CHAPTER III

EXPERIMENTAL PROCEDURE

3.1 Materials

3.1.1 Chemicals

- Acetone (AR grade), Mallinkrodt
- Dichloromethane (AR grade), Mallinkrodt
- Hexane (HPLC grade), Lab-Scan Analytical Science
- Silica gel 60 particle size 0.063-0.200 mm., Merck, Germany
- Sodium sulfate anhydrous, Merck, Germany
- Glass wool

3.1.2 Standard PAH

All 16 standard PAH were 99.5% purity purchased from Supelco (USA) and they are NAP, ACY, ACE, FLU, ANT, PHE, FLA, PYR, BbF, IP, CHR, BkF, BaP, BPER, DbA and BaA. The 99.5% purity of MPHE was used as internal standard and purchased from Chem survice (USA).

3.1.3 Glassware

- 250-ml round-bottom flask
- 2x10 cm. Glass column with a Teflon stopcock
- Separating funnel
- Beaker
- Erlenmeyer flask
- Volumetric flask

- Glass fiber filter (Whatman GF/C) and filtration equipment
- 5-ml and 10-ml pipette
- 10, 100, 250 and 500-µl syringes
- Vial with a Teflon liner crew cap

All glassware were soaked with 5% v/v of extran overnight, dried in oven and rinsed with hexane before use.

3.1.4 Instrument

- GC/FID, Agilent
- Rotary evaporator, Eyela
- Shaker, GFL 3017
- Nitrogen blower, Cole-Parmer
- Microwave, Milestone ETHOS SEL

3.2 Preparation of standard solutions

3.2.1 PAH test compounds

16 PAH were accurately weighed for determination of recoveries, calibration curves and partitioning experiments. For determination of recoveries and calibration curves, these compounds were prepared in hexane and made up in a volumetric flask. While the test compounds used for partitioning experiment were prepared in acetone then made up in another volumetric flask. The concentrations of both stock solutions were 250 (0.25S) mg/l and 2500(0.25S) mg/l, where S is aqueous solubility of each PAH. The 250 (0.25S) mg/l contained 10 PAH, which were NAP, ACY, ACE, FLU, ANT, PHE, FLA, PYR, BbF and IP. The 2500(0.25S) mg/l contained 6 PAH, which were CHR, BkF, BaP, BPER, DbA and BaA. The stock solutions were kept at -4°C. Each concentration of working solution contained 16 PAH was diluted from stock solutions to obtain the desired

concentrations in hexane. The four working concentrations in different proportion to S as 0.25S, 0.18S, 0.12S and 0.05S were shown in Table 3-1.

Table 3-1 Concentration of working solutions

Compounds	Aqueous solubilities (mg/l)	Working solution concentrations (μg/l)			
		0.25S	0.18S	0.12S	0.05S
NAP	31.7	7930	5706	3804	1585
ACY	3.93	983	710.00	471.60	196.50
ACE	3.93	983	710.00	471.60	196.50
FLU	1.98	495	360.00	237.60	99.00
PHE	1.29	322.30	240.00	154.80	64.50
ANT	0.073	18.30	13.00	8.76	3.65
FLA	0.26	65.00	50.00	31.20	13.00
PYR	0.135	33.70	25.00	16.20	6.75
BaA	0.014	3.50	2.52	1.68	0.70
CHR	0.002	0.50	0.36	0.24	0.10
BbF	0.062	15.50	11.16	7.44	3.10
BkF	0.00076	0.20	0.14	0.09	0.04
BaP	0.0038	1.00	0.68	0.46	0.19
IP	0.062	15.50	11.16	7.44	3.10
DbA	0.0005	0.13	0.09	0.06	0.03
BPER	0.0003	0.075	0.05	0.04	0.015

3.2.2 Internal standard solution

1-methylphenanthrene was used as internal standard. The internal standard was accurately weighed for 1.60 mg and dissolved in hexane, then made up to 100 ml in a volumetric flask to make a concentration of 250(0.25S) mg/l.

3.3 Extraction methods

3.3.1 Microwave extraction

Approximately 2 g of leaf samples were weighed in a crucible and transferred to a microwave vessel contained with 40 ml of hexane and 500 μl of internal standard. A weflon magnetic bar was placed in the vessel in order to distribute the heat from microwave to all of the leaves in the vessel. The sample was extracted for 20 min at 90°C. The extract was allowed to cool down and then filtered through a GF/C filter paper into a 250-ml round-bottom flask and rinsed with sufficient amount of hexane. The filtrated was then evaporated down to 2 ml of volume by a rotary evaporator at 35 °C.

