Review Article

Recent updates on analytical methods for detection of cyanide in human blood

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Abstract

Cyanide is a toxic chemical; its poisoning may be as a result of natural processes and/or legal or illicit uses. Cyanide exposure can be verified by analysis of cyanide or one of its breakdown products from biological samples. This verification may be important for medical, police & forensic investigation purposes. Various reported methods for determination of cyanide exposure for humans in biological matrix but blood is found to be the most versatile biological sample used to determine cyanide exposure because the analyses of cyanide, thiocyanate, ATCA, and cyanide-protein adducts can each be performed on blood samples. In this review efforts have been made to summarise the development of procedures to enable quantitation of cyanide ions or its break down products in blood samples that can provide an up to date reference document to the forensic toxicologists.

Keywords: Analytical techniques, Colorimetry, Fluorimetry, Spectrophotometric method, Capillary electrophoretic determination, High Performance Liquid Chromatography, Capillary gas chromatography, Gas chromatography-mass spectrometry, Liquid chromatography–tandem mass spectrometry

Introduction

Cyanide is poisonous to humans and animals and its exposure can occur in various ways. In the environment, cyanides can be found in many different forms. In industries cyanide is used to make paper, textiles, and plastics. It is found in the chemicals, which are used to develop photographs. Similarly cyanide salts are utilized in metallurgy for electroplating, metal cleaning, and removing gold from its ore. Toxicity of cyanogenic plant is one of the most common plants poisoning among the grazing livestock. Cyanide is contained in cigarette smoke and the combustion products of synthetic materials such as plastics. Combustion products are substances givenoff when things burn (https://emergency.cdc.gov/agent/cyanide/basics/facts.asp).

Along with many legal industrial uses of cyanide, multiple illegal uses of cyanide exist, with terrorist acts garnering the most publicity For example, in 1982, cyanide was placed in bottles of Tylenol in the Chicago area, killing seven people and in 1995, cyanide was found in several Tokyo subway restrooms in the weeks following the release of the nerve agent sarin (Eckstein, 2004; Logue et al., 2010). Cyanide is also used in fishing for subsequent sale in the live fish trade. This practice involves using cyanide at sub-lethal doses to temporarily stun fish, making them easier to catch. This practice has been found in a number of countries, and causes irreversible damage to coral reefs by killing the algae that are necessary for coral to survive. It also kills smaller fish species (Logue et al., 2010; Barber and Pratt, 1998). Cases related to cyanide poisoning are also occasionally reported & investigated for police, medico legal and forensic investigations. Various types of analytical techniques have been suggested & applied for the analysis of cyanide in blood. In the present review we have selected some of the analytical methods applied for the detection of cyanide and it's the breakdown products in blood to provide an up to date reference document to the forensic toxicologists.

The present review consist of detail about techniques including Colorimetry, Fluorimetry, Spectrophotometric method, Capillary electrophoretic determination, High Performance Liquid Chromatography (HPLC-MS, HPLC-Fluorescence Detection, HPLC- Fluorimetric Detection), Head-space gas chromatography (Headspace gas chromatography with electron-capture detection, Headspace

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Gas Chromatography coupled to Flame Ionization Detector, Headspace Gas Chromatography & Spectrophotometric method),Capillary gas chromatography with cryogenic oven trapping. Gas chromatography-mass spectrometry (Gas chromatography with an electron-capture detection gas chromatography, GC-MS, Gas chromatography-mass spectrometry –TOF, Headspace Gas Chromatography-Mass Spectrometry (HP- GC-MS), Chemical ionization gas chromatography mass-spectrometry (CI-GC-MS), HS-SPME and gas chromatography/ mass spectrometry, ID- mass spectrometry, Isotope-dilution gas chromatography– mass spectrometry (ID GC/MS), Solid-phase micro extraction and gas chromatography/mass spectrometry, Electrospray Ionization Tandem Mass Spectrometry (ESI-MS-MS), Solid phase, micro extraction and gas chromatography/mass spectrometry HS-GC/MS) and Liquid chromatography–tandem mass spectrometry (LC-MS-MS).

Colorimetric method for Cyanide determination in blood

A colorimetric method for detection of cyanide in whole blood method based on the reaction of hydrogen cyanide with vitamin B12 was proposed. The hydrogen cyanide is released on acidification of the blood sample. The vitamin B12 was loaded on a strip of a commonly used filter paper by impregnating the strip in a 1.0% (w/v) solution of vitamin B12 under alkaline conditions. The limit of detection of this method was found to be 1.0 mg L−1 that is lower than the reported lethal blood concentration of cyanide (2.5 mg L−1). According to authors suggested method is free from interferences from the common gases including ammonia, carbon dioxide, arsine, phosphine and carbon monoxide, thiocyanate, organophosphate pesticides and nitrile-containing pesticides. This method was successfully employed for detection of cyanide in several cases reported to the forensic toxicology (Chaudhary et al., 2016). A rapid colorimetry method of cyanide in blood developed, which was applicable to patients exposed to toxic levels of cyanide, based on the König reaction, which produces a chromophore from cyanide as well as thiocyanate. The latter compound that is normally present in blood is confined to plasma. Therefore, its interference with determination of cyanide was eliminated by performing the assay on the erythrocytes, which contain most of the blood cyanide. It was also explored that cyanide was trapped in the erythrocytes and stabilized during the initial washing steps by conversion of haemoglobin to methaemoglobin with inorganic nitrite (Lundquist and Sörbo, 1989). A micro diffusion technique was suggested to separate and concentrate the nanomole amount of cyanide in whole blood and to permit its simplified colorometric estimation. With this technique cyanide was evaluated in whole blood from normal smokers, normal non-smokers and patients suffering from tobacco amblyopia. Authors also measured cyanide was in whole blood from vitamin B12 deficient and normally fed Wistar rats. From the experimental animal results and results from patients it was suggested that a more suitable index of cyanide exposure may be the alteration of plasma thiocyanate rather than the direct determination of whole blood cyanide (Pettigrew and Fell, 1973).

