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RESEARCH PAPER

Determination of 13 endocrine disrupting chemicals in environmental solid samples using microwave-assisted solvent extraction and continuous solid-phase extraction followed by gas chromatography–mass spectrometry

Abdelmonaim Azzouz[1] · **Evaristo Ballesteros**[1]

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Abstract Soil can contain large numbers of endocrine disrupting chemicals (EDCs). The varied physicochemical properties of EDCs constitute a great challenge to their determination in this type of environmental matrix. In this work, an analytical method was developed for the simultaneous determination of various classes of EDCs, including parabens, alkylphenols, phenylphenols, bisphenol A, and triclosan, in soils, sediments, and sewage sludge. The method uses microwave-assisted extraction (MAE) in combination with continuous solid-phase extraction for determination by gas chromatography–mass spectrometry. A systematic comparison of the MAE results with those of ultrasound-assisted and Soxhlet extraction showed MAE to provide the highest extraction efficiency (close to 100 %) in the shortest extraction time (3 min). The proposed method provides a linear response over the range 2.0−5000 ng kg^{-1} and features limits of detection from 0.5 to 4.5 ng kg^{-1} depending on the properties of the EDC. The method was successfully applied to the determination of target compounds in agricultural soils, pond and river sediments, and sewage sludge. The sewage sludge samples were found to contain all target compounds except benzylparaben at concentration levels from 36 to 164 ng kg^{-1}. By contrast, the other types of samples contained fewer EDCs and at lower concentrations (5.6−84 ng kg^{-1}).

Keywords Endocrine disrupting chemicals · Environmental solid samples · Continuous solid-phase extraction · Microwave-assisted extraction · Gas chromatography–mass spectrometry

✉ Evaristo Ballesteros
eballes@ujaen.es

[1] Department of Physical and Analytical Chemistry, E.P.S. of Linares, University of Jaén, 23700 Linares, Jaén, Spain

Introduction

Endocrine disrupting chemicals (EDCs) are exogenous chemical substances or mixtures thereof that alter some functions of the endocrine system and have been postulated as the cause of a large number of adverse health effects most of which involve reproductive abnormalities potentially leading to population decline. The effects ascribed to EDCs in humans include temporary reductions in sperm count and quality; increased incidence of testicular, prostate, and breast cancer; altered sex ratios; neurological disorders; polycystic ovaries; endometriosis; cardiovascular disease; thyroid disorders and immune deficiencies [1]. A large variety of products commonly used in daily life (e.g., detergents, personal care products such as cosmetics, pharmaceuticals, industrial formulations) contain EDCs of diverse structure. As a result, EDCs are frequently encountered in the aquatic environment, which they reach largely through effluents from wastewater treatment plants (WWTPs) and run-off from farmland [2]. Because EDCs can potentially affect the environment at very low concentrations, their analysis requires methods with very low detection limits, which is especially difficult with complex matrices such as solid environmental samples (e.g., soils, sediments, sewage sludge). The EDCs 4-*tert*-octylphenol (4OP) and nonylphenol (NP) are ubiquitous contaminants—so much so that European Directive 2003/53/EC has established restrictions on the marketing, uses, and preparation of certain hazardous substances including both [3]. The 3rd Draft of Working Document on Sludge by the European Commission (EC) suggests a concentration limit of 50 mg kg^{-1} for NP in sludge [4]; also, the US Environmental Protection Agency

(EPA) has regulated NP at a maximum average level of 6.6 μg L^{-1} for fresh water [5] and, more recently, the EC has set a maximum allowable concentration of NP of 0.3 μg L^{-1} in inland and other surface waters, and 0.1 or 0.01 μg L^{-1} as maximum annual average concentration of 4OP in inland and other surface waters, respectively [6].

The greatest difference between the determination of EDCs in a solid matrix and water samples is in sample preparation. Thus, EDCs in dry solid samples are usually extracted with classical techniques such as Soxhlet extraction, which has been applied to a wide variety of compounds such as NP and bisphenol A (BPA) [7]. Ultrasound energy has also been widely used to leach organic and inorganic compounds from solid matrices. A wide range of EDCs have in fact been determined following ultrasound-assisted extraction (UAE) from a solid matrix [8–11]. Some authors, however, have used pressurized liquid extraction [12, 13] or microwave-assisted extraction (MAE) [14, 15] to isolate EDCs from soils, sludge, or sediments on the grounds of the improved extraction yields obtained.

Most of the solid–liquid techniques used for the extraction of EDCs from solid samples are unselective and require a clean-up step as a result. The clean-up treatment usually involves solid-phase extraction (SPE), gel permeation extraction, a combination of extraction and clean-up for selective pressurized liquid extraction, or some other effective approach [16]. SPE is a common choice for environmental samples. Thus, reversed-phase SPE has been widely applied to solid samples. This technique retains analytes as a result of the interaction of non-polar groups in them with non-polar functional groups on the sorbent via van der Waals forces. The interaction is facilitated by highly polar (i.e., scarcely non-polar) solvents [17]. Reversed-phase sorbents including silica-based C18 [8, 15, 18, 19] and polymeric phases such as Oasis-HLB [8, 9], Oasis-MAX [11], and Oasis MCX [20] have been used to determine parabens, alkylphenols, triclosan (TCS), and BPA. On the other hand, normal-phase SPE sorbents such as silica gel have been used to clean up extracts of river sediments for the determination of NP, 4OP, BPA, and hormones [14].

