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Method OIA-1677 Available Cyanide by Flow Injection, Ligand Exchange, and Amperometry

Acknowledgments

This method was developed by Michael Straka of OI Analytical in cooperation with Emil Milosavljevic and Ljiljana Solujic of the University of Nevada Reno Mackay School of Mines and guidance from William A. Telliard of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST). Additional assistance in preparing the method was provided by DynCorp Information and Enterprise Technology and Interface, Inc..

Disclaimer

This Method has been reviewed and approved for publication by the Analytical Methods Staff within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Introduction

Method OIA-1677 was developed by ALPKEM, a division of OI Analytical, in cooperation with the University of Nevada Reno Mackay School of Mines, as a way to measure available cyanide without the interference problems of the currently approved available cyanide methods. EPA proposed the use of Method OIA-1677 on July 7, 1998 (63 FR 36809). EPA is approving the use of Method OIA-1677 for compliance monitoring under Section 304(h) of the Clean Water Act. Method OIA-1677 is an additional test procedure for measuring the same cyanide species as are measured by currently approved methods for cyanide amenable to chlorination (CATC). In some matrices, CATC methods are subject to significant test interferences. Method OIA-1677 has been added to the list of approved methods because it is more specific for available cyanide, is more rapid, measures cyanide at lower concentrations, offers improved safety, reduces laboratory waste, and is more precise and accurate than currently approved CATC methods.

Requests for additional copies of this Method should be directed to:

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PB99-132011

Note: This Method is performance based. The laboratory is permitted to omit any step or modify any procedure provided that all performance requirements in this Method are met. The laboratory may not omit any quality control tests. The terms "shall" and "must" define procedures required for producing reliable data at water quality criteria levels. The terms "should" and "may" indicate optional steps that may be modified or omitted if the laboratory can demonstrate that the modified method produces results equivalent or superior to results produced by this Method.

Method OIA-1677

Available Cyanide by Flow Injection, Ligand Exchange, and Amperometry

1.0 Scope and Application

1.1 This method is for determination of available cyanide in water and wastewater by flow injection, ligand exchange, and amperometric detection. The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, Resource Conservation and Recovery Act, Comprehensive Environmental Response, Compensation and Liability Act, and Safe Drinking Water Act.

1.2 Cyanide ion (CN^-), hydrogen cyanide in water (HCN_{aq}), and the cyano-complexes of zinc, copper, cadmium, mercury, nickel, and silver may be determined by this method (see Section 17.2.1).

1.3 The presence of polysulfides may prove intractable for application of this method.

1.4 The method detection limit (MDL) is 0.5 µg/L and the minimum level (ML) is 2.0 µg/L. The dynamic range is approximately 2.0 µg/L (ppb) to 5.0 mg/L (ppm) cyanide ion using a 200 μL sample loop volume. Higher concentrations can be determined by dilution of the original sample or by reducing volume of the sample loop.

1.5 This method is for use by analysts experienced with flow injection equipment or under close supervision of such qualified persons.

1.6 The laboratory is permitted to modify the method to overcome interferences or to lower the cost of measurements, provided that all performance criteria in this method are met. Requirements for establishing method equivalency are given in Section 9.1.2.

2.0 Summary of Method

2.1 The analytical procedure employed for determination of available cyanide is divided into two parts: sample pretreatment and cyanide detection. In the pretreatment step, ligand-exchange reagents are added at room temperature to 100 mL of a cyanide-containing sample. The ligand-exchange reagents form thermodynamically stable complexes with the transition metal ions listed in Section 1.2, resulting in the release of cyanide ion from the metal-cyano complexes.

Cyanide detection is accomplished using a flow-injection analysis (FIA) system (Reference 15.6). A 200-μL aliquot of the pre-treated sample is injected into the flow injection manifold of the system. The addition of hydrochloric acid converts cyanide ion to hydrogen cyanide (HCN) that passes under a gas diffusion membrane. The HCN diffuses through the membrane into an alkaline receiving solution where it is converted back to cyanide ion. The cyanide ion is monitored amperometrically with a silver working electrode, silver/silver chloride reference electrode, and platinum/stainless steel counter

electrode, at an applied potential of zero volt. The current generated is proportional to the cyanide concentration present in the original sample. Total analysis time is approximately two minutes.

2.2 The quality of the analysis is assured through reproducible calibration and testing of the FIA system.

2.3 A flow diagram of the FIA system is shown in Figure 1.

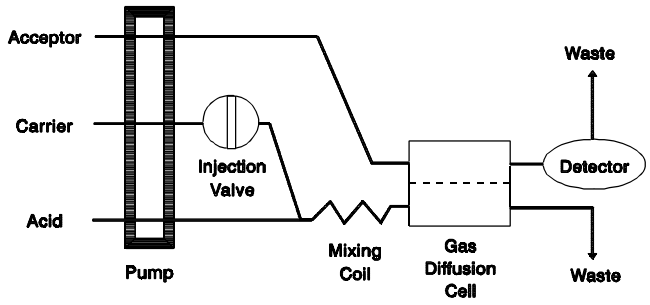

Figure 1. Flow injection Manifold used in the quantification of cyanide in the pretreated sample. Carrier (0.1 M HCl); Acid (0.1 M HCl); Acceptor (0.1 M NaOH).

