CHAPTER 2

EXPERIMENTAL

2.1 Apparatus

- 1) 10 mL polypropylene tubes, Nipro (Thailand) corporation Limited, Thailand
- 2) 1 mL disposable syringe, Nipro (Thailand) corporation Limited, Thailand
- 3) 0.45 µm cellulose acetate syringe filter, chrom Tech, Inc., England
- 4) 110 mm Ø GF/A filter papers, Whatman, USA.
- 5) 10, 50, 100, 1,000 mL volumetric flask, Duran, Germany
- 6) 50, 100, 250 mL beaker, Duran, Germany
- 7) 0.45 µm nylon syringe filter, , Agela Technologies, USA.
- 8) 47 mm Ø quartz filter paper, Whatman, USA.
- 9) 46.2 mm Ø 2 μ m PTFE filter paper, Whatman, USA.
- 10) 100, 200 µL reference pipette, Nichiryo Le, Japan
- 11) 2,500 µL reference pipette, Eppendorf, USA.
- 12) 1,000 µL reference pipette, Bran, Germany
- 13) Plastic bag (zip log)
- 14) Plastic boxes, Raaco, Denmark
- 15) Parafin oil film, Para film, USA
- 16) Quartz cuvettes, Starna Scientific Limited, UK.
- 17) Thermohygrometer, Testo, Germany
- 18) Aluminum foil, Diamond, Thailan
- 19) Desiccator, Duran, Germany

2.2 Chemicals

 Dichloromethane (CH₂Cl₂, MW 84.93 g/mol), HPLC grade, 99.9 %, Merck, Germany

- n-Hexane (CH₃(CH₂)₄CH₃, MW 86.18 g/mol), HPLC grade, 98.0 %, Merck, Germany.
- Mixed standard containing 16 priority PAHs at 2,000 µg/mL in Methylene Chloride, Restex, USA.
- 4) Standard reference material (SRM), Urban dust 1649b, NIST, USA.
- 5) Internal standard (Acenaphthene- D_{10} and pyrylene- D_{12}), Supelco, USA.
- 6) Sodium nitrite (NaNO₂, 69.00 g/mol), 97%, Ajax Finechem Pty Limited, Australia
- 7) Sulfanilamide (C₆H₈N₂O₂S, MW 172.21 g/mol), 99%, Sigma-Aldrich, China
- N-(1-Napthyl) ethylenediamine dihydrochloride (C₁₂H₁₄N₂. 2HCl, MW 259.18 g/mol), 98%, Sigma-Aldrich, Austria
- Triethanolamine (N(CH₂CH₂OH)₃, MW 149.19 g/mol), 80 %, Ajax Finechem Pty Limited, New Zealand
- 10) Ortho-Phosphoric acid (H₃PO₄, MW 98.00 g/mol), AR grade, 85%, QReC, New Zealand

2.3 Equipment

- 1) Ultrasonicator, P 300 H, Elma, Germany
- 2) Oven, model 100-800, Memmert, Germany
- 3) Analytical balance, AB304-S, Mettler Toledo, Switzerland
- 4) Microbalance weight, Mettler Toledo, Switzerland
- 5) Analytical balance, BB3, Mettler Toledo, Switzerland
- 6) Vortex (G-560F), Scientific Industries, INC., USA.
- 7) Rotary evaporator, Buchi Labortechnik AG, Switzerland
- 8) Chamber, consisting of
 - 8.1 Gas analyzer, 350-XL, Testo, Germany
 - 8.2 Thermohygrometer, Testo, Germany
 - 8.3 Mini-fan
 - 8.4 Evaporating dish
 - 8.5 Hot plate, C-MAG HS 4, IKA Company, USA.
 - 8.6 Incense burner

2.4 Instruments

- 1) Gas chromatograph-mass spectrometer, Hewlett Packard, USA consisting of
 - a) Gas chromatograph, Agilent 6890
 - b) Mass spectrometer, Hewlett Packard 5973 Mass selective Detector
 - c) Auto injector, Agilent 7683
 - d) Data processing system with MSD ChemStation software D.01.02.16, Agilent Technologies, 1989-2004
- 2) HP-5MS column (5% phenyl and 95% dimethyl polysiloxane), 30 m length,
 0.25 mm diameter, 0.25 µm film, Agilent Technologies, USA.
- 3) UV-Vis Spectrophotometer, PerkinElmer Lambda 25, Switzerland

2.5 Determination of Nitrogen dioxide (NO₂) by lab-made passive samplers and

spectrometer

2.5.1 Preparation of lab-made passive samplers

1) Absorbing solution

20 mL of triethanolamine (TEA) was added into a 100 mL volumetric flask and adjusted to 100 mL with de-ionized water.

