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Simultaneous Determination of Cyanide and Thiocyanate by High Performance Liquid Chromatography

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A sensitive, simple and specific method was developed for the simultaneous determination of cyanide and thiocyanate by high performance liquid chromatography with a strong base anion exchanger column. For detection, colorimetry based on the König reaction was employed with chloramine T, pyridine and barbituric acid as reagents. This method was applied to the determination of cyanide and thiocyanate in human urine.

Keywords——cyanide; thiocyanate; König reaction; high performance liquid chromatography; human urine

Cyanide is highly toxic to man and is metabolized *in vivo* to a much less toxic substance, thiocyanate.[1] Therefore, it is important to determine concentrations of cyanide and thiocyanate accurately for studies on the metabolic pathway of cyanide.

Numerous methods for the determination of low concentrations of cyanide and thiocyanate have been reported. These include colorimetric,[2] gas chromatographic[3] and electrochemical[4] methods. In these methods, colorimetry based on the König reaction has generally been used for detection.

Most of the methods for the determination of cyanide in various kinds of sample require a distillation step for clean-up and concentration of samples. However, distillation under acidic conditions causes errors due to the evolution of cyanide from thiocyanate, cyano-metal complexes and cyano compounds, and a method without a distillation step is desirable for the microdetermination of cyanide.

In this paper, we studied the simultaneous determination of cyanide and thiocyanate by high performance liquid chromatography (HPLC) with colorimetric detection.[5] Furthermore, we applied this method to the determination of cyanide and thiocyanate in human urine.

Experimental

Reagents——A standard solution of cyanide was prepared with potassium cyanide (Wako Pure Chem. Co., Ltd.) using 0.01 M NaOH and was tested for concentration by titration with $AgNO_3$ according to the Liebig–Dénigès method. A standard solution of thiocyanate was prepared by dissolving potassium thiocyanate (Wako Pure Chem. Co., Ltd.) in redistilled water. Other chemicals were of reagent grade.

Apparatus——Fig. 1 illustrates the flow diagram of HPLC. The system consists of reciprocating pumps (PSU-2.5, Seishin Seiyaku Co., Ltd.), a variable wavelength detector (UVILOG-7, Ōyō Bunkō Co., Ltd.), a variable input recorder (SS-250F, Sekonic Co., Ltd.) and a sample injector (VMU-6, Seishin Seiyaku Co., Ltd.).

The experimental conditions were as follows. Column: strong base anion exchange resin, TSK Gel LS-222 (6 μm, 3 mm × 150 mm, Toyo Soda Co., Ltd.). Eluent: 0.1 M acetate buffer (pH 5.0) containing 0.2 M

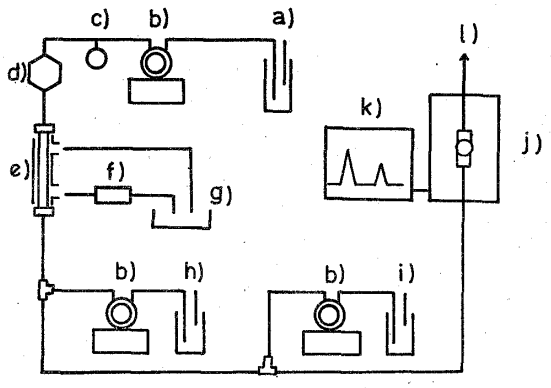

Fig. 1. Flow Diagram of High Performance Liquid Chromatography

a) eluent; b) pump; c) pressure gauge; d) sample injector; e) column; f) circulator; g) water bath; h) chloramine T solution; i) color developing reagent; j) detector; k) recorder; l) waste.

sodium perchlorate (flow rate, 0.5 ml/min). Chlorination reagent: 0.1% chloramine T aqueous solution (flow rate, 0.1 ml/min). Color developing reagent: a mixture of barbituric acid (3 g), pyridine (15 ml), concentrated hydrochloric acid (3 ml) and redistilled water (82 ml) (flow rate, 0.1 ml/min). Detector: set at 580 nm. Column temperature: 30°C. Sample size: 20 μl.

Procedure for Urine Sample——Urine was taken in a sampling bottle (100 ml), in which 0.5 ml of 4 M NaOH had been placed previously, and an aliquot was centrifuged at $1000 \times g$ for 15 min. Then, 20 μl of the supernatant was directly injected into the column.

Standard solutions of potassium cyanide and potassium thiocyanate were prepared in the range of 1—75 μM. Calibration curves for cyanide and thiocyanate were based on the peak heights obtained.

Results and Discussion

Separation of Cyanide and Thiocyanate

HPLC conditions for the separation of cyanide and thiocyanate were studied using TSK Gel LS-222 (polystyrene polymer, a strong base anion exchanger) and TSK Gel IEX 520 QAE (silica pellicular type, a strong base anion exchanger) with an acetate buffer containing sodium nitrate or perchlorate as the eluent. Nitrate and perchlorate are suitable for the elution of thiocyanate because they are strong chaotropic anions.[6]

The optimum conditions for the separation of cyanide and thiocyanate were established as described in "Experimental."

