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Simultaneous determination of tetrabromobisphenol A, tetrachlorobisphenol A, bisphenol A and other halogenated analogues in sediment and sludge by high performance liquid chromatography-electrospray tandem mass spectrometry

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Abstract

A high performance liquid chromatography–electrospray (negative) ionization-tandem mass spectrometry (HPLC–ESI(−)-MS–MS) based method has been developed for simultaneous determination of bisphenol A (BPA), tetrachlorobisphenol A (TCBPA), and tetrabromobisphenol A (TBBPA), as well as lower brominated BPA analogues in sediment and sludge samples. Samples were extracted with MTBE, target compounds were partitioned by aqueous solution of sodium hydroxide. The solution was subsequently acidified, and the enrichment and desalting were performed via solid phase extraction (SPE). After cleanup the target compounds were determined by HPLC–ESI(−)-MS–MS. The method limits of quantification (MLOQs) from sediment and sludge for BPA, monobromo-bisphenol A (mono-BBPA), dibromo-bisphenol A (di-BBPA), tribromo-bisphenol A (tri-BBPA), TBBPA and TCBPA were 0.15, 0.02, 0.02, 0.04, 0.05, and 0.03 ng/g (dry weight), respectively. Mean recovery of the analytes from spiked samples ranged from 70 to 105%, and the relative standard deviation ranged from 4.9 to 13.1%. The method was successfully applied to sediment and sludge samples analysis.

Keywords: Liquid chromatography tandem mass spectrometry; BPA; TCBPA; TBBPA; Sludge; Sediment

1. Introduction

Tetrabromobisphenol A (4,4′-isopropylidenebis(2,6-dibromophenol), TBBPA) is one of the primary components in a high volume, commercially-used flame retardant know as TBBPA. TBBPA is used as a reactive or additive flame retardant in polymers, such as ABS, epoxy and polycarbonate resins, high impact polystyrene, phenolic resins, adhesives, and others. In printed circuit boards TBBPA content may be as high as 34% by weight [1]. Despite the reactive properties of TBBPA, environmental release has been shown to occur for TBBPA and degradation products from both additive- and reactive-treated products [1]. TBBPA has been found in samples of air, soil, sediment and sludge, wildlife and human serum [2–10].

Similar to TBBPA, tetrachlorobisphenol A (4,4′-isopropylidenebis(2,6-dichlorophenol), TCBPA) is also used commercially as a flame retardant, but to a lesser extent than TBBPA. Recently investigation showed that chlorination of BPA in aqueous media may also result in the formation of TCBPA [11]. Regardless, there are very few published reports on TCBPA as an environmental residue in the literature [10,12].

Bisphenol A (BPA) is a major industry product and widely used in the production of epoxy resins and polycarbonate

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plastics, which are used as food-contact surface coatings for cans, metal jar lids, coatings, finishes, automobile parts, high-impact windows and others [13]. BPA is commonly found in sediment, sludge and environmental water samples, as well as drinking water, juice, milk and other food product [14,15].

Biodegradation studies have shown that TBBPA can be partly degraded to lesser brominated analogues under both aerobic and anaerobic conditions, in soil, and in river sediment [16]. This degradation is dependent on soil type, temperature, humidity, and the composition of the soil. It was reported that anaerobic incubation of the sediment with TBBPA and peptane–tryptone–glucose–yeast extract medium resulted in an 80% decrease in the TBBPA concentration and transformation to a non-brominated bisphenol A (BPA) metabolite [17]. TBBPA was reductively debrominated to BPA, and thus it is possible that degradation to other lesser brominated BPAs might occur [18].

The acute oral toxicity of BPA and its halogenated analogues for laboratory animals is low, but recent research indicates that these chemicals have high potential as endocrine disruptors in human and wildlife [19]. Although as much as 10,000 times less potent than the estrogen 17β-estradiol, BPA has been recognized as a relatively potent xenoestrogen in human and wildlife in vivo and in vitro studies [20]. TBBPA and lesser brominated analogues share structural similarities to thyroxine (T4), the major circulating form of thyroid hormone. With respect to T4, TBBPA competitively binds with human transthyretin (TTR) which is the major thyroid hormone transport protein in mammals and avian species [21]. It has also been reported that with a decreasing number of bromine atoms, polybrominated BPAs become poorer TTR binding competitors, but become increasingly potent agonists for estrogen receptor (ER)-mediated gene expression in, e.g. human breast and embroyonic kidney cell transfected with estrogen-responsive luciferase reporter gene construct (pEREtata-Luc) [22]. Like TBBPA, TCBPA has been shown to possess thyroid hormone disrupting potential [23].

