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A test to identify cyanide origin by isotope ratio mass spectrometry for forensic investigation

Illa Tea, Ingrid Antheaume, Ben-Li Zhang *

University of Nantes, Interdisciplinary Chemistry: Synthesis, Analysis, Modelling (CEISAM), UMR 6230, CNRS, 2 rue de la Houssinère, BP 92208, 44322 Nantes, France

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ABSTRACT

Cyanide is one of the common poisons in murders. When cyanide has been used, to identify the origin of cyanide may be necessary in the forensic investigation. We have examined the possibility of distinguishing different commercial cyanide samples through the $\delta^{13}C$ and $\delta^{15}N$ values and developed a protocol for the isotope analysis of cyanide extracted from several matrices as food and medicine. Several cyanide precipitates were tested for the isotope analysis. The results show that cupric ferrocyanide $Cu_2[Fe(CN)_6]$ is the most appropriate precipitate for the analysis. Thirteen batches of KCN and nine batches of NaCN chemicals were randomly chosen from different suppliers. The cyanides were converted to cupric ferrocyanide and then analysed by isotope ratio mass spectrometry coupled to elemental analysis (EA-IRMS). The isotopic signature of the commercial samples varied from −51.96 to −25.77‰ for $\delta^{13}C$ and from −4.51 to +3.81‰ for $\delta^{15}N$, highlighting the potential of applying EA-IRMS technique to identify cyanide from different batches and sources. The influence of the cyanide extraction and isolation from spiked matrix on the isotopic analysis was also studied. Three matrices: orange juice, yogurt drink and a medicine were tested. In many cases, the isotopic analysis results obtained from the original cyanides precipitates and those isolated from the matrices showed a good accordance, especially for $\delta^{15}N$. In some matrices, the ^{13}C analysis was interfered by co-precipitates. With carefully elaborated working protocol, determining the isotope ratio of N and C in cyanide by EA-IRMS is a promising method for forensic investigations.

1. Introduction

Cyanide is often used in murder because it is easily available at commercial or school laboratories. It can also be ordered from the web. Its high toxicity (1 g of cyanide can kill 250 people) makes it a popular choice of criminals. In certain cases, the identification of the source of cyanide is very important for the forensic investigation. One example is the famous "poisoned Josacine" crime case of France in 1994 [1]. In this case, a young girl was killed by sodium cyanide contained in an antibiotic medicine (Josacine). It was necessary to determine if the cyanide found in the residue of the medicine and that possessed by a suspect were of the same origin. In the investigation, the analysis was based on the determination and comparison of the impurities [1,2] contained in cyanide of the two samples by chemical method. Nevertheless, the analysis may be interfered by sample contamination. In addition, when the cyanide is mixed with a matrix, it is difficult to quantify its impurities. Stable isotope analysis has been widely used to identify substance source [3]. As cyanide is composed of two elements: carbon and nitrogen, it should be possible to characterizing the source of a cyanide sample by two characteristic parameters: the stable carbon and nitrogen isotope ratios, expressed as $\delta^{13}C$ and $\delta^{15}N$ values [4,5]. Through comparison with references in an isotope database, it is possible to identify its origin using theses parameters.

The commercial cyanides, in particular sodium and potassium cyanides are obtained by absorption of hydrogen cyanide by sodium and potassium hydroxide. The manufacturing process of hydrogen cyanide can be quite different [6]. It may be synthesized directly from ammonia and carbon monoxide or from ammonia, oxygen (or air), and natural gas. It is a by product of the production of coke from coal and is recovered (along with hydrogen sulfide) from coke-oven exhaust gases. It may also be prepared by thermal decomposition of formamide. Other methods can be cited too. In addition, for the fabricants using the same manufacturing process, the source of raw materials used may be different. Thus carbon and nitrogen isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) can be used as the fingerprints of the sample [7]. In this work, we have analysed a set of commercial cyanide samples and examined the possibility of distinguishing different samples through $\delta^{13}C$ and $\delta^{15}N$ values. We have also studied the method of extracting cyanide from several matrices (food and medicine) and evaluated the isotope

* Corresponding author. Tel.: +33 251125714; fax: +33 251125712.
E-mail address: Ben-Li.Zhang@univ-nantes.fr (B.-L. Zhang).

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fractionation and its influence during the isolation to develop a protocol for the analysis.

