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Application of on-line solid-phase extraction–gas chromatography–mass spectrometry to the determination of endocrine disruptors in water samples

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Abstract

We have applied a method based on solid-phase extraction (SPE), on-line coupled to gas chromatography–mass spectrometry through an on-column interface, to determine a group of endocrine-disrupting compounds in water samples. We have optimised the parameters affecting the SPE process and transfer step and used the method to analyse river, coastal and tap waters. In the full-scan acquisition mode, all the compounds were determined by preconcentrating only 15 ml of water sample. Di-*n*-butyl phthalate, benzylbutyl phthalate, bis(2-ethylhexyl) phthalate and bis(2-ethylhexyl) adipate at concentrations between 0.02 and 0.5 μg l^{-1} were determined in some real samples.

Keywords: Endocrine disruptors; Water analysis; Environmental analysis; Phthalates; Adipates; Pesticides; Polynuclear aromatic hydrocarbons

1. Introduction

In the last few years, a wide variety of chemicals have been identified as endocrine disruptors (EDs). These chemicals affect the endocrine system and cause alterations, such as breast cancer or reductions in sperm count, in the organism. They are either organochlorine pesticides (such as DDT, *p,p′*-DDE, endosulfan, dieldrin); natural or synthetic hormones; plasticisers or alkylphenols [1–3]. The number of compounds identified as endocrine disruptors is increasing [4]. Urban and industrial discharges [5] are the main source of ED distribution into the environment and they can cause adverse effects on aquatic life and contaminate ground water. Thus, interest has therefore recently grown in the development of analytical methods for determining such compounds in the aquatic environment.

Endocrine disruptors are usually present in the environment at low concentrations, so a preconcentration technique is usually needed to determine them. Solid-phase extraction (SPE) is one of the most common techniques [6–8], though solid-phase microextraction (SPME) has also been used [9,10].

Because they are so varied, endocrine disruptors are usually determined either by gas chromatography (GC) [6–12] or high-performance liquid chromatography (HPLC) [6]. More recently, immunoassay techniques have also been used [13,14]. However, methods developed are usually focused on families or groups of chemicals, but not on a large number of compounds with estrogenic activity, which usually belong to different families, in order to control their presence in real water samples.

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In this paper we aim to develop an automatic method for determining 14 endocrine disruptors in water samples using an on-line SPE–GC–mass spectrometry (MS) system with an on-column interface. These compounds include pesticides, phthalates and one polycyclic aromatic hydrocarbon. To our knowledge, this on-line combination has never before been used to determine such a wide variety of EDs.

2. Experimental

2.1. Reagents and standards

The compounds studied were atrazine, lindane (γ-hexachlorocyclohexane), vinclozolyn, aldrin, α-endosulfan, *p,p′*-DDE, *p,p′*-DDT, dieldrin, benzo[*a*]pyrene, hexachlorobenzene, di-*n*-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP) and bis(2-ethylhexyl) adipate. All were purchased from Riedel-de Häen (Seelze–Hannover, Germany) with a purity higher than 98%, except benzo[*a*]pyrene which was supplied by Aldrich (Steinheim, Germany) with a purity of 97% and hexachlorobenzene which was supplied by Fluka (Buchs, Switzerland) with a purity higher than 99%. A standard solution of 1000 mg l^{-1} of each pesticide was prepared in ethyl acetate and stored in the refrigerator. Every week, a solution of 40 mg l^{-1} in ethyl acetate was prepared from the standard solutions and used to prepare working solutions in Milli-Q water and to spike real water samples to the required concentrations.

Ethyl acetate and methanol, both trace analysis grade, were supplied by Merck (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Helium (carrier gas) and nitrogen were supplied by Carburos Metálicos (Tarragona, Spain) with a quality of 99.995%.