Fluorometric determination

A method was developed for the quantitative determination of cyanide ion in body fluids, especially blood, by fluorimetry. This method was based upon the transformation of cyanide ion into hydrocyanic acid, which then reacts with 2,3-naphthalenedialdehyde and taurine in a self-contained system. The 1-cyano-2-benzoisoindole derivate thus formed is suitable for fluorimetric measurement (lambda EX = 418 nm; lambda EM = 460 nm). According authors the fluorescence intensity can be determined by spectrophotometry or by high-performance liquid chromatography (HPLC) with fluorescence detection. The detection limit reported as 0.002 microg/mL. Linearity recorded from 0.002 to 1 microg/mL for spectrophotometry and from 0.002 to 5 microg/mL for HPLC with fluorescence detection. The coefficient of variation for repeatability was 8% or less. Authors claim that Thiocyanate and sulfide did not interfere, even at high concentrations (200 microg/mL). The method claimed to be suitable for both clinical and forensic purposes (Felscher and Wulfmeyer, 1998). A detailed procedure of a new simple fluorometric assay for cyanide in human blood, stomach contents and urine was presented which requires neither diffusion nor distillation of cyanide from samples; In this method small amounts of the samples were directly added to incubation mixtures, which were then heated at 80 degrees C for 45 min and acidified for fluorescence measurements. Suggested method said to be much more rapid than the previous ones by the authors. Some data was also presented on specificity and sensitivity of the present method (Suzuki et al., 1982).

A fluorometric method involving the catalytic conversion of pyridoxal to 4-pyridoxylactone was applied for use with biological fluids by employing micro diffusion analysis. The presence of the cyanide antagonist, sodium thiosulfate, interferes with the formation of the fluorophore. In order to circumvent this interference, the pH of the diffusion media was altered to selectively diffuse cyanide. After testing various acidifying agents, an acetate buffer (pH = 5.2) was determined to be satisfactory. The fluorometric method was then correlated with the classical colorimetric procedure by an in vivo study. Blood from mice treated with sodium nitrite and sodium thiosulfate, prior to receiving potassium cyanide, was analyzed by both procedures and no

significant difference was observed by the authors between the results of the two methods of analysis (Morgan and Way, 1980). Cyanide produces a fluorescent compound from pyridoxal (PAL) by a catalytic action. The velocity of the reaction could be measured by measuring the fluorescence intensity. For a concentration of cyanide below 10-5M, the apparent initial velocity of the reaction was maintained for 60 min at a concentration of 6×10-4M of PAL, and the initial velocity was proportional to the concentration of cyanide. A simple and sensitive fluorometric method for the determination of cyanide was devised based on the above findings. Cyanide was treated with PAL at pH 7.5 and the fluorescence intensity was measured at pH 10 (Takanashi and Tamura, 1970).

Several fluorogenic reagents such as p-benzoquinone and pyridoxal have been developed for the determination of cyanide ion. However their applicability is often limited by the low sensibility and by the necessity of the complicated procedure found in their previous study on the determination of sulfide ion it was found that cyanide ion showed fluorescence in the presence of taurine and o-phthalaldehyde (OPA). This fluorescence seems to be due to the formation of a fluorescent isoindole derivative because cyanide is known to give 1-cyano-2-methylisoindole when treated with methylamine and OPA. Therefore this fluorometric method for cyanide ion detection by using 0PA reaction was suggested. To 500µl of sample solution in a 1.5 µl glass stoppered test tube were added 100 µl of both mM taurine&1.5 mM OPA solution and 800 µl of 2-proponal. The fluorescence intensity was measured with excitation at 340nm and emission at 370nm (Sano et al., 1986).

Spectrophotometric method

A method based on the transformation, under the influence of a strong acid, of blood cyanide into very volatile hydrocyanic acid, followed by its diffusion in a Conway dish and stoichiometric complexation with hydroxocobalamin. The cyanocobalamin thus formed absorbs at a wavelength of 361 nm. The use of a standard curve prepared using the same biological matrix as the sample under investigation avoids several difficulties associated with the diffusion (e.g., time, temperature, biological matrix). Its rapidity, due to the coupling of the diffusion and coloration stages, allows the determination of cyanide in an emergency. Authors concluded that the technique proposed here, because of its rapidity, might be used in emergencies and allows the adaptation of treatment by hydroxocobalamin. Its advantages are that it is based on the formation of a very stable complex and that it is specific. Moreover, this reaction is used in the treatment of cyanide intoxications, where 5 g of hydroxocobalamin neutralizes 1 mg of cyanide. It is applicable to different situations, including hydrocyanic acid or cyanide intoxication, acetonitrile ingestion, antidotes (e.g., hydroxocobalamin, thiosulfate, dicobaltic EDTA), and it may be applied to other biological fluids, providing that the standard curve is prepared using the appropriate biological matrix and smoke inhalation due to fires (Lafarge et al.,1994). A simple, rapid, and highly sensitive method for determination of low parts per billion concentrations of CN- in equine blood and other biological fluids was developed. The analytical method is an adaptation of methods commonly in use and involves the evolution and trapping of gaseous hydrogen cyanide followed by spectrophotometric determination by autoanalyzer. The limit of quantitation of this method is 2 ng/mL in equine blood, and the standard curve shows a linear relationship between CN- concentration and absorbance ($r > .99$). The method throughput is high, up to 100 samples per day. Normal blood CN- concentrations in horses at pasture in Kentucky in October 2001 ranged from 3-18 ng/mL, whereas hayfed horses showed blood CN- levels of 2-7 ng/mL in January 2002. Blood samples from a small number of cattle at pasture showed broadly similar blood CN- concentrations. Intravenous administration of sodium cyanide and oral administration of mandelonitrile and amygdalin yielded readily detectable increases in blood CN- concentrations. This method is sufficiently sensitive and specific to allow the determination of normal blood CN- levels in horses, as well as the seasonal and pasture-dependent variations. The method should also be suitable for investigation of the toxicokinetics and disposition of sub acutely toxic doses of CN- and its precursor cyanogens in the horse as well as in other species (Hughes et al., 2003).

