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International Conference on
Natural and Applied Sciences-III
Villahermosa, Tabasco, Mexico

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October 4-6, 2022

# PROCEEDING BOOK

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# POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN SMOKED FISH AND HUMAN HEALTH RISK ASSESSMENT

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## ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are associated with risks to human health, especially carcinogenesis. One form of exposure to these compounds is through ingestion of smoked fish, which can occur during fish processing, involving high temperatures. Smoking is one of the oldest methods of fish preservation since smoke contains bactericidal and antioxidant properties. Depending on the smoking method, the amount of carcinogenic compounds in smoke varies. Several PAHs compounds represent carcinogenic, especially for smoked fish. The EU Scientific Committee on Food (SCF) has identified 15 PAHs compounds as carcinogenic genotoxic i.e. Benzo[a]anthracene, Benzo[b] fluoranthene, Benzo(j)fluoranthene, Benzo[k]fl fluoranthene, Benzo(a)pyrene, Benzo(ghi) perylene, Chrysene, Cyclopenta[cd]pyrene, Dibenz[a,h]anthracene, Dibenzo[a,e]pyrene Dibenzo[a,l]pyrene, Dibenzo[a,i]pyrene, Indeno[1,2,3-cd]pyrene, and 5-Methylchrysene. This research aimed to determine the content of polycyclic aromatic hydrocarbons (PAHs) in five species of smoked fish, namely brown trout (Salmo trutta), tuna (Thunnus albacares), mackerel (Scomber scombrus), Atlantic salmon (Salmo salar) and mullet (Mugil cephalus), obtained from markets of different countries. The levels of these compounds in smoked fish have been determined by a GC/MS technique. The content of all identified compounds, in each fish species, was below the permissible limits following European regulations for the maximum permitted amount of polycyclic aromatic hydrocarbons in smoked products.

**Keywords:** smoked fish, polycyclic aromatic hydrocarbons, risk assessment

## Introduction

According to [1], more than 100 polycyclic aromatic hydrocarbons (PAHs) have been characterized, sixteen of which were classified as priority pollutants because of their toxicity. [2] considered that PAHs have been reported to be highly mutagenic and carcinogenic in humans. One form of exposure to these compounds is through ingestion of smoked fish, which can occur during fish processing, involving high temperatures. Several PAHs compounds represent carcinogenic, especially for smoked fish. Smoking is one of the oldest methods of fish preservation since smoke contains bactericidal and antioxidant properties. Depending on the smoking method, the amount of carcinogenic compounds in smoke varies. [3] concluded that serious public health concerns could occur if PAHs residues present in smoked fish are above-recommended levels.

## MATERIALS AND METHODS

This research aimed to develop an analytical method for the determination of PAHs in samples of smoked fish. The method was proved using PAH standard Calibration MIX 1x1 ml, 10ug / ml - Acetonitrile.

### Materials

Samples of five species of smoked fish obtained from markets of different countries, namely brown trout (*Salmo trutta*) from Kosovo, tuna (*Thunnus albacares*) from Italy, mackerel (*Scomber scombrus*) from Great Britain, Atlantic salmon (*Salmo salar*) from Italy and mullet (*Mugil cephalus*) from Greece, were used for this research.


*Fig.1. Smoked fish samples*

Extraction of PAHs was carried out based on the method described by [4]. For this purpose, the following reagent and standards were used: Acetonitrile, water deionized, magnesium sulfate, sodium chloride 400 mg, C18 400 mg, Naphthalene, Acenaphtylene, Acenaphthene, Fluorene, Anthracene, Phenanthrene, Dimethyl, Fluoranthene, Pyrenees, Benzo (a) anthracene, Chrysene, Benzo (b) fluoranthene, Benzo (k) fluoranthene, Benzo (e) pyrene, Indeno (1,2,3-cd) pyrene and Benzo (g, h, i) perylene.

