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TECHNICAL NOTE

Determination of cyanide in blood by electrospray ionization tandem mass spectrometry after direct injection of dicyanogold

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Abstract An electrospray ionization tandem mass spectrometric (ESI-MS-MS) method has been developed for the determination of cyanide (CN^-) in blood. Five microliters of blood was hemolyzed with 50 μL of water, then 5 μL of 1 M tetramethylammonium hydroxide solution was added to raise the pH of the hemolysate and to liberate CN^- from methemoglobin. CN^- was then reacted with $NaAuCl_4$ to produce dicyanogold, $Au(CN)_2^-$, that was extracted with 75 μL of methyl isobutyl ketone. Ten microliters of the extract was injected directly into an ESI-MS-MS instrument and quantification of CN^- was performed by selected reaction monitoring of the product ion CN^- at *m/z* 26, derived from the precursor ion $Au(CN)_2^-$ at *m/z* 249. CN^- could be measured in the quantification range of 2.60 to 260 μg/L with the limit of detection at 0.56 μg/L in blood. This method was applied to the analysis of clinical samples and the concentrations of CN^- in the blood were as follows: 7.13±2.41 μg/L for six healthy non-smokers, 3.08±1.12 μg/L for six CO gas victims, 730±867 μg for 21 house fire victims, and 3,030±97 μg/L for a victim who ingested NaCN. The increase of CN^- in the blood of a victim who ingested NaN_3 was confirmed using MS-MS for the first time, and the concentrations of CN^- in the blood, gastric content and urine were 78.5±5.5, 11.8±0.5, and 11.4±0.8 μg/L, respectively.

Keywords Cyanide · Blood · Gold · Electrospray ionization · Tandem mass spectrometry

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Introduction

Cyanide (CN^-) is a toxic agent that inhibits the activity of cytochrome oxidase. CN^- in blood provides direct evidence of its intake, and healthy, toxic and fatal levels of CN^- in blood were reported to be 4.42–26.0 μg/L [1–3], 1.30 mg/L and 2.60 mg/L [4–8], respectively. CN^- and/or hydrogen cyanide (HCN) are reported to be trapped mostly in red blood cells, where they are bound to methemoglobin (MetHb) [1, 2, 9], and therefore should be liberated into the aqueous solution after hemolysis. Various methods have been employed for the determination of CN^- in blood including colorimetry [1, 4, 5], gas chromatography (GC) [2, 7, 9], GC-mass spectrometry (MS) [3, 6] and liquid chromatography (LC)-MS [8]. All of these methods took more than 40 min to complete since they had to be applied after the evaporation of CN^- as HCN from the blood matrix by acidification [1–5, 7–9] or the derivatization of CN^- to organic molecules (which were colored in some cases) [1, 4–6, 8]. Furthermore, these methods were performed using large amounts of blood from 0.4 to 2 mL [1–9], and their MS detections comprised only a single MS for the confirmation of CN^- and its derivatives [3, 6, 8].

Recently, we developed an electrospray ionization tandem mass spectrometric (ESI-MS-MS) method for the determination of CN^- in gastric content and urine after its chelate complex formation with Au ion [10]. According to this method, CN^- levels in healthy control urine could be determined using only 10 μL of urine within 10 min. When this method was applied to analyze a 10-μL blood sample spiked with CN^-, however, the recovery rate was <5% even at toxic levels, i.e., 260 μg/L. The reasons behind the low recovery of CN^- may be the disappearance of CN^- after its binding to albumin [1, 11, 12], the disappearance of Au ion after its binding to unknown red cell components [13], and/or the occlusion of target ions such as CN^-, Au ion and Au $(CN)_2^-$ by large amounts of coagulated proteins that were produced after the mixing of blood with methyl isobutyl ketone (MIBK) [10], that was used for the extraction of Au $(CN)_2^-$ from the hemolysate. Therefore, the blood was diluted with 10 volumes of water.

