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Journal of Chromatography A, 897 (2000) 393–397

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Determination of 1,3-, 1,6-, 1,8-dinitropyrene and 1-nitropyrene in airborne particulate by column liquid chromatography with electrochemical detection

Ching-Tang Kuo[a,*], Hong-Wen Chen[b]

[a]*Institute of Environmental Health, China Medical College, 91 Hsueh-Shih Road, Taichung 404, Taiwan*

[b]*Department of Environmental Engineering and Health, Yuanpei Technical College, 306 Yuanpei Street, Hsinchu, Taiwan*

Received 11 October 1999; received in revised form 26 July 2000; accepted 26 July 2000

Abstract

Nitrated polycyclic aromatic hydrocarbons (NPAHs) in airborne particulate were determined by column liquid chromatography with electrochemical detection. NPAHs were extracted ultrasonically prior to being injected into the separation system. A reversed-phase C_{18} column was used to separate the NPAHs with an aqueous eluent containing acetonitrile and sodium monochloroacetate as buffer. Calibration graphs were linear with very good correlation coefficients ($r>0.999$) and the detection limits were ca. 20 pg for all analytes. The proposed method provides a relatively simple and convenient procedure for determining the NPAHs in airborne particulate.

Keywords: Airborne particulate; Nitropyrenes; Nitrated polynuclear aromatic hydrocarbons

1. Introduction

Nitrated polycyclic aromatic hydrocarbons (NPAHs) are environmental pollutants that originate from the combustion of petrochemical fuel and other organic matter, or that form in the atmosphere from the photochemical reaction of pyrene with hydroxyl radicals and NO_2 or with NO_3^- radicals [1,2]. In the Ames test, the parent polycyclic aromatic hydrocarbons (PAHs) are direct mutagens [3]. The nitrated pyrenes [1,3-dinitropyrene (DNP), 1,6-DNP and 1,8-DNP] exhibit the strongest mutagenic activity [4–6]. Hence, developing a simple and convenient method to monitor NPAHs in airborne particulate is required for better understanding the mutagenicity in polluted air.

Several analytical techniques have been used for NPAH determination, such as gas chromatography with electron-capture detection (GC–ECD) [7] or with negative ion chemical ionization mass spectrometry (NCI-MS) [8], and column liquid chromatography (LC) with fluorescence detection (FL) [9]. NCI-MS seems to be the best method, however, it requires a high-cost instrument system and a skilled operator, thus it does not satisfy the requirement for routine analysis. LC–FL has a unique sensitivity for amino polycyclic aromatic hydrocarbons (APAHs), but does not work for NPAHs. Thus, a process is required to reduce NPAHs to APAHs before detection. For the NPAH-to-APAH reduction, Sigrar-

*Corresponding author. Tel.: +886-4-2053366 ext. 6306; fax: +886-4-2019901.

E-mail address: ctkuo@mail.cmc.edu.tw (C.-T. Kuo).

0021-9673/00/$ – see front matter
PII: S0021-9673(00)00809-8

dson and Birks [10] developed a convenient procedure by on-line zinc reduction which yielded excellent results on chemiluminescence detection. Recently, LC with electrochemical detection (EC) has been used as sensitive, and accurate method for reductive or oxidative compounds [11–13]. Therefore LC–EC is a worthwhile alternative method for the determination of NPAHs.

2. Experimental

2.1. Apparatus

High-performance liquid chromatography (HPLC) was performed with an LC-10A pump system (Shimadzu, Kyoto, Japan) equipped with a Rheodyne 7125 injection valve (10 μl sample loop) and a reversed-phase Kaseisorb LC ODS 60-5 analytical column (250×4.6 mm I.D.) (Tokyo, Japan). A BAS-LC-4A amperometric controller system with a glassy carbon working electrode (Lafayette, IN, USA), set in reducible mode at 0.60 V (vs. Ag/AgCl), in conjunction with a Chem Win 1.0 system (Taipei, Taiwan), was used to detect and collect chromatographic data. Stainless steel tubing was used throughout the system rather than PTFE to prevent the permeation of oxygen from mobile phase. The eluent flow-rate was 1.0 ml/min. The column was maintained at 40°C in the thermostatically controlled oven (Shimadzu Model CTO-6A).

2.2. Chemicals and reagents

Deionized water (MΩ/cm>18) was produced using a Millipore 60 system (Bedford, MA, USA) and was used for all aqueous solutions. All chemicals were of ACS reagent grade. 1,3-DNP, 1,6-DNP 1,8-DNP were purchased from Aldrich (Milwaukee, WI, USA). 1-Nitropyrene (1-NP) was obtained from TCI (Tokyo, Japan). Sodium monocholoroacetate was obtained from Fluka (Tokyo, Japan). Sodium hydroxide and sodium bisulfate were obtained from TCI (Tokyo, Japan). Benzene, ethanol and acetonitrile were purchased from Fisher (Springfield, MO, USA). Nitro-PAHs stock solution (1–15 m*M*) was prepared in acetonitrile. All of the solutions were stored in brown glass bottles, and kept in a −70°C refrigerator. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The HPLC eluent was prepared using 70% (v/v) acetonitrile, 30% 0.01 *M* sodium monochloroacetate buffer (pH 4.7). All eluents were purged of dissolved oxygen with helium for about 1 h with a degassed unit (Gasu Kuro Kogyo Model 546, Japan) and bubbles cleaned up with the filtration assembly prior to use.

