Journal of Chromatography, 490 (1989) 319–327
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4682

IMPROVED GAS CHROMATOGRAPHY WITH ELECTRON-CAPTURE DETECTION USING A REACTION PRE-COLUMN FOR THE DETERMINATION OF BLOOD CYANIDE: A HIGHER CONTENT IN THE LEFT VENTRICLE OF FIRE VICTIMS

CHIKATOSHI MASEDA

Criminal Investigation Laboratory, Shimane Prefectural Police Headquarters, Matsue 690 (Japan)

and

KAZUO MATSUBARA and HIROSHI SHIONO*

Department of Legal Medicine, Shimane Medical University, Izumo 693 (Japan)

(First received November 24th, 1988; revised manuscript received January 17th, 1989)

SUMMARY

We developed a head-space method for the determination of blood cyanide by gas chromatography with electron-capture detection. In this technique, a reaction pre-column, packed with chloramine-T, was used for the conversion of hydrogen cyanide into cyanogen chloride. Since the reaction pre-column eliminated the necessity for trapping hydrogen cyanide from the biological samples, blood cyanide was quickly analysed by acidification only. The reaction pre-column was durable for at least several months. The calibration curve gave good linearity when dichloromethane was used as the internal standard, and the lower detection limit taken from this plot was ca. 0.05 μg/ml. The relative standard deviation of spiked blood samples was in the range 0.6–3.9%. We determined blood cyanide levels at autopsy in victims who had died from fire using this method. A significantly higher cyanide content was detected in the left ventricular blood than in the right. There was a positive correlation between blood cyanide and carboxylhaemoglobin contents. This simple and sensitive technique could be very useful for the determination of cyanide in various samples.

INTRODUCTION

Cyanides are used in various industrial and chemical processes, such as electroplating, metal refining and the synthesis of cyanopolymer-based materials.

0378-4347/89/$03.50

The extreme toxicity of cyanide is well known, so cyanides are occasionally used as a mode of suicide. The recent introduction of sodium nitroprusside into therapy as a hypotensive agent has produced a case of iatrogenic cyanide poisoning [1]. Furthermore, the presence of hydrogen cyanide in tobacco smoke [2] and fire gas [3] has become an important social problem.

The detection of cyanide exposure is important, and various methods for the determination of cyanide in biological materials have been offered. A conventional method for the determination of cyanide in blood is the pyridine–pyrazorone method developed by Epstein [4]. This method requires complicated procedures and technical skills such as microdiffusion and/or aeration techniques for the separation of hydrogen cyanide from the biological matrix [5–7]. Recently, several gas chromatographic (GC) methods with electron-capture detection (ECD) or nitrogen–phosphorus detection (NPD) have been reported. These methods are sensitive for the determination of hydrogen cyanide. The ECD method [8], however, still has problems; the procedures for the collection of the evolved hydrogen cyanide and the extraction of halogenated cyanide by organic solvents take time and are presumably less reproducible. In the NPD method [9–11], hydrogen cyanide can be assayed directly without subsequent procedures. However, this method is not as reproducible, because there is no appropriate internal standard and the thermionic source of NPD is unstable.

We developed a GC–ECD method using a reaction pre-column packed with chloramine-T (sodium *p*-toluene sulphonchloramide) for the determination of cyanide in blood. We applied this technique for the determination of cyanide in the left and right ventricular blood of victims who had died from fire.

EXPERIMENTAL

Materials

All reagents used in the assay were of analytical grade and were from Wako (Osaka, Japan). PTFE-faced septa, aluminium crimp seals and 15-ml vials (actual capacity of 20 ml) were purchased from Shimadzu (Kyoto, Japan), and all syringes were from Hamilton (Reno, NV, U.S.A.).

Blood was collected from healthy subjects (non-smokers) and burned victims. The blood from the former was stored at 5 °C and used as normal blood samples. The blood from the latter was collected from the left and right ventricles at autopsy and stored at −80 °C until analysis. A solution of cyanide (8.87 mg/ml) was prepared by dissolving sodium cyanide in distilled water and standardized by titration with silver nitrate using *p*-dimethylaminobenzalrhodanine as an indicator. The solution was diluted with 0.1 *M* sodium hydroxide solution to make the stock cyanide solution (88.7 μg/ml). The standard solutions with various concentrations were prepared by diluting this stock cya-

nide solution. These standard solutions were used for making reference cyanide blood.

The solution for the internal standard (I.S.) was prepared by dissolving dichloromethane (5%, v/v) in dimethylformamide.

