A cost effective, sensitive, and environmentally friendly sample preparation method for determination of polycyclic aromatic hydrocarbons in solid samples

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ABSTRACT

A simple, cost effective, and yet sensitive sample preparation technique was investigated for determining Polycyclic Aromatic Hydrocarbons (PAHs) in solid samples. The method comprises ultrasonic extraction, Stir Bar Sorptive Extraction (SBSE), and thermal desorption–gas chromatography–mass spectrometry to increase analytical capacity in laboratories. This method required no clean-up, satisfied PAHs recovery, and significantly advances cost performance over conventional extraction methods, such as Soxhlet and Microwave Assisted Extraction (MAE). This study evaluated three operational parameters for ultrasonic extraction: solvent composition, extraction time, and sample load. A standard material, SRM 1649 a (urban dust), was used as the solid sample matrix, and 12 priority PAHs on the US Environmental Protection Agency (US EPA) list were analyzed. Combination of non-polar and polar solvents ameliorated extraction efficiency. Acetone/hexane mixtures of 2:3 and 1:1 (v/v) gave the most satisfactory results: recoveries ranged from 63.3% to 122%. Single composition solvents (methanol, hexane, and dichloromethane) showed fewer recoveries. Comparing 20 min with 60 min sonication, longer sonication diminished extraction efficiencies in general. Furthermore, sample load became a critical factor in certain solvent systems, particularly MeOH. MAE was also compared to the ultrasonic extraction, and results determined that the 20-min ultrasonic extraction using acetone/hexane (2:3, v/v) was as potent as MAE. The SBSE method using 20 mL of 30% alcohol-fortified solution rendered a limit of detection ranging from 1.7 to 32 ng L^{-1} and a limit of quantitation ranging from 5.8 to 110 ng L^{-1} for the 16 US EPA PAHs.

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1. Introduction

Polycyclic Aromatic Hydrocarbons (PAHs) comprise two or more fused aromatic rings, and physicochemical properties differ from one to another as chemical structure varies. As a result, each PAH has discreet fate, transport, and distribution patterns in the environment [1]. Among over 100 different PAHs, the US Environmental Protection Agency (US EPA) has identified 16 PAHs as priority pollutants based on their health and environmental risk concerns. Studying PAHs occurrence in environmental media has been focused on assessing human exposure to the pollutants and their impact on human health [2–4]; thus, measuring PAHs in the air, soil, and water attracted strong interests.

Soxhlet extraction is one of the major standard techniques for PAH determination in air or solid samples. The method uses dichloromethane or hexane/acetone mixture solvents and is followed by condensation, clean-up, and enrichment prior to instrumental analysis. However, the popular technique requires a high organic solvent volume, extensive extraction time (12–20 h), and intensive manpower, resulting in a high PAH analysis cost and a conflict with the Green Chemistry concept as well. Alternative techniques have been developed to overcome the impediments in the traditional method [5,6]. For instance, Microwave Assisted Extraction (MAE) was applied to extract PAHs in airborne particulate matter, wood samples, marine sediment, muscle samples of polluted fish, and sewage sludge [7–11]. Accelerated Solvent Extraction (ASE) was also utilized for extracting persistent organic pollutants, such as PAHs, PCBs, and pesticides from soil, marine sediment, and urban dust [12–14]. The US EPA has approved the above two methods for extracting hydrophobic organic compounds from solid materials (method 3545A and 2546). Indeed, the methods resolved the key issues of Soxhlet extraction, i.e. organic solvent consumption and extraction time; however, the methods raised another issue: a high capital cost. These environmentally friendly yet costly methods are, therefore, unavailable in many laboratories due to the capital cost. Consequently, science community promotes alternative techniques that are inexpensive, readily available, and

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easily used in many laboratories [15]. Ultrasonic extraction is one of those techniques that fit the criteria and has gained a reputation as a reliable method for extracting PAH from solid samples with satisfactory recovery [16,17]. In this study, we further postulate ultrasonic extraction using an ultrasonic bath for extracting PAHs effectively from solid samples without clean-up procedures.

An ultrasonic bath is accessible in most laboratories, and ultrasonic extraction favorably deals with relevant factors to analytical chemistry: capital and operating costs, environmental impact, and level of automation [18]. Using an ultrasonic bath has an advantage over a horn-type ultrasonic device described in EPA 3550C. An ultrasonic bath allows a multiple sample extraction in one process; thus, ultrasonic extraction using a bath significantly reduces preparation time. Although ultrasonic extraction demonstrates less extraction efficiency in some studies [16], the method was already exploited for PAH extraction from solid samples [19–22]. Still, relatively few studies have explored the extraction efficiency for operational parameters such as solvent selection, sonication duration, and sample load. Therefore, it is essential to investigate these parameters for the extraction efficiencies to determine the ultrasonic extraction potential and to provide meaningful comparison between ultrasonic extraction and other extraction approaches.

