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PAPER

Interlaboratory validation of an environmental monitoring method for trace analysis of endocrine disrupting compounds

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Environmental pollution continues to be an emerging study field, as there are thousands of anthropogenic compounds mixed in the environment whose possible mechanisms of toxicity and physiological outcomes are of great concern. Developing methods to access and prioritize the screening of these compounds at trace levels in order to support regulatory efforts is, therefore, very important. A methodology based on solid phase extraction followed by derivatization and gas chromatography-mass spectrometry analysis was developed for the assessment of four endocrine disrupting compounds (EDCs) in water matrices: bisphenol A, estrone, 17β-estradiol and 17α-ethinylestradiol. The study was performed, simultaneously, by two different laboratories in order to evaluate the robustness of the method and to increase the quality control over its application in routine analysis. Validation was done according to the International Conference on Harmonisation recommendations and other international guidelines with specifications for the GC-MS methodology. Matrix-induced chromatographic response enhancement was avoided by using matrix-standard calibration solutions and heteroscedasticity has been overtaken by a weighted least squares linear regression model application. Consistent evaluation of key analytical parameters such as extraction efficiency, sensitivity, specificity, linearity, limits of detection and quantification, precision, accuracy and robustness was done in accordance with standards established for acceptance. Finally, the application of the optimized method in the assessment of the selected analytes in environmental samples suggested that it is an expedite methodology for routine analysis of EDC residues in water matrices.

Introduction

In the last few years, a wide variety of environmental pollutants, of either natural or anthropogenic origin, have been mentioned to have effects on the endocrine system of vertebrates and invertebrates. These compounds, named endocrine disrupting chemicals (EDCs), were defined as a group of exogenous substances that are able to interfere with hormone-controlled physiological processes, causing adverse health effects in an intact organism, its progeny, or (sub)populations.[1,2]

The increasing widespread of EDCs in the environment leads to numerous studies concerning pollutants detection and possible harmful consequences of human and wildlife exposure, especially during prenatal and early postnatal development when organ and neural systems are forming.[3,4]

Primary pollutants that are regulated nowadays represent only a tiny fraction of contaminants that occur in the environment. Some of them, now being targeted by researchers, may come out with a clean slate, while others will require additional scrutiny. The United States Geological Survey, together with the Environmental Protection Agency, has identified sex and steroidal hormones and industrial and household products as two of the four groups of contaminants for further study and regulation.[5]

Among these substances, the natural hormones estrone (E1) and 17β-estradiol (E2), excreted by humans and animals, and the synthetic hormone 17α-etinylestradiol (EE2), commonly used as contraceptive and in some hormonal therapies, are supposed to have an important contribution to the estrogenic activity of domestic and industrial effluents and adjacent surface waters.[6,7] Many reasonable suspicions have been raised concerning the

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environmental presence of these substances, as their high physiological activity and high stability may lead to their ubiquitous occurrence in the environment.

Bisphenol A (BPA) is another environmentally omnipresent EDC.[8] It has been used for decades as an industrial monomer in the production of polycarbonate (PC) plastics, epoxy resins, unsaturated polyester resins and polyacrylate and polysulphone resins.[9–11] The European Commission places BPA in the list of priority substances and has registered it in line with the EU legislation for the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), as its migration from plastic materials is recognized worldwide and it has been associated with increased incidence of cardiovascular disease, diabetes, reproductive cancers, fertility problems, liver enzyme abnormalities and other endocrine-related endpoints.[12–14] The European Food Safety Authority (EFSA) also launches full re-evaluation focusing on exposure and possible low dose effects.[15]

As the detection of EDCs in environmental matrices can have serious financial, human health and environmental consequences, official organizations and scientific groups have devoted themselves to the development of appropriate analytical methods for their assessment. However, low concentrations and complex matrices are two major difficulties associated with the detection and quantification of these compounds.

One of the most critical steps involved in the determination of these emerging contaminants in environmental waters, or in liquid food/beverage matrices regarding BPA analysis, is the samples pre-treatment, which must include an accurate procedure for extraction, isolation and concentration of the analyte. Currently, there are several "environmental friendly" extraction procedures available as for example: solid-phase extraction (SPE), solid-phase microextraction (SPME), dispersive liquid–liquid microextraction (DLLME) or stir bar sorptive extraction. Among them, the SPE continues to be one of the best methodologies as, although time consuming, it offers an interesting alternative to liquid–liquid extraction, providing a drastic reduction of the amounts of organic solvents used, and allowing very high enrichment factors. Therefore, SPE coupled with gas chromatography/mass spectrometry (GC/MS or GC/MS-MS) is commonly accepted as one of the most powerful techniques for organic pollutants analysis, even in the nanogram range.[16,17] In order to improve chromatographic resolution, derivatization is sometimes required for increasing volatility and thermal stability of the analytes.[18–20]

Several methods have been published in the last ten years regarding the EDCs analysis[21–26] but robust analytical procedures are still required.

