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Determination of Cyanide in Whole Blood by Capillary Gas Chromatography with Cryogenic Oven Trapping

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Cyanide, one of the most important toxic substances, has been found measurable with high sensitivity by capillary gas chromatography (GC) with cryogenic oven trapping upon injection of headspace (HS) vapor samples. The entire amount of cyanide in the HS sample could be cryogenically trapped prior to on-line GC analysis. A 0.5-mL volume of blood in the presence or absence of cyanide and propionitrile (internal standard, IS) was added to a vial containing 0.25 mL of distilled water, 0.3 g of Na_2SO_4, 0.2 mL of 50% H_3PO_4, and 0.1 g of ascorbic acid (when needed), and the mixture was heated at 70 °C for 15 min. A 5-mL volume of the HS vapor was introduced into a GC capillary column in the splitless mode at −30 °C oven temperature that was programmed up to 160 °C for GC analysis with nitrogen−phosphorus detection. A sharp peak was obtained for cyanide under the present conditions, and backgrounds were very clean. The extraction efficiencies of cyanide and IS were 2.89−3.22 (100 or 500 ng/mL) and 2.42%, respectively. The calibration curve showed good linearity in the range of 25−1000 ng/mL and the detection limit was ~2 ng/mL. The coefficients of intraday and interday variations were 2.9 and 11.8%, respectively. The mean blood cyanide level measured for actual fire victims was 687 ± 597 ng/mL (mean ± SD, n = 9). Endogenous blood cyanide concentration for healthy subjects was 8.41 ± 3.09 ng/mL (mean ± SD, n = 6).

Cyanide is known as one of the most rapidly acting and powerful poisons; it inhibits cytochrome oxidase of the mitochondrial respiratory chain.[1] Suicidal, accidental, or homicidal death by cyanide salts is frequently experienced in forensic toxicological practice. Several researchers reported that cyanide occasionally played a significant role in the cause of death of fire cases.[2−7]

For analysis of cyanide, the most classical is a colorimetric method with microdiffusion;[8,9] fluorometric methods were also reported.[10−12] Methods using gas chromatography (GC) with electron capture detection (ECD)[13−15] and with nitrogen−phosphorus detection (NPD)[16] and mass spectrometry (MS),[17] after suitable derivatizations, were reported. GC measurements of cyanide with NPD without derivatization were usually made using the headspace (HS) method.[4,18−20] In most of these reports, conventional packed columns, which give relatively low sensitivity and poor separation, were used.[4,13,14,16−19] With wide-bore capillary columns, only a 0.5-mL volume of the HS vapor can be injected;[20] with medium-bore capillary columns, split injection giving less than 5% of efficiency has to be used.[15] Solid-phase microextraction [21] has been applied to analysis of cyanide in human whole blood.[22]