This research also took Green Chemistry into consideration. In the effort to reduce solvent and energy consumption and to eliminate harmful organic solvents such as dichloromethane, a solventless method called Stir Bar Sorptive Extraction (SBSE) was adopted in this study. SBSE is known for its high recovery and high extraction efficiency on hydrophobic and semivolatile organic pollutants including PAHs. SBSE has shown to be a rapid, environmentally friendly, and reliable analytical technique [23–27]. The fundamental SBSE methodology shares with solid phase microextraction (SPME). In the SBSE process, a Stir Bar (or Twister^{TM}) coated with 50–300 μL of polydimethylsiloxane (PDMS) is placed in the sample solution and is stirred for a pre-determined period. This process allows the organic compounds to be extracted from the solution phase to the Stir Bar. The equilibrium is controlled by the partition coefficient between the PDMS phase and the solution phase, $K_{PDMS/w}$. The value of $K_{PDMS/w}$ increases with analyte's octanol–water partition coefficient (K_{ow}). Compounds with high K_{ow}, such as PAHs, are prone to partition into the PDMS phase from the aqueous sample matrices; therefore, the PDMS phase on the Stir Bar enriches PAHs [23]. In general, a Stir Bar is placed in a vial with a sample solution and stirred for a pre-determined time ranging 0.5–24 h, depending on a sample matrix and a target compound. After stirring, the Stir Bar is removed from the sample solution, and the adsorbed compounds are thermally desorbed from the PDMS phase in a thermal desorption unit (TDU) and analyzed by gas chromatography and mass spectrometry (GC/MS). When a TDU is not available or thermally susceptible analytes are targeted, liquid desorption with liquid chromatography or capillary electrophoresis can be used as an alternative [28]. For example, García-Falcón et al. reported to use SBSE to couple with HPLC (high performance liquid chromatography) for the determination of free PAHs in run-off water samples. After SBSE extraction, the Stir Bar was placed in acetonitrile to allow PAHs desorption process to take place. Acetonitrile extracts were injected into the HPLC instrument [29].

As illustrated in Fig. 1, conventional techniques often require a clean-up step prior to instrumental analysis, which may compromise recovery of an analyte. In contrast, the SBSE method potentially eliminates the clean-up step and minimizes solvent consumption; consequently, the method decreases the complexity and interferences in the sample preparation processes. In a review paper, David and Sandra detailed the SBSE principles and included numerous SBSE applications for determination of PAH in aqueous samples without a clean-up step [25]. Even in samples with high
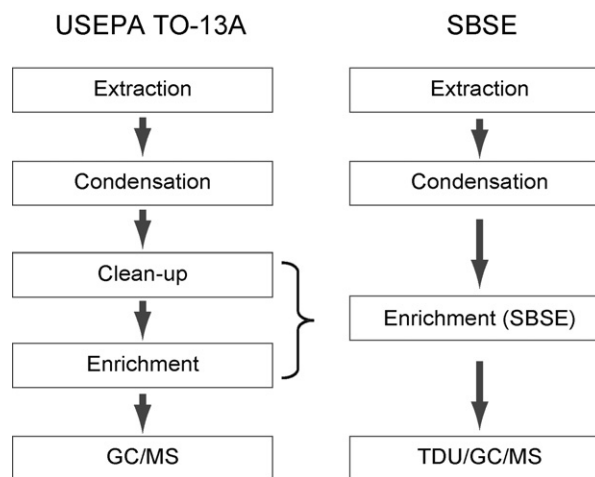

Fig. 1. Diagram of a conventional sample preparation process and the proposed method using SBSE.

organic content, such as vegetable, baby food, coffee and peats, SBSE was shown to have effective recovery of PAHs without or with minimal clean-up [30,15,17].

This study aimed to establish a simple, cost effective, environmentally friendly, and yet reliable technique for determining PAHs, using an ultrasonic bath followed by SBSE–TDU–GC/MS with no clean-up process. Effects of solvent composition, duration of ultrasonic extraction, and sample load were investigated to evaluate PAHs recoveries in solid samples.

2. Experimental

2.1. Materials

Standard Material SRM 1649a (urban dust) was purchased from the National Institute of Standards and Technology (NIST) (Gaithersburg, MD, USA). A mixture of PAH standards [phenanthrene (Phe), anthracene (Ant), fluoranthene (Ft), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbFt), benzo[k]fluoranthene (BkFt), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (InP), dibenz[a,h]anthracene (DahA), and benzo[ghi]perylene (BghiP) in methylene chloride/benzene (1:1)] was purchased from ULTRA Scientific (Kingstown, RI, USA). The mixture of internal standards, ASM-182 [1,4-dichlorobenzene-d4, acenaphthene-d10, chrysene-d12, naphthalene-d8, perylene-d12, and phenanthrene-d10 in dichloromethane/carbon disulfide (4:1)], was purchased from AccuStandard (New Haven, CT, USA). Solvents used in this study were of HPLC or higher grade: methanol: Burdick & Jackson (Muskegon, MI, USA); acetone: VWR International (West Chester, PA); hexane: J. T Baker (Phillipsburg, NJ, USA); 2-propanol: Sigma-Aldrich (St. Louis, MO, USA); and dichloromethane: VWR International.

2.2. Extraction of PAHs from SRM1649a

2.2.1. Ultrasonic extraction

Ultrasonic extraction was performed using an ultrasonic bath (Fisher Scientific FS 30H). To evaluate solvent effects, accurately weighed SRM 1649a (100–150 mg) was placed into a 40 mL amber vial along with 40 mL of organic solvent. The solvents used were methanol (MeOH), hexane (HEX), dichloromethane (DCM), methanol/acetone (MeOH/ACE (1:1, v/v)), hexane/isopropanol (HEX/PrOH (1:1, v/v)), acetone/hexane (ACE/HEX (1:1, v/v) and (2:3, v/v)), dichloromethane/acetone/methanol (DCM/ACE/MeOH (3:2:2, v/v/v)), and DCM followed by second extraction with

MeOH/ACE (1:1, v/v) (DCM–MeOH/ACE). The vial containing 40 mL of organic solvent with the sample was secured with a silicon cap and then sonicated in the ultrasonic bath for 20 or 60 min. After ultrasonic extraction, the extract was transferred to a Kuderna–Danish (K–D) condenser. A solvent exchange was performed during the condensation to methanol or isopropanol when non-alcohol base solvent was used for extraction. ASM-182 was added to the concentrated extracts after the condensation. The extract's final volume was adjusted to 10.0 mL by adding the same alcohol used for the solvent exchange. The extracts were stored in a refrigerator at 4 °C until following SBSE procedure. Blank samples were prepared for quality control purposes.

