 °C at 10 °C min^{-1}, and up to 250 °C at 8 °C min^{-1} (5 min). The carrier gas used was helium (purity 6.0) at 1.5 mL min^{-1}. The injection port and detector temperatures were kept at 250 °C. The sample injection was done in the splitless mode, using an injection volume of 1 μL.

GC–MS measurements were carried out using a Focus GC instrument interfaced to a DSQ II mass spectrometer and controlled by a computer running XCalibur software (Thermo Electron SA, Madrid, Spain). The MS was operated in both electron impact (EI) and

Table 1
Sorption efficiency (%) of carboxylic acids on different materials.[a]

	pKa	log $K_{o/w}$	Silica gel	Florisil	Discovery DSC-CN	Supelclean ENVI-18	LiChrolut EN	Oasis HLB	Amberlite XAD-2	Amberlite XAD-4	Amberlite XAD-7	Amberlite XAD-16	GCB	C_{60} fullerene	C_{70} fullerene	MWCNTs
Surface area (m^2/g)			400	300	480	500	1200	800	800	750	450	800	100	3000	<3000	–[b]
Particle size (μm)			60–200	590–1190	50	60–80	40–120	30–60	20–60	20–50	20–60	20–60	≤40	50–70	50–70	–[b]
Carboxylic acid																
Acetic	4.3	−0.2	21.2	27.0	28.4	28.0	99.5	82.1	75.5	50.9	74.1	74.2	50.0	0	0	0
Propionic	4.9	0.3	21.2	22.5	25.8	32.1	99.2	83.5	70.6	72.2	70.3	70.4	49.2	0	0	0
Butyric	4.8	0.8	19.1	18.3	25.0	30.9	99.9	98.5	90.9	90.9	99.9	99.9	42.5	0	0	0
2-Methylbutyric	4.8	1.2	27.1	20.7	26.6	29.7	99.9	98.6	90.9	90.9	92.9	94.9	39.5	0	0	0
Pentanoic	4.8	1.4	25.9	20.3	44.4	50.6	99.9	99.9	90.9	90.9	97.9	97.9	38.6	0	0	0
Hexanoic	4.9	2.0	25.7	28.1	52.3	84.5	98.1	98.9	80.0	80.1	80.2	80.2	31.3	0	0	0
Octanoic	4.9	–	28.9	20.0	82.5	85.5	97.5	95.7	81.3	79.5	81.2	80.3	32.0	0	0	0
Nonanoic	5.0	3.4	27.1	29.4	68.2	99.9	99.9	99.9	79.3	79.2	74.5	79.5	28.4	0	0	0
Decanoic	4.9	4.0	28.6	22.2	63.4	99.5	99.9	99.8	82.7	82.6	82.4	82.9	30.2	0	0	0
Dodecanoic	4.9	4.2	29.6	27.4	52.2	99.5	96.2	95.9	85.3	77.0	71.7	69.9	31.1	0	0	0
Myristic	4.9	6.0	23.6	20.4	62.6	99.2	75.2	74.7	60.4	40.5	40.2	41.5	30.9	0	0	0
Palmitic	–	–	25.5	25.3	42.2	99.5	54.2	58.2	55.2	55.4	55.3	54.4	31.4	0	0	0
Heptadecanoic	3.1	7.7	22.0	20.4	63.6	99.5	57.2	56.7	72.7	73.6	75.2	69.4	32.0	0	0	0
Stearic	5.0	8.0	21.5	25.6	69.2	99.5	55.9	50.2	67.1	72.3	71.4	53.4	30.7	0	0	0
Oleic	5.0	8.0	25.2	22.4	47.7	99.4	57.2	56.7	66.0	58.2	60.9	47.0	31.8	0	0	0
Linoleic	3.0	7.0	24.8	25.3	41.9	99.6	67.3	60.6	70.3	79.8	69.6	67.0	32.5	0	0	0
Benzoic	4.2	1.9	28.8	22.3	33.4	98.1	99.9	99.9	50.8	62.1	55.3	60.9	33.4	99.9	99.8	99.1
o-Toluic	4.3	2.4	28.3	20.9	36.6	90.3	99.9	94.4	61.2	66.3	50.1	63.5	30.2	60.0	60.1	59.9
m-Toluic	4.4	2.4	28.2	29.9	36.7	90.5	99.7	90.1	62.2	65.3	59.2	62.1	30.6	61.2	60.5	58.2
p-Toluic	4.4	2.4	28.3	25.8	36.5	88.7	99.8	92.3	62.3	64.6	51.4	62.5	32.4	62.0	61.3	60.0
Phenylacetic	4.3	1.4	27.2	28.3	34.4	63.6	99.6	92.8	42.6	57.9	55.2	54.1	31.8	71.2	70.2	69.2
Phthalic	2.9	0.7	29.3	20.2	34.6	65.9	99.9	93.5	45.2	90.2	55.4	55.2	29.7	74.9	71.2	69.4

[a] For 80 mg SPE sorbents.
[b] Not supplied by the manufacturer.

Table 2
Analytical figures of merit of the determination of carboxylic acids using the proposed GC (with FID, MS-PCI and MS-EI detection) method.

