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Trace determination of nitrated polycyclic aromatic hydrocarbons using liquid chromatography with on-line electrochemical reduction and fluorescence detection

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Abstract

An efficient clean-up procedure coupled with a high performance liquid chromatography (HPLC) with on-line electrochemical (EC) reduction and fluorescence detection (FLD) was developed to quantify nitrated polycyclic aromatic hydrocarbons (NPAHs) in the airborne particulate. In this process, NPAHs were extracted ultrasonically followed by analysis by using a reversed phase column with an aqueous eluent containing 70% aqueous acetonitrile and sodium monocholoroacetate as a buffer solution. The extraction efficiencies were above 83% for 1-nitropyrene and 1,3-dinitropyrene (1,3-DNP) 1,6-DNP, and 1,8-DNP, and calibration graphs were linear with very good correlation coefficients ($r > 0.999$) and the detection limits were in the range of 1.0–2.2 pg for dinitropyrenes and nitropyrene. The proposed method provides a relatively simple and convenient procedure for determining the NPAHs samples in airborne particulate.

Keywords: Nitrated polycyclic aromatic hydrocarbons; Ultrasonic extraction; High performance liquid chromatography; Electrochemical reduction; Fluorescence detection

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are widespread environmental pollutants. Formation of NPAHs results either from the combustion of petrochemical fuel, cigarette smoke, and cooking [1,2] or from the photochemical reactions of polycyclic aromatic hydrocarbons (PAHs) with hydroxyl radicals and NO_2 [3–5] in the atmosphere. The NPAHs are direct mutagens in the Ames test [6,7]. Among NPAHs the 1,3-dinitropyrene (DNP), 1,6-DNP, and 1,8-DNP exhibited the strongest mutagenic effects [8–10]. 1,6-Dinitropyrene and 1,8-dinitropyrene also are classified as the possible human carcinogens in IARC Monographs [11]. NPAHs are reported as the contributors are to the incidence of lung cancer [11–15]. On the other hand, the 1-nitropyrene (1-NP) is an important index of carcinogens relating to environmental degeneration in urban air particulate [9,10] due to high concentration with high mutagenicity. Therefore, developing a simple and convenient method to monitor the concentration of NPAHs in airborne particulate

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is required to evaluate their health risk of polluted air and to determine their toxicity mechanisms.

Several analytical techniques have been developed to analyze NPAHs in the environment, use such as: gas chromatography with flame ionization detection (FID) [16–18], with electron-capture detection (ECD) [19–21], with nitrogen–phosphorous detection (NPD) [22], or with mass spectrometry (MS) [23–27], and high performance liquid chromatography (HPLC) with fluorescence detection (FLD) [28–30] or with electrochemical detection [31–34]. Several detection methods for gas chromatography, such as FID, ECD, and NPD offer some degree of sensitivity and selectivity for nitrogen compounds. However, these detectors are often experience the severe interference as well as loss of sensitivity in the complex environmental matrices. Although the MS system possesses high sensitivity and high resolution, it is a sophisticated high-cost instrument system and requires a skillful operator, thus it does not satisfy the requirements for routine analysis. The HPLC/FLD method has a unique sensitivity for amino polycyclic aromatic hydrocarbons (APAHs), diaminopyrenes, and aminopyrenes [39]. Fluorescence detection method does not work for NPAHs, because NPAHs produced very low yield of fluorescence after irradiation by the UV light. Thus, when fluorescence detectors have been used, the reduction NPAHs to APAHs for detection is an essential step. Singvardson and Birks [35], Wing et al. [36], and Hayakawa et al. [37,38] developed a procedure to reduce NPAHs to APAHs by using on-line zinc or Pt/Rh reduction, which yielded the excellent results on chemiluminescense for detection. In the above method, an extra-column was required for packing the zinc powder or Pt/Rh-coated alumina. Recently, an electrochemical method has been applied as a simple, sensitive, and reproducible reduction method for the reducible chemical compounds [34]. Therefore, the use of the electrochemical (EC) reduction method is a worthwhile alternative method for NPAHs reduction.

