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SEPARATION AND IDENTIFICATION OF NAPHTHALENE, ACENAPHTHYLENE, PYRENE, BENZ{A} ANTHRACENE AND 1,3,2,4-DIBENZANTHRACENE

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Abstract

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) was coupled with ultraviolet absorption sepectoscopy (UV) for separation and identification of Naphthalene, Acenaphthylene, Pyrene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene.

RP-HPLC was performed on an ODS-C18 column (150×4.6 mm I.D) using acetonitrile–buffer phosphate as mobile phase. UV absorption spectra of the elutes was detected in 254 nm, and studying the chromatographic variables include organic modifier ratio, PH, column temperature and concentration of buffer to maximize resolution and minimize separation time. the results showed that using mobile phase(80:20) v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and column temperature 60 C° at wave length 254 nm would give ideal separation with good resolution. The separation time was (7.5) min.

Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) are environmental pollutants mainly formed during the incomplete combustion of organic matter, under pyroltic conditions (1,2). And they ara also formed by incomplete combustion of carbon-containing fuels such as wood, coal, diesel, fat, tobacoo, or incense (3). at high temperature the pyrolysis of organic yields molecule fragments and radicals which combine to give PAHs. polycyclic aromatic hydrocarbon known for their carcinogenic, mutagenic and teratogenic properties are benz [a] anthracene, chrysene ,benzo [b] fluoranthene, benzo [j] fluoranthene, benzo [k] fluoranthene, benzo [a] pyrene, benzo [ghi] perylene, coronene, dibenz [a,h] anthracene ($C_{20}H_{14}$), indeno [1,2,3-cd]pyrene ($C_{22}H_{12}$) and ovalene (4). The US EPA has identified 16 PAHs as priority pollutants, many of these PAHs are toxic, even pollutants in water, have received considerable attention, because of their documented carcinogenicity experimental animals of several species (5). Although PAHs clearly are aromatic compounds, the degree of aromaticity can be different for each ring segment. According to Clar's rule (formulated by Eric Clar in 1964) for PAHs the resonance structure with the most disjoint aromatic π-sextets—i.e. benzene-like moieties—is the most important for the characterization of the properties (6). however, there are several ansolved problems, including a practical analytical technique, under standards (7,8) adopted by the European Union (EU) for drinking water, the limit of the maximum concentration is 200 ng/l. PAHs are lipophilic, meaning they mix more easily with oil than water. The large compounds are less water-soluble and less volatile .In January 2004 (at the 203rd Meeting of the American Astronomical Society (9), it was reported (10) that a team led by A. Witt of the University of Toledo, Ohio studied ultraviolet light emitted by the Red Rectangle nebula and found the spectral signatures of anthracene and pyrene (no other such complex molecules had ever before been found in space). the advantage of HPLC for the fractionation of PAHs mixtures and sample clean-up are undoubted and validated. the technique of efficient separation has been described in several papers (11). The evalution and monitoring of trace levels of PAHs from authentic environmental matrices are imperative. in order to determine trace levels of ubiquitous environmental pollutants, effective, clean-up extraction and procedures are usually necessary, because of this, several analytical techniques have been developed to measure low concentration of PAHs in water. the analysis of these compounds can be performed by Variouse Gas Chromatography (GC) (12). High Performance Liquid Chromatography (HPLC) (13, 14) and electrophoretic methods. Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) on chemically bonded C18 has become the method of choice for the separation of PAHs and a review by Wise et al. (15) has described general protocols for the separation of PAHs using RP-HPLC. Schmit et al.(16) first described the separation of PAHs by liquid chromatography using a reverse phase column consisting of a chemically bonded C18 stationary phase. The structural and physicals characteristics of PAHs shown in Table (1) (17).

Experimental

Apparatus

The HPLC system consisted of two pumps (LC-6A) (shimadzu, Kyoto, Japan), a 7125 injector with a 20 µl loop(Rheodyne CA, USA), adopted with reverse phase analytical column(150×4.6 mm I.D Bellefont,PA,USA), the column was placed in a thermostatically costrolled oven(Shimadzue Model CTO-6A), instrument equipped with variable wave length UV spectrophotometric detector(Shimadzue Model CTO-6A).