2.2.2. Microwave Assisted Extraction (MAE)

MAE was performed with 100 mg of SRM 1649a and 40 mL of a mixture of ACE/HEX (2:3, v/v). A CEM MARS Xpress Microwave Accelerated Reaction System (CEM Corporation, Matthews, NC, USA) was used. The standard material and 40 mL of the solvent were placed into a 100 mL Teflon® vessel. Microwave energy was set at 600 W for the entire extraction process. Extraction was programmed based on the US EPA's recommended conditions in the method 3546: rising to a final temperate of 110 °C in 6 min and holding at 110 °C for 14 min. The extracts were cooled, transferred to K–D condensers, and concentrated to 10 mL; and solvent exchange was carried out with the same manner described in Section 2.2.1. The extracts were stored at 4 °C until subsequent SBSE process. For quality control purposes, blank samples were prepared.

2.3. Stir Bar Sorptive Extraction (SBSE)

Extracts from ultrasonic extraction and MAE were enriched by the SBSE technique. The optimized SBSE condition that are 30% alcohol-fortified solution with 4-h stirring was adopted from our previous work [31]. In a 20 mL amber vial, an aliquot of 0.5 or 1.0 mL of the extract from the ultrasonic extraction or the MAE was mixed with 14 mL of deionized (DI) water, and methanol or isopropanol was added to obtain a final volume of 20 mL at 30% alcohol content. A commercially available Stir Bars (Twister™, 10 mm × 1 mm, Gerstel, Mülheim an der Ruhr, Germany) was placed in the vial, and the solution was stirred for 4 h at 1000 rpm. The stir bar was removed from the solution, rinsed with DI water, dried with lint free paper, and placed into a thermal desorption tube for GC–MS analysis.

2.4. Thermal desorption–gas chromatography–mass spectrometry (TD–GC/MS)

PAHs were analyzed by a thermal desorption unit, TDU (Gerstel), coupled with a 6890 GC system and a 5973 N Mass Selective Detector (Agilent Technologies, Wilmington DE). The initial TDU temperature was 50 °C. After holding for 0.5 min, the TDU temperature was increased to 300 °C at 60 °C min^{-1} and held for 5 min. Desorption gas flow was set at 50 mL min^{-1}. During the desorption, desorbed compounds were concentrated in a cold injection system, CIS-4 (Gerstel), at −40 °C prior to GC injection. Once the desorption process was completed, the CIS temperature was ramped to 320 °C at 12 °C min^{-1} and held for 10 min in a solvent vent mode.

Splitless mode was employed for the GC analysis. A ZB-5ms capillary column (30 m × 0.25 mm × 0.25 μm with 5% phenyl–95% dimethylpolysiloxane, Phenomenex, USA) was used. The oven temperature was programmed as follows: held for 2 min at 50 °C; raised at 25 °C min^{-1} to 150 °C; increased at 3 °C min^{-1} to 230 °C; ramped at 8 °C min^{-1} to 300 °C; and held for 15 min at 300 °C. The US EPA 16 priority PAHs in samples were traced by Mass Selective detector using selected ion mode (SIM).

2.5. Determination of PAHs

The retention time for individual PAHs and internal standards were determined by mass spectra using a scan mode prior to sample analysis. Identification of individual PAHs was based on retention time comparison and mass-to-charge ratio (m/z). Analyte quantification was derived from analyte's peak area. At least a five-point standard calibration was carried out within the range of 10–2000 ng L^{-1} for PAHs, and a 10–500 ng L^{-1} range was applied to low level PAHs in the standard material. The r^2 (r: regression of coefficient) for PAHs varied between 0.9834 (DahA) and 0.999 (BaA and BeP). Although the US EPA 16 PAHs were analyzed, only 12 PAHs have certified values in SRM 1649a and were reported in this study.

