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Simultaneous determination of pesticides, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in seawater and interstitial marine water samples, using stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry

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Abstract

A method for the simultaneous determination of semi-volatile organic contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorine and organophosphorus pesticides) in marine samples has been developed, for the first time, using the stir bar sorptive extraction technique (SBSE) and thermal desorption coupled to capillary gas chromatography–mass spectrometry (SBSE-TD-GC–MS). Polydimethyl siloxane (PDMS) was used for the extraction of the selected analytes and two procedures have been optimised and validated, one for seawater samples (100 mL) and another for interstitial water samples (10 mL), using PDMS stir bars of 20 mm and 10 mm size, respectively. The extraction and analytical conditions, such as extraction time, matrix effects, sample volume and desorption time, were optimised. The proposed methods are sensitive, simple and show good linearity and detection limits lower than 1 ng L^{-1} with seawater and lower than 10 ng L^{-1} with interstitial marine water for the majority of compounds tested. Repeatability and reproducibility, expressed as relative standard deviation, have values lower than 20% for the majority of analytes considered. The recoveries for both sample volume procedures are higher than 60 and 70% for 10 and 100 mL, respectively, except for the more apolar (some PAHs and PCBs) and the more polar (some triazines) analytes which present lower values. The present SBSE/GC/MS method was applied for the analysis of trace organic contaminants in seawater and interstitial water samples from Cadiz Bay (SW of Spain). Terbutylazine, DDX and some PAHs were found at several seawater sampling points at ng L^{-1} levels, and some PAHs in interstitial water too.

Keywords: Stir bar sorptive extraction; GC/MS; Environmental analysis; Polycyclic aromatic hydrocarbons; Pesticides; Triazines; Polychlorinated biphenyl; Seawater; Interstitial water

1. Introduction

The marine environment is, in many cases, the ultimate "sink" for large volumes of many pollutants. In fact urban or industrial wastewater discharges and contamination of diverse types from urban and agricultural areas contribute significantly to pollution of the marine environment. As a result, a wide variety of organic contaminants are present in this system, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides . . . [1]. Most of these organic compounds have a tendency to bioaccumulate and present low rates of biodegradation and consequently they could represent a risk to environmental and human health. The European Environmental Agency (Directive 76/464/EEC and its daughter Directives) has drawn up a list of pollutants for priority monitoring, which need to be analysed with sensitive instrumental methods.

Organic pollutants are present at low concentrations in the environment and consequently it is necessary to use a preconcentration step prior to their analysis. Many authors have focused their research on developing techniques capable of improving

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this extraction step and reducing the use of organic solvents. In 1999, Baltussen et al. [2] developed a novel method for extracting volatile and semi-volatile compounds, termed stir bar sorptive extraction (SBSE). This technique consists of the sorption of apolar solutes present in aqueous samples onto a polydimethylsiloxane (PDMS) stir bar, and is based on the principles of solid-phase microextraction (SPME): partitioning of the analytes between the sample and an extracting phase [3]. Specifically a stir bar coated with PDMS is introduced in the sample and after stirring, it is removed, thermo-desorbed and cryofocused in a programmable temperature vaporisation (PTV) injector and finally analysed by GC/MS.

SBSE presents advantages with respect to SPME: lower detection limits (sub-ng L^{-1} to ng L^{-1}), higher capacity and recoveries, since extraction is performed with a larger amount of PDMS. The SBSE technique is a rapid and sensitive method that is being used for biomedical, environmental and food applications [4].

In relation to environmental analyses, SBSE has been applied to freshwaters for determining specific groups of pollutants, such as PAHs [5–7], PCBs [8,9] and organochlorine pesticides, carbamate and pyrethroid pesticides [10], xenoestrogens [11], alkylphenols and bisphenol A [11–13], polybrominated diphenyl ethers [14] and phthalates [15]. So far, however, to our knowledge, only one SBSE technique has been developed for application to seawater, for determining PAHs only [16].

Multirresidue analysis is of great interest and is especially relevant for environmental samples; for this SBSE offers considerable possibilities, as has been shown for fresh water samples [17,18]. However until now an SBSE method has not been developed for the simultaneous extraction of different types of contaminant present in seawater. SBSE could be a useful alternative as an enrichment stage for seawater samples because this matrix is complex and the organic compounds are extremely dilute; hence it is not possible to detect them by direct injection in GC. This procedure must be optimised for each application because different variables could affect its efficiency as an extraction technique; such variables include the sample and polymer volumes, the matrix composition and the physicochemical properties of the analytes considered [2].

In fact SBSE is less effective for polar than for more hydrophobic analytes [2]. The extraction efficiencies of polar compounds can often be improved by adding NaCl, since it reduces their solubility in water, as has previously been evaluated for triazines in freshwater samples [17]. Previous studies have optimised the efficiency of the SBSE technique for freshwater samples, which have an ionic strength less than that of marine waters [17,18]. Therefore, it is also necessary to evaluate if this effect could improve the extraction in seawater samples.