EDCs in soil, sediment, and sewage sludge extracts can be quantified by gas chromatography–mass spectrometry (GC–MS) [7, 14, 16, 18, 19], gas chromatography–tandem mass spectrometry (GC–MS/MS) [13, 21, 22], liquid chromatography–mass spectrometry (LC–MS) [23–25], or liquid chromatography–tandem mass spectrometry (LC–MS/MS) [10, 11, 15, 19–21]. GC–MS is more economical and operationally simple than LC–MS/MS for this purpose. However, the polar nature of most EDCs requires their derivatization in order to reduce adsorption on the chromatographic column or thermal decomposition at the injector port, and improve sensitivity and peak separation as a result. This is most often accomplished by acylation (usually acetylation) [7, 26–28] or silylation [8, 14, 16, 21, 22, 26].

The aim of this work was to develop a cost-effective, environmentally friendly analytical method for the simultaneous determination of 13 EDCs in environmental solid samples. To this end, three extraction methods (viz., Soxhlet extraction, MAE, and UAE) were compared in terms of extraction time and yield, and solvent consumption, in the simultaneous extraction of the target compounds from the sample matrix. Using a closed-circuit continuous SPE system was found to substantially boost selectivity and sensitivity, and also, possibly, to greatly reduce the volumes of reagents and solvents needed, and to minimize environmental pollution. The ensuing method was used to investigate the presence of EDCs in sewage sludge from wastewater treatment plants, as well as in agricultural soils, and river and pond sediments. Our results could provide a better understanding of the potential ecological and human health risks of these EDCs in environmental solid samples.

Experimental

Chemicals and materials

The chemicals used as analyte standards and reagents were all reagent-grade or better. The following EDCs were supplied by Sigma-Aldrich (St. Louis, USA): methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), butylparaben (BuP), isopropylparaben (iPrP), isobutylparaben (iBuP), benzylparaben (BzP), 2-phenylphenol (2PH), 4-phenylphenol (4PH), NP, 4OP, BPA, and TCS. As EDCs are potential or actual carcinogens, all products were handled with care, using efficient fume hoods and wearing protective latex gloves. Chromatographic grade solvents (methanol, acetonitrile, ethyl acetate, *n*-hexane, ethanol, dichloromethane, and isopropanol) and LiChrolut EN (particle size 40–120 μm) were supplied by Merck (Darmstadt, Germany). Derivatizing reagents (BSTFA and TMCS), hydrochloric acid (reagent-grade, 37 %), potassium hydroxide, and triphenylphosphate (internal standard, IS) were supplied by Fluka (St. Louis, USA).

Stock standard solutions of the individual EDCs (1 g L^{-1}) were prepared in methanol and stored at 4 °C. Intermediate standard solutions were prepared by appropriate dilution of the stocks in methanol and further used to spike soil samples to prepare standards containing all EDCs at the nanogram per gram level, before analysis.

Sampling and spiking of soils

The environmental solid samples (soil, sediment, and sewage sludge) samples were collected in Andalusia (southern Spain). Three river and two pond sediment samples were collected at a sediment depth of 5 cm and a distance of approximately

0.5 m from the bank. Agricultural soils were collected from four agricultural areas in Cordoba (sample 1, soil irrigated with well water), Seville (sample 2, soil irrigated with well water), and Jaen (samples 3 and 4, soil irrigated with well and pond water, respectively). To this end, a sampling area of ca. 1 m^2 was selected in each location to randomly collect three subsamples from each. Sewage sludge samples were obtained from the secondary sedimentation tanks of two municipal WWTPs in Andalusia (sample 1 from a city with a population of 50,000 and sample 2 from one of 500,000). All samples were collected in pre-cleaned 500-mL amber glass bottles and, once in the laboratory, passed through a 2-mm sieve, homogenized, and freeze-dried in a LyoQuest apparatus from Telstar (Madrid, Spain) at −55 °C under vacuum (0.05 bar) for 24 h. After freeze-drying, the samples were stored in stoppered vials at −20 °C until analysis (Fig. 1). Prior to analysis, soil, sediment, and sludge samples previously subjected to no freeze-drying were characterized for pH, moisture, and total organic carbon (Table 1). Soil pH was determined by using the AOAC Official Method 994.16 in ratios of 1:2.5 g soil/mL distilled water [29]. Moisture was determined by weighing, drying to a constant weight at 105 °C, and re-weighing [30]. Total organic carbon content was determined by wet oxidation with sulfuric potassium dichromate and titration of excess dichromate with $Fe(NH_4)_2(SO_4)_2$ [31].

Soil and sediment samples found to contain no EDCs (concentrations below of the limits of detection, LODs) in a preliminary test were used as blanks, and also to optimize and validate the proposed method. These samples were also freeze-dried and homogenized as described above.

Microwave extraction, ultrasound-assisted extraction, and Soxhlet extraction

The three extraction techniques used (MAE, UAE, and Soxhlet extraction) were compared and their operational variables optimized by processing representative samples, namely an agricultural soil sample containing 1.9 % organic carbon (OC) and a river sediment sample containing 2.0 % OC. Both were found to contain none of the EDCs in a preliminary test. Blanks of both samples (100 g) were spiked with 25 mL of methanol containing 100 ng of the 13 EDCs (1 ng analyte/g sample). After spiking, the samples were allowed to dry in air for about 5 h with shaking at hourly intervals. Then, the samples were stored in amber glass-stoppered bottles at 4 °C for 1 month before extraction. The EDCs were assumed to be uniformly distributed in the 100 g of sample; also, because both samples still retained residual moisture throughout the storage period, any analyte–matrix interactions were assumed to have occurred during the weathering period, to some extent a similar process to what happens in real contaminated soil of similar properties.