Detection System

In this study, cyanide and thiocyanate were detected by means of the modified König reaction.[6] Chloramine T was used for the halogenation and barbituric acid as the coupling reagent, because chloramine T is more stable than bromine water or sodium hypochlorite, and barbituric acid reacts with glutaconic aldehyde more rapidly than 1-phenyl-3-methyl-5-pyrazolone or benzidine.[7] It is well known that the reaction product formed from glutaconic aldehyde and barbituric acid decomposes rapidly, but, in the flow system, reproducible peak heights proportional to the concentrations of cyanide and thiocyanate (1—75 μM) were obtained on the chromatograms.

The optimum concentrations of chloramine T and color developing reagent were investigated with a flow injection system where the separation column was not employed. The flow rate of each reagent was minimized (0.1 ml/min), because the mixing coil length (duration of mixing) was required to be as short as possible to prevent broadening of the peak. Fifty μM cyanide and thiocyanate could be chlorinated quantitatively with 0.08% chloramine T, and thus 0.1% chloramine T was used in the procedure. The molar ratio of the components present

Table I. Effect of Foreign Anions on Determination of Cyanide and Thiocyanate

Anion	Added as	Recovery, %	
		CN^-	SCN^-
NO_2^-	$NaNO_2$	80.9	101.7
NO_3^-	$NaNO_3$	100.0	100.9
SO_3^{2-}	Na_2SO_3	95.2	100.0
SO_4^{2-}	Na_2SO_4	100.0	100.0
$S_2O_3^{2-}$	$Na_2S_2O_3$	76.2	110.2
Cl^-	NaCl	100.0	100.0
PO_4^{3-}	Na_2HPO_4	100.0	101.0
$B_4O_7^{2-}$	$Na_2B_4O_7$	100.0	100.0
$Fe(CN)_6^{4-}$	$K_4Fe(CN)_6$	95.2	101.7
$Fe(CN)_6^{3-}$	$K_3Fe(CN)_6$	103.2	100.9

CN^-, cyanide; SCN^-, thiocyanate.
Freshly prepared samples containing 25 μM cyanide, 50 μM thiocyanate and 10 mM foreign anion were subjected to the HPLC (pH, about 12; temperature, ambient).

in the color developing reagent was according to Lundquist,[5] and the optimum concentration of the reagent in the flow system was determined as described in "Experimental."

The effect of the mixing coil length was examined, and a mixing coil of 0.5 mm i.d. × 15 m gave the best analytical results.

Variability of the Present Method

The analytical interference caused by inorganic anions is shown in Table I. All the anions examined gave no absorbance when injected alone to the chromatograph and did not affect the chromatogram of cyanide and thiocyanate.

The detection limits of cyanide and thiocyanate in an injection volume of 20 μl are both 4 pmol, and are comparable to those of the gas chromatographic method.[8]

The recoveries of cyanide and thiocyanate (cyanide, 10 μM; thiocyanate, 50 μM) added to human urine samples were tested. The sample pH was adjusted to over 13 with 4 M NaOH as soon as possible to prevent cyanide from volatilizing as hydrogen cyanide. The mean recoveries of cyanide and thiocyanate from urine were 92.5% and 97.7%, respectively.

Analysis of Human Urine Samples

Thiocyanate is a detoxication product of cyanide, and the determinations of cyanide and thiocyanate in biological samples have consequently been used for monitoring exposure to hydrogen cyanide from tobacco smoke,[9] or fires.[10] In this paper, we investigated the concentrations of cyanide and thiocyanate in urine from tobacco smokers and nonsmokers.

Fig. 2 shows a typical chromatogram of urine from a smoker; cyanide could not be determined but was detectable. In the case of urine from nonsmokers, cyanide could not be detected. On the other hand, concentrations of thiocyanate in urine from smokers and nonsmokers were 171.0 μM (S.D., 42.1 μM, $n=6$) and 60.4 μM (S.D., 17.4 μM, $n=6$), respectively. These results are in good agreement with an earlier report.[9]

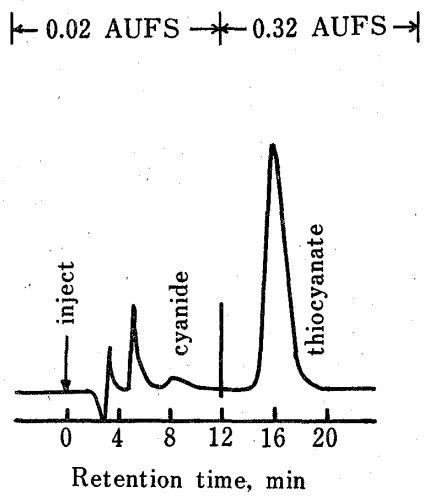

Fig. 2. Chromatogram of Urine from a Tobacco Smoker

Analytical conditions were as described in the text.

The present method does not require pretreatment such as distillation or microdiffusion of cyanide. Moreover, it appears to offer new possibilities for automated and simultaneous determinations of cyanide and thiocyanate in various scientific fields.

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