Optimisation of ASE for Determination of Organic Compounds Bound to Particulate Matter

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Abstract

Polycyclic aromatic hydrocarbons (PAHs), due to their harmful carcinogenic and mutagenic effects on human health, are significant organic pollutants in the air. Accelerated Solvent Extraction (ASE) at high temperature and pressure is a technique increasingly used to prepare samples for determining organic pollutants in environmental samples. To investigate ASE's efficacy in extracting organic pollutants bound to airborne particulate matter, model samples were prepared by spiking with known concentrations of a certified standard of PAHs. The following variables were optimised: solvent type, number of extraction cycles, extraction temperature, and flushing volume. ASE efficiency was compared with ultrasonic liquid extraction (ULE) using PAH certified reference material and real samples. All samples were analysed by high performance liquid chromatography with fluorescence detection and variable excitation and emission wavelength. Satisfactory efficiency for all PAHs were achieved using a solvent mixture of toluene and cyclohexane (7 : 3, *v/v*), two extraction cycles at 125 °C, and a flushing volume of 70 % of the cell volume, with recoveries exceeding 97 % for all PAHs except fluoranthene and pyrene (87 %).

Keywords

PAHs, liquid chromatography, benzo(a)pyrene, ultrasonic liquid extraction, human health

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are among the most recognised environmental pollutants due to their adverse effects on human health such as respiratory and cardiovascular diseases.[1–3] Previous studies have shown that some PAHs possess high carcinogenic and mutagenic potential.[4–6] These compounds are generated during various natural and anthropogenic processes, with approximately 90 % of PAH emissions stemming from anthropogenic sources.[7] Carcinogenic PAHs such as benzo(a)pyrene and dibenzo(ah)anthracene are predominantly associated with particulate matter.[8] In ambient air, PAHs with two to three aromatic rings are mostly present in the gaseous phase, while those with more than four aromatic rings are primarily bound to particulate matter.[9,10] Particulate matter (PM) consists of dispersed liquid and/or solid phases in the air. Particles with an aerodynamic diameter of less than 10 μm (PM_{10}) can remain airborne for several days and disperse over greater distances under the influence of wind. These particles are deposited in the respiratory tract of humans, while fine and ultrafine particles with aerodynamic diameters of less of than 2.5 μm and 1 μm, respectively ($PM_{2.5}$ and PM_1), can settle in the lung alveoli.[11,12] Several methods have been developed for the extraction of PAHs from particulate matter, including Soxhlet extraction, ultrasonic liquid extraction (ULE), and more recently, accelerated solvent extraction (ASE). Soxhlet extraction has a long duration (10 to 24 h), high consumption of organic solvents (300 ml *per* sample), and requires that the compounds to be extracted remain stable at the solvent's boiling temperature.[13,14] ULE uses less solvent volume and allows for the simultaneous processing of a larger number of samples.[15] ASE, a faster extraction technique, requires less solvent than the "classic" extraction techniques, and is easily automated.[16] An extraction method should be effective, practical, reliable, and steadfast because it forms the basis for successful analysis. Even nowadays, in many studies Soxhlet extraction is still used for extraction of PAHs (semi-volatile organic compounds) due to its high extraction efficiency.[17,18] To improve automation and decrease extraction times and solvent consumption, various extraction methods have been established. Among these techniques are ULE and ASE. Compared to Soxhlet extraction and ULE, ASE has shown to be the fastest extraction technique, achieving very high recoveries for PAH from aerosol with very small solvent volumes.[19] However, ASE involves high investment in equipment and servicing, and the assembly/disassembly of sample extraction cells can be difficult. Previous studies have demonstrated the applicability of ASE for extracting PAHs from various media, but data on the extraction of PAHs from aerosol samples remain scarce.[7,8,20] In this study, we

optimised the ASE method by testing variables such as solvent type, number of extraction cycles, extraction temperature, and flushing volume. The efficiency of ASE in extracting organic compounds from particulate matter was compared with the efficiency of ultrasonic liquid extraction (ULE), using certified reference material urban dust (Standard Reference Material NIST 1649b) and real airborne particulate matter samples.

2 Experimental

The experimental optimisation procedure included: (1) type of solvent, (2) number of extraction cycles, (3) extraction temperature, and (4) flushing volume. Three types of samples were used for method optimisation and efficiency testing: model filter samples spiked with known amounts of PAH standard solution, certified reference material urban dust, and real samples of the PM_{10} fraction of particulate matter (particulate matter with an aerodynamic diameter less than 10 µm).