2) Saltzmann reagent

Sulfanilamide solution was prepared by dissolving 10.75 g of sulfanilamide and in 28 ml of phosphoric acid and volume adjusted to 500 mL with deionized water in a volumetric flask. Moreover, N-(1Napthyl) ethylenediamine dihydrochloride (NEDA) solution was obtained by dissolving 0.1520 g of N-(1-Napthyl) ethylenediamine dihydrochloride in de-ionized water and adjusted to 100 mL in a volumetric flask. Sulfanilamide solution and NEDA solution were mixed in a ratio of 10:1. They must be refrigerated and protected from light.

3) Nitrite standard stock solution (1,000 mg/L)

0.150 g of sodium nitrite (NaNO₂) was dissolved in de-ionized water and adjusted to 100 ml in a volumetric flask.

5) Diffusion tube

The polypropylene (PP) diffusion tubes with a length of 7.7 cm and an internal diameter of 1.6 cm. were cleaned and sonicated for one hour, then rinsed with deionized water. Filter paper, Whatman GF/A, was cut into a circle with the same size of internal diameter of the tube. The cut filter papers were cleaned and sonicated for 30 minutes and rinsed twice with distilled water before drying at 60 degree Celsius for 24 hours and left in a desiccator prior to use (Bootdee et al., 2012; Khamkaew and Chantara, 2013).

2.5.2 Sampling of NO₂ by the passive sampler

Three sets of samplers were exposed at the sampling site for 8 and 24 hour. One set of sample was exposed in front of the shrines (outdoor) while two set were exposed to the indoor air of shrines. A set of sampling consisted of 5 sampling tubes and 3 blank tubes (un-opened tube). Steps of sample collection are described as follows:

1) The GF/A filter paper was placed at the bottom of the diffusion tube and 50 μ l of 20% TEA (absorbing solution) was added onto the filter paper. Tubes were then closed by a cap and paraffin film.

2) Five sampling tubes and three blank tubes (calculated as 1 sample) were fixed in a protective shelter (Figure 2.1) to protect them from meteorological effects i.e. wind, sunlight and rain. Next, it was hung at the sampling site above ground at 1.5 - 2.0 m. Sampling was started by removal of caps from the tubes.

3) After the sampling, the exposed passive diffusion tubes were collected. They were immediately closed with caps and tightened with parafilm. They were placed in plastic zip lock bags and kept in an airtight container then transported

and transferred into refrigerator. The samples were kept under -4.0 °C conditions until extraction.



Figure 2.1 Passive sampling tubes and protective shelter

2.5.3 Extraction and analysis of NO₂

After the sampling, NO₂ samples were extracted with 2 mL of de-ionized water (DIW), shaken and held for 15 minutes. The sample solution was filtered through 0.45 μ m cellulose acetate membrane. One mL of the extracted sample solution was mixed with 2 mL of Saltzmann reagent in a test tube and stood for 10 minutes until color development was completed. After that, the absorbance of the solution was measured with the spectrophotometer at 540 nm by using the reagent as blank (Bootdee et al., 2012).

2.6 Selection of shrines as sampling sites

Figure 2.2 shows the map of Chiang Mai and the location of sampling shrines. They are famous shrines located in the most crowded area in the city of Chiang Mai, namely Waroros market. The first shrine is the oldest shrine founded in 1876 by Chinese ancestors ($18^{\circ} 47' \quad 23.32 \text{ " N}$ and $99^{\circ} 0' \quad 6.75'' \text{ E}$) as shown Figure 2.3a. The size of the building is approximately $8 \times 18 \text{ m}^2$ and 4 m height. The ventilation system is

not well-equipped and the building was designed as almost a closed system with only one door open for visitors. The second ancestor shrine was established in 1897 ($18^{\circ} 47'$ 24.19" N and 99° 0' 0.245" E). It has an area of approximately 12×20 m². It is surrounded by high walls (~ 4 m height). The worship room (12×6 m²) is situated at one side of the area, while the other side is an open area. The ventilation system in the second shrine is better than the first one. It has 4 turbine ventilators on the roof of the worship room and its ceiling is open. This shrine is located near a narrow road with high traffic density, in particular motorcycles and small diesel trucks (pick-up), for almost the whole day (Figure 2.3b).