2. Experimental

2.1. Cyanide samples and matrices used

Nine sodium cyanide (NaCN) (Nos. 1–9) and thirteen potassium cyanide (KCN) (Nos. 1–13) chemicals of different batch were purchased from four chemical suppliers: Acros (www.acros.be), Aldrich (www.sigma-aldrich.com), VWR (www.vwr.com) and Alfa Aesar (www.alfa.com) (Table 1).

For the spiked matrices study, orange juice from Paquito, yogurt drink (Actimel) from Danone and an antibiotic medicine (Josacine) from Bayer Pharma were used.

2.2. Chemicals and instruments

2.2.1. Reagents and solutions for cyanide precipitation

Batch number for the products containing N and C is specified, since these products could interfere in isotope analysis.

NaOH (VWR International).
$AgNO_3$ (>99%, Acros Organics; batch number: A0260217).
Concentrated HCl (37%, Merck, www.merck.fr).
$FeCl_2$ solution: 3.81 g of $FeCl_2{\cdot}4H_2O$ (99%, Sigma–Aldrich) was dissolved in 15 mL of deionised water.
$CuCl_2$ solution: 6.54 g of $CuCl_2{\cdot}2H_2O$ (99%, Acros Organics) was dissolved in 15 mL of deionised water.
$FeSO_4$ solution: 1 g of $FeSO_4{\cdot}7H_2O$ (99%, Sigma–Aldrich) was dissolved in 10 mL of deionised water.
$FeCl_3$ solution: 3.4 g of $FeCl_3$ (98%, Acros Organics) was dissolved in 10 mL of deionised water.

2.2.2. Reagents for matrix treatment

Activated coal (Sigma–Aldrich). Diethyl ether (VWR International).

2.2.3. Instruments

pH-meter: Hanna Instruments pH 211-Microprocessor pH-meter using saturated Calomel electrode as reference.

2.3. Precipitation and isolation of cyanide from its aqueous solution

NaCN (No. 4) and KCN (No. 9) were used for the preparation of cyanide solutions. For AgCN and $Fe_4[Fe(CN)_6]_3$, 0.3 g of cyanide (NaCN or KCN) was dissolved in 100 mL of deionised water (pH 4.84). pH of the solution is 11.00. For $Cu_2[Fe(CN)_6$, 0.5 g of cyanide was dissolved in 100 mL of deionised water. pH of the solution is 11.10.

Table 1
Description of commercial cyanide samples used in the study.

Cyanide	No.	Supplier	Batch	Purity (%)
NaCN	1	Aldrich	1451296	97
(no. CAS: 143-33-9)	2	Aldrich	MKBD1745	95
	3	Aldrich	67296PJ-279	97
	4	Acros	A0279535	98
	5	Acros	A0253021	95
	6	Acros	815382	
	7	VWR	09G240976	95
	8	Alfa Aesar	10142263	98
	9	Alfa Aesar	A19S020	95
KCN	1	Aldrich	MKBB3134	96
(no. CAS: 151-50-8)	2	Aldrich	81710	96
	3	Aldrich	1408179	97
	4	Aldrich	90050	97
	5	Aldrich	1447235	98
	6	Acros	A0271047	97
	7	Acros	A0277558	97
	8	Acros	A0272679	97
	9	Acros	895741	99
	10	VWR	K40329067 948	97
	11	VWR	09G300973	98
	12	Alfa Aesar	B23U052	96
	13	Alfa Aesar	10142262	97

AgCN: 1 g of NaOH was added to 100 mL of cyanide solution to stabilize the cyanide. While adding 10 g of $AgNO_3$ to the solution under stirring, AgCN was precipitated [8].

Prussian blue $Fe_4[Fe(CN)_6]_3$: 1 g of NaOH was dissolved in 100 mL of cyanide solution under stirring. 0.5 mL of freshly prepared $FeSO_4$ solution and 5 mL of $FeCl_3$ solution were added successively. Prussian blue began to precipitate. The solution was acidified by adding concentrated HCl drop by drop until the solution just shows an acid reaction. $Fe_4[Fe(CN)_6]_3$ was obtained [9].

Copper(II) ferrocyanide $Cu_2[Fe(CN)_6]$: Dissolve 1 g of NaOH in 100 mL of cyanide solution under stirring. 5 mL of freshly prepared $FeCl_2$ solution and 5 mL of $CuCl_2$ solution were added successively. Cupric ferrocyanide began to precipitate. The solution was acidified with concentrated HCl till solution pH 1. $Cu_2[Fe(CN)_6]{\cdot}7H_2O$ was obtained [4,5].