Carboxylic acid	FID				MS (PCI)					MS (EI)				
	Linear range ($\mu g\,L^{-1}$)	Sensitivity (signal/ng L^{-1})	LOD ($\mu g\,L^{-1}$)	RSD (%)	Linear range ($\mu g\,L^{-1}$)	Sensitivity (signal/ng L^{-1})	LOD ($\mu g\,L^{-1}$)	RSD (%)	m/z[a]	Linear range (ng L^{-1})	Sensitivity (signal/ng L^{-1})	LOD (ng L^{-1})	RSD (%)	m/z[b]
Acetic	2.0–500	0.023	0.6	4.0	0.1–150	0.243	0.04	4.7	61	30–15,000	1.230	9	4.5	**43**, 45, 60
Propionic	7.0–500	0.004	2.1	4.6	1.1–150	0.017	0.34	4.7	76	120–15,000	0.164	40	4.8	45, **74**, 75
Butyric	1.3–500	0.028	0.4	5.7	0.1–150	0.263	0.03	6.0	89	80–15,000	0.410	25	6.2	**60**, 73, 88
2-Methylbutyric	1.6–500	0.020	0.5	4.7	1.2–150	0.025	0.40	4.8	103	35–15,000	1.161	10	4.4	57, **74**, 102
Pentanoic	1.0–500	0.029	0.3	5.3	0.8–150	0.036	0.24	5.9	88	7–15,000	4.926	2	5.5	**60**, 73, 87
Hexanoic	2.0–500	0.024	0.6	5.4	0.7–150	0.046	0.23	5.7	117	15–15,000	2.381	5	5.8	**60**, 73, 116
Octanoic	1.0–500	0.046	0.3	3.1	0.8–150	0.053	0.25	4.3	145	25–15,000	1.231	8	3.4	**60**, 73, 144
Nonanoic	2.0–500	0.033	0.6	5.3	1.2–150	0.038	0.40	3.2	159	50–15,000	0.745	15	5.2	**60**, 73, 158
Decanoic	1.3–500	0.038	0.4	5.7	0.8–150	0.042	0.26	5.9	173	45–15,000	0.821	14	5.8	**60**, 73, 172
Dodecanoic	0.7–500	0.046	0.2	4.7	0.7–150	0.043	0.20	4.3	201	10–15,000	3.283	3	4.5	60, **73**, 200
Myristic	1.5–500	0.028	0.5	5.5	0.4–150	0.084	0.12	5.3	229	20–15,000	1.642	7	5.6	60, **73**, 228
Palmitic	3.0–500	0.015	0.9	5.5	0.9–150	0.035	0.29	5.5	255	20–15,000	1.650	7	5.1	41, **55**, 254
Heptadecanoic	1.6–500	0.020	0.5	5.2	1.2–150	0.027	0.37	5.6	271	30–15,000	1.150	10	5.8	60, **73**, 270
Stearic	1.9–500	0.022	0.6	5.5	1.3–150	0.024	0.40	5.2	275	35–15,000	0.985	11	5.0	43, **73**, 274
Oleic	3.0–500	0.013	0.9	5.2	1.8–150	0.022	0.56	5.8	283	45–15,000	0.903	14	5.7	**55**, 69, 282
Linoleic	3.0–500	0.012	0.9	5.8	1.9–150	0.018	0.58	5.6	281	50–15,000	0.821	15	5.9	**67**, 81, 280
Benzoic	1.3–500	0.031	0.4	5.5	1.1–150	0.035	0.35	5.1	123	7–15,000	4.936	2	5.2	77, **105**, 122
o-Toluic	1.6–500	0.027	0.5	5.0	0.4–150	0.081	0.12	5.5	137	30–15,000	1.231	9	5.6	**91**, 119, 136
m-Toluic	2.0–500	0.020	0.6	4.3	0.4–150	0.070	0.12	4.8	137	30–15,000	1.234	9	4.9	**91**, 119, 136
p-Toluic	1.9–500	0.015	0.6	5.3	0.4–150	0.063	0.12	5.5	137	30–15,000	1.242	9	5.0	**91**, 119, 136
Phenylacetic	4.0–500	0.012	1.2	5.0	0.2–150	0.132	0.06	4.7	137	30–15,000	1.233	9	4.8	65, **91**, 136
Phthalic	1.0–500	0.034	0.3	5.3	0.9–150	0.038	0.27	6.0	149	50–15,000	0.769	15	5.8	76, **104**, 148

[a] Base peak: $[M+H]^+$.
[b] Base peaks used for quantification are boldfaced; m/z for IS (2-*tert*-butyl-4-methylphenol): 121, **149**, 164.

positive chemical (PCI) ionisations. To switch between ionisation modes, a vacuum interlock system allowed changing of the ion volume without breaking system vacuum. The chromatographic column and temperature program were the same as in GC–FID method. Samples were injected using an AI/AS 3000 Autosampler (Thermo Electron SA). A 10 μL syringe was washed 3 times with methanol before and after each injection and rinsed with 8 μL of sample solution before 1 μL was injected in the split mode (1:20). The time for solvent delay was set at 4 min. The injection port and transfer line temperatures were kept at 250 °C. CI mass spectra were obtained using methane (purity 5.5) as reagent gas at 2 mL min^{-1}. The source was kept at 150 and 200 °C for PCI and EI, respectively; the ionisation energy was 70 eV in all cases. In the scan mode, the acquisition range was in the 40–300 m/z range. Ions considered in the SIM mode are listed in Table 2.

The SPE system consisted of a Gilson Minipuls-3 peristaltic pump (Villiers-le-Bel, France) fitted with poly (vinylchloride) tubes, two Rheodyne (Cotati, CA, USA) 5041 injection valves and PTFE laboratory-made sorbent columns of variable lengths according to each sorbent material. The sorbent columns were conditioned as follows: 0.5 mL of *n*-hexane–acetonitrile (1:1) for fullerenes and MWCNTs or 1 mL of acetonitrile–methanol (1:1) for the other sorbents, and then 1 mL of purified water for all sorbents. All columns remain serviceable for at least 1–2 months with no change in their properties.

2.3. Sample collection and preservation

Water samples were collected in pre-cleaned amber glass bottles of 500 mL. An anti-biodegradation agent, benzalkonium chloride (10 mg L^{-1}) was employed as preservative as it referenced to determine carboxylic acids [22]. After collection the samples can be stored at 4 °C up to a week before analysis. Samples containing visible solids (i.e., wastewaters) should be filtered prior to analysis through a 0.45 μm membrane filter (mixed cellulose esters, Millipore Ibérica, Spain) to prevent suspended particles from reaching the continuous SPE unit.

