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Review

In-tube solid-phase microextraction coupled by in valve mode to capillary LC-DAD: Improving detectability to multiresidue organic pollutants analysis in several whole waters

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ABSTRACT

A simple and fast capillary chromatographic method has been developed to identify and quantify organic pollutants at sub-ppb levels in real water samples. The major groups of pesticides (organic halogens, organic phosphorous, and organic nitrogen compounds), some hydrocarbons (polycyclic aromatic hydrocarbons), phthalates and some phenols such as phenol and bisphenol A (endocrine disruptors) were included in this study. The procedure was based on coupling, in-tube solid-phase microextraction (IT-SPME) by using a conventional GC capillary column (95% methyl–5% phenyl substituted backbone, 80 cm × 0.32 mm i.d., 3 μm film thickness) in the injection valve to capillary liquid chromatography with diode array detection. A comparative study between the IT-SPME manifold and a column-switching device using a C_{18} column (35 mm × 0.5 mm i.d., 5 μm particle size) has been performed. The IT-SPME procedure was optimal, it allows reaching limits of detection (LODs) between 0.008 and 0.2 μg/L. No matrix effect was found and recoveries between 70 and 116% were obtained. The precision of the method was good, and the achieved intra- and inter-day variation coefficients were between 2 and 30%. This procedure has been applied to the screening analysis of 28 compounds in whole waters from several points of the Mediterranean coast (Valencia Community, Spain).

Contents

1. Introduction 2696
2. Experimental 2696
   2.1. Reagents and solutions 2696
   2.2. Apparatus and chromatographic conditions 2696
      2.2.1. Chromatographic system 2696
      2.2.2. Procedure A: in-tube solid-phase microextraction (IT-SPME) 2696
      2.2.3. Procedure B: column C_{18} coupled to capillary LC 2698
   2.3. Analysis of real water samples 2698
3. Results and discussion 2699
   3.1. In-tube SPME optimization 2699
   3.2. Analytical parameters 2700
   3.3. Application to real samples 2701
   3.4. Improving the detection limits (LODs) 2701
4. Conclusions 2702
   Acknowledgements 2702
   References 2702

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1. Introduction

The problem of diffuse pollution caused by industrial, agricultural and human activities has resulted in directives to control the sources of pollution, to contribute to the protection of the environment and to guarantee the utilization of natural resources. The 2008/105/CE Directive from European Union [1] and the Environmental Protection Agency of the United States (US EPA) [2] have listed the more toxic and persistent pollutants and the maximum permissible levels in the aquatic environment. From the analytical point of view, it is important to have cost-effective analytical methods for monitoring these compounds. The different chemical behaviours of the compounds lead to analyse them for family groups and no many procedures have been described in the literature capable to determine them by using a single method (Table 1). This fact and the low concentration limits established by the legislation, make difficult its chemical analysis. Thus, the current methods often require an enrichment step and the final determinations are usually carried out by liquid chromatography (LC) or gas chromatography (GC) in combination with one or more detectors. For these reasons a trend of analysis in the environmental field is the development of accurate, automated and sensitive methods that reduce sample handling.

In Table 1 are summarized several procedures described in the literature during the period of 2000–2009 for the analysis of some pollutants in water samples. As it can be seen many of these procedures are developed for single family compounds [11–13,16–24]. The GC technique is mainly used in the multiresidue methods [3–5,7–9]. There are less LC multiresidue procedures described, and most of them use mass detector [6,10]. Several sample treatments have been employed for the analysis of organic pollutants in waters, from more traditional techniques such as liquid/liquid extraction (L/L) [14], or solid-phase extraction (SPE) [3,5,6,15,18,22] to more moderns such as stir bar sorptive extraction (SBSE) [4,8,12], dispersive liquid–liquid microextraction (DLLME) [13,24] or solid-phase microextraction (SPME) [7,9,11,17,20,23]. Most of the procedures showed in Table 1 are performed off line, using high sample volume and a preconcentration step in order to reach low detection limits. The extraction time is ranged from 3 min [13] to 14 h [4].

On-line extraction techniques with sorbents are very useful because the samples can be often introduced with minimal preparation into the system and the preconcentration and clean-up steps are easily performed. Therefore, parameters such as time, analytical cost or sample preparation are reduced while others like reproducibility or sensitivity are increased. In this sense, in-tube SPME (IT-SPME) appears to be one of the most useful approaches for sample preparation. In IT-SPME is a mode of SPME which typically uses a GC capillary column with a proper coating to extract the analytes [11,23,25,26]. There are two fundamental approaches, passive or static and active or dynamic. In the passive mode the capillary is immersed into the sample, the analytes are extracted into the coating by diffusion or capillarity, and desorbed by introducing a moving stream of mobile phase or static desorption solvent [27]. In the dynamic mode the analytes are extracted by flushing the samples through the capillary. The sample can be injected with an autosampler [28] or manually using the extraction capillary as an injection loop [23]. In such way, the sample preparation can be performed on line in the LC device, and can be easily coupled to miniaturized chromatographic systems, thus enhancing sensitivity.

