Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. *86* (1) 77–90 (2021)
JSCS–5405
Original scientific paper

Headspace gas chromatography–mass spectrometry method for the determination of total cyanide concentration in water and *post-mortem* blood samples

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(Received 22 April, revised 2 July, accepted 27 September 2020)

Abstract: In this study, we aimed to develop a headspace gas chromatography––mass spectrometry method for determining the total cyanide concentration in the forensic evidences. Total cyanide content of the samples was calculated based on the hydrogen cyanide gas concentration evaporated from the liquid sample in the headspace vial. Hexacyanoferrate(II) was used for the optimization of headspace oven temperature. We have found that iron–cyanide bonds were completely degraded after 0.2 mL of the sample was treated with 1 mL of 1 M sulfuric acid under the optimized headspace conditions where the temperature and the heating time were 120 °C and 12.5 min, respectively. Satisfactory recovery results for both aqueous and blood samples were obtained. The method was linear in the range 0.05–10 μg mL^{-1} of cyanide which was a suitable range for toxicological investigations. The proposed method was validated and applied to the *post-mortem* blood samples, drinking waters, and the other forensic evidences. The proposed method can easily be performed not only in the forensic laboratories, but in the related laboratories where the total cyanide analysis is a critical issue.

Keywords: analytical toxicology; cyanide poisoning; evidence; forensic chemistry.

INTRODUCTION

Cyanides are the potent toxic agent for humans, animals and aquatic life. Hydrogen cyanide (HCN), which is a weak acid (pK_a 9.2), and CN^- can interconvert based on temperature and pH.[1] HCN is a colorless gas, and it has an almond-like odor, but half of the population is unable to smell it.[1,2] Acute toxic effect of cyanide mainly stems from inhibiting the final stage of the oxidative

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https://doi.org/10.2298/JSC200422063D

phosphorylation, which is essential for the aerobic cell respiration, by ligating to the haem group of the cytochrome c oxidase enzyme (EC 1.9.3.1).[3] In addition to cytochrome c oxidase, it disrupts activities of the many metalloenzymes such as catalase – Fe(III) (EC 1.11.1.6), iron–sulfur protein succinate dehydrogenase (EC 1.3.5.1), superoxide dismutase – Cu, – Zn (EC 1.15.1.1), carbonic anhydrase – Zn (EC 4.2.1.1), alkaline phosphatase – Zn (EC 3.1.3.1), alcohol dehydrogenase – Zn (EC 1.1.1.1), xanthine oxidase – Mo (EC 1.17.3.2), xanthine dehydrogenase – Mo (EC 1.17.1.4), aldehyde oxidase – Mo (EC 1.2.3.1), sulfite oxidase – Mo (EC 1.8.3.1) and glutathione peroxidase – Se (EC 1.11.1.9).[3,4] Moreover, it leads to the production of cyanohydrins by binding to carbonyl groups in the center of some enzymes.[3]

The most poisonous cyanide compounds are the hydrogen cyanide gas, the water-soluble salts of cyanide (sodium cyanide; potassium cyanide), and the weak acid dissociable (WAD) cyanide complexes of zinc, nickel, copper and cadmium. Whether voluntarily (committing suicide) or involuntarily (accidentally or fire exposure) ingestion of these cyanide solids or inhalation of HCN most likely results in death when the concentration of cyanide in the blood reaches about 4–5 mg L^{-1}.[2,5–7] 98 % of the total CN^- concentration in blood is distributed to erythrocytes (also called red blood cells) containing hemoglobin while the 2 % part of it could be present in the plasma either in the free form or bound to methemoglobin protein, the ferric form of hemoglobin.[2,8] It was reported that about 100 mg of HCN or 300 mg of KCN intake are the lethal doses for the humans.[2]

