

Forensic Toxicological Analysis in Cyanide Poisoning: Two Case Reports

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Abstract

Introduction: Cyanide toxicity has been well known for over two centuries and, although most cyanide intoxication cases are accidental, such as fire victims, cyanide poisoning is sometimes motivated by suicidal or homicidal purposes.

Methods and findings: The method proposed in this study uses Headspace extraction and Gas Chromatography coupled with a Nitrogen and Phosphorous Detector (HS-GC/NPD), which has presented good results for both detection and quantification of cyanide intoxication, with satisfactory sensitivity and reproducibility. Good linearity ($r^2 > 0.99$), accuracy and precision were achieved. Limits of detection and quantification were well below the lethal cyanide blood concentration.

Conclusions: The present method is suitable for detection of cyanide intoxication even at small concentrations, and has been successfully applied in two lethal cases of cyanide intoxication.

Keywords: Cyanide; Gas chromatography; Nitrogen and phosphorous detector; Intoxication

There are several sources of cyanide contamination, the majority of which are related to the use of cyanide salts for industrial purposes. Cyanide contamination can be found in the effluents from polyacrylonitrile industries, acrylic resins synthesis, nitriles and aldehydes production, drugs and dyes processing, pesticides production and handling, gold and silver extraction, electroplating, photographic applications and smoke from fires and tobacco products [1-4]. Although most cyanide poisoning cases are accidental, it can also be intentional motivated by suicidal or homicidal purposes [5,6].

The reported cyanide's lethal dose may differ in the literature, but concentrations as low as 1 µg/mL are generally considered toxic [2,7,8].

The toxicological analysis is always important for the diagnostic confirmation of clinical or forensic poisonings. Despite the difficulties in cyanide detection, mainly due to low concentrations involved in the poisoning cases and to the instability of cyanide in the biological specimens, several different analytical techniques have been described in the literature [1,2,4,8-11].

The aim of the present work was to detect and quantify cyanide in different biological matrices from two lethal intoxication cases using Headspace extraction and Gas Chromatography coupled with Nitrogen Phosphorous Detector (HS-GC/NPD).

Case Reports

A 56-year-old man that worked at home as a goldsmith was found dead on the floor of his kitchen. A plastic bottle which contents appeared to be a cyanide salt was found next to his body. A suicide note was also found in one of the rooms along with the jewelry equipment that the victim used for his work; cyanide salt is known to be used as a cleaning agent for jewelry. The external examination revealed severe cyanosis, nosebleed and no signs of violence. During the necroscopic

Introduction

Cyanide (CN) toxicity has been known for over two centuries. Cyanide inhibits cellular respiration by reacting with the iron-containing enzymes, such as cytochrome oxidase and catalase, thereby preventing oxygen consumption by cells and leading to rapid loss of the vital functions [1].

procedure, approximately 30 mL of femoral blood, 20 mL of urine and all the stomach content were collected and sent to the Laboratory of Forensic Toxicology of Vitória-ES, Brazil.

An 18-year old female, student from a chemistry technical course, was found unconscious in the hall of the school where she received first aid, but with no success. Examination of the crime scene was not carried out. In the cell phone of the victim, seized by the Police, pictures of the student holding a Potassium Cyanide flask were found. The medical examiner didn't report any apparent changes at the body, such as injuries and cyanosis. During the necroscopic procedure, 3.5 mL of blood, 2 mL of stomach content and 6 mL of hepatic exudate were collected and sent to the Chemical Forensic Laboratory at the National Criminalistics Institute of Federal Police, in Brasília/Brazil, and later analyzed at the Laboratory of Forensic Toxicology of Vitória.

Materials and Methods

Reagents and solutions

Potassium cyanide (KCN) was purchased from Isofar (Rio de Janeiro, Brazil). Acetonitrile was acquired from Cromoline (São Paulo, Brazil). Sulfuric acid was obtained from Impex (Novo Hamburgo, Brazil). All the solvents and reagents were analytical grade.

The internal standard (IS) solution was prepared by placing 1 mL of acetonitrile (ACN) in a 100 mL volumetric flask and diluting it with water. This solution was further diluted 1000-fold, to yield a working solution with a final concentration of 7.86 µg/mL of ACN.

