# Determination of Some Polychlorinated Biphenyls in River Tigris within Baghdad City

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## Abstract

A number of aqueous samples were collected from river Tigris in Baghdad city, enriched ~1000 times using solid phase extraction (SPE), then extracted the trace concentrations of some polychlorinated biphenyls (PCB) using an aqueous two-phase system (ATPS) composed of 1-Methylpyridinium chloride [MePy]Cl and $KH_2PO_4$ salt. High performance liquid chromatography technique coupled with ultraviolet (HPLC-UV) is used for the quantification.

Extraction under the optimized conditions of pH, solvent composition, duration and temperature has given with a yield of PCB about 91%. The limit of detection (LOD) and limit of quantification (LOQ) for analyses are 0.11-0.62 $\mu g.L^{-1}$ and 2.67–3.43 $\mu g.L^{-1}$ respectively with relative standard deviations (RSD) ranging from 2.9-7.6 %. The method can be applied for the analysis of other PCBs and bimolecular traces in aqueous media.

**Key words:** Polychlorinated Biphenyls (PCB), aqueous two-phase system (ATPS), 1-Methylpyridinium chloride [MePy]Cl, Solid Phase Extraction (SPE)

# Introduction

Polychlorinated Biphenyls (PCB) (Fig.1) are synthetic organic chemical compounds of chlorine attached to biphenyl. The chemical formula for a PCB is $C_{12}H_{10}{-}xCl_x$[1].

Polychlorinated biphenyls were widely used as dielectric and coolant fluids in electrical apparatus such as transformers and capacitors, cutting fluids for machining operations, carbonless duplicating paper and in heat transfer fluids[2].

PCB were produced and marketed as mixtures of many congeners. PCB mixtures are characterized by chlorine content. Less-chlorinated PCB are odorless, tasteless, clear to pale-yellow, viscous liquids, while highly chlorinated mixtures are more viscous and deeper yellow[3]. PCB compounds have toxic responses to humans and laboratory animals[4]. It appears to have relatively carcinogenic effect on human[5]. Their destruction by chemical, thermal, and biochemical processes is extremely difficult, and presents the risk of generating extremely toxic dibenzodioxins and dibenzofurans through partial oxidation[6].

Aqueous two-phase system (ATPS) is an organic-water solvent extraction system. The ATPS two phases mostly are composed of water and non volatile components. They have been used for years in biotechnological applications as non-denaturing and benign separation media. Recently, they have been used for metal ion separations, environmental remediation, metallurgical applications and as a reaction media[7]'[8].

Many methods are used to determine PCB such as column chromatography[9], high performance liquid chromatography[10] and gas chromatography coupled with mass detection[11]. Nevertheless, these approaches require complicated process with high cost of facilities and time-consuming course since the relatively low concentration in surroundings[11]. Thus, a fast and effective pre-concentration is required of quantitative analysis for PCB at trace levels. However liquid - liquid extraction, solid-phase column extraction, solid phase micro extraction and solid-phase membrane extraction have drawbacks not limited to using and wasting large amounts of noxious organic solvents[12].

Aqueous two-phase system was developed in Sweden during mid-1950s for the separation of macromolecules, cells and organelles[13]. It is considered environmentally benign compared to traditional solvents those harmful and hazardous to environment. ATPS that comprises ionic liquids (ILs) can retain and separate nonpolar compounds as well as polar compounds. However, it has other distinctive properties like negligible volatility, thermal stability, nonflammability and good solubility[14].

In this work, Ionic liquid aqueous two-phase system (ILATPS) based on pyridine ionic liquid 1-Methylpyridinium chloride [MePy]Cl in the presence of $KH_2PO_4$ salt was used as a green solvent to extract PCB from water samples[14]. Under the optimized conditions, the extracted PCB has analyzed by HPLC for detection and quantification.

