Trace level determination of bisphenol-A in wastewater and sewage sludge by high-performance liquid chromatography and UV detection
Bahman Banihashemi and Ronald L. Droste

ABSTRACT

The purpose of this study was to develop and optimize a simple and economical method for the extraction and determination of bisphenol-A (BPA), using high-performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection at environmentally relevant concentrations in both dissolved and particulate phases. To clean-up and pre-concentrate liquid samples, solid-phase extraction (SPE) method was optimized with regard to pH, volume, washing and elution solvents for high recovery of BPA and good clean-up. For sludge samples, four extraction methods, microwave-assisted extraction (MAE), ultrasonication extraction (USE), accelerated solvent extraction (ASE) and high-pressure homogenizer (HPH), were compared for isolation of BPA from activated sludge samples. Analysis was performed by optimized procedures using HPLC–UV. Recoveries of BPA from liquid and solid phases were determined to be 90–105 and 60–90%, respectively. MAE had the highest recovery among examined extraction methods. The method detection limits were 100 ng/L and 100 ng/g dry weight. To validate the method, a mass balance study was conducted with 100 mL spiked mixed liquor volatile suspended solids (VSS) samples from three laboratory-scale porous pot reactors and concentrations of BPA in liquid and solid phases were determined using the optimized conditions. The results had an average 86% overall recovery for all samples.

Key words | analytical method, bisphenol-A, extraction, sewage sludge, wastewater

INTRODUCTION

Bisphenol-A [2,2-bis(4-hydroxyphenyl)propane] (BPA) is a building compound for the manufacture of plastics, epoxy resins, and polycarbonate resins. BPA has also been incorporated into a number of products such as adhesives, construction materials, electrical and electronic parts, paints, coating of cans, automotive lenses, compact discs, and thermal paper (Cousins et al. 2002). BPA contamination in the environment may happen due to discharge from manufacturing facilities, effluents from wastewater treatment plants (WWTPs), as well as leaching from various BPA-containing products, particularly PVC plastics (Cousins et al. 2002). BPA contamination in Canadian sewage treatment plants (STPs) was reported in Lee & Peart (2000a) in which BPA was detected in all of 72 examined sewage samples with concentrations ranging from 0.08 to 4.98 μg/L for the influent, 0.01 to 1.08 μg/L for the effluent and 0.033 to 36.7 μg/g for sewage sludge. Mohapatra et al. (2011) measured BPA in samples from an urban community Quebec WWTP located in Quebec City, Canada, and showed the presence of BPA in the influent and the effluent samples with mean concentrations of 1.68 and 0.41 μg/L, respectively. BPA is also an endocrine disruptor (Krishnan et al. 1993; Fujimoto et al. 2006; Prasanth et al. 2010) that can cause acute toxicity to aquatic organisms between concentrations of 1 and 10 μg/L (Alexander et al. 1988) and may affect human development throughout the fetal period (Rubin & Soto 2009). Due to the occurrence of BPA in Canadian WWTPs and the environment, an adequate
analytical method for the detection of BPA in wastewater and sewage sludge is needed in order to establish a database for BPA, determine its concentrations in the WWTPs, and evaluate its fate in the Canadian environment. It has been found that laboratory tests using chemical concentrations greater than those found in nature may lead to erroneous conclusions about microbial transformations in nature or biotreatment systems (Alexander 1985; Berg & Nyholm 1996; Gaulke et al. 2008); therefore, it is important to establish the fate of any micropollutants at trace level concentrations. However, the trace determination of BPA in wastewater and activated sludge samples requires robust and complex processes that include various steps, such as sample collection, extraction, clean-up and quantification. In sludge samples, some molecules remain bonded with proteins and other biomolecules, which requires robust extraction procedures to remove it from particulates and solubilize it.