Concern continues to rise with respect to the environmental presence and fate of TBBPA, TCBPA and BPA, due to high volume production, widespread commercial use and ubiquitous occurrence in aquatic environments. BPA, TCBPA, and TBBPA and its halogenated analogues are expected to associate with solid matrices such as sediments and suspended particulate matter [5]. Because of its partition coefficient ($\log K_{ow} = 4.5$) and low water solubility (0.72 mg/L), TBBPA in generally adsorbed to organic matter and is frequently not detectable in water samples. BPA has a lower $\log K_{ow}$ of 3.4, but it has been predicated that about 50% of BPA in the environment is bound to sediments or soils [24].

Few analytical methods have been reported for determination of BPA in sediment and soil [14,15]. BPA can be spectroscopically determined using HPLC with ultraviolet (UV) detection, although it is often not an adequately sensitive technique for BPA determination in environmental samples [17]. With four bromine atoms, TBBPA can be determined at very low levels in sample fractions by gas chromatography-electron capture detection (GC-ECD) or mass spectrometry with electron capture negative ionization detection (GC–MS(ECNI)) [4,5,10,25]. However, derivatization is necessary for GC separation, and GC-ECD or GC–MS(ECNI) is generally insensitive for lower brominated BPAs unless an effective electron-capturing functional group is introduced into the molecule by derivatization.

High performance liquid chromatography (HPLC) with mass spectrometric detection, e.g. single quadrupole MS, tandem quadrupole MS–MS, ion trap MS–MS and TOF-MS, has been successfully employed in the determination of TBBPA in sediment, sludge and biological tissue samples [6–8]. To our knowledge, simultaneous determination of BPA, TCBPA and TBBPA and its lower brominated analogues by HPLC–MS has not yet been reported. We presently report on a high performance liquid chromatography–electrospray (negative) ionization-tandem mass spectrometry (HPLC–ESI(−)-MS–MS) based method for the sensitive, precise and simultaneous determination of BPA, TCBPA, TBBPA, tribromo-bisphenol A (tri-BBPA), dibromo-bisphenol A (di-BBPA) and monobromo-bisphenol A (mono-BBPA) for sediment and sewage sludge samples.

2. Experimental

2.1. Chemicals and materials

The molecular structures of target compounds studied in this work are shown in Fig. 1 Tetrabromobisphenol A, tetrachlorobisphenol A and bisphenol A were obtained from Aldrich Chemical Co. (WI, USA) and were of minimum 97% purity. Ring-$^{13}C_{12}$ labeled TBBPA was obtained from Cambridge Isotope Laboratories Inc. (MA, USA) and was of minimum 99% purity. mono-BBPA and tri-BBPA were a kind gift from Drs. Göran Marsh and Åke Bergman (Department of Environmental Chemistry, Stockholm University, Sweden). 4,4′-Isopropylidenebis(2-bromophenol) (di-BBPA) was synthesized in our lab according to the method reported by Eriksson et al. [18]. Briefly, 0.5 g of BPA was dissolved in acetic acid (200 mL) and 0.24 mL bromine solution was added. The reaction mixture was stirred at room temperature for 4 h. The solution was neutralized with sodium hydrogen carbonate after 100 mL water was added. The product was liquid–liquid extracted using dichloromethane, and was subsequently purified by three successive column chromatography steps using silica gel. HPLC–ESI(−)-MS–MS and GC–MS(ECNI) analysis demonstrated that the final di-BBPA purity was >97%.

Individual standard stock solutions of BPA, TCBPA, TBBPA, tri-BBPA, di-BBPA and mono-BBPA of 1 mg/mL of each were prepared by dissolving in methanol accurate amounts of pure standard compounds. Working solutions (10 μg/mL of BPA and 2 μg/mL of other compounds) were prepared by mixing individual stock solutions, followed by necessary serial dilution with methanol. The internal standard, $^{13}C_{12}$-labeled TBBPA, working solution concentra-