In nature, the primary source of CN^- comes from anthropogenic activities such as synthetic fibers, resins, herbicides, electroplating, mining, metal finishing, steel, petroleum and chemical industries, and gold extraction processes. Cyanide intoxication from HCN inhalation commonly arises from tobacco smokes and pyrolysis of nitrogen-containing polymers such as melamine, nylon, polyurethanes, polyamides, wool and silk. On the other hand, cyanide compounds are found in almonds, millet sprouts, lima beans, soy, spinach, bamboo shoots, sorghum and cassava roots. Due to its military use, authorities also identify HCN as a chemical terrorist agent.[1,4,9,10] In *post-mortem* blood samples, cyanide concentration can reach to 20 mg L^{-1} in the suicide cases or industrial exposure while it exceeds 1 mg L^{-1} in fire victims.[2] Although the concentration of cyanide seems to be low, the reason why the fire victims die is carbon monoxide and cyanide exhibiting synergistic effects.[4]

The toxicity of cyanide species differs depending on whether free cyanide or HCN is easily formed or not in the physiological conditions. Therefore, simple cyanide salts and WAD cyanide complexes are the most hazardous compounds, whereas strong acid dissociable (SAD) cyanide complexes like hexacyanoferrate(II,III) are the less toxic species. To degrade the metal–cyanide bonds of the

SAD complexes, a process needs more heavier conditions such as UV radiation, higher temperatures, and strong acids.[5,7,11]

Many methods have been developed for the determination of the cyanide species in different types of samples including ion chromatography (IC),[6,7] capillary electrophoresis (CE),[12,13] headspace gas chromatography (HS-GC),[14] GC–mass spectrometry (MS),[15–18] electrochemicalsensors,[8,19–21] headspace (HS)-single-drop microextraction (SDME)-NanoDrop® microspectrophotometry (ND),[11] HS-GC-atomic emission detector (AED),[22] HS-GC-electron capture detector (ECD),[23] HS-GC-ECD/photoionization detection (PID),[24] HS-GC-nitrogen–phosphorus detector (HS-GC-NPD),[25–30] HS-solid-phase microextraction (HS-SPME)-NPD,[31] HS-SPME-CE,[32] HS-GC–MS[9,15,33,34] and HS-SPME-GC–MS.[35]

Cyanide poisoning, which is an important issue in forensic sciences, is often caused by exposure to cyanide-containing compounds in committing suicide, homicide attempt, and accidental digestion cases. According to the Official Gazette of the Republic of Turkey, cyanide sales to the public have been banned because the number of cases of cyanide suicides increased in the past years.[36] The available cyanides (free and WAD species) rapidly act in the body, whereas the SAD species could not lead to death. Therefore, death does not occur in some cases where people who do not have any idea about toxic effect of the SAD species intentionally use these cyanides. Accordingly, even if someone else does not die, the suspect may be punished for attempting homicide.

Thus, a reliable method for the determination of total cyanide concentration in postmortem blood samples, environmental samples, and any evidence have vital importance, especially for forensic science. In this paper, as can be seen, we aimed to present an HS-GC–MS method for the total cyanide analysis in both postmortem blood samples and aqueous solutions. We carried out the method optimizations by using hexacyanoferrate(II) ($[Fe(CN)_6]^{4-}$) as SAD complex, and the temperature values were checked by calculating recovery values instead of optimizing HS conditions only by vapor pressure equilibrium.[9,33,34] Though the proposed method is highly accurate, it does not give any information relating to the cyanide species. Consequently, because the proposed method is fast (no derivatization) and reliable, it is very suitable for the related laboratories.

EXPERIMENTAL

Reagents

1000 mg L^{-1} of certified cyanide standard solution (CL01.0371.0100) was purchased from Chem-Lab (Zedelgem, Belgium). Aqueous certified reference material (CRM) of cyanide (lot: LRAA3393) was obtained from Sigma–Aldrich. Potassium hexacyanoferrate(II) trihydrate ($K_4[Fe(CN)_6]\cdot 3H_2O$, 99.5 %) was procured from Carlo Erba Reagents (Val de Reuil Cedex, France). Sulfuric acid (95–97 %) for analysis Emsure® ISO was supplied by Merck.

Ultrapure water was acquired from a New Human Power I Scholar UV system (Human Corporation, Seoul, South Korea).

