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Determination of cyanide and thiocyanate in blood by gas chromatography and gas chromatography–mass spectrometry

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Abstract

We devised a sensitive and simple method for determining cyanide and its major metabolite, thiocyanate, in blood using an extractive alkylation technique. Pentafluorobenzyl bromide was used as the alkylating agent, and tetradecyldimethylbenzylammonium chloride was used as the phase-transfer catalyst. The derivatives obtained were analyzed qualitatively by gas chromatography–mass spectrometry and quantitatively by gas chromatography with an electron-capture detection. The detection limits of cyanide and thiocyanate were 0.01 and 0.003 μmol/ml, respectively, while the gross recovery of both compounds was 80%. The calibration curve was linear over the concentration range from 0.02 to 1.0 μmol/ml for cyanide and from 0.01 to 1.0 μmol/ml for thiocyanate. The accuracy and precision of the method were evaluated, and the coefficients of variation were found to be within 10%. Using this method, the blood levels of two victims who had died from cyanide poisoning were determined.

Keywords: Cyanide; Thiocyanate

1. Introduction

Cyanide is used in electroplating, refining of precious metals and many other chemical processes. This compound is extremely toxic and causes rapid death. Hydrogen cyanide gas, produced by the pyrolysis and combustion of both natural and synthetic nitrogen-containing polymers, also causes severe poisoning [1,2]. Therefore, measurement of cyanide in the blood is of great importance.

Several reports have been published on the determination of cyanide by spectrometry [3,4], an electrochemical method using cyanide ion-selective electrode [5], gas chromatography (GC) [6–14] and gas chromatography–mass spectrometry (GC–MS) [15]. GC and GC–MS are the most widely used methods for biological samples, in combination with headspace extraction or derivatization of cyanide. Derivatization of cyanide is a superior technique for the specific analysis of cyanide by GC–MS, and several derivatization procedures have been described [6–8,10,11,14,15]. Their sensitivity and simplicity,

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however, do not appear to be satisfactory. Furthermore, analysis of cyanide and thiocyanate in whole blood is required.

We previously developed a sensitive and simple method to determine sulphide and polysulphide in whole blood using an extractive alkylation technique [16,17]. Using this technique, we tried to determine both cyanide and thiocyanate in whole blood.

2. Experimental

2.1. Reagents

Oxygen-free water was used throughout this study and was prepared by bubbling nitrogen into distilled water for 15 min.

A solution of internal standard (I.S.) was prepared by dissolving 1,3,5-tribromobenzene (TBB) in ethyl acetate to give a concentration of 10 μM. TBB was purchased from Wako Pure Chemical Industries (Osaka, Japan). An alkylating agent, pentafluorobenzyl bromide (PFBBr; Aldrich, Milwaukee, WI, USA), was dissolved in ethyl acetate with a concentration of 20 mM. Tetradecyldimethylbenzylammonium chloride (TDMBA), purchased from Tokyo Kasei Kogyo (Tokyo, Japan), was used as the counter-ion. Potassium cyanide was obtained from Wako. A standard solution of cyanide (100 μmol/ml) was prepared by dissolving potassium cyanide in a 0.1 M sodium hydroxide solution. The concentration of cyanide was determined by titration against silver nitrate, using p-dimethylaminobenzalrhodanine as an indicator. A standard solution of potassium thiocyanate (100 μmol/ml) was obtained from Wako.

2.2. Preparation of whole-blood samples

Samples to be tested were prepared by adding the standard solution of cyanide or thiocyanate to whole blood, which was collected from a healthy volunteer. For the analysis of cyanide, 0.4 ml of the sample was mixed with 0.1 ml of 0.5 M sodium sulphite solution and 0.4 ml of 10% ice-cold trichloroacetic acid solution in order to prevent the oxidation of cyanide and to precipitate protein, respectively. The mixture was shaken and centrifuged. A 0.4-ml aliquot of the supernatant was submitted to the extractive alkylation procedure for cyanide.

For the analysis of thiocyanate, 0.2 ml of the whole-blood sample was directly submitted to the extractive alkylation procedure, without any deproteinization steps.

2.3. Extractive alkylation procedure

A 0.5-ml volume of 20 mM PFBBr solution in ethyl acetate and 2.0 ml of ethyl acetate containing 10 μM of I.S. (TBB) were placed in a 10-ml glass-stoppered test tube, with 0.8 ml of 5 mM TDMBA solution in oxygen-free water saturated with sodium tetraborate.

