

Analytical Methods

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6 **Pressurized liquid extraction as an alternative to the Soxhlet extraction**
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8 **procedure stated in the US EPA method TO-13A for the recovery of**
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10 **polycyclic aromatic hydrocarbons adsorbed on polyurethane foam plugs**

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42 **Keywords**
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44 Pressurized liquid extraction (PLE)
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46 Soxhlet
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48 US EPA method TO-13A
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50 Polycyclic aromatic hydrocarbons (PAHs)
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52 Polyurethane foam plug (PUF)
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54 Air pollution
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Abstract

The aim of the present study was to develop a pressurized liquid extraction (PLE) method as an alternative to the relatively time consuming Soxhlet extraction procedure described in the United States Environmental Protection Agency (US EPA) method TO-13A for the extraction of PAHs adsorbed onto polyurethane foam plugs (PUFs). For this purpose PUF air samples were collected and split into two parts: one part extracted using PLE and the other one using Soxhlet extraction. Comparable PAH concentrations were obtained upon analysis of the extracts showing that the PLE method developed in this work is a more convenient choice than the commonly used Soxhlet extraction technique proposed by US EPA for the determination of PAHs in air samples. In fact, the developed PLE method required shorter assay times (minutes versus hours), less solvent consumption and simpler operational methods. The exhaustiveness of the developed PLE method was evaluated using repeat static extraction cycles, demonstrating an extraction efficiency for the PAHs of greater than 99 %. The PLE method was then applied to diesel exhaust and wood smoke PUF samples showing an extraction efficiency for the PAHs of greater than 93 % and 96 %, respectively. Furthermore, a PLE method for PUF cleaning was developed as well and employed as an alternative to Soxhlet extraction. The PLE methods developed for cleaning and extracting PUFs presented in this work are suitable to be used in mutagenicity studies using the Ames Salmonella assay as no mutagenicity was found in the PLE generated blanks.

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic environmental pollutants that cause or are suspected to cause severe adverse health effects in humans and animals including those from their mutagenic and carcinogenic activity.¹⁻⁴

PAHs are composed of two or more fused aromatic rings, and they either partition into the gas phase in the atmosphere or associate with particulate matter.⁵ In general, two- to three-ringed PAHs are gaseous, while PAHs that consist of five rings or more are almost entirely adsorbed onto particulate matter and four-ringed PAHs are distributed between both the gas phase and suspended particulate matter.⁶

Semi-volatile PAHs are subjected to long-range transboundary transport via deposition and re-volatilisation between air, soil and water bodies.^{7, 8} Some PAHs in the semi-volatile fraction are concerns for human health since they are present at relatively high concentrations in ambient air; these include phenanthrene, anthracene, methylated phenanthrenes/anthracenes, pyrene and fluoranthene.² Fluoranthene exhibits experimental carcinogenicity, and it has been suggested as an indicator complementary to benzo[a]pyrene (B[a]P) to assess the carcinogenic risk of PAHs in ambient air.² Furthermore, anthracene, phenanthrene, fluoranthene and pyrene are listed as Priority Pollutants by the United States (US) Environmental Protection Agency (EPA) and are considered in the Toxicological Profile for PAHs by the Agency for Toxic Substances and Disease Registry (ATSDR) at the US Department of Health and Human Services.^{9, 10} PAHs are formed during natural events, such as volcanic eruptions and forest fires, and from anthropogenic activities, and the two most dominant local sources of PAHs in ambient air in Sweden are domestic wood burning and vehicle transport.²

The collection of semi-volatile PAHs is generally achieved by passing large volumes of air through a sorbent, which is most commonly composed of polyurethane foam (PUF).¹¹

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Soxhlet extraction has traditionally been the most commonly utilised method for recovering the PAHs collected on PUFs ¹¹, and it is the extraction method described in the US EPA method TO-13A. ¹²

With Soxhlet extraction, samples undergo repeated extraction cycles with heated distilled solvents. It is one of the most widely used extraction techniques in PAH analysis because it provides high yields and is relatively inexpensive. ¹³ However, Soxhlet extraction also requires long analysis times, large solvent volumes and additional concentration steps ¹⁴, making it unsuitable for high-throughput protocols or for methods aiming for a low environmental impact. Furthermore, the operator is exposed to the risk of handling boiling solvents in the Soxhlet apparatus.

