O·I·Analytical **Methodology**

Method OIA-1677: Available Cyanide by Ligand Exchange and Flow Injection Analysis (FIA)

(Cartridge Part #A001982 or #A002654)

1.0 Scope and Application

1.1 This method is used for the determination of available cyanide in water and wastewater by ligand exchange, flow injection analysis, and amperometric detection according to USEPA Method OIA-1677 (References 15.3, 15.4, 15.9, and 15.10). This method is used in the USEPA'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. The presence of polysulfides and colloidal material may prove intractable for application of this method.

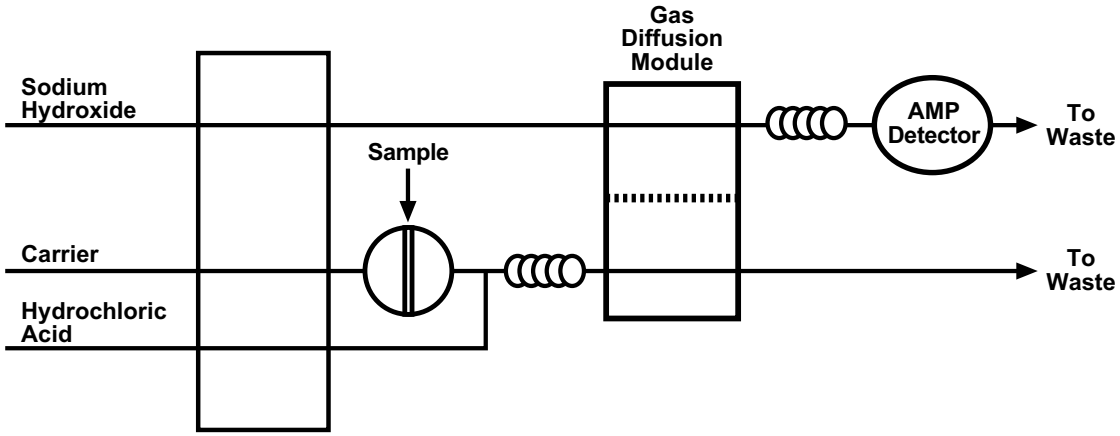
1.3 The Method Detection Limit (MDL) of this method is 0.5 µg/L cyanide (Reference 15.9). The applicable range of the method is 2.0 µg/L (ppb) to 5.0 mg/L (ppm) cyanide using a 200-µL sample loop. The range may be extended to analyze higher concentrations by sample dilution or changing the volume of the sample loop.

2.0 Summary of Method

2.1 Prior to analysis, the sample is treated to remove potential interferences (Section 4.0 and 8.0). Ligand exchange reagents are added to the sample. Thermodynamically stable complexes are formed with the transition metal ions listed in Section 1.2, releasing the cyanide ion from the cyano-complexes. An aliquot of the treated sample is injected into the Flow Injection Analysis (FIA) system (Reference 15.1). The addition of hydrochloric acid converts the cyanide ion to hydrogen cyanide gas (HCN), which passes under a gas diffusion membrane. The hydrogen cyanide gas 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.

2.2 The quality of the analysis is assured through reproducible calibration and testing of the Flow Injection Analysis (FIA) system.

2.3 A general flow diagram of the FIA system is shown below (see Section 17.0 for a detailed flow diagram).



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, "Glossary of Definitions and Purposes."

4.0 Interferences

4.1 Method interferences may be caused by contaminants in the reagents, reagent water, glassware, etc., which may bias the results. Care should be taken to keep all such items free of contaminants.

4.2 Sulfide is a positive interferant in this method (Reference 15.11). When sulfide is acidified, it forms hydrogen sulfide, which passes through the gas diffusion 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. Samples containing sulfide must be treated according to Section 8.4.

4.3 Sample containing water soluble aldehydes, such as formaldehyde or acetaldehyde, are treated by adding ethylenediamine solution (Section 8.5).