We propose an on-line analyte multiresidue procedure, without any previous sample treatment based on IT-SPME and capillary LC with diode array detection. This system has been compared with a column switching procedure formed by a packed C_{18} column. Different configurations have been compared in order to improve detectability. The criteria established in the Directive 2002/657/EC have been used for verification and identification [29]. The optimal IT-SPME procedure has been applied for the analysis of wastewaters discharged into the Mediterranean Sea (Valencia Community). Samples were collected from 22 different places in three different dates (April, July and November of 2007 and 2008). The compounds analysed depended on the area requirements. These compounds are included in the Directive 2008/105/CE [1]. The proposed method establishes a suitable protocol, to be followed by a research and routine laboratories, to analyse simultaneously 28 pollutant compounds in water samples according to water quality legislation. Although IT-SPME methods for several compounds tested in this study (triazines [23], phthalates [30], or organophosphorous compounds [11]) have been already proposed, the procedure described in this paper has several advantages such as: it provides lower detection limits and it allows to carry out the simultaneous determination of compounds from different families.

2. Experimental

2.1. Reagents and solutions

All the reagents were of analytical grade. Simazine, atrazine, propazine, ametryn, prometryn, terbutryn, parathion, fenitrothion, chlorfenvinphos, trifluralin, terbutylazine, phenol, bisphenol A, dibutylphthalate (DBP), di(2-ethylhexyl)phthalate (DEHP), dimethyl phthalate (DMP) and diethyl phthalate (DEP), were obtained from Sigma (St. Louis, MO, USA). Fenamiphos, fenthion, chlorpyrifos, fonofos, diuron, isoproturon, linuron, fluometuron, metobromuron, naphthalene, anthracene, fluoranthene, benzo[a]pyrene, indene[1,2,3-cd]pyrene, benzo[ghi]perylene, and DDT were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Acetonitrile of HPLC grade (Scharlau, Barcelona, Spain) and deionised water were used. All solvents were filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain) and degassed with helium before chromatographic use.

Stock standard solutions of individual compounds (10 μg/mL) were prepared in acetonitrile or water. Working solutions of these compounds were prepared by dilution of the stock solutions with water or acetonitrile depending on their solubility. All solutions were stored in the dark at 4 °C. Several mixtures of different compounds were performed by dilution of the stock standard solution in water at different concentration levels (1, 10 and 100 μg/L).

2.2. Apparatus and chromatographic conditions

2.2.1. Chromatographic system

The capillary chromatographic system consisted of a capillary pump (Agilent 100 Series, Waldbronn, Germany) equipped with an automate 2/6 Micro Switching Valve model 7725 high pressure six port valve and a Rheodyne model 7725 injection valve. The mobile phase was a mixture of acetonitrile–water in gradient elution mode. A photodiode array detector (DAD, Hewlett-Packard, 1040M Series II) was coupled to a data system (Agilent, HPLC ChemStation) for data acquisition and calculation. The signal was registered in the DAD detector and it was monitored at 230 nm. The corresponding spectra were saved. The different chromatographic conditions are shown in Table 2. A spectra library of the pure compounds was performed.

2.2.2. Procedure A: in-tube solid-phase microextraction (IT-SPME)

The injection loop consisted of a commercial GC capillary column connected to an injection valve. The extracting phase was a polysiloxane polymer with 95% methyl–5% phenyl substituted backbone (0.32 mm × 3 μm) (Teknokroma). Two different lengths were used, 40 cm (C1) and 80 cm (C2). The internal volumes of C1 and C2 were 28 and 57 μL, respectively. Capillary connections were

Table 1
Summary of different chromatographic procedures described in the literature between 2000 and 2008, for the analysis of organic pollutants in water samples (single and multiresidue procedures).

