AN EASY AND RELIABLE METHOD FOR PAH EXTRACTION FROM FOOD SAMPLES

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Abstract. *A simple and reliable method for the determination of 16 PAHs from meat and meat products is introduced. The method uses just 10 g of sample and has a high recovery of 70-85%. Prior to the liquid/liquid extraction with cyclohexane the meat samples are saponified using an alcoholic solution of KOH. The samples were purified using a Florosil column and analyzed with a HPLC-FLD instrument. The method was tested on sever meat products that are found on the Cluj-Napoca market with excellent results.*

Keywords: PAHs, HPLC-FLD, extraction method, meat and meat products

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of about 10,000 organic compounds containing two or more fused aromatic rings that are formed and released during incomplete combustion or pyrolysis of organic matter, during industrial processes and other human activities.[1,2]

Compounds that are relevant considering their effect on human health and there abundance in the environment are naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3,c,d]-pyrene, dibenz[a,h]anthracene, benzo[g,h,i] perylene [2, 3, 4, 5].

The most studied PAH is benzo[a]pyrene, which is often used as a marker for PAH in ambient air and food.[2]

Human's major routes of exposure to PAH are from inhaled polluted air and food products, especially the ones that suffer grilling, roasting and smoking processes. [2, 6, 7, 8] Vegetables may also contain high values of PAHs due to the air pollution and the deposit of these organic pollutants on the surface. [2, 6, 7]. PAHs are lipophilic compound and thus tend to from complex bound with the fatty part of meat products. [1, 9]

Considering the complexity of the sample matrix and the low concentration in which the analit is found, in order to have a sensitive, selective and stable method of analysis it is essential have an easy, reliable and rugged method of extraction.

MATERIAL AND METHOD

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.

Cyclohexane for HPLC (purity ≥99.9%), Ethanol and potassium hydroxid, 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.

Florisil (Merck) was used after heating overnight at 120°C. 0,45µm filtration cartridge for syringe where acquired from Phenomenex.

Samples

The extraction method was tested on 3 random choose meat products that were bought from the Cluj-Napoca market. The meat products had different fat content and different heat treatment during their production. The samples analyzed are: loin, bacon and baloney.

Liquid chromatography conditions

The method was developed using a Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with UV and FLD detectors.

System Parameters:

- Flow Rate: 1,6mL/min
- Mobile Phase: A (H2O)
  B (ACN)
- Column Temp: 25°C
- Injection Volume: 20 µL
- Column: ZORBAX Eclipse PAH 5µm, 4.6×150 mm column from Agilent Technologies
- Wavelength: 254 nm for the UV detector, different wavelenghts appropriate for each compound for the FLD detector

Recovery experiments and preparation of blank extracts

For the study of the recovery a 10 g of meat sample was spiked with 1 ml standard solution containing all the 15 PAH's in a concentration of 50µg/ml dissolved in acetonitrile. In the same time a blank sample from the same meat was analyzed in order to correctly calculate the recovery.

RESULTS AND DISCUSSION

The extraction method uses 10 g of sample that is homogenize in a laboratory blander.

After the homogenization the sample goes through a saponification step in order to dissolve all the fat that the sample contains. This is a very important step that ensures a high recovery. For the saponification step 50 ml of KOH solution 0,4 M in ethanol and water (9:1) was used. The sample was then put in a ultrasound bath for 30 minutes at 60^{o}C. Before the liquid/liquid extraction the sample was filter through a paper filter. The liquid/liquid extraction was done twice, using a separation funnel, each time using 15 ml of cyclohexane. The supernatant was purified with a Florosil column and the evaporated to dryness in a gentle nitrogen stream. The sample was reconstituted using 1 ml of acetonitrile. Before being injected the sample were filtered using a 0,45µm filtration cartridge.

20 µL of the samples where then injected in the HPLC-FLD. The gradient program is shown in table 1 and in table 2 is shown the wavelengths program for each of the 15 PAH's that were analyzed.

Table 1.

Gradient program for PAHs separation by HPLC

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

The following formula was used in order to establish the recovery:

$$R = \frac{A_s - A_b}{A_a} * 100$$

where:
R – recovery percentage,
A_s – amount of compound found in spiked sample,
A_b – amount of compound in sample,
A_a – amount of compound added.

The recovery for all 15 PAHs analyzed are shown in table 3.

Table 2.

Wavelengths program for PAHs determination by HPLC

Compound		Wavelength (nm)		Time (min)	Gain*
		Excitation	Emission		
1. 2. 3.	Naphthalene Acenaphthene Fluorene	224	330	0	3
4.	Phenanthrene	254	402	9,9	3
5. 6.	Anthracene Fluoranthene	237	440	10,9	4
7. 8. 9. 10. 11. 12.	Pyrene Benz[a]anthracene Chrysene Benzo[*b*]fluoranthene Benzo[*k*]fluoranthene Benzo[*a*]pyrene	270	390	13,4	3
13. 14.	Dibenz[*a,h*]anthracene Benzo[*ghi*]perylene	270	390	17,.6	4
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.