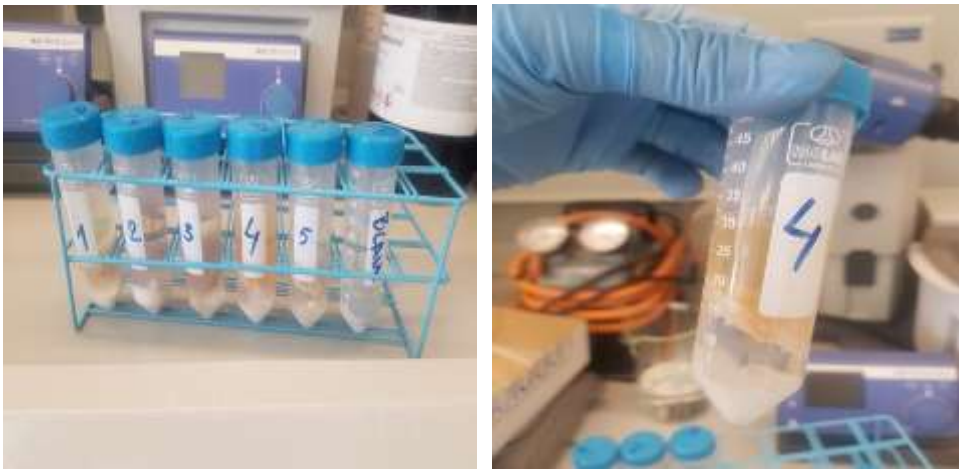


*Fig.2. Sample during quenchers (5982-6555) (Extraction containing 6 g magnesium sulfate and 1.5 g sodium chloride)*


*Fig.3. Sample during purification with quenchers (5982-5158) (contains 400 mg PSA, 400 mg C18EC, and 1200 mg $MgSO_4$)*

**Methods**

Below are the equipment and methods used for this research:

*General laboratory equipment*

- cups sized glass _ to MISCELLANEOUS
- tubes 50 ml
- tubes of 15 m
- Volumetric flasks with different sizes
- Balloons – different size

*Measuring and testing equipment*

- Electronic Scales s with weight and precision up to 0.01mg
- Centrifuge
- Mixer
- Vortex

*Main devices measurement*

- GCMS with MS detector
- Column per GCMS: DB-5

- GCMS - vials

*Centrifuge tube 50 ml with cap.*

*Column chromatographic DB-23 (30 Detector spectrometer mass capable of recording and transitions of at least GC / MS and equipped with ESI interface.*

- Centrifuge tube 15 ml with cap
- Glass tubes of 10 ml

*Computerized system for GCMS, and chromatographic data calculation.*

**Chromatographic Method - Gas chromatography**

The cleaned up extracts were analysed naphthalene, acenaphthylene, benzo[b]fluoranthene, phenanthrene, dibenzo[a,h]anthracene, chrysene, benzo[a]pyrene, acenaphthene, benzo[k]fluoranthene, fluorene, pyrene, benzo[a]anthracene, anthracene, fluoranthene, indeno[1,2,3-cd]pyrene, and benzo[g,h,i] anthracene, using Gas chromatography, programmed as follows:

*Table.1. MS - Operating conditions for testing PAHs in smoked fish meat*

| | |
|---|---|
| Column | HP-5 30mX |
| Injector temperature | temperature 280ºC |
| Carrier gas | Helium |
| Carrier gas flow | 1.2 mL / min |
| Split ratio | 50:02:00 |
| Oven Program | 60°C. 2.8 min 1°C |
| | 20 0°C / min 150°C 0 min |
| | 12 0°C / min 300°C 11.6 min |
| Total run time: | 29.6 min |
| Injection Volume | 2.0 ul |
| Diluent | Acetonitrile |
| **MS Parameters:** | |
| Ionization source | EI |
| Electron energy | 70 Ev |
| MS Source | 230°C |
| MS Quad | 150C |
| **SIM or SIR (Selective Ion Monitoring) Parameters:** | |
| Solvent delay | 5.0 min |

***Samples Preparation: meat sample of smoked fish***

These are the steps for sample preparation:

**Agilent Bond Elut QuEChERS Extraction Procedure for PAHs in Fish**

Weigh a 3g sample (±0.05g) in a 50 ml centrifuge tube

↓

Add 12ml of DI water and 2 ceramic bars to the sample

↓

Add 15ml of ACN vortex 1min

↓

Add original Agilent Bond Elut QuEChERS extraction salt packet for 15g samples (p/n 5982-6555)

↓

Shake vigorously for 1min on Geno/Grinder at 1500 pm

↓

Centrifuge at 4000 rpm for 5 min.

