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Recent advances in analytical methods for cyanide determination in different matrices: A Review

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Abstract

The extreme toxicity of cyanide, its wide industrial application as well as its continued illegal use generate research interest in different fields of science, imposing multidisciplinary approach to study cyanide poisoning. This seminar paper presents new data about cyanide exposure, toxicology, and antidote development. Cyanide concerned research in environmental and forensic sciences along with medicine closely depends on the recent achievements in cyanide determination methods. Newly reported cyanide detection systems and sample pretreatment procedures for environmental, biological and plant samples are summarized. The main requirements to analytical systems for cyanide determination and the trends in analytical research are also discussed.

Key words: analytical methods, cyanide, matrices

1. Introduction

Cyanide refers to a monovalent anion consisting of carbon and nitrogen atoms with triple covalent bonds. Cyanide is very reactive and readily forms metalloid cyanide complexes and organic compounds. The chemical composition of cyanide in environmental samples is affected by factors such as pH, temperature, trace metal content, and the presence of sulfur or sulfur

compounds. A multitude of cyanide species of varying stability may be present in a sample. For these reasons cyanide testing can be a challenging analytical task. Methods for the determination of cyanide are defined by the relative strength of metal complexes. From an analytical perspective cyanide compounds are broadly classified into three categories; total cyanide, available or weak acid dissociable (WAD) cyanide, and free cyanide.

Cyanides comprise a wide range of compounds of varying degrees of chemical complexity and toxicity, all of which contain a CN moiety, to which humans are exposed in gas, liquid, and solid form from a broad range of natural and anthropogenic sources. Daily, people may be exposed to low levels of cyanides from foods, smoking and other sources. Lethal exposures to cyanides result only from accidents, suicides or homicides. Inhalation of cyanide gas, especially within an enclosed space, poses a significant health risk. Ingestion of food and beverages containing cyanide can also cause serious health effects. Nowadays, sodium cyanide has been still used illegally for fishing in some south-east Asia countries. Cyanide fishing is a fast method to stun and collect fish, but practice causes irreversibly damaging of the coral reefs (Mak et al., 2005).

Although the cyanide containing water discharge is strictly regulated and pre-treatment procedures are strongly recommended, some industrial accidents and illegal wastes discharge have been reported. Cyanide poisoning presents one of the most difficult challenges in disaster medicine and forensic science, due to its high toxicity, fast acting, a number of possible sources of exposure and some limitations of analytical methods for cyanide determination. The aim of this seminar paper is to summarize the main anthropogenic and natural sources of cyanide releasing into environment, biochemical basis of cyanide poisoning and available antidotes for its treatment. The recent achievements in cyanide determination in biological fluids, environmental objects and plants are also reviewed and trends in method development are discussed.

2. Cyanide exposure

Cyanide containing compounds, mainly hydrogen cyanide and sodium or potassium cyanides, are widely used in the industry: in ore extracting processes for the recovery of gold and silver, electroplating, case-hardening of steel, base metal flotation, metal degreasing, dyeing, and printing, in the production of chelating agents, in the synthesis of organic and inorganic

chemicals. Hydrogen cyanide is also used as a fumigant in ships, railroad cars, large buildings, grain silos, and flour mills, as well as in the fumigation of peas and seeds in vacuum chambers. Anthropogenic sources of cyanide release to the environment are diverse: gaseous waste or waste water from manufacturing and processing industries, emissions from municipal solid waste incinerators, biomass burning, fossil fuel combustion, including vehicle emissions (Baum et al., 2007), fumigation operations, and the production of coke or other coal carbonization procedures. Hydrogen cyanide is formed during the incomplete combustion of nitrogen containing polymers, such as certain plastics, polyurethanes, and wool (Koskinen-Soivi et al., 2005).