3.0 Definitions

Definitions for terms used in this method are given in the glossary at the end of the method.

4.0 Interferences

4.1 Solvents, reagents, glassware, and other sample-processing hardware may yield artifacts that affect results. Specific selection of reagents or purification of these reagents may be required.

4.2 All materials used in the analysis shall be demonstrated to be free from interferences under the conditions of analysis by running laboratory blanks as described in Section 9.4.

4.3 Glassware is cleaned by washing in hot water containing detergent, rinsing with tap and reagent water, and drying in an area free from interferences.

4.4 Interferences extracted from samples will vary considerably from source to source, depending upon the diversity of the site being sampled.

4.5 Sulfide is a positive interferent in this method (References 15.3 and 15.4), because an acidified sample containing sulfide liberates hydrogen sulfide that is passed through the

membrane and produces a signal at the silver electrode. In addition, sulfide ion reacts with cyanide ion in solution to reduce its concentration over time. To overcome this interference, the sulfide ion must be precipitated with lead ion immediately upon sample collection. Sulfide ion and lead sulfide react with cyanide ion to form thiocyanate which is not detected in the analytical system. Tests have shown (Reference 15.7) that if lead carbonate is used for sulfide precipitation, the supernate containing cyanide must be filtered immediately to avoid loss of cyanide through reaction with precipitated lead sulfide (Section 8.2.1).

4.6 Though not interferences, substances that react with cyanide should also be removed from samples at time of collection. These substances include water soluble aldehydes that form cyanohydrins and oxidants such as hypochlorite and sulfite. Water soluble aldehydes react with cyanide to form cyanohydrins that are not detected by the analytical system; hypochlorite and sulfite oxidize cyanide to non-volatile forms. Procedures for the removal of these substances are provided in Sections 8.2.2 and 8.2.3.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been precisely determined; however, each chemical compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.2 Cyanides and cyanide solutions

WARNING: The cyanide ion, hydrocyanic acid, all cyanide salts, and most metal-cyanide complexes are extremely dangerous. As a contact poison, cyanide need not be ingested to produce toxicity. Also, cyanide solutions produce fatally toxic hydrogen cyanide gas when acidified. For these reasons, it is mandatory that work with cyanide be carried out in a well-ventilated hood by properly trained personnel wearing adequate protective equipment.

5.3 Sodium hydroxide solutions

CAUTION: Considerable heat is generated upon dissolution of sodium hydroxide in water. It may be advisable to cool the container in an ice bath when preparing sodium hydroxide solutions.

5.4 Unknown samples may contain high concentrations of volatile toxic compounds. Sample containers should be opened in a hood and handled with gloves to prevent exposure.

5.5 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses. Additional information on laboratory safety can be found in References 15.8 and 15.9.

6.0 Equipment and Supplies

NOTE: Brand names, suppliers, and part numbers are for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Flow injection analysis (FIA) system—ALPKEM Model 3000 (Reference 15.5), or equivalent, consisting of the following:

6.1.1 Injection valve capable of injecting 40 to 300 μL samples

6.1.2 Gas diffusion manifold with a microporous Teflon® or polypropylene membrane

6.1.3 Amperometric detection system with:

6.1.3.1 Silver working electrode

6.1.3.2 Ag/AgCl reference electrode

6.1.3.3 Pt/stainless steel counter electrode

6.1.3.4 Applied potential of 0.0 volt

6.2 Sampling equipment—Sample bottle, amber glass, 0.1-L, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110 - 150 °C for one hour minimum.

6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. all cleaned, rinsed and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Standards

7.1 Reagent water—Water in which cyanide and potentially interfering substances are not detected at the MDL of this method. It may be generated by any one of the methods listed below. Reagent water generated by these methods shall be tested for purity utilizing the procedure in Section 11.

7.1.1 Activated carbon—Pass distilled or deionized water through an activated carbon bed (Calgon Filtrasorb-300 or equivalent).

7.1.2 Water purifier—Pass distilled or deionized water through a purifier (Millipore Super Q, or equivalent).

7.2 Sodium hydroxide—ACS reagent grade.

7.3 Potassium cyanide—ACS reagent grade.

7.4 Mercury (II) cyanide, ≥99% purity—Aldrich Chemical Company Catalog No. 208140, or equivalent.

7.5 Potassium nickel (II) cyanide—Aldrich Chemical Company Catalog No. 415154, or equivalent.

7.6 Silver nitrate—ACS reagent grade. Aldrich Chemical Company Catalog No. 209139, or equivalent.

7.7 Hydrochloric acid—approximately 37%, ACS reagent grade.

7.8 Preparation of stock solutions. Observe the warning in Section 5.2.

7.8.1 Silver nitrate solution, 0.0192 N—Weigh 3.27 g of $AgNO_3$ into a 1-L volumetric flask and bring to the mark with reagent water.

7.8.2 Rhodanine solution, 0.2 mg/mL in acetone—Weigh 20 mg of p-dimethylaminobenzalrhodanine (Aldrich Chemical Co. Catalog No. 114588, or equivalent) in a 100-mL volumetric flask and dilute to the mark with acetone.

