# Determination of the extraction efficiency of polycyclic aromatic hydrocarbons from airborne particulate matter

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## Abstract

Polycyclic aromatic hydrocarbons are common environmental pollutants that reach atmosphere in the form of aerosol adsorbed on soot, ash and dust. Having in mind its carcinogenic and mutagenic effects most countries have regulations concerning their estimation in environmental samples. In this work we have investigated extraction efficiency of several polycyclic aromatic hydrocarbons from flying particles collected on glass fiber filters. Efficiency of extraction of different duration in Soxhlet apparatus, ultrasonic bath and boiling toluene under reflux were compared. It has been established that compounds having two or three condensed rings are lost during evaporation of toulene that was used as a solvent. For compounds having five or more condensed rings extraction in ultrasonic bath and boiling toluene using magnetic stirrer and reflux are the most efficient.

**Key words**: polycyclic aromatic hydrocarbons, extraction, Soxhlet apparatus, ultrasonic bath, reflux, gas chromatography

## Introduction

Polycyclic aromatic hydrocarbons (PAH) are group of organic compounds whose molecules are composed of two, three or more six-member, sometimes five-member rings condensed in linear or angular form. These compounds are formed during incomplete combustion of fossil fuels, in home furnaces and automobile exhaust, as well as in different industrial processes like production of coke, iron aluminium or crude oil refineries (1,2). Owing to its relatively high boiling points, they reach atmosphere in the form of aerosol, adsorbed on the ash, soot or dust. PAHs having two or three rings might be present in gas phase and solid phase simultaneously. Polycyclic aromatic hydrocarbons are very stabile in the environment showing tendency to cumulate and they can be transferred through food chain. Particular interest that these compounds attract is due to their distinct carcinogenic and mutagenic effect. In fact, they are the first described chemical carcinogens. By the end of the 18th century British physician Percival Pott described increased incidence of scrotum cancer among London chimneysweepers, which was attributed to soot. Later, during the second decade of 20th century PAHs were identified in the soot (3). Mechanism of carcinogenic impact of PAHs is based on its metabolic transformation involving cytochrom-dependent monooxygenazes that give dihydrodioles which can be further oxydized giving rise to substances that can have stable carbonium ion (4). Due to expressive nucleophylic properties of carbonium ions, these metabolites are covalently bonded to cell macromolecules including nucleic acids, which can give mutations and further can be converted in pre-neoplastic or neoplastic lesion (5).

Nowadays the most of developed countries have strict regulations concerning PAHs. At the same time they are classified among persisting organic pollutants that have specific treatment (6). All of this demand application of sensitive and reliable analytical methods for its determination in food, water and environmental samples. Extraction procedure is the first step in every analytical method that strongly influence on the overall sensitivity, accuracy and precision. Up to date different extraction procedures of PAHs from particulate matter have been published (7, 8). These methods involve the use of different solvents in Soxhlet apparatus (9), use of ultrasonic bath (10, 11), extraction with supercritical fluids (12,13), exraction with ionic tenzides (14) or extraction assisted by micro waves (15). However, only few publications deal with comparison of efficiency of different extraction procedures. This is why we have investigated and compared extraction efficiencies of relatively simple procedures suitable to be used in modestly equipped laboratories.

## Material and methods

In this work we have investigated extraction in ultrasonic bath lasting 30 minutes and 3 hours, extraction in boiling toluene with magnetic stirrer under the reflux lasting 30 minutes and 3 hours and extraction in Soxhlet apparatus lasting 5 hours. In all experiments was used toluene supplied by Kemika, Zagreb. Ultrasonic bath of 100 W power and 30 kHz frequencies was supplied by Iskra, Kranj and magnetic stirrer with hot plate MM 510 by Tehtnica, @eleznik. Following polycyclic aromatic hydrocarbons were investigated: naphthalene, acenaphthylene, acenaphtene, fluorene, phenatrene, anthracene, pyrene, fluorantene chryzene, benz(a)anthracene, benzo(a)pyrene and dibenz(a,h)anthracene. All standards were purity pro analysis, supplied by Fluka. Methyl stearate was used as internal standard and was purchased through Micromass, Manchester.

Extract clean up was performed using XAD-2 resin, according to modified method, originally described by Spitzer (16). The resin was purchased from Sigma Feinbiochemica, Heidelberg and was prepared according to procedure previously described by [ober and Nikolin (17, 18). Clean up was performed as follows. Fraction of polar compounds was first eluted using ethanol (supplied by Kemika, Zagreb), followed by fraction of aliphatic compounds eluted by n-hexane (Merck, Darmstadt) and ethanol. Finally, fraction with aromatic compounds was eluted with toluene. Toluene fraction was spiked with internal standard, evaporated to dryness at 40 $^{o}$C under the stream of nitrogen, reconstituted in exactly 100 µL of toluene and subjected for further chromatographic analysis.

Quantification of polycyclic aromatic hydrocarbons was performed by gas chromatography on semi polar capillary column DB-5, 30 m x 0.32 mm purchased from J&W Scientific. Gas chromatograph was Dani 3800 HR equipped with flame ionisation detector and helium was used as a carrier gas with flow rate of 1 mL/min. Temperature program started at 110 $^{o}$C, hold for 10 minutes, then heated to 280 $^{o}$C with rate of 3 $^{o}$C/min. Chromatograms were registered on Perkin Elmer 56 recorder with 10 mV input sensitivity.

Determination of all compounds was performed using internal standard method, with calibration curve.

Results of extraction efficiency were analysed by analysis of variance to find out whether significant difference between groups exists or not.

