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Application of Dispersive Liquid–Liquid Microextraction Followed by High-Performance Liquid Chromatography/Tandem Mass Spectrometry Analysis to Determine Tetrabromobisphenol A in Complex Matrices

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Abstract: An accurate and sensitive ultrasound-dispersive liquid–liquid microextraction technique followed by high-performance liquid chromatography separation coupled with electrospray ionization tandem mass spectrometry detection method to determine the presence of tetrabromobisphenol A (TBBPA) in complex environmental matrices is proposed. The miniaturized procedure was used to extract and quantify the analyte in domestic sewage, anaerobic sludge, and the aquatic test organism species *Daphnia magna* and *Chironomus sancticaroli*, which are standardized organisms for ecotoxicity bioassays. Limits of detection of 2 ng L^{-1} (domestic sewage), 2 ng g^{-1} (anaerobic sludge), 0.25 ng g^{-1} (*D. magna*), and 5 ng g^{-1} (*C. tentans*) were obtained. The presence of TBBPA was determined in domestic sewage and anaerobic sludge from an anaerobic batch bioreactor at a concentration of 0.2 ± 0.03 μg L^{-1} and 507 ± 79 ng g^{-1}, respectively. In *D. magna* and *C. sancticaroli* exposed to TBBPA in an acute toxicity bioassay, the micropollutant accumulated at 3.74 and 8.87 μg g^{-1}, respectively. The proposed method is a simple and cost-effective tool to determine TBBPA environmental occurrence and biomagnification potential compared with conventional extraction methods. To the best of our knowledge, this is the first liquid–liquid miniaturized extraction method to be applied to *D. magna* and *C. sancticaroli*. *Environ Toxicol Chem* 2020;39: 2147–2157. © 2020 SETAC

Keywords: Domestic sewage; Anaerobic sludge; *Daphnia magna*; *Chironomus sancticaroli*; Bioaccumulation; Ultrasound-dispersive liquid–liquid microextraction/high-performance liquid chromatography/electrospray ionization tandem mass spectrometry detection

INTRODUCTION

Tetrabromobisphenol A (TBBPA) is the most common flame retardant applied to electric and electronic equipment, epoxy resins, and plastic products to meet fire safety requirements. This organic compound is an environment micropollutant and has been detected in air, dust, sediment, biota, and water. In water bodies, its concentrations range from undetectable to 4870 ng L^{-1} (Yang et al. 2012; Ni and Zeng, 2013; Wang et al. 2014; Zhou et al. 2014; Qu et al. 2016; Daso et al. 2017; Rothenbacher and Pecquet 2018). Although the toxic effects of TBBPA on living beings are not yet defined, some studies indicate that this micropollutant may cause endocrine changes in organisms, and it may be associated with the development of cancer in the uterus of rats, maternal transmission between fish generations, and genetic mutation in frogs (Veldhoen et al. 2006; Nyholm et al. 2008; Yang et al. 2012; National Toxicology Program 2014).

Sample preparation is one of the most important steps in identifying and quantifying micropollutants in environmental samples including the elimination of matrix interferents, preconcentration, and isolation of analytes. Extraction and clean-up procedures that align green analytical chemistry (GAC) principles with good sensitivity, speed, precision, accuracy and efficiency, providing low limit of detection (LOD) values and relative recoveries, have been developed over the past decades (Rutkowaska et al. 2016; Kabir et al. 2017;

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Primel et al. 2017; Burato et al. 2020). Minimizing or eliminating toxic organic solvent consumption and reducing sample volume and extraction time while maintaining or enhancing extraction efficiency are some of the concerns of GAC (Rutkowska et al. 2019; Burato et al. 2020). Even though liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the best conventional techniques for sample preparation, the aim of using minimum solvent and sample volumes leads to miniaturized concepts, such as ultrasound-dispersive liquid–liquid microextraction (US-DLLME), that are more environmentally friendly (Rutkowaska et al. 2016; Kabir et al. 2017; Burato et al. 2020).

To determine the presence of TBBPA in complex matrix samples, extraction and clean-up methods are still based on nonminiaturized techniques. Analytical methods to determine the presence of tetrachlorobisphenol A, pentabromophenol, bisphenol A, TBBPA, hexabromocyclododecane, polybrominated diphenyl ether, and bromophenols in complex solid and aqueous samples based on the use of a larger volume of organic solvent, SPE cartridges, time-consuming steps throughout the procedure, and a complex apparatus compared with DLLME procedures have been extensively reported (Deceuninck et al. 2014; Chen et al. 2016; Zhang et al. 2016; Chi et al. 2017; Li et al. 2017). For complex environmental samples, the DLLME technique may be combined with other extraction and/or clean-up procedures depending on the nature of the sample. Generally, more attention has been given to aqueous environmental samples for the application of DLLME; solid samples remain underexplored.

Domestic sewage and anaerobic sludge are important matrices for determining the environmental occurrence of hazardous organic chemicals because wastewater treatment plants receive contaminants through industrial, hospital, and domestic sewage discharges, and mostly do not effectively remove and/or degrade micropollutants, which contaminate water bodies and ecosystems (Gorga et al. 2013). Predicting the possible impact of substances in the environment and their interaction with organisms is one of the aims of ecotoxicological bioassays. The use of invertebrates in these studies is highly relevant because they are primary consumers in aquatic ecosystems and are used as prey for high-level consumers (Baun et al. 2008; Newman 2008; Cattaneo et al. 2009; Chaumot et al. 2014; O'Brien et al. 2016).

Daphnia magna Straus 1820 and *Chironomus sancticaroli* Strixino & Strixino 1981 are freshwater aquatic invertebrate representative species of the water column and benthic habitat, respectively. They are representative of zooplankton organisms and recommended for ecotoxicological tests to assess water quality (Müller 1980; Koivisto 1995; Baumann et al. 2014; Besseling et al. 2014; Colombo-Corbi et al. 2017; Horton et al. 2018; Richardi et al. 2018; Bernegossi et al. 2019; Corbi et al. 2019; Dornfeld et al. 2019). The effective concentration (EC) of TBBPA that affects 50% of daphnid mobility was less than 1 mg L^{-1} (48-h median EC [EC50] of 0.6 mg L^{-1}; Waaijers et al. 2013). Even though the concentration of TBBPA in the body fluid of *D. magna* has been reported (Choi et al. 2020), to the best of our knowledge, there is no report on analytical methods based on DLLME using the whole organism for determining TBBPA bioaccumulation.

In the Chironomidae family, the TBBPA lethal concentration that affects 50% of the organisms in water was determined to be 0.13 mg L^{-1} for the *Chironomus tentans* species (14-d exposure). In sediments, the lowest-observed-effect concentration for larvae emergence ratio and development time was determined to be 250 mg TBBPA kg^{-1} of dried sediment (28-d exposure; US Environmental Protection Agency 2005). Moreover, there is no published information regarding the detection or bioaccumulation of TBBPA in *C. sancticaroli*. Several studies have assessed the potential of bioaccumulation of flame retardants in biological matrices (animals and plants) in the laboratory and field (Gustafsson et al. 1999; Bragigand et al. 2006; Sun et al. 2007; Tian and Zhu 2011; Wu et al. 2011; Mansouri et al. 2012); this is the first step in determining the bioconcentration and biomagnification capacity of hazardous substances (Law et al. 2006; Sormo et al. 2006; Choo et al. 2019).

Thus, the present study applies a US-DLLME technique followed by high-performance liquid chromatography (HPLC) separation coupled to electrospray ionization–tandem mass spectrometry (ESI–MS/MS) detection to identify and quantify TBBPA in domestic sewage, in anaerobic sludge, and in the aquatic invertebrates *D. magna* and *C. sancticaroli*. Therefore, this method was used to determine TBBPA in real samples. To the best of our knowledge, this technique has not been used to determine the presence of TBBPA in the matrices studied, and we have found no report on TBBPA bioaccumulation in *C. sancticaroli*.