Figure 1 depicts the procedures used to determine the 13 target EDCs in freeze-dried samples or spiked soil, sediment, or sewage sludge samples. For MAE, the vials containing the samples were tightly sealed and placed in front of the magnetron of a household microwave oven for extraction at 350 W for 3 min. For UAE, the vials were secured with a silicon cap and then sonicated in an ultrasonic bath for 30 min. Soxhlet extraction was performed in a 30-mL Soxhlet apparatus operating at 4–6 cycles/h according to reported recommendations [32]. Soxhlet, MAE, and UAE extracts were centrifuged on a

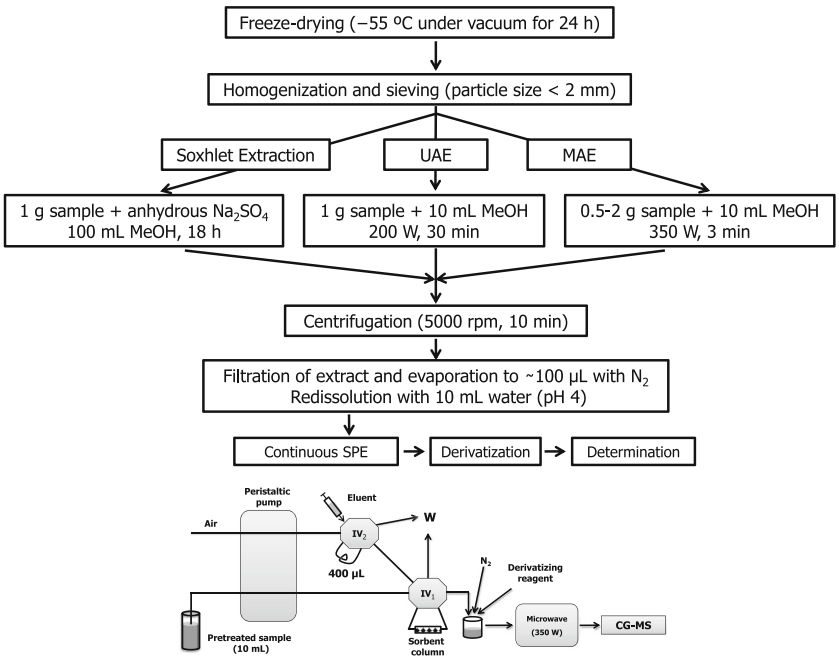

Fig. 1 Flow diagram depicting the overall protocol for the determination of endocrine disrupting chemicals in freeze-dried samples or spiked soil, sediment, and sewage sludge samples. *UAE* ultrasound-assisted extraction, *MAE* microwave-assisted extraction, *SPE* solid-phase extraction, *GC–MS* gas chromatography with mass spectrometric detection, *IV* injection valve, *W* waste

Table 1 Physicochemical characterization of environmental solid samples

		Organic carbon (% dry wt)	pH	Water content (%)
Agricultural soil	1	3.0	6.5	0.12
	2	1.8	8.1	0.13
	3	1.9	6.8	0.13
	4	2.6	7.4	0.14
Pond sediment	1	2.5	7.5	2.3
	2	2.3	7.2	2.5
River sediment	1	1.6	7.7	1.6
	2	2.5	7.6	1.7
	3	2.0	7.8	1.5
Sewage sludge	1	13.8	6.5	6.7
	2	12.4	6.7	6.6

Centrofriger BL-II apparatus from JP Selecta (Barcelona, Spain) at 5000 rpm for 10 min, the supernatant being transferred into a glass tube. The pooled extracts were concentrated to ca. 100 μL under a gentle stream of nitrogen and diluted with 10 mL of ultrapure water at ca. pH 4.0 adjusted with dilute HCl for passage through the continuous solid-phase extraction system.

Clean-up procedures and derivatization

The continuous flow system for preconcentration/clean-up of the EDCs extracted from the sediments, soils, and sewage sludge samples was constructed by using a peristaltic pump (Gilson minipuls-3, Villiers-le-Bel, France) fitted with poly(vinylchloride) tubes, two Rheodyne 5041 injection valves (Cotati, CA, USA), and a laboratory-made PTFE column (3 mm i.d.) containing 80 mg of LiChrolut EN sorbent. The sorbent column was conditioned with 1 mL of acetonitrile and 1 mL of water purified with a Milli-Q system (Millipore, Bedford, MA, USA). Under these conditions, the sorbent column was serviceable for about 1–2 months with no change in its properties.

The final extract was passed through the continuous SPE unit in several steps (Fig. 1). In the preconcentration step the EDCs were completely retained in the sorbent column and the matrix was sent to waste. Residual water in the column and connections was dried by passing through a stream of air for 2 min. In the elution step, the loop of the second valve IV_2 was filled with the eluent (acetonitrile containing 500 μg L^{-1} triphenylphosphate as IS) by using a syringe and then the valve was switched to pass its contents (400 μL) through the column in the opposite direction as sample aspiration. The organic extract was manually collected in an air-tight 0.5-mL conical glass insert and evaporated to a volume of ca. 25 μL under a gentle stream of ultrapure N_2. Potential errors in measuring the final extract volume were avoided by using the internal standard. Next, 70 μL of a mixture of BSTFA containing 1 % TMCS (derivatizing agent) was added to the sample extract. After that the vial was tightly sealed and the analytes were derivatized using a household microwave oven for 3 min at 350 W. Finally, 1 μL of the silylated derivatives was determined by GC–MS.

