CHAPTER III

EXPERIMENTAL

1. Apparatus

A Waters™ Controller, model 600

A Waters™ Autosampler, model 717

A Waters™ Pump, model 600

A Waters™ Photodiode Array Detector, model 996

A COMPAQ Computer

A LaserJet 4L Printer, Hewlett Packard, USA.

A Silicon Power Supply, SR-111

A Nova-Pak® C_{18} Column 150 x 3.9 mm I.D., 4 µm, Waters, Millipore, USA.

A Vydac™, C_{18} Column 250 x 4.6 mm I.D., 5 µm, The Separations Group, USA.

A Vydac™, C_{18} High performance guard column 30 x 2 mm I.D., 5 µm, The Separations Group, USA.

A Guard Cartridge Holder, Hewlett Packard, USA.

A Waters Vacuum Pump, model DOA-V130-BN, with Pressure Regulator, Millipore, USA.

A Glass Filter Holder Set (300 mL Funnel, 1 L Flask, Glass base and tube cap, and 47 mm Spring clamp) for HPLC mobile phase filtration xx1504700, Millipore, USA.

Membrane Filters, type HA 0.45 µm, Millipore, USA.

Membrane Filters, type FH 0.50 µm, Millipore, USA.

Swinny Stainless Steel Filter Holders, 13 mm xx3001200, Millipore, USA.

'BAKER'-10 SPE™ System, J.T. Baker, USA.

'BAKER' Disposable Extraction Columns Resorvoir 75 mL, J.T. Baker, USA.

An Alba Watch, sw01-x001, Japan

Helium Gas 99.99% purity, TIG, Thailand

Empore™ Extraction Disks (Bakerbond Extraction Disks) with Bakerbond C_{18}, 47 mm I.D., Production of 3M Co. USA., Distributed by J.T. Baker Inc., USA., were cut to 13 mm I.D. using the tool shown in Appendix A.

A Syringe Pumps 10 mL, Kloehn, USA.

A Microsyringe 25 μL, Hamilton, USA.

A Microsyringe 100 μL, ITO corporation, Japan

Graduated pipettes 1.00, 5.00,and 10.00 mL

Volumetric pipette 5.00 mL

Volumetric flasks 5.00, 10.00, 25.00, 50.00, 100.00, and 250.00 mL

Beakers 10, 50, 150, 250, 500, and 1000 mL

Vials 1 mL with caps, Millipore, USA.

Vials 4 mL, Millipore, USA.

Caps for 4 mL vials, Vidhyasom Co.,Ltd., Thailand

All glass apparatus were washed in detergent, throughly rinsed with double distilled water and then soaked in dilute HNO_3 (1:1) overnight. The glass apparatus were then rinsed with double distilled water and baked in an oven at 150°C for at least 3 hours, except volumetric flasks and pipettes. In the last step, all glass apparatus were double rinsed with elution solvents.

2. Chemicals

2.1 The Standard of PAHs

PAHs mixture certified chemical standard 500 ppm in acetonitrile was purchased from Hewlett Packard, USA. comprising Acenaphthene (Acent), Acenaphthylene (Acenl), Anthracene (Ant), Benzo[a]anthracene (BaA), Benzo[b] fluoranthene (BbF), Benzo[k]fluoranthene (BkF), Benzo[g,h,i]perylene (Bghi), Benzo [a]pyrene (BaP), Chrysene (Chry), Dibenzo[a,h]anthracene (Dah), Fluoranthene (Flt),

Fluorene (Flu), Indeno[1,2,3-cd]pyrene (Ind), Naphthalene (Naph), Phenanthrene (Phen) and Pyrene (pyr).

2.2 Organic Solvents

Dichloromethane and acetonitrile were obtained from J.T. Baker, USA. Methanol was obtained from Mallinckrodt, France and 2-propanol was obtained from BDH, England.

All solvents were analytical grade (AR Grade) and they were purified by fraction distillation in all glass apparatus and the distilled was checked for purity by high performance liquid chromatography prior to use.

2.3 Reagents

Nitric acid was analytical grade from J.T. Baker, USA., Brij-35 was purchased from Fluka, Switzerland, and NANOpure® analytical grade water was obtained from Barnstead Ultrapure Water System.

3. Preparation of the Standard Solutions

3.1 The Stock Standard Solution of PAHs Mixture in Acetonitrile

The 100.00 ppm stock standard solution of PAHs mixture was prepared by pipetting 1.00 mL of 500 ppm PAHs mixture certified chemical standard into 5.00 mL volumetric flask and then filling it to the mark with acetonitrile.