3. Results and discussion

3.1. Effects of solvent composition

Solvent effect was studied for the following composition: methanol (MeOH), hexane (HEX), dichloromethane (DCM), methanol/acetone (MeOH/ACE (1:1, v/v)), hexane/isopropanol (HEX/PrOH (1:1, v/v)), acetone/hexane (ACE/HEX (1:1, v/v) and (2:3, v/v)), dichloromethane/acetone/methanol (DCM/ACE/MeOH (3:2:2, v/v/v)), and DCM followed by second extraction with MeOH/ACE (1:1, v/v) (DCM–MeOH/ACE). The recovered total 12 PAHs [$\sum$PAHs = $\sum$(Phe, Ant, Ft, Pyr, BaA, Chry, BbFt, BkFt, BaP, InP, DahA, and BghiP)] in SRM 1649a ranged from 17.32 to 33.25 mg kg^{-1} with an average of 28.13 mg kg^{-1}. The values were compared to the SRM certified value, 39.92 mg/kg, and individual and total PAHs recoveries were reported. The lowest and the highest recovered $\sum$PAHs were extracted in MeOH and ACE/HEX (2:3) mixtures, respectively. Mixtures of polar and non-polar solvents had higher recoveries than single solvents in general: the results exhibited a similar trend reported by other studies [8,32,33]. The order of $\sum$PAHs recovered by various solvent compositions were MeOH < HEX < DCM < DCM/MeOH/ACE (2:3:3) < HEX/PrOH (1:1) < HEX/ACE (1:1) < MeOH/ACE (1:1) < HEX/ACE (3:2). PAHs recoveries in various solvent systems were compared to the certified values and shown in Fig. 2. Mixtures of polar and non-polar solvent showed higher recoveries than what was obtained using single solvents, except DahA. The high recovery of DahA in hexane could be due to the DahA's low concentration in SRM 1649a. DahA presented the lowest concentration (0.288 ± 0.02 mg kg^{-1}) in the standard material: it is significantly lower than InP (3.18 ± 0.72 mg kg^{-1}) and BghiP (4.01 ± 0.91 mg kg^{-1}). Therefore, even with the same degree of variation in extract, the low concentration of DahA will render a greater difference in the recovery than those derived from InP or BghiP, which have higher concentrations. Further study on solvation parameter and other distribution properties would be needed to reach a conclusive discussion.

Two sample Student's t-tests (α = 0.05) were performed to compare extraction efficiencies in sets of two solvent systems; Table 1 summarizes the result. A positive value denotes that the solvent on the left column exhibited higher extraction efficiency than that on the top row for the number of PAHs. For example, to compare extraction efficiency between MeOH and ACE/HEX (2:3), one could look up MeOH on the left column and then find ACE/HEX (2:3) on the uppermost row. The value, −11, indicates that MeOH showed significantly less efficiencies in extracting 11 PAHs against ACE/HEX (2:3). We postulate that the sum of each row expresses a provisional figure of the solvent system's extracting efficiency. Based on the values, the least and the most effective solvent compositions are MeOH and ACE/HEX. Closely examining the PAH recoveries in
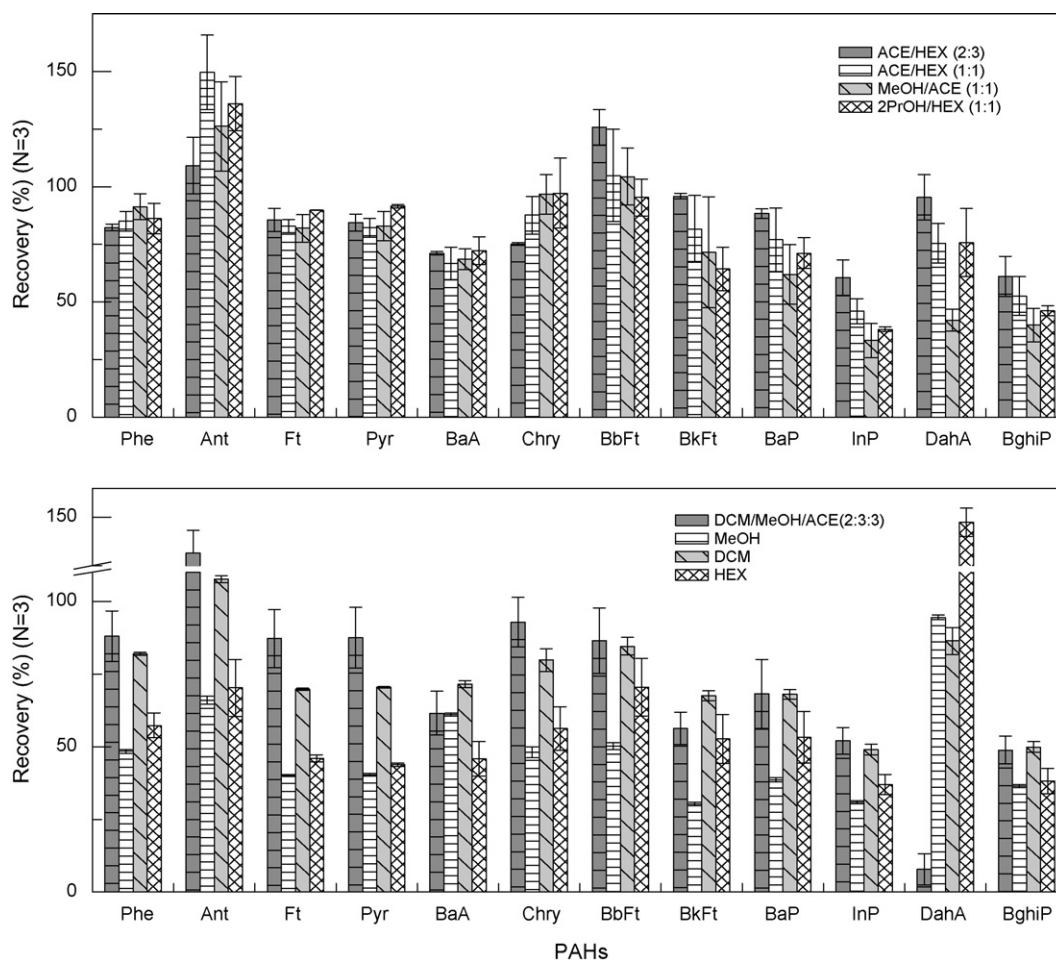
Fig. 2. Effect of solvent on extraction efficiency. Ultrasonic extraction for 20 min followed by 4-h SBSE at 1000 rpm. Error bars indicate the standard error ($N = 3$).