The aim of the present work was the optimization and validation of a multi-residue method for detection and quantification of trace-levels of two distinct groups of EDCs in water matrices. Unequivocal analytical data require a specific set of validation criteria and method performance verification for ensuring international acceptation of analytical results and a common level of quality. So, the method was validated following mainly the International Conference on Harmonization (ICH)[27–29] but also according to other official documents based on ICH decisions, regulatory authorities and major international organizations like the European Union,[30,31] the Food and Drug Administration (FDA),[32] the Clinical and Laboratory Standards Institute (CLSI), formerly NCCLS,[33,34] the EURACHEM group,[35,36] the International Union of Pure and Applied Chemistry,[37] the International Organization for Standardization (ISO),[38] the National Health Surveillance Agency (ANVISA)[39] and the National Institute of Metrology, Standardization and Industrial Quality (INMETRO).[40]

Matrix effect may cause a withdrawal or increased efficiency of ionization that may lead to method's sensitivity alterations.[41,42] Matrix-standard calibration solutions were used to correct matrix-induced chromatographic response enhancement and derivatization was carried out to ensure the stability of the compounds during the analytical process. Verified heteroscedasticity has been overtaken by a weighted least squares linear regression model application (WLSLR)[31,41,43] that provides unbiased estimative for prediction, calibration and optimization when standard deviation of the data random errors is not constant across all levels of the explanatory variables.[43]

To ensure the reliability of the analytical method the following parameters were regarded as essential: specificity, linearity, limit of quantification (LOQ), limit of detection (LOD), range, precision, accuracy, extraction efficiency and stability of the method.

Interlaboratory procedures are very important for validation purposes in order to evaluate the robustness of the method and increase the quality control over its application in routine analysis, thus the study was performed in two different laboratories using different GC-MS equipments and chromatographic columns.

Experimental

Reagents and chemicals

Bisphenol A, d_{16}-bisphenol A (BPA-d_{16}), estrone (3-hydroxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[α]phenanthren-17-one), 17β-estradiol ((17β)-estra-1,3,5(10)-triene-3,17-diol), 17α-ethinylestradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol) and 2,2,2-trifluoro-*N*-methyl-*N*-(trimethylsilyl)acetamide (MSTFA) were supplied by Sigma-Aldrich (Steinheim, Germany). Methanol and ethyl acetate were of organic trace analysis grade (SupraSolv) and were supplied by Merck (Darmstadt, Germany). Acetic acid (glacial) 100% was from Carlo Erba (Rodano, Italy). Ultrapure water was highly purified by a Milli-Q gradient system (18.2 mΩ cm) from Millipore (Milford, MA, USA).

Standard solutions preparation

Individual stock standard solutions of the studied compounds were prepared in ethyl acetate by exact weighing of the high-purity substances and accurate dilution. A mixture was then prepared, in the same solvent, containing 1.5 mg L^{-1} of each compound. Stock standard solutions were stored in glass-stoppered flasks at 4 °C, in the dark.

One of the most reliable approaches is the use of matrix matched calibration standards, *i.e.*, standards with the same matrix composition as the samples to be analyzed.[31,44,45] Matrix-standard calibration solutions (residue-free matrix spiked with standards), at concentration levels ranging from 0.075 μg L^{-1} to 0.750 μg L^{-1} per compound, were prepared by spiking 1000 mL

of water with different volumes of the 1.5 mg L^{-1} mixture just before extraction. Water was added to 0.5% (v/v) methanol and pH adjusted to 4 with glacial acetic acid.

The use of a pre-treatment step, more or less complex, made essential a quality control internal standard, the so-called procedural or instrument internal standard, for assessing the effectiveness of the extraction technique and monitoring the recovery during sample analysis. Deuterated-BPA (BPA-d_{16}), at a concentration of 0.750 μg L^{-1}, was chosen for that purpose.

Optimization of the solid phase extraction procedure

SPE was conducted in a SPE vacuum manifold system from Phenomenex (Torrance, California, USA). Six different cartridges (LiChrolut® RP-18, LiChrolut® EN/RP-18, Strata™ X, Strata™ SDB-L, GracePure™ C18-Fast and GracePure™ C18-Low) were tested.