Table 3

Recovery for the 15 PAHs by liquid/liquid extraction of food sample

Nr. Crt.	Name of compound	Recovery (%)
1	Naphthalene	81,2
2	Acenaphthene	75,4
3	Fluorene	73,2
4	Phenanthrene	69,8
5	Anthracene	77,9
6	Fluoranthene	73,8
7	Pyrene	71,3
8	Benz[a]anthracene	84,3
9	Chrysene	78,4
10	Benzo[*b*]fluoranthene	75,0
11	Benzo[*k*]fluoranthene	79,5
12	Benzo[*a*]pyrene	77,1
13	Dibenz[*a,h*]anthracene	75,8
14	Benzo[*ghi*]perylene	69,9
15	Indeno[1,2,3-cd]pyrene	84.5

The chromatogram obtained from the sample spiked with 50µg/ml standard solution containing all the 15 PAHs is shown in Figure 1.

All 3 samples of loin, bacon and baloney where prepared using the liquid/liquid extraction method and then analyzed using the HPLC-FLD instrument (Figure 2).

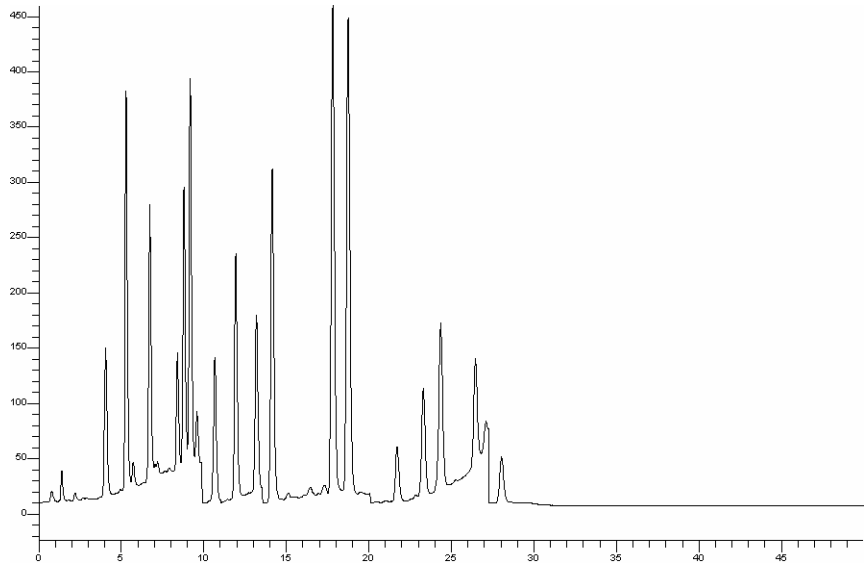

Figure 1. Chromatogram from the meat sample spiked with 50µg/ml standard solution containing all the 15 PAHs

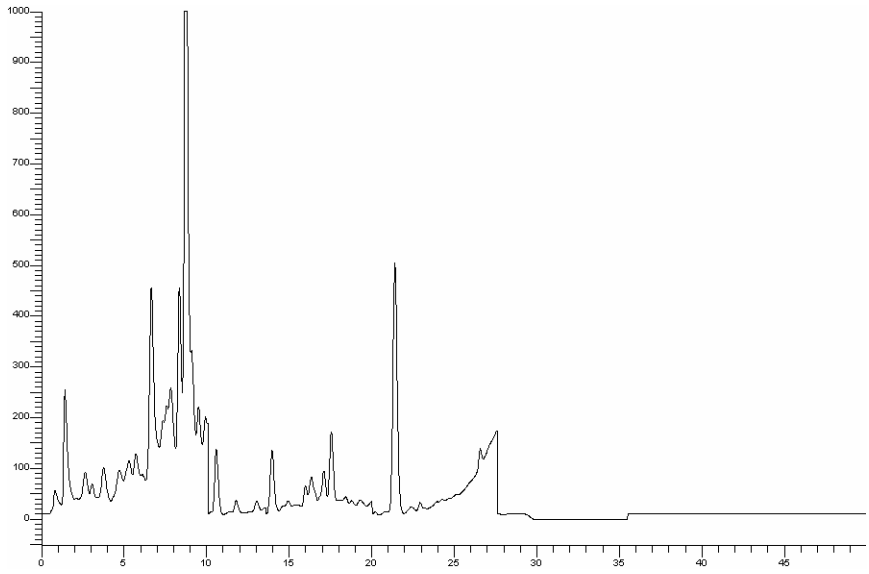

Figure 2. Chromatogram of bacon obtained using HPLC-FLD

Conclusions

The recoveries obtained with the liquid/liquid extraction are very good. It is an easy, reliable and rugged method, optimal for the extraction of meat and meat products. The amount of solvents used is low. The Florosil column retains all the fat that is let after the extraction, assuring an impurity free sample. No matrix effect was observed for the compounds of interest in the analyzed samples, they have the same retention time as the standard solution.

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