Determination with gas chromatography–mass spectrometry

The EDCs in the prepared samples were determined by gas chromatography (GC) with mass spectrometric detection (MS) on a Focus GC instrument interfaced to a DSQ II mass spectrometer and controlled by a computer running XCalibur software (Thermo Electron SA, Madrid, Spain). The instrument was equipped with a DB-5 MS GC column (30 m×0.25 mm i.d. × 0.25 μm film thickness) obtained from J&W (Folson, CA, USA) for chromatographic separation with helium (purity>99.999 %) as the carrier gas at a constant flow rate of 1.2 mL/min. Injector temperature was 285 °C. The GC oven temperature was programmed from 70 °C (held for 1 min) to 150 °C at 14 °C min^{-1}, raised to 215 °C at 6 °C min^{-1} and then to 285 °C at 10 °C min^{-1}. A 1-μL sample was injected in pulsed split mode (1:20) and the total analysis time for a GC run was 24.5 min. MS was operated in EI ionization mode (70 eV) with SIM mode and a solvent delay time of 8 min. The GC–MS interface, ion source, and quadrupole temperatures were set at 285, 280, and 200 °C, respectively. The MS was set in full scan modem (60–400 amu) for identification purposes. The retention time and fragment ions were identified by injecting a single compound standard under the full scan. For each silyl derivative, $M^{\bullet+}$, [M−15], and other additional ions were monitored which are included in Table 2, where $M^{\bullet+}$ is the parent ion and $[M-15]^+$ is the parent ion minus 15 corresponding to the loss of a $CH_3^\bullet$ of the $Si(CH_3)_3$ group.

Table 2 Limits of detection (LOD), linearity, correlation coefficient (r^2), precision (RSD, $n=11$), retention times, and mass values used for MS detection of the 13 endocrine disrupting chemicals

Compounds	LODs (ng kg^{-1})[a]	LOQ (ng kg^{-1})[a]	Linear range (ng kg^{-1})[a]	r^2	RSD (%)		t_R (min)	m/z[b]		
					Within-day	Between-day		$[M]^{\bullet+}$	$cM-15]^+$	Additional ions
Methylparaben (MeP)	4.3	15	15–5000	0.999	6.0	6.9	10.15	224	**209**	135,149,177, 193
Ethylparaben (EtP)	4.4	15	15–5000	0.995	5.8	6.1	11.16	238	**223**	135, 151, 193
Isopropylparaben (iPrP)	3.0	10	10–5000	0.998	3.1	4.5	11.65	252	237	151, **193**, 195, 210
2-Phenylphenol (2PH)	0.5	2.0	2.0–5000	0.994	3.8	5.1	11.69	242	**227**	105, 152, 211
4-*tert*-Octylphenol (4OP)	1.0	3.5	3.5–5000	0.993	6.0	6.8	12.27	278	263	151, 191, **207**
Propylparaben (PrP)	4.4	15	15–5000	0.997	5.6	6.0	12.65	252	237	**193**, 195, 210
Isobutylparaben (iBuP)	3.1	10	10–5000	0.993	5.5	6.2	13.59	266	251	151, **193**, 195, 210
Nonylphenol (NP)	0.5	2.0	2.0–5000	0.999	4.5	5.9	13.73	292	277	179, **207**, 221, 263
Butylparaben (BuP)	4.3	15	15–5000	0.997	5.0	5.8	14.25	266	251	193, 195, **210**
4-Phenylphenol (4PH)	0.5	2.0	2.0–5000	0.994	5.3	6.1	14.47	**242**	227	113,152, 207, 211
Triclosan (TCS)	0.5	2.0	2.0–5000	0.998	4.7	5.8	19.65	362	**347**	200, 310
Benzylparaben (BzP)	4.5	15	15–5000	0.995	5.2	6.0	20.24	300	285	91, **193**, 255
Bisphenol A (BPA)	0.5	2.0	2.0–5000	0.993	4.2	5.4	20.58	372	**357**	207, 285

[a] To 2 g of sample

[b] The base peaks used for quantification are in bold typeface. m/z for IS (triphenylphosphate): 77, 170, 325, **326** (t_R, 22.79 min)

Results and discussion

Comparison of MAE, UAE, and Soxhlet extraction of EDCs

Selectivity and sensitivity were initially boosted by using a system described elsewhere [33] to clean up and preconcentrate the MAE, UAE, and Soxhlet extracts. Table 3 shows the optimum operating range for each variable affecting extraction of the 13 EDCs (Fig. 1). As noted earlier, their GC–MS determination required their prior derivatization. The reagents used for this purpose included a mixture of BSTFA and 1 % TMCS, which proved the most efficient. Conducting the reaction in a microwave oven was found to ensure quantitative derivatization of all analytes in a shorter time (3 min) than in a water bath (20 min) or ultrasonic bath (15 min).

The potentially adverse effects of freeze-drying the soil, sediment, and sludge samples on recovery of the target analytes from them were examined by spiking uncontaminated samples of agricultural soil and river sediment with the EDCs in order to compare the analytical results for the original soil and sediment samples (moisture content ca. 0.13 and 1.5 %, respectively) with those for their freeze-dried counterparts. For this purpose, 100 g of each type of sample was spiked with 25 mL of methanol containing 100 ng of each EDC (1 ng analyte/g sample), homogenized, and stored at 4 °C for 1 month as described in "Sampling and spiking of soils". Then, 1-g aliquots ($n=5$) were placed in stoppered lyophilization vials and freeze-dried at −55 °C under vacuum for 24 h. Finally, the freeze-dried and wet pond sediment samples ($n=5$) were subjected to MAE, preconcentrated in the continuous SPE system, and analyzed by GC–MS. The recoveries thus obtained (94–99 %) were similar for all EDCs, which allowed us to conclude that freeze-drying caused no analyte losses.