Model samples were prepared by spiking quartz filters (47 mm, Whatman), typically used for collection of airborne particulate matter, with known concentrations of eleven PAHs (EPA 610 PAH standard). These model samples were used for optimisation of the ASE method. The extraction efficiencies were tested considering the type of solvent (dichloromethane (DCM) and acetone (1 : 1, *v/v*), DCM and n-hexane (1 : 1, *v/v*), toluene and cyclohexane (7 : 3, *v/v*), number of extraction cycles (1, 2, 3, and 4 cycles), extraction temperatures (60 °C, 80 °C, 100 °C, 125 °C, 150 °C), and flushing volumes (50 %, 60 %, 70 %, 80 %, and 90 % of cell volume). In the next step, the efficiency of the optimised ASE method was compared with ULE.

To determine the accuracy of the method, certified reference material (Standard Reference Material NIST 1649b, CRM) urban dust was used. CRM was weighed on two sets of quartz filters, each set containing six filters. One set of filters was placed in a 10 ml stainless steel ASE cell, and the other set of filters was placed into ULE tubes. The efficiency of ASE and ULE was also compared using real samples. PM_{10} samples were collected for 20 days at urban-industrial location, 24 h/day. PM_{10} particle samples were collected on quartz filters using low-volume sequential automatic samplers (LVS3, Sven Leckel, Ingenieurbüro GmbH, Germany), set according to EN 12341 standard (flow rate 2.3 m^3 h^{-1}). The quartz filters were cut into two pieces: one part for ASE extraction and the other for ULE extraction. ASE was performed using a Dionex ASE 350 instrument (Thermo Fisher Scientific, SAD) with 10-ml extraction cells. For the ASE procedure, each filter sample was added to a 10-ml stainless steel cell with a cellulose filter placed at the bottom end of the cell and filled with diatomaceous earth to the top. The samples

were extracted at temperatures ranging from 60 to 150 °C under a pressure of 103 bar. The extraction period was set to 5 min with a flush volume from 50 to 90 % of cell volume, purged with N_2 for 60 s. This extraction step was repeated for four cycles. A mixture of different solvents was used. All extracts were evaporated to dryness using a Rocket evaporator (Thermo Fisher Scientific, SAD) and redissolved in acetonitrile.

The ULE procedure was optimised in previous studies conducted in the laboratory.[21,22] For ULE, cyclohexane/toluene solvent mixture was used according to the procedure described by *Jakovljević et al.*[23] Filters were extracted in an ultrasonic bath for 1 h, centrifuged, and evaporated to dryness under a mild stream of N_2. Samples were then redissolved in acetonitrile.

For PAH analysis, an Agilent Infinity high-performance liquid chromatography (HPLC) system with a fluorescence detector was used (Agilent Technology, SAD). The analysis included the following PAHs: fluoranthene (Flu), pyrene (Pyr), benzo(a)antracene (BaA), chrysene (Chry), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(ah)antrachene (DahA), benzo(ghi)perilene (BghiP), and indeno(1,2,3,cd)pyrene (IP). The detailed analytical procedure is described in *Jakovljević et al.*[23] Calibration curves for Pyr, BaA, Chry, BkF, BaP, and IP ranged from 0.005 to 0.08 ng μl^{-1}, whereas Flu, BbF, DahA, and BghiP ranged from 0.01 to 0.16 ng μl^{-1}. The method's accuracy was assessed using NIST 1649b urban dust, varying from 88 % for Flu to 109 % for BkF. The limit of detection (LOD) ranged from 0.001 to 0.03 ng m^{-3} for BaA and BjF.