The recovery rates of CN^- spiked into the diluted blood at 0.26–26.0 μg/L were about 90% of those spiked into 0.1 M tetramethylammonium hydroxide (TMAH) solution, respectively. Although large amounts of hemoglobin (Hb) and other components were still contained in the hemolysate, they neither appreciably interfered with $Au(CN)_2^-$ formation, nor MS-MS detection when Au $(CN)_2^-$ was extracted with MIBK from the hemolysate. Using only 5 μL of blood, CN^- levels from 2.60 to 260 μg/L could be determined within 10 min by the method presented in this study. This novel method is therefore the most rapid, sensitive, and conclusive method reported to date that is applicable to the determination of CN^- in the blood of healthy subjects, as well as intoxicated victims.

In the present work, the lower limit of quantification (LOQ) of CN^- in aqueous solution has been improved from 10^{-7} M (2.60 μg/L) in our previous work [10] to 10^{-8} M (0.260 μg/L) although the LOQ in blood is 2.60 μg/L. The following treatments have contributed to improve the sensitivity ten times. That is, the concentration of Au^{3+} has been lowered because they act not only as chelating ions but also oxidizing agents against CN^-. The centrifugal force at the extraction of the chelate complex with MIBK has been weakened to the lowest limit because the extraction may be performed most efficiently when the complex has no electric charge and hence precipitates easily. The LOQs reported for toxic negative ions such as SCN^-, N_3^-, and S^{2-} were rather high concentrations as 10^{-5} M for SCN^- [6], 2×10^{-6}–10^{-5} M for N_3^- [14, 15] and 2×10^{-4} M for S^{2-} [6] since they are easily oxidized and/or reduced as well as they are resistant to be electrosprayed like CN^-. The above treatments in the detection of CN^-, therefore, may be applicable to improve the sensitivity in the detection of other weak negative ions.

Materials and methods

Materials

MIBK of atomic absorption grade and other chemicals of analytical grade were obtained from Wako Pure Chemicals, Osaka, Japan. Pure water with a specific resistance of 18 MΩcm was used (Millipore, Bedford, MA, USA).

Control blood samples were collected by venipuncture into EDTA-containing vacuum containers from healthy non-smokers (n=6) under permission in January 2010, and some of the control blood samples were separated into three portions: whole blood, plasma and red cells washed with saline. These samples were placed into tubes with lids (Eppendorf AG, Hamburg, Germany) and stored at −20°C. Heart blood samples were collected at autopsy from suicide victims (n=6) who had inhaled CO gas from December 2009–April 2010, from house fire victims (n=21) from September 9, 2009–September 6, 2010, from a suicide victim who ingested NaCN in January 2009, respectively, and blood, gastric content and urine were collected at autopsy from a suicide victim who ingested NaN_3 in September 2010, and these samples were also stored at −20°C.

Standard solutions

The 1 M CN^- stock solution was prepared by dissolving potassium cyanide into water and stored at −20°C. The concentration of the stock solution was determined using the pyridine–pyrazolone method [4]. The standard CN^- solutions were prepared just prior to use by diluting the stock solution with 0.01 M TMAH solution and they were spiked into blood samples that had been hemolyzed and alkalized.

Assay procedures

To 5 μL of blood placed in a tube (Eppendorf AG) using a MICROMAN M10 (Gilson S.A.S., Villiers-le-Bel, France), 50 μL of water was added to hemolyze the blood. Five microliters of 1 M TMAH solution was added and mixed for 1 s, and 1 μL of standard CN^- solution was added when necessary. After 30 s, 2 μL of 10^{-4} M $NaAuCl_4$ solution and 75 μL of MIBK were added and mixed together for 20 s using a vortex-mixer. After centrifugation at 100×g for 10 s, the upper MIBK layer was placed into a new tube for auto sampler and used for the detection of $Au(CN)_2^-$.

Instruments

ESI-MS-MS was performed using a TSQ 7000 LC-quadrupole mass spectrometer (Thermo Quest, Japan) in the negative ion mode. Methanol was used as the mobile

phase at a flow rate of 300 μL/min and the capillary temperature was set at 230°C. The electrospray voltage was set at 4.5 kV, the multiplier voltage at 1.3 kV and the collision voltage at 35 V. Nitrogen was used as the sheath gas (469 kPa) and also as an auxiliary gas (8 units), and argon was used as the collision gas (134 kPa). Ten microliters of the MIBK sample layer was injected with time interval of 1.5 min using an auto sampler (Agilent 1100 series, Waldbronn, Germany) into the ESI-MS-MS apparatus via direct injection. ESI-MS-MS quantification was performed by the integration of the peak area of the product ion at *m*/*z* 26.1±0.2, derived from the precursor ion at *m*/*z* 248.9±0.3, using a calibration curve constructed from spiked blood samples at different concentrations.