2.2. Equipment

A Hewlett-Packard (HP; Waldbronn, Germany) HP 6890 series gas chromatograph equipped with an on-column injector and a HP 5973 mass-selective detector was used. To inject large volumes and perform the chromatographic separation, we installed a 5 m×530 μm I.D. retention gap, a HP-5 MS 0.25 μm retaining precolumn of 2 m×250 μm I.D. and a solvent vent valve. The analytical column was a HP-5 MS 0.25 μm of 30 m×250 μm I.D. All connections were made with conventional glass press-frits. Chromatographic data were recorded using an HP G1701CA Chemstation, which was controlled by Windows NT (Microsoft) and equipped with the Hppest and Wiley 138 mass spectral libraries.

Three six-port Valco valves (Houston, TX, USA), automatically controlled by the GC software, were used for the SPE process. The precolumn (10×2 mm I.D.) was hand-packed with a styrene–divinylbenzene copolymer (PLRP-S, 100 Å, 20 μm particle size) (Polymer Labs., Shropshire, UK). A HP 1100 pump was used to deliver the sample and the solvents needed to clean and activate the sorbent. The ethyl acetate used in the elution step was delivered with a syringe pump (Cole-Parmer, IL, USA). A fused-silica capillary (30 cm×0.10 mm I.D.) permanently mounted in the on-column injector transferred the analytes from the precolumn to the GC system. A 100-μl loop of polyether ether ketone (PEEK) tubing (HP) was used instead of the precolumn for direct injection. The set up of the equipment for the on-line SPE–GC–MS method has been reported in a previous paper [15].

2.3. Chromatographic conditions

The oven temperature was held constant at 60 °C until the analytes were preconcentrated and transferred to the GC system. The temperature was then increased to 270 °C at 40 °C min^{-1} and kept constant for 2 min. Finally, it was increased to 290 °C at 60 °C min^{-1} and kept constant for 3 min. The on-column injector worked in the track oven mode and the carrier gas was maintained at a flow-rate of 1.2 ml min^{-1}.

The MS transfer line was kept at 280 °C to prevent the analytes from recondensing. The ion energy used for the electron impact (EI) ionization was 70 eV and the MS was tuned to m/z 69, 219 and 502 for the EI corresponding to perfluorotributylamine (PFTBA). The mass range was 50–350 in the full scan mode with a rate of 4.72 scans s^{-1}.

2.4. On-line trace enrichment

Three six-port valves connected in series were used for on-line sample enrichment. The precolumn was conditioned by flushing 3 ml of methanol and 3 ml of water. Then 50% (v/v) of methanol was added to the sample and 15 ml of the sample was preconcentrated. The tubes were purged with the corresponding solution and this was then passed through the precolumn. The flow-rate throughout the process was 2 ml min^{-1}. Before the elution, the precolumn was dried with 3 bar nitrogen for 15 min. Finally, the analytes were desorbed in the backflush mode with 100 μl ethyl acetate pumped at 47 μl min^{-1} with a syringe pump and on-line transferred to the GC system via the transfer line. The solvent vapor exit (SVE) was opened a few seconds before the transfer started and closed 1 min after the transfer finished to eliminate the ethyl acetate vapours without losing analytes. The GC temperature programme was started 7 min after the SVE was closed and data acquisition was started 2 min later to ensure that solvent peak eluted. The total analysis time was 55 min.

2.5. Sampling

River and coastal water samples were collected in 2.5 l precleaned amber glass bottles and filtered through a 0.45 μm filter (MSI, Westboro, MA, USA). They were kept at 4 °C in the dark until analysis. Tap water was collected just before the analysis.

The glass material used such as vials, bottles and volumetric material was carefully cleaned to avoid contamination problems.

3. Results and discussion

3.1. Chromatographic separation

The chromatographic separation was optimised by GC–MS by manually injecting 0.5 μl of a standard solution containing 10 mg l^{-1} of each compound in ethyl acetate into the on-column injector. The optimal conditions are described in Section 2.3.

We selected the ions for quantifying the pesticides in the full-scan mode from the spectrum obtained for each compound under EI ionization and selected the base peak of each pesticide. These ions are shown in Table 1.