7.8.3 Potassium cyanide stock solution, 1000 mg/L

7.8.3.1 Dissolve approximately 2 g (approximately 20 pellets) of sodium hydroxide in approximately 500 mL of reagent water contained in a one liter volumetric flask. Observe the caution in Section 5.3. Add 2.51 g of potassium cyanide (Aldrich Chemical Co. Catalog No. 207810, or equivalent), dilute to one liter with reagent water, and mix well. Store KCN solution in an amber glass container at 0-4°C.

7.8.3.2 Standardize the KCN solution (Section 7.8.3.1) by adding 0.5 mL of rhodanine solution (Section 7.8.2) to 25 mL of KCN solution and titrating with $AgNO_3$ solution (Section 7.8.1) until the color changes from canary yellow to a salmon hue. Based on the determined KCN concentration, dilute the KCN solution to an appropriate volume so the final concentration is 1.00 g/L, using the following equation:

EQUATION 1

$$x \times v = 1\ g/L \times 1\ L$$

where:
x=concentration of KCN solution determined from titrations
v=volume of KCN solution needed to prepare 1 L of 1 g/L KCN solution

If the concentration is not 1.00 g/L, correct the intermediate and working calibration concentrations accordingly.

7.8.4 1M sodium hydroxide—Dissolve 40 g of sodium hydroxide pellets in approximately 500 mL of reagent water in a 1-liter volumetric flask, observing the caution in Section 5.3. Dilute to one liter with reagent water. Store in an amber bottle at room temperature.

7.9 Secondary standards.

7.9.1 Cyanide, 100 mg/L—Dilute 100.0 mL of cyanide stock solution (Section 7.8.3.2) and 10 mL of 1M sodium hydroxide (Section 7.8.4) to one liter with reagent water (Section 7.1). Store in an amber glass bottle at 0-4°C.

7.9.2 Cyanide, 10 mg/L—Dilute 10.0 mL of cyanide stock solution and 10 mL of 1M sodium hydroxide to one liter with reagent water. Store in an amber glass bottle at 0-4°C.

7.9.3 Cyanide, 1 mg/L—Dilute 1.0 mL of cyanide stock solution and 1 mL of 1M sodium hydroxide to one liter with reagent water. Store in an amber glass bottle at 0-4°C.

7.9.4 Cyanide working calibration standard solutions (2 - 5000 µg/L as cyanide)—Working calibration standards may be prepared to cover the desired calibration range by adding the appropriate volumes of secondary standards (Sections 7.9.1, 7.9.2, 7.9.3) to 100 mL volumetric flasks that contain 40 mL of reagent water (Section 7.1) and 1 mL of 1M sodium hydroxide (Section 7.8.4). Dilute the solutions to 100 mL with reagent water. Prepare working calibration standards daily. The following table provides the quantity of secondary standard necessary to prepare working standards of the specified concentration.

Working Calibration Standard Concentration (μg/L)	Secondary Standard Solution Volume		
	Secondary Standard Concentration (Section 7.8.3) 1 mg/L	Secondary Standard Concentration (Section 7.8.2) 10 mg/L	Secondary Standard Concentration (Section 7.8.1) 100 mg/L
0.000			
2.0	0.200		
5.0	0.500	0.050	
10.0	1.00	0.100	
50.0	5.00	0.500	0.050
100	10.0	1.00	0.100
200	20.0	2.00	0.200
500	50.0	5.00	0.500
1000		10.0	1.00
3000		30.0	3.00
5000		50.0	5.00

If desired, the laboratory may extend the analytical working range by using standards that cover more than one calibration range, so long as the requirements of Section 10.3 are met.

7.10 Sample Preservation Reagents

7.10.1 The presence of sulfide may result in the conversion of cyanide to thiocyanate. While lead acetate test paper has been recommended for determining the presence of sulfide in samples, the test is generally unreliable and is typically not usable for sulfide concentrations below approximately 1 ppm. The use of lead carbonate (Aldrich Chemical Co. Catalog No. 336378, or equivalent), followed by immediate filtration of the sample is required whenever sulfide ion is present. If the presence of sulfide is suspected but not verifiable from the use of lead acetate test paper, two samples may be collected, one without lead carbonate addition and another with lead carbonate addition followed by immediate filtration. Analyze both samples. If sulfide is present, the preserved sample should contain higher levels of cyanide than the unpreserved sample. Lead acetate test paper may be used, but should be tested for minimum level of sulfide detection by spiking reagent water aliquots with decreasing levels of sulfide and determining the lowest level of sulfide detection attainable. The spiked samples are tested with lead acetate test paper moistened with acetate buffer solution. The buffer solution is prepared by

dissolving 146 g anhydrous sodium acetate, or 243 g sodium acetate trihydrate in 400 mL of reagent water, followed by addition of 480 g concentrated acetic acid. Dilute the solution to 1 L with reagent water. Each new batch of test paper and/or acetate buffer should be tested to determine the lowest level of sulfide ion detection prior to use.

7.10.2 Ethylenediamine solution—In a 100 mL volumetric flask, dilute 3.5 mL pharmaceutical-grade anhydrous ethylenediamine (Aldrich Chemical Co. Catalog No. 240729, or equivalent) with reagent water.