Results and discussion

In this study, "blank" is defined as the signal that derived from blood without the addition of Au ions, since even control blood from healthy subjects contains small amounts of endogenous CN^- [1–3] and shows signals corresponding to $Au(CN)_2^-$ when Au ions are added.

Suitable conditions for the production and extraction of $Au(CN)_2^-$ in blood hemolysates

Dilution of blood Prior to the detection of CN^-, hemolyzation is necessary since CN^- is contained predominantly in red blood cells [1, 2, 11, 16]. The dilution of blood is also necessary since large amounts of coagulated proteins produced after the addition of MIBK occlude the target ions such as CN^-, Au ion and $Au(CN)_2^-$, and the recovery from undiluted blood spiked with CN^- at 260 μg/L was <5% [10]. To determine a suitable dilution, the recoveries from some diluted bloods were examined as follows: diluted blood samples were assayed after the addition of 5 μL of TMAH solution, 1 μL of 1.30 mg/L CN^- solution, 2 μL of 10^{-4} M Au ions and 75 μL of MIBK, respectively. The recoveries of fourfold-diluted blood, sixfold-diluted blood, and 11-fold-diluted blood were 30%, 60%, and 90% that of CN^- spiked into 55 μL of 0.1 M TMAH solution, respectively. The 11-fold dilution and the extraction with 75 μL of MIBK were adopted in the following assay, since the quantification of endogenous CN^- in control blood became difficult when a higher dilution, or extraction with a larger volume of MIBK, was employed.

Concentration of TMAH It is difficult to detect CN^- in 11-fold-diluted blood added with TMAH at a concentration of 0.01 M, although 0.01 M TMAH aqueous solution was the best solution in which to detect CN^-. Raising the pH of the hemolysate may be necessary to liberate CN^- from MetHb. The maximum recovery was obtained by the addition of 5 μL of 1 M TMAH solution to 55 μL of 11-fold-diluted blood.

Concentration of Au ion and condition at centrifugation To detect CN^- levels in control blood samples, i.e., concentrations of 4.42–26.0 μg/L [1–3], the LOQ should be in the order of 0.4 μg/L since blood was diluted to 11-fold in the present method. In our previous report, the quantification range was from 2.60 μg/L to 1.30 mg/L CN^-, where the concentration linearity deviated at <2.60 μg/L CN^- [10].

The following two treatments were found to be suitable for the sensitive detection at <2.60 μg/L CN^-. Firstly, the concentration of Au ion in the reacting solution has been lowered from 10^{-3} M in the previous work [10] to 4×10^{-6} M in the present work since extra larger amounts of Au^{3+} may have oxidized some portions of CN^-. $NaAuCl_4$ at 4×10^{-6} M was found to be sufficient for the detection of CN^- at the highest concentration, 10^{-6} M (26.0 μg/L) in the diluted blood, and hence 2 μL of 10^{-4} M $NaAuCl_4$ solution was added to 55 μL of 11-fold-diluted blood in the following assay. Secondly, the centrifugal force at the separation of MIBK from the aqueous solution has been lowered from 5,000×*g* for 30 s to 100×*g* for 10 s, i.e., the lowest limit that can separate MIBK from the aqueous solution. The extraction of the chelate complex, $MAu(CN)_2$ (here, M is an unidentified cation) from the aqueous solution with an organic solvent, MIBK may be performed most efficiently when $MAu(CN)_2$ has no electric charge. On the other hand, $MAu(CN)_2$ with no charge is easily precipitated in aqueous solution. This may be the reason why the lowering of the centrifugal force has improved the detection.

Solvent Isoamyl alcohol and octanol dissolved large amounts of blood components when the blood hemolysates were denatured in 0.1 M TMAH solution, and hence only MIBK could be used as the extraction solvent for $Au(CN)_2^-$ from the hemolysate, although the extraction efficiencies of $Au(CN)_2^-$ from 0.01 M TMAH solution with MIBK, isoamyl alcohol and octanol were 100:80:60 [10].

