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### Rapid and sensitive determination of biphenyl and biphenyl oxide in water samples using dispersive liquid-liquid microextraction followed by gas chromatography

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## Rapid and sensitive determination of biphenyl and biphenyl oxide in water samples using dispersive liquid–liquid microextraction followed by gas chromatography

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A rapid and sensitive method has been developed for the determination of biphenyl and biphenyl oxide in water samples using dispersive liquid–liquid microextraction followed by gas chromatography. This method involves the use of an appropriate mixture of extraction solvent (8.0  $\mu\text{L}$  tetrachloroethylene) and disperser solvent (1.0 mL acetonitrile) for the formation of cloudy solution in 5.0 mL aqueous sample containing biphenyl and biphenyl oxide. After extraction, phase separation was performed by centrifugation and biphenyl and biphenyl oxide in sedimented phase ( $5.0 \pm 0.3 \mu\text{L}$ ) were determined by gas chromatography-flame ionisation (GC-FID) system. Type of extraction and disperser solvents and their volumes, salt effect on the extraction recovery of biphenyl and biphenyl oxide from aqueous solution have been investigated. Under the optimum conditions and without salt addition, the enrichment factors for biphenyl and biphenyl oxide were 819 and 785, while the extraction recovery were 81.9% and 78.5%, respectively. The linear range was (0.125–100  $\mu\text{g L}^{-1}$ ) and limit of detection was (0.015  $\mu\text{g L}^{-1}$ ) for both analytes. The relative standard deviation (RSD,  $n=4$ ) for 5.0  $\mu\text{g L}^{-1}$  of analytes were 8.4% and 6.7% for biphenyl and biphenyl oxide, respectively. The relative recoveries of biphenyl and biphenyl oxide from sea, river water and refined water (Paksan company) samples at spiking level of 5.0  $\mu\text{g L}^{-1}$  were between 85.0% and 100 %.

**Keywords:** dispersive liquid–liquid microextraction; biphenyl; biphenyl oxide; water samples; gas chromatography

### 1. Introduction

Biphenyl and biphenyl oxide are widely used in production of heat-transfer fluids, plastics, optical brighteners, hydraulic fluids, high-temperature lubricants and in perfumery industries [1–5]. Workers exposed to these toxins experience nausea, vomiting, irritation of respiratory tract and later result in damage to the liver and nervous system [6]. Toxins enter the aquatic environment through waste effluents from textile mills, industrial processes and from leaking heat exchangers [5]. In order to determine trace amounts of biphenyl and biphenyl oxide in water samples, new sample preparation methods, especially in the microextraction category, are always of great interest. Therefore, new sample-preparation

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techniques which are fast, easy to use, inexpensive, environmentally friendly and compatible with a range of analytical instruments are required. Recently, efforts have been made towards miniaturisation of the liquid–liquid extraction procedure by greatly reducing the solvent to aqueous phase ratio, leading to the development of the liquid–phase microextraction (LPME) methodology [7–9]. Rezaee and co-workers [10–15] have developed a simple and rapid microextraction method, named dispersive liquid–liquid microextraction (DLLME) and so far it has been applied for extraction of different compounds. DLLME is based on a ternary component solvent system. The dispersion of the extraction solvent (assisted by the disperser solvent) within the aqueous solution leads to the generation of a significantly large contact area between the extraction solvent and the aqueous phase. In this paper, DLLME method has been optimised for the biphenyl and biphenyl oxide analysis in water samples. The analytes were extracted from the water samples and then, determined using a gas chromatography–flame ionisation (GC–FID) system.

## 2. Experimental

### 2.1 Reagents and standards

Biphenyl and biphenyl oxide were provided by Paksan Company (Tehran, Iran). Carbon tetrachloride, tetrachloroethylene, chloroform and chlorobenzene as extraction solvents and acetone, acetonitrile and methanol as disperser solvents were obtained from E. Merck (Darmstadt, Germany). Reagent grade NaCl was also obtained from E. Merck. Double distilled water was used for preparation of aqueous solutions. 0.001 g of biphenyl and biphenyl oxide were dissolved in 10.0 mL of methanol to obtain standard stock solution with a concentration of 100 mg L<sup>-1</sup>. A fresh 10 mg L<sup>-1</sup> Standard solution containing biphenyl and biphenyl oxide was prepared in methanol every week and stored at 4°C. Sea, river water and refined water (Paksan Company) samples, used for evaluation of the method were collected in glass bottles from Caspian Sea (Tonekabon, Iran), Cheshmehkileh River (Tonekabon, Iran) and Paksan Company (Tehran, Iran), respectively and stored at 4°C. Water samples were filtered through 0.45 µm cellulose acetate syringe filter (Osmonics, California, USA).