Several extraction procedures have been developed for the isolation of BPA from environmental samples. For liquid samples the most common method used is solid-phase extraction (SPE) (Kuch & Ballschmiter 2007; Lee et al. 2005), although liquid–liquid extraction (González-Casado et al. 1998; Varelis & Balafas 2000), Soxhlet extraction (Pryor et al. 2002), and solid-phase microextraction (Helaleh et al. 2001; Chang et al. 2005; Huang et al. 2005) have also been reported. Among these extraction techniques, SPE has the advantage of relatively low solvent usage, good reproducibility and repeatability, and convenient operation (Gang et al. 2005). For solid samples, several extraction methods have been used, including pressurized liquid extraction (Agüera et al. 2003; Gang et al. 2005; Ferrer et al. 2011), supercritical fluid extraction (SFE) (Lee & Peart 2000b), microwave-assisted extraction (MAE) (Pedersen & Lindholst 1999; Liu et al. 2004), Soxhlet extraction (Zhao et al. 2008), Soxtec extraction (Jeannot et al. 2002), and ultrasonic extraction (USE) (Petrovic & Barcel 2001; Petrovic et al. 2002). The MAE technique has proven to be more efficient than the Soxhlet extraction due to less solvent consumption and quicker extraction time (Diagne et al. 2002). The determination of BPA in WWTPs has been analyzed mostly by chromatographic methods either by liquid chromatography (LC) (Motoyama et al. 1999; Benijs et al. 2004; Carabias-Martínez et al. 2004) or by gas chromatography (GC) (Meesters & Schröder 2002; Urase & Kikuta 2005) with or without mass spectrometric (MS) detection. GC/MS as a commonly used method has been reported to have good sensitivity and low detection limit for BPA trace determination (Petrovic & Barcel 2001; Hernando et al. 2004; Gatidou et al. 2007); however, it requires a derivatization procedure, which generally takes up to several hours to be completed. On the contrary, the micropollutant analyses using high-performance liquid chromatography (HPLC) with a choice of detectors, such as ultraviolet (UV), fluorescence and electrochemical detection, only require a simple and rapid pre-treatment method (Sajiki 2001). Mohapatra et al. (2011) have reviewed the analytical methods in the literature and concluded that among the possible detection methods, MS detection, especially LC/MS, showed significant selectivity when the target compound existed in a complex matrix such as sludge and sediments. High sensitivity and popularity of the LC–MS/MS system makes it a powerful tool for quantifying BPA in very low concentrations (Laganà et al. 2004; Baugros et al. 2008; Mohapatra et al. 2010; Seyhi et al. 2011). However, an MS detector is a complex machine that requires skilled personnel for operation. Also, it is not easy to maintain high stability of analytical results, especially for low concentrations of analyte (Hammarling et al. 2000; Le Blanc et al. 2009). Additionally, this detection method is not available in every environmental laboratory and it is costly. Therefore researchers have tried to develop analytical methods using widely used HPLC systems to make it more feasible for laboratory technicians to detect BPA in environmental samples (Naassner et al. 2002; Clara et al. 2004; Zhao et al. 2008; Hadjmohammadi & Saeidi 2010). Nevertheless, most of these researchers have looked at higher BPA concentrations than those found in the environment, and mainly in liquid samples, or they have used other detection methods such as fluorescence instead of widely used UV detection.

The purpose of this study was to develop, optimize and fully validate a simple, fast and precise integrated analytical method for the trace determination of BPA in the dissolved and particulate phases of wastewater, as well as in sewage sludge. SPE and HPLC-UV were used for liquid samples based on their availability and ease of operation, while MAE, high-pressure homogenizer (HPH), accelerated solvent extraction (ASE), USE, followed by SPE were applied and compared for solid samples before the HPLC analysis.
MATERIAL AND METHODS

Chemicals

Methanol (MeOH), hexane, acetonitrile (ACN), dichloromethane, and acetone, used for cleaning and extraction purposes, were purchased from Fisher Scientific (ON, Canada) and were all of HPLC grade. BPA (>97% purity assay) was obtained from Sigma-Aldrich (ON, Canada). BPA stock solution was prepared in 45% ACN/55% Milli-Q water at 1 mg/L and was used to regularly prepare working standard solutions for calibration and spiking experiments. Supelclean LC-18 (3 mL, 500 mg) cartridges used for extraction and clean-up samples were supplied by Sigma-Aldrich (Oakville, ON, Canada). The Zorbax Eclipse plus C18 chromatography column (4.6 × 150 mm, 5 μm) was purchased from Chromatographic Specialties Inc. (ON, Canada). All other chemicals used in this study were supplied by Fisher Scientific (ON, Canada) and were of analytical grade. Milli-Q water was prepared in the laboratory using a Milli-Q/Millipore system with Super-C carbon, Ion-Ex, and Orgonex-Q cartridges.

