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Separation and Determination of Some Poly Aromatic Compounds in Samples of Vegetables in Salahuddin Governorate, Using High-Performance Liquid Chromatography Technique (HPLC)

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Abstract

The Reversed Phase High Performance Liquid Chromatography (RP-HPLC) has been used for the separation of Poly aromatic hydrocarbons (PAHs) by using column Reprosil C_{18} which was found to be a suitable one for this purpose.

The result showed that using mobile phase of (Acetonitrile – water) Reversed Phase HPLC, flow rate of (1.2 ml/min), column temperature (30 C°) , using fluorescence Detector and wave length of (Ex.365 - Em.355), give a complete separation with a good resolution. The total separation time was less than 20 min. The result of the study showed that the vegetables of Salahuddin Governorate were polluted by poly aromatic hydrocarbons (PAHs) in deferent place where samples taken because of drainage of heavy water, industrial trash and trash of oil colanders.

Naphthalene, Acenaphthylene, Fluorene,Acenaphthene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo(a)anthracene, Benzo(b)Fluoranthene, Benzo(k)Fluoranthene, Dibenzo(a,h)Anthracene, Indeno(1,2,3-cd)Pyrene Benzo(a)pyrene and Benzo(g,h,i)perylene.

Keywords: Poly aromatic hydrocarbons (PAHs), Pollution, HPLC.

Introduction

Poly Aromatic Hydrocarbons (PAHs) are known as a group of organic compounds which classified within the petrol polluted hydrocarbons . PAHs involve more than thousand compounds , a few numbers of these compounds are taken into account depending on its spreading in environment and its effect on mankind and other live organisms health .These compounds have the ability to transfer for long distance through air [1,2] . PAHs relatively have low solubility in water and high solubility in non-polar organic solvents , PAHs consisting of carbon and hydrogen elements which form two benzene rings or more , these compounds may react with other elements and compounds like ozone , nitrogen oxides , carbon dioxide and sulfur dioxide [3]. The least poly aromatic hydrocarbon is Naphthalene , another example for this compounds is Benzo(a)Pyrene which contain five aromatic rings **(Figure 1)** , most dangerous compound with intensified ring system [4,5] .

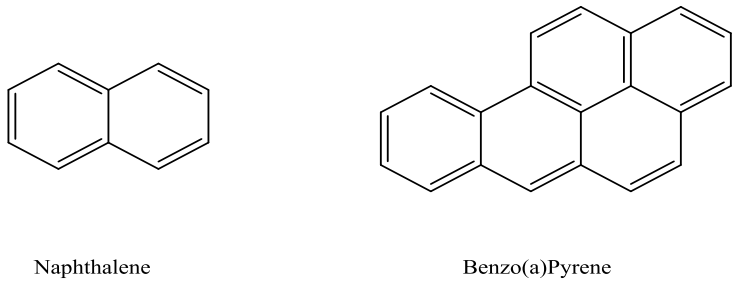

Fig. (1) *Structure of some PAHs compounds* .

PAHs with two to four rings called light PAHs and have low molecular weight , while that of five rings and more called heavy PAHs and have high stability due to their high molecular weight [6]. Sixteen compounds among one hundred PAHs were classified by US Environmental

Protection Agency (U.S . EPA) as a dangerous compounds which effect the human health through consumption polluted vegetables , animal products , drinking water and breathing polluted air , these compounds have been selected for research due to their availability more than the other PAHs compounds [7]. There is a concentration limit of these compounds in food , if the concentration exceed this limit the food consider polluted food . The German Society for Fat Science determine the allowed limit in vegetables oil for light PAHs (five rings or less) concentrations to be 25 ppm and 5 ppm for heavy PAHs (six rings or more)[8] . Some European countries like Germany , Austria and Poland determined the allowed limit of Benzo(a)pyrene in food to don't exceed 1 ppm [9] . Our study aim is to show the severity of PAHs (a dangerous pollutants on environment especially human health) and find a method to separate them and determine their quantities by HPLC , and open the door for future studies about the ways to get rid or reduce these compounds .Table 1 shows the above compounds with their physical properties and carcogenic effect [10-12] . The prosed method will using in determination of the mentioned poly aromatic hydrocarbons in the samples of vegetables in some areas in Salahuddin governorate in Iraq . This is the first study using in Salahuddin governorate to determine PAHs in vegetables . Previous studies have been used to determine PAHs in Baghdad governorate /Iraq , where Jamal found the less quantity of PAHs as 0.11 ppm in Malva sample , while the high quantity was 25.13 ppm in Dill sample [5] .