7.10.3 Ascorbic acid—Crystals—Aldrich Chemical Co. Catalog No. 268550, or equivalent.

7.11 FIA Reagents.

7.11.1 Carrier and acid reagent (0.1M hydrochloric acid)—Dilute 8 mL of concentrated hydrochloric acid to one liter with reagent water.

7.11.2 Acceptor reagent (0.1M sodium hydroxide)—Dilute 100 mL of sodium hydroxide solution (Section 7.8.4) to 1000 mL with reagent water.

7.11.3 Ligand-exchange reagent A-ALPKEM part number A001416, or equivalent.

7.11.4 Ligand-exchange reagent B-ALPKEM part number A001417, or equivalent.

7.12 Quality control solutions

7.12.1 Mercury (II) cyanide stock solution (1000 mg/L as cyanide)—Weigh 0.486 g of mercury (II) cyanide (Section 7.4) in a 100-mL volumetric flask. Add 10 - 20 mL of reagent water and 1 mL of 1M sodium hydroxide solution (Section 7.8.4). Swirl to mix. Dilute to the mark with reagent water.

7.12.2 Laboratory control sample (LCS)—Place 0.20 mL of the mercury (II) cyanide stock solution (Section 7.12.1) in a 100-mL volumetric flask and dilute to the mark with reagent water to provide a final cyanide concentration of 2.00 mg/L.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection and preservation—Samples are collected using manual (grab) techniques and are preserved immediately upon collection.

8.1.1 Grab sampling—Collect samples in amber glass bottles with PTFE-lined caps cleaned according to the procedure in Section 6.2. Immediately after collection, preserve the sample using any or all of the preservation techniques (Section 8.2), followed by adjustment of the sample pH to $\geq$12 by addition of 1M sodium hydroxide and refrigeration at 0-4°C.

8.1.2 Compositing—Compositing is performed by combining aliquots of grab samples only. Automated compositing equipment may not be used because cyanide may react or degrade during the sampling period. Preserve and refrigerate each grab sample immediately after collection (Sections 8.1.1 and 8.2) until compositing.

8.1.3 Shipment—If the sample will be shipped by common carrier or mail, limit the pH to a range of 12.0 - 12.3. (See the footnote to 40 CFR 136.3(e), Table II, for the column headed "Preservation.")

8.2 Preservation techniques

8.2.1 Samples containing sulfide ion

8.2.1.1 Test the sample with lead acetate test paper (Section 7.10.1) to determine the presence or absence of sulfide ion. If sulfide ion is present, the sample must be treated immediately (within 15 minutes of collection) with sufficient solid lead carbonate (Section 7.10.1) to remove sulfide (as evidenced by the lead acetate test paper), and immediately filtered into another sample bottle to remove precipitated lead sulfide.

8.2.1.2 If sulfide ion is suspected to be present, but its presence is not detected by the lead acetate paper test, two samples should be collected. One is treated for the presence of sulfide and immediately filtered, while the second is not treated for sulfide. Both samples must be analyzed. (Tests conducted prior to the interlaboratory validation of this method showed significant and rapid losses of cyanides when lead sulfide was allowed to remain in contact with the sample during holding times of three days or less. As a result, the immediate filtration of samples preserved with lead carbonate is essential (Reference 15.6)).

8.2.1.3 If the sample contains particulate matter that would be removed upon filtration, the sample must be filtered prior to treatment with lead carbonate to assure that cyanides associated with the particulate matter are included in the measurement. The collected particulate matter must be saved and the filtrate treated using the sulfide removal procedure above (Section 8.2.1.1). The collected particulate and treated filtrate must be recombined and homogenized, and then sent to the laboratory for analysis.

8.2.2 Samples containing water soluble aldehydes—Treat samples containing or suspected to contain formaldehyde, acetaldehyde, or other water soluble aldehydes with 20 mL of 3.5% ethylenediamine solution (Section 7.10.2) per liter of sample.

8.2.3 Samples known or suspected to contain chlorine, hypochlorite, and/or sulfite—Treat with 0.6 g of ascorbic acid (Section 7.10.3) per liter of sample. EPA Method 330.4 or 330.5 may be used for the measurement of residual chlorine (Reference 15.1).

8.3 Sample holding time—Maximum holding time for samples preserved as above is 14 days. Unpreserved samples must be analyzed within 24 hours, or sooner if a change in cyanide concentration will occur. (See the footnotes to Table II at 40 CFR 136.3(e).)

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.9). The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of LCSs and MS/MSDs as a continuing check on performance. Laboratory performance is compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable precision and accuracy with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to allow the laboratory to overcome sample matrix interferences, the laboratory is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or immuno-assay techniques, and changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in this method is used, then that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analytes of interest.

9.1.2.1 Each time a modification is made to this method, the laboratory is required to repeat the procedure in Section 9.2. If the detection limit of the method will be affected by the change, the laboratory must demonstrate that the MDL is equal to or less than the MDL in Section 1.4 or one-third the regulatory compliance level, whichever is greater. If calibration will be affected by the change, the laboratory must recalibrate the instrument per Section 10.3.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the information in this subsection, at a minimum.

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification.