4.4 Oxidizing agents that decompose cyanides are removed by adding ascorbic acid (Section 8.6)

4.5 High concentrations of carbonate may result in a negative response in the amperometric detector when carbon dioxide diffuses across the gas diffusion membrane into the alkaline receiving solution, reducing its pH. Effluents from high-carbonate containing wastes, such as coal gasification waste and atmospheric emission scrub water, can be treated with hydrated lime to stabilize the sample (Reference 15.11).

4.6 Tests conducted on samples containing large amounts of colloids indicate rapid cyanide losses. Filtration can be used to remove colloids, but measured cyanide levels may be adversely affected.

5.0 Safety

5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.

5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.

5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.

5.3.1 Acetic Acid, glacial, $C_2H_4O_2$ (FW 60.05)

5.3.2 Acetone, C_3H_6O (FW 58.08)

5.3.3 5-[4-(Dimethylamino)benzylidene]rhodanine, $C_{12}H_{12}N_2OS_2$ (FW 264.37)

5.3.4 Ethylenediamine, anhydrous, $C_2H_8N_2$ (FW 60.10)

5.3.5 Mercury(II) Cyanide, $Hg(CN)_2$ (FW 252.63)

5.3.6 Nickel(II) Cyanide, $Ni(CN)_2$ (FW 110.73)

5.3.7 Potassium Cyanide, KCN (FW 65.12)

5.3.8 Silver Nitrate, $AgNO_3$ (FW 169.88)

5.3.9 Sodium Acetate, anhydrous, $C_2H_3O_2Na$ (FW 82.03)

5.3.10 Sodium Hydroxide, NaOH (FW 40.00)

5.3.11 WAD Cyanide Reagent A (Part #A001416)

5.3.12 WAD Cyanide Reagent B (Part #A001417)

5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.

5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.

5.6 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.

6.0 Apparatus, Equipment, and Supplies

6.1 Flow Injection Analysis (FIA) System (OI Analytical CNSolution™ 3000) consisting of the following:

6.1.1 120-Place Autosampler

6.1.2 Amperometric detection system with:

6.1.2.1 Silver working electrode

6.1.2.2 Silver/silver chloride reference electrode

6.1.2.3 Platinum/stainless steel counter electrode

6.1.2.4 Applied potential of zero volt

6.1.3 Data Acquisition System (PC or Notebook PC) with WinFLOW™ software

6.1.4 Available Cyanide, Method OIA-1677 Cartridge (Part #A001982 or #A002654)

6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with deter gent and water, rinsing with two aliquots of reagent water , and drying by baking at 1 10°–150°C for a minimum of one hour .

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

7.0 Reagents and Calibrants

7.1 Raw Materials

7.1.1 Acetic Acid, glacial, $C_2H_4O_2$ (FW 60.05)

7.1.2 Acetone, C_3H_6O (FW 58.08)

7.1.3 Ascorbic Acid, $C_6H_8O_6$ (FW 176.12)

7.1.4 Deionized Water (ASTM Type I or II)

7.1.5 5-[4-(Dimethylamino)benzylidene]rhodanine, $C_{12}H_{12}N_2OS_2$ (FW 264.37)

7.1.6 Ethylenediamine, anhydrous, $C_2H_8N_2$ (FW 60.10)

7.1.7 Mercury(II) Cyanide, $Hg(CN)_2$ (FW 252.63)

7.1.8 Nickel(II) Cyanide, $Ni(CN)_2$ (FW 110.73)

7.1.9 Potassium Cyanide, KCN (FW 65.12)

7.1.10 Silver Nitrate, $AgNO_3$ (FW 169.88)

7.1.11 Sodium Acetate, anhydrous, $C_2H_3O_2Na$ (FW 82.03)

7.1.12 Sodium Hydroxide, NaOH (FW 40.00)

7.1.13 WAD Cyanide Reagent A (Part #A001416)

7.1.14 WAD Cyanide Reagent B (Part #A001417)

7.2 Reagent Preparation

Note: For best results, filter and degas all reagents prior to use.