The purpose of this study was to explore the potential of the HPLC/EC/FLD method for determining the concentration of NPAHs in airborne particulate. A heart-cutting technique combined a pre-column and switching valve [37] was used to clean up the interferences of environmental matrices. In this report, the clean-up method and optimum conditions for HPLC/EC/FLD analysis of NPAHs are discussed.

2. Experimental

2.1. Apparatus

The instrumental assembly was described as in Fig. 1. A HPLC system consisted of two pumps (LC-10AD and SIL-10 A) (Shimadzu, Kyoto, Japan) equipped with a six-port switching valve (FCV-2AH, Shimadzu, Kyoto, Japan) adapted with a Tosh ODS-80TM pre-separated column (150 mm × 4.6 mm I.D.) (Tokyo, Japan) and a reversed-phase Kaseisorb LC ODS 60-5 analytical column (250 mm × 4.6 mm I.D.; Tokyo, Japan). A BAS-100A amperometric controller system with a glassy carbon working-electrode (Lafayette, Ind. USA), in a reducible mode at −1.5 V (versus Ag/AgCl) was used to achieve reduction. A Chem Win 1.0 system (Taipei, Taiwan) was used to control a programmable fluorescence detector (RF-10 AXL; Shimadzu, Kyoto, Japan), and to process the analytic data. Stainless steel tubing was used throughout the system rather than Teflon to prevent the permeation of oxygen into the mobile phase. The column was maintained at 40 °C in a thermostatically controlled oven (Shimadzu model CTO-6A). Some prospective interference species in the sample matrix were pre-separated with the pre-column using a heart-cutting technique. The flow rate of eluent was programmed based on column switching steps.

2.2. Chemicals and reagents

Deionized water purified through a Millipore 60 system (Bedford, MA, USA), was used for all aqueous solution. All chemicals were of ACS reagent grade. The standard compounds, 1,3-DNP (99%, Aldrich), 1,6-DNP (100%, Accustandard Inc., USA) and 1,8-DNP (98%, Aldrich), and 1-NP (>98%, TCI, Japan) were used without further purification. Sodium monocholoroacetate was obtained from Fluka (Switzerland). Cyclohexane, and acetonitrile were purchased from Fisher (Springfield, MO, USA). Stock solutions of NPAHs were prepared by dissolving 1–5 mg of compounds in 100 ml acetonitrile. All of the solutions were stored in brown glass bottles, and kept in a refrigerator (−70 °C). Fresh working solutions were prepared daily using appropriate dilution of the stock solutions. The HPLC eluent was prepared using 70% (v/v) acetonitrile, and 30% 0.01 M