Family compound	Sample	Sample treatment	Sample volume (mL)	Final (mL)	Technique	Ext. time	Number compounds	LD	%Recovery	Reference
Multiresidue (benzidines, chloroanilines, PAHs, PCBs, pesticides, phenylurea, triazines)	Surface water	SPE	200	0.1	GC–EI-MS	30 min	109	ppt–ppb	70–120	3
Multiresidue (OCPs, OPPs, triazines, PAHs)	Tap water	SBSE	100		TD-GC–MS	>14 h	35	0.1–36.1		4
Multiresidue (OCPs, OPPs, carbamates, fungicides, phthalates, alkylphenols, bisphenol A)	Surface and ground	SPE	300	0.2	GC–MS and LC–MS/MS	Approx 50 min	33	0.2–88.9 ppt	84–118	5
Multiresidue (pesticides)	Surface water	SPE	100	0.3	LC/TOF-MS	Approx 30 min	101	0.04–120 ppb		6
Multiresidue (OCPs, OPPs, triazines, pyrethroids)	Ground and drinking	SPME	3		CG-ECD and CG-TSD	>60 min	36	2-20 ppt	73–130	7
Multiresidue (PAHs, PCBs, Pes, NPs)	Sea, estuarine water	SBSE	20		TD-GC–MS	12 h	37	0.1–10 ppt	84–124	8
Multiresidue (PAH, OPPs, OCPs)	River, mineral, underground	In-tube and on fiber SPME	60		GC–MS	38	17	6.1–21.8 ng/L	27–78	9
Multiresidue (pesticides)	Mineral water	On-line	0.1		LC–MS/MS		300	0.1 ppb	20–87%	10
Screening										
Multiresidue (PAHs, OPPs, triazines, phenylureas. . .)	Water, river and sea	On-line SPME	2000		LC-capillary/DAD		32	0.008–0.2 ppb	70–110%	This paper
OPPs	Sea water	On-line SPME	1000		LC-UV	On-line	10	0.1–10 ppb	Around 100	11
OPPs		SPME	5	0.0009	GC-FPD	40 min	13	1–5 ppt	91–104	12
OPPs	River, well and farm	DLLME	5		GC-FPD	<3 min	13	3–20 ppt	78.9–107	13
OPPs and OCPs	Surface water	LLE	500	0.3	CG–MS	>30 min	14	5–50 ppt	84–102	14
Sulfonyl and phenylurea herbicides	River	SPE	500	0.5	LC–MS and LC-UV diode	>70 min	10	50 ppt	70–95	15
Phenylurea herbicides	Lakes, waste,	SPE-derivatization_SPME	1000	0.1-0.15	GC–MS	>3 h	6	0.1–1 ppt	>80	16
Phenylurea herbicides	Lakes	SPME	3		LC-UV	>40 min	9	0.5–5.1 ppb	85–111	17
PAHs	Ground water	SPE	200	0.25	GC–MS		16	0.3–15 ppt	35–112	18
		SPE-MWCNTs	500	4	HPLC–UV-DAD		10	5–58 ppt	78–118	19
	Beach, river, waste	SPME	18		GC–MS–MS		27	0.07–0.76 ppt	73–102	20
	River	MASE	15		LVI-GC–MS		16	3–40 ppt	72–114	21
		SPE	100		HPLC–UV-DAD		9	1–30 ppt	66–91	22
Triazines herbicides	Field, river, irrigation, ground, waste	On fiber SPME	25		LC-UV	>45 min	6	25–125 ppb	0.27–1.96	23
		In-tube SPME-open column (on-line)	1		Capillary LC-UV		6	0.1–0.5	0.74–9.1	23
		In-tube SPME-C_{18} (on-line)	0.1		Capillary LC-UV		6	0.025–0.5 ppb	41–131	23
	Drinking, river water	DLLME	5	0.002	GC–MS	<3 min	8	0.021–0.12 ppb	24.2–115.6	24

Table 2
Experimental conditions optimized for the different procedures assayed. For the IT-SPME procedure, different conditions were assayed (1, 2 and 3) depending on the length of GC capillary column, the sample volume injected and particle diameter of capillary chromatographic column.

Procedure	Conditions	
(A) In-tube SPME with an open column-capillary column	Sample volume: 1000 μL, 50 μL H_2O[1] or 2000 μL, 100 μL H_2O[2,3]	
	GC capillary column polysiloxane polymer with 95% methyl–5% phenyl substitute backbone 40 cm × 0.32 mm i.d., 3 μm coating thickness column [1] 80 cm × 0.32 mm i.d., 3 μm coating thickness column[2,3]	
	Chromatographic conditions Analytical column Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter)[1,2] Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 3.5 μm particle diameter)[3] Flow: 20 μL/min[1,2] or 15 mL/min[3] Stop time: 30 min[1,2]	
	Mode gradient (MeCN:H_2O)	
	Time (min)	% MeCN
	0	40
	3	40
	11	60
	13	100
	16	100
	18	100
	23	40
(B) Column switching (C_{18}) capillary column	Sample volume: 100 μL	
	Valve 1t: 0 position 2, *t*: 5 min position 1 Valve 2 Zorbax SB C_{18} pre-column (35 mm × 0.5 mm i.d., 5 μm particle diameter)	
	Chromatographic conditions Analytical column: Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) Flow: 20 μL/min Stop time: 24 min	
	Mode gradient (MeCN:H_2O)	
	Time (min)	% MeCN
	0	45
	7	45
	15	60
	17	100
	20	100
	22	45
	24	45

[1]Procedure 1 (in-tube SPME 40 cm length), [2]procedure 2 (in-tube SPME 80 cm length), and [3]procedure 3 (in-tube SPME 80 cm length).

facilitated by the use of a 2.5 cm sleeve of 1/16 in. polyether ether ketone (PEEK) tubing at each end of the capillary.

Sample volumes between 25 and 3000 μL were processed in the load position of the injection valve in order to study the maximum preconcentration factor. After sample processing the GC capillary column was cleaned by flushing volumes of water (50 or 100 μL) for C1 and C2, respectively. Two different columns were used as analytical column, a Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) column (Agilent) or a Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 3.5 μm particle diameter) column (Agilent). The chromatographic conditions are shown in Table 2. Three different procedures were assayed. Procedure 1: 40 cm (C1) CG capillary length and Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) column. Procedure 2: 80 cm (C2) CG capillary length and Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) column. Procedure 3: 80 cm (C2) CG capillary length and a Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 3.5 μm particle diameter) column.

2.2.3. Procedure B: column C_{18} coupled to capillary LC

The injector valve (V1), with a 100 μL external loop, was connected to a Zorbax SB C_{18} pre-column (35 mm × 0.5 mm i.d., 5 μm particle diameter) (Agilent) by using a programmable switching microvalve (V2). A Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) column (Agilent) was used as analytical column.