# Experimental

## Reagents, standard solutions and equipment

- EPA 525, 525.1 PCB Mix, 500 μg/mL each component in hexane [2-Chlorobiphenyl (1-PCB), 2,3-Dichlorobiphenyl (2-PCB), 2,4′,5-Trichlorobiphenyl (3-PCB), 2,2′,4,4′-Tetrachlorobiphenyl (4-PCB), 2,2′,3′,4,6-Pentachlorobiphenyl (5-PCB), 2,2′,4,4′,5,6′-Hexachlorobiphenyl (6-PCB), 2,2′,3,3′,4,4′,6-Heptachlorobiphenyl (7-PCB), 2,2′,3,3′,4,5′,6,6′-Octachlorobiphenyl (8-PCB)], (from SUPELCO).
- 1-Methylpyridinium chloride 98% was from (TCI America, USA).

- Monopotassium phosphate 99% ($KH_2PO_4$) was from (Sigma-aldrich, USA).
- Water and methanol (both HPLC grade) were from (SGS, Spain).
- Dichloromethane was from (SGS, Spain).
- Solid phase extraction cartridges (SiliaPrep C18 nec (23%) 6mL, Silicycle, Canada).
- Set of standard solutions in methanol were prepared from the stock solution of PCB.
- Chromatographic analyses were performed on a Shimadzu Prominence UFLC (Japan) high-performance liquid chromatograph HPLC, equipped with a quaternary pump LC-20 AD, degasser DGU-20A5, Column EC 250/4.6 Nucleodur 100-5 C18 ec, and a UV/Vis detector SPD-20A. Data collection and the performance of all components in the system were controlled by LC-Solution chromatography software of Shimadzu.

## Sample collection

Sampling from Tigris River up to downstream designated to be close to the drainages of some main industrial premises suspected to discharge wastewater to the river without enough treatment (Table.1).

## Sample enrichment by Solid Phase Extraction (SPE)

The matrices of aqueous samples were filtered through 0.45 µm Whatman filter paper, then 100mL of methanol is added to 1L of each water sample and stirred for homogenization. While each SPE cartridge is conditioned with 12mL followed by 12mL of deionized water/methanol mixture (v/v) 85/15 at flow rate 2mL/min. Then samples were loaded to the conditioned SPEs at flow rate 6mL/min. The loaded SPEs then washed with 1mL of the mixture of deionized water/methanol (v/v) 85/15 at flow rate 1mL/min[15]. The PCBs analytes eluted dropwise from the readily washed SPEs with 3mL of Dichloromethane. Then the eluted analytes were left to evaporate at room temperature to 1mL volume. Enrichment factor for each aqueous sample was 1000 times.

$$Pre\ concentration\ factor = \frac{Sample\ volume\ before\ inrichment}{Sample\ volume\ after\ inrichment} \cong \frac{1000}{1} \cong 1000\ times$$

# Results

## Constructing a phase diagram by the cloud point titration method[16]

A salt standard solution dropped wise to a glass test tube containing a weighed amount of [MePy]Cl solution until the development of a cloudy biphasic solution. Then drop-wise ultra-pure water until the formation of a clear solution corresponding to the monophasic regime.

The composition of the two-phase system is determined by the ratio of the weight of a component added to the total weight of all added components. The same procedure was repeated to obtain sufficient data for the construction of an equilibrium phase diagram.

The vessel temperature is controlled by surrounding it with a glass jacket with circulating water inside it to maintain a constant temperature with an uncertainty of ±0.05℃.

In a 10mL centrifuge tube, a mixture of $KH_2PO_4$ with 1.0ml [MePy]Cl is mixed then a standard solution of PCB is added and diluted with deionized water to the mark. The mixture was stirred thoroughly at ambient temperature and centrifuged at 2,000 rpm for 30min to complete phase separation. In order to achieve phase equilibrium, the tubes were kept at temperature 25±0.5℃. The top phase which contains the majority of PCB and [MePy]Cl was aspirated with a micro-syringe (Fig.2), then injected into the HPLC.

Extraction efficiency (E%) of PCB was calculated as follows[17]:

$$E = \frac{C_t . V_t}{m_s} \times 100\%$$

Where $C_t$ is the concentration of PCB in top layer, $V_t$ is the volume of the top layer, $m_s$ is the amount of PCB added.