7.2.1 Reagent Water

7.2.1.1 Degassed and deionized reagent water can be prepared in one of the following manners:

7.2.1.1.1 Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonification will aid in the degassing process.

7.2.1.1.2 Purge distilled/deionized water with a stream of nitrogen gas (or other inert gas) through a glass frit for approximately 5 minutes.

7.2.1.1.3 Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker , and allow it to cool to room temperature.

7.2.1.2 After preparing the degassed reagent water , store the reagent water in a tightly sealed container to protect it from reabsorption of atmospheric gases. For best results, store degassed reagent water under a slight vacuum when not in use.

7.2.2 Sample Preservation Reagents

7.2.2.1 Acetate Buffer (1 L)

7.2.2.1.1 Dissolve 146 g of sodium acetate anhydrous in 400 mL of reagent water (Section 7.2.1).

7.2.2.1.2 Add 480 g of glacial acetic acid. Dilute to 1,000 mL with reagent water in a 1-L volumetric flask and mix well.

7.2.2.2 Ethylenediamine Solution, 3.5% v/v (100 mL)

7.2.2.2.1 Dilute 3.5 mL of ethylenediamine to 100 mL with reagent water in a 100-mL volumetric flask and mix well.

7.2.2.3 Ascorbic Acid—Crystals

7.2.3 Quality Control Reagents

Warning: Cyanide ion, hydrocyanic acid, all cyanide salts, and most metal-cyanide complexes are extremely dangerous. As a contact poison, cyanide does not need to 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.

7.2.3.1 Sodium Hydroxide, 1 M (1 L)

7.2.3.1.1 While stirring, carefully add 40 g of sodium hydroxide in 700 mL of reagent water in a 1-L volumetric flask.

7.2.3.1.2 Cool to room temperature. Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide with water releases a great amount of heat. Take appropriate precautions, such as cooling the solution while adding the sodium hydroxide.

Note: Store in an amber bottle at room temperature.

7.2.3.2 Nickel(II) Cyanide Stock Solution, 1,000 mg/L Cyanide (100 mL)

7.2.3.2.1 Dissolve 0.213 g of nickel(II) cyanide in 25 mL of reagent water and 1 mL of 1 M sodium hydroxide (Section 7.2.3.1) in a 100-mL volumetric flask.

7.2.3.2.2 Dilute to 100 mL with reagent water and mix well.

7.2.3.3 Mercury(II) Cyanide Stock Solution, 1,000 mg/L Cyanide (100 mL)

7.2.3.3.1 Dissolve 0.486 g of mercury(II) cyanide in 25 mL of reagent water and 1 mL of 1 M sodium hydroxide in a 100-mL volumetric flask.

7.2.3.3.2 Dilute to 100 mL with reagent water and mix well.

7.2.3.4 Nickel(II) Cyanide Working Solution, 2 mg/L Cyanide (100 mL)

7.2.3.4.1 Add 0.2 mL of nickel(II) cyanide stock solution (Section 7.2.3.2) to 25 mL of reagent water and 1 mL of 1 M sodium hydroxide (Section 7.2.3.1) in a 100-mL volumetric flask.

7.2.3.4.2 Dilute to 100 mL with reagent water and mix well.

7.2.3.5 Mercury(II) Cyanide Working Solution, 2 mg/L Cyanide (100 mL)

7.2.3.5.1 Add 0.2 mL of mercury(II) cyanide stock solution (Section 7.2.3.3) to 25 mL of reagent water and 1 mL of 1 M sodium hydroxide in a 100-mL volumetric flask.

7.2.3.5.2 Dilute to 100 mL with reagent water and mix well.

7.2.4 FIA Reagents

7.2.4.1 Carrier and Hydrochloric Acid Solution (1 L)

7.2.4.1.1 Add 8 mL of concentrated hydrochloric acid to approximately 800 mL of reagent water in a 1-L volumetric flask.

7.2.4.1.2 Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing hydrochloric acid with water releases a great amount of heat. Take appropriate precautions.

