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Determination of endocrine-disrupting compounds in water samples by on-line solid-phase extraction–programmed-temperature vaporisation–gas chromatography–mass spectrometry

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Abstract

We developed an automated on-line solid-phase extraction (SPE)–gas chromatography–mass spectrometry method to determine a group of endocrine disruptors in water samples. The interface device used for connecting SPE with GC was a programmed-temperature vaporiser (PTV) whose liner was packed with Tenax. We optimized the parameters that affected both SPE and PTV working in solvent vent mode. The performance of the method was tested with several environmental water samples. The limits of detection of the method were between 0.001 and 0.036 μg l^{-1} under full-scan acquisition mode. We determined phthalates and adipate in all real samples at concentrations between 0.11 and 8.10 μg l^{-1}. Atrazine and *p,p′*-DDE were also found in an irrigation stream water sample at 0.16 and 0.04 μg l^{-1}, respectively.

Keywords: Water analysis; Sample preparation; Injection methods; Programmed-temperature vaporiser; Automation; Environmental analysis; Endocrine disruptors; Pesticides; Phthalates

1. Introduction

Endocrine disruptors (EDs) are compounds that can have adverse effects on the living organism because they interfere with the endocrine system. Some of them are suspected of causing abnormalities in sperm and increasing hormone-related cancers in humans [1–3]. Some studies have also been published on the estrogen-like responses of EDs in wildlife, such as birds, amphibians, reptiles and fish [4]. These compounds include a wide variety of pollutants such as pesticides, polycyclic aromatic hydrocarbons, phthalate plasticizers, alkylphenols, and natural and synthetic hormones [5]. They have many industrial and household uses and therefore they may appear in environmental samples such as waste, river and sea waters. Analytical methods must be developed so that these contaminants can be determined in aqueous environmental samples and their possible health and ecological effects evaluated [6].

These compounds are present in the environment at low levels of concentration and they can only be determined by using a preconcentration technique. They are generally determined by solid-phase extraction (SPE), liquid–liquid extraction (LLE) or

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solid-phase microextraction (SPME) [7–9] followed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) [7,8,10–12]. Nowadays, mass spectrometry (MS) is the detection technique generally used because it enables quantitative and qualitative analysis simultaneously [8,10,12–14]. Some EDs have also been determined with immunochemical techniques, such as enzyme-linked immunosorbent assay (ELISA) [15]. But there is now increasing interest in developing on-line techniques for water analysis that combine sample preparation, separation and detection. The most frequently used of these hyphenated techniques is on-line SPE–HPLC [16,17] because of its simplicity. These techniques can be fully automated, and they can improve reproducibility, increase sensitivity and minimize contamination from external sources. Consequently, these techniques are suitable for performing monitoring studies.

In this paper, we tested the applicability of an on-line SPE–programmed-temperature vaporisation–GC–MS method for determining a group of EDs in water samples collected in the south of Catalonia. A programmed-temperature vaporiser (PTV) interface was used because it allows multiple injections in the GC and the analysis of dirty samples when a packed liner is used [18].

2. Experimental

2.1. Reagents and standards

The compounds studied were atrazine, lindane (γ-hexachlorocyclohexane), vinclozolin, malathion, parathion ethyl, aldrin, α-endosulfan, *p,p′*-DDE, *p,p′*-DDT, dieldrin, benzo[*a*]pyrene, hexachlorobenzene (HCB), 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 (Steinhem, 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 ED was prepared in ethyl acetate and stored in the refrigerator. Every week, a solution of 50 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 was supplied by Merk (Darmstadt, Germany) and methanol was supplied by SDS (Peypin, France), both trace analysis grade. 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

We used an Agilent (Waldbronn, Germany) HP 6890 Series gas chromatograph equipped with a PTV injector and an HP 5973 mass spectrometry detector. To perform the chromatographic separation we used an analytical column 0.25-μm of 28 m×250 μm I.D. HP-5 MS supplied by Agilent. 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). An Agilent 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 metallic tubing (25 cm×0.25 mm I.D.) permanently mounted in the PTV injector transferred the analytes from the precolumn to the GC system. The PTV liner (7 cm×2 mm I.D.) was packed with 0.014±0.002 g of Tenax (Supelco, Bellefonte, PA, USA) to reduce losses of volatile solutes. A 100-μl loop of polyethylene ether ketone (PEEK) tubing (Agilent) was used instead of the precolumn for direct injection.