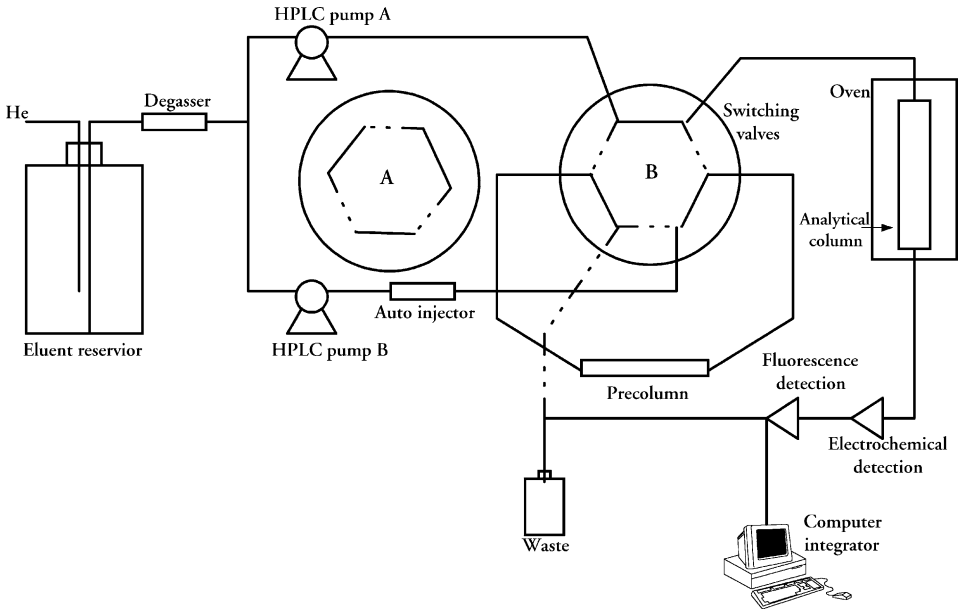

Fig. 1. Experimental arrangement schematic and layout of the switching system for determining nitrated polycyclic aromatic hydrocarbons.

sodium monochloroacetate buffer solution (pH 4.7). All eluents were purged of dissolved oxygen using a helium for about 1 h with a degassed unit (Gasu Kuro Kogyo model 546, Japan), and then were cleaned up with 0.45 μm membrane filter (Millipore) prior to using.

2.3. Sampling collection and preparation

A high volume for particles (<10 μm in diameter, PM_{10}) were collected by the air sampler (Kimoto Denshi, Osaka, Japan). The samplers were set up on the campus of China Medical College near an urban district, in Taichung city, Taiwan. It is one of the representative areas of the city where is direct exposure to near by the emission exhaust sources. The altitude of sampler was about 10 m. Airborne particulate was collected on a glass fiber filter (10 in. × 8 in., Gelman Science, MI, USA) for 1 month (30 days). The samplers were run for 24 h continuously at a flow rate of approximately 1 m^3/min which were calibrated approximately once per week. After being conditioned and dried in the dark, the filters were weighed. Finally, each filter was covered with tinfoil and then stored in refrigerated (−70 °C) until use.

2.4. Sample pretreatment

The filter from the air sampler was cut into halves: one of the cut filters was divided into small pieces (7 cm × 1 cm) and then put in a centrifugal tube (Pyrex) contained 50 ml cyclohexane. The tube was placed in an ultrasonic ice-bath (4 °C) for 40 min and then centrifuged for 10 min (3000 rpm). A volume of 40 ml of the upper solution in the tube was taken and then reduced to 2 ml using rotary evaporation. These resultants were dried under nitrogen stream at ambient temperature. The residue was extracted with cyclohexane (3 ml), washed with sodium hydroxide (5%, 4 ml) followed by partitioning in the organic-aqueous phase solution. A 2.5 ml volume of cyclohexane phase was removed to a 10 ml tube and dried under nitrogen stream. Acetonitrile solution (0.5 ml) was used to dissolve the residue for chromatographic analyses.

2.5. Recovery test

The NPAHs were spiked on the sampling filter as the following: the filters, which had been used to collect airborne particulate for 24 h, were cut into small sections of equal size (7 cm × 1 cm). Aliquots (0.5 ml)

of standard solution NPAHs with concentration of 0.46 μg/ml were added to the tubes, individually. A piece of cut filter was placed in each tube to absorb the NPAHs. The filter was then dried under darkness at room temperature. The spiked filters were treated by the same procedure as described above. The recovery yield was calculated using dividing the slope of the linear regression for spiked samples by that for standard solutions in the same concentration range.

3. Results and discussion

3.1. Selection of wavelength for fluorescence detection of APAHs

It is essential to select a suitable wavelength with the best sensitivity for measuring the amounts of APAHs individually.

The detection parameters were pre-set and calibrated the programming because a real-time imaging fluorescence detector with a rapid scanning function was used in this method. Table 1 lists the optimal sensitivity parameters for measuring APAHs. The highest intensities of fluorescence were observed at the following wavelength pairs of excitation and emission; 1,3-DNP 375/480 nm, 1,6-DNP 372/445 nm, 1,8-DNP 394/450 nm, and 1-NP 356/436 nm. The chosen conditions were based on the compromise with the optimal detection conditions with retention behaviors of measuring species and relative fluorescence intensity. Finally, detection conditions were pre-set at $\lambda_{ex} = 385$ nm, $\lambda_{em} = 450$ nm for three diaminopyrenes (DAPs) at initial and then at $\lambda_{ex} = 356$ nm, $\lambda_{em} = 436$ nm after 21.7 min of elution for monitoring 1-aminopyrene.