3 Results and discussion

3.1 Optimisation of ASE extraction method

To determine the most suitable conditions for the ASE methods, four key parameters were optimised: the type of extraction solvent, temperature, number of extraction cycles, and extraction flushing volume. These conditions were varied, while others – such as pressure (103 bar), purge time (60 s), and static time (5 min) – remained constant. The recoveries determined from spiked filters with known concentrations of eleven PAHs (EPA 610 PAH standard) are presented in Fig. 1. Considering factors such as solvent saving, reduction in toxic waste, energy efficiency, and environmental preservation (green chemistry principles), two extraction cycles were found to be optimal despite the small loss. Previous studies have shown that increasing in extraction temperature enhances the solubility and diffusion rate of analytes in the solvent, which, coupled with the decrease in solvent viscosity, improves extraction efficiency.[24,25]
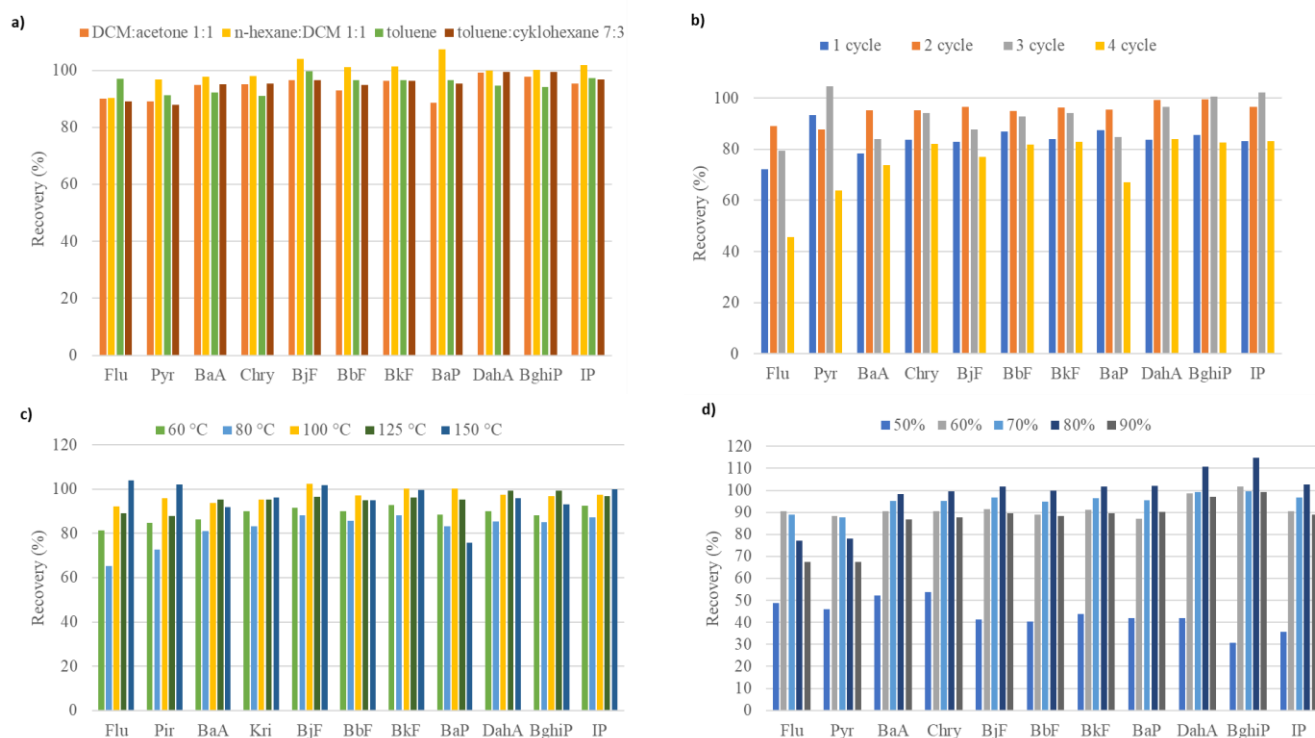
Fig. 1 – Optimisation of ASE extraction method: extraction efficiency with regard to: a) type of extraction solvent, b) number of extraction cycles, c) extraction temperature, and d) extraction flushing volume

Slika 1 – Optimizacija ASE metode: učinkovitost ekstrakcije s obzirom na: a) vrstu otapala za ekstrakciju, b) broj ciklusa ekstrakcije, c) temperaturu ekstrakcije i d) volumen ispiranja

To determine other variable conditions (temperature, flushing volume), a mixture of cyclohexane and toluene (3 : 7, *v/v*) with two extraction cycles was used. Extraction temperatures were tested across a range from 60 to 150 °C.

As shown in Fig. 1c, the best recoveries were obtained at 100 and 125 °C, with slightly higher results for 5- and 6-ring PAHs at 125 °C, which was subsequently selected for further analysis. At that temperature, the flushing volume was optimised (Fig. 1d).