MATERIALS AND METHODS

Chemicals and materials

The TBBPA (4,40-isopropylidenebis (2,6-dibromophenol; 97% purity, CAS 79-94-7) and the stable isotope labeled internal standard ($^{13}C_{12}$-TBBPA) were purchased from Sigma-Aldrich and from Wellington Laboratories, respectively. All organic solvent methanol (from JT Baker), acetonitrile (from JT Baker), tetrahydrofuran (from Merk Millipore), and chloroform (from JT Baker) were HPLC grade. Ammonium acetate salt was purchased from Sigma-Aldrich. All other chemicals were at least of analytical grade. Deionized water (18.2 MΩ cm) was generated by a Milli-Q Advantage A10 system (Millipore) and used throughout the experiment.

Stock solutions were prepared in acetonitrile independently at a concentration of 100 mg L^{-1} and serially diluted to the proper concentrations of the spiking solutions (5000, 1000, 500, 200, 10 and 1 μg L^{-1}). All solutions were stored at –20 °C.

The laboratory-made domestic sewage was adapted from Santos et al. (2016), with the following composition (mg L^{-1}): beef extract (260), sucrose (45), soluble starch (142.5), NaCl (500), $MgCl_2{\cdot}6H_2O$ (14), and $CaCl_2{\cdot}2H_2O$ (9). A micronutrient solution added to avoid limitations arising from a shortage of micronutrients in anaerobic processes was also included in the composition of the medium (Touzel and Albagnac 1983). The anaerobic sludge was collected from an Up-flow Anaerobic Sludge Blank (UASB) reactor treating poultry slaughterhouse

wastewater (Avícola Dacar). Samples were filtered in Combi syringe filters with a coarse glass fiber prefilter and a small-pore membrane as the main filter (1.0/0.20 μm).

Instrumentation

The TBBPA was identified and quantified using an HPLC Agilent Technologies 1260 Infinity device coupled to a hybrid triple-quadrupole–linear ion trap mass spectrometer AB Sciex QTrap® 5500 equipped with an ESI source (TurboV™). Chromatographic separation was performed on an InfinityLab Poroshell 120 EC-C18 device (3.0 × 50 mm, 2.7 μm) preceded by a guard column (precolumn HPLC, Sb-C18, 20 × 4.6 mm, 1.8 μm) kept at 30 °C, and the injection volume was 15 μL. The mobile phase consisted of water (10%) and acetonitrile (90%) in isocratic mode, and the pumps were set to a 300 μL min^{-1} flow rate. The mass spectrometer equipped with an ESI source was operated in negative-ion mode (–ESI), and the Turbo V™ ion source parameters were optimized by flow injection as follows: curtain gas 20 volts, collision gas, source temperature 500 °C, ion source gas (GS1) 50 psi, ion source gas (GS2) 40 psi, and ion spray voltage 4000 volts. The declustering potential, collision energy, and cell exit potential were optimized by direct infusion of TBBPA and $^{13}C_{12}$-TBBPA for each transition (Table 1). The equipment was operated under selective reaction monitoring (SRM) mode with a dwell time of 200 ms. Two MS/MS ion transitions (product ion) were monitored for TBBPA (447.7 and 417.7 *m/z*) and ^{13}C-TBBPA (457.8 and 428.7 *m/z*); the most intense transition was used for quantification, and the second one was used for confirmation. The initial MS and separation parameters were based on the methods proposed by Saint-Louis and Pelletier (2004) and Liu L. et al. (2017).

C. sancticaroli *and* D. magna *culture and sampling*

Chironomus sancticaroli and D. magna cultures were maintained at the Aquatic Ecology Environment Laboratory, University of São Paulo (São Paulo, Brazil). *Chironomus sancticaroli* was cultivated in plastic trays following the recommendations of Dornfeld et al. (2019) and the Organisation for Economic Co-operation and Development (2004). The culture was maintained in dechlorinated tap water with constant aeration and the presence of inorganic fine sediment; organisms were fed once a week with macerated Tetramin® vitamin and fish food. The organisms were kept in a temperature-controlled room (25 ± 2 °C) and a 12:12-h light:-dark cycle. The *D. magna* culture was maintained as outlined by the Brazilian Association of Technical Standards (2016) and the Organisation for Economic Co-operation and Development (2004), in a 2-L glass bottle containing reconstituted water, vitamins (VitaChem® and Seachem Prime®) and food (1 mL L^{-1} of Tetramin® at a concentration of 5 g L^{-1} and 3×10^5 *Raphidocelis subcaptata* cells mL^{-1}). The culture medium was replaced, and the organisms were fed 3 times a week. Crustaceans were kept in a Solab SL-224 incubator with a 16:8-h light:dark cycle and a temperature of 19 ± 2 °C.

To compose each sample before it was spiked with the corresponding stock solution of TBBPA to final concentrations from 5 to 1000 ng g^{-1}, *C. sancticaroli* organisms (larvae from IV instar) and *D. magna* neonates (less than 24 h of life) were sampled directly from the culture and weighed on an analytical balance until a wet weight of 10 ± 1 mg was reached, which corresponded to approximately 5 to 8 larvae for *C. sancticaroli* and 60 to 80 for *D. magna* neonates. The organisms were macerated and dried in an oven at 50 °C before contamination.

TABLE 1: Selective reaction monitoring parameters for quantitative and qualitative determination of TBBPA and mass-labeled internal standard

Compound	Precursor ion (Q_1; *m/z*)	Product ion (Q_3; *m/z*)	DP (volts)	CE (volts)	CXP (volts)
TBBPA	543.0	447.7	−190	−42	−11
TBBPA	543.0	417.7	−140	−50	−25
$^{13}C_{12}$-TBBPA	555.0	457.8	−140	−46	−21
$^{13}C_{12}$-TBBPA	555.0	428.7	−140	−54	−17

TBBPA = tetrabromobisphenol A; DP = declustering potential; CE = collision energy; CXP = cell exit potential.

Solid matrix preparation

An aliquot of the anaerobic sludge was washed in ultrapure water 3 times by vortex homogenization followed by centrifugation, to wash out any residual soluble organic matter. Each sample had 0.5 g wet weight of the centrifuged sludge, which had approximately 90% of water (dry wt of 50 mg). For the *D. magna* and the *C. sancticaroli* matrices, each sample had 10 mg wet weight. Samples were spiked with TBBPA to final concentrations of 5, 50, 100, 250, 500, 750, 1000, and 10 000 ng g^{-1} by adding different solutions in acetonitrile, left at room temperature (25 °C) for 24 h to evaporate the organic solvent, and kept sealed at 3 °C for 24 h to equilibrate. Before the spiking experiment, all samples were ground and dried overnight in an oven at 50 °C to preserve the adsorptive surface.

Chloroform and methanol (1:4, v/v) were used to perform the pre-extraction of the analyte. This mixture was the most appropriate for TBBPA extraction among the nonpolar extraction (dichloromethane and chloroform) and dispersive (acetonitrile and methanol) solvents that were tested. Then 1 mL of the extraction solution was added to the spiked samples and sonicated for 15 min at 25 °C. Samples were centrifuged and filtered through syringe filters with a coarse glass fiber prefilter and a small-pore membrane as the main filter (1.0/0.20 μm). This procedure was performed 3 times. The organic extracts were combined and concentrated to approximately 100 μL and then diluted with ultrapure water to a final volume of 1 mL, which was subjected to the US-DLLME.