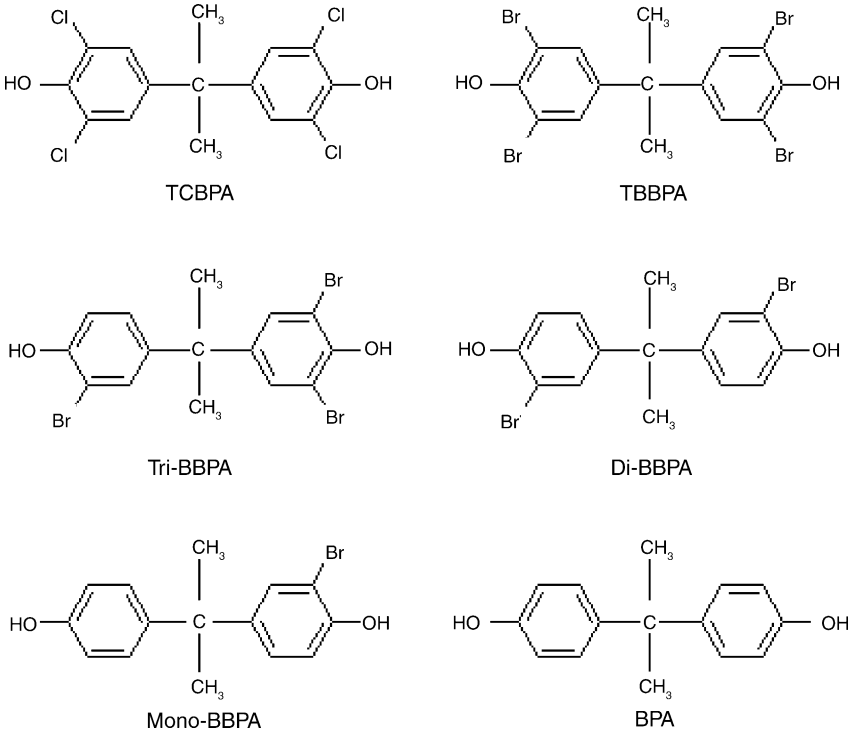

Fig. 1. Molecular structures of TCBPA, TBBPA, tri-BBPA, di-BBPA, mono-BBPA and BPA.

tion of 200 ng/mL was prepared by appropriate dilution in methanol.

Anhydrous sodium sulfate was purchased from VWR International Inc. (ON, Canada) and pretreated in a muffle furnace at 650 °C for 12 h to destroy all possible organic contamination. All other solvents used (anhydrous ethanol, hexane, dichloromethane, acetonitrile, methanol, acetone, *tert*-butyl methyl ether (MTBE)) were OMNISOLV grade solvent and purchased from VWR International Inc. (Mississauga, ON, Canada). For chromatographic and liquid–liquid partitioning purposes, water was purified (18 MΩ/cm quality) by a Milli-Q system (Millipore, Bedford, MA, USA).

The 12-port Visiprep SPE Vacuum Manifold were purchased from Supelco (PA, USA). All glassware, except volumetric equipment, was washed with detergent solution, rinsed with distilled water and then heated at 450 °C overnight prior to use. Glass volumetric equipments were rinsed with acetone and hexane after they were washed and air dried.

2.2. Samples

Surface sediments, which included 46 samples from a 0 to 10 cm depth and 9 from a 10 to 20 cm depth, were collected from 48 locations in Lake Erie during May–June 2004, and subsequently frozen at −20 °C for storage. In preparation for chemical analysis, the frozen sediment was thawed overnight in a refrigerator (4 °C). Sewage effluent samples were collected from the Little River Wastewater Treatment Plant (LR) and the West Windsor Pollution Control Plant (WW) located in the City of Windsor, Ontario, Canada. Sludge was separated from the liquid phase by centrifugation at 3000 rpm for 10 min. The moisture in the sediment and sludge samples were measured gravimetrically depending on the weight difference after heating a sub-sample at 105 °C overnight.

2.3. Sample preparation

A flow diagram illustrating the sample preparation procedure is shown in Fig. 2. A sample size of 10 g of sediment or sludge was ground with 30 g anhydrous sodium sulfate in glass mortar. The mixture was then transferred to extraction thimble and 100 μL of internal standard solution (200 ng/mL of $^{13}C_{12}$-labeled TBBPA in methanol) was spiked into sample. After 2 h the sample was Soxhlet extracted for 12 h with 150 mL of MTBE. The extract was rotary evaporated to 2 mL and transferred to a 50 mL centrifuge tube with 10 mL hexane. A volume of 4 mL of sodium hydroxide solution (1 M) was added for liquid–liquid separation of the neutral and phenolic compound fractions, which was repeated three times. The phenolic fractions were combined and acidified with 12 M HCl (pH = 2–3). The enrichment and desalting were performed by SPE using LC-C18 cartridges (500 mg × 3 mL, Supelco, USA) on SPE Vacuum Manifold. SPE cartridges were conditioned successively with 10 mL of water, 10 mL of acetone, 10 mL of methanol and 10 mL of water at a flow rate of 3 mL/min. After the sample was loaded on the cartridge and subsequently washed with 30 mL of water, the cartridges were dried on the vacuum system for 30 min. The analytes were eluted from the SPE cartridges with 10 mL acetone, and the collected eluent was concentrated to an approxi-