To this mixture was added 0.4 ml of the above supernatant (for cyanide analysis) or 0.2 ml of whole blood (for thiocyanate analysis), and the preparation was vortex-mixed for 1 min at room temperature. The mixture was maintained at 55°C in a water bath for 30 min and then centrifuged at 1400 g for 15 min. A 0.1-ml aliquot of the supernatant was diluted with 2.0 ml of n-hexane to prevent the decrease of sensitivity with a concentrated solution of PFBBr, and a 0.2-μl aliquot of the solution was injected into the GC–ECD apparatus. For GC–MS analysis, a 1-μl aliquot of the supernatant was used without dilution. GC–ECD was used for quantitative determination, while GC–MS was used for confirmation. The alkylation of cyanide (or thiocyanate) is explained by the following formula:

$$\text{R-Br} + \text{CN}^- \text{ (or } \text{SCN}^-) \rightarrow \text{R-CN (or R-SCN)} + \text{Br}^-$$

where R- = pentafluorobenzyl-

2.4. Preparation of calibration graphs

Whole-blood samples were prepared that contained cyanide or thiocyanate at concentrations of 0.01–1.0 μmol/ml. These samples were extracted and derivatized in the same manner as described above. Calibration graphs were ob-

tained by plotting the peak-area ratio of the derivative of cyanide or that of thiocyanate to the I.S. versus the concentration of cyanide or thiocyanate using GC.

2.5. GC conditions

The apparatus used was a Shimadzu Model GC-14AE gas chromatograph (Kyoto, Japan), equipped with a ^{63}Ni electron-capture detector, connected to a computerized recorder, Shimadzu Model C-R5A Chromatopac. The column was a glass tube of 2.1 m × 3 mm I.D. packed with 5% OV-225 on Uniport HP, 60–80 mesh. The temperatures of the column, the injection port and the detector were kept at 170, 220 and 220°C, respectively. Nitrogen was used as the carrier gas at a flow-rate of 30 ml/min.

2.6. GC–MS conditions

GC–MS was carried out on a Hewlett-Packard HP 5790A gas chromatograph (Palo Alto, CA, USA) interfaced to a JEOL AX505A mass spectrometer. The column was a J&W fused-silica capillary tube of DB-225 (30 m × 0.32 mm I.D., 0.25 μm film thickness). A splitless injection mode was selected with a valve off-time of 1.5 min. The initial temperature of the column was held at 80°C for 3 min, programmed at 10°C/min to 200°C. The injection port, separator and ion source were kept at 220, 200 and 220°C, respectively. Helium was used as the carrier gas at a flow-rate of 2 ml/min. The ionization energy was 70 eV.

3. Results

3.1. Analysis by GC

A gas chromatogram of alkylated extracts from deproteinized whole blood containing 0.2 μmol/ml of cyanide is shown in Fig. 1.

Sharp and symmetrical peaks of the derivative of cyanide and I.S. were observed, with retention times of 3.5 and 5.2 min, respectively. There were no interfering peaks of the derivative of cyanide in the control blood sample. A gas chromatogram of alkylated extracts from whole blood containing 0.2 μmol/ml of thiocyanate is shown in Fig. 2. Each peak of I.S. and the derivative of thiocyanate was clearly separated on the chromatogram, with retention times of 5.2 min and 7.1 min, respectively. There was a small peak of the derivative of thiocyanate in the control whole blood obtained from a non-smoker containing 0.02 μmol/ml of thiocyanate.

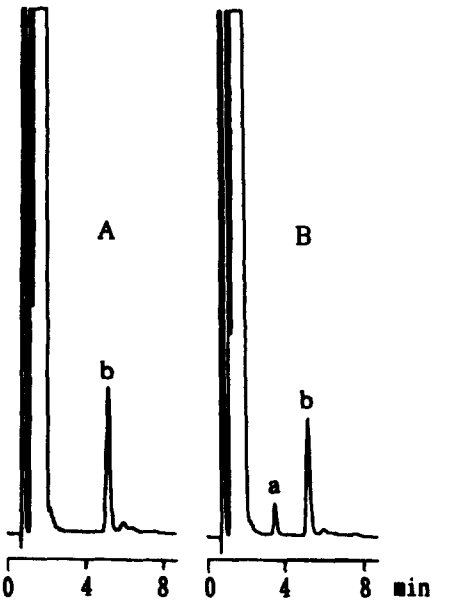

Fig. 1. Gas chromatograms of the alkylated extracts from deproteinized whole-blood samples: (A) control whole blood, (B) whole blood containing 0.2 μmol/ml of cyanide. a = pentafluorobenzyl cyanide; b = TBB (I.S.).