Measurement of a stable, toxic metabolite, 2-aminothiazoline-4-carboxylic acid (ATCA), in an attempt to circumvent the challenge of directly determining cyanide concentrations in aqueous media was carried out. This study was focused on the spectrophotometric ATCA determination in the presence of cyanide, thiocyanate (SCN (-)), cysteine, rhodanese, thiosulfate, and other sulfur donors. The method involves a thiazolidine ring opening in the presence of p-(hydroxy-mercuri)-benzoate, followed by the reaction with diphenylthiocarbazone (dithizone). The product is spectrophotometrically analyzed at 625 nm in carbon tetrachloride. The calibration curve was linear with a regression line of $Y = 0.0022x$ ($R(2) = 0.9971$). Interference of cyanide antidotes with the method was determined. Cyanide, thiosulfate, butanethiosulfonate (BTS), and rhodanese did not appreciably interfere with the analysis, but SCN (-) and cysteine significantly shifted the standard curve. According to authors this spectrophotometric method has shown promise as a substitute for the measurment of the less stable cyanide (Baskin et al., 2006). It was pointed that conventional methods do not meet the

clinical need for rapid cyanide measurements and they reported a procedure which can provide a result in 10 min. n the proposed method a sample of blood (100 µL) was mixed with H3P04, containing a surfactant, HCN is trapped in an alkaline mixture of 1,2-dinitrobenzene and 4-nitrobenzaldehyde in 2-methoxyethano!. The catalytic action of cyanide, which produces purple 2-nitrophenylhydroxylamine, is stopped with acetone after 6 min. The absorbance measured at 560 nm against a water blank using a CE 2010 spectrophotometer (Cecil Instruments Ltd, Cambridge, UK). Shows a linear relationship with the cyanide concentration, but the slope varies with the ambient temperature. Since KCN added to both 50 mmol/L NaOH and blood gives similar assay results, any inaccuracy arising from changes in temperature can be avoided by running standards at the same time as the blood sample (Vesey et al., 1999). A spectrophotometric method proposed for the determination of hydrogen cyanide in air. Hydrogen cyanide present in air is absorbed in 0.002 M sodium hydroxide solution, which is then treated with bromine. The cyanogen bromide so formed reacts with pyridine to form glutaconic aldehyde. The latter is condensed with anthranilic acid to form a yellowish orange polymethine dye, which shows a maximum absorbance at 400 nm. Beer's law is obeyed in the range 0.4–4 p.p.m. Other analytical parameters have been investigated. The method has been successfully applied to the determination of hydrogen cyanide in biological samples, *e.g.*, cysteine and whole blood (Kaur et al., 1987).

Capillary electrophoretic determination

A new method involving headspace single-drop micro extraction (SDME) with in-drop derivatization and CE was developed for the preconcentration and determination of free cyanide. An aqueous microdrop (5 µL) containing Ni(II)-NH(3) (as derivatization agent), sodium carbonate and ammonium pyromellitate (as internal standard) was used as the acceptor phase. The extracted cyanide forms a stable Ni (CN)(4) (2-) complex, which is then determined by CE. Common experimental parameters (sample and acceptor phase pH, extraction temperature, extraction time and sample ionic strength) affecting the extraction efficiency were investigated. Using headspace SDME, free cyanide can be effectively extracted from the neutral solutions, i.e. without the acidification of the sample, which often is prone to errors due to incomplete liberation and artefactual cyanide production. Proposed SDME-CE method provided about 58-fold enrichment in 20 min. The calibration curve was linear for concentrations of CN (-) in the range from 0.25 to 20 micromol/L (R (2) = 0.997). The LOD (S/N = 3) was estimated to be 0.08 micromol/L of CN (-). Such detection sensitivity is high enough for free cyanide determination in common environmental and physiological samples. Finally, headspace SDME was applied to determine free cyanide in human saliva and urine samples with spiked recoveries in the range of 91.7-105.6%. The main advantage of this method is that sample clean up, preconcentration and derivatization procedures can be completed in a single step. In addition, the proposed technique does not require any sample pre-treatment and thus is much less susceptible to interferences compared to existing methods (Jermak et al., 2006). Determination of thiocyanate in human serum, urine and saliva was reported. The determinations were performed in a fused-silica capillary [64.5 cm (56 cm effective length) x 75 microm] using 0.1 M beta-alanine-HCl (pH 3.50) as a background electrolyte, separation voltage 18 kV (negative polarity), temperature of capillary 25 degrees C and direct detection at 200 nm. Serum samples were 10-times diluted with deionised water and deproteinised with acetonitrile in the ratio 1:2. Urine and saliva samples need only 20-fold dilution with deionised water. The proposed method was successfully applied to the determination of thiocyanate in various human serum, saliva and urine samples (Glatz et al., 2001).

