Cyanide poisoning:
Mass spectrometric analysis of forensic evidences

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Abstract: Cyanide poisoning may result from a variety of exposures including structural fires, industrial exposures, medical exposures and certain plant products. Cyanide poisoning cases may be accidental and intentional motivated by suicidal or homicidal purposes. Cyanogenic plant toxicity is one of the most common plant poisonings among the grazing livestock. Such cases are occasionally reported and investigated for medico legal, police and forensic settings. Forensic evidences, such as stomach contents and whole blood of the victims, are usually collected and analysed in order to confirm the cause of death. Various analytical techniques have been suggested for the toxicological detection and measurement of hydrocyanic acid (syn. hydrogen cyanide, prussic acid, HCN) and its metabolites from biological and environmental samples. However. the volatility and reactivity of cyanide leaves direct measurements highly susceptible to errors introduced during the sample collection and separation step. An alternative approach, that can help to minimize false positive and false negative results, is to detect stable biomarkers of cyanide metabolism, rather than cyanide itself. Some studies proved 2-aminothiazoline-4-carboxylicacid (ATCA) to be a stable cyanide metabolite and thus ATCA has been considered a promising biomarker for cyanide poisoning. Some important aspects of cyanide poisoning and its detection, mainly by mass spectrometric methods, are discussed in this article.

1. Introduction

Cyanide is commonly known as a toxic chemical that has been used as a poison for thousands of years. Since the time of ancient Egypt, plants containing cyanide (e. g., cassava (Maniok, *Manihot esculenta* Crantz), bitter almonds (*Prunus dulcis var. amara*), cherry laurel leaves (*Prunus laurocerasus* L., Fig. 1), and peach pits have been used as poisons [1].

Romans added cherry laurel leaves (which contain cyanogenic glycosides like amygdalin) into a tea and used it as a method of execution [1,2]. The first description of cyanide poisoning was by Wepfer in 1679 from its extraction from bitter almond [3]. Even though plants containing cyanide have been used for centuries as poisons, cyanide was not identified until 1782, when German-Swedish pharmacist and chemist Carl Wilhelm Scheele isolated cyanide from Prussian blue dye, a dark blue synthetic pigment [1]. In 1815 the French chemist Joseph Louis Guy-Lussac discovered cyanogen (HCN, a colourless, poisonous gas) that smelled like almonds and was considerably thermally stable [3]. Alzhrani Elaf [4] reviewed the use of cyanide as a chemical weapon and stated that cyanide delivery devices are inexpensive and easy to produce and that cyanide is relatively easy to procure.

Fig. 1. Cherry laurel (*Prunus laurocerasus* L.) reproduced from “Köhler's Medizinal-Pflanzen in naturgetreuen Abbildungen und kurz erläuterndem Texte (Band 2)", 1887, Gera-Untermhaus, Verlag von Franz Eugen Köhler with kind permission from www.biolib.de (http://www.biolib.de/koehler2/high/DSC_3179.html; 02.06.2020).

Thus, even small terrorist groups can use cyanide to create mass casualties. Alzhrani discussed also some famous cases of cyanide poisoning [4].

Cyanide is one of the most famous poisons - from spy novels to murder mysteries; it has developed a reputation for causing an almost immediate death. But in real life, cyanide is a little more complicated. Cyanides contain a cyano group. In inorganic cyanides, the cyanide group is present as the anion (CN^-). Cyanide can refer to any chemical that contains a carbon-nitrogen (CN) bond, and it can be found in some surprising places [5] (see below).

2. Cyanide poisoning exposures

Cyanide poisoning may result from a variety of exposures including structural fires, industrial exposures, medical exposures such as sodium nitroprusside, and certain foods. Cyanide is also used in a number of industrial applications such as electroplating, photography, plastics and rubber manufacturing, and pesticides.

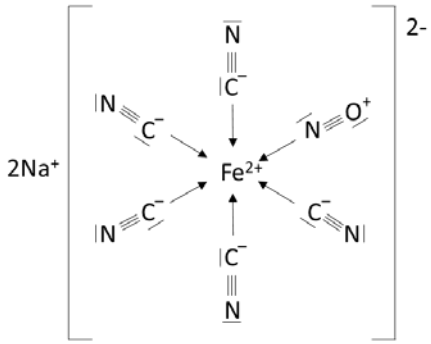
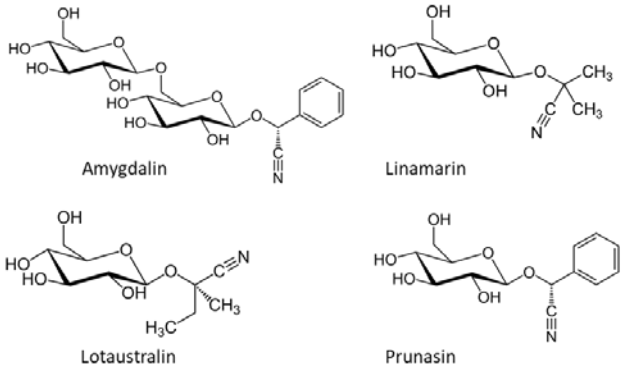


Fig. 2. Structure of disodium nitroprusside.

Sodium nitroprusside, a medication used to treat a hypertensive emergency, contains five cyanide groups per molecule (Fig. 2). Toxic levels of cyanide may be present in patients who receive prolonged infusions of sodium nitroprusside [6]. In air, cyanide ions are present mainly as HCN. Miners, fire fighters and workers of metallurgical chemical and galvanic industries are exposed largely to cyanide poisoning [7]. Cyanides are present in various environmental compartments such as water, soil, air, food and biological materials like blood, urine and saliva at concentration levels of µg/L to mg/L [8,9].

3. Cyanide poisoning

Cyanogenic plant toxicity is one of the most common plant poisoning among the grazing livestock. Prussic acid poisoning is caused by cyanide production in several types of plants under certain growing conditions. Sorghums and closely related species are the plants most commonly associated with prussic acid poisoning. Prussic acid precursors are degraded by the animal and poisonous HCN is released.



Poisoning occurs when livestock consume young or drought stressed or damaged plants with high HCN content [10]. Cyanogenic plants contain cyanide as cyanogenic glycosides (Fig. 3). Normally, these glycosides are nontoxic but can become toxic for animals and humans when hydrolyzed.

Fig. 3. Structures of some cyanogenic glycosides (wikipedia.de; 02.06.2020).