3.2. Transfer conditions

We optimised the transfer conditions such as transfer flow-rate, transfer temperature and SVE open time following the standard procedure [15], so that partially concurrent solvent evaporation (PCSE) could be used with an on-column interface. The optimal conditions are described in Section 2.4.

3.3. SPE process

First of all, we established certain parameters in accordance with our previous experience in this field and the literature. We selected PLRP-S as the sorbent because it can retain some pesticides with similar characteristics to the compounds studied. Ethyl acetate was selected as the elution solvent because it desorbs analytes with a wide range of polarities from PLPR-S and it is compatible with the transfer conditions we used. Before the elution step, the sorbent was dried with nitrogen to avoid the deactivation of the retention gap to be destroyed. Although 3 bar N_2 for 30 min are the most common conditions [15], we only used 15 min because we experimentally demonstrated that it was enough to dry the sorbent as was shown in the paper of Koning et al. [16].

After selecting these parameters, we optimised the elution volume, sample pre-treatment and sample volume step by step. First, we optimised the elution volume by preconcentrating 10 ml of a working solution of 0.5 μg l^{-1} and eluting with volumes from 100 to 300 μl of ethyl acetate. We chose a volume of 100 μl of ethyl acetate because recoveries were no greater when the volume was higher. Second, to increase the low recoveries that were probably caused by the adsorption of the analytes in the system, we optimised the sample pre-treatment conditions by preconcentrating 10 ml of working solution of 0.05 μg l^{-1} and eluting with 100 μl of ethyl acetate. Recoveries were calculated by comparing the areas with those obtained with 100 μl loop injections. We added several volumes of methanol to the sample before the enrichment step to check for

Table 1
Selected ions and analytical data obtained in the full-scan acquisition mode for river water analysed by SPE–GC–MS

Compound	Selected ions (relative abundances)	Linear range ($\mu g\ l^{-1}$)	r^2	LOD ($ng\ l^{-1}$)	RSD (%, $n=3$)[b,c]	RSD (%, $n=3$)[a,c]
Hexachlorobenzene	284 (100)	0.006–0.6	1	1	14	24
Atrazine	200 (100)	0.05–5.0	0.9999	20	12	27
Lindane	181 (100)	0.06–6.0	0.9999	10	8	28
Vinclozolyn	212 (100)	0.05–5.0	1	10	14	14
DnBP	149 (100)	0.007–3	0.9996	0.2[d]	14	22
Aldrin	263 (100)	0.005–0.5	0.9999	2	19	26
α-Endosulfan	195 (100)	0.06–6.0	1	20	12	20
p,p′-DDE	246 (100)	0.006–0.6	0.9994	2	22	24
Dieldrin	79 (100)	0.005–0.5	0.9997	2	16	8
BBP	149 (100)	0.05–5	0.9997	7[d]	10	15
Adipate	129 (100)	0.05–5	1	7[d]	12	18
p,p′-DDT	235 (100)	0.05–0.6	0.9999	8	28	32
DEHP	149 (100)	0.05–3	0.9998	0.1[d]	16	20
Benzo[*a*]pyrene	252 (100)	0.005–0.5	0.9993	2	14	20

[a] Under repeatability conditions.
[b] Under reproducibility between-days conditions.
[c] Spiking level: 0.1 $\mu g\ l^{-1}$.
[d] LODs estimated by calibration curves of direct injection.

the adsorption problems. As Table 2 shows, methanol increased the recovery of all compounds except atrazine. Due to its elution, the recovery of this compound decreased when the percentage of methanol increased. Taking into account the recoveries of all the analytes, we chose 50% of methanol as the best condition. Before preconcentration we also added acetic acid to the sample to study how this affected recoveries. However, recoveries did not increase and acetic acid was not used in further experiments.