In the case of environmental samples, sometimes it is not possible to obtain large enough volumes; this is a particular difficulty for samples of interstitial water. In view of this, it is also necessary to optimise a method for low sample volumes; for this reason two extraction procedures have been optimised in this study. This is a relevant variable because total analytes extracted depends on the sample water volume and on the volume of PDMS utilised, as other authors have shown previously [2,4]. For example, in the case of apolar compounds, a higher sample volume increases the amount of analyte extracted but volume has little effect for other compounds [2].

This article proposes a multi-residue extraction method using SBSE coupled to GC/MS, to determine 44 non-regulated and priority persistent pollutants, including 14 polycyclic aromatic hydrocarbons (PAHs), 16 organochlorinated pesticides, two organophosphorus pesticides, seven triazines and five polychlorinated biphenyls (PCBs) in marine samples (seawater and interstitial water) at low levels (ng L^{-1}). A significant number of these compounds (19 pollutants) are included in the European Union list of priority pollutants: the majority of PAHs (except naphthalene and acenaphthene), atrazine and several organochlorinated pesticides. Extraction (ionic strength, sample volume, PDMS volume) and desorption (desorption time) procedures have been optimised for marine water matrices. Two procedures have been developed, one for low sample volumes (interstitial water) and other for higher sample volumes (seawater). This is the first multi-residue method proposed for marine water samples using SBSE/TDS/GC/MS, and to confirm its practical application, it has been applied to interstitial and seawater samples from Cadiz Bay (SW of Spain).

2. Experimental

2.1. Materials and reagents

Seawater free of pollutants was obtained 10 miles from the coast, filtered (0.22 μm) and maintained at −20 °C until use. The commercial PDMS stir bars employed (Gerstel, Mulheim a/d Ruhr, Germany) were 10 mm × 0.5 mm (length × film thickness) and 20 mm × 0.5 mm. A 15 position magnetic stirrer (Gerstel, Mulheim a/d Ruhr, Germany) was used to stir samples at 900 rpm. HPLC-grade methanol and sodium chloride (Merck, Darmstadt, Germany) were used for ionic strength experiments. The absence of the considered pollutants from seawater was confirmed using standards and blanks performed with bi-distilled water with added NaCl.

2.2. Standard solutions

A standard containing organochlorine pesticides (α-HCH, β-HCH, δ-HCH, lindane, heptachlor epoxide isomer B, α-endosulfan, β-endosulfan, endosulfan sulfate, endrin ketone, endrin, dieldrin, methoxychlor, aldrin, *p*,*p*′-DDD, *p*,*p*′-DDT, *p*,*p*′-DDE) was purchased from Dr. Ehrenstorfer GmH (Augsburg, Germany). Polycyclic aromatic hydrocarbons (acenaphthylene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, benzo[ghi]perylene), triazines (atrazine, propazine, terbutylazine, ametryn, prometon, terbutryn and prometryn) and organophosphorus pesticides (malathion, parathion) were purchased from Supelco (Oakville, Canada). Polychlorinated biphenyls (PCB 28, PCB52, PCB 138, PCB 153, PCB 180) were obtained from Heidel-de Haen (Seelze, Germany). The spiked samples were

prepared daily using seawater and adding the corresponding working solution containing all target compounds (Table 1) in methanol (HPLC grade methanol, Merck, Darmstadt, Germany) at 500 μg L^{-1}. The MeOH content was lower than 0.5% in 10 mL protocol and lower than 0.1% in 100 mL protocol, with no significant differences observed between several concentrations tested.

Deuterated terbutylazine, deuterated PAH mix (chrysene D12 and phenanthrene D10) and quintozene were obtained from Dr. Ehrenstorfer (Augsburg, Germany).

Table 1
Octanol/water partition coefficients and mass/charge (*m*/*z*) ratios used to identify the target compounds

Compounds	Log $K_{o/w}$	TIon-Q1-Q2-Q3
Polycyclic aromatic hydrocarbons		
Acenaphthylene	3.17	152
Fluorene	4.02	166
Anthracene	4.35	178-192
Phenanthrene	4.35	178-193
Pyrene	4.93	202-228-252
Fluoranthene	4.93	202
Benzo[a]anthracene	5.52	228
Chrysene	5.52	228
Benzo[b]fluoranthene	6.11	252-126
Benzo[k]fluoranthene	6.11	252-127
Benzo[a]pyrene	6.11	252
Dibenzo[a,h]anthracene	6.7	278
Indeno[1,2,3-cd]pyrene	6.7	276-138
Benzo[ghi]perylene	6.7	276-139
Organochlorinated pesticides		
β-HCH	3.78	219-181-183-217
δ-HCH	3.78	181-219-183-217
α-HCH	3.8	181-219-183-217
Lindane	4.14	181-183-219-11
Heptachlor epoxide isomer B	4.98	353-355-351-357
Endosulfan sulfate	3.66	272-274-229-237
α-Endosulfan	3.83	241-195-239-237
β-Endosulfan	3.83	195-237-207-241
Endrin ketone	4.99	317-67-315-319
Methoxychlor	5.08	227
Endrin	5.2	317-263-229-237
Dieldrin	5.4	79-263-277-279
4,4′-DDE	6.02	246-318-316-248
4,4′-DDD	6.51	235-237-165-236
4,4′-DDT	6.91	235-237-165-237
Aldrin	6.5	263-66-265-261
Triazines		
Atrazine	2.82	200-215-202-58
Propazine	3.24	214-229-172-58
Terbutylazine	3.27	214-173-216-229
Ametryn	3.32	227-212-170-185
Prometon	3.57	210-225-168-183
Terbutryn	3.77	226-185-241-170
Prometryn	3.73	241-184-226-105
Organophosphorus pesticides		
Malathion	2.29	173-127-125-93
Parathion	3.73	291-109-97-139
Polychlorinated biphenyls		
PCB 28	5.71	256
PCB 52	5.79	292
PCB 138	6.82	360
PCB 153	7.29	360
PCB 180	7.21	396
Internal standards		
Terbutylazine-D5		129
Phenanthrene-D10		188
Chrysene-D12		240
Quintozene		249