2.3. Sampling collection and preparation

A high volume for particles of <10 μm diameter (PM_{10}) air sampler (Kimoto Denshi, Osaka, Japan) was used for the collection of airborne samples. The samplers were set up in Ser-Chia junior high school near the industrial districts, Kaohsiung, Taiwan. The elevation of sampling is about 9 m. Airborne particulate was collected on a glass fiber filter [10×8 in. (1 in.=2.54 cm), Gelman Science, Michigan, MI, USA] for 1 month. The samplers were run for 24 h continuously at a flow-rate of approximately 1 m^3/min and were calibrated about every 2 weeks. After being conditioned and dried in the dark, the filters were weighed. Finally, each filter was covered with tinfoil and then stored in a refrigerator (−70°C) until use.

2.4. Sample pretreatment

The NPAHs in particulate were extracted ultrasonically. The filter was cut into halves; one of the cut filters was divided into small pieces (7×1 cm^2) in a centrifugal tube (Pyrex, 50 ml). Filter was extracted with 52 ml benzene–ethanol (3:1, v/v). The tube was placed in an ultrasonic ice-bath (0°C) for 40 min and then centrifuged for 5 min (3000 rpm). Then a 40-ml volume of the upper solution was taken and reduced to 2 ml by rotary evaporation. Concentrated extracts were dried under nitrogen at ambient temperature. The residue was eluted with benzene (3 ml), sodium hydroxide (4 ml) and partitioned in the organic–aqueous phase solution. A 2.5-ml volume of benzene phase was removed to a 10 ml-tube and dried under nitrogen; 0.5 ml of benzene was used to dissolve the residue and perform LC–EC.

2.5. Recovery

The NPAHs were spiked on the sampling filter as follows: filters which had collected airborne particulate for 24 h were cut into small sections of equal area (7×1 cm^2). Aliquots (0.5 ml) of standard solution of 1,3- 1,6- 1,8-DNPs, and 1-NP with concentrations of 1.38, 4.16, and 12.50 μg/ml were prepared and 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 and room temperature. The spiked filters were treated by the same procedure as described above.

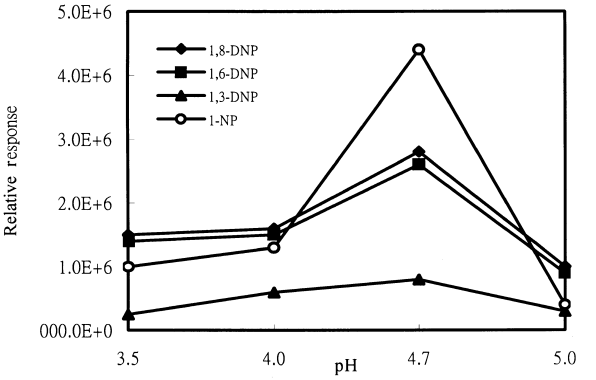

Fig. 2. Effect of pH on detection response.

3. Results and discussion

3.1. Selection of reduction potential of the electrochemical detector

In order to obtain the best sensitivity, the detection is better set at the potential, which compromises with the four NPAHs of interest. With the cyclic voltammograms for the NPAHs tested, the S/N ratio increases with the cathodic potential (Fig. 1) and reaches a maximum at −0.6 V. Considering the sensitivity and the stability in detection, a reduction potential of −0.6 V (vs. Ag/AgCl) was selected for monitoring the four NPAHs.

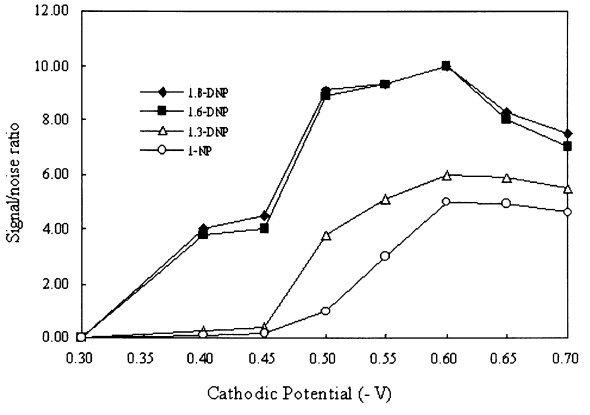

Fig. 1. Effect of reduction potential on NPAHs and S/N ratios for the cyclic voltammograms.