High Performance Liquid Chromatography

High performance liquid chromatography method was developed, using cyanide derivatisation, to the determination of plasma pyridoxal-5-phosphate (PLP) concentrations as an indicator of vitamin B6 adequacy. Blood samples were taken at the Institute, at Health Centres, or in the volunteer's home. Subjects: 51 adolescent, 131 adult, 68 non-institutionalized elderly and 44 aged (>73 y) volunteers were recruited from local authority schools, local Health Centres and General Practitioners. The mean PLP recovery was 92.8%. The intra- and inter-assay coefficients of variation were 2.8% and 5.2% respectively. Mean PLP concentrations for males and females, respectively, were: adolescents (13 ± 14 y), 36.4 and 43.5 nM; adults (20 ± 64 y), 39.2 and 40.0 nM; elderly (68 ± 73 y), 34.8 and 35.3 nM; aged (>73 y), 57.8 and 49.0 nM. Percentages of subjects with PLP concentrations < 34.4 nM were over 26% in all population groups. Mean vitamin B6 intakes (mg=g protein intake), as assessed by weighed dietary records, were all above reference nutrient intakes (15 mg=g protein). It was concluded these authors that an HPLC method, using cyanide derivitisation, has been applied to the determination of plasma PLP. Comparisons of results for local population groups with current cut-off values for plasma PLP, show large numbers of volunteers at risk of vitamin B6 deficiency although this is not reacted by vitamin B6 intakes calculated from food tables. The 34.4 nM cut-off value for value for plasma PLP indicating deficiency is questioned (Bailey et al, 1999). High-performance liquid chromatographic-mass spectrometric procedure for the determination of cyanide (CN) in whole

blood was proposed and reported. After the addition of K13C15N as internal standard, blood was placed in a micro diffusion device, the inner well of which was filled with a mixture of taurine (50mM in water)/ naphthalene-2, 3-dicarboxaldehyde (NDA, 10mM in methanol)/methanol/ concentrated (approximately 20%) ammonia solution (25:25:45:5, v/v). Concentrated H2SO4 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 vial were pipetted and directly injected onto a Nova Pak C18 HPLC column. Separation was performed by a gradient of acetonitrile in 2mM NH4COOH, 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 13C15N, respectively. Limits of detection and quantitation for blood CN recorded 5 and 15 ng/mL, respectively (Tracqui et al., 2002).

Simultaneous determination of inorganic ions including cyanide by photometric ion chromatography was useful for a cyanide analysis in drinks, but not applicable to that in blood, because of its poor resolution for cyanide and chloride. A method was suggested to determine cyanide in blood, Authors adopted a selective and sensitive method for cyanide based on a fluorometric reaction with 2,3-naphthalenedialdehyde (NDA) and taurine to afford 1-cyanobenz [*f*] isoindole derivative. Cyanide was extracted from blood by adding water and methanol to whole blood, and then derivatized with NDA and taurine. The cyanide derivative was analyzed on a reversed-phase high performance liquid chromatograph system with fluorescence detector. In the analysis of standard solutions, the reagent blank showed a minor peak of cyanide corresponding to ca. 0.04ng/ml. Thus the lower detection limit for cyanide standard solution was 0.1ng/ml as 2.5-fold concentration of the reagent blank peak. The peak seemed to be due to trace cyanide in reagents however, it was so minor peak that it didn't interfere with cyanide determination in blood. The calibration curve for cyanide standard solution was linear in the range 0.1-200 ng/ml. In the blood analysis, the method enabled us to determine cyanide from healthy persons level (ca. 10 ng/ml) to fatal level (ca. 3000 ng/ml) employing the same treatment (Chinaka and Takayama, 1977). A sensitive method was developed for the simultaneous determination of oxidizable inorganic anions (sulphide, thiocyanate, thiosulphate and nitrite) was developed by use of high-performance liquid chromatography and fluorimetric detection based on the formation of fluorescent cerium (III) by a redox reaction with cerium (IV). The detection limits are 0.1 nmol for both thiocyanate and nitrite, 0.3 nmol for thiosulphate and 0.8 nmol for sulphide per 10-µl injection volumes. This system can be utilized for the determination of salivary thiocyanate and nitrite and serum thiocyanate (Tanabe et al., 1988).

High-performance liquid chromatography and fluorometric detection

Method for the determination of cyanide and thiocyanate in blood plasma and red cells of humans was established. It involved high-performance liquid chromatography and fluorometric detection by the König reaction. Calibration curves for cyanide and thiocyanate were linear in the range 1–200 pmol and 2–300 pmol, respectively. Clean-up methods for the determination of cyanide and thiocyanate in red cells were also developed. These methods were applied for the determination of cyanide and thiocyanate in the blood of smokers and non-smokers (Toida et al., 1984).

A simple and sensitive high-performance liquid chromatographic (HPLC) method was established for the trace determination of thiocyanate anion as a fluorogenic derivative. It is based on the chemical derivatization of thiocyanate anion with 3-bromomethyl-7-methoxy-l,4-benzoxazin-2-one. The resulting derivative was separated by a Nova-Pak C18 reversed-phase column. Optimization conditions for the derivatization of thiocyanate anion were investigated & reported by HPLC with fluorimetric detection. The linear range for the quantitation of thiocyanate anion was 1-0.05 nmol in 0.1 mL of sample; the detection limit (with a signal-to-noise ratio of 5) of a 20-pt injected aliquot found approximately 3.3 ± 1.2 fmol. Authors claimed application of the method to the analysis of thiocyanate anion in saliva and plasma proved to be feasible (Chen et al., 1996).

