INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

DEVELOPMENT OF A NEW MODIFIED METHOD FOR POLYAROMATIC HYDROCARBONS (PAHS) MEASUREMENT IN SERA SAMPLES OF HEALTHY INDIVIDUALS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TECHNIQUE

Omar F. Abdul-Rasheed[1] and Noor M. Ali[2*]

[1]Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad-Iraq.

[2]Medical research unit, College of Medicine, Al-Nahrain University, Baghdad-Iraq.

ABSTRACT

To develop a new rapid sensitive high performance liquid chromatography method for polyaromatic hydrocarbons compounds**.** This study was conducted at chemistry and biochemistry department, college of medicine, Al-Nahrain University, Baghdad, Iraq from July 2010 to March 2011; fifty healthy volunteers aged 23.45±6.54 years were included in this study. All participant, were non-smokers, and under no any medication for the last two months. Naphthalene, Anthracene and phenanathrene were measured using by improved gradient reversed phase HPLC technique. Reproducible determination with highly sensitive detection was attained by HPLC with UV-Visible detection. The detection limit of Naphthalene, Anthracene and phenanathrene were 0.1, 0.1 and 0.5mg/ml respectively. The method gives a good linearity ranges between 0.1 to 10mg/ml for Naphthalene and phenatherene and 0.5 to 10mg/ml for Anthracene. The CVs% of within-day precision for Naphthalene was 0.59-8.14%, Anthracene was 0.37-3.89% and phenanathrene was 0.78-3.34%. While the analytical recoveries were 98.25% for Naphthalene, 96.15 % for Anthracene and 97.21% for phenanathrene.

Keywords: Polyaromatic hydrocarbons, Naphthalene, Anthracene, Phenanathrene, HPLC.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) originate from the incomplete combustion or pyrolysis of organic matter such as fuel oils [1]. Polycyclic aromatic hydrocarbons represent a complex mixture of chemicals, some of which have been recognized as cytotoxic, carcinogenic and mutagenic in humans[2] and causing irreversible changes in structures and functioning of living organisms[2-4]. As the molecular weight increases, the carcinogenicity of PAHs also increases, and acute toxicity decreases[3]. PAHs known for their carcinogenic and teratogenic properties are benzo(a) anthracene, chrysene, benzo(b) fluoranthene, benzo(j)fluorathene, benzo(k) fluoranthene and benzo(a)pyrene, indeno(1,2,3 cd) pyrene and dibenzo(a,h) anthracene[3]. Because PAHs are abundant in many petroleum and coal derived products, such as kerosene, diesel and jet fuels, coal tar, and coke, workers are exposed to PAHs in industries that produce, process, or consume these fuels [2]. Exposures to PAHs are greatest in the aluminum, iron and steel industries [2]. Major sources of environmental exposure to PAHs are from inhalation of engine exhaust and tobacco smoke, cooking fires, and from ingested of smoked foods [2,3,5].

PAHs are highly lipid soluble and thus readily absorbed from gastrointestinal tract of mammals. They are rapidly distributed in a wide variety of tissues with marked tendency for localization in body fat [5] or may be excreted in urine or feces as hydroxylated metabolites [1].

The aim of this study was to develop a new rapid and sensitive HPLC method for measurement of PAHs in sera of healthy individuals.

MATERIAL AND METHODS

This case-control study was conducted during the period from July 2010 to March 2011 in the Chemistry and Biochemistry Department/ College of Medicine/ Al-Nahrain University/ Al-Khadhimiya/ Baghdad- Iraq. HPLC technique with new modified method was used to estimate serum naphthalene, phenanthrene and anthracene in serum samples of 50 male healthy volunteers aged 23.45±6.54 years participated in this study. All participants were non-smokers and not underwent to any treatment for the last two months.

Preparation of calibrations

A stock solution of 10ppm of standard **PAHs** were prepared by dissolving 1mg of naphthalene in ethanol and diluted to 100ml. The same procedure for phenanathrene and anthracene was followed in the preparation of their stock solutions. Other standard solutions were prepared by subsequent dilution of the stock solutions. The solvent used to prepare these solutions before injection into HPLC was usually used as the mobile phase employed for their separation.

Serum sample preparation

In order to analyze **PAHs** in serum, the samples were deproteinized by adding 50µl of 15% sulphosalicylic acid to 400µl of serum, then mixed well and let to stand for 15min and centrifuged at 3000rpm for 10min. The supernatant was taken and diluted three folds with ethanol and filtered using millipore filter (0.22µm).