ACE/HEX (2:3) and ACE/HEX (1:1), both demonstrated no statistical difference for the 12 PAHs, yet $\sum$PAHs were slightly different. A Tukey-test was performed for the solvent systems (supplementary data can be found in the Appendix). The Tukey-test warranted the previously stated results: single solvents in ultrasonic extraction produced the least effective recovery for the PAHs, except for DahA. The solvent system combining three solvents, i.e. DCM/MeOH/ACE (2:3:3), impaired extraction efficiency.

Table 1

The result of paired t-test ($\alpha = 0.05$) for 12 individual PAHs (Phe, Ant, Ft, Pyr, BaA, Chry, BbFt, BkFt, BaP, InP, DahA, and BghiP) extracted by different solvent systems. A positive value denotes that the solvent on the left column exhibited higher extraction efficiency than that on the top row for the number of PAHs. A negative value indicates that the solvent on the left column is less effective for the number of PAHs. $\sum$PAHs (mg kg^{-1}) is the total 12 PAHs extracted from NIST 1649a. In cells where two numbers are present, this indicates that the recovery of individual PAH by the solvent system shows mixed outcome: significantly more efficient for certain PAHs but less so for others.

	MeOH	ACE:HEX (2:3)	ACE/HEX (1:1)	MeOH/ ACE (1:1)	PrOH/ HEX (1:1)	HEX	DCM	DCM/Me OH/ACE (2:3:3)	$\sum$PAHs (mg/kg)
MeOH		-11	-6	1 -6	-10	-3	-11	1 -8	17.32±0.30
ACE:HEX (2:3)	11		0	2	3	10 -1	6	2	34.63±0.41
ACE/HEX (1:1)	6	0		1	0	5 -1	3	1	32.05±5.91
MeOH/ ACE (1:1)	6 -1	-2	-1		0	5 -5	-1	1	30.90±5.68
PrOH/ HEX (1:1)	10	-3	0	0		4 -4	3 -1	1	31.80±2.21
HEX	3	1 -10	1 -5	5 -5	4 -4		1 -8	1 -5	20.71±1.73
DCM	11	-6	-3	1	1 -3	8 -1		1	28.34±0.44
DCM/MeOH/ACE (2:3:3)	8 -1	-2	-1	-1	-1	5 -1	-1		30.65±6.03

Table 2
Analysis of PAHs in NIST SRM 1649a (urban dust) ($N=3$). Acetone/hexane (2:3, v/v) was used in ultrasonic extraction for 20 min; SBSE method was carried out in 30% alcohol/water with 4 h extraction.

PAHs	pK_{ow}	Certified value (mg kg^{-1})	Measured results Mean ± SD (mg kg^{-1})	% Recovery Mean ± SD	Literature results Mean ± SD (Karthikeyan et al.)
Phenanthrene	4.35	4.14 ± 0.37	3.43 ± 0.08[*]	82.3 ± 1.35	4.37 ± 0.34
Anthracene	4.35	0.432 ± 0.09	0.49 ± 0.08	109 ± 12.3	0.54 ± 0.01
Fluoranthene	4.93	6.45 ± 0.18	5.49 ± 0.40	85.5 ± 5.07	5.65 ± 0.41
Pyrene	4.93	5.29 ± 0.25	4.46 ± 0.24[*]	84.4 ± 3.74	4.95 ± 0.38
Benz[a]anthracene	5.52	2.208 ± 0.07	1.66 ± 0.15	71.2 ± 0.85	2.17 ± 0.16
Chrysene	5.52	3.049 ± 0.06	2.31 ± 0.04[*]	75.1 ± 0.57	3.44 ± 0.44
Benzo[b]fluoranthene	6.11	6.45 ± 0.64	7.85 ± 0.76	125 ± 7.76	6.43 ± 0.44
Benzo[k]fluoranthene	6.11	1.913 ± 0.17	1.85 ± 0.04	95.9 ± 1.18	1.50 ± 0.11
Benzo[a]pyrene	6.11	2.509 ± 0.09	2.24 ± 0.08[*]	88.3 ± 2.01	2.21 ± 0.17
Indeno[1,2,3-cd]pyrene	6.70	3.18 ± 0.72	2.00 ± 0.33[*]	60.5 ± 7.72	3.86 ± 0.76
Dibenz[a,h]anthracene	6.70	0.288 ± 0.02	0.28 ± 0.04	95.3 ± 9.83	0.34 ± 0.10
Benzo[ghi]perylene	6.70	4.01 ± 0.91	2.56 ± 0.47[*]	61.0 ± 8.76	3.42 ± 0.26

Karthikeyan et al. [8].

[*] Statistically different from certified value.

The 20 min ultrasonic extraction efficiency using ACE/HEX (2:3) is shown in Table 2. The table also includes corresponding values reported by Karthikeyan et al. using a low temperature MAE and ACE/HEX (1:1) [8]. Statistical analysis suggests that the amounts of Ant, Flt, BaA, BbFt, BkFt, and DahA extracted with our method did not significantly differ from the corresponding certified values while other PAH recoveries are significantly fewer. Overall, ACE/HEX (2:3) and ACE/HEX (1:1) are suitable for PAH extraction. The PAH recoveries using ACE/HEX (2:3) solvent system ranged from 60.5% to 125.7%.