DLLME

This technique was applied based on the liquid–liquid extraction optimized by Wang et al. (2013). In the present study,

sample and dispersive solvent volumes were reduced by 80%, with the aim of minimizing the residues generated and the costs incurred by the method without losing its efficiency. In addition, the time procedure was also reduced (ultrasonication and centrifugation duration). The optimum volumes of dispersive and extraction solvents were evaluated by analysis of variance (ANOVA) and the Tukey test to determine whether the tested volumes led to significantly different peak areas. Thus, 1 mL of the samples was placed in a 1.5-mL Eppendorf® microtube with 120 µL of tetrahydrofuran (dispersive solvent) and 25 µL of chloroform (extraction solvent). The mixture was manually shaken for 5 s, ultrasonicated for 2 min, and centrifuged at 14 000 rpm for 1 min. The sedimentary phase (25 µL) was transferred using an automatic pipette to a vial containing 925 µL of acetonitrile and 50 µL of the stable isotope internal standard solution prior to analysis by HPLC–MS/MS. Every sample set included a quality assurance/quality control check of a matrix blank, a blank sample (ultrapure water), and 3 samples containing the magic mix solution (25% methanol, 25% acetonitrile, 25% propanol, and 25% ultrapure water acidified with formic acid, 1%) in LC–MS vials, at a 10% frequency (one quality control run/10 matrix samples).

Method validation

Linearity, limits of detection and quantification, repeatability, precision, accuracy, recovery, and storage effect were measured for method validation. The LOD and limit of quantitation (LOQ) were determined by injecting samples to obtain signal-to-noise ratios of 3 and 10 times, respectively. Precision, accuracy, and recovery were assessed according to Matuszewski et al. (2003). Intraday and interday precision (relative standard deviation [RSD]) were determined by the replicate analyses ($n = 3$) at low, medium, and high concentrations of each calibration curve. The linearity of each standard curve was confirmed by plotting the peak area ratio of the analyte to the internal standard versus TBBPA nominal concentration. The residuals were evaluated for homoscedasticity via the Breusch–Pagan test (Breusch and Pagan 1979) and for autocorrelation via the Durbin–Watson test (Durbin and Watson 1951). Analysis of variance, *F* tests, and *t* tests were conducted to verify the linear regression quality and lack-of-fit significance (Snedecor and Cochran 1989).

The accuracy of the method was expressed by the calculated concentration/spiked concentration ratio percentage. The recovery was determined by comparing the mean peak area ratio of samples in which the analyte was spiked before extraction with the samples that were spiked after extraction (Matuszewski et al. 2003). The matrix effect was not investigated because the slope of the calibration curve obtained using the area ratio analyte signal/internal standard signal versus analyte concentration is independent of the matrix composition, considering that the analyte and the internal standard co-elute (Hewavitharana 2011).

The storage period test was conducted to evaluate the stability of the extracts for the domestic sewage matrix. Two sets of extracts at low, medium, and high concentrations each were analyzed by HPLC–ESI–MS/MS ($n = 3$). One was stored at 3 °C and the other at −20 °C for 10 d. After this period, the vials were ultrasonicated for 10 s and re-analyzed.

Real sample analysis

To evaluate the applicability of the proposed method, samples of domestic sewage, anaerobic sludge, and *D. magna* and *C. sancticaroli* were analyzed. For the aquatic organisms, acute toxicity bioassays were carried out in triplicate by exposing 60 neonates of *D. magna* and 6 larvae of *C. sancticaroli* in 250 mL of TBBPA aqueous solution at a concentration of 100 µg L^{-1} for 48 and 96 h, respectively (unpublished data). After exposure, the organisms were collected, and the proposed method was performed to investigate the bioaccumulation.

The biodegradation of TBBPA by anaerobic digestion was evaluated in domestic sewage (unpublished data). The experiment was conducted in batch reactors maintained in constant agitation at 150 rpm at 30 °C for 10 d. The TBBPA was added to the medium at a concentration of 100 µg L^{-1}. At the end of the experiment, TBBPA was quantified in both aqueous and solid matrices.

RESULTS AND DISCUSSION

Optimizing HPLC conditions and ESI–MS parameters

Different combinations of organic solvents (acetonitrile and methanol) and aqueous solvents (ultrapure water and ammonium acetate 5 mM) were tested, as well as different injection volumes (5, 10, 15, and 20 µL), for optimal separation conditions and analytical efficiency. No gradient elution procedure was necessary, and ammonium acetate (5 mM; A) and acetonitrile (B; 10/90%, v/v) in isocratic mode yielded better results. The column temperature was 30 °C for a total run time of 5 min (TBBPA and $^{13}C_{12}$-TBBPA retention time of 1.4 min). The TBBPA was quantified by the determination of product ions in negative-ion mode (ESI–), and the SRM mode was carried out for the acquisition. The transitions 417.7 *m/z* $[M\text{-}H\text{-}CH_4Br\text{-}CO]^-$ and 447.7 *m/z* $[M\text{-}H\text{-}CH_4Br]^-$ were used for quantification and confirmation, respectively. These product ions were selected considering the highest signal obtained in the SRM optimization of transition, chromatogram stability, and previous reports in the literature (Saint-Louis and Pelletier 2004; Liu A. et al. 2017). Figure 1A shows the mass spectra of TBBPA and the possible structures of product ions according to their molecular formulas and the similar fragmentation properties, as previously reported (Liu A. et al. 2017). Figure 1B shows the mass chromatogram of TBBPA and the internal standard under the optimized condition. Further mass chromatograms of TBBPA and $^{13}C_{12}$-TBBPA for each studied matrix are presented in the Supplemental Data.

Method performance

Dispersive and extraction solvents volume in the DLLME. To evaluate the ideal dispersive solvent volume, samples were prepared ($n = 3$) with 25 µL of extraction solvent,