SPE conditions were as follows: (a) conditioning step, by the sequential addition of 7 mL of ethyl acetate, 7 mL of methanol and 7 mL of Milli-Q water at a flow rate of 1 mL min^{-1} (LiChrolut® RP-18 and LiChrolut® EN/RP-18 cartridges) or 7 mL of methanol and 7 mL of Milli-Q water at a flow rate of 1 mL min^{-1} (Strata™ X, Strata™ SDB-L, GracePure™ C18-Fast and GracePure™ C18-Low cartridges); (b) loading step, by passing the sample/standard through the cartridges at a flow rate of 5 mL min^{-1}; (c) washing step, by rinsing the cartridge with 5 mL water and dried by vacuum pressure for approximately 60 min; and (d) elution performed with 2 × 2.5 mL of methanol and 2 × 2.5 mL of ethyl acetate, at a flow rate of 1 mL min^{-1}, for all cartridges. After elution, the extracts were evaporated to dryness in a rotative evaporator (Buchi/Brinkman Rotavapor RE-111 and Water Bath B-461), reconstituted within ethyl acetate to a final volume of 500 μL and then derivatized.

Derivatization

MSTFA was used for derivatization and different protocols from the literature were tested. Derivatization conditions described by Quintana *et al.*[46] were selected due to better yield. Calibration matrix standards and water sample extracts were derivatized in 1.5 mL vials. 75 μL of the extracts were mixed with 150 μL of MSTFA. Vials were closed and placed in an oven at 85 °C for 30 min. The reaction time was reduced from original 100 min to 30 min without losing analytical signals enabling faster samples pre-treatment. After that, they were cooled down to room temperature and analyzed.

Instrumental conditions (GC-MS or GC-MS/MS)

The two laboratories involved were a governmental laboratory and a university one, located in Oporto. Each laboratory analyzed the same standards and samples under repeatability conditions and followed the same methodology.

Laboratory 1. Chromatographic analyses were carried out in a Shimadzu GCMS-QP2010 Gas Chromatograph Mass Spectrometer equipped with an auto injector AOC-5000. Injections (1 μL) were made in the splitless mode with a 1.0 min purge-off time and the injector temperature was set at 275 °C. Helium (99.9999%) at a constant flow rate of 1.5 mL min^{-1} was used as the carrier gas. Samples were analyzed using a fused-silica capillary column coated with 5% diphenylmethylsiloxane (30 m × 0.25 mm ID, 0.25 μm film thickness) from Teknokroma with the following oven program temperature: initial temperature 50 °C (held for 1 min), increased by 20 °C min^{-1} to 220 °C (held for 17 min), increased again by 20 °C min^{-1} to 250 °C and held at this temperature for 10 min. In this device the temperature of the GC-MS interface was maintained at 250 °C with an electron impact ionization of 70 eV. The transfer line was set at 275 °C and source at 200 °C. Positive fragment ions (*m*/*z*—ions mass–charge ratio) were analyzed over the 45–500 *m*/*z* mass range in full scan mode and in selected-ion monitoring (SIM) mode.

Instrument control and mass spectrometry data were managed by a personal computer with the LabSolutions GCMS software (2.50 SU3 version).

Laboratory 2. In Laboratory 2 a Trace GC Ultra device coupled to a MS from Thermo Polaris Q was used with a Zebron ZB-XLB (30 m × 0.25 mm ID × 0.25 μm film thickness) from Phenomenex. The carrier gas used was high purity helium (99.9999%), with a constant flow rate of 1.3 mL min. An AI-3000 automatic injector was used. The injections (1 μL) were made in splitless mode with a purge time of 0.5 min and the injector temperature was set at 270 °C. The program of the oven temperatures used was similar to the program of Laboratory 1 being necessary to keep the final temperature of 250 °C for 20 minutes. The ion trap mass spectrometer from Thermo Polaris Q and interface were maintained at 250 °C with an electron impact ionization of 70 eV. The positive ion fragments (*m*/*z*) were analyzed over the 50–650 *m*/*z* mass range in full scan mode and SIM mode.

The instrumental control and data from mass spectrometry were managed by a computer with GCMS Xcalibur software (version 1.3).

In the analysis by GC-MS/MS, ions from the first electron impact ionization (EI) of the target compounds were selected and fragmented a second time with collision-induced dissociation (CID) of helium gas in the ion trap, using a voltage collision excitation of 1.00 V. The resulting spectra of these fragments were scanned from the resulting ions with *m*/*z* belonging to the selected mass range. The selection of ions was organized according to different segments. The proposed fragmentation pattern of the target silylated EDCs is shown in Table 1.