After processing the sample in the injection loop, valves 1 and 2 were rotated and the gradient elution program was started (see Table 2). The loop content was transferred to the C_{18} pre-column. Next, the valve V1 was rotated to the load position at 5.5 min. The flow direction method was used in the column-switching device. The chromatographic conditions are shown in Table 2.

2.3. Analysis of real water samples

Different real wastewater samples flowing to the sea were collected at different points of the Comunidad Valenciana coast. In order to analyse the real water samples the next sequence was followed: blank (processing 100-μL nanopure water), blank standard (processing 2000-μL nanopure water), standard solution (processing 2000 μL of nanopure fortified with standard solution of analytes at concentration level of 1 ppb), and the whole water samples after centrifugation (processing 2000 μL). In all cases, 100 μL of nanopure water was introduced into the system before the rotating of the valve to the inject position. The samples, which were suspected of any analyte, were fortified with a known concentration of standard solution and were processed following the same procedure.

3. Results and discussion

3.1. In-tube SPME optimization

Two different GC capillary lengths were tested 40 cm (C1) and 80 cm (C2), for the in-tube extraction (SPME) of the different organic pollutants. For this purpose, standard solutions containing a mixture of a representative group of the different compounds at a level of 25 ng/mL were processed. Several sample volumes of standard solution, in the range between 25 and 3000 μL, were processed. As expected, higher analyte signals were obtained by increasing the injected sample volume (Fig. 1). However different behaviours was observed depending on the analyte polarity. The signal of polar analytes such as metobromuron (tr: 7.6) and bisphenol A (tr: 8.2) starts to decrease at high volumes (>1 mL), while it increases for more non-polar analytes such as chlorpyrifos (tr: 16.6). That fact was related to the analyte polarity. According to this, 1.0 mL was the sample volume selected for further work. Similar behaviour was observed when 80 cm (C2) column length was used. In this case the selected sample volume was 2 mL. The water volume required to clean the capillary column was optimized for both column lengths being 50 and 100 μL for C1 and C2, respectively. No significant losses of analytes were observed by including this step.

The limits of detection reached with both capillary lengths were calculated to compare both procedures. The LODs were experimentally established as concentrations that produced a peak with a signal to noise ratio of 3. The values obtained are listed in Table 3. The fact that higher sample volume could be processed (using 80 cm

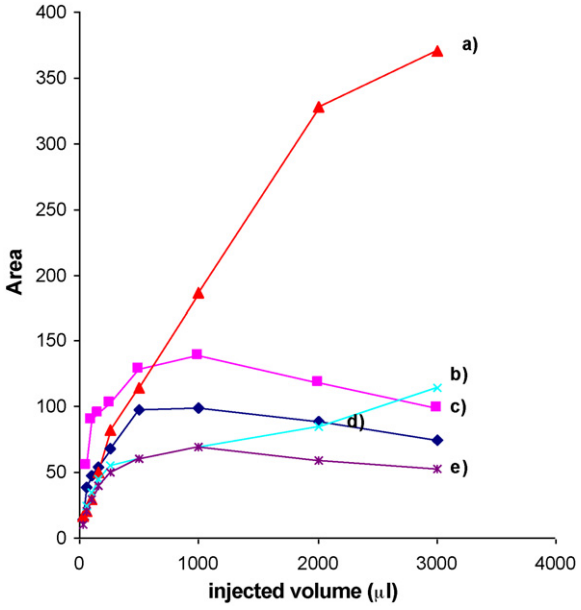

Fig. 1. Influence of sample injection volume by using 40 cm (C1) length GC capillary column (see Section 2 and Table 2) for compounds with different polarities: chlorpyrifos (a); prometryn (b); metobromuron (c); bisphenol A (d); terbutryn (e).

Table 3
LODs and retention times (tr) of the different analytes assayed by using different procedures. (A) Column switching C_{18} pre-column and (B) in-tube SPME—procedure 1: with GC capillary of 40 cm (C1), procedure 2: with GC capillary of 80 cm (C2), procedure 3: with GC capillary of 80 cm (C2).