Instrumentation

We carried out the analysis of cyanide by utilizing a Perkin Elmer Clarus 680 gas chromatograph equipped with a Clarus SQ 8 T mass spectrometer and HS40 headspace (HS) autosampler. A Perkin Elmer Elite – free fatty acid phase (FFAP) GC column providing appropriate separation of acidic compounds with a crossbond carbowax-PEG structure was used. Dimensions of the column were 30 m long, 0.25 mm i.d. and 0.5 μm df. The constant flow rate of the carrier gas (He) was adjusted at 1 mL min^{-1} with an HS pressure of 30 psi.

HCN gas liberated from all of the possible cyanide species by keeping the vials in the HS oven set at 120 °C for 13 min. The HS needle temperature and the transfer line temperatures were 130 and 150 °C, respectively. The authors used a stainless-steel needle jet to achieve minimum carryover from the adsorption of cyanide on the needle surface.[33] After the incubation time finished, pressure was the applied to the vial by the needle for 1.0 min, and then the gas sample was injected for 0.1 min (loopless). HCN was successfully separated from the other peaks on the column by the optimized GC oven temperature program which was set initially 40 °C for 7 min, then ramped at 25 °C min^{-1} to 220 °C, and finally, held at 220 °C for 2 min. The equilibration time of the GC oven was 30 s. GC injector temperature was 200 °C, total analysis time was 16.20 min.

Electron energy was 70 eV of the EI+ source. Mass detection was performed at 200 °C. The authors utilized a TurboMass (version 6.1.0.1963) software for data acquisition and instrumental control for GC and MS, while the headspace autosampler was controlled by computer using PerkinElmer HS Driver v2.5.0.0125 software. The software simultaneously collected the data by both the full scan between 12–150 atomic mass units (amu) for identification of the peaks during 5-8 min and the selected-ion recording (SIR) of *m*/*z* 27 ($^{1}H^{12}C^{14}N^{+}$) for quantitative analysis at a dwell time of 40 ms between 5-7.5 min. Solvent delay was set for the first 5 min. We measured the concentration of HCN in the samples using a standard calibration curve prepared by plotting the peak area against the designated concentrations (0.05-10 μg mL^{-1}).

Preparation of the $[Fe(CN)_6]^{4-}$ solutions

To prepare the stock solution of $[Fe(CN)_6]^{4-}$ in which the CN^- equivalent concentration was 100 mg L^{-1}, 27.1 mg of $K_4[Fe(CN)_6]\cdot 3H_2O$ was dissolved in a 100 mL volumetric flask. Then, we diluted this solution to obtain an aqueous $[Fe(CN)_6]^{4-}$ solution to be equivalent to 5 mg L^{-1} of CN^-. Similarly, we spiked the blood samples from the blood stocks with same concentration of $[Fe(CN)_6]^{4-}$.

Samples and sample preparation

The *post-mortem* blood samples were sent to our laboratory after the autopsies conducted at the Council of Forensic Medicine (ATK). We carried out this study by the permission of ATK Chairmanship, Education and Scientific Research Commission (decision number: 2018/737; date: September 18, 2018). The informed consent was waived. We pledged to comply with all ethical rules during the application. The authors confirm that this research was conducted according to the principles expressed in the Declaration of Helsinki.

The authors prepared the standard solutions and the samples as follows: we added 0.2 mL of the sample solution into 22 mL headspace vial, which was in a tube holder placed on an icebox with an appropriate size. Then, 1 mL of 1 M H_2SO_4 was added above the cold sample. After we instantly sealed the vial with a gas-tight polytetrafluoroethylene (PTFE)-lined rubber

septum cap, it was vortexed for 1 min. Finally, we loaded the vials to the HS autosampler for analyzing.