The extraction conditions were initially optimized by using MAE and UAE for agricultural soil and pond sediment samples spiked with the target EDCs at a 1 ng g^{-1} level each (see Fig. 1). Recoveries were calculated by assigning 100 % to a standard solution of the analytes in 10 mL of ultrapure water (ca. pH 4). The MAE and UAE extracts were centrifuged, dried to ca. 100 μL, and redissolved in 10 mL of ultrapure water (ca. pH 4) as described in "Microwave extraction, ultrasound-assisted extraction, and Soxhlet extraction". Then, the extracts were cleaned up in the continuous system and derivatized as described in "Clean-up procedures and derivatization". MAE performance was influenced mainly by (a) solvent nature and volume, (b) microwave power, and (c) irradiation time. As in most solid–liquid extraction techniques, the solvent was one of the most important variables to be optimized in MAE. For this purpose, seven solvents, namely methanol, acetone, acetonitrile, isopropanol, ethyl acetate,

Table 3 Variables affecting the continuous solid-phase extraction and derivatization processes of endocrine disrupting chemicals (EDCs) in environmental solid samples

Variable	Optimum range (selected value)
Continuous solid-phase extraction	
Sample pH	3.5–4.5 (4.0)
Amount of sorbent (LiChrolut EN, mg)	75–85 (80)
Volume of eluent (ethyl acetate, μL)	350–450 (400)
Sample flow rate (mL min^{-1})	0.5–4.5 (4)
Air flow rate (mL min^{-1})	0.5–4.5 (4)
Breakthrough volume (mL)	1–250 (10)
Derivatization (silylation of EDCs)	
Percentage of TMCS in BSTFA[a]	1–15 (1)
Reaction time (min)	1–5 (3)
Irradiation power of microwave (W)	300–400 (350)

[a] *TMS* trimethylchlorosilane, *BSTFA N,O*-bis(trimethylsilyl)trifluoroacetamide

dichloromethane, and *n*-hexane, were assessed for extraction efficiency. The highest recoveries of all EDCs were obtained by using methanol as the organic solvent in the MAE step. The other solvents were approximately 3−9 times less efficient, possibly as a result of the high polarity of the alcohol. In addition, methanol is miscible with water and hence potentially more effective in mixing well with wet sediments or sludge and enhancing extraction from sediment or sludge matrices. Methanol was therefore chosen as the best solvent for further testing. The next variables to be examined were the solvent volume, microwave power, and extraction time. For this purpose, 1-g aliquots of spiked agricultural soil or river sediment were extracted with variable volumes of methanol (2–15 mL) at a variable microwave power (70–500 W) for an also variable time (1–10 min). In order to accurately determine the optimum extractant volume, all extracts were diluted to a final volume of 15 mL with methanol. The highest extraction efficiency was obtained by using a methanol volume of 8–12 mL; thus, 10 mL was selected for subsequent tests. Similarly, the optimum microwave power and extraction time were found to be 350 W and 3 min, respectively. Higher power levels led to lower yields through overheating of the vials and degradation of EDCs as a result. The influence of the ratio of the amount of sample to extractant volume (10 mL methanol) was examined by using aliquots of 0.5, 1, 2, 3, or 4 g of agricultural soil or river sediment under the optimum conditions for MAE described above. The maximum efficiency in the MAE of target compounds with 10 mL of extractant was obtained for sample amounts of up to 2 g. In fact, larger amounts required increased volumes of sample (e.g., 15 mL for 3 g of soil or sediment). This led us to select an amount of sample of 2 g and a volume of extractant (methanol) of 10 mL for MAE.

The variables optimized for ultrasound-assisted extraction were the nature and volume of solvent, and the extraction time. To this end, an amount of 1 g of agricultural soil or river sediment sample containing the analytes at a 1 ng g^{-1} level each was mixed with 10 mL of each of the seven solvents used to optimize the MAE procedure and placed in an ultrasonic bath at 200 W for a variable time (1–60 min), after which the resulting extract was evaporated and redissolved as described above. As with MAE, the extraction efficiency was greatest with methanol; however, matching its efficiency required using extraction times exceeding 30 min. Finally, Soxhlet extraction was optimized by subjecting portions of 1 g of agricultural soil or river sediment spiked with a 1 ng g^{-1} concentration of each EDC to EPA 3540C method but using 100 mL of methanol for 18 h (see Fig. 1).

The optimum conditions for MAE, UAE, and Soxhlet extraction were used to determine the EDCs in agricultural soil and pond sediment samples. The results are compared in Fig. 2. As can be seen, the best results were obtained with MAE, with EDC recoveries of 93–99 %. UAE was somewhat less efficient (recoveries of 81–95 %) and Soxhlet extraction even less so (recoveries of 70–89 %). On the basis of the volume of organic solvent and extraction time used, MAE was the best choice; in fact, it required only 10 mL of extractant and 3 min of extraction time for near-quantitative recovery of all analytes. This led us to select it for the extraction of EDCs from soil, sediment, and sewage sludge samples.

Analytical performance

The performance and reliability of the proposed method were assessed by determining its linear range, analyte detectability, and precision for the 13 target EDCs. To this end, uncontaminated agricultural soil samples (2 g) spiked with 2.0−5000 ng of each EDC and processed as described in the "Experimental" were used to construct calibration curves. The equations for the standard curves were obtained by plotting the analyte-to-

