

Measurement of selected polychlorinated biphenyls (PCBs) in water via ultrasound assisted emulsification–microextraction (USAEME) using low-density organic solvents

Begum Yurdakok-Dikmen, Ozgur Kuzukiran, Ayhan Filazi and Erdem Kara

ABSTRACT

Despite bans and restrictions for their adverse health effects including endocrine disruption, due to their stability in the environment, polychlorinated biphenyls (PCBs) are still of concern for their residues in several matrices. This study employed low-density ultrasound-assisted emulsification–microextraction (USAEME) to measure selected PCBs (28, 52, 101, 118, 138, 153, and 180) in water samples for gas chromatography–mass spectrometry analysis. Among tested solvents (isooctane, chloroform, hexane, and cyclohexane), 200 μ L isooctane resulted in the highest yield for a 10 mL sample. The optimized method was validated and yielded recoveries of 87.29–92.83% with the limit of detection and limit of quantification (LOQ) values 3–12 ng/L and 10–40 ng/L, respectively. Twelve tap water samples collected in September 2014 were screened using this simple, rapid, and validated method. PCB concentrations in two samples were above the LOQ values; one sample contained 1,380 ng/L of PCB 118, 530 ng/L of PCB 138, and 152 ng/L of PCB 153, and the other contained 444 ng/L of PCB 138. Despite the city water supply being clean and the municipality employing all available measures to ensure clean water supply, the general public must be made aware of the regular maintenance of local water pipelines and storage tanks for the prevention of PCB contamination.

Key words | Ankara, gas chromatography–mass spectrometry, low density solvent, polychlorinated biphenyls, ultrasound-assisted emulsification–microextraction, water

Begum Yurdakok-Dikmen (corresponding author)
Ozgur Kuzukiran
Ayhan Filazi
Erdem Kara
Department of Pharmacology and Toxicology,
Ankara University Faculty of Veterinary Medicine,
06110 Ankara,
Turkey
E-mail: byurdakok@yahoo.com

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of chlorinated aromatic hydrocarbon chemicals that have been used in heat exchangers, transformers, and hydraulic fluids, and as additives in paints, oils, joint caulking, and floor tiles (Agency for Toxic Substances and Disease Registry (ATSDR) 2000). PCBs have been linked to cancer, endocrine disruption and reproductive disorders. PCB congeners released into the environment can bio-accumulate in animal tissues and thereby enter the human food chain (Centers for Disease Control and Prevention (CDC) 2013). Due to the environmental toxicity and classification as a persistent organic pollutant, PCB production was banned by the

United States Congress in 1979 and by the Stockholm Convention on Persistent Organic Pollutants in 2001. Nonetheless, PCBs are still produced and used in many developing countries. PCBs also continue to be released into the environment from old equipment and waste disposal sites, posing an ongoing threat to human health and the environment (Porta & Zumeta 2002; Yurdakok *et al.* 2015).

The detection of trace levels of PCB residues in water is generally performed via the chromatographic method combined with mass spectrometry (MS); however, before PCBs can be analyzed they must be extracted from source material using one of a variety of extraction methods. Extraction is

very important as the method chosen can affect the quality of measurement of the analyte obtained (Chen *et al.* 2013a, 2013b). The most common extraction techniques used for PCB analysis are traditional liquid–liquid extraction (LLE) (Zaater *et al.* 2005) and solid-phase extraction (Aydin *et al.* 2004); however, these methods are time-consuming, require a large volume of organic solvent, and are relatively expensive (Rezaee *et al.* 2006; Regueiro *et al.* 2008; Ozcan 2011). Recent research has focused on the development of efficient, economical, and miniaturized sample preparation methods. As a result, solid phase microextraction (SPME) (Yang *et al.* 1998), liquid phase microextraction (LPME) (Sarafraz-Yazdi & Amiri 2013), hollow fiber-LPME (Xiao *et al.* 2007), polymer-coated hollow fiber membrane extraction (PC-HFME) (Basheer *et al.* 2007), and stir bar sorptive extraction (SBSE) (Popp *et al.* 2005) have been developed. These techniques are of interest to many researchers, but require longer extraction times than older methods for obtaining good extraction efficiency. Efforts to overcome these limitations led to the development of dispersive liquid–liquid microextraction (DLLME), which is characterized by short extraction times, ease of application, and use of a small volume of solvent. Since its introduction in 2006 (Rezaee *et al.* 2006), DLLME has been used extensively as a simple and effective method for the extraction and preconcentration of a wide variety of compounds from aqueous samples (Rezaei *et al.* 2008; Rezaee *et al.* 2010).

