

Supercritical fluid extraction of polynuclear aromatic hydrocarbons from <75- μ M wet-sieved sediments: A method development and validation study

Alma Oberes-Gonzales¹ and Mario B. Capangpangan*

*Department of Chemistry
Mindanao State University-Iligan Institute of Technology
9200 Iligan City, Philippines*

An analytical method for the isolation, detection, and quantitation of selected polynuclear aromatic hydrocarbons (PAHs) namely naphthalene, phenanthrene, fluoranthene, and pyrene from <75- μ m wet-sieved and closed-jar dried sediment samples has been developed and validated. Supercritical Fluid Extraction (SFE) and High Performance Liquid Chromatography (HPLC) were utilized as the sample preparation and analyte detection techniques, respectively. The developed method was evaluated in comparison with Soxhlet extraction as the reference method.

In the method development phase, the SFE method achieved an overall accuracy (% recovery) of 94.59%, with an overall precision of 1.02% RSD, while Soxhlet extraction had an overall accuracy of 67.99%, and an overall precision of 13.89% RSD. These preliminary results showed that SFE method has much better performance than Soxhlet extraction.

Moreover, in the method validation phase, the developed SFE method achieved an overall accuracy of 93.94%, with an overall precision of 3.47% RSD, while Soxhlet extraction had an overall accuracy of 68.66%, and an overall precision of 23.93% RSD. These results indicate that SFE method is more accurate and more precise than Soxhlet extraction, thus, proving it was highly acceptable for application purposes. Therefore, the developed SFE method has been validated.

Keywords: PAHs; sediments; supercritical; SFE; HPLC; method development; method validation

INTRODUCTION

Polynuclear aromatic hydrocarbons (PAHs) are identified as priority toxic pollutants by the United States Environmental Protection Agency (US-EPA) because of their carcinogenic and mutagenic properties, and adverse health effects have been linked [1]. The determination of PAHs in environmental samples is essential because they are described to be persistent as well as ubiquitous environmental pollutants [2].

PAHs appear to be widely distributed in the sea and coastal areas, as well as in river waters, soils, and sediments, since they are naturally occurring and are also formed by the pyrolysis of carbonaceous materials at high temperatures. Although

PAH levels in open lake or marine surface waters are low, they are readily adsorbed and accumulated by sediments and particulate matter [3]. The assessment of PAHs levels in sediments has attracted great interest, since PAHs are widely distributed in the marine sediments that serve as the source for toxic uptake of the benthic invertebrates, fish, and seaweed which represent an important food source for man [4]. Also, benthic organisms live in close association with sediments and detrital materials that constitute the storage depot of PAH in the aquatic system [5]. Moreover, they present a particular threat to marine life in the aquatic bodies and may exert effects on human health since these contaminants can concentrate in various food chains.

*Author to whom correspondence should be addressed.

Many researchers recommend that preference be given to the fine-grained fraction of sediments for sampling and analyses. The obvious reason for this selection is that a greater proportion of the volume of a particle is occupied by a surface coating as the particle size decreases. In addition, smaller particles such as clay minerals frequently have a greater reactivity to form organic coatings than do larger particles such as silicate sands [6]. Thus, this research study utilized <75- μ m wet-sieved sediments separated using sieve # 200 as the sampler for analysis.

There are several methods available for the extraction of PAHs from sediment samples. PAHs may be extracted with liquid organic solvents using conventional methods, such as Soxhlet extraction, mechanical shaking, ultrasonic extraction, and homogenization. These methods require a considerable amount of time and result in different efficiencies [7]. On the other hand, recognition of the health and safety hazards of commonly used organic solvents and their increasing purchase and disposal costs have resulted in additional interest in supercritical fluid extraction (SFE), despite the relatively large capital costs of this instrument [8]. SFE method is of particular importance for environmental monitoring where extraction of the target analytes from a complex and solid matrix can be the most difficult and time-consuming analysis step. This modern extraction technique takes advantage of the enhanced solvent power of supercritical fluids (SCF). The great solvent power of these fluids is due to its density and viscosity that are intermediate between those of a gas and a liquid. Moreover, solute diffusivity in SCF tends to be much higher than that of liquids, which allows relatively fast extraction kinetics [9].

Drying is an essential step in SFE analyses as moisture content of the samples influences recovery; high moisture yields lower recoveries [10]. The commonly used drying techniques for sediment samples are air-drying and freeze-drying. However, air-drying increases potential losses, cross-contamination when drying multiple samples, and exposure to air contaminants, while freeze-drying is time consuming, involves expensive equipment and is not compatible with volatile analytes [7].