The 5.00 ppm stock standard solution of PAHs mixture was prepared by pipetting 0.50 mL of 100.00 ppm stock standard solution into 10.00 mL volumetric flask and then filling it to the mark with acetonitrile.

The 1.00 ppm stock standard solution of PAHs mixture was freshly prepared by pipetting 2.00 mL of 5.00 ppm stock standard solution into 10.00 mL volumetric flask and then filling it to the mark with acetonitrile.

3.2 The Solution of Brij-35 in Water

The 10 mM Brij-35 solution was prepared by dissolving 6.0000 g of brij-35 and filling it to the mark with water in 500.00 mL volumetric flask.

All of the solutions were stored at 4°C except 1.00 ppm stock standard solution of PAHs mixture.

4. The Study of High Performance Liquid Chromatographic Conditions

The HPLC conditions in EPA Method 550.1 were modified by vary flow rate, percent of mobile phase, and equilibration time in order to obtain the optimum HPLC conditions for this study.

All of the standard solutions and extracted from spiked standard solutions and sample solutions were analysed by the optimum HPLC conditions.

5. The Creation of Spectral Library

The spectral library was created from the chromatogram of 1.00 ppm standard solution of PAHs mixture that injected into HPLC with the optimum conditions.

6. The Study of Linearity of Standard PAHs

The procedure for the study of linearity of standard PAHs could be described as follows :

6.1 The concentration of standard solutions of PAHs mixture 0.10, 0.20, 0.40, 0.60, 0.80, 5.00, 10.00, 20.00, and 40.00 ppm were prepared from the 100.00 ppm stock standard solutions of PAHs mixture.

6.2 The concentration of standard solutions of PAHs mixture 0.10, 0.20, 0.40, 0.60, 0.80, 5.00, 10.00, 20.00, 40.00,and 100.00 ppm were injected respectively into HPLC.

6.3 The relationships between concentration and peak area were plotted.

7. The Study of Calibration Curve of Standard PAHs

The procedure for the study of calibration curve of standard PAHs could be described as follows :

7.1 The concentration of standard solutions of PAHs mixture 100.00, 200.00, 400.00, 600.00, and 800.00 ppb were injected respectively into HPLC.

7.2 The relationships between concentration and peak area were plotted.

8. The Study of Detection Limit

The detection limit of the instrument was defined as the amount of analyte in standard solutions that yields a peak at signal-to-noise ratio equal to 3 (71). In this study would be triplicate analysis. The procedure could be described as follows :

8.1 The standard solutions were prepared by pipetting each volume of 1.00 ppm standard solution of PAHs mixture into the series of 5 mL volumetric flasks and then filling it to the mark with acetonitrile.

8.2 Each standard solutions prepared in step 8.1 were injected into HPLC.

9. The Procedure for Extraction of Spiked Standard Solution

The procedure for extraction of spiked standard solution could be described as follows :

9.1 A 13 mm diameter disk was inserted into the 13 mm swinny stainless steel holder. The holder was placed on 'BAKER'-10 SPE^{TM} System and fitted with a 75 mL 'BAKER' Disposable Extraction Columns Resorvoir that were shown in Appendix A.

9.2 The disk was cleaned with 5.00 mL dichloromethane by adding the dichloromethane to the disk, drawing about half of dichloromethane through the disk and allowing it to soak for about a minute, then drawing dichloromethane through the disk. After that, vacuum was maintained for 5 min to remove all dichloromethane.

9.3 The disk was conditioned with 5.00 mL methanol by adding the methanol to the disk, drawing about half of methanol through the disk and allowing it to soak for about a minute, then drawing methanol through the disk. When the methanol was just above the top surface of the disk, immediately pipetted 5.00 mL water onto the disk and drew it through the disk. Do not allow any air to pass through the disk or to reach the top surface of the disk in this step.

9.4 The spiked standard solution was poured into the disk, directly onto the film of water left on the disk from the last conditioning step and drew it through the disk. The disk must not go dry until the entire solution has been processed. After that, vacuum was maintained for 5 min to dry disk.

9.5 Once the disk was dry, it was removed from its support and transferred directly to a 4 mL vial by using forcep. Acetonitrile 0.50 mL was pipetted into this vial. The vial was then capped and standed for 15 min to allow for complete elution before injected into HPLC.

10. The Study of Various Effects on the Percent Recovery of Small Disk Extraction Method

The various effect on the percent recovery of small disk extraction method including the concentration of 2-propanol, the concentration of Brij-35, the volume of eluting solvent, the elution time, and the breakthrough volume were studied in order to determine the optimum condition of this method. All determinations were performed in triplicate.