Apparatus

A Shimadzu GC/7A gas chromatograph equipped with a ^{63}Ni electron-capture detector was used. GC separation was achieved with a glass column (3 m×3 mm I.D.) packed with 7% Halcomid M-18 on 80–100 mesh Chromosorb W (AW-DMCS). Pure nitrogen was used as the carrier gas at a constant flow-rate of 30 ml/min. The detector and injection port temperatures were kept at 100°C. The column temperature was maintained at 55°C. Data were recorded using a Shimadzu C-R6A printer–plotter integrator.

As a reaction pre-column, a 2.5 cm×3 mm I.D. glass column packed with chloramine-T was used. This column was covered with aluminium foil and was directly connected to the GC, as illustrated in Fig. 1.

Procedure

The normal blood (0.5 ml) was pipetted into a 15-ml glass vial, and 50 μl of standard solution were spiked with a 100-μl syringe. After the vial was sealed with a PTFE-faced septum and an aluminium crimp seal, 50 μl of I.S. solution and 0.3 ml of phosphoric acid solution (50%, v/v) were taken into the vial using syringes of 100 and 500 μl capacity, respectively. The vial was agitated for 1 min in a water-bath at 50°C, and was then allowed to incubate for 10 min. After incubation, 50 μl of the head-space gas were withdrawn with a gas-tight syringe for GC analysis. The samples from the fire victims were similarly analysed without the spiking step.

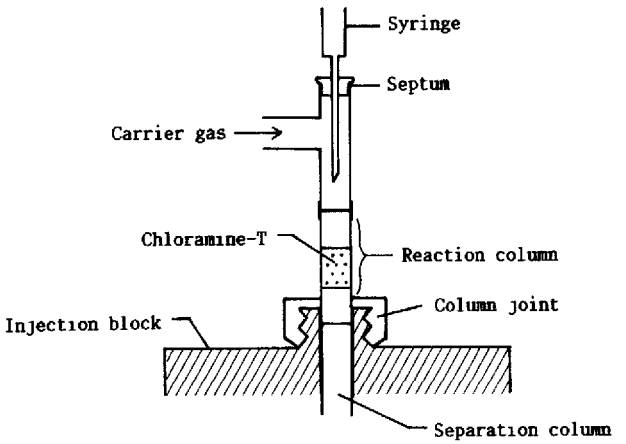

Fig. 1. Setting of reaction pre-column.

The peaks on the gas chromatogram were identified by gas chromatography–mass spectrometry (GC–MS) with electron-impact ionization using a Shimadzu GCMS-QP1000A. The carboxyhaemoglobin (CO-Hb) concentrations were measured by the method previously reported [12,13]. Statistical significances of difference were determined by Wilcoxon's signed ranks test and regression analysis.

RESULTS

GC–ECD analysis

In order to test the capacity for the conversion of hydrogen cyanide into cyanogen chloride (CNCl), the length of the reaction pre-column (2.5, 5 and 10 cm) was examined using the reference cyanide blood (1 μg/ml). There was no significant difference in the peak area of detected CNCl among the three columns. However, broadening and tailing of the peaks were observed when longer columns were employed (Fig. 2). Thus, the reaction pre-column packed with chloramine-T at a length of 2.5 cm was used in this work. This column was covered with aluminium foil and set on the fore part of the GC injection block to prevent the predictable decomposition of chloramine-T due to light or heat (Fig. 1). In a typical gas chromatogram (Fig. 2A), sharp peaks corresponding to CNCl and I.S. appeared with the retention times of 2.1 and 5.2 min, respectively. These peaks were identified by electron-impact MS. The

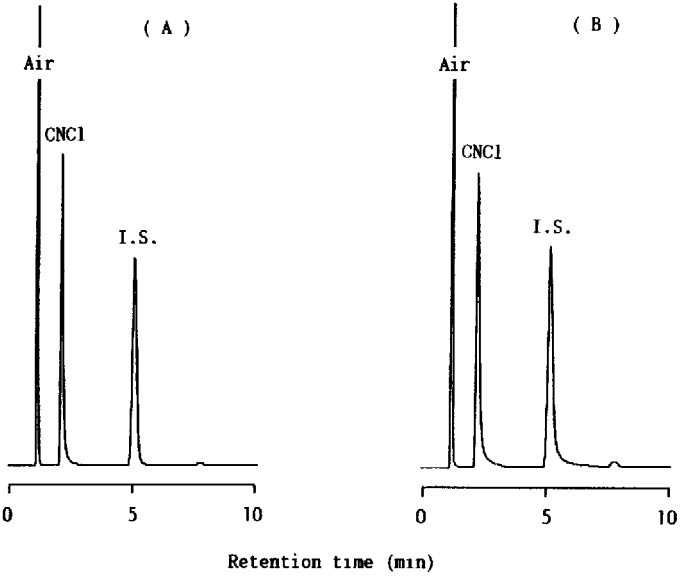

Fig. 2. Gas chromatograms obtained by a reaction pre-column of 2.5 cm long (A) and 10 cm long (B). The blood cyanide content was prepared at 1 μg/ml.