It is reported that if the plant is freezed, chopped or chewed, damaged plant cells can release enzymes in their vacuoles which release cyanide from cyanogenic precursors like for example amygdalin, prunasin, linamarin, lotaustralin, dhurrin, taxiphylin, vicianin, proteacin or gyno-

cardin (as the most common glycosides in cyanogenic plants). Kutay et al. [11] reviewed the lethal dose of HCN for cattle and sheep. It is considered to be about 2 mg/kg body weight. Plants are considered as toxic when containing more than 200 ppm of these glycosides. Ingested cyanide is rapidly absorbed and circulated and e. g. bound to hemoglobin to form cyanomethemoglobin. Cyanide interferes with enzymes of the cytochrome oxidase family and thus inhibits oxidative phosphorylation causing death by histotoxic anoxia.

4. Cyanide poisoning and forensic evidence

Forensic evidence, such as stomach contents, whole blood and urine of the victims, is usually collected and analysed [12]. The toxicological detection of cyanide involves extraction and measurement of HCN from biological extracts. Additionally, the presence of cyanide at the crime scene and the source of cyanide is commonly investigated during the crime scene search. When the body undergoes decomposition, no diagnostic signs of cyanide poisoning can be observed. Livor mortis and scent should not be used solely to include or exclude cyanide poisoning. The observation of gastric burns and blood that has a cherry red color are sound forensic factors to confirm acute cyanide poisoning cases. Usually, the requirement to confirm a cyanide poisoning case is based on the medical examiner's diagnosis of the totality of the case. The forensic factors associated with cyanide poisoning should be considered, but not all of them need to be collected and tested. Each case is unique in its own way [13].

It has been reported that the most common source of cyanide poisoning in humans arises from exposure to fire smoke. In fires, cyanide is produced at temperatures of 315°C (600°F) or more. Cyanide is released from the toxic fumes in the gaseous form, i.e. hydrogen cyanide (HCN) that may be inhaled by the victim. HCN is developed from an incomplete combustion of any material containing carbon and nitrogen such as plastic, wool or silk [14].

The toxicological and post-mortem analysis of fire victims' blood and tissue can disclose the type and quantity of toxic species, such as carbon monoxide or hydrogen cyanide that they inhaled prior to death. In fire cases, these toxicological data can reveal objective data about the nature and circumstances of a fire, and thus assist both the medical examiner and the fire investigator in their investigations [15].

Cyanides can contaminate the water through discharges of factory wastes and can be washed down from fields and urban areas. As a component of wastewater, they are present in the effluents from electroplating processes, gold and silver extraction and production of medicines and plastic [16,17].

5. Analytical methods: challenges and solutions

A number of analytical challenges can occur when determining cyanide in environmental samples and biological materials. These have be taken into account already at the stage of developing and implementing new analytical procedures [18].

Samples of biological material, wastewater and food are complex matrices and thus require adequate preparation for analysis. Interfering substances within the sample can react with cyanide and thus can cause wrong analysis results. Thus, sample preparation should aim on the decomposition of stable metal cyanide complexes and the elimination of interfering substances [19,20]. Sample preservation and storage are always crucial for cyanide analysis. In the case of biological samples, the storage temperature is very important as it may change the cyanide concentration up to 66% [21,22].

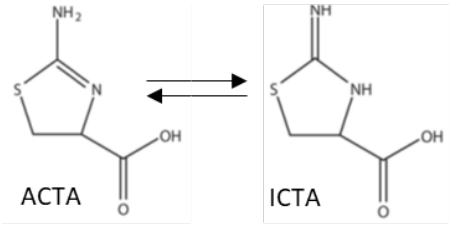
Cyanide is a swift and powerful poison. Suicidal, accidental, or homicidal death involving the determination of cyanide is encountered frequently in forensic toxicological practice [23]. In crime scene investigations, two indicators of acute cyanide poisoning are a “bitter almond” odor emanating from the victim and the presence of pink lividity in the post mortem examination [24]. Due to the relatively short half-life of cyanide (from minutes to hours depending on the matrix), direct analysis of cyanide to confirm cyanide poisoning may only be feasible within the first few hours following exposure [15,25,26]. Therefore, alternative methods have been developed to confirm and measure cyanide exposures.
Note: Care should be taken when working with cyanide containing material, since approx. 30% of human beings can not smell cyanide due to a genetic defect.

6. Detection of stable biomarkers of cyanide

Natural dietary and pulmonary intake of cyanide from the environment provides a non-zero cyanide background level in the body. Smoke inhalation from fires greatly increases background cyanide levels [27]. The volatility and reactivity of cyanide leaves direct measurements highly susceptible to errors introduced during the sample collection and separation step [14]. An alternative approach, that can help to minimize false positive and false negative results, is to detect stable biomarkers of cyanide, rather than cyanide itself.

Thiocyanate (SCN^-), 2-aminothiazoline-4-carboxylic acid (ATCA), and cyanide-protein adducts in biological fluids and tissues have been reported as alternative biomarkers for cyanide exposure and poisoning [28,29]. In vivo detoxification of cyanide by cystine to produce ATCA was reported by Wood and Cooley [30]. This pathway accounts for approximately 20% of the cyanide metabolism and the quantity of ATCA production is directly proportional to the amount of cyanide metabolised [30]. ATCA is stable in the samples for months in the freezer [31]. Therefore, ATCA has been considered a promising marker for the detection of cyanide poisoning.

Li et al. [32] reviewed the analytical methods proposed for the determination of ATCA from biological samples and also evaluated the potential use of ATCA as a forensic marker in death investigations. They described that direct analysis of cyanide may be indicated immediately following exposure but analysing cyanide metabolites may be more feasible after significant time has passed between intoxication and sampling.



Upon entering the biological system, cyanide is readily metabolized to thiocyanate (SCN^-), 2-aminothiazoline-4-carboxylic acid (ATCA) and its tautomer 2-iminothiazoline-4-carboxylic acid (ITCA) (Fig. 4) as well as protein adducts, α-ketoglutarate cyanohydrin (α-KgCN) and cyanocobalamin.

Fig. 4. Structures of 2-aminothiazoline-4-carboxylic acid (ATCA) and its tautomer 2-iminothiazoline-4-carboxylic acid (ITCA), according to [38].

Thiocyanate is the major metabolite of cyanide. Advantages of targeting SCN^- include its nonvolatility and much longer half-life. However, the detection of SCN^- is nonspecific for cyanide exposure, because it is present endogenously at high concentrations and is involved in metabolic pathways other than cyanide. According to the current state of knowledge, ATCA has not yet been associated with other metabolic pathways except for cyanide detoxification. Moreover, ATCA is stable under various conditions over time.