Although the instrumentation may require a higher capital investment than a Soxhlet apparatus, the use of pressurized liquid extraction (PLE) for PAH analysis has gained popularity due to its lower solvent consumption and its significantly shorter extraction times compared with Soxhlet extraction. ^{11, 15} In the PLE technique, samples are enclosed in stainless steel extraction cells and are extracted with organic solvents at high pressures (500–3000 psi) and temperatures (50–200 °C). ¹⁶ The use of elevated pressures and temperatures enhances the extraction yields compared to traditional extraction methods by increasing the solubility and mass transfer effects and by disrupting surface equilibria. ¹⁶ PLE has proven to be an equivalent or superior alternative for the extraction recovery of PAHs from various environmental matrices, such as particulate matter from ambient air and diesel exhausts, sediments and soils, compared with other commonly used techniques, such as Soxhlet extraction, ultrasonically assisted extraction and microwave-assisted extraction. ^{15, 17-20} For example, Rosa Ras *et al.* reported higher recovery and repeatability values when extracting PUF plugs that were spiked with PAH standards using PLE compared with ultrasonically assisted extraction. ²¹ Primbs *et al.* evaluated the extraction recoveries and co-extraction of

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3 sorbent interferences using PLE as an extraction technique during the analysis of PAHs and
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5 other semi-volatile organic compounds in PUFs that were fortified with standard compounds.
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8 ²² In several studies, PLE has been used to recover semi-volatile PAHs collected from
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10 ambient air on PUF plugs. ²²⁻²⁴ However, to the best of our knowledge, there are no
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12 previously published studies that have compared PLE to Soxhlet for the extraction of PAHs
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14 from PUFs using real atmospheric samples, i.e., from air samples other than PUFs spiked with
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16 PAH standards. The use of real samples is essential in evaluating extraction methods as
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18 samples fortified with standards may not behave in a similar way during the analysis.¹⁵
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22 In the present study, PLE was evaluated as an alternative to the Soxhlet extraction protocol
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24 described in the US EPA method TO-13A for the extraction of airborne PAHs collected on
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26 PUF cartridges. The optimised PLE methodology developed herein was applied to wood
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28 smoke and diesel engine exhaust samples collected on PUF plugs as well. The PLE and
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30 Soxhlet extraction techniques were compared in terms of the extraction recovery of PAHs.
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32 Method blanks were also tested for suitability using the Ames Salmonella mutagenicity assay.
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35 Furthermore, a PLE method for cleaning the PUFs was developed and employed in place of
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37 the time-consuming Soxhlet extraction procedure previously used in our research group.
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2 Experimental

2.1 Chemicals

Toluene, hexane, diethyl ether and acetone (HPLC grade, Rathburn Ltd, Scotland), anhydrous dimethyl sulfoxide (DMSO) (> 99 %, VWR international BDH Prolabo, Sweden) anhydrous dodecane (> 99 %, Sigma-Aldrich, St. Louis, MO, USA) and ethanol (95 %, Kemetyl, Haninge, Sweden) were used as solvents. A solution containing the native PAHs phenanthrene, anthracene, 3-methylphenanthrene, 2-methylphenanthrene, 2-methylanthracene, 9-methylphenanthrene, 1-methylphenanthrene, 4H-cyclopenta[def]phenanthrene, 2-phenylnaphthalene, fluoranthene and pyrene and the deuterated internal standards phenanthrene-d₁₀ and pyrene-d₁₀ was used to identify the analytes by means of their chromatographic retention times and to calculate the instrumental response factors. Information regarding the suppliers and purities of the PAH standards and deuterated internal standards are provided in detail elsewhere.²⁵

2.2 Cleaning of PUFs

PUFs were cleaned prior to sampling by machine-washing at 90 °C for approximately three hours with tap water, immersing in ethanol, and being manually squeezed dry. Subsequently, the PUFs were cleaned using PLE (three PUFs per cell in a 33 mL cell volume) with three sequential cycles using toluene (first cycle) and acetone (second and third cycles) as the extraction solvents. The instrument used was an ASE 200 (Dionex Corp., Sunnyvale, CA, USA) and the details regarding the PLE parameters are shown in Table 1. The cleaned PUFs were allowed to air dry then stored in sealed glass jars away from light at ambient temperature prior to sampling.