2.4. Analytical procedure

The continuous SPE unit employed for the preconcentration of carboxylic acids from water samples is depicted in Fig. 1. Firstly (preconcentration step), 50 mL of water or standard solutions at pH 1.3 (adjusted with 0.5 mL of 5 M HCl) containing between 0.7–500 μg L^{-1} (FID), 0.1–150 μg L^{-1} (MS-PCI) and 7–15,000 ng L^{-1} (MS-EI) of each acid were aspirated through a sorbent column (5 cm × 3 mm I.D.) containing 80 mg of the mixture LiChrolut EN/Supelclean ENVI-18 (1:1). The retention of carboxylic acids was instantaneous and the sample matrix was sent to waste. Next, IV_1 was switched and the sorbent column dried for 2 min with an air stream at 3 mL/min; simultaneously the loop of IV_2 (200 μL) was filled with the eluent, methanol containing the IS according to the detection employed, by means of a syringe. Secondly (elution step) IV_2 was switched to pass 200 μL of the eluent, carried out by the air stream, through the column in the opposite direction of the sample aspiration. The organic extract was collected in a conical glass insert (0.3 mL) inside a 2 mL amber glass GC vial which was tightly sealed and aliquots of 1 μL were injected into the gas chromatograph for analysis.

3. Results and discussion

3.1. Optimization of the sorption/elution process

Solid-phase extraction has gradually replaced classic liquid–liquid extraction and become the most common sam-

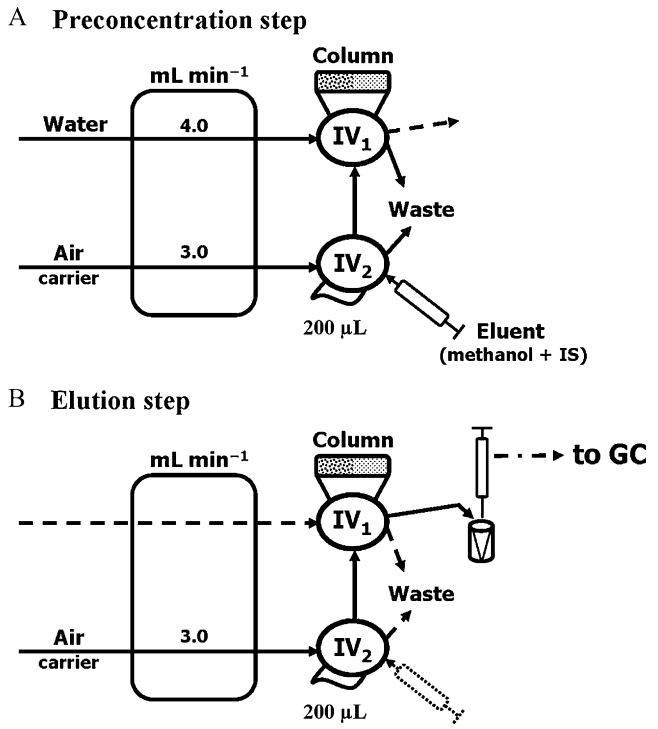

Fig. 1. Continuous flow system for the on-line preconcentration of carboxylic acids and off-line determination by gas chromatography. IV, injection valves; sorbent column, 80 mg of LiChrolut EN/Supelclean ENVI-18 (1:1). See text for details.

ple preparation technique in the environmental area. The choice of sorbent is a key point in SPE because it can control parameters such as selectivity, affinity and capacity [23]. Before selecting a sorbent for SPE, it is necessary to take into account some physico-chemical considerations such as the functional groups of the analytes, the nature of the bonded phase, the interactions between the analyte–sorbents or sorbent–components of the sample matrix and the analyte–sample matrix, and the energetic force of these interactions [24]. Anion-exchange sorbents such as aliphatic quaternary amine groups covalently bonded to a silica surface [25] and polymeric sorbents [16,26,27] have been employed in the SPE of carboxylic acids from water samples. In the present work, an exhaustive study was performed about the efficiency of several sorbent materials for the retention of aliphatic and aromatic carboxylic acids. For this purpose, polar sorbents (silica gel and Florisil), silica-reverse phase sorbents with octadecyl (non-polar) and cyanopropyl (polar) functional groups (Supelclean ENVI-18 and Discovery DSC-CN, respectively), polymeric sorbents [Amberlites (XAD-2, XAD-4, XAD-7 and XAD-16), Oasis HLB and LiChrolut EN], graphitised carbon black (GCB) and fullerenes and derivates (C_{60} and C_{70} fullerenes and nanotubes, MWCNTs) were assayed using columns packed with 80 mg of each material included in a continuous SPE unit. As can be seen in Table 1, aliphatic and aromatic carboxylic acids have variable logs of octanol–water partition coefficient (to predict analyte behaviour based on its hydrophobicity) and pK_a values (to predict analyte behaviour based on its polar interactions). Carboxylic acids require a pH value two units below their pK_a values for adequate sorption as neutral compounds. In order to increase the sorption efficiency of all acids, the aqueous sample was adjusted at pH 1.3 (pK_a values of the acids ranged between 2.9 and 5.0, Table 1) by adding 0.5 mL of 5 M HCl per 50 mL of sample. The sorption efficiency was assessed by comparing the amount of each carboxylic acid present in fractions of 5 mL of aqueous standard solutions at pH 1.3 containing 15 ng of each analyte before (fraction completely sorbed) and after preconcentration (fraction unsorbed) on the sorbent column. Each aqueous solution fraction was extracted by hand with 1 mL of ethyl acetate, and 1 µL aliquots were injected into a GC–FID for analysis.