Interferences

Albumin When CN^- was spiked into the control blood and control plasma at 260 μg/L and stored at 4°C for 1 day, the detected signals were >95% and <1%, respectively, that of the signal of CN^- that was spiked into the control blood just before the assay. One of the reasons for this may be the gradual irreversible binding of CN^- to albumin, as previously suggested [1, 11, 12], and the preferential binding in alkaline solution [12]. We observed that albumin did not interfere with the detection of CN^- when it was contained together with red blood cells in a solution at neutral pH, as previously reported

[12]. These results may indicate that the binding of CN^- to MetHb is much faster than the binding to albumin [1, 11, 12]. The conversion of CN^- to OCN^- and the modification of proteins by carbamylation have also been suggested to occur in alkaline solution [11]. In the present assay, the recovery of CN^- decreased appreciably with time in alkaline solution produced by the addition of TMAH, although CN^- bound to MetHb was stable at neutral pH. The addition of Au ions as well as MIBK, 30 s after the addition of TMAH is therefore recommended.

In the present assay, however, MetHb may be denatured and may liberate CN^- completely within 30 s in alkaline solution where chelate complex formation occurs, as demonstrated by the complete formation of $Au(CN)_2^-$ in hemolysate spiked with CN^- at a concentration of 0.26–26.0 μg/L. The concentration of $Au(CN)_2^-$ in MIBK was maintained >95% for more than 3 h after the extraction from the alkaline hemolysate at ambient temperature.

Thiocyanate (SCN^-) The endogenous SCN^- level in control plasma was 3.3×10^{-5} M [2]. Some previous studies have reported the production of CN^- from SCN^- by large amounts of oxyhemoglobin and/or other components [1–3, 7, 9, 16], whereas other studies refute this [11]. In the present assay, an increase in CN^- was not observed even though SCN^- was added to control blood at ten times the endogenous SCN^- level. One of the reasons for this may be our assay conditions, as the assay was performed at room temperature and pH>7. The production of CN^- from SCN^- has previously been reported to be maximal at 50°C and pH 4–4.5 [9, 16], adjusted by the addition of acids such as acetic acid, phosphoric acid and trichloroacetic acid [9, 16], whereas the conversion was not observed at room temperature and pH>7 [9, 16].

Ion suppression In ESI-MS-MS detection the signal of the target ions decreases due to ion suppression by impurities although they do not give signals. That is, the signals of CN^- at 0.26–26.0 μg/L in 0.1 M TMAH solution containing 0.15 M NaCl were about 90% of those in 0.1 M TMAH solution, respectively, although the concentration linearity was maintained in both solutions. The recovery rates of CN^- spiked at 0.26, 2.60, and 26.0 μg/L in 11-fold-diluted blood were about 90% of those spiked in 0.1 M TMAH solution, respectively. That is, 11-fold-diluted blood components interfere neither the production nor the extraction of $Au(CN)_2^-$ but suppress the signal in ESI-MS-MS under the present method.

MS-MS spectra

According to our previous work, the suitable precursor ion, product ion and collision voltage for the detection were: Au $(CN)_2^-$ at *m/z* 249, CN^- at *m/z* 26 and 35 V, respectively [10]. The MS-MS spectra are shown in Fig. 1a for control blood, and **b**, for control blood spiked with CN^- at a concentration of 260 μg/L. The blank did not exhibit the signals at *m/z* 249 and *m/z* 26.

Precision and accuracy

Calibration of standard blood samples were prepared by spiking standard solutions of CN^- to 11-fold-diluted control blood to set the concentrations of CN^- spiked in undiluted blood to be 0, 2.60, 26.0, and 260 μg/L, respectively. The MS-MS product ions from these samples were shown in Fig. 2b and c. Blood containing no added Au ions was considered as a blank and shown in Fig. 2a. Concentrations determined from the peak area (*y*) were linear to the spiked concentrations (*x* micrograms per liter in undiluted blood) up to 260 μg/L (i.e., $y=12.3\ x+35.1$), with a correlation coefficient of 0.9999. Here, the value calculated from the *y*-intercept divided by the *slope*, 35.1/12.3=2.85, indicates the endogenous CN^- level (in micrograms per liter) in the blood used for the calibration, and the CN^- level of a sample can be calculated in a similar way.