spectrum of the isolated CNCl showed M^+ peaks at 61 and 63 with an intensity ratio of 3:1. The apparent cyanide concentration in the blood of ten healthy subjects was found to be less than 0.05 μg/ml.

Conditions of procedure

The conditions of the procedure, including the equilibration time, equilibration temperature and sealing septum, were determined using reference cyanide blood (2 μg/ml). Fig. 3 shows the peak-area ratio of CNCl against I.S. obtained from head-space analysis using two kinds of septum, a PTFE-faced septum and butyl rubber septum, at 50°C. Using the former septum, no significant difference in the peak-area ratio was found among the various incubation times from 3 to 60 min; it gave good reproducibility with a relative standard deviation of 1.4%. On the other hand, the butyl rubber septum showed a rise in the peak-area ratio resulting from the decrease of I.S. along with the time.

Accuracy and precision study

A calibration curve was made using normal blood spiked with various concentrations of cyanide. Good linearity was obtained over the range 0.1–5 μg/ml blood cyanide. The equation parameters were $y=0.541x+0.073$ ($r=0.99$, $n=18$). The lower detection limit taken from this plot was ca. 0.05 μg/ml. The reproducibility of this technique was examined by replicate analyses of spiked blood samples. The data are summarized in Table I.

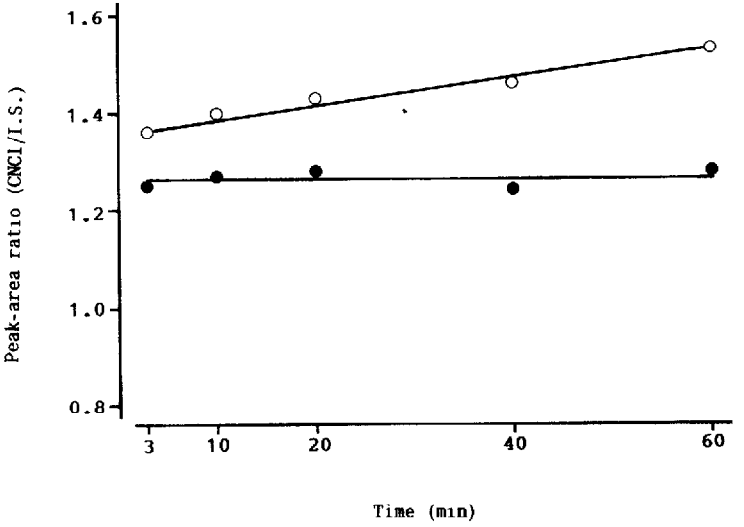

Fig. 3. Effect of time and septum on the equilibration of head-space. The plots represent the mean values of triplicate analyses. (●) PTFE-faced septum; (○) butyl rubber septum.

TABLE I

ACCURACY AND PRECISION OF BLOOD CYANIDE DETERMINATION

Prepared (μg/ml)	n	Detected (μg/ml)		Coefficient of variation (%)
		Mean	S.D.	
0.22	5	0.18	0.007	3.9
1.11	5	1.15	0.019	1.7
2.22	5	2.28	0.013	0.6

TABLE II

BLOOD CYANIDE AND CARBOXYHAEMOGLOBIN CONTENT IN AUTOPSY CASES

Significant difference for blood cyanide ($p<0.01$) and carboxyhaemoglobin ($p<0.05$) contents between left ventricle (l.v.) and right ventricle (r.v.) by Wilcoxon's signed ranks test.

Subject	Cyanide (μg/ml)[a]		CO-Hb (%)[a]	
	r.v.	l.v.	r.v.	l.v.
A	9.44	18.12	91.7	94.7
B	3.04	—[b]	52.1	—
C	2.29	—	78.2	—
D	1.96	2.70	76.6	81.3
E	1.24	1.32	36.4	36.5
F	1.07	2.23	59.6	61.0
G	0.91	1.69	59.4	62.8
H	0.85	—	38.3	—
I	0.64	0.66	37.5	38.2
J	0.54	1.83	60.6	60.1
K	0.20	0.49	41.5	45.4

[a]Mean values of duplicate analyses.
[b]Not determined.