7.2.4.2 Acceptor Stock Solution, 5 M Sodium Hydroxide (1 L)

7.2.4.2.1 While stirring, carefully add 200 g of sodium hydroxide in 700 mL of reagent water in a 1-L volumetric flask.

7.2.4.2.2 Cool to room temperature. Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide with water releases a great amount of heat. Take appropriate precautions, such as cooling the solution while adding the sodium hydroxide.

Note: Store in an amber bottle at room temperature.

7.2.4.3 Acceptor Reagent, 0.1 M Sodium Hydroxide (1 L)

7.2.4.3.1 Dilute 20 mL of acceptor stock solution (Section 7.2.4.2) with reagent water to 1,000 mL in a 1-L volumetric flask and mix well.

Note: Store in an amber bottle at room temperature.

7.2.4.4 WAD Reagent A (Part #A001416)

Note: Store at 4°C. If stored properly , the WAD Reagent A is stable for approximately 6 months after opening.

Note: After opening, check the WAD Reagent A monthly. Adjust a sample of 2 mg/L nickel(II) cyanide working solution (Section 7.2.3.4) to pH 12. Treat the nickel(II) cyanide sample with the WAD Reagent A according to Section 11.1 and proceed with the analysis to confirm the cyanide recovery.

7.2.4.5 WAD Reagent B (Part #A001417)

Note: Store at 4°C. If stored properly, the WAD Reagent B is stable for approximately 6 months after opening.

Note: After opening, check the WAD Reagent B monthly. Adjust a sample of 2 mg/L mercury(II) cyanide working solution (Section 7.2.3.5) to pH 12. Treat the mercury(II) cyanide sample with the WAD Reagent B according to Section 11.1 and proceed with the analysis to confirm the cyanide recovery.

7.3 Calibrant Preparation

Warning: Cyanide ion, hydrocyanic acid, all cyanide salts, and most metal-cyanide complexes are extremely dangerous. As a contact poison, cyanide does not need to 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.

7.3.1 Stock Solutions

7.3.1.1 Silver Nitrate Solution, 0.0192 N (1 L)

7.3.1.1.1 Dissolve 3.27 g of silver nitrate in 700 mL of reagent water in a 1-L volumetric flask.

7.3.1.1.2 Dilute to 1,000 mL with reagent water and mix well.

Note: Store in an amber bottle at room temperature.

7.3.1.2 Rhodanine Solution, 0.2 mg/mL in Acetone (100 mL)

7.3.1.2.1 Dissolve 20 mg of 5-[4-(dimethylamino)benzylidene]rhodanine in 80 mL of acetone in a 100-mL volumetric flask.

7.3.1.2.2 Dilute to 100 mL with acetone and mix well.

7.3.1.3 Potassium Cyanide Stock Solution, 1,000 mg/mL (1 L)

7.3.1.3.1 Dissolve 2 g of sodium hydroxide in 500 mL of reagent water in a 1-L volumetric flask.

7.3.1.3.2 Add 2.51 g of potassium cyanide. Dilute to 1,000 mL with reagent water and mix well.

Warning: Mixing sodium hydroxide with water releases a great amount of heat. Take appropriate precautions, such as cooling the solution while adding the sodium hydroxide.

Note: Store in an amber bottle at 4°C. If stored properly , this reagent is typically stable for 2 months.

7.3.1.4 Standardized Potassium Cyanide Stock Solution

7.3.1.4.1 Add 0.5 mL of rhodanine solution (Section 7.3.1.2) to 25 mL of potassium cyanide stock solution (Section 7.3.1.3).

7.3.1.4.2 Titrate with silver nitrate solution (Section 7.3.1.1) until the color changes from canary yellow to a salmon hue.

7.3.1.4.3 Based on the determined potassium cyanide concentration, dilute the potassium cyanide stock solution to the final concentration of 1.00 g/L using Equation 1. If the concentration is not 1.00 g/L, correct the intermediate and working calibration concentrations accordingly .

