etermination of Some Phenols in Tigris River by HPLC

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Abstract

This study describes the determination of some phenols in four different zones of Tigris river in Iraq including, AL-Krieat, AL-Kadhimiya, AL-Jadiriyah, and AL-Adhamiyah. The phenolic compounds analyzed were (2,3-dimethylphenol, 4-chlorophenol, 3-nitrophenol and 2,4-dinitrophenol) using reverse phase high performance liquid chromatography (RP-HPLC) with a UV detector on ODS-C_{18} column(150×4.6 mm I.D) and a mobile phase consisted of methanol-water(30:70)%(v/v) at pH 5.0, and a column temperature at 30C° with 20 µL injection. UV detection helps to identify different phenols at wave length at 280 nm with a flow rate at 0.5 ml/min. The separation time was (< 6) min.The results indicated that the AL-Adhamiyah zone contains the highest concentration of 3-nitrophenol (0.094 µg/mL), while the AL-Jadiriyah zone recorded the lowest concentration of 3-nitrophenol (0.056 µg/mL).On the other hand the other phenolic compounds studied did not record any concentration in four zones of Tigris river. The values of correlation coefficient and accuracy were calculated for 3-nitrophenol, the graph was linear with very good correlation coefficient (R^2>0.9999).

Key words: Phenols, Tigris river, High performance liquid chromatography.

Introduction

Phenolic compounds, one kind of priority pollutants, often occur in the aquatic environment due to their widespread use in many industrial processes such as the manufacture of plastics, dyes, drugs, antioxidants, and pesticides[1]. Phenols, even at concentrations below 1 µg/L, can affect the taste and odor of water. Hence, identification and monitoring of these compounds at trace level in drinking water and surface waters are imperative. Priority phenols consist of a number of substituted phenolic compounds including halogenated (e.g., chlorophenol), nitrated (e.g., 2-nitrophenol), alkylated (e.g., 2,4-dinitrophenol), and ether (e.g., methoxyphenol). Priority phenols are used (or produced) in several industrial processes. They are commonly used as preservatives, disinfectants, in pulp processing, in the manufacture of pesticides and other intermediates. Unfortunately, priority phenols are now common environmental pollutants found in potable water, sediments and soil. Many priority phenols especially the chlorophenols, are known for their toxicity, carcinogenicity and persistence in the environment[2]. Pentachlorophenol is widely used as a wood preservative and has been found to be an indoor air contaminant[3]. Many phenol derivatives are now included in the lists of priority pollutants in many countries. The European Community (EC) directive specifies a legal tolerance level of 0.5 µg/L for each phenol in water intended for human consumption and Japan's Ministry of Health, Labour, and Welfare specifies a maximum contaminant level (MCL) of 5 µg/L for phenols in drinking water. The U.S. EPA specifies a MCL of 1 µg/L for pentachlorophenol, and eleven common phenols are on the U.S. EPA priority pollutants list[2,4]. The U.S. EPA lists eleven phenols on its list of priority pollutants. The European Union has set the maximum total and individual phenol permitted concentrations in water used for human consumption at 0.5 µg/L and 0.1 mg/L, respectively[2]. Gas chromatography(GC) is commonly used for detection of phenols[5,6], but nonvolatiles in many water samples can poison GC columns. Liquid chromatography (LC) methods require preconcentration[7] and matrix elimination to detect low concentrations. Solid-phase extraction (SPE) is highly effective, but is time consuming and expensive.

On-line SPE combined with HPLC reduces the labour and expense of manual SPE and allows phenols to be determined at low concentrations in real samples[8]. High performance liqid chromatography(HPLC) is widely used for the determination of phenolic compounds. HPLC with electrochemical detection(ECD) provides superior sensitivity for most of the phenols of interest with the exception of the dinitrophenols which are measured by UV absorbance. Previous studies[9,10] have shown that reversed-phase liquid chromatography (RP-LC) coupled to atmospheric pressure chemical ionization mass spectrometry (APCI-MS) can effectively separate and detect a range of phenolic compounds at low ppb levels.

Generally, HPLC was often used to determine phenols because of its stable sensitivity[11–14]. Prior to HPLC analysis, an effective preconcentration step is always necessary. Recently, LPME has shown to be an attractive alternative for sample preparation. Till date, LPME has been successfully applied for the extraction of organic pollutants from a variety of matrices [15–20]. In the present study we use the HPLC technique for the determination of various phenolic compound in different samples.

