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Miniaturized Solvent Extraction and Cleanup Method for Polycyclic Aromatic Hydrocarbons in Air Particulate Matter by Gas Chromatography/Mass Spectrometry

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are one of the priority pollutants measured in atmospheric particles due to their potential health risks to exposed individuals. A miniaturized solvent extraction and cleanup method has been developed for the determination of 14 types of PAHs in air particulate matter (APM) collected through the use of a five-stage stainless steel Berner sampler with aluminum foil as substrates. The analytical method employed sonication of the impacted APM in the aluminum foil substrates with dichloromethane, extract purification through silica cleanup in miniaturized glass columns, and analysis of the PAHs by gas chromatography/mass spectrometry. The performance characteristics of the method such as linearity, range, detection limits and quantification limits, recovery and precision for each of the 14 PAHs were established using procedures suggested by Eurachem and/or the Association of Official Analytical Chemists. The method has direct advantages, i.e., use of minimal volume of solvents, and non-utilization of disposable silica solid phase extraction cartridges, but with performance characteristics that are within acceptable limits−thus resulting in a low-cost, practical, and reliable protocol that can quantify PAHs in APM.

Key words: *PAHs, solvent extraction, sonication, particulate matter*

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INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are among the hazardous air pollutants classified as a Group1 carcinogen by the International Agency for Research on Cancer (*IARC 2012*). In urban environments, PAHs can be detected in the atmosphere mainly due to anthropogenic sources such as vehicle emissions (C*hang et al. 2006; Zhang and Tao 2009; Shi et al. 2009; Riddle et al. 2007 in Wu et al. 2010*).

The determination of PAHs in the atmosphere, or airborne particulate matter (APM), is usually performed by the following the steps: sample collection in a suitable substrate, solvent extraction from the matrix, and separation using chromatographic techniques. Among these steps, the solvent extraction procedure requires longer laboratory time and generates more solvent wastes. The United States Environmental Protection Agency (US EPA) published the *Compendium Method TO-13A* (*1999*) which employs filter and sorbent cartridges, high-volume flow rate air sampler (approximately 300 m^3 in 24 hours), traditional Soxhlet extraction, Kuderna-Danish (K-D) evaporator, silica gel cleanup and GC/MS analysis Ultrasonication techniques are also known to extract PAHs in APM with lower cost, less time, and small volumes of solvents without the need for intricate instrumentation and glasswares (*Pozzoli et al. 2004; Liu et al. 2007*). Among the many traditional and modern techniques of extracting PAHs, Soxhlet extraction is considered the standard, but requires large volumes of organic reagents and can generate additional chemical wastes.

Liu et al. (*2007*) described the other methods that have been developed to extract atmospheric PAHs, but majority of published studies that use high-volume sampling employ almost the same procedures as Method TO-13A (*USEPA 2009*), with modifications such as ultrasonication, rotary evaporation and analysis by HPLC

The other solvent based extraction methods that are also employed to extract particulate PAHs are: microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE), subcritical water extraction (SWE) and the CO_2-based supercritical fluid extraction (SFE) (*Pozzoli et al. 2004; Lee 2010; Albinet et al. 2013*). To remove matrix effects and other contaminants, cleanup procedures such as solid phase extraction (SPE) can also be performed, with the use of silica gel columns or C^{18} cartridges. Extracts are then mainly qualified and quantified either through gas chromatography (GC) coupled with detection by mass spectrometry (MS), or through high-pressure liquid chromatography (HPLC) in tandem with fluorescence and UV detectors (*Pozzoli et al. 2004; Dzepina et al. 2007*). In order to further reduce the amount of solvent and time of analysis, other techniques have also been developed to simplify the extraction, purification and quantification steps. Some of these studies include the QuEChERS procedure (*Albinet et al. 2013*); the use of an aerosol mass spectrometer (*Dzepina et al. 2007*); and the coupling of a pyrolysis system with GC/MS (*Spindler et al. 2012*)−all of which requiring equipment that requires higher costs for set-up, operation and maintenance.

The objective of this research is to develop a miniturized PAH extraction method that is relatively simple to implement, and minimizes the requirements in extraction time (non-labor-intensive) and organic solvent (non-resource-consuming), as compared to other conventional solvent-based methods. In this study, the developed miniturized method employed sonication with dichloromethane (DCM), an improvised silica cleanup and GC/MS analysis. The miniaturized method will be applied to atmospheric ultrafine particulate matter collected through the use of a five-stage stainless steel Berner impactor sampler with aluminum foil substrates (separate paper, in preparation).