10.1 The Effect of The Concentration of 2-Propanol

The concentrations of 2-propanol were studied at 0, 5, 10, 15, and 20% in spiked standard solution. The procedure for the study of this effect on the percent recoveries of each PAHs could be described as follows :

10.1.1 The spiked standard solutions were prepared by pipetting 2-propanol 0, 5, 10, 15, and 20 mL respectively into the series of 100.00 mL volumetric flasks spiked with 40.00 μL of 5.00 ppm standard solution of PAHs mixture and then

filling it to the mark with water. The 2.00 ppb spiked standard solutions were already prepared.

10.1.2 Each spiked standard solution prepared in step 10.1.1 were extracted by procedure in 9.

10.1.3 The percent recoveries of each PAHs were calculated.

10.1.4 The relationships between the percent recoveries and each of PAHs for each of 2-propanol concentrations were plotted in a curve.

10.2 The Effect of The Concentration of Brij-35

The concentrations of Brij-35 were studied at 0, 0.05, 0.11, 0.30, and 0.50 mM in spiked standard solution. The procedure for the study of this effect on the percent recoveries of each PAHs could be described as follows :

10.2.1 The spiked standard solutions were prepared by pipetting 10 mM Brij-35 solution 0, 0.5, 1.1, 3.0, and 5.0 mL respectively into the series of 100.00 mL volumetric flasks spiked with 40.00 µL of 5.00 ppm standard solution of PAHs mixture and then filling it to the mark with water. The 2.00 ppb spiked standard solutions were already prepared.

10.2.2 Each spiked standard solution prepared in step 10.2.1 were extracted by procedure in 9.

10.2.3 The percent recoveries of each PAHs were calculated.

10.2.4 The relationships between the percent recoveries and each of PAHs for each of Brij-35 concentrations were plotted in a curve.

The optimum concentration of Brij-35 found in this section would be used in the next studies.

10.3 The Effect of The Volume of Eluting Solvent

The volumes of eluting solvent were studied at 0.50, 0.75, and 1.00 mL. The procedure for the study of this effect on the percent recoveries of each PAHs could be described as follows :

10.3.1 The spiked standard solutions were prepared by pipetting optimum volume of 10 mM Brij-35 solution (from 10.2) into the series of 100.00 mL volumetric flasks spiked with 40.00 μL of 5.00 ppm standard solution of PAHs mixture and then filling it to the mark with water. The 2.00 ppb spiked standard solutions were already prepared.

10.3.2 Each spiked standard solution prepared in step 10.3.1 were extracted by procedure in 9 but used various volumes of eluting solvent.

10.3.3 The percent recoveries of each PAHs were calculated.

10.3.4 The relationships between the percent recoveries and each of PAHs for each of volumes of eluting solvent were plotted in a curve.

10.4 The Effect of The Elution Time

The elution time were studied at 5, 15, 30, 45, and 60 min. The procedure for the study of this effect on the percent recoveries of each PAHs could be described as follows :

10.4.1 The spiked standard solutions were prepared by pipetting optimum volume of 10 mM Brij-35 solution (from 10.2) into the series of 100.00 mL volumetric flasks spiked with 40.00 μL of 5.00 ppm standard solution of PAHs mixture and then filling it to the mark with water. The 2.00 ppb spiked standard solutions were already prepared.

10.4.2 Each spiked standard solution prepared in step 10.4.1 were extracted by procedure in 9 but used various elution times.

10.4.3 The percent recoveries of each PAHs were calculated.

10.4.4 The relationships between the percent recoveries and each of PAHs for each of elution times were plotted in a curve.

10.5 The Effect of The Breakthrough Volume

The breakthrough volumes were studied at 25.00, 50.00, 100.00, and 250.00 mL. The procedure for the study of this effect on the percent recoveries of each PAHs could be described as follows :

10.5.1 The spiked standard solutions were prepared by pipetting 10 mM Brij-35 solution that gave same optimum concentration from 10.2 into the series of volumetric flasks 25.00, 50.00, 100.00, and 250.00 mL respectively spiked with 40.00 μL of 5.00 ppm standard solution of PAHs mixture and then filling it to the mark with water.

10.5.2 Each spiked standard solution prepared in step 10.5.1 were extracted by procedure in 9.

10.5.3 The percent recoveries of each PAHs were calculated.

10.5.4 The relationships between the percent recoveries and each of PAHs for each of solution volumes were plotted in a curve.