7. Massspectrometry

Various mass spectrometric methods have been developed even for the analysis of cyanide and mainly its metabolites, like 2-aminothiazoline-4-carboxylic acid (ATCA) as a novel biomarker and forensic biomarker for cyanide poisoning [33-39]. Modification of originally published procedures aimed e. g. at the improvement of the detection limits as well as of the accuracy and imprecision.

Mass spectrometric applications for the determination of cyanide(metabolites) in biological samples (plasma, urine, exhailed air) and water are given in the Appendix of this review

8. Discussion

Logue et al. [40] correctly pointed out that analytical determination of cyanide and its metabolites is not an easy task due to the chemical properties of the analytes, the in vivo and in vitro metabolism and limited research. Numerous methods, each with its own advantages and limitations have been developed and provided useful information how to detect a cyanide exposure and how to interprete the analysis results, e. g. from biological samples. Care should be taken for choosing an analytical method for the specific analytical task and aside from analytical parameters the toxicokinetics of cyanide and its metabolites should be considered. Mass spectrometry combined with different kinds of separation techniques has shown its power to improve the determination of cyanide and its metabolites in different (biological) matrices.

9. Acknowledgements

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Addendum to Cyanide poisoning: Mass spectrometric analysis of Forensic evidences by Mahesh Prasad Goutam and Poonam Yadav

The intent of this addendum is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. In addition to this, analytical methods are included that modify previously reported and used methods to obtain lower detection limits and/or to improve accuracy and precision. This article is based on the survey of publications related to cyanide identification using mass spectophotometry and reviews the analysis of cyanide and ATCA as well as studies related to potential use of ATCA as a marker for the diagnosis of cyanide exposure.

There are numerous analytical techniques to determine cyanide and thiocyanate or both in biological samples, but only a few techniques are available to measure ATCA. Derivatization steps were needed in those studies due to the use of either fluorometric detection or gas chromatography [1]. Molecularly imprinted stir bar sorption extraction (MISBSE) of ATCA was reported [2] but this technique enables the selective extraction of ATCA from urine samples followed by direct detection of ATCA (without derivatization) by a tandem mass spectrometer. Then after to determine the optimal conditions for the quantification of ATCA in various biological fluids and organs using SPE, and high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was proposed [1]. This study also compared the two sample preparation methods of SPE and MISBSE suggesting the priority of SPE over MISBSE. Because of the importance for clinical, forensic and very likely, security and antiterrorism applications, it has become urgent to establish rapid, sensitive, specific and robust point of care cyanide analysers. Application of Mass Spectrometry proved its significance in this field. Mass Spectrometry is used for both qualitative and quantitative chemical analysis. It may be used to identify the elements and isotopes of a sample, to determine the masses of molecules, and as a tool to help identify chemical structures. It can measure sample purity and molar mass. The availability of inter phases made it possible to hyphenate this sophisticated technique with other chromatographic techniques that has opened the new horizons of its applicability like GC-MS, CI-GC-MS, ID GC/MS, HPLC-MS, LC-MS, LC–MS–MS, ESI-MS-MS, SIFT-MS.

Cyanide determination in blood

LC–MS–MS: Lacroix, et al. [3] developed liquid chromatography–tandem mass spectrometry (LC–MS–MS) method coupled to online extraction for cyanide determination in blood. These authors mentioned that a method involving fluorimetric detection after naphthalene-2,3-dicarboxyaldehyde (NDA) complexation by taurine in the presence of cyanide was previously described. Its performance was limited because of the absence of an internal standard (IS). Using cyanide isotope ^{13}C ^{15}N as IS allowed quantification in MS–MS. After the addition of ^{13}C ^{15}N, 25 µL of blood were diluted in water and deproteinized with methanol. Following derivatization with NDA and taurine for 10 min at 4°C, 100 µL was injected into the online LC–MS–MS system. An Oasis HLB was used as an extraction column, and a C18 Atlantis was the analytical column. The chromatographic cycle was performed with an ammonium formate (20 mM, pH 2.8) (solvent A) and acetonitrile/solvent A (90:10, v/v) gradient in 6 min. Detection was performed in negative electrospray ionization mode (ESI^-) with a Quattro Micro. For quantification, transitions of derivatives formed with CN and ^{13}C ^{15}N were monitored, respectively, as follows: 299.3/191.3 and 301.3/193.3. The procedure was fully validated, linear from 26 to 2600 ng/mL with limit of detection of 10 ng/mL. Authors suggested that this method, using a small blood sample, is not only simple, but also time saving. The specificity and sensitivity of LC–MS–MS coupled to online extraction and using ^{13}C ^{15}N as the IS make this method very suitable for cyanide determination in blood and could be useful in forensic toxicology

HPLC-MS: Tracqui et al [4] developed a High-performance liquid chromatographic-mass spectrometric procedure for the determination of cyanide (CN) in whole blood and reported that after the addition of $K^{13}C^{15}N$ as internal standard, blood was placed in a micro diffusion device, the inner well of which was filled with a mixture of taurine (50 mM in water)/naphthalene-2,3-dicarboxaldehyde (NDA, 10 mM in methanol)/methanol/ concentrated (approximately 20%) ammonia solution (25:25:45:5, v/v). Concentrated H_2SO_4 was added to the blood sample, and the micro diffusion chamber was sealed. After 30 min of gentle agitation, 2 microL of the contents of the inner vials were pipetted and directly injected onto a Nova Pak C18 HPLC column. Separation was performed by a gradient of acetonitrile in 2 mM NH_4COOH, pH 3.0 buffer (35-80% in 10 min). Detection was done with a Perkin-Elmer Sciex API-100 mass analyzer with an ion spray interface, operated in the negative ionization mode. MS data were collected as either TIC or SIM at m/z (299 + 191) and (301 + 193) for the derivatives formed with CN and $^{13}C^{15}N$, respectively. Limits of detection and quantitation for blood CN recorded 5 and 15 ng/mL, respectively.

ID-MS: A direct and sensitive method for the determination of blood cyanide by isotope dilution was developed by Dumas et al. [5] the blood is placed in a headspace vial, and $K^{13}C^{15}N$ is added as internal standard. Addition of phosphoric acid liberates the cyanide as HCN. The detection is accomplished by mass spectrometry after a fine mass calibration tuning. The detection limit obtained is 0.3 micromol/L. The within- and inter-run coefficients of variation are 4.4% (for a concentration of 2.5 micromol/L) and 3.9% (for a concentration of 4.7 micromol/L), respectively. The observed recovery reported as 98%. Authors claimed that round-robin exercise was carried out to compare the performance of this method with others currently in use in other clinical laboratories.