The study of Capangpangan and Suffet (1997) [11] used a closed-jar drying method wherein filtered suspended sediments were dried in a closed jar with CaCl_2 as the desiccant at the bottom. This method is most appropriate when the target analytes include volatile PAH compounds such as naphthalene. Closed-jar drying method is comprised of tightly sealed system, minimizing volatilization of analytes and preventing contamination from the surroundings. This method of drying with modification was adapted in this study.

There have been no studies involving the SFE analysis of PAHs from <75- μ m wet-sieved and closed-jar-dried sediment samples. Thus, this study was conducted to develop a supercritical fluid extraction method for the isolation, detection, and quantitation of the concentrations of naphthalene, phenanthrene, fluoranthene, and pyrene from marine sediments

described previously, and to validate the developed method by comparison with Soxhlet extraction as the reference method. PAH concentration measurements were done using EPA Method 610, which specifies the use of reversed-phase liquid chromatography (LC) with fluorescence detection for the determination of PAHs [12, 13]. Sediment samples taken from a control site considered to be PAH-free, were used as PAH analyte matrix.

In the method development phase, recovery studies were done to optimize the different individual steps involved in the overall SFE method of analysis, and also, to optimize the overall SFE method itself. For sediment extraction, recovery studies are usually done by direct spiking of known amount of analyte onto an analyte-free sediment, and comparing the amount of analyte recovered after the analysis to the amount spiked. However, extraction of matrices to which analytes have been directly added or "spiked" is inadequate to evaluate a method's effectiveness at determining native concentrations because of the absence of typical analyte/matrix interactions in the laboratory spiked samples [14]. Thus, in this phase of the study, it was necessary to "load" reference sediments with known amounts of PAHs from an aqueous solution through actual sorption process. Here, the sediments were in continuous contact for 24 h with an aqueous solution of PAHs of known concentrations, during which time equilibrium sorption of the PAHs onto the sediments occurred. Such PAH-loaded reference sediments, after separation from the aqueous solution followed by drying, were subsequently used in the SFE recovery studies during method development and validation phase.

On the other hand, method validation is an integral part of the development of an analytical protocol. It is the process of proving that an analytical method is acceptable for its intended purpose. In the absence of a standard reference material, method validation is achieved by comparing the results from the developed method to in-house results obtained using a different and previously accepted method such as Soxhlet extraction [15]. Such was done in this study, using the same relatively clean sediment sample loaded with known amounts of PAH analytes. However, the method to be validated should depend on the findings from the recovery studies in the method development phase. Development is then considered complete and successful once the method has been tested and found to demonstrate acceptable analytical performance.

EXPERIMENTAL

Analytes and HPLC analysis. All individual primary stock solutions (10,000 ppm) of the PAH analytes (AR Grade from AJAX and MERCK) were prepared in acetonitrile (HPLC Grade). Then, a 4-component PAH stock mixture consisting of naphthalene (1006 ppm), phenanthrene (50 ppm), fluoranthene (1511 ppm), and pyrene (50 ppm) was subsequently prepared from the individual primary stock solutions.

An optimization of the HPLC operating parameters was done. A SPD-10AW Shimadzu HPLC with shrinkpack ODS stationary system band coupled with FR-551 spectrofluorometric detector was used for PAH analysis. The mobile phase (isocratic) was 80 + 20 acetonitrile water (HPLC) grade at 1.0 mL/min flow rate. The detector wavelength was set at 280 nm excitation and 389 nm emission [13]. Ten- μ L aliquots of the sample were injected into the HPLC at a stop time of 15 min.

Construction of the calibration curve. The standard calibration curve was constructed using the 4-component PAH mixture. The calibration solutions at different concentrations of naphthalene, phenanthrene, fluoranthene, and pyrene, were prepared by taking aliquots of 4, 8, 12, 14, and 16 μ L, respectively from the 4-component PAH stock mixture and diluting to 1.0 mL with acetonitrile solvent. Peak height ratios relative to the selected internal standard (5 ppm of 2-methylnaphthalene) were determined for each compound at each concentration, using identical injection volumes and the optimized operating conditions for the HPLC system. Least-squares analyses of the plot of concentration versus mean relative peak height were used to find the best straight line among the points. Given the mean peak height for the specified PAH, the concentration was extrapolated from the corresponding calibration curves.