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(1) Way, J. L. *Annu. Rev. Pharmacol. Toxicol.* **1984**, *24*, 451−481.
(2) Anderson, R. A.; Harland, W. A. *Med. Sci. Law* **1982**, *22*, 35−40.
(3) Becker, C. E. *Vet. Hum. Toxicol.* **1985**, *27*, 487−490.
(4) Zamecnik, J.; Tam, J. *J. Anal. Toxicol.* **1987**, *11*, 47−48.
(5) Levin, B. C.; Rechani, P. R.; Gurman, J. L.; Landron, F.; Clark, H. M.; Yoklavich, M. F.; Rodriguez, J. R.; Dros, L.; Mattos de Cabrera, F.; Kaye, S. *J. Forensic Sci.* **1990**, *35*, 151−168.
(6) Matsubara, K.; Akane, A.; Maeda, C.; Shiono, H. *Forensic Sci. Int.* **1990**, *46*, 203−208.
(7) Mayes, R. W. *J. Forensic Sci.* **1991**, *36*, 179−184.
(8) Feldstein, M.; Klendshoj, N. C. *J. Lab. Clin. Med.* **1954**, *44*, 166−170.
(9) Holzbecher, M.; Ellenberger, H. A. *J. Anal. Toxicol.* **1985**, *9*, 251−253.
(10) Ganjeloo, A.; Isom, G. E.; Morgan, R. L.; Way, J. L., *Toxicol. Appl. Pharmacol.* **1980**, *55*, 103−107.
(11) Morgan, R. L.; Way, J. L. *J. Anal. Toxicol.* **1980**, *4*, 78−80.
(12) Suzuki, O.; Hattori, H.; Oya, M.; Katsumata, Y. *Forensic Sci. Int.* **1982**, *19*, 189−195.
(13) Maseda, C.; Matsubara, K.; Shiono, H. *J. Chromatogr.* **1989**, *490*, 319−327.
(14) Shiono, H.; Maseda, C.; Akane, A.; Matsubara, K. *Am. J. Forensic Med. Pathol.* **1991**, *12*, 50−53.
(15) Odoul, M.; Fouillet, B.; Nouri, B.; Chambon, R.; Chambon, P. *J. Anal. Toxicol.* **1994**, *18*, 205−207.
(16) Funazo, K.; Tanaka, M.; Shono, T. *Anal. Chem.* **1981**, *53*, 1377−1380.
(17) Thomson, I.; Anderson, R. A. *J. Chromatogr.* **1980**, *188*, 357−362.
(18) Darr, R. W.; Capson, T. L.; Hileman, F. D. *Anal. Chem.* **1980**, *52*, 1379−1381.
(19) McAuley, F.; Reive, D. S. *J. Anal. Toxicol.* **1983**, *7*, 213−215.
(20) Seto, Y.; Tsunoda, N.; Ohta, H.; Shinohara, T. *Anal. Chim. Acta* **1993**, *276*, 247−259.

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Recently, a microcomputer-controlled device for cooling oven temperatures below 0 °C has become available for new types of GC instruments. It was originally designed for rapid cooling of the oven to reduce time for analysis. This new device has been applied for determining chloroform and methylene chloride in blood.[23]

In this paper, we have established a new GC technique using the cryogenic oven for measuring cyanide in whole blood without any complicated pretreatment; as much as 5 mL of the HS vapor for cyanide can be introduced without any loss into a medium-bore capillary column by use of a low oven temperature. This means that 10–100 times higher sensitivity can be obtained by this method as compared with that of the previous methods.[4,8–20,22]

EXPERIMENTAL SECTION

Materials. Potassium cyanide and methyl acetate were obtained from Wako Pure Chemical Industries (Osaka, Japan), and propionitrile was from Aldrich (Milwaukee, WI). Other chemicals were of analytical grade. Human whole blood samples were obtained from healthy volunteers or cadavers that were dissected or examined in our department.

Procedure. A stock solution of a mixture of cyanide (5 μg/mL) and propionitrile (internal standard, IS, 8 μg/mL) was prepared in 0.1 N NaOH solution. To a 7.0-mL screw-cap vial containing 0.5 mL of whole blood were added 0.25 mL of distilled water, 0.05 mL of a stock solution of cyanide and propionitrile (250 and 400 ng/vial, respectively), 0.3 g of Na_2SO_4, and 0.2 mL of 50% H_3PO_4 solution. For measurements of very low endogenous cyanide in whole blood, 0.1 g of ascorbic acid should be added to the vial in order to inhibit conversion of a minor amount of thiocyanate into cyanide.[24–26] The vial was rapidly sealed with a Teflon-lined silicone septum cap and then put on an aluminum block heater (Reacti-Therm Heating/Stirring model, Pierce, Rockford, IL) after vortexing for 30 s. After heating at 70 °C for 15 min, a 24-gauge needle of a plastic syringe (10-mL volume) was inserted into the vial; a 5-mL volume of the HS vapor was drawn into the syringe and injected into the GC port in the splitless mode at −30 °C of the oven temperature.