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3 The cleaning method using PLE was compared with the Soxhlet extraction method that has
4 previously been used in our research group for cleaning PUFs.²⁶ Briefly, in this set-up, the
5 PUFs were machine-washed at 90 °C with tap water, rinsed with distilled water (step omitted
6 in the PLE clean-up protocol, as it did not impact the blanks) immersed in ethanol and then
7 manually squeezed dry. Then, the PUFs were Soxhlet extracted (three plugs per extractor)
8 with toluene for 12 h, acetone for 12 h and finally with acetone again for 12 h. The PUFs
9 cleaned using Soxhlet extraction were dried and stored in the same manner as those cleaned
10 using PLE.
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25 **2.3 Sample collection**

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27 All of the collected and analysed samples consisted of a filter sample with a corresponding
28 downstream PUF plug. However, the filter samples were not analysed since the filter analysis
29 was outside the scope of the present study, which was concerned only with the development
30 of PLE cleaning and extraction methods for PUF samples for the analysis of semi-volatile
31 PAHs.
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39 The PUFs used were of the polyether type, as recommended by the US EPA¹², and possessed
40 the following dimensions: length, 25 mm; diameter, 70 mm; density, 0.023 g/cm³ (Special-
41 Plast Produktion, Vallentuna, Sweden). The flow during sampling ranged from 16 to 18
42 m³/hour for a total sampling time comprised between 27 and 92 hours. The sampling site was
43 located in Stockholm on the rooftop of the Arrhenius Laboratory building at the Stockholm
44 University campus. This collection site was previously used to collect particulate matter for
45 PAH analysis, as described in detail elsewhere.²⁷ The wood smoke samples were collected
46 from the Umeå University exposure chamber, where diluted wood smoke was generated using
47 a common chimney stove fed with birch logs (moisture content of 16-18 %). The
48 experimental set-up has been described in detail elsewhere.²⁸ The diesel vehicle exhaust
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3 samples were collected as described in detail elsewhere²⁹ and generated from heavy- and
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5 light-duty vehicles. The heavy-duty vehicle was a Euro V diesel truck tested in the world
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7 harmonized transient cycle (WHTC) with Swedish environmental class 1 (typical aromatic
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9 values ranging from 3.0 vol % to 5.0 vol %) and European EN 590 (typical aromatic values
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11 ranging from 15 vol % to 30 vol %) diesel fuels.²⁹ The light duty vehicle was a Euro V
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13 passenger car tested in the Artemis road and motorway driving cycles with Swedish
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15 environmental class 1 diesel fuel.³⁰

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18 Following sampling, all PUFs were stored individually in airtight glass jars with Teflon-
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20 coated lids in a freezer at -18 °C. A set of blanks was also prepared, treated and analysed in
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22 the same manner as the samples.
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31 Each air sample PUF was weighed and then cut into two sub-sections using a stainless steel
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33 surgical blade. The PUFs were split in the direction of the air flow. Each sub-section was
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35 weighed ($d = 0.1$ mg) to calculate the percentage of corresponding sample volumes. One half
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37 was subjected to Soxhlet extraction, and the other half was extracted using PLE. Both PUF
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39 sub-sections were spiked with an internal surrogate standard solution containing
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41 phenanthrene- d_{10} and pyrene- d_{10} immediately prior to extraction.
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48 49 **2.4.1 Soxhlet extraction**

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51 The PUF sub-sections sampled in ambient air underwent Soxhlet extraction using
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53 approximately 350 mL of 1:9 diethyl ether:hexane (v/v) for 18 hours at a minimum of 3
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55 cycles per hour as described by the US EPA.¹²
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2.4.2 PLE

The PLE method was developed with minimal environmental impact. Correspondingly, chlorinated solvents were not used since they are considered hazardous to health and the environment.^{22, 31} Hexane was the solvent of choice for the extractions using PLE. Diethyl ether was not used due to its high flammability and the risk of forming explosive peroxides upon storage. The ambient air PUF samples and the wood smoke and diesel exhaust PUF samples were extracted using PLE as described in detail in Table 1. To investigate the number of cycles needed for an exhaustive extraction, three additional air samples were extracted with PLE using four consecutive five-minute extraction cycles with hexane, and the generated extracts were spiked with the internal surrogate standard solution. Furthermore, the wood smoke and diesel exhaust samples were subjected to repeat extractions using the same conditions as the analytical extraction. The generated extracts were fortified with the internal surrogate standard solution to verify the exhaustiveness of the method.