Note: Store in an amber bottle at 4°C. If stored properly , this reagent is typically stable for 2 months

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EQUATION 1

$$xV_1 = CV_2$$

Where:
x = Concentration of potassium cyanide stock solution determined from titrations
C = 1.00 g/L potassium cyanide
V_1 = Volume (in L) of potassium cyanide stock solution needed to prepare 1 L of 1.00 g/L standardized potassium cyanide stock solution
V_2 = 1 L, final volume (in L) of standardized potassium cyanide stock solution

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7.3.2 Secondary Calibrants

7.3.2.1 Cyanide, 100 mg/L (1 L)

7.3.2.1.1 Add 100 mL of standardized potassium cyanide stock solution (Section 7.3.1.4) and 10 mL of 1 M sodium hydroxide (Section 7.2.3.1) to a 1-L volumetric flask.

7.3.2.1.2 Dilute to 1,000 mL with reagent water and mix well.

Note: Store in an amber bottle at 4°C. If stored properly, this reagent is typically stable for 2–4 weeks.

7.3.2.2 Cyanide, 10 mg/L (1 L)

7.3.2.2.1 Use a volumetric pipet to add 10 mL of standardized potassium cyanide stock solution and 10 mL of 1 M sodium hydroxide to a 1-L volumetric flask.

7.3.2.2.2 Dilute to 1,000 mL with reagent water and mix well.

Note: Store in an amber bottle at 4°C. If stored properly, this reagent is typically stable for 2–4 weeks.

7.3.2.3 Cyanide, 1 mg/L (1 L)

7.3.2.3.1 Use a volumetric pipet to add 1 mL of standardized potassium cyanide stock solution (Section 7.3.1.4) and 1 mL of 1 M sodium hydroxide (Section 7.2.3.1) to a 1-L volumetric flask.

7.3.2.3.2 Dilute to 1,000 mL with reagent water and mix well.

Note: Store in an amber bottle at 4°C. If stored properly, this reagent is typically stable for 2–4 weeks.

7.3.3 Working Calibrants

7.3.3.1 Add the designated volumes of secondary calibrant (see Equation 2) to the required number of 100-mL volumetric flasks that each contain approximately 40 mL of reagent water.

7.3.3.2 Dilute each solution to the mark with reagent water and mix well.

Note: Prepare working calibrants fresh daily.

EQUATION 2

$$C_1V_1 = C_2V_2$$

Where:
C_1 = *Concentration (in mg/L) of stock solution (or calibrant)*
V_1 = *Volume (in L) of stock solution (or calibrant) to be used*
C_2 = *Desired concentration (in mg/L) of working calibrant to be prepared*
V_2 = *Final volume (in L) of working calibrant to be prepared*

By solving this equation for the volume of stock solution to be used (V_1), the following equation is obtained:

$$V_1 = \frac{C_2V_2}{C_1}$$

Since the desired concentration (C_2), the final volume (V_2), and the concentration of the stock solution (C_1) are all known for any given calibrant concentration in a defined volume, the volume of stock solution to be used (V_1) is easily calculated.

7.3.3.3 Calibrants covering the entire range of this analysis can be prepared from the following table.

Working Calibrant Final Concentration (µg/L)	**Volume of Secondary Calibrant Solution (mL)**		
	Cyanide 1 mg/L	**Cyanide 10 mg/L**	**Cyanide 100 mg/L**
2.0	0.20		
5.0	0.50	0.05	
10	1.0	0.10	
50	5.0	0.50	0.05
100	10	1.0	0.10
200	20	2.0	0.20
500	50	5.0	0.50
1,000		10	1.0
3,000		30	3.0
5,000		50	5.0

8.0 Sample Collection, Preservation, and Storage

8.1 Samples should be collected in plastic or glass bottles that have been thoroughly cleaned and rinsed with reagent water (Section 7.2.1).

8.2 The volume of sample collected should be sufficient to ensure that a representative sample is obtained, replicate analysis is possible, and waste disposal is minimized.