2.3. Marine samples

Seawater samples from different sampling points in Cadiz Bay (less than half mile from coast), Andalusia, Spain were collected in bottles of amber-glass (500 mL), filtered (0.45 μm) and placed in a cooler to be maintained at 4 °C. Interstitial waters were obtained from sediment cores collected at a sampling point in Cadiz Bay, by centrifugation at 4500 rpm during 30 min (5 °C) and the supernatant water was obtained and placed into 20 mL vials. Internal standards were used with samples to correct the sorption of some analytes onto vial walls, losses from the stir bar after extraction or variations in equipment sensitivity.

2.4. Factors affecting SBSE efficiency

Two extraction procedures have been optimised for marine water samples, one for samples of large volume (100 mL), like seawater, and another for samples of low volume (10 mL), like interstitial waters. The marine water sample was placed in the corresponding flask, sodium chloride (NaCl) was added, and the mixture was and stirred at 900 rpm using the PDMS stir bar. After the extraction time, the stir bar was removed with magnetic tweezers, rinsed with Milli-Q water and dried with a paper tissue. Lastly the sorptive stir bar was placed in the liner of a thermodesorption system (TDS-2) and was thermally desorbed.

2.4.1. Optimisation of desorption conditions

The effect of desorption time was evaluated by analysing aqueous samples with 500 ng L^{-1} of standard solution for different periods of time (7, 10 and 12 min); this test was performed with two replicates. Carryover was also evaluated in each case by desorbing the stir bar for 15 min and analysing by GC/MS.

2.4.2. Optimisation of the extraction conditions

Extraction efficiency with stir bar sorptive extraction (SBSE) has been optimised by evaluating the effect of the variables: sample volume, PDMS volume, extraction time and ionic strength concentration. The effect of sample and PDMS volume on method sensitivity and on extraction efficiency was evaluated by comparing the signal intensity using 100 and 10 mL of sample volume and two sizes of stir bar (20 and 10 mm length) for the same analyte mass and for the same sample. Specifically, in the large volume extraction procedure, 100 mL of seawater sample were placed in 100 mL-Erlenmeyer flasks and the 20 mm stir bar was used for extraction. The 10 mL extraction procedure was performed using 20 mL headspace vials and 10 mm stir bars**.** The effect of extraction time on the GC/MS response of the compounds analysed was evaluated over different periods of times (1, 2, 4, 6, 8, 14, 24, 48 h). The effect of ionic strength on extraction efficiency was evaluated, by adding dif-

Table 2
Main experimental and instrumental parameters optimised

Parameter	Value
Stirring speed (rpm)	900
Extraction time (h)	14
NaCl added (g L^{-1})	100
Desorption temperature (°C)	280
Desorption time (min)	7
Helium gas flow (mL min^{-1})	75
Cryofocusing temperature (°C)	20

ferent amounts of sodium chloride to get 0.7, 2.42 and 4.14 M (0, 100 and 200 g L^{-1} of NaCl, respectively).

Recoveries of the procedures for the two volumes were also determined by comparing GC/MS response for the same amount extracted (100 ng L^{-1}) with a standard solution spiked directly in a glass wool thermodesorption tube. Naphthalene and acenaphthene were also present in the mix of PAHs, but the extraction efficiency of these compounds was low, due probably to their high volatility and/or competition with the rest of the analytes.

Once desorption and extraction conditions had been optimised for marine samples, the complete SBSE/TDS/GC/MS method was validated; linearity, repeatability, reproducibility, quantification and detection limits were determined at the conditions shown in Table 2. Repeatability and reproducibility were evaluated at two concentration levels (50 and 200 ng L^{-1}) for 10 and 100 mL sample volumes. Four replicate measurements were analysed with the proposed method to determine repeatability, and these results have been compared with those obtained on 3 different days (reproducibility). The linearity of the proposed method was studied in the range of 5–500 ng L^{-1}. The limits of detection (LODs) and quantification (LOQs) were established for signal-to-noise ratios of 3 and 10, respectively, using low concentration standard solutions (20, 10 and 1 ng L^{-1}) for both extraction procedures.