Head-space gas chromatography

A method describing determination of cyanide in blood proposed i.e. Headspace gas chromatography with electron capture detector applied and reported. This method involved transformation of cyanide into cyanogen chloride by reacting hydrogen cyanide with chloramine-T on a stick of filter paper in the space above the blood in the headspace vial. The recovery was 84-96% and the coefficient of variation was 3.3-7.2%. The limit of quantitation was about 0.01 mg cyanide/l (Felby, 2009). A rapid headspace (HS) gas chromatographic (GC) assay for the determination of cyanide in human whole blood obtained from both cadaveric and intoxicated subjects was proposed. The poison was extracted from whole blood samples by HS technique after 10 min incubation at 80°C with an HCl (1N) acidic solution supplemented with 30 g L−1 of sodium chloride and 10 mg L−1 of isopropanol as internal standard. A SGE WAX capillary column (30 m × 0.32 mm ID × 0.5µm df) was used. The Flame Ionization Detector (FID) was set at 250°C. The

detection and the quantification limits were 0.02 and 0.2 mg L^{-1}, respectively. In the within-day study, the coefficients of variation for three different whole blood cyanide concentrations varied between 3.2 and 6.5%, whereas those in the day-to-day study varied between 4.8 and 6.3%. The assay was performed in less than 20 min per sample, which appears to be suitable for emergency toxicological tasks involved in diagnosis of cyanide poisoning (Sadeg and Belhadj-Tahar, 2009).

Headspace gas chromatography with electron-capture detection

An improved method has been developed for the quantitative determination of cyanide in human blood by headspace gas chromatography with electron-capture detection. In this novel method, cyanide was detected after conversion of hydrogen cyanide into cyanogen chloride by a reaction with chloramine T. The advantage of this new procedure lies in the fact that hydrogen cyanide formation and chlorination are carried out in a single step and in the same reaction medium. This method is simple, rapid, and specific for cyanide and does not suffer from any interference by cyanate and thiocyanate. The detection limit is 5 micrograms/L. The detection response is linear from 5 to 1000 micrograms/L, and the within-run coefficient of variation in this range is 8% or less. This method is particularly useful for routine diagnostic analysis of biological samples in case of acute cyanide poisoning (Odoul et al., 1994).

Headspace Gas Chromatography coupled to Flame Ionization Detector

A simple, rapid and reliable method for quantitation of cyanide was developed on a headspace gas chromatograph coupled to a flame ionization detector using a HP-Innovax (Polyethylene glycol bonded) column on an Agilent 7890A GC. Cyanide in blood or other matrices was liberated by conversion of potassium cyanide to the volatile hydrogen cyanide (HCN) through addition of 5N sulfuric acid in a headspace vial and analyzed using an Agilent G1888 headspace auto-sampler. HCN gas diffuses into the headspace above the specimen in a sealed vial based on Henry's Law of partial pressure (Shafi et al., 2015).

Headspace Gas Chromatography & Spectrophotometric method

Blood samples of two cases were analysed and reported, preliminarily by a classical spectrophotometric method (VIS) and by an automated headspace gas chromatographic method with nitrogen-phosphorus detector (HS-GC⁄NPD). In the former, hydrogen cyanide (HCN) was quantitatively determined by measuring the absorbance of chromophores forming as a result of interaction with chloramine T. In the automated HS-GC⁄NPD method, blood was placed in a headspace vial; internal standard (acetonitrile) and acetic acid were then added. This resulted in cyanide being liberated as HCN. The spectrophotometric (VIS) and HS-GC⁄NPD methods were validated on post-mortem blood samples fortified with potassium cyanide in the ranges 0.5–10 and 0.05–5 lg⁄mL, respectively. Detection limits were 0.2 lg⁄mL for VIS and 0.05 lg⁄mL for HS-GC⁄NPD. This study showed that results obtained by means of the two procedures were insignificantly different and that they compared favorably. They are suitable for rapid diagnosis of cyanide in post-mortem cases as reported by authors (Gambaro et al., 2007).

Capillary gas chromatography with cryogenic oven trapping

Cyanide, one of the most important toxic substances, has been found measurable with high sensitivity by capillary gas chromatography (GC) with cryogenic oven trapping upon injection of headspace (HS) vapour samples. The entire amount of cyanide in the HS sample could be cryogenically trapped prior to on-line GC analysis. A 0.5-mL volume of blood in the presence or absence of cyanide and propionitrile (internal standard, IS) was added to a vial containing 0.25 mL of distilled water, 0.3 g of Na2-SO4, 0.2 mL of 50% H_3PO_4, and 0.1 g of ascorbic acid (when needed), and the mixture was heated at 70 degrees C for 15 min. A 5-mL volume of the HS vapour was introduced into a GC capillary column in the split less mode at -30 degrees C oven temperature that was programmed up to 160 degrees C for GC analysis with nitrogen-phosphorus detection. A sharp peak was obtained for cyanide under the present conditions, and backgrounds were very clean. The extraction efficiencies of cyanide and IS were 2.89-3.22 (100 or 500 ng/mL) and 2.42%, respectively. The calibration curve showed good linearity in the range of 25-1000 ng/mL and the detection limit was approximately 2ng/mL. The coefficients of intraday and interlay variations were 2.9 and 11.8%, respectively. The mean blood cyanide level measured for actual fire victims was 687 ± 597 ng/mL (mean ± SD, n = 9). Endogenous blood cyanide concentration for healthy subjects was 8.41 ± 3.09 ng/mL (mean +/- SD, n = 6) (Ishii et al., 1998).