GC Conditions. GC analyses were carried out on an HP 6980 series gas chromatograph equipped with an NPD and with a cryogenic oven temperature device (Hewlett-Packard Co., Wilmington, DE). An electrically operated solenoid valve introduced liquid carbon dioxide at a rate appropriate to cool the oven to desired temperatures. The GC conditions were as follows: column used a Supel-Q PLOT fused-silica capillary (30m × 0.32 mm i.d., Supelco Inc., Bellefonte, CA); column temperature −30 to 160 °C (1-min hold at −30 °C, 10 °C/min from −30 to 160 °C); injection temperature 150 °C; detection temperature 260 °C; and helium flow rate 3 mL/min. The vapor samples were injected in the splitless mode, and the splitter was opened 1 min after completion of the injection.

MS Conditions. To identify endogenous cyanide in human whole blood, we used a quadrupole GC/MS system QP 5050A connected to a GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan). MS conditions were as follows: ionization current 60 μA; ion source temperature 200 °C; electron energy 70 eV; and accelerating voltage 1.5 kV. GC conditions were the same as described above. To avoid the interference by nitrogen (m/z 28) coexisting in the HS vapor, the measurements of m/z 27 ion were initiated 5 min after the injection of the HS vapor.

Comparison with the Previous HS-GC Method. To reproduce the previous HS-GC method, we followed the conditions reported by Seto et al.[20] using a GS-Q wide-bore capillary column (30 m × 0.53 mm i.d., J & W Scientific, Folsom, CA); we performed the analyses of cyanide in both split (1:3.7) and splitless modes. To a 0.5-mL volume of whole blood, 100 ng of cyanide was added and analyzed by both our method and the previous method for comparison. A 0.5-mL volume of HS was introduced into GC for the previous method.

Cyanide in Whole Blood of Actual Fire Victims and of Healthy Subjects. Whole blood or cerebrospinal fluid (CSF) samples were obtained from victims of actual fires at forensic autopsies or medical examinations. They were analyzed less than 1 h after the samplings. When immediate analyses were impossible, the samples were stored at −80 °C until analysis. Carboxyhemoglobin (COHb) was measured by the spectrophotometric method of Katsumata et al.[27] For measuring samples at high concentrations of cyanide, 0.5 mL of whole blood diluted 10-fold was used.

For measurements of endogenous cyanide in blood of healthy subjects, six healthy volunteers refrained from smoking for 24 h before samplings. Whole blood (~6 mL) was drawn and subjected to cyanide measurements immediately. In this kind of experiment, the amount of IS was 40 ng/vial; 0.1 g of ascorbic acid was added to the vial.

RESULTS AND DISCUSSION

Analytical Conditions. Various conditions for the HS extraction of cyanide and propionitrile from whole blood were tested. Heating at 70 °C gave more vapor pressure when compared to heating at 50 °C; this was advantageous to draw as much as 5 mL of HS vapor. We heated the vials at 70 °C for 5, 10, 15, and 30 min; peak areas of cyanide and propionitrile (IS) were almost constant after 15 min. Thus, we heated the vials at 70 °C for 15 min.

We have tested various initial oven temperatures for trapping cyanide and propionitrile vapor (Figure 1). At 20 °C, the peak of cyanide (filled arrows) was quite broad and became sharper upon lowering the oven temperature to −30 °C, while the lowering of initial oven temperature did not affect the peaks of IS (open arrows). At −40 °C, the peak area of cyanide was almost the same as those at −30 °C (data not shown). Thus, −30 °C was selected for trapping both compounds.

Identification of Endogenous Cyanide. Figure 2 shows GC profiles for nonextracted authentic cyanide and IS (12.5 and 10 ng on-column, respectively) dissolved in methyl acetate and for HS extracts from 0.5 mL of whole blood in the presence (250 ng

(21) Arthur, C. L.; Pawliszyn, J. *Anal. Chem.* **1990**, *62*, 2145–2148.
(22) Takekawa, K.; Oya, M.; Kido, A.; Suzuki, O. *Chromatographia* **1998**, *47*, 209–214.
(23) Watanabe, K.; Seno, H.; Ishii, A.; Suzuki, O.; Kumazawa, T. *Anal. Chem.* **1997**, *69*, 5178–5181.
(24) Versey, C. J.; Wilson, J. *J. Pharm. Pharmacol.* **1978**, *30*, 20–26.
(25) Toida, T.; Togawa, T.; Tanabe, S.; Imanari, T. *J. Chromatogr.* **1984**, *308*, 133–141.
(26) Seto, Y. *Arch. Biochem. Biophys.* **1995**, *321*, 245–254.
(27) Katsumata, Y.; Aoki, M.; Sato, K.; Oya, M.; Yada, S.; Suzuki, O. *Forensic Sci. Int.* **1981**, *18*, 175–179.

