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Journal of Chromatography A, 664 (1994) 177–182

JOURNAL OF CHROMATOGRAPHY A

Determination of mono- and di-nitro polycyclic aromatic hydrocarbons by on-line reduction and high-performance liquid chromatography with chemiluminescence detection

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(First received October 20th, 1993; revised manuscript received December 17th, 1993)

Abstract

The determination of mono- and di-nitro polycyclic aromatic hydrocarbons (PAHs) was accomplished by on-line reduction to the corresponding amino PAHs, which were then separated and detected using high-performance liquid chromatography (HPLC) and chemiluminescence detection. The on-line reduction was carried out in a methanol–water mobile phase by the use of a catalyst column packed with material originating from a grained automobile three-way catalyst. HPLC separation was completed with acetonitrile–buffer mobile phase at pH = 7.5. Bis(2,4,6-trichlorophenyl) oxalate and hydrogen peroxide was used as the reagent for chemiluminescence detection. The detection limits for some mono- and di-nitro PAHs were in the range of 1 to 10 picogram. The developed method was demonstrated on samples originating from diesel exhaust particle material extracts.

1. Introduction

Diesel and gasoline engine exhaust emissions are complex mixtures containing thousands of chemical components. Among these components, some nitrated polycyclic aromatic hydrocarbons (nitro PAHs) have been identified as mutagens and possible carcinogens [1]. Nitro PAHs have been estimated to be responsible for up to 40% of the total mutagenic activity determined in diesel particulate extracts [2]. Among the nitro-PAHs, great attention has been directed to 1-nitropyrene and 1,3-, 1,6- and 1,8-dinitropyrenes. This is because 1-nitropyrene is one of the most abundant of these compounds, and the three dinitropyrenes are found to be much more mutagenic than 1-nitropyrene [3]. The amount of dinitropyrenes present in motor vehicle exhaust samples is lower than mono-nitro-PAHs. The determination of nitro PAHs in complex sample matrices is usually carried out using analytical methods, such as gas chromatography with nitrogen–phosphorus detection (GC–NPD) or a chemiluminescence detection (GC–CD), and gas chromatography with negative-ion chemical ionisation mass spectrometry (GC–NICIMS) [4,5]. HPLC with fluorescence detection [6] and electrochemical and fluorescence detection [7] has also been developed. The peroxyoxalate chemiluminescence reaction was originally developed by Rauhut *et al.* [8] and combined with HPLC by Kobayashi and Imai [9]. It is used for the detection of fluorescent compounds such as PAHs, amino PAHs and

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0021-9673/94/$07.00
SSDI 0021-9673(93)E1302-G

nitro PAHs [10–13]. Detection limits are in the range of picomole to subfemtomole [14,15]. Two review papers, which present progress and developments in this field of research, were recently published by Robards and Worsfold [16] and Kwakman and Brinkman [17]. The peroxyoxalate chemiluminescence reaction mechanism was previously described by Sigvardson *et al.* [18]. The detector used is usually a photo multiplier tube (PMT) without a light source which gives an improved signal-to-noise ratio when compared with conventional fluorescence detection [19].

The objective of the present study is to develop a sensitive and routine analytical method by on-line reduction and combining HPLC separation and peroxyoxalate chemiluminescence detection for determining mono- and di-nitro PAHs in engine exhaust emission. The major advantages of this approach are: (1) the catalyst column shows enough reduction capacity and lifetime, (2) both mono- and di-nitro PAHs can be identified and (3) the detection limits are in the range of 1–10 picogram.

2. Experimental

2.1. Chemicals

All the organic solvents used were HPLC grade. Bis(2,4,6-trichlorophenyl) oxalate (TCPO), H_2O_2, imidazole, and nitro PAH standards (1,8-dinitropyrene, 2-nitroanthracene, 1-nitropyrene, 6-nitrochrysene, 3-nitroperylene and 1-nitroperylene) were obtained from either Aldrich Chemical Co. (Steinheim, Germany) or Sigma Chemical Co. (St. Louis, MO, USA). Water was purified by an Elgastat UHQ II unit (Elga Ltd., UK). All solvents were degassed using reduced pressure conditions and an ultrasonic bath before use.