Table 1
Fluorescence optimal characteristics of selected NPAH compounds after reduction electrochemical detector

NPAH compounds	Maxima (nm)	
	Excitation	Emission
1-Nitropyrene	356	436
1,3-Dinitropyrene	375	480
1,6-Dinitropyrene	372	445
1,8-Dinitropyrene	394	450

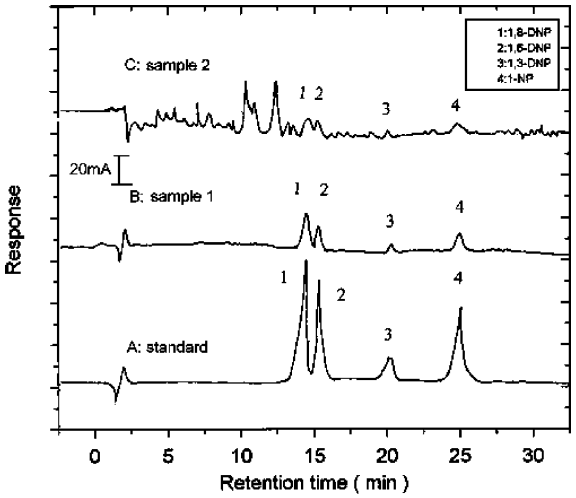

Fig. 2. LC-FLD chromatograms of standards sample and authentic samples. (A) Standard samples: 1,8-DNP, 1,6-DNP, 1,3-DNP, 1-NP, respectively; (B) run 1: the chromatogram of sample obtained by using column-switching method; and (C) run 2: the chromatogram of sample obtained without passing through a pre-column.

3.2. Selection of reduction potential for NPAHs

As in our previous studies [34], the best reduction potential for measuring NPAHs was selected at − 0.6 V (versus Ag/AgCl) by compromising both the sensitivity and the stability in detection. However, the electrochemical detector serviced as the reduction apparatus for reducing NPAHs to their amino counterparts in this work. In order to obtain the best sensitivity for the measurements, the reductive potential must be investigated to obtain the optimal yields for APAHs. Figs. 2 and 3 shows the relative fluorescence intensities under varied reductive potentials for NPAHs. We can find that relative intensities of the four APAHs increase with more negative potential. The relative intensities become to the maximum at potential of −1.5 V, and then decrease. Therefore, a reduction potential of −1.5 V (versus Ag/AgCl) was selected for the reduction the four NPAHs.

3.3. Removal of interferences in sample matrix

A number of interferences existed in the sample matrix which worsened the ability of separation of NPAHs from other species and increased the