Compound	(A) Column switching C_{18} pre-column		(B) In-tube SPME						Legislation [1] AA/MAC-EQS
			Procedure 1 (40 cm, C1)		Procedure 2 (80 cm, C2)		Procedure 3 (80 cm, C2)[a]		Unit (ppb)
	tr (min)	LOD (ppb)	tr (min)	LOD (ppb)	tr (min)	LOD (ppb)	tr (min)	LOD (ppb)	
Atrazine	7.5	0.05	7.9	0.1	9.3	0.2	12.8	.2	0.6/2
Simazine	7.3	0.05	6.2	0.1	7.3	0.1	11.2	0.08	1/4
Propazine	8.0	0.05	10.4	0.1	11.9	0.2	15	0.2	
Ametryn	7.7	0.025	10.2	0.1	11.8	0.2	14.6	0.2	
Prometryn	8.7	0.05	12.7	0.1	14.6	0.1	17.1	0.08	
Terbutryn	8.9	0.025	13.5	0.1	15.1	0.1	18	0.08	
Trifluoralin					20	0.01	18.2	0.008	0.03/n.a.
Naphthalene	9.1	2	14.6	0.5	16.2	0.1	18.8	0.08	n.a.
Anthracene	12.4	10	18	0.05	19.4	0.02	21.9	0.02	0.1/0.4
Fluoranthene	13.0	5	18.2	1	19.9	0.05	22.2	0.04	0.1/1
Dimethylphtalate	8.8	0.5	7	0.5	8.4	0.2	12	0.2	
Diethylphtalate	8.4	0.5	10.9	0.5	12.8	0.2	15.7	0.2	
Dibutylphthalate	13.2	1	18.2	0.1	19.5	0.1	21.8	0.08	
Di(2-ethylhexyl)phthalate	22.43	1	21.1	0.5	23	0. 25	25.9	0.2	1.3/n.a.
Diuron	7.4	0.5	8.5	0.5	10.1	0.2	13.1	0.2	0.2/1.8
Isoproturon	7.9	1	8.4	0.1	9.7	0.2	13.5	0.2	0.3/1
Linuron	8.7	0.5	12	0.3	13.4	0.2	16.4	0.2	
Fluometuron	7.3	0.5	8.2	0.5	9.4	1	12.6	0.8	
Metobromuron	8	1	7.6	1	11	0.1	14	0.08	
Chlorfenvinphos					17.5	0.1	19.4	0.08	0.1/3
Chlorpyrifos	14.4	5	16.6	0.5	20.1	0.025	22.6	0.02	0.03/0.1
Fonofos	12.5	0.5	17.6	0.25	18.7	0.1	21.2	0.08	
Fenamiphos	9.08	1	12.3	1	13.8	0.2	16.8	0.2	
Fenthion	11.7	2.5	17	1	18.6	0.2	20.8	0.2	
Fenitrothion	10.14	1	15.4	0.5	16.6	0.2	19.4	0.2	
Parathion	9	5	13.9	0.5	15.5	0.25	17.6	0.2	
Phenol	8.83	1	5.6	1	6	0.5	7.0	0.4	
Bisphenol A	6.31	1.5	8.2	1.5	9.7				

AA-EQS: This parameter is the Environmental Quality Standard (EQS) expressed as annual average values. Unless otherwise specified it applies to the total concentration of all isomers.
MAC-EQS: This value expressed as a maximum allowable concentration. n.a., not applicable. The AA-EQS values are also protective against short-term pollution peaks since they are significantly lower than the values derived on the basis of acute toxicity.
[a] See Table 2 for the details of the different procedures.

(C2)), allowed to increase the LODs in a factor between 20 and 1 for the determination of the most of the analytes. Thus analytes such as chlorfenvinphos or chlorpyrifos could be determined according to the legislated concentration limits (see Table 3). However, using procedure 2, the LODs obtained for more polar analytes (such as triazines), were lower than those obtained by using the shorter length (C1 40 cm), although it was sufficient to reach the legislated limits.

The IT-SPME procedure has been compared with the column switching procedure which was initially proposed for triazines [23] and in this case has been adapted to 28 analytes. It consists in a pre-column C_{18} coupled to a capillary LC (see Section 2.2.3). In Table 3 are also listed the LODs obtained with this procedure. For the most of the analytes (except triazines) the LODs were markedly reduced by the IT-SPME approach in a factor up to 40. This can be probably explained by the affinity of the analytes for the apolar coating and by the large sample volume passed through the extraction GC capillary. However, the more polar analytes presented higher LODs, due probably their co-elution with the sample in the processing through the GC capillary.

In Table 3 are also shown the retention time (tr) of the different compounds by using the different procedures assayed. As it can be seen, they are shorter by using the column switching system, especially for the most polar compounds. As it can be seen for the IT-SPME procedure, tr increases by increasing the length of the capillary column. However the total chromatographic time required for the different conditions was similar.

Based on the results showed in Table 3 (tr and detection limits), and in order to reach the legislated concentrations, the procedure selected was the IT-SPME with a capillary loop of 80 cm (C2) length (procedure 2).

The absolute recoveries of the IT-SPME were calculated by comparing the amount of analyte extracted, which is the amount of the analyte transferred to the analytical column, with the total amount of analyte passed though the GC extraction capillary. The amount of analyte extracted was established from the peak areas in the resulting chromatograms and form the calibration equations constructed through the direct injection of 2 μL of standard solution of the analytes in the 10–100 ng/mL concentration range. The recoveries obtained are listed in Table 4. The values obtained are in accordance with those reported in Ref. [11].

Table 4
Absolute recoveries obtained with the IT-SPME (procedure 2) ($n = 3$). Values established from standard solutions containing 0.1 μg/mL of each analyte; volume of sample passed through GC capillary: 2 mL.

Compound	Recovery (%)	Compound	Recovery (%)
Simazine	4.0 ± 0.5	Naphthalene	37 ± 4
Atrazine	7.1 ± 0.7	Anthracene	45 ± 4
Propazine	1.12 ± 0.06	Fluoranthene	42 ± 2
Ametryn	4.8 ± 0.1	Metobromuron	3.0 ± 0.2
Fenthion	5.4 ± 0.7	Flumeturon	3.5 ± 0.1
Fonofos	9 ± 1	Isoproturon	3.6 ± 0.1
Chlorpyrifos	14 ± 1	Linuron	3.6 ± 0.2
Chlorfenvinphos	61 ± 5	Diuron	1.4 ± 0.1
Trifluralin	9.2 ± 0.6	DDT	19 ± 2

3.2. Analytical parameters

The calibration equations obtained are presented in Table 5. The values obtained indicated that this procedure provides adequate linearity in the working concentration interval (0.001–0.1 μg/mL). The repeatability and reproducibility were evaluated by calculating the intra- and inter-day relatively standard deviation, respectively. These coefficients were established by processing standard solutions of several analytes. In all instances the results obtained were <15% (see Table 5). Thus it can be considered acceptable at the concentration levels tested and in accordance with published SPME papers [11,23,30–32].