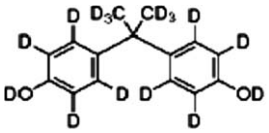
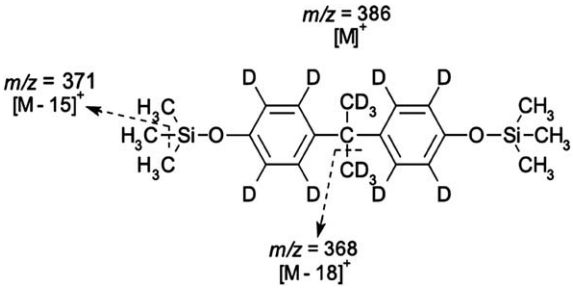
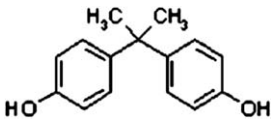
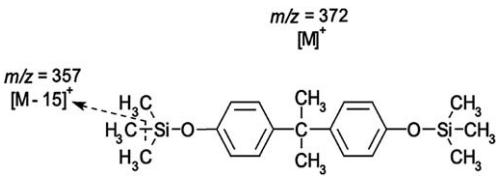
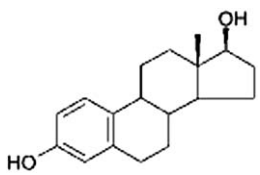
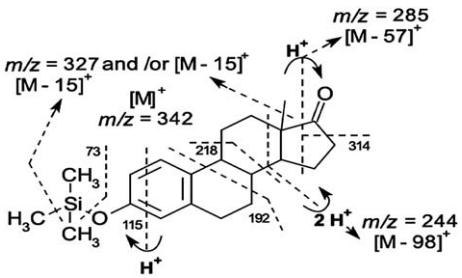
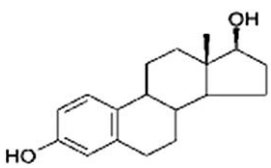
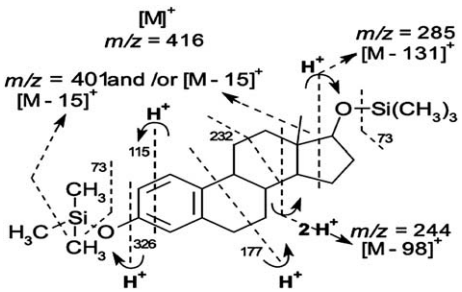
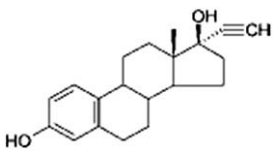
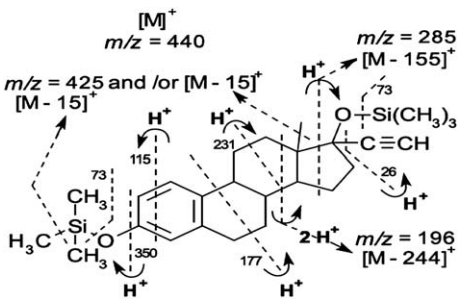
Validation of the analytical method parameters

Validation was done mainly according to the International Conference on Harmonization (ICH) recommendation[29] as well as some European and American validation guidelines.[31–33,35]

As the assumption of homoscedasticity was not met for analytical data, the weighted least squares linear regression procedure was applied as a simple and effective way to counteract the greater influence of the greater concentration on the fitted regression line, improving accuracy at the lower end of the calibration curve.[41,47]

In order to develop and optimize the SPE procedure followed by GC-MS for an effective and reproducible detection of low EDCs concentration, several parameters such as specificity and selectivity, linearity and linear range, limits of detection and

Table 1 Common names, chemical structure and proposed fragmentation pattern of the target silylated EDCs

Free compound	Derivatized compound
 Bisphenol A-d_{16} (BPA-d_{16})	 Di-trimethylsilyl (TMS) BPA-d_{14}
 Bisphenol A (BPA)	 Di-TMS BPA
 Estrone (E1)	 Mono-TMS E1
 17β-Estradiol (E2)	 Di-TMS E2
 17α-Ethinylestradiol (EE2)	 Di-TMS EE2

quantification, precision, accuracy, trueness (recovery), stability and robustness were determined and accessed.

Real samples analysis

The optimized methodology was applied for environmental water samples analysis, namely superficial water, groundwater and wastewater. Samples were also spiked with the tested compounds at levels near LOQ to perform recovery tests.

The selection of sampling sites was primarily focused on areas considered susceptible to be contaminated by human, industrial or agricultural wastewaters. Samples from four rivers located in the north of Portugal were collected near the estuary. Samples were collected in precleaned amber glass bottles (1 L), previously rinsed several times with the river water, acidified with glacial acetic acid (1%, v/v) immediately after collection and stored at 4 °C. Samples were processed in 24 h.

A groundwater sample and an urban wastewater sample were also collected under the same conditions.

Results and discussion

GC-MS method optimization

Silylated individual standards were first injected in the full-scan mode. Technical parameters such as injection conditions, flow and temperature gradients were optimized for a better resolution of the chromatographic peaks (Fig. 1).

The analytes were identified by both their chromatographic characteristics, as the retention time (RT), and through their specific fragmentation. Ion transitions and specific intensity ratios of the product ions were compared with library standards included at NIST, Wiley or PEST with an acceptance criterion of a match above a critical factor of 80%. A private library of our standards mass spectra was then created.

