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Determination of tetrabromobisphenol-A, tetrachlorobisphenol-A and bisphenol-A in soil by ultrasonic assisted extraction and gas chromatography–mass spectrometry

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ABSTRACT

In this work, an isotope dilution method for the determination, in agricultural and industrial soil samples, of tetrabromobisphenol-A, tetrachlorobisphenol-A and bisphenol-A by gas chromatography–mass spectrometry was developed. The compounds were extracted from soil by sonication assisted extraction in small columns (SAESC) with a low volume of ethyl acetate as extraction solvent. For dirty soil samples, such as industrial soils, a simultaneous clean-up on an acidified Florisil–anhydrous sodium sulfate mixture was carried out to remove interferences. After extraction, solvent was evaporated and analytes were derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and determined by isotope dilution gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MS–SIM), using $^{13}C_{12}$ labeled compounds as internal standards. Recoveries from spiked samples were between 88% and 108% and the estimated limits of detection (S/N = 3) varied from 30 pg g^{-1} to 90 pg g^{-1}. The response obtained with this method was linear over the range assayed, 5–300 ng ml^{-1}, with correlation coefficients equal or higher than 0.999. The validated method was used to investigate the levels of these phenolic compounds in soil samples collected from different locations in Spain. Bisphenol-A was detected in all samples at concentrations from 0.7 ng g^{-1} to 4.6 ng g^{-1} in agricultural soils and from 1.1 ng g^{-1} to 44.5 ng g^{-1} in industrial soils. Tetrabromobisphenol-A was found in various soil samples at levels in the range of 3.4–32.2 ng g^{-1} in industrial soils and at 0.3 ng g^{-1} in one agricultural soil, whereas tetrachlorobisphenol-A was not detected.

1. Introduction

Tetrabromobisphenol-A (TBBPA) and tetrachlorobisphenol-A (TCBPA) are probably the most consumed organic flame retardants worldwide [1–3] and, as a consequence, they are widely distributed in the environment. It has been reported that TBBPA may degrade to bisphenol-A (BPA) during anaerobic soil processes [4] and, therefore, the BPA potential risks to sediment and soil have been assessed for additive flame retardant uses [5]. Moreover, BPA is a major industry product widely used in the production of resins and polycarbonate plastics [6]. Although the acute oral toxicity of BPA for laboratory animals is low, this compound has a high potential as endocrine disruptor in human and wildlife [7]. The chemical structures of these compounds are depicted in Fig. 1.

BPA, TBBPA and TCBPA have been mainly detected in sewage sludge [8–12], biological matrices [13,14], air [15] and water [16,17], but very few data are available for soils [18], where these compounds may be found as a consequence of point source contamination, atmospheric deposition and agricultural practices.

The determination of these compounds in environmental samples has been carried out by liquid chromatography with tandem mass spectrometry (LC-MS/MS) [8,19–21] and gas chromatography–mass spectrometry (GC–MS) [15,17,22–24]. Due to the high polarity of the studied compounds, poor chromatographic peaks were obtained and derivatization was necessary when they were determined by gas chromatography. The derivatization reagents most frequently used are N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and N-(ter-butyldimethylsilyl)-N-methyl-trifluoro acetamide (MTBSTFA), which lead to the formation of trimethylsilyl (TMS) and tributylsilyl derivatives, respectively.

Several traditional extraction methods have been reported for the determination of some halogenated phenolic compounds, used as flame retardants, in sediments and sludge samples, including Soxhlet extraction [19] and mechanical shaking extraction [8]. More recently, some alternative techniques have been developed, such as solid-phase extraction (SPE) [17] and solid-phase microextraction (SPME) [22] for water analysis, and matrix solid-phase dispersion

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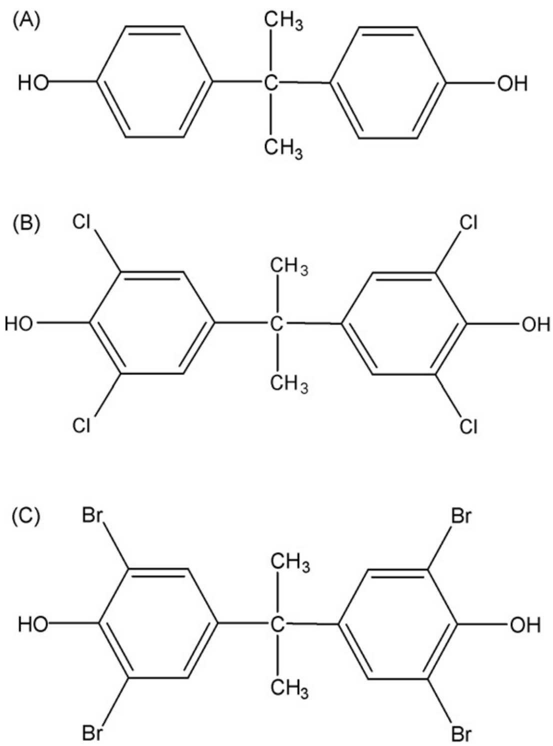

Fig. 1. Chemical structures of phenolic compounds (A) BPA, (B) TCBPA and (C) TBBPA.