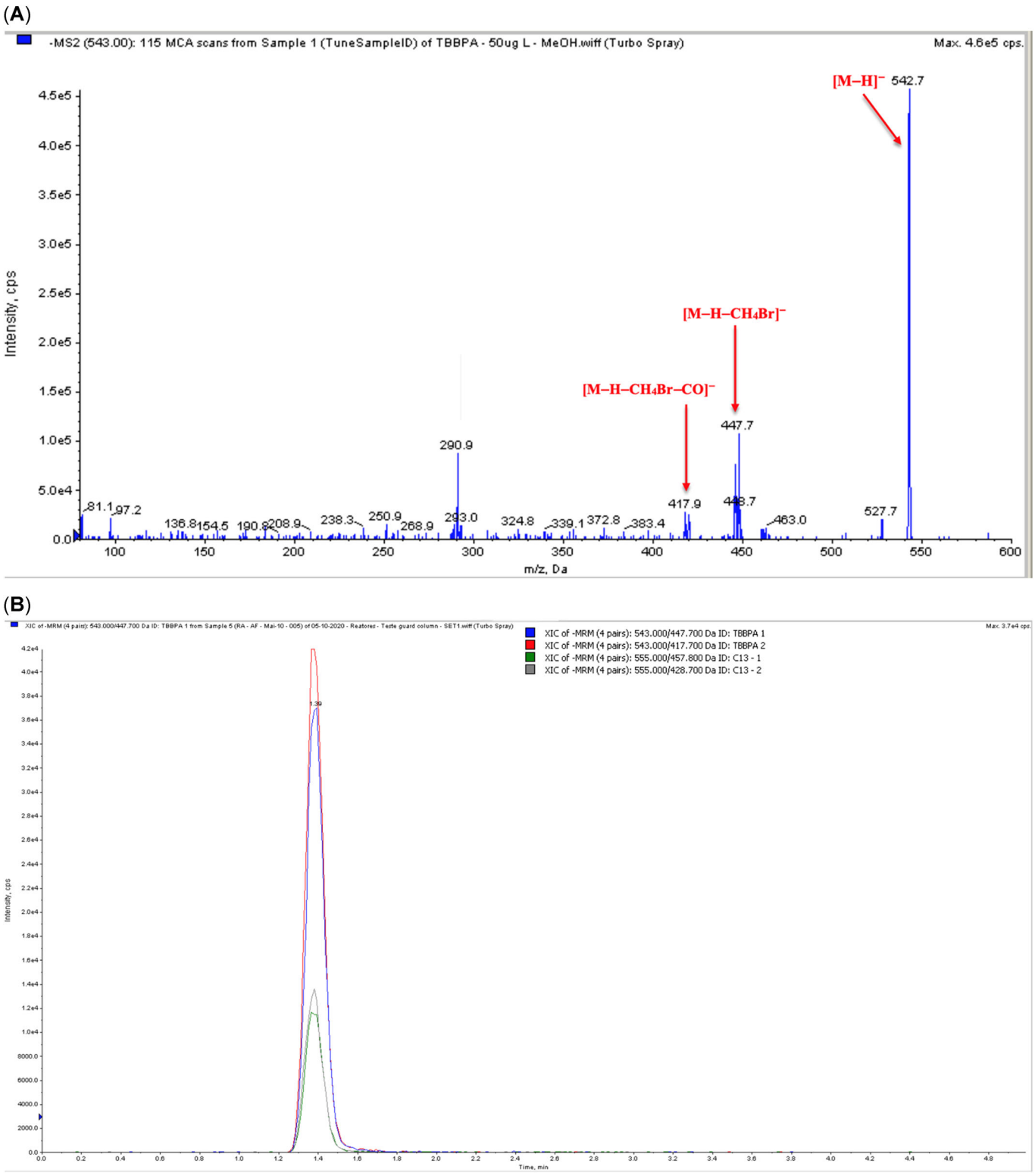

FIGURE 1: Mass spectra of tetrabromobisphenol A (TBBPA; **A**) and mass chromatogram (**B**) of TBBPA and $^{13}C_{12}$-TBBPA in optimized instrumental conditions.

which were optimized by Wang et al. (2013), testing the volumes of 60, 120, 180, and 240 µL. The tested dispersive solvent volumes led to statistically different peak areas (ANOVA, $p = 3.41E\text{-}5$). The Tukey test showed that 60 µL of dispersive solvent led to a TBBPA peak area that was statistically different from the results obtained with 120, 180, and 240 µL ($p = 0.0247$, 0.0079, and 0.0010, respectively). However, the greatest recovery of TBBPA (highest signal intensity) was obtained using 120 µL of dispersive solvent (Figure 2A). Afterward, different volumes of the extraction solvent were tested

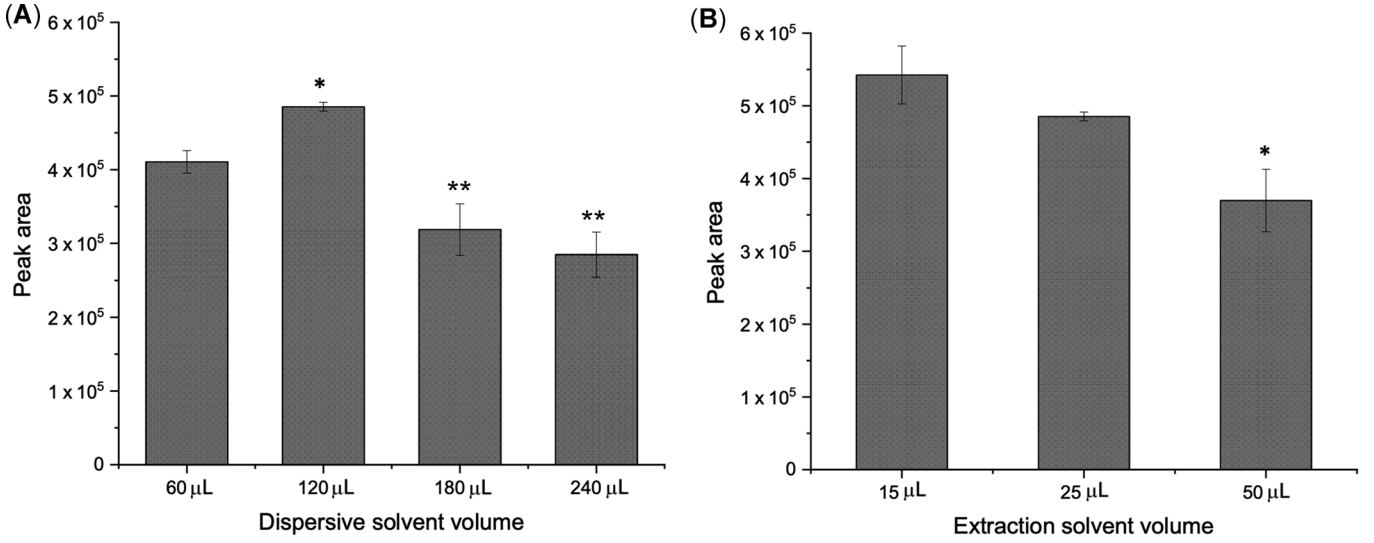

FIGURE 2: Average tetrabromobisphenol A (TBBPA) peak area and respective standard deviation obtained for different volumes of dispersive (**A**) and extraction (**B**) solvent in the dispersive liquid–liquid microextraction (DLLME) method. *, **, presence of statistical difference ($p < 0.05$).

(15, 25, and 50 µL, $n = 3$; Figure 2B), which led to statistically different results (ANOVA, $p = 0.0022$). It was observed that the average peak areas obtained using 50 µL of extraction solvent were statistically different from the averages obtained using 15 and 25 µL by Tukey test analysis. Even though 15 µL led to the analyte's highest signal intensity, it was difficult to accurately remove the sedimentary phase from the bottom of the centrifuged microtube. For this reason, 25 µL was used in the proposed method.

Merit figures for method validation. Linear regression analysis was carried out by plotting the peak area ratio (peak area of the analyte divided by the internal standard peak area) versus analyte concentration, using 5 levels in triplicate experiments. The linear range was determined based on the environmental occurrence of TBBPA in each studied matrix and is presented in Table 2. The values of correlation (R) and regression coefficients (R^2) were considered adequate, demonstrating good linearity for the studied intervals. The residuals of the linear regression were examined for obvious patterns, and homoscedasticity was confirmed by the Breusch–Pagan test (Breusch and Pagan 1979) for the domestic sewage and anaerobic sludge matrices only, in which the residual variability across all concentration levels was constant ($p > 0.05$). For the *D. magna* and *C. sancticaroli* matrices, Breusch–Pagan statistics were significant ($p = 0.001$ and 0.004, respectively). Heteroscedasticity is commonly related to a wide linear range (0.05–10 μg^{-1}), and performing weighted linear regression is an appropriate way to better adjust heteroscedastic data (Deng et al. 2016; Valese et al. 2016). The weighting factor chosen for each matrix (Table 2) yielded the smaller relative error among the empirical weighting factors mostly used. Even though homoscedasticity was observed in the domestic sewage and anaerobic sludge matrices, weighted linear regression was applied to enhance accuracy.

The Durbin–Watson test (Table 2) was performed to evaluate the autocorrelation of residuals. These test values ranged from 1.3 to 2.4, demonstrating that residuals are uncorrelated (Durbin and Watson 1951; Pastor-Belda et al. 2018).

Table 3 presents the ANOVA statistics for the weighted linear regressions, in which the F test was performed to evaluate the quality of the regression for each matrix. For each curve, a highly significant regression ($p < 0.01$) was obtained. The lack of fit was also evaluated and was nonsignificant ($F_{exp} < F_{2,10}$) for a significance level of 95%.

Table 4 presents the coefficients that describe the calibration equations for all matrices. The slope coefficients were statistically significant ($p < 0.01$) by the t test performed at the 99% significance level.