A modified DLLME technique known as ultrasound-assisted emulsification–microextraction (USAEME) was developed for the detection of synthetic musk fragrances, phthalate esters and lindane in aqueous samples (Regueiro *et al.* 2008). USAEME was shown to be an efficient, simple, rapid and inexpensive extraction procedure for gas chromatography (GC) analysis. USAEME uses ultrasonic radiation instead of a disperser solvent to assist emulsification which was consolidated as a simple and efficient extraction and preconcentration procedure for minor compounds in aqueous matrices. USAEME requires only a small volume of extraction solvent and the extraction solvent is emulsified by ultrasound waves that boost mass transfer from the aqueous phase to the organic phase by promoting the formation of a large surface area. To date, USAEME has been successfully used to analyze fungicides (Vinas *et al.* 2010), polycyclic aromatic hydrocarbons (PAHs) (Saleh *et al.* 2009), organochlorine pesticides

(Ozcan *et al.* 2009a, 2009b; Zhang & Lee 2012), carbamate pesticides (Chen *et al.* 2013a, 2013b), polybrominated diphenyl ethers (PBDE) (Fontana *et al.* 2009), pyrethroids (Xu *et al.* 2013), PCBs (Ozcan *et al.* 2009a, 2009b), and non-steroidal anti-inflammatory drugs (Lee *et al.* 2014) in water samples. Water analysis has been the primary target of USAEME, but it has also been used for other matrices, including wine (Jofre *et al.* 2010), beer (Chung *et al.* 2012), and biological samples (Seidi & Yamini 2012).

The time required for emulsification via the USAEME method is in the range of 5–10 min. This time range is significantly greater than what is necessary to disperse an organic solvent via the DLLME method. Moreover, in both USAEME and DLLME due to the difficulty collecting micro volumes of floated organic solvents, the selected extraction solvent must be denser than the aqueous sample (Rezaee *et al.* 2006; Regueiro *et al.* 2008). In the case of all the extraction methods mentioned, extraction solvents with higher densities than water have been most widely used, although some researchers have attempted to use low-density organic solvents (Saleh *et al.* 2009; Hu *et al.* 2010; Su & Jen 2010; Ebrahimzadeh *et al.* 2011; Guo & Lee 2011; Kamarei *et al.* 2011; Wei *et al.* 2011; Zhang & Lee 2012; Chen *et al.* 2013a, 2013b). For example, two of them (Saleh *et al.* 2009; Su & Jen 2010) used USAEME with an organic solvent with lower density than water (toluene) to detect PAHs and organophosphorus pesticides in water samples, and very high enrichment factors were obtained; however, the methods they employed required a special extraction device to collect the floating organic solvent, after centrifugation.

Based on a search of the literature, there is only one study on the use of USAEME for detecting PCBs in water samples (Ozcan *et al.* 2009a, 2009b), but literature is available for the use of USAEME with low density solvents (LD-USAEME) to detect PCBs. Therefore, the present study was aimed at determining if USAEME, assumed as an innovative and environmentally friendly technique, with low-density solvents could be used for extraction and preconcentration of selected PCBs from water samples using a syringe by GC-MS. Several factors, including solvent type, solvent volume, extraction time, centrifugation time and addition of salt were studied and optimized over the relative response of the PCBs. For this purpose a special extraction device was not used, only a simple syringe was

used and compared to high-density solvent, chloroform. The analytical performance of the procedure was evaluated in terms of detection limits (LODs), quantification limits (LOQs), inter-day and intra-day repeatability, precision and linear working range. All samples analyzed were tap water.