Cyanide working solutions were prepared by weighing 25.0 mg of KCN and diluting with 0.1 N of NaOH in a 100 mL volumetric flask to obtain a solution with a concentration of 100 µg/mL of CN⁻. Then, two successive dilutions were performed, so the final concentrations of 10 and 1 µg/mL were achieved.

Instrumentation

Cyanide analysis was performed on a Varian 450 Gas Chromatographer (GC) equipped with a Nitrogen and Phosphorous Detector (NPD) (Varian, Palo Alto, CA, USA) with a VF 624 capillary column (30 m × 0.32 mm i.d., 1.8 µm film thickness) (Agilent Technologies, Palo Alto, CA, USA). The headspace oven and syringe temperatures were set at 60°C. Samples were heated for 10 min with continuous shaking at 500 rpm. The column oven temperature was programed as follows: 30°C for 0.25 min, increased to 40°C (3°C/min) and then increased to 150°C (40°C/min) and held for 1 min (total run time of 7.3 min). The injector and detector temperature were 200 and 300°C, respectively. The injector operated in split mode (20:1), and the carrier gas flow (nitrogen) was 2 mL/min, with pressure pulse (25 psi for 0.25 min).

Sample preparation

A pool of blood from seven different deceased victims was analyzed to confirm the absence of cyanide and then used as blank matrix. Fire victims were the only exclusion criteria. Calibrators and quality controls (QC's) samples were prepared fortifying the pooled cyanide-free blood samples with the proper amount of cyanide working solutions at concentrations of 0.1 (low), 0.7 (medium) and 3.0 µg/mL (high).

Half milliliter of the calibrators, QC's and case samples (blood, urine, stomach content and hepatic exudate) were placed into a 20 mL headspace vial along with 0.5 mL of the IS working solution. Finally, 50 µL of sulfuric acid was added, and the vials were immediately capped and analyzed by the HS-GC/NPD method.

Results and Discussion

The method presented good chromatographic separation without any interference. The Linearity study was carried out by using six calibrators over a concentration range of 0.1-4.0 µg/mL, with six replicates for each concentration. A coefficient of determination greater than 0.99 was obtained ($r^2=0.9984$). The limit of detection (LOD) was measured by the analysis of decreasing cyanide concentrations and was determined at 0.05 µg/mL. The achieved limit of quantification (LOQ) of the method was 0.1 µg/mL and the acceptance criteria was based on the accuracy of five independent determinations at this concentration with a maximum deviation from the nominal value within 20%.

The precision and accuracy of the method was ascertained by measurements of the three CQ's levels, over a period of three days with five replicates of each concentration. Inter and intra-day precision (%RSD) ranged from 82.4 to 91.7% and from 86.3 to 95.2%, respectively. Accuracy values (%bias) lay between 82.4 (CQ low) and 91.7%.

Case 1

Cyanide was quantified at 30.7 µg/mL in blood, 100.5 µg/mL in the stomach content and 0.1 µg/mL in the urine. Blood and stomach content were diluted 10 and 100 times, respectively, for a correct measurement. The low concentration detected in the urine sample can be explained in cases of acute intoxication resulting in rapid death, since there is no time for elimination of the toxic agent. Cyanide was also detected in the white salt found at the crime scene.

Case 2

Cyanide was quantified at 26.7 µg/mL in blood, 12.8 µg/mL in the hepatic exudate and 41.5 µg/mL in the stomach content. Blood and hepatic exudate had to be diluted 10 times, while the stomach content was diluted 200 times. The cyanide flask allegedly used by the victim was not subjected to chemical analysis.

Conclusions

The present method showed good results for the detection and quantification of cyanide intoxication, with good sensitivity and reproducibility. Both LOD and LOQ are well below the lethal cyanide blood concentration, making the present method suitable for detection of cyanide intoxication even at small concentrations.

The toxicological analysis of both cases showed high concentrations in the blood, stomach content and in the hepatic exudate (only Case 2), allowing the conclusion that these deaths occurred due to a cyanide acute intoxication. Case 1 has the manner of death well characterized as a suicide, mainly because of the findings at the crime scene, such as position of the body, indications of self-administration of the cyanide salt and the suicide note. For case 2, the manner of death has yet to be determined, pending investigation (accidental death and suicide are the main hypothesis).

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