Subsequently, programs were developed in the SIM mode, based on the detection of selected ions for each analyte (Table 2), with a significant increase in sensitivity, elimination of interfering compound signals and lower limits of detection. A multi-residue method was chosen as it allows the qualitative and quantitative monitoring of several analytes simultaneously.

Matrix-induced chromatographic response and extraction efficiency

Different SPE cartridges were tested and the SPE procedure was optimized using ultrapure water spiked with the compounds under study. Matrix-induced effects during GC-MS determination were evaluated by matrix-standard solution recoveries, calculated considering the areas obtained by direct injection of standards solution, at the same concentration, as 100% (Fig. 2). Strata™ SDB-L cartridges presented good performance in the extraction procedure with greater retention capacity and were chosen due to their efficiency–cost ratio.

To counterbalance the observed matrix effects, method validation was performed using standards prepared under the same experimental conditions applied for the samples, *i.e.*, matrix-matched standards.[31,44,48]

Method validation

To ensure that the optimized procedure was suitable for application in routine analysis, the basic analytical performance parameters such as specificity and selectivity, linearity and linear range, limits of detection and quantification, precision, accuracy,

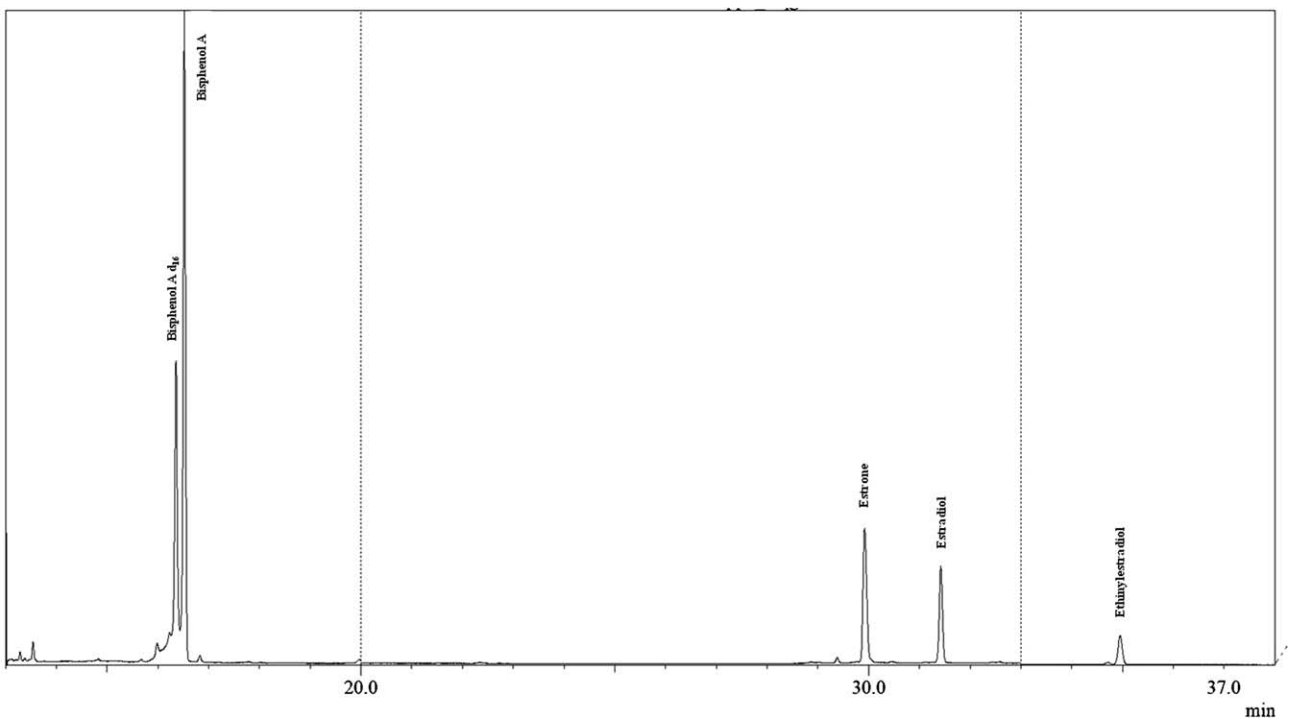


Fig. 1 Chromatogram of derivatized BPA-d16, BPA, estrone, estradiol and ethinylestradiol (extract concentration 150 μg L^{-1}).