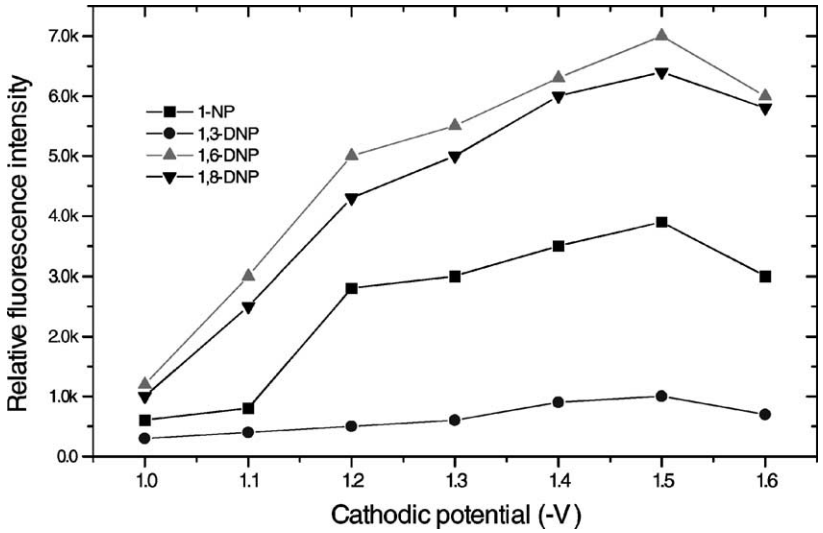

Fig. 3. Relationship for relative fluorescence intensity and reduction potential.

fluctuation of the baseline. A pre-column in this heart-cutting technique was used to clean up the interferences prior to analytical column separation. The process for removing the interferences was controlled by switching valves and both HPLC-pump systems. The timetable of switching and pump flow rates of both pumps are listed in Table 2. As shown in Fig. 1 and Table 2, the sample aliquot was injected into B status and eluted at the beginning. After 5.9 min, the switching valve was switched to A status until 9.1 min. During this period, the effluent was passed through the pre-column first and then to the analytical column. The status was switched again, and the chromatographic system was run in B status from 9.1 to 25.3 min and in A status from 25.3 to 30 min. The flow rate of pump B was 1.0 ml/min for the first 5.9 min, and kept at 2.0 ml/min during the periods of 5.9–25.3 min, then back to 1.0 ml/min until the end. The flow rate of pump A was kept at 1.0 ml/min through the whole run. After each analysis, the pre-column was back-flushed using pump A in A status of switching valve while the new sample aliquot was injected into the sample loop. Using the switching plan as described in Table 2, the best condition for removing interference was obtained. The reliability of the heart-cutting method was confirmed by comparing the chromatogram with that obtained from the analysis without the presence of pre-column, which is demonstrated in Fig. 2. It can be seen the chromatogram of NPAHs in an authentic sample is clear, well resolved, and with stable baseline, which was similar to that of the standard sample, while the chromatogram obtained without the presence of pre-column is still somewhat interfered with other species and the baseline is still unstable.

Table 2
Column-switching analytical procedure using the scheme according Fig. 1

	Time (min)			
	0–5.9	5.9–9.1	9.1–25.3	25.3–30
Switching position	B	A	B	A
Flow rate of pump A (ml/min)	1.0	2.0	2.0	1.0
Flow rate of pump B (ml/min)	1.0	1.0	1.0	1.0

3.4. Optimization of eluent condition and identification of NPAHs

According to our previous studies [34], the aqueous solution containing 70% acetonitrile and sodium monochloroacetate buffer solution (pH 4.7) offered an acceptable retention time and resolution of these four NPAHs. The proper concentration and pH of buffer solution not only enhanced the separation, but also

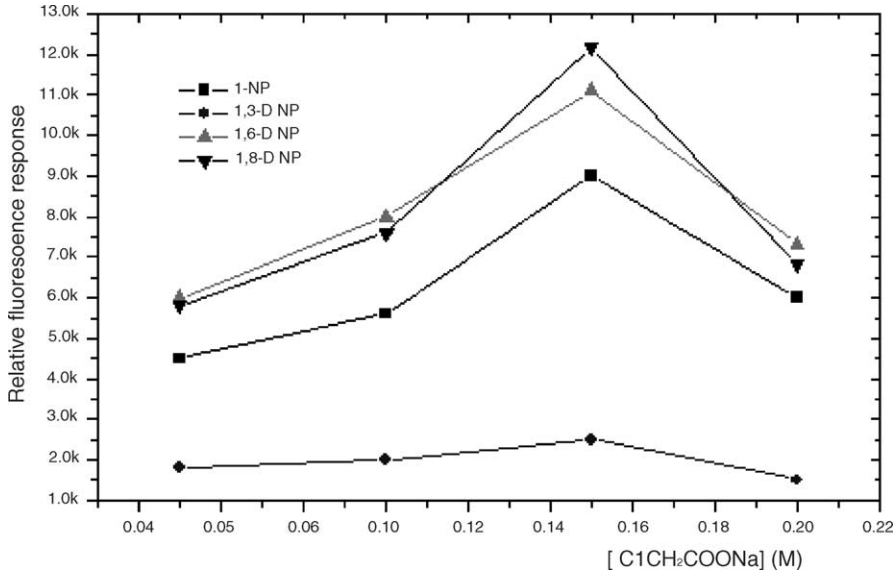

Fig. 4. Effect of the buffer concentration on detection response.

