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Determination of cyanide by high-performance liquid chromatography using postcolumn derivatization with *o*-phthalaldehyde

Koichi Sumiyoshi[a,*], Takao Yagi[a], Hiroshi Nakamura[b]

[a]*Tokyo Customer Support Centre, Shimadzu Corporation, 380-1 Horiyamashita Hadano-city, Kanagawa 259-13, Japan*
[b]*Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan*

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Abstract

A simple, highly sensitive and selective method for the determination of cyanide ion by high-performance liquid chromatography (HPLC) with postcolumn fluorescence derivatization is described. Cyanide ion was separated on an ion-exclusion chromatographic column and derivatized with two reagents, ammonium ion in the mobile phase and *o*-phthalaldehyde in the postcolumn reagent solution at pH 9.5. Sulfite ion, 2-mercaptoethanol and 3-mercaptopropionic acid did not interfere with the analysis. The average recovery of cyanide from spiked environmental water samples (river water) was 99.0% ($n = 6$). The relative standard deviation was 2.5% (based on peak area) for cyanide standard solutions (0.1 μg/ml, $n = 10$). The linear determination range was from 2.5 ng/ml to 1 μg/ml. The detection limit was 0.1 ng/ml (signal-to-noise ratio = 3, injection volume 200 μl).

1. Introduction

Cyanide ion is found in industrial wastewater and river water. Because of its extreme toxicity, the development of a sensitive and selective analytical method is highly desirable. HPLC and ion chromatography (IC) using various detection methods have been reported for this purpose. The electrochemical method, which has been considered the most sensitive, has interference problems [1]. The conductivity method [2], based on the formation of cyanate ion, and the spectrophotometric method [3], based on the König reaction, are often limited by low sensitivity. The precolumn fluorescence derivatization method, based on the reaction of cyanide with 1,4-benzoquinone, which has high selectivity, has the problem that the calibration graph is non-linear below 1 μg/ml for a potassium cyanide solution [4]. It is known that *o*-phthalaldehyde (OPA) reacts with cyanide ion and primary amines or primary amino acids to afford highly fluorescent isoindole derivatives [5,6]. These methods can be utilized in precolumn or postcolumn derivatization modes, but for the former a complicated procedure is necessary to

* Corresponding author.

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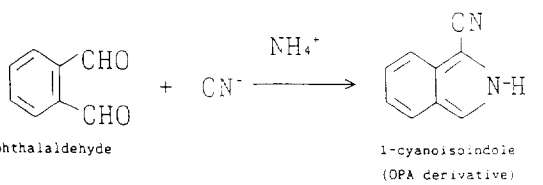

Fig. 1. Fluorogenic reaction of cyanide ion with OPA and ammonium ion.

remove contaminants and block thiols, sulfite and sulfide ion prior to derivatization, whereas for the latter it is necessary to use two pumps for the derivatizing reagents, i.e., OPA and primary amines or primary amino acids [5,6].

Cyanide ion can be derivatized to a fluorescent substance by reaction with ammonium ion and OPA according to the scheme in Fig. 1 [5]. This work was aimed at establishing a simple and sensitive fluorimetric method for determining cyanide ion by utilizing this reaction. Specifically, cyanide ion was separated from related compounds on an ion-exclusion chromatographic column and was then derivatized with two reagents, ammonium ion in the mobile phase and OPA in the postcolumn reagent solution, requiring only two pumps. The method described is simple and highly effective in decreasing the noise level cause by pumping the two postcolumn reagent solutions in addition to the pumping of the mobile phase.

Additionally, we describe its application to the determination of cyanide ion in river water and industrial wastepaper, demonstrating the utility of the proposed method.

2. Experimental

2.1. Reagents

OPA, potassium cyanide, boric acid, diammonium hydrogencitrate, perchloric acid, sodium hydroxide, 2-mercaptoethanol, 3-mercaptopropionic acid, sodium sulfite and methanol were obtained from Wako (Osaka, Japan).

A standard solution of cyanide (0.10 μg/ml) was prepared by dissolving potassium cyanide in 0.1 *M* sodium hydroxide solution. The concentration of cyanide was calibrated by titration with silver nitrate according to the Japanese Industrial Standard (JIS) method [7].