RESULTS AND DISCUSSION

Optimization of the HS temperature

In the first experiments, we calculated the recoveries from the aqueous iron cyanide complex solutions. By this means, we prepared a series of solutions with the same concentration: 200 μL of the $[Fe(CN)_6]^{4-}$ of which CN^- concentration was equivalent to 5 mg L^{-1} and 200 μL 1 M H_2SO_4 solutions were added into a cold 22 mL headspace vial, respectively. After immediate sealing, each vial was vortexed. The solutions were analyzed after 20 min of incubation at 80, 90, 100, 110, 120 and 130 °C. Three of the solutions containing the complex and three standard solutions of 5 mg L^{-1} CN^- were analyzed in parallel for each temperature point. The recoveries of the parallel measurements were calculated. CN^- have been measured until a plateau was observed. As seen in Fig. 1, 120 °C was found to be the adequate temperature with 102 % recovery to completely degrade the Fe–CN bonds. Therefore, all subsequent experiments were carried out at 120 °C. It is noteworthy that the available cyanide species could be measured with good recoveries at the lower temperatures, but this does not allow accurate analyzing of the SAD species in the complex matrices possessing transition metals. Furthermore, approximately 10 % recovery values were obtained when the temperatures were exerted between 80–90 °C while 50 % recovery was calculated at 100 °C. These recovery values indicated not only that merely controlling the vapor pressure equilibrium was unsufficient, but they could contribute to finding positive cyanide concentrations when the available cyanides were analyzed in complex matrices as well. Therefore, it should be taken into account not to be given misleading results for both SAD and WAD cyanide species.

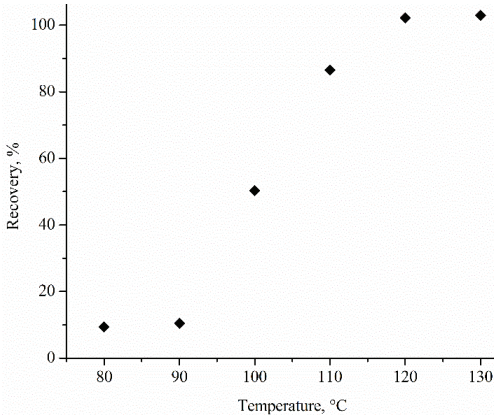

Fig. 1. Effect of HS oven temperature time on the recovery of 5 mg L^{-1} CN from $[Fe(CN)_6]^{4-}$ complex in aqueous solution.

To calculate the recovery from the blank blood samples, three different HS vials containing 200 µL of the blood sample spiked with $[Fe(CN)_6]^{4-}$ to obtain 5 mg L^{-1} of CN^- and 200 µL of 1 M H_2SO_4 were prepared, and also three different standard solutions with 5 mg L^{-1} of CN^- (*vide supra*). Recovery was 71.5±1.5 %. The low recovery value resulted from the vapor pressure difference between the standard solution and the sample solution. Hereafter, we decided to increase the volume of the acid. In the next study, 200 µL of the blood sample similarly spiked with $[Fe(CN)_6]^{4-}$ and 1.0 mL of 1 M H_2SO_4 were mixed. The recovery rose to 96.0±2.9 %. Besides, we have found the recovery value to be 100.6±1.7 %.

Optimization of the HS heating time

To optimize the heating time of the HS oven, we analyzed the solutions containing 200 µL of same aqueous $[Fe(CN)_6]^{4-}$ solution and 1.0 mL of 1 M H_2SO_4 after keeping in the HS oven throughout 1, 2.5, 5, 7.5, 10, 12.5, 15, 17.5 and 20 min. As can be seen from Fig. 2, the peak areas reached a plateau after 12.5 min. Hence, we optimized the heating time to 13 min of the HS oven.

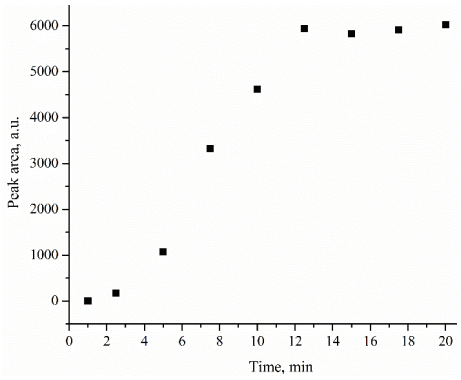

Fig. 2. Effect of heating time of the HS oven on HCN peak area. Aqueous solutions containing 200 µL of $[Fe(CN)_6]^{4-}$ (equivalent to 5 mg L^{-1} of CN^-) and 1 mL of 1 M H_2SO_4 were injected.