2.3. On-line SPE–PTV–GC–MS

Three six-port valves connected in series were used for on-line sample enrichment. The precolumn was conditioned by flushing with 3 ml of methanol and 3 ml of water. We added 50% of methanol to the sample to avoid adsorption problems and 15 ml of this mixture was preconcentrated. The tubes were purged with this solution, which 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. The analytes were desorbed in the backflush mode with three ethyl acetate fractions of 100 μl pumped at 48 μl min^{-1} with a syringe pump and on-line transferred to the GC system via the transfer line. A PTV injector, working in solvent vent mode, was used as the interface.

PTV involved three steps: sampling/venting, analyte transfer and purge/clean-up. During the first step, sampling/venting, the inlet temperature was 65 °C, the vent flow was 30 ml min^{-1} and the split valve was kept open. Between the multiple injections and the second step (vent end time) there was a period of 2 min so that most of the solvent vapours were vented. The pressure through the column (6 p.s.i.; 1 p.s.i.=6894.76 Pa) was lower than the working pressure to accelerate the solvent elimination and to reduce the amount of carrier gas and solvent vapour that entered to the column. In the second step, analyte transfer, the split valve was closed and the inlet was rapidly heated to 300 °C at 720 °C min^{-1} and kept constant for 3 min. In this way the analytes were vaporised and introduced into the column. The inlet pressure was set at a variable working pressure so that the flow-rate was a constant 1 ml min^{-1}. In the third step, purge/clean-up, the split valve was opened again and Tenax was flushed with the carrier gas at 50 ml min^{-1} to prevent carryover.

The oven temperature was held constant at 60 °C until 1 min before the analyte transfer from the Tenax had finished. Then, it was increased to 270 °C at 40 °C min^{-1} and kept constant for 2 min; subsequently it was increased to 290 °C at 60 °C min^{-1} and kept constant for 3 min. The optimal conditions and time relationships between these parameters are shown in Fig. 1. The chromatographic separation was optimized by GC–MS by manually injecting 1 μl of a standard solution containing 10 mg l^{-1} of each compound in ethyl acetate. The PTV injector worked in splitless mode at 300 °C and its liner was empty. The total analysis time, including sample preparation as well as chromatographic analysis, was 63.3 min.

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 75–400 with a rate of 4.72 scans s^{-1}. Table 1 shows the ions for quantifying the EDs, obtained in the full-scan mode from the spectrum of each compound. All ions corresponded to the base peak, except for ethyl parathion for which we selected a higher m/z peak to increase selectivity.

2.4. Sampling

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

All the glass material (e.g. vials, bottles and volumetric material) was carefully cleaned with methanol to avoid contamination problems.

3. Results and discussion

3.1. Large volume injection

In accordance with the literature, we set parameters such as the inlet pressure during the sampling/venting step and the liner sorbent. The sorbent selected was Tenax because it has a good capacity for retaining compounds with a wide range of volatility, polarity and thermostability [18]. The inlet pressure during the sampling/venting step was set at 6 p.s.i., the minimum value for the GC equipment.

First, we optimized the time needed to quantitatively desorb the analytes from Tenax (purge time) by manually injecting 1 μl of a 1 mg l^{-1} working solution in the packed liner at 300 °C. Fig. 2 shows

