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Research Article

Analyses of polychlorinated biphenyls in waters and wastewaters using vortex-assisted liquid–liquid microextraction and gas chromatography-mass spectrometry

A method was developed for viable and rapid determination of seven polychlorinated biphenyls (PCBs) in water samples with vortex-assisted liquid–liquid microextraction (VALLME) using gas chromatography-mass spectrometry (GC-MS). At first, the most suitable extraction solvent and extraction solvent volume were determined. Later, the parameters affecting the extraction efficiency such as vortex extraction time, rotational speed of the vortex, and ionic strength of the sample were optimized by using a 2^3 factorial experimental design. The optimized extraction conditions for 5 mL water sample were as follows: extractant solvent 200 μL of chloroform; vortex extraction time of 2 min at 3000 rpm; centrifugation 5 min at 4000 rpm, and no ionic strength. Under the optimum condition, limits of detection (LOD) ranged from 0.36 to 0.73 ng/L. Mean recoveries of PCBs from fortified water samples are 96% for three different fortification levels and RSDs of the recoveries are below 5%. The developed procedure was successfully applied to the determination of PCBs in real water and wastewater samples such as tap, well, surface, bottled waters, and municipal, treated municipal, and industrial wastewaters. The performance of the proposed method was compared with traditional liquid–liquid extraction (LLE) of real water samples and the results show that efficiency of proposed method is comparable to the LLE. However, the proposed method offers several advantages, i.e. reducing sample requirement for measurement of target compounds, less solvent consumption, and reducing the costs associated with solvent purchase and waste disposal. It is also viable, rapid, and easy to use for the analyses of PCBs in water samples by using GC-MS.

Keywords: Factorial design / Polychlorinated biphenyls / Vortex-assisted liquid–liquid microextraction / Wastewater / Water
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1 Introduction

Polychlorinated biphenyls (PCBs) are a class of synthetic organic compounds that are known as persistent organic pollutants (POPs) and they consist of 209 different congeners. PCBs are hazardous substances due to their persistence, hydrophobic character, and toxic properties [1]. PCBs have been produced commercially since 1929. They have been used in plasticizers, surface coatings, inks, adhesives, flame retardants, pesticide extenders, and paints. PCBs have been used in dielectric fluids in transformers and capacitors because of their chemical and heat stability [2]. Although they have been banned in the industrialized countries for years and in some instances for decades, PCBs are still routinely found throughout the world and continue to cause many ecotoxicological problems [3]. Because of their persistence and hydrophobicity, PCBs accumulate in sediments and soils where they are likely to be retained for many years. Consequently, soils are an important reservoir for these compounds [4]. PCBs may enter the environment from transformers, incinerators, landfills, and sludge drying beds and also disposal of old electrical equipments [5–7]. Additionally, these compounds can cause various human health problems, such as neurotoxicity, dermatological, and pulmonary diseases [1, 8]. Therefore, the analysis of PCBs as

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Abbreviations: **DLLME**, dispersive liquid–liquid microextraction; **LLE**, liquid–liquid extraction; **LPME**, liquid-phase microextraction; **PCB**, polychlorinated biphenyl; **POP**, persistent organic pollutant; **PTV**, programmed temperature vaporizing; **SBSE**, stir bar sorptive extraction; **SPME**, solid-phase microextraction; **USAEME**, ultrasound-assisted emulsification microextraction; **VALLME**, vortex-assisted liquid–liquid microextraction well as developing analytical methods in environmental samples should be continued.

The European Union Drinking Water Directives and Turkish Drinking Water Regulation have established maximum admissible concentrations of PCBs for drinking water which is 0.1 μg/L for individual PCBs and 0.5 μg/L for total concentrations [9, 10]. PCBs are also listed by Stockholm Convention on POPs that aims to eliminate or restrict the production and use of POPs. Turkey signed the Stockholm Convention on 2001 and has been required to monitor and reduce POPs in environment. Therefore, determination and monitoring of PCBs in water samples are important in environmental and human health point of view as well as our National Implementation Plan for the management of POPs.