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method including:

(a) calibration (Section 10.3)
(b) calibration verification (Section 9.5)
(c) initial precision and recovery (Section 9.2)
(d) analysis of blanks (Section 9.4)
(e) laboratory control sample (Section 9.6)
(f) matrix spike and matrix spike duplicate (Section 9.3)
(g) MDL (Section 1.4)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:

(a) sample numbers and other identifiers
(b) analysis dates and times
(c) analysis sequence/run chronology
(d) sample weight or volume
(e) sample volume prior to each cleanup step, if applicable
(f) sample volume after each cleanup step, if applicable
(g) final sample volume prior to injection (Sections 10 and 11)
(h) injection volume (Sections 10 and 11)
(i) dilution data, differentiating between dilution of a sample or modified sample (Sections 10 and 11)
(j) instrument and operating conditions
(k) other operating conditions (temperature, flow rates, etc.)
(l) detector (operating condition, etc.)
(m) printer tapes, disks, and other recording of raw data
(n) quantitation reports, data system outputs, and other data necessary to link raw data to the results reported

9.1.3 Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in Section 9.3.

9.1.4 Analyses of blanks are required to demonstrate freedom from contamination and that the compounds of interest and interfering compounds have not been carried over from a previous analysis. The procedures and criteria for analysis of a blank are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS (Section 7.12.2) that the analysis system is in control. This procedure is described in Section 9.6.

9.1.6 The laboratory should maintain records to define the quality of data that is generated. Development of accuracy statements is described in Sections 9.3.8 and 9.6.3.

9.1.7 Accompanying QC for the determination of cyanide is required per analytical batch. An analytical batch is a set of samples analyzed at the same time, to a maximum of 10 samples. Each analytical batch of 10 or fewer samples must be accompanied by a laboratory blank (Section 9.4), an LCS (Section 9.6), and a matrix spike and matrix spike duplicate (MS/MSD, Section 9.3), resulting in a minimum of five analyses (1 sample, 1 blank, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 blank, 1 LCS, 1 MS, and 1 MSD) in the batch. If greater than 10 samples are analyzed at one time, the samples must be separated into analytical batches of 10 or fewer samples.

9.2 Initial demonstration of laboratory capability

9.2.1 Method Detection Limit (MDL)—To establish the ability to detect cyanide at low levels, the laboratory shall determine the MDL per the procedure in 40 CFR Part 136, Appendix B (Reference 15.4) using the apparatus, reagents, and standards that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.4 must be achieved prior to practice of this method.

9.2.2 Initial Precision and Recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the laboratory shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 7.12.2) according to the procedure beginning in Section 10.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (s) for cyanide. Use Equation 2 for calculation of the standard deviation of the percent recovery.

EQUATION 2

$$s = \sqrt{\frac{\sum x^2 - \frac{(\sum x)^2}{n}}{n - 1}}$$

where:
n = Number of samples
x = Percent recovery in each sample

9.2.3 Compare s and x with the acceptance criteria specified in Table 1. If s exceeds the precision limit or x falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before analyses can begin.

9.3 Matrix spike/matrix spike duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10 percent of all samples (one sample in duplicate in each batch of ten samples) from a given discharge.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of cyanide in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of cyanide in a sample is not being checked against a limit, the spike shall be at the concentration of the LCS or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2 Analyze one sample aliquot out of each set of ten samples from each discharge according to the procedure beginning in Section 11 to determine the background concentration (B) of cyanide.

9.3.2.1 Spike this sample with the amount of mercury (II) cyanide stock solution (Section 7.12.1) necessary to produce a cyanide concentration in the sample of 2 mg/L. If necessary, prepare another stock solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery of cyanide in each aliquot using Equation 3.

EQUATION 3

$$P = \frac{100\ (A - B)}{T}$$

where:
P = Percent recovery
A = Measured concentration of cyanide after spiking
B = Measured background concentration of cyanide
T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Table 1. If recovery is outside of the acceptance criteria, and the recovery of the LCS in the ongoing precision and recovery test (Section 9.6) for the analytical batch is within the acceptance criteria, an interference is present. In this case, the result may not be reported for regulatory compliance purposes.

9.3.5 If the results of both the MS/MSD and the LCS test fail the acceptance criteria, the analytical system is judged to be out of control. In this case, the problem shall be identified and corrected, and the analytical batch reanalyzed.

9.3.6 Calculate the relative percent difference (RPD) between the two spiked sample results (Section 9.3, not between the two percent recoveries) using Equation 4.

EQUATION 4

$$RPD = \frac{|D_1 - D_2|}{(D_1 + D_2)/2} \times 100$$

where:
RPD = Relative percent difference
D_1 = Concentration of cyanide in the spiked sample
D_2 = Concentration of cyanide in the spiked duplicate sample

9.3.7 Compare the precision to the RPD criteria in Table 1. If the RPD is greater than the acceptance criteria, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected, and the analytical batch reanalyzed.

9.3.8 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained. After the analysis of five spiked samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery

interval from $P_a - 2s_p$ to $P_a + 2s_p$. For example, if $P_a = 90\%$ and $s_p = 10\%$ for five analyses, the accuracy interval is expressed as 70 – 110%. Update the accuracy assessment on a regular basis (e.g., after each five to ten new accuracy measurements).