The LODs, LOQs, accuracy, intraday and interday RSDs, and method recoveries are presented in Table 5 for low, medium,

TABLE 2: Weighted linear regression coefficients and Durbin–Watson test values for residuals analysis

Matrix	Weighting factor	R	R^2	Adjusted R^2	SE of the estimate	Durbin–Watson
DS (1–120 µg L^{-1})	1/x	0.998	0.996	0.996	0.177	2.1
AS (0.05–1 µg $gVSS^{-1}$)	1/x	0.985	0.971	0.969	0.005	1.3
Dm (0.1–10 µg g^{-1})	1/x	0.999	0.998	0.998	0.001	1.4
Cs (0.1–10 µg g^{-1})	Ln(x)	0.986	0.973	0.971	0.159	2.4

SE = standard error; DS = domestic sewage; AS = anaerobic sludge; Dm = *Daphnia magna*; Cs = *Chironomus sancticaroli*; VSS = volatile suspend solids.

TABLE 3: Analysis of variance statistics for linear regression of calibration curves

		SS	*df*	MS	*F*	*p* value
DS	Regression	97.37	1	97.379	3099.97	7.45E-17
	Residual	0.41	13	0.031		
	Total	97.79	14			
AS	Regression	9.97E-03	1	9.97E-03	433.38	2.29E-11
	Residual	2.99E-04	13	2.30E-05		
	Total	1.03E-02	14			
Dm	Regression	7.46E-03	1	7.00E-03	5898.87	1.15E-18
	Residual	1.60E-05	13	0.00E + 00		
	Total	7.48E-03	14			
Cs	Regression	1.17E + 01	1	1.17E + 01	463.55	1.50E-11
	Residual	3.29E-01	13	2.50E-02		
	Total	1.21E + 01	14			

SS = sum of square; MS = mean square; DS = domestic sewage; AS = anaerobic sludge; Dm = *Daphnia magna*; Cs = *Chironomus sancticaroli*.

TABLE 4: Calibration curves parameters and *t* test values for slope significance

		Coefficients			
Matrix		B	SE	*t* test	*p* value
DS	(Constant)	−1.14E-01	7.20E-02	−1.58	1.37E-01
	Slope	5.95E-02	1.00E-03	55.68	7.45E-17
AS	(Constant)	−5.29E-02	1.79E-02	−2.95	1.12E-02
	Slope	1.65E-03	7.90E-05	20.82	2.29E-11
Dm	(Constant)	−1.32E-02	3.58E-03	−3.70	2.67E-03
	Slope	4.82E-04	6.00E-06	76.80	1.15E-18
Cs	(Constant)	1.36E-02	2.10E-02	0.655	0.523991
	Slope	8.20E-05	0.00E + 00	21.53	1.50E-11

SE = standard error; DS = domestic sewage; AS = anaerobic sludge; Dm = *Daphnia magna*; Cs = *Chironomus sancticaroli*.

and high concentrations within the calibration curve of each matrix. These parameters ranged in agreement with international acceptance criteria (Munch et al. 2005).

For the storage period test performed for domestic sewage extracts, the average intraday repeatability values were 14.9 and 4.2% for the sets stored at 3 °C and –20 °C for 10 d, respectively, demonstrating good stability of the extracts stored at –20 °C. Accuracy and RSD greater than 120, and 15%, respectively, were obtained for the *C. sancticaroli* matrix, which may be related to the complexity of the matrix and the wide linearity range. In addition, these results are comparable to the RSD (%) values and correlation coefficients reported for a magnetic solid-phase extraction–high-performance liquid chromatography–ultraviolet method to determine TBBPA in fish samples (Hu et al. 2019) and a slug-flow microextraction–paper spray–mass spectrometry method for analyzing perfluorooctanosulfonate and perfluorooctanoic acid in the body fluid of *D. magna* (Deng et al. 2016). The LOD of 0.002 μg L^{-1} for the sewage sludge matrix was at least 40-fold smaller compared with results reported for liquid samples of lower complexity, demonstrating the method's sensitivity (Wang et al. 2013; Zhang et al. 2016; Liu L. et al. 2017). For the other matrices, the LODs we obtained were comparable to other limits reported for a DLLME–gas chromatography (GC)–MS method in environmental matrices (Erarpat et al. 2019). The method recovery (Table 5) for each matrix was relatively lower than the method recovery (%) reported in the literature. Greater recovery (84.2–88.6%) was obtained by Erarpat et al. (2019) possibly due to the derivatization process and the greater sample volumes reported by these authors to determine organotin compounds in fish and mussel samples. Generally, extraction methods for environmental matrices such as wastewaters, sludge, animal tissues, and aquatic organisms require more robust and expensive techniques, using greater volumes of organic solvents and longer sample preparation times (Zhang et al. 2016; Kotthoff et al. 2017; Liu L. et al. 2017). Most environmental samples are pretreated or extracted with conventional procedures such as SPE or liquid phase extraction, leading to excellent results (Saint-Louis and Pelletier 2004; Kabir et al. 2017; Burato et al. 2020). It should be noted that the costs and waste generated by the methods just described are the real limitations of these processes. In addition, sample digestion is not required in the method we present, reducing the time required to process samples and technical complexity. Table 6 presents a more detailed

TABLE 5: Performance of the US–DLLME–LC–MS method for different matrices[a]

Matrix	LOD	LOQ	Accuracy (%)	RSD intraday (%)	Repeatability, RSD interday (%)	Recovery (%)
DS	2.0 ng L^{-1}	1 μg L^{-1}	L: 113.9	L: 4.1	L: 8.2	L: 50.7
			M: 95.2	M: 6.6	M: 7.7	M: 48.5
			H: 106.3	H:2.6	H: 3.9	H: 48.7
AS	2.0 ng $VSSg^{-1}$	0.1 μg $SSVg^{-1}$	L: 98.3	L: 3.1	L: 11.9	L: 32.5
			M: 89.5	M: 9.6	M: 8.7	M: 30.9
			H: 108.0	H: 6.2	H: 5.8	H: 35.5
Dm	2.5 ng g^{-1}	0.05 μg g^{-1}	L: 107.6	L: 3.9	L: 8.1	L: 36.5
			M: 98.6	M: 4.6	M: 5.7	M: 43.5
			H: 100.3	H: 5.0	H: 8.7	H: 42.9
Cs	5 ng g^{-1}	0.05 μg g^{-1}	L: 127.9	L: 3.1	L: —	L: —
			M: 99.5	M: 16.2	M: 9.6	M: 13.6
			H: 97.4	H: 8.5	H: —	H: —

[a]Low (L), medium (M), and high (H) concentrations for each matrix: DS (1, 40, and 120 μg L^{-1}), AS (0.1, 0.25, and 1 μg $SSVg^{-1}$), Dm (0.1, 1, and 10 μg g^{-1}), and Cs (0.1, 0.5, 10 μg g^{-1}).
US–DLLME–LC–MS = ultrasound-dispersive liquid–liquid microextraction–liquid chromatography–mass spectrometry; LOD = limit of detection; LOQ = limit of quantification; RSD = relative standard deviation; DS = domestic sewage; AS = anaerobic sludge; Dm = *Daphnia magna*; Cs = *Chironomus sancticaroli*; VSS = volatile suspended solids.