2.4.3 Sample clean-up

The Soxhlet extracts were reduced to approximately 5 mL using a Büchi Rotavapor (R-114, Büchi Labortechnik AG, Switzerland). The extracts from both methods were then reduced to a final volume of 0.5 mL under a gentle stream of nitrogen in a water bath (Zymark Ltd, Runcorn, Cheshire, England). The crude extracts were finally subjected to solid-phase extraction (SPE) on silica cartridges (100 mg Isolute, IST, England) using hexane as the mobile phase, as detailed elsewhere.³² An aliquot of each eluate was then transferred to an individual micro-vial for chromatographic analysis.

2.5 Chromatographic analysis

The purified samples were analysed using a hyphenated high-performance liquid chromatography-gas chromatography/mass spectrometry (HPLC-GC/MS) system constructed in house and described in detail previously.^{18, 32} Briefly, the HPLC system consisted of a 9012 pump (Varian, Palo Alto, CA, USA), an autosampler (CMA/200 Microsampler; CMA Microdialysis AB, Sweden), a UV detector (SPD-6A; Shimadzu, Japan) and a nitrophenylpropylsilica column (4.0 mm i.d. x 125 mm, 5 µm particle size; Phenomenex, Torrance, CA, USA). The HPLC analysis was performed isocratically using hexane (1/1000 dodecane v/v) as a mobile phase and in back-flush mode for further sample clean-up. The GC (6890N; Agilent Technologies, Palo Alto, CA, USA) was equipped with a programmed temperature vaporiser injector (CIS-3; Gerstel, Germany) and a DB-17 MS column (60 m x 250 µm i.d., 0.15 µm film thickness, J & W Scientific, Folsom, CA, USA). Mass selective detection was performed using a quadrupole mass analyser (MSD 5973N; Agilent Technologies) that was operated in electron ionisation and selected ion monitoring (SIM) mode. The instrumental parameters used are provided in detail elsewhere.²⁵

2.6 Mutagenicity tests

In order to check if the PUF extracts were suitable to be used in mutagenicity studies employing the Ames Salmonella assay, blanks from PUFs cleaned and extracted using the PLE methods described above were tested at three different concentrations corresponding to the amounts used in samples tested previously as detailed in Westerholm *et al.*²⁶ The blanks were analysed using Salmonella typhimurium TA 98 and TA100 with and without a metabolising system (10 % S9) according to Maron and Ames³³, with the modifications reported by Sjögren *et al.*³⁴

3 Results and Discussion

3.1 PUF cleaning

The cleaning step for the PUFs using PLE was compared with the Soxhlet extraction method previously used in our research group, and the two methods did not show any difference with respect to their PAH content (blanks containing less than 3 % of the sample amounts). However, the use of PLE offered substantial reductions in operational time and solvent consumption. In fact, the time required to clean three plugs using the Soxhlet method was 12 hours per day for each extraction step, for a total of 36 hours, or three days (12 hours x 3 days), whereas the cleaning of three PUFs required less than 30 minutes using the PLE method. A considerable difference in solvent consumption was also observed between the two techniques, with the Soxhlet cleaning requiring more than 1.5 L of solvent compared with approximately 200 mL for PLE.

In addition, mutagenic potentials were also evaluated for the PLE blanks using the Ames Salmonella test. The blanks showed no mutagenicity in either of the test strains compared with the solvent (DMSO) control (data not shown).

The blank extracts originating from Soxhlet extraction were not evaluated for mutagenicity in the Ames test in the present work as this has been done previously by Westerholm *et al.*²⁶

3.2 Extraction recovery of PLE versus Soxhlet for ambient air samples

The number of static extraction cycles needed to exhaustively extract the analytes from the PUFs was investigated by extracting three additional air samples with hexane using four sequential extraction cycles, thereby generating four extracts from each PUF. The results demonstrated that one cycle was sufficient to extract > 98 % of the analytes except for anthracene and 2-methylanthracene for which slightly lower recoveries were obtained (96 %