As can be expected, carboxylic acids were poorly retained on silica gel and Florisil (sorption efficiency lower than 30%, Table 1) since the water deactivates these sorbents to such an extent that only weak interactions are possible. Thus, it is necessary for the silica surface to be hydrophobic in nature for it to be functional with water [23]. Reverse-phase SPE involves the partitioning of organic solutes from a polar mobile phase (water) into a non-polar phase such as silica-based, carbon-based or polymeric sorbents. In this case, the partitioning involves Van der Waals or dispersion forces, which is a low energy process (5 kcal mol^{-1}) analogous to a molecule being removed from water in a liquid–liquid extraction. Also hydrophobic interactions favour retention of the carboxylic acids in the reversed-phase sorbent. Silica reverse-phase sorbents are available in both monofunctional and trifunctional groups. The latter phases are more stable to acid because organosilane is attached to the silica surface at several locations. Thus, when the sample pH is adjusted to acid values, it is critical to use trifunctional phases such as Supelclean ENVI-18 in order to prevent hydrolysis of the hydrocarbon group from the surface of the sorbent [23,27,30]. The sorption efficiency in the silica reverse-phase with octadecyl groups (Supelclean ENVI-18) was over 85% for the more hydrophobic analytes (medium and long chain aliphatic and aromatic acids), whereas polar analytes (C_2–C_5, phenylacetic and phthalic acids) retained less. Similar behaviour was observed for silica-bonded cyanopropyl sorbent although the retention of the analytes was lower.

Polymeric sorbents have been used to extract polar organic pollutants from environmental waters [26,27]. The most widely used are the styrene-divinylbenzene copolymers (PS-DVB) which have a hydrophobic structure with variable surface area up to 1200 m^2/g. These materials contain aromatic rings which permit electron-donor interactions between the sorbent and π bonds of the solute; they are often "doped" with a hydrophilic group, such as sulphonic acid, which imparts a somewhat polar character to the matrix of the sorbent. Therefore, they can act as mixed-mode sorbents with more capacity for polar compounds. As can be seen in Table 1, Oasis HLB and LiChrolut EN showed similar behaviour, providing a higher sorption efficiency for C_2–C_{12} and aromatic carboxylic acids (~100%) whereas long-chain carboxylic acids (C_{14}–C_{18}) retained less (50–75%). With regard to Amberlites, the mean sorption efficiency was lower than that obtained with Oasis HLB and LiChrolut EN except for the less polar analytes (C_{16}–C_{18}). The differences between the sorption of polymeric materials can be ascribed to the differences in particle or mesh sizes and polarity [27].

Finally the present study was completed with several carbon derivatives. GCB is a non-specific and non-porous sorbent with positively charged chemical heterogeneities on its surface which can act as a mixed-mode sorbent (reverse-phase and anion exchange) [23]. Surprisingly, the sorption efficiency of the different carboxylic acids was ~30%, which can be ascribed to the fact that the bond strength is generally lower in GBC than in polymeric sorbents [28]. In contrast to the previous sorbents, fullerenes and MWCNTs can only establish π–π interactions with aromatic compounds [29] and therefore aliphatic carboxylic acids were not retained in these materials. The average sorption efficiency for aromatic carboxylic acids was ~70% for the three sorbents (omitting benzoic acid ~100%).

According to the results listed in Table 1, it can be concluded that LiChrolut EN was the most adequate for retaining the most polar carboxylic acids (C_2–C_{12} and aromatics), whereas for long-chain carboxylic acids (C_{14}–C_{18}) the highest sorption efficiency was achieved when using Supelclean ENVI-18. From the foregoing, a sorbent column packed with both materials, separated by a piece of

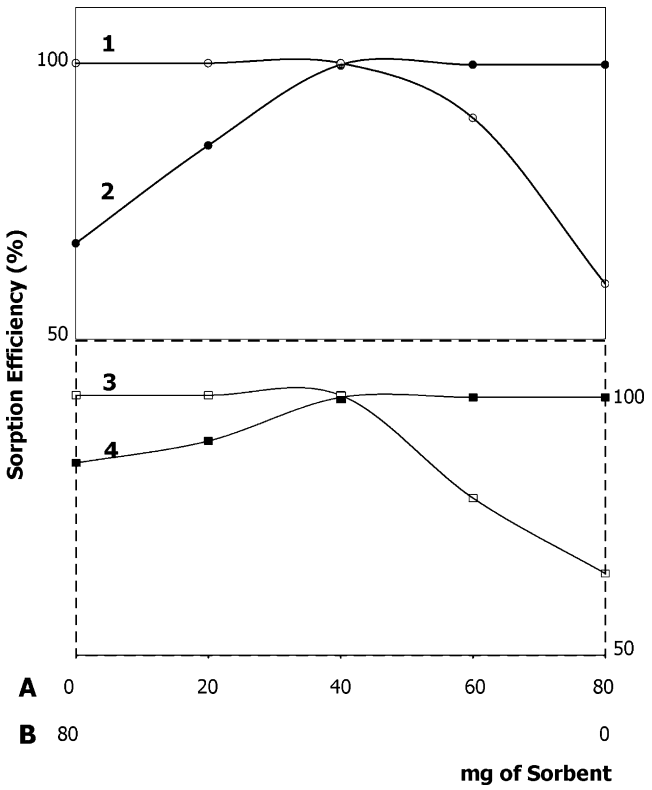

Fig. 2. Influence of the amount of Supelclean ENVI-18 (A) and LiChrolut EN (B) sorbents on the sorption efficiency of 4 carboxylic acids. 1, acetic; 2, linoleic; 3, phenylacetic; 4, myristic acids. The sorption efficiency of analytes 3 and 4 should be read on the right axis.

glass wool, was selected as the best option for further experiments. In this context, the optimum amount of each sorbent in the mixture was studied using several columns containing between 0 mg of LiChrolut EN and 80 mg of Supelclean ENVI-18 and vice versa. As can be seen in Fig. 2 for four representative acids of each group, the highest sorption percentage was achieved using a column packed with 40 mg of each sorbent. Experiments carried out with more or less than 80 mg of both sorbents (ratio 1:1) showed that better results were obtained in the interval 75–85 mg and therefore 80 mg was then adopted for further tests. The order of these sorbents within the column does not affect on the resolution of the column.

