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Evaluation of greener solvent options for polycyclic aromatic hydrocarbon extraction

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are widespread environmental pollutants with known carcinogenic and mutagenic effects. In order to monitor their presence in complex food and environmental matrices, liquid-liquid extraction (LLE) was employed as a reliable sample preparation technique. To minimize environmental impact, green solvents are used as alternatives to more hazardous traditional solvents. Quantification and confirmation of 16 target PAHs were performed using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS), ensuring high sensitivity and selectivity. This study compared toluene and ethyl acetate as PAH extraction solvents in water samples at lower and higher concentrations (10 µg/L and 100 µg/L). Both solvents extracted similar amounts of the 16 target PAHs from water, although the AGREE tool favours ethyl acetate over toluene, due to the former's environmental and safety benefits.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic compounds composed of two and more fused aromatic rings. One of the main ways they are created is through the combustion of coal, wood and waste. As such, they can be found in soil, water and air, both through human activity and natural processes (*Agency for Toxic Substances and Disease Registry - ATSDR*, 1995; *Iko Afé et al.,* 2021). Due to their mutagenic and carcinogenic properties, these compounds pose a significant risk to human health. First, several PAHs are classified by IARC as known or potential carcinogens. Second, their metabolites can cause mutations in DNA molecules (*Wilson and Jones,* 1993; *Agency for Toxic Substances and Disease Registry - ATSDR*, 1995; *Liu et al.*, 2015).

Because of their demonstrated toxicity and high abundance, regular analysis of PAHs is very important, primarily for human health and environmental safety. This is accomplished through regular monitoring of water, air, and soil. Similarly, food safety is ensured by routinely checking the levels of PAHs in, e.g., smoked and grilled products (*Kumar et al.*, 2021; *Imrigha et al.*, 2022).

Modern and routine analysis and quantification of these compounds is mainly performed using gas chromatography with mass detection or high-performance liquid chromatography with fluorescence detection (*Zhang et al.*, 2020). However, it is also essential to use mass detection, especially as

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detection limits and regulatory requirements continue to become stricter over time.

Alongside the requirements for increasingly lower detection limits, there is a rising demand for the implementation of environmentally friendly solvents in the extraction of these compounds from diverse matrices. Commonly used organic solvents for the extraction of PAHs, e.g., toluene, dichloromethane, chloroform and hexane, can present very serious health risks, primarily due to their toxicity, volatility and high carcinogenic potential (*Jinadasa et al.*, 2020).

In order to reduce health and environmental risks, there is an increasing need to use greener solvents, i.e., chemicals that are less toxic, more biodegradable and can be produced from renewable sources (*U.S. Environmental Protection Agency - EPA*, 2005). Green chemistry aims at sustainable development, reduction of pollution and better use of resources. Green analytical chemistry focuses on reducing the harmful impact of analytical methods on human health and the environment, while on the other hand, ensuring the high quality of the results is not impaired. Some of the principles of green analytical chemistry are reduction of the use of dangerous chemicals, replacement of toxic solvents, miniaturization and automation of processes and analysis of multiple analytes with one method (*Capello et al.*, 2007; *Kaya et al.*, 2022).

Ethyl acetate is one of the more acceptable solvents for the environment, being a safer solution compared to commonly used solvents, because of its lower toxicity and higher biodegradability. Its volatility prevents persistence and bioaccumulation in the environment. Additionally, it can be sustainably produced from bioethanol and acetic acid (*Armenta et al.*, 2008).

Liquid-liquid extraction (LLE) is a one of a widely used technique for isolating PAHs prior to chromatographic analysis. Liquid-liquid extraction is a separation technique based on the differential solubility of compounds in two immiscible liquids, mainly between the organic and aqueous phases. The analytes of interest, such as PAHs, partition preferentially into the organic phase due to their non-polar characteristics. Some of the parameters that influence extraction efficiency (EE) are choice of solvent, pH adjustment and sample volume (*Manoli and Samara*, 1999; *Peña-Pereira et al.*, 2009; *Rezaee et al.*, 2006).

The aim of this study was to compare toluene and ethyl acetate as extraction solvents for PAHs in water samples at two concentration levels, lower and higher (10 µg/L and 100 µg/L). A non-polar aromatic solvent (toluene), is traditionally favoured for its high affinity for hydrophobic PAHs. Ethyl acetate, a moderately polar solvent, is a greener alternative due to its lower toxicity and better biodegradability.

