DETERMINATION OF 1,3-, 1,6-, 1,8-DINITROPYRENES AND 1-NITROPYRENE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH CHEMILUMINESCENCE DETECTION

KAZUICHI HAYAKAWA and MOTOICHI MIYAZAKI

Faculty of Pharmaceutical Sciences, Kanazawa University, 13-1 Takara-machi, Kanazawa-shi 920, Japan

A HPLC method with peroxyoxalate-chemiluminescence (CL) detection has been developed for the determination of dinitropyrenes (DNPs) and nitropyrene (NP). Both DNPs and NP, which are CL-insensitive, were initially reduced to the corresponding amino-derivatives with sodium hydrosulfide. They were separated on a reversed-phase column and chemilumigenically determined with detection limits in the sub-fmol range. Utilizing this method, DNPs and NP were determined in gasoline and diesel particulate samples.

Key words dinitropyrene, nitropyrene, high performance liquid chromatography, peroxyoxalate-chemiluminescence detection, gasoline and diesel particulates

Polycyclic aromatic hydrocarbons (PAHs) and their nitro-derivatives (NPAHs) have attracted much attention due to their potent mutagenic and carcinogenic effects. The mutagenicity of 1,3-, 1,6-, 1,8-dinitropyrenes (DNPs) and 1-nitropyrene (NP), especially, are much higher than that of any PAH. They are formed as undesirable by-products during the combustion of materials such as petroleum. In the past, both gas chromatography/mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC) with fluorescence (FL) detection have been used to determine them. However, the former method requires complex enrichment and clean-up procedures and both methods are not sensitive enough to detect trace levels of NPAHs, which are present at much lower concentrations in the environment than PAHs themselves. HPLC with peroxyoxalate-chemiluminescence (CL) detection is a highly sensitive method for several fluorophores. A mixture of bis(2,4,6-trichlorophenyl)oxalate (TCPO) and hydrogen peroxide is used as a post-column CL reagent solution [2]. We found that diaminopyrenes (DAPs) and aminopyrene (AP), which are the reduction products of DNPs and NP, respectively, were sensitive to CL detection, although they were unstable in the presence of metals and/or light. Trace levels of DAPs and AP were stabilized by adding reducing agents to sample solutions, thus allowing determination [3]. In this report, we have developed a HPLC determination method for 1,3-, 1,6-, 1,8-DNPs and 1-NP after off-line chemical reduction with sodium hydrosulfide. Using this method, all four compounds were detected in gasoline and diesel particulates and their concentrations compared.

EXPERIMENTAL

Chemicals DNPs and NP were purchased from Aldrich. DAPs and AP were prepared according to previous reports [4,5]. All other chemicals were of analytical grade.

HPLC conditions Fig. 1 shows a schematic diagram of the HPLC system used in this work. The system consisted of a pump (P_1) for the mobile phase (MP), a short stroke pump (P_2) for the CL reagent solution (RS), an injector (I), an analytical column (A), a mixing device (MD), a reaction coil (C) and a chemiluminescence detector (CL). HPLC conditions were as follows: analytical column, Cosmosil 5C18 (4.6 mm i.d. x 25 cm); mobile phase, 10 mM imidazole: perchloric acid (pH 7.6) - acetonitrile (1:1, v/v) at a flow rate of 1 ml/min; CL reagent solution, acetonitrile containing 0.02 mM bis(2,4,6-trichlorophenyl)oxalate (TCPO) - 15 mM hydrogen peroxide at a flow rate of 1

ml/min.

Sample preparations Car emission particulates were collected on a glass filter (cut off size over 10 um) for 30 min or 2 hr at a flow rate of 1,800 l/h. Emission particles (2-5 mg) on the filter were firstly extracted with benzene-ethanol (3:1, v/v). After evaporating, the remaining residue was redissolved in ethanol and refluxed with sodium hydrosulfide. The compounds of interest (DAPs and AP) were extracted into benzene containing ascorbic acid. After evaporating to dryness, the residue was redissolved in acetonitrile containing ascorbic acid. An aliquot of this solution was injected into the HPLC system. Also, benzene-ethanol extracts were washed with sodium hydroxide and sulfonic acid and then treated in the same manner as described above.

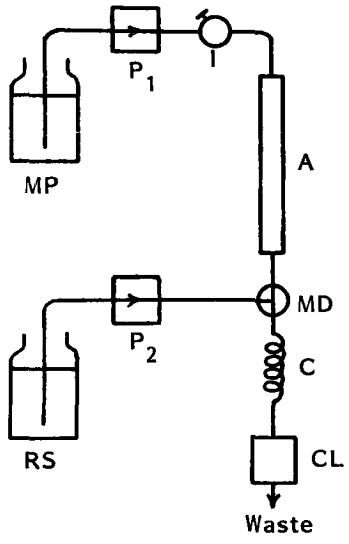

Fig. 1 Schematic diagram of HPLC-CL system

RESULTS AND DISCUSSION

HPLC system In the past, post-column CL detection systems required a pump for each of the two reagents, the aryl oxalate and hydrogen peroxide. We found that the mixture of TCPO and hydrogen peroxide was the most stable among possible aryl oxalate-hydrogen peroxide combinations when dissolved in acetonitrile and kept in a borosilicate or polyethylene bottle [6]. This mixture was stable for over 8 h, thus enabling a one-pump system to be used as shown in Fig. 1. Furthermore, upon decreasing the volume per pump stroke, the noise level decreased. By using a short-stroke pump instead of a conventional long-stroke pump, the signal-to-noise ratio was increased [7].