Chemicals and Reagents

The compounds include(Naphthalene, Acenaphthylene, Pyrene, Benz {a} anthracene and 1,3,2,4-Dibenzanthracene) were purchased from Supelco(Bellefont, PA, USA) and chem.service(BDH-British). All chemicals were HPLC-grade reagents. The solvents were HPLC-grade include Acetonitrile obtained from (BDH-British) and(Fluka-Swiss) and Methanol from(Riedel-Germany) and (Fluka-Swiss). The other compounds were grade including potassium phosphate was obtained from (BDH-British), phosohoric acid and sodium hydroxide were obtained from (Fluka-Swiss).

Preparation of Solution

1-The buffer solutions prepared by dissolving 15.6 gm from potassium phosphate (KH_2PO_4) in 1 liter distillation water for prepared 0.1M, and dissolving 1.56 gm in 1 liter for prepared 0.01M. and stable the PH of buffer by using PH-meter and addition phosphoric acid and sodium hydroxide concentrated.

2-The stock standard solution was prepared (100 ppm) by dissolving 1mg for each compound in 10 ml methanol, and other solution prepared from stock solution.

3-Standard solution of mixture of PAHs was prepared from stock solution (Naphthalene 10 PPm by dilution 10 ml to 100 ml methanol as final volume, Pyrene 2PP m by dilution 2 ml to 100 ml methanol as final volume, Acenaphthylene 5PP m by dilution 5 ml to 100 ml methanol as final volume, Benz {a} anthracene 0.5PPm by dilution 0.5 ml to 100 ml methanol as final volume and 1,3,2,4- Dibenzanthracene 1 PPm by dilution 1 ml to 100ml methanol as final volume).

Preliminary Test

1-Inject 20 µl for each compound on column ODS-C18 at wave length 254 nm, and elution the mixture by using 55% of acetonitrile in 0.01M buffer phosphate at PH 4.7, and column temperature 25C° at flow rate 1 ml/min, and determine the retention time for each compound.

2-Inject 20 µl of mixture on column ODS-C18 at wave length 254 nm, and elution the mixture by using 55% of acetonitrile in 0.01M buffer phosphate at PH 4.7, and column temperature 25 C° at flow rate 1ml/min, and identificate all compounds by the retention time for each compound Table (2).

3-Inject 20 µl of mixture on column ODS-C18 at wave length 254 nm, and elution the mixture by using different ratio of acetonitrile between(55-80)% in 0.01M buffer phosphate at PH 4.7,and column temperature 25C° at flow rate 1ml/min.

4-Inject 20 µl of mixture on column ODS-C18 and elution the mixture by using mobile phase (80:20) v/v acetonitrile : 0.01 M buffer phosphate at different PH between (3.5-6.5) and column temperature 25C° at flow rate 1ml/min.

5-Inject 20 µl of mixture on column ODS-C18 and elution the mixture by using mobile phase (80:20) v/v acetonitrile : 0.01M buffer phosphate at PH 6 and different column temperature between (30-60) C° at flow rate 1 ml/min.

6-Inject 20 µl of mixture on column ODS-C18 and elution the mixture by using mobile phase (80:20) v/v acetonitrile : different concentration of buffer phosphate between (0.01-0.1) M of at PH 6 and column temperature 60 C° at flow rate 1 ml/min.

The Application In Water Using RP-HPLC With 0ptimized Conditions Of PAHs :

During the winter, water samples were collected from Dijla river of Baghdad. water sample were collected in glass and filtered through a membrane filter under vacume to obtain dissolved samples for polycyclic aromatic hydrocarbons.

Separation And Analysis Of Polycyclic Aromatic Hydrocarbons In The River :

1-Inject 20 µl of the sample on ODS-C18 column at wave length 254 nm using optimized conditions.
2-Inject 20 µl of the mixture of PAHs on ODS-C18 column at wave length 254 nm using optimized conditions.
3-Identificate the PAHs in the sample by corresponding the retention time for each compound in chromatogram of sample with its retention time in chromatogram of mixture.
4-Measured peak height of compounds in chromatogram of sample and comparison it with peak height for these compounds in chromatogram of mixture, then determination the concentration of the compound in sample.