MATERIALS AND METHODS

Chemicals and materials

Standards of the fourteen PAHs (fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[g,h,i]perylene) were sourced from a commercially prepared stock PAHs Standard Mix (Supelco). The surrogate standard perylene-d12 and the internal standards (IS) phenanthrene-d10, pyrene-d10 and chrysene-d12, were all supplied by Cambridge Isotope, Inc. The solvents DCM, hexane and acetone were all analytical grade and were purchased from JT Baker and Merck. The silica gel powder (60-200 mesh; JT Baker) and the glass wool were baked at 400°C prior to use. Pre-baked blank aluminum foils, and foils with test air particulate matter (APM) collected through a five-stage stainless-steel Berner cascade impactor (Hauke type), were used for method development. The certified reference material (CRM) used was obtained from the National Metrology Institute of Japan (NMIJ CRM 7308-a).

Method evaluation

In validating the miniaturized solvent extraction and cleanup method for PAHs in APM, the performance characteristics such as instrument detection limits (IDL), method detection limits (MDL), accuracy and precision of each PAH were established. The IDL of each PAH was obtained by injecting low level concentrations (0, 5 and 10 μgL^{-1}) of PAHs (n=8) and multiplying the standard deviation of the resulting concentrations by 3 (IDL=3SD; Eurachem, 2014). The MDL (MDL=3SD), reliable DL (RDL=2MDL) and limit of quantitation (LOQ=10SD=3.33MDL; *AOAC 1998*) were determined through recovery experiments with 100 $\mu g\ L^{-1}$ spiked foils. A clean, pre-baked aluminum foil was used as laboratory blank, and was included in every batch of samples tested using the developed method. Accuracy of the method was measured from the recovery of PAHs spiked into the foils with APM and from the results of the analysis of the tunnel dust NMIJ CRM 7308-a.

Extraction procedure

Aluminum foils containing APM were transferred to 8 mL glass test tubes by using stainless-steel tweezers. PAHs were extracted from the foil by adding 2 mL of DCM and sonicating (Rocker Ultrasonic Cleaner SONER 210H Model) for 15 min. in a water bath at room temperature. Using a glass Pasteur pipette, the extract was quantitatively transferred to another test tube, leaving the foil in the test tube for the second extraction with another 2 mL of DCM under the same conditions. The second extract was quantitatively transferred and combined with the first extract. To ensure complete extraction of the PAHs, the test tube with the foil was washed with 1 mL of DCM, and the washing was combined with the first and second extracts for a total volume of ~5 mL. The extracts were concentrated to ~1 mL using a gentle stream of N_2 gas.

To purify the extracts, an improvised silica solid-phase extraction (SPE) column, made from the standard glass Pasteur pipette measuring 105 mm in length with and ID of 5 mm plugged with a small amount of glass wool and packed with 100 mg of silica powder corresponding to a height of ~1 cm. The column was pre-conditioned with 1 mL DCM and 2 mL hexane before passing the ~1 mL of concentrated extract. PAHs were eluted from silica column using 5 mL of 25% DCM in hexane. The purified extract was then concentrated to almost dryness using N^2 and resolved with 500 µL of 50 µg L^{-1} IS mixture.

Instrumental analysis

Final extracts of 2 µL in volume were injected into the high precision gas chromatograph/mass spectrophotometer (GC/MS; Shimadzu GC/MS QP2010) equipped with electron impact (EI) ion source, quadruple ion focusing and auto injector (Shimadzu AOC-20i). The GC/MS used is equipped with a Supelco SPB-5 (30 m, 0.32 mm ID, 0.25 µm thickness) column and operated with helium as carrier gas at 3 mL min^{-1} and 50 kPa. The injector, detector, and interface temperatures of the GC/MS for analysis of PAHs were set at 280, 200 and 280˚C, respectively. The column for PAH analysis was operated at eight ramp temperature programs to ensure separation of PAHs peaks (**Table 1**). The initial temperature was set to 55˚C with hold for 2 min, rise of 20˚C min^{-1} to 135˚C with hold for 1.5 min, 5˚C min^{-1} to 175˚C with hold of 1 min, 10˚C min^{-1} to 220˚C, 5˚C min^{-1} to 235˚C with hold for 1.5 min, 5˚C min^{-1} to 240˚C with hold for 5 min, 8˚C min^{-1} to 250˚C with hold for 1 min, 15˚C min^{-1} to 285˚C, 10˚C min^{-1} to 300˚C with hold for 10 min. The total run time is 48 min. The samples were introduced into the GC by splitless injection, and the MS was set in the selected ion monitoring (SIM) mode for the target and reference mass ion charge ratios of each PAH (**Table 2**), with a sampling rate of 0.2 sec.

Table 1. The GC/MS temperature program for the analysis of PAHs extracted through the developed method (Shimadzu QP2010).