The determination of PCBs in water samples is typically extraction with solvent prior to analysis by gas chromatography (GC). Up to now, in order to determine the PCBs in aqueous solution, the most developed analytical methods, i.e. liquid–liquid extraction (LLE) [11, 12], solid-phase extraction (SPE) [13, 14], solid-phase microextraction (SPME) [15] and the more recently developed liquid-phase microextraction (LPME) [16], dispersive liquid–liquid microextraction (DLLME) [3, 17], ultrasound-assisted emulsification microextraction (USAEME) [18], membrane-assisted solvent extraction [19, 20], and stir bar sorptive extraction (SBSE) [21, 22] have been used. LLE and SPE are the oldest and the most widely used procedures for the extraction of PCBs from aqueous matrices. However, LLE and SPE need relatively larger volumes of organic solvents and samples and they are time consuming as well as labor-intensive methods. The LLE method has some complications such as the formation of stable emulsions. SPE is also a relatively expensive method and analytes may be adsorbed, and complex matrices can cause settling in cartridges. LLE and SPE methods are complicated and difficult for automation. Using large amounts of organic solvents can cause environmental pollution and health hazards for laboratory personal and extra operational costs for waste treatment [23]. Therefore, in order to overcome disadvantages of these methods, an efficient, fast, easy, economical, and comparable sample preparation method such as SPME and different modes of LLME, termed as LPME or solvent microextraction (SME) for example SDME, HF-LPME, headspace LPME, dynamic LPME, DLLME, and USAEME have been developed in recent years. Among these methods, SPME is based on the partitioning of analytes between the sample matrixes and the polymer-coated fiber. Although SPME has some important advantages such as rapidity, simplicity, and being solvent free, the main disadvantages of SPME method are their relatively high price and fragile coating layer of fiber. Fiber can also degrade with time and the partial loss of stationary phase can cause coelution with the analytes. In addition, sample carryover has been frequently reported for SPME method [24, 25]. LLME is based on the distribution of the analytes between a micro-volume of organic solvent and the aqueous solution [16, 26, 27]. These alternative techniques such as SDME, LPME, and DLLME have advantages, such as short extraction time, small volumes of solvent and water requirement, rapid, easy, and low cost. Compared with the SPME, SDME has many advantages including no sample carryover, wide selection of available solvents, simplicity and ease of use, short preconcentration time, requiring no conditioning, no need for instrument modification, etc. Nevertheless, these techniques also have some drawbacks. For example, SDME method has difficulty to automate, instability of droplet, and relatively low precision [28]. In comparison to the traditional LLE and SPE, LPME procedure has many advantages including wide selection of available solvents, low cost, simplicity and ease of use, minimal solvent use, short preconcentration time, and possibility of automation. Furthermore, compared with the SPME, LPME has also advantages, such as no sample carryover, requiring no conditioning, no need for instrument modification, etc. [29]. Nevertheless, some drawbacks, such as instability of droplet and relatively low precision were reported for LPME procedure [28]. The advantages of DLLME could be given as rapid, simple, short extraction time, low cost, and high recovery of analytes. However, general drawbacks of this method are difficult to automate and it requires using a dispersive solvent which usually decreases the partition coefficient of analytes into the extraction solvent [30, 31]. Some advantages of USAEME are viable, simple, rapid, low cost, and it needs less amount of sample and extraction solvent [18, 32–34]. However, the most important disadvantage of this method is that excessive ultrasound energy may degrade the analytes in water and may cause irreversible damages to the properties of analytes [32, 35].

Yiantzi et al. [36] have recently introduced a novel extraction technique, which is called as vortex-assisted liquid-liquid microextraction (VALLME). In this method, dispersion of the solvent phase into the aqueous solution has been provided by using vortex mixing and miniaturization approach has been achieved using a micro volume of extraction solvent. They used this new microextraction technique for the determination of trace amount of octylphenol, nonylphenol, and bisphenol-A in water samples. In addition, this method was optimized and used for the residue analysis of organochlorine pesticides in water and wastewater samples [37]. They demonstrate that VALLME is a fast, repeatable, and efficient method and it requires quite small volume of extraction solvent and water sample. According to the literature survey, there is no report on the usability of VALLME procedure for the residue analysis of PCBs in water samples. Therefore, objective of this study was to develop a VALLME procedure followed by gas chromatography-mass spectrometry (GC-MS) for the determination of selected PCBs in water and wastewater samples. After the determination of the most suitable extraction solvent and solvent volume, parameters such as vortex extraction time, rotational speed of the vortex, and ionic strength of the sample were optimized by using a 2^3 factorial experimental design. The efficiency of the suggested procedure was compared with traditional LLE method on real water and wastewater samples such as tap, well, surface, bottled water, municipal wastewater, treated municipal wastewater, and industrial wastewaters.

2 Materials and methods

2.1 Reagent and solvent

All chemicals used were of analytical grade. PCBs mixed standard including 2,4,4′-trichlorobiphenyl (PCB 28), 2,2′,5,5′-tetrachlorobiphenyl (PCB 52), 2,2′,4,5,5′-pentachlorobiphenyl (PCB 101), 2,3′,4,4′,5′-pentachlorobiphenyl (PCB 118), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB 138), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180) were purchased from Accustandard (USA). Residue grade of solvents including dichloromethane, chloroform, 1,2-dichlorobenzene, bromoform, *n*-hexane, cyclohexane, petroleum ether, 2-propanol, isooctane, *n*-pentane, toluene, diethylether, 1-octanol, methanol, and ethyl acetate were purchased from Merck, Darmstadt, Germany. Sodium chloride and sodium sulfate were also from Merck. All solutions were stored in the dark at 4°C. Working solutions were prepared by dilution of standard stock solutions with deionized water.

2.2 Real water samples

To determine the matrix effects, optimized VALLME procedure was applied on the real water samples including tap, well, surface, bottled waters, municipal wastewater, treated municipal wastewater, and industrial wastewaters. Tap water was obtained from the laboratory and well water from deep-ground water in Konya (Turkey). Surface water was taken from Ihlara stream in Konya. Bottled water was taken from the market in Konya. Municipal and industrial wastewater samples were taken from the sewage system in residential and industrial areas of Konya, respectively. Treated municipal wastewater sample was also obtained from the wastewater treatment plant in Konya. The sampling and storage of the samples were performed according to the previously published method [38]. All samples were collected free of air bubbles in glass containers and they were stored in the dark at 4°C. Tap, well, surface, and bottled water samples were analyzed without previous treatment or filtration. The wastewater samples were filtered through 0.45 μm pore size membrane filters before the extraction procedures. In order to investigate the matrix effect, all real water samples were fortified with level of 1 μg/L for each PCB.