8.3 Determine cyanide in unpreserved samples within 24 hours or as soon as possible to eliminate loss of analyte.

8.4 Samples Containing Sulfide Ion

8.4.1 Precipitate sulfide ion with lead ion immediately upon sample collection. Sulfide ion and lead sulfide will react with cyanide ion to form thiocyanate, which is not detected in the analytical system. 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 (Reference 15.11).

8.4.2 Test samples with lead acetate test paper to determine the presence or absence of sulfide ion. Note that the lead acetate test paper can be unreliable and is typically not usable for sulfide concentrations below approximately 1 ppm. If the presence of sulfide is suspected but not verifiable by the 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 should be tested for minimum level of sulfide detection by spiking reagent water aliquots with decreasing levels of sulfide. The spiked samples are tested with lead acetate test paper moistened with acetate buffer (Section 7.2.2.1). 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.

8.5 Treat samples containing or suspected to contain formaldehyde, acetaldehyde, or other water soluble aldehydes with 20 mL of 3.5% ethylenediamine solution (Section 7.2.2.2) per liter of sample.

8.6 Treat samples containing chlorine, hypochlorite, and/or sulfite with 0.6 g of ascorbic acid per liter of sample.

8.7 Treat samples containing high concentrations of carbonate with hydrated lime.

8.7.1 Slowly add hydrated lime while stirring to raise the pH of the sample to 12.0–12.5.

8.7.2 Decant the sample after the precipitate has settled.

8.8 The holding time for preserved samples is 14 days from the time of collection (Reference 15.8). Sample analysis should be performed as soon as possible to eliminate loss of analyte.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 15.6). The minimum requirements of this program consist of an initial demonstration of laboratory capability and the periodic analysis of Laboratory Control Samples (LCSs) and Matrix Spike/Matrix Spike Duplicates (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 analyst 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 analyst to overcome sample matrix interferences, the analyst is permitted certain options to improve performance or lower the costs of measurements. Alternate determinative techniques, such as the substitution of spectroscopic or other techniques, and changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in this method for the analyte(s) of interest.

9.1.2.1 Each time a modification is made to this method, the analyst 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 is required to demonstrate that the MDL is lower than one-third the regulatory compliance level or as low as or lower than that listed in Section 1.3. If calibration will be affected by the change, the analyst must recalibrate the instrument per Section 10.4.

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 10.4)

b) calibration verification (Section 9.5)

c) initial precision and recovery (Section 9.2.2)

d) analysis of blanks (Section 9.4)

e) ongoing precision and recovery (Section 9.6)

f) matrix spike and matrix spike duplicate (Section 9.3)

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

h) injection volume

i) dilution data, differentiating between dilution of a sample or modified sample

j) instrument and operating conditions

k) other operating conditions

l) detector

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 MS/MSD 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 laboratory reagent blanks (LRBs) 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 an LRB are described in Section 9.4.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through the analysis of the LCS that the analytical 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 reagent blank (LRB, Section 9.4), a laboratory control sample (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 LRB, 1 LCS, 1 MS, and 1 MSD) and a maximum of 14 analyses (10 samples, 1 LRB, 1 LCS, 1 MS, and 1 MSD) in the batch. If more 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 analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B (Reference 15.2) 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 1.3 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 analyst shall perform the following operations:

9.2.2.1 Analyze four samples of the LCS (Section 9.6) according to the procedure beginning in Section 10.0.

9.2.2.2 Using the results of the set of the four analyses, compute the average percent recovery (x) and the standard deviation of the percent recovery (s) for cyanide. Use Equation 3 for the calculation of the standard deviation of the percent recovery (s).

EQUATION 3

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

Where:
s = Standard deviation
n = Number of samples
x = Percent recovery in each sample

9.2.2.3 Compare s and x with the precision and percent recovery acceptance criteria specified in Section 13.0. If the value of s exceeds the precision limit or the value of x falls outside the range for recovery, system performance is unacceptable and the problem must be found and corrected before the analysis may continue.