Chromatographic analysis

All the prepared serum samples, standard solutions have been chromatographically analyzed with the SD-ODS columns, with 250mm length and 4.6mm internal diameter (i.d.) using different sets of mobile phases. The mobile phase used was gradient ethanol: acetonitrile (80% : 20% , by volume) at 1.2ml/min flow rate and detection at wavelength 254 nm for PAHs.

Quantification was done by comparing the areas under curves (AUC) of samples with those obtain of standards. The measurement of **PAHs** depended on the standard addition methods. The HPLC-UV-VIS system used in this work was Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, a degasser model DGU-12A, two liquid delivery pumps model LC-8AVP, UV-Visible detector model SPD-10AVP, and injector model SIL-10A, equipped with 20 µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer; Epson LQ-300 printer model P852A (Japan).

Data analysis

Measurements were repeated three times for each sample and the results were averaged. Results were compared by use of student's t-test for inedependent variables. Significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Traditional methods used for PAHs measurements are Soxhlet, solid- phase and liquid-liquid extraction, with previous saponification with KOH- methanol solution [6]. These methods are, however, very difficult and time and solvent- consuming, and, because they involve long and complex procedures, are unsuitable for routine analysis [7,8]. Nowadays, the analytical methods most frequently used for determination of PAHs are HPLC with fluorescent detection [9-11] and GC-MS [12-14]. The improvement used throughout this study is by the use of UV- Vis detection for the three measured PAHs instead of fluorescence detection previously used with very good recovery and accuracy.

A simple, rapid, sensitive and suitable procedure for determination of three important PAHs by HPLC using C18 column with UV detection has been developed. The limits of detection at λ= 254 nm with gradient elution of ethanol: acetonitrile (80%: 20% by volume) as mobile phase and flow rate of 1.2 mL/min. were found to be 0.1 µg/mL for naphthalene and phenanathrene and 0.5 µg/mL for anthracene. Separation for these compounds was about 15 minutes. The developed method demonstrates excellent linearity (r=0.999 for naphthalene, phenanathrene and anthracene). Results of analysis of PAHs by HPLC showed a concentration range of 0.1- 10 µg/mL for naphthalene and phenanathrene and 0.5-10 µg/mL for anthracene. Analytical recoveries were 98.25% for naphthalene, 97.21% for phenanathrene and 96.15% for anthracene.

Table (1) shows the time programming of gradient elution of PAHs separation using two solvents (ethanol and acetonitrile) with different proportions throughout the separation time.

Table 1: The mobile phase gradient

Time(min)	% B (Acetonitrille)	% A(Ethanol)
0	20	80
3	20	80
6	0	100
9	20	80
12	20	80
15	20	80

Table 2: Recovery and percentage relative error of PAHs, using SD-ODS column (25×0.4 cm (id))

Compounds	Conc. Intended (µg/ml)	Conc. measured* ((µg/ml))	Recovery %**	Relative error%***
Naphthalene	5.00	4.91	98.25	1.80
Phenanathrene	5.00	4.86	97.21	2.80
Anthracene	5.00	4.77	96.15	4.60

* using the linear equation for each PAH.
**calculated by (concentration measured/concentration intended)*100
***calculated by ((concentration measured-concentration intended)/ concentration intended)*100