(MSPD) [9] and pressurized liquid extraction (PLE) [25] for solid samples. These alternative procedures have shown clear advantages in terms of time and solvent consumption, although some of them require the use of expensive instrumentation.

The analytical methods used for the determination of some flame retardants in environmental samples, particularly for TBBPA, have been reviewed recently [26,27], however, no method has been found for the simultaneous extraction and determination of BPA, TBBPA and TCBPA in soil samples.

Sonication assisted extraction in small columns (SAESC) is a sample preparation method developed in our laboratory for the extraction of various organic contaminants from soil samples [28,29], which has a number of advantages like a low solvent consumption and a short processing time.

The main objective of this study is to develop a fast, selective and sensitive method for the analysis of BPA, TBBPA and TCBPA in soil by ultrasonic assisted extraction in small columns, using a low volume of organic solvent, and their subsequent determination by isotope dilution gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MS-SIM). The linearity, precision and detection limits will be evaluated in order to asses the performance of the proposed method, which will be applied to the analysis of the mentioned compounds in several agricultural and industrial soils collected from different areas of Spain. To our knowledge, this is the first reported method for the simultaneous determination of BPA, TBBPA and TCBPA in soil.

2. Experimental

2.1. Reagents and standards

Ethyl acetate, methanol, acetonitrile and n-hexane, residue analysis grade, were purchased from Scharlab (Barcelona, Spain). Florisil, a magnesium silicate adsorbent, 150–250 μm (60–100 mesh) for chromatography and anhydrous sodium sulfate were obtained from Aldrich (Steinheim, Germany), heated for 24 h at 180 °C and then allowed to cool down in a desiccator before use. Bondesil-C_{18} and Bondesil-PSA, particle diameter of 40 μm, were purchased from Scharlab (Barcelona, Spain). Hydrochloric acid (37%), analytical grade reagent, was purchased from Panreac (Barcelona, Spain). A Milli-Q water purification system from Millipore (Bedford, MA, USA) was used to provide ultrapure water in this study.

A mixture of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (99:1, v/v) purchased from Aldrich (Steinheim, Germany) was used as silylation reagent.

BPA, TCBPA, TBBPA and the $^{13}C_{12}$-internal standards (purity >99%, 50 μg ml^{-1} in methanol) were provided by Cambridge Isotope Laboratories (Andover, MA, USA).

Separate stock solutions of individual compounds were made up at 5 μg ml^{-1} level in ethyl acetate. From these solutions, a mixture of working standards containing 150 ng ml^{-1} was prepared weekly by dilution of the stock solution in ethyl acetate, and used to spike samples. Standard solutions of $^{13}C_{12}$-internal standards were prepared by diluting an appropriate amount of each substance in ethyl acetate. All the standard solutions were stored at −4 °C prior to use.

A series of six calibration solutions of the standards were prepared in a concentration range of 5–300 ng ml^{-1}, containing 50 ng ml^{-1} of the $^{13}C_{12}$-internal standards (half concentrations were used for BPA due to its higher response). Standard solutions were derivatized with BSTFA before GC injection.

2.2. Apparatus

GC–MS analysis was performed with an Agilent 6890 (Waldbronn, Germany) gas chromatograph equipped with an automatic injector, Model HP 7683, and a mass spectrometric detector (MSD), Model HP 5973N, equipped with an inert ion source. A fused silica capillary column ZB-5MS, 5% phenyl polysiloxane as nonpolar stationary phase (30 m × 0.25 mm i.d. and 0.25 μm film thickness), from Phenomenex (Torrance, CA), was used. Operating conditions were as follows: injector port temperature 280 °C; helium (purity 99.995%) as carrier gas at a flow-rate of 1.0 ml min^{-1} and pulsed splitless mode (pulsed pressure 45 psi = 310 kPa for 1.5 min) with the splitless injector purge valve activated 1.5 min after sample injection, in a double-taper glass liner with a nominal volume of 800 μl. The column temperature was maintained at 75 °C for 1 min,

Table 1
Retention times, limits of detection and quantification (LOD, LOQ ng g^{-1}), selected ions and SIM program of the compounds studied.

Compound	t_R	m/z [a]	Group	Time	LOD	LOQ
1 BPA–TMS	9.838	357 (100), 359 (20), 372 (15)	1	5	0.03	0.12
2 $^{13}C_{12}$-BPA–TMS	9.840	369 (100), 371 (40), 384 (20)			–	–
3 TCBPA–TMS	12.051	493 (100), 495 (80), 508 (20)	2	10.3	0.05	0.14
4 $^{13}C_{12}$-TCBPA–TMS	12.053	505 (80), 507 (100), 520 (20)			–	–
5 TBBPA–TMS	13.326	673 (100), 675 (70), 688 (25)	3	12.8	0.09	1.1
6 $^{13}C_{12}$-TBBPA–TMS	13.328	685 (100), 687 (70), 700 (12)			–	–

[a] Target ions are underlined and their relative abundances in the mass spectra are in brackets.

then programmed at 20 °C min^{-1} to 310 °C and held for 5 min. The total analysis time was 17.75 min and the equilibration time 2 min.