3.2. Effects of ultrasonic extraction time

The extraction time effect was studied for three solvent systems: MeOH, MeOH/ACE (1:1), and ACE/HEX (2:3). ACE/HEX (2:3) previously exhibited the highest recovery; therefore, this solvent system was also selected in this section. MeOH/ACE (1:1) and MeOH were also chosen to simplify the sample preparation process using the SBSE. During SBSE process, methanol is added to sample matrices to prevent adsorption of non-polar organic compounds onto the glass wall in many studies [25,34]. By using methanol or a mixture of methanol and acetone as an extraction solvent, solvent exchange process can be eliminated; therefore, using MeOH/ACE (1:1) or MeOH has an advantage to simplify the whole process. The sample was 100 mg of SRM 1649a, and 40 mL of solvent was used in extraction. A solvent exchange to

isopropanol was performed during K-D condensation for ACE/HEX (2:3).

Two extraction periods, 20 and 60 min, were compared, and the results for MeOH/ACE (1:1) and ACE/HEX (2:3) are presented in Fig. 3 (MeOH is not shown). Statistical analyses found that no significant difference exists between 20 min and 60 min extraction time for the three solvent compositions, except for InP in ACE/MeOH (1:1) and for Chry and BkFt in ACE/HEX (2:3). In these cases, 60 min extraction time provided higher recoveries for the PAHs indicated. Taking into consideration of efficiency, 20 min sonication suffices PAHs extraction from the solid material.

3.3. Effects of extraction method

MAE is a well-studied technique for PAHs extraction due to its low quantity of organic solvent consumption and its high efficiency [35]. MAE using ACE/HEX (2:3) was compared to the ultrasonic extraction. The results of statistical analyses and recoveries are shown in Table 3 and Fig. 4, respectively. Statistically significant difference was not observed between MAE and sonication method. Slightly higher recovery was obtained by MAE for higher molecular weight PAHs, i.e. BkFt, BaP, InP, DahA, and BghiP, although the sonication method demonstrated better recoveries for lower molecular weight PAHs to some extent. The results suggest that the more volatile PAHs are subject to poor recovery with MAE. This could be explained
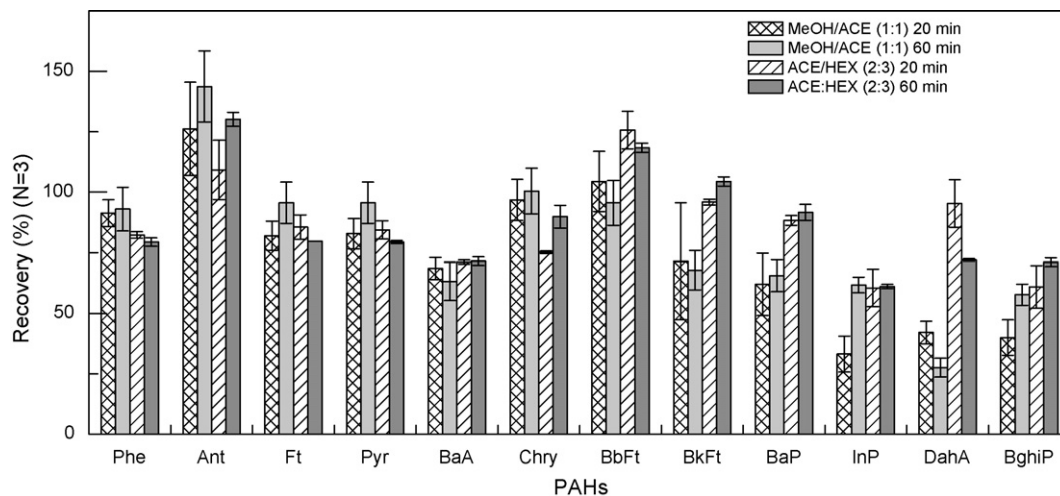

Fig. 3. Effect of ultrasonic extraction periods, 20 min and 60 min, on extraction efficiency. Error bars indicate the standard error ($N=3$).

Table 3
Paired t-test ($\alpha = 0.05$) results for 12 PAHs recovery. Ultrasonic extractions in various solvent systems and MAE. MAE: results from the MAE performed in ACE/HEX (2:3, v/v); 20 min: results from the ultrasonic extraction for 20 min in ACE/HEX (2:3, v/v); 60 min: results from the ultrasonic extraction for 60 min in ACE/HEX (2:3, v/v); 2-step: ultrasonic extraction by DCM and followed by a second extraction using MeOH/ACE (1:1, v/v) each for 20 min. $\sum$PAHs (mg kg^{-1}) is the total 12 PAHs from NIST 1649a.

	MAE	20 min	60 min	2-step	$\sum$PAHs (mg kg^{-1})
MAE		0	-1	2	35.48±2.14
20 min	0		-2	2 -1	34.63±0.41
60 min	1 (Chry)	2		1 -2	34.44±0.26
2-step	-2	1 -2	2 -1		35.39±5.60

by their evaporation into the headspace during the heating process in MAE [35,36], resulting in the loss of these compounds.

DCM extraction followed by a second ultrasonic extraction with MeOH/ACE (1:1) was studied to compare if the two-step extraction (DCM–MeOH/ACE) has an advantage over single or mixture solvent systems. A low recovery was observed for higher molecular weight PAHs. Lower molecular weight PAHs, such as Phe and Pyr, were more effectively recovered. Based on the relative standard deviation, the greater data discrepancy was apparent in the two-step process when compared to any other methods.