2.2. Instrumentation

Fig. 1 illustrates the system by means of a schematic diagram. Three pump units were used in this study. The first pump (P1), a Varian Star 9010 (Varian, Walnut Creek, CA, USA), was

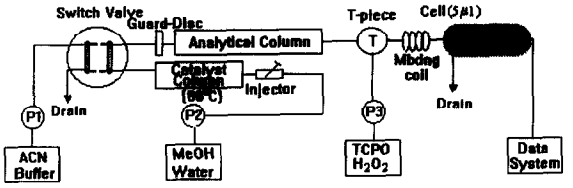

Fig. 1. Schematic diagram of the HPLC–CL system. The details are described in the text.

used for acetonitrile–buffer solution (65:35) eluent delivery, the second pump (P2), a BAS 400 (Bioanalytical Systems Inc., Lafayette, USA) was used for on-line reduction solution supply, methanol–water (65:35) as mobile phase, and the third pump (P3), Waters M45 (Waters, Milford, MA, USA), was used for reagent (TCPO–H_2O_2) delivery. A catalyst column was used for on-line reduction of nitro PAHs to corresponding amino PAHs. A heater was used for heating the catalyst column to 80°C, and a four-port switch valve was used for transferring the eluent. The analytical column was a C_{18} column (150 × 4.6 mm I.D., 3 mm particles) which was protected by a C_{18}-A Guard-Disc (1.0 × 4.6 mm I.D., 0.2 mm pore size) (Sarasep, Inc., USA). A Rheodyne Model 7125 syringe injector with a 20-μl loop was used for injection. The analytical column outlet was connected to a T-piece, which was connected to the detector flow cell by way of a 12-cm (0.25 mm I.D.) stainless-steel coil. The detector used was a Kratos FS970 fluorescence detector (Schoeffel Instruments, USA) with a 5-μl flow cell. The detector was operated with its light off and without an emission cut-off filter. Emitted light was detected using the photomultiplier tube (PMT). An ELDS 900 laboratory chromatography data system (Chromatograph Data Systems AB, Kungshög, Sweden) was used for recording and integrating the chromatograms.

2.3. On-line reduction

On-line reduction of nitro PAHs to amino PAHs was carried out using a catalyst column packed with grained three-way catalyst material, which was originally designed to reduce gasoline fuelled vehicle exhaust emissions (0.25% platinum and 0.05% rhodium, preheated to

800°C, Volvo Personvagnar AB, Sweden). The column was prepared by dry packing (40.0 × 3.0 mm I.D., 140–160 mm particle size). The catalyst was tested from ambient temperature up to 80°C with flow rates from 0.20 ml/min to 1.0 ml/min, using methanol–water (65:35) solution as the carrier stream. The catalytic column reduction dynamic range was examined from 0.012 ng to 120.0 ng 1-nitropyrene. On-line reduction was carried out by holding sample in the catalyst column for 90 s at stop-flow, the elution was then accomplished with methanol–water (65:35) at a flow rate of 0.3 ml/min for 60 s, in order to transfer the amino PAHs peak into the analytical column; during the procedure the temperature was held at 80°C. Because the methanol–water solution is not appropriate to the chemiluminescence detection system, it is necessary to change to an acetonitrile–buffer solution as mobile phase by using a switch valve, Fig. 1.

2.4. HPLC separation and chemiluminescence detection

Chromatographic separation of the amino compounds was carried out using an acetonitrile–buffer solution (65:35) at flow rate of 1.0 ml/min. The nitrated imidazole buffer (pH = 7.5, 50 m*M*) provides the necessary base catalysis for the peroxyoxalate reaction to run. Since acetonitrile was used in the chromatography separation as mobile phase and TCPO has a high solubility in ethyl acetate, the reagent solution of TCPO–H_2O_2 was prepared in acetonitrile–ethyl acetate that was mixed in the ratio of 3:1. The concentrations of TCPO and H_2O_2 were 0.25 m*M* and 25 m*M*, respectively, the flow rate was set to 0.7 ml/min. A standard mixture containing six mono- and di-nitro PAHs was examined by the system, the chromatogram is presented in Fig. 2A.

2.5. Diesel exhaust samples

Two samples were investigated in this present study: a diesel particulate material and a diesel exhaust emission particulate sample. The samples are described in detail elsewhere [20,21]. The samples were Soxhlet extracted with dichloromethane and the crude extract was fractionated on a silica column into five fractions, according to polarity. This is described in detail elsewhere [22]. The fractions containing mono-nitro PAHs, fraction III and di-nitro PAHs, fraction IV, were analysed by the system, the