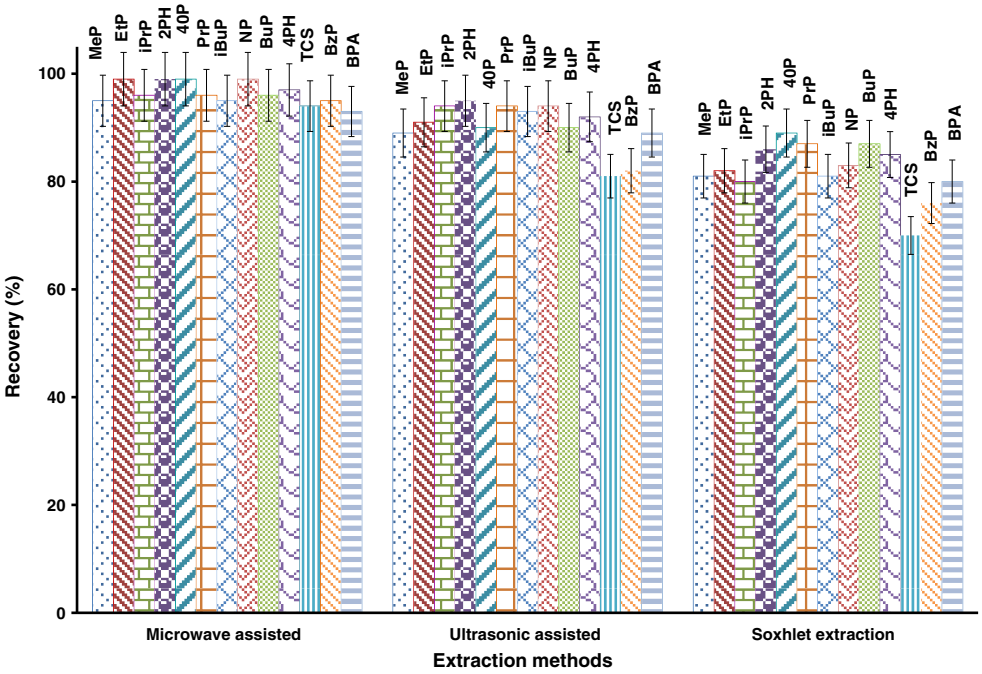

Fig. 2 Comparison of the efficiency of microwave-assisted, ultrasound-assisted, and Soxhlet extraction in isolating EDCs from soil and sediments. For compound abbreviations, see Table 2

internal standard peak area ratio against the amount of EDC and their correlation coefficients ranged from 0.993 to 0.999. The limits of detection (LODs), calculated as the standard deviation of the residuals ($S_{y/x}$) divided by the slope of the calibration curve, ranged from 0.5 to 4.5 ng kg^{-1}. LODs were also calculated as the smallest detectable amounts of analytes with a signal-to-noise ratio of 3:1 (means±standard deviations) by analyzing 12 uncontaminated agricultural soil samples containing a 20 ng kg^{-1} concentration of each EDC. The LODs thus obtained were quite similar to the previous ones. The precision of the proposed method, as a relative standard deviation (RSD), was calculated by measuring 11 uncontaminated agricultural soil samples spiked with a 25, 100, or 1000 ng kg^{-1} concentration of each EDC. A comparative study of within-day and between-day precision was conducted—the latter over 7 days—at the previous three analyte concentration levels; the former statistic ranged from 3.1 to 6.0 % and the latter from 5.4 to 6.9 % (Table 2).

Table 4 Recoveries (%) of endocrine disrupting compounds from spiked environmental solid samples[a]

Compounds[b]	Agricultural soil (ng/kg)			Pond sediment (ng/kg)			River sediment (ng/kg)			Sewage sludge (ng/kg)		
	25	100	1000	25	100	1000	25	100	1000	25	100	1000
MeP	100(6)	102(6)	97(6)	94(6)	94(6)	101(6)	93(5)	95(6)	99(6)	97(6)	98(5)	98(6)
EtP	93(5)	101(6)	95(6)	92(5)	100(5)	97(6)	96(5)	93(5)	102(6)	96(5)	100(6)	98(6)
iPrP	95(4)	97(4)	102(4)	93(4)	92(4)	101(5)	94(4)	93(4)	96(4)	92(4)	102(5)	94(5)
2PH	92(4)	100(4)	98(5)	98(4)	95(5)	99(5)	101(4)	97(4)	102(4)	95(5)	95(4)	101(5)
4OP	101(6)	100(6)	96(6)	94(5)	98(6)	96(6)	92(5)	92(5)	100(6)	92(5)	99(6)	94(6)
PrP	95(6)	92(5)	102(6)	101(5)	97(6)	98(6)	95(5)	101(6)	97(6)	93(5)	97(5)	102(6)
iBuP	92(5)	101(6)	100(6)	92(4)	93(5)	102(5)	97(6)	94(5)	100(6)	95(6)	102(5)	95(6)
NP	93(4)	97(5)	101(5)	95(5)	102(5)	99(5)	92(4)	97(5)	99(5)	93(4)	101(5)	96(6)
BuP	94(4)	102(6)	100(6)	98(5)	96(5)	102(6)	93(4)	95(4)	101(6)	94(5)	93(5)	97(5)
4PH	97(5)	101(6)	99(5)	92(5)	98(5)	97(5)	94(5)	102(5)	95(5)	102(6)	95(6)	101(6)
TCS	100(5)	95(5)	98(5)	94(4)	93(4)	98(4)	93(4)	100(5)	101(5)	95(5)	92(4)	100(5)
BzP	92(4)	101(5)	95(5)	96(5)	94(5)	99(5)	95(5)	101(5)	96(5)	100(6)	96(5)	99(6)
BPA	93(4)	98(5)	102(5)	93(4)	100(5)	102(5)	102(5)	95(4)	98(4)	92(4)	102(5)	92(4)

[a] (±SD, n=3)

[b] For compound abbreviations, see Table 2

Table 5 Determination of endocrine disrupting chemicals in soil, sediment, and sewage sludge samples by the proposed MAE-SPE/GC–MS method