Optimization of the H_2SO_4 concentration

We put 1 mL of H_2SO_4 solutions with increasing concentrations from 0.1 to 5.0 M in the HS vials containing 200 µL of aqueous $[Fe(CN)_6]^{4-}$ which was equivalent to 5 mg L^{-1} of CN^-. We determined the optimal H_2SO_4 concentration to be 1.0 M (see Fig. 3).

Method performance characteristics

The authors investigated the method validation parameters, which were sensitivity, linearity, selectivity, accuracy and precision.

Linearity and sensitivity. Table I summarizes the linear range, retention time, regression equation, the limit of detection (*LOD*), and limit of quantification (*LOQ*) values. We calculated *LOD* and *LOQ* concentrations as 3×standard devi-

ations (*SD*) and 10*SD* of the very low concentration (signal-to-noise ratio: 3) of cyanide, respectively, obeying the Eurachem Guide.[37]

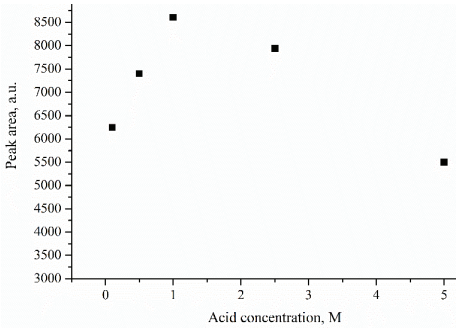

Fig. 3.++ Effect of H_2SO_4 concentration on HCN peak area. 1 mL of 0.1, 0.5, 1.0, 2.5 and 5.0 M H_2SO_4 were added onto the HS vial containing aqueous $[Fe(CN)_6]^{4-}$ which was equivalent to 5 mg L^{-1} of CN^- solutions, respectively.

TABLE I. The linearity, retention time, *LOD* and *LOQ* parameters of the proposed method

Analyte	Linear calibration range, μg mL^{-1}	Regression equation	Regression coefficient	Retention time, min	*LOD* ng mL^{-1}	*LOQ* ng mL^{-1}
Cyanide	0.05–10	$y = 3917.6x - 55.4$	0.9996	6.20	27	41

Linear range (0.05–10 μg mL^{-1}) was useful for the routine toxicological analyses. The calibration curves were prepared monthly. We allowed every sequence to start as long as we find QC (total cyanide 0.76 μg mL^{-1}) measurements in the acceptable range. We reconstructed the calibration without waiting for monthly repetition if QC controlling did not pass somehow. Also, after each blood sample run, a blank measurement was conducted owing to the observation of carry-over. We encountered no carry-over problem at all since we employed the stainless steel needle jet, and adjusted (loopless) injection time to above 0.05 min. Even though increasing injection time gave rise to both elevated sensitivity and decreased carry-over it caused expansion, of the width of the HCN peak, above 0.12 minutes. Thereby, we optimized 0.1 minute for the injection time. On the other hand, sensitivity of the method could be enhanced either by adding Na_2SO_4 leading to salt-out effect or by increasing sample volume. Yet, we did not need more sensitivity.