Apparatus

HPLC analysis was carried out on an LC-10AT liquid chromatography (Shimadzu, Japan), with two LC-10ATVP pumps and an SPD-10A UV/VIS detector. The analytical column was avp-ODS-C_{18} column (150×4.6 mm I.D) practical size 5 µm; Shimadzu. A 7125 injector with 20 µL loop (Rheodyne CA, USA). AM 420 pH meter (Hanna, Italy) was used to measure the PH of mobile phase.

Experimental

Materials and Reagents

2,3-Dimethylphenol was obtained from (Fluka AG, Germany); 3-nitrophenol was obtained from (Riedel-De, Germany); 2,4-dinitrophenol and 4-chlorophenol were obtained from (BDH Chemicals, England). Methanol was used as a component of the mobile phase was obtained from (Tedia Chemical Co. Germany GFS) and deionised water.

Water samples

In this work, four water samples were used for evaluation including AL_Krieat, AL_Kadhimiya, AL_Jadiriyah and AL_Adhamiyah were collected from Tigris river in Baghdad city (500 mL of each sample). Before the environmental water samples were used, they were filtered through 0.45µm micropore membranes.

Method for preparing standard solution and sample solution, separation of each compounds and determination of four phenols

Each phenol was dissolved in methanol to obtain a standard stock solution with the concentration of 100 µg/mL. A mixed standard solution containing four phenols was prepared in methanol. Working solutions were prepared daily by appropriate dilution of the standard solutions with ultrapure water with the concentration of 1 µg/mL. Phenols were eluted by using mobile phase consisted of methanol: water(30:70)v/v at flow rate of 0.5 mL/min. The injection volume and detection of wavelength were 20 µL and 280 nm, respectively. The retention times(t_{R1} and t_{R2}) were used to calculate the capacity factors of the first and second eluted phenols, k'_1 and k'_2, respectively, by use of the equations ($k'_1 = (t_1 - t_0)/t_0$) and ($k'_2 = (t_2 - t_0)/t_0$), the number of theoretical plates(N) is calculated by use of the equation $N=(16(t_R/w)^2)$, the selectivity factor was calculated by use of the equation ($\alpha = k'_2/ k'_1$) and the resolution factor by the use of the equation ($R_S = 2(t_2 - t_1)/(w_1 + w_2)$), where w_1 and w_2 are the widths of the two peaks, where t_R is the retention time of the sample and t_0 is the of mobile phase(1.60 min.).

Phenols were initially identified by the retention times, and the concentrations were determined by comparing the peak areas of the samples to the phenols standard.

Results and discussion

The isocratic method enables the separation and detection of four different standard phenols in a 6 minute run (Fig.1). A good separation can be achieved in a short separation time . In this selected solvent using methanol:water(30:70)% as an eluent gave a good peak shapes and a good resolution of phenolic compounds.

As a consequence of the separation, it was found that the elution order of the four compounds were as follows; 2,3-dimethylphenol, 4-chlorophenol, 3-nitrophenol and 2,4-dinitrophenol.

Initial investigations into the change in the capacity factors(k′) of phenols with pH of eluent, and the percentage of an organic modifier showed that for full separation of phenols with avp-ODS-C_{18} column the pH should be kept at 5 and the organic modifier at 30% v/v methanol can be used under isocratic conditions.

After multiple preliminary assay an ODS-C_{18} column an isocratic elution program by using methanol-water as solvent was chosen.The phenolic compounds have been identified according to their retention times of standard and samples solutions.

Table 1 gives the retention times, peak height and peak area for a number of the phenols, and table 2 shows the retention time(t_R), capacity factor($\bar{K}$), number of theoretical plates(N), separation factor(α) and resolution(Rs) of the phenolic compounds.

Application

Four environmental water samples, including AL_Krieat, AL_Kadhimiya, AL_Jadiriyah and AL_Adhamiyah in Tigris river. The proposed procedure has been successfully applied to the determination of phenols in river water. A sep-pak C_{18} cartridge was preconditioned for phenolic compounds by passing 500 ml of each sample through the cartridge, after the sample passed completely then washed with 5 ml methanol. The final volume of each sample was 5 mL that means it is concentrated one hundred times. Fig. 2 shows the chromatogram for separation of 3-nitrophenol in Tigris river (AL-Krieat zone), fig.3 shows the chromatogram for separation of 3-nitrophenol in Tigris river(AL-Kadhimiya zone), fig.4 shows the chromatogram for separation of 3-nitrophenol in Tigris River (AL-Jadiriyah zone), and fig.5 shows the chromatogram for separation of 3-nitrophenol in Tigris river (AL-Adhamiyah zone). All of these sample analysis were by using λ max 280 nm (UV-Detector), detection on column ODS-C_{18} (150 × 4.6 mm I.D) and mobile phase (30:70)% methanol : water at PH 5 with flow rate 0.5 mL/min and column temperature 30 C°.