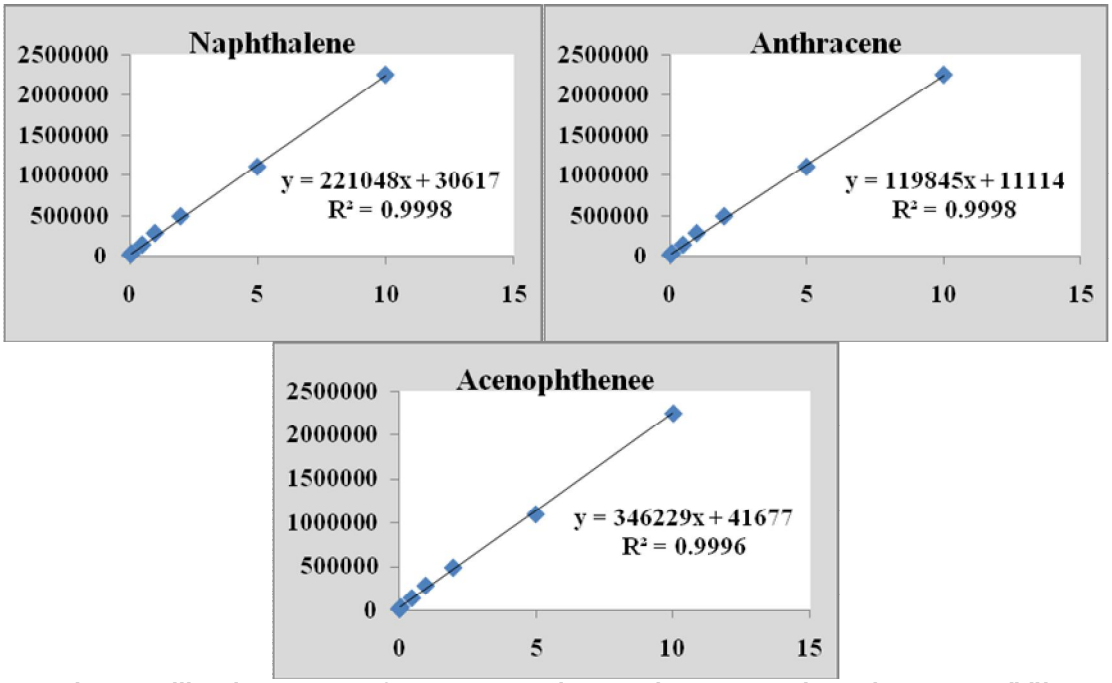

Fig. 1: Calibration curves of PAHs separation used SD-ODS column (25×0.4 cm (id))

Table 3: Linear equation, correlation coefficients R, and detection limit of PAHs using SD-ODS column (25×0.4 cm (id))

Compounds	Linear Equation Y*=mx*+b	Conc. range(µg/ml)	R	Detection Limit (µg/ml)
Naphthalene	Y=221048x+30617	0.1-10	0.9998	0.1
Phenanathrene	Y=119845x+11114	0.1-10	0.9997	0.1
Anthracene	Y=346229x+41677	0.5-10	0.9998	0.5

***y represent peak area, * x represent concentration**

Table 4: Retention time t_R, capacity factor Ḱ, separation factor α, resolution, peak asymmetry, number of theoretical plates N and height to a theoretical plate HETP for PAHs using SD-ODS column (25×0.4 cm (id))

Compounds	$t_{R(min.)}$	Ḱ	α	R_S	A_S	N	HETP
Naphthalene	4.91	2.30			1.04	2356	0.134
Phenanathrene	9.97	2.65	1.51	1.56	1.05	2387	0.165
Anthracene	13.24	3.02	1.33	1.62	1.01	2372	0.141

Table 5: Recovery of PAHs

Compounds	Serum pool	Intended conc. (µg/ml) mean±SD	Measured conc. (µg/ml) mean±SD	Recovered conc. (µg/ml)	CV%
Naphthalene	A	0	0.167±0.0136	-	8.143713
	B	2	2.193±0.0197	1.156	1.651299
	C	5	5.227±0.0133	1.176	0.597216
Phenatherene	A	0	0.248±0.0083	-	3.346774
	B	2	2.251±0.0098	1.012	0.783373
	C	5	5.264±0.018	1.051	0.795053
Anthracene	A	0	0.398±0.0155	-	3.894472
	B	2	2.462±0.0162	1.161	1.108071
	C	5	5.529±0.0095	1.145	0.375643

Table 6: Reproducibility of naphthalene, phenanathrene and anthracene in human sera

Sample	Naphthalene (µg/ml) (Mean±SD)	CV%	Phenatherene (µg/ml) (Mean±SD)	CV%	Anthracene (µg/ml) (Mean±SD)	CV%
1	0.158±0.004	2.53	0.246±0.006	2.44	0.396±0.005	1.26
2	0.162±0.002	1.23	0.244±0.004	1.64	0.381±0.002	0.52
3	0.165±0.001	0.60	0.249±0.006	2.41	0.384±0.002	0.52
4	0.172±0.002	1.16	0.233±0.008	0.343	0.322±0.001	0.31
5	0.167±0.003	1.79	0.240±0.009	0.37	0.343±0.001	0.29
6	0.169±0.001	0.59	0.247±0.004	1.62	0.363±0.002	0.55
7	0.168±0.003	1.78	0.242±0.003	1.24	0.369±0.003	0.81
8	0.166±0.003	1.80	0.239±0.0009	0.37	0.378±0.003	0.79
9	0.167±0.004	2.39	0.244±0.003	1.23	0.389±0.004	1.03
10	0.162±0.002	1.23	0.237±0.002	0.84	0.411±0.006	1.46
RSD %		**1.5**		**1.25**		**0.75**