Figure 2.2 Map of sampling sites in Chiang Mai City

At the first shrine, each visitor would purchase 8 large incense sticks (~ 9 mm diameter, ~ 48 cm long and ~ 34 cm coated combustible part). After each individual ceremony, the burned incense sticks are not allowed to be kept inside the worship area, but are kept outside. At the second shrine, one visitor would purchase a package of 28 smaller incense sticks. Twenty-six of them are burned in a worship room and the rest are burned outdoors. The size of incense is much smaller than at the first shrine. It has a

diameter of ~2.4 mm with a length of ~ 32 cm and ~ 21 cm coated with combustible part. The amount of incense burned each day depends on the number of visitors, which was counted during the sampling period. Apart from incense, some candles and lanterns filled with vegetable oil were also burned inside the shrine. However, the amount of incense used was much higher than the number of candles and lanterns.



Figure 2.3 Sampling sites in Chiang Mai City; a: shrine 1 and b: shrine 2





Figure 2.4 Chinese New Year festival; a: shrine 1 and b: shrine 2

Copyright[©] by Chiang Mai University

The sampling was carried out during special occasions and over normal periods (background value). The special occasions in this study were those associated with the Chinese lunar calendar including Chinese New Year (January 22-24, 2012 and February 9-11, 2013), the Chinese Ghost festival (August 31, 2012), the moon festival (September 30, 2012), a vegetarian festival (October 15-16 and 19-20, 2012) and the celebration of establishment's anniversary (February 3-6, 2012 for shrine 1, and December 30-31, 2012 and January 01, 2013 for shrine 2). The sampling in the normal periods was randomly carried out during a non-event for the whole year (2012). The amount of incense being burned on each occasion depends on the number of visitors.

During special occasions, more visitors come and a high amount of incense is used. Figure 2.4(a-b) shows the visitors or pilgrims attending to celebrate the Chinese New Year at the shrines.

2.7 Sampling of PM_{2.5} in shrines

Indoor PM_{2.5} samples were collected on Teflon fiber filters (2 µm PTFE, 46.2 mm diameter, Whatman's filter paper) using Minivol Air Samplers (Air metric, USA) at a flow rate of 5 L/min (Figure 2.5) for 8 hrs (8 am – 4 pm) and 24 hrs (8 am – 8 am) on the same day for shrine 2, while shrine 1 was collected in the alternated day. The filters were pre-weighed by a microbalance in a controlled room (25.4 ± 2.8 °C, $41.3\pm5.4\%$ RH). After the sampling, the filters were kept on aluminium foil plates inside a desiccator for 48 h before being re-weighed and stored in a freezer (-4.0 °C) until analysis. The indoor temperature and humidity were measured for the whole sampling period using a hygrometer (Sato keiryoki MEG. CO., LTD.). Their ranges were $24.8\pm3.5 - 33.5\pm14.3$ °C and $56.1\pm6.5 - 65.7\pm10.1\%$ for shrine 1 and 27.0 - 35.5 °C and 51.5 - 75.7 % for shrine 2, respectively.



Figure 2.5 Mini-volume air sampler

2.8 Design of an experimental chamber for testing of incense and raw materials burning

2.8.1 The chamber for burning experiment

A chamber was constructed for testing emissions of PM_{2.5}, PAHs and pollutant gases from incense burning. The schematic diagram of the incense burning of the experimental chamber is shown in Figure 2.6. The chamber was made from acrylic plates (thickness 0.5 cm). The size of the chamber was 0.5 m (W)×0.5 m (L)×0.6 m (H) accounting for a total of 0.15 m³. The chamber was equipped with a gas analyzer, incense holder, a digital thermohygrometer (Testo), a mini-fan, a hot plate, evaporating dish, gas analyzer (350-XL, Testo) and a mini-volume air sampler. The temperature inside the chamber was kept at 32.6±2.5 ° C, while the relative humidity was recorded at 41.9±11.0 %. The incense sample was placed in the chamber and lighted with a lighter and the door was immediately closed.

2.8.2 Preparation of incense and raw materials samples for burning experiment

Type of incense and details are showed as Table 2.1 and Figure 2.8. Incense samples were smoldered to emission $PM_{2.5}$ and pollutant gases. Until, the burning process burned out. As for the raw materials of producing incense, the raw material sample was put into evaporating dish in the chamber and the door was immediately closed. The raw materials were lighted by a hot plate at a temperature of 400 °C. The raw materials were smoldered to burn out shown as Figure 2.7.

All rights reserved



b.



a) diagram of chamber

b) chamber apparatus: (1) gas sensor (2) mini-volume air sampler (3) gas analyzer (4) mini-fan and (5) thermo hygrometer



Figure 2.7 Burning experiment in the chamber using hot plate and evaporating dish

c.