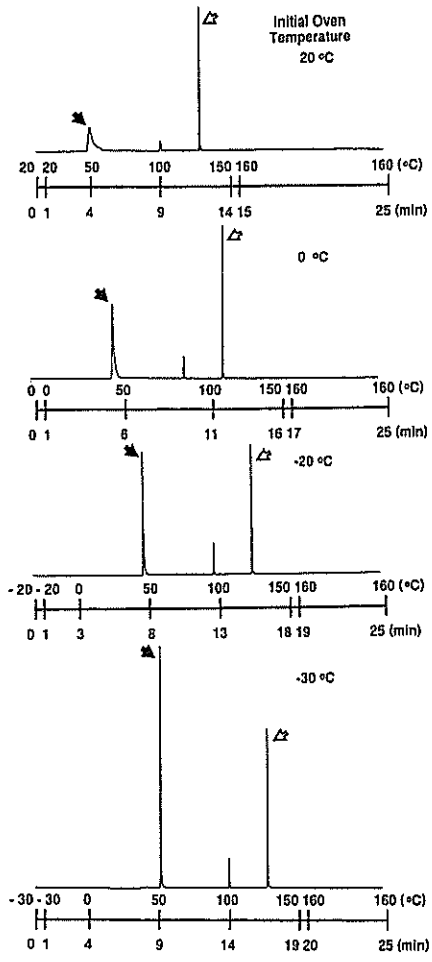

Figure 1. Headspace capillary GC for cyanide (filled arrow) and propionitrile (IS, open arrow) in whole blood as a function of various initial oven temperatures. To 0.5 mL of human whole blood were added 250 ng of cyanide and 400 ng of IS for HS extraction.

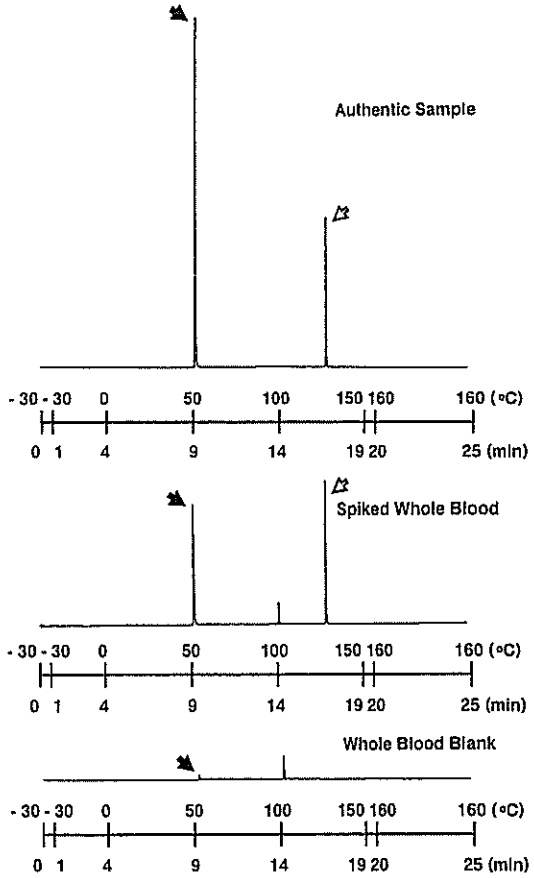

Figure 2. Capillary GC chromatograms with cryogenic oven trapping at −30 °C for 12.5 ng (in the form of CN^-) of the authentic cyanide (filled arrow) and 10 ng of IS (open arrow) dissolved in methyl acetate with direct injection (top panel), for whole blood spiked with 250 ng of cyanide and 400 ng IS (middle panel), and for whole blood in the absence of the compounds (bottom panel).