influenced the efficiencies of electrochemical reduction for following fluorescence detection. As shown in Fig. 4, we can find that the fluorescence intensity increased upon increasing the concentration of sodium monochloroacetate, but it decreased after the concentration of buffer solution higher than 0.15 M. The effect of pH on the relative fluorescence response is shown in Fig. 5, which suggest that the best detection sensitivity was the 0.15 M sodium monochloroacetate buffer solution with pH 4.7. Therefore, the elution

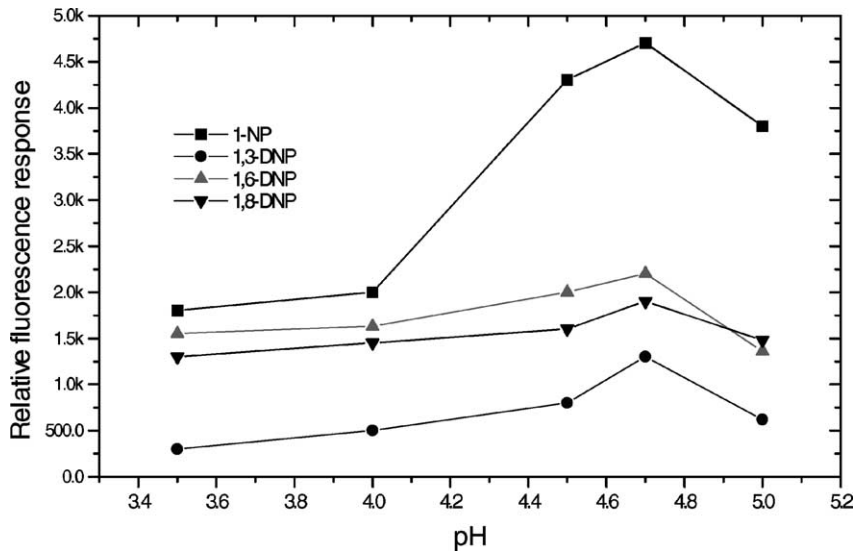

Fig. 5. Effect of pH on fluorescence response.

was performed under the conditions of 70% (v/v) acetonitrile and 30% 0.15 M sodium monochloroacetate buffer solution with pH 4.7. In addition, the temperature was controlled at 40 °C based on the consideration of the response sensitivity, band broadening, and resolution. Under the optimal separation and detection conditions, chromatograms of the standard sample and an authentic sample are shown in Fig. 2. These species were confirmed only by comparing the retention time with that of the standards, but also by using a fluorometry for the following fraction collection. As shown in the chromatograms, the four NPAHs were well separated within 27 min. The retention times for peaks 1–4 showed the agreement with 1,8-DNP, 1,6-DNP, 1,3-DNP, and 1-NP, respectively.

3.5. Validation and detection limits

The calibration plots for these four analytes over concentration ranges that are summarized in Table 3. Excellent linear relationships obtained for the four analytes. The repeatability was obtained from measuring the peak areas with 10 replicated injections of 10 μl (concentration 0.03 μg/ml) of analytes. The relative standard deviations (R.S.D.s) were all within 5%. They are acceptable in an environmental analysis. The detection limit are defined as the total amount of APAHs at a signal/noise ratio of three (S/N = 3) and the blank level. The detection limits were ca. 2 pg (S/N = 3) for all analytes. These detection limits are better than those data obtained in previous studies using HPLC with either electrochemical detection [31,34] or comparable to HPLC with chemiluminescent detection [35,38].

