Comparison of Extraction Techniques for the Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil

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Abstract. The development of a simple method for the extraction and determination polycyclic aromatic hydrocarbons (PAHs) from soil by applying the clean-up, and pre concentration technique has been validated with limits of detection and limit of quantification (LOQ), recovery and others factors. The implemented techniques were Soxhlet, ultrasonication and mechanical shaking and followed by solid phase extraction C-18 (SPE) to clean up and pre-concentrated was investigated. The results were compared to determine the method with the highest extraction efficiency. Chromatographic conditions for the separation of PAHs using High Performance Liquid Chromatography (HPLC) using Ultraviolet – Diode Array Detector (UV-DAD) were also optimised. Analysis of standard spiked soil blank resulted in recoveries 70 – 107% for ultrasonic, 55 – 110% for mechanical shaker and 57 – 99% for Soxhlet.

1. Introduction
Polycyclic aromatic hydrocarbon (PAHs) are organics compound which are mostly colorless, white or pale-yellow solids is form both natural and anthropogenic sources, typically formed during incomplete combustion of biomass or fossil fuels. [1][2] Small amount of PAHs have also been found in geological formations such as hard coal, brown coal, crude oil bituminous shales [3]. Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds with two or more fused aromatic rings. They have a relatively low solubility in water, but are highly lipophilic. Most of the PAHs with low vapor pressure in the air are adsorbed on particles [4]. PAHs are thermodynamically stable and recalcitrant organic pollutants in the environment because of their negative resonance.[5] The nature of PAHs causes in migration through the atmosphere from places where PAHs is high such a industrial, urban areas or burning land. (Malawska et.al, 2002).

The mutagenic and carcinogenic of PAHs, and also the toxicity the study of PAHs in environmental matrix including air, water and soil are great importance (Titato et.al, 2006). The 16 priority PAHs designated by U.S.EPA because of toxicity and easy chemical detectable, and also wide range of potential structure were frequently found. [6]

Polycyclic Aromatic Hydrocarbon (PAHs) determination methods rely on extraction, purification and detection technique. The extraction method become one of the time consuming and require chemicals and complex technique. Ultrasonic, Soxhlet and mechanical shaker are widely used to day to extract PAHs in soil. [7][2][8]. PAHs compound is non polar and hydrophobic, soluble in most of organic solvents, US EPA 3540, 3550C suggested dichloromethane or mixture of dichloromethane with other organic solvent such as acetone. The detection technique also one of the factors in
The measurement of PAH. Solid phase extraction technique as pre concentration and clean up helps to eliminate interference and also to improve detection of PAHs that mostly have low in nature. [9][10].

The assessment of level PAHs become interesting topic. The chromatographic are the most method that is used for detecting. High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are the recommended by USEPA to detect the PAHs compound in water, waste water or in sediment. Because of the sensitivity and selectivity and also the availability, HPLC with diode array detector (DAD) and Fluorescence detector (FLD) method widely use for measurement PAHs.[11]

2. Methods
This study is intended to find the most efficient method without ruling out the results with the highest recovery from the three extraction methods used. The application of the SPE technique is also of concern in recovering clean extracts for measurement in HPLC. Optimization of the method for measuring the concentration of PAHs in HPLC, allows the method to be used to measure concentrations at low level.

2.1. Reagent and Material
A standard mixture of the USEPA 16 priority PAHs in acetonitrile: methanol: 500 µg/mL of naphthalene (Nap), 500 µg/mL of acenaphthylene (Acy), 1000 µg/mL of acenaphthene (Ace), 100 µg/mL of fluorene (Flu), 40 µg/mL of phenanthrene (Phe), 20 µg/mL of anthracene (Ant), 50 µg/mL of fluoroanthene (Flt), 100 µg/mL of Pyrene (Pyr), 50 µg/mL of benzo[an]thracene (BaA), 50 µg/mL of chrysene (Chr), 20 µg/mL of benzo[b]fluoroanthene (BbF), 20 µg/mL of benzo[k]fluoroanthene (BkF), 50 µg/mL of benzo[a]pyrene (BaP), 200 µg/mL of dibenzo[a,h]anthracene (DaA), 80 µg/mL of benzo[g,h,i]perylene (BgP) and 50 µg/mL of indeno[123-cd]pyrene (IP) was obtained from SUPELCO, Bellefonte, PA, USA. Serial dilutions of the standard solution using HPLC grade acetonitrile were made. HPLC-grade acetonitrile, methanol, water and dichloromethane suprasolv were purchased from Merck (Millipore-Merch, USA), and acetone was purchased from Fisher.