In order to compare the analytical performance of the two solvents and their impacts on the environment, the EE of the solvents was monitored, while the analytical greenness (AGREE), proposed by *Pena-Pereira et al.* (2020), was calculated. AGREE is a quantitative measure of method greenness comparing 12 principles of green analytical chemistry.

2. Materials and methods

The CRM mixture of 16 US EPA PAHs, i.e., Naphthalene (Naph), Acenaphthylene (Acy), Acenaphthene (Ace), Fluorene (Flu), Phenanthrene (Phen), Anthracene (Ant), Fluoranthene (Flt), Pyrene (Pyr), Benzo(a)anthracene (BaA), Chrysene (Chry), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Dibenzo(a,h)anthracene (DahA), Benzo(g,h,i)perylene (BghiP) and Indeno(1,2,3-cd)pyrene (IcdP), was purchased from Supelco (Bellefonte, PA, USA). Deuterated Benzo(a)pyrene Internal Standard (IS) was purchased from HPC Standard GmbH (Cunnersdorf, Germany). Stock standard solutions were prepared by dissolving the reference standard in acetonitrile and stored in a freezer at -20°C. Ethyl acetate and toluene were purchased from Honeywell Specialty Chemicals (Seelze, Germany), water was purchased from VWR International (Leuven, Belgium). Solvents used were HPLC grade.

The simplified Small Volume Liquid-Liquid Extraction (SV-LLE) method was used for sample preparation (*ISO*, 2005). A standard solution of PAHs was prepared in acetonitrile (ACN). Serial dilutions were made to achieve concentrations of 1, 10, 20, 50, and 100 µg/L and were prepared in two solvents, ethyl acetate and toluene. In order to test the EE, two PAH concentration levels were prepared, 10 and 100 µg/L in water. For each extration, aqueous PAH solution (250 µL) was dispensed into a 2 mL screw top vial and extracted two times with 1 mL of extraction solvent. The vials prepared in this way were vortexed for 1 min, after which organic phase was removed and another portion of extraction solvent was added. After 1 min of vortexing, the second organic phase was added to first one and then evaporated under a current of N_2. After evaporation, 250 µL of ethyl acetate or toluene was added, as appropriate.

All extracts were analysed using GC-MS/MS. All data were acquired using a Shimadzu GCMS-TQ8050NX gas chromatograph with triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan), with the sampling unit consisting of an autoinjector (AOC-20i) and autosampler (AOC-20s) from the same manufacturer. A fused silica SH-I-5ms capillary column (Length 30 m × I.D. 0.25 mm × film thickness 0.25 µm, Shimadzu, USA) was used for GC separation. The injector operated in spitless mode, injecting 1 µL at 280°C. The oven temperature program was: 40°C (hold for 1.0 min), ramped at 25°C/min to 120°C (no hold), ramped at 10°C/min to 160°C (hold for 10.0 min), ramped at 15°C/min to 220°C (hold for 5.0 min), and ramped at 5°C/min to 325°C (hold for 7.0 min). Helium was used as the carrier gas with flow of 2 mL/min in a linear velocity mode of 51.0 cm/sec. Acquisition mode was MRM. The temperature of transfer line was set to 300°C, ion source temperature was set at 240°C. MRM transitions and collision energies (CE) of analysed compound were set according to *U.S. Environmental Protection Agency - EPA* (2007) and *Wang et al.* (2019).

All measurements were carried out in triplicate. EE was used as a quantitative measure to evaluate the effectiveness of an extraction process in isolating PAHs from water. EE was calculated as the ratio of the concentration of the analyte in the extract to its initial concentration in the sample, multiplied by 100 to express the result as a percentage (*European Commission Directorate-General for Health and Food Safety*, 2023).

$$Extraction\ Efficiency = \frac{C_{ex}}{C_{ini}}\ x\ 100$$

where C_{ex} is concentration of PAHs in the extracted organic phase and C_{ini} is initial concentration of PAHs in the water sample before extraction.

Statistically significant differences between the EE of the two solvents were tested by two-way ANOVA with factors (solvent and concentration) using Minitab 17.