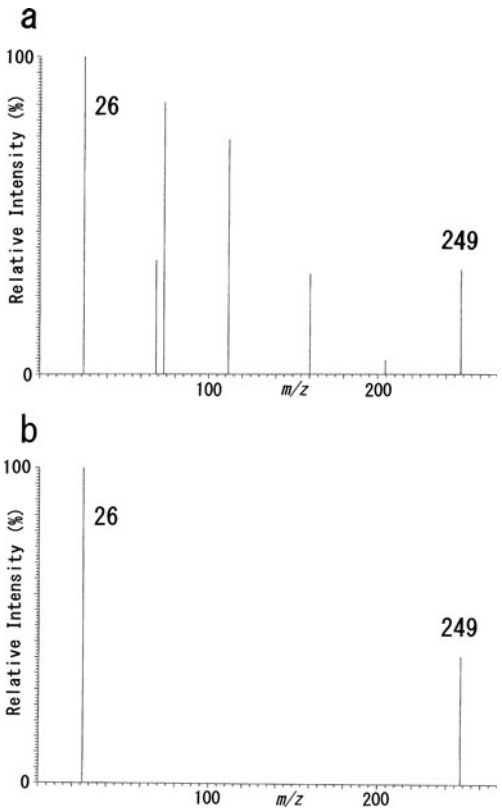

Fig. 1 Product ion spectra at a collision voltage of 35 V from the precursor ion $Au(CN)_2^-$ at *m/z* 249 extracted: **a** from a control blood having endogenous CN^- at 6.61 μg, and **b** from the control blood spiked with CN^- at 260 μg/L

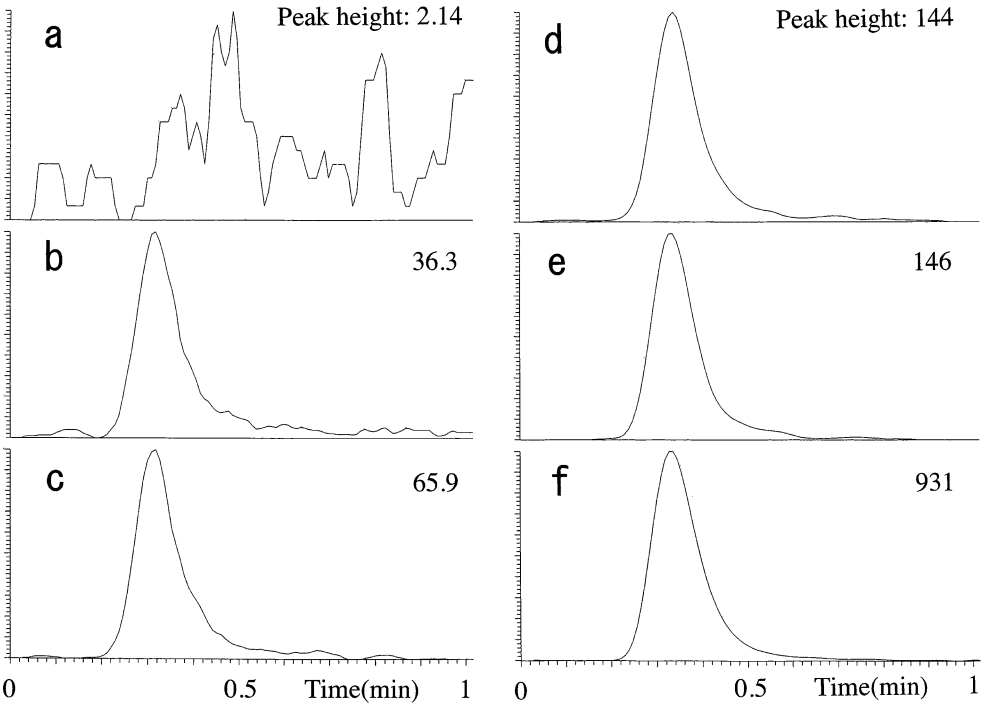

Fig. 2 The amounts of the product ion, CN^- at *m/z* 26, after direct injection of methyl isobutyl ketone layer extracted: **a** from blank, **b** from a control blood having endogenous CN^- at 2.70 μg, **c** from the control blood spiked with CN^- at 2.60 μg/L, respectively, and **d**, **e**, and **f** were from the urine, gastric content, and blood, respectively, of a victim who ingested NaN_3. The height of each peak is indicated at the *right* and the height of the maximum noise is indicated in blank since it is a control blood to which Au ions were not added