Hydrogen cyanide is present in cigarette smoke (Xu et al., 2006). It is one of the 44 harmful substances in cigarette smoke which inhibits several respiratory enzymes and is a major ciliatoxic agent, which cause changes in the epithelial lining of certain organs of the body. The amount of cyanide in cigarette smoke might directly affect people's health, especially the central nervous system. Studies of workers exposed chronically to hydrogen cyanide have reported a range of nonspecific neurological effects that include headache, dizziness and paresthesiae (Pritchard, 2007).

Principal natural sources of cyanides are over 2600 plant species, including fruits and vegetables that contain cyanogenic glycosides (cyanogens), which can release cyanide on hydrolysis when ingested. Among them, cassava (tapioca, manioc) and sorghum are staple foods for millions of people in many tropical countries. Hydrogen cyanide is released into the atmosphere also from natural biogenic processes from higher plants, bacteria, and fungi (Ganjewala et al., 2010).

The majority of the population is exposed to very low levels of cyanide in the general environment. There are, however, specific subgroups with higher potential for exposure. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogen glycosides (WHO, 2004). The cassava root (tapioca) contains a sufficient amount of cyanogens to require special processing to reduce the danger of toxicity. The maximum permissible limit of cyanogen content in cassava flour is 10 mg HCN/kg (WHO, 2004). The edible portions of dietary plant species

commonly used in the European countries contain relatively low levels of cyanogen glycosides, although some pits and seeds of common fruits contain significantly higher concentrations.

The cyanogens content of apricot and choke cherry kernels are high enough to cause acute intoxication, especially in children (WHO, 2004). Cyanide is also found in canned stone fruits. A dangerous dose of 20 almond kernels containing 29 mg HCN/kg have been recently reported (Morandini, 2010). Livestock poisoning due to cyanogenic glycoside dhurrin in sorghum and in sudangrass is well documented (Goff et al., 2011). Flaxseed is a multi-purpose crop and its consumption is beneficial for human health, but some cultivars contain high concentration of cyanogens that restrict their daily dose or their use in animal feed mixtures (Herchi et al., 2012). Other subgroups with greatest potential for exposure include those in the vicinity of accidental or intended releases from point sources, active and passive smokers, and fire-related smoke inhalation victims. Workers may be exposed to cyanides during fumigation operations and the production and use of cyanides in many industrial processes (WHO, 2004). Probably the commonest cause of cyanide poisoning in the world is through inhaled smoke in confined spaces during fires affecting domestic and industrial buildings (Lindsay et al., 2004).

The majority of recent studies support blood cyanide concentration of less than 0.026 μg/mL in healthy subjects. Raised blood cyanide concentration is a clinical feature of smoke inhalation and inhaled hydrogen cyanide gas may prove to be fatal. In fire death cases, toxicological data from the victims, such as their carboxyhemoglobin and blood cyanide levels, can provide the Fire Investigator with important scientific evidence to further a determination of the origin and cause of the fire. As discussed in Guide for Fire and Explosion Investigations (NFPA 921, 2008): a relationship exists between the nature of a fire, i.e., smoldering, flaming, post-flashover, and the production of toxic gases such as carbon monoxide and hydrogen cyanide. However, cyanide stability comes into question when the investigator has to interpret toxicological results from fire victims due to changes in cyanide concentration over time in postmortem victims and stored blood samples (McAllister et al., 2008).

Different cyanide exposure models to car emissions in open and closed areas have also been proposed by (Baum et al., 2007). The cyanide concentration in air above the acute toxicity level was obtained for a residential garage model: 192 μg HCN/m^3 over 3 h of an idle-running car. Some drugs contain cyanide or substances which can be converted to cyanide within the body, for example, sodium nitroprusside ($Na_2Fe(CN)_5NO$) which is sometimes administered