MATERIAL AND METHODS

All chemicals used were of analytical grade. The PCBs mixed standard including PCB 28, 52, 101, 118, 138, 153 and 180 was obtained from Dr Ehrenstorfer Laboratories (Augsburg, Germany). Solvents including chloroform (density 1.48 g mL^{-1} at 20°C), n-hexane (density 0.66 g mL^{-1} , at 20°C), isooctane (density 0.69 g mL^{-1} , at 20°C) and cyclohexane (density 0.78 g mL^{-1} at 20°C) were obtained from Merck (Darmstadt, Germany). Ultra-pure water was obtained using a Millipore system (Millipore, Molfheim, France). Standard stock solutions of target compounds (1 mg L^{-1} of each PCB) were prepared in methanol and stored in the dark at 4°C . Water samples were prepared daily by spiking ultrapure water with analytes at known concentrations ($25 \text{ }\mu\text{g L}^{-1}$ of each), so as to access extraction performance under various conditions.

A modified USAEME procedure was used (Ozcan *et al.* 2009a, 2009b). Recovery experiments were performed to determine the efficiency of USAEME using low-density organic solvents instead of high-density chloroform. First, the extraction efficiency of n-hexane, isooctane, cyclohexane and chloroform was determined. Thus, 10 mL of fortified distilled water ($25 \text{ }\mu\text{g L}^{-1}$ of each PCB) with no ionic strength adjustment was placed in a 15-mL glass-centrifuge tube. Next, 200 μL of extraction solvent was added and mixed. The resulting mixture was immersed in an ultrasonic bath (frequency 35 kHz, 0.32 kW, Super RK 510, Sonorex, Bandelin, Germany) for 10 min at $25 \pm 2^\circ\text{C}$. An emulsion was formed during ultrasonication due to the dispersion of fine solvent droplets into the aqueous bulk. The emulsion was centrifuged at 4,000 rpm for 10 min to disrupt the emulsion and to separate the solvent from the aqueous phase. Following centrifugation, low-density extraction solvent floated on the aqueous phase; the floating extraction phase was collected using a 250- μL Hamilton syringe (Hamilton Bonaduz AG, Switzerland) and was transferred into a microvial. Then, GC-MS analysis was performed as described below.

Secondly, to estimate the effect of extraction solvent volume on USAEME, 100, 200, 300 and 400 μL of extraction solvent (isooctane) were used. Then, the optimum ultrasonication time was determined using 200 μL of isooctane, which yielded the highest recovery for the studied PCBs. For this, 10 mL aliquots of fortified distilled water ($25 \text{ }\mu\text{g L}^{-1}$ of each PCB) with no ionic strength adjustment were ultrasonicated for 5, 10, 15 and 20 min. Following the selection of the most suitable extraction solvent and volume, and ultrasonication time, along with the other factors affecting the extraction efficiency of the studied procedure, such as centrifugation times (3, 5, 10 and 15 min) and ionic strength of the sample (0, 15, 30 and 50 g L^{-1} of NaCl), the method was optimized.

Instrumentation consisted of a Thermo Finnigan (San Jose, CA, USA) Trace GC gas chromatograph equipped with a split/splitless (SSL) injection port and a Thermo Finnigan Polaris Q ion trap-mass spectrometric detector (electronic ionization 70 eV, external ion source temperature 200°C , interface temperature 250°C , injector temperature 250°C). The injector was equipped with a 12 cm \times 5 mm i.d. Silcoseeve liner (Thermo Finnigan) and was used in splitless mode. Two μL of sample were injected onto the SSL injector in the constant flow mode set at 1 mL min^{-1} . Chromatographic separation was performed using an Agilent Technologies (Palo Alto, CA, USA) HP-5MS capillary column (30 m \times 0.25 mm i.d., 0.25 μm film thickness). The carrier gas was He (purity 99.995%) at a constant flow of 1.0 mL min^{-1} .