Temperature	Hold time (min)	Cumulative run time (min)
55 ˚C	2.0	2.00
20˚C min^{-1} up to 135 ˚C	1.5	7.50
5˚C min^{-1} up to 175˚C	1.0	16.5
10˚C min^{-1} up to 220 ˚C	0.0	21.0
5˚C min^{-1} up to 235˚C	1.5	25.5
5˚C min^{-1} up to 240˚C	5.0	31.5
8˚C min^{-1} up to 250˚C	1.0	33.8
15˚C min^{-1} up to 285˚C	0.0	35.8
10˚C min^{-1} up to 300˚C	10	48.0

Table 2. The retention time (RT), target and reference ions of each PAH analyzed through the developed Solvent Extraction-GC/MS method.

PAH	Retention Time (min)	Target Ions (m/z)	Reference ions (m/z)
Fluorene	11.42	166.10	165.05
Phenanthrene	15.08	178.05	176.05
Anthracene	15.36	178.05	179.10
Fluoranthene	19.98	202.05	200.05
Pyrene	20.57	202.05	200.05
Chrysene+ Benz[a] anthracene	24.89	228.10	229.10
Benzo[b] fluoranthene	30.13	252.10	250.10
Benzo[k] fluoranthene	30.29	252.05	126.10
Benzo[e]pyrene	31.80	252.10	250.05
Benzo[a]pyrene	32.37	252.05	250.10
Indeno[c,d]pyrene	37.05	276.10	274.15
Dibenz[a,h] anthracene	37.13	278.10	139.10
Benzo[ghi]perylene	37.60	276.10	274.15

RESULTS AND DISCUSSION

Analysis of the Certified Reference Material (CRM)

Fluorene, fluoranthene and pyrene were the only PAH species that were recovered from the analysis of the CRM tunnel dust (NMIJ 7308-1), with percent recoveries of 75%, 31% and 28%, respectively (CRM mass = 0.02 g). Reduction to half of the mass of the CRM tunnel dust analyzed (CRM mass = 0.01 g) resulted in lower (half) percent recoveries as well (**Table 3**). The recovery of the three low-molecular weight PAHs from the CRM used in this study can be explained in conjuction with *Itoh et al.* (*2011*) which focused on the certification of the PAHs in this sample. *Itoh et al.* (*2011*) used Soxhlet, microwave-assisted, and pressurized liquid extraction of the NMIJ 7308-a, and have found that in general, the recovery yields became lower with high-molecular weight PAHs due to the strong π-π overlap of the high-molecular weight PAHs with the matrix. The peaks of the PAHs in the chromatograms were therefore masked in the CRM samples due to more pronounced matrix effects.

The distinct matrix effect can be attributed to the main composition of the NMIJ CRM 7308-a which is char (*Itoh et al 2008*). Furthe, the particle size of the said CRM is <106 µm, which may have affected the efficiency of the miniturized method to extract PAHs, in contrast to the ultrafine particles (size <0.14 µm) that were used in the recovery tests of the miniturized method. It can also be inferred that the miniature silica cleanup employed is not

Table 3. The percent recoveries of PAHs extracted from the certified reference material (CRM) 7308-a samples using the developed method.

PAHs	Percent Recoveries of Certified Reference Material (CRM) samples									
	CRM mass = 0.02 g					CRM mass = 0.01 g				
	1	2	3	Ave.	SD	1	2	3	Ave.	SD
Fluorene	64.72	73.02	86.01	74.59	10.73	26.51	29.97	35.91	30.80	4.75
Fluoranthene	26.41	33.48	33.91	31.27	4.21	12.78	15.22	17.34	15.11	2.28
Pyrene	25.26	27.48	31.40	28.05	3.11	12.43	11.77	13.04	12.41	0.63

sufficient to remove or minimize the matrix effect from the tunnel dust CRM sample. However, the developed method is nonetheless suitable for ambient air particulates with very low estimated masses and smaller particle diameters, as supported in the next section of this paper.

Performance characteristics of the method

In the analysis of actual air particulate samples spiked with 100 µg L^{-1} native PAH standards, the correlation coefficients (r) of each PAH in the calibration curves for 8 standards used (0, 10, 20, 40, 60, 80, 100, 250 µg L^{-1}) show good linearity (**Table 4**) together with the instrument detection limits (IDL). On the other hand, the recoveries of the PAHs ranged from 84% to 153%, with %RSD ranging from 5.2% to 36.8%. The very high percent recovery for indeno[1,2,3-cd]pyrene (153%) can be attributed to possible peak overlap with that of dibenz[a,h]anthracene; their retention times are 37.05 and 37.13 min, respectively. A summary of the recoveries and the rest of the quality control parameters for the method developed in the study (**Table 5**) are acceptable for most PAH species, as per the Association of Official Analytical Chemists Peer-Verified Methods Program manual (*AOAC 1998*).