Finally, we optimised the sample volume by

Table 2
Effect of methanol, acetic acid and sample volume on recoveries ($n=3$) by preconcentrating a working solution

Compound	Recovery, % (RSD, %, $n=3$)							
	10 ml sample[a]						15 ml sample[b], 50% methanol	20 ml sample[a], 50% methanol
	10% Methanol	20% Methanol	40% Methanol	50% Methanol	60% Methanol	60% Methanol+ 1% acetic acid		
Hexachlorobenzene	–	–	16 (11)	68 (2)	82 (3)	64 (9)	68 (4)	62 (5)
Atrazine	100 (4)	44 (9)	35 (8)	10 (11)	9 (10)	–	10 (10)	3 (10)
Lindane	70 (6)	64 (8)	66 (5)	78 (3)	74 (5)	68 (10)	80 (4)	64 (6)
Vinclozolyn	64 (7)	65 (6)	62 (4)	63 (6)	64 (5)	59 (9)	64 (7)	48 (7)
DnBP	6 (25)	25 (14)	58 (6)	62 (2)	63 (8))	60 (7)	65 (9)	50 (6)
Aldrine	–	–	40 (7)	73 (1)	73 (3)	71 (2)	63 (6)	54 (6)
α-Endosulfan	9 (13)	25 (11)	58 (4)	72 (1)	79 (2)	80 (2)	73 (3)	57 5)
p,p′-DDE	–	–	43 (11)	71 (1)	66 (9)	66 (10)	70 (8)	53 (3)
Dieldrin	2 (14)	19 (11)	56 (5)	68 (2)	64 (7)	65 (11)	70 (8)	57 (9)
BBP	–	18 (4)	50 (2)	56 (1)	50 (1)	59 (2)	58 (3)	41 (7)
Adipate	–	–	–	42 (1)	46 (3)	58 (2)	41 (4)	38 (3)
p,p′-DDT	–	–	25 (9)	42 (4)	27 (5)	52 (2)	45 (4)	25 (8)
DEHP	–	–	2 (14)	48 (9)	57 (8)	83 (6)	50 (10)	43 (11)
Benzo[*a*]pyrene	–	–	61 (14)	68 (10)	90 (5)	85 (7)	67 (16)	46 (17)

[a] Working solutions spiked at different concentration levels so that the theoretical final amount preconcentrated would be 5 ng.

preconcentrating several volumes between 10 and 20 ml of working solutions (50% methanol) at different concentrations so that the theoretical final amount preconcentrated (5 ng) would remain constant. The retained compounds were eluted by 100 μl of ethyl acetate. As Table 2 shows, the recoveries corresponding to 10 ml and 15 ml of sample were quite similar and at 20 ml they started to decrease. Consequently, we chose a sample volume of 15 ml. Further experiments showed that there were no carry over effects with these conditions.

3.4. On-line SPE–GC–MS

After optimising the SPE parameters, we checked the performance of the method by analysing 15 ml of working solutions by SPE–GC–MS (full scan). We used full-scan acquisition mode because the mass spectra confirm the presence of the EDs and because the levels of concentration are low enough.

When we analysed a blank of Milli-Q water, some peaks appeared at retention times corresponding to DnBP, BBP, DEHP and adipate. That these peaks were indeed those of these four EDs was confirmed by their spectra. We therefore checked the linear range of the method for these compounds by subtracting the areas obtained in the blank chromatograms ($n=4$) from the areas obtained in the working solutions spiked at different levels of concentration.

The linearity of the response in Milli-Q water was checked in the 0.005 to 6 μg l^{-1} range. Linearity was good in this range for most of the compounds whose correlation coefficients were higher than 0.997. We calculated the limits of detection (LODs), taking into account a signal/noise ratio of 3, were between 0.1 and 20 ng l^{-1} for all compounds except for DnBP, BBP, DEHP and adipate. The LODs for these compounds were estimated taking into account a signal/noise of 3 but subtracting signals obtained in the blank chromatograms from the signals obtained in the working solutions. The repeatability and reproducibility between days were also checked by analysing three working solutions spiked with 0.1 μg l^{-1} of each analyte. The results, expressed as relative standard deviation (RSD), varied from 8 to 15% for repeatability, except for *p,p′*-DDE (20%) and *p,p′*-DDT (25%) and from 10 to 25% for reproducibility, except for *p,p′*-DDT (30%).