The mass spectrometric detector (MSD) was operated in electron impact ionization mode with an ionizing energy of 70 eV, scanning from *m/z* 150 to 750 at 3.62 s per scan, an ion source temperature of 300 °C and a quadrupole temperature of 150 °C. The electron multiplier voltage (EM voltage) was maintained 100 V above autotune with a solvent delay of 5 min.

Table 1 lists the compounds studied and the internal standards along with their retention times, selected ions, limits of detection and quantification. The SIM program used to determine and confirm these phenolic compounds in soil has three acquisition windows with an ion dwell time of 100 ms and 1.69 cycles s^{-1}. The target and qualifier abundances were determined by injection of standards under the same chromatographic conditions using full-scan with the mass/charge ratio ranging from 150 to 750 *m/z*. The analytes were confirmed by their retention times, the identification of target and qualifier ions and the determination of qualifier to target ratios. Retention times must be within ±0.3 min of the expected time and qualifier-to-target ratios within a 20% range for positive confirmation.

The quantification of the studied compounds was based on their relative response factor to the internal labeled standards.

2.3. Samples

2.3.1. Sample collection

Soil used in the recovery assay was collected from the plough layer (0–10 cm) of an experimental plot located in the region of Madrid (Spain), sieved (2 mm) and stored at room temperature until fortified. The characteristics of the soil were: pH 7.69, total organic matter content 0.97%, sand 44.34%, silt 37.44% and clay 18.22%.

Surface soil (0–10 cm) was sampled from agricultural fields (horticultural and forested) located in different Spanish regions and from industrial soils in the area of Bilbao, an important industrial region. Soil samples were air dried, sieved (2 mm) and stored frozen (−18 °C) in glass containers.

2.3.2. Sample preparation

2.3.2.1. Extraction. The extraction method was adapted from a procedure developed in our laboratory for the analysis of pesticides in soil [28]. Two filter paper circles of 2 cm diameter were placed at the end of a glass column (20 ml) and anhydrous sodium sulfate (2 g) was added as a layer over the paper filter, then sieved soil (5 g ± 0.001) was weighed into a 10 ml weighing funnel and placed in the column. For recovery studies, soil samples were previously fortified with 0.5 ml of a mixture of BPA, TCBPA, TBBPA and the $^{13}C_{12}$-internal standards to reach final concentrations of 3 ng g^{-1}, 15 ng g^{-1}, 30 ng g^{-1} and 50 ng g^{-1} (half concentrations were used for BPA) and left at room temperature for 2 h to allow solvent evaporation.

Soil samples were extracted with 5 ml of ethyl acetate for 15 min in an ultrasonic water bath at room temperature. The water level in the bath was adjusted to equal the extraction solvent level inside the columns, which were supported upright in a tube rack and closed with 1-way stopcocks. After extraction, the columns were placed on a multiport vacuum manifold where the solvent was removed and collected in graduated tubes. Soil samples were extracted again with another 5 ml of ethyl acetate (15 min). The extracting solvent was removed and soil samples washed with 1 ml of additional solvent. The total extract collected in 10 ml graduated tubes was concentrated with a gentle stream of nitrogen to an appropriate volume, 1 ml, and 0.1 ml were transferred into a 2 ml reaction minivial for the derivatization step.

2.3.2.2. Clean-up. Florisil (2 g) was acidified with 0.5 ml of 37% aqueous hydrochloric acid, mixed thoroughly with anhydrous sodium sulfate (2 g) and left 20 min at room temperature. This mixture was transferred to a 20 ml glass column containing two filter paper circles at the end, before placing the sieved soil on the column (5 g ± 0.001). The extraction of the studied compounds (including the simultaneous clean-up step) was carried out as indicated above. After extraction, extracts were concentrated and an aliquot (0.1 ml) transferred to the reaction minivial for the derivatization step.

2.3.2.3. Derivatization. An aliquot (0.1 ml) of the standard or extract solution was transferred into a 2 ml reaction vial, followed by the addition of 50 μl of BSTFA containing 1% TMCS. The vials were closed and the mixture left react for 10 min at 60 °C. After the derivatization process, an aliquot (2 μl) of these solutions was injected in GC–MS.

2.4. Quality assurance/quality control

The quality assurance and quality control criteria used for this method with each set of samples included analyses of laboratory blanks (solvent blank), laboratory control samples and surrogate standard recoveries.

One laboratory blank was run with each set of samples to check for contamination from the preparative steps and to demonstrate laboratory background levels. Using the proposed procedure, background levels of laboratory blanks were below the detection limits.

Laboratory control samples (LCS) were used in the recovery assay and the concentrations of the studied compounds determined in blank samples were subtracted.