Selectivity. The Elite-FFAP was a suitable column for the separation of polar substances. No interference on the HCN peak (*m*/*z* 27) has been observed in the samples at optimized conditions, even it contained many volatile compounds. As the filament switched off before 5th min and after the 8th min of the analysis, data were collected between 5th to 8th min. Therefore, acetaldehyde, diethyl ether, acetone, ethyl acetate, methanol, 2-propanol, ethanol and benzene eluted before 5th min and 1-butanol, 3-methyl-1-butanol, *o*-,*m*-,*p*-xylenes, acetic acid and formic acid eluted after 8th min were not detected. Only chloroform, toluene and 1-propanol with the retention times of 5.86, 6.50 and 6.62 min, respectively, were

observed between 5th and 8th min. Some of these compounds, especially alcohols, could be formed endogenously in the postmortem blood samples while the source of the other compounds might be huffing glue or paint thinners (by abusers) due to their psychoactive effects. To check out the interference effect, a solution containing 0.5 μg mL^{-1} of CN^- and 100 μg mL^{-1} of the other compunds, described above, was prepared. No HCN peak was observed between chloroform and toluene peaks on the total ion current (TIC) chromatogram (see Fig. 4c) while HCN was successfully separated on the SIR chromatogram (*m*/*z* 27) as seen in Fig. 4a with 103.3 % of recovery value. In other words, cyanide could be determined by the proposed method, even if a sample had high concentrations of chloroform and toluene.

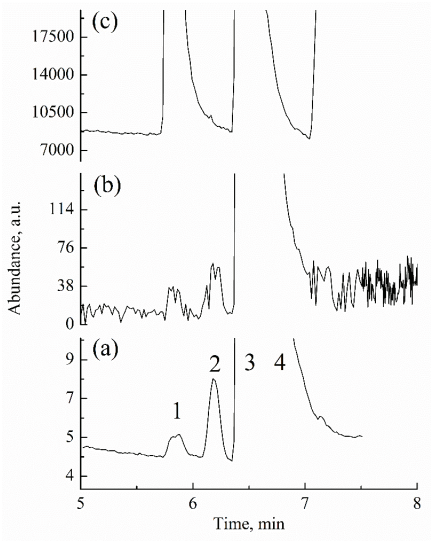

Fig. 4. Overlaid chromatograms of: a) SIR (*m*/*z* 27), b) *m*/*z* 27 ion in the full scan and c) TIC (12–150 amu); 1: chloroform, 2: HCN, 3: toluene, 4: 1-propanol.

It can be assumed that CN^- is formed as a result of the conversion of SCN^- at low levels in the blood. Though the addition of ascorbic acid inhibits this conversion, we did not use it because this conversion at the trace level is negligible for toxicological analysis.[33] Eventually, it was clear that the proposed method was quite selective for cyanide present in many sample matrices.

Trueness. The authors calculated the bias (*b*) of the method by using the reference material. The significance test (*t*-test) exerted to certified reference material (CRM) and recovery values obtained from two spike levels applied to a positive *post-mortem* blood sample with 6.40 μg mL^{-1} of mean concentration of CN^-. As seen in Table II, after the post-mortem blood sample was spiked with cyanide at 3.00 and 6.00 μg mL^{-1}, the average recovery values were found to be 101.1 and 94.5 %, respectively. Eq. (1) was employed for calculation of recovery, %, as:

$$\text{Recovery} = 100\frac{C_f - C_m}{C_a} \quad (1)$$

where C_f is the total found concentration value of the spiked blood sample; C_m is the mean concentration of the unspiked sample; C_a is the added concentration value of standard cyanide in the blood sample. We could not perform more than three times for the number of analyses of the positive *post-mortem* blood sample and its spiked solutions since the sample volume was limited. According to the results exhibited in Table II, calculated t values were well below the critical *t*-values. It indicated that there was no difference between our results and the reference concentrations at the 95 % confidence level.

TABLE II. Results of the accuracy experiments. For CRM, *t*-values were calculated by using certificate value and our results while recoveries were used for calculating *t*-values of spiked blood sample

Sample	Number of analyses	Measured mean concentration of cyanide ± *SD* μg mL^{-1}	*RSD* %	CRM results			Spike results of blood			*t*-Test results	
				Certified concentration of cyanide μg mL^{-1}	Bias	Bias %	Added μg mL^{-1}	Found μg mL^{-1}	Recovery %	*t* Value calculated	*t* Value reference
CRM	10	0.760 ± 0.039	5.19	0.757	0.003	0.40	–	–	–	0.24	2.26
Blood	3	6.40 ± 0.27	4.22	–	–	–	3.00	9.43	101.1	0.3	4.3
							6.00	12.27	94.5	2.0	

Precision. To investigate the precision of the developed method, the researchers carried out inter-day (three days×six replicates) and intra-day (one-day×six replicates) repeatability studies for the concentration and t_R by using CRM. Table III presents the repeatability results expressed as *RSD*. *RSD* values found <11 both in the trueness and in the precision studies were acceptable according to the AOAC guideline.[38] Consequently, the proposed method was successfully validated.