of CN^- and 400 ng of IS) and absence of the compounds. The retention times of cyanide and IS were 9.0 and 16.7 min, respectively. The backgrounds gave only one small impurity peak and a small peak appearing at the same retention time as that of cyanide. We thus tried to identify the latter small peak by GC/MS. We obtained selected ion monitoring (SIM) chromatograms with a channel at m/z 27 (HCN) for human whole blood samples spiked and not spiked with 100 ng of CN^-. A small but distinct peak at m/z 27 was found at the same retention time in the sample of the whole blood blank; it can therefore be concluded that the small peak found in the GC chromatogram (Figure 2) of the blank blood sample is due to endogenous cyanide.

Reliability of the Method. The extraction efficiencies of cyanide and IS were determined by external calibration. Peak areas of whole blood spiked with known amounts (50 or 250 ng for CN^- and 400 ng for IS) of the compounds (after cryogenic trapping of the HS prior to GC analysis) were compared with peak areas obtained by direct injection of the authentic compounds into GC. The extraction efficiencies for cyanide were 2.89 ± 0.34% (mean ± SD, n = 5, CV = 12%) at 250 ng/0.5 mL of blood and 3.22 ± 0.39% (n = 5, CV = 12%) at 50 ng/0.5 mL of blood. That for IS was 2.42 ± 0.37% (n = 10, CV = 15%).

A calibration curve for cyanide was drawn by plotting six different concentrations using 400 ng of IS. It gave good linearity in the range of 25−1000 ng/mL whole blood. The equation and r value for the curve were $y = 0.00280x - 0.0188$ and $r = 0.991$. Its detection limit giving a signal-to-noise ratio of 3 was estimated to be 2 ng/mL. In the previous reported methods, the detection limits were 25−135 ng/mL by colorimetric or fluorometric methods,[8-12] ~50 ng/mL by HS GC−NPD,[18,19] 5−50 ng/mL by GC-ECD,[13-15] ~100 ng/mL by GC/MS,[17] and ~20 ng/mL by GC−NPD using SPME,[22] which are much larger than our result.

To check reproducibility of the present method, we have added 250 ng of CN^- and 400 ng of IS to 0.5 mL of whole blood and determined them with each calibration curve. The coefficients of intraday and interday variations were 2.9 and 11.8%, respectively (n = 5 for each experiment).

We compared our own method with a reported method by HS-GC.[20] To a 0.5-mL volume of whole blood, 100 ng of CN^- was added and analyzed by the previous method. The peak height of cyanide by our method was ~15 times higher than that by the previous method with split injection; the peak area was ~30 times greater.

Cyanide in Actual Fire Victims. We measured cyanide of whole blood in actual fire cases to validate the present method. Table 1 shows the summary of the cases; COHb levels are also shown. The mean blood cyanide level was 687 ± 597 ng/mL

Table 1. Blood Cyanide Concentrations in Fire Victims

case no.	note	COHb (%)	cyanide (ng/mL)
1	death by fire with self-ignition using petrol	16	421
2	found after a fire of a house	56	2090
3	found after a fire of a house	23	44
4	found in a burnt-out car	34	625
5	found after a fire of a house	59	582
6	found after a fire of a house	79	641
7	found in the same house of case 6	11	561
8	death by fire with self-ignition using petrol inside a house	17	193 [32][a]
9	found in the next room in case 8	58	1030 [91][a]

[a] The numbers in the brackets represent cyanide concentrations in CSF.

(mean ± SD, $n = 9$). We could detect cyanide even in whole blood of victims who committed suicide by self-ignition using petrol (cases 1 and 8). In these cases, cyanide seems to have been produced when their clothing burned; the victims inspired CO and cyanide. We could also detect cyanide in the CSF of cases 8 and 9. We present typical chromatograms of whole blood and CSF of case 9 in Figure 3. Toxic and fatal ranges for whole blood cyanide are considered to be ≥0.1 and ≥1 μg/mL, respectively.[3] The cyanide levels in most cases (except cases 2 and 9) presented in Table 1 are within the toxic range.