The calibration curves were linear in the concentration range from 0.02 to 1.0 μmol/ml for cyanide, and from 0.01 to 1.0 μmol/ml for thiocyanate, with a correlation coefficient of 0.999. The gross recoveries of both compounds from whole blood were 80%. The lower limits of detection, based on a concentration giving a signal three times stronger than the average noise intensity, were ca. 0.01 μmol/ml for cyanide, and ca. 0.003 μmol/ml for thiocyanate in the whole-blood samples. Within-day precisions were obtained using different concentrations (0, 0.1, 0.25 and 0.5 μmol/ml) by adding cyanide or thiocyanate to blank whole blood.

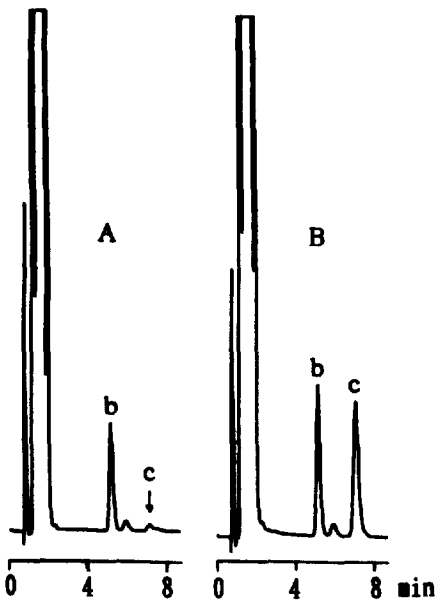

Fig. 2. Gas chromatograms of the alkylated extracts from whole-blood samples without deproteinization: (A) control whole blood, (B) whole blood containing 0.2 μmol/ml of thiocyanate. b = TBB (I.S.); c = pentafluorobenzyl thiocyanate.

The coefficients of variation (C.V.) ranged from 7.3% to 9.1% for cyanide and from 3.3% to 6.9% for thiocyanate (see Tables 1 and 2).

3.2. Analysis by GC–MS

The peaks of the cyanide derivative, TBB (I.S.) and thiocyanate derivative were analyzed by GC–MS. Their mass spectra are shown in Fig. 3.

The molecular ion of the cyanide derivative (Fig. 3a) was observed at m/z 207 (M^+), the fragment ion at m/z 188 (M − F). The mass spectral pattern indicated that the derivative was pentafluorobenzyl cyanide. The molecular ion of TBB (Fig. 3b) was observed at m/z 312, the fragment ion at m/z 233 (M − Br). The molecular ion of the thiocyanate derivative (Fig. 3c) was observed at m/z 239 (M^+), the fragment ion at m/z 181(M − SCN), and the mass spectral pattern indicated that the derivative was pentafluorobenzyl thiocyanate.

Selected-ion monitoring was carried out by monitoring ions at m/z 207 and 188 for the

Table 1
Accuracy and precision of blood cyanide determination

CN added (μmol/ml)	n	Detected (mean ± S.D.) (μmol/ml)	C.V. (%)
0.00	5	ND	
0.10	5	0.08 ± 0.007	9.1
0.25	5	0.25 ± 0.020	7.8
0.50	5	0.50 ± 0.037	7.3

ND = not detected.

Table 2
Accuracy and precision of blood thiocyanate determination

SCN added (μmol/ml)	n	Detected (mean ± S.D.) (μmol/ml)	C.V. (%)
0.00	5	0.02 ± 0.001	4.1
0.10	5	0.11 ± 0.007	5.8
0.25	5	0.28 ± 0.019	6.9
0.50	5	0.52 ± 0.017	3.3

