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Author(s)
Kishikawa, Naoya; Nakashima, Hajime; Ohyama, Kaname; Nakashima, Kenichiro; Kuroda, Naotaka

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Determination of 9, 10-phenanthrenequinone in airborne particulates by high-performance liquid chromatography with post-column fluorescence derivatization using 2-aminothiophenol

Naoya Kishikawa, Hajime Nakashima, Kaname Ohyama, Kenichiro Nakashima, Naotaka Kuroda*

Graduate School of Biomedical Sciences, Course of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

*Corresponding author.
Tel.+81-95-819-2894; Fax:+81-95-819-2444.
E-mail address: n-kuro@nagasaki-u.ac.jp (N. Kuroda)

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Abstract

9, 10-Phenanthrenequinone (PQ) is regarded as a harmful environmental pollutant and its presence has been reported in atmospheric environment. The measurement of PQ in environment should be necessary to evaluate the influence of PQ on human health. We found that PQ reacted with 2-aminothiophenol under acidic condition to form fluorescent derivative which emits green fluorescence at 510 nm. Based on this reaction, a simple and rapid determination method for PQ was developed by HPLC with post-column derivatization and fluorescence detection. By the proposed HPLC system, PQ was detected at 24 min and the detection limit was 67 fmol/injection (S/N=3). The proposed method was able to determine the atmospheric PQ concentrations by the direct injection of extract from airborne particulates.
Introduction

Quinones are regarded as harmful environmental pollutants which have been detected in environment samples including airborne particulates [1-3]. Among the quinones detected in environment, 9, 10-phenanthrenequinone (PQ) should be hazardous quinone because it has been reported that PQ possess significant toxicity compared with other quinones [4,5], and the atmospheric levels of PQ are relatively high among the quinones [6,7]. It has been reported that PQ can react with functional groups of enzymes such as nitric oxide synthase to form covalent enzyme adducts, therefore it can alter the enzyme activity [8,9]. Additionally, PQ can cause oxidative damage to biological components because PQ generates reactive oxygen species through their redox cycle in biological systems [4,10,11]. It was considered that inhalation of PQ in pollutant air was one of the contributing factors of serious diseases such as lung cancer, asthma and allergic inflammation [12-14]. Therefore, a sensitive and reliable method for the determination of PQ in environment is necessary to evaluate the potential health risks of PQ.

For the determination of PQ in environmental samples, the methods based on gas chromatography with mass spectrometry (GC-MS) [7] or high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) [15] have been reported. However, the
equipment used for mass spectrometry has not spread due to its expensiveness.

We developed a highly sensitive method for determining PQ using HPLC with fluorescence detection and pre-column derivatization [16]. The method was based on the conversion of non-fluorescent PQ to the highly fluorescent benzimidazole derivative by the reaction with benzaldehyde and ammonium acetate. Although the method allowed the sensitive determination of PQ in airborne particulates [17], the method involved the moderately laborious and time-consuming pre-column derivatization procedure prior to the injection to HPLC system. Considering the simplicity and rapidness of the method, a post-column derivatization procedure is more favorable for routine analysis of environmental samples. However, it is difficult that the derivatization reaction by using benzaldehyde and ammonium acetate is applied to a post-column derivatization method because the reaction requires a long reaction time under high temperature.

In the course of the related work, we found that PQ was rapidly converted to the highly fluorescent derivative by the reaction with 2-aminothiophenol (2-ATHP) which is known as a fluorogenic derivatization reagent for aromatic aldehydes [18]. Based on this finding, we attempted to establish a determination method for PQ by HPLC with post-column derivatization using 2-ATHP and fluorescence
detection. Furthermore, the developed HPLC method was applied to the
determination of PQ in airborne particulates collected in Nagasaki city.

2. Experimental

2.1. Material and reagents

PQ and 2-ATHP were obtained from Tokyo Chemical Industry (Tokyo, Japan). H₂SO₄ (95%), NaH₂PO₄ and Na₂HPO₄ were obtained from Nacalai Tesque (Kyoto, Japan). An HPLC grade of methanol was obtained from Kanto Chemical (Tokyo). Purified water was prepared by a Simpli Lab UV (Millipore, Bedford, MA, USA). The other chemicals were of analytical reagent grade.