Wang et al. reported that MAE extraction was as effective as Soxhlet [37]; therefore, the results suggest that the ultrasonic extraction method is also comparable to Soxhlet when the same solvent load is applied. Considering the capital cost, the ultrasonic extraction method provides compatible results for the recoveries of PAHs to MAE extraction.

3.4. Effects of sample load

Sample load effect on PAHs recovery was studied for 20 and 100 mg sample amounts using 40 mL of MeOH. The sample to solvent ratio selected was comparable to the value (1 g of sample in 200 mL of solvent) reported in the NIST Certificate of Analysis of SRM 1649a. Although methanol was incompetent among previously studied solvent systems, using methanol offers a significant advantages of simple extraction process; therefore, methanol was studied anew for this section. Extraction times of 20 and 60 min

were examined. As illustrated in Fig. 5, statistical analysis verified a significant difference ($\alpha = 0.05$) between 20 and 100 mg of sample load. The smaller sample load, i.e. 20 mg, showed significantly higher recoveries for Ft, Pyr, BaA, BkFt, BaP, InP, DahA, and BghiP. On the other hand, the longer extraction time had a negative impact for BaA, BaP, and DahA. Yet 100 mg of samples in ACE/HEX (2:3) demonstrated a higher extracting efficiency than 20 mg sample in MeOH.

The average of 12 PAHs' recoveries for the 100 mg-ACE/HEX (2:3) was 86% while that for the 20 mg-MeOH system showed 65%. Additionally, ACE/HEX (2:3) solvent system satisfactory dealt with a higher sample load ranging 130–150 mg with no statistically significant difference (data not shown). In conclusion, MeOH is deficient in extracting PAHs from SRM 1649a.

3.5. LOD and LOQ in SBSE

As mentioned previously, SBSE coupled with thermal desorption has shown sufficient recovery and high extraction efficiency on PAHs. The limits of detection (LOD) and the limits of quantification (LOQ) for PAHs in SBSE–TD–GC–MS were tested. Using a 30% alcohol-fortified solution and a final volume of 20 mL, spiked PAH solutions were extracted by a Stir Bar with 4-h stirring time at 1000 rpm. Because the solution in the SBSE procedure was fixed volume (20 mL), LODs are reported in ng L^{-1}. Analyzed by GC/MS, LOD and LOQ values were determined by a signal-to-noise ratio of three-to-one and ten-to-one, respectively. As listed in Table 4,
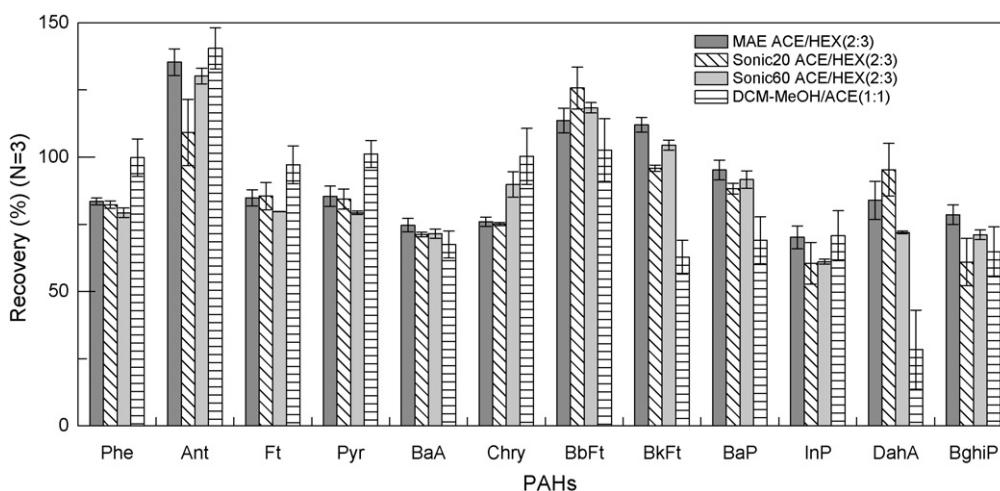

Fig. 4. Effect of extraction method on extraction efficiency. MAE ACE/HEX (2:3): MAE extraction with ACE/HEX (2:3); Sonic 20 ACE/HEX (2:3): sonication for 20 min with ACE/HEX (2:3); Sonic 60 ACE/HEX (2:3): sonication for 60 min with ACE/HEX (2:3); DCM–MeOH/ACE (1:1): sonication for 20 min with DCM followed by additional 20 min with MeOH/ACE (1:1). All methods were followed by 4-h SBSE at 1000 rpm. Error bars indicate the standard error ($N = 3$).
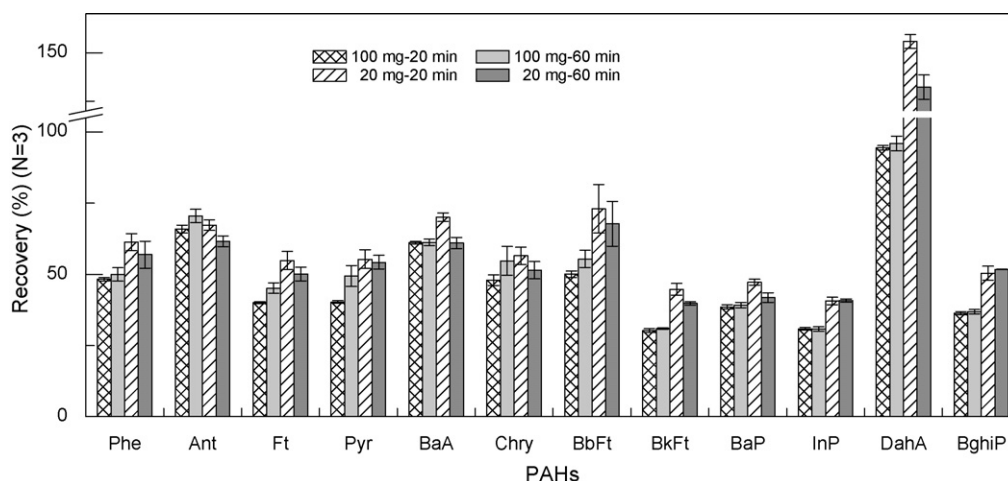
Fig. 5. Effect of sample load on extraction efficiency using methanol as extraction solvent. Error bars indicate the standard error ($N = 3$).