intravenously during the critical care treatment of hypertension. However, toxic effects of this drug have been reported (Sani et al., 2011), originally ascribed to the nitroso moiety or to various decomposition products such as cyanide, thiocyanate, and nitrite. It was postulated that the iron atom of the nitroprusside complex reacts with the free sulfhydryl groups (SH) in erythrocytes and releases cyanide in vivo by nonenzymatic reaction. Cyanide salts such as sodium cyanide (NaCN) and potassium cyanide (KCN) are associated with ingestive poisoning. Cyanides are used as suicidal but also as homicidal agents (Gill et al., 2004) particularly among healthcare and laboratory workers, and they can potentially be used in a terrorist attack. It is also still used in cases of illegal euthanasia. Recently, a case report on a person who was not very familiar with chemicals, especially not with cyanides, has demonstrated the acquisition of professional information via the internet, enabling a suicide with a complex procedure by inhalation of HCN (Musshoff et al., 2011).

3. Biochemical basis for cyanide poisoning

Cyanides are well absorbed via the gastrointestinal tract or skin and rapidly absorbed via the respiratory tract. Once absorbed, cyanide is rapidly and ubiquitously distributed throughout the body, although the highest levels are typically found in the liver, lungs, blood, and brain. There is no accumulation of cyanide in the blood or tissues following chronic or repeated exposure (Baskin et al., 2008).

High concentrations of cyanide may produce giddiness, headaches, unconsciousness and convulsions with paralysis of the central respiratory center. Clinical features include coma, respiratory arrest, and cardiovascular collapse. Cyanide ion toxicity is mediated primarily by its high affinity for the ferric moiety of cytochrome C oxidase in mitochondria, a key component in oxidative respiration. This stable but reversible interaction blocks the last stage in the electron transfer chain, resulting in cellular hypoxia and shift from aerobic to anaerobic cellular respiration, leading to cellular ATP depletion, lactic acidosis as well as cell and tissue death (Pritchard, 2007).

The most important route of cyanide excretion is by formation of thiocyanate, which is subsequently excreted in the urine. Thiocyanate formation is catalyzed directly by the enzyme rhodanese and indirectly via a spontaneous reaction between cyanide and the persulfide sulfur products of the enzymes 3-mercaptopyruvate sulfurtransferase and thiosulfate reductase. Minor pathways for cyanide detoxification involve reaction with cystine to produce aminothiazoline- and iminothiazolidinecarboxylic acids and combination with hydroxycobalamin (vitamin B_{12a}) to form cyanocobalamin (vitamin B_{12}); these end-products are also excreted in the urine (WHO, 2004). Combined, these metabolic routes detoxify 0.017 mg of cyanide per kilogram of body weight per minute in the average human (1.19 mg/min in a 70-kg person). After a single brief exposure to a low concentration of hydrogen cyanide from which an individual recovers quickly, no long term health effects are anticipated. Intoxication following deliberate ingestion of sodium or potassium cyanide has been reported to cause severe neurological impairment. A slow recovery from severe dystonia syndromes arising from cyanide intoxication has been noted in some cases (Pritchard, 2007).

Hydrogen cyanide in breath has been also suggested as a diagnostic tool for cyanide poisoning and for cyanide-producing bacterial infections (Stamyr et al., 2009). The major metabolite of cyanide, thiocyanate, is considered to be more stable than cyanide in vivo, but it can be introduced by routes other than cyanide metabolism, making it difficult to use as a marker of cyanide exposure. Cyanide also forms a minor metabolite, 2-amino-2-thiazoline-4-carboxylic acid, which is relatively stable and has good potential as a biomarker for cyanide exposure (Logue et al., 2005). Recently, thiocyanate protein adducts has been proposed as a long-term repository for information regarding cyanide exposure (Youso et al., 2010).