3.6. Recovery studies of spiked samples by ultrasonic extraction

The Soxhlet extraction with the organic solvents is a common method for extraction from solid samples. Recently, ultrasonic extraction has been widely applied because of the time-saving and less amount of organic solvent used. In order to test the efficiency of the ultrasonic extraction with cyclohexane for analysis of NPAHs, 0.5 ml of each standard solution with concentration of 0.46 μg/ml, respectively, was spiked into filter paper. After ultrasonic extraction, pretreatment, and chromatographic analysis, the average recovery yields ranged from 83 to 94% for these four analytes. In order to study the possible matrix effect on the extraction 0.46 μg/ml of the standards solution was spiked into the airborne particulate. Similar recovery yield were obtained, which were 83% (R.S.D. 2.5%), 83% (R.S.D. 2.1%), 84% (R.S.D. 2.7%), and 88% (R.S.D. 3.8%) for 1,3-DNP, 1,6-DNP, 1,8-DNP, and 1-NP, respectively. The extraction efficiencies of the ultrasonic method with cyclohexane as a solvent were high and greater than 83% for all four analytes, which met the requirement for accuracy and precision for the purpose of environmental determination. Therefore, cyclohexane was chosen as a solvent rather than using a benzene ethanol solution in our previous works based on the consideration of toxicity of benzene and good recovery yields for cyclohexane.

The effect of temperature on ultrasonic extraction efficiency was examined for temperature that ranged from 4 to 40 °C under ambient pressure (1 atm). The results are shown in Fig. 6. It is obvious that elevating the extraction temperature decreased the extraction efficiency. Therefore, ultrasonic extraction

Table 3
Calibration plots for nitrated polycyclic aromatic hydrocarbons in standards solutions

	Substance			
	1-Nitropyrene	1,3-Dinitropyrene	1,6-Dinitropyrene	1,8-Dinitropyrene
Concentration range (μg/ml)	0.001–7			
Regression equation $f(x)$	$y = -4.70 + 145.78x$	$y = 7.20 + 111.01x$	$y = -1.39 + 189.67x$	$y = -2.18 + 275.37x$
Linear regression relationship (r)	0.999	0.999	0.999	0.999
Detection limit (pg)	2.20	1.03	1.61	1.71

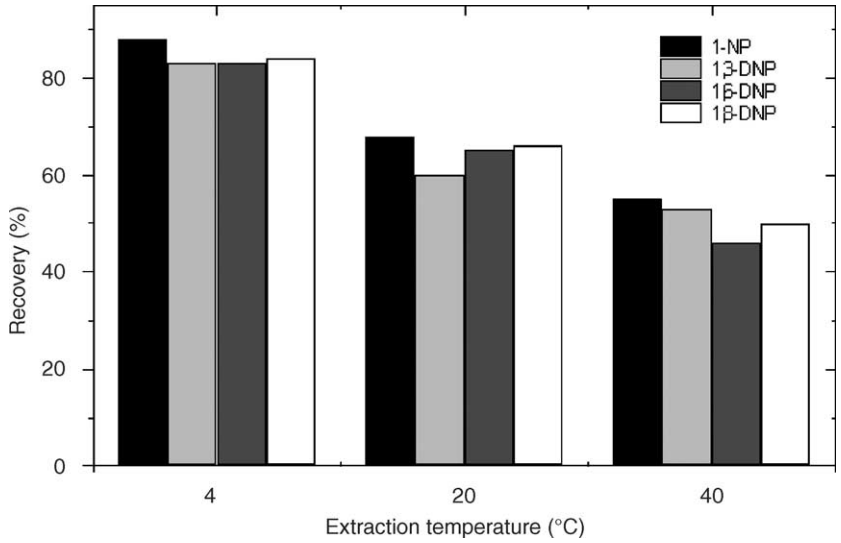

Fig. 6. Effect of extraction temperature on the recovery (%) of nitrated polycyclic aromatic hydrocarbons.