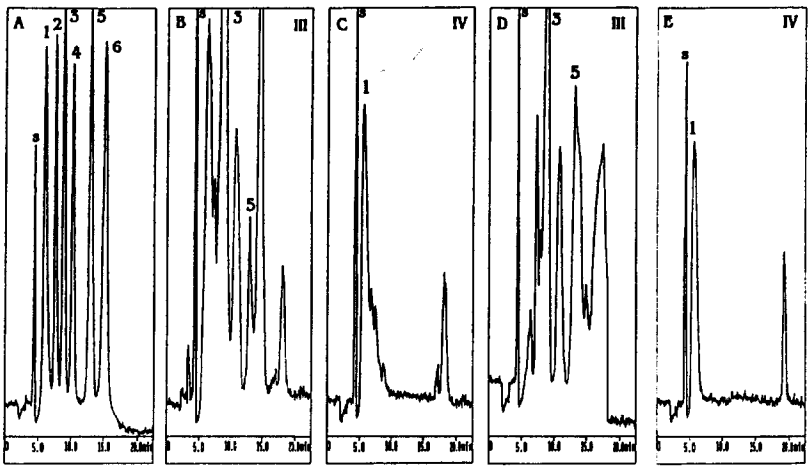

Fig. 2. (A) Chromatogram of a standard mixture. Peaks: 1 = 1,8-dinitropyrene, 2 = 2-nitroanthracene, 3 = 1-nitropyrene, 4 = 6-nitrochrysene, 5 = 3-nitroperylene and 6 = 1-nitroperylene. Detection limits are presented in Table 1. (B) and (C) Chromatograms of a diesel particulate extract from fractions containing mono-nitro PAHs and di-nitro PAHs, respectively. (D) and (E) Chromatograms of a diesel exhaust emission filter extract sample from fractions containing mono-nitro PAHs and di-nitro PAHs, respectively. Chromatographic conditions are described in the text.

chromatograms are presented in Fig. 2B, 2C, 2D and 2E, respectively.

3. Results and discussion

3.1. On-line reduction

Initial experiments with zinc powder mixed with silica as the catalyst material were performed but, due to a limited lifetime (3–5 days), the catalyst was excluded as inappropriate. Instead, a three-way catalyst column was selected and investigated in the present study. It was observed that the reduction reaction needs a certain temperature and time to reach high reduction efficiency. The catalyst was tested from ambient temperature up to 80°C with flow rates from 0.20 ml/min to 1.0 ml/min of methanol–water (65.35) as carrier stream. Initially the methanol–water ratio varied from 50:50 to 90:10, however, the best response was obtained at the ratio of 65:35. The best efficiency was obtained when the sample was held in the catalyst column for 90 s at stop-flow, then the elution was carried out with flow rate of 0.3 ml/min, the temperature was held at 80°C. Six replicate injections of 1.0 ng of 1-nitropyrene gave relative standard deviations (R.S.D.s) of less than 2.4%, showing that the reduction of nitro PAHs to amino PAHs is reproducible. The catalytic reduction dynamic range was examined from 0.012 ng to 120.0 ng 1-nitropyrene. Repeated injections ($n \geq 2$) gave a R.S.D. of less than 5%, the correlation coefficient r was 0.995 at the 95% confidence level. Because the amount of nitropyrene in the injected exhaust samples is less than 100 ng [6], the maximum capacity for the catalyst column was not determined.

In order to examine and to check the efficiency of the catalyst column, a test mixture containing known concentrations of mono- and dinitro PAHs (see Table 1, however, no 2-nitroanthracene present) and 2-aminoanthracene was analyzed. On a regular basis the test mixture was analysed with respect to retention times, absolute and relative chemiluminescence (CL) intensity in comparison to 2-aminoanthracene. The catalyst column has been employed for more than six months of daily use, and is still active. These results strongly suggest that the reduction process is catalytic. The zinc catalyst column having a finite lifetime was previously observed by Sigvardson and Birks [12] and MacCrehan *et al.* [7]. Hayakawa and co-workers [13] have reported that in determination of 1-nitropyrene and three dinitropyrenes in vehicle exhaust particulate, an off-line hydrosulphide reduction method was used. The disadvantages of this method are tedious sample treatment procedure and a considerable reduction time.

3.2. HPLC separation and chemiluminescence detection.

The peroxyoxalate-CL reaction is known to be base-catalysed so the pH has a significant influence on the chemiluminescence kinetics. Two

Table 1
Retention times, injected amounts and detection limits of a standard mixture consisting of mono- and di-nitro PAHs

Peak no. [a]	Compound	R_s [b]	Retention time (min)	Injected amount (picogram)	Detection limit [c] (picogram)
1	1,8-Dinitropyrene		6.01	75	< 3
2	2-Nitroanthracene	1.57	7.57	75	< 3
3	1-Nitropyrene	1.44	8.85	50	< 2
4	6-Nitrochrysene	1.38	10.21	250	< 10
5	3-Nitroperylene	2.32	12.94	25	< 1
6	1-Nitroperylene	1.56	15.13	25	< 1

[a] Peak numbers refer to the standard compounds which are presented in Fig. 2A.
[b] Resolution is defined as the ratio between the two peak maxima and the average base width of the two peaks.
[c] ($S/N > 3$).

buffer compounds were tested in the system, imidazole and tris(hydroxymethyl)aminomethane. Initial experiments were performed ranging from pH 5 to 8 using both buffer compounds, however, imidazole gave the highest relative CL intensity at pH 7.5. Below pH 7.5, the intensity decreased dramatically and no attempt to measure above pH 8.0 was made due to damage to the analytical column used. The imidazole buffer was selected in present system, at a concentration of 50 m*M* and the pH was adjusted to 7.5 with HNO_3. The imidazole buffer concentration was investigated in the range of 10 to 200 m*M* and the best response was obtained at 50 m*M*.