4. Cyanide antidotes

Cyanide produces a rapid onset of toxicity and thus requires vigorous and immediate treatment to prevent the toxic syndrome. Rapid removal from further exposure, administration of general support measures including 100% oxygen, and administration of specific antidotes in critically impaired casualties effectively reverse the effects of exposure. A series of newer antidotes both

alone or in conjunction with sodium thiosulfate treatment have been examined and classified into three major groups (Hall et al., 2009): (i) methemoglobin inducers: sodium nitrite, amyl nitrite, and 4-dimethylaminophenol promote the formation of methemoglobin which binds cyanide and so keeps it from binding to cellular cytochrome oxidase. However, they are reported to be very slow acting and associated with severe side effects (Bhattacharya and Vijayaraghavan, 2002): (ii) cobalt containing compounds: dicobaltedetate (cobalt EDTA) and hydroxocobalamin. Cobalt acts as a chelating agent for cyanide, and bounded cyanide is excreted in the urine. Dicobaltedetate have been shown to be potentially toxic, but hydroxocobalamin has recently been approved as a safe and effective cyanide antidote (Des Lauriers et al., 2006): (iii) cyanohydrin formers: alpha-ketoglutarate reacts with cyanide to form nontoxic cyanohydrin derivatives and its promising role as an alternative treatment for cyanide poisoning has been reported (Bhattacharya and Vijayaraghavan, 2002).

Many of the existing antidotes for cyanide poisoning are highly toxic themselves particularly when they are given at such doses that there is no remaining cyanide on which they can act (Lindsay et al., 2004). Sometimes the antidotes are given before obtaining the results from blood tests and thus they are in inappropriate quantity. Sometimes the antidote administration is delayed and the damage is done either by cyanide or by antidotes. During the delay between diagnosis and administration, cyanide has been metabolized and the required dose of antidote has invariably altered.

5. Analytical methods for cyanide determination

5.1 Cyanide determination in environmental samples

The specificity of cyanide as an environmental pollutant is of special concern, due to the different toxicity of cyanide-containing substances, from one side, and from other side, to the fact that the cyanide quantification depends on the analytical method used (Zheng et al., 2003). Cyanide pollutants have been officially classified into three main groups depending of their toxicity and environmental fate: (i) free cyanide - including HCN, alkaline and alkaline earth cyanides; (ii) weak acid dissociable cyanide (WAD) - a collective term for free cyanide and metal-cyanide complexes ($Ag(CN)^{2-}$, $Cu(CN)_4^{3-}$, $Cd(CN)_4^{2-}$, $Zn(CN)_4^{2-}$, $Hg(CN)_4^{2-}$, $Ni(CN)_4^{2-}$),

which easily release HCN under slightly acidic environmental conditions; and (iii) total cyanide each potential source of HCN regardless of its originion (US EPA, 2004).

The term “cyanide” refers to all CN groups that can be determined analytically as cyanide ion (CN) via spectrophotometric or electrochemical measurements, usually following appropriate sample pre-treatment to release cyanide ion. The Environmental Protection Agencies have imposed maximum contaminant levels (MLC) for cyanide discharge into the environment. The MLC for WAD cyanide vary from 0.05 to 0.07 μg/L for drinking water and in the range between 200-500 μg/L for waste water. The MCL for total cyanide is much higher – 1 mg/L. The group of WAD cyanide has been a subject of special consideration as the assessment of environmental risk and efficiency of detoxification procedures depend on its analytical quantification.

The facts mentioned above highlight the main demands to cyanide determination methods in environmental objects: (i) high sensitivity to reach the low MLC; (ii) high selectivity to analyze a great variety of matrices; (iii) capability for speciation to quantify toxic cyanides; (iv) implementation in portable analytical devices to allow on-site analysis in real time. In the past few years, a variety of new cyanide sensors and improved cyanide determination methods have been reported. Nevertheless, it is not easy to respond to all of the requirements above. Recently, a review presenting the available methods for cyanide determination and assessing their flexibility to application in automated portable analyzers has been published (Surleva, 2009).

The potential of electrochemical detection is specially emphasized in view of its suitability for automation and miniaturization. In portable devices the amperomeric detection has been given preference regardless its low selectivity, which calls for cyanide separation and an on-line method by flow-injection, ligand exchange, and amperometric detection has been officially approved (US EPA, 2004). New flow-injection cyanide selective detectors obtained by thin-layer electrochemical deposition technique have been recently proposed (Neshkova et al., 2006).