The accuracy of the described procedure was studied by processing standard solutions. The analyte concentrations were established from the calibration equations listed in Table 5. The results obtained provided suitable accuracy, with relative errors (%) < 20%.

The parameters used to confirm the presence or absence of any analyte in the samples was: tr, spiked samples and the UV–vis

Table 5
Analytical characteristics of the in-tube SPME (80 cm length) coupled to capillary liquid column (for more details see Section 2 and Table 2 procedure A2).

Compound	Linearity ($n = 5$)[a] $y = a + bx$ (ng/mL)	Precision RSD (%)		Accuracy relative error (%) ($n = 3$)
		Intra-day ($n = 3$)[b]	Inter-day[c] ($n > 5$)	
Simazine	$a \pm s_a$: 17 ± 10; $b \pm s_b$: 2176 ± 177; R^2 = 0.9970	3.8	5.05	−13
Atrazine	$a \pm s_a$: −11 ± 9; $b \pm s_b$: 7638 ± 159; R^2 = 0.9999	2.0		−18
Propazine	$a \pm s_a$: 15 ± 13; $b \pm s_b$: 7024 ± 226; R^2 = 0.9989		5.8	6
Ametryn	$a \pm s_a$: 9.7 ± 0.6; $b \pm s_b$: 19,200 ± 20; R^2 = 0.9999	11		−19
Prometryn	$a \pm s_a$: 24 ± 23; $b \pm s_b$: 25,267 ± 473; R^2 = 0.999	6.2	9.6	8
Terbutylazine	$a \pm s_a$: 10 ± 10; $b \pm s_b$: 23,971 ± 19; R^2 = 0.9999			
Terbutryn	$a \pm s_a$: 27 ± 10; $b \pm s_b$: 5350 ± 233; R^2 = 0.9999	6.02	7.6	−3
Fluometuron	$a \pm s_a$: 5.1 ± 0.2; $b \pm s_b$: 3936 ± 20; R^2 = 0.9968			
Isoproturon	$a \pm s_a$: 8 ± 8; $b \pm s_b$: 3600 ± 20; R^2 = 0.9970			20
Linuron	$a \pm s_a$: 29 ± 31; $b \pm s_b$: 5155 ± 220; R^2 = 0.9949			−17
Diuron	$a \pm s_a$: 3 ± 1; $b \pm s_b$: 1764 ± 29; R^2 = 0.9987			−12
Metobromuron	$a \pm s_a$: 3 ± 3; $b \pm s_b$: 2580 ± 80; R^2 = 0.9969			−3
Anthracene	$a \pm s_a$: 12 ± 2; $b \pm s_b$: 43,540 ± 31; R^2 = 0.9999		15.5	
Naphthalene	$a \pm s_a$: −13 ± 13; $b \pm s_b$: 85,871 ± 236; R^2 = 0.9999	5.3		16
Indene[1,2,3-cd]pyrene	$a \pm s_a$: 15 ± 7; $b \pm s_b$: 18,431 ± 148; R^2 = 0.9999			
Fluoranthene	$a \pm s_a$: −0.8 ± 0.3; $b \pm s_b$: 108,427 ± 590; R^2 = 0.9999	4.4		1
Chlorfenvinphos	$a \pm s_a$: 4 ± 2; $b \pm s_b$: 16,850 ± 30; R^2 = 0.9999	3.72	4	−13
Chlorpyrifos	$a \pm s_a$: 22 ± 9; $b \pm s_b$: 18,182 ± 151; R^2 = 0.9999	0.7		3
Fenthion	$a \pm s_a$: 20 ± 11; $b \pm s_b$: 8608 ± 168; R^2 = 0.9999			
Fonofos	$a \pm s_a$: 22 ± 6; $b \pm s_b$: 15,633 ± 100; R^2 = 0.9999	0.3	3.9	6
4.4-DDT	$a \pm s_a$: −7 ± 1; $b \pm s_b$: 14,784 ± 21; R^2 = 0.9999	6.5		
Phenol	$a \pm s_a$: 3.6 ± 1.1; $b \pm s_b$: 1060 ± 11; R^2 = 0.9999	9		
Trifluralin	$a \pm s_a$: 68 ± 42; $b \pm s_b$: 23,067 ± 738; R^2 = 0.999			

[a] Concentration interval 1 ng/mL to 0.1 μg/mL.
[b] Established at concentration of 2 ng/mL.
[c] Established at concentration of 10 ng/mL.

