DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR POLYCYLIC AROMATIC HYDROCARBONS IN FINE PARTICULATE MATTER ($PM_{1.0}$ AND $PM_{2.5}$) SAMPLES BY GC-MS/MS

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TÓM TẮT

PHÁT TRIỂN VÀ THẨM ĐỊNH PHƯƠNG PHÁP PHÂN TÍCH CÁC HYDROCARBON THƠM ĐA VÒNG TRONG MẪU BỤI MỊN $PM_{1.0}$ VÀ $PM_{2.5}$ BẰNG PHƯƠNG PHÁP SẮC KÍ KHÍ GHÉP KHỐI PHỔ (GC-MS/MS)

Trong nghiên cứu này, chúng tôi tiến hành phát triển và thẩm định phương pháp phân tích hàm lượng 15 hydrocarbon thơm đa vòng (PAHs) trong mẫu bụi mịn ($PM_{1.0}$ và $PM_{2.5}$) bằng phương pháp sắc ký khí ghép nối khối phổ (GC-MS/MS). Mẫu bụi được chiết với hỗn hợp dung môi acetone và n-hexane (1:1, v/v) bằng kĩ thuật chiết siêu âm. Sau đó dịch chiết mẫu được làm sạch trên cột chiết pha rắn silica gel đa lớp và PAHs được rửa giải bằng hỗn hợp dichloromethane và n-hexane (1:1, v/v). Các PAHs được tách trên cột mao quản DB-5MS (30 m × 0.25 mm × 0.25 µm) với khí mang là heli. Sự có mặt và hàm lượng của các PAHs được xác định trên hệ thống GC-MS/MS dựa trên phổ khối lượng và cường độ mảnh khối đặc trưng. Các thông số để đánh giá phương pháp phân tích như giới hạn phát hiện, giới hạn định lượng, phương trình đường chuẩn, độ thu hồi và độ lặp lại đã được khảo sát. Giới hạn phát hiện của 15 PAHs nằm trong khoảng 0.0001 đến 0.049 ng/m^3, độ thu hồi từ 70% đến 110% và độ lệch chuẩn tương đối của các thí nghiệm lặp lại nhỏ hơn 10%. Phương pháp phân tích đáp ứng được các yêu cầu về khả năng phát hiện, độ đúng và độ lặp lại đã được áp dụng để xác định hàm lượng PAHs trong một số mẫu bụi mịn thực tế.

***Từ khóa**: polycyclic aromatic hydrocarbons, particulate matter, gas chromatography mass spectrometry.*

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of hazardous pollutants due to their omnipresence and high toxicity [1]. PAHs can be released into the atmosphere from natural processes such as volcanoes and forest fires. However, the main sources of PAHs in the environment are caused by human activities. They are the product of incomplete combustion or pyrolysis of materials such as petroleum, coal, wood, solid waste, and several industrial processes [2]. In addition, large amounts of

PAHs can be released into the environment through traffic activities [3]. PAH has the ability to spread long distances in the environment. Many PAH-related compounds are potential carcinogenic and mutagenic. Some PAH transformation products (i.e., substituted PAHs) are even more toxic than their parent compounds. The toxicity of PAHs depends on their molecular structure. The impact of PAHs on human health depends on exposure doses and toxicity profiles of PAHs [4,5]. PAHs are harmful even at low exposure doses over a long period. In addition, PAHs can also cause other non-carcinogenic effects on the skin, body fluids, and resistance. Humans can be exposed to PAHs through consumption of food and water, breathing air, or direct contact with materials containing PAHs. A number of studies determined PAHs in fine dust samples (e.g., $PM_{2.5}$ and PM_{10}), thereby demonstrating harmful effects of PAHs associated with this media [6]. Inhalation of polluted air is a significant exposure source of these pollutants in human.

Chromatographic methods such as gas chromatography (GC) and high-performance liquid chromatography (HPLC) are commonly used to analyze PAH mixtures. However, GC coupled with mass spectrometry (MS) has been considered as the most effective method to detect PAHs at trace levels in environmental samples due to their high separation efficiency and low detection limits [7-8]. In GC-MS methods, PAHs are usually quantified by using internal standard/isotope dilution method with deuterated PAHs as surrogate and internal standard compounds. A typical PAH analytical procedure consists of several steps such as: solvent extraction, extract purification, concentration, followed by chromatographic separation and quantification by using GC. In general, a conventional procedure for PAH analysis is still time and labor consuming. Although single quadrupole MS detectors are frequently used in many studies on PAHs, triple quadrupole MS/MS detectors exhibit outstanding selectivity and sensitivity. The high selectivity and sensitivity of the MS/MS detectors allow to simplify the sample preparation process, and the peak integration is easier and faster, thereby simplifying data processing, eliminating noise, increasing analysis efficiency, and generating more reliable results [8].

