

## REZALIM-ICIA'S METHOD FOR DETERMINATION OF PAHs FROM WATER SAMPLES

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### ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are a class of pollutant chemical compounds consisting of fused aromatic rings with great impact on human health and environment. A modern method for determining these pollutants from water at nanoscale level was developed in REZALIM-ICIA laboratory using an HPLC-FLD instrument. The column, its temperature, the mobile phase and the excitation and emission wavelengths were optimized in order to achieve the proposed goal. Calibration curves for each compound were obtained in 5-60 ng/ml concentration range. The method was tested on spiked samples and the results proved to be satisfying.

### INTRODUCTION

Polycyclic aromatic hydrocarbons are formed as a result of burning of organic material and naturally as a result of thermal geological reaction [1, 2]. These compounds are hydrophobic and have low water solubility leading to their persistence in the environment [2, 3]. Polycyclic aromatic hydrocarbons are undergoing degradation through chemical oxidation, photolysis and microbial action; the last is the most efficient [4]. The European Environment Agency as well as other Environmental Protection Agency have listed 16 PAHs as priority pollutants because of their toxicity, mutagenicity and carcinogenic properties [5-7].

Because they have such negative impact on the environment and especially human health a sensitive method for analyzing these extremely dangerous pollutants at nanoscale level is of great interest.

### MATERIALS and METHODS

#### Reagents and standards

PAH Calibration Mix containing 10µg/ml of each compound (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenz[a,h]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene) in Acetonitrile was acquired from Supelco. Acetonitrile Chromasolv gradient grade for HPLC (purity ≥99.9%) was acquired from Sigma – Aldrich. The ultra-pure water was obtained with a Milli-Q water purification system from Millipore.

### Liquid chromatography conditions

The method was developed using a Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with UV and FLD detectors. 20  $\mu$ L sample volumes were injected using a manual injection system into the system. For separation, a ZORBAX Eclipse PAH 5  $\mu$ m, 4.6  $\times$  150 mm column from Agilent Technologies was used. The column was kept during the analysis at 25°C. The mobile phase was formed from water (A) and Acetonitrile (B) in variable proportions. The UV detector was set at 254 nm and the FLD detector was set at different wavelengths appropriate for each compound.

### Preparation of standards and samples

A stock solution of 200 ng/ml was prepared from an ampoule of PAH Mix. The standard solutions for calibration curves were made in dark glass volumetric flasks of 5 ml using 2 pipettes of 1 and 5 ml. The working standards for calibration curves were developed in Acetonitrile at a 5-60 ng/ml concentration range. A spiked sample of 50ng/ml concentration was extracted by liquid – liquid extraction method according to SR EN ISO 17993 [8].

## RESULTS

In order to obtain the retention times for each compound, a standard solution of 100ng/ml was injected into the system using a mobile phase of A/B 50:50 v/v. The retention times of the compounds were established using the UV detector set at 254 nm. For obtaining a good separation of the compound and a shorter method the flow rate was set at 1.6 ml/min. Starting from these parameters a gradient of mobile phase was developed (Table 1). The elution of PAHs takes place in steps 1 to 5. The 6<sup>th</sup> and 7<sup>th</sup> steps assure the cleaning and the reconditioning of the column. The optimal value of the thermostat's temperature was determined to be 25°C. A 6 steps wavelength program was developed for the FLD detector (Table 2). Using this method a solution of 50ng/ml standard solution was injected into the system and the obtained chromatogram is presented in Figure 1.