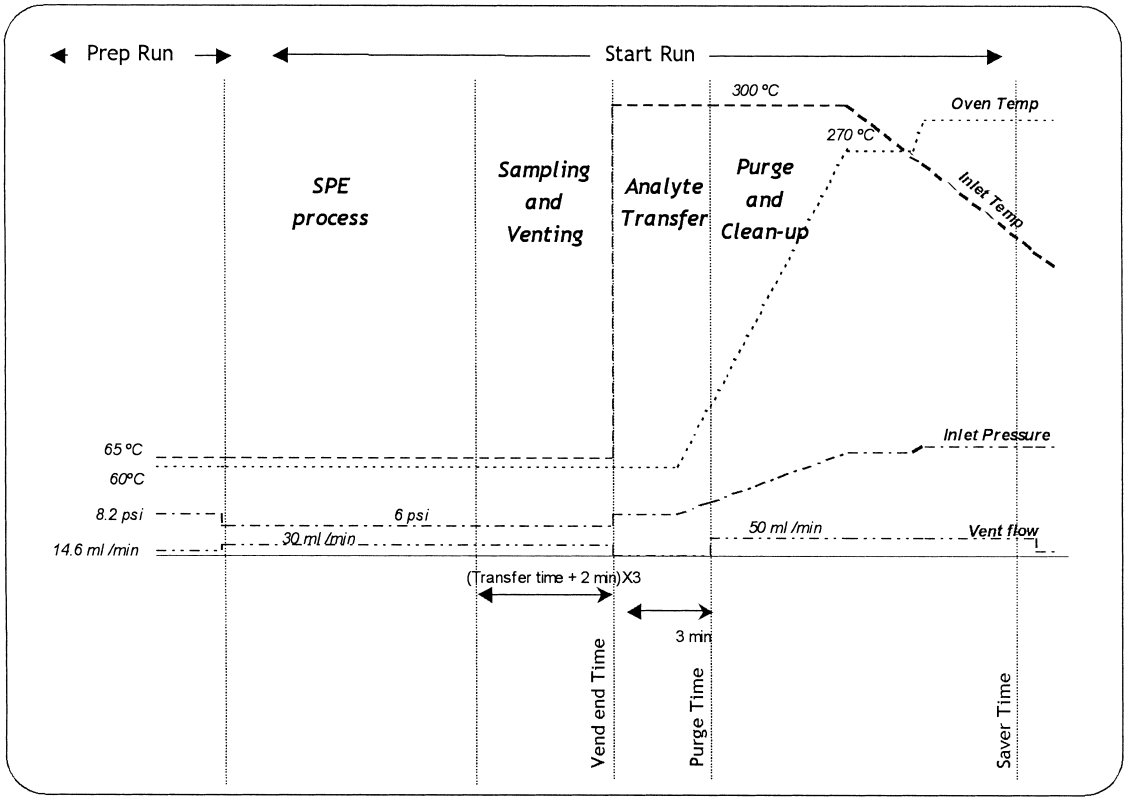

Fig. 1. Optimal conditions and time relationships between the parameters for the PTV injector in a solvent vent mode and some GC parameters.

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

Compounds	Selected ions (relative abundances)	Linear range ($\mu g\ l^{-1}$)	r^2	LOD ($ng\ l^{-1}$)	RSD (%, $n=3$)[a]	RSD (%, $n=3$)[b]
Hexachlorobenzene	284 (100)	0.01–5	0.9900	1	2	10
Atrazine	200 (100)	0.05–1	0.9973	20	5	30
Lindane	181 (100)	0.05–5	0.9997	20	7	15
Vinclozolin	212 (100)	0.05–5	0.9994	10	3	6
DnBP	149 (100)	0.05–4	0.9916	8	4	26
Malathion	173 (100)	0.10–10	0.9990	30	11	18
Parathion ethyl	291 (88)	0.05–10	0.9989	10	3	5
Aldrin	263 (100)	0.02–5	0.9990	5	3	3
α-Endosulfan	195 (100)	0.05–5	0.9984	10	2	17
p,p′-DDE	246 (100)	0.003–5	0.9990	1	2	9
Dieldrin	79 (100)	0.05–5	0.9996	10	4	10
BBP	149 (100)	0.05–5	0.9994	5	9	23
Adipate	129 (100)	0.05–5	0.9995	5	5	29
p,p′-DDT	235 (100)	0.02–5	0.9975	7	8	25
DEHP	149 (100)	0.10–10	0.9913	10	32	35
Benzo[*a*]pyrene	252 (100)	0.10–5	0.9990	36	1	9

RSD (%) has been calculated by spiking all compounds at their LOQ except HCB, *p,p′*-DDE and *p,p′*-DDT at 0.05 $\mu g\ l^{-1}$.
[a] Under repeatability conditions.
[b] Under reproducibility between-days conditions.