Determination of DAPs and AP Both DAPs and AP were unstable in the presence of several metals and/or light. Metals such as molybdenum, copper, manganese and iron, which are contained in stainless-steel, had a particularly significant effect, suggesting that this metal catalysis may be the main reason for degradation of DAPs and AP during HPLC separation. This degradation prevented sensitive and accurate determination by CL detection. This problem was completely removed when reducing agents were added. Among the agents tested, ascorbic acid was found to be the most effective. Adding ascorbic acid to the sample solution enabled sub-fmol levels of DAPs to be detected, with straight calibration curves down to the fmol level. As can be seen in TABLE 1, detection limits with CL detection were 30-60 times lower than those with FL detection, which had been the most sensitive method in the past [8].

TABLE 1. Detection limits (fmol) for DAPs and AP with CL and FL detections

Method	Detection limit			
	1,3-DAP	1,6-DAP	1,8-DAP	1-AP
CL	0.25	0.25	0.35	1.5
FL	15	8	25	25

S/N = 3.

Sample preparation and typical chromatogram DNPs and NP were quantitatively reduced to DAPs and AP by refluxing the sample solution with sodium hydrosulfide. Although the resulting DAPs and AP were unstable in the presence of metals and/or light as described above during sample preparation and HPLC analysis, it was resolved completely by the addition of ascorbic acid. Fig. 2 shows a typical chromatogram obtained from the particulate emissions of a gasoline-engine car. Four peaks, 1,3-, 1,6-, 1,8-DAPs and 1-AP, were detectable in the fmol/mg range. These peaks are ascribable to the DNPs and NP originally contained in the particulates. These trace levels of DNPs and NP in gasoline particulates were not obtainable using FL detection due to its low sensitivity. Another advantage is that the selectivity of the CL detection reduces tedious sample-cleanup procedures (necessary in GC/MS, for example). DNPs and NP were determined as DAPs and AP, respectively, without any interfering peaks after only a benzene-ethanol extraction.

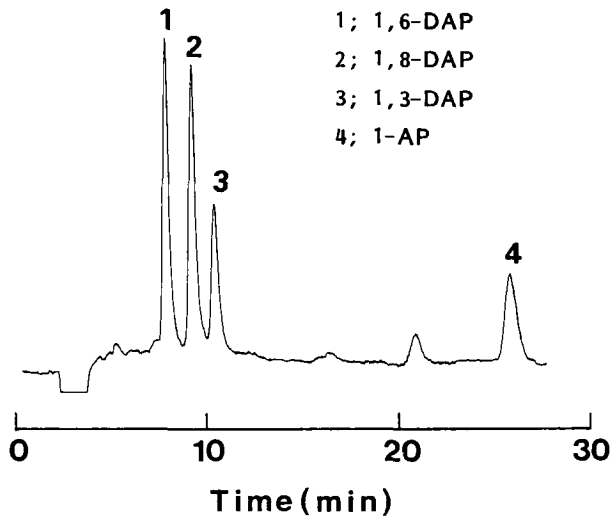

Fig. 2 Chromatogram of gasoline particulates

Comparison of gasoline- and diesel-engine cars The concentrations of the four compounds in gasoline and diesel particulates determined by this method are compared in TABLE 2. The concentration of 1-NP was much higher in diesel particulates than in gasoline particulates. In contrast to this, the concentrations of DNPs seemed to be higher in gasoline particulates than in diesel particulates. The concentrations of the three DNP isomers were similar to each other in both samples.

TABLE 2. Concentrations (pmol/mg) of 1,3-, 1,6-, 1,8-DNPs and 1-NP in gasoline and diesel particulates

Engine type	Concentration			
	1,3-DNP	1,6-DNP	1,8-DNP	1-NP
gasoline (n = 8)	0.23 ± 0.15	0.44 ± 0.36	0.35 ± 0.18	1.8 ± 0.8
diesel (n = 3)	0.16 ± 0.09	0.17 ± 0.16	0.19 ± 0.18	72.3 ± 71.0

Mean ± SD.

CONCLUSION

These results indicate that this HPLC-CL detection method can be used in the sensitive and selective determination of both DNPs and NP in environmental samples. Considering their high mutagenicity, DNPs in car emission particulates as well as airborne particulates should be monitored, although their concentrations in gasoline and diesel particulates were lower than that of NP in diesel particulates.

REFERENCES

1. S. Kobayashi and K. Imai, Anal. Chem., 52, 424 (1980).
2. K. Hayakawa, K. Hasegawa, N. Imaizumi, O. S. Wong and M. Miyazaki, J. Chromatogr., 464, 343 (1989).
3. K. Hayakawa, R. Kitamura, M. Butoh, N. Imaizumi and M. Miyazaki, Anal. Sci., 7, 573 (1991).
4. Y. Hashimoto and K. Shudo, Chem. Pharm. Bull., 32, 1992 (1984).
5. M. J. Edwards, J. M. Parry, S. Bathmanghelich and K. Smith, Mutation Res., 163, 81 (1986).
6. N. Imaizumi, K. Hayakawa, M. Miyazaki and K. Imai, Analyst, 114, 161 (1989).
7. K. Hayakawa, N. Imaizumi and M. Miyazaki, Biomed. Chromatogr., 5, 148 (1991).
8. K. Tanabe, H. Matsushita, C-T. Kuo and S. Imamiya, Taikiosen-gakkaishi, 21, 535 (1986).