Environmental sample	Compound[a]	Concentration found (±SD, ng kg^{-1}, n=3)
Agricultural soil 1	4PH	15±1
	TCS	29±2
	BPA	7.1±0.4
Agricultural soil 2	TCS	17±1
	BPA	15±1
Agricultural soil 3	TCS	8.2±0.4
	BPA	5.6±0.3
Agricultural soil 4	none	
Pond sediment 1	MeP	18±1
	iPrP	16±1
	2PH	30±1
	4OP	31±2
	NP	48±3
	BuP	17±1
	4PH	8.0±0.5
	TCS	43±2
	BPA	65±3
Pond sediment 2	MeP	35±2
	iPrP	37±2
	2PH	27±1
	4OP	26±2
	NP	43±2
	BuP	35±2
	4PH	19±1
	TCS	58±3
	BPA	15±1
River sediment 1	2PH	33±2
	NP	54±3
	4PH	19±1
	TCS	48±3
	BPA	70±4
River sediment 2	MeP	21±1
	EtP	16±1
	iPrP	17±1
	2PH	25±1
	NP	84±5
	BuP	19±1
	4PH	22±1
	TCS	53±3
	BPA	9.7±0.5
River sediment 3	none	
Sewage sludge 1	MeP	130±8
	EtP	126±7
	iPrP	134±5
	2PH	141±7
	4OP	109±7
	PrP	95±5
	iBuP	56±3
	NP	120±7
	BuP	36±2
	4PH	65±4
	TCS	145±8
	BPA	164±9
Sewage sludge 2	MeP	112±7
	EtP	109±7
	iPrP	87±4
	2PH	89±5
	4OP	130±8
	PrP	145±8
	iBuP	96±6
	NP	95±6
	BuP	85±5
	4PH	102±7
	TCS	150±8
	BPA	128±7

[a] For compound abbreviations, see Table 2

In order to validate the proposed method, a recovery study was conducted to analyze agricultural soil, river, and pond sediment and sewage sludge samples spiked with a 25, 100, or 1000 ng kg^{-1} concentration of each target EDC in triplicate (n=3) by following the procedure described in the "Experimental" (Table 4). Some samples contained only a few EDCs and allowed recoveries to be calculated by subtracting the previously quantified endogenous compounds from the total contents. Recoveries ranged from 92 to 102 % of the spiked analyte concentrations, which testifies to the applicability of the proposed method for any type of soil, sediment, or sewage sludge sample and also to the fact that matrix interferences are reduced or completely suppressed by the sample pretreatment and clean-up step in the SPE system.

Analysis of environmental samples

The proposed method was used to determine the target compounds in four agricultural soil, five sediment, and two sewage sludge samples from various locations. To this end, 2-g aliquots of freeze-dried samples were analyzed in triplicate by following the analytical procedure described in the "Experimental". When negative results were obtained (agricultural soil 3 and river sediment 3), the amount of sample was increased to 4 g and extracted with 20 mL of methanol to confirm the absence of the 13 EDCs at detectable levels.

Table 5 shows the analyte concentrations in the contaminated samples.

The EDC present at the highest concentrations in the agricultural soil samples was TCS (8.2–29 ng kg^{-1}), followed by BPA