HS-SPME: Frison et al. [6] reported an improved qualitative and quantitative method for cyanide determination in blood using solid phase, micro extraction and gas chromatography/mass spectrometry .The method claimed to be simple, fast and sensitive enough for the rapid diagnosis of cyanide intoxication in clinical and forensic toxicology It involves the conversion of cyanide into hydrogen cyanide and its subsequent headspace solid phase micro extraction (HS-SPME) and detection by gas chromatography/mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode. Optimizing the conditions for the GC/MS (type of column, injection conditions, temperature program) and SPME (choice of SPME fiber, effect of salts, adsorption and desorption times, adsorption temperature led to the choice of a 75µm carboxen/polydimethylsiloxane SPME fiber, with D3-acetonitrile as internal standard, and a capillary GC column with a polar stationary phase. Method validation was carried out in terms of linearity, precision and accuracy in both aqueous solutions and blood. The limit of detection (LOD) and limit of quantitation (LOQ) were determined only in aqueous solutions. The assay is linear over three orders of magnitude (water 0.01–10, blood 0.05–10 µg/mL); and the LOD and LOQ in water were 0.006 and 0.01 µg/mL, respectively. Good intra and inter assay precision was obtained, always <8%.

ID GC/MS: Murphy et al. [7] determined Cyanide in blood applying isotope-dilution gas chromatography– mass spectrometry (ID GC/MS). In this method a known amount of isotopically labeled potassium cyanide ($K^{13}C^{15}N$) was added to 0.5 g of whole blood in a headspace vial. Hydrogen cyanide was generated through the addition of phosphoric acid, and after a 5-min incubation, 0.5 mL of the headspace was injected into the GC/MS at an oven temperature of 15 °C. The peak areas from the sample, 1 $H^{12}C^{14}N$, at m/z 27, and the internal standard, $^{1}H^{13}C^{15}N$, at m/z 29, were measured, and ID quantified the CN concentration. The analysis time recoded was 15 min for a single injection. Authors reported method accuracy by measuring the CN content of unfrozen whole blood samples fortified with a known amount of CN. Intermediate precision was demonstrated by periodic analyses over a 14-month span. Relative expanded uncertainties based on a 95% level of confidence with a coverage factor of 2 at CN concentrations of 0.06, 0.6, and 1.5 g/g were 8.3%, 5.4%, and 5.3%, respectively. The mean deviation from the known value for all concentrations was reported <4% and claimed that automated ID GC/MS method can accurately and rapidly quantify nanogram per gram to microgram per gram concentrations of CN in blood.

ESI-MS-MS: Determination of Cyanide in Blood by Electrospray Ionization Tandem Mass Spectrometry After Direct Injection of Dicyanogold (ESI-MS-MS) reported by Minakata et al. [8]. In this method five micro liters of blood was haemolysed 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 $Au(CN)^-$ 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. Authors applied this method 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. Authors reported that 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^- ins the blood, gastric content and urine were 78.5 ± 5.5, 11.8 ± 0.5, and 11.4 ± 0.8 µg/L, respectively.

GC/MS: Kage et al. [9] devised a sensitive and simple method for determining cyanide and its major metabolite, thiocyanate, in blood using an extractive alkylation technique. Pentafluorobenzyl bromide was used as the alkylating agent, and tetradecyldimethyl benzyl ammonium chloride was used as the phase-transfer catalyst. The derivatives obtained were analysed qualitatively by gas chromatography-mass spectrometry and quantitatively by gas chromatography with electron-capture detection. The detection limits of cyanide and thiocyanate were 0.01 and 0.003 µmol/ml, respectively, while the gross recovery of both compounds was 80%. The calibration curve was linear over the concentration range from 0.02 to 1.0 µmol/ml for cyanide and from 0.01 to 1.0 µmol/ml for thiocyanate. The accuracy and precision of the method were evaluated, and the coefficients of variation were found to be within 10%. Using this method, the blood levels of two victims who had died from cyanide poisoning were reported.

Løbger et al. [10] reported automated procedure for the analysis of low cyanide concentrations in whole blood using headspace gas chromatography and mass spectrometry in the ($^{1}H^{12}C^{14}N$) and *m/z* 29 ($^{1}H^{13}C^{15}N$). Developing a new method that enabled automated flushing of the needle in between each cyanide analysis prevented carryover from cyanide adsorption onto the surface of the needle. Authors compared results of ordinary calibrations and those of isotope dilutions and calculated 18 min for a single cyanide analysis. Lundquist et al. [11] described a method for determination of cyanide in whole blood, erythrocytes, and plasma after stabilization of cyanide by addition of silver ions. The cyanide is then transferred from the acidified sample, by aeration, into sodium hydroxide and quantified by the König reaction, with sodium hypochlorite as the chlorinating agent. A rapid loss of measurable cyanide found when cyanide was added to plasma in the absence of silver ions was attributed to a reaction with serum albumin. Cyanide added to whole blood was bound to a saturable component in erythrocytes, which can be identified as methemoglobin.

GC/MS-TOF: In a case of cyanide poisoning Roda et al. [12] reported that a man was found dead in a hotel located near Rome (Italy). The man was still holding a syringe attached to a butterfly needle inserted in his left forearm vein. The syringe contained a cloudy pinkish fluid. In the hotel room the Police found a broken propofol glass vial plus four

sealed ones, an opened NaCl plastic vial and six more still sealed, and a number of packed smaller disposable syringes and needles. An opened plastic bottle containing a white crystalline powder labeled as potassium cyanide was also found. Systematic toxicological analysis (STA), carried out on blood, urine and bile, evidenced only the presence of propofol in blood and bile. So the validated L-L extraction protocol and the GC/MS-TOF method for the confirmation of propofol in the biological fluids optimized in the laboratory was applied to blood, urine and bile. The concentration of propofol resulted to be 0.432 μg/mL in blood and 0.786 μg/mL in bile. The quantitative determination of cyanide in blood was carried out by micro diffusion technique coupled to spectrophotometric detection obtaining a cyanide concentration of 5.3 μg/mL. The quantitative determination was then confirmed by GC/NPD and the concentration of cyanide resulted to be 5.5 μg/mL in blood and 1.7 μg/mL in bile. Data emerging from autopsy findings, histopathological exams and the concentrations of cyanide suggested that death might be due to poisoning caused by cyanide; however, respiratory depression caused by propofol could not be excluded.