Table 2 Quantitation and identification ions for the GC-MS and GC-MS/MS analyses of selected derivatized EDCs[a]

	Laboratory 1	Laboratory 1 and Laboratory 2		Laboratory 2		
Derivatized EDCs	RT (min)	Quantitation ions (m/z)	Identification ions (m/z)	RT (min)	Precursor ions $(m/z)_{MS/MS}$	2nd Fragmentation ions $(m/z)_{MS/MS}$
BPA-d_{16}	14.32	386	369, 368, 217	14.22	368	197 > 260
BPA	14.48	372	357, 217	14.46	357	191 > 175
E1	30.19	342	285, 257, 244	28.07	342	257 > 314, 244
E2	30.36	416	298, 285, 244	28.94	285	285 > 270, 256, 229, 205
EE2	33.70	440	425, 368, 285	31.89	425	193 > 257, 242

[a] RT –retention time.

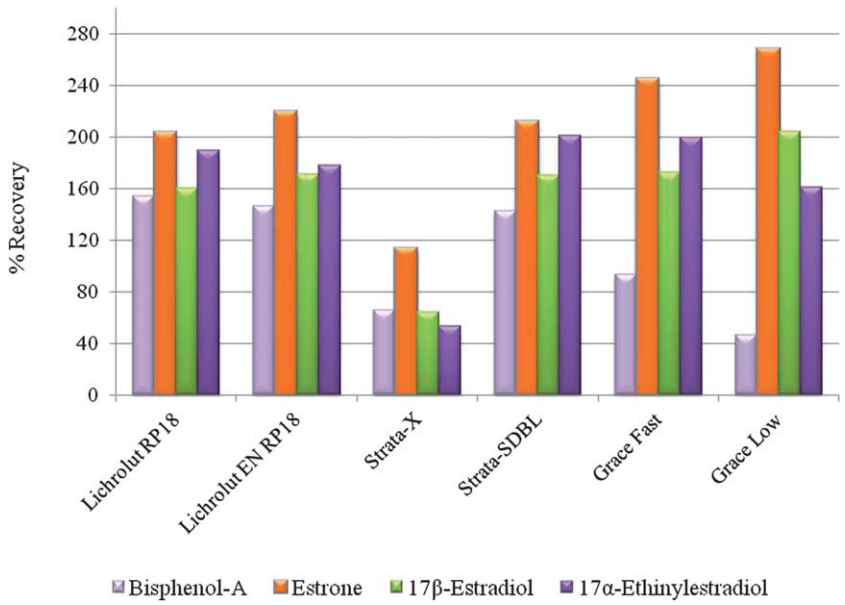


Fig. 2 Matrix-standard solution recoveries (extracts concentration of 500 μg L^{-1}) calculated considering the areas obtained by direct injection of 500 μg L^{-1} standards solution as 100%.

trueness (recovery), stability, robustness as well as measurement uncertainties were determined and assessed.

Selectivity/specificity. Specificity and selectivity were evaluated by comparing the chromatograms of matrix-blank samples (different samples of ultrapure water and glass bottled mineral water) with an aqueous solution of the analytes at concentrations near the limits of quantification.[29,31,32,49] No significant interferences have been detected at the RT of the estrogens. Regarding BPA, it was observed a small chromatographic peak even in the glass bottled mineral water that was used as blank during samples analysis, being subtracted from samples BPA concentration. This fact was probably due to the equipment used in sample preparation, namely the SPE plastic cartridges.

Selectivity was also assessed by comparison of the analytes mass spectra with spectra from libraries, which gave the evidence that the proposed method has a selectivity/specificity in accordance with the standards set forth by the validation authorities.

Linearity and detection and quantification limits. Six matrix-calibration standards (0.075, 0.150, 0.375, 0.450, 0.525 and 0.750 μg L^{-1} of each analyte) were prepared and injected in triplicate.

Calibration graphs showed good linear responses for the concentration range of all target compounds with correlation coefficients (r_w) higher than 0.997. Regression parameters obtained after application of the weighting factors were calculated and are presented in Table 3. Calibration in the SIM mode was therefore performed using external standardization.

Good sensitivity (LOD), ranging from 2.49 to 11.79 ng L^{-1} in Laboratory 1 and 3.15 to 11.64 ng L^{-1} in Laboratory 2, was found for the 4 compounds under the optimized experimental conditions. Method limits of quantification (LOQ) were also in the ng L^{-1} range, with values below 39.31 ng L^{-1} for Laboratory 1 and 38.80 ng L^{-1} for Laboratory 2, and were thus considered suitable for the detection and quantification of the target compounds. The analysis of the calibration results revealed that a better sensitivity was achieved in Laboratory 1.

Precision and accuracy. Precision and accuracy were determined according to the ICH[29] and FDA recommendations.[32] For repeatability or intraday precision there were performed 9 determinations in the same day (3 concentrations/3 replicates each).[28] For intermediate precision or inter-assay precision there were performed 9 determinations in 3 independent days.