## HPLC-UV analysis

Sets of tests were conducted for the adjustment of the HPLC optimum conditions to separate and determine PCB in its aqueous matrices. The optimized parameters are listed in (Table.2).

# Discussion

In this study, PCB were extracted from water specimens and determined using a green and sensitive method by an Ionic liquid aqueous two-phase system (ILATPS) as a sample preparation procedure. The same procedure can be applied for other hazardous materials present in water samples at trace amount. Following the ILATPS with HPLC technique improved the sensitivity for the whole method. The parameters affecting the extraction efficiency were optimized.

The increase in the salting-out strength of a salt can be observed by the decrease in salt amount required for the formation of ILATPS. The salting-out effect of the separation system is based on $KH_2PO_4$ concentration (Figure.3). $KH_2PO_4$ salt has a valence of three, which gave it the ability to interact strongly with water molecules. Thus salting out with $KH_2PO_4$ facilitates the aqueous two-phase formation.

A set of $KH_2PO_4$ standards with a certain amount of [MePy]Cl were investigated on the extraction efficiency E% of PCB, the results were clarified in (Figure.4). The extraction efficiency increased from 11% to 89% with the increase of $KH_2PO_4$ amount from 11% to 16% of the total mass, then reached the peak of 93% with an invariant state. The impact of [MePy]Cl percentage in the mixture has been investigated against the extraction efficiency (Figure.5). The E% rises upon the gradual addition of $KH_2PO_4$ but dropped down after reaching the vertex on 98% at 70% [MePy]Cl mass of the mixture. Thus the best mass percentage of $KH_2PO_4$ and [MePy]Cl were 16.5% (w/w) and 70% (w/w) respectively to maintain high extraction efficiency of PCB.

The study perceived that the change in the amount of $KH_2PO_4$ has a greater effect on the extraction efficiency than the amount of [MePy]Cl, which is due to the strong salting-out effect of $KH_2PO_4$. While on the contrary, deficiency in $KH_2PO_4$ consequence in less free water available for dissolving the PCB, which impairs the salting-out effect of the salt $KH_2PO_4$.

Temperature has a critical effect on ILATPSs, i.e. controlling temperature affects the enrichment behavior of the analyte PCB based on [MePy]Cl/ $KH_2PO_4$ system. The best extraction efficiency obtained at ambient temperature (Figure.6).

The increase in temperature resulted in migration of water molecules from the top (IL) to the bottom (salt) phase in ATPS[18]. Consequently when the temperature increases, the concentration of IL increases in the top phase while the concentration of the salt decreases in the bottom phase.

As a result, the salting-out effect, the phase-forming ability, decreases in salt-rich phase and the extraction efficiency of PCB decreased.

The required duration to fulfil a simple ILATPS extraction process is found to be 2.5h at ambient temperature ~298K° (Figure.7). To enhance accuracy, each extraction is repeated three times for average. The limit of detection (LOD) and limit of quantification (LOQ) for the analyte are 0.11-0.62 µg.L$^{-1}$ and 2.67–3.43 µg.L$^{-1}$ respectively. In addition, the relative standard deviations (RSD) ranged from 2.9-7.6 % with an average extraction efficiency of 96%.

The effect of the acidity function on the extraction efficiency can be attributed to the hydrophobic interactions between IL and the analyte PCB. As any change in pH will result in a change in the charge of PCB, and will terminate the electrostatic equilibrium. Therefore in an acidic medium, the analyte PCB will tend to stay in the IL phase. The extraction on PCB was studied using buffer solutions at different pH values (4 – 9) at room temperature. The optimal pH was 5.5 (Figure.8).

Under the optimum conditions of HPLC separation, the resolutions and parameters (Table.3) of the standard mixture of eight PCBs were excellent at a total run time of 12 min (Figure.9):

Real water samples were injected to the HPLC system for quantification (Table.4). The exact HPLC retention times for the targeted analytes were determined by spiking a distilled water with the standard mixture of PCBs. The dominant PCBs in all samples were 4-PCB then 3-PCB and 7-PCB comes next (Figure.10).