2.5. Instrumentation and analytical conditions

The analyses were performed using a TDS-2 thermodesorption unit mounted on a 6890 Agilent GC system, which is coupled to an Agilent 5973 mass spectrometric detector (Agilent Technologies, Little Falls, DE, USA). The cryofocusing and chromatographic conditions applied are those previously optimised for PAHs and pesticides in surface waters [17]. Consequently desorption was achieved at 280 °C under a helium flow of 75 mL min^{-1} in the splitless mode and the transfer line at 250 °C (TDS-2). The effect of desorption time on the GC/MS response was evaluated by analysing seawater samples spiked with 500 ng L^{-1} for different times (7, 10 and 12 min). Carryover was also evaluated in each case by desorbing the stir bar for 15 min and analysing by GC/MS. The desorbed compounds were cryofocused in programmable temperature vaporisation (PTV) injector (CIS-4, Gerstel) at 20 °C and the analytes were transferred to the HP-5MS column (30 m × 0.25 mm i.d. × 0.25 μm film thickness of 5%phenyl, 95% polydimethylsiloxane). The column temperature was maintained at 70 °C for 2 min, ramped at 30 °C/min to 200 °C, held for 1 min, and finally increased 3 °C min^{-1} to 280 °C and held for 2 min. The mass spectrometer was operated in the full-scan mode (60–400 *m/z*) and the quantification was performed for the selected target ion. The use of full-scan mode is adequate for a multi-residue method and useful for screening other pollutants that could be present in real samples. The compounds were quantified using an external calibration curve (5–500 ng L^{-1}), and deuterated terbutylazine, deuterated PAH mix and quintozene were used as internal standards for triazines, PAHs and the rest of the analytes, respectively.

3. Results and discussion

3.1. Optimisation of the desorption and extraction processes

3.1.1. Effect of desorption time

In a previous study, the desorption time for a multi-residue extraction method in freshwater samples (6 min) was optimised [17]. In this study the effect of desorption time (7–12 min) has been evaluated for all tested pollutants in seawater, with special attention being given to PCBs and some PAHs not included in the previous procedure. A slight improvement in responses of the more polar PAHs was observed for 10 and 12 min desorption

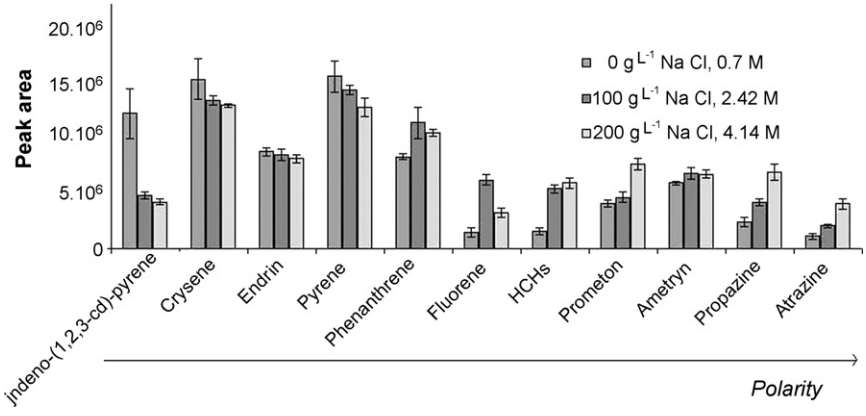

Fig. 1. Effect of NaCl addition (0, 100, 200 g L^{-1}) on the peak areas for selected compounds.

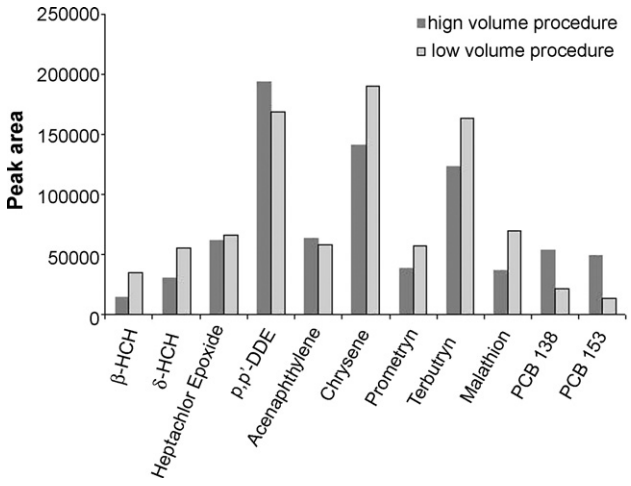

Fig. 2. Signal intensities obtained using different sample volumes (10 and 100 mL) and stir bar sizes (10 and 20 mm) with the same amount of analytes added.

times (data not shown). However, the response of other compounds such as lindane, α-HCH, α-endosulfan, malathion and triazines showed decreases with longer desorption times. Consequently 7 min was selected as the time duration for performing desorption in the optimum conditions, attending especially to lower response analytes. Carryover was evaluated for all tested desorption times and concentrations (500, 100 and 5 ng L^{-1}), by desorbing the stir bars again at 280 °C for 15 min. The detected carryover was less than 0.1% with 7 min of desorption for all analytes; consequently the length of time selected for desorption is adequate.