Preparation of aqueous PAH solution of known concentration. Before preparing the aqueous PAH solutions, a trial 100- μ L spike of the prepared 4-component PAH stock mixture in acetonitrile was made into 10-mL volumetric flask with acetonitrile to quantitate the concentration of the PAH stock mixture by HPLC. From the calculated concentration, the spike volume of PAH stock mixture into 1 L solution was calculated such that the maximum saturation level of any of the analytes did not exceed 80%, to ensure a true solution.

Using the calculated spike volume of the 4-component PAH mixture, known amounts of PAH analytes were spiked into 1000 mL filtered artificial seawater in 2-L wide mouth jar. (The artificial seawater was prepared by dissolving 38 g iodized salt in 1000 mL deionized water [16]). The resulting aqueous PAH solution was used in the simulation of the actual sorption of the analytes onto the sediments.

Efficiency studies for silica gel clean-up step. The recovery studies for the silica gel clean-up step were done using three sets of 4-component PAH mixtures at four trials each. The mixtures were prepared by taking aliquots of 9, 13, and 15 μ L, from the PAH stock mixture used in preparing the calibration solutions, and diluting to 3.0 mL each with methylene chloride solvent. A pasteur pipette (230 \times 5 mm) with glass-wool plug and prepared silica gel (1.0 g; activated at 130°C for 24 h before use) was used as the clean-up column. Three mL each of the previously prepared 4-component test PAH mixtures were passed through the column. The column was then eluted using the following sequence of solvents (4 mL each): n-hexane, n-hexane:methylene chloride mixture (2:1), and methylene chloride. The last fraction which should contain the PAH

analytes was added with 1.5 mL acetonitrile to exchange the methylene chloride solvent (cyclohexane for Soxhlet) with acetonitrile by purging with nitrogen gas stream to 1.0 mL final volume. After spiking a known amount (volume) of internal standard (2-methylnaphthalene), the sample was injected into the HPLC [13].

The reference PAH mixtures for the clean-up recovery studies were prepared in quadruplicate by obtaining aliquots of 9, 13 and 15 μ L from the 4-component PAH stock mixture and diluting each to 1.0 mL with acetonitrile. The PAH mixtures were directly spiked with the internal standard (resulting in a 5-ppm final concentration) prior to HPLC analysis. The % recovery of the PAH analytes was subsequently calculated relative to the reference solution.

Efficiency studies for solvent exchange step by purging with ultrahigh purity N_2 gas. The recovery studies for the solvent exchange step through purging with N_2 were done in four trials utilizing two sets of PAH mixtures. The mixtures were prepared by taking aliquots of 7 and 11 μ L from the PAH stock mixture used in preparing the calibration solutions. The test PAH mixtures were added with 1.5 mL acetonitrile to exchange the methylene chloride solvent with acetonitrile by purging with N_2 gas. The resulting 1 mL PAH mixture after purging was spiked with the internal standard to a final 5-ppm concentration prior to HPLC analysis.

The reference PAH mixtures for the solvent exchange step were prepared in quadruplicate by obtaining aliquots of 7 and 11 μ L from the 4-component PAH stock mixture and diluting to 1.0 mL each with acetonitrile. The PAH mixtures were directly spiked with the internal standard resulting in a 5-ppm final concentration prior to HPLC analysis.

Collection of sediment samples. The reference sediment samples were randomly collected from the coastal area of Barangay Pangasihan in Gingoog Bay during low tide 20 m away from the highest high-tide level. There are only few industries located in this area and it is a good site for fishing, thus, it could be considered a relatively clean area. The 2–10 cm portion from the surface of the sediment bed load phase was collected with a modified corer using PVC pipe with 14-cm diameter. The sediment sample was first wet-sieved using a screen with an opening of 1/8 in to remove the larger particle size portion of the sediment. Subsequent wet-sieving of the previously sieved sediment sample was done using a screen with a 1/32-in opening followed by sieve # 100 (with 150 μ m screen opening). Final wet-sieving was done using sieve # 200 (with 75 μ m screen opening). The sieve pan or catch basin containing the sieved sediment sample with the water was allowed to stand for 1 h. The water portion was separated by siphoning using plastic tubing with internal diameter of 1/4 in to avoid disturbance of the settled very fine particles. The sieved sediment samples were then placed in a glass container, stored in an icebox and transported to the MSU-IIT Chemistry Laboratory.

The water content of the sieved sediment samples was determined prior to further chemical analysis as follows: The wet/dry wet-sieved sediment sample was transferred to a porcelain crucible and weighed. The sample was dried in an oven at a temperature of 105 to 110°C, transferred to a desiccator to cool-off, and then weighed to constant weight to the nearest 0.0010 g in an analytical balance.