### 2.2 Instrumentation

A gas chromatograph (Agilent GC-7890) equipped with a split/splitless injector system and flame ionisation detector was used for separation and determination of biphenyl and biphenyl oxide. Ultra pure helium gas (99.999%, Air Products, UK) was passed through a molecular sieve and oxygen trap (Crs, USA) and was used as a carrier gas with a flow rate of 2 mL min<sup>-1</sup>. The injection port was held at 250°C and operated in the splitless mode for 1 min then split valve was opened and split ratio of 1 : 5 was applied. Separation was carried out on a DB5, 25 m × 0.32 mm i.d. and 0.25 µm film thickness from SGE (Victoria, Australia) Capillary column. The oven temperature was kept at 100°C for 2 min and then increased to 250°C at the rate of 10°C min<sup>-1</sup>, and was held for 3 min. The FID oven temperature was maintained at 270°C. Hydrogen was generated by hydrogen generator (OPGU-2200S, Shimadzu) for FID at a flow rate of 40 mL min<sup>-1</sup>. The flow of air (99.999%, Air Products) for FID was 400 mL min<sup>-1</sup>. The model 2010 D Centurion Scientific centrifuge (Centurion Scientific, West Sussex, UK) was used for separation of sediment phase from sample solution.

### 2.3 Dispersive liquid–liquid microextraction procedure

A quantity 5.0 mL of double distilled water was placed in a 10 mL screw cap glass test tube with a conical bottom and spiked at the level of  $100\ \mu\text{g L}^{-1}$  by biphenyl and biphenyl oxide. An amount of 1.0 mL of acetonitrile (as disperser solvent) containing  $8.0\ \mu\text{L C}_2\text{Cl}_4$  (as extraction solvent) was injected into a sample solution by using 1.0 mL syringe rapidly and then the mixture was gently shaken. A cloudy solution was formed in a test tube (the cloudy state was stable for a long time). Then it was centrifuged for 2 min at 5000 rpm and the dispersed fine particles of extraction phase were sedimented in the bottom of the conical test tube. After that  $2.0\ \mu\text{L}$  of the sedimented phase was injected into GC for analysis. The volume of the sediment phase was measured using a  $10\ \mu\text{L}$  microsyringe which was about  $5.0\ \mu\text{L}$ .

### 3. Results and discussion

In this work, DLLME followed with GC-FID has been applied for extraction and determination of biphenyl and biphenyl oxide in water samples. In order to obtain a high extraction recovery and enrichment factors, the effect of different parameters such as kind of extraction and disperser solvents, their volumes and salt addition on the extraction recovery were examined and optimal conditions were chosen. The enrichment factor and extraction recovery were calculated based on the following equations:

$$EF = \frac{C_{\text{sed}}}{C_0} \quad (1)$$

where  $EF$ ,  $C_{\text{sed}}$  and  $C_0$  are the enrichment factor, concentration of analyte in sedimented phase and initial concentration of analyte in aqueous sample, respectively.

$$ER = \frac{C_{\text{sed}} \times V_{\text{sed}}}{C_0 \times V_{\text{aq}}} = EF \times \frac{V_{\text{sed}}}{V_{\text{aq}}} \quad (2)$$

where  $ER$ ,  $V_{\text{sed}}$  and  $V_{\text{aq}}$  are the extraction recovery, volumes of sedimented phase and aqueous sample, respectively.  $C_{\text{sed}}$  was calculated from suitable calibration curve that was obtained by direct injection of biphenyl and biphenyl oxide standard solutions with concentration in the range of  $10\text{--}100\ \text{mg L}^{-1}$ .