Cyanide in burned body

The cyanide levels in the fire victims are shown in Table II. The CO-Hb concentrations in their blood were measured at the same time. The values of cyanide in the blood were 18.12 μg/ml at the highest and 0.20 μg/ml at the lowest. The CO-Hb levels were 34.6–94.7%. The cyanide levels in the left ventricular blood were higher than those in the right ($p<0.01$). The correlation between blood cyanide and CO-Hb content is shown in Fig. 4. The regression analysis indicated a linear correlation with some statistical significance.

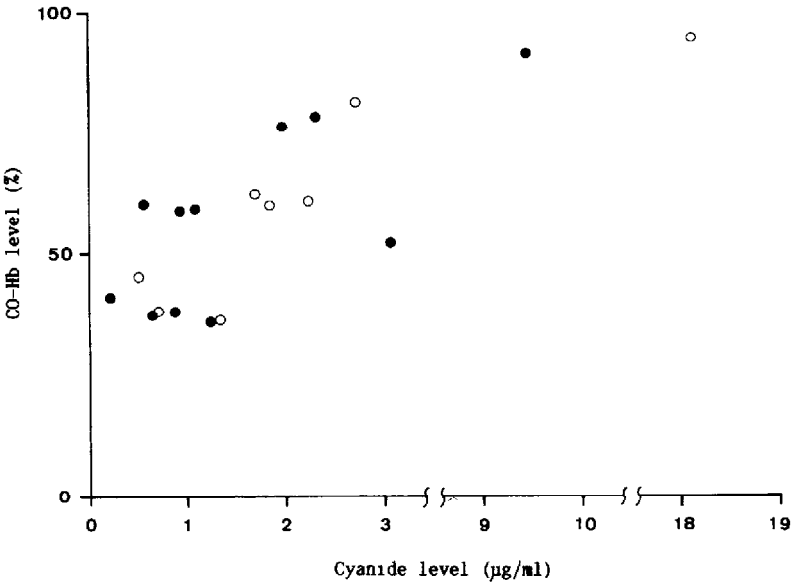

Fig. 4. Scatter diagram showing cyanide versus CO-Hb levels in autopsy cases. (●) From right ventricular blood; (○) from left ventricular blood. Correlation coefficients in all samples, right ventricular blood and left ventricular blood were 0.694 ($p<0.001$), 0.700 ($p<0.05$) and 0.761 ($p<0.05$).

DISCUSSION

For the determination of cyanide in biological specimens, the GC–ECD method was reported as a very sensitive detection technique by Valentour et al. [8]. In their method, the microdiffusion cell was used for the separation of cyanide from biological samples. The separated cyanide was converted into CNCl with chloramine-T, and its extract with organic solvent was then analysed by GC. These complicated procedures were employed in order to prevent the reaction of chloramine-T with endogenous compounds. If the hydrogen cyanide released from a sample by only acidifying can be analysed using GC–ECD, the method is the most desirable.

In this study, a GC–ECD system was directly coupled with the reaction pre-column. The pre-column converted hydrogen cyanide into CNCl before a separation column. This conversion was carried out easily and quantitatively on a GC flow-line. The aluminium foil cover and setting at room temperature prevented the predictable decomposition of chloramine-T by light or heat. This column under these conditions was lasted for at least six months.

Since the use of the reaction pre-column enabled the head-space analysis of cyanide in blood by GC–ECD, several conditions for preparation of the sample were investigated. The acidifying agent must be strong enough to release the

hydrogen cyanide and yet have little influence on the subsequent GC–ECD analysis. Sulphuric acid, which has been used in the microdiffusion method, caused coagulation of the blood resulting in unfavourable data (unpublished data). Phosphoric acid was very suitable for the releasing agent in the head-space analysis.

The equilibration time of hydrogen cyanide between the head-space and liquid phase has been determined by several investigators [9–11]. It was specified as 60 min at 60 °C by Darr et al. [9] and 30 min at room temperature by McAuley and Reive [10]. Zamecnik and Tam [11], who used acetonitrile as an I.S., employed 30 min at room temperature for optimal equilibration. Acetonitrile has a higher boiling point (81.6 °C) than hydrogen cyanide (25.7 °C). In this study, the equilibration of hydrogen cyanide and dichloromethane in the head-space gas was performed for 10 min at 50 °C after hand agitation for 1 min at the start of incubation. The agitation was suggested for the rapid equilibration of halocarbons [14]. A lower equilibration temperature reduced the peak response for cyanogen chloride and a higher temperature caused water to condense in the syringe.