The sensors are fully competitive with amperometric detection as far as the lower linear limit, sample throughput, and sensitivity are concerned. Moreover, the potentiometric detectors offer

additional advantages: selective response (so that the separation step could be omitted and thus the equipment simplified) and cyanide speciation. Due to the high sensitivity of UV-Vis spectroscopy a lot of research was done in attempt to improve selectivity, analysis time or to develop environmentally friendly procedures. A comparative study of some new and some established spectrophotometric assays for environmental cyanide was reported by (Drochioiu et al., 2008): (i) the Aldridge method and its variants with pyridine and pyrazolone; (ii) isonicotinate-barbiturate method that was useful to detect minute amounts of cyanide in vivo and in vitro; (iii) the reaction of cyanide ion with ninhydrin, which was proved to be fast, simple, highly selective, and free from most interference, but under reducing conditions; (iv) picric acid-based assay which was described to be highly selective, but yet less sensitive; (v) combined resorcinol-picric acid method which showed improved sensitivity. Although a lot of work has to be done to propose a robust method, these sensors show very low detection limit coupled with good selectivity, small sample volumes and rapid response. They work on "turn-off and–on" principle and are extremely suitable for portable signaling devises in dangerous environment. Analytical methods for cyanide determination in environmental samples are summarized in Table 1.

Table 1. Analytical methods for cyanide determination in environmental samples

Methods	Samples	LOD, µg/mL	Range, µg/mL	RSD, %	Recovery, %	Reference
Spectrophotometry	Tap, mineral and waste water	0.007	0.01-0.5	2-4	97-109	Abbasiet al., 2010
Spectrophotometry	Drinking water	0.11	0.26-6.5	2	-	Absalan et al., 2010
Spectrophotometry naked eye detection	Drinking water	0.03	4-8	-	95-105	Isaad et al., 2011
Voltammetry	Industrial waste	0.0002	0.001-3.9	1.4	98-104	Noroozifal et al., 2011
Spectrofluorimetry naked eye detection	Drinking water	0.008	0.5-4.7	2	99	Li et al., 2011

UV-Vis	Tap, bottle and ground water	16	50-2000	2.3	99-109	Ma and Dasgupta, 2010

5.2 Cyanide determination in biological samples

Human fluids contain cyanide due to different sources of cyanide exposure. Apart from sodium nitroprusside therapy (as a hypotensive agent) and ingestion of cyanide salt in the context of suicidal or homicidal attempts, the main sources of exposure are smoke from fires or cigarette smoking, accidental inhalation of hydrocyanic acid in the metal and plastic industries, and ingestion of various types of food such as cassava, cherry, or almond. Blood cyanide concentration is essential information in medicine and forensic science. Although the state of the objects for analysis is completely different, medical and forensic cyanide analyses have the same difficulties:

(i) First, sample storage and pretreatment significantly affect the results of the analysis. Prior to detection, cyanide needs separation from hemoglobin. This separation is most commonly performed by acidification using microdifusion in a Conway cell or nitrogen carrying into an alkaline trap solution. The acidification process is prone to errors due to incomplete releasing or artificial cyanide production. (ii) Second, standard methods for cyanide determination in blood are time consuming and cannot provide adequate data on real time basis. Many of the methods described in the literature are highly sensitive but do not have upper calibration limits high enough to be used in cyanide fatalities. Besides cyanide assay has to differentiate between bound and unbound cyanide to provide data for cyanide antidotes administration.

The postmortem specimens most frequently analyzed for cyanide in forensic toxicology are blood, spleen, liver, and brain. Blood cyanide concentrations lower than 0.25 μg⁄mL are considered normal, and those between 0.25 and 2–3 μg/mL as elevated, but not ordinarily causing death. Concentrations above 3 μg⁄mL are consistent with death in the absence of other relevant or toxicological findings (Gambaro et al., 2007). Animal tissues are other forensic targets for analyzing, especially when illegal use of cyanide compounds in the environment is

concerned (Mak et al., 2005). Therefore, cyanide determination in forensic analysis and cyanide monitoring at very low levels are of great importance (Meng et al., 2009).