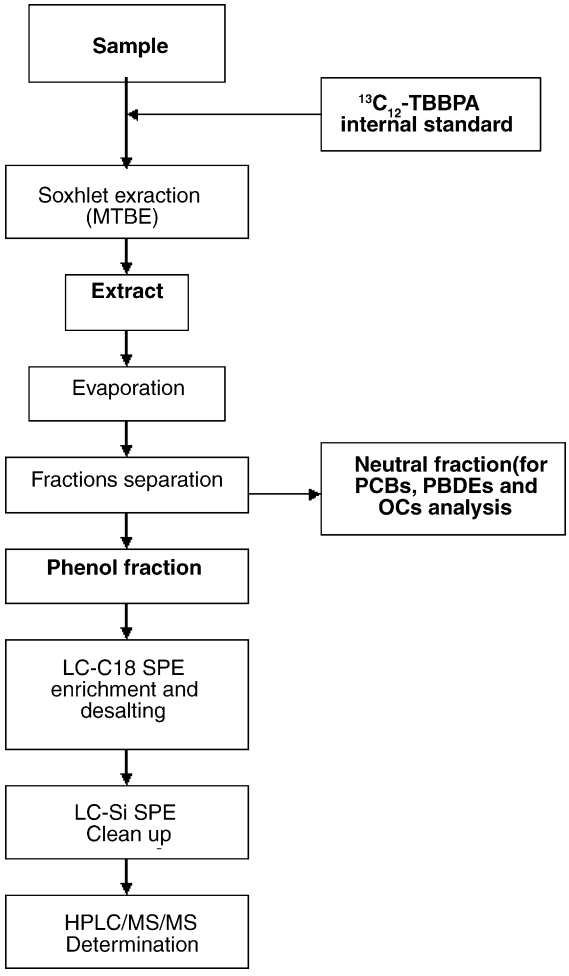

Fig. 2. Flow diagram of the analytical method for the extraction, isolation and determination of BPA and halogenated BPA analogues in sediment and sludge samples.

mate volume of 0.5 mL under a gentle stream of nitrogen. After 2 mL of anhydrous ethanol was added to the solution, the sample was taken to dryness under a gentle stream of nitrogen and reconstituted in 0.2 mL of dichloromethane:*n*-hexane (1:9, v/v). A SPE clean-up step was then performed using LC-Si SPE cartridges (500 mg × 3 mL; Supelco, USA). The LC-Si SPE cartridges were conditioned by successive washes of 10 mL dichloromethane and then 10 mL of *n*-hexane, followed by the loading of sample solution. The cartridge was first washed with 10 mL dichloromethane:*n*-hexane (1:9, v/v), which was discarded. The analytes were then eluted with 10 mL of MTBE:dichloromethane (1:9, v/v) and collected. This eluent was concentrated to dryness under a gentle steam of nitrogen and reconstituted with methanol to a final volume of 100 μL for HPLC–ESI(−)-MS–MS analysis.

2.4. HPLC–ESI(−)-MS–MS analysis

A Quattromicro triple-quadropole mass spectrometer (Micromass, Manchester, UK) coupled to a Waters 2695 (Waters Corporation, Milford, MA) HPLC system was used for all HPLC–ESI(−)-MS–MS analysis. The data were processed using Masslynx NT software (v 4.0).

Table 1
ESI(−)-MS–MS parameters for the determination of bisphenol A (BPA) and halogenated-BPA analogues

BPA compound	Cone voltage (V)	Collision energy (eV)	MRM channels	
			Precursor ion	Product ion
BPA	30	15	227	212
mono-BBPA	40	40	307	81
di-BBPA	40	40	385	81
tri-BBPA	40	40	465	81
TBBPA	40	40	543	81
TCBPA	40	30	365	286
$^{13}C_{12}$-labeled TPPBA	40	40	555	81

HPLC separation was performed using a 150 mm × 2.1 mm i.d., 4 μm particles Genesis C18 120A column (Jones Chromatography Limited, Hengoed, UK) preceded by a Phenomenex C18 guard column (4 mm length, 2.0 mm i.d., 4 μm particles) (Phenomenex, CA, USA) at a flow rate of 0.2 mL/min. A sample volume of 20 μL was injected into the separation system using an autoinjector. Separation was performed using 25% methanol/75% water (A) and methanol (B) as mobile phases. The elution profile started with an initial condition of 100% A, after injection the mobile phase was changed to 100% B and held constant for 15 min, then decreased linearly to the initial condition (100% A) over a 5 min period, and finally held 100% A for 10 min.

The HPLC–ESI-MS–MS was operated in the ESI negative mode and in multiple reaction monitoring (MRM) mode. The MS–MS settings were optimized by using 10 μL injections of individual standard solutions at concentrations of 1 mg/mL for selection of suitable monitoring ions (product ions and precursor ions). The working solution (10 μg/mL of BPA and 2 μg/mL of other individual target compounds) was used to optimize HPLC separation parameters. The potential of the electrospray needle was held at 3.5 kV. The nebulizing and cone gas flow rates were 300 and 50 L/h, respectively, while the desolvation and source temperatures were 200 and 130 °C, respectively, during MS–MS analysis. Ultra high pure argon (99.999%) (BOC Canada Limited, ON, Canada) was used as the collision gas at 3×10^{-3} mbar pressure for multiple reaction monitoring. The collision energy, cone voltage, precursor ion and product ion were adjusted to optimize performance for each compound and summarized in Table 1.