Extraction of liquid samples

To clean-up and pre-concentrate the liquid samples, the SPE method using a commercial SPE cartridge (Supelclean LC-18) was optimized with regard to sample pH, sample volume, washing and elution solvents, for high recovery of BPA and good clean-up. The LC-18 cartridge was chosen in this study due to its higher recovery and reproducibility found in the literature (Gatidou et al. 2007).

For the optimization experiments, a set of 100 mL wastewater effluent samples were collected from laboratory-scale porous pot reactor and filtered through a pre-ashed glass fiber filter (GF/C, pore size 0.45 μm). Samples were then spiked with the 1 mL of 1 mg/L BPA standard solution. The spiked samples were mixed in an ultrasonic bath for 15 min to ensure efficient distribution of the compounds in the solution. LC-18 cartridges fitted on a vacuum apparatus (Chromabond, Fisher Scientific) were used to isolate BPA from the spiked wastewater effluent samples. SPE requires four main steps: conditioning, sample loading, washing and elution. Each step can be optimized based on the compound properties and matrix effect to increase the recovery rate. Many SPE procedures are introduced in the literature, such as EPA method 3535A; however, these methods are not specific for every compound nor for all cartridges and can only be used as a very good starting point. Therefore, the optimization of each step in the SPE method is important if highest recovery of a compound of interest is of concern. Conditioning the reversed phase cartridges such as LC-18 is usually proceeded with two tube-volumes of water-miscible organic solvent such as MeOH, and followed by two tube-volumes of buffer solution or water. Sample loading was performed by applying vacuum pressure at flow rate of 5 mL/min. Studies have shown that the sample pH and total sample volume affect the extraction efficiency and sample recovery (Hennion 2000; Gatidou et al. 2007; Hadjmohammadi & Saeidi 2010); consequently, the sample pH and the breakthrough volume were optimized in this study. To remove unwanted impurities from the sample and enhance the selectivity in the separation step, the washing solution needs to be stronger than the sample matrix to remove impurities, while it must not be able to extract and remove the compound of interest. Hence, the method was optimized based on the solvent composition in the washing step. In the final step, the elution solvent was chosen to remove the compounds of interest from the tube packing and increase the recovery of BPA. It is generally accepted that using small aliquots is more efficient than using larger aliquots in the elution step; therefore, three aliquots of tube size volume (3 × 3 mL) were chosen with dropwise flow in the elution step.

Extraction of solid samples

To find the proper extraction method with the highest recovery, low cost and ease of operation, extraction of the target compounds from sewage sludge was performed and compared by ASE, MAE, USE, and HPH. Initially, a set of 100 mL mixed liquor suspended solids (MLSS) samples was homogenized and deactivated using sodium azide solution (0.2% w/w) to prevent biodegradation. This concentration has been shown to inhibit the biodegradation process without causing any cell lysis, and also it does not cause change to sludge hydrophobicity (Guellil et al. 1998;
were mixed on an orbital shaker for 2 h at 200 rpm, and then were centrifuged for 30 min at 10,000 rpm. Two grams of sludge cake were collected, dried at 60 °C for 12 h and spiked with 1 mL of 200 μg/L BPA standard solution and allowed to accumulate in the biomass for 12 h. These prepared dried sludge samples (0.2 g) were used for MAE and ASE extraction while wet sludge samples (2 g) were collected for USE and HPH extraction. Prior to extraction, 5 g of anhydrous sodium sulfate was added to each sample to remove the moisture. Two additional control samples were prepared with the same procedure using Milli-Q water with no biomass and un-spiked biomass to measure the BPA loss to the glassware, as well as calculating the background BPA level. To investigate any BPA loss during the drying procedure, wet sludge samples were also prepared. All samples were extracted using ASE, USE, HPH and MAE, and then extracts were filtered through 0.22 μm filters (Millipore), evaporated by gentle nitrogen gas to dryness and re-dissolved in 10 mL of MeOH for further treatment by SPE. Since the compound of interest is already in MeOH and might be eluted along with other impurities existing in the sample, the SPE extraction method was modified to increase the reaction time between the sample and bonding silica inside the cartridge by eliminating the vacuum pressure and using the gravity force in the sample loading step.