TABLE III. Inter-day and intra-day precision values of the concentration and retention time parameters. CRM solution of cyanide was analyzed six times in one day and three different days with six replicates for intra-day and inter-day repeatability studies, respectively

Examination	Parameter	*RSD* / %
Intra-day study	Concentration	3.66
	Retention time	0.14
Inter-day study	Concentration	5.93
	Retention time	0.21

Analysis of the real samples

The specialists should transfer the postmortem blood samples of the cyanide poisoning cases into the grey-colored tubes containing fluoride and EDTA during the autopsy. Then, they should quickly deliver the blood samples to toxicology laboratories for analysis. Bacteria and fungi might increase or decrease the cyanide concentration in the body or the other samples. Fluoride can inhibit *post-mortem* bacterial enzymatic reactions. Although fluoride and EDTA help to keep cyanide concentration stable, one should conduct the analysis as soon as possible to prevent the loss of analyte. In this study, we applied the proposed method to the real samples, and we found that total cyanide concentrations were in the range of 3.67-24 μg mL^{-1} and 0.66–2.40 μg mL^{-1} in the *post-mortem* blood samples of victims who ingested cyanide salts or were exposed to the smoke of the fire, respectively (see Table IV). In general, after the cyanide concentration was about 1–2 μg mL^{-1} in a blood sample with no case story, the stomach content was qualitatively analyzed for the presence of cyanide. If the result was negative, the reason for death most probably was that the victim has been exposed to carbonmonoxide also. Here, cyanide and carbonmonoxide, which are the two strong field ligands, could synergistically result in the death of the fire victims. The affinity of carbon monoxide for haemoglobin is 200–300 times higher than that of oxygen. Therapeutic and toxic concentration of carboxyhaemoglobin (COHb) are 1–5 % and > 20 %, respectively. Elevated COHb causes nausea, headache, gastrointestinal upset, hypertension, hyperventilation and drowsiness to coma. When the concentration of COHb reaches to 40–50 %, reddish colour on skin and cyanosis can occur. We observed when cyanide was not detected, the concentration of COHb was found up to 80–90 % in the *post-mortem* blood samples. However, its concentration range was between 25–45 % when the concentration of cyanide was determined at 1–2 μg mL^{-1}.

TABLE IV. Distribition of cyanide concentration in the *post-mortem* blood samples by cyanide intake and fire cases

Case	Number of cases	Concentration of cyanide, μg mL^{-1}		
		Range	Mean	Median
Cyanide intake	13	3.7–24.0	9.1	6.4
Fire	9	0.7–2.4	1.4	1.3

People who commit suicide at home sometimes leave a note in front of the door or on walls so that nobody could be harmed from the HCN gas. 6.40 μg mL^{-1} of cyanide was measured in the blood of a person found dead in an industrial zone. On the other hand, ten commercial drinking water samples, purchased from Istanbul markets, were analyzed and cyanide was not detected in the samples. The chromatograms, of the blood sample with 6.40 μg mL^{-1} of cyanide and a

drinking water sample, are given in Fig. 5. To sum up, the validated method was successfully applied to the real samples.