Table 3 Method validation data. Calibration parameters and related uncertainties[a]

Derivatized EDCs	$b_w \pm t_{(n-2)} \cdot S_{(b)w}$ (95%)	$a_w \pm t_{(n-2)} \cdot S_{(a)w}$ (95%)	$S_{(y/x)w}$	$S_{(b)w}/b_w$ %	r_w	LOD (ng L^{-1})	LOQ (ng L^{-1})	U_{50ppb} (%)	U_{500ppb} (%)
Laboratory 1									
BPA	1500.41 ± 19.41	−803.23 ± 5808.37	2588.30	0.47	0.9998	7.76	25.88	3.97	1.13
E1	557.09 ± 13.59	−949.14 ± 4067.05	1812.33	0.88	0.9994	4.88	16.27	7.13	1.63
E2	207.72 ± 2.58	−641.93 ± 773.86	344.84	0.45	0.9997	2.49	8.30	3.60	1.07
EE2	36.97 ± 0.83	−32.63 ± 248.53	290.70	0.81	0.9959	11.79	39.31	18.39	4.39
Laboratory 2									
BPA	7468.76 ± 205.59	45 971.22 ± 61 533.68	17 755.29	0.64	0.9993	10.70	35.66	5.83	1.47
E1	1724.45 ± 77.35	1967.91 ± 23 150.48	10 316.18	1.62	0.9975	8.97	29.91	13.30	3.10
E2	1314.92 ± 76.50	43 722.98 ± 22 896.89	10 203.19	2.10	0.9982	11.64	38.80	17.35	2.54
EE2	615.89 ± 3.70	−664.64 ± 1106.07	1293.74	0.22	0.9997	3.15	10.50	4.80	1.60

[a] b_w – weighted slope; a_w – weighted intercept; r_w – weighted correlation coefficient; $S_{(b)w}$ and $S_{(a)w}$ – standard deviations of the weighted slope and weighted intercept; $S_{(y/x)w}$ – standard deviation of y-residuals of weighted regression line; LOD – limit of detection of the method; LOQ – limit of quantification of the method; *U* – uncertainties associated with calibration.

Washington Conference, as well as FDA and ANVISA, required precision to be within 15% (RSD) except at the LOQ, which can assume a value of ±20%.[32,39,42] Accuracy was expressed as absolute bias[34,40] and was inferior to ±15% of the accepted true value.[32] The results are shown in Table 4.

To evaluate the instrumental precision, four replicates of a standard at 0.450 μg L^{-1} were analyzed under optimal experimental conditions, presenting an acceptable value regarding the criteria of acceptance of 15% for this assay.[32]

Stability. The stability of the calibration standard solutions prepared in ethyl acetate and stored at 4 °C was evaluated by their periodic analysis for more than 6 months, with an averaged CV ≤ 15%. However, after derivatization, it was found that the compounds retained their stability at room temperature for a very short period of time (24 h). So, when it was not possible to inject the extracts immediately after preparation, the derivatized standard solutions and sample extracts were stored at −18 °C.

Recovery. Deuterated bisphenol A (BPA-d_{16}) was used as a quality control internal standard (IS) for process evaluation through recovery studies, being added in a constant amount to blanks, samples and calibration standards prior to extraction.

Control charters of media and amplitude of samples fortified with the IS were used to verify deviations, which was important for evaluating the efficiency of extraction and provides the indication of tendency and dispersion. The tendency of the data was expressed by the sample standard deviation (*s*) and the dispersion was expressed by amplitude. There was no relevant tendency in the BPA-d_{16} area of peaks, so, it was not necessary for any review.

Robustness. The robustness of the method evaluates the sensitivity to variations, *i.e.*, determines its ability to produce accurate results despite experimental variations that may occur during its implementation. The interlaboratory study was performed in order to assess robustness. Concerning SPE, different lots of SPE cartridges (S214-38/57770-2-24 and S214-39/66655-1-21) and solvents were tested during the validation procedure as well as different derivatization reagents (bottles of 5 mL: lots 000 1441282 and 000 1436553 or ampoules 10 × 1 mL: BCBB9448) and no differences were found regarding the obtained results.

As temperatures and flow-rates were kept constant, robustness was also assessed by testing two different equipments, a quadrupole and an ion trap, with different columns, without any observed discrepancy in chromatographic data.

Environmental water samples analysis

Different matrices of water samples, namely superficial water, groundwater and waste water, were directly analyzed and also

Table 4 Inter-day, intraday and instrumental precision (% CV) and accuracy (% Bias) of the method