Closed-jar drying method: Determination of the optimum weight of the sediment sample at constant amount of desiccant for efficient drying. The closed-jar drying method was first developed by Capangpangan and Suffet (1996) to dry filtered suspended sediments for subsequent supercritical fluid extraction [11]. It consists of CaCl_2 as the desiccant placed at the bottom of a wide-mouth jar with an air-tight lid. However, it has not been used to dry wet-sieved sediments for subsequent SFE. Thus, the method was optimized for wet-sieved sediment samples.

1 g, 5 g, 10 g, 20 g, and 30 g of the wet-sieved sediment samples were placed in five different drying jars for 24 h. The jars contained a fix amount (100 g) of calcium chloride as the desiccant. The optimum weight of the sediment sample that 100 g of desiccant could dry was determined by evaluating the % moisture remaining in the sediment samples after 24 h. The resulting dry sediment was extracted immediately through SFE or Soxhlet after drying.

Calcium chloride was used as the desiccant because in the study of Capangpangan and Suffet (1996), this drying agent resulted in the fastest removal of water compared to calcium sulfate, magnesium sulfate, and sodium sulfate. In that study, CaCl_2 removed 99.8% of the water content from the suspended sediments after only one day [11].

Sorption of analytes (Loading) onto sediment samples by equilibration with aqueous PAH solution. For the sorption process, 5-g portions of the sediment samples were exposed in the aqueous PAH solution on a shaker for 24 h prior to extraction. This loading method was based from the study of Capangpangan and Suffet in 1996 [11]. Results from that study showed that the amount of analytes sorbed onto the sediment samples during the 24-h contact period was sufficient for subsequent SFE analysis. Thus, in this study, the 24-hour sorption was followed as the optimum number of hours of sorption. Furthermore, the reference aqueous PAHs solution with no sediments was also allowed to stand on a shaker for 24 h. The aqueous solution, which was in contact with the sediments during sorption of the analytes, was allowed to stand for 30 min and the sorption solutions were subsequently pipetted out. Each decantate was channeled directly into a I-L bottle for subsequent analysis by liquid-liquid extraction as described below. The sediment samples were collected by a spatula and then dried by closed-jar drying method. The dried sediment samples were directly analyzed through SFE and Soxhlet as described below.

Liquid-liquid extraction (LLE) of the aqueous PAH solutions. Quadruplicate liquid-liquid extractions of reference solutions and of the decantates were completed with methylene chloride as described in Reference 18. The final extracts were subjected to the clean-up step using silica gel column, then quantitated through HPLC analysis to determine the initial and final concentration of the PAH analytes. The amount of analytes sorbed onto the sediment sample during the contact period was calculated based from the difference in the amounts extracted from both the reference and sorption solutions.

Supercritical fluid extraction of dry sediment samples. The SFE experiments were done using a SFX 2130 Supercritical Fluid Extractor System (ISCO, Lincoln, Nebraska, U.S.A.), with carbon dioxide as the solvent. Two-gram samples of dry wet-sieved sediments loaded and not loaded (blank samples) with the PAH analytes were extracted using a pressure (P) of 355 atm., a temp. (T) of 120°C, and a modifier (M) mixture comprised of 250 μL each of water, methanol, and methylene chloride. This set of SFE conditions was proven by Capangpangan and Suffet in 1997 [11] as optimum conditions and resulted in highest percentage recoveries in the extraction of PAHs from suspended solids. Each sample was extracted sequentially two times at the same P-T-M combination mentioned above, for 5 min static and 20 min dynamic extraction, adding the modifier before each extraction sequence. The collection solvent consisted of pre-chilled methylene chloride. After two sequential extractions, the extracts were combined. Then, the resulting extract was concentrated using a Kuderna-Danish (K-D) evaporator to a final volume of 3 mL. The concentrated extract was passed through a silica gel column for the clean-up step prior to HPLC analysis. Amounts of PAHs extracted were then calculated.

Soxhlet extraction of the dry sediment samples. Two-gram samples of sediments loaded and not loaded (blank samples) with the PAH analytes was Soxhlet-extracted with 200 mL cyclohexane for 6 h at a rate of five cycles per hour. The extract collected in a round bottom flask was evaporated to 3 mL using Kuderna-Danish evaporator to a final volume of 3 mL. The clean-up of the concentrated extract was done using silica gel clean-up method prior to HPLC analysis. Amounts of PAHs extracted were then calculated.