In this study, we developed and validated a simple, rapid, and effective analytical method for the determination of 15 PAHs in fine dust samples ($PM_{1.0}$ and $PM_{2.5}$) by using a combination of ultrasonic extraction, solid-phase extraction (SPE) clean-up, and GC-MS/MS quantification. The validated method was applied to analyze PAHs in $PM_{1.0}$ and $PM_{2.5}$ samples collected from some areas in Bac Ninh Province, northern Vietnam.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Native standards include 15 target compounds: naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fln), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo [a] pyrene (BaP), indeno(1,2,3-cd)pyrene (IcdP), dibenz[a,h]anthracene (DahA), and benzo[ghi] perylene (BghiP). Internal Standards Mix 33 including naphthalene-D8, acenaphthene-D10, fluoranthene-D10, phenanthene-D10, chrysene-D12, and perylene-D12 (2000 ppm in toluene) was purchased from Dr. Ehrenstorfer (Germany). Solvents including acetonitrile (ACN), n-hexane, methanol (MeOH), and dichloromethane (DCM) with purities >99.8% were purchased from Merck (Germany). Other chemicals including anhydrous sodium sulfate, silica gel, and sulfuric acid impregnated silica gel were obtained from Sigma-Aldrich (USA).

2.2. Sample collection

Sixteen fine dust samples were collected from different areas in Bac Ninh Province, northern Vietnam. The Kanomax nano sampler with pump speed of 40 L/min operated in vacuum pump mode was used. The sampling period from July 2021 to October 2021, sampling time for one sample is around 21:00 to 23:00. During sampling, the device was manually adjusted and controlled in time from the beginning and the end of the sampling. After collection, the samples were stored in clean Petri dishes, wrapped in aluminum foil, and taken to a standard weighing room to equilibrate temperature

(20-25°C) and humidity (RH: 30-40%) at least 1 day prior to mass determination. There was a total of 16 samples were collected including 8 $PM_{1.0}$ samples and 8 $PM_{2.5}$ samples.

2.3. Sample preparation

The filters containing fine dust particles will be chopped and then transferred to a 15-mL test tube with a stopper. After that, 20 µL of 10 ppm internal standard was spiked to each test tube and allowed to stand for 20 min. After adding 10 mL of a mixture of acetone and n-hexane (1:1, v/v), the sample was extracted in an ultrasonic water bath for 30 min. The sample tube was then centrifuged and the supernatant was transferred into a new 50-mL tube. The extraction procedure was repeated in total 3 times. The extract fractions were pooled and evaporated. The clean-up SPE column comprise anhydrous sodium sulfate, silica gel, and 20% and 40% sulfuric acid impregnated silica gel. The column was activated by 10 mL of methanol and 10 mL of n-hexane. After loading the extract onto the column, PAHs were eluted by 12 mL of DCM in n-hexane (1:1, v/v). The eluate was concentrated under a gentle nitrogen stream and reconstituted in 1 mL n-hexane. This final solution was then transferred to a vial before GC-MS/MS analysis.

2.4. Instrumental analysis

The GC-MS/MS system (GC Trace 1310, MS TSQ 8000) from Thermo was operated with a DB-5MS capillary column (stationary phase 5% phenyl 95% methyl polysiloxane, 30 m × 0.25 mm × 0.25 µm; Agilent, USA). The sample pump chamber temperature was 280°C. The sample injection volume was 1 µL. Carrier gas was helium (99.999%) at a flow rate of 1 mL/min. The column oven temperature program was initially set at 80°C (keep 3 min), increased to 200°C (15°C/min, keep 3 min), and finally increased to 310°C (8°C/min, keep 3 min). The ionization source temperature was 280°C. The interface temperature was 300°C. The mass sweep range was adjusted from 50 to 500 amu. The acquisition mode was selected reaction monitoring (SRM). TraceFinder-Thermo software was applied for data evaluation and reporting. The quantification of PAHs was performed using the standard curve method.