9.4 Laboratory blanks—Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1 Analyze a reagent water blank initially (i.e., with the tests in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

9.4.2 If cyanide is detected in the blank at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination.

9.5 Calibration verification—Verify calibration of the analytical equipment before and after each analytical batch of 14 or fewer measurements. (The 14 measurements will normally be 10 samples, 1 reagent blank, 1 LCS, 1 MS, and 1 MSD). Verification is accomplished by analyzing the mid-range calibration standard and verifying that it is within the QC acceptance criteria for recovery in Table 1. (The concentration of the calibration verification depends on the calibration range being used.) Failure to verify calibration within the acceptance criteria requires recalibration of the analysis system.

9.6 Laboratory control sample (LCS)—To demonstrate that the analytical system is in control, and acceptable precision and accuracy is being maintained with each analytical batch, the laboratory shall perform the following operations.

9.6.1 Analyze a LCS (Section 7.12.2) with each analytical batch according to the procedure in Section 10.

9.6.2 If the results for the LCS are within the acceptance criteria specified in Table 1, analysis of the batch may continue. If, however, the concentration is not within this range, the analytical process is not in control. In this event, correct the problem, repeat the LCS test, and reanalyze the batch.

9.6.3 The laboratory should add results that pass the specification in Section 9.6.2 to IPR and previous LCS data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for cyanide by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if $R = 95\%$ and $s_r = 5\%$, the accuracy is 85% to 105%.

9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory should periodically test an external reference sample, such as a Standard Reference Material (SRM) if an SRM is available from the National Institutes of Standards and

Technology (NIST). The reference sample should be analyzed quarterly, at a minimum. Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Calibration and Standardization

This section describes the procedure to calibrate and standardize the FIA system prior to cyanide determination.

10.1 Instrument setup

10.1.1 Set up the FIA system and establish initial operating conditions necessary for determination of cyanide. If the FIA system is computerized, establish a method for multi-point calibration and for determining the cyanide concentration in each sample.

10.1.2 Verify that the reagents are flowing smoothly through the FIA system and that the flow cell is purged of air bubbles.

10.2 Instrument Stabilization

10.2.1 Load a 10 mg/L KCN standard (Section 7.8.3) into the sampling valve and inject into the FIA system.

10.2.2 Continue to inject 10 mg/L KCN standards until 3 successive peak height or area results are within 2% RSD, indicating that the electrode system is stabilized.

10.2.3 Following stabilization, inject the highest concentration calibration standard until 3 successive peak height or area results are within 2% RSD indicating stabilization at the top of the calibration range.

10.3 External standard calibration

10.3.1 Inject each of a minimum of 3 calibration standards. One of the standards should be at the minimum level (ML) unless measurements are to be made at higher levels. The other concentrations should correspond to the expected range of concentrations found in samples or should define the working range of the FIA system.

10.3.2 Using injections of a constant volume, analyze each calibration standard according to Section 11 and record peak height or area responses against the concentration. The results can be used to prepare a calibration curve. Alternatively, if the ratio of response to amount injected (calibration factor) is constant over the working range (<10% RSD), linearity through the origin can be assumed and the averaged calibration factor (area/concentration) can be used in place of a calibration curve.

11.0 Procedure

This section describes the procedure for determination of available cyanide using the FIA system.

11.1 Analysis of standards, samples, and blanks

11.1.1 Ligand-exchange reagent treatment of standards, samples, and blanks.

11.1.2 To 100-mL of cyanide-containing sample (or standard or blank) at pH of approximately 12, add 100 µL of ligand-exchange reagent Part B (Section 7.11.5), 50µL of ligand-exchange reagent Part A (Section 7.11.4), and mix thoroughly. Load the sample, standard, or blank into the sample loop.

NOTE: The ligand-exchange reagents, when added to 100 mL of sample at the specified volume, will liberate cyanide from metal complexes of intermediate stability up to 5 mg/L cyanide ion. If higher concentrations are anticipated, add additional ligand-exchange reagent, as appropriate, or dilute the sample. The ligand-exchange reagents have an approximate lifetime of 6 months after opening. The reagents should be stored in a refrigerator at 4°C. Samples should be analyzed within 2 hours of adding the ligand-exchange reagents. The reagents should always be used in solutions similar to cyanide samples (pH 12 adjusted). It is recommended that the ligands be checked monthly. This can be done by preparing pH 12 adjusted 2 mg/l solutions of mercury(II) cyanide (Section 7.4) and of potassium nickel(II) cyanide (Section 7.5). Add ligand-exchange reagent B to the mercury(II) standard and ligand-exchange reagent A to the potassium nickel(II) cyanide standard and confirm cyanide recovery.

11.1.3 Inject the sample and begin data collection. When data collection is complete, analyze the next sample, standard or blank in the batch until analyses of all samples in the batch are completed.

12.0 Data Analysis and Calculations

12.1 Calculate the concentration of material in the sample, standard or blank from the peak height or area using the calibration curve or calibration factor determined in Section 10.3.

12.2 Reporting

12.2.1 Samples—Report results to three significant figures for cyanide concentrations found above the ML (Section 1.4) in all samples. Report results below the ML as <2 μg/L, or as required by the permitting authority or permit.

12.2.2 Blanks—Report results to three significant figures for cyanide concentrations found above the MDL (Section 1.4). Do not report results below the MDL unless required by the permitting authority or in the permit.

13.0 Method Performance

13.1 Method detection limit (MDL)—MDLs from nine laboratories were pooled to develop the MDL of 0.5 μg/L given in Section 1.4 (Reference 15.12).