2.5. Recovery experiments and quantification

The recovery and overall method reproducibility were investigated with spiked sediment samples ($n = 6$) at the 100 ng/g level for BPA and 20 ng/g level for the other analytes. The spiked samples were subjected to the described

workup procedure (Fig. 2). The identification of analytes was based on retention times and their respective MRM channels. An internal standard method was used for quantification. Quantitative determinations were done using a six multi-level calibration curve spanning the range of anticipated analyte concentrations in real samples.

3. Results and discussion

3.1. Sample extraction and cleanup

Several extraction solvents have been used for BPA or TBBPA analysis [5–7,14,15]. We tested several of these extraction solvents, and the results showed that MTBE was superior relative to other solvents, e.g. *n*-hexane/acetone, for BPA or TBBPA isolation. MTBE has been used for BPA or TBBPA extraction in the analysis of plasma or blood samples, but has rarely been applied in extraction from solid matrices [9]. For most reported methods to determine TBBPA or BPA by HPLC–MS, optimal sample cleanup was noted as being dependent only on the C-18 SPE cartridge step. However, in our experiments, C-18 SPE cartridge cleanup was not always beneficial with sediment samples. C-18 SPE cleanup may be more suitable for sediment samples where the organic compound contamination is low. When target compounds are pre-concentrated, any potentially interfering contaminants will also be concentrated, which are often present in far greater quantities than the target compounds in the sample. Since we intended to expand this method to the analysis of different sediment samples, a cleanup process using SPE silica cartridge was employed for all sample analysis.

3.2. HPLC separation

Various mobile phase compositions have been recently reported for BPA or TBBPA analysis by HPLC–MS based approaches [6,7,10,12,14,18,26]. In a few cases, additives are present in the mobile phase to optimize the chromatographic separation and/or ionization response. Different solutions and additives were presently tested in our experiments. We found that when using methanol as the mobile phase, the response of target compounds was about one-third greater than when using acetonitrile. Formic acid or tris(hydroxymethyl)aminomethane additives in the methanol mobile phase were found to give decreased ESI(−) response for all the target compounds. Using methanol with an additive of ammonium acetate (1 mM) instead of just methanol as mobile phase resulted in a 37% increase in ESI(−) response for BPA, but the TCBPA response was deceased 49% relative to a methanol mobile phase alone. Furthermore, using a methanol mobile phase alone resulted in a more stable detector baseline and thus lowering the method limit of quantification (MLOQ). Therefore, using methanol and water as mobile phase was concluded to be more advantageous for this quantitative analysis.

An on-column concentration technique was applied in our experiments to improve the separation and peak sharpness when a relatively large volume of sample solution (20 μL) was injected into micro-HPLC column. The principle of this on-column pre-concentration method, referred to as a two-sided bracketing of the sample injection [27], is described as follows. In this injection technique, the introduced sample plug is completely bracketed by the mobile phase with water, or low concentration of methanol in water (in our method 25% methanol was used). The analytes are first concentrated at the top of the column during the injection stage, then separation of the pre-concentrated analytes is performed with a suitable mobile phase (in this method 100% methanol was used). Experiments have shown that if the initial composition of mobile phase is kept at 25% methanol (in water) for 5 min before the mobile phase is changed to 100% methanol, the retention time of all the target compounds will shift about 5 min longer. This assures that at the beginning no separation will occur and the analytes can be focused on the top of column with the 25% methanol aqueous solution. When using 100% methanol as mobile phase, all the target compounds co-eluted as one peak. Different gradient elution conditions were also tested to optimize the peak separation, which was found to be unnecessary. The separation can be accomplished with a mobile phase change to methanol after the on-column pre-concentration step. Using this technique a relatively large volume of sample solution (up to 40 μL) could be directly injected into the micro-column without causing a reduction in chromatographic peak resolution or the quality of the peak shape. For all target compounds the peaks were sharp with widths <0.25 min at the baseline, and therefore all analytes could be resolved with the exception of TCBPA and tri-BBPA. However, TCBPA and tri-BBPA had different MS–MS reaction channels (both precursor ion and product ion), and thus the signal could be resolved into its two components for analyte quantification.