Precision and accuracy were assessed by the analysis of diluted control blood samples spiked with CN^- at 2.60, 26.0, and 260 μg/L in undiluted blood, respectively (Table 1). These samples were analyzed three times a day, as well as on three different days. The coefficient of variation was <10.8% and the accuracy was 94.2–103.8% for intra- and inter-day variations. The LOQ of this method was therefore 2.60 μg/L for undiluted blood.

The lower limit of detection (LOD) based on a concentration giving a signal three times the back ground noise [6] was 0.56 μg/L from the calculation of 2.60×3×2.14/29.6=0.56, since the height of noise level was 2.14 as shown in Fig. 2a and the increment of the peak height after the addition of CN^- at 2.60 μg/L in undiluted blood was 29.6 from the calculation of 65.9–36.3=29.6, as shown in Fig. 2c and b.

Determination of CN^- in control blood samples and victim's samples

Samples were divided into five groups: the blood samples from healthy non-smokers (n=6), those from suicide victims who inhaled CO gas (n=6), those from house fire victims (n=21), the blood from a suicide victim who ingested NaCN (n=1), and the blood, gastric content, and urine from a suicide victim who ingested NaN_3 (n=1).

The percentage of carboxyhemoglobin (COHb) was measured in the blood of CO gas victims and house fire victims at autopsy using a CO-oximeter.

The apparent postmortem alcohol level was measured in the blood of all the victims at autopsy using a GC. The vitreous humor was not collected although its alcohol level is less affected by the postmortem production of alcohol and the diffusion of alcohol from gastric content. Only four fire victims showed a low blood alcohol level among all the victims autopsied.

Although the levels of COHb, alcohol, and CN^- in the blood of the autopsied victims were determined using the blood from the left and right ventricles, the mean values were adopted since the differences between the two samples were <10%. The quantification of CN^- and more precise data for house fire victims are listed in Table 2, where burns are graded from 1 to 4°.

The concentrations of CN^- in the blood samples were as follows: the values varied from 2.70±0.19 to 10.20±0.53 μg/L with the mean, 7.13±2.41 μg/L for control subjects; from 1.68±0.26 to 5.14±0.34 μg/L with the

Table 1 Intra-day (three times) and inter-day (3 days) accuracy/precision for CN^- spiked into control blood

CN^- spiked (μg/L)	Intra-day		Inter-day	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
2.60	103.5	10.8	94.2	2.4
26.0	103.8	6.8	100.4	2.7
260.	99.2	3.6	101.9	6.9

Table 2 Postmortem findings in house fire victims in 2009 September–2010 September

Case	Age (years)	Sex	Burns	PI (days)	COHb (%)	CN^- μg/L
1	78	F	3–4	5.5	68.2	86.2±1.7
2	76	F	2–3	2.5	19.4	111±4
3	67	M	2–3	2.5	80.7	184±11
4	46	M	3	1.5	76.5	3,100±18
5	58	M	3	1.5	57.0	697±87
6	65	M	3	2.5	53.2	1,193±38
7	59	M	3–4	6.0	60.0	816±78
8	48	M	4	6.5	55.0	270±1
9	90	F	3	2.5	5.2	172±11
10	27	M	4	4.0	65.0	900±39
11	53	M	2–3	3.5	62.2	613±27
12	48	F	2–3	2.5	82.1	595±11
13	49	M	3	2.5	5.0	270±4
14	79	M	4	7.0	50.7	12.3±0.2
15	95	M	4	7.0	52.3	5.97±0.17
16	80	M	3–4	7.0	70.0	424±1
17	68	M	4	1.5	32.5	2,500±6
18	77	M	3	4.5	60.0	2,510±42
19	75	F	3	4.5	42.5	257±7
20	71	M	3–4	2.0	75.0	400±15
21	65	F	2–3	3.0	74.0	204±4

PI postmortem interval after fire

Blood ethanol (mg/mL): Case 3 (0.6), Case 4 (0.2), Case 16 (0.1), Case 20 (0.6), others (0)

mean, 3.08±1.12 μg/L for CO gas victims; from 5.97±0.17 to 3,100±18 μg/L with the mean, 730±867 μg/L for house fire victims; 3,030±97 μg/L for the victim who ingested NaCN, respectively. The concentrations of CN^- in the blood, gastric content and urine were 78.5±5.5, 11.8±0.5, and 11.4±0.7 μg/L, respectively, for the victim who ingested NaN_3.