2.8.3 Operation of the chamber

A gas analyzer was used to measure gases emitted from incense burning including carbon monoxide (CO), nitrogen monoxide (NO), nitrogen oxide (NO_x), nitrogen dioxide (NO₂) and sulfur dioxide (SO₂). The stabilization of pollutant gas concentrations emitted from the burning took approximately 5 minutes. The PM_{2.5} collecting was started by the incense, which was burned out. Each PM_{2.5} sample was collected for 1 hour with a flow rate of 5.0 L/min from the storage chamber using a mini volume air sampler. The samples were collected on quartz fiber filters (\emptyset 47 mm, Whatman). The filters were pre- and post-weighed by microbalance. After PM_{2.5} collection, the filters were kept on a plate covered with aluminium foil, and then transferred into a desiccator for 24 hours before being weighed and stored in a freezer until analysis. The chamber was cleaned every time before the next burning experiment. Blank samples were collected after every five samples. PM_{2.5} samples collected from incense burning in the chamber were performed after background sampling (blank sample).

2.9 Preparation of incense samples for burning experiment in the chamber

Before and after the burning experiment, the incense samples were measured for length and mass by ruler and two digit balance, respectively.

2.9.1 Types of incense samples

There are various forms of incense including sticks, cones, coils, powders, rope and rocks/charcoal. The incense stick is the most popular in Thailand. Incense sticks and cones were selected for testing of emission of pollutants. There were various brands of incense produced in Chiang Mai and Lamphun provinces, Thailand. The selected incense types included longan peels incense, dried flower incense, aromatic incense and traditional incense. Figure 2.8 shows various types of incense sell in the market. The information of these types of incense is given in Table 2.1.



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่ Copyright[©] by Chiang Mai University All rights reserved



Figure 2.8 Incense samples; A: Traditional incense stick, B: Traditional incense stick, C: Longan peels, D: Dye-dried flower, E: Dried flower, F: Aromatic incense stick, G: Mosquito stick, H: Smoke-free incense, I: Aromatic incense cone/ citronella oil and J: Chili-liked shape incense cone / white musk.

				018	ายหล				
ID	Genetic name	Forms	Type of incense	Scent/ burned time	Length (cm)		Weight of burned part	Ingredients and details	
			5		Whole stick	Coated part	(g)		
А	Traditional	stick	Traditional incense,	NA/	41.77±0.06	27.27±0.06	10.07 ± 0.10	Unknown materials	
	incense		machine based	~120 min	and a straight of the straight	11	- 1		
В	Traditional	stick	Traditional incense,	NA/	32.07±0.12	21.90±0.26	1.19±0.02	Unknown materials	
	incense		hand-made	~35 min	E SY		恐ち		
С	Longan peels	stick	Traditional incense,	NA/	28.00±0.26	15.83±0.59	0.840±0.03	Longan peels, plant-based	
			eco-friendly incense,	~43 min	NV	λ / λ	21	glutinous powders, dried flower,	
			hand-made		MAN	1 2		wood powder and bamboo	
D	Dye-dried flower	stick	Traditional incense,	NA/	28.37±0.47	16.23±0.49	0.75±0.07	Sawdust, dried flower, plant-based	
			eco-friendly incense,	~38 min	0000	SV/		glutinous powders, wood powder,	
			hand-made	MAI	INIVI	ERS		bamboo and light blue color	
Е	Dried flower	stick	Traditional incense,	NA/	28.40±0.61	15.87±0.93	0.67 ± 0.04	Sawdust, dried flower, plant-based	
			eco-friendly incense,	~42 min			0 1	glutinous powders, wood powder	
			hand-made	ธิบหาวิ	ัทยาล่	ัยเชีย	งเหม	and bamboo	
			Copyrig	nt [©] by (Chiang I	Mai Uni	versity		
			AII	righ t	ts r	eser	ved		