Table 4
Recovery yields and validation of peak areas for these four nitrated polycyclic aromatic hydrocarbons

Compound	The spiked filter		The spike air particulate sample		Peak areas R.S.D. (%)
	Recovery yield (%)	R.S.D. (%)	Recovery yield (%)	R.S.D. (%)	
1-Nitropyrene	94	1.1	88	3.8	1.4
1,3-Dinitropyrene	83	1.5	83	2.5	2.3
1,6-Dinitropyrene	84	2.7	83	2.1	2.3
1,8-Dinitropyrene	85	1.7	84	2.7	3.2

at a lower-temperature is recommended to obtain a higher-recovery yield of NPAHs. It is possible that NPAHs are semi-volatile compounds.

3.7. NPAHs measurement

The proposed method including airborne particulate collection, extraction, and pre-treation as described previously was applied to determine the NPAHs in metropolitan airborne particulate. Thirty samples were collected during a month period. Chromatogram of an authentic sample is shown in Fig. 2. The monitored results for PM_{10} particulates for airborne particulate in Taichung City from mid-November to -December were the range of 4.14–264.44 pg/m^3 for NPAHs and listed in Tables 4 and 5. The mean concentrations of 30 samples were 64.72, 38.14, 61.84, and 222.28 pg/m^3 for 1,8-DNP, 1,6-DNP, 1,3-DNP, and 1-NP, respectively. As shown in Table 5, although

Table 5
Comparison of the concentrations of NPAHs between Taiwan and Japan

Compound	Concentration range	Reference
1-Nitropyrene		
Taichung, Taiwan	65.98–229.00	This study
Chierding, Taiwan	240.00–1220.00	[39]
Kanazawa, Japan	145.48–230.20	[38]
1,3-Dinitropyrene		
Taichung, Taiwan	4.14–264.22	This study
Chierding, Taiwan	140.00–250.00	[39]
Kanazawa, Japan	0.50–0.80	[38]
1,6-Dinitropyrene		
Chierding, Taiwan	13.33–124.65	This study
Chierding, Taiwan	270.00–4900.00	[39]
Kanazawa, Japan	0.80–1.72	[38]
1,8-Dinitropyrene		
Taichung, Taiwan	18.36–233.33	This study
Chierding, Taiwan	230.00–5390.00	[39]
Kanazawa, Japan	0.68–1.54	[38]

the concentration ranges for nitropyrenes in this work are higher than that reported by Japanese groups, but they are still lower than the data reported for another Taiwan's city. In general, higher concentration of NPAHs presence in Taiwan infers more air pollution and the tropical climate leading more photochemical nitration to yield more NPAHs. The results indicated that this was a feasible method to detect the trace concentrations of 1,8-DNP, 1,6-DNP, 1,3-DNP, and 1-NP, for the urban airborne particulates.

4. Conclusion

Capillary gas chromatography with mass spectrometry [23–27] and HPLC with chemiluminescent detection [35,36] has been used as a selective and sensitive analytical technique for complex mixtures such as airborne particulates. However, these instruments are not readily available in most laboratories. The major advantages of the methods developed in this study include: (1) expensive equipment and laborious work are not necessary; (2) recovery yields of NPAHs during clean-up are quite high and the process is simple; and (3) sensitivities are high enough to detect the trace amounts of NPAHs in airborne particulates. In this study, LC/EC/FLD provides a relatively simple, sensitive, accurate, and convenient procedure for the determination of four NPAHs via analyses of APAHs (1,8-DNP, 1,6-DNP, 1,3-DNP, and 1-NP) at pg trace levels in airborne particulate. The chromatograms were clean by using a time programmed heart-cutting technique. It has been proven to be an applicable method for monitoring urban air quality. Ultrasonic extraction is a rapid procedure for monitoring urban air quality. Our technique is able to offer a large database on NPAHs presence in urban air particulates in relative short term for local environmental policy makers.

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