Cyanide determination in biological matrices

HS GC–MS: Desharnais et al. [13] reported a method for Cyanide quantification in post-mortem biological matrices, using headspace gas chromatography coupled to mass spectrometry with a GS-GASPRO column on an HP-6890 gas chromatograph with an HP-5973N mass detector. The biological sample was treated with an internal standard, frozen, glacial acetic acid was added and the sample was then incubated at 60 8C for 15 min. The headspace was sampled with a disposable syringe, and analysed to quantify hydrogen cyanide. Isotopically labeled cyanide ^{13}C ^{15}N was used as the internal standard to minimize matrix effect and sampling error. The method produced an extended linear dynamic range (0.07–50 mg/mL), and a method detection limit of 0.02 mg/mL. Identical calibration curves were obtained when blood, gastric contents and aqueous solutions were used as the calibration standard matrix. This method was also successful in quantitative detection of cyanide in gastric contents, and this method have been validated and used for forensic cases such as fire victims and suicides.

ESI-MS-MS: A rapid and sensitive electrospray ionization tandem mass spectrometric (ESI-MS-MS) procedure was developed by Minakata et al. [14] for the determination of cyanide. CN^- in biological fluids was reacted with Na $[AuCl_4]$ to produce dicyanogold Au $(CN)^{2-}$ which was extracted with methyl isobutyl ketone (MIBK). One microliter of the extract was injected directly into an ESI-MS-MS instrument. Quantification of CN^- was performed by selected reaction monitoring of the product ion CN^- at m/z 26 that derived from precursor ion Au CN^{2-} at m/z 249. CN^- could be measured in the quantification range of 10(-7) to 5x10(-5) M with the limit of detection at 4x10(-8) M using 10 microL of urine within 10 min. A victim's urine and gastric content were diluted with water to 4-fold and 500-fold and measured, respectively.

HS-GC/MS: HS-GC/MS method with a capillary column HP-624 of 30 was developed by Asselborn and Wennig [15]. The column temperature is programmed from 60°C (2-min hold) to 120°C (7-min hold) at 50°C/min. Total GC-run time is 10 min. The sample volume used in the photometric method could be reduced from 2 ml to 1 ml. HCN is liberated during an incubation step for 60 min at 60 °C by concentrated phosphoric acid from the matrix in a headspace vial and subsequently transformed to cyanogen chloride Cl-CN by reaction with chloramine T. The ions m/e = 61 and 63 for Cl-CN are monitored by SIM/MS and 1-BuOH is used as internal standard (m/e = 56, 31 and 41). Moreover full MS-scan is also possible, if higher amounts of HCN are present. Thus not only a more rapid, but also a more specific method is now available. The LOD is = 0,1 mg CN^-/L and the calibration curve is linear from 0,4 to 4 mg CN^-/L. The method can be also applied to other biological tissues and may be useful for a routine clinical and/or forensic toxicology laboratory in case of acute cyanide poisoning.

Yu et al. [1] demonstrated the potential of using 2-aminothiazoline-4-carboxylic acid (ATCA) as a novel bio-marker/forensic biomarker for cyanide poisoning. Authors suggested a sensitive method was developed and employed for the identification and quantification of ATCA in biological samples, where the sample extraction and clean up were achieved by solid phase extraction (SPE). After optimization of SPE procedures, ATCA was analysed by high performance liquid chromatography-tandem mass spectrometry. ATCA levels following the administration of different doses of potassium cyanide (KCN) to mice were measured and compared to endogenous ATCA levels in order to study the significance of using ATCA as a biomarker for cyanide poisoning. A custom made analytical method was established for a new (mice) model when animals were exposed to increasing KCN doses. The application of this method provided important new information on ATCA as a potential cyanide biomarker. ATCA concentration in mice plasma samples were increased from 189 ± 28 ng/mL (n = 3) to 413± 66 ng/mL (n = 3) following a 10 mg/kg body weight dose of KCN introduced subcutaneously. The sensitivity of this analytical method proved to be a tool for measuring endogenous level of ATCA in mice organs as follows: 1.2 ± 0.1 μg/g for kidney samples, 1.6 ± 0.1 μg/g for brain samples, 1.8 ± 0.2 μg/g for lung samples, 2.9 ± 0.1 μg/g for heart samples, and 3.6 ± 0.9 μg/g for liver samples.

Giebultowicz et al. [16] reported that 2-aminothiazoline-4-carboxylic acid (ATCA) is a hydrogen cyanide metabolite that has been found to be a reliable biomarker of cyanide poisoning, because of its long-term stability in biological material. There are several methods of ATCA determination; however, they are restricted to extraction on mixed mode cation exchange sorbents. To date, there has been no reliable method of ATCA determination in whole blood, the most frequently used material in forensic analysis. This novel method for ATCA determination in post mortem specimen includes protein precipitation, and derivatization of interfering compounds and their later extraction with ethyl acetate.

ATCA was quantitatively analyzed via high performance liquid chromatography-tandem mass spectrometry with positive electrospray ionization detection using a hydrophilic interaction liquid chromatography column. The method satisfied all validation criteria and was tested on the real samples with satisfactory results. Therefore, this analytical approach has been proven to be a tool for measuring endogenous levels of ATCA in post mortem specimens. To conclude, a novel, accurate and sensitive method of ATCA determination in post mortem blood was developed. The establishment of the method provides new possibilities in the field of forensic science

Jackson et al. [2] reported that in forensic casework, a stable and quantifiable marker is desirable for the determination of cyanide poisoning in biological fluids. 2-Aminothiazoline-4-carboxylic acid (ATCA) is a chemically stable urinary metabolite of cyanide that has been considered to be a reliable biological marker for cyanide exposure. However, endogenous ATCA is always present in low quantity originating from either dietary intake of cyanide or from normal metabolism of amino acids. A selective and sensitive analytical method is needed to determine the endogenous level of ATCA in order to identify cyanide poisoning. The objective of this research was to prepare molecularly imprinted polymers (MIPs) on the surface of a silica stir bar for molecularly imprinted stir bar sorption extraction (MISBSE). Under optimal extraction conditions, the MISBSE could selectively preconcentrate ATCA from urine samples. The binding capacity of one MISBSE stir bar for ATCA was determined to be 35 ± 3 ng (n = 3). Combining MISBSE with electrospray ionization tandem mass spectrometry (ESI/MS/MS), ATCA was detected without derivatization at the 400-ng/mL-concentration level. This new strategy of MISBSE-ESI/MS/MS enhanced the selectivity and sensitivity for the detection of ACTA in urine samples.