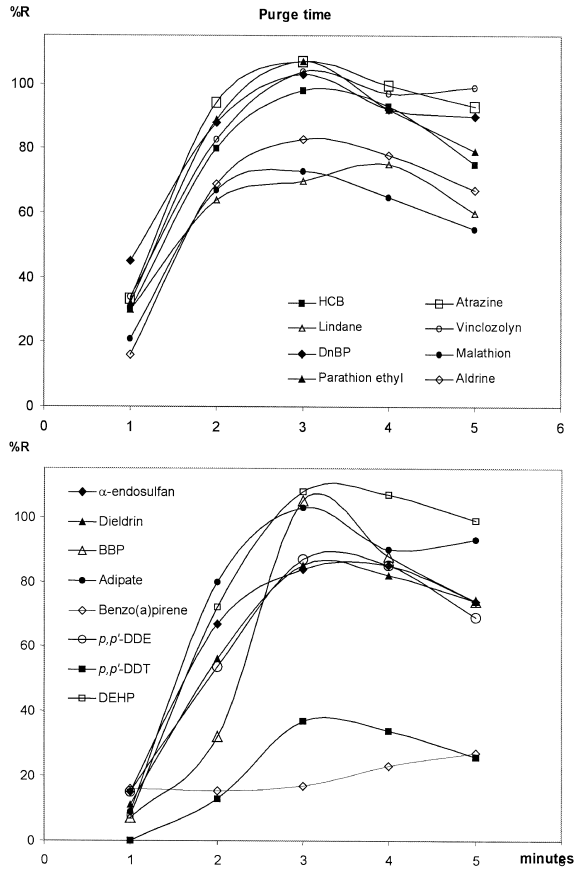

Fig. 2. Effect of purge time on recoveries.

the results. A purge time of 3 min was selected because at longer times recoveries did not increase.

Secondly, we optimized the vent flow, vent end time and the initial inlet temperature by introducing 100 μl of a working solution of 0.01 mg l^{-1} at 48 μl min^{-1}. As Fig. 3 shows the optimum vent flow was 30 ml min^{-1}. Subsequently, 2 min was the optimum vent end time and 65 °C the optimum initial inlet temperature. We calculated the relative recoveries of these parameters by comparing the areas with those obtained with direct injections of 1 μl in a PTV injector with an empty liner working in splitless mode at 300 °C.

Finally, we optimized the carrier gas flow-rate to purge the Tenax and the optimum value was 50 ml min^{-1}.

3.2. SPE process

We selected PLRP-S as the sorbent and ethyl acetate as the elution solvent because we had used them successfully in a previous study [19]. We preconcentred 15 ml of working solution, 50% of which was methanol. Before the elution step, the sorbent was dried with nitrogen at 3 bar for 15 min.

We optimized the elution volume from 100 to 300 μl of ethyl acetate, doing multiple injections of 100 μl. We chose a volume of 300 μl of ethyl acetate because, as shown in Table 2, the higher the volume, the higher the recoveries. Also, further experiments showed that there were no carry over effects in these conditions. In general, these recoveries are better than those obtained in the previous study [19].

3.3. On-line SPE–PTV–GC–MS

We checked the performance of the method by analyzing 15 ml of working solutions by SPE–PTV–GC–MS in full-scan acquisition mode.

When we analyzed a blank of Milli-Q water, some small peaks appeared at retention times corresponding to DnBP, BBP, DEHP and adipate and they were identified by their spectra. Due to their ubiquitous presence, contamination with phthalates during experimentation might well interfere with analyses. This contamination hinders the interpretation of measured concentrations. But, using the Milli-Q water collected on the same day, the areas obtained in the blank chromatograms ($n=4$) had good repeatability (RSD below 8%) for the four compounds. So, we checked the linear range of the method for these compounds by subtracting the areas obtained in the blank chromatograms 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 0.003 to 10 μg l^{-1} range. Linearity was good in several ranges with correlation coefficients higher than 0.9900. To calculate the limits of detection (LODs) we took into account a signal/noise ratio of 3. They were between 1 and 36 ng l^{-1} for all compounds. The LODs for DnBP, BBP, DEHP and adipate were estimated taking into account a signal/noise ratio of 3 but the signals obtained in the blank chromatograms were subtracted