A 500 μl aliquot (equivalent to 15 ng of each analyte, half amount for BPA) was added to each LCS prior to analysis. The recoveries of the internal standards in these samples, as measured by the external standard method, ranged from 101.0% to 107.2% for $^{13}C_{12}$-BPA, from 99.8% to 104.2% for $^{13}C_{12}$-TCBPA, and from 100.8% to 105.0% for $^{13}C_{12}$-TBBPA.

3. Results and discussion

3.1. Sample preparation

Compared with traditional methods, such as Soxhlet extraction and mechanical shaking, the method proposed in this work, based on sonication assisted extraction in small columns (SAESC), has the advantage of using a low volume of solvent over a short period of time [28]. Several extraction solvents, such as methanol, ethyl acetate and mixtures of ethyl acetate–methanol (50:50, v/v), were initially assayed. Recoveries obtained from soil samples, at the 5 ng g^{-1} fortification level, with methanol or ethyl acetate-methanol mixtures were lower than 60%, whereas those achieved with ethyl acetate were higher than 80%. Therefore, ethyl acetate was the solvent selected for the extraction of the phenolic compounds studied.

Selective extraction of analytes from complex matrices, such as industrial soils, is recognized as a very complicated task because these matrices contain a large variety of contaminants that may difficult analysis and make necessary lengthy purification processes. For industrial soils, the SAESC method produced dark extracts and a clean-up to remove co-extracted materials was necessary. In the analysis of some phenolic compounds, the sorbents most commonly used for the clean-up of extracts from sewage sludge and sediment samples were Florisil, alumina and C_{18} [9,19] and Florisil and C_{18} provided the best results. Therefore, these SPE sorbents were initially tested, as well as PSA, due to the good results obtained for other organic compounds [30].

Table 2
Recoveries (%) obtained from industrial soil samples by using different sorbents[a].

Compound	Recovery (%) (mean ± SD)[b]				
	PSA	Florisil	C_{18}	Florisil-acidified soil	Acidified Florisil
BPA A	100.4 ± 2.3	102.5 ± 4.1	100.5 ± 1.4	20.5 ± 5.8	99.8 ± 4.0
TCBPA	99.8 ± 4.0	56.6 ± 2.5	93.8 ± 4.5	38.8 ± 6.1	98.7 ± 3.7
TBBPA	10.6 ± 3.8	20.2 ± 1.9	101.1 ± 6.1	28.7 ± 4.3	100.3 ± 5.2

[a] Soil samples were spiked at 30 ng g^{-1}.
[b] Results are the mean of four replicates.

Table 2 shows the results obtained for the studied adsorbents at the 5 ng g^{-1} fortification level. The best recoveries were obtained with $C_{18,}$ although industrial soils yielded dark yellow extracts. Florisil provided results similar to those of PSA regarding the cleanliness of extracts, although low recoveries were obtained for the halogenated compounds (Table 2). In order to improve the recovery (especially for TBBPA, the most polar compound) and to reduce the number of interferences, the influence of acid addition on the extraction was investigated.

Different amounts of HCl were added to the adsorbent or to the soil samples and their effect on the recovery was studied. When the acid addition was done to the soil, low recoveries and dirty extracts were obtained. Nevertheless, cleaner extracts were produced when the adsorbent, Florisil, was acidified, although the addition of 0.5 ml of 20% HCl provided low recoveries (<70%). The use of a more concentrated hydrochloric acid (37%) allowed to increase the extraction efficiency and a recovery near (100%) was obtained (Table 2). The experimental results showed that Florisil acidified with 0.5 ml of 37% HCl yielded good recoveries and clean extracts for the determination of BPA, TCBPA and TBBPA in soil samples and it is was the adsorbent selected. The analytical results achieved by this optimized procedure applied to industrial soil samples provided recoveries in agreement with other extraction methods for solid matrices, such as sediment and sludge samples [8,9,19]. Fig. 2 shows a chromatogram of an industrial soil extract with or without a clean-up using acidified Florisil.