Rużycka et al. [17]: Cyanides are infamous for their highly poisonous properties. Accidental cyanide poisoning occurs frequently, but occasionally, intentional poisonings also occur. Inhalation of fumes generated by fire may also cause cyanide poisoning. There are many limitations in direct analysis of cyanide. 2-Aminothiazoline-4-carboxylic acid (ATCA), a cyanide metabolite, seems to be the only surrogate that is being used in the detection of cyanide because of its stability and its cyanide-dependent quality in a biological matrix. Unfortunately, toxic kinetic studies on diverse animal models suggest significant interspecies differences; therefore, the attempt to extrapolate animal models to human models may be unsuccessful. The aim of the present study was to evaluate the use of ATCA as a forensic marker of cyanide exposure. For this purpose, post-mortem materials (blood and organs) from fire victims (n = 32) and cyanide-poisoned persons (n = 3) were collected. The distribution of ATCA in organs and its thermal stability were evaluated. The variability of cyanides in a putrid sample and in the context of their long-term and higher temperature stability was established. The presence of ATCA was detected by using an LC-MS/MS method and that of cyanide was detected spectrofluorimetrically. This is the first report on the endogenous ATCA concentrations and the determination of ATCA distribution in tissues of fire victims and cyanide-poisoned persons. It was found that blood and heart had the highest ATCA concentrations. ATCA was observed to be thermally stable even at 90 °C. Even though the cyanide concentration was not elevated in putrid samples, it was unstable during long-term storage and at higher temperature, as expected. The relationship between ATCA and cyanides was also observed. Higher ATCA concentrations were related to increased levels of cyanide in blood and organs (less prominent). ATCA seems to be a reliable forensic marker of exposure to lethal doses of cyanide.

Cyanide determination in urine and plasma samples

Logue et al. [18] considered the cyanide metabolite 2-aminothiazoline-4-carboxylic acid (ATCA) is a promising biomarker for cyanide exposure because of its stability and the limitations of direct determination of cyanide and more abundant cyanide metabolites. They suggested a simple, sensitive, and specific method based on derivatization and subsequent gas chromatography-mass spectrometry (GC-MS) analysis was developed for the identification and quantification of ATCA in synthetic urine and swine plasma. The urine and plasma samples were spiked with an internal standard (ATCA-d (2)), diluted, and acidified. The resulting solution was subjected to solid phase extraction on a mixed-mode cation exchange column. After elution and evaporation of the solvent, a silylating agent was used to derivatize the ATCA. Quantification of the derivatized ATCA was accomplished on a gas chromatograph with a mass selective detector. Authors reported the suggested method produced a coefficient of variation of less than 6% (intra- and interassay) for two sets of quality control (QC) standards and a detection limit of 25 ng/ml. The applicability of the method was evaluated by determination of elevated levels of ATCA in human urine of smokers in relation to non-smokers for both males and females.

CI-GC-MS: An analytical method utilizing chemical ionization gas chromatography-mass spectrometry was developed Bhandari et al. [19] by for the simultaneous determination of cyanide and thiocyanate in plasma. Sample preparation for this analysis required essentially one-step by combining the reaction of cyanide and thiocyanate with pentafluorobenzyl bromide and simultaneous extraction of the product into ethyl acetate facilitated by a phase-transfer catalyst, tetrabutylammonium sulfate. The limits of detection for cyanide and thiocyanate were 1 μM and 50 nM, respectively. The linear dynamic range was from 10 μM to 20 mM for cyanide and from 500 nM to 200 μM for thiocyanate with correlation coefficients higher than 0.999 for both cyanide and thiocyanate. The precision, as measured by %RSD, was below 9 %, and the accuracy was within 15 % of the nominal concentration for all quality control standards analyzed. The gross recoveries of cyanide and thiocyanate from plasma were over 90 %. Using this method, the toxicokinetic behavior of cyanide and thiocyanate in swine plasma was assessed following cyanide exposure.

GC-MS: Exposure to cyanide can occur in a variety of ways, including exposure to smoke from cigarettes or fires, accidental exposure during industrial processes, and exposure from the use of cyanide as a poison or chemical warfare agent. Confirmation of cyanide exposure is difficult because, in vivo, cyanide quickly breaks down by a number of pathways, including the formation of both free and protein-bound thiocyanate. Youso et al. [20] developed a simple method to confirm cyanide exposure by extraction of protein-bound thiocyanate moieties from cyanide-exposed plasma proteins. Thiocyanate was successfully extracted and subsequently derivatized with pentafluorobenzyl bromide for GC-MS analysis. Thiocyanate levels as low as 2.5 ng mL^{-1} and cyanide exposure levels as low as 175 μg kg^{-1} were detected. Samples analyzed from smokers and non-smokers using this method showed significantly different levels of protein-bound thiocyanate ($p<0.01$). Authors claimed these results demonstrate the potential of this method to positively confirm chronic cyanide exposure through the analysis of protein-bound cyanide in human plasma.

Cyanide determination in breath samples

SIFT-MS: Chandran et al. [21] suggested selected ion flow tube mass spectrometry (SIFT-MS) technology to identify breath VOCs for the detection of HNSCC. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common form of cancer worldwide, with approximately 630,000 new cases diagnosed each year. Breath samples were obtained from HNSCC patients (N = 23) and healthy volunteers (N = 21). Exhaled alveolar breath samples were collected into FlexFoil®PLUS (SKC Limited, Dorset, UK) sampling bags from newly diagnosed, histologically confirmed, untreated patients with HNSCC and from non-cancer participants. Breath samples were analyzed by Selected Ion Flow Tube-Mass Spectrometry (SIFT-MS) (Syft Technologies, Christchurch, New Zealand) using Selective Ion Mode (SIM) scans that probed for 91 specific VOCs that had been previously reported as breath biomarkers of HNSCC and other malignancies. Authors analyzed the 91 compounds; the median concentration of hydrogen cyanide (HCN) was significantly higher in the HNSCC group (2.5 ppb, 1.6–4.4) compared to the non-cancer group (1.1 ppb, 0.9–1.3; Benjamini–Hochberg adjusted $p < 0.05$). A receiver operating curve (ROC) analysis showed an area under the curve (AUC) of 0.801 (95% CI, 0.65952–0.94296), suggesting moderate accuracy of HCN in distinguishing HNSCC from non-cancer individuals. There were no statistically significant differences in the concentrations of the other compounds of interest that were analyzed. Authors claimed the feasibility of SIFT-MS technology to identify VOCs for the detection of HNSCC.