## Conclusion

A simple, green and sensitive method was developed to extract and determine the concentration of trace level of PCB in aqueous samples. The sample enrichment by SPE increased the method sensitivity. The ILATPS extraction procedure demonstrates high sensitivity to trace level of the analyte and uses benign extraction solvents, followed by PCB determination using HPLC-UV technique. The method is a good alternative for the analysis of PCBs where GC instrumentation is not available. Furthermore, it is applicable for other hazardous organic substances found in water in trance levels.

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**Table (1). Sample collection locations from Tigris river (up to downstream)**

| Water samples | Up to downstream collection locations/ River Tigris |
|---|---|
| X1 | Al-Quds thermal power plant |
| X2 | Raw river water from DEWTP (from river) |
| X3 | Raw river water from DEWTP wells |
| X4 | Drinking water supplied by DEWTP |
| X5 | Drinking water supplied by DEWTP |
| X6 | Nu'aman hospital |
| X7 | Al-Karkh wastewater treatment plant |
| X8 | Drainage1, the city of medicine |
| X9 | Drainage2, the city of medicine |
| X10 | Southern Baghdad power plant |
| X11 | Al-Rustumiya wastewater treatment plant |

***DEWTP** Dijla East water treatment plant, capacity more than 200,000 m3/day*

**Table (2): HPLC optimum conditions used for the separation and determination of PCB**

| | | | |
|---|---|---|---|
| **Column** | EC 250/4.6 Nucleodur 100-5 C18 ec | | |
| **Eluent A** | Water/Acetonitrile 25:75 (v/v) | | |
| **Eluent B** | Acetonitrile | | |
| **Gradient elution** | **Time [min]** | **% A** | **% B** |
| | 0.0 | 100 | 0 |
| | 5.0 | 100 | 0 |
| | 12.0 | 0 | 100 |
| **Flow rate** | 0.7 mL/min | | |
| **Column temperature** | 35 ºC | | |
| **Injection volume** | 5 µL | | |
| **Run time** | 12 min | | |
| **Post time** | 3 min | | |
| **Detection wavelength** | UV at 225nm | | |

**Table (3): Chromatographic analytical parameters for the standard mixture of PCBs**

| PCBs | $t_R$ (min) | W (min) | k | N | H | Rs | α | LOD (μg.L$^{-1}$) | % RSD |
|---|---|---|---|---|---|---|---|---|---|
| 1-PCB | 3.6 | 0.2 | 1.29 | 5184 | 0.0482 | | | 0.11 | |
| 2-PCB | 4.4 | 0.2 | 1.57 | 7744 | 0.0323 | 4.0 | 2.00 | 0.20 | 7.60 |
| 3-PCB | 5.75 | 0.2 | 2.05 | 13225 | 0.0189 | 6.8 | 1.84 | 0.16 | 3.10 |
| 4-PCB | 6.35 | 0.15 | 2.27 | 28674 | 0.0087 | 3.4 | 1.20 | 0.31 | 3.20 |
| 5-PCB | 7.6 | 0.3 | 2.71 | 10268 | 0.0243 | 5.6 | 1.35 | 0.19 | 3.00 |
| 6-PCB | 8.1 | 0.2 | 2.89 | 26244 | 0.0095 | 2.0 | 1.10 | 0.10 | 3.40 |
| 7-PCB | 9.95 | 0.3 | 3.55 | 17600 | 0.0142 | 7.4 | 1.35 | 0.38 | 2.90 |
| 8-PCB | 10.97 | 0.2 | 3.92 | 48136 | 0.0052 | 4.1 | 1.14 | 0.62 | 4.60 |

