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Short communication

Development of an analytical method for polycyclic aromatic hydrocarbons and their derivatives

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Abstract

The possibilities of utilising pressurised liquid extraction for five nitro-polycyclic aromatic hydrocarbons from an inert matrix are shown. Different extraction temperatures and pressures were tested. The highest recoveries were obtained at extraction pressure 14 MPa and temperature 100 °C. Separation of non-polar, aromatic and polar fractions by the silica gel column chromatography is shown. *n*-Hexane, cyclohexane and dichloromethane as a solvent were tested. The best separations of monitored fractions were obtained, when extract was dissolved in cyclohexane. Non-polar and aromatic fractions eluted together when the extract was dissolved in dichloromethane.

Keywords: Extraction methods; Polynuclear aromatic hydrocarbons

1. Introduction

Nitrated derivatives of the polycyclic aromatic hydrocarbons (nitro-PAHs) are the class of polyaromatic compounds with at least one nitro (NO_2) functional group on the aromatic ring. These compounds are widespread semi-volatile environmental pollutants. Nitro-PAHs are emitted to the environment from a wide range of combustion sources [1,2]. They occur in the atmosphere in concentration of 10–1000 times lower then PAH and they can arise in situ from PAH and $^{\bullet}NO_3$ radical in presence of radical $^{\bullet}OH$ [3–6].

About 30 years ago, many researchers have pointed out that organic extracts of the atmospheric particulate matter and extracts of the diesel exhaust exhibit strong direct mutagenicity when the extracts were tested at animals [7] or in Ames assay [8–11]. The mutagenicity of these compounds was also tested on mammalian cells [12–16].

Liquid extraction methods are mostly used for isolation of nitro-PAHs from the solid samples. The most popular are Soxhlet extraction [17–20] and ultrasonic extraction [21–23]. Pressurised liquid extraction (PLE) [24,25] can be used, too. Dichloromethane (DCM) or its mixture with acetone, methanol or other polar solvents is used for extractions.

Some of the purification and clean-up steps of PAHs and their derivatives extracts are necessary. These steps exploit differences in the chemical or physical properties of extracted compounds. One of the most used clean-up methods is an acid–base treatment exploiting various acid–base properties [10,16]. Chromatography, especially liquid chromatography (LC), is also used for clean-up of extracts. The normal phase LC exploit different polarities of compounds. This technique is used in two forms. The first is open-column liquid chromatography [26–30] or high-performance liquid chromatography [2,11,31–33] with silica gel or aluminium oxide as a stationary phase. The second variant is solid-phase extraction (SPE) with silica gel or modified silica gel [34–37]. The polar interactions are the main for elution of compounds. For differently polar compound groups, elution solvents with increasing polarity is used. Non-polar solvents such as hexane (nC_6) or pentane are used for elution of the first fraction. More polar solvents or their mixtures then follow and the last is methanol or acetonitrile. The fractionation enables decrease of the detection limits to a level necessary for environmental sample analysis. The clean-up technique for separation of PAHs from their derivatives is shown in literature [28,30]. Unfortunately, it is not shown separation PAHs from the fraction contains alkanes that can interfere when gas chromatography is used as a following separation system.

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The effect of solvent used for extract dissolution is shown in the presented work. The efficiency of the separation system is affected by individual solvent.

2. Experimental

2.1. Reagents and chemicals

Dichloromethane, methanol, *n*-hexane, isooctane (all HPLC gradient grade), cyclohexane (analytical-reagent grade purified by rectification before use) were from Merck (Darmstadt, Germany). 1-Nitronaphthalene (1-NN, 99%), 1-nitropyrene (1-NP, 98%), 9-nitroanthracene (9-NA, 97%), 2-nitrofluorene (2-NFl, 98%), 6-nitrochrysene (6-NCh, 95%) were supplied by Sigma–Aldrich, USA. Silica gel 60 (70–230 mesh, activated 4 h at 180 °C) was from Fluka, USA. Also anhydrous sodium sulphate (analytical-reagent grade, Onex, Czech Republic, dried 1 h at 650 °C), helium (99.999%, Messer, Czech Republic) and nitrogen (99.99%, Linde, Czech Republic) were used.