2.3 Instruments and GC-MS conditions

Vortex agitator (WiggenHauser, Germany) was used for VALLME process. The determination of PCBs was carried out using a gas chromatograph (GC, Agilent 6890 N, Agilent Technologies, Palo Alto, CA, USA) equipped with MS (Agilent 5973, Agilent Technologies, Foster City, CA, USA). The features and operating conditions of GC-MS system were as follows: GC, equipped with programmed temperature vaporizing (PTV) injector, DB-5 MS 5% phenylmethyl siloxane fused silica capillary column (30 m length, 0.25 mm id, and 0.25 μm film thickness) and helium (purity 99.999%) was used as a carrier gas at a constant flow rate of 2.1 mL/min. PTV program was as follows: 80°C, 12°C/s to 350°C and hold at 350°C for 2 min, 3.33°C/s to 80°C. The volume injected was 2 μL. PTV was operated in splitless mode. Injections were performed by an Agilent 7683 B Series automatic injector (Agilent Technologies). The temperature of the ion source and MS transfer line were maintained at 150 and 270°C, respectively. The oven program was as follows: initial temperature 70°C for 2 min, 25°C/min to 150°C, 3°C/min to 200°C, 8°C/min to 280°C, hold at 280°C for 5 min (run time: 41.87 min). MS detector was operated in selected ion monitoring (SIM) mode. Ions monitored for the quantification and confirmation of PCBs and corresponding retention times are summarized in Table 1. The quantitation of PCBs was performed using the external standard calibration. Analytical curves were drawn using seven pure PCBs standards in methanol within the concentration range between 0.001 and 0.8 μg/L.

Table 1. Retention times of PCBs for DB-5MS fused silica capillary column and ions selected for MS analysis

PCBs	Structures	Retention times (min)[a]	Ions selected for MS analysis (*m/z*)
PCB 28	2,4,4′-Trichlorobiphenyl	14.96	258[b], 256[c], 150[c]
PCB 52	2,2′,5,5′-Tetrachlorobiphenyl	16.43	292, 290, 257
PCB 101	2,2′,4,5,5′-Pentachlorobiphenyl	20.94	326, 324, 254
PCB 118	2,3′,4,4′,5′-Pentachlorobiphenyl	24.16	326, 328, 324
PCB 153	2,2′,4,4′,5,5′-Hexachlorobiphenyl	24.85	360, 358, 292
PCB 138	2,2′,3,4,4′,5′-Hexachlorobiphenyl	25.81	360, 358, 292
PCB 180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	27.93	394, 390, 326

a) Obtained under described temperature program.
b) Ions used for quantification.
c) Ions used for the confirmation.

2.4 The optimized VALLME procedure

The recovery experiments were carried out for the determination of the VALLME efficiency. At the beginning of the experiments, extraction efficiency of different solvents such as dichloromethane, chloroform, 1,2-dichlorobenzene, bromoform, *n*-hexane, cyclohexane, petroleum ether, 2-propanol, isooctane, *n*-pentane, toluene, diethylether, 1-octanol, methanol, and ethyl acetate was determined. For that determination, an aliquot of 5 mL distilled water sample spiked with PCBs compounds (1 μg/L of each PCB) was placed in a 10 mL glass centrifuge tube and 50 μL extraction solvent was injected. The resulting mixture was shaken for 2 min at 1800 rpm by vortex agitator. During the vortex process, fine solvent droplets into the aqueous bulk were formed. The vortex phenomenon favored the mass-transfer process of PCBs from the aqueous bulk to the organic phase. Then, the solution was centrifuged at 4000 rpm for 5 min to separate solvent phase from aqueous phase. After centrifugation, extraction solvent was removed from the bottom of the tube by using a 50 μL Hamilton syringe (Hamilton Bonaduz AG, Switzerland) and transferred into the micro-vial. Then, GC-MS analysis was performed as described in Section 2.3. In the second set of experiments, the optimum volume of solvent was determined. This optimization experiment was carried out using chloroform because of the highest recovery obtained for the PCBs studied. In order to determine the optimum volume of chloroform, an aliquot of 5 mL fortified distilled water sample without ionic strength adjustment was extracted with 50, 100, and 200 μL chloroform by means of vortex agitator for 2 min at 1800 rpm.

After choosing the most suitable extraction solvent and extraction solvent volume, the other factors affecting the extraction efficiency of the proposed procedure, such as extraction time (denoted as factor 1), rotational speed of the vortex (denoted as factor 2), and ionic strength of the sample (denoted as factor 3) were optimized by means of a factorial experimental design at two levels (2^3). As summarized in Table 2, the corresponding levels (low and high level) for factors 1–3 were 2 and 5 min, 1800 and 3000 rpm, 0 and 5%, respectively. All the experiments were performed in duplicate and randomized. The experimental design matrix in factorial design is summarized in Table 3. After each extraction, the solution was centrifuged at 4000 rpm for 5 min and then extraction solvent was removed from the bottom of the tube by using syringe and transferred into the micro-vial and GC-MS analysis was performed as described in Section 2.3.

Table 2. Variable and their levels for factorial experimental design

Variables		Variable levels	
		(−)	(+)
Extraction time	Factor 1 (min)	2	5
Rotational speed of the vortex	Factor 2 (rpm)	1800	3000
Ionic strength of the sample	Factor 3 (%)	0	5

2.5 Traditional LLE

Traditional LLE procedure was carried out according to the US EPA Method 3510C [39]. Aliquot of 200 mL water sample spiked with PCBs compounds (1 μg/L of each PCB) was placed in a 250 mL capacity separatory funnel and extraction was carried out three times with 20 mL of dichloromethane. The extracts were combined and dried with anhydrous sodium sulfate. The concentration of resulting extract was carried out by rotary evaporator (Buchi B-160 Vocabox, Flawil 1, Switzerland) and gentle nitrogen stream until volume of extract became less than 1 mL. After that, this volume of extract was transferred into 1 mL capacity of volumetric flask and adjusted to exactly 1 mL. Then, GC-MS analysis was performed as described in Section 2.3.