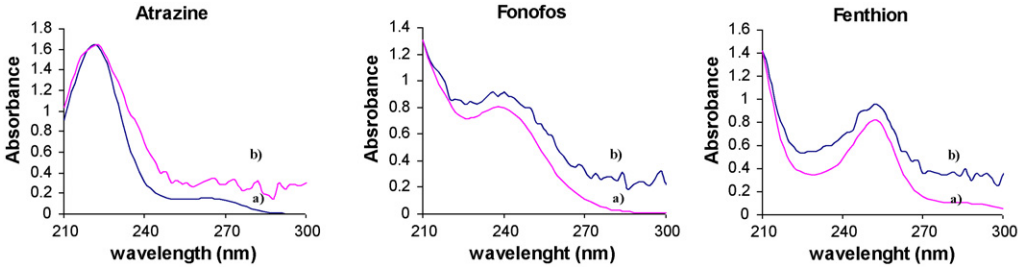

Fig. 2. UV–vis spectra of several tested analytes at LODs: (a) at 10 ppb level and (b) at LODs.

spectra. Although some analytes present similar tr, the UV–vis spectra allowed to distinguish them. The criteria followed were those established in 2002/627/CE [29] corresponding to a LC and full-scan UV–vis detection. The relative tr of the analytes presented the same as that of the calibration standard in the water matrix, within a margin of ±2.5%. The reproducibility in the tr was satisfactory. The UV–vis spectra of all the pure compounds were registered and included in a library that was used to identify the compounds in the sample. The criterion followed was firstly that the same maximum should be presented and secondly that the differences between both spectra, sample and library had to be not superior to 10% of the standard spectra. Thus the matching factor was established during the validation process for every analyte. Fig. 2 shows the UV–vis spectra corresponding to several analytes at 10 ng/mL and at the LODs. As it can be seen, the match between both spectra (10 ppb and LODs) is acceptable, being superior of 90% for all the tested analytes.

The proposed procedure was also applied to spiked real water samples. As illustrative example, Fig. 3 shows the chromatogram obtained for the blank (100 μL distilled water), one waste sample, and such sample fortified with some of the tested analytes (three replicates). As it can be seen in Table 4, the proposed procedure provides good repeatability with RSD% lower than 10% for the spiked samples. As well it was describe above, the criteria followed was that established in 2002/627/CE Directive [29]. The recoveries percentage of the analytes found in these samples ranged between 70 and 100% which confirmed that the proposed method provided suitable accuracy (calculated by using the calibration graphs listed in Table 5). No differences were observed depending on the water source (waste, river or sea). Table 6 compares the recoveries (%) obtained by using the IT-SPME optimized procedure and the procedure using the column-switching device. As it can be seen, the recoveries obtained by both procedures were similar, and in both cases the recoveries were close to 100%. The reproducibility results were better by using the IT-SPME procedure.

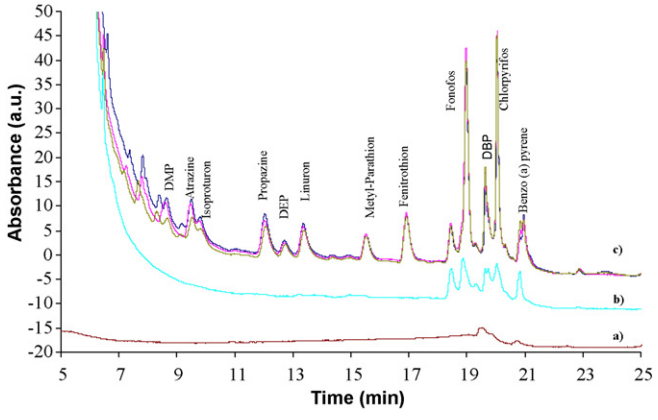

Fig. 3. Chromatogram obtained under the optimum conditions with IT-SPME procedure (80 cm CG capillary) for (a) blank (100 μL distilled water), (b) a wastewater sample, (c) three replicates of sample spiked with different analytes (10 ng/mL each). For other experimental details see text.

3.3. Application to real samples

Different real samples collected from different points along the coast of the Comunidad Valenciana area (Spain) were screened using the optimized procedure.

In Fig. 4 are depicted the chromatograms obtained for different water samples that contained some analytes. The criteria used for confirmation and quantification were the same that those established above. The analytes screened and its concentrations for the samples shown in Fig. 4 were: (a) simazine (0.1 ppb (LOD)) and DEP (2.5 ± 0.5 ppb), (b) ametryn (0.20 ± 0.02 ppb), (c) naphthalene (0.4 ± 0.1), and (d) simazine (4 ± 0.2 ppb) and DEP (LOD).

3.4. Improving the detection limits (LODs)

The IT-SPME optimized procedure provided satisfactory results. However, in order to increase the sensitivity, some chromatographic conditions were modified (see Table 2). As it can be seen in Fig. 5, the peak shape and the resolution of the compounds improved by using the column with 3.5 μm particle diameter. Due to the small particle size, the flow solvent was decreased to 15 μL/min and the chromatogram time increased in a few minutes. In these conditions the sensitivity increased by a factor from 1 up to 2.5, and lower LODs could be reached (see Table 3). A lineal regression was obtained by relating the tr obtained by using the two different conditions ($tr_2 = -4.9 \pm 0.4 + 1.1 \pm 0.2\ tr_1$, $r = 0.999$, $n = 8$) (1, particle size 5 μm; 2, particle size 3.5 μm). This procedure has

Table 6
%Recovery obtained for fortified water samples, by using the (A) IT-SPME optimized procedure (procedure A2, Table 2) and (B) column switching C_{18} device. Concentration level: 2 ng/mL. n.d., not determined.