Six of PAHs CRM (99.7% Nap, 99.0% Ant, 99.2% Pyr, 98.5% BaA, 96.7% BaP and 98.0% IP was purchased from Sigma-Aldrich. The stock standard was made in acetonitrile: methanol (9:1) and used to spike soil blank for optimization the extraction procedure.

Standard spiked soil matrix soil was made by adding 6.0 mL spike standard to 60 g of blank soil, additional acetonitrile was added until a layer of solvent above the soil can be seen to ensure homogeneity of standard PAHs in soil. The solvent allowed evaporated in room temperature for usually overnight [12]. The recovery of the analytes and other quality control parameters were determined for each batch of extraction.

2.2. Selection of method of extraction of polycyclic aromatic hydrocarbons (PAHs)
2.2.1. Soxhlet extraction
The spiked blank soil was put into extraction thimbles (after washed in dichloromethane). The samples were extracted using the Soxhlet extractor with 350 mL DCM: Acetone for 16 h. The extracts were further reduced to ± 5.0 mL using a rotary evaporator and kept it in vials and put in the refrigerator for clean up and analysis.

2.2.2. Ultrasonication extraction
The spiked blank soil in bottle glass with Teflon screw cap added 30 mL of solvent DCM: Acetone (9:1). The bottles then sonicated in a high-performance ultrasonic bath with microprocessor control for precision time operation (Bransonic CPX3800H, Emerson, Branson Ultrasonic Corporation USA) for 20 min. After 20 minutes allow to separated and the clear phase is pipetted in the bottle glass. These processes were performed three times by adding 30 ml solvent each time to ensure all the PAHs completely extracted. The extraction solutions were then centrifuged and the supernatant decanted into
bottle glass. Rotary evaporator was used to reduced the volume to ± 5 mL and kept in the refrigerator for clean up and analysis.

2.2.3. **Mechanical shaking extraction**

The spiked blank soil in bottle glass with Teflon screw cap added 30 mL of solvent DCM:Acetone (9:1) and shaken in multistube vortex-shaker at 2000 rpm for 30 minutes. The extract was centrifuged and the supernatant decanted to glass container and repeated 3 times by each time adding 30 mL solvent. The extract then reduced using a rotary evaporator to ± 5 mL and kept in refrigerator for clean up and analysis.

2.3. **Solid phase extraction (SPE) clean up of extracts**

SPE clean-up of the extracts was carried out using a vacuum with 6 ml Agilent C18 SPE cartridges. The sorbents of SPE cartridges were conditioned with 5 ml of methanol and 1 ml of 40% of the extracting solvent in water respectively. The extraction solutions were each loaded and aspirated through the cartridge under gentle vacuum at a flow rate of less than 2 ml/min then eluted by 2 x 1 mL dichloromethane and 3 x 1ml mixture of dichloromethane and acetone (9: 2) at flow rate less than 2 ml/min. The eluates allowed to dryness using a gentle stream of nitrogen. The residues were dissolved in 1 mL mixture of acetonitrile and methanol (9:1) for HPLC measurement.[13]

2.4. **High Performance Liquid Chromatography (HPLC)**

PAHs were analyzed with Agilent 1260 Infinity quaternary pump system with a programmable wavelength diode array detector. The HPLC system was equipped with a reverse phase C18 column (250 mm × 4.6 mm, 5 μm particle size, Eclipse LC-PAH). The column temperature was maintained at 30°C. The injection volume was 15 μL and the flow rate was 1 mL/min. Total run time 30 minutes with 7 minutes for equilibration of column before next injection before next sequence injection. Gradient mobile phase shown in table 1. The PAHs peaks were identified by its retention time. The quantification of PAHs concentration was using external calibration of PAHs standard plot.

| Time Lapse | % Water (H₂O) | % Acetonitrile (ACN) |
|-----------|--------------|---------------------|
| 0 - 20    | 60           | 40                  |
| 20 - 25   | 5            | 95                  |
| 25.1      | 60           | 40                  |

The external calibration was made by preparing a serial dilution of PAH standards ranging from 0.02 to 5.0 mg/L except acenaphthene which have lower sensitivities was calibrated from 0.1 to 10.0 mg/L. The linearity was evaluated at different concentrations using peak areas.

3. **Result and Discussion**

3.1. **Optimized detection PAHs**

The measurement PAHs using HPLC and GC methods have been considered to be equally valid by USEPA and other environmental agencies. In this study, the separation of PAHs was performed in reverse-phase column with acetonitrile-water as mobile phases using ultraviolet-diode array (UV-DAD) detection. [14][15][16]. To optimize the selectivity and sensitivity the parameter setting chromatographic was setting up, such as mobile phase composition, column temperature and detector wavelength.