 Table 2.1 Ingredients and details of incense samples burned in the chamber

44

				Scont/	Length (cm)		Weight of		
ID	Genetic name	Forms	Type of incense	burned time	Whole stick	Coated part	burned part (g)	Ingredients and details	
F	Aromatic incense	stick	Aromatic incense, machine based	Sandalwood/ ~65 min	33.00±0.00	20.20±0.00	1.95±0.04	Sawdust, tree bark powders and sandalwood perform and bamboo: no dust	
G	Mosquito stick	stick	Aromatic incense, machine based	citronella oil/ ~65 min	25.23±0.06	17.60±0.10	4.94±0.04	Sawdust, wood powders, ground coconut shell, plant-based glutinous powders bamboo and mixed color: safety and non-toxic, ingredients from natural extraction	
Н	smoke-free incense	stick	Aromatic incense, hand-made	Jasmine/ ~30 min	33.17±0.06	20.97±0.25	0.71±0.02	Charcoal, corncob, plant-based glutinous powders and bamboo: no dust and smoke, reduced carcinogen	
Ι	Aromatic incense	cone	Aromatic incense, hand-made	citronella oil/ ~17 min	4.00±0.00	ເຮີຍວ	2.44±0.03	Unknown materials	
J	Chili-liked shape	cone	Aromatic incense, hand-made	white musk/ ~15 min	6.40±0.26	3.60±0.17	0.73±0.08	Unknown materials and red color	

 Table 2.1 Ingredients and details of incense samples burned in the chamber (continued).

2.9.2 Raw materials / ingredients for incense production

Figure 2.9 shows raw materials used to produce incense such as sawdust, wood powders (Chankhao), plant-based glutinous powders (tree bark), bamboo stick and dye powders were collected from manufactures. The raw materials were dried by oven at 60 °C at 24 hours and weighed a 1.00 g per sample on a 2-digit balance.



Figure 2.9 The raw materials for incenses production

2.10 Determination of PM_{2.5}-bound PAHs by GC-MS

2.10.1 Preparation of mixed 16 PAHs standard solutions

polycyclic aromatic hydrocarbons (16-PAHs), including Sixteen naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benz[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IND), dibenz[a,h]anthracene (DBA) and benzo[g,h,i]perylene (BPER), were used as standards for analysis (Restex, USA). A 1 mL of 2,000 mg/L mixed 16 PAHs stock solution was diluted in 100 mL volumetric flask with dichloromethane (DCM) : nhexane (1:1) to a concentration of 20 mg/L as an intermediate standard solution. Working solutions of 16 PAHs in a range of 0.002 - 1.0 mg/L were prepared from intermediate and used for calibration curves.

2.10.2 Preparation of mixed internal standards

Five mg of each internal standards (Acenaphthene- D_{10} and Perylene- D_{12} ; Supelco, USA) were weighed using a 4-micro balance. They were added into a 100 mL volumetric flask, followed by dichloromethane (DCM) : n-hexane to result in a concentration of 50 mg/L.

1) Preparation of mixed internal standards for sampling of indoor air

A 10 mL of 50 μ g/mL internal standard solution was pipetted, and then adjusted with acetonitrile to 100 mL in volumetric flask to obtain a concentration of 5 mg/L. The 5 mg/L of 0.2 mL of mixed solution was spiked into the mixed PAHs standard solutions and the extracted samples to achieve 0.5 mg/L of the internal standard in 2 ml final volume. 2) Preparation of mixed internal standards for incense and raw material burning in chamber

A 1 mL of 25 mg/L acenaphthene- D_{10} and 1 mL of 50 mg/L perylene- D_{12} solution was pipetted, and then adjusted with DCM : n-hexane (1:1) to mix in 50 mL volumetric flask to obtain a concentration of 0.5 mg/L acenaphthene- D_{10} and 1.0 mg/L perylene- D_{12} . The 200 µl of mixed internal standards solution was spiked into the mixed PAHs standard solutions and the extracted samples to achieve 0.05 mg/L acenaphthene- D_{10} and 0.1 mg/L perylene- D_{12} of the internal standard in 2 mL final volume.

2.10.3 Extraction of PM_{2.5} samples

The filter containing $PM_{2.5}$ were extracted in 25 ml of DCM : n-hexane (1:1) using an ultrasonicator (Elma, Germany) for 45 minutes under controlled temperature (~15°C) as shown in Figure 2.10. The extracted solution was filtered through a 45 µm nylon filter (Agela Technologies, USA) prior to being evaporated by vacuum rotary until it was almost dry. The mixture of internal standards (acenaphthene-d12 and perylene-d10) was spiked into the extracted solution and the volume was adjusted to 2 mL with DCM : n-hexane.



Figure 2.10 Extraction of PM_{2.5} by sonicator

48

2.10.4 Analysis for PM2.5-bound PAHs by GC-MS

1) GC-MS conditions

The GC-MS condition for 16-PAHs analysis was optimized in terms of temperature program, injection mode and injection parameter. MS was used as the GC detector to achieve satisfactory separation and detection. A solution of 0.5 μ g/mL of 16 PAHs solution mixed with 5 μ g/mL of internal standards was injected into the GC-MS in the splitless mode. The optimum GC-MS condition is shown in Table 2.2.