In Milli-Q water, DnBP and DEHP were found in concentrations of 0.5 μg l^{-1} and BBP and adipate were found in concentrations of 0.02 and 0.03 μg l^{-1}, respectively ($n=4$).

3.5. Application to real samples

We tested the performance of this method with real samples of water from the Ebro river, and real samples of coastal and tap waters.

We first analysed a blank of Ebro river water. This showed several peaks at the same retention times as some of the compounds we studied. The spectra of these peaks confirmed that they corresponded to DnBP, BBP, DEHP and adipate.

The recoveries for Ebro river water were similar to those obtained with working solutions. With river water spiked with 0.005–6 μg l^{-1} of all the analytes, linearity was similar to that for Milli-Q water. The linear ranges for each compound are given in Table 1. We calculated the linearity of the four compounds identified in the blank of river water blank in the same way as with Milli-Q water. The limits of detection, calculated with a signal/noise ratio of 3, were between 0.1 and 20 ng l^{-1}. Repeatability and reproducibility between days were also checked by analysing three samples of river water spiked with 0.1 μg l^{-1} of each analyte. The results, expressed as RSD, ranged from 8 to 28% for repeatability and from 8 to 32% for reproducibility. The results for river water obtained under full-scan acquisition are given in Table 1. Fig. 1 shows the extracted ion chromatogram of a river-water sample spiked with 0.1 μg l^{-1} of each compound.

In river water, DnBP and adipate were each found at a concentration of 0.08 μg l^{-1} ($n=3$). However, BBP and DEHP could not be quantified because their concentrations lay between the detection limit and the quantification limit of the method. Fig. 2 shows the extracted ion chromatograms for phthalates (m/z 149) and adipate (m/z 129), from the analysis of an unspiked river-water sample in the full-scan acquisition mode.

We also analysed several samples of coastal and tap waters. When methanol was added to the coastal-water sample to 50%, a filtration step was needed before SPE to eliminate the salts precipitated. For example, in one of sample of coastal water BBP,