The measurement in three different wavelengths of UV-DAD showing different detection limit of HPLC. The injection volume sample 15 μL was increased the detection limit in HPLC. The
Wavelength 220 nm [17] showing the highest response for Naphthalene (Nap), Acenaphthene (Acy) only detected in 220 and 230 nm with the highest response at 230 nm and 254 nm [15] for another 14 PAHs. Figure 1 shows the HPLC chromatogram of the PAH standards.

**Figure 1.** HPLC-UV-DAD chromatograms separation of PAHs in three different wavelengths: 230 nm, 254 nm and 220 nm

### 3.2. Calibration Range and Linearity

In this method, the serial standard PAHs were prepared in five different concentration levels. The interval from lower to upper levels was demonstrated to determine the precision, accuracy and linearity. The correlation coefficients \([R^2]\) ranged from 0.997 to 1.000 for all the 16 PAHs which is mean in acceptable range \(\geq 0.995\) or have good correlation.[18]

The detection limit in HPLC was calculated from the lower concentrations (the blank was not giving any numerical result) of PAHs responding in instrument [7][19]. Limit of quantification (LOD) and limit of quantification were calculated.

Limit of detection (LOD) = Std Dev \(\times t_{\text{student}}\)

Limit of quantification (LOQ) = Std Dev \(\times 10\)

The detection of quantification showed chrysene (Chr), anthracene (Ant), fluoranthene (Flt) and phenanthrene (Phe) have the lowest and benzo[g,h,i]perylene (BgP) has the highest. The results are shown in table 2.
3.3. Optimized extraction and detection method in soil matrix

The extraction and detection PAHs in soil matrix was investigated in blank soil spiked with six CRM standards (Nap, Ant, Pyr, BaA, BaP and IP). The concentration added varies according to the detection limit of the instrument (9840, 1080, 10080, 1995, 1995, 1980 µg/L respectively with total PAHs 26,970 µg/L). The additional solvent the soil Spiked CRM so that the water layer is visible above the soil, to ensure standard CRM is distributed throughout the soil matrix. After evaporating for one night, this sample is stirred with a shaker to ensure the uniformity of soil particles.

The non polar structure of PAHs inhibits them from dissolving in water. Nevertheless, PAHs are not completely insoluble, particularly the lower molecular weight PAHs but very poor solubility in water.[1]. The hydrophobic properties that increase as the number of rings require an organic solvent that can dissolve these components [20] and for extraction from soil or sludge requires a miscible solvent so that it can enter into soil / sludge particles, the addition of acetone is needed with a smaller ratio. [8]

Table 2. HPLC performance parameters for detection of sixteen PAHs

| PAH compounds            | UV - DAD Wavelength | Cal. Range [µg/L] | Regression Equation | Linearity [R²] | LOD [µg/L] | LOQ [µg/L] |
|--------------------------|---------------------|-------------------|---------------------|----------------|------------|------------|
| Naphtalene               | 220 nm              | 250 - 5000        | Y=0.0938X+0.1584    | 1.0000         | 27.24      | 86.74      |
| Acenaphthylene           | 230 nm              | 247 - 4949        | Y=0.1918X-9.1857    | 0.9996         | 50.62      | 151.85     |
| Acenaphthene             | 254 nm              | 992 - 9924        | Y=0.0069X-0.5515    | 0.9994         | 73.50      | 220.50     |
| Fluorene                 | 254 nm              | 99.4 - 994        | Y=0.0947-1.5382     | 0.9993         | 11.00      | 33.01      |
| Phenanthrene             | 254 nm              | 39.6 - 396        | Y=0.241X-0.9618     | 0.9990         | 5.00       | 15.01      |
| Anthracene               | 254 nm              | 20 - 200          | Y=0.4957X-0.9513    | 0.9995         | 3.77       | 11.30      |
| Fluoranthene             | 254 nm              | 49.1 - 491        | Y=0.0488-0.5064     | 0.9980         | 3.84       | 11.51      |
| Pyrene                   | 254 nm              | 100 - 1000        | Y=0.0271-0.2691     | 0.9980         | 99.31      | 297.94     |
| Benz(a)anthracene        | 254 nm              | 49.1 - 491        | Y=0.1299X-1.4534    | 0.9987         | 7.04       | 21.13      |
| Chrysene                 | 254 nm              | 50 - 500          | Y=0.2142X-3.0315    | 0.9993         | 3.29       | 9.86       |
| Benzo(b)fluoranthene     | 254 nm              | 19.8 - 198        | Y=0.1317X-0.7087    | 0.9999         | 17.35      | 52.04      |
| Benzo(k)fluoranthene     | 254 nm              | 20 - 200          | Y=0.0804X-0.0609    | 1.0000         | 25.24      | 75.71      |
| Benzo(a)pyrene           | 254 nm              | 49.6 - 496        | Y=0.1046X-2.1292    | 0.9980         | 42.61      | 127.82     |
| Dibenz(a,h)anthracene    | 220 nm              | 99.7 - 997        | Y=0.1273x+3.0551    | 0.9970         | 31.24      | 99.24      |
| Benzo(g,h,i)perylene     | 254 nm              | 200 - 791         | Y=0.0285X+0.5       | 0.9995         | 107.86     | 323.57     |
| Indeno(1,2,3-cd)pyrene   | 254 nm              | 49.8 - 498        | Y=0.1139X-1.3581    | 0.9992         | 24.60      | 78.35      |