On the other hand, several organic solvents of variable polarity were assayed as eluent, namely: methanol, ethanol, 2-propanol, ethyl acetate, acetonitrile, acetone, diethyl ether and dichloromethane. For this purpose, 50 mL of aqueous standard solutions containing 25 $\mu g\,L^{-1}$ of each carboxylic acid were passed through the sorbent column at 4 $mL\,min^{-1}$, being eluted with 200 μL of each solvent and 1 μL of the extract injected into a GC–FID for analysis. Methanol provided the strongest chromatographic peaks as an effect of its increased eluting efficiency – the other solvents were approximately 1.5 times less efficient, and was thus selected as eluent.

The flow rates of the sample (50 mL containing 25 $\mu g\,L^{-1}$ of each acid) and the eluent (200 μL) had very little influence on the sorption/elution efficiency throughout the 0.5–4.0 $mL\,min^{-1}$ range. A sample flow rate of 4.0 $mL\,min^{-1}$ and an air flow rate (eluent carrier) of 3.0 $mL\,min^{-1}$ were chosen in order to increase the sample throughput. The effect of the eluent volume was varied between 50 and 400 μL of methanol using several loops in the IV_2 valve (see Fig. 1). The chromatographic signal increased on increasing the volume up to 200 μL (desorption efficiency prevails over dilution), above which it started to decrease, probably because the acids were diluted. A second injection with 200 μL of methanol revealed the absence of carryover; thus, complete elution of all acids was obtained with one injection of 200 μL of methanol.

The breakthrough volume is crucial in SPE methods because it is directly related to the enrichment factors, and therefore to the sensitivity of the method. The effect of this variable was examined using aqueous standard solutions at pH 1.3 containing 1.5 μg (FID detection) of each carboxylic acid at different volumes (from 10 to 300 mL), for insertion into the SPE system. Sorption efficiency of ~100% was obtained with volumes up to 60 mL above which it started to decrease because the capacity of the sorbent was overloaded and/or the proper sample matrix eluted the acids.

3.2. Comparison of FID and MS (EI and PCI modes) gas chromatographic detectors

Gas chromatographic methods to determine carboxylic acids in water include FID [5,31] and ECD detectors (previous to their conversion into halogenated derivates) [3,17], but these detectors provide no evidence for compound identification whereas this occurs with MS. For these reasons, GC–MS is the most widely used technique in environmental laboratories. For carboxylic acid determination, the most common practice is to perform GC–MS in the EI mode [2,16,32] in conjunction with a library search for its unequivocal identification, whereas the PCI mode [2,16,20] can provide additional molecular mass information. In this work, the potential of FID and MS in two ionisation modes was assessed to establish the optimum conditions for the determination and identification of carboxylic acids in water samples. A GC capillary column, coated with a 0.25 μm film of poly-ethylene glycol as the stationary phase, was used since it provided the best chromatographic resolution. The MS was set in full scan mode (40–300 amu) for identification purposes, and quantification fragments (m/z) for each acid (SIM mode) were selected following abundance (highest sensitivity) and specific criteria. In the EI mode, the base peak was selected for quantification while two qualifier ions, which are listed in Table 2, were also used for confirmation. In the PCI mode, $[M+H]^+$ was the most abundant ion and it was selected as base peak for all acids (see Table 2). The most important variables in the PCI mode were the methane flow rate and the ion source temperature. The reagent gas flow rate was evaluated throughout the 1–3 $mL\,min^{-1}$ range. The ionisation process of carboxylic acids was favoured at flow rates between 1.5 and 2.5 $mL\,min^{-1}$; above this value, the response was lower owing to the scattering of electron and ion beams [33]. With regard to the ion source temperature, the maximum response was obtained at 150 °C (the range studied was 100–220 °C). Fig. 3 shows the chromatograms obtained using the three detection modes from 50 mL of aqueous solutions at pH 1.3 containing 50 $\mu g\,L^{-1}$ (FID), 10 $\mu g\,L^{-1}$ (MS-PCI) or 0.5 $\mu g\,L^{-1}$ (MS-EI) of each carboxylic acid. As can be seen in Fig. 3, the resulting chromatograms (SIM in both MS modes) were very clean, the 22 acids being separated in 26 min. At first sight it is observed that MS-EI was the most sensitive option because the peak heights were of the same order of magnitude for three detectors whereas solute contents were quite different.

Finally, two organic compounds (viz. triphenylphosphate and 2-*tert*-butyl-4-methylphenol) were evaluated as internal standards, adding them to the eluent (methanol). Triphenylphosphate was strongly retained in the chromatographic column appearing in the chromatogram after the analytes (40 min), whereas 2TB4MP was located in the middle of the chromatogram (~15.5 min). Solutions of 2TB4MP as the internal standard were used as the eluent at concentrations of 100 $mg\,L^{-1}$ (FID), 10 $mg\,L^{-1}$ (MS-PCI) or 1 $mg\,L^{-1}$ (MS-EI) in methanol.