↓

Transfer 8ml of the ACN layer to Agilent AOAC fatty sample type 15ml tube (p/n 5982-5258)

↓

Vortex 1min. Centrifuge at 4000 rpm for 5min

↓

Analyze extract by GC/MS

The samples have been tested within 24 hours from the moment of preparation.

**5. RESULTS AND Discussion**

Polycyclic Aromatic Hydrocarbons (PAHs) levels in five commonly consumed smoked fish species, namely, brown trout (*Salmo trutta*) from Kosovo, tuna (*Thunnus albacares*) from Italy, mackerel (*Scomber scombrus*) from Great Britain, Atlantic salmon (*Salmo salar*) from Italy and mullet (*Mugil cephalus*) from Greece were assessed to evaluate possible human health risks associated with consumption.

Testing is performed using the analytical method of Gas chromatograph with a detector with a spectrometer of mass (GC-MS). Methods are accurate in detecting PAH - in smoked fish meat. The calibration curve ranged from 10-1000 ng/ml.

*Table 2. PAH compounds Calibration MIX 1x1 ml, 10 ug / ml – Acetonitrile*

| **Compounds** | **MM g / mol** | **Target ion** | **Q1** | **Q2** | **RT (min)** | **Correlation coefficient (R2)** | **Calibration Curve - range (ng / ml)** | **LOD (ng / ml)** | **LOQ (ng /ml)** |
|---|---|---|---|---|---|---|---|---|---|
| **NAPHTHALENE ($C_{10}H_8$)** | 128.17 | 128 | 129 | 127 | 4.45 | 1 | 10-1000 | 8.09 | 24.5 |
| **ACENAPHTHYLENE ($C_{12}H_8$)** | 152.2 | 152 | 151 | 153 | 6.35 | 0.99 | 10-1000 | 61.54 | 186.5 |
| **ACENAPHTHENE ($C_{12}H_{10}$)** | 154.2 | 154 | 153 | 152 | 6.6 | 1 | 10-1000 | 24.49 | 74.22 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **FLUORES (C $_{13}$ H $_{10}$ )** | 166.22 | 166 | 165 | 167 | 7.34 | 1 | 10-1000 | 31.22 | 94.61 |
| **PHENANTHRENE (C $_{14}$ H $_{10}$ )** | 178.23 | 178 | 179 | 176 | 8.87 | 0.99 | 10-1000 | 46.56 | 141.08 |
| **ANTHRACENE (C $_{14}$ H $_{10}$ )** | 178.23 | 178 | 179 | 176 | 8.95 | 0.99 | 10-1000 | 50.14 | 151.94 |
| **FLORANTHENE (C $_{16}$ H $_{10}$ )** | 202.26 | 101 | 202 | 203 | 11.02 | 0.99 | 10-1000 | 53.47 | 162.03 |
| **PYRENE (C $_{16}$ H $_{10}$ )** | 202.26 | 202 | 200 | 203 | 11.4 | 0.99 | 10-1000 | 53.1 | 160.9 |
| **BENZO (A) ANTHRACENE (C $_{18}$ H $_{12}$ )** | 228.29 | 228 | 229 | 226 | 13.75 | 0.95 | 10-1000 | 107.5 | 325.9 |
| **CHRYSENE (C $_{18}$ H $_{12}$ )** | 228.29 | 228 | 226 | 229 | 13.82 | 0.99 | 10-1000 | 44.64 | 135.28 |
| **BENZO (B) FLUORANTHENE (C $_{20}$ H $_{12}$ )** | 252.31 | 252 | 126 | 253 | 15.71 | 0.97 | 10-1000 | 81.59 | 247.25 |
| **BENZO (K) FLUORANTHENE (C $_{20}$ H $_{12}$ )** | 252.32 | 123 | 252 | 253 | 15.76 | 0.97 | 10-1000 | 80.78 | 244.78 |