The selection of extraction of soil were using three different methods (Soxhlet, ultrasonication and mechanical shaking) on the blank soil that have been spiked with the CRM PAHs standard and the results were compared to determine the method with the highest extraction efficiency.

The clean up process and also pre concentrating carried out by using solid phase extraction (SPE) technique. Using two types of eluant (dichloromethane and mixture of dichloromethane: acetone (9:2) to reduce the rate of evaporation due to low boiling point of dichloromethane [21].

The extraction efficiencies for most of the PAHs in the standard spiked soil using the sonication method were higher than Soxhlet and shaking except for benzo[a]pyrene (BaP) (Shaking 93%, Sonication 92%) and indeno[123-cd] pyrene (IP) (Shaking 84%, Sonication 82%). All method show recovery below 80% for naphthalene. Figure 2 shows the recovery of six PAHs in soil by the three extraction methods.
The time spent for ultrasonication was 20 minutes, mechanical shaker 30 minutes while Soxhlet took about 16 hours. Also, ultrasonication and shaker requires less solvent (30 ml) than Soxhlet which needs 350 ml. Soxhlet gave the lowest recoveries for four out of six investigated PAHs (Naph: 57%, Anth: 41%, BaP 72% and IP: 64%).

This study has shown that ultrasonication and mechanical shaking are a more efficient technique when compared to other traditional methods for extracting trace organics from soil and sediments (EPA 3540 and 3550). While mechanical shaker still needs 30 minutes for shaking, other study around 1 hour, ultrasonic only need 20 minutes. This is in line with previous studies showing ultrasonic extraction yields comparable or higher recovery in hydrocarbon extraction than other extraction techniques [21]. Table 3 showing the reproducibility test of PAHs using ultrasonic, shown good recovery > 80% except for Naphthalene.

Table 3. Recovery and reproducibility of PAHs in soil using ultrasonic method

| PAHs | Concentration µg/Kg | Average Concentration µg/Kg | Assign Value µg/Kg | % Recovery |
|------|---------------------|-----------------------------|--------------------|------------|
|      | I       | II      | III     |              |            |               |                  |
| Naph | 100.04  | 83.38   | 83.40   | 88.93 ± 7.85 | 125.44 ± 12.54 | 70.90          |
| Anth | 640.27  | 783.42  | 531.75  | 651.81 ± 103.07 | 633.83 ± 63.38 | 102.84         |
| Pyr  | 545.11  | 659.26  | 532.76  | 579.04 ± 56.95 | 618.74 ± 61.87 | 93.58          |
| BaA  | 148.15  | 126.55  | 131.87  | 135.52 ± 9.19 | 125.44 ± 12.54 | 108.03         |
| BaP  | 55.57   | 69.89   | 59.58   | 61.68 ± 6.03  | 67.91 ± 6.79  | 90.82          |
| IP   | 95.13   | 117.91  | 99.72   | 104.25 ± 9.84 | 124.50 ± 12.45 | 83.74          |

Losses of PAHs in Soxhlet extraction can be attributable to high temperatures used which can result in losses of hydrocarbons due to volatilization and/or oxidation of highly volatile and thermally labile species. The limit of quantification (POQ) of PAHs in soil was investigated in CRM standard spiked blank soil. Ultrasonic method gives most of the lowest detection from six investigated PAHs. Table 4 shown the limit of detection result in method for six PAHs.
Table 4. Limit of quantification 6 PAHs in soil

| PAHs                  | Concentration µg/Kg |
|-----------------------|---------------------|
| Naphthalene           | 35.55               |
| Anthracene            | 29.80               |
| Pyrene                | 27.98               |
| Benz[a]anthracene     | 52.89               |
| Benzo[a]pyrene        | 29.87               |
| Indeno(1,2,3-cd)Pyrene | 39.65               |

4. Conclusion

Extracting PAHs in soil method by using ultrasonic shows the most efficient method, coupled with SPE clean-up technique improves the method detection capability in HPLC.

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