Table 2 summarizes the figures of merit in the calibration graphs for the 22 carboxylic acids selected using FID and MS (PCI and EI

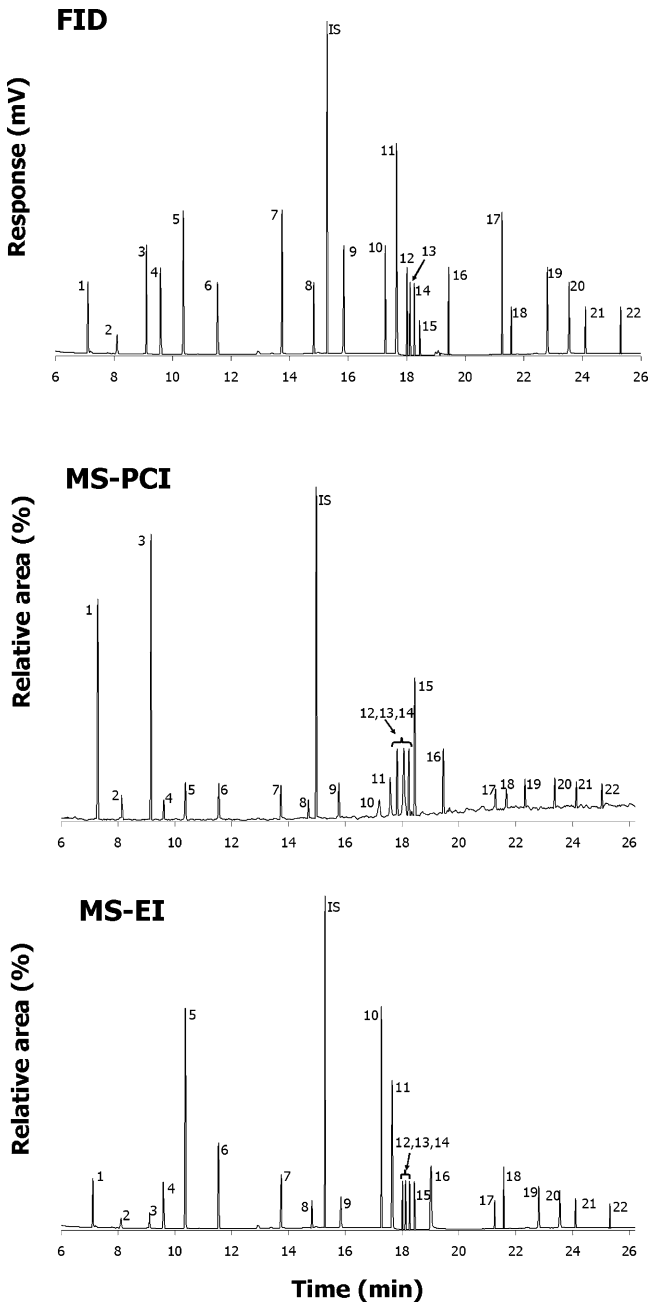

Fig. 3. Gas chromatograms obtained from 50 mL of aqueous sample solutions at pH 1.3 spiked with 50 (FID), 10 (MS-PCI) and 0.5 μg L^{-1} (MS-EI) of each carboxylic acid. 1, acetic; 2, propionic; 3, butyric; 4, 2-methylbutyric; 5, pentanoic; 6, hexanoic; 7, octanoic; 8, nonanoic; 9, decanoic; 10, benzoic; 11, dodecanoic; 12, o-toluic; 13, m-toluic; 14, p-toluic; 15, phenylacetic; 16, myristic; 17, phthalic; 18, palmitic; 19, heptadecanoic; 20, stearic; 21, oleic; 22, linoleic acids; IS, internal standard (2-*tert*-butyl-4-methylphenol).

modes). Using the continuous system depicted in Fig. 1, several analytical curves were constructed using aqueous standards at pH 1.3 (50 mL) containing variable concentrations of the analytes from 0.7 to 500 μg L^{-1}, 0.1 to 150 μg L^{-1} and 7 to 15,000 ng L^{-1} for FID, MS-PCI and MS-EI detection, respectively. Regression coefficients were greater than 0.994 in all cases. The sensitivity (slope of the calibration graph) was related to the detection technique since the volumes of the sample (50 mL) and the eluent (200 μL) were common in all cases. The limits of detection (LODs) listed in Table 2 were calculated as three times the standard deviation of residuals $S_{y/x}$, divided by the slope of each calibration graph [34]. Similar

Table 3
Analysis of water samples by SPE-GC/MS (EI mode). Sample volume, 50 mL.

Water	Carboxylic acid	Concentration found ± SD (μg L^{-1}, n = 3)
Well 1	Propionic	7.5 ± 0.4
	Butyric	3.7 ± 0.2
	2-Methylbutyric	0.25 ± 0.01
	p-Toluic	0.61 ± 0.04
Well 2	Propionic	10.1 ± 0.5
	Butyric	9.2 ± 0.6
	2-Methylbutyric	0.80 ± 0.04
River 1–2	nd[a]	nd
Pond 1	Acetic	9.1 ± 0.5
	Propionic	20 ± 1
	Butyric	4.7 ± 0.3
	2-Methylbutyric	0.68 ± 0.04
Pond 2	Acetic	15.7 ± 0.8
	Propionic	6.2 ± 0.3
	2-Methylbutyric	0.27 ± 0.02
Rain 1	Acetic	10.8 ± 0.5
	Propionic	9.4 ± 0.6
	Butyric	1.5 ± 0.1
Rain 2	nd	nd
Waste 1	Acetic	2.1 ± 0.1
	Propionic	0.56 ± 0.03
	Butyric	0.83 ± 0.06
	Octanoic	0.40 ± 0.02
	Decanoic	0.21 ± 0.01
	Dodecanoic	0.18 ± 0.01
	Palmitic	0.86 ± 0.05
	Oleic	0.96 ± 0.06
	Linoleic	0.64 ± 0.04
	Benzoic	0.060 ± 0.004
	Phenylacetic	0.28 ± 0.01
Waste 2	Acetic	19.1 ± 0.9
	Propionic	17.7 ± 0.9
	Butyric	1.5 ± 0.1
	2-Methylbutyric	0.97 ± 0.05
	Hexanoic	0.030 ± 0.002
	Decanoic	6.8 ± 0.4
	Dodecanoic	0.18 ± 0.01
	o-Toluic	0.26 ± 0.01
	m-Toluic	0.37 ± 0.02
	p-Toluic	0.25 ± 0.01
	Palmitic	4.1 ± 0.2
	Oleic	1.6 ± 0.1
	Linoleic	1.7 ± 0.1
	Benzoic	0.64 ± 0.04
	Phenylacetic	2.3 ± 0.1

[a] Not detected.