3.2. Optimization of eluent condition and identification of NPAHs

After several test runs, a water–acetonitrile (30:70, v/v) mixture was found to obtain an acceptable run time and resolution of the four NPAHs of interest. A 0.01 *M* buffer of sodium monochloroacetate was used due to the increase buffer concentration decreasing the detection response but increasing peak resolution. Fig. 2 shows that the 0.01 *M* sodium monochloroacetate buffer at pH 4.7 offers the best detection sensitivity. The temperature was controlled at 40°C based on the consideration of response sensitivity, band broadening, and resolution. Chromatograms of a standard and an authentic sample are shown in Fig. 3. To identify the analytes, they were reconfirmed using cyclic voltammetry, following fraction collection. As shown in the chromatograms, the four NPAHs are well separated within 16 min.

3.3. Validation

Calibration plots were constructed for these four analytes over concentration ranges summarized in Table 1. The linear relationships were very good for the four analytes. The repeatability was examined with 10 replicated injections of 10 μl (concentration 0.25 μg/ml) of analytes for peak height. The relative standard deviations (RSDs) were all within 4.6%.

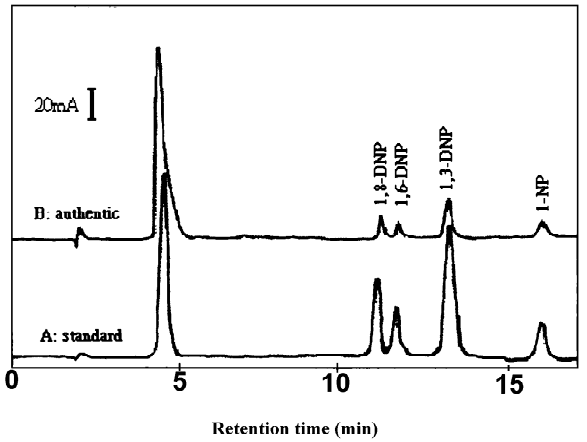

Fig. 3. LC–EC chromatograms of standard sample and an authentic sample. (A) Standard sample: concentrations of 0.04, 0.09, 0.04, 0.22 μg/ml for 1,8-DNP, 1,6-DNP, 1,3-DNP, 1-NP, respectively.

They are acceptable in an environmental analysis. The detection limits for all four NPAH analytes were ca. 20 pg.

In order to test the efficiency of the ultrasonic extraction for the quantitative determination of NPAHs, 0.5 ml of each standard solution with 1.38, 4.16 and 12.5 μg/ml, respectively, was spiked into filter paper. After sonic extraction, pretreatment, and chromatographic determination, the average recoveries were 88% (RSD 1.1%), 90% (RSD 2.1%), 91% (RSD 0.8%) and 83% (RSD 2.5%) for 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP, respectively. In the matrix effect studies, similar recoveries were obtained: 84% (RSD 0.6%), 83% (RSD 1.3%), 86% (RSD 1.5%) and 90% (RSD 1.9%) for 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP, respectively. They meet the requirements for accuracy and precision in an environmental determination.

3.4. Analysis of NPAHs in metropolitan airborne particulate

The proposed method was applied to determine the NPAHs in metropolitan airborne particulate. Airborne particulate was collected, extracted and pretreated as described previously; 30 samples were collected in 1 month. A chromatogram of an authentic sample is shown in Fig. 3. The monitored results for particles of <10 μm diameter (PM_{10}) airborne particulate showed that at Kaohsiung City in November, the NPAHs were in the concentration ranges of ND to 55 pg/m^3, ND to 72 pg/m^3, ND to 84 pg/m^3 and ND to 208 pg/m^3, for 1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP, respectively. The data were similar to that in the literature [7,8]. It indicates that it is a feasible method to detect the trace concentrations of 1,3-, 1,6-, 1,8-DNP and 1-NP for mutagens in airborne particulates. The amount of DNPs in particulate samples from Taiwan is much higher than that from other countries [7,14], probably due to the presence of much heavy petrochemical industry in southern Taiwan. To correlate the 17–26 pg absolute limits of detection with the measured concentrations demonstrate that also much lower concentrations found in these counties can be determined by the presented procedure.

4. Conclusion

In this study, LC–EC provides a relatively simple, sensitive and convenient procedure for simultaneous determination of four NPAHs (1,3-DNP, 1,6-DNP, 1,8-DNP and 1-NP) at pg trace level in airborne particulate. After ultrasonic extraction it is a rapid procedure for monitoring urban air quality.

Table 1
Calibration plots for four NPAHs in standard solutions

Substance	Concentration range (μg/ml)	Linear regression relationship	Detection limit (pg)	RSD (%)
1,3-Dinitropyrene	0.006–0.47	0.999	17	4.6
1,6-Dinitropyrene	0.012–0.95	1.000	17	3.9
1,8-Dinitropyrene	0.005–0.38	0.999	15	1.5
1-Nitropyrene	0.020–1.60	1.000	26	5.3

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