Table 4
Limit of detection (LOD) and limit of quantification (LOQ) in ng L^{-1} for investigated PAHs in 20 mL of SBSE solution followed by GC/MS. Method Detection Limit (MDL) was calculated based on 20 mg of sample load.

PAHs	LOD (ng L^{-1})	LOQ (ng L^{-1})	MDL (μg kg^{-1})
Naphthalene	31.9	106	
Acenaphthylene	3.59	12.0	
Acenaphthene	3.07	10.2	
Fluorene	2.65	8.83	
Phenanthrene	7.01	23.4	8.52
Anthracene	1.73	5.77	1.59
Fluoranthene	4.79	16.0	5.60
Pyrene	4.33	14.4	5.13
Benz[a]anthracene	1.98	6.60	2.78
Chrysene	4.65	15.5	6.19
Benzo[b]fluoranthene	20.9	69.7	16.7
Benzo[k]fluoranthene	9.40	31.3	9.80
Benzo[a]pyrene	5.23	17.4	5.92
Indeno[1,2,3-cd]pyrene	5.15	17.2	8.51
Dibenz[a,h]anthracene	4.66	15.5	4.89
Benzo[ghi]perylene	7.09	23.6	11.6

the LOD for PAHs ranged between 1.7 and 32 ng L^{-1}, and the LOQ ranged from 5.8 to 110 ng L^{-1} for the US EPA 16 PAHs. García-Falcón et al. reported similar analytical capacity of using SBSE to determine PAHs in water [29].

Describing MDLs in μg kg^{-1} without citing sample amounts used is frequently invalid for practical procedure [38]. This method was optimized to use ACE/HEX (2:3) for 20–150 mg of solid samples. LODs in Table 4 stated the instrument's sensitivity based on alcohol-fortified samples in SBSE–TD–GC–MS for individual PAHs. Recoveries express ultrasonic extraction efficiencies for these compounds in SRM 1649a (Table 2). Method Detection Limit (MDL) was readily estimated by using recovery and LOD [39] and was summarized in Table 4. MDLs were determined by (LOD × final volume)/(sample weight × extraction recovery). Upon specifying the sample load at 20 mg and using 20 mL as the final volume, the MDLs in this particular study were estimated to be from 1.59 μg kg^{-1} (Ant) to 16.7 μg kg^{-1} (BbFt).

4. Conclusions

This study demonstrated that ultrasonic extraction using an ultrasonic bath is a rapid, reliable, and environmental friendly approach in recovering PAHs from a solid matrix. Ultrasonic extraction using ACE/HEX (2:3 and 1:1, v/v) solvents provided sufficient extraction efficiencies. Compared to commonly used sample prepa-

ration techniques, such as Soxhlet and MAE, ultrasonic extraction is fast and effective. The postulated method requires only a 20 min extraction and a common device in laboratories; this method significantly improves cost and extraction time over 2–4 h for MAE (including the cooling time) and 12–24 h for Soxhlet extraction.

The solvent effect study demonstrated that combination of non-polar and polar solvent ameliorate extracting PAHs from SRM 1649a. Acetone/hexane mixtures at 2:3 and 1:1 (v/v) ratios gave the most satisfactory results for PAHs recovery from SRM 1649a. Longer sonication duration diminished extraction efficiencies in most cases; therefore, 20 min extraction suffices PAHs extraction. The comparison of the two extraction methods, ultrasonic extraction and MAE, rendered the similar result. The ultrasonic sonic extraction gave higher recoveries on PAHs that are more volatile while MAE was more effective for less volatile PAHs. In addition, sample load becomes a critical factor on extraction efficiency when methanol is used. Regardless, the solvent system is still more crucial than sample load. LOD ranged from 1.7 ng L^{-1} for anthracene to 32 ng L^{-1} for naphthalene, and LOQ ranged from 5.8 ng L^{-1} for anthracene to 110 ng L^{-1} for naphthalene under the SBSE conditions: 20 mL of 30% alcohol-fortified solution with 4 h stirring at 1000 rpm. Although SBSE was utilized in this study, SBSE is not a compulsory process for determining of PAHs: an orthodox clean-up process substitutes SBSE. Similarly, when SBSE is available but not a TDU, solvent desorption may replace the TDU. The presented method is simple, fast, use lower volumes of inexpensive and non-halogenated solvents. It incorporated Green Chemistry in environmental analysis, and presents to be particularly useful when estimated level of PAHs is desired for screening purposes or for preliminary study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2010.08.055.

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