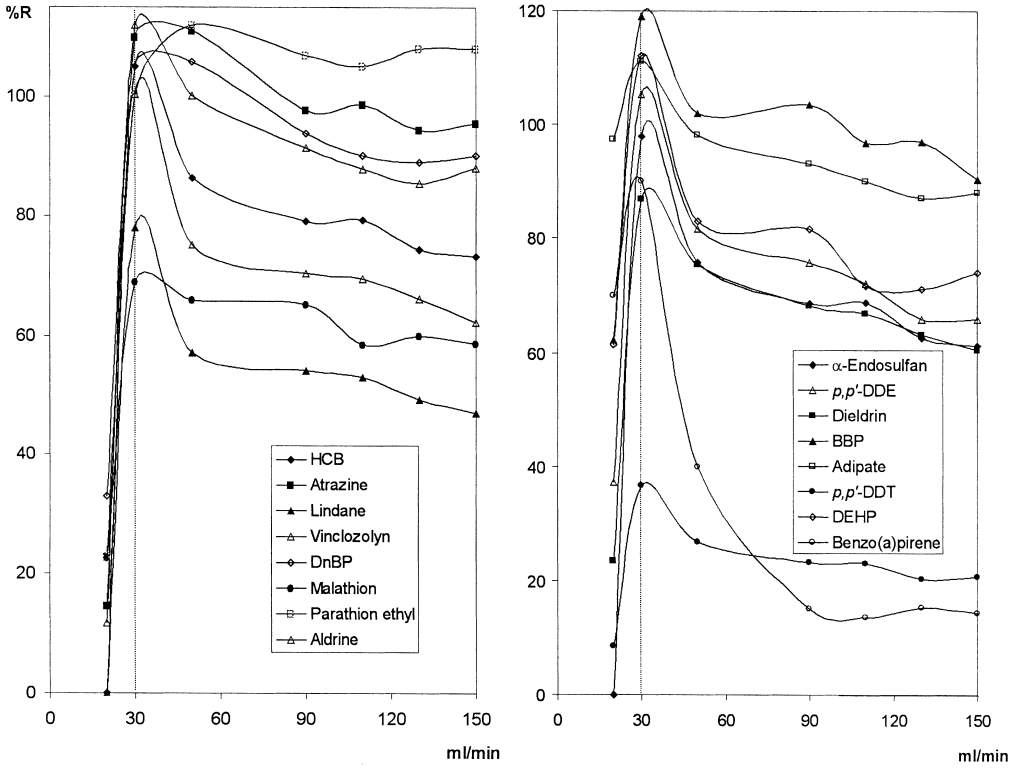

Fig. 3. Effect of split vent flow on recoveries.

Table 2
Effect of elution volume on recoveries ($n=3$) by preconcentrating a working solution

Compounds	% Recovery (RSD, %, $n=3$)		
	15 ml sample, 50% methanol		
	100 μl Ethyl acetate	200 μl Ethyl acetate[a]	300 μl Ethyl acetate[a]
Hexachlorobenzene	41 (14)	54 (3)	64 (7)
Atrazine	12 (8)	13 (8)	29 (25)
Lindane	64 (8)	81 (15)	111 (19)
Vinclozolin	67 (7)	64 (12)	89 (8)
DnBP	70 (6)	87 (5)	93 (9)
Malathion	68 (14)	80 (18)	111 (12)
Ethyl parathion	63 (13)	69 (11)	105 (10)
Aldrin	47 (4)	53 (7)	80 (5)
α-Endosulfan	53 (3)	65 (15)	83 (15)
p,p′-DDE	55 (12)	49 (6)	69 (14)
Dieldrin	59 (5)	51 (8)	80 (12)
BBP	74 (8)	73 (11)	97 (15)
Adipate	51 (3)	92 (7)	98 (4)
p,p′-DDT	48 (8)	66 (9)	78 (15)
DEHP	51 (7)	63 (11)	72 (10)
Benzo[*a*]pyrene	–	96 (19)	102 (18)

[a] By multiple injections of 100 μl.

from the signals obtained in the working solutions. To check the repeatability and reproducibility between days, we analyzed three working solutions spiked at their limits of quantification (LOQs) for all the compounds, except HCB, *p,p′*-DDE and *p,p′*-DDT, which were spiked at 0.05 μg l^{-1}. The results, expressed as RSD, varied from 1 to 8% for repeatability, except for DEHP (20%), and from 3 to 25% for reproducibility.

3.4. Application to real samples

We tested the performance of this method with real samples of water collected in the south of Catalonia (from the Ebro river, the sea, the tap, an irrigation stream and a sewage treatment plant).