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3 and 97 %, respectively) whereas two static extraction cycles were sufficient to extract > 99 %
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5 of all the analytes, as shown in Table 2.
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8 These results agreed with the study of Primbs *et al.*, which showed extraction recoveries of
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10 83.0 %, 83.3 % and 82.5 % for phenanthrene, pyrene and fluoranthene, respectively, spiked
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12 onto PUFs and extracted using PLE with 75:25 hexane:acetone (v/v) and two five-minute
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14 static extraction cycles at 100 °C.²²
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17 The bar graph shown in Figure 1 presents the percent ratios between the PAH concentration
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19 values (pg/m³) of the air samples obtained by using the US EPA Soxhlet extraction method
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21 with 10 % diethyl ether in hexane (set as 100 %) and those obtained by PLE using hexane and
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23 two static extraction cycles (Table 1). These findings were statistically verified with the
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25 Wilcoxon signed-rank test and Student's t-test, and no significant differences between the two
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27 techniques were encountered at the 5 % probability level (calculated using the freeware
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29 WinStat3 <http://www.anchem.su.se>). The obtained results demonstrate the developed PLE
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31 method to be equivalent in terms of determined PAH concentrations in a fraction of the time
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33 (15 min vs 18 hours) and consuming much less solvents (approximately 60 mL vs 350 mL) as
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35 compared to the US EPA Soxhlet method. In addition, the possibility of loading up to 24 cells
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37 into the PLE system for automated extraction significantly improves the sample throughput
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39 using the developed PLE method over the US EPA Soxhlet procedure. Furthermore, the lower
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41 extract volumes generated by the PLE method (approximately 30 mL) allowed for a quicker
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43 sample processing (solvent evaporation using a nitrogen flow of up to 12 sample vials
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45 simultaneously in a water bath) whereas the higher extract volumes (approximately 350 mL)
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47 obtained from the Soxhlet extractions were serially reduced using a Büchi Rotavapor.
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3.3 PLE of wood smoke and diesel exhaust samples

To evaluate whether the PLE method developed for air sampling was suitable for other types of PUF samples, the method was applied to wood smoke and diesel exhaust samples from a passenger and a heavy-duty vehicle. These PUF samples were subjected to an analytical PLE extraction followed by a repeat extraction with the same parameters used for the extraction of PUFs generated from the air sampling (Table 1). The results obtained showed the analytical extraction to recover more than 93 % and 96 % for the diesel and wood smoke samples, respectively, as shown in Table 3. The slightly higher standard deviations obtained from the wood and diesel samples as compared to the air samples could potentially be a result of the different experimental conditions used for these samples (i.e. different vehicles, fuels and combustion conditions etc.). This suggests that the extraction recovery could be dependent on the experimental set-up and it is therefore advisable to perform repeat extractions for these types of samples to assure quantitative extraction conditions.

4. Conclusions

A rapid and exhaustive PLE method was developed for the analysis of semi-volatile PAHs from different PUF samples such as ambient air, diluted wood smoke and diluted diesel exhaust. The efficiency of the method was established by comparison with the US EPA Soxhlet method TO-13A applied to real air PUF samples split in two parts. Comparable PAH concentrations were obtained from the two extraction procedures making the PLE method developed herein a more suitable choice than the US EPA Soxhlet method. In fact, the extraction with PLE was significantly faster (15 minutes versus 18 hours per sample) and required less solvent (less than 60 mL versus 350 mL per sample) and less operator handling compared to Soxhlet extraction, consequently allowing higher sample throughput. In addition, a PLE method was also developed for cleaning of PUFs prior to sampling, further reducing