The ASE system, Dionex ASE 200, was used to extract BPA from sludge samples. The samples were extracted in a 1:1 (v/v) mixture of acetone/hexane solvent solution (15 mL). The procedure for ASE system for BPA detection was reported in Gang et al. (2005). It consists of 5 min static time, two extraction cycles, 50% flush, 120 s purge, and repeating the flush and purge twice. For ultrasound extraction, a sonifier system (Branson model S-450D) with a frequency of 20 KHz was used. A procedure for extracting nonvolatile and semi-volatile organic compounds from solids such as soils, sludges, and wastes is presented in EPA method 355 (February 2007). A 2 g wet sludge sample was extracted with 1:1 (v/v) acetone/hexane solvent solution (100 mL) three times, using ultrasonic extraction with 50% energy cycle for 10 min. MW extraction was carried out with a Mars 5® (MW Accelerated Reaction System; CEM Corporation) MW oven. This extraction method uses MW energy to produce elevated temperature and pressure conditions in a closed vessel containing the sample and organic solvents to achieve analyte recoveries equivalent to those from Soxhlet extraction, using less solvent and taking significantly less time than the Soxhlet procedure. It is also validated on commercially available solvent extraction systems in EPA method SW-846-3546 (February 2007). The sample was extracted in a 1:1 (v/v) mixture of acetone/hexane solvent solution (20 mL) with MW power of 1,200 W (100%) and the extraction was performed in the temperature-controlled mode. The extraction procedure was a modified protocol based on the method reported by Mohapatra et al. (2011). The temperature was ramped to 110 °C for 10 min, and then held at 110 °C for 10 min. The extract was removed from the cell and the procedure was repeated three times. High pressure homogenizing extraction was carried out with the EmulsiFlex-C5 homogenizer which is powered by an electric motor, homogenizing valve, pressurized tank (adjustable between 500 and 30,000 psi or 35 and 2,000 bar) and temperature control system by heat exchanger. Two grams of wet spiked sludge was extracted with 30 mL of 1:1 (v/v) acetone/hexane solvent solution three times at 15,000 psi.

BPA was detected by HPLC (Hewlett-Packard, HP 1100). The system consisted of the degasser (G1322A), a quaternary pump (G1311A), an ALS auto sampler (G1315A), a Colcomp column oven (G1314A) and multi-wavelength UV-VIS detector (G1365A). A ZORBAX Eclipse Plus C18 (4.6 × 150 mm, 5 μm) column was used. The HPLC detection method was optimized as a part of this study based on the organic mobile phase selection and injection volume.

RESULTS AND DISCUSSION

Optimization of solid-phase extraction

Effluent samples of 100 mL volume were collected, filtered and spiked with 1 mL of 1 mg/L BPA standard solution to investigate the effect of sample pH on recovery of BPA. Generally, the solution properties, such as its pH value, plays a major role in speciation of weakly acidic compounds in aqueous solutions. To decrease the dissociation of weakly acidic
analytes, the water solution can be acidified; this could increase the extraction efficiency of the compound of interest if the non-dissociated form binds strongly to the SPE cartridges. In addition, if the molecule is polar and ionizable, the extraction efficiency using SPE columns can be improved by restraining the ionization of the analytes and by pH control of the sample. Since the pK_a value of BPA is around 9.8–10, at least a 2 pH difference between the pH of the sample solution and pK_a value of BPA is needed in order to prohibit the BPA from ionization. pH of samples was modified by adding HCl or NaOH to each sample before spiking with standard solution, and the recoveries of BPA samples with different pHs (2, 7, and 11) were compared (Figure 1). pH adjustment of the sample may also enhance the extraction efficiency of interested compound if ionizable polar interferences can be removed from the SPE column by ionizing the interferences and eliminating the binding force between interferences and the SPE column. This can be the reason why a pH of 2 had a higher recovery than a pH of 7 for BPA.