**Table (4): Results of the analyses for real water samples by HPLC**

| Water samples | Concentration of PCBs (μg.L$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 2-PCB | 3-PCB | 4-PCB | 5-PCB | 6-PCB | 7-PCB | 8-PCB |
| X1 | 0.90 | 3.75 | 6.11 | ND | 5.44 | 1.20 | ND |
| X2 | ND | ND | 1.40 | ND | ND | ND | ND |
| X3 | ND | ND | ND | ND | ND | ND | ND |
| X4 | ND | ND | ND | ND | ND | ND | ND |
| X5 | ND | ND | ND | ND | ND | ND | ND |
| X6 | 0.29 | 3.02 | 3.22 | 0.22 | 0.31 | 2.00 | ND |
| X7 | ND | 0.18 | 3.40 | ND | 0.11 | 0.57 | ND |
| X8 | 0.30 | ND | 5.10 | 0.43 | 0.28 | 1.91 | ND |
| X9 | 0.25 | 0.13 | 4.93 | 0.30 | 0.20 | 2.42 | ND |
| X10 | 1.60 | 4.14 | 6.70 | ND | ND | ND | ND |
| X11 | ND | ND | 2.35 | ND | ND | ND | ND |

*ND. Not Detected*

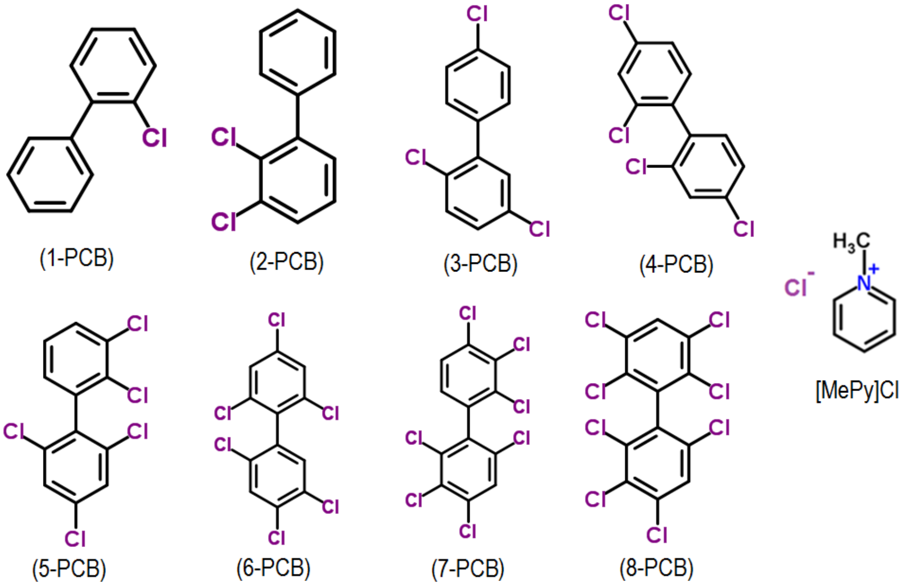

**Figure.(1) Chemical structures of PCB and [MePy]Cl**

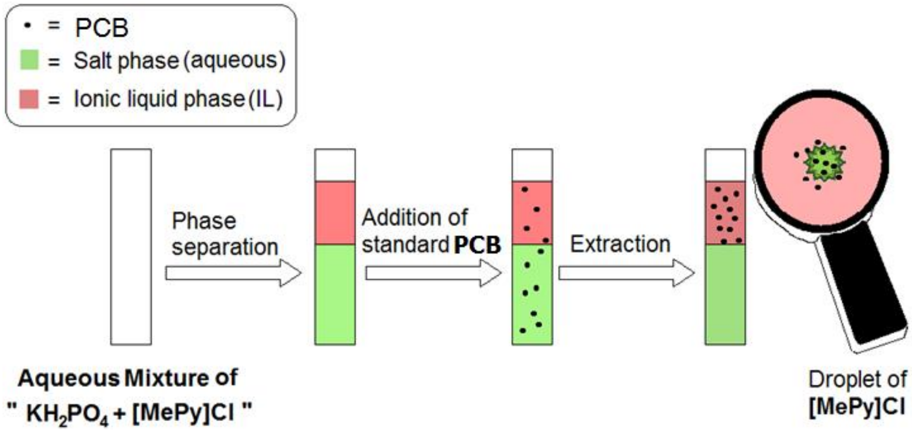

**Figure.(2) The steps of Ionic liquid aqueous two-phase extraction**

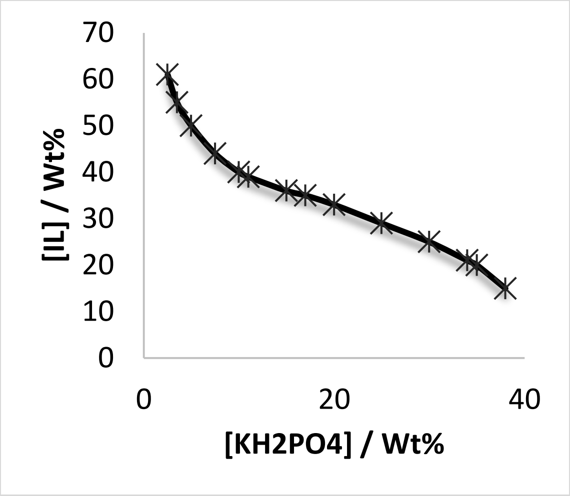

**Figure.(3) The phase diagrams for [MePy]Cl + salt $NaH_2PO_4$ + $H_2O$ water at 298.15K°**

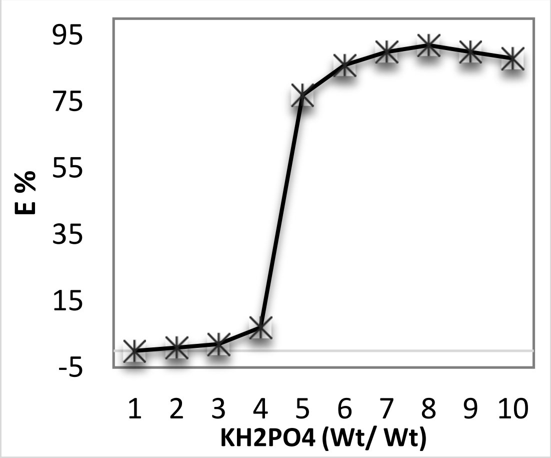

**Figure.(4) Influence of $KH_2PO_4$ concentration on Extraction efficiency**

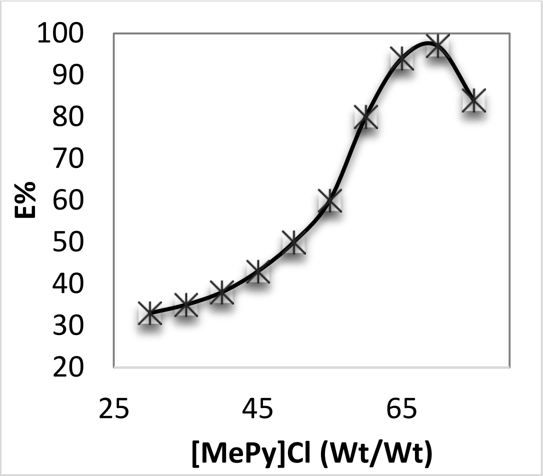

Figure.(5) Influence of [MePy]Cl mass percentage on E%

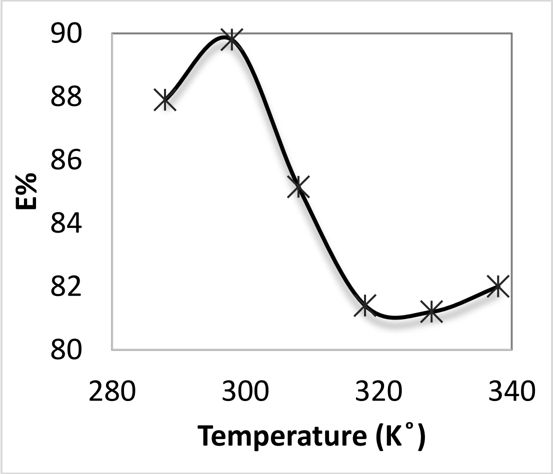

Figure.(6) Influence of temperature on the extraction efficiency

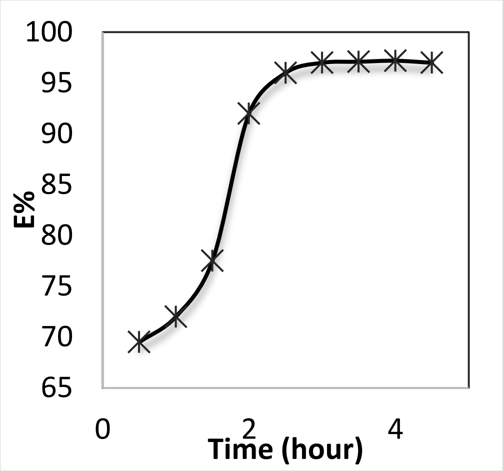