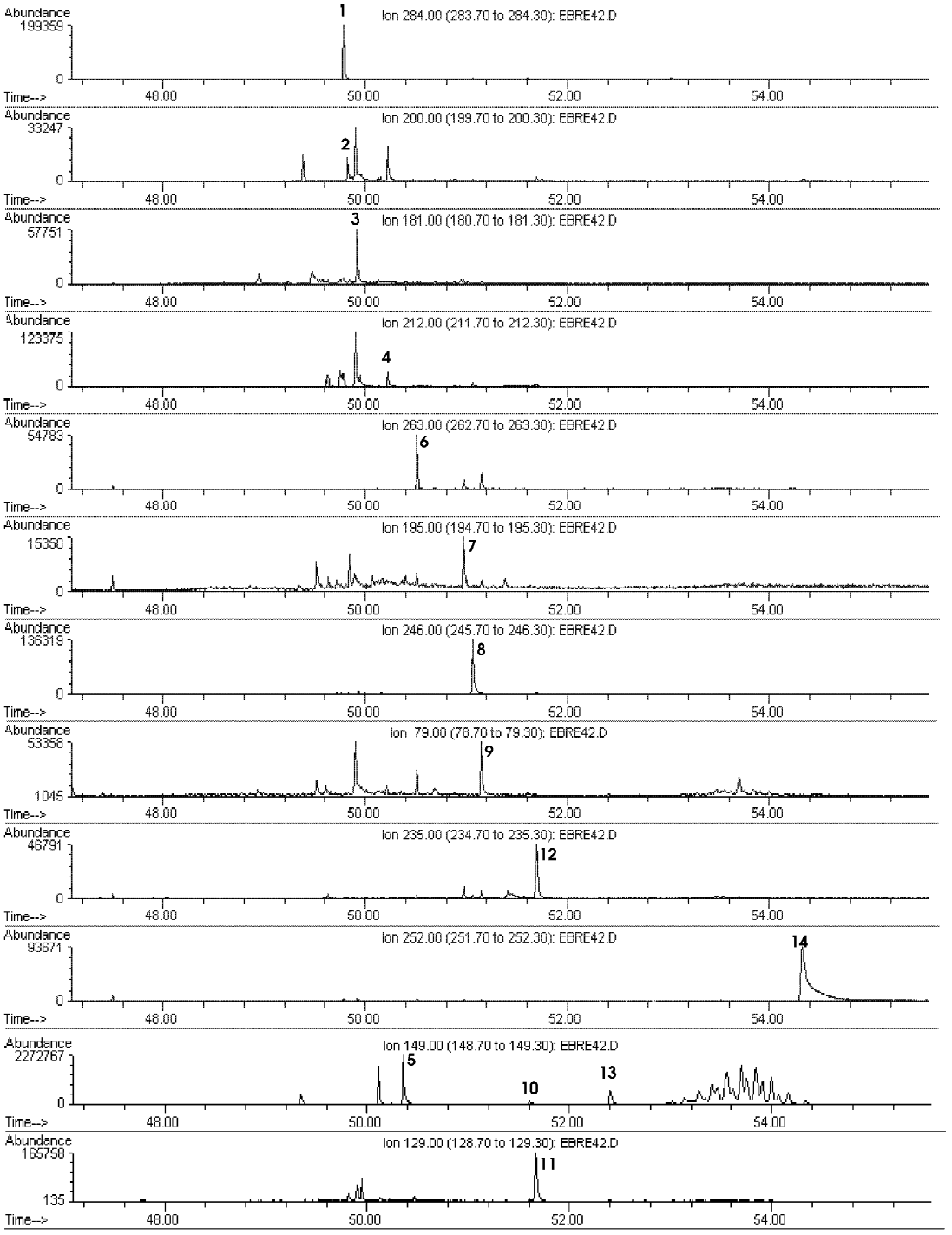

Fig. 1. Extracted ion chromatograms for a Ebro river water sample spiked at 0.1 μg l^{-1}. Peaks: (1) hexachlorobenzene, (2) atrazine, (3) lindane, (4) vinclozolyn, (5) DnBP, (6) aldrin, (7) α-endosulfan, (8) *p,p*-DDE, (9) dieldrin, (10) BBP, (11) adipate, (12) *p,p*-DDT, (13) DEHP, (14) benzo[*a*]pyrene. Time scales in min.

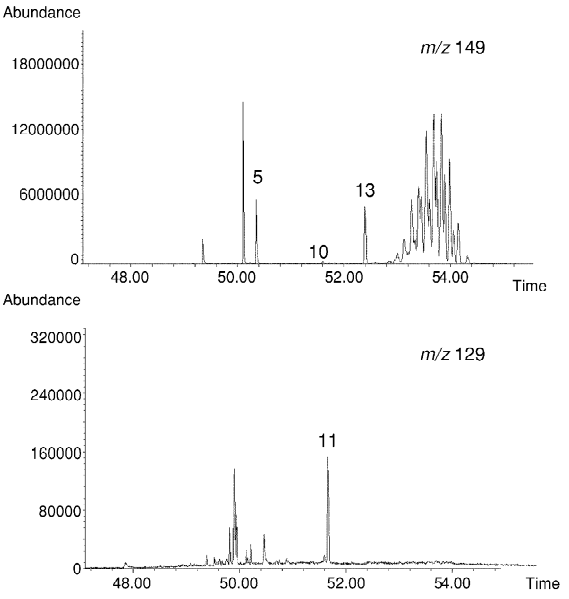

Fig. 2. Extracted ion chromatograms at m/z 149 and 129 obtained by on-line SPE–GC–MS of 15 ml Ebro river water. For peak assignation see Fig. 1. Time scale in min.