Table 3
Recovery (%) of some selected analytes at 100 ng L^{-1} for the two extraction procedures optimised

Compounds	High volume procedure[a]	Low volume procedure[b]
α-HCH	78.7 ± 10	110.4 ± 4.2
Dieldrin	101.8 ± 6.0	115.2 ± 7.7
Endrin	94.8 ± 5.2	106.2 ± 6.4
p,p′-DDD	91.4 ± 12.0	115.5 ± 4.3
p,p′-DDT	91.7 ± 1.1	123.0 ± 10.5
Heptachlor epoxide	96.6 ± 1.9	101.4 ± 0.6
Acenaphthylene	55.6 ± 2.9	78.5 ± 7.0
Pyrene	88.1 ± 3.7	90.9 ± 6.0
Fluoranthene	85.2 ± 4.1	86.3 ± 5.7
Anthracene	65.2 ± 0.5	72.7 ± 1.6
Phenanthrene	83.0 ± 2.3	73.6 ± 1.0
Fluorene	72.4 ± 4.6	86.6 ± 5.7
Chrysene	101.6 ± 4.8	72.6 ± 3.0
Benzo[a]pyrene	78.1 ± 15.3	75.3 ± 3.4
Indeno[1,2,3-cd]pyrene	65.8 ± 14.3	67.2 ± 15.6
Ametryn	52.6 ± 1.0	79.3 ± 2.3
Prometryn	76.1 ± 0.9	79.0 ± 5.4
Propazine	43.0 ± 1.5	79.9 ± 8.0
PCB 28	56.3 ± 2.7	83.4 ± 4.2
PCB 52	56.4 ± 3.6	47.6 ± 2.9

[a] 100 mL sample volume, 20 mm size stir bar.
[b] 10 mL sample volume, 10 mm size stir bar.

3.1.2. *Effect of salting-out*

The salting-out effect on the signal improvement has been evaluated (Fig. 1), by adding different amounts of NaCl (0, 100 and 200 g L^{-1}). The higher ionic strength (200 g L^{-1} NaCl, corresponding to 4.14 M) increases the signal intensities for more

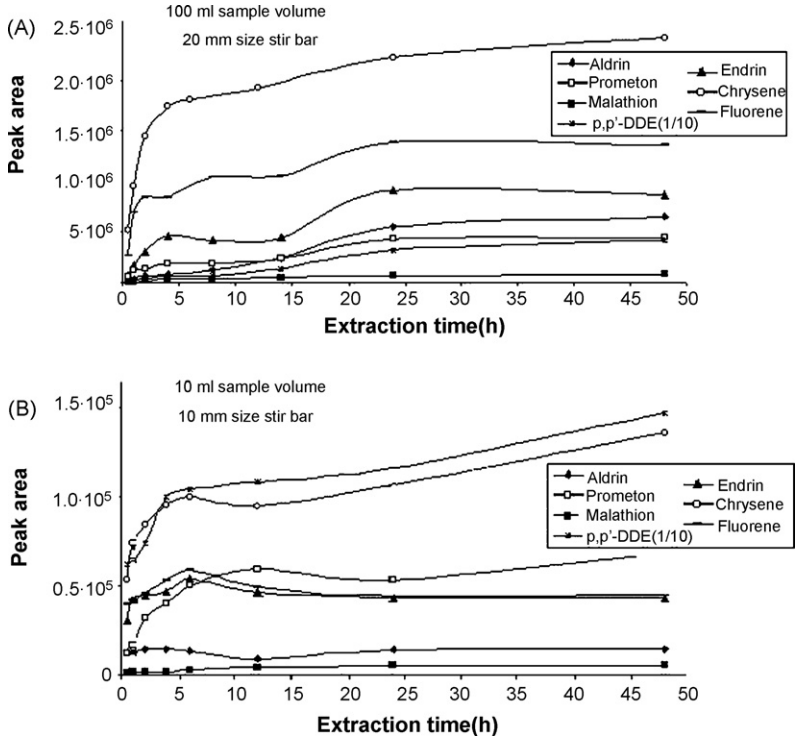

Fig. 3. Extraction profiles of some of the compounds studied, at 400 ng L^{-1} and 100 g L^{-1} NaCl. (A) 100 mL sample volume + 20 mm stir bar. (B) 10 mL sample volume + 10 mm stir bar.

polar compounds, especially for triazines (atrazine, propazine, ametryn, prometon) and HCHs. The addition of salt affects the activity coefficients of the analytes considered, increasing the concentration of water-soluble compounds sorbed onto PDMS. On the other hand, most apolar PAHs and other apolar compounds show lower extraction efficiency when ionic strength increases, due to their sorption onto the walls of the extraction receptacle as was previously verified. A concentration of 100 g L^{-1} NaCl was finally selected with the object of improving the response, especially for compounds presenting lower peak areas.

3.1.3. Sample volume and size stir bar

The responses obtained for 10 and 100 mL sample volumes, spiked with the same amount of analyte in both cases, are shown in Fig. 2. For the majority of compounds, higher intensity signals were obtained with 10 mL extraction volumes than with 100 mL. Consequently a larger analyte mass is sorbed onto PDMS when the analyte concentration in water is higher or the same absolute amount of analyte is present in a lower volume, according to SBSE theory under equilibrium conditions [2]. However there are some exceptions, such as PCBs of higher molecular weight and the more volatile PAHs (fluorene, anthracene . . .), for which responses were higher using 100 mL sample volume (Fig. 2).