Endogenous Cyanide Levels in Healthy Subjects. We could measure endogenous blood cyanide levels in healthy subjects because of the very high sensitivity of our method. Some previous papers suggested that a minor part of endogenous thiocyanate was converted to cyanide by acidification and/or heat [24–26] and this reaction was completely suppressed by the addition of ascorbic acid;[25,26] we thus added it in this system. The amounts of endogenous cyanide found in healthy subjects were 8.41 ± 3.09 ng/mL (mean ± SD, $n = 6$). Several papers had reported endogenous cyanide levels;[28–31] the values were 1–12,[31] 3.51 ± 2.16,[30] 16 ± 2,[28] and 78.3 ± 64.8 ng/mL.[29] For routine toxicological analysis of cyanide, however, the addition of ascorbic acid does not seems to be necessary, because the amounts of cyanide converted from thiocyanate are negligible as compared to the toxic levels of cyanide in blood samples.

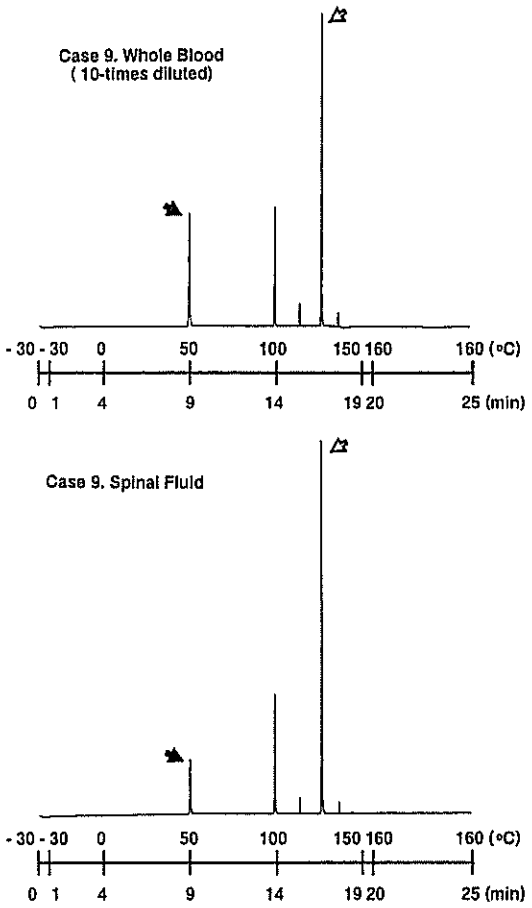

Figure 3. Typical chromatograms of whole blood and cerebrospinal fluid samples in an actual fire case (case 9). For whole blood, 0.5 mL of 10-fold diluted whole blood was used. As IS, 400 ng of propionitrile was added to each sample. Filled and open arrows show peaks of cyanide and IS, respectively.

CONCLUSION

To our knowledge, this is the first report for detecting cyanide from whole blood using GC with cryogenic oven trapping; this is the most sensitive GC method for cyanide measurement so far reported. The high sensitivity even enabled us to measure endogenous cyanide in whole blood. It is recommended for use in forensic and environmental toxicology, because it is simple, requires no special GC operations, and gives very low background noises in addition to such high sensitivity.

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(28) Ballantyne, B. *Clin. Toxicol.* **1977**, *11*, 173–193.
(29) Symington, I. S.; Anderson, R. A.; Thomson, I.; Oliver, J. S.; Harland, W. A.; Kerr, J. W. *Lancet* **1978**, (2), 91–92.
(30) Lundquist, P.; Rosling, H.; Sörbo, B. *Clin. Chem.* **1985**, *31*, 591–595.
(31) Uges, D. R. A. In *Analytical Toxicology for Clinical, Forensic and Pharmaceutical Chemists*; Brandenberger, H., Maes, R. A. A., Eds.; Walter de Gruyter; Berlin, 1997; p 710.