DEHP and DnBP were found at concentrations of 0.08, 0.12 and 0.48 μg l^{-1}, respectively ($n=3$). Fig. 3 shows the extracted ion chromatogram (m/z 149) of the blank of coastal water and the spectrum corresponding to DEHP. Adipate, DEHP and DnBP were found in tap water at concentrations of 0.02, 0.1 and 0.3 μg l^{-1}, respectively ($n=3$). Some of these phthalates had been found at levels of around 0.3 and 2 μg l^{-1} in similar matrices (such as Ebro river water and port fishing water) in previous studies [17,18].

The precolumn packed with PLRP-S and the retention gap analysed at least 100 real samples without any change in performance.

4. Conclusions

SPE has been on-line coupled to GC–MS through an on-column interface to determine a group of endocrine-disrupting compounds in several water samples.

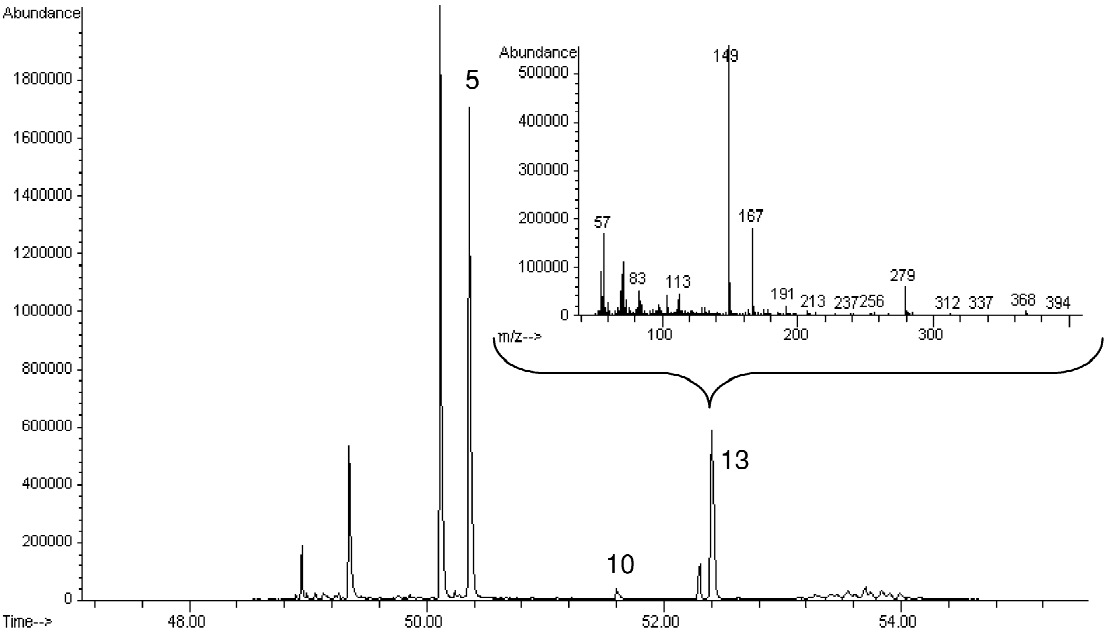

Fig. 3. Extracted ion chromatogram (m/z 149) of a coastal-water sample. The insert shows the spectrum of DEHP. For peak assignation see Fig. 1. Time scale in min.

Parameters affecting the SPE process were optimised and 50% of methanol was added to the samples before the preconcentration step to minimise adsorption problems and enhance the recoveries of the analytes. Optimal transfer conditions were: a syringe pump flow-rate of 47 μl min^{-1}, transfer temperature of 60 °C and closure of the SVE 1 min after the transfer.

With this method we quantified all the analytes at low levels by preconcentrating only 15 ml of sample and simultaneously identified them using the full-scan acquisition mode. The total analysis time was 55 min.

Three phthalates and adipate were found in several real samples. In river water DnBP and adipate were found at concentrations of 0.08 μg l^{-1}. In coastal and tap waters the three phthalates and adipate were found at concentrations of between 0.02 and 0.5 μg l^{-1}.

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