3.3. ESI(−) and MRM parameters

Since the use of a single MS can give rise to false positives as a result of lower analyte selectivity, quantitative overestimates are possible, and thus HPLC–MS–MS is advantageous. Using individual standard solutions (1 mg/mL) of BPA, TCBPA, TBBPA, tri-BBPA, di-BBPA and mono-BBPA, full-scan ESI(−)-MS mass spectra (*m*/*z* of 50–600 amu) were obtained. In all cases, the pseudo-molecular isotopic ion cluster $[M-H]^-$ dominated the mass spectra at high mass range (not shown). Therefore, the $[M-H]^-$ isotope ions were chosen as precursor ions. For these $[M-H]^-$ precursor ions, the collision induced dissociation (CID) mass spectra are shown in Fig. 3 (the cone voltage and collision energy are listed in Table 1). For BPA, the $[M-H]^-$ ion from BPA at *m*/*z* 227 gave major product ions at *m*/*z* 212 and 133, which are $[M-CH_3]^-$ and $[M-C_6H_5O]^-$, respectively (Fig. 3A). Therefore, $[M-CH_3]^-$ ion was chosen for the quantitative

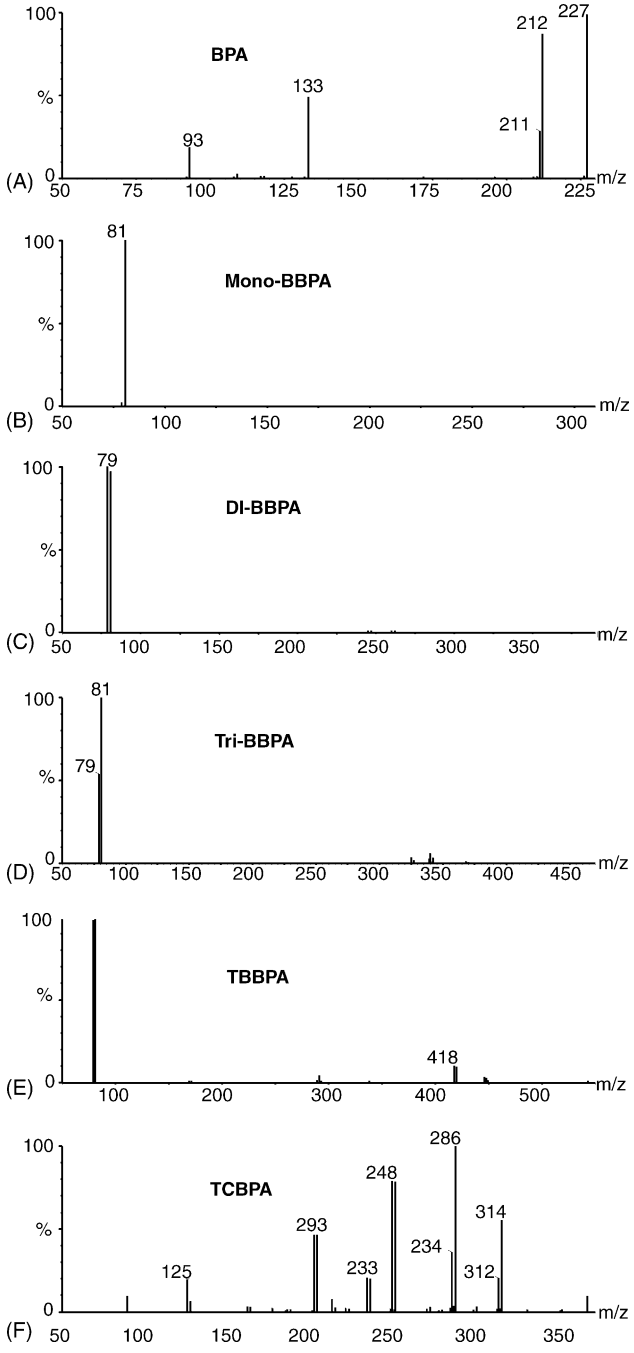

Fig. 3. Collisionally-induced dissociation, full-scan mass spectra for electrospray negative ionization, tandem MS–MS analysis of: (A) bisphenol A (BPA), (B) monobromo-BPA (mono-BBPA, (C) dibromo-BPA (di-BBPA), (D) tribromo-BPA (tri-BBPA), (E) tetrabromo-BPA (TBBPA) and (F) tetrachloro-BPA (TCBPA). See Table 1 for CID energies.

MRM channel for BPA. For mono-BBPA, di-BBPA, tri-BBPA and TBBPA the dominant product ions were m/z 81 and 79, which corresponds to isotopic Br^- anions (Fig. 3B–E), and m/z 81 was chosen for the quantitative MRM channels for these analytes. This result differs from that reported by Saint-Louis and Pelletier [6], where for TBBPA determination, m/z 528 and 448 were used as product ions for MRM. In the present study, the sensitivity of MS–MS using 543 > 81 for the determination of TBBPA was about 10 times more sensitive than using 543 > 528. For TCBPA determination, the ion at m/z 314 corresponds to $[M - ClOH]^-$, and m/z 286 was chosen for the quantitative MRM channel, which most probably represents a rearrangement after an aromatic ring is ruptured.