Chromatographic parameters in terms of retention time and peak area of PAHs compounds were integrated using ChemStation software. Quantitation was based on the ratio of the peak area of PAHs standard to a denudated internal standard. Moreover, the MS was operated in the SIM mode and was set at 5 minute solvent delay. Characteristic ions of target PAHs were used for identification purpose (Table 2.3).

2) Detection limit of GC-MS for 16-PAHs analysis

Detection limit is the smallest concentration that can be quantitatively measured with a particular technique. The detection limit was checked by injecting 7 times of the lowest concentration (0.004 mg/L) of mixed 16-PAHs into the GC-MS system under the optimum condition. The detection limit was 3 times more than the standard deviation according to the detector output (US EPA, 2010).

> Copyright[©] by Chiang Mai University All rights reserved

GC Parameter	Condition				
GC					
Column	HP-5MS capillary column				
Carrier Gas	Helium, Flow-rate 1 ml/min				
Injection Mode	Splitless, 0.7 min				
Injection Temperature	275 °C				
Temperature Program	SPE 131				
Initial Column Temperature	75 °C				
Initial Hold Time	0.5 min				
Temperature Program	25 °C/min to 245°C				
NEI I	3 °C/min to 285°C and held 6 min				
Post run Temperature	290°C				
Post run Hold Time	2 min				
MS	UNIVER				
Detection Mode	SIM mode				
Transfer Line Temperature	290°C				
Source Temperature	230°C Mai University				
Eletron Energy	70 eV				
Ionization Mode	EI				

Table 2	2.3	Charact	eristic	ions	for	PAI	Hs
1 4010 1		Charace	ernoure	10110	101		

DAIL	Abbroviation	Molecular	Primary	Secondary	Retention
I AIIS	ADDIEviation	weight	Ion	Ions	Time
Naphthalene	NAP	128.17	128	127, 129	4.06
Acenaphthylene	ACY	152.19	152	151, 153	5.59
D ₁₀ -Acenaphthene*	D ₁₀ -ACE	164.17	162	164, 160	5.74
Acenaphthene	ACE	154.21	154	153, 152	5.78
Fluorene	FLU	166.22	166	167, 165	6.28
Phenanthrene	РНЕ	178.23	178	176, 179	7.22
Anthracene	ANT	178.23	178	176, 179	7.27
Fluoranthene	FLA	202.25	202	200, 203	8.57
Pyrene	PYR	202.25	202	203, 200	8.87
Benzo[a]anthracene	BaA	228.29	228	226, 229	11.19
Chrysene	CHR	228.29	228	226, 229	11.27
Benzo[b]fluoranthene	BbF	252.31	252	253, 250	14.19
Benzo[k]fluoranthene	BkF	252.31	252	253, 250	14.26
Benzo[a]pyrene	BaP	252.31	252	253, 250	15.18
D ₁₂ -Perylene*	D ₁₂ -PER	264.15	264	260, 265	15.36
Indeno[1,2,3-c,d]pyrene	IND	276.33	276	274, 277	d 19.04
Dibenzo[a,h]anthracene	DbA	278.35	278	276, 279	19.21
Benzo[g,h,i]perylene	BPER	276.33	276	138, 277	20.02

Note: *Internal standards

2.10.5 Quality control of PAHs analysis by GC-MS

1) Testing of accuracy

1.1) Use of standard reference materials (SRM)

Standard reference material (SRM; Urban Dust 1649b, U.S.A.) provided by the National Institute of Standards and Technology (NIST) was used for the conditioning of quality control for PAHs analysis. The composition of PAHs in the SRM is shown in Table 2.4. Approximately 50 mg of the SRM was weighted and put into in a flask. Three replications were performed in each batch. They were extracted and analyzed as described in sub-sections 2.10.3.

1.2) Use of spiking method

A 500 μ L of 0.08 mg/L and 2.00 mg/L of the mixed PAHs standard solution were spiked onto a Teflon fiber filter (2 μ m PTFE, 46.2 mm diameter, Whatman). It was left for ~ 30 minutes until it was nearly dry. Three replications were prepared. They were extracted and analyzed for PAHs using the same conditions as described in section 2.10. The recovery was calculated using Equation 2.1.