Table 3. Design matrix in factorial design[a)]

No.	Codified variables			No codified variables			Average recovery (%)
	Factor 1 (min)	Factor 2 (rpm)	Factor 3 (%)	Factor 1 (min)	Factor 2 (rpm)	Factor 3 (%)	
1-9	−	−	−	2	1800	0	79
2-10	+	−	−	5	1800	0	81
3-11	−	+	−	2	3000	0	96
4-12	+	+	−	5	3000	0	97
5-13	−	−	+	2	1800	5	48
6-14	+	−	+	5	1800	5	50
7-15	−	+	+	2	3000	5	58
8-16	+	+	+	5	3000	5	59

a) Factor 1, extraction time; factor 2, rotational speed of the vortex; and factor 3, ionic strength of the sample.

3 Results and discussion

3.1 Effect of extraction solvent type and the volume

The selection of an extraction solvent is very important in liquid–liquid microextraction methods in order to obtain an efficient extraction. In vortex-assisted extraction, extraction solvent should be water immiscible and it should remain at the bottom or surface of the centrifuge tube. Moreover, the chosen extraction solvent presents good affinity for target compounds, and it should have excellent gas chromatographic behavior and high extraction capability for interesting analytes [33, 34, 37, 40–42]. Therefore, different extraction solvents denser than the water (dichloromethane, chloroform, 1,2-dichlorobenzene, and bromoform) and lower than the water (*n*-hexane, cyclohexane, ethyl acetate, petroleum ether, 2-propanol, isooctane, *n*-pentane, toluene, diethyl ether, and 1-octanol) were examined. The density values of the selected organic solvents are 1.32 g/mL (dichloromethane), 1.30 g/mL (1,2-dichlorobenzene), 1.48 g/mL (chloroform), 2.89 g/mL (bromoform), 0.65 g/mL (*n*-hexane), 0.78 g/mL (cyclohexane), 0.90 g/mL (ethyl acetate), 0.60 g/mL (petroleum ether), 0.78 g/mL (2-propanol), 0.69 g/mL (isooctane), 0.81 g/mL (*n*-pentane), 0.86 g/mL (toluene), 0.71 g/mL (diethylether), and 0.83 g/mL (1-octanol) [43].

Aliquot of 5 mL of the deionized water including 1 μg/L of each PCB compounds was extracted by using 50 μL of each solvent by vortex agitator for 2 min at 1800 rpm. After centrifugation for 5 min at 4000 rpm, high-density solvents, chloroform, 1,2-dichlorobenzene, and bromoform were retained at the bottom of the centrifugation tube. Among the low-density solvents, only 1-octanol was gathered at top surface of the water in centrifugation tube. In addition, among all studied extraction solvents, the remaining solvents were completely dissolved in the aqueous solution. Similar observations were reported for VALLME of octylphenol, nonylphenol, bisphenol-A, and organochlorine pesticides from the aqueous solution [36, 37]. Therefore, 1-octanol, chloroform, 1,2-dichlorobenzene, and bromoform were tested for the extraction of PCBs in waters. The extraction efficiencies for chloroform, bromoform, 1,2-dichlorobenzene, and 1-octanol are shown in Fig. 1. As shown in Fig. 1, the highest recoveries of PCBs were obtained in the range of 16 ± 3 to 72 ± 3% when chloroform was used. This result may be explained with the lower dipole moment of the chloroform (1.15 D at 25°C) than the other used solvents, because lower dipole moment allows a better interaction between solvent molecules and non-polar compounds. Similar observation for using chloroform in the USAEME of PCBs from water was reported [18]. Therefore, chloroform was selected as an optimum extraction solvent for further optimization studies. In addition, the water samples were purged by air or nitrogen after being extracted with chloroform in order to avoid the water contamination with this solvent.

To examine the effect of the extraction solvent volume, different volumes of chloroform in the range of 50–200 μL were examined in the same VALLME procedure. With the increase of chloroform volume from 50 to 200 μL, the volume of sedimented phase increased from 42 to 145 μL. The extraction efficiencies for different extraction solvent volumes are shown in Fig. 2. As shown in Fig. 2, solvent volume affected the sensitivity of the VALLME procedure and the recoveries for all PCB compounds were increased with chloroform volume from 50 to 200 μL. As the numbers of submicron droplets increase with increasing the volume of chloroform, the contact surface between extractant and water increases. Hence, higher mass transfer from aqueous phase to extractant phase is obtained. In the further optimization experiments, 200 μL of chloroform was selected as an optimum volume of the extraction solvent.

3.2 Factorial experimental design

After choosing chloroform and 200 μL as the optimum extraction solvent and solvent volume, respectively, several other parameters influencing the efficiency of the VALLME procedure, such as extraction time, rotational speed of the vortex, and ionic strength of the sample, were studied and