*Table 3. PAH compounds in smoked fish samples*

| | Compounds | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
|---|---|---|---|---|---|---|---|
| **1** | NAPHTHALENE (C10H8) | n/d* | n/d | n/d | n/d | n/d | n/d |
| **2** | ACENAPHTHYLENE (C12H8) | n/d | n/d | n/d | n/d | n/d | n/d |
| **3** | ACENAPHTHENE (C12H10) | n/d | n/d | n/d | n/d | n/d | n/d |
| **4** | FLUORENE (C13H10) | n/d | n/d | n/d | n/d | n/d | n/d |
| **5** | PHENANTHRENE (C14H10) | n/d | n/d | n/d | n/d | n/d | n/d |
| **6** | ANTHRACENE (C14H10) | n/d | n/d | n/d | n/d | n/d | n/d |
| **7** | FLORANTHENE (C16H10) | 8.36 | n/d | n/d | 0.38 | 1.07 | n/d |
| **8** | PYRENE (C16H10) | 7.94 | n/d | 0.59 | 0.36 | 1.21 | n/d |
| **9** | BENZO (A) ANTHRACENE (C18H12) | 1.61 | 0.16 | 0.38 | 0.3 | 0.18 | n/d |
| **10** | CHRYSENE (C18H12) | n/d | n/d | n/d | n/d | n/d | n/d |
| **11** | BENZO (B) FLUORANTHENE (C20H12) | n/d | n/d | n/d | n/d | n/d | n/d |
| **12** | BENZO (K) FLUORANTHENE (C20H12) | n/d | n/d | n/d | n/d | n/d | n/d |
| **13** | BENZO (A) PYRENE (C20H12) | n/d | n/d | n/d | n/d | n/d | n/d |
| **14** | BENZO (G, H, I) ANTHRACENE (C22H12) | n/d | n/d | n/d | n/d | n/d | n/d |
| **15** | DIBENZO (A, H) ANTHRACENE (C22H14) | n/d | n/d | n/d | n/d | n/d | n/d |
| **16** | INDENO (1, 2, 3-CD) PYRENE (C22H12) | n/d | n/d | n/d | n/d | n/d | n/d |

*Sample 6 is the control

*Table 4. Levels of contamination with PAH compounds in smoked fish samples*

| Compounds | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | Sample 6 |
|---|---|---|---|---|---|---|
| **Floranthene (C16H10)** | 8.36 | n/d | n/d | 0.38 | 1.07 | n/d |
| **Pyrene (C16H10)** | 7.94 | n/d | u | 0.36 | 1.21 | n/d |
| **Benzo (A) Anthracene (C18H12)** | 1.61 | 0.16 | 0.38 | 0.3 | 0.18 | n/d |
| **According to the order of contamination** | **1** | **5** | **4** | **3** | **2** | **6** |