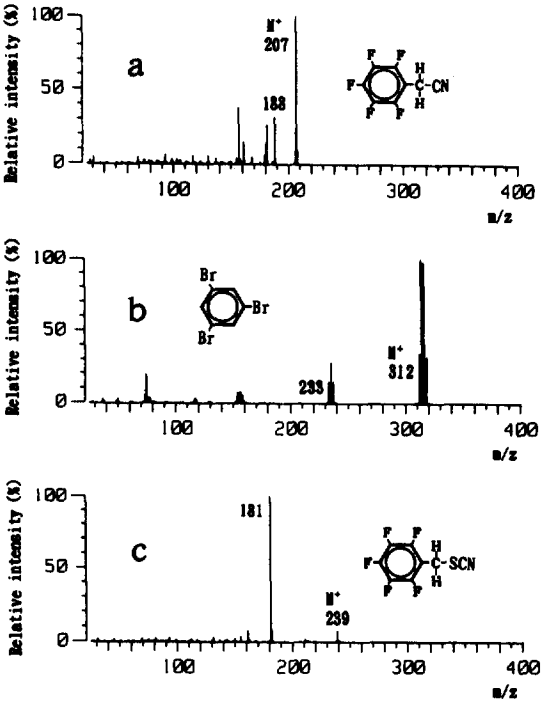

Fig. 3. Mass spectra of the cyanide derivative (a), the internal standard, TBB (b), and the thiocyanate derivative (c).

cyanide derivative, at m/z 239 and 181 for the thiocyanate derivative, and at m/z 314 (M + 2) and 235 (M + 2 − Br) for the I.S. All the peaks were clearly separated with no interference in the blood samples. Selected-ion monitoring proved to be useful for the identification of derivatives based on its sensitivity and selectivity.

4. Discussion

Wu et al. [7] alkylated cyanide in industrial waste water, using PFBBr in acetone, by stirring for 90 min at 30°C. Since their method, however, produces the same derivative from thiocyanate, cyanide in biological samples cannot be analyzed. Funazo et al. [8] reported the derivatization of cyanide with pentafluorobenzyl *p*-toluenesulphonate by extractive alkylation, where the sensitivity is limited to levels of over 5 μg/ml. Their method is not practical for small amounts of cyanide in blood. Recently, Chen et al. [14] reported a sensitive derivatization procedure for cyanide in waste water from electroplating works with PFBBr by extractive alkylation. Their method, however, requires a long reaction time of over 3 h. Miki et al. [11] derivatized cyanide and thiocyanate in plasma with PFBBr to 2,3-bis-(pentafluorophenyl)propionitrile and pentafluorobenzyl thiocyanate, respectively. In forensic cases where postmortem samples are being dealt with, the plasma can rarely be obtained, and so decayed, hemolyzed or frozen blood needs to be examined in practice. In order to overcome the above problems, our extractive alkylation procedures for sulphide and polysulphide [16,17] were applied to cyanide analysis. Derivatization of cyanide was found to be successful after precipitating protein in whole blood. On the other hand, thiocyanate was derivatized without any deproteinization steps in whole blood, but was not derivatized after precipitating protein. Therefore, different procedures were required for the analysis of cyanide and thiocyanate. Sulphide was derivatized by our method, but the retention time of sulphide derivative (15.0 min) was completely different from the times of cyanide and thiocyanate. Therefore, sulphide does not interfere in this method. Our procedure is advantageous in that each of the derivatives of cyanide and thiocyanate are produced, while the reaction time is reduced to 30 min, so that the analytical problems described above can be practically overcome.

4.1. Application

We examined two blood samples collected from a 61-year-old man who had died after drinking a sodium cyanide solution, and from a 60-year-old man who had died after inhaling fire gas, which contained hydrogen cyanide produced at the time of pyrolysis of a nitrogen-containing polymer urethane foam, used for the insulation of a cold-storage warehouse. Toxicological examinations were carried out using our method. The blood levels of cyanide and thiocyanate were 0.52 and 0.10 μmol/ml in the former case, and 0.28 and 0.13 μmol/ml in the latter case, respectively. As the toxic and fatal levels of cyanide were reported to be 0.05 and 0.1 μmol/ml, respectively [18,19], the cause of death of the two victims was concluded to have been cyanide poisoning. Thiocyanate levels of the victims were twice the mean level (0.0598 μmol/ml) of smokers, and were lower than those of cyanide. This observation can be explained due to the sudden death of the victims.

5. Conclusion

A sensitive and simple method for analyzing cyanide and thiocyanate in whole blood was devised, using an extractive alkylation technique. This method enabled forensic analyses of whole blood to be made in cases of cyanide poisoning.

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