The AGREE metric was used to evaluate the methods greenness, and it was calculated by AGREE software (*Pena-Pereira et al.*, 2020). This green chemistry assessment tool based on the 12 principles of green analytical chemistry (sample treatment, sample amount, device positioning, sample preparation procedure, degree of automation, derivatization, amount of waste, number of analytes determined, energy consumption, source of reagents, toxicity, operator's safety). A standardized approach was used to compare the ecological impact of different extraction procedures quantitatively. It is visually represented as circular clock-like diagram, where each hour position (1 to 12) around the edge corresponds to one of the 12 principles of green analytical chemistry. AGREE scores from 0 to 1 are, respectively, not green at all and fully green (*Pena-Pereira et al.*, 2020).

3. Results and discussion

In this study, toluene and ethyl acetate were evaluated as extraction solvents for PAHs in water samples at two concentration levels (10 µg/L and 100 µg/L). The results of PAH EE with two different extraction solvents at two concentration levels are shown in Table 1. Two-way ANOVA was performed for each PAH to test the effects of solvent, concentration, and their interaction on extraction recovery. Table 1 summarizes the p-values for all tested PAHs. There was no statistically significant difference in recovery between toluene and ethyl acetate, between low and high concentration levels at a confidence level of 95%, using the previously described method of extraction and in a water matrix (i.e., in samples that were not loaded with matrix compounds). When the interaction term was not statistically significant ($p > 0.05$), there was no indication that the solvent's influence fluctuated with concentration. This means that variations among solvents were uniform across both concentration levels, while variations among concentrations were uniform across both solvents. Under these circumstances, the primary impacts of solvent and concentration were analysed directly.

The EE was higher than 70% for all analytes and in both solvents, except for naphthalene, probably due to its high volatility. The lowest EE was observed for naphthalene at the lower concentration level when using ethyl acetate as the extraction solvent. The highest EE at the lower spiking level was recorded for benzo(b)fluoranthene with ethyl acetate, while for toluene, the highest EE was 86.9% for both benzo(a)pyrene and benzo(a)anthracene. At the higher spiking level, the lowest EE was again observed for naphthalene, with a value of 65.6% and 70.6, in ethyl acetate and toluene, respectively. The highest EE at this level was obtained for pyrene when using toluene, and for chrysene when using ethyl acetate, reaching 94.1%. For the lower PAHs, compounds with 2-3 rings in their structure, we detected lower EEs than in higher PAHs (four and more rings), probably due to the number of rings and its correlation to the higher volatility of those compounds (*Satouh et al*., 2021).

In this study, we decided to readjusts weights (Figure1) for criteria number 10 (source of reagents, is it possible to be renewable) and number 12 (operator's safety), so the weights of these criteria were set to four due to their importance and ability to differentiate chromatographic methods. Based on the AGREE evaluation, ethyl acetate with a score of 0.56 demonstrated a more favorable environmental and analytical greenness profile than toluene, scoring 0.37. While neither solvent achieved optimal green chemistry standards (score closer to 1.0), the higher-scoring solvent aligns better with the principles of green analytical chemistry, particularly in terms of lower toxicity, biodegradability and safety.

Table 1. Extraction efficiency of poly aromatic hydrocarbons (%) dissolved in water using two different solvents at two different levels