GC conditions were as follows: initial temperature 70°C , 2 min isothermal, $25^\circ\text{C min}^{-1}$ to 150°C , 3°C min^{-1} to 200°C , 5 min isothermal, 8°C min^{-1} to 260°C , 5 min isothermal (total 39.37 min). PCBs were analyzed in selective ion monitoring mode in splitless mode for quantitative measurement of the analytes. The solvent delay time was adjusted to 12 min so as to bypass the solvent peak.

To evaluate the proposed USAEME method such parameters as selectivity, linearity, LODs, LOQs, repeatability and recovery were determined using fortified distilled water samples. The linearity of the method was assessed based on a series of analyte solutions varying in concentration (from 0.5 to $5.0 \text{ }\mu\text{g L}^{-1}$). The LOD and LOQ for all compounds were calculated on the same blank extracts used to assess the specificity of the method. LODs and LOQs were expressed as the analyte concentration corresponding to the mean value of 20 blank measurements

plus three-fold and 10-fold their standard deviation, respectively. Intra-day and inter-day precision were determined via evaluation of six replicates at five different concentrations representative of the intended validation range across 4 d of analysis. The results were expressed as relative standard deviation (RSD%) of the measurements.

Tap water samples were collected in September 2014 from 12 sites connected to the drinking water supply network in Ankara, Turkey. Samples were stored in the dark at 4 °C before unfiltered use, as proposed by the method. To eliminate any potential matrix effect for the quantitative measurement of the PCBs, samples were spiked with the standards. In parallel, three aliquots of each of tap water sample were analyzed. RSD and recovery were assessed in spiked tap water samples at three different concentrations (0.5, 1.5 and 5 µg L⁻¹).

RESULTS AND DISCUSSION

The selection of an extraction solvent was the most important experimental parameter for the described method. An appropriate extraction solvent must have low toxicity, a high boiling point, and low vapor pressure in order to reduce the risk of evaporation, and ensure immiscibility with water, good chromatographic behavior and high extraction ability for the target compounds. Finally, high purity organic solvents should be selected (Chen *et al.* 2013a, 2013b). PCBs are hydrophobic compounds that are highly soluble in most organic solvents (Yurdakok *et al.* 2015). As

such, chloroform, n-hexane, cyclohexane and isooctane (with a boiling points of 61–99 °C) were investigated.

Following centrifugation, n-hexane, cyclohexane and isooctane floated but chloroform remained at the bottom of the centrifuge tube after phase separation, because the density values of the selected organic solvents – except chloroform – were lower than that of water. Figure 1 shows the extraction efficiency obtained with each solvent. N-hexane, cyclohexane and isooctane yielded better analytical signals; however, n-hexane and cyclohexane did not form a clear interface with the water sample after centrifugation, making them more difficult to retrieve. Additionally, n-hexane and cyclohexane evaporated more quickly than the other solvents due to their relatively higher vapor pressure. Isooctane did not suffer from these problems and its repeatability was the best. The polarity indexes for isooctane, n-hexane, cyclohexane and chloroform were 0.1, 0.1, 0.2 and 4.1, respectively (Merck leaflet). As is known, the polarity index is a measure of the relative polarity of a solvent and is useful for identifying suitable mobile phase solvents (Bash-eer *et al.* 2004); therefore, as isooctane has a low polarity, a better distinction was observed in the current study. Isooctane also has low toxicity (oral median lethal dose in rat >5 g kg⁻¹ body weight), a high boiling point (99 °C) and low vapor pressure (5.1 kPa at 20 °C) (Merck leaflet), which is why it was used in the present study. Similar observations with organochlorine pesticides in water have been reported (Zhang & Lee 2012).