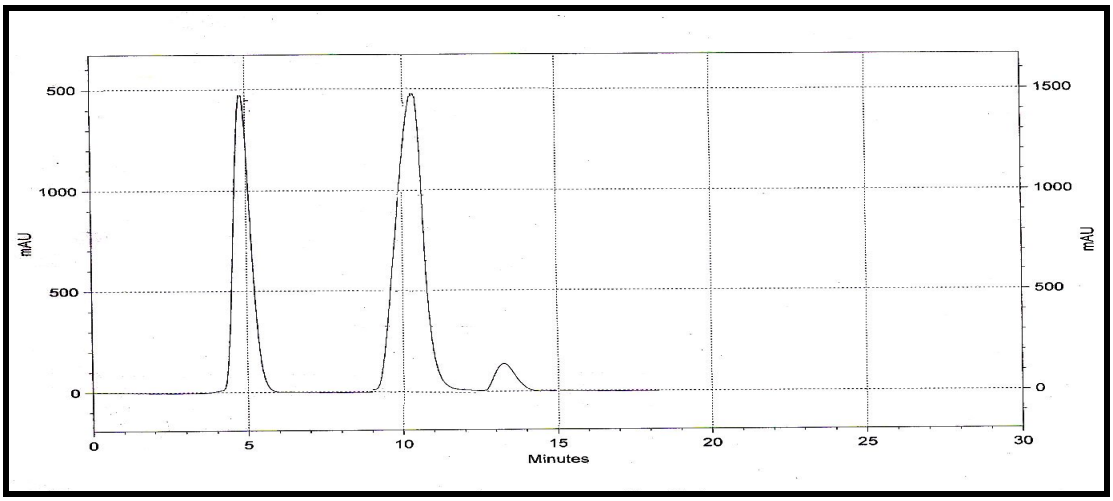

Fig. 2: Chromatogram of Polyaromatic compounds using gradient mobile phase Ethanol: Acetonitrille (80:20), *SD-ODS* column (25×0.4 cm (id))

CONCLUSION

A simple, gradient, stability- indicating reversed phase high performance chromatography method has been developed for measurement of Naphthalene, Anthracene and phenanathrene in human blood serum

REFERENCES

1. Kishikawa N, Wada M, Kuroda N, Akiyama, Nakashima K. Determination of polycyclic aromatic hydrocarbons in milk samples by high- performance liquid chromatography with fluorescence detection. Journal of Chromatography 2003; 789: 257-264.
2. Waidyanatha S, Zheng Y, Serdar B, Rappaport SM. Albumin adducts of naphthalene metabolites as biomarkers of exposure to polycyclic aromatic hydrocarbons. Cancer epidemiology, biomarkers and prevention 2004; 13: 117-124.
3. Ogala JE, Iwegbue CMA. Occurrence and profile of polycyclic aromatic hydrocarbons in coals and shales from eastern Nigeria. Petroleum and Coal 2011; 53: 188-193.
4. Saleh NMD, Sanagi MM. pressurized liquid extraction of polycyclic aromatic hydrocarbons from soil samples. Pertanika J Sci and Technology 2011; 19: 25-32.
5. Gomare KS, Lahane MN. Degradation of polycyclic aromatic hydrocarbons by isolated cultures from contaminated soils at petrol pump stations. International Journal of recent trends in science and technology 2011; 1: 9-13.
6. Nieva-Cano MJ, Rubio-Barroso, Santos- Delgado. Analyst 2001; 126: 1326.
7. Moret S, Conte LS, J. Sep sci 2002; 25:96.
8. Wenzel KD, Hubert A, Manz M, Weissflog L, Engewald W, Schurmann G. Anal Chem 1998;70: 4827.
9. Wegrzyn E, Grzes'kiewicz S, Poplawska W, Glo'd BK. Modified analytical method for polycyclic aromatic hydrocarbons, using SEC for sample preparation and RP-HPLC with fluorescence detection. Application to different food samples. Acta Chromatographica 2006; 17:233-249.
10. Barranco A, Alonso- Salces RM, Crespo I, Burreta LA, Gallo B, Vicente F, et al,. J. Food Prot 2003; 67: 2786.
11. Dissanayake A, Galloway TS., Marine Environ. Res. 2004; 58: 281.
12. Lodovici M, Dolara P, Casalini C, Ciapellano S, Testolini G. Food anal contam 1995; 12: 703.
13. Jira W. Eur food res. Technol 2004; 218: 208.
14. Chen BH, Wang CY, Chin CP. J. agri. Food chem.. 1996; 44: 2244.