Figure.(7 )Influence of extraction time on E%

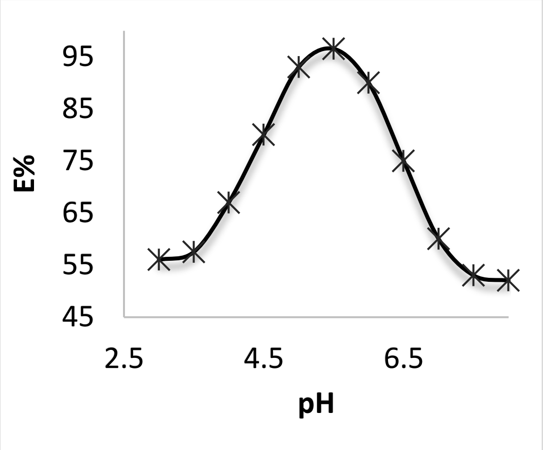

Figure.(8) Influence of pH on the extraction efficiency

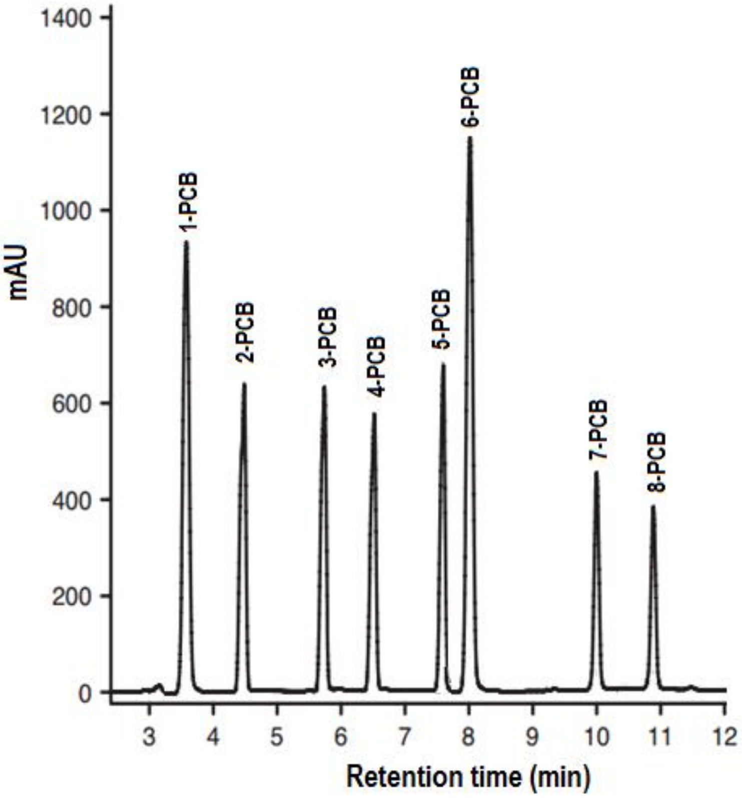

Figure.(9) HPLC Chromatogram of the standard mixture of PCBs

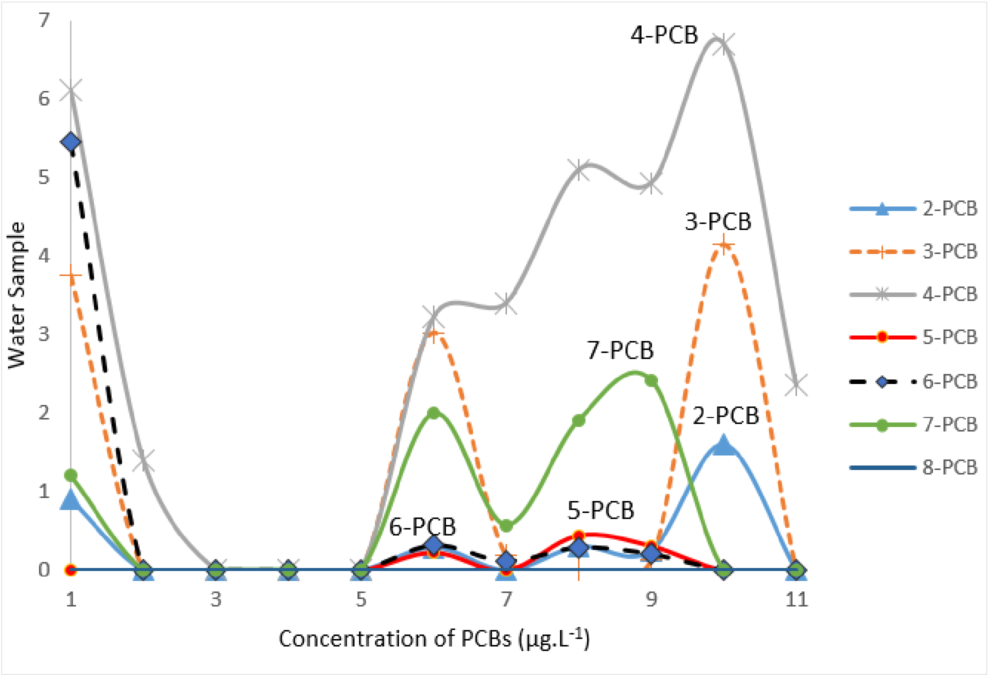

Figure.(10) Availability of different PCBs in water samples

# تعيين بعض ثنائي الفنيلات متعددة الكلوريد في نـهر دجلة ضمن مدينـة بغداد

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**استلم في:14/شباط/2016،قبل في:30/اذار/2016**

## الخلاصة

جمعت مجموعة من العينات المائية من نهر دجلة في مدينة بغداد، وتم تركيزها 1000~ مرة بإستعمال الإستخلاص بالطور الصلب (SPE)، ومن ثم استخلصت التراكيز الضئيلة من ثنائي الفنيلات متعددة الكلوريد (PCB) باستعمال