2.2. HPLC apparatus and analytical conditions

Fig. 2 shown a flow diagram of the HPLC postcolumn derivatization system, which consists of two microvolume double-plunger pumps (LC-10AD) for the eluent and the postcolumn derivatization reagent, a variable-wavelength fluorimetric detector (RF-10A) equipped with a xenon lamp and a 12-μL flow cell, a sample autoinjector (SIL-10A), a column oven (CTO-10A) and PTFE tubing (5–30 m × 0.5 mm I.D.) for the mixing coil (all from Shimadzu, Kyoto, Japan).

The HPLC conditions were as follows: column, Shim-pack SCR-102H polystyrene matrix strong cation exchanger (d_p = 7 μm, 300 mm × 8.0 mm I.D.) (Shimadzu); eluent, 10 m*M* ammonium citrate buffer (pH 3.3) at a flow-rate of 0.8 ml/min; postcolumn derivatization reagent,

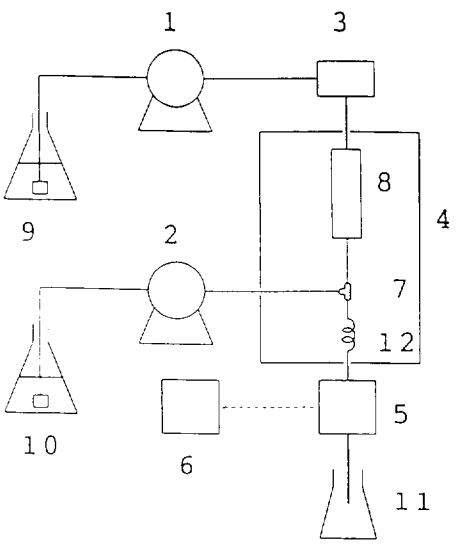

Fig. 2. Flow diagram for the HPLC postcolumn derivatization of cyanide ion. 1, 2 = Pumps; 3 = sample injector; 4 = column oven; 5 = fluorescence detector; 6 = data processor; 7 = mixing joint; 8 = analytical column; 9 = mobile phase; 10 = OPA reagent; 11 = waste; 12 = mixing coil (15 m × 0.5 mm i.d.).

0.5 *M* sodium borate buffer (pH 7–13) containing 54 m*M* OPA methanolic solution (flow-rate 0.15 ml/min); excitation and emission wavelengths of the spectrofluorimeter set at 328 and 370 nm, respectively; column and mixing coil maintained at 30–50°C in the column oven. Chromatograms were recorded with a Shimadzu Model C-R7A recording integrator.

3. Results and discussion

Diammonium hydrogencitrate was selected as the source of ammonium ions because it contained essentially no contaminants such as sulfide or sulfite, which gave interfering fluorescence on reaction with OPA in the presence of ammonium ion and thus caused interference.

In the following investigation, the reaction coil length, the pH of the OPA buffer, the reaction temperature and the excitation and emission wavelengths were optimized. Sano et al. [5] reported the optimum pH for the reaction of cyanide with OPA and taurine to be 9.0. Using flow-injection fluorimetry, with ammonium in place of taurine, the effect of the pH of the borate solution on the fluorescence intensity of the derivatized cyanide ion was investigated. The reaction conditions were as follows: reaction time, 15 min at room temperature; 1.0 mg/ml cyanide standard solution 50 μL; 0.5 *M* sodium borate buffer (pH 7–13, adjusted with concentrated sodium hydroxide) containing 54 mmol/l OPA methanolic solution, 750 μl; 10 m*M* diammonium hydrogencitrate (pH 3.3), 4 ml; wavelengths, $\lambda_{ex} = 328$ nm, $\lambda_{em} = 370$ nm. In the pH region above 9, the fluorescence intensity increased, as was reported using taurine [5], and the maximum intensity was achieved at pH 9.5.

The effect of the reaction coil length on the peak height and the peak area was examined. As shown in Fig. 3, the peak height and peak area increased with increasing reaction coil length up to 15 m. On the other hand, when a coil length of 15 m or more was used, the peak height decreased significantly for the diffusion, but the peak area remained stable and high. Consequently, a reaction coil length of 15 m was suitable in this method. When using a coil length of 15 m at a total flow-rate of 0.95 ml/min, it took 3.1 min (186 s) for the eluate to reach the detector after exiting the column.