#### 3.1 Selection of extraction solvent

The suitable extraction solvent should have extraction capability of interested compound. Carbon tetrachloride, tetrachloroethylene, chloroform and chlorobenzene were tested as extraction solvents. A series of sample solutions containing  $100\ \mu\text{g L}^{-1}$  of biphenyl and biphenyl oxide were prepared. A amount of 1.0 mL of acetonitrile containing different volumes of extraction solvent to achieve  $5.0\ \mu\text{L}$  volume of sedimented phase ( $12.0$ ,  $13.0$ ,  $8.0$  and  $45.0\ \mu\text{L}$  volumes of  $\text{C}_6\text{H}_5\text{Cl}$ ,  $\text{CCl}_4$ ,  $\text{C}_2\text{Cl}_4$  and  $\text{CHCl}_3$ , respectively) were injected into the sample solution rapidly. The extraction recoveries using different extraction solvents have been given in Table 1. The results show that  $\text{C}_2\text{Cl}_4$  has the highest extraction efficiency ( $81.9\%$  and  $78.5\%$  for biphenyl and biphenyl oxide, respectively) in comparison with other solvents. Therefore,  $\text{C}_2\text{Cl}_4$  was chosen as the extraction solvent in further experiments.

### 3.2 Selection of disperser solvent

Miscibility of disperser solvent with extraction solvent and aqueous phase is the main point in selection of disperser solvent. Thereby, acetone, acetonitrile and methanol were examined as disperser solvent. A series of sample solutions containing  $100.0 \mu\text{g L}^{-1}$  of biphenyl and biphenyl oxide were prepared and extracted using 1.0 mL of each disperser solvent containing  $8.0 \mu\text{L}$  of  $\text{C}_2\text{Cl}_4$ . The extraction recoveries using acetone, acetonitrile and methanol for biphenyl and biphenyl oxide are given in Table 2. According to the results, the extraction recoveries slightly vary when different disperser solvents are used. It is because in presence of water, minute amounts of these solvents are dissolved in extraction solvent and transported to sediment phase, they later appear as small peaks in chromatograms of the analytes. When acetonitrile is used as disperser solvent, sharp and cleaner chromatograms of analytes are obtained compared to when acetone or methanol is used. Due to this, acetonitrile is selected as disperser solvent.

### 3.3 Effect of extraction solvent volume

To examine the effect of extraction solvent volume on the extraction recovery, solutions containing different volumes of  $\text{C}_2\text{Cl}_4$  were subjected to the same DLLME procedure. The experimental conditions were fixed and included the use of 1.0 mL acetonitrile containing different volumes of  $\text{C}_2\text{Cl}_4$  (8.0, 13.0, 18.0 and  $23.0 \mu\text{L}$ ). By increasing the volume of  $\text{C}_2\text{Cl}_4$

Table 1. Extraction recovery (%) of different extraction solvents evaluated for the extraction of biphenyl and biphenyl oxide<sup>a</sup>.

Compound	Extraction recovery			
	$\text{CHCl}_3$	$\text{CCl}_4$	$\text{C}_2\text{Cl}_4$	$\text{C}_6\text{H}_5\text{Cl}$
Biphenyl	24.7	61.1	81.9	66.3
Biphenyl oxide	24.4	60.3	78.5	63.6

<sup>a</sup>Extraction conditions: water sample volume, 5.00 mL; disperser solvent (acetonitrile) volume, 1.00 mL; extraction solvent volumes,  $45.0 \mu\text{L}$   $\text{CHCl}_3$ ,  $12.0 \mu\text{L}$   $\text{C}_6\text{H}_5\text{Cl}$ ,  $13.0 \mu\text{L}$   $\text{CCl}_4$ ,  $8.0 \mu\text{L}$   $\text{C}_2\text{Cl}_4$ ; concentration of analytes,  $100 \mu\text{g L}^{-1}$ .

Table 2. Extraction recovery (%) of different disperser solvents evaluated for extraction of biphenyl and biphenyl oxide<sup>a</sup>.