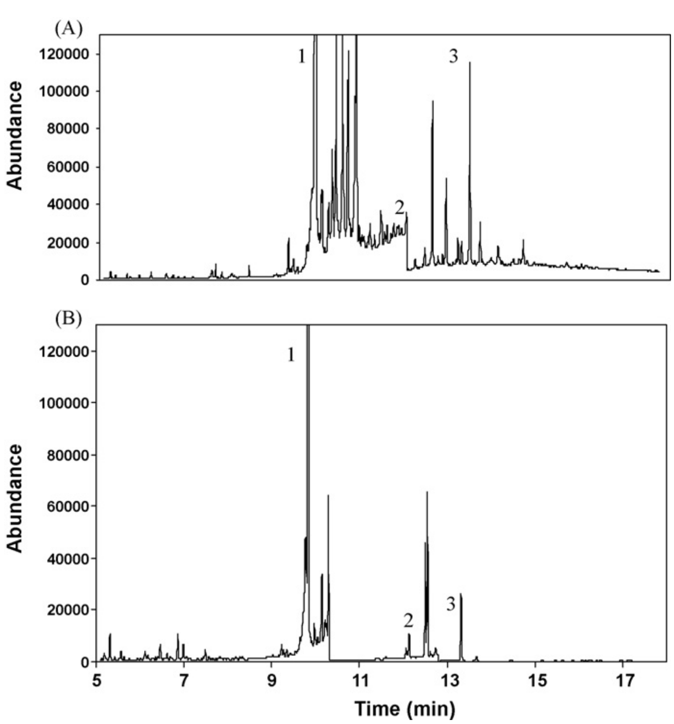

Fig. 2. GC–MS–SIM chromatograms of an industrial soil extract (A) without clean-up and (B) after a clean-up with acidified Florisil. See Table 1 for peak identification.

3.2. Derivatization and mass spectrometric determination

Compounds containing functional groups with active hydrogen atoms, such as phenols, are difficult to analyze by GC because of their insufficient volatility and thermal instability. Those compounds are generally derivatized prior to GC analysis to increase their volatility, reduce thermal degradation and increase detector response. TMS (trimethylsilyl) are the derivatives most frequently used for GC–MS analysis and (N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and (N-(tertbuthyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA) are the reagents most widely used to derivatize compounds bearing hydroxyl groups. Nevertheless, when MTBSTFA is used, the major ion of the TBBPA silylated derivative (804 amu) is beyond the mass range of bench-top MS (<700 amu), and, therefore, BSTFA was selected as derivatization reagent.

In order to check the increment in the signal-to-noise ratio, 1000 ng ml^{-1} and 100 ng ml^{-1} of each compound were injected before and after derivatization, respectively, and a signal increment of about 3–5 times was obtained when analytes were derivatized. In general, good results were obtained using BSTFA with a catalyst such as trimethylchlorosilane (TMCS) for the simultaneous derivatization of the three phenolic compounds studied. The influence of several derivatization parameters, such as the reaction time, temperature and volume of BSTFA on the silylation reaction was evaluated. The effect of temperature was studied at 50 °C, 60 °C and 70 °C, and several reaction times (10 min, 30 min and 60 min) and BSTFA volumes (50 μl and 100 μl) were also assayed. Long derivatization times (30–60 min) and high volumes (100 μl) did not improve the derivatization efficiency, and consequently 10 min and 50 μl of BSTFA were chosen for derivatization. Regarding temperature, the results obtained at 60 °C and 70 °C were similar, but a somewhat lower response, near 80%, was obtained at 50 °C. Therefore, the experimental conditions selected for the simultaneous derivatization of the three compounds were as follows: 50 μl of BSTFA + 1% TMCS added to a 100 μl sample and kept at 60 °C during 10 min. Relative standard deviations (RSD) in the range of 2–6% were obtained in these conditions for the compounds studied, which indicate the satisfactory reproducibility of the derivatization step.

The stability of the derivatized analytes was evaluated at different times after preparation, and they were found stable for at least one week when stored at 4 °C (RSD <5%).

Although a solvent effect on the silylation reaction has been reported [15], similar responses were found when acetonitrile, hexane or ethyl acetate was used. Therefore, ethyl acetate was chosen because it was the extraction solvent.

The studied compounds were determined, after their derivatization, by gas chromatography–mass spectrometry with selected ion monitoring (SIM) and electron impact ionization mode (EI). Fig. 3 represents a SIM chromatogram of a standard mixture solution of BPA–TMS, TCBPA–TMS and TBBPA–TMS with their $^{13}C_{12}$-labeled derivatives.

The molecular ions of these compounds are present in their spectra at m/z 372, 508 and 688, and their major fragments at m/z 357, 493 and 673 for BPA–TMS, TCBPA–TMS and TBBPA–TMS,

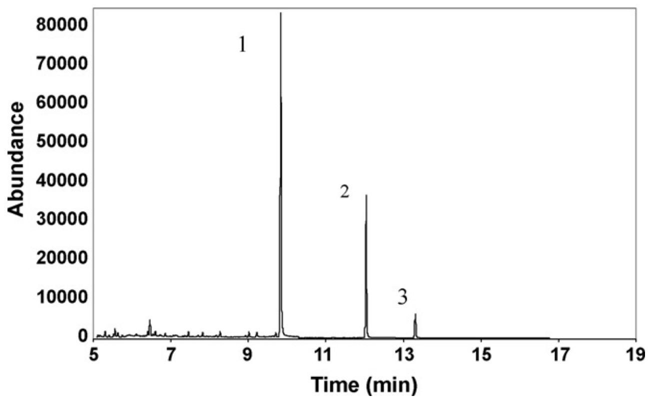

Fig. 3. A GC–MS–SIM chromatogram of a standard mixture solution of BPA–TMS, TCBPA–TMS and TBBPA–TMS (5 μg l^{-1}) and their $^{13}C_{12}$ labeled derivatives (50 μg l^{-1}, except for $^{13}C_{12}$-BPA which was 25 ng ml^{-1}). See Table 1 for group identification.

respectively, which are formed by losing a methyl group from their molecular ions. The protonated ions of their major fragments were also chosen, but the peaks formed by losing $C_6H_3Br_2OSiMe_3$ were not selected for the GC–MS–SIM method due to the interferences observed in the analysis of real samples.