In the chromatogram of the Ebro river water, several peaks appeared at the same retention times as DnBP, BBP, DEHP and adipate and they were confirmed with the spectra. Some of these phthalates, such as DnBP and DEHP, had also been determined in an Ebro river water sample using SPME–GC–MS by our group [20].

The recoveries for Ebro river water were similar to the recoveries with working solutions. With river water spiked with 0.003–10 μg l^{-1} of all the analytes, linear range was similar to that for Milli-Q water. We calculated the linear range of the four compounds identified in the river water blank in the same way as with Milli-Q water. We calculated the limits of detection with a signal/noise ratio of 3, and checked the repeatability and reproducibility between days by analyzing three samples of river water spiked at their LOQs for all the compounds, except HCB, *p,p′*-DDE and *p,p′*-DDT, which were spiked at 0.05 μg l^{-1}. The analytical data for river water obtained under full-scan acquisition are given in Table 1.

In a river water sample, DnBP, DEHP and adipate were found at a concentration of 0.80, 2.10 and 0.11 μg l^{-1}, respectively ($n=3$). However, BBP could not be quantified because its concentration was between the detection limit and the quantification limit of the method. These values found are similar to these obtained for samples from the same origin analysed by SPME [20].

We also analyzed several samples of water from the sea, the tap, irrigation streams and an effluent sewage treatment plant. As we showed in a previous study [19], when methanol was added to sea-water samples, a filtration step was needed before SPE to eliminate the precipitated salt. By analyzing a lot of samples with a high salt concentration, we proved that this method with a PTV interface was more robust than the method with an on-column interface. The packing material of the liner serves as a filter, protects the GC column and is easier to change than the retention gap in the on-column interface. Table 3 shows the contaminants found in these samples. Phthalates and adipate were determined at different concentration levels in most of the samples analyzed. Fig. 4 shows the extracted ion chromatograms in merged format for phthalates and adipate (*m/z* 149 and 129, respectively) from the analysis of an unspiked irrigation stream water sample. In this sample, two peaks appeared and were identified as other phthalate compounds because the base peak (*m/z* 149) of their spectra is a typical fragment of this kind of compound. The first peak was identified by the Wiley138 library as di-*n*-ethyl phathalate

Table 3
Levels (μg l^{-1}) of some EDs found in several real samples ($n=3$)

Compounds	River water	Sea water	Irrigation stream water	Waste water (effluent from treatment plant)	Tap water
Atrazine	–	–	0.16	–	–
DnBP	0.80	2.09	3.57	2.10	1.34
p,p′-DDE	–	–	0.04	–	–
BBP	l.q.	0.15	0.10	l.q.	l.q.
Adipate	0.11	0.59	0.24	l.q.	0.20
DEHP	2.10	8.10	5.21	3.97	4.26

l.q. = below limit of quantification.