In all studied zones of Tigris river (AL_Krieat, AL_Kadhimiya, AL_Jadiriyah and AL_Adhamiyah) contained 3-nitrophenol at different concentrations,(0.073 µg/mL), (0.084 µg/mL), (0.056 µg/mL) and (0.094 µg/mL) respectively.While the other phenolic compounds studied did not record any concentration in four zones of Tigris river (table 3). Fig.6 shows the linear relationships between peak area and the concentrations of 3-nitrophenol in all studied zones. The concentration, retention time, peak area, peak height, corresponding regression equation, correlation coefficient and accuracy of 3-nitrophenol are summarized in table 4.

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Table (1): Retention time, peak height, and peak area of phenols by using RP-HPLC on column ODS-C_{18} and mobile phase methanol-water(30:70)%(v/v) at pH 5.0, and column temperature 30C°.

No.	Phenols	t_R (min)	peak height cm	Peak area µv	Peak area %
1	2,3-Dimethylphenol	2.644	7.00	295528	38.344
2	4-Chlorophenol	3.688	5.50	219471	28.476
3	3-Nitrophenol	4.094	3.50	155330	20.154
4	2,4-Dinitrophenol	5.118	1.00	44175	5.732
Total			17.00	714504	92.706

Table (2): Retention time(t_R),capacity factor($\bar{K}$), number of theoretical plates(N), separation factor(α) and resolution(Rs) of phenols using RP-HPLC on column ODS-C_{18} and mobile phase methanol-water(30:70)%(v/v) at pH 5.0, and column temperature 30C°.

No.	Phenols	Dead time (to) min	t_R min	$\bar{K}$	N	α	Rs
1	2,3-Dimethylphenol	1.6	2.644	0.65	310.69	—	—
2	4-Chlorophenol		3.688	1.30	604.50	2.00	1.74
3	3-Nitrophenol		4.094	1.55	419.02	1.19	0.58
4	2,4-Dinitrophenol		5.118	2.19	1164.17	1.41	1.46

Table (3):Concentrations of phenols in Tigris river using RP-HPLC on column ODS-C_{18} and mobile phase methanol-water(30:70)%(v/v) at pH 5.0,and column temperature 30C°.

No.	Phenols	AL-Krieat zone µg/mL	AL-Kadhimiya zone µg/mL	AL-Jadiriyah zone µg/mL	AL-Adhamiyah zone µg/mL
1	2,3-Dimethylphenol	-	-	-	-
2	4-Chlorophenol	-	-	-	-
3	3-Nitrophenol	0.073	0.084	0.056	0.094
4	2,4-Dinitrophenol	-	-	-	-

Table (4): Concentrations, retention time(tR), peak area, peak height, corresponding regression equation, correlation coefficient and accuracy of 3-nitrophenol for four water samples in Tigris river using RP-HPLC on column ODS-C18 and mobile phase methanol-water(30:70)%(v/v) at pH 5.0, and column temperature 30C°.

No.	Water sample	3-Nitrophenol					
		Conc. µg/mL	t_R (min)	Peak Area (µv)	Peak Height(cm)	Peak area %	Peak Height %
1	AL-Krieat zone	0.073	3.991	285963	6.00	48.038	42.664
2	AL-Kadhimiya zone	0.084	3.961	329182	6.50	56.886	60.138
3	AL-Jadiriyah zone	0.056	3.960	219433	6.80	58.031	60.950
4	AL-Adhamiyah zone	0.094	4.187	368388	6.80	63.423	57.290
Total		0.307					
Mean		0.076					
R.S.D. %		21.20					
Regression equation		y = 3.9E + 6x –142.30					
Correlation coefficient (R^2)		1.0000					