2.2 Fluorescence measurement of PQ after reaction with 2-ATHP

To a 100-µL portion of methanol solution of PQ in a screw-capped vial, 50 µL of 0.1 M 2-ATHP in methanol and 50 µL of 3.0 M H₂SO₄ aqueous solution were added. After vortex-mixing, the reaction mixture was heated at 100°C for 30 min. The reaction mixture was diluted 15 times with methanol and was measured the fluorescence by a Hitachi 650-10S spectrofluorometor.
2.3. HPLC system and conditions

The HPLC system consisted of a two LC-6A liquid chromatographic pumps (Shimadzu, Kyoto), an RF-10AxL fluorescence detector (Shimadzu), a column oven CTO-6A (Shimadzu), a 7125 injector with a 20-µL loop (Rheodyne, Cotati, CA, USA), a noise cleaner UNI-1s (Union, Tokyo) and an FBR-1 recorder (Tosoh, Tokyo). Chromatographic separation was performed on a Cosmosil 3C18MS (250 x 4.6 mm, i.d., 5 µm; Nacalai Tesque) by an isocratic elution with a mixture of methanol-1 mM phosphate buffer (pH 6.86) (=60:40, v/v) at a flow rate of 0.5 mL/min. The post-column derivatization reagent was methanol solution containing 0.02 M ATHP and 0.15 M H₂SO₄. The column eluent was merged with the derivatization reagent solution pumped at 0.2 mL/min and the mixture was heated in the reaction coil of PTFE tubing (10 m x 0.5 mm, i.d.) at 60 ºC to form fluorescent derivative. The excitation and emission wavelengths for the derivative were set at 390 and 510 nm, respectively.

2.4. Airborne particulates sample

Sampling of airborne particulates was carried out at the main avenue of Nagasaki City. Airborne particulates were collected on Q-R100 silica-fiber filters (Advantec Toyo, Tokyo, Japan) for 24h at a flow rate of 1200 L/min by a Model No. 120 FT type high-volume air
sampler (Kimoto Electro. Kogyo, Osaka, Japan). The filters involving collected airborne particulates were stored in a refrigerator at –20°C until analysis. The filter (1.0 x 1.0 cm²) was extracted ultrasonically with 4 mL of methanol for 10 min. After taking the organic layer (3 mL), the extraction was repeated again. These organic layers were combined and evaporated to dryness, and the resultant residue was dissolved in 100 µL of methanol. The 20 µL of reconstituted solution was injected into the HPLC system after the filtration with 0.45 µm of membrane filter.

Results and discussion

3.1 Fluorescence spectra of PQ after reaction with 2-ATHP

Fig. 1 shows the fluorescence spectra obtained from the reaction mixture PQ with 2-ATHP. The fluorescence was detected at excitation and emission maxima of 390 nm and 510 nm, respectively. It was thought that PQ reacted with 2-ATHP under acidic condition to produce the fluorescent derivative. On the other hand, the significant fluorescence was not observed from the each of PQ and the reagent blank (2-ATHP) solutions because they did not have intrinsic fluorescence. Since the proposed reaction was fluorogenic derivatization reaction, 2-ATHP can be used as a post-column derivatization reagent for the HPLC determination method for PQ.

In order to elucidate the structure of the fluorescent derivative,
the precipitate from the reaction mixture of PQ and 2-ATHP was analyzed by EI-MS (JMS-DX 303 electron impact mass spectrometer, JEOL, Tokyo). The most abundant ion peak was found at $m/z$ 299. It was reported that the reaction products of 1,4-naphthoquinone with 2-ATHP was phenothiazine derivatives [19]. In these aspects, the fluorescent derivative was suggested to be the phenothiazine derivative as shown in Fig. 2.

3.2 Optimization of the conditions of the HPLC system.

Figure 3 shows the typical HPLC chromatogram of standard PQ solution obtained by the developed post-column derivatization method. The fluorescent derivative of PQ was detected at 24 min on the chromatogram. To achieve higher sensitivity, post-column derivatization conditions including reaction coil length, reaction temperature and reagent concentrations were optimized. The effect of kind of the acid was examined with H$_2$SO$_4$, HCl, HClO$_4$, HNO$_3$ and CH$_3$COOH. Among these acids, the optimal result was obtained with H$_2$SO$_4$ and it was selected for subsequent work. The effect of reaction coil length on peak height of the derivative was examined over the range of 5 to 14 m (Fig. 4). The peak height increased with the increase in the coil length up to 9 m and then reached constant. The effect of reaction temperature was examined over the range of 30 to 70 °C. The peak
height increased with elevation of the temperature up to 55 °C and then reached constant. As a result, 10 m and 60 °C were selected as reaction coil length and reaction temperature, respectively. The concentration of 2-ATHP was examined over the range of 0.005 to 0.1 M, and the peak height was reached maximum at the concentration of 0.02 M and then decreased (Fig. 5): thus, 0.02 M 2-ATHP was selected. The concentration of H$_2$SO$_4$ was also optimized over the range of 0.01 to 0.5 M. The maximum and constant peak heights were obtained more than 0.1 M: 0.15 M was employed as optimal H$_2$SO$_4$ concentration.