نظام مائي ثنائي الطور (ATPS) مكوناً من كلوريد-1-مثيل بريدينيوم [MePy]Cl و ملح $KH_2PO_4$. وإستعملت تقنية كروماتوغرافيا السائل عالي الأداء مع كاشف الأشعة فوق البنفسجية (HPLC-UV) لتعيين التركيز.

تم الإستخلاص تحت ظروف مثلى من دالة حامضية، تركيب مذيب الإستخلاص، مدة الإستخلاص ودرجة الحرارة. كانت حصيلة استخلاص PCB قُرابة 91%. وكان حد الكشف LOD وحد تعيين التركيز LOQ للتحليلات 0.11-0.62 $\mu g.L^{-1}$ و $2.67–3.43\ \mu g.L^{-1}$ على التوالي، والإنحراف القياسي النسبي RSD كان % 2.9-7.6. يمكن تطبيق الطريقة لتحليل مركبات PCB أخرى ومركبات ثنائية الجزيئة بتركيز واطئ جداً في وسط مائي.

**الكلمات المفتاحية:** الفنيلات الثنائية متعددة الكلوريد PCB، النظام المائي ثنائي الطور ATPS، كلوريد-1-مثيل بريدينيوم [MePy]Cl، الاستخلاص بالطور الصلب SPE.