Nitro-PAHs solution (10 μg/ml) was prepared in methanol and it was used as a solution for spiking of inert matrix.

2.2. PLE of nitro-PAHs from inert matrix

Extraction of nitro-PAHs was carried out by a pressurised liquid extractor (Fastex, Unikovo, Czech Republic). Dichloromethane was used as an extraction solvent. About 5 g of sodium sulphate was spiked with 1 μg of each nitro-PAH and filled into 11 ml extraction vessel. Then DCM was pumped into the vessel. After half of set extraction pressure achievement the flow of solvent was stopped. The vessel with solvent and matrix was preheated for 2 min to reach the selected extraction temperature. Then DCM was pumped into the vessel to reach the set extraction pressure, followed by a static extraction step at this temperature. A duration of static extraction step was 5 min. The extract was discharged to the vial and extraction was repeated. To ensure that all extracted analytes reach the collection vial, the vessel was rinsed with fresh solvent. Finally, pure nitrogen was purged through the extraction vessel for 1 min to assure that the solvent is completely transferred to the collection vial. Total time for one sample extraction was less than 30 min. Different extraction temperatures (60, 80 and 100 °C) and pressures (10, 12 and 14 MPa) were tested. Extracts were collected into 40 ml glass vials with PTFE–silicon septa. Extracts were evaporated to dryness by vacuum rotary evaporating at 30 °C after filtration through anhydrous sodium sulphate, redissolved in 500 μl of methanol and analysed by GC–MS. To exclude possible losses of analytes, all these samples were analysed without any clean-up step. Five extractions were carried out for each of extraction conditions.

2.3. Real samples and their extraction

A roadside dust was collected from tunnel in a middle-size town by brush and a trowel. Dust sample was dried at the room temperature for 24 h. Dry sample was sieved to remove particles of ≥0.6 mm. Sieved dust sample was homogenised by mixing and kept in 40 ml glass vials with PTFE–silicon septa and stored at −18 °C in dark.

Extraction of nitro-PAHs from the roadside dust was carried out by a pressurised solvent extractor (Fastex). Dichloromethane was used as an extraction solvent. About 2 g of roadside dust was mixed with 3 g of anhydrous sodium sulphate and filled into 11 ml extraction vessel. Free capacity of the extraction vessel was filled with glass beads. Two extraction cycles of 5 min at 100 °C and 14 MPa were performed for each sample. Extracts were collected into 40 ml glass vials, evaporated to dryness by vacuum rotary evaporator at 30 °C and redissolved in 2 ml of *n*-hexane, cyclohexane or DCM. Non-soluble particles in the evaporation flasks were homogenised ultrasonically.

2.4. Clean-up of organic extracts

A 1000 μl of the raw extract in an organic solvent was applied on the top of the silica gel column. *n*-Hexane, cyclohexane and DCM were tested as an organic solvent. A glass column (250 mm × 10 mm i.d., Merci, Czech Republic) was filled with 5 g of activated silica gel and on the top of silica gel bed 1 g of anhydrous sodium sulphate was applied. The column was washed by 10 ml of *n*-hexane before the application of raw extract. Elution was performed by *n*-hexane, DCM, methanol and their mixtures to get non-polar, aromatic and polar fractions. Each fractions was eluted by 1 ml of the organic solvent or mixture of solvents. The fraction eluted by DCM was collected together. The total volume of eluting solvents was: 10 ml of *n*-hexane, 15 ml *n*-hexane–DCM (1:1, v/v), 10 ml DCM and 10 ml DCM–methanol (1:1, v/v). All fractions were dried, redissolved in 100 μl of isooctane and analysed by GC–MS.