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3 solvent consumption and operational times compared to the use of Soxhlet extraction for the
4 same purpose. The extraction efficiencies obtained for selected semi-volatile PAHs with the
5 PLE method were greater than 99 %, 96 % and 93 % for air, wood smoke and diesel exhaust
6 samples, respectively. Additionally, the developed PLE methods are suitable to be used for
7 mutagenic testing with the Ames Salmonella assay as no mutagenicity was observed in the
8 blanks obtained from PLE cleaning and extraction.
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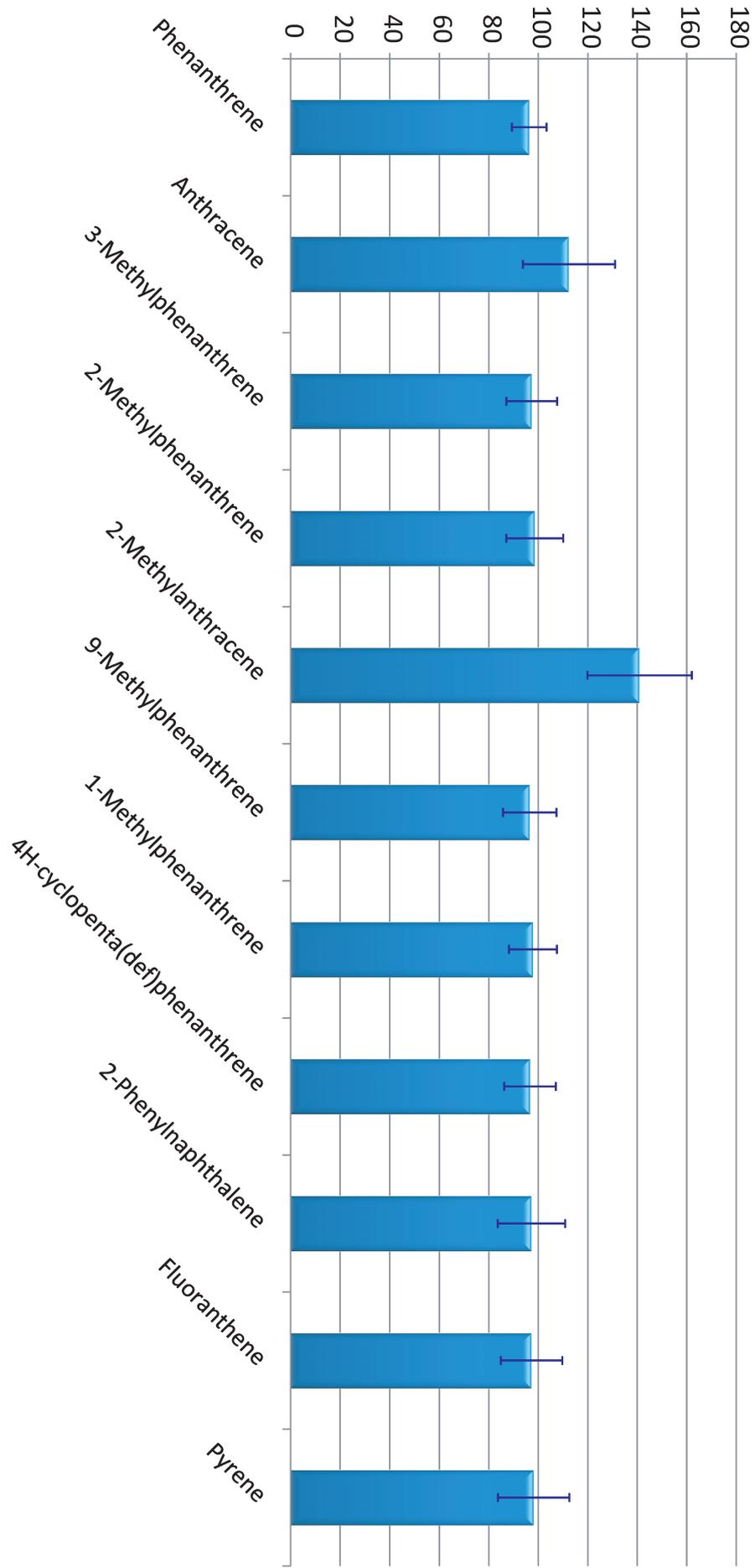
References