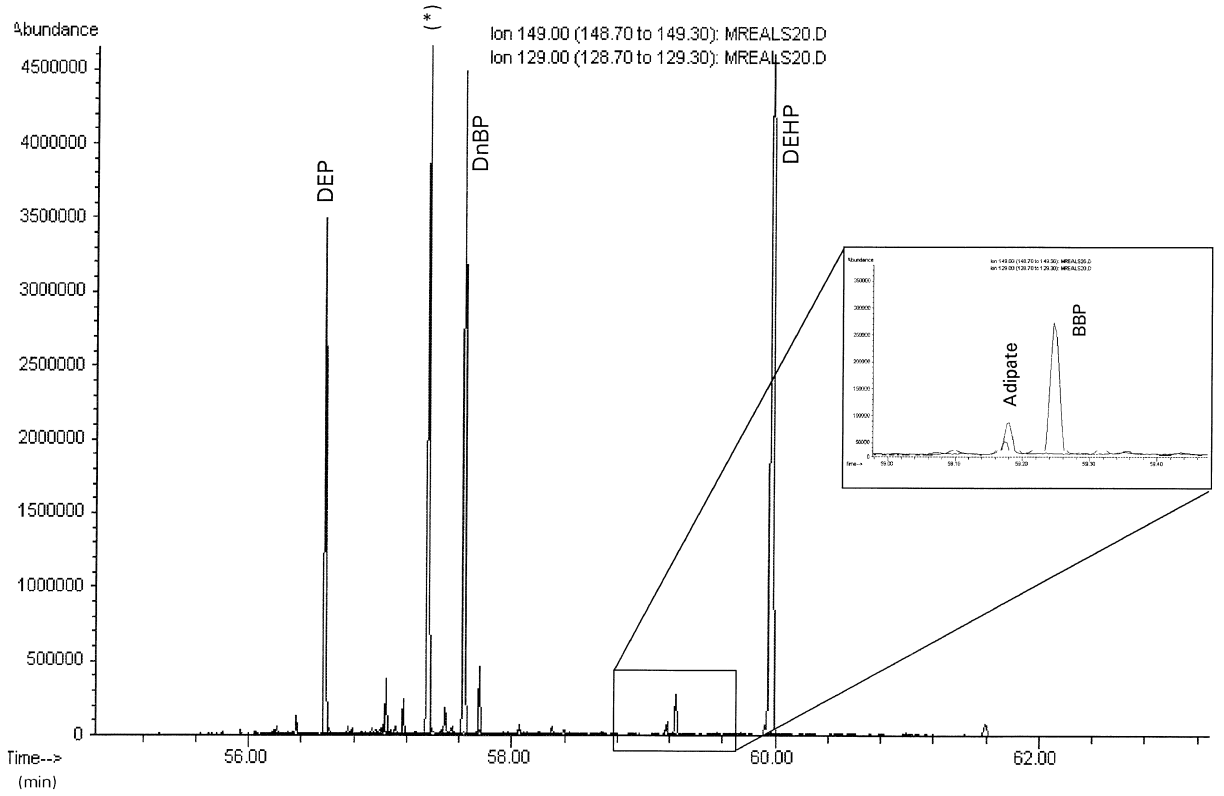

Fig. 4. Extracted ion chromatograms, displayed in merged format, at m/z 149 and 129 obtained by on-line SPE–PTV–GC–MS of 15 ml irrigation stream water. (*) Not confirmed.

(DEP) (95%) and was confirmed with a standard. The second peak was identified by the library as bis(2-methylpropyl) phthalate (72%), but we could not confirm this because no standard was available. The other small peaks could not be assigned to any contaminant.

Although phthalates have several degradation pathways and therefore are not considered to be persistent chemicals, DnBP and DEHP fluxes in the environment are high because of the considerable direct and indirect emissions. In the Netherlands, Van Wezel et al. [21] derived the environmental risk limits (ERLs) for these compounds in water to 10 μg l^{-1} for DnBP and 0.19 μg l^{-1} for DEHP. They used these ERLs as the estimated ecosystem no-effect concentration concluded that they give sufficient protection against endocrine disruptive effects. The levels found in various surface waters for DnBP are below the ERL, but for DEHP are generally 5 to 40 times higher than the ERL (see Table 3).

Atrazine and p,p'-DDE were determined in an irrigation stream water sample at 0.16 and 0.04 μg l^{-1}, respectively. The sample was collected in spring and in a zone where atrazine is usually used as a herbicide. Its concentration is lower than the maximum admissible concentration suggested by the European Union (EU) for surface water (1–3 μg l^{-1}). The presence of p,p'-DDE in the environment is due to DDT degradation. Nowadays, DDT is banned by an EU Directive and by the US Environmental Protection Agency (EPA), but it was extensively used for decades and it is a persistent organic chemical. Fig. 5 shows the extracted ion chromatograms of atrazine and p,p'-DDE. The insert