The results of extraction of 10 mL of aqueous solution containing 25 µg L⁻¹ of each PCB and 100–400 µL of isooctane via USAEME for 10 min and centrifugation for

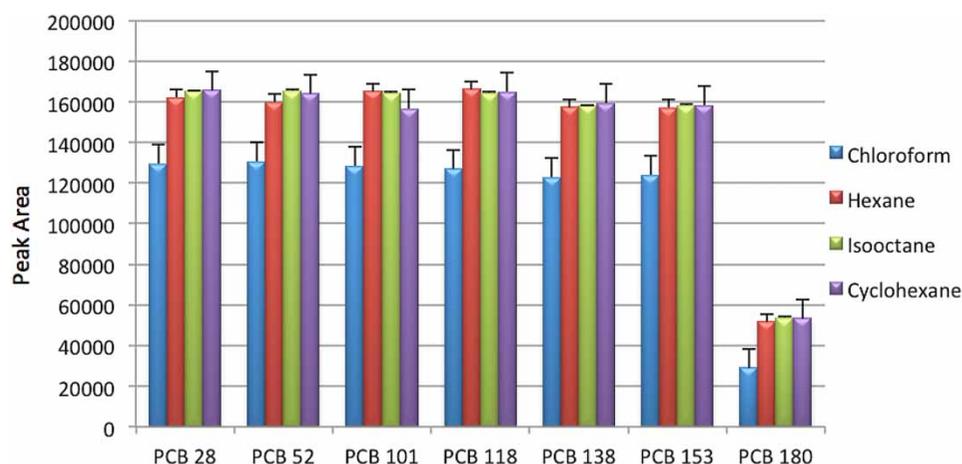


Figure 1 | Effect of solvents (chloroform, hexane, isooctane, cyclohexane) on extraction efficiency.

10 min are shown in Figure 2. The analytical signals first increased when the extraction solvent volume was increased from 100 to 200 μL , and then decreased when the volume was further increased to 400 μL ; the highest extraction efficiency was obtained with 200 μL of extraction solvent.

USAEME is based on the use of ultrasonic energy to disperse an organic solvent, forming an emulsion of submicron droplets in aqueous solution. Emulsions can markedly increase the contacting surface between an organic solvent and aqueous solution, and can increase the mass transfer rate of an analyte; therefore, it was necessary to investigate the effect of ultrasonication time (Xu *et al.* 2013), the results of which are shown in Figure 3. Maximum recovery was achieved with ultrasonication for 10 min and no significant

variation was observed with an extraction time of 15 min. This was probably due to the fact that the ultrasonication water bath generated the emulsion quickly, rapidly making a very large contact surface area between the extraction solvent and the aqueous phase; as such, 10 min of ultrasonication was used in the present study.

The objective of centrifugation is to break down an emulsion and collect the extract at phase. Centrifugation time also plays an important role in extraction recovery of selected PCBs (Kamarei *et al.* 2011). The results are shown in Figure 4. There was no significant change in signal intensity between 5, 10 and 15 min of centrifugation. As the best results were obtained with 10 min of centrifugation, this was considered sufficient to break down the emulsion and result in phase separation.

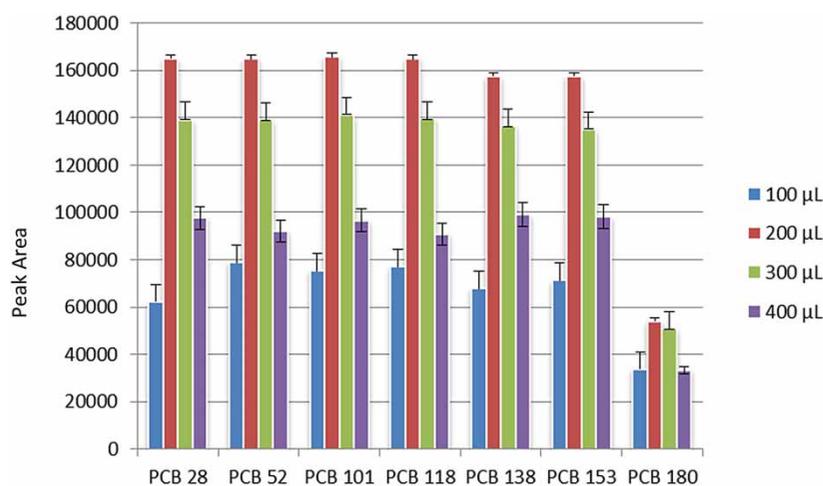


Figure 2 | Effect of extraction solvent volume on the preconcentration of PCBs.