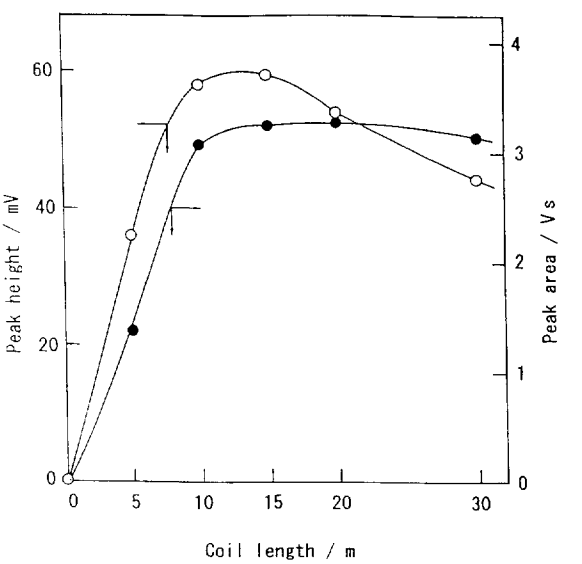

Fig. 3. Effects of reaction coil length on (○) peak height and (●) peak area. pH of OPA reagent, 9.5; 0.1 μg/ml cyanide standard solution, 10 μl; other conditions as in Section 2.2.

The effect of the reaction temperature on the fluorescence peak area was investigated. The reaction conditions were as follows: reaction time, 3.1 min; pH of OPA reagent, 9.5; 1.0 μg/ml cyanide standard solution, 10 μl; other conditions as those given in Section 2.2. The fluorescence peak area for the cyanide derivative increased with increasing temperature from 30 to 50°C. However, when the reaction temperature was 45°C or above, the background noise level of the eluent itself was relatively high. In consideration of the sensitivity, the optimum reaction temperature was determined to be 40°C.

Following the fluorescence scanning technique using the stopped-flow method, the excitation and emission spectra of OPA-derivatized cyanide were investigated under the above-selected conditions. The maximum excitation and omission wavelengths obtained were at 328 and 370 nm, respectively, which are close to the reported maximum excitation (330 nm) and emission

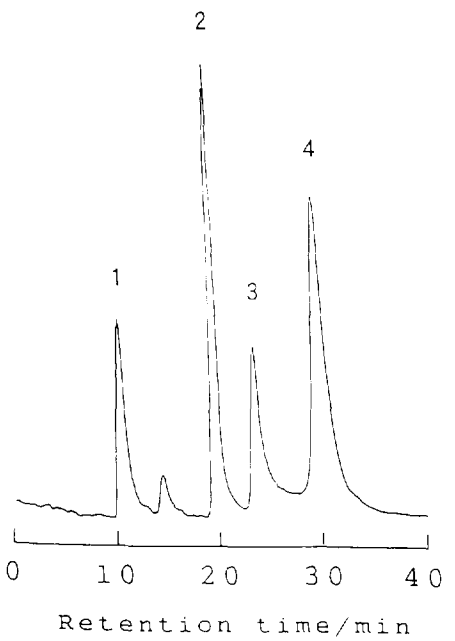

Fig. 4. Chromatogram of sulfite ion, cyanide ion, 2-mercaptoethanol and 3-mercaptopropionic acid, derivatized with OPA and ammonium ion. Peaks: 1 = sulfite ion (1 ng); 2 = cyanide ion (1 ng); 3 = 2-mercaptoethanol (100 ng); 4 = 3-mercaptopropionic acid (100 ng). Chromatographic conditions as in Section 2.2.

wavelengths (371 nm) of OPA-derivatized cyanide using taurine [5].

The proposed method should show no interference from the presence of primary amines or primary amino acids in the samples, because of the difference in excitation and emission wavelengths normally used in the determination of these substances with OPA derivatization (λ_{ex} = 360 nm and λ_{em} = 450 nm, respectively) [8]. It is well known that OPA also reacts with organic thiols such as 2-mercaptoethanol and 3-mercaptopropionic acid and with sulfite ion in the presence of primary amines or primary amino acids to afford the fluorescent isoindole derivatives [9,10]. Therefore, the separation of those compounds and cyanide ion was investigated. A typical chromatogram of the above compounds and cyanide ion in standard solution is depicted in Fig. 4. It was found that the determination of cyanide ion was not interfered with by the sulfur compounds because they were completely separated in the ion-exclusion mode. This also suggests that it is possible to determine these compounds and cyanide ion simultaneously.