Validation of the developed SFE method. Following the same procedure done in the method development phase, the reference sediment samples were collected, wet-sieved, closed-jar dried, and loaded with the PAH analytes from aqueous solution. LLEs of the reference and sorption solutions were done. SFE and Soxhlet analysis of the reference samples loaded and not loaded with the PAH analytes were subsequently done. Resulting extracts were concentrated, subjected to clean-up step and quantitated through HPLC analysis. Evaluation and calculation of data are similar to the steps done in the method development phase. Results were then compared to the results from method validation phase.

RESULTS AND DISCUSSION

Method development phase. An important step in the success of the study was the preparation of a true PAH aqueous solution to be used in loading the PAH analytes onto the sediment samples by actual sorption process. A maximum saturation level of 80% was targeted for any of the analytes, in order to safely prevent precipitation of the PAHs in the aqueous solution, thereby ensuring a true PAH solution. Results would certainly be erroneous if precipitation of the analytes would occur during the sorption process.

Table 1 shows the calculated amounts and saturation levels of PAHs in the prepared aqueous PAH solution. Note that the percent saturation levels are all below 80%, hence, precipitation of the PAH analytes was possibly prevented, resulting in a true solution. The results are highly acceptable based from the %RSD values which are less than 5% for the mean amount calculated.

Silica gel clean-up method was conducted prior to quantitation of PAHs in the extracts in order to investigate whether there might be losses of PAH analytes after this step or other chemical compounds that will interfere in the detection of PAH analytes. Table 2 shows that the recoveries from silica gel clean-up method are above 90%. Thus, silica gel clean-up method is a highly efficient step because losses are insignificant and results are statistically reliable as shown from the %RSD values obtained which are significantly less than 10%.

After the clean-up step, the extracting solvent (methylene chloride or cyclohexane) had to be exchanged with acetonitrile prior to HPLC analysis. To evaluate whether there might be losses of PAH analytes after exchanging the extracting solvents with acetonitrile (by purging with ultrahigh purity N_2 gas), a recovery study was done. Results from Table 2 show that reduction of the amount of PAH analytes after this step was insignificant. The mean recoveries obtained from four trials for two sets of PAH mixtures were above 90%. The data are reliable as seen from their %RSD values which are less than 10%.

Table 1. Calculated amounts and saturation levels of PAHs in the aqueous PAH solution.

| PAHs | Mean Amount or Total μ g Spiked Into 1 L H_2O [%RSD] | Concentration in H_2O (μ g/L) [C_{H_2O}] | Solubility (μ g/L) [S_{ol}] | Saturation Level (%) ¹ |
|--------------|--|---|--------------------------------------|-----------------------------------|
| Naphthalene | 258.270 [1.67] | 258.27 | 31700 | 0.81 |
| Phenanthrene | 223.343 [1.73] | 223.34 | 1290 | 17.31 |
| Fluoranthene | 199.627 [4.32] | 199.63 | 260 | 76.78 |
| Pyrene | 107.719 [4.84] | 107.72 | 135 | 79.79 |

¹%Saturation Level = $([C_{H_2O}]/[S_{ol}]) \times 100$

Values represent the mean from four trials and values in bracket represent the mean percent relative standard deviation.

Table 2. Percent recoveries from the silica gel clean-up method and the solvent-exchange-to-acetonitrile step by purging with ultrahigh purity N_2 gas.

| PAH Mixtures | Overall Mean %Recovery [%RSD] | |
|--------------|---------------------------------------|------------------------------------|
| | Silica Gel Clean-up Step ¹ | Solvent Exchange Step ² |
| Naphthalene | 96.79 [0.62] | 98.68 [0.73] |
| Phenanthrene | 98.18 [3.62] | 97.35 [0.67] |
| Fluoranthene | 96.30 [2.94] | 98.48 [1.71] |
| Pyrene | 96.92 [2.53] | 97.50 [1.87] |

¹Values represent the mean from three measurements in four trials.

²Values represent the mean from two measurements in four trials. Values in bracket represent the mean percent relative standard deviation.

Table 3. Results from optimization of closed-jar drying method by exposing varied weights of wet-sieved sediment samples to 100 g $CaCl_2$ for 24 h.

| Mean Weight of Sediment Sample (g) [% RSD] | % Moisture Remaining |
|--|----------------------|
| 1.0978 [2.15] Mean (% RSD) | 0.06 ([13.61]) |
| 5.0879 [0.77] Mean (% RSD) | 1.28 ([5.20]) |
| 10.0766 [0.49] Mean (% RSD) | 3.11 ([4.08]) |
| 20.1028 [0.03] Mean (% RSD) | 22.52 ([9.86]) |
| 30.1025 [0.04] Mean (% RSD) | 23.24 ([4.43]) |

Values represent the mean from four trials and values in bracket represent the mean percent relative standard deviation.