3. RESULTS AND DISCUSSION

3.1. Identification of 15 PAHs in standard mixtures

With the instrumental conditions mentioned in Section 2.4, the chromatogram of a PAH standard mixture obtained by the GC-MS/MS system is shown in Figure 1. From this chromatogram we see that all peaks are sharp and clear.

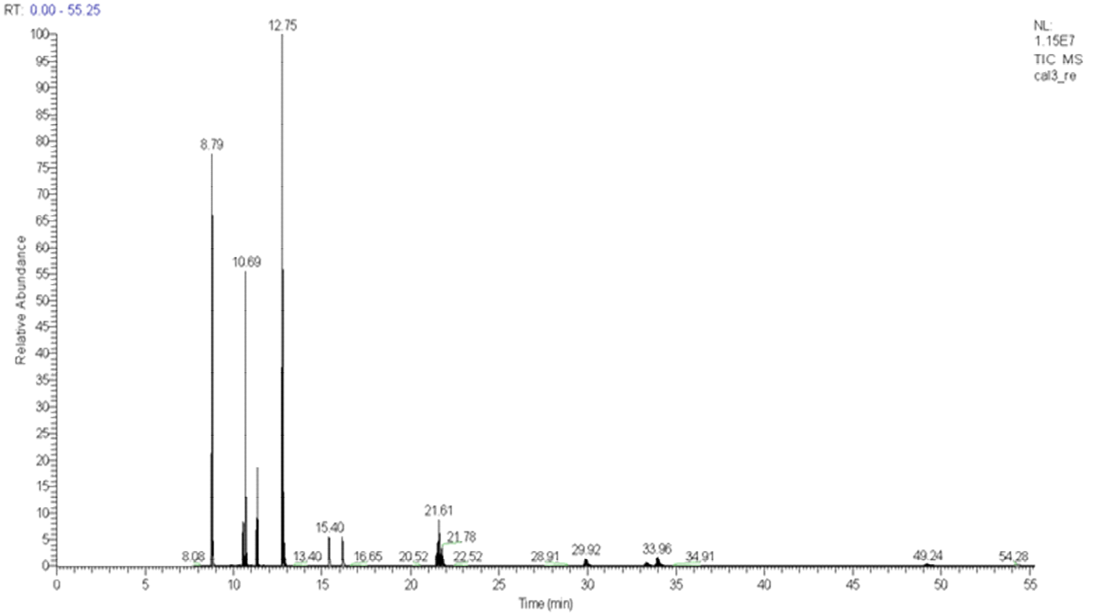

Figure 1. Chromatogram of 15 PAHs at concentration of 50 ppb

The retention times, mass transition patterns, and collision energies of 15 PAHs are presented in Table 1.

Table 1. Retention time and MS/MS conditions for PAHs analysis

	Retention time (min)	**Transition (m/z)**		**CE (eV)**
Nap	8.80	128.08	102.06	15
Acy	10.55	152.08	151.10	15
Ace	10.72	153.11	152.11	15
Fln	11.34	166.10	165.10	15
Phe	12.78	178.11	152.09	20
Ant	12.88	178.11	152.08	20
Flu	15.38	202.08	200.10	30
Pyr	16.14	202.11	200.10	35
BaA	21.48	228.11	226.12	35
Chy	21.73	228.12	226.11	30
BbF	29.82	252.12	250.12	30
BaP	33.21	252.11	250.12	35
IcdP	48.88	276.15	274.12	40
DahA	48.93	278.15	276.12	35
BghiP	53.83	276.14	274,10	40

3.2. Calibration curve, method detection limits, and method quantitation limits

In the GC-MS/MS method, it is recommended to build calibration curve using internal standards to ensure the accuracy of the measurement. To match the concentration of PAHs in fine dust, the calibration curve was made in the concentration range of 5–2000 ppb. In addition, results showed that the correlation coefficient R^2 for all analyzed compound was around 0.99. Linearity was validated by calculating the correlation coefficient (R^2). Method detection limits (MDL) and method quantitation limits (MQL) were estimated based on instrumental limits of detection (LOD) and limits of quantitation (LOQ), final extract volume, and air volume. The LOD and LOQ values were determined by adding a mixture of 15 PAHs to the blank at the lowest concentration that could be observed by the instrument, giving a signal-to-noise ratios of 3 and 10, respectively. The MDLs and MQLs of this method ranged from 0.0001 to 0.049 ng/m^3, and from 0.0002 to 0.165 ng/m^3, respectively. The detailed results for 15 PAHs are showed in Table 2.