3.3.2 Liquid-liquid extraction

2 L of water from the partitioning experiments was extracted in a separating funnel using 25 ml of hexane, 500 μl of internal standard was also spiked in the separating funnel. The separating funnel was shaken until the two layers of lipid and water were occurred. The lower layer of water was repeatedly extracted with hexane as described above. The combined hexane was dried over anhydrous sodium sulfate, and evaporated down to 5 ml of volume using a rotary evaporator. The extract was concentrated again to 1 ml of volume under a gentle stream of nitrogen.

3.4 Isolation

Silica gel 60 was activated by heating at 500 °C for 4 hours. It was then left to cool in a desiccator. A slurry of activated silica gel was prepared using 15 g of silica gel in 50 ml of hexane. This slurry was filled into a glass column and pre-eluted with 100 ml of hexane. The elution was drained until the silica gel was almost exposed to the air. The 2 ml of sample extract (from section 3.3.1) was gradually transferred onto the column. A 70 ml of 20% dichloromethane in hexane was used as an eluent. The eluent was collected into a 250-ml round bottom flask. The collected fraction was evaporated down to 5 ml of volume by using a rotary evaporator. The extract was concentrated again to 1 ml of volume under gentle stream of nitrogen.

3.5 Quantification

Standard solution and the sample extracts were each injected into the column of the GC/FID using an auto-injector with the injection volume of 2 μl.

GC Parameters

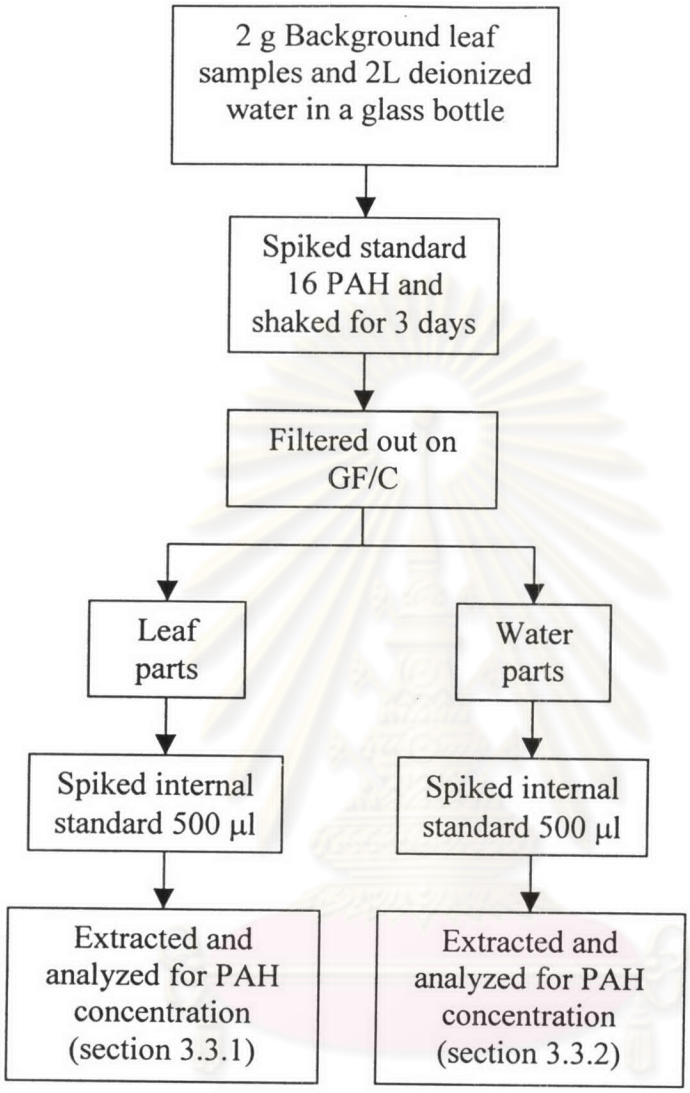
- GC instrument: Agilent 6890N
- Column: 30-m HP-5 5% Phenyl Methyl Siloxane, Agilent 19091J-413 diameter 320 μm and film thickness 0.25 μm
- Column temperature: 325°C
- Temperature program

step	Temperature (°C)	Temp Rate (°C/min)	Time (min)
1	80	-	1
2	160	25	3
3	300	3	2