TABLE 6: Latest extraction methods for determining the presence of tetrabromobisphenol A (TBBPA) and other flame retardants on environmental matrices

Analyte	Sample		Technique	Solvent vol/sample		Extraction time (min)[a]	LOQ	LOD	Reference
	Nature	Amount		Dispersive	Extraction				
Butyltin compounds (MBT, DBT, TBT),[b]	Fish and mussel	8 mL	DLLME–GC–MS	1.64 mL	300 µL	30	1.3–2.1 0.4–0.6 µg kg^{-1}	0.4–0.6 µg kg^{-1}	Erarpat et al. 2019
TBBPA, BPA, and OP	Urine	10 mL	LLE–LC–MS	32 mL	8 mL	36	0.05–0.25 µg L^{-1}	0.01–0.08 µg L^{-1}	Zhang et al. 2016
TBBPA	Fish	–	MSPE–HPLC–UV	N/A	15 m[c]	65	50 ng g^{-1}	15.2 ng g^{-1}	Hu et al. 2019
PFOS and PFOA	Body fluid of *Daphnia magna*	10 µL	SFME–PS–MS	N/A	N/A	N/A	0.13–0.3 µg L^{-1}	0.04–0.09 µg L^{-1}	Deng et al. 2016
TBBPA/S	Water and seawater	200 mL	LLE–HPLC–MS/MS	N/A	300 m[c]	N/A	5 µg L^{-1}	0.12–0.19 µg L^{-1}	Liu L. et al. 2017
TBBPA and derivatives	Urine and fruit juice	5 mL	DLLME–HPLC–UV	600 µL	25 µL	7	0.43–2.1µg L^{-1}	0.13–0.63 µg L^{-1}	Wang et al. 2013
TBBPA	Soil	1000 mg	HTDMAE–LLE–LC–MS/MS	N/A	12.5 m[c]	20	4.6 ng g^{-1}	1.4 ng g^{-1}	Zhao et al. 2016
Bisphenols	Beverages	10 mL	SPE–LC–MS/MS	N/A	30 m[c]	75	5.2–75.1 ng L^{-1}	1.6–17.6 ng L^{-1}	Regueiro and Wenzl 2015
TBBPA, HBCD and BDE	Human serum	1 mL	QuEChERS–GC/LC– MS/MS	N/A	18 m[c]	12	0.25–5 µg L^{-1}	4–19 ng L^{-1}	Li et al. 2017
Bisphenol A	Food items	5000 mg	SPE–GC–MS/MS	N/A	80 m[c]	N/A	0.03 µg kg^{-1}	0.1 µg kg^{-1}	Deceuninck et al. 2014
Bromophenols	Water	100–300 mL	SPE–HPLC–MS/MS	N/A	12 m[c]	20	–	0.1–21.9 ng L^{-1}	Chi et al. 2017
PeBP, TCBPA, and TBBPA	Seafood	500 mg	VH–MSPD–GC–MS/MS	N/A	12 m[c]	N/A	0.5 ng g^{-1}	0.2 ng g^{-1}	Chen et al. 2016

[a]Time of main extraction procedure described.
[b]Butylin compounds are not flame retardants.
[c]Methods based on solid-phase extraction.
LOQ = limit of quantification; LOD = limit of detection; MBT = monobutyltin; DBT = dibutyltin; BPA = bisphenol A; OP = 4-tert-octylphenol; PFOS = perfluorooctanesulfonic acid; PFOA = perfluorooctanoic acid; HBCD = hexabromocyclododecane; BDE = brominated diphenyl ether; PeBP = pentabromophenol; TCBPA = tetrachlorobisphenol A; DLLME–GC–MS = dispersive liquid–liquid microextraction–gas chromatography–mass spectrometry; LLE–LC–MS = liquid–liquid extraction–liquid chromatrography–mass spectrometry; MSPE–HPLC–UV = magnetic solid-phase extraction–high-performance liquid chromatography–ultraviolet; SFME–PS–MS = slug-flow microextraction–paper spray–mass spectrometry; HTDMAE = high-throughput dynamic microwave-assisted extraction; SPE = solid-phase extraction; QuEChERS = Quick Easy Cheap Effective Rugged Safe extraction; VH–MSPD = vortex-homogenized matrix solid phase dispersion; N/A = not available.

description of published studies regarding the use of extraction methods based on liquid–liquid and SPE in environmental samples, especially for TBBPA and related flame retardants; we obtained equivalent LOD and LOQ results. Compared with the proposed method, even the miniaturized extraction techniques require a greater solvent volume and procedure time (Wang et al. 2013; Erarpart et al. 2019) and/or a more complex and time-consuming apparatus (Deng et al. 2016; Zhao et al. 2016). The results we obtained suggest that the sample preparation before the DLLME procedure and the miniaturization of what was proposed by Wang et al. (2013) led to satisfactory results for TBBPA quantification even in complex matrices. For solid environmental matrices, most of the methods rely on SPE, with conventional (C_{18} cartridges; Deceuninck et al. 2014; Regueiro and Wenzl 2015; Chi et al. 2017; Li et al. 2017) or alternative sorbent materials (Chen et al. 2016; Hu et al. 2019), requiring a large volume of solvents for cartridge activation and analyte elution.

Real sample analysis

To evaluate the applicability of the proposed method, domestic sewage, anaerobic sludge, *D. magna*, and *C. sancticaroli* samples were analyzed. In the anaerobic batch reactor, the final concentration of the contaminant in the domestic sewage and in anaerobic sludge was $0.2 \pm 0.03\ \mu g\ L^{-1}$ and $507 \pm 79\ ng\ g^{-1}$, respectively. For the aquatic organisms subjected to acute toxicity bioassays, TBBPA accumulated at 3.74 and $8.87\ \mu g\ g^{-1}$ in *D. magna* (48-h exposure) and *C. sancticaroli* (96-h exposure), respectively, indicating that TBBPA is highly bioaccumulative. Generally, TBBPA bioaccumulation is investigated in fish and fish tissues, with values ranging from 0.01 to $2.85\ ng\ g^{-1}$ (wet wt) found (Ashizuka et al. 2008; Tang et al. 2015). Quantifying this micropollutant in *D. magna* and *C. sancticaroli* is highly relevant because they are primary consumers in aquatic ecosystems and are used as prey for high-level consumers, allowing earlier detection of environmental contamination.

CONCLUSIONS

The present study contributes to the need to develop ecofriendly and miniaturized analytical methods to determine TBBPA in environmental samples. Contrary to what has been used to detect micropollutants in complex matrices, the proposed US–DLLME–LC–MS/MS method allows for a simple, fast, accurate, sensitive, and cost-effective quantification procedure. The method was able to successfully quantify TBBPA in domestic sewage and anaerobic sludge, which are matrices that indicate the environmental occurrence of hazardous chemicals. In addition, to our knowledge, the present study is the first to determine bioaccumulation of TBBPA in the experimental species *D. magna* and *C. sancticaroli*, which are used for ecotoxicological assessment, suggesting the feasibility of the method for environmental monitoring.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4837.

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REFERENCES

Ashizuka Y, Nakagawa R, Hori T, Yasutake D, Toblishi K, Saaki K. 2008. Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan. *Mol Nutr Food Res* 52:272–282.

Baumann J, Köser J, Arndt D, Filser J. 2014. The coating makes the difference: Acute effects of iron oxide nanoparticles on *Daphnia magna*. *Sci Total Environ* 484:176–184.

Baun A, Hartmann NB, Grieger K. 2008. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: A brief review and recommendations for future toxicity testing. *Ecotoxicology* 17:387–395.

Bernegossi AC, Cardoso BNP, Felipe MC, de Lima e Silva MR, Corbi JJ. 2019. *Chironomus sancticaroli* generation test: A new methodology with a Brazilian endemic insect. *MethodsX* 6:92–97.

Besseling E, Wang B, Lürling M, Koelman AA. 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. *Environ Sci Technol* 48:12336–12343.

Bragigand V, Amiard-Triquet C, Parlier E, Boury P, Marchand P, Hourch ME. 2006. Influence of biological and ecological factors on the bioaccumulation of polybrominated diphenyl ethers in aquatic food webs from French estuaries. *Sci Total Environ* 368:615–626.

Brazilian Association of Technical Standards. 2016. ABNT NBR 12713: Ecotoxicologia aquática–Toxicidade aguda–Método de ensaio com *Daphnia* spp. (Crustacea, Cladocera). Rio de Janeiro, Brazil.

Breusch TS, Pagan AR. 1979. A simple test for heteroscedasticity and random coefficient variation. *Econometrica* 47:1287.

Burato JSS, Vargas Medina DA, Toffoli AL, Vasconcelos Soares Maciel E, Mauro Lanças F. 2020. Recent advances and trends in miniaturized sample preparation techniques. *J Sep Sci* 43:202–225.