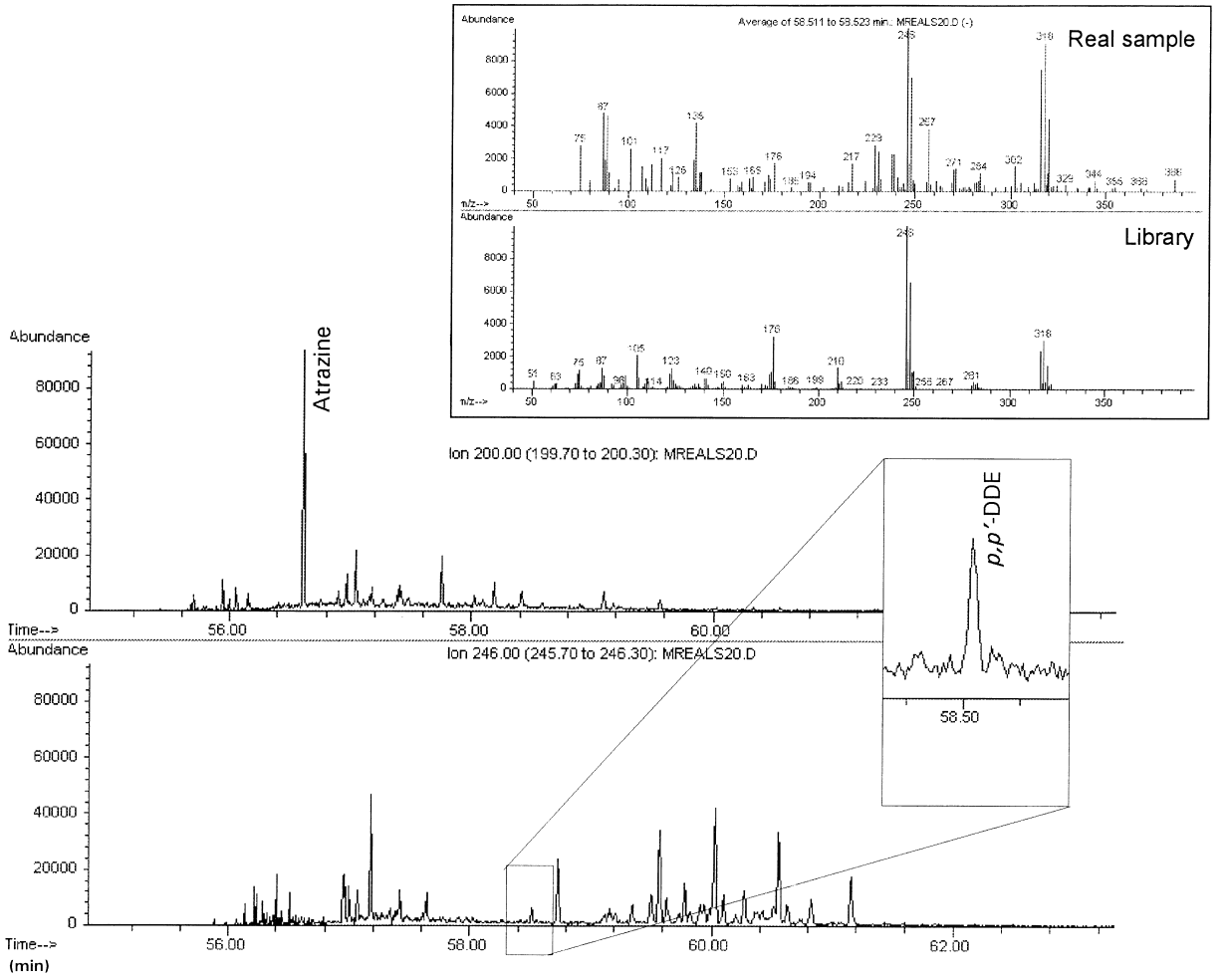

Fig. 5. Extracted ion chromatograms at m/z 200 and 246 obtained by on-line SPE–PTV–GC–MS of 15 ml irrigation stream water. The insert shows the spectra of p,p'-DDE of the real sample and the library.

shows the p,p'-DDE spectrum that was identified with a quality higher than 90%.

4. Conclusions

An automatic on-line SPE–PTV–GC–MS method was successfully applied to determine a group of EDs in several water samples.

The method quantified the analytes at ng l^{-1} levels by preconcentrating only 15 ml of sample and identified them by using full-scan acquisition mode.

The recoveries obtained by this method were better than the recoveries obtained with a SPE–GC–MS using an on-column interface. Although the analytical data were similar in both methods, the PTV interface proved to be more robust than the on-column interface when samples with a high salt concentration were analysed.

DnBP, BBP, DEHP and adipate were found in all real samples. The DEHP concentration was generally

between 5 and 40 times higher than the ERL and the DnBP concentration was below its ERL. Atrazine and p,p'-DDE were found in an irrigation stream water sample at concentrations of 0.16 and 0.04 μg l^{-1}, respectively.

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