The analytical techniques for cyanide detection in blood published before 2004 have been critically reviewed by (Lindsay et al., 2004). Here we present the latest achievements in cyanide determination in biological samples reported (Table 2). In attempt to improve efficiency and accuracy of the sample pre-treatment procedures a hollow fiber-protected headspace liquid-phase microextraction, a headspace single-drop microextraction or solid-supported liquid-liquid extraction combined either with capillary electrophoresis or chromatographic separation were proposed. Interesting approach for cyanide liberation without acidification is an enzymatic degradation of free and complexed cyanide (Mak et al., 2005).

Another research direction is aimed at the development of sensitive and selective detection systems. The lowest detection limit of 0.3 ng/mL was reported for capillary electrophorese with UV detection (Meng et al., 2009). The widest linear concentration range is reported for gas chromatography/mass spectrometry: 0.05–10 μg/mL (Frison et al., 2006) and 0.1–20 μg/mL (Liu et al., 2009). A high selective nafion-modified electrochemical sensor for cyanide determination at physiological pH without separation was described by (Lindsay and O'Hare, 2006), but additional validation in blood samples is needed. Cyanide instability in post-mortem blood samples was studied and sodium fluoride was proposed to be added to blood samples obtained from fire victims to reduce cyanide instability due to bacteriological activity (McAllister et al., 2011).

Chromatography, notably gas chromatography, has been particularly important in the measurement of cyanide in complex, especially biological samples. Uses with MS detectors have already been discussed in the foregoing; here we discuss use with two selective detectors, the nitrogen-phosphorus detector (NPD), and the ECD (Ma and Dasgupta, 2010).

Matrix isolation of analyte cyanide is typically achieved by acidification of the sample to produce HCN. The headspace can then be sampled either directly or via an SPME fiber. Further derivatization is not needed because the NPD responds sensitively to HCN. The GC-NPD approach has been widely used for cyanide determination in clinical and forensic needs. Many methods based on this principle and incremental improvements thereof have been published in the past. While during the period of this review no major novelties in GC-NPD based approaches were reported, the following are noteworthy. Compared results from a classical spectrophotometric method with those from an automated headspace GC-NPD approach and found them to be statistically equivalent. Headspace SPME sampling followed by GC-NPD analysis for the simultaneous determination of cyanide, acetonitrile, cis- and trans- crotononitrile, allylnitrile and butyronitrile at low µg/L concentration on rat and mice blood. The maximum RSD was less than 12% across the analytes and LOD less than 3 µg/L throughout (Ma and Dasgupta, 2010).

The ECD is more sensitive for appropriately derivatized analytes and tends to be more robust and stable than the NPD. HCN does not directly respond to the ECD and must be derivatized. While there was no dramatic new development, there were some important application papers. Applied headspace GC-ECD to measure cyanide and reported on cyanide distribution in various in human blood, kidney, brain, urine, and stomach content. Used chloramine-T to convert cyanide in cigarette smoke to cyanogen chloride, which is taken up in n-hexane, this was then analyzed by capillary GC coupled to a micro ECD. Ma and Dasgupta (2010) utilized the same derivatization principle to determine cyanide in blood except that the derivatization was conducted on a strip of filter paper in the sample vial headspace.