3.4. Assessment of matrix effects

Although HPLC–MS based approaches can be highly analyte selective in discriminating from signal interferences, interferences present during the ESI process must be eliminated to yield accurate quantitative data, which is a fundamental requirement in HPLC–ESI-MS method development [28]. Sample cleanup is necessary to minimize or eliminate matrix effects on ionization suppression or enhancement. Ionization suppression (or enhancement) is a phenomenon in any type of HPLC–MS, especially incorporating ESI, which occurs in the ionization process and modifies the ionization yield [29]. The mechanism of ion suppression is not fully understood. Ion suppression is mostly attributed to the competition that occurs between matrix components (e.g. humic acids and inorganic salts) and analytes for ionization or access to the droplet surface for the gas phase emission [26]. This effect may reduce or increase the intensity of analyte ions and affect the reproducibility and accuracy of the assay [30].

Elimination of ion suppression/enhancement effect is essential for trace level quantification of pollutants in complex environmental matrices such as sediment and sludge. In the present sediment and sludge samples, the concentration of interfering components may be in the order of several magnitudes higher relative to the target compounds. The use of isotope labeled internal standards (ILIS) can overcome the matrix effect problem, but isotope labeled standards are not always available for all the target compounds. On another hand, this cannot improve the MLOQ. Therefore, incorporation of effective sample clean up and HPLC parameters is necessary while an appropriate internal standard is used.

It is well known that some inorganic or organic salts show serious ionization suppression in HPLC–MS analysis, especially for some polar compounds [6,30,31]. Because there is a large amount of inorganic salt (NaCl) introduced in the partition process, a desalting process is necessary for sample preparation. In our present method, during the enrichment and desalting process target compounds absorbs on the cartridge and salts in the sample pass through the SPE. Without desalting process by LC-C18 cartridges, our preliminary spiked sample recovery was no more than 20% for BPA and consistently >120% for TBBPA (not internal standard recovery correction). To assess the effect of salts in sample solution, a series of standard solutions with varying NaCl concentrations were analyzed with an isocratic methanol/water (80:20, v/v) mobile phase. In this experiment the retention times for

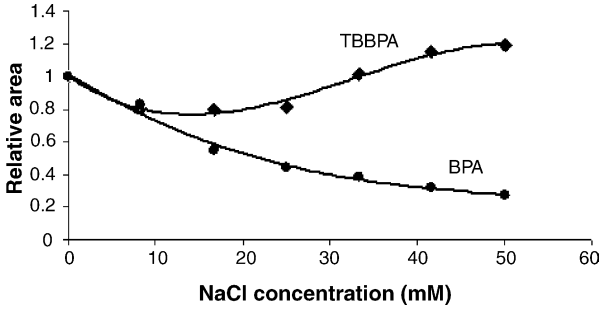

Fig. 4. A plot of relative responses of BPA and TBBPA by HPLC–MS–MS(ESI−) with increasing concentrations of sodium chloride in sample solution.

BPA and TBBPA were 2.9 and 4.4 min, respectively. The results were shown in Fig. 4. At a NaCl concentration of 50 mM the ESI(−) response of BPA was reduced to about 27%.

Ionization suppression could also be derived from large amount of anhydrous sodium sulfate using during the sample drying step, which may result in residuals in the final volume (0.1 mL) for HPLC–MS–MS determination. Therefore, anhydrous ethanol is added to the sample solution and then blown it to dryness, because ethanol and water can be evaporated as binary azeotrope. To test the potential matrix interferences, a known amount of target compounds (0.1 μg of BPA and 0.02 μg of other analytes) was spiked into sample extract (from two different sediments), which had been cleaned up with the method described above, and analyzed with HPLC–MS–MS. The peak areas difference from the spiked extract and corresponding standard solution is in the error range by repeated injection of standard solution (within 8% difference).

3.5. Method validation

Method recovery is influenced by both analyte losses from the extraction/cleanup procedure and from matrix interferences. Because there is presently no reference material available for these pollutants, the method was validated using sediment samples spiked at levels of 100 ng/g for BPA and 20 ng/g for other individual analytes. The internal standard-corrected mean recoveries ranged from 70% (di-BBPA) to 105% (TCBPA). The percent relative standard deviation (%RSD) ranged from 4.9 to 13.1% (Table 2), and the inter-day variations were within 5.2% (TBBPA) to 21.4% (TCBPA). The ESI(−)-MS–MS response range linearity was assessed for all analytes from the method limits of quantification (MLOQ) to 5 μg/mL for BPA and 1 μg/mL for the other halogenated BPAs corresponding to 50 ng/g and 10 ng/g in real samples (for 10 g sample). The response was found to be linear in the validated range, with correlation coefficient (r^2) ranging from 0.9956 (di-BBPA) to 0.9969 (TBBPA). MLOQs were estimated as the signal of the blank plus 10-fold the standard deviation of the blank [6]. The MLOQs ranged from 0.02 ng/g (mono-BBPA) to 0.15 ng/g (BPA) sample dry weight.