3.1. Calibration graph and accuracy

The reliability of the proposed method was tested by the following experiments. A linear calibration graph was obtained over the concentration range 2.5–1000 ng/ml cyanide ion per 20 μl of sample. Linear regression analysis resulted in the following equation: $y = -1.06 + 3.821x$, where y = peak area (mVs) and x = concentration (ng/ml). The regression coefficient was 0.9999. The relative standard deviation was 2.5% for 0.1 ng/ml cyanide standard solution (n = 10) (Table 1).

A series of recovery tests were performed by adding one volume of cyanide standard (1 μg/ml) to nine volumes of the river water and comparing the results with those for an equivalent cyanide standard solution (0.1 μg/ml). The mean recovery of cyanide ion was 99% with a relative standard deviation of 2.2% (n = 6) (Table 1). Before the recovery tests, metal ions in the river water were determined by cation-exchange chromatography. As the concentration of Fe(III) from the river water was below about 10 ng/ml and other metal-related complex

Table 1
Accuracy and precision of the postcolumn derivatization system

Sample	n	Peak area (Vs) (mean ± S.D.)	R.S.D. (%)	Recovery (%)
Standard	10	2.37 ± 0.06	2.5	
Spiked river water	6	1.11 ± 0.02	2.2	99.0

cyanides were not detected, spiked cyanide (100 ng/ml) was completely recovered from the river water.

One of the standard methods for determining of cyanide is molecular absorptiometric analysis (pyridine–pyrazolone spectrophotometric method), which is the recommended JIS method [7]. The cyanide in water is classified into free cyanide (HCN, CN^-) and complex cyanide [$Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$, $Cu(CN)_4^{3-}$, etc.]. The cyanide in an environmental sample is determined after converting cyanide ion by pretreatment distillation in acidic solution in accordance with the JIS method.

The analytical data for the proposed method were in fair agreement with those for the JIS method in the range above 0.1 ppm. Consequently, using the proposed method without the distillation pretreatment, the analytical data for an environmental sample indicated the concentration of free cyanide. As the detection limit of the JIS method is 0.1 ppm, the proposed method could not be compared with it in the range below 0.1 ppm.

It was found that the resolving power of the analytical column and the stability of the pumping pressure were unchanged over the ca. 1000 samples used during the investigation.

The detection limit for cyanide ion in standard solution obtained by the present method is about 0.1 ng/ml for 200 μl of sample with a signal-to-noise ratio of 3. The sensitivity reported here is approximately two orders of magnitude higher than that reported for the taurine and OPA system [5].

3.2. Application

One of the major advantages of the present method is its extremely high sensitivity and selectivity compared with other HPLC and IC techniques for cyanide ion. The method is particularly well suited for the determination of cyanide ion in environmental waters such as industrial wastewater and river water. In order to test the utility of the proposed method, such samples were analysed and typical chromatograms are shown in Fig. 5. By use of ion-exclusion chromatography as described above, a sample could be injected without clean-up steps. Cyanide ion was not detected in the river water. In contrast, cyanide ion was detected in industrial wastewater from a plating bath prior to treatment with wastewater treatment equipment. The proposed method showed no interference from complex cyanides, because the latter in environmental water eluted at the retention volume of the exclusion limit and separated completely with free cyanide in the ion-exclusion mode.

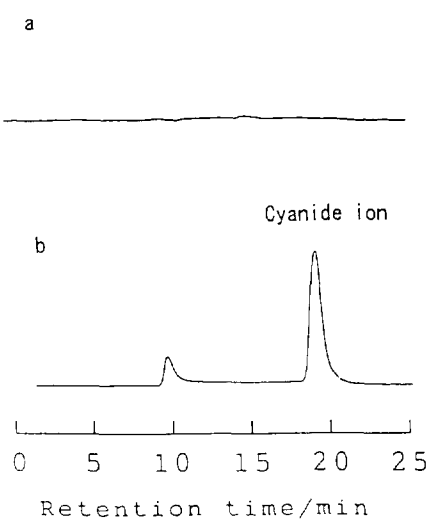

Fig. 5. Chromatograms of (a) river water and (b) industrial wastewater from a plating bath. (a) Sampling from the River Nogawa, Japan; (b) sampling prior to treatment with wastewater treatment equipment. Chromatographic conditions as in Section 2.2.

4. Conclusions

In conclusion, a highly sensitive, reproducible and selective determination of cyanide was performed using ion-exclusion chromatography employing a simplified postcolumn derivatization with OPA and ammonium ion. This method allows cyanide ion to be detected at the level of 0.1 ppb in environmental waters.

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