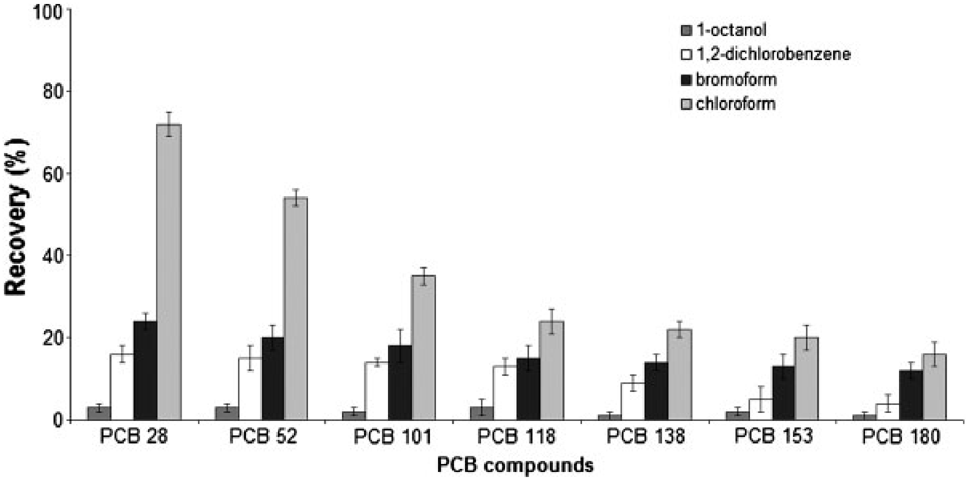

Figure 1. Recoveries of PCBs for different extraction solvents ($n = 5$) (extraction conditions: sample volume, 5 mL; concentration level, 1 μg/L; extraction solvent volume, 50 μL; extraction time, 2 min at 1800 rpm; centrifugation time, 5 min (4000 rpm); ionic strength, 0%).

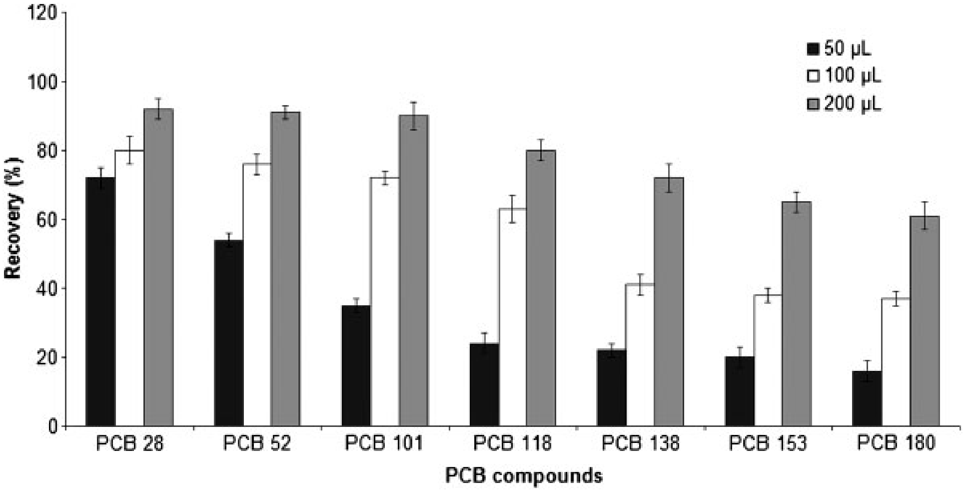

Figure 2. Recoveries of PCBs for different extraction solvent volumes ($n = 5$) (extraction conditions: extraction solvent, chloroform; sample volume, 5 mL; concentration level, 1 μg/L; extraction time, 2 min at 1800 rpm; centrifugation time, 5 min (4000 rpm); ionic strength, 0%).

optimized using a 2^3 factorial design with two levels for each parameter. After processing the data by means of the analysis of variance (ANOVA) using Tool Pak in Microsoft Excel, the analysis of variance tables were constructed to test the significance of the effect of each factor on the extraction efficiency. The effect of a factor on the extraction efficiency is determined by comparison of *F*-value with critical *F*-value at 5% significance level. Table 4 summarizes the *p*- and *F*-values for each factor, both significance and direction of the factor's effect on the extraction efficiency. As summarized in Table 4, factor 1 (extraction time) was determined as not significant factor for the extraction of PCBs in water. Factor 2 (rotational speed of the vortex) and interaction between factor 1 (extraction time) and 2 (rotational speed of the vortex) were found as significant factors with positive effect, whereas factor 3 (ionic strength of the sample), interactions between factor 1 (extraction time) and 3 (ionic strength of the sample), factor 2 (rotational speed of the vortex) and factor 3 (ionic strength of the sample) were found as significant factors with negative effect.

Extraction time is one of the most important factors for solvent microextraction process because it affects mass transfer of compounds from aqueous phase to the organic solvent phase. Therefore, to increase the sensitivity of the VALLME procedure, extraction time was selected as factor. When the extraction time was increased from 2 to 5 min, recoveries of studied PCBs were not changed. Average recoveries for investigated PCB compounds were determined as 79% for 2 min extraction time, whereas average recoveries were determined as 81% for 5 min extraction time. Namely, 2-min extraction time was adequate to the mass transfer of analytes from aqueous phase to the organic solvent phase. The effect of vortex extraction time on the efficiency of VALLME was investigated by Yiantzi et al. [36] and Ozcan [37] and they reported that increasing the extraction time up to 2 min increased the extraction efficiency for studied phenols and organochlorine pesticide compounds and extraction efficiency constant after 2 min. Therefore, 2 min was chosen as an optimal extraction time for further studies. Rotational speed of the vortex is an important factor affecting the extraction efficiency of the analytes because of dispersing the extractant phase into aqueous phase. As summarized in Table 4, rotational speed of the vortex was significant factor with positive effect on the extraction of investigated PCB compounds. An increase in the recoveries for all PCBs was observed with increase in the rotational speed from 1800 to 3000 rpm. Average recovery of studied PCB compounds for 1800 rpm rotational speed was obtained as 79%, whereas at 3000 rpm rotational speed average recovery was obtained as 96%. Therefore, 3000 rpm was chosen as a rotational speed of the vortex in further experiments in the study. The effect of ionic strength of the sample on the recoveries of PCBs was evaluated with 0% and 5 NaCl (w/v) in the water sample. Ionic strength of the sample had negative sign for the studied PCBs. In the study, increasing NaCl concentration of the sample from 0 to 5% decreased the average recoveries of the studied PCBs from 79 to 48%. This situation can be explained with increasing the viscosity and density of the medium as the ionic strength of the medium increases. Also, the addition of the salt decreases the solubility of organic phases in the aqueous phase. Therefore, this causes a diminishing in the efficiency