Cattaneo AG, Gornati R, Chiriva-Internati M, Bernardini G. 2009. Ecotoxicology of nanomaterials: The role of invertebrate testing. *Invertebr Surviv J* 6:78–97.

Chaumot A, Ferrari B, Geffard O, Garric J. 2014. Ecotoxicology, aquatic invertebrates. In Wexler P, ed, *Encyclopedia of Toxicology*, 3rd ed. Elsevier, New York, NY, USA, pp 284–288.

Chen J-M, Yang C-C, Chung W-H, Ding W-H. 2016. Vortex-homogenized matrix solid-phase dispersion coupled with gas chromatography–electron-capture negative-ion mass spectrometry to determine halogenated phenolic compounds in seafood. *RSC Adv* 6:96510–96517.

Chi X, Liu J, Yu M, Xie Z, Jiang G. 2017. Analysis of bromophenols in various aqueous samples using solid phase extraction followed by HPLC-MS/MS. *Talanta* 164:57–63.

Choi Y, Jeon J, Choi Y, Kim SD. 2020. Characterizing biotransformation products and pathways of the flame retardant triphenyl phosphate in *Daphnia magna* using non-target screening. *Sci Total Environ* 708: 135106.

Choo G, Lee I-S, Oh J-E. 2019. Species and habitat-dependent accumulation and biomagnification of brominated flame retardants and PBDE metabolites. *J Hazard Mater* 371:175–182.

Colombo-Corbi V, Gorni GR, Sanzovo-Falcoski T, Costa PI, Corbi JJ. 2017. Genetic diversity loss in *Chironomus sancticaroli* (Diptera: Chironomidae) exposed to pyrimethanil fungicide: An analysis using RAPD technique. *Water Air Soil Pollut* 228:399.

Corbi JJ, Bernegossi AC, Moura L, Felipe MC, Issa CG, Silva MRL, Gorni GR. 2019. *Chironomus sancticaroli* (Diptera, Chironomidae) as a sensitive test species: Can we rely on its use after repeated generations, under laboratory conditions? *Bull Environ Contam Toxicol* 103:213–217.

Daso AP, Rohwer ER, Koot DJ, Okonkwo JO. 2017. Preliminary screening of polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCDD) and tetrabromobisphenol A (TBBPA) flame retardants in landfill leachate. *Environ Monit Assess* 189:418.

Deceuninck Y, Bichon E, Durand S, Bemrah N, Zendong Z, Morvan ML, Marchand P, Dervilly-Pinel G, Antignac JP, Leblanc JC, Le Bizec B. 2014. Development and validation of a specific and sensitive gas chromatography tandem mass spectrometry method for the determination of bisphenol A residues in a large set of food items. *J Chromatogr A* 1362:241–249.

Deng J, Wang W, Yang Y, Wang X, Chen B, Yao Z-P, Luan T. 2016. Slug-flow microextraction coupled with paper spray mass spectrometry for rapid analysis of complex samples. *Anal Chim Acta* 940:143–149.

Dornfeld CB, Rodgher S, Negri RG, Espíndola ELG, Daam MA. 2019. *Chironomus sancticaroli* (Diptera, Chironomidae) as a sensitive tropical test species in laboratory bioassays evaluating metals (copper and cadmium) and field testing. *Arch Environ Contam Toxicol* 76:42–50.

Durbin J, Watson GS. 1951. Testing for serial correlation in least squares regression. II. *Biometrika* 38:159–178.

Erarpat S, Bodur S, Öz E, Bakırdere S. 2019. Determination of butyltin compounds in fish and mussel samples at trace levels by vortex assisted dispersive liquid-liquid microextraction-gas chromatography mass spectrometry. *J Food Compos Anal* 82:103248.

Gorga M, Martínez E, Ginebreda A, Eljarrat E, Barceló D. 2013. Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain). *Sci Total Environ* 444:51–59.

Gustafsson K, Bjork M, Burreal S, Gilek M. 1999. Bioaccumulation kinetics of brominated flame retardants (polybrominated diphenyl ethers) in blue mussels (*Mytilus edulis*). *Environ Toxicol Chem* 18:1218–1224.

Hewavitharana AK. 2011. Matrix matching in liquid chromatography–mass spectrometry with stable isotope labelled internal standards—Is it necessary? *J Chromatogr A* 1218:359–361.

Horton AA, Vijver MG, Lahive E, Spurgeon DJ, Svendsen C, Heutink R, van Bodegom PM, Baas J. 2018. Acute toxicity of organic pesticides to *Daphnia magna* is unchanged by co-exposure to polystyrene microplastics. *Ecotoxicol Environ Saf* 166:26–34.

Hu L, Zhou T, Luo D, Feng J, Tao Y, Zhou Y, Mei S. 2019. Bioaccumulation of tetrabromobisphenol A in a laboratory-based fish–water system based on selective magnetic molecularly imprinted solid-phase extraction. *Sci Total Environ* 650:1356–1362.

Kabir A, Locatelli M, Ulusoy H. 2017. Recent trends in microextraction techniques employed in analytical and bioanalytical sample preparation. *Separations* 4:36.

Koivisto S. 1995. Is *Daphnia magna* an ecologically representative zooplankton species in toxicity tests? *Environ Pollut* 90:263–267.

Kotthoff M, Rüdel H, Jürling H. 2017. Detection of tetrabromobisphenol A and its mono- and dimethyl derivatives in fish, sediment and suspended particulate matter from European freshwaters and estuaries. *Anal Bioanal Chem* 409:3685–3694.

Law K, Halldorson T, Danell R, Stern G, Gewurtz S, Alaee M, Marvin C, Whittle M, Tomy G. 2006. Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. *Environ Toxicol Chem* 25:2177–2186.

Li J, Chen T, Wang Y, Shi Z, Zhou X, Sun Z, Wang D, Wu Y. 2017. Simple and fast analysis of tetrabromobisphenol A, hexabromocyclododecane isomers, and polybrominated diphenyl ethers in serum using solid-phase extraction or QuEChERS extraction followed by tandem mass spectrometry coupled to HPLC and GC. *J Sep Sci* 40:709–716.

Liu L, Liu A, Zhang Q, Shi J, He B, Yun Z, Jiang G. 2017. Determination of tetrabromobisphenol-A/S and their main derivatives in water samples by high performance liquid chromatography coupled with inductively coupled plasma tandem mass spectrometry. *J Chromatogr A* 1497: 81–86.

Liu A, Shi J, Qu G, Hu L, Ma Q, Song M, Jing C, Jiang G. 2017. Identification of emerging brominated chemicals as the transformation products of tetrabromobisphenol A (TBBPA) derivatives in soil. *Environ Sci Technol* 51:5434–5444.

Mansouri K, Consonni V, Durjava MK, Kolar B, Oberg T, Todeschini R. 2012. Assessing bioaccumulation of polybrominated diphenyl ethers for aquatic species by QSAR modeling. *Chemosphere* 89:433–444.

Matuszewski BK, Constanzer ML, Chavez-Eng CM. 2003. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC–MS/MS. *Anal Chem* 75:3019–3030.

Müller HG. 1980. Experiences with test systems using *Daphnia magna*. *Ecotoxicol Environ Saf* 4:21–25.

Munch DJ, Wasko M, Flynt E, Wendelken SC, Scifres J, Mario JR, Hunt M, Gregg D, Schaeffer T, Clarage M, Lumpkin MS. 2005. Validation and peer review of US Environmental Protection Agency chemical methods of analysis. Forum on Environmental Measurements, US Environmental Protection Agency, Washington, DC.

National Toxicology Program. 2014. Technical report on the toxicology studies of tetrabromobisphenol A (CAS NO. 79–94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogenesis study of tetrabromobisphenol A inWistar Han [Crl:WI(Han)] rats and B6C3F1/N mice. Public Health Service, US Department of Health and Human Services, Research Triangle Park, NC, USA.