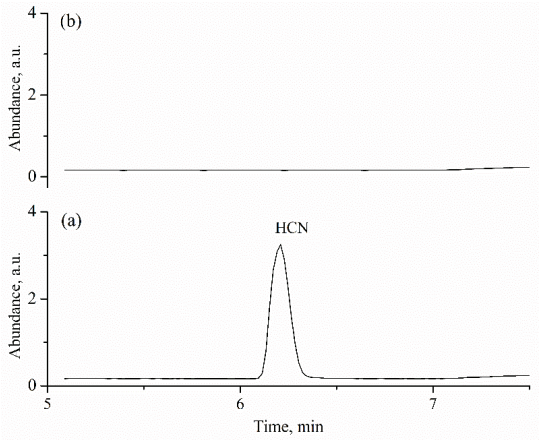

Fig. 5. Overlaid chromatograms (SIR acquisition *m*/*z* 27) of: a) blood sample with 6.40 μg mL^{-1} of cyanide and b) drinking water sample (cyanide was not detected).

A male, falling deeper into debt, have decided to commit suicide by intaking a cyanide compound. He drank the solution containing a cyanide compound from a small bottle, after parking his car. The statement told that after a while, he was nauseated and then his health got worse, but he survived and called the emergency. The crime scene investigation team took a swab sample from the bottle and sent it to our laboratory. We mixed the yellowish tip of the swab with 5 mL of 0.1 M NaOH. After analysis, we found 11 mg $swab^{-1}$ cyanide in the sample. We decided to carry out an experiment by ICP–MS for analysis of iron in the extracted solution. About six to one of molar ratio was determined as expected. We found out that the reason for his survival was that he drank a solution containing iron cyanide complex, which was most likely purchased as a cyanide-containing pesticide.

In addition to the analysis of the forensic evidences, the proposed method could be performed for the determination of total cyanide content in the environmental samples and in the industrial wastewaters. It is crucial that HCN may be released to the air from the industrial wastewater when acidic wastes are mixed with cyanide-containing wastes or when the wastewaters are directly exposed to the sunlight during the treatment processes. Thus, the related laboratories may easily adapt our method to control total cyanide concentration in the wastewaters.

CONCLUSION

In this work, a reliable HS-GC–MS method was developed for the determination of total cyanide in the forensic evidences such as postmortem blood

samples and aqueous samples. We attained very good recovery values from the whole blood sample, even $[Fe(CN)_6]^{4-}$ was used as a SAD complex, so we successfully validated the method. The proposed method has many advantages: the sample preparation procedure is simple, it does not require any derivatization step or clean-up procedure, and it is adequately rapid, sensitive, and selective for the routine toxicological investigations.

Acknowledgment. The authors are grateful to the Council of Forensic Medicine (ATK) Chairmanship, Turkey, for permitting, supporting and encouraging us to conduct this research.

ИЗВОД

МЕТОДА „HEADSPACE“ ГАСНЕ ХРОМАТОГРАФИЈЕ–МАСЕНЕ СПЕКТРОМЕТРИЈЕ ЗА ОДРЕЂИВАЊЕ УКУПНЕ КОНЦЕНТРАЦИЈЕ ЦИЈАНИДА У ВОДИ И *POST-MORTEM* УЗОРЦИМА КРВИ

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У овој студији имали смо за циљ да развијемо методу *headspace* гасне хроматографије–масене спектрометрије за одређивање укупне концентрације цијанида у форензичким доказима. Укупни садржај цијанида у узорцима израчунат је на основу концентрације цијановодоника испареног из течног узорка у вијали. Током оптимизације температуре пећи коришћен је хексацијаноферат(II). Утврђено је да се везе гвожђе-цијанид у потпуности разлажу након што је 0,2 mL узорка третирано са 1 mL 1 M сумпорне киселине, при температури од 120 °C и времену загревања од 12,5 min. Добијани су задовољавајући резултати процента приноса (*recovery*) и из водених и узорака крви. Метода је била линеарна у распону 0,05–10 μg mL^{-1} цијанида, што је било погодно за токсиколошка испитивања. Предложени метод је валидиран и примењен на узорке крви после смрти, воде за пиће и остале форензичке доказе. Предложена метода се може применити не само у форензичким, већ и у другим лабораторијама у којима је се анализирају узорци на садржај цијанида.

(Примљено 22. априла, ревидирано 2. јула, прихваћено 27. септембра 2020)

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