	Inter-day						Intraday						Instrumental precision (% CV)
Derivatized EDCs	Precision (% CV)			Accuracy (% Bias)			Precision (% CV)			Accuracy (% Bias)			
Nominal conc./ng L^{-1}	75	450	750	75	450	750	75	450	750	75	450	750	450
Laboratory 1													
BPA	19.07	14.90	10.67	−1.13	1.42	−0.75	12.74	1.39	4.88	12.93	12.92	7.28	9.12
E1	19.69	13.04	11.04	13.71	9.94	5.50	11.53	6.16	9.63	−5.39	14.47	15.43	7.83
E2	8.78	10.66	8.20	1.68	3.10	−11.50	4.36	2.35	2.39	13.83	8.87	3.33	11.10
EE2	19.63	14.97	15.95	6.31	6.20	−0.92	14.45	1.22	2.45	9.27	−3.23	7.69	10.09
Laboratory 2													
BPA	18.58	13.09	8.08	13.94	−3.51	8.21	8.25	4.20	5.57	6.80	5.94	−8.78	4.20
E1	17.13	13.74	7.88	1.93	14.61	1.48	4.49	5.04	12.16	−4.62	4.06	−6.49	5.04
E2	19.37	11.38	12.88	10.58	7.85	9.40	9.12	12.70	13.77	−6.75	8.16	6.67	6.38
EE2	13.66	17.73	14.25	13.84	12.98	10.02	12.00	5.48	9.40	−13.57	2.20	−15.13	9.52

Table 5 Environmental samples analysis. Results of BPA, E1, E2 and EE2 obtained in Laboratory 1 and in Laboratory 2

Derivatized EDCs	BPA (ng L^{-1})		E1 (ng L^{-1})		E2 (ng L^{-1})		EE2 (ng L^{-1})	
Samples	Lab 1	Lab 2	Lab 1	Lab 2	Lab 1	Lab 2	Lab 1	Lab 2
River 1	29.8	<LOQ	<LOD	nd	<LOQ	nd	<LOD	nd
River 2	<LOQ	<LOQ	<LOD	<LOD	<LOQ	nd	<LOD	nd
River 3	41.0	49.1	<LOQ	nd	<LOQ	nd	<LOD	<LOQ
River 4	46.2	35.5	<LOD	nd	<LOQ	nd	<LOD	nd
Groundwater	<LOQ	nd	nd	nd	<LOQ	nd	nd	nd
Wastewater	1144.8	792.5	<LOQ	<LOD	nd	nd	<LOD	nd

spiked with the tested compounds at levels near LOQ. Results suggested an excellent applicability of the developed methodology, with recoveries between 80 and 120%.

The identification of the analytes in the samples was done according to the following conditions: (1) the relative or the absolute retention time of the sample component matched that of the authentic compound within a limit deviation of ±0.02 min in the chromatogram of the latest calibration standard, measured under identical conditions; (2) the selected diagnostic ions were present at the substance specific retention time; and (3) the relative intensities of two of the chosen diagnostic ions measured in the sample do not deviate by more than $I_2 \pm (0.1\ I_2 + 10)\%$ from the relative intensities determined in the reference standard working solution (I_2 is the relative intensity of the diagnostic ion in the reference standard working solution). The maximum allowed deviation for I_2 in the sample shall lie between 12.5% and 37.5%.[50] Finally, the comparison with comprehensive mass-spectral libraries allowed an unequivocal identification of target compounds.

It is shown that BPA was detected in all river samples ranging between <LOQ and 49.1 ng L^{-1} and in the wastewater. The estrogens were detected in some samples but behind the LOQ. The results of the two laboratories were considered to be consistent. Data are displayed in Table 5.

Conclusions

The results showed that the developed methodology SPE/GC-MS could be established as a suitable protocol for the simultaneous screening of ultra-trace levels of the selected EDCs in water matrices, which is very important for future national and international regulation actions.

The two laboratories satisfied the requirements for the analysis of these EDCs. Both laboratories have shown identical precision and accuracy, although in Laboratory 1 LODs were lower for three compounds.

To avoid and minimize any ambiguity related to the matrix-effect in chromatographic response, calibration curves for all quantification purposes were generated from matrix-matched standards. Instrumental conditions in the SIM mode showed excellent linear responses for the studied analytes.

As the assumption of homoscedasticity was not satisfied, leading to improper estimation and inference in the statistical quantification model, a weighted least squares calibration procedure was applied revealing useful improvements in accuracy, particularly at the lower end of the range where percentage bias was considerably greater than the acceptable limits of ±20% when simple least squares regression was used. Although weighted least squares regression is more complex and laborious than ordinary linear regression, involving the use of additional statistical tests and mathematical operations, it should be performed in order to reduce uncertainties and to obtain lower limits of quantification.

Good reproducibility and high sensitivity were achieved and the method was considered efficient, precise and accurate for all analytes in accordance with the suggested standards of acceptance, providing a good possibility of simultaneous screening for hazardous compounds in cases of suspected water poisoning. The fact of being developed simultaneously in two different laboratories, under different conditions, also confirms its robustness and its interlaboratory quality performance.

The applicability of the developed methodology to environmental samples analysis revealed a widespread contamination of the water samples, especially by BPA, which suggests the necessity of complementing these preliminary results with a more complete evaluation of the water quality condition.

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