Results and Discussions

Effect of the Variation in the Modifier Content

Generally, using HPLC, the increase in the organic solvent content in necessary of reduce the retention time of Naphthalene, Acenaphthylene, Pyrene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene. (shown in Table (3). Because the silanophilic intraction between the solute and free silanole groups at the stationary phase, where decrease this interactions by increase the organic modifier ratio in mobile phase. The Fig. (1) show. Chromatogram for sepration of PAHs by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D and mobile phase (80:20) v/v acetonitrile: 0.01M buffer phosphate at PH 4.7 and temperature 25 C°.

Effect of PH

The PH of the sample can be adjusted to provide better selectivity. table 4 shows the retention time of Naphthalene, Acenaphthylene, Pyrene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene. Which measured at different PH of phosphate buffer solution. Studies by Yang(18) and Powliszyn(19) had confirmed that adjusting the PH of an aqueous woud change capacity factor for dissociable species, and effect the extraction efficiency. the Fig.(2) show chromatogram for sepration of PAHs. by using RP- HPLC on column ODS-C18 (150×4.6 mm I.D and mobile phase (80:20) v/v acetonitrile : 0.01M buffer phosphate at PH 6 and temperature 25 C°.

Effect of Temperature

Increase of the temperature can strongly reduce the retention time of Table (5). The results show that Naphthalene, Acenaphthylene, Pyrene, Benz {a} anthracene and 1,3,2,4-Dibenzanthracene .increasing the temperature decrease the capacity factor(K^{-}), because decrease the viscosity of mobile phase then increase the column efficicency. The Fig. (3) show chromatogram for sepration of PAHs. by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20) v/v acetonitrile : 0.01 M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm.

Effect of Buffer Concentration

The results show that increasing of buffer concentration decrease the capacity factor, because of change the surface strain of salt, then retention time was decreased. Table (6) shows retention time of Naphthalene, Acenaphthylene, Pyrene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene. Which measured at different concentration of phosphate buffer solution. The Fig. (4) show chromatogram for sepration of PAHs by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20) v/v acetonitrile : 0.01 M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm.

Separation of Naphthalene , Acenaphthylene, Pyrene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene :

Fig. (5) show chromatogram for sepration of Pyrene, Naphthalene, Acenaphthylene, Benz{a} anthracene and 1,3,2,4-Dibenzanthracene. By using RP-HPLC, on column ODS-C18 (150×4.6 mm I.D), and mobile phase (80:20) v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1 ml/min and temperature 60 C° at wave length 254 nm, by using isocratic elution. The separation time was 7.5 min. Table (7) : show retention time (tR), capacity factor ($\bar{K}$), number of theoretical plates (N), separation factor(α) and resolution (Rs) of these compounds. These chromatographic variables calculated by using the following equations:

$Rs = (tR_2 - tR_1)/0.5(W_1+W_2)$ ....(1)

W1,W2: peak width

$\bar{K} = tR - to/to$ ....(2)

to : Dead time

$\alpha = \bar{K}_2 / \bar{K}_1$ ....(3)

$N = 16\left(\frac{tR}{w}\right)^2$ ....(4)

The Application In Water Using RP-HPLC With 0ptimized Conditions Of PAHs :

Used RP-HPLC for separation and determination of polycyclic aromatic hydrocarbons in Dijla river.the monitored results for sample by RP-HPLC determination (2.40 µg/L) for Naphthalene and (2.66 µg/L) for Pyrene. The concentrations of these compounds were higher. Fig.(6) shows chromatogram for sepration of Naphthalene and Pyrene in Dijla river by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20) v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm.

Table (1)
Structural, physicals characteristics of PAHs.