LODs were obtained using 12 individual standard solutions containing 10 μg L^{-1} (FID), 2.5 μg L^{-1} (MS-PCI) or 0.2 μg L^{-1} (MS-EI) of each carboxylic acid through their mean values and standard deviations. The precision (also included in Table 2 as the relative standard deviation, RSD) was checked in 11 individual samples containing 20 μg L^{-1} (FID), 4 μg L^{-1} (MS-PCI) and 0.4 μg L^{-1} (MS-EI) of each analyte.

Some interesting conclusions can be drawn from the results listed in Table 2. First, the sensitivity (the average value for 22 carboxylic acids was (0.030, 0.062 and 1.55 signal/ng L^{-1} for FID, MS-PCI and MS-EI, respectively) was higher with MS-EI, providing LODs between 25 and 60 times lower than those achieved using MS-PCI or FID, respectively. Secondly, the linear ranges also differed with the type of detection used: MS-EI provided the widest linear ranges (viz. 7–15,000 ng L^{-1}), while this value was shorter for MS-PCI (viz. 0.1–150 μg L^{-1}) owing to the saturation of the electron population at high concentrations [33]. The interval for FID, although it is wide, was the least sensitive which is consistent with the fact that MS is normally most sensitive for organic compounds. Third, the precision of the method was similar when using the three detectors (RSD, 3.4–6.2%), since it was mainly due to the ruggedness of the continuous system employed for sample

Table 4
Analysis of tap waters disinfected by chlorination (1–4) or ozonation plus chlorination (5-8) by SPE-GC/MS (EI mode). Sample volume, 50 mL.

Water	Carboxylic acid	Concentration found ± SD ($\mu g\,L^{-1}$, $n=3$)
Tap 1	Butyric	0.095 ± 0.005
	2-Methylbutyric	0.080 ± 0.004
Tap 2	Butyric	0.11 ± 0.01
	2-Methylbutyric	0.040 ± 0.002
Tap 3–4	–	–
Tap 5	Acetic	3.1 ± 0.2
	Propionic	9.6 ± 0.5
	Butyric	3.7 ± 0.2
	2-Methylbutyric	2.6 ± 0.1
	Pentanoic	0.13 ± 0.01
	Hexanoic	0.050 ± 0.003
	Octanoic	0.86 ± 0.04
	Decanoic	3.3 ± 0.2
	Dodecanoic	0.80 ± 0.04
	o-Toluic	0.22 ± 0.01
	m-Toluic	0.21 ± 0.01
	p-Toluic	0.16 ± 0.01
	Benzoic	0.45 ± 0.03
	Phenylacetic	2.3 ± 0.1
Tap 6	Acetic	19.3 ± 0.9
	Propionic	6.5 ± 0.4
	Butyric	0.93 ± 0.05
	Octanoic	0.10 ± 0.01
	Decanoic	2.7 ± 0.2
	o-Toluic	0.22 ± 0.01
	m-Toluic	0.20 ± 0.01
	Phenylacetic	1.1 ± 0.1
Tap 7	Acetic	7.7 ± 0.4
	Propionic	6.6 ± 0.4
	Butyric	1.7 ± 0.1
	2-Methylbutyric	0.20 ± 0.01
	Hexanoic	0.13 ± 0.01
	Octanoic	0.41 ± 0.02
	Decanoic	6.1 ± 0.4
	o-Toluic	0.26 ± 0.02
	m-Toluic	0.33 ± 0.02
	Benzoic	0.44 ± 0.03
	Phenylacetic	1.7 ± 0.1
Tap 8	Acetic	8.9 ± 0.5
	Propionic	18.4 ± 0.9
	Butyric	4.3 ± 0.3
	2-Methylbutyric	0.25 ± 0.01
	Hexanoic	1.2 ± 0.1
	Octanoic	1.3 ± 0.1
	Decanoic	5.8 ± 0.4
	o-Toluic	0.37 ± 0.02
	m-Toluic	0.42 ± 0.03
	p-Toluic	0.041 ± 0.003
	Phenylacetic	3.6 ± 0.2
	Phthalic	12.2 ± 0.8