Compound	Extraction recovery		
	Acetone	Acetonitrile	Methanol
Biphenyl	81.9	79.8	73.6
Biphenyl oxide	78.5	78.2	80.3

<sup>a</sup>Extraction conditions: water sample volume, 5.00 mL; disperser solvent (acetone, acetonitrile and methanol) volume, 1.00 mL; extraction solvent ( $\text{C}_2\text{Cl}_4$ ) volume,  $8.0 \mu\text{L}$ ; concentration of analytes,  $100 \mu\text{g L}^{-1}$ .

from 8.0 to 23.0  $\mu\text{L}$ , the volume of extraction solvent increases, and thereby the enrichment factor decreases due to dilution of sedimented phase as shown in Figure 1. Therefore the highest sensitivity was achieved by using 8.0  $\mu\text{L}$  of  $\text{C}_2\text{Cl}_4$ .

### 3.4 Effect of disperser solvent volume

Variation of the volume of acetonitrile (as disperser solvent) causes change in the volume of sedimented phase, hence it is impossible to consider the influence of the volume of acetonitrile on the extraction efficiency. In order to achieve a constant volume of sedimented phase, the volume of acetonitrile and  $\text{C}_2\text{Cl}_4$  were varied, simultaneously. The experimental conditions were fixed and included the use of different volumes of acetonitrile 0.5, 1.0, 1.5 and 2.0 mL containing 7.0, 8.0, 10.0 and 12.0  $\mu\text{L}$  of  $\text{C}_2\text{Cl}_4$ , respectively. Under these conditions, the volume of sedimented phase was constant ( $5.0 \pm 0.3 \mu\text{L}$ ). The results are shown in Figure 2. Accordingly the extraction recovery increases by increasing acetonitrile volume up to 1.0 mL and then decreases by increasing the volume of acetonitrile. It seems that at low volume of acetonitrile, cloudy state is not formed well, thereby, the extraction

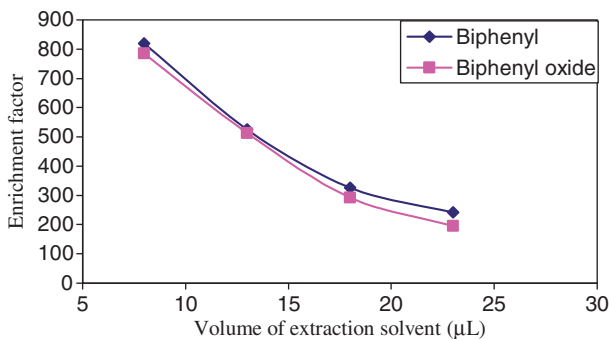


Figure 1. Effect of the extraction solvent ( $\text{C}_2\text{Cl}_4$ ) volume on the enrichment factor of analytes. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (acetonitrile) volume, 1.0 mL; extraction solvent ( $\text{C}_2\text{Cl}_4$ ) volumes, 8.0, 13.0, 18.0, 23.0  $\mu\text{L}$ ; concentration of analytes,  $100 \mu\text{g L}^{-1}$ .

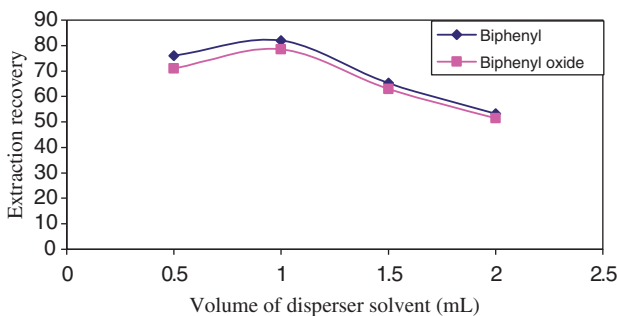


Figure 2. Effect of the disperser solvent (acetonitrile) volume on the extraction recovery of analytes. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (acetonitrile) volumes, 0.50, 1.0, 1.5 and 2.0 mL; extraction solvent ( $\text{C}_2\text{Cl}_4$ ) volumes, 7.0, 8.0, 10.0 and 12.0  $\mu\text{L}$ ; concentration of analytes,  $100 \mu\text{g L}^{-1}$ .