Breakthrough volume is the volume at which a particular solute passing continuously through a column begins to elute and it is useful in determining the total column sorption capacity for a specific compound. The breakthrough volume determination is a very important step in BPA extraction and determination level since it demonstrates the pre-concentration factor and capacity of the SPE cartridge used. In this study, Hennion’s procedure (Hennion 2000) was used to determine the breakthrough volume; therefore, the same amount of BPA standard solution was added to 10–1,000 mL of wastewater effluent samples (adjusted to pH 2) and passed through the cartridge. The retained analyte was then eluted with 3 mL of MeOH three times, dried by a gentle stream of nitrogen gas reconstituted in 1 mL of MeOH, and analyzed by HPLC system. Recoveries of samples were measured based on the height of the response chromatograph. The results showed that the recovery of BPA declined after the breakthrough volume of 100 mL (Figure 2).

The washing solution needs to be stronger than the sample matrix to remove impurities, while it must not be able to extract and remove the compound of interest; therefore, the proper percentage of solvent is needed to obtain the highest recovery of the compound of interest. Seven different MeOH/Milli-Q water ratios (10, 20, 30, 40, 50, 60, 70%) were chosen for optimizing the washing solution to remove the impurities and increase the recovery of BPA from wastewater effluent samples. The results show that the 30% MeOH solution did not wash out the BPA from the cartridge bonding material while it removed the impurities of sample; therefore, the maximum recovery was found when 30% MeOH and 70% Milli-Q water was used (Figure 3).

Analytes can be eluted using an organic solvent such as MeOH, tetrahydrofuran, isopropanol, ACN, acetone or ethyl acetate. In this study, three common water miscible organic solvents, MeOH, ACN and acetone were used in the SPE elution step, and the recoveries of BPA using these solvents were studied. The recovery of BPA was compared based on the result of HPLC analysis (Figure 4). MeOH and ACN were both found suitable for elution based on their strong polarity characteristics; however, MeOH was chosen due to its lower cost.

MeOH and ACN with or without buffer solution were reported as an adequate mobile phase to detect BPA. In this study, ACN was shown to have a lower noise level and increased the detection limit of BPA compared to MeOH; therefore, it was chosen for optimization studies. To detect the optimum mobile phase solution, different ratios of Milli-Q water/ACN with the same injection volume were used and samples with the same BPA concentration (20 μg/L) were compared for best peak shape, lower noise level and better calibration curve (Figure 5). In addition, sample volumes of 10, 20, 40 and 100 μL with the same concentration of BPA were injected into the HPLC and the effect of injection volumes were investigated (Figure 6).

Table 1 summarizes the SPE and HPLC-UV optimized condition, which was found as a part of this work to detect

Figure 1 | Effect of sample pH on recovery of BPA from wastewater effluent sample.
Figure 2 | Effect of sample volume on recovery of BPA in SPE sample loading step.

Figure 3 | Effect of volume of MeOH in solvent solution on recovery of BPA in washing step.

Figure 4 | Effect of solvent selection on recovery of BPA in SPE elution step.
Figure 5 | Effect of solvent ratio on recovery of BPA in HPLC–UV system.

Figure 6 | Effect of injection volume on recovery of BPA in HPLC–UV system.
BPA in environmentally relevant concentrations in liquid samples. To study the efficiency of the optimized SPE method for liquid samples, three replicate wastewater effluent samples, spiked at two trace level concentrations of 100 ng/L and 1 μg/L, were cleaned-up and analyzed based on the described method. Recovery rates were determined to be 93 ± 4.8 and 102 ± 3.3 for samples with 100 ng/L and 1 μg/L BPA concentrations, respectively.