In addition, the double sonication step employed in this procedure only takes 30 min, and uses as little as 15 mL of solvent, compared to the traditional Soxhlet extraction that can take up 16 to 24 hours and use up to 150 mL of solvent for the initial extraction step alone (*Liu et al. 2007; Lau et al. 2010; Itoh et al. 2011; Oluseyi et al. 2011*). Consequently, the extracts are of small volumes, and the pre-concentration step employed N_2 blowdown. It does not necessitate the use of rotary evaporation that entails the use of more volumes of solvents and consequently more solvent wastes, and can lead to higher occurrence of analyte losses, especially for the low-molecular weight PAHs (*Cheng 2003*). Since the cleanup step in this developed method use reusable glass Pasteur pipettes, plastic containers from commercial SPE silica cartridges are avoided. Although microwave-assisted extraction (MAE), pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE), subcritical water extraction (SWE) and the CO_2-based supercritical fluid extraction (SFE) are also described as efficient (*Pozzoli et al. 2004; Liu et al 2007; Lee 2010; Albinet et al. 2013*), these extraction methods require more steps and elaborate (as well as costly) instruments while the developed miniturized method requires simple equipment that are commonly found in chemical laboratories.

Table 4. Instrument detection limits and linearity coefficients of the calibration curves of the PAHs[a].

PAH	IDL (µgL^{-1}; n=8)	Linearity (Correlation Coefficient, r) (n=8)	SD (n=8)
Fluorene	2	0.9984	0.0009
Phenanthrene	2	0.9982	0.0008
Anthracene	6	0.9960	0.0012
Fluoranthene	2	0.9982	0.0016
Pyrene	3	0.9970	0.0015
Chrysene+ Benz(a)anthracene	1	0.9952	0.0024
Benzo(b)fluoranthene	4	0.9972	0.0016
Benzo(k)fluoranthene	4	0.9976	0.0017
Benzo(e)pyrene	3	0.9984	0.0009
Benzo(a)pyrene	4	0.9964	0.0014
Indeno(1,2,3-cd)pyrene	1	0.9982	0.0008
Dibenzanthracene	1	0.9960	0.0012
Benzo(ghi)perylene	2	0.9982	0.0006

[a] Obtained using the Shimadzu GCMS QP2010.

Table 5. Quality control parameters of the developed method for extracting PAHs from air particulates.

PAH	Accuracy (%Recovery) (n=7)	Precision (%RSD) (n=7)	MDL[b], ng m^{-3} (n=7)	LOQ[c], ng m^{-3} (n=7)	RDL[d], ng m^{-3} (n=7)
Fluorene	83.7	15.7	0.3	1	0.6
Phenanthrene	91.3	14.2	0.3	1	0.6
Anthracene	84.3	5.2	0.2	0.7	0.4
Fluoranthene	95.1	22.9	0.4	2	0.8
Pyrene	81.9	11.5	0.3	1	0.6
Chrysene+ Benz(a)anthracene	114.8	6.8	0.2	0.7	0.4
Benzo(b)fluoranthene	137.3	36.8	0.7	3	2
Benzo(k)fluoranthene	118.2	11.1	0.3	1	0.6
Benzo(e)pyrene	105.5	7.6	0.2	0.7	0.4
Benzo(a)pyrene	141.5	18.7	0.4	2	0.8
Indeno(1,2,3-cd)pyrene	153.2	14.4	0.4	2	0.8
Dibenzanthracene	136.3	18.2	0.4	2	0.8
Benzo(ghi)perylene	116.5	21.8	0.4	2	0.8

Notes: [b.] MDL=3SD; [b.] LOQ=3.33MDL; [d.] RDL=2MDL (*Eurachem 2014*).

SUMMARY AND CONCLUSION

In this study, a simple miniturized extraction method was developed to extract PAHs particularly from air particulate matter (APM) collected through a Berner impactor. The results from the recovery and linearity tests, and the determination of the quantification and detection limits, have shown acceptable validation of the method. Compared to other methods, the developed method employs minimal time and resources, and generates less wastes (solid containers and solvents), without sacrificing precision and accuracy. Due to its simplicity and relatively low-cost, this procedure can be done in any organic chemistry laboratory–allowing the analysis of PAHs in APM even with limited resources. The application of this method can thus lead to more research that can give information on the concentration of PAHs in the atmosphere, providing scientific basis for future studies such as understanding of the relationship between PAH exposure from roadway emissions, and possible detrimental health effects on exposed populations.

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