Table 2. Analytical methods for cyanide determination in biological samples

Methods	Samples	LOD, µg/mL	Range, µg/mL	RSD, %	Recovery, %	Reference
Gas chromatography	Whole blood	0.01	0.01-0.2	3-7	84-96	Felby, 2009
Gas chromatography/mass spectrometry	Plasma and urine	0.04	0.1-20	7	91-116	Liu et al., 2009

Electron captured electrophoresis	Urine and saliva	0.26	2.6-520	5.3-7	92-103	Ma and Dasgupta, 2010

5.3 Cyanide determination in plants

The human's health authorities pay special attention on cyanogens as toxic food constituents, as some cyanogenic plants are staple food in some countries and the population is exposed to high level risk of cyanide intoxication. The analysis of plant and the estimation of its cyanogen content have specific problems related to the need of: (i) hydrolysis and separation of cyanogens or produced cyanide from complex matrices, and (ii) sensitive detection systems. Most of the cyanide related diseases are reported in developing countries, so the availability of the analytical devises to small plants farms is of special importance.Some summaries of the methods for cyanogenic glycoside determination (although not exhaustive ones) can be found in (Herchi et al., 2012). Methods for determination of cyanogenic glycosides in plants and cyanide in foods are presented in Table 3.

Table 3. Methods for determination of cyanogenic glycosides in plants and cyanide in foods.

Methods	Samples	Cyanogenic compounds	Analyte	Range, µg/mL	Reference
Spectrophotometry enzymatic assay	Cassava roots	Amygdalin linumarin	Total cyanide	0.08-2.6	Tatsuma et al., 2000
Spectrophotometry picrate method	Cassava flour	Linumarin	Total cyanide	0.1-50	Bradury, 2009
GC spectrophotometry	Sorghum, sudan grass, forage	Dhurrin	Total cyanide	0.04-1.8	Goff, et al 2011

The main trends in the research on cyanogen determination could be summarized as: (i) development of sample pre-treatment procedure suitable for large range of matrices and a great number of cyanogens; (ii) development of efficient cyanide liberation and separation procedures; (iii) development of sensitive and selective detection systems suitable for analyzing small quantities of samples; (iv) development of low cost and easy to maintain equipment.

Cyanogenic glycosides can be determined directly by various chromatographic methods. An advantage of chromatographic method is the quantification of cyanogenic glycosides in their native form. Its wide application is limited for a lack of cyanogenic glycoside standards or their high cost. Indirect cyanogenic glycosides determination, also referred as determination of the plant cyanogenic potential, is based on quantification of HCN released after acidic or enzymatic hydrolysis of cyanogen glycosides (Table 3).

Efficient extraction and complete hydrolysis is the key for accurate determination of plant cyanogens. Spectrophotometric detection after different color formation reactions is the most widely used in total cyanogens determination: picrate paper assay (Bradbury and Denton, 2011), picrate based solid state detection (Abban et al., 2011); combined picrate/resorcinol method. Recently, the nynhidrin based method has specially modified for determination of total cyanogens in plants (Surleva and Drochioiu, 2012). A spontaneous enzymatic hydrolysis (at pH 6-8) was combined with extraction using bicarbonate solution or microdiffusion separation. The method is fast, cheap and environmentally friendly. Non-toxic reagents have been used. No special training or sophisticated instrumentation was needed.

6. Conclusions

This seminar paper provides a good example of how the demands of ecology, forensic science and medicine motivate the research and development of new analytical methods and instrumentation. Rapid cyanide analysis in blood or breath is ripe for new attractive approaches. There are fast acting antidotes for cyanide poisoning, whether from smoke inhalation or exposure to a weapon of terrorism. It is vital to determine blood or breathe cyanide levels fast and accurately so that an appropriate dose of the antidote can be readily determined. Physiological

half-life of free cyanide is short and concentration can be affected by storage conditions and many other factors. It is crucial to rapidly analyze such samples, if it possible in situ. The same demand is imposed also by ecology. Due to different toxicity of industrial cyanide containing pollutants, different detoxification procedures have to be applied so that the ecological equilibrium will not be disturbed at a large scale.

Quickly available and highly reliable information about cyanide contamination is required for this purpose. 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 analyzers.

The new colorimetric/fluorimetric probes working on “turn-of-and-on” principle have a lot of promise to be used in small alarm devices or spot tests. However, a lot of research is needed to validate them in real samples, e.g., air, natural waters, industrial wastewater, biological fluids like urine, blood, saliva, etc.

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