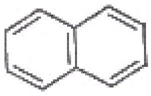
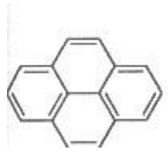
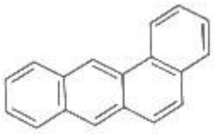
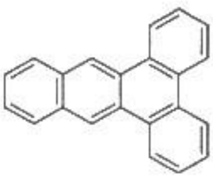
	Solute	**Molecullar Weight (g/mol)**	**Melting Point (C°)**	**Boling Point (C°)**	**Molecullar Structure**
1	Naphthalene	128.17	81-83	217.7	
2	Acenaphthylene	152.20	88-91	280	
3	Pyrene	202.26	149-151	399	
4	Benz {a} anthracene	228.29	157-159	437.6	
5	1,3,2,4-Dibenzanthracene	278.35	205-207	518	

Table (2)

Retention time of PAHs by using RP-HPLC on column ODS-C18 and mobile phase 55% acetonitrile: 0.01M buffer phosphate at PH 4.7 and temperature 25 C° .

	Solute	**tR (min)**
1	Naphthalene	5.29
2	Acenaphthylene	6.29
3	Pyrene	9.80
4	Benz {a} anthracene	1.81
5	1,3,2,4- Dibenzanthracene	16.81

Table (3)

The effect of solvent modifier on retention time of PAHs by using RP-HPLC on column ODS-C18 and mobile phase between (55-80)% acetonitrile: 0.01M buffer phosphate at PH 4.7 and temperature 25 C° .

		tR					
		Conc. Of Acetonitrile %					
	Solute	55	60	65	70	75	80
1	Naphthalene	5.29	4.69	4.46	4.17	3.96	3.49
2	Acenaphthylene	6.29	5.69	5.05	4.87	4.33	4.49
3	Pyrene	9.80	8.50	7.77	7.07	6.35	5.90
4	Benz {a} anthracene	11.81	10.53	9.70	8.11	7.85	6.88
5	1,3,2,4- Dibenzanthracene	16.81	15.00	14.50	12.68	10.50	9.68

Table (4)

The effect of PH on retention time of PAHs by using RP-HPLC on column ODS-C18 and mobile phase (80:20)% acetonitrile: 0.01M buffer phosphate at PH between (4-6.5) and temperature 25 C .

		tR					
		PH					
	Solute	4	4.5	5	5.5	6	6.5
1	Naphthalene	3.98	3.81	3.52	4.51	4.37	4.12
2	Acenaphthylene	6.00	5.68	5.52	5.12	5.12	4.29
3	Pyrene	8.17	7.86	7.68	6.22	6.15	5.99
4	Benz {a} anthracene	12.18	11.50	11.25	9.25	8.02	7.54
5	1,3,2,4- Dibenzanthracene	17.75	15.18	12.22	10.00	9.22	7.15

Table(5)
The effect of temperature on retention time of PAHs by using RP-HPLC on column ODS-C18 at temperature between (30-65)C°, and mobile phase (80:20)% acetonitrile : 0.01M buffer phosphate at PH 6.

		tR							
		Temperature (C°)							
	Solute	30	35	40	45	50	55	60	65
1	Naphthalene	4.00	3.90	3.52	3.30	3.52	3.27	3.22	3.22
2	Acenaphthylene	4.80	4.72	4.55	4.45	4.22	4.27	4.22	3.99
3	Pyrene	7.12	7.00	7.00	6.75	5.90	5.90	5.33	5.33
4	Benz {a} anthracene	8.30	7.87	7.72	7.60	6.99	6.16	6.16	6.00
5	1,3,2,4-Dibenzanthracene	10.58	9.96	9.90	9.57	9.40	8.00	7.50	7.01

Table (6)
The effect of buffer concentration on retention time of PAHs by using RP-HPLC on column ODS-C18 and and mobile phase (80:20)% cetonitrile : buffer phosphate between (0.01-0.1)M at PH 6 and temperature 60C°.

		tR				
		Conc. Of buffer (M)				
	Solute	0.01	0.025	0.05	0.075	0.1
1	Naphthalene	3.22	3.52	3.44	2.18	2.53
2	Acenaphthylene	4.22	4.17	4.13	4.00	4.00
3	Pyrene	5.33	5.90	5.78	5.72	5.36
4	Benz {a} anthracene	6.16	6.18	5.75	5.50	5.18
5	1,3,2,4- Dibenzanthracene	7.50	7.50	7.48	7.08	6.94

Table (7)
Show retention time(tR,capacity factor (K^{-}), number of theoretical plates(N),separation factor (α)and resolution(Rs) of PAHs by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20)v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm .

	Solute	**Dead time (to) min**	**tR min**	**K^{-}**	**N**	**α**	**Rs**
1	Naphthalene	1.6	3.22	1.01	1036	—	—
2	Acenaphthylene		4.22	1.63	2326	1.61	2.66
3	Pyrene		5.33	2.33	5050	1.42	3.17
4	Benz {a} anthracene		6.16	2.85	4956	1.22	2.21
5	1,3,2,4- Dibenzanthracene		7.50	3.68	7347	1.29	3.57

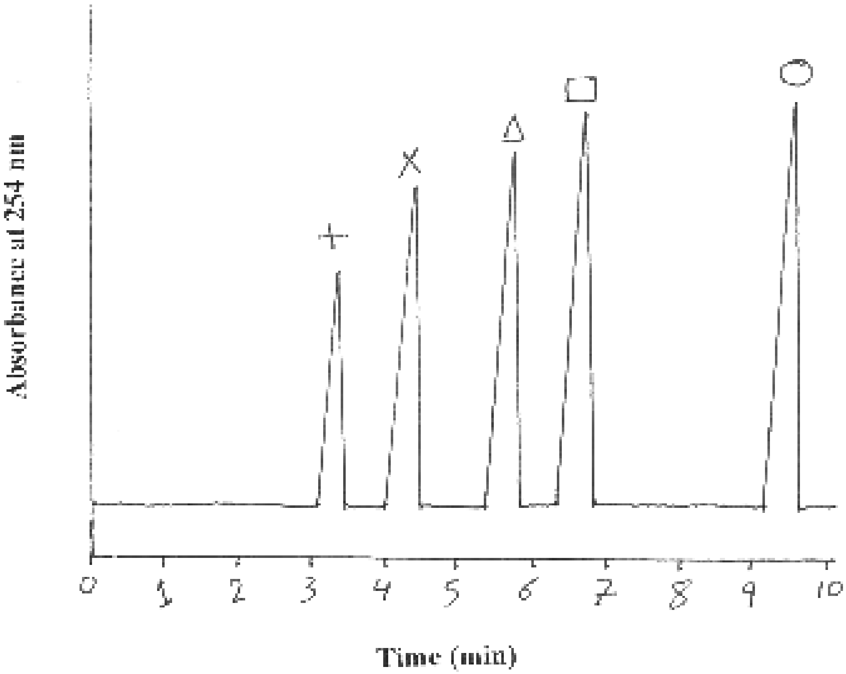


Fig. (1): chromatogram for sepration of Naphthalene(+) (10ppm), Acenaphthylene(×) (5ppm), Pyrene(Δ) (2ppm), Benz {a} anthracene(□) (0.5ppm) and 1,2,3,4-Dibenzanthracene(○) (1ppm). by using RP- HPLC on column ODS-C18 (150×4.6 mm I.D and mobile phase (80:20)v/v acetonitrile: 0.01M buffer phosphate at PH 4.7 and temperature 25 C°.