Table 2
Method recovery, repeatability (RSD for $N = 6$) and the method limits of quantification (MLOQs) for the determination of BPA and halogenated BPA analogues in sediment and sludge samples

Compound	Recovery (RSD)	MLOQ (ng/g, d.w.)
BPA	71.5 (9.7)	0.15
mono-BBPA	72.3 (9.6)	0.02
di-BBPA	70.3 (4.9)	0.02
tri-BBPA	96.6 (9.5)	0.04
TBBPA	102.2 (5.1)	0.05
TCBPA	105 (13.1)	0.03

3.6. BPA and halogenated BPAs in sediment and sewage sludge samples

In the sewage sludge samples (LR and WW, City of Windsor), BPA, TCBPA, TBBPA, tri-BBPA, di-BBPA and mono-BBPA were all detected and quantifiable (Fig. 5), with the exception of mono-BBPA in the LR2 sample (Table 3). The order of concentration in the sludge samples were generally BPA > TBBPA > TCBPA, > tri-BBPA > di-BBPA > mono-BBPA. To our knowledge, this is the first published report of the multiple determination of mono-BBPA,

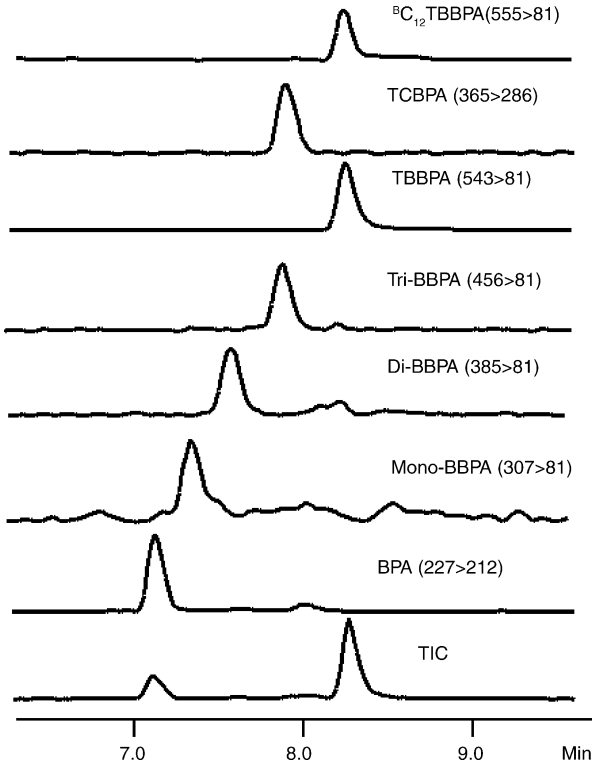

Fig. 5. The individual MRM chromatograms for BPA and halogenated BPA analogues, and the total ion chromatogram (TIC) for all the MRM channels in a typical sludge samples from waste treatment plant (WW) in the City of Windsor, Ontario, Canada.

Table 3
Concentration of bisphenol A (BPA) and halogenated BPA analogues found in sludge samples (ng/g d.w.) collected from the Little River Wastewater Treatment Plant (LR) and the West Windsor Pollution Control Plant (WW) in Windsor, Ontario, Canada

Sample	BPA	mono-BBPA	di-BBPA	tri-BBPA	TBBPA	TCBPA
LR 1B	7.01	1.00	0.29	0.26	5.75	0.47
LR 2B	3.78	0.10	0.18	0.11	2.09	0.42
WW 1B	37.49	0.25	0.52	0.55	28.30	0.54
LR 2A	74.38	N.D.	0.22	0.23	5.34	0.14

di-BBPA, tri-BBPA and TCBPA in an environmental sample. Furthermore, in the present sludge samples, it is clear that TBBPA can undergo degradation by debromination, although it is not clear whether such degradation is the consequence of debromination of TBBPA during the wastewater/sludge treatment process, or perhaps other abiotic (e.g. photocatalyzed) debromination processes. Our findings are consistent with previous studies that have suggested that dehalogenation of TBBPA in environmental systems is an important degradation path for TBBPA [17].

In 65% of the 55 surface sediment samples from Lake Erie, BPA was quantifiable up to concentrations of 6.1 ng/g (d.w.). In contrast, TBBPA was detected only in three of these sediment samples (S/N = 3), and only in one sample could TBBPA be quantitatively determined with concentration of 0.51 ng/g (d.w.). tri-BBPA was also found in the same sediment sample at a concentration of 0.34 ng/g (d.w.). Neither di-BBPA nor mono-BBPA was detectable in any of the samples from Lake Erie down to sub-ng/g (d.w.) levels. We are currently investigating in more detail the spatial distribution and fate of these BPAs and other brominated and chlorinated contaminants in sediment samples from sites spanning Lake Erie.

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