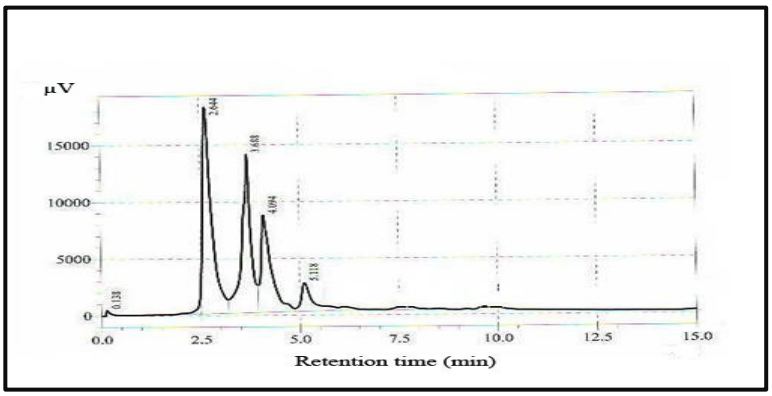

Fig.(1):Chromatogram for separation of 2,3-dimethylphenol, 4-chlorophenol,3-nitrophenol and 2,4-dinitrophenol respectively by using RP-HPLC on column ODS-C_{18} and mobile phase methanol-water(30:70)%(v/v) at pH 5.0, and column temperature

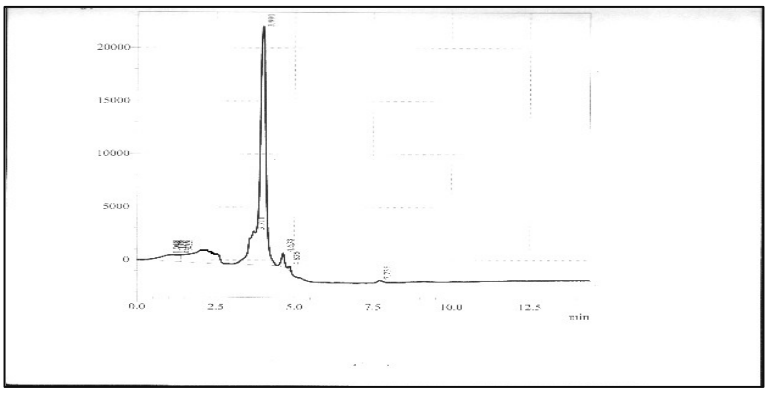

Fig.(2): Chromatogram for separation of 3-nitrophenol in Tigris river(AL-Krieat zone) on column ODS-C_{18}(150 × 4.6 mm I.D) and mobile phase(30:70)% methanol : water at pH 5 with flow rate 0.5 mL/min and column temperature 30 C°.

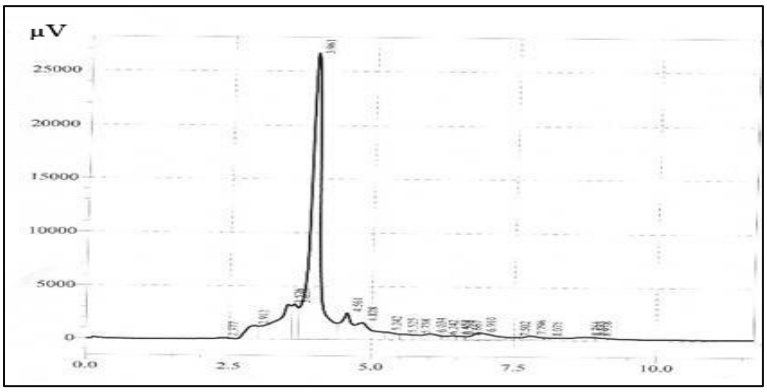

Fig.(3): Chromatogram for separation of 3-nitrophenol in Tigris river(AL-Kadhimiya zone) on column ODS-C_{18}(150 × 4.6 mm I.D) and mobile phase(30:70)% methanol : water at pH 5 with flow rate 0.5 mL/min and column temperature30 C°.

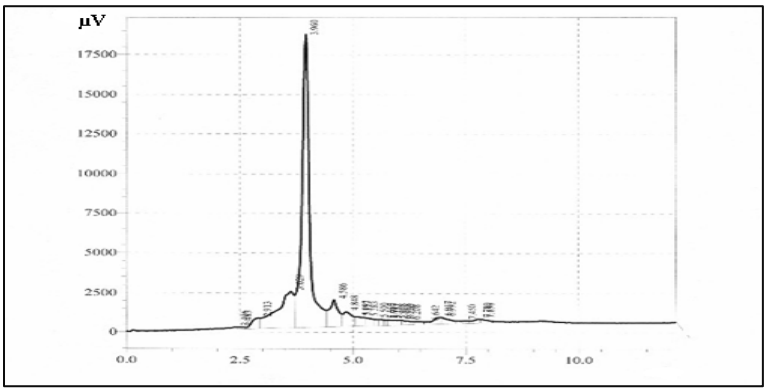

Fig.(4): Chromatogram for separation of 3-nitrophenol in Tigris river(AL-Jadiriyah zone) on column ODS-C_{18}(150 × 4.6 mm I.D) and mobile phase(30:70)% methanol : water at pH 5 with flow rate 0.5 mL/min and column temperature 30 C°.