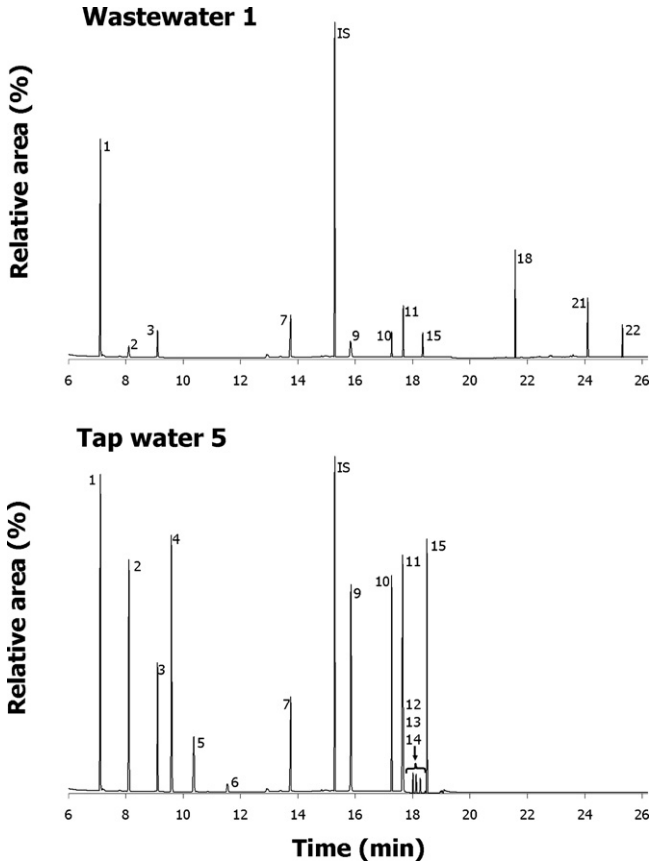

Fig. 4. GC–MS-EI chromatogram (SIM mode) obtained in the analysis of 50 mL of wastewater 1 (Table 3) and tap water 5 (Table 4). For peaks identification, see Fig. 3.

preparation. On the other hand, a comparison with other methods described in the bibliography demonstrates that the LODs of the proposed method using MS-EI (2–40 $ng\,L^{-1}$) were lower than that achieved by another SPME–GC–MS-EI method for fatty acids (0.280 $mg\,L^{-1}$) [31]. With respect to the MS-PCI method, free volatile fatty acids have been extracted by SPME from aqueous samples and determined by GC–MS-PCI with LODs within the range 10–115 $\mu g\,L^{-1}$ [20], which is higher than that obtained in the method proposed (35–580 $ng\,L^{-1}$). Finally for GC–FID determination, the LODs obtained in the method proposed (0.2–2.1 $\mu g\,L^{-1}$) were also lower than those achieved by other alternatives, by example for volatile fatty acids determined by HS-GC–FID methods (LODs between 0.3 and 3.7 $mg\,L^{-1}$) [5] or SPME–GC–FID (LODs between 6 and 675 $\mu g\,L^{-1}$) [20].

Recoveries of the method were calculated using various types of water including drinking, pond, river, swimming pool, well and wastewater (no certified material was available). Most samples contain carboxylic acids, and therefore concentrations in the spiked samples were quantified and compared to those calculated as the sum of the native concentration in unspiked samples and spiked concentrations. Each water sample was fortified with three different concentrations (0.1, 0.5 and 1 $\mu g\,L^{-1}$) of each carboxylic acid to 50 mL of sample in triplicate ($n=3$). All compounds were determined using the most sensitive method (GC–MS-EI) with average recoveries of 93–102% for all types of water, which testified to the applicability of this method to any water sample, which can be attributed to the efficiency of the SPE system employed for sample preparation.

3.3. Application for the determination of carboxylic acids in water

In order to verify the effectiveness of the SPE–GC–MS-EI method proposed for the application in question, over twenty water samples were analysed including samples subjected to oxidative treatment with ozone in addition to chlorination. If the concentration of some analyte lay out-side the linear range (Table 2), then the sample concerned was diluted with purified water to bring it within. Tables 3 and 4 list the acid concentrations found in different samples. As can be seen in Table 3, in environmental waters (pond, well and rain) short-chain carboxylic acids (mainly butyric and propionic) were found at higher amounts; in wastewaters the number of acids increased notably. The number and high concentrations of carboxylic acids in these waters can be explained for the following reasons. Carboxylic acids in pond and well water can be found close to the seeping of naturally occurring hydrocarbons or in the proximity of contaminated sites. Biodegradation of these compounds leads to a variety of metabolic intermediates, including low molecular weight organic acids. These compounds

can be detected also in rainwater due to emissions from vegetation, biomass and incomplete fuel combustion or through photochemical reactions in the atmosphere [1]. With regard to wastewaters, short and medium chain carboxylic acids were found as products of the anaerobic fermentation of organic material. Some aromatic and long chain carboxylic acids were also detected since the samples were collected near olive oil industries. Table 4 shows the results obtained from water treated by chlorination or ozonation plus chlorination. In practice, the concurrent oxidation process with ozone makes the resulting data quite complex. Several acids were proven to be present in chlorinated water but their concentration and number increased as the treatment with ozone proceeded. In such cases, these substances can be classified as ozonation by-products even if they were already present at time zero of the process. The large number of carboxylic acids detected in ozonated drinking water samples comes from the oxidation of natural organic matter or pollutants present in the source water during ozonation [2]. By way of example, Fig. 4 shows the SIM mode chromatograms obtained in the analysis of wastewater 1 (Table 3) and tap water 5 treated by ozonation plus chlorination (Table 4) with the proposed SPE–GC–MS-EI method. As can be seen, the chromatograms were very clean since only a few peaks from the matrix components were detected.

4. Conclusions

An exhaustive study has been performed with several sorbent materials for the simultaneous preconcentration of aliphatic and aromatic carboxylic acids. For the first time a column packed with two materials (a polymeric sorbent and a silica-reverse phase sorbent with octadecyl groups) was employed for the continuous SPE of 22 carboxylic acids using methanol as eluent. The potential of two GC detectors, FID and MS in both PCI and EI modes was assessed in order to establish optimum conditions for the identification and quantification of 22 carboxylic acids in water, obtaining the highest sensitivity with MS-EI. In this study it has been demonstrated that in spite of the polarity of carboxylic acids, they were easily extracted using a SPE unit and determined by GC without prior derivatisation when a poly-ethylene glycol column was used, providing a good chromatographic resolution. The concentrations of carboxylic acids found in the wastewaters were higher than in environmental ones due to the higher amount of organic matter from anthropogenic sources (drains from industries and cities). In the study of water treatment, highly polar disinfection by-products originated from the reaction of ozone with organic matter (i.e., humic and fulvic acids), the most frequently found being acetic, propionic, butyric, decanoic and phenylacetic acids.

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