The same extraction method was applied to extracted solid samples prior to HPLC analysis for more clean-up and pre-concentration purposes with slightly modified steps. The vacuum pressure sample loading step was replaced by the gravity pressure loading to increase the retention time. In addition, to eliminate the sludge matrix effect and the solvent front peak effect on the BPA response peak, the mobile phase solvent was replaced with less ACN content solvent (45%ACN/55%Milli-Q water) to decrease the polarity of the solution and increase the elution time of BPA. These changes are shown in parentheses in Table 1.

### Results of method comparison for extraction of BPA in sludge samples

The results of the recovery study for BPA are presented in Table 2. Recoveries were evaluated by analyzing three replicates and then applying the following equation:

\[
\text{Recovery(\%)} = \frac{(C_m - C_0)}{C_s} (1)
\]

where \(C_m\) is the measured concentration of the BPA in the spiked sample, \(C_0\) is the initial concentration of BPA in sludge sample, and \(C_s\) is the concentration of BPA in the spiked sample.

The results showed that the MAE method had the highest BPA recovery percentage followed by USE. Therefore, the MAE method was chosen in this study for further method validation and limit of detection (LOD) calculation. For the determination of the method LOD, 2 g of sludge sample was extracted and then spiked with 200 ng of BPA. The LOD of BPA was calculated as three times the signal-to-noise ratio of the relative chromatograph. Limits of quantification (LOQ) were determined as 3.3 times LOD. For the sludge samples, using the MAE method, the obtained LOD and LOQ were found to be 100 ng/g dry weight and 330 ng/g, respectively.

### Validation of the method

In order to evaluate the trueness of the method, mass balance experiments were conducted and concentrations of BPA in both liquid and solid phases were determined based on the analytical procedure presented in this study (Figure 7).

The mass balance study was carried out with 100 mL of MLSS spiked samples. Three 100 mL aliquots of MLSS samples were collected from three porous pot reactors with varied solid retention time (5, 10, and 15 days) and mean biomass concentration of 3,145, 2,763, and 1,733 mg

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**Table 1** SPE and analytical condition for LC-UV analysis of BPA

<table>
<thead>
<tr>
<th>SPE conditions</th>
<th>HPLC conditions</th>
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<tbody>
<tr>
<td>Conditioning: 5 mL twice MeOH and 5 mL twice Milli-Q water</td>
<td>Column: ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm</td>
</tr>
<tr>
<td>Loading: 100 mL at 10 mL/min</td>
<td>Injection volume: 10 μL</td>
</tr>
<tr>
<td>Washing: 5 mL twice MeOH/Water (30/70)</td>
<td>Mobile phase: 55%ACN/45% Milli-Q water (45%ACN/55% Milli-Q water for sludge samples)</td>
</tr>
<tr>
<td>Dry with vacuum</td>
<td>Flowrate: 1 mL/min</td>
</tr>
<tr>
<td>Elution: 3 mL MeOH three times</td>
<td>UV detection: 230 nm</td>
</tr>
<tr>
<td>Dry with nitrogen gas</td>
<td>Elution time: 2.88 min (4.21 min for sludge samples)</td>
</tr>
<tr>
<td>Dissolved in 1 mL 45%ACN/55%Milli-Q water</td>
<td>LOD: 100 ng/L</td>
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</table>