- Detector: FID
- Carrier gas: Hydrogen (ultra high purity)
- Make-up gas: Nitrogen (high purity)
- Injection volume: 2 μl

3.6 Leaf/water partitioning experiments

A 2 g of background leaf samples and 2 L of deionized water were added in a screw-cap glass bottle. Then the standard solution was spiked into the bottle. Each bottle was shaked by a shaker of varying time (1, 2, 3 and 6 days). When each bottle reach to the time, the leaves were filtered out on GF/C, and dried with anhydrous sodium sulfate. The leaves and water parts were extracted and analyzed for PAH as detailed in Section 3.3.1 and 3.3.2. The equilibrium time was determined by plotting the concentration of PAH in leaves parts against the time. At equilibrium time the concentration of PAH in leaves were steady. From the experiment steady state was 3 days (as shown in Appendix B).



Figures 3-1 A diagram shown the leaf/water partitioning steps

3.7 Sample Collection

Plant leaves were collected from orange jasmine (*Murraya paniculata* L. Jack) in four sites with different traffic volumes (as shown in Figure 3-2 and Table 3-2). The collected fresh leaves were a mature and fully expanded leaves from the second pairs of stem in the middle of trunk (shown in Figure 3-3 and 3-4).

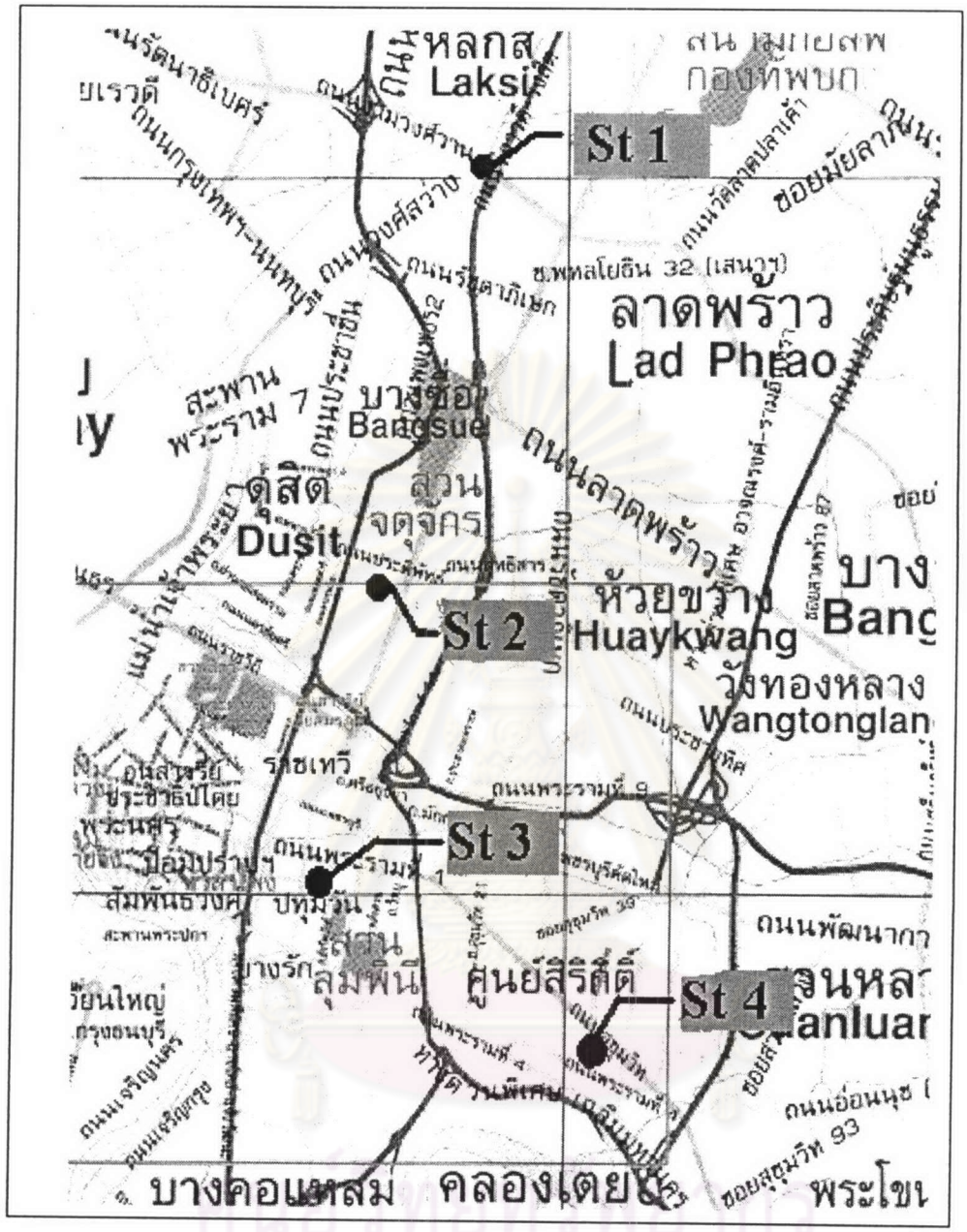
The sites were located in urban area of Bangkok including Patumwan, Phongphet, Saphan Khwai and Kasemraj junction. Background leaf sample was collected inside Asian Institute of Technology (AIT) which is situated about 40 kilometers north of Bangkok. Leaf samples were collected from May to June 2002. At each site, leaves were collected everyday for 7 days and combined to one sample with three replicates (The atmospheric condition has shown in Table 3-3). All samples had been stored at - 4°C in cleaned glass bottle.