The molecular ions of the $^{13}C_{12}$-labeled derivatives were present at m/z 384, 520 and 700 and their major fragments at m/z 369, 507 and 685 for BPA–TMS, TCBPA–TMS and TBBPA–TMS, respectively. Table 1 shows the main fragments selected for the SIM program.

3.3. Method validation

3.3.1. Assessment of matrix effect

Blank sample extracts, prepared according to the method described above, were fortified and used to compare the response of analytes in sample extracts with that of external standards prepared in pure solvents. In our case, the matrix effect was studied using fortified blank extracts in the range of 3 ng g^{-1} to 30 ng g^{-1} and a response enhancement of 21%, 15% and 22% for BPA, TCBPA and TBBPA, respectively, in agricultural soil samples and an increase of 45%, 20% and 30% in industrial soils was obtained.

The matrix effect occurring in the GC analysis of some organic compounds has a negative impact on the accuracy of the generated results. Therefore, the elimination of this effect is essential for the quantification of pollutants in complex environmental matrices at trace levels. A further sample clean-up is a possible way to minimize the matrix effect, but it increases the time and complexity of analysis. Other possibility is to employ fortified blank extracts as standards, although some regulatory agencies, like the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) in the USA, do not permit matrix-matched standardization for enforcement purposes.

An alternative method to counteract the matrix effect is to use isotope dilution mass spectrometry for the determination of analytes, which takes advantage of the fact that the chemical and physical properties of the isotope labeled analogues are nearly identical to those of non-labeled analytes. In the present work, the use of ^{13}C isotope labeled internal standards in fortified blanks extracts yielded responses similar to those of external standards in pure solvent, which indicates that the matrix effect was counteracted.

3.3.2. Recovery

Recovery through the method was tested by adding known amounts of BPA, TCBPA and TBBPA (3 ng g^{-1}, 15 ng g^{-1}, 30 ng g^{-1} and 50 ng g^{-1}, half concentrations were used for BPA because of its higher response in GC–MS) to four replicates of “blank” soil samples. These fortified samples were allowed to stand for 2 h to allow solvent evaporation before extraction and then they were analyzed

Table 3
Recoveries[a] of the studied phenolic compounds in soils.

Compound	Fortification levels (ng g^{-1}) ± SD							
	Agricultural soils				Industrial soils			
	50	30	15	3	50	30	15	3
BPA–TMS	100.5 ± 3.5	99.8 ± 4.2	107.7 ± 2.0	88.1 ± 2.1	99.3 ± 2.4	99.3 ± 3.6	102.8 ± 4.2	90.5 ± 4.1
TCBPA–TMS	102.1 ± 2.5	102.0 ± 1.6	100.2 ± 2.7	97.0 ± 2.5	98.9 ± 3.3	98.5 ± 2.2	99.3 ± 1.6	95.0 ± 3.5
TBBPA–TMS	99.7 ± 3.0	103.2 ± 1.1	100.8 ± 2.9	97.8 ± 3.6	99.3 ± 1.0	101.1 ± 2.6	100.7 ± 1.1	96.9 ± 2.9

[a] Results are the mean of four replicates ± standard deviation.

Table 4
Calibration data and repeatability[a] of the studied phenolic compounds.

Compound	Calibration data		Repeatability (RSD, %)[b]	
	Equation	r	Peak area	t_R
BPA–TMS	$y = 2.98x + 1.58 \times 10^{-3}$	1.000	9.6	0.02
TCBPA–TMS	$y = 1.07x - 2.79 \times 10^{-2}$	0.999	6.5	0.01
TBBPA–TMS	$y = 9.06 \times 10^{-1}x - 1.25 \times 10^{-4}$	1.000	4.0	0.01

[a] Repeatability of the chromatographic method.
[b] RSD of retention times and peak areas ($n = 10$).

Table 5
Concentration[a] of the studied phenolic compounds (ng g^{-1}) in soils collected in various areas of Spain.

Compounds	Agricultural Soils[b]					Industrial Soils[c]					
	1	2	3	4	5	1	2	3	4	5	6
BPA–TMS	4.6 ± 0. 32	3.0 ± 0.07	0.7 ± 0.06	0.8 ± 0.06	3.0 ± 0.22	10.2 ± 1.2	1.6 ± 0.12	0.2 ± 0.02	1.1 ± 0.3	44.5 ± 3.0	10.1 ± 1.5
TCBPA–TMS	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TBBPA–TMS	n.d.	0.3 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	32.2 ± 5.2	4.3 ± 0.5	3.4 ± 0.11

[a] Results are the mean of four replicates, expressed in ng g^{-1}; n.d.: <Method detection limit.
[b] Agricultural soils were collected in several horticultural and forest fields.
[c] Industrial soils were sampled in the area of Bilbao.