When sample volume was not a limiting factor (i.e. with seawater), and the pollutants were at low concentrations, the procedure with 100 mL volume is recommended**.** This is because a higher response is obtained for 100 mL extraction when the same sample (concentration) is extracted by the two procedures. For this reason a sample volume of 100 mL and stir bar size of 20 mm × 0.5 mm were selected as optimum for the extraction of seawater samples; only in the case of samples for which that volume is difficult to obtain (i.e. interstitial water), should the 10 mL sample volume and 10 mm stir bar be applied Fig. 3.

3.1.4. Effect of extraction time

The extraction time profiles were obtained over a range of time periods from 1 to 48 h, for sample volumes of 100 and 10 mL are studied. First, in the case of higher volume sam-

Table 4
Repeatability and reproducibility (expressed as % RSD) calculated at 50 and 200 ng L^{-1} levels, using 10 mm size stir bars for interstitial water samples (10 mL) and 20 mm size stir bars for seawater samples (100 mL) for some representative compounds

Concentration	Low sample volume procedure		High sample volume procedure	
50 ng L^{-1}	Repeatability	Reproducibility	Repeatability	Reproducibility
Heptachlor epoxide	16.3	19.9	5.8	7.3
α-HCH	4.9	7.2	5.8	8.1
Lindane	6.4	14.6	5.1	9.3
α-Endosulfan	9.2	17.0	8.8	10.4
Fluorene	6.0	10.2	8.3	10.1
Phenanthrene	6.8	19.3	1.1	6.5
Anthracene	10.2	19.7	2.1	7.4
Benzo-a-anthracene	8.7	14.8	6.0	23.1
Chrysene	11.5	23.0	10.6	25.2
Prometryn	11.2	12.8	3.4	9.8
Ametryn	13.3	14.7	5.1	11.5
Atrazine	9.7	14.4	8.2	14.2
Prometon	10.5	14.3	3.3	10.5
Malathion	n.d.	n.d.	14.2	29.0
PCB 52	14.7	19.1	11.9	9.3
PCB 138	12.4	26.1	6.3	15.9
Concentration	Low sample volume procedure		High sample volume procedure	
200 ng L^{-1}	Repeatability	Reproducibility	Repeatability	Reproducibility
Heptachlor epoxide	0.6	4.3	1.9	5.6
α-HCH	3.6	3.9	13.4	19.6
Lindane	3.5	3.6	4.9	5.7
α-Endosulfan	16.1	17.2	2.0	7.5
Fluorene	6.9	15.7	6.4	8.5
Phenanthrene	1.4	7.8	2.8	5.9
Anthracene	14.2	16.4	0.8	6.0
Benzo-a-anthracene	7.3	26.6	4.1	20.2
Chrysene	4.3	22.0	5.7	21.7
Prometryn	5.0	6.1	1.1	4.5
Ametryn	4.7	6.2	1.8	5.3
Atrazine	11.7	13.0	5.2	6.2
Prometon	1.8	10.5	1.7	5.4
Malathion	13.2	13.5	2.5	8.7
PCB 52	5.6	12.1	1.5	10.0
PCB 138	6.0	22.9	2.2	20.2

ples, most compounds required 24 h to reach steady state. In the extraction of low volume samples, the majority of compounds tested show a constant response after 14 h.

We have selected 14 h as the optimum exposure time for samples of 100 mL, too, as a compromise between efficiency and run time, because full equilibrium is not essential for accurate quantification [2]. In our case, the extraction time selected for all compounds studied was adequate for high and low sample volumes; and for the less volatile analytes, the signals were strong enough to enable determination with low detection limits at this extraction time.

3.1.5. Recoveries

In general recoveries are around 70% for the majority of compounds studied. The recoveries obtained, shown in Table 3, are higher when the extraction is performed with the low sample volume procedure, in agreement with the theory of sorptive extraction [2]. As has been stated previously, extraction could be improved with longer extraction periods (24 h) in the case of the 100 mL sample procedure. Recoveries of PAHs and PCBs (50–90%) are lower than for the rest of the analytes due to their higher hydrophobicity, which favours their sorption onto the surfaces of the extraction receptacle, as has been shown previously

Table 5
Limits of detection and quantification at ng L^{-1} and correlation coefficients of the calibration curves for 100 and 10 mL sample volumes and 20 and 10 mm stir bar sizes