Gas chromatography and mass spectrometry

A sensitive and simple method for determining cyanide and its major metabolite, thiocyanate, was devised 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 analyzed 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 (Kage et al., 1996). An 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 ($^{1}H^{13}C^{15}N$) was proposed by 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 (Lobger et al., 2008).

A method for determination of cyanide in whole blood, erythrocytes, and plasma after stabilization of cyanide by addition of silver ions was suggested. 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 (Lindquist et al., 1985). Cyanide, its metabolite thiocyanate and azide in human biological fluids are commonly analyzed by gas chromatography–mass spectrometry (GC–MS) after derivatization with pentafluorobenzyl bromide using extractive alkylation. However, the reported methods have some drawbacks. These authors examined these reported methods and attempted to establish a more reliable method to determine the levels of the above compounds in human whole blood. They also examined the applicability of the established method to NAGINATA–GC–MS screening. The deproteinization method, internal standard (IS), the cause of column damage, and the effect of the addition of ascorbic acid were examined, and then the best procedure was selected. The obtained data, including mass spectra, retention times and calibration curves were registered to the database of NAGINATA software. Authors reported that the analysis of cyanide in whole blood was possible only when the blood was deproteinized with trichloroacetic acid. A high recovery of thiocyanate and azide was obtained without the deproteinization step. K13C15N (for cyanide) and tribromobenzene (for thiocyanate and azide) were selected as ISs. The column damage caused by the phase transfer catalyst was successfully eliminated by passing the catalyst containing solution through an ethyl benzoic sulfonic silica gel column. By these improvements, a more reliable determination method was established. All anions were rapidly identified using NAGINATA software, and the approximate concentration of each compound in whole blood was obtained at the same time. In conclusion Authors mentioned that because NAGINATA–GC–MS screening can rapidly identify these poisons without using toxic compounds as reference standards, it should be useful in forensic and emergency medicine laboratories (Kudo et al., 2017).

GC/MS-TOF

In a case of cyanide poisoning 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 labelled 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 0.432µg/mL in blood and 0.786µg/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 5.3µg/mL. The quantitative determination was then confirmed by GC/NPD and the concentration of cyanide resulted to be 5.5µg/mL in blood and 1.7µg/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 (Roda et al., 2018)

Chemical ionization gas chromatography mass-spectrometry (CI-GC-MS)

An analytical method utilizing chemical ionization gas chromatography-mass spectrometry was developed 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 1µM and 50 nM, respectively. The linear dynamic range was from 10 µM to 20 mM for cyanide and from 500 nM to 200µM 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. Authors reported the gross recoveries of cyanide and thiocyanate from plasma were over 90 %. Using this method the toxicokinetic behaviour of cyanide and thiocyanate in swine plasma was assessed following cyanide exposure (Bhandari et al., 2012).

HS-SPME and gas chromatography/mass spectrometry

An improved qualitative and quantitative method for cyanide determination in blood using solid phase, micro extraction and gas chromatography/mass spectrometry was developed. The method claimed to be simple, fast and sensitive enough for the rapid diagnosis of cyanide intoxication in clinical and forensic toxicology by the authors. 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. Authors validated this method 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 and reported only in aqueous solutions. The assay was 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. It was claimed that good intra and interassay precision was obtained, always <8% (Frison, 2006).

Isotope dilution-mass spectrometry (ID-MS)

A direct and sensitive method for the determination of blood cyanide by isotope dilution was developed. Ion this method the blood is placed in a headspace vial, and K13C15N 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 & reported 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%. A round-robin exercise was carried out to compare the performance of this method with others currently in use in other clinical laboratories (Dumas et al., 2005).

Isotope-dilution gas chromatography-mass spectrometry.(ID GC/MS)

An automated procedure based on isotope-dilution gas chromatography– mass spectrometry (ID GC/MS) for the accurate and rapid determination of CN in whole blood was proposed. In this method a known amount of isotopically labelled 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 5min 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 was 15 min for a single injection. Authors demonstrated 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 <4%. The automated ID GC/MS method can accurately and rapidly quantify nanogram per gram to microgram per gram concentrations of CN in blood as mentioned by authors (Murphy et al., 2006).

ESI-MS-MS

Determination of Cyanide in Blood by Electrospray Ionization Tandem Mass Spectrometry after direct injection of Dicyanogold (ESI-MS-MS) was developed. In this method five micro liters of blood was hemolyzed with 50 µL of water, then 5 µL of 1 M tetramethylammonium hydroxide solution was added to raise the pH of the hemolysate and to liberate CN (-) from methemoglobin. CN (-) was then reacted with NaAuCl (4) to produce dicyanogold, Au (CN)(2)(-), that was extracted with 75 µL of methyl isobutyl ketone. Ten microliters of the extract was injected directly into an ESI-MS-MS instrument and quantification of CN (-) was performed by selected reaction monitoring of the product ion CN (-) at m/z 26, derived from the precursor ion Au (CN)(2)(-) at m/z 249. CN (-) could be measured in the quantification range of 2.60 to 260µg/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 ± 2.41µg/L for six healthy non-smokers, 3.08 ± 1.12µg/L for six CO gas victims, 730 ± 867µg for 21 house fire victims, and 3,030 ± 97µg/L for a victim who ingested NaCN. Authors explained 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 (-) in the blood, gastric content and urine were 78.5 ± 5.5, 11.8 ± 0.5, and 11.4 ±

0.8µg/L, respectively (Minakata et al., 2011).