Table 4. Results obtained in the factorial experimental design: significance and direction of the factors' effect on the VALLME efficiency (significance level: 5%)[a)]

	p-Value	*F*-value	Effect
Factor 1	0.7617	0.1	NS
Factor 2	**0.0065**	**10.8**	S (+)
Factor 3	**0.001**	**18.46**	S (−)
Factors 1 and 2	**0.005**	**11.78**	S (+)
Factors 1 and 3	**0.0012**	**17.75**	S (−)
Factors 2 and 3	**0.0018**	**15.98**	S (−)

a) Factor 1, extraction time; factor 2, rotational speed of the vortex; factor 3, ionic strength of the sample; S(+), significant with positive effect; S(−), significant with negative effect; and NS, not significant.

of the mass-transfer process and consequently the extraction efficiency of the procedure diminishes. Similar results were reported in the literature by other researchers [18, 33, 41, 43]. Therefore, further experiments in the study were performed without addition of NaCl into the water samples. As summarized in Table 4, interactions between extraction time and rotational speed of the vortex (factors 1 and 2) were found as significant factors with positive effect on the efficiency of VALLME. The average recoveries of investigated PCBs increased from 79 to 97%. However, interactions between extraction time and ionic strength of the sample (factors 1 and 3) and rotational speed of the vortex and ionic strength of the sample (factors 2 and 3) were significant with negative affect. As a result, the optimum conditions for the development of VALLME method, the studied PCBs from water were used: extraction solvent 200 μL chloroform; vortex extraction time of 2 min at 3000 rpm; centrifugation 5 min at 4000 rpm; without addition of NaCl into the water sample.

3.3 Evaluation of analytical performance of the optimized method

Analytical performances of the proposed VALLME method for the extraction of PCBs are summarized in Table 5. Analytical curves were drawn using seven points in the concentration range of 0.001–0.8 μg/L. The calibration curves gave a good linearity for all PCB compounds with correlation coefficients ranging between 0.9996 and 0.9998. The precision was obtained from six injections of a standard with corresponded concentration of 0.1 μg/L for each PCB congener and the RSD was in the range of 1.25 and 2.50% for MS. Limits of detection (LODs) and limits of quantification (LOQs) were calculated at a signal-to-noise ratio (S/N) of 3 and 10, respectively [44]. LODs and LOQs were in the range of 0.36–0.73 and 1.21–2.46 ng/L, respectively. In order to validate, the developed VALLME procedure was examined on the fortified distilled water with three different fortification levels (level 1, 0.1 μg/L; level 2, 1 μg/L; and level 3, 5 μg/L) and the results of recoveries are also summarized in Table 5. It can be seen that recoveries for fortification level 1 (0.1 μg/L) ranged from 85 ± 5 to 100 ± 4%. Comparable and satisfactory recoveries were also obtained from fortification levels 2 (1 μg/L) and 3 (5 μg/L). For fortification levels 2 and 3, recoveries were in the range of 87 ± 3 to 102 ± 3% and 88 ± 3 to 101 ± 2%, respectively. When statistical evaluations were carried out between fortifications levels 1–2, 1–3, and 2–3, significant differences ($p < 0.05$) were not observed. The repeatability of the proposed method was found to vary between 2 and 5% for the fortified water samples. According to these results, extraction of PCBs from water samples optimized VALLME method can be efficiently used.

3.4 Real water analysis

To evaluate applicability of the optimized VALLME method in real water and wastewater samples and to determine the matrix effects, the optimized procedure was applied on the real water samples including tap, well, surface, bottled waters, municipal wastewater, treated municipal wastewater, and industrial wastewaters. A procedural chemical and sample blanks were also checked to be sure that interfering compounds were absent. According to blank analysis results, tap, well, surface, and bottled waters were free of PCBs contamination. However, PCB 52 was determined in municipal wastewater (0.50 ± 0.10 μg/L) and treated municipal wastewater (0.48 ± 0.12 μg/L). PCB 52 and PCB 153 were also determined in industrial wastewater with concentrations of 0.58 ± 0.08 and 0.42 ± 0.07 μg/L, respectively. The 0.1 ng/μL mix of PCB standard and the real sample chromatograms are shown in Figs. 3 and 4, respectively.

Comparable results were also obtained when traditional LLE were applied to the same unfortified real samples. The real water and wastewater samples were fortified with 1 μg/L of each PCBs and they were extracted using optimized VALLME method and analyzed by GC-MS. The efficiency of the suggested procedure was compared with traditional LLE method on the same fortified real water and wastewater samples. The results of the recovery are summarized in Table 6. As summarized in Table 6, the

Table 5. Analytical performances of the proposed VALLME method for the extraction of PCBs

PCBs	Linear range (μg/L)	r^2	RSD	LOD (ng/L)	LOQ (ng/L)	Recovery[a)] (%)		
						Level 1 (0.1 μg/L)	Level 2 (1 μg/L)	Level 3 (5 μg/L)
PCB 28	0.001–0.8	0.9998	1.82	0.52	1.73	100 ± 3	101 ± 2	100 ± 3
PCB 52	0.001–0.8	0.9998	1.65	0.65	2.17	100 ± 4	100 ± 2	101 ± 2
PCB 101	0.001–0.8	0.9997	2.1	0.73	2.46	99 ± 5	102 ± 3	100 ± 3
PCB 118	0.001–0.8	0.9998	1.8	0.66	2.21	97 ± 4	98 ± 4	100 ± 3
PCB 138	0.001–0.8	0.9996	2.5	0.65	2.16	95 ± 3	96 ± 5	98 ± 4
PCB 153	0.001–0.8	0.9997	1.34	0.36	1.21	88 ± 4	90 ± 4	91 ± 3
PCB 180	0.001–0.8	0.9998	1.25	0.67	2.25	85 ± 5	87 ± 3	88 ± 3

a) Extraction conditions: extraction solvent, chloroform; extraction time, 2 min at 3000 rpm; sample volume, 5 mL; extraction solvent volume, 200 μL; centrifugation time, 5 min at 4000 rpm; ionic strength, 0%.