**Table 2** Spiked concentration of BPA in sludge samples, mean recovery, and relative standard deviation (RSD)

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Conc. in unspiked sludge (ng/g)</th>
<th>Spiked conc. (ng/g)</th>
<th>Determined concentration (ng/g)</th>
<th>Mean recovery rate (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASE 180</td>
<td>1,000</td>
<td>725</td>
<td>55</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>MAE 135</td>
<td>1,000</td>
<td>1,025</td>
<td>90</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>USE 125</td>
<td>1,000</td>
<td>845</td>
<td>73</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>HPH 160</td>
<td>1,000</td>
<td>700</td>
<td>53</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>
volatile suspended solids (VSS)/L, respectively. Samples were homogenized, and deactivated using sodium azide solution (0.2% w/w). Deactivated samples were mixed on the orbital shaker for 2 h at 200 rpm, and spiked with BPA standard solution to the total concentration of 10 μg/L. Samples were then mixed for 12 h to accumulate BPA in the biomass and subsequently were centrifuged for 30 min at 10,000 rpm. The sludge cakes were carefully removed from the centrifuged tube and dried at 60°C for almost 12 h until constant weight was reached. The similar MLSS samples were collected and VSS concentrations were measured for calculating biomass concentration. The dried biomass was placed into microwave vessels and 5 g of sodium sulfate anhydrous were added and mixed to form a free floating powder. Later, 20 mL of acetone/hexane (1:1, v/v) solvent solution was imported to each vessel which was then put in the microwave for BPA extraction. The extraction procedure was carried out based on the MAE method described previously. After microwave extraction, the samples were evaporated to dryness, re-dissolved in 1 mL of 45%ACN/55% Milli-Q water, and filtered through 0.22 μm Millipore syringe filters before analyzing with HPLC. A set of un-spiked samples was collected for determination of background BPA level from each reactor and all recoveries were adjusted based on those results. Blank samples using distilled water were prepared to evaluate any BPA loss during the mass balance study for both liquid and solid samples. The results of blank sample analysis showed a mean recovery of 106 ± 2% for all blank samples. Precision was assessed by analyzing repeatability and reproducibility of three replicates (n = 3) of MLSS samples from three different reactors (k = 3). All samples were analyzed three times using HPLC–UV. Precision and mean recovery data of the extraction and analytical procedure for MLSS samples are given in Table 3. The results demonstrate satisfactory precision of the analytical procedure for MLSS samples. RSDs were less than 8% for BPA in all samples, indicating good precision of the developed analytical methods for both liquid and solid samples. The recoveries ranged between 80 and 92% for all measured samples with an average recovery of 86%. The results from the validation experiments were compared for any significant differences using one-way analysis of variance (ANOVA) and post-hoc analysis (Tukey) (IBM SPSS for Windows, 2010). Statistical analysis of the results had shown that there is no significant difference between the recoveries of samples taken from the same reactor, also from reactors with highest and lowest biomass concentration (sludge retention times (SRTs) of 15 and 5 days); however, the recovery of samples from a reactor with SRT of 10 days was slightly higher than the other two reactors (Table 4). This was in agreement with Yi & Harper (2007) and could be due to a lower BPA sorption partition coefficient in sludge samples at SRT of 10 days and higher recovery level in liquid samples opposed to solid samples.

Figure 7 | Schematic diagram of analytical procedure for analysis of BPA in liquid and solid phases.

Table 3 | Precision and mean recovery data of the extraction and analytical procedure to detect BPA in solid and liquid samples

<table>
<thead>
<tr>
<th>Spiked level (μg/L)</th>
<th>Precision RSD (%)</th>
<th>Recovery (%)</th>
<th>Standard deviation</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>7.2</td>
<td>86</td>
<td>4.3</td>
</tr>
</tbody>
</table>
CONCLUSION

The combination of SPE and HPLC–UV analyses permitted a sensitive, reliable, and rapid determination of low level BPA concentration in wastewater and sludge samples. SPE parameters and HPLC conditions were optimized concerning sample pH, volume, washing, elution solvents, HPLC injection volume and mobile phase. Four solid extraction techniques were compared in this study for trace determination of BPA in sludge samples and the results showed that the recovery of BPA in spiked samples ranged from 60 to 90% for ASE, HPH, USE, and MAE extraction methods. The MAE was proven to have the highest recovery and low solvent consumption. The optimized SPE condition for LC-18 cartridges was also reported in this work and demonstrated high recoveries for BPA detection in liquid samples. To validate the extraction and HPLC–UV analysis of BPA at trace level concentration, a mass balance study was performed and the results showed good recovery of BPA in all mass balance samples (average of 86%). RSDs were found to be less than 8% for BPA in all samples, indicating the good precision of the developed analytical methods for both liquid and solid samples. The LOD for liquid and solid samples were found to be 100 ng/L and 100 ng/g, respectively. Low-cost, fast, simple sample preparation, simplicity of HPLC–UV detection, as well as good repeatability and reproducibility make this method a useful tool for the routine analysis of BPA in wastewater and sludge samples.

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