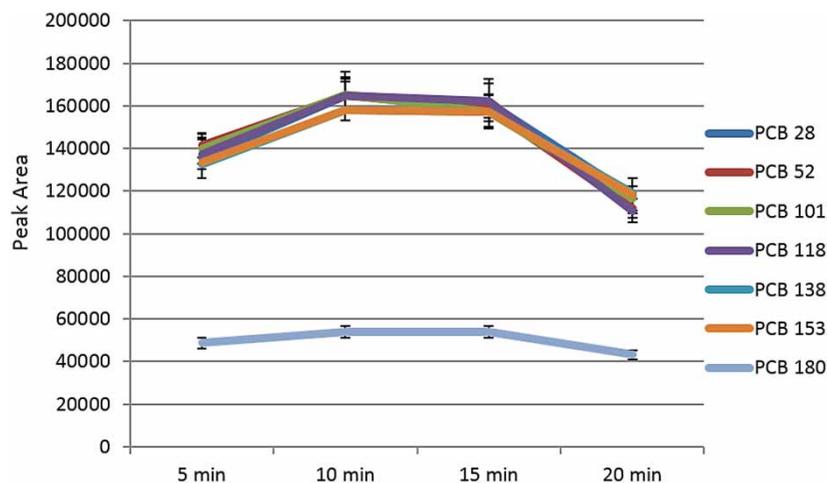


Figure 3 | Effect of ultrasonication time on extraction efficiency.

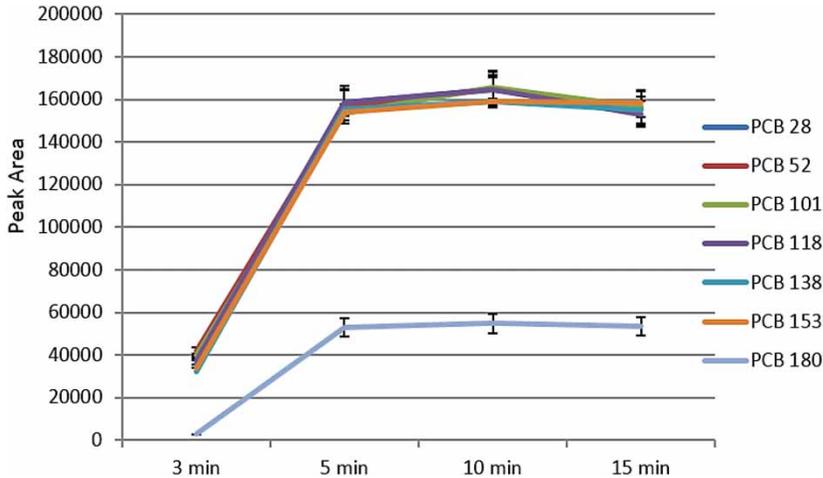


Figure 4 | Effect of centrifugation time on extraction efficiency.

In the present study, the results of the salting-out effect are not shown since no significant results were observed. The salting-out effect has been universally used in LLE methods. The addition of salt to an analytical sample can potentially increase analyte extraction recovery when using microextraction procedures (Saleh *et al.* 2009). The addition of salt during the extraction procedure increases ionic strength, which leads to the salting-out phenomenon. The presence of salt decreases the solubility of the target analytes in the aqueous phase and improves their partition from the aqueous to organic layer. On the other hand, ultrasound waves can be absorbed and dispersed in a viscous medium as calorific energy; thus, the cavitation process can be withdrawn, reducing emulsification

(Fontana *et al.* 2009; Saleh *et al.* 2009; Chen *et al.* 2013a, 2013b; Xu *et al.* 2013). In the present study, the addition of salt did not result in a more floating phase; therefore, salt was not considered beneficial to the extraction procedure. The ionic strength values of water samples can vary depending on the type of samples. Similar observations with organochlorine pesticides (Zhang & Lee 2012) and PBDE in water (Fontana *et al.* 2009) have been reported.