Although drying is achieved at the expense of losing the low molecular mass (volatile) analytes, such loss is relatively small when closed-jar drying is used [17]. Table 3 shows the profile of water removal from varied weights of the sediment samples exposed to 100 g $CaCl_2$ for 24-h. Note that the sample corresponding to 1 g was efficiently dried, resulting in the least % moisture remaining. This low moisture content satisfies the requirement for SFE analysis since water affects the extraction efficiency of the supercritical fluid CO_2 .

On the other hand, the samples of greater weights (5 g, 10 g, 20 g, 30 g) had greater % moisture remaining. Thus, subsequent closed-jar drying was done by using 1-g samples of sediment per 100 g of $CaCl_2$ desiccant. The percent moisture remaining was determined prior to further analysis because all of the amounts calculated were based on a dry-weight basis.

Table 4. Preliminary results from the recovery study for the overall SFE method as obtained in the method development phase.

| PAHs | Amount Extracted (μg) [%RSD] | | Amount Sorbed ¹ (μg) [%RSD] | Amount Extracted (μg) [%RSD] | | % Recovery (μg) [%RSD] | |
|--------------|--|-------------------|---|--|-------------------|--|------------------|
| | Reference Solution | Sorption Solution | | SFE | Soxhlet | SFE | Soxhlet |
| Naphthalene | 238.906 [0.51] | 215.678 [0] | 23.227 [5.24] | 21.682 [13.39] | 12.554 [24.22] | 93.15 [8.19] | 54.46 [29.26] |
| Phenanthrene | 168.673 [2.60] | 110.645 [1.60] | 58.028 [5.15] | 55.138 [2.69] | 41.140 [5.66] | 95.02 [6.15] | 70.99 [6.74] |
| Fluoranthene | 186.869 [5.31] | 60.122 [7.55] | 126.747 [8.51] | 119.928 [8.94] | 88.297 [4.43] | 95.08 [12.01] | 70.11 [11.03] |
| Pyrene | 94.756 [7.69] | 26.746 [5.38] | 68.012 [8.59] | 64.358 [1.59] | 52.028 [11.51] | 94.92 [7.06] | 76.41 [2.93] |
| Overall Mean | | | | | | 94.59 [1.02] | 67.99 [13.89] |

¹Amount Sorbed = Amount in Reference Solution – Amount in Sorption Solution

²% Recovery = (Amount Extracted SFE/Soxhlet)/(Amount Sorbed) \times 100

Table 4 shows the initial performance data of the overall SFE method as obtained in the method development phase of the study. The fourth column shows the amount of PAHs sorbed onto the sediments during the 24-h contact period. The sorption data showed acceptable precision with %RSD ranging from 2 to 14%. The amount sorbed was based from the difference of the reference solution and the sorption solution, and was the basis in the evaluation of percent recoveries of the extraction process such as SFE and Soxhlet. Any loss of analyte not due to sorption (e.g., volatilization during the contact period, separation or extraction) was accounted for by the fact that both the sorption solutions (with sediment samples) and the reference aqueous solution (without sediment samples) were subjected to the same shaking, settling after shaking, separation by pipetting out, and extraction procedures.

The PAH analytes sorbed (loaded) onto the sediment sample were extracted through both SFE and Soxhlet methods. However, native PAHs may be initially present in the reference sediment sample. Thus, the reference sediment samples not loaded with the PAH analytes were also analyzed through both SFE and Soxhlet methods. The amounts of PAHs obtained were then deducted from the corresponding amounts extracted from the sediments loaded with the analytes.

Table 4 (5th and 6th columns) shows the amounts extracted through SFE and Soxhlet analysis after deduction of the amount of PAHs initially present in the sediment samples. Note that the values obtained are acceptable as shown from the %RSD values which ranged from 1.59 to 13.39% for SFE and 4.43 to 24.22% for Soxhlet analysis.

The recoveries for SFE and Soxhlet based from the sorbed amount (difference from LLE of reference and sorption solutions) were subsequently calculated. Table 4 (7th and 8th columns) shows the recoveries obtained based from SFE and Soxhlet analysis. Results show that SFE analysis is much more

efficient, with individual recoveries greater than 90%, compared to the classical Soxhlet method, with recoveries ranging from 54.46% to 71.40%. The values obtained are acceptable as seen from the individual %RSD values which ranged from 6.15 to 12.01 for SFE and 6.74 to 29.26 for Soxhlet analysis. Furthermore, the overall SFE accuracy or % recovery of 94.59% and overall precision of 1.02% showed the superiority of SFE over Soxhlet, with 67.99% overall accuracy and 13.89% overall RSD.