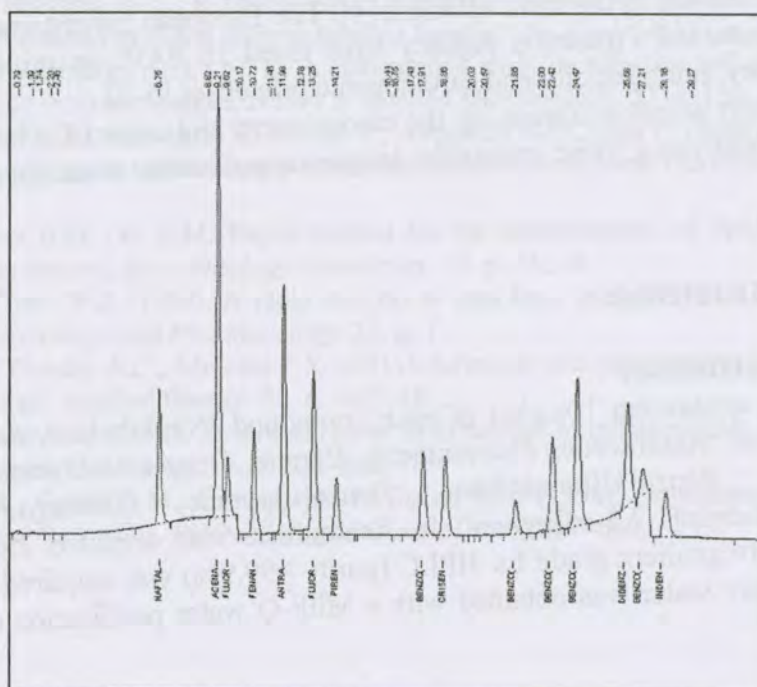


Figure 1. Chromatogram of 50 ng standard solution by HPLC using the FLD detector

Table 1. HPLC gradient program for PAHs determination

No.	Time (min.)	Flow (ml/min)	Water A (%)	Acetonitrile B (%)
Step 1	1	1.6	55	45
Step 2	5	1.6	40	60
Step 3	15	1.6	10	90
Step 4	4	1.6	0	100
Step 5	2	1.6	0	100
Step 6	6	1.6	55	45
Step 7	17	1.6	55	45

Table 2. HPLC wavelengths program for PAHs determination

Compound	Wavelength (nm)		Time (min)	Gain*
	Excitation	Emission		
1. Naphthalene	224	330	0	3
2. Acenaphthene				
3. Fluorene				
4. Phenanthrene	254	402	9.9	3
5. Anthracene	237	440	10.9	4
6. Fluoranthene				
7. Pyrene	270	390	13.4	3
8. Benz[a]anthracene				
9. Chrysene				
10. Benzo[b]fluoranthene				
11. Benzo[k]fluoranthene				
12. Benzo[a]pyrene				
13. Dibenz[a,h]anthracene	270	390	19.9	4
14. Benzo[ghi]perylene				
15. Indeno[1,2,3-cd]pyrene	300	500	27.4	3

\*Gain order ranges from 1-5 where 1 is the highest and 5 is the lowest.

Using the method developed earlier a spike sample with a 50ng/ml concentration was extracted and injected into the HPLC system. The recovery ranges for each compound are presented in Table 3.

Table 3. Recovery range for each of the 15 PAHs analyzed

No.	Compound	Recovery range (%)	Recovery (%)
1	Naphthalene	[88.3-95.4]	92.1
2	Acenaphthene	[50.9-55.3]	53.0
3	Fluorene	[52.4-58.7]	56.6
4	Phenanthrene	[83.3-88.6]	85.0
5	Anthracene	[61.1-66.9]	63.0
6	Fluoranthene	[58.4-63.7]	60.0
7	Pyrene	[69.4-73.3]	71.3
8	Benz[a]anthracene	[61.7-65.2]	64.3
9	Chrysene	[67.4-71.7]	69.1

No.	Compound	Recovery range (%)	Recovery (%)
10	Benzo[ <i>b</i> ]fluoranthene	[102.3-107.5]	105.0
11	Benzo[ <i>k</i> ]fluoranthene	[75.4-80.1]	78.3
12	Benzo[ <i>a</i> ]pyrene	[84.9-88]	86.3
13	Dibenz[ <i>a,h</i> ]anthracene	[65.2-69.1]	67.6
14	Benzo[ <i>ghi</i> ]perylene	[64.4-69.7]	66.9
15	Indeno[1,2,3- <i>cd</i> ]pyrene	[82.4-86.7]	84.3

## CONCLUSIONS

- The HPLC-FLD method developed in REZALIM-ICIA laboratory allows the determination at nanoscale level of 15 priority PAHs.
- The instrument parameters were optimized in order to obtain the best selectivity and sensitivity.
- The method is used in REZALIM-ICIA laboratory for environmental pollution assay.

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