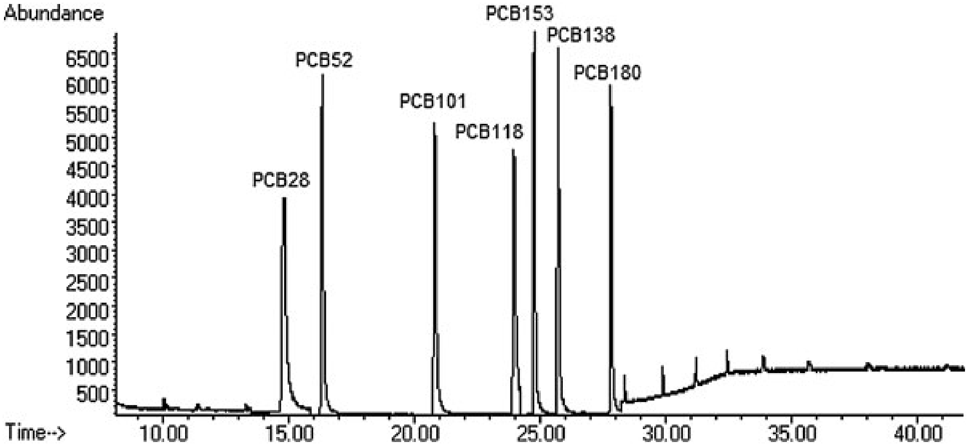

Figure 3. A mixed PCBs standard chromatogram (concentration of each PCB: 0.1 ng/μL).

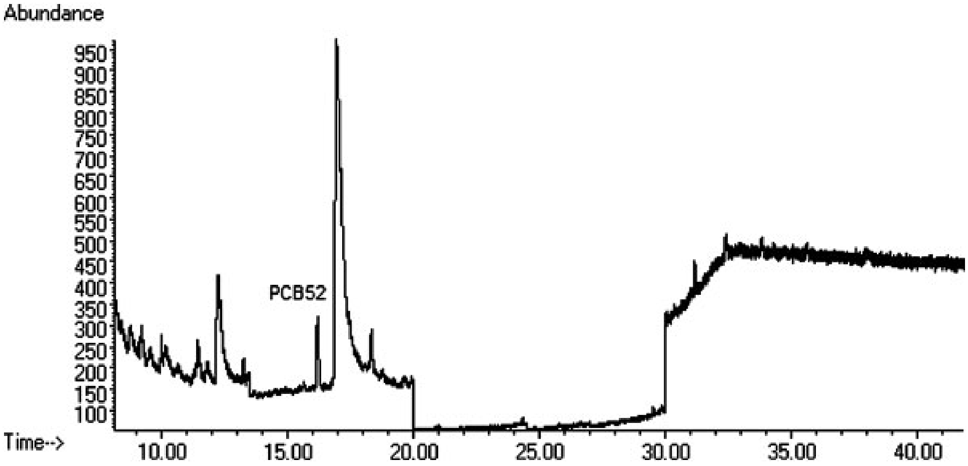

Figure 4. A real municipal wastewater sample chromatogram.

recoveries of studied PCBs were changed from 87 ± 3 to 104 ± 5% in water samples, whereas the recoveries were changed from 84 ± 5 to 100 ± 3% in wastewater samples. These results demonstrated that water and wastewater sample matrixes were not adversely affected on the efficiency of the VALLME procedure. Also, the recoveries of studied PCBs obtained with the proposed VALLME comply with the limit value of 70–130% given by US EPA [45]. Traditional LLE showed comparable recoveries with proposed VALLME. The recoveries for the PCBs were in the range of 86 ± 3 and 96 ± 2% in water samples and 83 ± 5 and 93 ± 3% in wastewater samples. Moreover, in comparison to the traditional LLE, optimized VALLME method is not time-consuming procedure and requires much lower solvent and water sample volumes. Additionally, it is not necessary to reconcentrate the extract prior to the GC analysis.

3.5 Comparison of VALLME-GC-MS with other extraction methods

The performance of proposed methods (VALLME-GC-MS) for PCBs determination in water and wastewater samples was compared with the other previously reported extraction methods. Table 7 summarizes reported LODs, linear ranges, RSDs, extraction time, sample, and solvent volumes found in the literature for the analysis of PCBs in water samples when using for LLE-GC-ECD [46], SPE-GC-ECD [13], SPME-GC-MS [47], LLME-GC-ECD [48], SBSE-GC-MS [21], HFMME-GC-MS [49], DLLME-GC-ECD [3], and USAEME-GC-MS [18]. As it can be seen, the analytical performance for proposed method is comparable with other methods. In comparison with other extraction methods, the proposed VALLME has low RSD and wide linear range values. Although the obtained LODs of the proposed method were significantly lower than LLE-GC-ECD, SPME-GC-MS, and LLME-GC-ECD, LODs obtained for SPE-GC-ECD and DLLME-GC-ECD were comparable with our optimized procedure. As summarized in Table 7, VALLME-GC-MS compared with other methods possessed the shortest extraction time with 2 min. In addition, the volume of sample and extraction solvent required in proposed method was lower than methods listed in Table 7 such as LLE, SPE, LLME, HFMME, and USAEME.