Figure 1 shows the graphical comparison of SFE to Soxhlet. This graph shows the degree of discrepancy between the two extraction procedures. SFE resulted in higher yields and better results compared to Soxhlet. Therefore, the method for the supercritical fluid extraction of PAH analytes from <75- μm wet-sieved and closed-jar dried sediment sample has been developed, and thus, was now ready for validation stage.

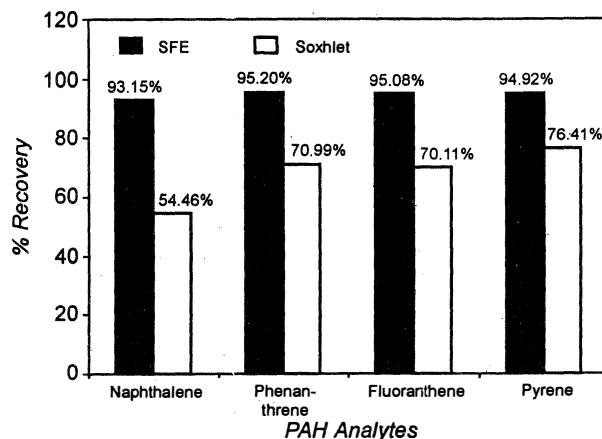
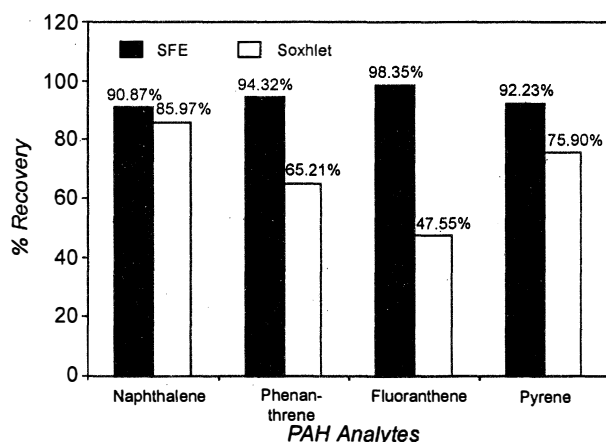


Fig. 1. Graphical comparison of percent recoveries from SFE method and Soxhlet extraction analysis as obtained in the method development phase.

Table 5. Final results from the recovery study for the overall SFE method as obtained in the method validation phase.

| PAHs | Amount Extracted (μ g) [%RSD] | | Amount Sorbed ¹ (μ g) [%RSD] | Amount Extracted (μ g) [%RSD] | | % Recovery ² (μ g) [%RSD] | |
|--------------|---------------------------------------|-------------------|--|---------------------------------------|-------------------|--|------------------|
| | Reference Solution | Sorption Solution | | SFE | Soxhlet | SFE | Soxhlet |
| Naphthalene | 142.667 [7.72] | 128.448 [7.18] | 14.219 [5.24] | 12.965 [18.03] | 12.270 [18.42] | 90.87 [5.45] | 85.97 [5.85] |
| Phenanthrene | 156.341 [1.09] | 116.212 [0.57] | 40.129 [2.59] | 37.304 [2.52] | 26.116 [1.11] | 94.32 [3.39] | 65.21 [4.12] |
| Fluoranthene | 109.601 [7.33] | 30.178 [13.59] | 79.423 [6.47] | 77.822 [2.24] | 37.713 [4.62] | 98.35 [8.68] | 47.55 [4.51] |
| Pyrene | 48.479 [12.71] | 9.593 [4.64] | 38.887 [14.73] | 35.382 [6.67] | 29.061 [4.39] | 92.23 [13.82] | 75.90 [14.34] |
| Overall mean | | | | | | 93.94 [3.47] | 68.66 [23.93] |

¹Amount Sorbed = Amount in Reference Solution – Amount in Sorption Solution²% Recovery = (Amount Extracted SFE/Soxhlet)/(Amount Sorbed) \times 100**Fig. 2. Graphical comparison of percent recoveries from SFE method and Soxhlet extraction analysis as obtained in the method validation phase.**

Method validation. Table 5 shows the final validation results for the overall SFE method, as obtained in the method validation phase. As in the method development phase, the values for the amount sorbed in Table 5 (4th column) have acceptable precision, as shown from the %RSD values which range from 2.59 to 14.73%.