2.5. Analytical instrumentation

Analyses of the extracts were performed using the gas chromatograph (GC 8060, Carlo Erba, Milan, Italy) connected to the mass spectrometer (Trio 1000, Fisons Instruments, USA) operating in selected ion monitoring (SIM) mode for nitro-PAHs or in scanning mode (50–350 units) for the fraction analysis. Monitored ions in the SIM mode were M^+ and $[M-30]^+$ for nitro-PAHs. Interface and ion source temperatures were 220 and 200 °C, respectively. Electron impact ionisation with electron energy 70 eV was used. The GC column (DB-5MS, 30 m × 0.25 mm i.d., 0.25 μm phase thickness, J&W Scientific, USA) was used for the separation. Injector temperature was 250 °C; helium was used as a carrier gas at the constant head pressure 150 kPa; samples were injected in the splittless mode (splitter was closed for 1 min); sample injected volume was 2 μl. The temperature program for nitro-PAHs analysis was: injection temperature 70 °C for 1 min, 25 °C/min up to 180 °C, 10 °C/min up to 290 °C, 25 min at 290 °C. GC oven temperature program for

fraction analysis was: injection temperature 90 °C for 1 min, 25 °C/min up to 180 °C, 10 °C/min up to 290 °C, 25 min at 290 °C.

3. Results and discussion

3.1. PLE of nitro-PAHs

Spiked sodium sulphate was extracted by PSE at varying conditions. Recoveries of the extraction of nitro-PAHs at 60 °C and three different extraction pressures 10, 12 and 14 MPa are shown in Table 1. At the lowest extraction pressure, the recoveries between 44 and 71% were obtained. The relative standard deviations (R.S.D.) of five extractions were in the range 18–23%. At the extraction pressure 12 and 14 MPa, recoveries 50 and 75% were obtained with R.S.D. from 3 to 20%. Extraction recoveries at 60 °C seem to be too low to extract all amounts of analytes from matrix. When extracts were analysed in the scan mode, no degradation products were found. Increasing pressure had no influence on the extraction efficiency of nitro-PAH at 60 °C. Recoveries obtained at the extraction temperature 100 °C and extraction pressures 10, 12 and 14 MPa are shown in Table 2. Recoveries ranged from 33 to 51% with R.S.D. in the range of 20–30% at 10 MPa and increased with increasing extraction pressure. The highest recoveries were obtained at 14 MPa (51–112%) with R.S.D. in the range of 6–18%. Decrease of recovery with increasing temperature at 10 MPa was observed (Table 3). It could be due to low thermostability of analytes. The prolongation of the static extraction time or more extraction cycles had no effect on the increasing of the recoveries.

Table 1
Effect of the extraction pressure at 60 °C on PLE recovery (%)[a]

Compound	Recovery, % (R.S.D., %)		
	10 MPa	12 MPa	14 MPa
1-NN	56 (22)	56 (3)	58 (16)
2-NFl	58 (20)	50 (15)	55 (15)
9-NA	73 (14)	61 (10)	63 (17)
1-NP	74 (17)	63 (12)	75 (20)
6-NCh	70 (22)	75 (5)	69 (20)

[a] Extraction solvent was dichloromethane, average recovery values of five extractions were used.

Table 2
Effect of the extraction pressure at 100 °C on PSE recovery (%)[a]

Compound	Recovery, % (R.S.D., %)		
	10 MPa	12 MPa	14 MPa
1-NN	40 (29)	59 (9)	61 (6)
2-NFl	45 (22)	48 (9)	51 (10)
9-NA	44 (20)	69 (19)	72 (6)
1-NP	51 (23)	103 (15)	106 (14)
6-NCh	33 (30)	114 (16)	112 (18)

[a] Extraction solvent was dichloromethane, average recovery values of five extractions were used.

Table 3
Effect of the extraction temperature at 10 MPa on PLE recovery (%)[a]

Compound	Recovery, % (R.S.D., %)		
	60 °C	80 °C	100 °C
1-NN	56 (3)	52 (9)	40 (29)
2-NFl	58 (13)	46 (13)	45 (22)
9-NA	73 (8)	50 (12)	44 (20)
1-NP	74 (10)	68 (21)	51 (23)
6-NCh	70 (6)	48 (26)	33 (30)

[a] Extraction solvent was dichloromethane, average recovery values of five extractions were used.