11. The Study of Method Detection Limit

The method detection limit was defined as the amount of analyte in spiked standard solutions that yields a peak at signal-to-noise ratio equal to 3 (71). In this study would be triplicate analysis. The procedure could be described as follows :

11.1 The spiked standard solutions were prepared by pipetting optimum volume of 10 mM Brij-35 solution (from 10.2) into the series of 100.00 mL volumetric flasks spiked with each volume of 1.00 ppm standard solution of PAHs mixture and then filling it to the mark with water.

11.2 Each spiked standard solution prepared in step 11.1 were extracted by procedure in 9.

11.3 Blank solution was prepared the same as spiked standard solution but was not added the standard solution of PAHs mixture.

12. The Study of Precision of Small Disk Extraction Method and Recoveries of Each PAHs

The procedure for the study of precision of small disk extraction method and recoveries of each PAHs could be described as follows :

12.1 Eleven spiked standard solutions were prepared by pipetting optimum volume of 10 mM Brij-35 solution (from 10.2) into the series of 100.00 mL volumetric

flasks spiked with 40.00 μL of 5.00 ppm standard solution of PAHs mixture and then filling it to the mark with water. The 2.00 ppb spiked standard solutions were already prepared.

12.2 Each spiked standard solution prepared in step 12.1 were extracted by procedure in 9.

12.3 The precision of this method was calculated and reported in form of percent relative standard deviation (% R.S.D.) of each PAHs.

12.4 The percent recoveries of each PAHs were calculated.

13. The Study of Accuracy of Small Disk Extraction Method

The unknown PAHs mixture solution was prepared by instructor. This solution was used to prepare the synthetic unknown PAHs mixture solutions for the evaluation of the accuracy of small disk extraction method. The procedure for this study could be described as follows :

13.1 Three synthetic unknown mixture solutions were prepared by pipetting optimum volume of 10 mM Brij-35 (from 10.2) into the series of 100.00 mL volumetric flasks spiked with a known quantity of the unknown PAHs mixture solution and then filling it to the mark with water.

13.2 Each synthetic unknown mixture solutions prepared in step 13.1 were extracted by procedure in 9.

13.3 The accuracy of this method was calculated and reported in form of percent error of each PAHs.

14. The Determination of PAHs in Real Water Samples

Fifteen collected water samples from several places could be described as follows :

1. SPM drinking water [polypropylene bottle], manufactured by SPM Foods and Beverages Co., Ltd., Bangkok, Thailand, collected from a store.

2. Fresh drinking water [polypropylene bottle], manufactured by Fresh drinking water, Bangkok, Thailand, collected from a store.

3. Puntip drinking water [polypropylene bottle], manufactured by Puntip drinking water, Bangkok, Thailand, collected from a store.

4. Crystal drinking water [polypropylene bottle], manufactured by Serm Suk Public Company Limited, Patumtanee, Thailand, collected from a store.

5. Sprinkle drinking water [polypropylene bottle], manufactured by M. Water Co., Ltd., Bangkok, Thailand, collected from a store.

6. Idea drinking water [polypropylene bottle], manufactured by Sahaidea Intertrading Co., Ltd., Bangkok, Thailand, collected from a store.

7. Crystal drinking water [poly(ethylene terephthalate) bottle], manufactured by Serm Suk Public Company Limited, Nakornsawan, Thailand, collected from a store.

8. Sprinkle drinking water [poly(ethylene terephthalate) bottle], manufactured by M. Water Co., Ltd., Bangkok, Thailand, collected from a store.

9. Singha drinking water [poly(ethylene terephthalate) bottle], manufactured by Boon Rawd Brewery Co., Ltd., Bangkok, Thailand, collected from a store.

10. Volvic natural mineral water, manufactured by STE Volvic, France, collected from a store.

11. Vittel natural mineral water, manufactured by Vittel S.A. company, France, collected from a store.

12. Aura natural mineral water, manufactured by Thorraneepipat Co., Ltd., Chiangmai, collected from a store.

13. Ice collected from a store near Chemistry 3 Building, Chulalongkorn University. It was standed at room temperature to dissolve before use.

14. Tap water collected from fourth floor, Chemistry 3 Building, Chulalongkorn University.

15. Water collected from Chulalongkorn University Pool near Phyathai Road.

The procedure for determination of PAHs in each water sample can be described as follows :

14.1. Three 100.00 mL of each water samples were prepared. Optimum volume of 10 mM Brij-35 (from 10.2) was pipetted into 100.00 mL volumetric flasks and filled it to the mark with this water sample.

14.2. Each water samples prepared in step 14.1 was extracted by procedure in 9.

14.3. The peaks obtained from chromatogram were matched against the spectral library.

14.4. The concentration of each PAHs was calculated by using the external standardisation method.