SFE and Soxhlet analysis of the reference sediment samples not loaded with the PAH analytes were again done to evaluate the presence of native PAHs. These values were deducted from the amount extracted through SFE and Soxhlet respectively. Table 5 (5th and 6th columns) shows the amount of PAHs extracted from SFE and Soxhlet analysis. The values obtained have acceptable precision as seen from their %RSDs which range from 2.24 to 18.03 for SFE and 1.11 to 18.42% for Soxhlet.

On the other hand, Table 5 (7th and 8th columns) shows that the recoveries from SFE are above 90% while that from Soxhlet are below 86%, confirming that SFE is a better technique for extracting PAH analytes from <75- μ m wet-sieved and closed-jar dried sediment samples. Figure 2 shows the graphical comparison of PAH recoveries from SFE and Soxhlet analysis. The values obtained have acceptable precision, as shown from the individual %RSD values of 3.39 to 13.82% in Table 5.

Furthermore, as in the method development phase, SFE showed a more superior overall accuracy or % recovery of 93.94% and overall precision of 3.47% RSD compared to Soxhlet, with 68.66% and 23.93% RSD, respectively. Note also that these overall values agree closely with the overall values obtained in the method development phase of the study (Table 4), indicating excellent reproducibility of the results obtained in the two phases of the study. This in turn indicates high reliability of the results of the entire study.

Therefore, based on the results, a method for the isolation, detection, and quantitation of target polynuclear aromatic hydrocarbons (PAHs) namely naphthalene, phenanthrene, fluoranthene, and pyrene, from <75- μ m wet-sieved and closed-jar dried sediment sample, has been developed and validated. supercritical fluid extraction (SFE) and high performance liquid chromatography (HPLC) were used as the sample preparation and analyte detection techniques, respectively. The validated method is environment-friendly, faster, and more efficient than the conventional Soxhlet extraction method. Thus, this method will surely be of great use in the determination and assessment of PAHs contamination in sediments in different parts of the country.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Science and Technology-Philippine Council for Advanced Science and Technology Research and Development (DOST-PCASTRD) for the thesis financial assistance, and the Commission on Higher Education (CHED) for providing the ISCO Supercritical Fluid Extractor (SFX 2130) System under the Center of Excellence (Chemistry) Project.

REFERENCES

1. Polynuclear Aromatic Hydrocarbons: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vols. 32–35 (IARC, Lyon, France, 1984-1985).
2. Dipple, A. *American Chemical Society*, ACS Monograph 173, pp. 245–314. (Washington DC, 1976).
3. Griger, W. and Schaffner, C. *Anal. Chem.* 50, 243 (1978).
4. Eisler, R. *U.S. Fish Wildlife Serv. Biol. Rep.* 85 (1.11), (1987).
5. De Leon, I. R., Ferrario, J. B., and Byrne, C. J. *Bull. Envir. Contam. Toxicol.* 41, 872 (1988).
6. Trask, P. D., *Recent Marine Sediments, A Symposium*, Ed., pp. 428–453. (Tulsa, O.K.; American Association of Petroleum Geologists, 1939).
7. Greibrokk, T. *J. Chromatogr.* 33, 626 (1992).
8. Levitz, P. and van Damme, H. J. *Phys. Chem.* 90, 1302 (1986).
9. Mira, B., Blasco, M., and Subirats, S. *The Journal of Supercritical Fluids.* 9, 238 (1996).
10. McNally, M. D. *Analytical Chemistry.* 67 (7), 1995.
11. Capangpangan, M. B. and Suffet, I. H. *J. Chromatogr.* 738, 253 (1996).
12. Wise, S. A. et al. *Analysis Magazine.* 1, 20 (1992).
13. Lee, M. L., Novotny, M., and Bartle, K. D. *Analytical Chemistry of PAHs.* (Academic Press: New York, 1981).
14. Bowadt, S. and Johansson, B. *Anal. Chem.* 66, 667 (1994).
15. Green, J. M. *Analytical Chemistry News and Features.* pp. 305A–307A (1 May 1996).
16. McLaughlin, R. Jr. B. and Neff, J. M. *Mar. Biol.* (1979).
17. Capangpangan, M. B. and Suffet, I. H. *J. Chromatogr. A* 782, 247 (1997).
18. APHA, AWWA, and WEF. *Standard Methods for the Examination of Water and Wastewater*, 19th Edition, pp. 6–103. (American Public Health Association, 1995).