Flushing volumes ranged from 50 to 90 % of the extraction cell volume. The lowest PAH recoveries were observed with a solvent filling 50 % of cell volume. The highest recoveries were achieved at an 80 % cell volume; however, this method yielded slightly lower recoveries for Flu (77 %) and Pyr (78 %). Therefore, a 70 % flushing volume was selected as the optimal condition, providing recoveries ranging from 87.7 % for Pyr to 99.3 % for DahA and BghiP. In summary, the optimal recoveries for individual PAHs were obtained with a solvent mixture of toluene:cyclohexane (7 : 3, *v/v*), 2 cycles at 125 °C with 70 % flashing volume.
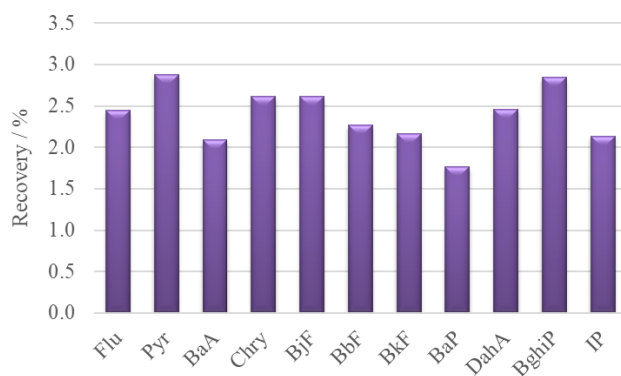

Fig. 2 – Share of PAHs concentration in the third extraction cycle

Slika 2 – Udio koncentracije PAU u trećem ekstrakcijskom ciklusu

3.2 Comparison of ULE and ASE extraction methods

After optimising the ASE method, certified standard reference materials of urban dust (NIST SRM 1649b) and real PM$_{10}$ samples were used to compare the extraction efficiencies of ASE and ULE. The real filter samples were cut into two equal pieces, while the samples of certified standard reference materials (urban dust) were weighted

(approximately 20 mg). Samples were extracted using both ULE and ASE methods and analysed as real samples. The mass concentrations of individual PAHs in NIST 1649b urban dust, determined by ULE and ASE, are shown in Fig. 3. The results were in good agreement with the certified PAH concentrations in NIST standard. The comparison of the concentrations determined in this study and the certified NIST concentrations showed good correlation, with slopes of 1.15 (R^2 = 0.98) for ULE, and 1.21 (R^2 = 0.98) for ASE. ASE generally demonstrated better recovery efficiencies for four-ring PAHs (Flu, Pyr, BaA, and Chry), which are prone to evaporation during sample preparation, and because the ASE instrument's bottle vials are compatible with Rocket evaporator, there is no loss of samples by switching samples to another vial for evaporation. The relative standard deviation (RSD) determined from consecutive measurements of 10 samples, for the ASE method, ranged from 1.8 % for BaP to 12.2 % for DahA, while for the ULE method, it ranged from 2.2 % for Flu to 11.6 % for DahA. According to the standardised method norms for the determination of PAHs in particulate matter in ambient air (EN 15549:2008, CEN/TS 16645:2016), extraction recoveries should be within 80 % to 120 % with an RSD of less than 20 %. This study shows that both extraction methods (ASE and ULE) are suitable for extracting PAHs from particulate matter and urban dust. However, the ASE method appears to be the better choice, particularly for more volatile four-ring PAHs.
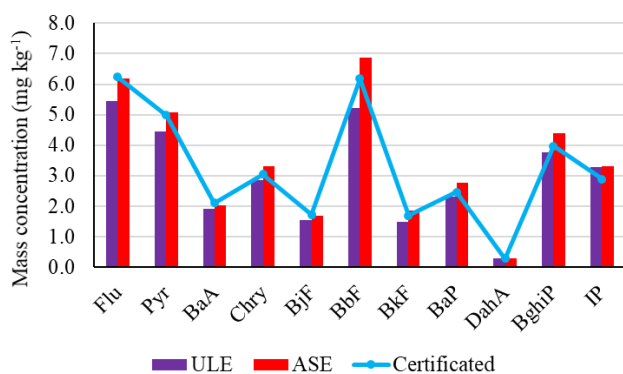
Fig. 3 – Mass concentrations of individual PAHs in NIST SRM 1649b reference material extracted using the ASE and ULE methods; blue line represents certified concentration of PAH in NIST

Slika 3 – Masena koncentracija zasebnih PAU u NIST SRM 1649b referentnom materijalu dobivena ULE i ASE ekstrakcijom; plava linija prikazuje certificiranu vrijednost PAU u NIST-u

Both extraction methods were also used for the extraction of PAHs from real PM_{10} samples collected from an urban-industrial area. The ASE method yielded higher results for all PAHs, especially for Flu and Pyr, which are PAHs with smaller molecular masses and primarily originate from wood combustion.[17,18] Only for DahA were the extraction

efficiencies comparable between the ASE and ULE methods, but this PAH was found at the lowest concentrations in ambient air. Overall, ASE extraction appears to be a more effective method for extracting PAHs from particulate matter compared to ULE. This advantage might be even more significant for the more volatile 2- and 3-ring PAHs, which are typically present in the gaseous phase and should be a focus of future research. The mass concentrations of all PAHs measured in the PM_{10} particulate fraction using both ASE and ULE methods are shown in Fig. 4.