13.2 Data obtained from single laboratory testing of the method are summarized in Table 2 and show recoveries and reproducibility for "free" forms of cyanide, including the recovery and reproducibility of silver, nickel, and mercury cyanide species. Determination of these species tends to be problematic with other methods for the determination of available cyanide. As it is the case with other methods used for available cyanide, iron cyanide species were not recovered and recoveries for gold and cobalt species were zero or very low. The complete results from the single laboratory study are available in the Report of the Draft OIA Method 1677 Single Laboratory Validation Study (Reference 15.11).

13.3 Listed in Table 1 are the QC acceptance criteria developed from an interlaboratory validation study of this method. This study was conducted following procedures specified in the Guide to Method Flexibility and Approval of EPA Water Methods (Reference 15.10). In this study, a total of nine laboratories performed analyses for various water matrices. Table 3 shows a summary of the interlaboratory results which include the accuracy and precision data as % recoveries and relative standard deviations. In addition to spikes of easily dissociable cyanides, some samples contained known amounts of cyanides that are not recoverable (e.g., Pt and Fe complexes) and thiocyanate was spiked to one sample to investigate the potential for interference. The complete study results are available in the Report of the Draft OIA Method 1677 Interlaboratory Validation Study (Reference 15.12).

14.0 Pollution Prevention and Waste Management

14.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

14.2 Samples containing cyanide, certain metals, and acids at a pH of less than 2 are hazardous and must be treated before being poured down a drain or must be handled as hazardous waste.

14.3 For further information on waste management, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, Reference 15.8.

15.0 References

15.1 Environmental Monitoring Systems Laboratory. EPA Method 335.1. In: *Methods for the Chemical Analysis of Water and Wastes* (EPA/600/4-79-020). Environmental Protection Agency, Cincinnati, Ohio. Revised March 1983.

15.2 American Public Health Association, American Waterworks Association, Water Pollution Control Board. Methods Section 4500-CN in *Standard Methods for the Examination of Water and Wastewater*, 19th Edition. American Public Health Association, Washington, DC, 1995.

15.3 Ingersol, D.; Harris, W.R.; Bomberger, D.C.; Coulson, D.M. *Development and Evaluation Procedures for the Analysis of Simple Cyanides, Total Cyanides, and Thiocyanate in Water and Waste Water* (EPA-600/4-83-054), 1983.

15.4 *Code of Federal Regulations*, Title 40, Part 136, Appendix B. U.S. Government Printing Office, Washington, D.C., 1994.

15.5 ALPKEM CNSolution Model 3000 Manual. Available from ALPKEM / OI Analytical, Box 9010, College Station, TX 77842-9010.

15.6 Milosavljevic, E.B.; Solujic, L.; Hendrix, J.L. *Environmental Science and Technology*, Vol. 29, No. 2, 1995, pp 426-430. Rapid Distillationless "Free Cyanide" Determination by a Flow Injection Ligand Exchange Method.

15.7 Wilmont, J.C.; Solujic, L.; Milosavljevic, E. B.; Hendrix, J.L.; Reader, W.S. *Analyst*, June 1996, Vol. 121, pp 799-801. Formation of Thiocyanate During Removal of Sulfide as Lead Sulfide Prior to Cyanide Determination.

15.8 *Less is Better: Laboratory Chemical Management for Waste Reduction*. Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC 20036.

15.9 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories* (EPA-600/4-79-019), USEPA, NERL, Cincinnati, Ohio 45268 (March 1979).

15.10 *Guide to Method Flexibility and Approval of EPA Water Methods*, December, 1996, (EPA-821-D-96-004). Available from the National Technical Information Service (PB97-117766).

15.11 *Report of the Draft OIA Method 1677 Single Laboratory Validation Study*, November 1996. Available from ALPKEM / OI Analytical, Box 9010, College Station, TX 77842-9010.

15.12 *Report of the Draft OIA Method 1677 Interlaboratory Validation Study*, March 1997. Available from ALPKEM / OI Analytical, Box 9010, College Station, TX 77842-9010.

16.0 Tables

Table 1. Quality Control Acceptance Criteria

Criterion	Required Recovery Range (%)	Precision
Initial Precision and Recovery	92 - 122	<5.1% RSD
Ongoing Precision and Recovery (Laboratory Control Sample)	82 - 132	N/A
Calibration Verification	86 - 118	N/A
Matrix Spike/Matrix Spike Duplicate	82 - 130	<11% RPD

Table 2. Species-Dependent Cyanide Recoveries Using Draft Method 1677[1]

Species	0.20 µg/mL CN^-	2.00 µg/mL CN^-
$[Zn(CN)_4]^{2-}$	97.4 (0.7)	98.5 (0.7)
$[Cd(CN)_4]^{2-}$	100.0 (0.8)	100.0 (0.2)
$[Cu(CN)_4]^{2-}$	100.9 (1.3)	99.0 (0.6)
$[Ag(CN)_4]^{3-}$	101.8 (0.9)	100.0 (0.5)
$[Ni(CN)_4]^{2-}$	104.3 (0.2)	103.0 (0.5)
$[Hg(CN)_4]^{2-}$	100.0 (0.6)	99.0 (0.3)
$Hg(CN)_2$	103.4 (0.4)	98.0 (0.3)
$[Fe(CN)_4]^{4-}$	0.0	0.0
$[Fe(CN)_6]^{3-}$	0.0	0.0
$[Au(CN)_2]^-$	1.3[2] (0.0)	0.0
$[Co(CN)_6]^{3-}$	2.9[2] (0.0)	2.0[2] (0.0)

[1] Values are % recoveries; numbers in parentheses are percent relative standard deviations.
[2] Commercial product contains some free cyanide.