Concerning the fact that standard reference material was not available, it was necessary to prepare samples of airborne particulate matter with exact concentration of polycyclic aromatic hydrocarbons. To do so, we collected airborne particles in city of Zenica during winter period, when emission is the highest. Samples were collected by LIB sampling device supplied by SAG Apparate und Gerateban, Ottobrun, Freilinger und Ritschel, GmbH. Flying particles were collected on glass fibber filters Sartorius type SM 134 00. Each sample was collected for 24 hours with 350 $m^3$ mean volume of air passed through the filter. After sampling, filters were extracted in Soxhlet apparatus for 24 hours using toluene, with approximately 5 cycles per hour. After drying in the air and dessicator, each filter was cut out in 8 equal parts. Two parts were separately extracted and extracts checked for residues of organic compounds by gas chromatography. Other parts of the filter were spiked by the means of Hamilton RNE 25 precision syringe with standard solution containing amounts of PAHs listed in table 1.

**Table 1:** Amounts of substances used in experiment

| **Substance** | **Amount** |
|---|---|
| Naphthalene | 3,46 µg |
| Acenaphthylene | 3,09 µg |
| Acenaphtene | 3,58 µg |
| Fluorene | 3,55 µg |
| Phenantrene | 3,70 µg |
| Anthracene | 3,48 µg |
| Fluorantene | 3,41 µg |
| Pyrene | 3,17 µg |
| Benz(a)anthracene | 3,21 µg |
| Chryzene | 3,95 µg |
| Benz(a)pyrene | 3,25 µg |
| Dibenz(a,h)anthracene | 3,73 µg |

Each sample prepared on such way was dried at room temperature and kept in dessicator until extraction.

Extraction in ultrasonic bath was performed in duration of 30 minutes and 3 hours. Due to technical limitations, ultrasonic vibrations were applied in 15 minutes intervals, followed by 10 minutes break. That means that 30 minutes of effective extraction lasted 40 minutes and 3 hours of effective extraction lasted almost 6 hours.

Extraction in boiling toluene was performed using magnetic stirrer with hot plate, under reflux, lasting 30 minutes and 3 hours.

Extraction in Soxhlet apparatus was also performed using toluene, with approximately 5 cycles per hour.

In each case extract was protected from direct sunlight, to avoid photo oxidation.

## Results and discussion

In this experiment toluene was chosen as solvent due to its similarity with investigated substances. In few other publications authors used benzene, but we chose toluene for reasons of its lover toxicity. Unfortunately, due to toluene's relatively high boiling point (111 $^{o}$C) most of low volatile PAHs were lost during procedure of sample evaporation. It has been established that naphthalene, acenaphthylene and acenaphthene are completely lost as well as most of phenantrene and anthracene and significant amount of pyrene and fluorene. Fortunately, these compounds have negligible biological activity, so proposed extraction procedures still might be used for analy-

**Table 2**: Extraction efficiency of five different procedures for PAHs extraction

| Compound name | Percent of recovery | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| **Phenantrene** | **9.98** | **11.07** | **11.12** | **12.48** | **12.15** |
| **Anthracene** | **12.25** | **12.76** | **16.64** | **16.74** | **14.81** |
| **Fluorantene** | **29.05** | **28.55** | **29.65** | **29.66** | **29.05** |
| **Pyrene** | **27.85** | **28.00** | **30.25** | **36.74** | **29.18** |
| **Benz(a)anthracene** | **66.41** | **70.83** | **76.93** | **77.55** | **65.90** |
| **Chyzene** | **65.95** | **66.41** | **80.52** | **71.34** | **70.46** |
| **Benzo(a)pyrene** | **42.55** | **41.94** | **58.34** | **54.50** | **51.19** |
| **Dibenz(a,h)anthracene** | **34.33** | **34.87** | **65.03** | **62.37** | **55.76** |

**Legend:**
A = Extraction in boiling toluene, using magnetic stirrer and reflux lasting 30 minutes
B = Extraction in ultrasonic bath lasting 30 minutes
C = Extraction in boiling toluene, using magnetic stirrer and reflux lasting 3 hours
D = Extraction in ultrasonic bath lasting 3 hours
E = Extraction in Soxhlet apparatus lasting 5 hours

sis of carcinogenic PAHs. These are compounds with 5 or more condensed rings and it was shown that their extraction efficiency ranges from 42.55% to 80.52%.
In table 2 are shown mean results of six individual samples from each group, analysed in duplicate (table 2).

Analysis of variance was used to test significance of difference among results in one group and between groups. It has been established that results of groups C and D significantly differ from groups A and B, but there is no significant difference among them. Results of group E are significantly different from results of groups C and D in the case of benz(a)anthracene, benzo(a)pyrene and dibenz(a,h)anhtracene. Extraction efficiency of chrysene in group C is significantly different from all other results. In other words it means that extraction in boiling toluene lasting 3 hours is the most appropriate for extraction of chrysene. But in case of pyrene, extraction in ultrasonic bath lasting 3 hours is the most effective.
Results for extraction efficiency of phenantrene differ from all other. However it was previously stated that most of the phenantrene was lost during evaporation procedure, so figures in table might reflect this lost more then difference in extraction procedure. To avoid lose of low boiling substance it would be necessary to switch between toluene to another low volatile solvent. Methylene chloride might be appropriate choice.
In the experiment described in this work it has been established that the most effective procedures are extraction in boiling toluene using magnetic stirrer and reflux lasting 3 hours and extraction in ultrasonic bath lasting 3 hours. However, extraction in boiling toluene has priority over extraction in ultrasonic bath, due to previously described technical limitations that make actual extraction time almost 6 hours. Extraction in Soxhlet apparatus gives somewhat cleaner extracts compared with extraction in boiling toluene, since magnetic stirrer destroys glass fibber filter and extract contains particles of filter.
Due to significant loses of low volatile substances through evaporation procedure; it should be reconsidered switch between toulene and another solvent with lower boiling point.

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