- 1 International agency for research on cancer (IARC), *IARC monographs*, 2010, **92**.
- 2 C. Boström, P. Gerde, A. Hanberg, B. Jernström, C. Johansson, T. Kyrklund, A. Rannug, M. Törnqvist, K. Victorin and R. Westerholm, *Environ. Health Perspect.*, 2002, **110**, 451-488.
- 3 K-H. Kim, S. A. Jahan, E. Kabir and R. J.C. Brown, *Environ. Int.*, 2013, **60**, 71-80 .
- 4 U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program, Report on Carcinogens, Twelfth Edition, 2011.
- 5 S. O. Baek, M. E. Goldstone, P. W. W. Kirk, J. N. Lester and R. Perry, *Chemosphere*, 1991, **22**, 503-520.
- 6 K. Srogi, *Environ. Chem. Lett.*, 2007, **5**, 169-195.
- 7 G. Lammel, A. M. Sehili, T. C. Bond, J. Feichter and H. Grassl, *Chemosphere*, 2009, **76**, 98-106.
- 8 I. J. Keyte, R. M. Harrison and G. Lammel, *Chem. Soc. Rev.*, 2013, **42**, 9333-9391
- 9 US Environmental Protection Agency (US EPA), <http://water.epa.gov/scitech/methods/cwa/pollutants.cfm>, 2013, (accessed 16/06/2014).
- 10 The Agency for Toxic Substances and Disease Registry (ATSDR), <http://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=122&tid=25>, 1995, (accessed 16/06/2014).
- 11 S. K. Pandey, K. Kim and R. J. C. Brown, *TrAC, Trends Anal. Chem.*, 2011, **30**, 1716-1739.
- 12 US Environmental Protection Agency (US EPA), *Determination of polycyclic aromatic hydrocarbons (PAHs) in ambient air using gas chromatography/mass spectrometry (GC/MS)*, EPA/625/R-96/010b, 1999.
- 13 A. Colmsjö, *Concentration and Extraction of PAHs from Environmental Samples*, in *The Handbook of Environmental Chemistry*, Neilson, 1997, **3**, 55-76.
- 14 L. Liu, Y. Liu, J. Lin, N. Tang, K. Hayakawa and T. Maeda, *Journal of Environmental Sciences*, 2007, **19**, 1-11
- 15 M. M. Schantz, *Anal. Bioanal. Chem.*, 2006, **386**, 1043-1047.
- 16 B. E. Richter, B. A. Jones, J. L. Ezzell, N. L. Porter, N. Avdalovic and C. Pohl, *Anal. Chem.*, 1996, **68**, 1033-1039.
- 17 M. M. Schantz, J. J. Nichols and S. A. Wise, *Anal. Chem.*, 1997, **69**, 4210-4219.

- 1
2
3 18 C. Bergvall and R. Westerholm, *Anal. Bioanal. Chem.*, 2008, **391**, 2235-2248.
4
5
6 19 L. Turrio-Baldassarri, C. L. Battistelli and A. L. Iamiceli, *Anal. Bioanal. Chem.*, 2003,
7 **375**, 589-595.
8
9 20 N. Barco-Bonilla, J. L. M. Vidal, A. Garrido Frenich and R. Romero-González, *Talanta*,
10 2009, **78**, 156-164.
11
12 21 M. R. Ras, F. Borrull and R. M. Marce, *J. Sep. Sci.*, 2009, **32**, 1051-1059.
13
14
15 22 T. Primbs, S. Genualdi and S. M. Simonich, *Environ. Toxicol. Chem.*, 2008, **27**, 1267-
16 1272.
17
18 23 A. Albinet, E. Leoz-Garziandia, H. Budzinski and E. Villenave, *Sci. Total Environ.*, 2007,
19 **384**, 280-292.
20
21
22 24 K. Ravindra, R. Sokhi and R. Van Grieken, *Atmos. Environ.*, 2008, **42**, 2895-2921.
23
24
25 25 I. Sadiktsis, J. H. Koegler, T. Benham, C. Bergvall and R. Westerholm, *Fuel*, 2014, **115**,
26 573-580.
27
28 26 R. N. Westerholm, J. Almén, H. Li, J. U. Rannug, K. E. Egebäck and K. Grägg, *Environ.*
29 *Sci. Technol.*, 1991, **25**, 332-338.
30
31 27 C. Bergvall and R. Westerholm, *Environ. Sci. Technol.*, 2007, **41**, 731-737.
32
33
34 28 J. Unosson, A. Blomberg, T. Sandström, A. Muala, C. Boman, R. Nyström, R.
35 Westerholm, N. L. Mills, D. E. Newby, J. P. Langrish and J. A. Bosson, *Part Fibre Toxicol*,
36 2013, **10**, 20.
37
38 29 R. Westerholm, A. Christensen, C. De Serves and J. Almen, *Soc. Automot. Eng., [Spec.*
39 *Publ.] SP*, 1999, **SP-1477**, 231-238.
40
41
42 30 Swedish Department for Transport (Trafikverket), *Government commission to illustrate*
43 *the differences in the health and environmental impact of using diesel of environmental class*
44 *1 and EURO 3 (in Swedish)*, **178**, 2012.
45
46
47 31 A. M. Ruder, *Ann. N. Y. Acad. Sci.*, 2006, **1076**, 207-227.
48
49
50 32 A. Christensen, C. Östman and R. Westerholm, *Anal. Bioanal. Chem.*, 2005, **381**, 1206-
51 1216.
52
53 33 D. M. Maron and B. N. Ames, *Mutat. Res.*, 1983, **113**, 173.
54
55
56 34 M. Sjögren, H. Li, C. Banner, J. Rafter, R. Westerholm and U. Rannug, *Chem. Res.*
57 *Toxicol.*, 1996, **9**, 197-207.
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RATIO %