Table 3. Cyanide Recoveries From Various Aqueous Matrices

Sample	Sample CN Concentration	Added CN(1) Concentration	Average % Recovery	% RSD
Reagent water w/0.01M NaOH	0 µg/L	100 µg/L as KCN	108	4.0
POTW secondary effluent	3.0 µg/L	100 µg/L as KCN; 2 mg/L as $[Pt(CN)_6]^{4-}$	102	7.0
Petroleum Refinery Secondary Effluent	9.9 µg/L	2 mg/L as KCN; 5 mg/L as $[Fe(CN)_6]^{4-}$	87	21
Coke Plant Secondary Effluent	14.0 µg/L	50 µg/L as KCN	95	4.0
Rolling Mill Direct Filter Effluent	4.0 µg/L	none	80	41
Metals Finishing Indirect Primary Effluent	1.0 µg/L	200 µg/L as KCN; 2 mg/L as KSCN	92	16
Reagent water w/0.01M NaOH	0 µg/L	200 µg/L as KCN	101	8.0
Reagent water w/0.01M NaOH	0 µg/L	10 mg/L as KCN; 10 mg/L as $[Pt(CN)_6]^{4-}$	103	2.0
Mining Tailing Pond Effluent	842 µg/L	4 mg/L as KCN	98	3.0

1 Cyano-complexes of Pt and Fe were added to the POTW and petroleum refinery effluents, respectively; and thiocyanate was added to the metals finishing effluent to demonstrate that the FI/LE system does not determine these forms of cyanide.

17.0 Glossary of Definitions and Purposes

The definitions and purposes are specific to this method but have been conformed to common usage as much as possible.

17.1 Units of weights and measures and their abbreviations

17.1.1 Symbols

°C	degrees Celsius
%	percent
±	plus or minus
≥	greater than or equal to

17.1.2 Alphabetical characters

g	gram
L	liter
mg	milligram
mg/L	milligram per liter
µg	microgram

μg/L	microgram per liter
mL	milliliter
ppm	parts per million
ppb	parts per billion
M	molar solution

17.2 Definitions

17.2.1 Available cyanide consists of cyanide ion (CN^-), hydrogen cyanide in water (HCN_{aq}) and the cyano-complexes of zinc, copper, cadmium, mercury, nickel, and silver.

17.2.2 Calibration blank—A 100 mL volume of reagent water treated with the ligand-exchange reagents and analyzed using the FIA procedure.

17.2.3 Calibration standard (CAL)—A solution prepared from the dilution of stock standard solutions. A 100 mL aliquot of each of the CALs are subjected to the analysis procedure. The resulting observations are used to calibrate the instrument response with respect to the analyte concentration.

17.2.4 Discharge—Specific discharge (also known as "matrix type") means a sample medium with common characteristics across a given industrial category or industrial subcategory. Examples include: C-stage effluents from chlorine bleach mills in the Pulp, Paper, and Paperboard industrial category; effluent from the continuous casting subcategory of the Iron and Steel industrial category; publicly owned treatment work (POTW) sludge; and in-process streams in the Atlantic and Gulf Coast Hand-shucked Oyster Processing subcategory.

Specific discharge also means a discharge with characteristics different from other discharges. Therefore, if there are multiple discharges from a facility all with the same characteristics, these are the same discharge for the purpose of demonstrating equivalency of a method modification. In this context, "characteristics" means that results of the matrix spike and matrix spike duplicate (MS/MSD) tests with the unmodified method meet the QC acceptance criteria for recovery and relative percent difference (RPD).

17.2.5 Initial precision and recovery (IPR)—Four aliquots of the LRB spiked with the analytes of interest and used to establish the ability to generate acceptable precision and accuracy. An IPR is performed the first time this method is used and any time the method or instrumentation is modified.

17.2.6 Laboratory control sample (LCS)—An aliquot of LRB to which a quantity of mercury (II) cyanide stock solution is added in the laboratory. The LCS is analyzed like a sample. Its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

17.2.7 Laboratory reagent blank (LRB)—An aliquot of reagent water that is treated like a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if the method analyte or other interferences are present in the laboratory environment, reagents, or apparatus.

17.2.8 Matrix spike/matrix spike duplicate (MS/MSD)—An aliquot of an environmental sample to which a quantity of the method analyte is added in the laboratory. MS/MSDs are analyzed like a sample. Their purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentration of the analyte in the sample matrix must be determined in a separate aliquot and the measured values in the MS/MSD corrected for the background concentration.

17.2.9 Minimum level (ML)—The level at which the entire analytical system shall give a recognizable signal and acceptable calibration point, taking into account method specific sample and injection volumes.

17.2.10 Ongoing precision and recovery (OPR)—See Laboratory control sample