Table (1) Some physical properties and cacogenic activity of poly aromatic hydrocarbons used in this study [5 , 12] .

Compound	Molecular weight g/mole	Melting point C^o	Boiling point C^o	Solubility in water µg/l at 25 C^o	Carcinogen effect
Naphthalene	128.00	81	200	31700	**0**
Acenaphthylene	152.21	92	265-275	16100	**0**
Fluorene	166.22	115	298	1980	**0**
Acenaphthene	154.21	96.2	279	3930	**0**
Phenanthrene	178.23	99-101	340	1290	**0**
Anthracene	178.23	216.4	340	73	**0**
Fluoranthene	202.26	107	384	260	**0**
Pyrene	202.26	156	393	135	**0**
Chrysene	228.29	254	448	2	**+**
Benzo(a)anthracene	228.3	158.4	400	14	**+**
Benzo(b)fluoranthene	252.32	168	-	-	**++**
Benzo(k)flouranthene	252.32	217	480	-	**++**
Di benzo(a,h)anthracene	278.36	269	-	-	**+**
Benzo(a)pyrene	252.3	179	495	3800	**++**
Indeno(1,2,3-cd)Pyrene	276	161-163.5	-	-	**+**
Benzo(g,h,i)perylene	276.34	277-279	500	0.3	**+**

(0) Non Carcinogen , (+) Carcinogen percentage up to 33% ,
(++)Carcinogen percentage more than 33% .

Experimental part :

1. Apparatus :

1.1 High Performance Liquid Chromatography (HPLC) .

Skyam type , S 1122 model with Reprosil C_{18} Column special for PAHs separation .

1.2 Soxhlet extractor .

It was applied to samples extraction .

1.3 Rotary Evaporator .

Büchi RE 121 Evaporator type was used to concentrate sample extract and evaporate the solvents used in the extraction .

2. Chemicals .

2.1 PAHs .

The standard PAHs with the physical properties listed in table 1 with purity not less 99.92 % have been supplied from OMA Germany company .

2.2 Organic solvents .

Acetonitrile from BDH British company , Acetone and Hexane from Romil British company , all these solvents have been supplied with high purity . Deionized water supplied from Schar Lau Spanish company .

2.3 Identification of standard samples of PAHs by HPLC technique .

We had prepared a standard solution for each compound of PAHs with concentration of 50 ppm and 100 ml volume , then each compound was analyzed by HPLC according to analytical program list in table 2 to know the retention time for each compound . A standard solutions of a mixture of poly aromatic hydrocarbons with a different concentrations for each compound (0.5 , 1 , 2 , 4 , 8 , 10) ppm have been prepared . 100 µl of each solution of standard solutions with above concentration have been injected (according to analytical program in table 2) on Reprosil C_{18} column , which its properties list in table 3 . Each compound peak high in the mixture chromatogram have been determined by the retention time of compound separately beside that each peak high area have been determined .

Table (2) Gradual changing program .

Time (min)	**CH_3CN**	**H_2O**
0	40	60
7	60	40
11	100	0
17.9	100	0
18	40	60

Table (3) Optimum separation condition of PAHs by RP-HPLC

Column	**Reprosil C_{18} with dimension 250 ×4.6**
Flow rate (ml / min)	1.2
Temperature (C^o)	30
Used detector	Fluorescence Detector
Wave length (nm)	Ex . 365 , Em. 355

2.4 Vegetable samples preparation .

All vegetable samples have been collected from Salahuddin farms in different areas . The samples were dried at 70 C^o for six hours , then grinded to homogeneous small pieces by special mill . 250 gram of each sample put in cellulose finger which fixed in soxhlet . 200 ml of Acetone and hexane mixture (1:1) as extraction solvents was added and the extraction achieved at mixture boiling point 50 C^o for 20 hours [11]. Each sample concentrated via evaporate the solvents mixture till drying and then we added 10 ml of Acetonitrile. We put the container of the sample on a magnetic stirrer for 15 minutes the sample was filtered by filter syringe .After that we put the sample in opaque container load its name and the date of extraction , the sample measurement then done by HPLC .