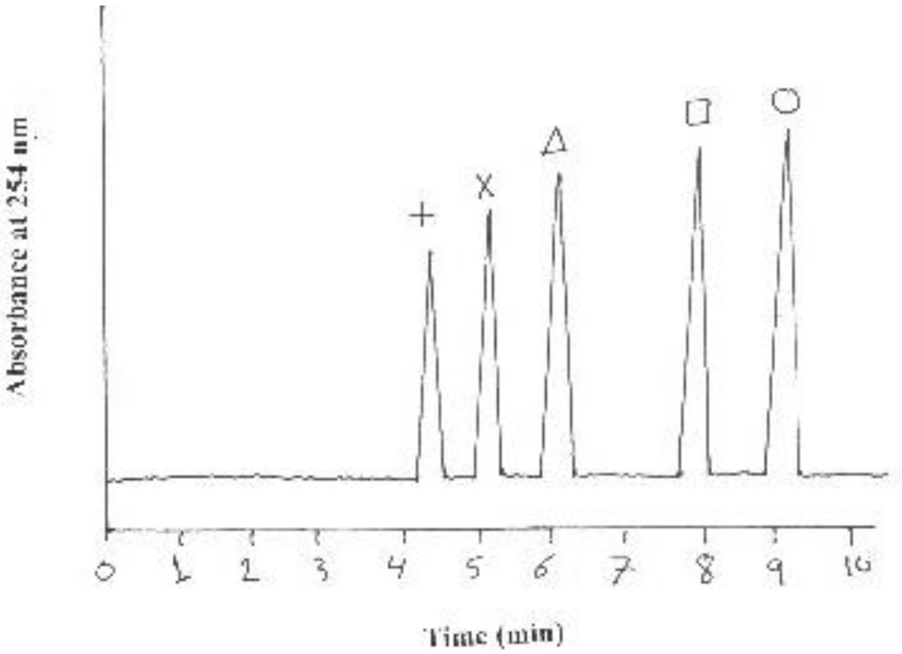


Fig.(2): chromatogram for sepration of Naphthalene(+) (10ppm), Acenaphthylene(×) (5ppm), Pyrene(Δ) (2ppm), Benz {a} anthracene(□) (0.5ppm) and 1,2,3,4-Dibenzanthracene(○) (1ppm). by using RP- HPLC on column ODS-C18 (150×4.6 mm I.D and mobile phase (80:20) v/v acetonitrile: 0.01M buffer phosphate at PH 6 and temperature 25 C°.

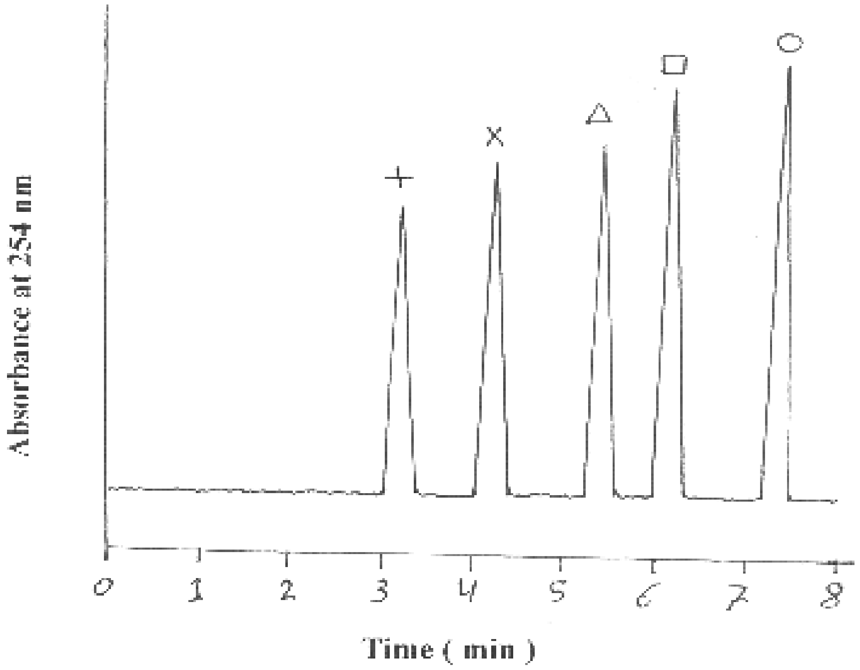


Fig.(3): chromatogram for sepration of Naphthalene(+) (10ppm), Acenaphthylene(×) (5ppm), Pyrene(Δ) (2ppm), Benz {a} anthracene(□) (0.5ppm) and 1,2,3,4-Dibenzanthracene (○) (1ppm). by using RP- HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20)v/v   acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm .

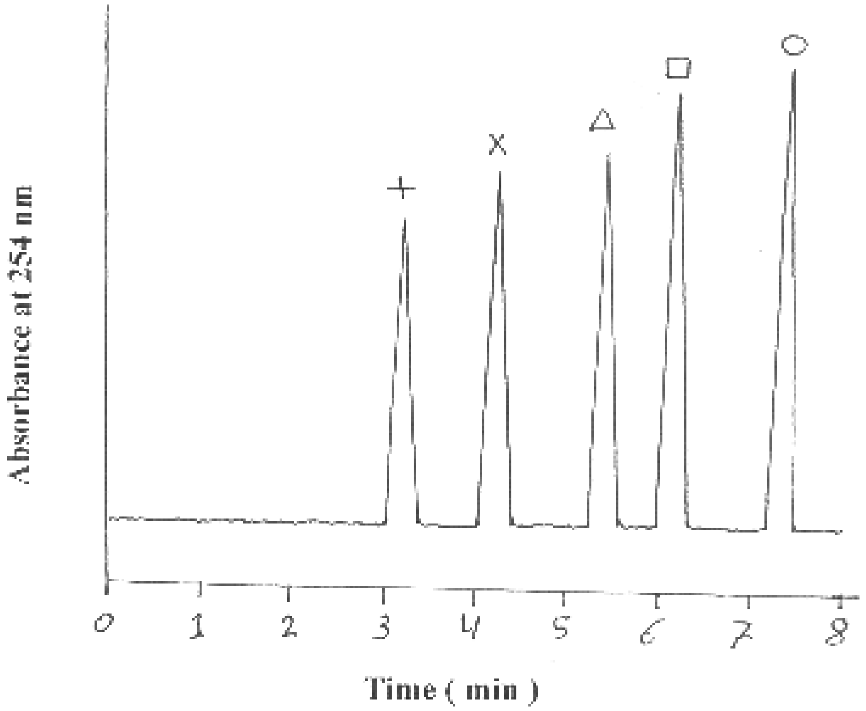


Fig. (4): chromatogram for sepration of Naphthalene(+) (10ppm), Acenaphthylene(×) (5ppm), Pyrene(Δ) (2ppm), Benz {a} anthracene(□) (0.5ppm) and 1,2,3,4-Dibenzanthracene (○) (1ppm). by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20) v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm .

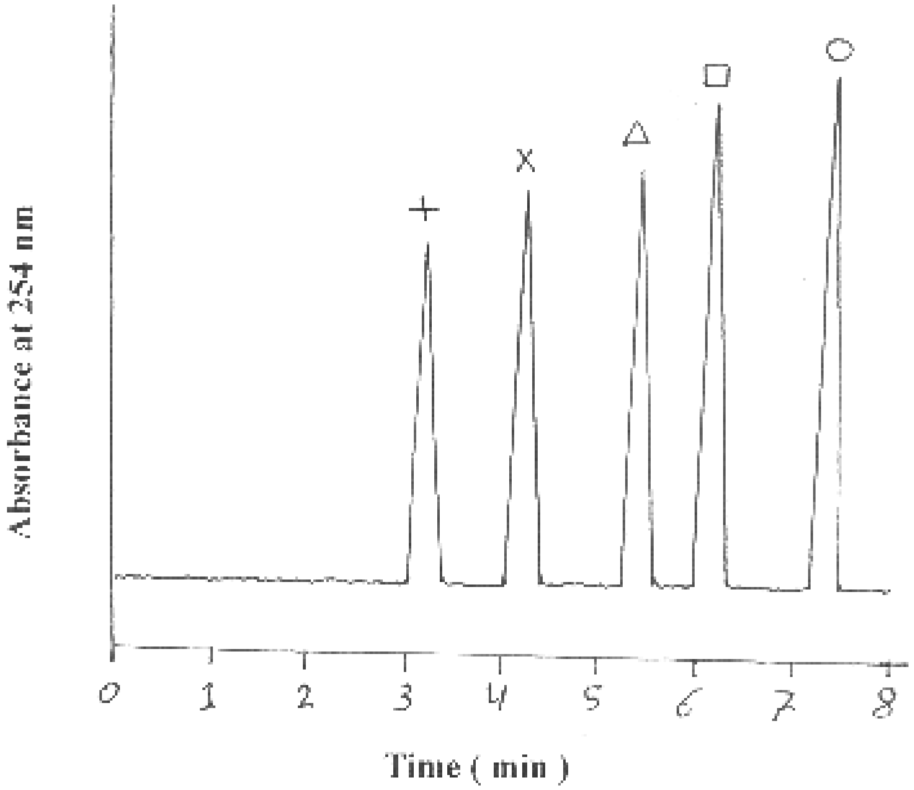


Fig. (5): chromatogram for sepration of Naphthalene(+) (10ppm), Acenaphthylene(×) (5ppm), Pyrene (△) (2ppm), Benz {a} anthracene(□) (0.5ppm) and 1,2,3,4-Dibenzanthracene (○) (1ppm). by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20) v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm .

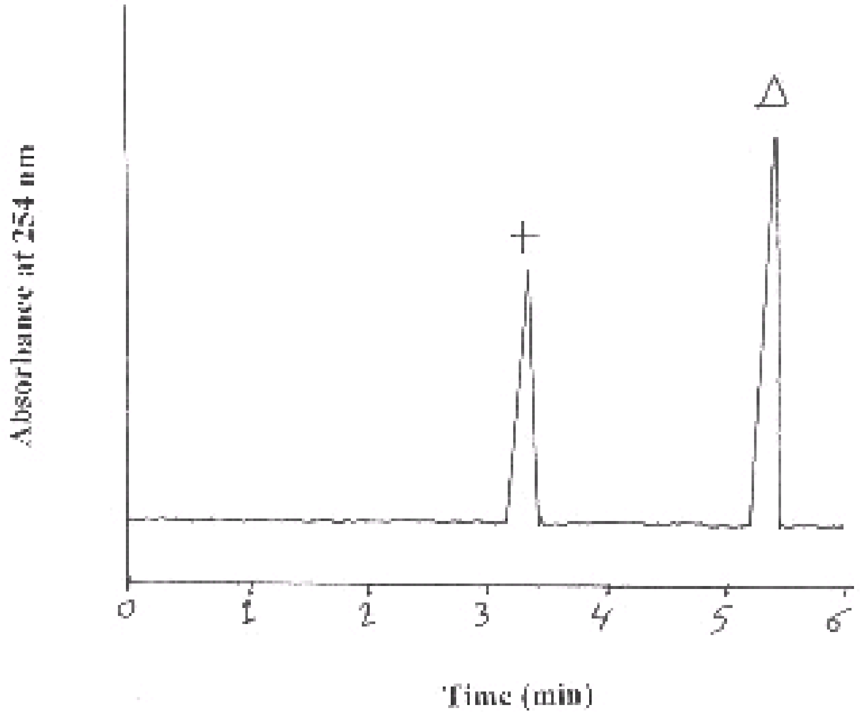


Fig. (6): chromatogram for sepration of Naphthalene(+)(2.40 µg/l) and Pyrene(△)(2.66 µg/l) in Dijla river by using RP-HPLC on column ODS-C18 (150×4.6 mm I.D) and mobile phase (80:20)v/v acetonitrile:0.01M phosphate buffer solution at PH 6 with flow rate 1ml/min and temperature 60 C° at wave length 254 nm .

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الخلاصة

تضمن البحث استخدام تقنية كروموتوغرافيا السائل ذات الأداء العالي – الطور المعكوس لفصل المركبات التالية : بايرين ، نفثالين ، اسينافثالين ، بنزاثراسين و 4،3،2،1− ثنائي بنزاثراسين . وذلك باستخدام عمود ODS-C18 بأبعاد (150 × 4.6) ملم ، وطور متحرك من الأسيتونتريل ومحلول الفوسفات المنظم . وتمت دراسة المتغيرات الكروموتوغرافية من نسبة المحور العضوي ، الأس الهيدروجيني ، درجة حرارة العمود الكروموتوغرافي وتركيز المحلول المنظم المستخدم في الطور المتحرك لغرض الحصول على أفضل تفريقية وبأقل زمن فصل.

أظهرت النتائج بأن استعمال طور متحرك (80: 20) v/v اسيتونتريل : 0.01 مولاري محلول الفوسفات المنظم ذي PH 6 بمعدل جريان 1 مل / دقيقة عند درجة حرارة 60 م° وطول موجي 254 نانوميتر تعطي أفضل فصل خلال زمن قدره (7.5) دقيقة ، وحسب الترتيب التالي : نفثالين ، اسينافثلين ، بايرين ، بنزاثراسين و 1،2،3،4− ثنائي بنزاثراسين .