Traditional LLE and SPE techniques require large volume of organic solvents and they are time-consuming procedure. SPE is also a relatively expensive method. These

Table 6. Comparison for the efficiency of the optimized VALLME procedure with traditional LLE of PCBs fortified real water samples ($n = 4$)

PCBs	Recovery (%)													
	Tap water		Well water		Surface water		Bottled water		Municipal wastewater		Treated municipal wastewater		Industrial wastewaters	
	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method	Optimized VALLME method	Traditional LLE method
PCB 28	100 ± 3	95 ± 4	102 ± 4	95 ± 2	99 ± 3	96 ± 2	100 ± 3	94 ± 3	98 ± 5	90 ± 3	99 ± 4	92 ± 4	100 ± 3	92 ± 4
PCB 52	102 ± 4	93 ± 4	104 ± 3	94 ± 2	99 ± 4	93 ± 2	100 ± 4	92 ± 3	NC[a]	NC	NC	NC	NC	NC
PCB 101	104 ± 5	90 ± 2	100 ± 3	93 ± 3	100 ± 4	88 ± 3	100 ± 4	90 ± 3	96 ± 5	93 ± 3	97 ± 3	93 ± 4	99 ± 4	93 ± 4
PCB 118	100 ± 4	94 ± 2	101 ± 4	95 ± 4	98 ± 4	90 ± 2	99 ± 4	92 ± 2	95 ± 4	90 ± 3	96 ± 4	91 ± 3	97 ± 4	91 ± 3
PCB 138	98 ± 5	92 ± 3	99 ± 3	94 ± 3	97 ± 4	90 ± 3	98 ± 5	91 ± 2	91 ± 6	90 ± 4	95 ± 3	90 ± 4	95 ± 5	89 ± 4
PCB 153	95 ± 4	90 ± 3	96 ± 3	90 ± 3	95 ± 5	89 ± 4	92 ± 3	92 ± 3	87 ± 5	92 ± 3	87 ± 5	92 ± 3	NC	NC
PCB 180	90 ± 4	92 ± 4	88 ± 2	90 ± 2	87 ± 3	86 ± 3	88 ± 4	92 ± 2	84 ± 5	90 ± 3	85 ± 4	89 ± 3	86 ± 6	83 ± 5

a) NC, not considered because these compounds were detected in real wastewater samples.

Table 7. Comparison of VALLME-GC-MS with other extraction methods for determination of PCBs in water samples

Method	LOD (ng/L)	Linear range (μg/L)	RSD (%)	Extraction time (min)	Sample volume (mL)	Solvent volume (mL)	Reference
LLE-GC-ECD	<200	0.3–2.4	1.1–9.8	>90	500	100	[46]
SPE-GC-ECD	0.25–1	1–40	3–8	>30	1000	30	[13]
SPME-GC-MS	30–110	0.2–0.6	8–14	50	30	Not required	[47]
LLME-GC-ECD	40	–	1.7–26.9	>30	500	2	[48]
SBSE-GC-MS	0.05–0.15	0.0001–0.1	3.3–29.7	>120	8	Not required	[21]
HFMME-GC-MS	0.04–0.21	0.05–5	1–9	>40	10	0.1	[49]
DLLME-GC-ECD	1–2	0.005–2	4.1–11	<3	5	0.01	[3]
USAEME-GC-MS	14–30	0.001–1	3–10	10	10	0.2	[18]
VALLME-GC-MS	0.36–0.73	0.001–0.8	2–6	2	5	0.2	Present study

methods are complicated and difficult in automation. In SPME technique, fibers are rather expensive and have a limited lifetime, as they tend to degrade with increased usage. Before using a fiber for the first time, a thermal conditioning step is required. In addition, sample carryover between runs has often been reported with fiber in SPME technique. SDME and LPME methods are difficult to automate and instability of droplets may cause relatively low precision. DLLME method is difficult to automate and it requires using a dispersive solvent which usually decreases the partition coefficient of analytes into the extraction solvent. The most important disadvantage of USAEME method is that excessive ultrasound energy may degrade the analytes in water and may cause irreversible damages to the properties of analytes. The main disadvantage of the SBSE technique is that it requires a special device for thermal desorption. The proposed VALLME method for the determination of PCBs in water and wastewaters samples is very simple, rapid, and easy and also it does not require special approach and equipments.

4 Concluding remarks

In the study, an efficient microextraction method combined with GC-MS for the determination of PCBs at trace levels in environmental samples is described. VALLME method under the optimized extraction conditions provides high recovery and repeatability. Comparing to the other methods, the method requires only small volume of sample (5 mL) and consumption of toxic organic solvent (0.2 mL) and shorter extraction time (2 min). The performance of proposed method in the extraction of PCBs from real water and wastewater samples was investigated and it was obtained that sample matrix had no adverse effect on the efficiency of proposed VALLME method. In addition, the developed method is viable, rapid, and easy to use for the qualitative and quantitative analyses of PCBs in waters. Additionally, minimum organic solvent consumption in the developed method diminishes environmental pollution and extra operational costs for waste treatment. Therefore, VALLME-GC-MS method can be efficiently used for the determination of PCBs in different water and wastewater samples alternatively to the other methods.

The authors have declared no conflict of interest.

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