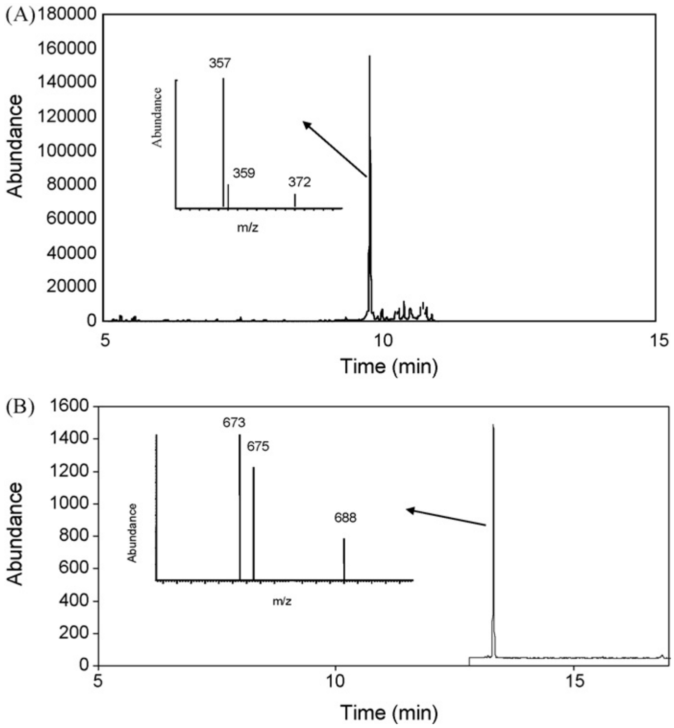

Fig. 4. An ion chromatogram of an agricultural soil extract containing (A) BPA ($3.0\,ng\,g^{-1}$) and (B) TBBPA ($0.3\,ng\,g^{-1}$) with the main ions of their mass spectra.

following the method described above. Unspiked "blank" samples were previously analyzed to determine the possible presence of these phenolic compounds and the levels found were subtracted. Recoveries of BPA, TCBPA and TBBPA ranged between 88.1% and 107.7% for agricultural soil samples and between 90.5% and 102.8% for industrial soil samples (Table 3), with standard deviations equal or lower than 4.2%, thus fulfilling the requirements of the IUPAC [31]. The range of recoveries achieved is similar to that obtained by other authors for the analysis of some of these phenolic compounds in sewage sludge and sediments [9,23,32].

3.3.3. Repeatability

The repeatability of the chromatographic determination was determined by injecting 10 times a standard solution of $50\,ng\,ml^{-1}$ with an automatic injector. The relative standard deviations (RSD) obtained for the retention times ranged from 0.01% to 0.02%, whereas for peak areas those values ranged from 4.0% to 9.6% (Table 4). Within-laboratory reproducibility of the chromatographic determination was evaluated at different days during three consecutive weeks and it was found to be lower than 13% for all of the compounds, expressed as RSD.

3.3.4. Linearity

Linearity was studied by performing a multipoint calibration curve with six standard solutions at different concentration levels, in the range of the content expected in soil samples. Each calibration level of the curve ($5\,\mu g\,l^{-1}$, $10\,\mu g\,l^{-1}$, $50\,\mu g\,l^{-1}$, $100\,\mu g\,l^{-1}$, $200\,\mu g\,l^{-1}$, $300\,\mu g\,l^{-1}$) was spiked prior to analysis with $50\,\mu g\,l^{-1}$ of $^{13}C_{12}$-internal standards. Due to its higher response, half concentrations were used for BPA.

The calibration data, correlation coefficients and regression equations of the calibration curves are listed in Table 4, which shows a good linearity of the results obtained with correlation coefficients equal or higher than 0.999.

3.3.5. Limits of detection (LODs) and quantification (LOQs)

The limits of detection (LODs) and quantification (LOQs), considered as the minimum amount of target analyte that produces a chromatogram peak with a signal-to-noise ratio (S/N) of three and ten times the background chromatographic noise, respectively, following IUPAC recommendations [31], were determined. The S/N was measured at the lowest spiked level in the validation study. Low