Compounds	%Recovery		
	(A) IT-SPME[a]		(B) Column switching (intra-day)
	Inter-day ($n = 3$)	Intra-day ($n = 12$)	
Atrazine	99 ± 8	n.d.	101 ± 21 ($n = 17$)
Simazine	116 ± 5	112 ± 30	94 ± 30 ($n = 20$)
Ametryn	105 ± 2	109 ± 6	81 ± 18 ($n = 18$)
Propazine	103 ± 10	86 ± 15	125 ± 11 ($n = 14$)
Terbutryn	115 ± 6	102 ± 8	116 ± 23 ($n = 12$)
Prometryn	n.d.	n.d.	113 ± 12 ($n = 9$)
Fenitrothion	85 ± 3	n.d.	70 ± 22 ($n = 3$)
Fenamiphos	70 ± 9	71 ± 15	99 ± 2 ($n = 3$)
Fenthion	95 ± 8	92 ± 17	96 ± 29 ($n = 4$)
Fonofos	100 ± 8	91 ± 12	98 ± 17 ($n = 12$)
DEP	104 ± 3	n.d.	100 ± 26 ($n = 8$)
DBP	97 ± 4	n.d.	83 ± 14 ($n = 7$)
Chlorpiryfos	111 ± 7	95 ± 10	133 ± 23 ($n = 5$)

[a] Optimized procedure (capillary column 80 cm).

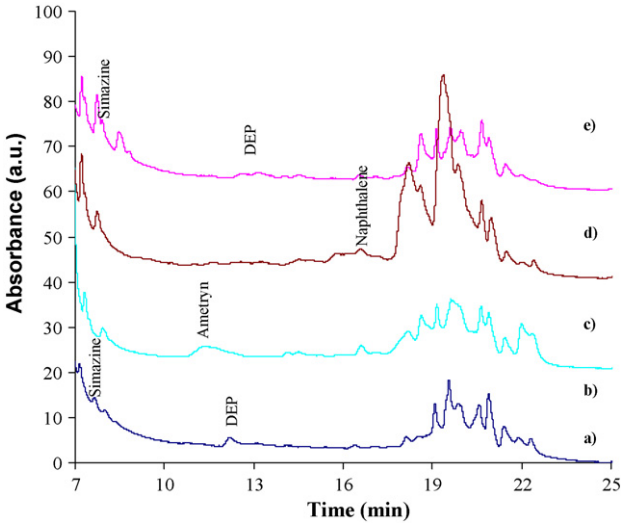

Fig. 4. Chromatograms corresponding of different waste real samples processed according to the optimized procedure (see Section 2).

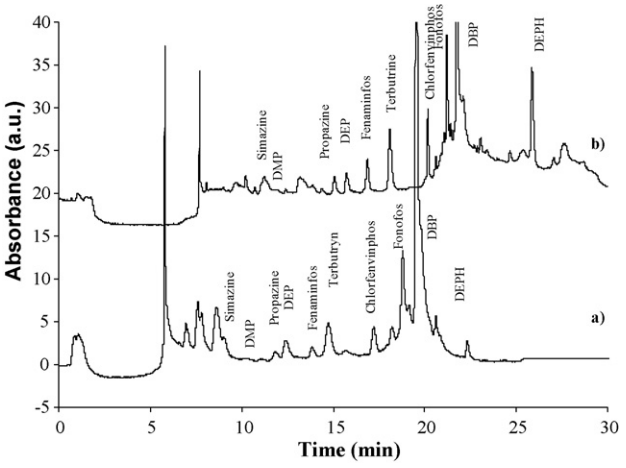

Fig. 5. In-tube SPME/LC chromatograms of standard solution (mixed of different analytes at 10 μg/L) with 80 cm CG capillary column and as analytical column. (a) Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 5 μm particle diameter) column and (b) Zorbax SB C_{18} (150 mm × 0.5 mm i.d., 3.5 μm particle diameter).

been applied to the analysis of pollutants in river and sea samples with satisfactory results.

4. Conclusions

It has been demonstrated that the in-tube SPME in combination with LC-UV-DAD offers high sensitivity, accuracy and reproducibility for identification and quantification of some organic pollutants in water samples in the range of low parts-per-billion. Therefore it is suitable to control the surface water quality for different pollutants according to the maximum concentration levels established in the legislation. The in-tube SPME assembly used permits the on-line enrichment of the analytes with advantages of minimum sample manipulation (samples only need to be centrifuged if necessary), sensitivity (lower detection limits can be reached with the processing of large sample volumes in capillary liquid chromatography) and high speed (the total analysis time is less than 25 min.) Alternatively, some other strategies (such as increasing the loop (GC capillary) length or reducing the particle size of the column), could be performed depending on the analysis requirements, particularly if lower detection limits want to be reached.

The developed and validated method has been successfully used for the analysis of real water samples (waste, river and sea water). No matrix effect or interferences of other compounds were observed in the real water samples under the proposed conditions.

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