Kasemraj leaf sample was collected at Rama IV road near Kasemraj junction. Sampling period was between May 21 - May 27, 2002. This area has the highest intensity of traffic volume among four sites of the study area.

Saphan Khwai site is located on Paholyothin road near Saphan Khwai junction. Sampling period was between May 17 - May 23, 2002. It is a residential and commercial area and has six-lanes of road. There are high buildings standing along both sides of the road. This area usually has high traffic volume and the buildings around this junction.

Phongphet site is located on Ngamwongwan road that has little traffic volume. Sampling period was between May 17 - May 23, 2002. It is a residential and commercial area and has eight-lanes.

Patumwan site is located on Phaya Thai road in front of Chula soi 62, which is the lowest intensity of traffic volume. Sampling period was between May 28 – June 3, 2002. It is a commercial area and has six-lanes.



St 1 : Phongphet site	St 3 : Patumwan site
St 2 : Saphan Khwai site	St 4 : Kasemraj site

Figure 3-2 Sampling sites in Bangkok

Figure 3-3 Leaves in sampling site

Figure 3-4 Leaves sampling

Table 3-2 Traffic volume (vehicles/day) in four study sites

Areas	Traffic volume (vehicles/day)
Patumwan	14,158
Saphan Khwai	34,265
Phongphet	77,122
Kasemraj	101,572

Source: Traffic Information System Division, Department of Traffic and Transportation, 2000

Table 3-3 Atmospheric condition at four sites during May 2002

Mean pressure (Hectopascal)	Mean temperature (°C)	Mean wind speed (knots)	Number of rainy days	Total rainfall (mm)
1007.35	29.8	2.5	19	229.3

Source: Climatological data for the year 2002, Climatology Division, Meteorological Department, 2002

3.7.1 Determination of percentage of moisture

2 g of leaf sample was weighed on a tarred crucible. The sample was dried overnight at 105 °C in oven. And then allowed to cool in a desiccator and weighed. The percentage of moisture was calculated as following.

$$\%\text{Moisture} = \frac{\text{weight of sample - weight of dry sample}}{\text{weight of sample}} \times 100$$

3.7.2 Determination of percentage of wax

Leaf samples were weighed for 2 g, then transferred to a microwave vessel added with hexane 40 ml and put the weflon into the vessel. The sample was extracted by Microwave extraction for 20 min at 90°C. The extract was allowed to cool after extraction and filtered through a GF/C filter paper into a preweighed drying round-bottom flask. The flask was evaporated by rotary evaporator at 35 °C until the solvent dried out. The round-bottom flask was reweighed, and the percentage of wax was calculated as following.

$$\%\text{Wax} = \frac{\text{reweighed flask - preweighed flask}}{\text{weight of sample}} \times 100$$

Note: %Wax is defined as all those dissolved in hexane.