*Sample 6 is the control

*1 – the highest level; 5 – the lowest level

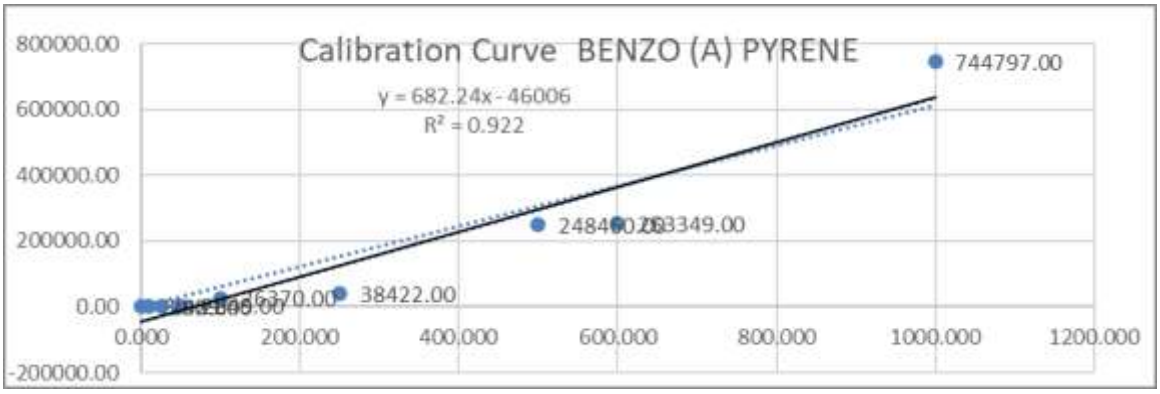


*Fig. 4. Calibration curve – BENZO (A) PYRENE -10-1000ng/ml*

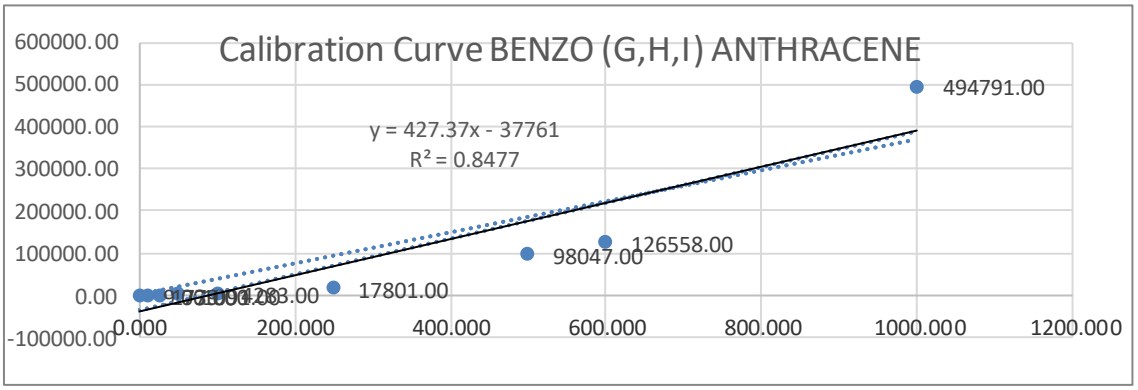


*Fig. 5. Calibration curve – BENZO (G, H, I) ANTHRACENE -10-1000 ng/ml*

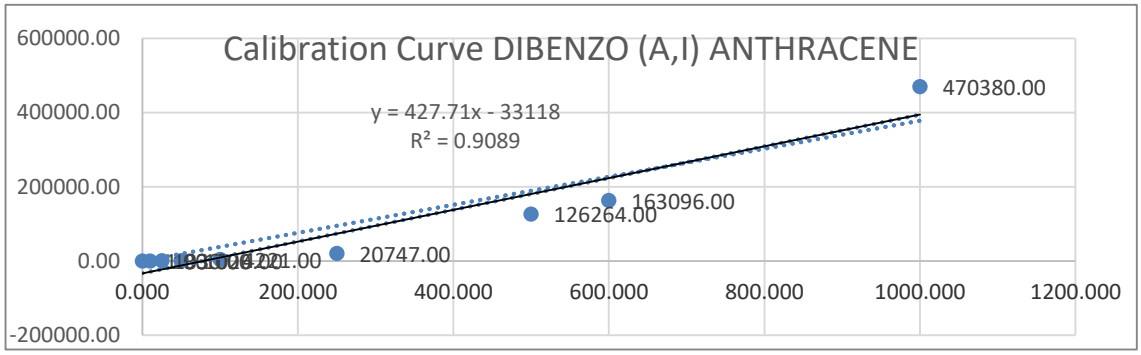