Table 2. Calibration curves, MDLs and MQLs of 15 PAHs

	Linear regression equation	**R^2**
Nap	Y = 0.001X – 0.004	1.0000
Acy	Y = 0.003X – 0.049	0.9989
Ace	Y = 0.005X – 0.041	0.9997
Fln	Y = 0.007X – 0.072	0.9980
Phe	Y = 0.001X – 0.01	0.9998
Ant	Y = 0.001X– 0.017	0.9986
Flu	Y = 0.001X – 0.046	0.9996
Pyr	Y = 0,002X – 0.037	0.9983
BaA	Y = 0.008X – 0.011	0.9999
Chy	Y = 0.009X – 0.021	0.9998
BbF	Y = 0.014X – 0.016	0.9999
BaP	Y = 0.012X – 0.135	0.9995
IcdP	Y = 0.011X – 0.187	0.9991
DahA	Y = 0.011X – 0.275	0.9980
BghiP	Y = 0.007X – 0.193	0.9978
	MDL (ng/m^3)	**MQL (ng/m^3)**
Nap	0.0004	0.001
Acy	0.0003	0.001
Ace	0.0045	0.015
Fln	0.0001	0.0002
Phe	0.004	0.014
Ant	0.031	0.102
Flu	0.0035	0.012
Pyr	0.016	0.055
BaA	0.021	0.069
Chy	0.019	0.063
BbF	0.005	0.016
BaP	0.009	0.031
IcdP	0.026	0.087
DahA	0.017	0.055
BghiP	0.049	0.165

3.3. Recovery and repeatability

Recovery rates and deviations of replicate experiments are important parameters to assess the reliability of an analytical method. In this method, recoveries and deviations of the analytical procedure were estimated by using a matrix spiked with PAH standards at a concentration of 100 ppb each compound. The recoveries and relative standard deviations ranged from 70% to 110%, and from 3% to 9%, respectively, indicating adequate accuracy and precision of the method. The detailed results are showed in Table 3.

Table 3. Recovery efficiency of the method with standard increments of 100 ppb

	Recovery (%)	SD	RSD (%)
Nap	90	7.17	8
Acy	76	6.81	9
Ace	99	3.32	3
Fln	79	5.08	6
Phe	70	9.82	8
Ant	75	4.80	6
Flu	95	8.54	9
Pyr	75	6.7	8
BaA	72	3.58	6
Chy	83	7.20	9
BbF	79	2.50	3
BaP	110	6.32	6
IcdP	85	6.16	7
DahA	100	9.44	9
BghiP	75	6.16	8

3.4. Method applicability

The analytical procedure mentioned in Section 2.3 was applied to determine concentrations of 15 PAHs in fine dust samples collected from some industrial zones and traffic intersections in Bac Ninh Province. PAHs were detected in all the samples with total concentrations ranging from 15 to 21 ng/m^3, and from 14 to 73 ng/m^3 for $PM_{1.0}$ and $PM_{2.5}$ samples, respectively. The average concentrations of 15 PAHs in two sample types (i.e., $PM_{1.0}$ and $PM_{2.5}$) are shown in Figure 2. Interestingly, concentrations of lighter PAHs (e.g., 2 to 4 rings) were generally higher in $PM_{2.5}$ samples, whereas concentrations of heavier compounds (e.g., 5 and 6 rings) were slightly higher in $PM_{1.0}$ samples. This observation suggests different behavior of these compounds in the atmosphere due to their molecular weights and emission sources.

Profiles of 15 PAHs in $PM_{1.0}$ and $PM_{2.5}$ samples collected from industrial zones and traffic intersections in Bac Ninh Province are shown in Figure 3. The profiles observed in the two sample types were quite similar. Phe was the most abundant compound, which accounted for 20% and 22% of total 15 PAHs in $PM_{1.0}$ and $PM_{2.5}$ samples respectively. Other predominant compounds were Flu, Pyr, and Nap. Concentrations and proportions of the remaining compounds were relatively low.