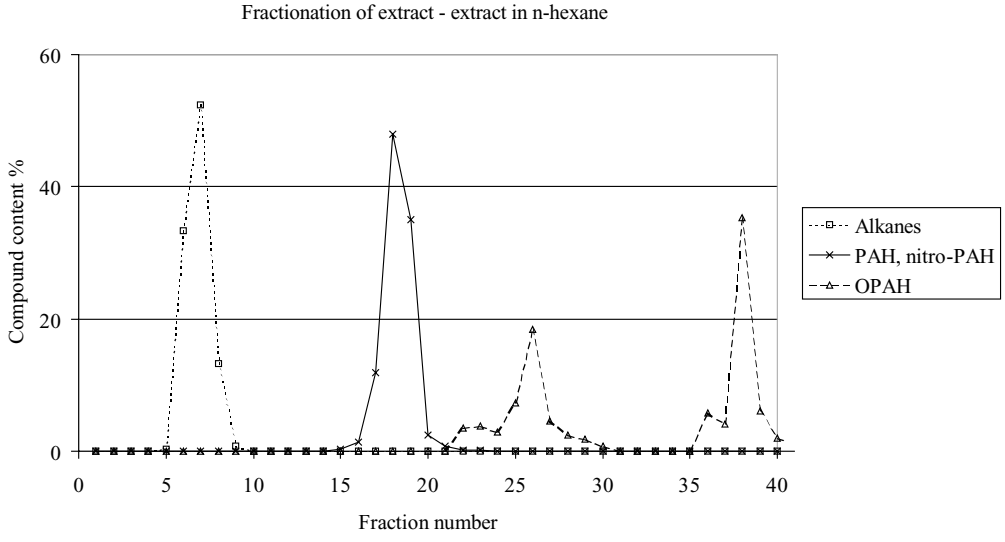

Fig. 1. Separation of the fractions by silica gel column chromatography, when the extract is dissolved in *n*-hexane. OPAH: keto and oxy derivatives of PAHs.

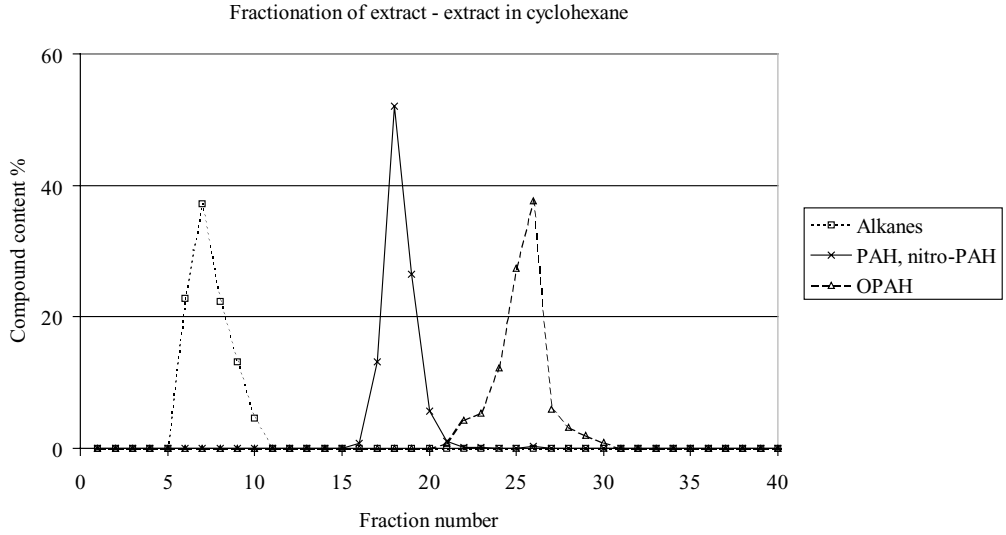

Fig. 2. Separation of the fractions by silica gel column chromatography, when the extract is dissolved in cyclohexane. OPAH: keto and oxy derivatives of PAHs.