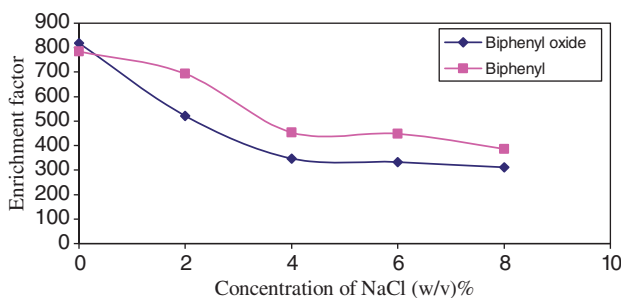


Figure 3. Effect of salt addition on the enrichment factor of analytes. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (acetonitrile) volume, 1.0 mL; extraction solvent ( $C_2Cl_4$ ) volume, 8.0  $\mu$ L; concentration of analytes, 100  $\mu$ g  $L^{-1}$ .

recovery decreases. At the high volume of acetonitrile, the solubility of biphenyl and biphenyl oxide in water increases, therefore, the extraction recovery decreases. Thus 1.0 mL of acetonitrile was chosen as optimum volume in further work.

### 3.5 Effect of ionic strength

To investigate the influence of ionic strength on the extraction recovery of biphenyl and biphenyl oxide using DLLME, various experiments were performed by adding different amount of NaCl (0–8% (w/v)) where other experimental conditions were kept constant. The volume of the sedimented phase was increased from 5 to 7  $\mu$ L by increasing the amount of NaCl from 0 to 8 (w/v)%, because of decreasing solubility of extraction solvent in aqueous phase. According to Figure 3, the enrichment factor decreases, with the increase in the volume of sedimented phase. Therefore, the extraction experiments were carried out without salt addition.

### 3.6 Effect of extraction time

In DLLME, extraction time is defined as interval time between injection of the mixture of disperser solvent (acetonitrile) and extraction solvent ( $C_2Cl_4$ ), before starting to centrifuge. The effect of extraction time was examined in the range of 0–40 min with constant conditions. The extraction recoveries ranged from 80.5% to 82.8% for analytes in that period of time. According to this, time has no significant impact on the extraction efficiency. This outcome is similar to other reported works on DLLME method [10–15]. Thereby, transition of analytes from aqueous phase (sample) to extraction solvent is fast. Subsequently, equilibrium state is achieved quickly. This is the main advantage of DLLME technique. In this method centrifuging step of sample solution in extraction procedure is about 2 min.

### 3.7 Quantitative analysis

Figures of merit of the developed method under optimised conditions are shown in Table 3. Linearity was observed over the range 0.125–100  $\mu$ g  $L^{-1}$  for both analytes. Coefficients of



Table 3. Quantitative results of DLLME and GC-FID method for biphenyl and biphenyl oxide.

Analyte	Linear range ( $\mu\text{g L}^{-1}$ )	LOD <sup>a</sup> ( $\mu\text{g L}^{-1}$ )	RSD (%) <sup>b</sup>	ER (%) <sup>d</sup>	EF <sup>c</sup>	R <sup>2e</sup>
Biphenyl	0.125–100	0.015	8.4	819	81.9	0.9997
Biphenyl oxide	0.125–100	0.015	6.7	785	78.5	0.9979

<sup>a</sup>LOD, limit of detection for  $S/N=3$ .

<sup>b</sup>RSD, relative standard deviation ( $n=4$ ).

<sup>c</sup>Enrichment factor at the concentration analytes of  $100 \mu\text{g L}^{-1}$ .

<sup>d</sup>Extraction recovery.

<sup>e</sup>Coefficient of determination.

Table 4. Determination of biphenyl (BP) and biphenyl oxide (BPO) in sea, river and refined water C<sup>a</sup> and relative recovery of spiked biphenyl and biphenyl oxide in them.

Sample	Concentration of BP and BPO ( $\mu\text{g L}^{-1}$ ) $\pm$ SD, $n=3$		Added BP and BPO ( $\mu\text{g L}^{-1}$ )		Found BP and BPO ( $\mu\text{g L}^{-1}$ ) $\pm$ SD, $n=3$		Relative recovery (%)	
	BP	BPO	BP	BPO	BP	BPO	BP	BPO
River water <sup>b</sup>	0.64 $\pm$ 0.06	n.d. <sup>d</sup>	5	5	4.9 $\pm$ 0.4	4.4 $\pm$ 0.5	85	88
Sea water <sup>c</sup>	0.83 $\pm$ 0.06	n.d. <sup>d</sup>	5	5	5.4 $\pm$ 0.5	4.8 $\pm$ 0.4	91	96
Refined water C <sup>a</sup>	0.76 $\pm$ 0.06	n.d. <sup>d</sup>	5	5	5.0 $\pm$ 0.4	5.0 $\pm$ 0.3	58	100

<sup>a</sup>Paksan Company (Tehran, Iran).