Newman MC. 2008. Ecotoxicology: The history and present directions. In Jorgensen SE, Fath BD, eds, *Encyclopedia of Ecology*. Elsevier, New York, NY, USA, pp 1195–1201.

Ni H, Zeng H. 2013. HBCD and TBBPA in particulate phase of indoor air in Shenzhen, China. *Sci Total Environ* 458–460:15–19.

Nyholm JR, Norman A, Norrgren L, Haglund P, Andersson PL. 2008. Maternal transfer of brominated flame retardants in zebrafish (*Danio rerio*). *Chemosphere* 73:203–208.

O'Brien AL, Morris L, Keough MJ. 2016. Rapid invertebrate responses to macroalgal wrack: Two novel field experiments on intertidal mudflats in Southern Australia. *Mar Ecol* 38:1–17.

Organisation for Economic Co-operation and Development. 2004. Test No. 202: *Daphnia* sp., acute immobilisation test. *OECD Guidelines for the Testing of Chemicals*. Paris, France.

Pastor-Belda M, Marín-Soler L, Campillo N, Viñas P, Hernández-Córdoba M. 2018. Magnetic carbon nanotube composite for the preconcentration of parabens from water and urine samples using dispersive solid phase extraction. *J Chromatogr A* 1564:102–109.

Primel EG, Caldas SS, Marube LC, Escarrone ALV. 2017. An overview of advances in dispersive liquid–liquid microextraction for the extraction of pesticides and emerging contaminants from environmental samples. *Trends Environ Anal Chem* 14:1–18.

Qu G, Liu A, Hu L, Liu S, Shi J, Jiang G. 2016. Recent advances in the analysis of TBBPA/TBBPS, TBBPA/TBBPS derivatives and their transformation products. *Trends Anal Chem* 83:14–24.

Regueiro J, Wenzl T. 2015. Determination of bisphenols in beverages by mixed-mode solid-phase extraction and liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr A* 1422:230–238.

Richardi VS, Vicentini M, Morais GS, Rebechi D, da Silva TA, Fávaro LF, Navarro-Silva MA. 2018. Effects of phenanthrene on different levels of biological organization in larvae of the sediment-dwelling invertebrate *Chironomus sancticaroli* (Diptera: Chironomidae). *Environ Pollut* 242: 277–287.

Rothenbacher KP, Pecquet AM. 2018. Summary of historical terrestrial toxicity data for the brominated flame retardant tetrabromobisphenol A (TBBPA): Effects on soil microorganisms, earthworms, and seedling emergence. *Environ Sci Pollut Res* 25:17268–17277.

Rutkowska M, Płotka-Wasylka J, Sajid M, Andruch V. 2019. Liquid–phase microextraction: A review of reviews. *Microchem J* 149:103989.

Saint-Louis R, Pelletier E. 2004. LC-ESI-MS-MS method for the analysis of tetrabromobisphenol A in sediment and sewage sludge. *Analyst* 129:724.

Santos CED, Moura RB, Damianovic MHRZ, Foresti E. 2016. Influence of COD/N ratio and carbon source on nitrogen removal in a structured-bed reactor subjected to recirculation and intermittent aeration (SBRRIA). *J Environ Manage* 166:519–524.

Snedecor GW, Cochran WG. 1989. *Statistical Methods*, 8th ed. Iowa State University, Ames, IA, USA.

Sormo EG, Salmer MP, Jenssen BM, Hop H, Baek K, Kovacs K, Lydersen C, Falk-Petersen S, Gabrielsen GW, Lie E, Skaare JU. 2006. Biomagnification of polybrominated diphenyl ether and hexabromocyclododecane flame retardants in the polar bear food chain in Svalbard, Norway. *Environ Toxicol Chem* 25:2502–2511.

Sun Y, Guo H, Yu H, Wang X, Wu J, Yuqun X. 2007. Bioaccumulation and physiological effects of tetrabromobisphenol A in cootail *Ceratophyllum demerson* L. *Chemosphere* 70:1787–1795.

Tang B, Zeng Y-H, Luo X-J, Zheng X-B, Mai BX. 2015. Bioaccumulative characteristics of tetrabromobisphenol A and hexabromocyclododecanes in multi-tissues of prey and predator fish from an e-waste site, South China. *Environ Sci Pollut Res Int* 22:12011–12017.

Tian S, Zhu L. 2011. Bioaccumulation kinetics of sediment-associated DE-83 in benthic invertebrates (*Nereis succinea*, polychaete). *Chemosphere* 84:160–165.

Touzel JP, Albagnac G. 1983. Isolation and characterization of *Methanococcus-mazei* strain MC3. *FEMS Microbiol Lett* 16:241–245.

US Environmental Protection Agency. 2005. TSCA work plan chemical, problem formulation and initial assessment: Tetrabromobisphenol A and related chemicals cluster flame retardants. EPA 740-R1-4004. Office of Chemical Safety and Pollution Prevention. Washington, DC.

Valese AC, Molognoni L, de Sá Ploêncio LA, de Lima FG, Gonzaga LV, Górniak SL, Daguer H, Barreto F, Oliveira Costa AC. 2016. A fast and simple LC-ESI-MS/MS method for detecting pyrrolizidine alkaloids in honey with full validation and measurement uncertainty. *Food Control* 67:183–191.

Veldhoen N, Boggs A, Walzak K, Helbing CC. 2006. Exposure to tetrabromobisphenol-A alters TH-associated gene expression and tadpole metamorphosis in the Pacific tree frog *Pseudacris regilla*. *Aquat Toxicol* 78:292–302.

Waaijers SL, Hartmann J, Marieke Soeter A, Helnmus R, Kools AE, de Voogt P, Admiraal W, Parsons JR, Kraak MHS. 2013. Toxicity of new generation flame retardants to *Daphnia magna*. *Sci Total Environ* 463:1042–1048.

Wang X, Liu J, Liu Q, Du X, Jiang G. 2013. Rapid determination of tetrabromobisphenol A and its main derivatives in aqueous samples by ultrasound-dispersive liquid–liquid microextraction combined with high-performance liquid chromatography. *Talanta* 116: 906–911.

Wang Y-Q, Zhang H-M, Cao J. 2014. Exploring the interactions of decabrominated diphenyl ether and tetrabromobisphenol A with human serum albumin. *Environ Toxicol Pharmacol* 38:595–606.

Wu J-P, Guan Y-T, Zhang Y, Luo X-J, Zhi H, Chen S-J, Mai B-X. 2011. Several current-use, non-PBDE brominated flame retardants are highly bioaccumulative: Evidence from field determined bioaccumulation factors. *Environ Int* 37:2010–215.

Yang S, Wang S, Liu H, Yan Z. 2012. Tetrabromobisphenol A: Tissue distribution in fish, and seasonal variation in water and sediment of Lake Chaohu, China. *Environ Sci Pollut Res* 19:4090–4096.

Zhang S-H, Zhang Y-X, Ji G-X, Xu H-Z, Liu J-N, Shi L-L. 2016. Determination of bisphenol A, Tetrabromobisphenol A and 4-tert-octylphenol in children and adults urine using high performance liquid chromatography-tandem mass spectrometry. *Chin J Anal Chem* 44:19–24.

Zhao J, Yan X, Li H, Zhang P, Zhou T, Li Y, Chen Y, Ding L. 2016. High-throughput dynamic microwave-assisted extraction coupled with liquid–liquid extraction for analysis of tetrabromobisphenol A in soil. *Anal Methods* 8:8015–8021.

Zhou X, Guo J, Zhang W, Zhou P, Deng J, Lin K. 2014. Tetrabromobisphenol A contamination and emission in printed circuit board production and implications for human exposure. *J Hazard Mater* 273: 27–35.