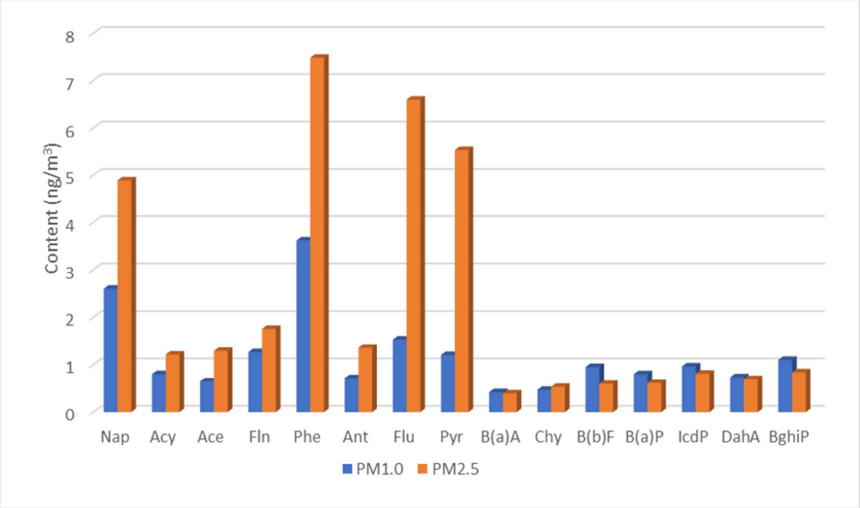

Figure 2. Average concentrations of PAHs in $PM_{1.0}$ and $PM_{2.5}$ samples

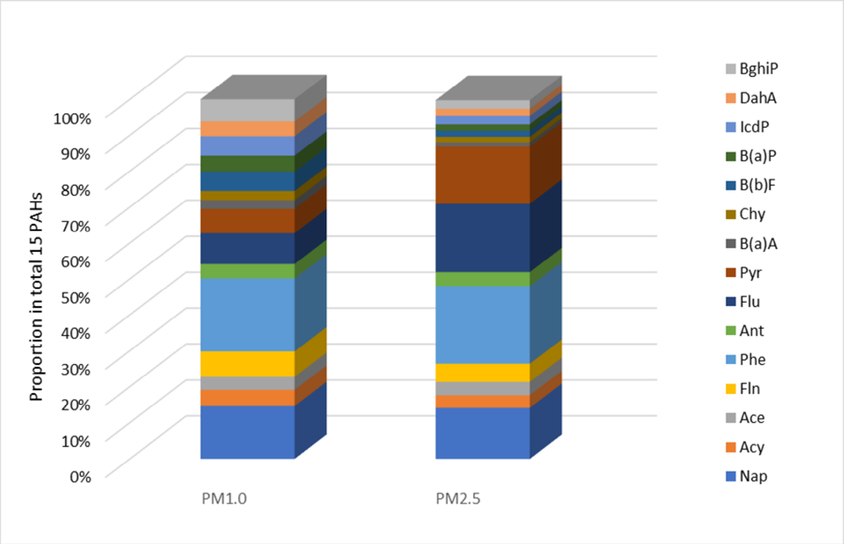

Figure 3. Profiles of PAHs in $PM_{1.0}$ and $PM_{2.5}$ samples.

4. CONCLUSION

In this study, the GC-MS/MS method was developed for the analysis of 15 PAHs in $PM_{1.0}$ and $PM_{2.5}$ dust samples. The analytical procedure consisted of ultrasonic extraction with acetone/n-hexane (1:1) mixture, multilayer silica gel SPE clean-up, and GC-MS/MS quantification. The recovery of the analytical method was in the range of 70% to 110%, RSD < 10%, and method quantitation limits ranging from 0.0002 to 0.165 ng/m^3. The validated method was successfully applied to analyze 15 PAHs in real $PM_{1.0}$ and $PM_{2.5}$ samples collected from some industry zones and traffic intersections in Bac Ninh Province. The total PAH concentrations in $PM_{2.5}$ samples (14–73 ng/m^3) were slightly higher than those in $PM_{1.0}$ samples (15–21 ng/m^3). Phenanthrene was the most abundant compound found in both $PM_{1.0}$ and $PM_{2.5}$ samples and the distribution profiles of PAHs in the two sample types were quite similar.

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