GC–MS: Dong et al. [22] developed a simple gas chromatography–mass spectrometry (GC–MS) technique for the analysis of vapor phase mainstream cigarette smoke. The analysis includes two parts: (A) separation and identification of as many as possible compounds in vapor phase smoke, and (B) quantitative analysis of a selected number of analytes. Authors reported that the cigarettes are smoked using a Borgwaldt RM20/CS smoking machine using Federal Trade Commission (FTC), International Organization for Standardization (ISO), or other recommended conditions. The vapor phase smoke is separated from particulate phase smoke with a standard Cambridge pad. The vapor phase is collected in a gasbag, and then a precise volume (1 or 5 mL) is injected in a GC–MS system for separation and analysis. About 90 compounds are separated and identified in vapor phase smoke. A quantitative procedure was developed for acetaldehyde, 1-3 butadiene, acrolein, propionaldehyde, acetonitrile, acetone, isoprene, propionitrile, benzene, croton aldehyde, hydrogen cyanide (HCN), and styrene. Using appropriate standards, most of the other compounds identified in vapor phase smoke could also be quantitatively analysed.

SIFT-MS: Hydrogen cyanide (HCN) in exhaled breath has been proposed by Dummer et al. [23] as a biomarker for airway inflammation, and also a marker of the presence in the airways of specific organisms, especially Pseudomonas aeruginosa. However the production of HCN by salivary peroxidase in the oral cavity increases orally exhaled concentrations, and may not reflect the condition of the lower airways. Using SIFT-MS. Authors aimed to determine an appropriate single-exhalation breathing maneuver, which avoids the interference of HCN, produced in the oral cavity. Authors reported that the SIFT-MS Voice200™ is suitable for the online measurement of HCN in exhaled breath. In healthy volunteers, a significantly higher end exhaled HCN concentration was measured in oral exhalations compared to nasal exhalations (mean ± SD) 4.5 ± 0.6 ppb versus 2.4 ± 0.3 ppb, $p<0.01$. For the accurate and reproducible quantification of end exhaled HCN in breath a nasal inhalation to full vital capacity and nasal exhalation at controlled flow is recommended. This technique was subsequently used to measure exhaled HCN in a group of patients with chronic suppurative lung disease (CSLD) and known microbiological colonization status to determine utility of HCN measurement to detect P. aeruginosa. Median nasal end exhaled HCN concentrations were higher in patients with CSLD (3.7 ppb) than normal subjects (2.0 ppb). However no differences between exhaled HCN concentrations of subjects colonized with P. aeruginosa and other organisms were identified, indicating that breath HCN is not a suitable biomarker of P. aeruginosa colonization.

Španel et al. [24] measured the concentrations of ammonia, acetone, methanol, ethanol, propanol and hydrogen cyanide in the exhaled breath of 26 young adults of age 17/18 years, using selected ion flow tube mass spectrometry (SIFT-MS). Thus the concentration distributions have been constructed and are seen to be essentially log normal with median values in parts per billion (ppb), being ammonia, 317; acetone, 363; methanol, 238; ethanol, 104; propanol, 13; hydrogen cyanide (HCN), 8. There is a clear separation in the median breath ethanol levels between those volunteers who had ingested sugary food/drinks (109 ppb) and those who had not (48 ppb). These data are compared with the results of a study of the same breath compounds, excepting HCN, for a similar sized cohort of healthy volunteers within the age range of 20 to 60 years, which shows that the median levels of these compounds are lower in the young

adult volunteer cohort. These HCN measurements are the first to be made in the breath of healthy individuals. The potential implications of these combined results for clinical diagnosis are alluded to.

Smith and Spaněl [25] reviewed various aspects of SIFT-MS and described that selected ion flow tube mass spectrometry performs ambient analyses both accurately and rapidly. The underlying ion chemistry underpinning SIFT-MS through a discourse on the reactions of different classes of organic and inorganic molecules with H_3O^+, NO^+ and O^{2+} studied using the SIFT technique. Rate coefficients and ion products of these reactions facilitate absolute SIFT-MS analyses and can also be useful for the interpretation of data obtained by the other ambient analysis methods mentioned above. The essential physics and flow dynamics of SIFT-MS are described that, together with the reaction kinetics, allows SIFT-MS to perform absolute ambient analyses of trace compounds in humid atmospheric air, exhaled breath and the headspace of aqueous liquids. Authors reviewed the several areas of research that, through pilot experiments, are seen to benefit from ambient gas analysis using SIFT-MS.

Determination of hydrogen cyanide in air

SPME- GC–MS: Zain et al. [26] quantified the ambient HCN concentration in a residential area close to a gold mine using solid-phase micro extraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) and calculated it's potential health risks. Authors reported that air samples were collected at a distance of 0.1, 1.0 and 2.0 km away from the gold mine. All cyanide compounds were extracted using 75 μm carbowax/ polydimethylsiloxane-coated SPME fibre and analysed using GC–MS. Calibration curve was constructed using standard concentrations ranged between 5 and 500 μg L^{-1}. This method showed good linearity (r^2 = 0.999) and accuracy (recoveries = 84–119%), reproducibility (relative standard deviation < 11.5%) and the LOD was 0.16 ppbv. HCN was detected in 68% of samples ranging between 0.16 and 8.56 ppbv. HCN concentration was significantly higher ($p < 0.05$) for samples taken at 0.1 km away from the gold mine compared to concentrations at 1.0 and 2.0 km. The non-carcinogenic risk of HCN from air inhalation was negligible as the calculated hazard quotient was less than 1. Suggested method used in this study was sensitive enough to detect ambient HCN concentrations at levels which were below the reference concentration for long term inhalation exposure by the U.S. Environmental Protection Agency (0.72 ppbv) .The method found to be simpler and less time consuming method compared to the conventional sample preparation method, enabling rapid community exposure assessment.

GC-MS: Analysis of hydrogen cyanide in air in a case of attempted cyanide poisoning reported by Magnusson et al. [27]. A 32-year-old man attempted to poison his ex-girlfriend with hydrogen cyanide by hiding the pesticide Uragan D2 in her car. During the police investigation, chemical analysis of the air inside the car was performed. Hydrogen cyanide was detected through on-site air analysis using a portable Fourier transform infrared (FTIR) spectroscopy gas analyzer and colorimetric gas detection tubes. Furthermore, impinger air-sampling was performed for off-site sample preparation and analysis by gas chromatography-mass spectrometry (GC-MS). All three independent techniques demonstrated the presence of hydrogen cyanide, at concentrations of 14-20 ppm. Owing to the high volatility of hydrogen cyanide, the temperature and the time since exposure have a substantial effect on the likelihood of detecting hydrogen cyanide at a crime scene. The prevailing conditions (closed space, low temperature) must have supported the preservation of HCN in the car thus enabling the identification even though the analysis was performed several days after the hydrogen cyanide source was removed. The authors mentioned the applicability of combining on-site FTIR measurements and off-site GC-MS analysis of a crime scene in order to ensure fast detection as well as unambiguous identification for forensic purposes of hydrogen cyanide in air.