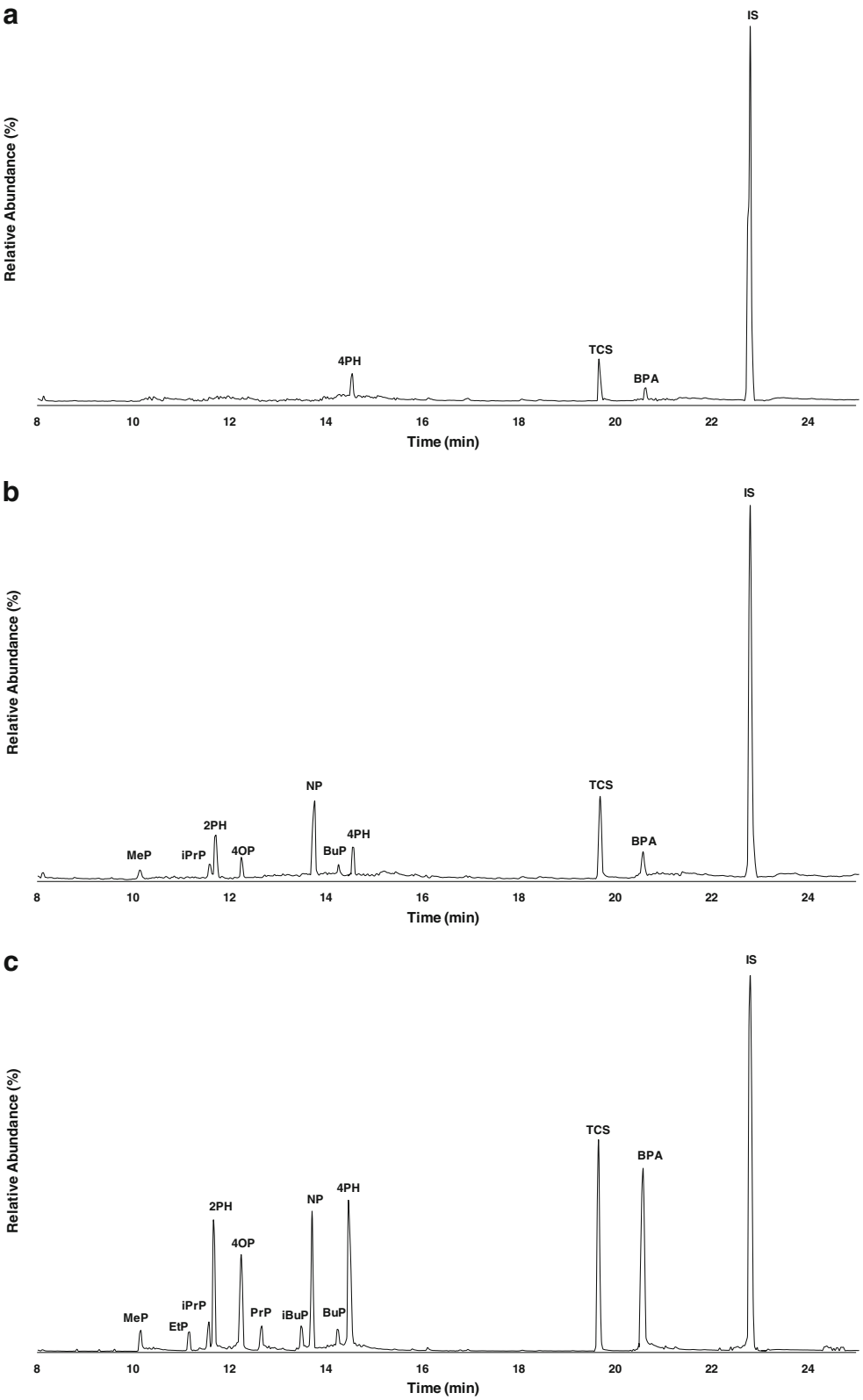

Fig. 3 GC–MS-EI chromatogram in the SIM mode obtained in the analysis of 2 g of agricultural soil 1 (**a**), pond sediment 2 (**b**) and sewage sludge 2 (**c**). For peak and sample identification, see Table 2 and Table 5, respectively

(5.6−15 ng kg^{-1}). By contrast, 4PH was present in only one soil sample and none of the remaining EDCs were detected at levels above the sensitivity threshold for the proposed method. Our concentration values are lower than those previously found by Albero et al. in agricultural soils from different areas of Spain [13]; these authors detected MeP, PrP, TCS, and BPA at levels from 0.8 to 70.2 ng kg^{-1}. By way of example, Fig. 3a shows the chromatograms for agricultural soil 1 (Table 5).

The sediment samples included three from rivers and two from ponds in southern Spain. The pond sediment samples contained three of the parabens studied (MeP, iPrP, and BuP) at concentrations from 17 to 37 ng kg^{-1}, the phenylphenols 2PH and 4PH at 8.0−30 ng kg^{-1}, and the alkylphenol 4OP at 26−31 ng kg^{-1}. The EDCs present at the highest concentrations in these samples were TCS (43−58 ng kg^{-1}), BPA (15−65 ng kg^{-1}), and NP (43−48 ng kg^{-1}) (Fig. 3b). Two of the three river sediment samples contained EDCs, the specific compounds present including parabens (9.1−21 ng kg^{-1}), phenylphenols (19–33 ng kg^{-1}), NP (54–84 ng kg^{-1}), TCS (48−53 ng kg^{-1}), and BPA (9.7−70 ng kg^{-1}). The EDC levels found in all samples were lower than previously reported values [2, 10, 11, 14, 20, 21].

We also analyzed sewage sludge from two wastewater treatment plants in Andalusia. These samples were found to contain all but one of the target analytes at levels above those in the soil and sediment samples; however, BzP was not detected at the sensitivity level of the proposed method. The concentrations of parabens, phenylphenols, and alkylphenols in the sludge samples spanned the ranges 36–145, 65–141, and 95−130 ng kg^{-1}, respectively. The concentrations of TCS (145−150 ng kg^{-1}) and BPA (128−164 ng kg^{-1}) in both samples were higher than those of the other EDCs. However, our values were lower than previously reported values for sewage sludge samples, which suggests that the two WWTPs studied were more efficient in degrading EDCs than those with which they were compared [2, 10, 20, 21]. By way of example, Fig. 3c shows the chromatogram obtained in the analysis of sewage sludge 2 with the proposed method. As can be seen, the chromatogram was quite clean, mainly as the result of potential interferences being removed during the clean-up step in the SPE system.

Conclusions

A method based on MAE followed by SPE clean-up and determination by GC–MS for the accurate, precise quantitation of major EDCs in soil, sediment, and sewage sludge samples was developed. The optimum extraction conditions were found to be a volume of 10 mL of methanol as solvent, a microwave power of 350 W, and an extraction time of 3 min. These conditions ensured near-quantitative extraction the target analytes. By contrast, UAE required a longer time (30 min), and Soxhlet extraction a large solvent volume (100 mL) and a rather long time (18 h). Using continuous SPE resulted in substantially increased selectivity and sensitivity. Thus, the sensitivity of the proposed method (LOD 0.5−4.5 ng kg^{-1}) exceeded that of PLE and GC–MS/MS with in situ derivatization for the determination of parabens, BPA, and TCS among other contaminants (0.1−1.3 ng g^{-1}) [13]. Although the combination MAE-SPE-GC–MS has been used in some previous research to determination of EDCs in solid samples [14, 34], LODs are about 1000 times higher (0.5–1.0 ng g^{-1} and 6.7–140 μg kg^{-1}, respectively) than those reported in our work. The proposed method also has several salient advantages over other previous methodologies for the determination of EDCs in environmental solid samples, namely (i) the pretreatment module allows the isolation-preconcentration of EDCs with high preconcentration factors, (ii) the risk of environmental contamination is minimal because it works with a closed system, (iii) low consumption of organic solvents and reagents, and (iv) MAE provided high EDC recoveries with low solvent consumption and short extraction times.

The proposed method was validated for routine analyses by application to agricultural soil, river and pond sediment, and sewage sludge samples. On the basis of the results, the sewage sludge samples contained all target compounds except BzP, albeit at much lower levels than those previously reported for sludge from WWTPs. This can be ascribed to wastewater treatments being highly effective in removing EDCs. However, the studied water purification plants failed to completely eliminate these compounds.

On the other hand, the agricultural soil samples studied contained few EDCs (only 4PH, TCS, and BPA) and at very low concentrations (5.6−29 ng kg^{-1}). This suggests that the soils are exposed to little contamination from these chemicals. By contrast, river and pond sediments contained greater numbers of EDCs, albeit at lower concentrations than in sewage sludge. The EDCs were probably present in river or pond water, from which they were deposited into the sediments; in fact, a number of environmental studies have shown that it is common to find EDCs in surface waters [35, 36].

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

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