Results and discussion .

To know the possibility of HPLC in separating PAHs , a primary test have been done to get optimal separation for each aromatic compound

.These optimal conditions list in table 3 after a series of experiments [12] . The concentration of PAHs in vegetable samples calculated according to the following equation :

$$C_c = \frac{\text{Analytical concentration (ppm)} \times \text{volume of the added solvent (ml)}}{\text{weiht (g) of taken sample}}$$

C_c : represent the concentration of PAH compound .

We can get the analytical concentration from straight line equation :

y = mx + b , x : represent the concentration of PAH (ppm) , m : represent the slope , b: represent the intercept and y: represent the peak high area . For example the equation for Naphthalene y=2.891 – 1.181 , correlation coefficient R^2 = 0.9967 , R represent the deviation from straight line R = y-(mx + b) .

The application of optimal condition to separate the standard mixture of poly aromatic hydrocarbons by HPLC technique . In this study RP-HPLC have been devolved to separate the slandered poly aromatic hydrocarbons through examination the optimal conditions of separation operation . Some chromatographic variables have been studied like the effect of mobile phase in the separation operation for these compounds and then we got typical chromatograms for the separation of sixteen poly aromatic hydrocarbon compounds as in figures 15 and 16 ,using optimal conditions according to the program of gradual changing table 3 .

Separation and determination Poly aromatic hydrocarbons in vegetables samples by RP-HPLC technique at optimal conditions .

The concentration of poly aromatic hydrocarbons have been measured in vegetables samples which have been selected from different areas of Salahuddin governorate .Table 4 presents the concentrations of PAHs in vegetable samples in Salahuddin governorate areas .These concentrations exceed the allowed limits of PAHs concentrations in that areas , the concentrations were at the range (9.95 ppm – 67.15 ppm) . British food standard agency had published that the allowed concentration for each four compounds of PAHs should not exceed 0.14 ppm [13 ,14] . The increasing of PAHs concentration may attribute to the sources of environment pollutions , oil refineries , thermal station generator of electricity , cars exhausts and private electricity generators .The

concentrations of PAHs was 67.15 ppm in the chromatogram of samples of celery in Biji city , indicates a clear pollution because the vicinity of the celery farms to Biji oil refinery and thermal station generator of electricity . The chromatogram of parsley sample in Tikrit city shows that the concentration of PAHs at 25.15 ppm , this high value due to the Tigris river water which contain the waste of Biji oil refinery and also because the private electricity generators . The concentration of PAHs in the sample of Basil in Samarra city appears in the chromatogram at the value of 23.94 ppm , this value of PAHs came from the pollution of electricity generator station which is site near the farms and also from the cars exhaust in the main street which is located near the farms . The concentration of PAHs is 12.8 ppm in watercress sample which taken from the city of Balad , while the concentration of PAHs is 16.84 ppm as depict in the chromatogram of the sample of leek in Dajeel city . The pollution of PAHs is high in Balad and Dajeel cities but still lower than the pollution in other cities , because these two cities relatively are far from the sources of pollution .

Table (4) Concentration in ppm of PAHs in vegetable samples in some areas in Salahuddin governorate measured by PR-HPLC technique .