Determination of halides and cyanide ions

ESI-MS: Hiroki Hotta et al. [28] developed a electrospray ionization mass spectrometry (ESIMS) method for determination of halide ions (X^- = F^-, Cl^-, Br^- and I^-), where negative ions of the ternary complexes of group-13 elements, nitrilotriacetic acid (NTA), and halides were measured. In particular, these halides were simultaneously determined by measuring $[InX(nta)]^-$ and the limits of detection (LODs) were 1.1 μmol dm^{-3} for F^-, 0.32 μmol dm^{-3} for Cl^-, 3.8 nmol dm^{-3} for Br^-, and 1.6 nmol dm^{-3} for I^-, respectively. Authors extended this approach to the determination of CN^-, where the ternary complex of Cu^{II}, CN^- and 4-(2-pyridylazo) resorcinol (PAR), i.e., $[^{63}Cu^{II}(CN)(par)]^-$ (m/z 302) was measured. The LOD for CN^- was 20 nmol dm^{-3}.

Cyanide determination in water samples

GC–MS/MS: Kang and Shin [29] established a gas chromatography–tandem mass spectrometric (GC–MS/MS) method for the determination of cyanide in surface water. This method is based on the derivatization of cyanide with 2-(dimethylamino) ethanethiol in surface water. The following optimum reaction conditions were established by the authors: reagent dosage, 0.7 g L^{-1} of 2-(dimethylamino)ethanethiol; pH 6; reaction carried out for 20 min at 60 °C. The organic derivative was extracted with 3 mL of ethyl acetate, and then measured by using GC–MS/MS. Under the established conditions, the detection and quantification limits were 0.02 μg L^{-1} and 0.07 μg L^{-1} in 10-mL of surface water, respectively. The calibration curve had a linear relationship relationship with $y = 0.7140x + 0.1997$ and $r2 = 0.9963$ (for a working range of 0.07–10 μg L^{-1}) and the accuracy was in a range of 98–102%; the precision of

the assay was less than 7% in surface water. It was claimed by the authors that the common ions Cl^{-}, F^{-}, Br^{-}, NO_3^{-}, SO_4^{2-}, PO_4^{3-}, K^{+}, Na^{+}, NH_4^{+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Mn^{4+}, Mn^{2+}, Fe^{3+}, Fe^{2+} and sea water did not interfere in cyanide detection, even when present in 1000-fold excess over the species. Cyanide was detected and reported in a concentration range of 0.07–0.11 μg L^{-1} in 6 of 10 surface water samples.

Kang and Sang [30] developed novel derivatization method of free cyanide (HCN + CN^{-}) including cyanogen chloride in chlorinated drinking water with d-cysteine and hypochlorite. The optimum conditions (0.5 mM d-cysteine, 0.5 mM hypochlorite, pH 4.5, and a reaction time of 10 min at room temperature) were established by the variation of parameters. Cyanide (C^{13} N^{15}) was chosen as an internal standard. The formed β-thiocyano-alanine was directly injected into a liquid chromatography-tandem mass spectrometer without any additional extraction or purification procedures. Under the established conditions, the limits of detection and the limits of quantification were 0.07 and 0.2 μg/L, respectively, and the interlay relative standard deviation was less than 4% at concentrations of 4.0, 20.0, and 100.0 μg/L. The method was successfully applied to determine CN^{-} in chlorinated water samples. Authors reported the detected concentration range and detection frequency of CN^{-} were 0.20-8.42 μg/L (14/24) in source drinking water and 0.21-1.03 μg/L (18/24) in chlorinated drinking water.

HS-GC/MS: Jim Eaton [31] established an official test method for the State of Maine and claimed that to determine the concentration of dissolved cyanide in drinking water, the most suitable technique is headspace gas chromatography using mass selective detection (HS-GC/MS). The technique captures all cyanide compounds that form HCN under acidic conditions. Method ME355.01 is entitled Determination of cyanide in drinking water by GC/MS headspace analysis. The method was originally made available by the Centers of Disease Control (CDC) for the analysis of cyanide in whole blood, a method that is part of the technology transfer group at CDC involved with chemical terrorism. Method ME 355.01 has now been promulgated by the US EPA. The method involves the following steps: Samples are taken in 40 mL brown glass vials. 1 mL of a 1 M NaOH solution is added to preserve the sample, which is then stored at 4 °C in darkness until it is analysed. The sample must be analysed within seven days. Prior to the analysis, a 2-methyl aniline solution is added to the preserved sample. If the sample turns yellow it is discarded. To ensure efficient and accurate analysis, a suitable auto sampler capable of automated sample preparation should used, the Dual Rail Prep Station version of the GERSTEL Multipurpose Sampler (MPS) is listed in the official method. The MPS Prep Station is a two-in-one auto sampler, capable of performing both headspace sampling and liquid handling. The liquid handling steps can include, for example, the addition of an internal standard, a reagent, or a diluent. A standard GC/MS system was used for separation and determination of the analytes. Experimental conditions were selected: GC column: PLOT Q Column from Agilent, part number: #19091P-Q04 or equivalent Carrier gas: Helium (1.1 mL/min), constant flow Oven: 110 °C (0 min) – 4 °C/ min –130 °C (0 min) – 99 °C/min – 250 °C (1.79 min) MS Mode: Selected Ion Monitoring mode: (SIM) Mass traces: 0, 29 (internal standard), m/z: 27, and 26.

Conclusion

Exposure to cyanide can be verified by analysis of cyanide or one of its breakdown products from biological samples. This verification is important for medical, law-enforcement, military, forensic, research, or veterinary purposes. In the present scenario cyanide poisoning presents one of the most difficult challenges in disaster medicine and forensic science due to its high toxicity, fast acting, different sources of exposure and limitations of analytical methods for cyanide determination. However efforts have been made by the scientific community to over come with these challenges and generating research interest in different fields of science imposing multidisciplinary approach to study cyanide poisoning. Researches and suggested techniques is indicative of the interest in this field not only from forensic toxicologists and analytical chemists, but also researchers from diverse environmental, medical, and clinical arena.

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