Liquid chromatography–tandem mass spectrometry

LC–MS–MS method coupled to online extraction for cyanide determination in blood. Authors mentioned that method involved 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, 25µL of blood were diluted in water and deproteinized with methanol. Following derivatization with NDA and taurine for 10 min at 4°C, 100µL 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 2600ng/mL with limit of detection of 10ng/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 13C15N as the IS make this method very suitable for cyanide determination in blood and could be useful in forensic toxicology (Lacroix et al., 2011).

Micro-chemiluminescence determination

A reactant volume self-controlled micro-device developed and applied to the flow injection chemiluminescence (CL) for determination of cyanide in whole blood. A mini distiller was fabricated for cyanide extraction from the blood samples with the extraction efficiencies of cyanide > or = 98%. A fluidic control platform with air driving was fabricated. The described system showed the features of easy fabrication, undiluted sample injection, safe analysis operation, and suitability for automatic cyanide analysis. The calibration curve showed linearity in the cyanide concentration range of 5.0 x 10(-7) to 5.0 x 10(-5) mol l(-1) with the detection limits (3sigma) of 2.3 x 10(-7) mol l(-1). CL peak-height precision was 1.9% R.S.D. (n = 11) at the 1.0 x 10(-6) mol l(-1) cyanide level. Authors claimed that suggested devices were applied to the analysis of cyanide in rabbit whole blood samples and the results agreed well with those obtained from official method (Zhang and Luo, 2005).

Forensic Marker of Cyanide Exposure

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, toxicokinetic studies on diverse animal models suggest significant interspecies differences; therefore, the attempt to extrapolate animal models to human models may be unsuccessful. A method was proposed to study 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 (Rużycka et al., 2017).

Potential of using 2-aminothiazoline-4-carboxylic acid (ATCA) as a novel biomarker/forensic biomarker for cyanide poisoning was demonstrated. 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 analyzed 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 ± 28ng/mL (n = 3) to 413-± 66ng/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 ± 0.1µg/g for kidney samples,

1.6 ± 0.1µg/g for brain samples, 1.8 ± 0.2µg/g for lung samples, 2.9 ± 0.1 µg/g for heart samples, and 3.6 ± 0.9 µg/g for liver samples Authors suggested their findings shows that ATCA has the potential to serve as a plasma biomarker / forensic biomarker for cyanide poisoning (Yu et al., 2012).

Discussion and Conclusion

Various analytical techniques suggested for the analysis of cyanide in human blood or biological matrix is very much significant for forensic, clinical, research, law enforcement,

Table 1. Summary of different techniques used for cyanide detection

Sample matrix	Methods	Limit of detection	Advantage	References
Whole Blood	HPLC-MS	5 and 15 ng/mL	Sensitive Suitable for toxicological analysis	Tracqui et al., 2002
Blood	Head space GC/ECD	5 micrograms/L	Rapid, and specific for cyanide and does not suffer from any interference by cyanate and thiocyanate	Odoul et al., 1994
Blood	Spectrophotometry	0.07µg/mL	Its rapidity, might be used in emergencies & may be used for other biological fluids	Laforge et al., 1994
Plasma	Spectrophotometry (thiocyanatecyanide determination)	0.07 ppm	96% thiocyanate recovery	Pettigrew and Fell, 1972
Whole blood	Colorimetry	1.0 mg L^{-1}	Suitable for forensic toxicology, free from interferences from the common gases	Chaudhary et al., 2016
Blood	Fluorimetry	0.002 microg/mL	More rapid than the previous ones	Suzuki et al., 1982
Blood	Spectrophotometry	2 ng/mL in equine blood,	Suitable for investigation of the toxicokinetics	Hughes et al., 2003
Physiological samples Saliva urine	SDME-CE method	0.08 micromol/L	technique does not require any sample pre-treatment and thus is much less susceptible to interferences compared to existing methods	Jermak et al., 2006
Blood	HPLC-MS	0.1 ng/ml	Free from interferences	Chinaka and Takayama, 1977
Saliva	HPLCwith Fluorimetric Detection	0.1 nmol	Suitable for the determination of salivary thiocyanate and nitrite and serum thiocyanate	Tanabe et al., 1988
Saliva plasma	HPLCwith Fluorimetric Detection	3.3 ± 1.2 fmol.	Sensitive and suitable for forensic toxicological analysis	Chen et al., 1996
Blood	Head-Space gas chromatography	0.2 mg L^{-1}	Suitable for both cadaveric and intoxicated subjects	Sadeg and Belhadj-Tahar, 2009
Blood	Headspace gas chromatography with electron capture detector	0.01 mg/L	84-96% recovery	Felby, 2009
Blood	Capillary gas chromatography with cryogenic oven trapping	2ng/mL	Sensitive & suitable for toxicological assay.	Ishii et al., 1998
Blood	Gas chromatography and mass spectrometry	0.01 and 0.003 µmol/ml	A sensitive and simple method for determining cyanide and its major metabolite	Kage et al., 1996
Blood, urine and bile	GC/MS-TOF	0.432 µg/mL in blood and 0.786 µg/mL in bile	Suitable for toxicological analysis	Roda et al., 2018
Plasma	CI-GC-MS	Cyanide and thiocyanate 1 µM and 50 nM,	Toxicokinetic behaviour of cyanide and thiocyanate in swine plasma can be assessed	Bhandari et al., 2012.
Blood	Isotope dilution-mass spectrometry (ID-MS)	0.3 micromol/L	Better performance with comparison to other clinical laboratories	Dumas et al., 2005
Blood, Gastric content and Urine	ESI-MS-MS	Blood, gastric content and urine 78.5 ± 5.5, 11.8 ± 0.5, and 11.4 ± 0.8 µg/L,	Suitable for toxicological analysis	Minakata et al., 2011
Blood	LC–MS–MS	10 ng/mL	Suitable for small blood sample& time saving.	Lacroix et al., 2011
Blood	Micro-chemiluminescence determination	2.3 x 10(-7) mol l(-1)	Results agreed well with those obtained from official method	Zhang and Luo, 2005
Blood	Colorimetry	Toxic level of cyanide	Applicable to patients exposed to toxic levels of cyanide	Lundquist and Sörbo, 1989
Blood	Fluorometric determination	0.002 microg/mL	Suitable for both clinical and forensic purposes	Felscher and Wulfmeyer, 1998
Plasma	HPLC	92.8% recovery	Suitable for Plasma pyridoxal-5-phosphate (PLP) determination	Bailey et al., 1999
Blood	HS-GC/NPD	0.2 lg/mL for VIS and 0.05 lg/mL for HS-GC/NPD.	Suitable in post-mortem cases	Gambaro et al., 2007
Blood	HS-SPME and gas chromatography/mass spectrometry	0.05–10µg/mL	Good intra and interassay precision was obtained, always <8%. Simple, fast and sensitive method suitable for diagnosis of cyanide intoxication in clinical and forensic toxicology	Frison, 2006
Biological samples	LC-MS/MS	1.2 ± 0.1µg/g for kidney	Suitable for ATCA in biological samples	Yu et al., 2012