Each precipitate (AgCN, $Fe_4[Fe(CN)_6]_3$ or $Cu_2[Fe(CN)_6]$) was isolated from the solution by centrifugation (4082 rpm for 5 min), washed twice with deionised water (each time 50 mL of deionised water was used) and then dried in an incubator under 50 °C for 48 h before isotopic analysis.

2.4. Precipitation and isolation of cyanide from spiked matrices

2.4.1. Preparation of contaminated matrices

Three matrices (orange juice, yogurt drink and an antibiotic medicine) were used. The orange juice and yogurt drink were applied as is. Deionised water was added to the bottle containing Josacine granula to obtain a suspension according to the instruction of the supplier. The final concentration of the antibiotic in the suspension is 500 mg per 5 mL. Four commercial cyanides (NaCN Nos. 3 and 9, KCN Nos. 1 and 9) were used in sample preparation. 1 g of each cyanide was added to 10 mL of orange juice (pH 3.71), yogurt drink (pH 4.11) or the medicine suspension (pH 6.90). These are spiked matrices of which pH of orange juice = 10.15, pH of the yogurt drink is 10.23 and pH of the medicine suspension is 10.92.

2.4.2. Treatment of matrices and precipitation of cyanides

10 mL of each spiked matrices was mixed with 10 mL of deionised water. 1 g of activated coal was added. After homogenization, the solution was centrifuged (4082 rpm for 5 min) three times (each time with the supernatant) and then filtered by suction filtration. The filtrate (20 mL) was washed twice by equal volume of diethyl ether to remove organic impurities. Then centrifuge (4082 rpm for 5 min) again, the top 20 mL aqueous solution (supernatant) was diluted with deionised water (1:20, v:v) and filtered by suction filtration. After adding 2 g of NaOH into the limpid filtrate (400 mL), $Cu_2[Fe(CN)_6]{\cdot}7H_2O$ was precipitated with 10 mL of $FeCl_2$ solution and 10 mL of $CuCl_2$ solution as described above. The solid was dried in an incubator at 60 °C for 24 h and then analysed by EA-IRMS.

2.4.3. Blank test

Repeat the above procedure for the three matrices without spiking cyanide. Any solids obtained final were dried and analysed by EA-IRMS further.

2.5. Sample preparation for IRMS analysis

Weigh 0.6 mg of NaCN, KCN, precipitates AgCN, $Fe_4[Fe(CN)_6]_3$ or $Cu_2[Fe(CN)_6]$ with a microbalance (Ohaus Discovery DV215CD, Pine Brook, New Jersey, USA) and transfer into a tin capsules (solids "light" 3.3 mm × 5 mm, Thermo Fisher Scientific). Each cyanide sample was analysed in duplicate.

2.6. Isotope ratio mass spectrometer (IRMS) analysis

The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ values for each reference compound and standard were determined by encapsulation and analysis using a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific) coupled on-line via a Conflo III interface to an elemental analyser Flash EA 1112 HT (Thermo Fisher Scientific). Sample combustion and reduction took place in a single quartz tube reactor set at 1020 °C. The tube was packed with chromium oxide, reduced copper and silvered cobalt oxide granules. The gases produced were carried in a stream of He at 90 mL min^{-1} through a $Mg(ClO_4)_2$ water trap. N_2 was completely separated from CO_2 at 45 °C using a Porapak Q gas chromatography stainless steel column (3 m × 6.35 mm). N_2 and CO_2 were transferred to the ion source of the isotope ratio mass spectrometer at a pressure of 1.2×10^{-6} mbar. Ion currents were measured continuously for m/z 28, 29, 30 and m/z 44, 45, 46, for N_2 and CO_2 respectively, using triple Faraday cups connected to high-gain amplifiers.

Reference pulse peaks of laboratory N_2 and CO_2 gases were calibrated against the international standards IAEA-N1 ($\delta^{15}N$ = 0.46‰, SD 0.17‰) and IAEA-N2 ($\delta^{15}N$ = 20.31‰, SD 0.19‰) (IAEA, Vienna, Austria) for N_2 and IAEA 305A ($\delta^{13}C$ = 39.8‰, SD 0.25‰) and NBS 22 ($\delta^{13}C$ = −30.031‰, SD 0.043‰) for CO_2.

The N and C isotope compositions were determined in the same run using the 'Dual Gas Acquisition' feature of the ISODAT3.0 software (Thermo Fisher Scientific). The working standard, glutamic acid, was measured under identical conditions every ten sample. Blank determinations were performed routinely in duplicate before each batch of samples (including working standards) by running empty tin capsules.