The choice of septum for sealing the equilibration vial was also important in order to achieve the reproducible head-space analysis; the butyl rubber septum gave an increase in the peak-area ratio, owing to loss of the dichloromethane used as I.S. via adsorption into or onto the septum. On the other hand, constant values for the peak-area ratio were observed when the PTFE-faced septum was used.

On the basis of the above results, a rapid, simple and sensitive method for the determination of cyanide in blood was developed. It was applied to the determination of blood cyanide levels in victims who had died from domestic fires. The measurement in most samples was carried out immediately on arrival because of the instability of the cyanide in blood [6,15,16]. When storage of the samples was required, the samples were stored at −80 °C and analysed within two days. The level of cyanide in the blood of subject A was very high and considered as a fatal level and the levels in subjects B, C and D were toxic [17]. From this result, it was concluded that the presence of hydrogen cyanide in fire gas is very dangerous. However, the blood samples from the left and right ventricles gave different cyanide levels in the same victims. The cyanide levels in the left ventricle were higher than those in the right. This effect could have been due to circulation, diffusion and metabolization in the living body just before death. We also observed this phenomenon with petroleum fuel in burned victims [18]. The data presented here also showed that there were several victims with cyanide levels in the left ventricular blood double those in the right. This suggested that the collection point of the blood sample is an important factor for the detection of cyanide poisoning as the result of the inhalation of cyanide gas.

The blood CO-Hb levels in these fire victims were measured at the same

time. The relationship between cyanide and CO-Hb levels was examined by regression analysis. A positive correlation with some statistical significance was observed. However, the scatter of the points was very wide and no meaningful finding was obtained from this result, except that victims with high CO-Hb levels were likely to have high cyanide levels.

In conclusion, our technique described here provides superior simplicity, sensitivity and reproducibility for the determination of blood cyanide by acidification only. The data from the fire victims revealed a higher content of cyanide in the left ventricular blood than in the right.

ACKNOWLEDGEMENT

The authors thank Prof. Y. Fukui, Kyoto University, for his valuable advice.

REFERENCES

1 M. Bogusz, J. Moroz, J. Karski, J. Gierz, A. Regieli, R. Witkowska and A. Golabek, Clin. Chem., 25 (1979) 60.
2 K.D. Brunnemann, L. Yu and D. Hoffmann, J. Anal. Toxicol., 1 (1977) 38.
3 E. Urbas and E. Küllık, J. Chromatogr., 137 (1977) 210.
4 J. Epstein, Anal. Chem., 19 (1947) 272.
5 A.R. Pettigrew and G.S. Fell, Clin. Chem., 19 (1973) 466.
6 M. Holzbecher and H.A. Ellenberger, J. Anal. Toxicol., 9 (1985) 251.
7 P. Lundquist, H. Rosling and B. Sörbo, Clin. Chem., 31 (1985) 591.
8 J.C. Valentour, V Aggarwal and I. Sunshine, Anal. Chem., 46 (1974) 924.
9 R.W. Darr, T.L. Capson and F.D. Hileman, Anal. Chem., 52 (1980) 1379.
10 F. McAuley and D.S. Reive, J. Anal. Toxicol., 7 (1983) 214.
11 J. Zamecnik and J. Tam, J. Anal. Toxicol., 11 (1987) 47.
12 Y. Fukui, M. Matsubara, S. Takahashi and K. Matsubara, J. Anal. Toxicol., 8 (1984) 277.
13 Y. Fukui, M. Matsubara, A. Akane, K. Hama, K. Matsubara and S. Takahashi, J. Anal. Toxicol., 9 (1985) 81.
14 E.A. Dietz, Jr. and K.F. Singley, Anal. Chem., 51 (1979) 1809.
15 B. Ballantyne, J. Bright and P. Williams, J. Forensic Sci. Soc , 13 (1973) 11.
16 B. Ballantyne, J. Forensic Sci. Soc., 16 (1976) 305.
17 R.A. Anderson and W.A. Harland, Med. Sci. Law., 22 (1982) 35
18 K. Matsubara, A. Akane and S. Takahashi, H. Shiono, Y. Fukui, M. Kagawa and C. Maseda, J Chromatogr., 424 (1988) 49.