Compound	Parsley sample/ Tikrit city	Celery sample / Biji city	Basil sample / Samaara city	Watercress sample/ Balad city	Leek sample / Dajeel city
Naphthalene	–	43.189	2.998	2.116	–
Acenaphthylene	5.483	–	–	–	–
Fluorene	–	–	–	–	–
Acenaphthene	–	6.267	–	–	–
Phenanthrene	–	–	4.216	–	–
Anthracene	–	–	–	2.969	–
Fluoranthene	0.652	–	–	–	–
Pyrene	–	–	–	–	8.762
Chrysene	–	9.304	0.599	–	–
Benzo(a)anthracene	–	–	–	–	2.102
Benzo(b)fluoranthene	–	–	4.020	–	1.538
Benzo(k)flouranthene	–	8.392	5.422	–	–
Di benzo(a,h)anthracene	8.528	–	8.893	–	4.439
Benzo(a)pyrene	–	–	–	7.723	–
Indeno(1,2,3-cd)Pyrene	10.487	–	3.221	–	–
Benzo(g,h,i)perylene	–	–	–	–	–
Sum	**25.15**	**67.152**	**23.947**	**12.808**	**16.841**

Theoretical treatments in chromatography .

The results show the possibility of PAHs separation in the examination conditions according to the sequence in table 5 .The retention time of PAHs increase with the increasing the number of aromatic rings , one of the important treatments deal with the calculation in this study are capacity factor , number of theoretical plates and separation ability . Capacity factor (K') describe the transfer of solute on column which represent the degree of retention , we can calculate it through :

$$K = \frac{t_R - t_O}{t_O} \quad \text{------ 1}$$

t_o represents the dead time and almost equal the required time for the transfer of a molecule through a mobile phase in the column , and from that value we can calculate the retention time t_R in accurate way .When the value of K' for a solute less than one , the recovery will done in a fast way and the retention time determination become so difficult , at range 20-30 of K' values the recovery time become so long , so the best separation of a component in a mixture be at the values of K' in the range 2-10 . Number of theoretical plates N , it is a measure of column efficiency , at which the repetition equilibrium for the solutes distribution between the two phases occurs .The greater value of N lead to narrowest peak and increase the approaching from perfect behavior , we can calculate the N from :

$$N = 16 \left(\frac{t_R}{W}\right)^2 \quad \text{-------- 2}$$

w : represents the band width at the base line and measure by the time unit [14].

Separation ability R_S , represents the degree of separation of one component from another and decide whether the interference will happen or not . R_S of two neighbor peaks equal to the difference between the two peaks height divided by the width of the two bands .

$$Rs = \frac{t_{R_2} - t_{R_1}}{1/2\ (W_1 + W_2)}$$

W_1 and W_2 : represent the widths of the two bands at the base line [15] .

The separation factor α which represent the ratio between the capacity factors of the two components :

$$\alpha = \frac{\grave{k}_2}{\grave{k}_1} \text{ -------- 3}$$

The separation factor affected by temperature and the changing of the components of the two phases , its value more than one . When the value of α equal one , the value of separation degree become zero and there is no separation between components [16] .

Table(5) Using the separation column Rerosil C_{18} with (250 × 4.6mm) dimensions at optimal separation conditions , temperature (30 C^{o}) , flow rate (1.2 ml/min) and wave length (Ex.365 – Em.455) .

No.	PAH	t_O(min)	t_R(min)	` K	N	α	Average ` K	Average N	R_S
1.	Naphthalene	1.1	12.16	10.05	25283.2	-	-	-	-
2.	Acenaphthylene	-	12.66	10.51	34701.1	1.045	10.28	29992.15	1.747
3.	Fluorene	-	13.33	11.12	34077.5	1.058	10.82	34389.3	2.348
4.	Acenaphthene	-	13.44	11.22	156360.9	1.008	11.17	95219.2	0.497
5.	Phenathrene	-	13.64	11.40	60989.0	1.016	11.31	108674.9	1.124
6.	Anthracene	-	13.81	11.55	9697.9	1.013	11.48	35343.47	0.431
7.	Fluoranthene	-	14.20	11.90	13288.4	1.030	11.73	11493.15	0.763
8.	Pyrene	-	14.49	12.17	59345.8	1.022	12.04	36317.1	0.787
9.	Chrysene	-	14.97	12.60	253269.6	1.035	12.39	156307.7	0.596
10.	Benzo(a)anthracene	-	14.98	12.62	73560.8	1.001	12.61	163415.2	0.076
11.	Benzo(b)fluoranthene	-	15.11	12.73	64558.7	1.009	12.68	69059.75	0.576
12.	Benzo(k)fluoranthene	-	15.80	13.36	16451.0	1.049	13.05	40504.8	1.879
13.	Dibenzo(a.h)anthracene	-	16.24	13.76	17373.4	1.029	13.56	16912.2	0.888
14.	Benzo(a)pyrene	-	16.42	13.92	149367.4	1.012	13.84	83370.4	0.554
15.	Indeno(1,2,3-cd)pyrene	-	17.23	14.66	20978.0	1.053	14.29	85172.7	2.560
16.	Benzo(g,h,i)perylene	-	17.50	14.90	46997.9	1.016	14.78	33987.9	0.688