<sup>b</sup>From Cheshmehkileh (Tonekabon, Iran).

<sup>c</sup>From Caspian Sea (Tonekabon, Iran).

<sup>d</sup>Not detected.

determination ( $R^2$ ) were 0.9997 and 0.9979 for biphenyl and biphenyl oxide, respectively. The extraction recoveries were 81.9% and 78.5%, and enrichment factors were 819 and 785 in this method for biphenyl and biphenyl oxide, respectively. The relative standard deviations (RSDs,  $n=4$ ) at the level of  $5.0 \mu\text{g L}^{-1}$  of biphenyl and biphenyl oxide were 8.4% and 6.7%, respectively. The limit of detections (LODs), based on signal to noise ratio ( $S/N$ ) of 3 were  $0.015 \mu\text{g L}^{-1}$  for both of analytes.

### 3.8 Water samples analysis

In order to test the applicability of the proposed method, three different water samples (sea, river and refined water) were extracted and analysed. The results are recorded in Table 4. All the water samples were spiked with biphenyl and biphenyl oxide standard solution ( $5.0 \mu\text{g L}^{-1}$  concentration level) to assess the matrix effects. The obtained relative recoveries were between 85 and 100.0% (Table 4). The results show that matrix has negligible effect on DLLME of biphenyl and biphenyl oxide. Figure 4 shows GC-FID chromatograms of sea water prior (a) and after (b) spiking with biphenyl and biphenyl oxide at  $5.0 \mu\text{g L}^{-1}$  level.

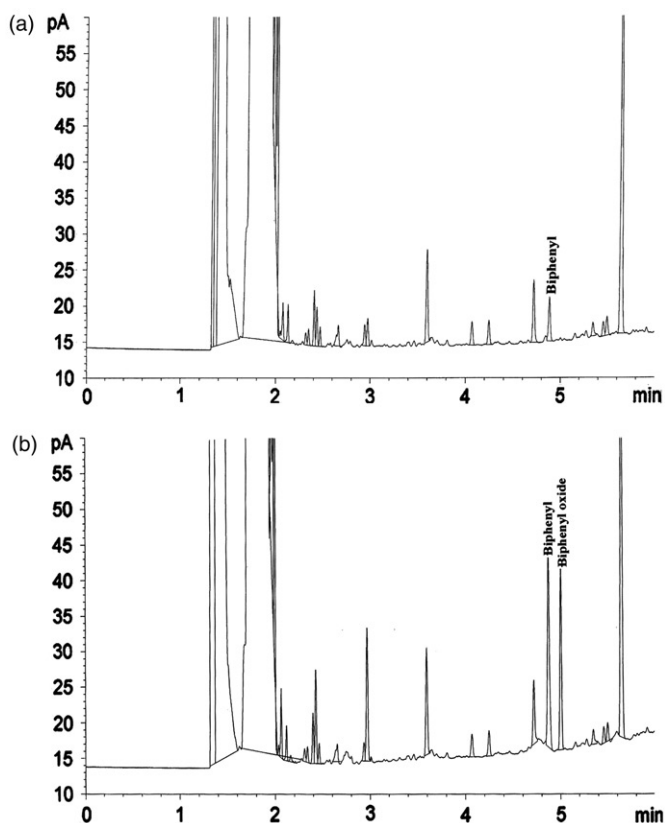


Figure 4. GC-FID chromatograms of analytes in sea sample before spiking (a) and after spiking with  $5.0 \mu\text{g L}^{-1}$  (b) using DLLME method combined with GC-FID under optimum conditions.

#### 4. Conclusion

In the present study, DLLME combined with GC-FID has been optimised for determination of biphenyl and biphenyl oxide in water samples. This method provides good repeatability, high recovery and enrichment factor within a much short time. The performance of this procedure in the extraction of biphenyl and biphenyl oxide from sea, river water and refined water (Paksan Company) is excellent. The present developed microextraction technique has distinct advantages over conventional method with respect to extraction time, volume of solvents required and low detection limits are readily achieved.

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