*Fig. 6. Calibration curve – DIBENZO (A, I) ANTHRACENE -10-1000 ng/ml*

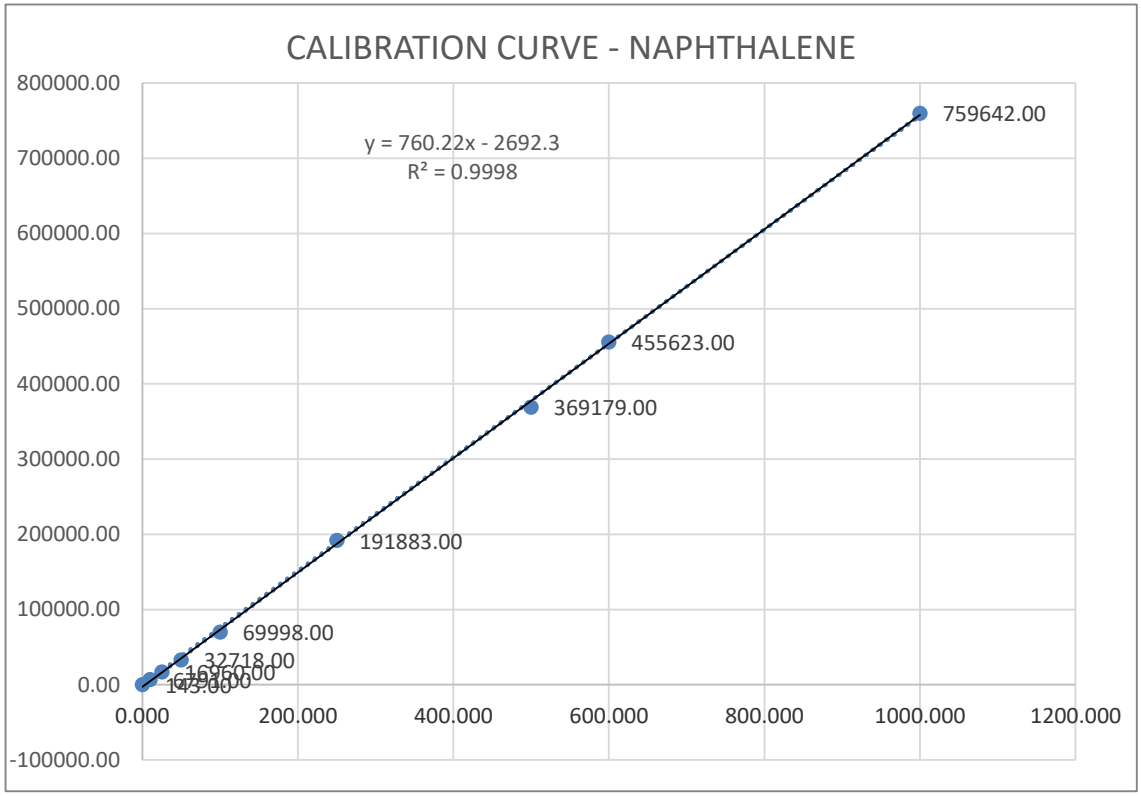


*Fig.7. Calibration Curve - Naphthalene -10-1000 ng /ml*

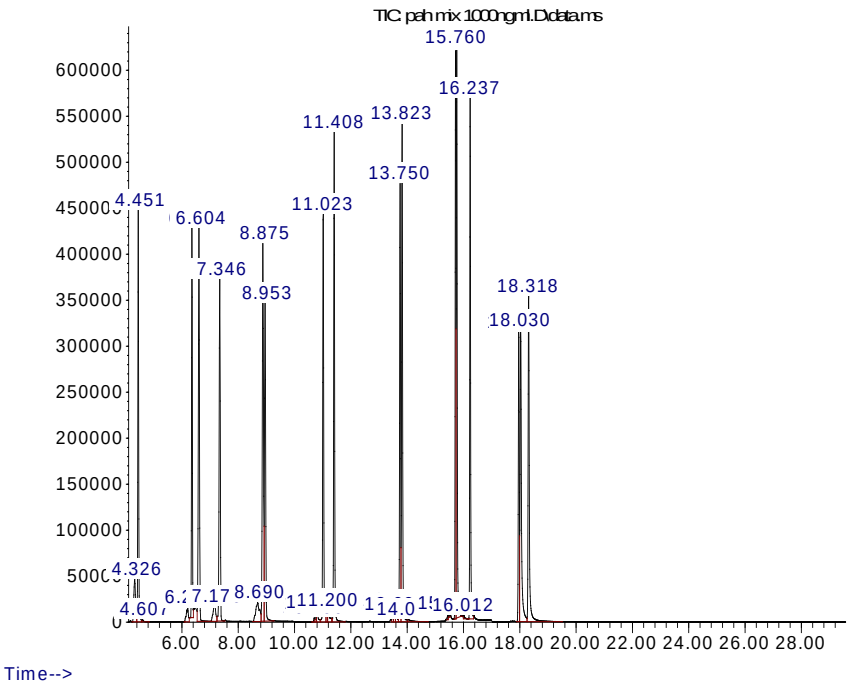


*Fig. 8. Separation of PAH compounds STAND. MIX 1000ng / ml with column (SCAN) HP-5 30 m x 0.320 mm x 0.25m*