Compounds	High volume procedure[a]			Low volume procedure[b]		
	LOD	LOQ	r^2	LOD	LOQ	r^2
PCB 28	0.4	1.3	0.830	6.0	20.0	0.974
PCB 52	2.0	6.7	0.914	5.0	16.7	0.972
PCB 138	2.4	8.0	0.991	5.0	16.7	0.982
PCB 153	2.7	9.0	0.995	5.0	16.7	0.968
PCB 180	1.1	3.5	0.984	15.0	50.0	0.987
α-HCH	0.3	8.3	0.982	6.0	20.0	0.991
β-HCH	0.3	4.0	0.966	6.0	20.0	0.991
Lindane	0.3	10.0	0.975	6.0	20.0	0.985
δ-HCH	0.3	6.0	0.974	6.0	20.0	0.991
Aldrin	0.9	3.0	0.932	7.5	25.0	0.960
Dieldrin	1.3	4.3	0.997	6.0	20.0	0.989
Endrin	3.0	10.0	0.997	7.5	25.0	0.993
β-Endosulfan	1.2	4.0	0.993	2.5	8.3	0.995
Heptachlor epoxide	2.4	8.0	0.986	7.5	25.0	0.969
Endosulfan sulfate	0.3	0.8	0.994	10.0	33.3	0.981
α-Endosulfan	0.3	1.0	0.996	6.0	20.0	0.984
p,p′-DDE	0.2	0.6	0.937	4.3	14.3	0.912
p,p′-DDD	0.0	0.1	0.965	7.5	25.0	0.989
p,p′-DDT	0.1	0.3	0.949	7.5	25.0	0.990
Methoxychlor	0.5	1.7	0.997	5.0	16.7	0.916
Endrin ketone	0.3	1.1	0.988	5.0	16.7	0.973
Fluoranthene	0.2	0.5	0.992	2.5	8.3	0.991
Acenaphthylene	0.1	0.3	0.937	1.9	6.3	0.965
Phenanthrene	0.1	0.5	0.967	2.1	7.1	0.999
Anthracene	0.2	0.6	0.954	2.1	7.1	0.991
Fluorene	0.1	0.3	0.918	3.0	10.0	0.996
Benzo[a]anthracene	0.2	0.6	0.996	3.0	10.0	0.981
Chrysene	0.2	0.6	0.996	3.0	10.0	0.982
Pyrene	0.2	0.5	0.990	1.7	5.6	0.982
Benzo[b]fluoranthene	0.1	0.2	0.980	3.8	12.5	0.963
Benzo[k]fluoranthene	0.1	0.4	0.976	3.8	12.5	0.978
Benzo[a]pyrene	0.1	0.4	0.973	5.0	16.7	0.969
Indeno[1,2,3-cd]pyrene	0.3	1.0	0.968	2.5	8.3	0.811
Dibenzo[a,h]anthracene	0.3	1.0	0.968	2.7	9.1	0.838
Benzo[ghi]perylene	0.3	0.8	0.964	2.3	7.7	0.859
Terbutylazine	0.6	4.7	0.976	2.5	8.3	0.991
Ametryn	0.3	0.8	0.971	1.9	6.3	0.996
Atrazine	0.2	0.6	0.920	3.0	10.0	0.939
Prometryn	0.3	1.0	0.989	1.9	6.3	0.995
Prometon	2.1	7.0	0.983	3.0	10.0	0.995
Propazine	0.4	1.3	0.959	3.0	10.0	0.974
Terbutryn	0.3	1.0	0.990	3.8	12.5	0.998
Parathion	7.5	25.0	0.965	50.0	167.0	0.850
Malathion	3.8	12.5	0.985	50.0	167.0	0.981

[a] 100 mL volume sample, 20 mm size stir bar.
[b] 10 mL volume sample, 10 mm size stir bar.

for fresh waters [17]. For this reason the sorption of analytes on the walls of the Erlenmeyer flasks has been evaluated by rinsing the flask walls with methanol after SBSE, and analysing the methanol extract by direct injection in GC/MS. Sorption was only detected for some PAHs (benzo[b]fluoranthene, benzo[k] fluoranthene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene) and varied between 20 and 40% of total analyte. However, some polar compounds (triazines) have also shown recoveries below 50%. This result could be attributed to their higher polarity, which reduces their affinity with PDMS (as a result of the lower octanol–water partitioning coefficient), to the insufficient sorption capacity of the coated stir bar, to a process of competition between analytes [19] or to losses by volatilisation of these compounds during extraction.

In all cases recoveries for all compounds tested were repeatable and reproducible, as shown in the next section, and consequently the procedure is adequate for application to environmental samples.

3.2. Validation of the SBSE method

3.2.1. Repeatability and reproducibility

Table 4 shows the results obtained for both concentrations expressed as a percentage of relative standard deviation (% RSD). The results for repeatability show the precision of the method at 50 and 200 ng L^{-1} with mean values that varied between 0.5–17% and 1–12%, for 10 and 100 mL volumes, respectively. Results for reproducibility indicate the robustness of the extraction method with mean values of RSD around 15% for both volumes at 50 and 200 ng L^{-1}. Repeatability and reproducibility are good for both extraction volumes, but are slightly better for 100 mL procedure and decrease slightly at lower concentrations (50 ng L^{-1}), especially for lower sample volumes. The reproducibility data for PCBs and PAHs with four or more rings in their molecular structure (15–25%) are not as good as for the rest of the compounds. However, these values improve significantly when internal standard corrections are applied; this allows repeatabilities and reproducibilities to be obtained with RSD, expressed as a percentage, of less than 10%. Consequently both procedures are adequate for the extraction of marine (100 mL) and interstitial water samples (10 mL).

3.2.2. Calibration curves and linearity

The linear range varied between 5 and 500 ng L^{-1} for the compounds tested with 100 mL and 10 mL extraction procedures. Correlation coefficients (r^2) were higher than 0.98 for the majority of analytes studied (Table 5).