The roadside dust spiked with nitro-PAH was extracted at the pressure 14 MPa and temperature 100 °C due to best results obtained with spiked inert matrix. The recoveries of extraction were from 55% for 1-nitronaphthalene to 107% for 1-nitropyrene with R.S.D. in the range of 9–20%.

Obtained results can be compared with results that were obtained by Soxhlet extraction applied to samples of atmospheric particles with recoveries in the range of 52% for 1-nitronaphthalene and 73% for 1-nitropyrene (R.S.D. 21–33%) [19].

3.2. Clean-up of organic extracts

The pressurised liquid extracts obtained from the roadside dust were evaporated and redissolved in different solvents and were fractionated on silica gel column. Fractions of non-polar (alkanes), aromatic (PAH and nitro-PAHs) and polar (oxy and keto derivatives of PAH) compounds were monitored. Elution curve of individual fractions separated on silica gel column are displayed in Figs. 1–3. The *x*-axis represent the fraction number, the average content of compounds in the individual fractions is displayed on the *y*-axis. Fig. 1 shows elution curves of the extract dissolved in *n*-hexane. All fractions are separated the fraction containing polar derivatives of PAHs is divided into two subfractions. Fig. 2 shows the elution curves of extracts dissolved in cyclohexane. All fractions are separated a faster elution of polar derivatives of PAHs was obtained. Fig. 3 shows the elution curves of extracts dissolved in dichloromethane. The elution of fraction containing PAHs and polar derivatives of PAHs is faster, but non-polar and PAHs fractions elute together.

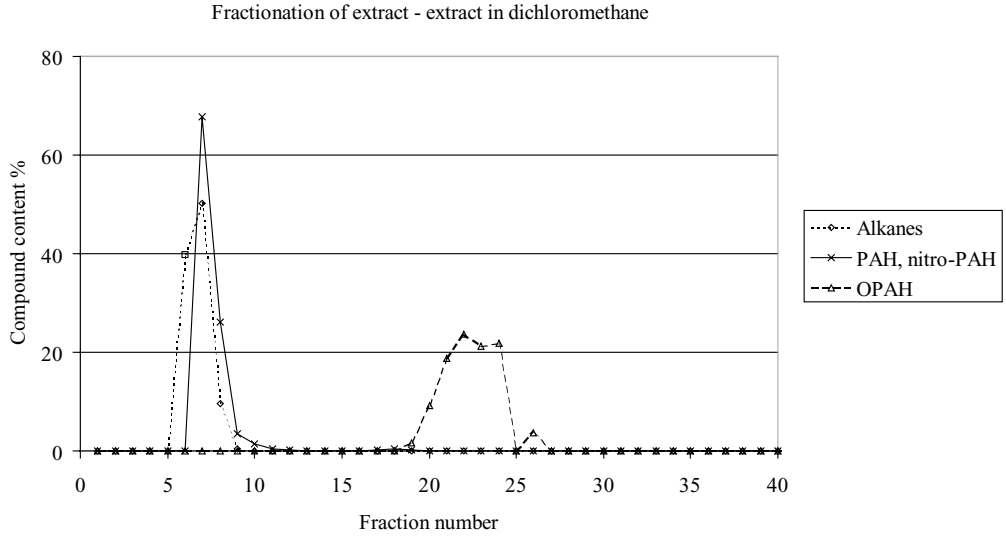

Fig. 3. Separation of the fractions by silica gel column chromatography, when the extract is dissolved in dichloromethane. OPAH: keto and oxy derivatives of PAHs.

4. Conclusion

This work showed the possibilities of extraction of nitro-PAHs from the solid samples as exemplified by extraction from an inert matrix when using PLE. The highest recoveries were obtained at the extraction pressure 14 MPa and temperature 100 °C. The best separations of monitored fractions were obtained when the extract was dissolved in cyclohexane. When extract was dissolved in dichloromethane, non-polar and aromatic fraction eluted together.

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