% Recovery =
$$\frac{MV}{SV}$$
 x 100 (Eq. 2.1)
Where MV is a measured value (mg/kg)
SV is a spiked value (mg/kg)

PAHs	Certified Value
	(mg/kg)
Naphthalene	1.120 ± 0.420
Acenaphthylene	0.184 ± 0.026
Acenaphthene	0.192 ± 0.036
Fluorene	0.222 ± 0.016
Phenanthrene	3.941 ± 0.047
Anthracene	0.403 ± 0.002
Fluoranthene	6.140 ± 0.120
Pyrene	4.784 ± 0.029
Benzo(a)anthracene	2.092 ± 0.048
Chrysene	3.008 ± 0.044
Benzo(b)fluoranthene	5.990 ± 0.200
Benzo(k)fluoranthene	1.748 ± 0.083
Benzo(a)pyrene	2.470 ± 0.170
Indeno(1,2,3-cd)pyrene	2.960 ± 0.170
Dibenzo(a,h)anthracene	0.290 ± 0.004
Benzo(g,h,i)perylene	3.937 ±0.052
Copyright [©] by Chiang	; Mai University
All rights i	reserved

Table 2.4 Certified values of SRM urban dust NIST 1649b

2) Testing of Precision

2.1) Repeatability

Repeatability of GC-MS system was determined with 7 repeating measurements of a 0.02 mg/L mixed standard solution by GC-MS under the optimum condition.

2.2) Reproducibility

Reproducibility of GC-MS system was determined by repeated measurements of a 0.02 μ g/mL mixed standard solution by GC-MS under the optimum condition for 7 continuous weeks.

The results of the repeatability and reproducibility were estimated by relative standard deviation (RSD) as calculated by using Equation 2.2.

% RSD =
$$\frac{SD}{x} \ge 100$$

(Eq. 2.2)

Where % RSD is a percentage relative standard deviation

SD is a standard deviation

x is an average value **Mai University** Copyright[©] by Chiang Mai University All rights reserved

2.11 Data analysis of PM_{2.5} and PAHs emitted from incense and raw materials burning

2.11.1 Emission factors (EFs) of pollutants

An emission factor (EF) is a representative value that relates to the quantity of a pollutant released to the atmosphere with an activity associated with the release of that pollutant. These factors are usually expressed as the weight of pollutant divided by a unit weight, volume, distance, or duration of the activity emitting the pollutant (US EPA, 2011). EFs are calculated based on the measurements of flow rate and pollutant mass concentration using Equation 2.3 (Kim Oanh et al., 2011).

 $EF = \frac{[Conc. (ng/m^3) \times Flow rate (m^3/h) \times Sampling time (h)]}{Biomass burned (kg)}$

(*Eq* 2.3)

2.11.2 Emission rates (ER) of pollutants

Emission rates (ERs) are emission of air pollutant in terms of mass of pollutant emitted per time (Jetter ei al., 2002). It used to quantify air pollutant emission from incense burning. Traditionally, ERs for a specific incense types are calculated as shown in Equation 2.4.

$$ERs = \frac{Amount \ of \ pollutants \ mass}{Burning \ time \ (hr)}$$

(*Eq 2.4*)

2.11.3 Statistical data analysis

The correlation coefficient was assessed by the Spearman's rank correlation (r_s) . The Spearman's rank-correlation was implemented to identify the relationships between the various PM_{2.5} concentrations and the total PAHs (t-PAHs) bounded with

 $PM_{2.5}$, which included carcinogenic PAHs (c-PAHs) and non-carcinogenic PAHs (nc-PAHs). Moreover, the correlation between concentrations of $PM_{2.5}$, NO_2 and the number of visitors worshiped at shrines was investigated. The various emission factors of $PM_{2.5}$, the total PAHs bounded with $PM_{2.5}$ and gaseous pollutants emitted from each type of incense and raw materials burning in the chamber, which were calculated by One-way ANOVA test. Furthermore, One-way ANOVA was used to compare concentrations of $PM_{2.5}$, $PM_{2.5}$ -bound PAHs, NO_2 inside the shrines on each occasion.

กมยนดิ

2.11.4 Isomeric ratios

The isomeric ratios are concentration ratios of some PAHs considered as fingerprint of an emission source (Orecchio, 2011; Tobiszewski and Namienik, 2012; Wiriya et al., 2013). PAHs isomer pair ratios have extensively been used to explain the characteristics of the specific source. The FLA/(FLA+PYR) and IND/(IND+BPER) ratios have been used to specify the source of PAHs. Value of FLA/(FLA+PYR) and IND/(IND+BPER) higher than 0.5 indicated grass, wood, coal combustion, while value lower than 0.5 indicated fossil fuel combustion or petroleum input (Hischenhuber and Stijve, 1987; Yunker et al., 2002). Ratio of BaA/(BaA+CHR) implied petroleum (<0.20), petroleum or combustion (0.20 to 0.35) and combustion (>0.35) (Hischenhuber and Stijve, 1987). The ratio value of ANT/(ANT+PHE) < 0.10 was referred to petroleum, while a ratio > 0.10 indicated a high temperature source (combustion) (Yunker et al., 2002).