3.2.3. Limits of detection and quantification

The limits of detection (LOD) and quantification (LOQ) have been established for a signal to noise ratio of 3 and 10, respectively, using different concentrations of standard solutions (20, 10 and 1 ng L^{-1}), for both extraction procedures. In the case of the 100 mL extraction procedure, the limits of detection were lower than 1 ng L^{-1} for the majority of compounds tested; the lowest detection limit (0.03 ng L^{-1}) was for *p,p′*-DDD and the highest LOD (7.50 ng L^{-1}) for parathion (Table 5). For the 10 mL extraction procedure, the mass of analyte extracted is less than with the 100 mL extraction procedure, therefore limits of detection are below 10 ng L^{-1} for all cases, except organophosphorus pesticides (parathion, malathion) which showed higher values (Table 5). In spite of these results, the response obtained is adequate for determining the presence of these compounds in interstitial seawater, where these contaminants are usually present at higher concentration levels than in seawater.

3.2.4. Environmental marine samples

The SBSE procedure was applied for determining traces of semi-volatile compounds in environmental samples. Five seawater samples from different zones of Cadiz Bay were extracted, using stir bar, and their content of contaminants was analysed by the proposed method. The results are given in

Table 6
Concentration (ng L^{-1}) of organic pollutants in seawater samples from Cadiz Bay

Compound/sampling point	Puerto Sta Maria	Valdelagrana	Ventorrillo	Camposoto	Suazo
p,p′-DDD	3	11	n.d.	n.q.	n.d.
p,p′-DDT	n.d.	n.q.	n.d.	n.q.	n.d.
Acenaphthylene	21	n.q.	n.q.	48	62
Phenanthrene	344	266	377	359	344
Anthracene	n.q.	273	389	376	356
Fluorene	n.q.	n.q.	71	n.d.	n.q.
Pyrene	97	93	90	80	90
Fluoranthene	14	15	14	16	17
Benzo[a] anthracene	n.d.	n.q.	n.d.	n.q.	n.q.
Chrysene	n.d.	n.q.	n.d.	n.q.	n.d.
Benzo[b]fluoranthene	n.d.	43	n.d.	n.q.	69
Benzo[k]fluoranthene	n.q.	n.q.	n.d.	n.d.	n.d.
Benzo[a]pyrene	n.d.	50	n.d.	71	n.q.
Indeno[1,2,3-cd]pyrene	n.d.	36	n.d.	386	n.d.
Dibenzo[a] anthracene	n.d.	n.d.	n.d.	299	n.d.
Benzo[ghi]perylene	n.d.	157	n.d.	295	n.d.
Terbutylazine	23	n.d.	n.d.	n.d.	n.d.

Extraction procedure: 100 mL sample volume, 100 g L^{-1} NaCl, 20 mm size stir bar and 14 h extraction time. n.d.: not detected. n.q.: detected, not quantified.

Table 6 and show that terbutylazine was found only in seawater from El Puerto de Santa Maria at levels of around 20 ng L^{-1}; this area in particular is influenced by industrial and agricultural discharges. In the other hand phenanthrene, anthracene and pyrene, were detected at all sampling points, at levels of 100–400 ng L^{-1}; these are the most extensive pollutants found in the area studied. Other PAHs (acenaphthylene, fluorene, fluoranthene, benzo[a] anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenzo[a] anthracene) and some organochlorined pollutants (DDX) were detected at several sampling stations; the maximum values of 10 ng L^{-1} found were in samples taken close to wastewater discharge points. In the case of interstitial waters, phenantrene was detected at maximum levels of between 100 and 300 ng L^{-1} at medium depth (5–20 cm), with the concentration showing a decrease with sediment depth. Other PAHs such as fluoranthene and pyrene were found in some interstitial waters with values lower than quantification limits.

4. Conclusions

Procedures for the simultaneous analysis of pesticides, PAHs and PCBs, developed for 10 and 100 mL sample volumes, have been optimised and validated for marine water samples. The key parameters of extraction have been optimised to obtain a method adequate for compounds of very different polarities ($\log K_{o/w}$ from 2 to 7). The proposed method for marine water samples consists of a stir bar sorptive extraction stage lasting 14 h with 100 g L^{-1} NaCl; subsequently the analytes taken up by the stir bar are desorbed at 280 °C for 7 min, cryofocused in a PTV injector at 20 °C and analysed by GC/MS in full-scan mode. Under these conditions the method is sensitive, robust and shows a good linearity between 5 and 500 ng L^{-1} for all compounds tested. The method presents detection limits lower than 1 and 10 ng L^{-1} for 100 mL and 10 mL samples, respectively, and recoveries ranged from 20 to 90%.

In summary, the proposed analytical method is easy, fast and presents low detection limits, good reproducibility and repeatability, and good sensitivity. It has been successfully applied for the simultaneous determination of semi-volatile trace pollutants in seawater and interstitial water at ng L^{-1} levels. The use of full-scan mode with MS spectrometer permits screening for other pollutants that could be present in real samples, an option which can also be incorporated in the proposed method.

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