3.3 Calibration curve, detection limit and repeatability.

The calibration curve obtained with the standard PQ showed good linear relationship ($r = 0.999$) between the concentrations and peak heights in the range from 0.013 to 50 µM. The slope and intercept of the regression equation (mean ± standard error, n = 3) were 17.49 ± 0.15 and -0.15 ± 0.35, respectively. The detection limit for standard PQ at a signal-to-noise (S/N) ratio of 3 was 3.4 nM (67 fmol/injection). The sensitivity of the proposed method was 14 times higher than that of GC-MS [7], 10 times higher than that of HPLC-MS/MS with positive ionization [15], and almost same as that of HPLC-MS/MS with negative ionization [15]. Although the sensitivity of the proposed method was 10 times lower than that of our previous pre-column derivatization method
[16], the proposed method allowed the direct injection of the sample solution with shorter analysis time. The repeatability of the proposed method was examined using different concentrations (0.05, 1 and 30 µM) of standard PQ solution: the relative standard deviations (R.S.D.) for intra-day (n = 5) analyses were 2.2, 2.3 and 0.4%, respectively and for inter-day (n = 5) analyses were 8.8, 5.8 and 5.4%, respectively.

3.4 Application to airborne particulates samples

The proposed method was applied to the determination of PQ in the airborne particulates collected in Nagasaki city. The peak derived from PQ was detected at 24 min by the injection of the extract solution (Fig. 6A), while the peak at 24 min was not detected in the absence of 2-ATHP (Fig. 6B). Therefore, it revealed that the peak could be attributed to PQ derivative. The average concentration (mean ± S.D., n = 11) of PQ found in airborne particulates collected at Nagasaki city was 0.28 ± 0.09 ng/m³. Furthermore, the concentrations of obtained by the proposed post-column derivatization method were found to be well correlated with those determined by the pre-column derivatization method [16] as shown in Fig. 7. This result confirmed the reliability of the determination values obtained by the proposed method.
4. Conclusion

We found that 2-ATHP could convert a non-fluorescent PQ to the fluorescent derivative. Based on this finding, we developed a sensitive method for the determination of PQ by HPLC with post-column derivatization and fluorescence detection. The proposed method was successfully applied to the determination of PQ in airborne particulates. This method should be useful especially for the routine monitoring system for PQ in environmental samples.

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References


Figure captions

Fig. 1. Fluorescence spectra obtained from the reaction mixture of PQ with 2-ATHP and the reagent blank. Excitation spectra with the emission wavelength at 510 nm of (A) the reaction mixture and (B) the reagent blank. Emission spectra with the excitation wavelength at 390 nm of (C) the reaction mixture and (D) the reagent blank.

Fig. 2. Suggested structure of the fluorescent derivative obtained by the reaction of PQ with 2-ATHP

Fig. 3 Chromatogram of standard PQ solution (2 µM).

Fig. 4 Effect of reaction coil length for post-column derivatization on the peak height of the derivative. The concentration of PQ was 2.5 µM.

Fig. 5 Effect of 2-ATHP concentration on the peak height of the derivative. The concentration of PQ was 2.5 µM.

Fig. 6 Chromatograms of PQ in the extract from airborne particulates (A) with and (B) without post column derivatization reaction.

Fig. 7 Correlation of determination values between pre-column derivatization method and post-column derivatization method.
Fig. 1 Fluorescence spectra obtained from the reaction mixture of PQ with 2-ATHP and the reagent blank. Excitation spectra with the emission wavelength at 510 nm of (A) the reaction mixture and (B) the reagent blank. Emission spectra with the excitation wavelength at 390 nm of (C) the reaction mixture and (D) the reagent blank.
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