All of the suicide victims inhaled CO gas that was produced from incomplete combustion of so-called Renntann, a charcoal briquet, in a closed car (n=3) or in a closed room (n=3). Renntann is sometimes used in domestic outer heaters in Japan and is known not to produce appreciable amounts of N compounds such as CN^-. That is the reason why the concentrations of CN^- in these victims were similar to those recorded in the control subjects. The concentration of COHb in all of these victims were >60%.

Fire victims included in this study were limited to house fire victims, and all of them showed apparent tracheal soot. Blood alcohol was observed in only four of the cases (3, 4, 16, and 20) and all of these levels were ~0.6 mg/mL, indicating a weak alcohol intoxication and/or merely its postmortem production and diffusion. The concentrations of CN^- in the 21 house fire victims varied from that of the control to 3,100 μg/L, a fatal concentration. The concentration of COHb in three of the cases (2, 9, and 13) was <20%, but the concentration of CN^- in these cases was >10 times that of the control. The concentration of CN^- in two of the cases (14 and 15) was the same as that of the control, but the concentration of COHb was >50%, a fatal concentration. Although the concentrations of CN^- and COHb decrease with time [1, 11], even the blood examined several days after the fire showed sufficiently high levels to suggest the cause of death.

The blood of a suicide victim who ingested NaN_3 was also examined in the present work since the conversion of N_3^- to CN^- in the blood of victims intoxicated with NaN_3 was disputed between two works [14, 15]. NaN_3 is a toxic substance that inhibits cytochrome oxidase like CN^-, but it also exerts a hypotensive action. The MS-MS product ions from the urine, gastric content, and blood were shown in Fig. 2d, e, and f, respectively. The MS-MS spectra of those samples from the NaN_3 victim showed both two signals at m/z 26 and m/z 249. The CN^- level in the victim's blood was 11 times that in the control blood, and that in the victim's urine was comparable to that in the control urine [10], respectively. The CN^- level in the victim's blood was seven times higher than that in the gastric content although the N_3^- level in the blood was lower than 1% of that in the gastric content, respectively, indicating that CN^- in the victim's blood may be produced by some metabolic pathway.

Comparison of the methods

Detection of CN^- in the present method can be performed using as little as 5 μL of blood within 10 min, whereas

previous methods were performed using 0.4–2 mL of blood and took at least 40 min to complete [1–9]. The present LOD, 0.56 μg/L, was lower than the lowest value, 2 μg/L [7], among the values previously reported [1, 3, 6–8].

For the detection of strongly hydrophilic ions using our electrospray MS-MS method [10, 17–20], it is necessary to choose a suitable buffer, chelating agent, and organic solvent. As shown here, MIBK can be used for the extraction of $Au(CN)_2^-$ from hemolysate denatured in strong alkaline solution, but alcohol cannot be used for this purpose. The extraction of $Au(CN)_2^-$ with MIBK from hemolysate saved time (ca. 30 min) that was previously required for the vaporization of HCN from the blood [1–5, 7–9]. MS-MS detection [10, 17–20] can afford not only more robust data by detecting *m*/*z* values twice but also more improved sensitivity by eliminating once again the interfering ions that might be still contained in a single MS [3, 6, 8]. That is, MS-MS offers effective purification of the target ion within a rapid time scale.

Conclusion

Forensic drug analysis often starts from specifying the fatal drug. According to the "Manual for Forensic Analysis of the Japanese Society of Legal Medicine" issued in 1999 [21], the screening of drugs as well as the quantification of the fatal drug should be made using only half of the forensic specimen and the other half should be left for a future diagnosis. There are many drugs to be screened as soon as possible, and the screening of cyanide is essential since cyanide poisonings are often encountered in forensic relevance. The present method can afford the most sensitive, rapid and decisive diagnosis of cyanide poisoning so far reported.

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