The effects of the selection of solvent on the chemiluminescence reaction have been reported by Kobayashi and Imai [9]. The stability of TCPO–H_2O_2 has been measured in methanol, acetone, dioxane, acetonitrile and ethyl acetate, as well as mixtures of them. Due to the use of acetonitrile as the mobile phase and the fact that TCPO dissolves easily in ethyl acetate, acetonitrile and ethyl acetate (3:1) were selected as the TCPO–H_2O_2 solvent. The concentrations of TCPO and H_2O_2 were optimised in the range of 0.05–1.0 *M* and in the range of 15–150 m*M*, respectively. The most favourable concentrations of TCPO and H_2O_2 are 0.25 m*M* and 25 m*M*, respectively. The reagent flow rate was optimised to be 0.7 ml/min which was introduced into the T-piece and mixed with the mobile phase. The highest CL intensity was obtained using a mixing coil length of 12 cm (0.25 mm I.D.) at present conditions.

The HPLC separation was carried out by an acetonitrile–buffer mobile phase (65:35). The flow rate was set to 1.0 ml/min, and a standard mixture was examined by this system. The chromatogram is presented in Fig. 2A. The separation took less than 20 min. The resolution (R_s) value for the least separated peaks, *i.e.* 1-nitropyrene and 6-nitrochrysene, is 1.38. Other R_s values are presented in Table 1. A good separation for the six compounds can be observed from Fig. 2A. A blank sample was investigated which was at baseline level. Detection limits are defined as the mass of analyte that provides a signal equal to three times that of the baseline. As can be seen from Table 1, the individual nitrated PAHs have different detection limits under the applied conditions; for the six standards, the detection limits range from less than 1 to less than 10 picogram. The detection limits in the present study are better than those data obtained using HPLC with fluorescence detection [6,7] and electrochemical detection [7], and as good as those data obtained by other researchers by means of HPLC with chemiluminescence detection [12,13].

3.3. Diesel exhaust samples

The chromatograms originating from the diesel particulate material extract fraction, fractions III (mono-nitro PAHs) and IV (di-nitro PAHs) are presented in Fig. 2B and 2C, respectively. The results obtained show that the concentrations of 1-nitropyrene and 3-nitroperylene are about 4.5 μg/g and 1.1 μg/g, respectively. In fraction IV the dinitropyrene content was 0.3 μg/g. The chromatograms of the diesel exhaust particulate filter extract fractions III and IV are presented in Fig. 2 D and 2 E, respectively. It can be seen that 1-nitropyrene and 3-nitroperylene can be identified in fraction III, the emission rates being 1.2 μg/km (2 μg/g particulate material) and 0.5 μg/km (0.8 μg/g particulate material), respectively. In fraction IV the emission factor is 0.18 μg/km (0.3 μg/g particulate material). The quantification was made with 1-nitroperylene as internal standard. The present results are in the same order as previously published data [23], *i.e.* 1-nitropyrene 6.5 μg/g particulate material.

4. Conclusions

The three-way catalyst has a lifetime of more than 6 months of daily use with methanol–water as the carrier stream which suggests that the reduction process is catalytic. The on-line catalyst column has a capacity for quantitative on-line reduction of mono- and di-nitro PAHs to the corresponding amino PAHs. The HPLC separation with chemiluminescence detection has a high sensitivity. The major advantages of this

approach are: (1) the catalyst column shows enough reduction capacity and lifetime, (2) both mono- and di-nitro PAHs can be quantified and (3) the detection limits are in the range of 1–10 picograms of the standard compounds injected. Compounds determined using this method are: 1,8-dinitropyrene, 2-nitroanthracene, 1-nitropyrene, 6-nitrochrysene, 3-nitroperylene and 1-nitroperylene.

5. Acknowledgements

The authors wish to thank Lennart Ekman, Volvo Personvagnar AB, Göteborg, for providing the vehicle three-way catalyst material, Lena Elfver for laboratory assistance and Anders Dyremark for fruitful discussions, and Professor Lars Blomberg and Professor Björn Josefsson for critical comments.

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