Selectivity is the ability to differentiate between compounds that is achieved via separation or detection. The selectivity of the present study's method is shown in Figure 5. A column that facilitates maximum selectivity was used in the present study. Depending on the compounds, calibration

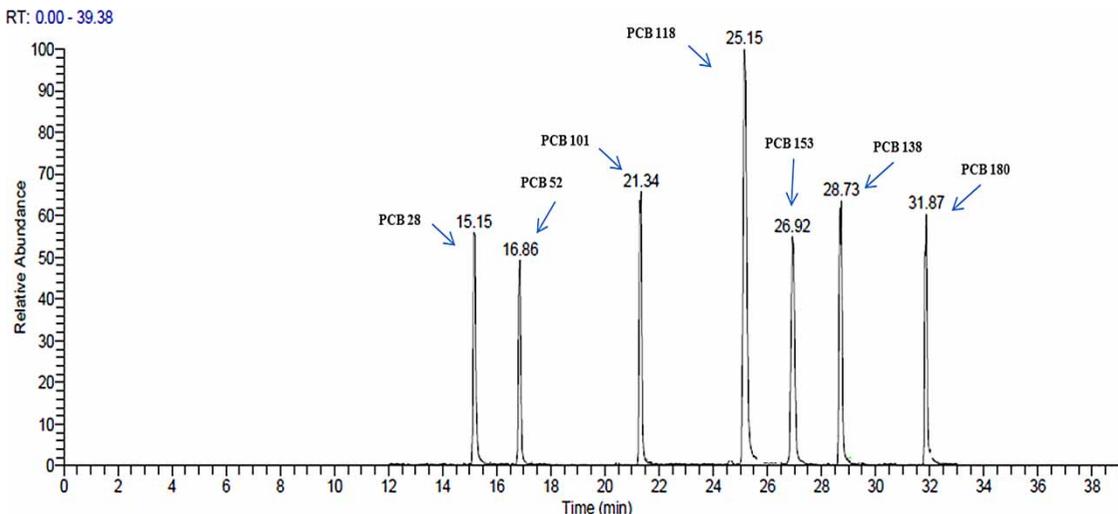


Figure 5 | The selectivity of the method.

curves yielded satisfactory linearity in the range of 0.5–5.0 $\mu\text{g L}^{-1}$ for the selected PCBs with correlation coefficients >0.999 . LODs ranged from 3 to 12 ng L^{-1} , and LOQs ranged from 10 to 40 ng L^{-1} . The optimized procedure was validated and yielded recovery between 87.29 and 92.83%, with RSD values of 2.24–4.14%. The method's validation parameters are given in Table 1.

A comparison between the low-density USAEME technique described herein and other published techniques for the extraction of PCBs using conventional USAEME (Ozcan *et al.* 2009a, 2009b), SPME (Cortazar *et al.* 2002), vortex-assisted liquid–liquid microextraction (VALLME) (Ozcan 2011), PC-HFME (Basheer *et al.* 2007) and SBSE (Popp *et al.* 2005) is presented in Table 2. Isooctane, used as an extraction solvent in the present method, is less toxic than other solvents. Extraction time (Table 2) for the present method was shorter than that for SPME, PC-HFME and SBSE methods, but longer than for conventional USAEME and VALLME methods. Additionally, LOD values for the present method are better than those associated with conventional USAEME. The most important disadvantage of USAEME is the high level of ultrasound energy required, which can degrade analytes in water and can irreversibly damage analytes. To overcome the potential analyte degradation associated with USAEME, Yiantzi *et al.* (2010) developed the method in which the extraction solvent is dispersed into aqueous samples via vortex mixing (a powerful, but mild emulsification procedure); however, extraction of PCBs via VALLME is performed using chloroform.