and veterinary purpose. It is therefore selection of the analytical method is important factor in forensic toxicological analysis. Apart from this one of the most important aspects of the cyanide ion analysis is preparation of samples for analysis because it is related to the fact that cyanide ions are not stable ones, and they occur in various forms. The presence of matrix interferences must be also considered in the preservation procedure (Ewa Jaszczak, 2014). To get accurate results, careful sample preparation and storage of biological samples containing cyanide or its metabolites is an important key element. Additionally a considerable problem in the analysis of cyanide and thiocyanate is their inter conversion, which can be during sample preparation and storage and leads to inaccurate result (Pettigrew and Fell, 1973, Boxer and Rickards, 1952). One more factors that is artificial formation of thiocynate from HCN, there are a number of methods to help prevent artificial formation of cyanide during storage, and if samples containing cyanide are to be stored before analysis, methods to prevent cyanide formation should be considered. It was demonstrated that artificial formation of HCN from thiocyanate in blood and later observed that ascorbic acid prevents artifactual cyanide formation at temperatures below 63◦C (Seto et al., 1993; Seto, 1995; Seto, 1996). It was also found that pre-treatment of blood samples with water and methanol was successful in preventing artifactual formation of cyanide from thiocyanate (Sano et al., 1992). The artifactual formation or degradation of ATCA does not appear to be a problem as the stability of ATCA in biological samples under a number of storage conditions has been established. Some factors that will influence the analytical technique to use are cellular absorption and detoxification kinetics, sampling and analysis time, sample storage time and conditions, sample matrix, interferences, sensitivities. Careful sample preparation and storage of biological samples containing cyanide or its metabolites is a important issue to get authentic results. Cyanide is most often analysed for the determination of cyanide exposure and for breakdown products of cyanide. It was also suggested that although cyanide metabolites may offer longer half-lives, they also have a number of drawbacks. Care should be taken when selected any method to consider not just the parameters of the analytical method but also the toxicokinetics of cyanide and its metabolites (Logue Hinkens, 2008). Some suggestions have been given for the analysis of delayed analysis of biological samples, because of the rapid detoxification of cyanide from blood samples, a sample for cyanide analysis should be collected quickly after exposure and analysis should be performed as soon as possible. However if analysis of cyanide cannot be performed quickly and storage of biological samples is necessary, the following should be considered: Volatility and nucleophilicity of cyanide, Cyanide concentration varies in biological components and Potential for cyanide formation during storage. Additionally, production and transformation of cyanide must be considered when interpreting results for post-mortem cyanide analysis (Logue, 2010).

So far as the wide range of methodologies proposed for the determination of CN in biological specimens, older colorimetric and spectrophotometric techniques still remain useful, especially for screening purposes in clinical situations. Now several separation procedures have become available, and they have substantially improved both the sensitivity and specificity of this analysis. Mostly there is headspace gas chromatography (HS-GC) with electron capture, nitrogen-phosphorus, or mass spectrometric detection. Some times as an alternatively liquid chromatography has seldom been applied for CN determination, except for a few methods involving fluorometric detection after complexation of the analyte by the pyridine/barbiturate reaction or by naphthalene-2, 3-dicarboxaldehyde in the presence of taurine. An unequivocal and accurate determination of CN in whole blood by means of high-performance liquid chromatography with mass spectrometric detection (HPLC-MS) was proposed (Tracqui et al., 2002). 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 An alternative approach that can help to minimize false positive (no cyanide is involved but cyanide is detected) and false negative (cyanide is involved but cyanide is not detected) results, is to detect stable biomarkers of cyanide, rather than cyanide itself. Thiocyanate (SCN-), 2-aminothiazoline-4-carboxylic acid (ATCA), and cyanide-protein adduct in biological fluids and tissues have been reported as alternative biomarkers for cyanide exposure and poisoning. SCN- is the major cyanide metabolite found in blood. However, SCN- is also a natural metabolite of non-cyanide mediated pathways and thus is not a good marker for cyanide exposure (Yu et al, 2012). In the present paper we have reviewed some of the methods that will explore advantages and disadvantages of the suggested methods that may be informative as well as useful to the forensic toxicologists.

Conflict of interest: Not declared

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