No.	Compound	Spike$_t$ 10	Spike$_{EA}$ 10	Spike$_t$ 100	Spike$_{EA}$ 100	p-value$_S$	p-value$_c$	p-value$_{cxs}$
1	Naph	67.4 ± 15.0	63.5 ± 4.4	70.6 ± 9.9	65.6 ± 6.5	0.122	0.246	0.520
2	Acy	73.6 ± 9.1	72.9 ± 8.8	72.2 ± 6.0	74.5 ± 4.8	0.872	0.753	0.993
3	Ace	75.3 ± 12.5	73.7 ± 7.8	76.1 ± 5.9	75.4 ± 3.2	0.743	0.737	0.896
4	Flu	77.9 ± 4.3	78.2 ± 9.4	70.3 ± 12.4	79.1 ± 3.4	0.102	0.464	0.120
5	Phen	74.3 ± 4.8	77.8 ± 7.4	72.1 ± 7.4	81.2 ± 4.1	0.071	0.847	0.377
6	Ant	75.3 ± 5.8	81.2 ± 4.8	82.0 ± 3.6	83.7 ± 8.2	0.233	0.153	0.492
7	Flt	80.9 ± 6.1	80.1 ± 9.7	79.6 ± 6.2	89.2 ± 4.0	0.149	0.192	0.092
8	Pyr	81.0 ± 7.4	87.3 ± 6.8	94.4 ± 4.9	89.4 ± 5.5	0.835	0.052	0.096
9	BaA	86.9 ± 9.5	85.5 ± 6.7	83.8 ± 10.5	80.9 ± 9.0	0.519	0.267	0.824
10	Chry	84.4 ± 2.5	90.8 ± 6.3	83.3 ± 5.9	94.1 ± 8.4	0.088	0.819	0.635
11	BbF	81.6 ± 9.9	95.1 ± 8.3	87.1 ± 4.7	89.2 ± 4.1	0.786	0.112	0.786
12	BkF	81.1 ± 7.7	89.4 ± 8.5	83.6 ± 7.4	88.6 ± 4.2	0.094	0.815	0.660
13	BaP	86.9 ± 3.2	84.0 ± 6.7	88.5 ± 8.6	84.6 ± 9.5	0.376	0.781	0.896
14	DahA	80.1 ± 10.1	81.9 ± 9.3	86.2 ± 10.6	86.2 ± 6.2	0.838	0.283	0.843
15	IcdP	82.4 ± 9.7	80.9 ± 8.1	85.5 ± 8.1	85.9 ± 6.0	0.103	0.345	0.537
16	BghiP	83.7 ± 6.7	80.5 ± 7.7	81.5 ± 7.3	81.8 ± 4.2	0.074	0.200	0.363

Legend: T – Toluene; EA – Ethyl acetate; ± Standard deviation; s - solvent, c - concentration, cxs – concentration × solvent. The PAH compounds are listed in Materials and Methods; p-values from two-way ANOVA regarding the effects of solvent type (*Solvent*), concentration level (*Concentration*), and their interaction *Solvent* × *Concentration* on extraction recovery for each PAH. *Solvent* evaluates the overall disparity between toluene and ethyl acetate, averaged across various concentrations. *Concentration* evaluates the total disparity between low ($10\ \mu g\ L^{-1}$) and high ($100\ \mu g\ L^{-1}$) values, averaged over tested solvents. The *Solvent* × *Concentration* analysis examines if the solvent impact varies with different concentration levels.

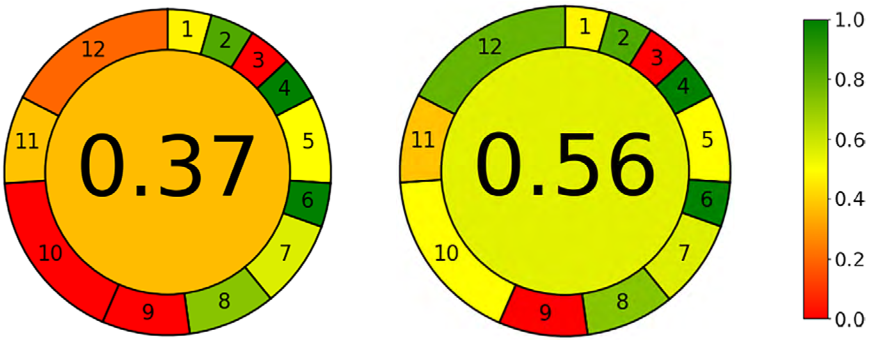

Figure 1. Evaluation of the solvents' greenness using the AGREE method

Therefore, from a sustainability perspective, ethyl acetate, the solvent with the 0.56 AGREE score, is the preferred choice, provided it maintains acceptable analytical performance.

4. Conclusion

PAHs are persistent, bioaccumulative, and toxic pollutants that pose serious risks to human health and ecosystems. Their testing and monitoring are essential for assessing exposure risks, guiding regulatory policies, and implementing effective environmental and public health protections. Continued research and improved analytical methods are key to managing and mitigating the impact of PAHs in our environment.

Quantitatively, both solvents achieve similar extraction efficiencies. However, the AGREE tool clearly favours ethyl acetate due to its better environmental and safety profile. Thus, from a green chemistry standpoint, ethyl acetate is the preferred solvent for sustainable and responsible analytical workflows. Additional investigations are necessary to thoroughly evaluate the EE of green solvents, especially for more complex matrices—such as food products, feed and environmental samples—where matrix effects and co-extractives may considerably affect performance.

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