The applicability of the described extraction method with a tap water sample was investigated, and the results are

Table 1 | The validation parameters of the method

Analyte	Mean recovery (%)	Intra-day precision (RSD%) ^a	Inter-day precision (RSD%) ^a	LOD ^b (ng L^{-1})	LOQ ^c (ng L^{-1})
PCB 28	87.29	2.24	3.04	12	40
PCB 52	91.60	3.23	4.14	9	32
PCB 101	90.79	3.35	3.12	11	39
PCB 118	91.18	3.18	3.18	6	20
PCB 138	90.24	3.22	3.09	10	34
PCB 153	92.83	3.04	3.06	5	17
PCB 180	89.86	3.55	2.93	3	10

^aRSD.

^bLOD.

^cLOQ.

Table 2 | Comparison of USAEME-GC-MS with other extraction methods for determination of selected PCBs in water samples

Method	Solvent and volume (mL)	Extraction time (min)	LOD (ng L^{-1})	Reference
Low density solvent-USAEME-GC-MS	Isooctane, 0.2	20	3–12	This study
USAEME-GC-MS	Chloroform, 0.2	15	14–30	Ozcan <i>et al.</i> (2009a, 2009b)
SPME-GC-MS	Not required	50	30–110	Cortazar <i>et al.</i> (2002)
VALLME-GC-MS	Chloroform, 0.2	7	0.36–0.73	Ozcan (2011)
PC-HFME-GC-MS	Hexane, 0.1	>40	0.04–0.21	Basheer <i>et al.</i> (2007)
SBSE-GC-MS	Not required	>120	0.05–0.15	Popp <i>et al.</i> (2005)

presented in Table 3. Mean recovery values were 89.47–103.01%, and RSDs were $\leq 6.29\%$. Both RSDs and recovery values were satisfactory. The PCB concentration in two samples was above the LOQ values; one sample contained 1,380 ng L^{-1} of PCB 118, 530 ng L^{-1} of PCB 138 and 152 ng L^{-1} of PCB153, and the other contained 444 ng L^{-1} of PCB 138. The source of contamination of all PCB-positive samples was investigated, and old plumbing systems and water storage tanks were assumed to be the source. It was concluded that even though the city water supply is clean and the municipality employs all available measures to ensure a clean

Table 3 | Analytical results and recoveries obtained from analysis of tap water samples by the proposed method

Analyte	Mean recovery (%)			RSD (%) ^a		
	0.5 $\mu\text{g L}^{-1}$	1.5 $\mu\text{g L}^{-1}$	5 $\mu\text{g L}^{-1}$	0.5 $\mu\text{g L}^{-1}$	1.5 $\mu\text{g L}^{-1}$	5 $\mu\text{g L}^{-1}$
PCB 28	97.85	95.73	90.12	2.08	1.01	0.64
PCB 52	90.11	95.10	89.47	2.11	1.82	4.05
PCB 101	101.92	98.59	91.95	3.82	2.76	6.29
PCB 118	96.26	100.16	91.81	2.58	4.19	3.67
PCB 138	100.15	99.36	93.95	2.79	4.95	4.33
PCB 153	100.14	103.01	95.81	2.37	1.12	4.87
PCB 180	95.81	98.56	98.83	1.97	3.79	1.62

^aRelative standard deviation.

water supply, the general public must be made aware of the importance of regular maintenance of local pipelines and storage tanks for the prevention of PCB contamination.

CONCLUSIONS

The present study demonstrated the practical application of a low-density solvent USAEME procedure for measuring PCBs in tap water samples using GC-MS. A 250- μ L syringe was used to easily collect floated organic solvent on the surface of aqueous samples. The optimized extraction conditions for 10 mL of sample were as follows: 200 μ L of iso-octane as extraction solvent; ultrasonication for 10 min; centrifugation for 10 min and without added sodium chloride at 25 °C. The proposed method was also used to analyze tap water samples. As there were no matrix effects observed, quantification was easily performed via calibration, using extraction from samples including standards in doubly distilled water. Finally, based on the findings that the proposed method is an efficient, rapid, simple and inexpensive microextraction method, we think it can be used as an alternative to DLLME and conventional USAEME methods both of which use organic solvents that are denser than water.

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