When the investigated samples calculated by isomeric ratios were not in agreement with each other, a total index was used to confirm emission of PAHs. Mannino and Orecchio (2008) suggested total index Equation (Eq.2.5), which defined the total index as the sum of the above mentioned isometric ratios (Yunker et al, 2002). The total index higher than 4 indicated PAHs from high temperature processes (combustion), while the value less than 4 indicated low temperature (petroleum products).

$$Total index = \frac{FLA/(FLA + PYR)}{0.4} + \frac{IND/(IND + BPER)}{0.5} + \frac{BaA/(BaA + CHR)}{0.2} + \frac{ANT/(ANT + PHE)}{0.1}$$

$$(Eq. 2.5)$$

2.12 Heath risk assessment of PAHs

2.12.1 Toxicity equivalent concentration (TEQ)

The toxicity equivalent concentration (TEQ) is widely used to assess the risk of carcinogenic potency of each individual PAH. This parameter is calculated from the total of individual concentrations of each PAH multiplied by their toxic equivalent factor (TEF) relative to the carcinogenic potency of BaP (Eq.2.6), which was used as a reference carcinogenic compound. The toxicity equivalent concentration (TEQ) was calculated by Nisbet and LaGoy (1992).

$$\Gamma EQ = \sum (PAH_i \times TEF_i)$$

(Eq. 2.6)

Where PAH_i is concentration of individual PAHs, TEF_i is the toxic equivalent factors (Table 2.5).

Compound	TEF	Compound	TEF	Compound	TEF
NAP	0.001	FLA	0.001	BaP	1.0
ACY	0.001	PYR	0.001	IND	0.1
ACE	0.001	BaA	0.1	DbA	1 .0
FLU	0.001	CHR	0.01	BPER	0.01
PHE	0.001	BbF	0.1	1ai Univ	ersity
ANT	0.01	BkF	0.1	serv	/ e d

Table 2.5 Toxic equivalency factors (TEFs) for PAHs (Nisbet and LaGoy, 1992)

2.12.2 Mutagenic equivalent concentration (MEQ)

The mutagenic equivalent concentration (MEQ) was calculated by multiplying the concentration of each PAH compound with the mutagenic equivalent factor (MEF) relative to the mutagenic potency of BaP (Eq. 2.6) (Durant et al., 1999 and Jung et al., 2010). The mutagenic activity is not uniformly implicated for cancer (Zeiger, 2001) but it is associated with some adverse health effects such as pulmonary

diseases (DeMarini et al., 2004). However, the carcinogenic potency is used more worldwide than mutagenic potency.

MEQ = 0.082(BaA) + 0.017 (CHR) + 0.25(BbF) + 0.11(BkF) + 0.31(IND) + 0.29(DbA) + 0.19(BPER) + BaP(Eq. 2.7)

2.12.3 Inhalation cancer risk (ICR)

The inhalation cancer risk (ICR) was used to estimate the value of cancer risk from PAHs exposure during the different periods. Several studies (Jung et al., 2010; Jia et al, 2011; Sarkar and Khillare, 2012; Wiriya et al., 2013) reported the equation for this calculation as follows (Eq. 2.7 and 2.8):

$$ICR = [\Sigma(PAH_i \times TEF_i)] \times IUR_{BaP}$$
(Eq. 2.8)
$$ICR = TEQ \times IUR_{BaP}$$
(Eq. 2.9)

Where, IUR_{BaP} is the inhalation unit risk. Two different IUR_{BaP} values were used in this study to estimate the inhalation cancer risk. One IUR_{BaP} of the World Health Organization (WHO) recommends the value at 8.7×10^{-5} m³/µg (WHO, 2000) and another IUR_{BaP} of the Office of Environmental Health Hazard Assessment (OEHHA) of California Environmental Protection Agency (CalEPA) recommends the value at 1.1×10^{-6} m³/µg (Collins et al., 1998; OEHHA, 2003) for lifetime (70 years) PAHs exposure. The mean TEQ value was obtained from the calculations of each period based on Equation 2.5.