| **Sample Name: PAH MIX 1000 ng / ml** | |
|---|---|
| **Compound Name** | **RT (min)** |
| **Naphthalene** | 4.451 |
| **Acenaphthylene** | 6.356 |
| **Acenaphthene** | 6.604 |
| **Fluorene** | 7.346 |
| **Phenanthrene** | 8.875 |
| **Anthracene** | 8.953 |
| **Floranthene** | 11.023 |
| **Pyrenees** | 11.408 |
| **Benzo (a) Anthracene** | 13.75 |
| **Chrysene** | 13.823 |
| **Benzo (b) Fluoranthene** | 15.717 |
| **Benzo (k) Flioranthene** | 15.76 |
| **Benzo (a) Pyrenees** | 16.237 |
| **Benzo (g, h, i) Anthracene** | 17.96 |
| **Dibenzo (a, i) Anthracene** | 18.03 |
| **Indeno (1,2,3-cd) Pyrenees** | 18.318 |

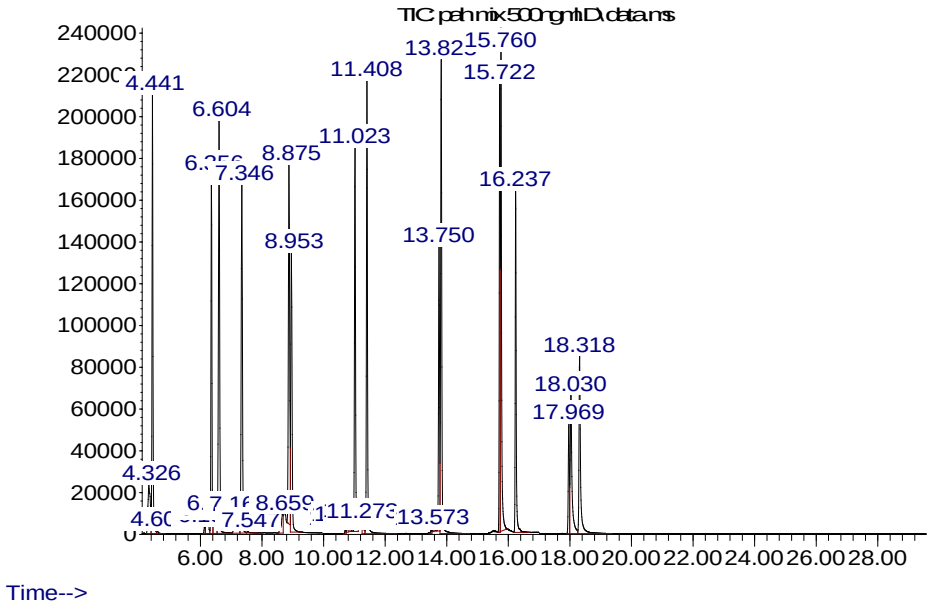


*Fig. 9. Separation of PAH compounds STAND. MIX 1000ng / ml with column HP-5 30 m X – (SIM)*

## CONCLUSIONS

The major source of contamination by PAHs are processing procedures, such as smoking, drying, and cooking of food. PAHs compounds are formed in the smoked food, depending on a variety of parameters, such as time of exposure, type of wood, distance from the heat source and fat drainage, way of cooking (smoking, grilling, frying, roasting), etc. The impact of PAHs on human health depends mainly on the length and route of exposure, the amount or concentration of PAHs one is exposed to, as well as the relative toxicity of the PAHs. Pre-existing health status and age, as subjective factors can also affect human health. In our research, the content of all identified compounds, in each fish species, was below the permissible limits following European regulations for the maximum permitted amount of polycyclic aromatic hydrocarbons in smoked products.

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