Fig. 4 – PAHs concentrations in PM_{10} particulate fraction obtained by ASE and ULE extraction methods

Slika 4 – Koncentracija PAU u PM_{10} frakciji čestica dobiveni s ASE i ULE ekstrakcijskom metodom

In general, the average concentrations of the measured PAHs were lower when ULE was used. In both extraction methods, BbF was the dominant compound, followed by BghiP, with the lowest concentration recorded for DahA. Concentrations of PAHs extracted by ASE ranged from 0.069 ng m^{-3} for DahA to 0.622 ng m^{-3} for BbF, and from 0.065 ng m^{-3} for DahA to 0.547 ng m^{-3} for BbF. The differences in PAH mass concentrations in PM_{10} samples obtained by both ASE and ULE methods were tested using a T-test, with variables treated as dependent samples, $p < 0.05$. Concentrations of Flu and Pyr were statistically significant ($p < 0.001$), while the rest of the PAH concentrations were not statistically significant.

4 Conclusion

Both studied extraction methods, ASE and ULE, provided satisfactory results in terms of efficiency and compliance with PAHs norms. However, a comparison between ASE and ULE demonstrated several advantages of ASE: it is faster, easier to automate, more economical, eco-friendly, and more effective in extracting PAHs from particulate matter. The ASE apparatus is user-friendly and versatile, making it suitable for the extraction of organics from various sample matrices and sizes. In this study, ASE proved to be the superior method for determining volatile PAHs in particulate matter and urban dust samples.

List of abbreviations and symbols
Popis kratica i simbola

PAH   – polycyclic aromatic hydrocarbon

PM   – particulate matter

PM_{10} – particles with an equivalent aerodynamic diameter less than 10 μm

HPLC   – high-performance liquid chromatography

ULE   – ultrasonic liquid extraction

ASE   – accelerated solvent extraction

CRM   – certified reference material

DCM   – dichloromethane

LVS3   – low-volume sequential automatic samplers

RSD   – relative standard deviation

Flu   – fluoranthene

Pyr   – pyrene

BaA   – benzo(a)anthracene

BbF   – benzo(b)fluoranthene

BkF   – benzo(k)fluoranthene

BjF   – benzo(j)fluoranthene

BaP   – benzo(a)pyrene

DahA   – dibenzo(a,h)anthracene

BghiP   – benzo(ghi)perylene

IP   – indeno(1,2,3-cd)pyrene

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SAŽETAK

Optimiranje ASE pri određivanju organskih spojeva vezanih na lebdeće čestice

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Policiklički aromatski ugljikovodici (PAU) zbog svojeg štetnog karcinogenog i mutagenog djelovanja na ljudsko zdravlje predstavljaju važnu skupinu organskih onečišćujućih tvari u zraku. Ubrzana ekstrakcija otapalom (ASE) pri visokoj temperaturi i tlaku tehnika je koja se sve više primjenjuje za pripravu uzoraka pri određivanju organskih onečišćujućih tvari u uzorcima iz okoliša. Da bi se odredila učinkovitost ASE organskih zagađivala vezanih na lebdeće čestice, pripravljeni su modelni uzorci nakapavanjem filtara od kvarcnih vlakana poznatom koncentracijom certificiranog standarda policikličkih aromatskih ugljikovodika. Optimirane su sljedeće varijable: vrsta otapala, broj ekstrakcijskih ciklusa, temperatura ekstrakcije i volumen ispiranja. Učinkovitost ASE je uspoređena s ultrazvučnom ekstrakcijom otapalom (ULE) upotrebom certificiranog referentnog materijala i realnih uzoraka. Uzorci su analizirani tekućinskom kromatografijom visoke učinkovitosti s fluorescentnim detektorom promjenjivih valnih duljina ekscitacije i emisije. Zadovoljavajuća djelotvornost metode dobivena je uporabom smjese otapala toluen i cikloheksan (7 : 3, *v/v*), dva ekstrakcijska ciklusa pri temperaturi 125 °C i s volumenom ispiranja od 70 % volumena ćelije, pri čemu je iskorištenje iznosilo više od 97 % za sve PAU-e, osim za fluoranten i piren (87 %).

Ključne riječi
PAU, tekućinska kromatografija, benzo(a)piren, ultrazvučna ekstrakcija otapalom, ljudsko zdravlje

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