Conclusion

According to our study results we are suggesting an annual survey to ensure the concentration of PAHs compounds in vegetables in different areas in Salahuddin Governorate . Apply this study to determine poly aromatic compounds in Tigris water and in soil samples in Salahuddin Governorate also measure the concentration of these compounds in the air in the areas far and near the sources of pollution like oil refinery and implement so studies to find a ways to reduce the PAHs pollution and treat it . 200 ml of Acetone and hexane mixture (1:1) as extraction solvents was added and the extraction achieved at mixture boiling point 50 C^o for 20 hours [11]. Each sample concentrated via evaporate the solvents mixture till drying and then we added 10 ml of Acetonitrile. We put the container of the sample on a magnetic stirrer for 15 minutes the sample was filtered by filter syringe .After that we put the sample in opaque container load its name and the date of extraction , the sample measurement the done by HPLC .

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فصل وتقدير بعض المركبات الاروماتية متعددة الحلقات في عينات من الخضراوات في محافظة صلاح الدين باستخدام تقنية كروماتوغرافيا السائل عالي الأداء HPLC

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الخلاصة

استعملت تقنية كروماتوغرافيا السائل عالي الأداء ذات الطور العكوس RP-HPLC لفصل وتقدير مركبات الهيدروكاربونات الاروماتية متعددة الحلقات PAHS في عينات من الخضراوات مأخوذة من مناطق مختلفة من محافظة صلاح الدين (تكريت، بيجي، سامراء، بلد، الدجيل) إذ تم استعمال العمود Reprosil C_{18} (250 × 4.6mm)، و باستعمال طور متحرك من (Acetonitrile – Water) في تقنية RP-HPLC وفق برنامج التغير التدريجي وبمعدل جريان (1.2ml/min)، ودرجة حرارة 30C°، واستعمل مكشاف الفلورة بطول موجي (Ex.365nm – Em.455nm)، حيث تعطي أفضل فصل بزمن احتجاز لا يتجاوز (20 min). وتمت عملية الاستخلاص للنماذج بجهاز الاستخلاص السوكسليت Soxhlet واستعمل المبخر الدوار لتركيز المستخلص والتخلص من المذيبات.

أظهرت نتائج الدراسة تلوث الخضراوات بمركبات الهيدروكاربونات الاروماتية متعددة الحلقات في المناطق التي أُخذت منها العينات وذلك بسبب المخلّفات الصناعية، ومخلفات مصفاة نفط بيجي، ومحطات الطاقة الكهربائية، فضلاً عن عوادم السيارات. إذ بينت النتائج وجود تراكيز تجاوزت الحدود المسموحة لبعض مركبات PAHs الشائعة الانتشار وفقاً للحدود المسموحة حسب وكالة الأغذية القياسية البريطانية ومن هذه المركبات التي تم فصلها وتقديرها هي:

Naphthalene, Acenaphthylene, Fluorene,Acenaphthene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Chrysene, Benzo(a)anthracene, Benzo(b)Fluoranthene, Benzo(k)Fluoranthene, Dibenzo(a,h)Anthracene, Benzo(a)pyrene, Indeno(1,2,3-cd) Pyrene and Benzo(g,h,i)perylene.

الكلمات المفتاحية: الهيدروكاربونات الاروماتية متعددة الحلقات (PAHs)، التلوث، HPLC.