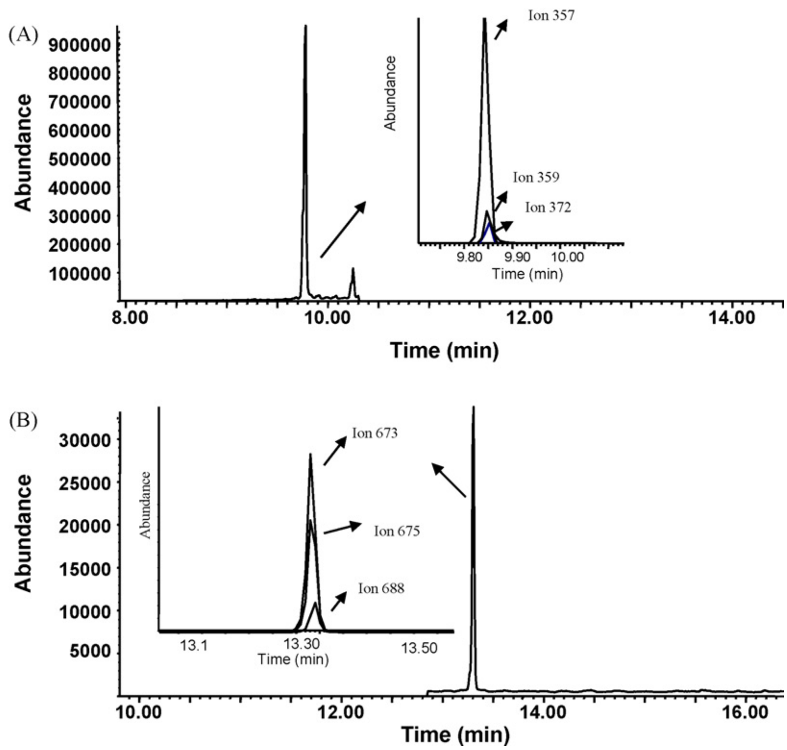

Fig. 5. An ion chromatogram of an industrial soil extract containing (A) BPA ($1.1\,ng\,g^{-1}$) and (B) TBBPA ($32.2\,ng\,g^{-1}$) with the main ions of their mass spectra.

limits were obtained due to the selectivity of the analytical procedure and the high sensitivity of the GC–MS–SIM technique, allowing the determination of these phenolic compounds at the trace levels found in soil samples. These limits are shown in Table 1 and range from 0.03 ng g^{-1} to 0.09 ng g^{-1} for LODs and from 0.12 ng g^{-1} to 1.1 ng g^{-1} for LOQs, being TBBPA the compound with the highest limits.

3.4. Application to real samples

The developed method was applied to the analysis of BPA, TCBPA and TBBPA in various types of agricultural and industrial soils collected in different areas of Spain. Table 5 shows the concentrations found, expressed as ng g^{-1} dry weight. In agricultural soils, all of the examined samples contained BPA, which was the main compound detected, with concentrations ranging from 0.7 ng g^{-1} to 4.6 ng g^{-1}. In contrast, TBBPA was detected in only one soil at a concentration of 0.3 ng g^{-1}, and TCBPA was not detected. This result is similar to the value reported (0.5 ng g^{-1}) for TBBPA in Chinese agricultural soils [18]. No information on concentrations of BPA and TCBPA in agricultural soils has been found.

A representative chromatogram of an agricultural soil sample containing BPA (3.0 ng g^{-1}) and TBBPA (0.3 ng g^{-1}) is depicted in Fig. 4.

Regarding industrial soils, all of the examined samples showed detectable amounts of BPA with concentrations ranging from 0.2 ng g^{-1} to 44.5 ng g^{-1}. These results are in same range of those reported for this compound in sediments and sewage sludge (0.6–50.3 ng g^{-1}) [19,33,34], although higher concentrations have been found in some soil samples collected from known point sources of contamination [27].

TBBPA was detected in three samples at concentrations ranging from 3.4 ng g^{-1} to 32.2 ng g^{-1} (Table 5). These values are lower than those reported for sewage sludge (28–220 ng g^{-1} [10,19,35] and for levels of TBBPA in industrial soils from China (1360–1780 ng g^{-1} [36].

A chromatogram of an industrial soil containing BPA (1.1 ng g^{-1}) and TBBPA (32.2 ng g^{-1}) is depicted in Fig. 5.

TCBPA is also used as a flame retardant, although to a lesser extent than TBBPA, and no published report on TCBPA in soils samples have been found. In our case, TCBPA was not detected in any of the samples.

4. Conclusions

The developed method allows the determination of the three studied phenolic compounds in agricultural and industrial soils by GC–MS after derivatization. Ultrasonic assisted extraction was used in the analysis of agricultural samples, whereas a simultaneous clean-up was necessary to remove co-extracted compounds in industrial soil samples. The analytical procedure showed good recoveries and sensitivity for the determination of these compounds in soil. Accurate quantification was achieved by isotope dilution analysis, using stable isotopes of these phenolic compounds as internal standards. To our knowledge, this is the first work reporting the simultaneous determination of BPA, TCBPA and TBBPA in soil by GC–MS.

This method was applied to the analysis of BPA, TCBPA and TBBPA in soil samples collected from various areas of Spain, and BPA was detected in the range from 0.2 ng g^{-1} to 4.7 ng g^{-1}in agricultural soils and from 1.1 ng g^{-1} to 44.5 ng g^{-1}in industrial soils, along with TBBPA in some soils at a similar range of concentrations. The presence of these compounds in sewage sludge has been previously reported, but very scarce data are available for their occurrence in soils.

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