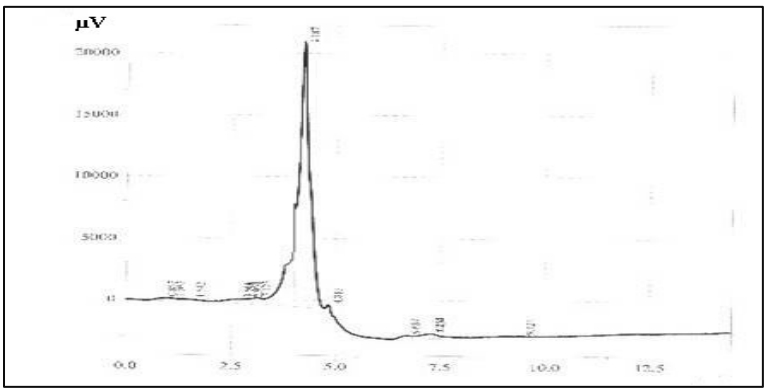

Fig.(5): Chromatogram for separation of 3-nitrophenol in Tigris river(AL-Adhamiyah zone) on column ODS-C_{18}(150 × 4.6 mm I.D) and mobile phase(30:70)% methanol: water at pH 5 with flow rate 0.5 mL/min and column temperature 30 C°.

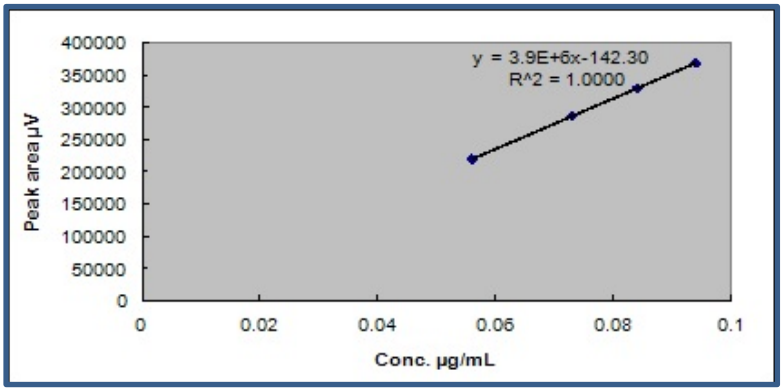

Fig.(6): The relationship between peak area and concentrations of 3-nitrophenol in all studies zones of Tigris river including AL-Jadiriyah, AL-Krieat, AL-Kadhimiya and AL-Adhamiyah zone respectively.

تقدير بعض الفينولات في مياه نهر دجلة بإستعمال تقنية كروموتوغرافيا السائل ذي الأداء العالي

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استلم البحث في : 28 تشرين الثاني 2012 ، قبل البحث في :16 كانون الثاني 2013

الخلاصة

تناولت هذه الدراسة تقدير بعض الفينولات في أربع مناطق مختلفة من نهر دجلة في العراق التي تضمنت الكريعات، والكاظمية، والجادرية والأعظمية. تم تحليل المركبات الفينولية المدروسة(٢،٣- ثنائي مثيل فينول، ٤- كلورو فينول، ٣- نايترو فينول و ٢،٤- ثنائي نايترو فينول) باستعمال تقنية كروموتوغرافيا السائل ذي الأداء العالي-الطور المعكوس مع كاشف للأشعة فوق البنفسجية على عمود ODS-C_{18} بأبعاد (150×4.6) ملم وطور متحرك من الميثانول والماء(30 : 70)% ذي pH 5.0 ودرجة حرارة العمود الكروموتوغرافي (30 C°) بمعدل جريان 0.5 مل/دقيقة وطول موجي 280 نانوميتر خلال زمن قدره (٦>) دقيقة . بينت النتائج ان منطقة الأعظمية تحتوي على أعلى تركيز من ٣- نايترو فينول (0.094 مايكروغرام/مل)، بينما سجلت منطقة الجادرية اقل تركيز من ٣- نايترو فينول (0.056 مايكروغرام/مل). في حين لم تسجل المركبات الفينولية المدروسة الاخرى أي تركيز في المناطق الاربعة من النهر. حسبت قيم الدقة، ومعادلة الخط المستقيم، ومعامل الارتباط للمركب ٣- نايترو فينول في المناطق المدروسة.

الكلمات المفتاحية: الفينولات ؛ نهر دجلة، كروموتوغرافيا السائل ذات الأداء العالي.