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5 **Fig. 1** Ratios (%) between the PAH concentration values obtained by Soxhlet extraction with
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8 10% diethyl ether in hexane (set as 100%) and by PLE with hexane (extraction conditions
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10 reported in Table 1). The error bars in the graph represent the standard deviation.
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Table 1. PLE conditions during cleaning and extraction.

	PUF cleaning	PUF extraction
Temperature	110 °C	110 °C
Pressure	500 psi	500 psi
Heat Time	6 min	6 min
Static Time	5 min	5 min
Flush Volume	60 %	60 %
Purge Time	60 s	60 s
Static Cycles	1	2
Solvent (mL)		Hexane (30)
step 1	Toluene (60)	
step 2	Acetone (60)	
step 3	Acetone (60)	

Table 2. Recoveries of PAHs from ambient air samples using PLE with sequential 5 min extraction cycles. Recoveries are expressed as percentage of total extracted amount.

Repeat extraction	Mean values (n=3)				1 st + 2 nd % of total	sd
	1 st % of total	2 nd % of total	3 rd % of total	4 th % of total		
Phenanthrene	98.1	1.72	0.08	0.06	99.9	0.04
Anthracene	95.9	3.56	0.30	0.25	99.4	0.04
3-Methylphenanthrene	98.6	1.27	0.09	0.08	99.8	0.07
2-Methylphenanthrene	98.7	1.17	0.09	0.07	99.8	0.08
2-Methylanthracene	96.8	2.45	0.31	0.43	99.3	0.98
9-Methylphenanthrene	98.6	1.20	0.10	0.09	99.8	0.07
1-Methylphenanthrene	98.7	1.22	0.07	0.04	99.9	0.13
4H-cyclopenta(def)phenanthrene	98.9	1.04	0.03	0.03	99.9	0.02
2-Phenylnaphthalene	99.3	0.62	0.03	0.03	99.9	0.05
Fluoranthene	98.7	1.22	0.05	0.04	99.9	0.05
Pyrene	98.4	1.45	0.08	0.06	99.9	0.09

Abbreviations: sd=standard deviation

Table 3. PAH extraction recoveries from air, diesel exhaust and wood smoke PUF samples using the PLE method developed for ambient air PUF samples. Extraction recoveries are expressed as percentage of determined amounts in the analytical extraction relative to the sum of the total amounts determined in analytical and repeat extractions (extraction conditions reported in Table 1).

PAH	Air (n=6)		Diesel (n=5)		Wood (n=5)	
	mean	sd	mean	sd	mean	sd
Phenanthrene	99.9	<0.1	93.7	3.6	98.3	0.8
Anthracene	99.5	<0.1	97.3	3.1	98.3	2.0
3-Methylphenanthrene	99.8	0.1	96.0	3.0	97.2	1.8
2-Methylphenanthrene	99.8	0.1	95.3	2.3	97.5	1.6
2-Methylanthracene	99.3	1.0	99.6	0.9	99.4	1.2
9-Methylphenanthrene	99.8	0.1	95.7	2.6	97.3	2.1
1-Methylphenanthrene	99.9	0.1	94.7	3.5	97.7	2.0
4H-Cyclopenta(def)phenanthrene	99.9	<0.1	95.3	4.3	99.4	1.3
2-Phenyl-naphthalene	99.9	0.1	99.1	1.4	99.1	1.1
Fluoranthene	99.9	0.1	93.8	4.3	99.2	0.7
Pyrene	99.9	0.1	95.7	1.7	99.1	0.8

Abbreviations: sd=standard deviation