9.3 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory shall spike, in duplicate, a minimum of 10% of all samples (one sample in duplicate in each batch of 10 samples) from a given sampling site.

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 one to five 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 least four times greater than the MDL.

9.3.2 Analyze one sample aliquot out of each set of 10 samples from each site or discharge according to the procedure beginning in Section 10.0 to determine the background concentration of cyanide.

9.3.2.1 If necessary, prepare a stock solution appropriate to produce a concentration level in the sample at the regulatory compliance limit or at one to five times the background concentration of cyanide (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution (Section 9.3.2.1) and analyze these aliquots to determine the concentration after spiking.

9.3.3 Calculate the percent recovery of cyanide in each aliquot using Equation 4.

EQUATION 4

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

Where:
P = Percent recovery
A = Measured concentration of cyanide after spiking (Section 9.3.2.2)
B = Measured background concentration of cyanide (Section 9.3.2)
T = True concentration of the spike

9.3.4 Compare the recovery to the QC acceptance criteria in Section 13.0. If percent 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 must be reanalyzed.

9.3.6 Compute the relative percent difference (RPD) between the two spiked sample results (Section 9.3.2.2, not between the two percent recoveries) using Equation 5.

EQUATION 5

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

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 If the RPD is greater than 10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected. The analytical batch must be 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 70–1 10%. Update the accuracy assessment on a regular basis (e.g., after each 5–10 new accuracy measurements).

9.4 Laboratory Reagent Blanks (LRB)—Laboratory reagent blanks are analyzed to demonstrate freedom from contamination.

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

9.4.2 If cyanide is detected in the LRB at a concentration greater than the ML, analysis of samples is halted until the source of contamination is eliminated and consequent analysis of another LRB 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 LRB, 1 LCS, 1 MS, and 1 MSD). This can be accomplished by analyzing the midrange calibration standard and verifying that it is within the QC acceptance criteria for recovery in Section 13.0. (The concentration of the calibration verification depends on the calibration range being used.) Failure to attain recoveries within the acceptance criteria requires recalibration of the analytical system (Section 10.4).

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 analyst shall perform the following operations:

9.6.1 Analyze an LCS (Section 7.2.3.4) with each analytical batch according to the procedure in Section 10.0.

9.6.2 If the precision and recovery for the LCS are within the acceptance criteria specified in Section 13.0, 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 85–105%.

9.7 Reference Sample—To demonstrate that the analytical system is in control, the laboratory may wish to periodically test an external reference sample, such as a Standard Reference Material (SRM) available from the National Institute of Standards and Technology (NIST). Corrective action should be taken if the measured concentration significantly differs from the stated concentration.

10.0 Configuration and Start-up

10.1 Instrument Configuration

10.1.1 Configure the OI Analytical CNSolution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Available Cyanide, Method OIA-1677 Cartridge (Part #A001982 or #A002654) is configured as illustrated in the flow diagram shown in Section 17.0.

10.1.3 Connect the appropriate pump tubes to the cartridge and to their appropriate reagent containers according to the flow diagram.

10.2 Instrument Stabilization

10.2.1 Start the pump, allowing the reagents to flow through the entire system.

10.2.2 Verify that the flowcell of each detector is purged of all bubbles and that the flow is stable and free from surging.

10.2.3 Load a 10 mg/L cyanide calibrant into the sampling valve and inject it into the FIA system.

10.2.4 Continue to inject 10 mg/L cyanide calibrant until three successive peak heights or area results are within 5% RSD, indicating that the electrode system is stabilized.

10.2.5 Following stabilization, inject the highest concentration calibration standard until three successive peak heights or area results are within 5% RSD, indicating stabilization at the top of the calibration range.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.12) for help on creating a Method.

10.3.2 Create and save a Sample Table in WinFLOW that will be used to generate a calibration curve using at least three calibrants that cover the full range of expected concentrations in the samples to be analyzed. This Sample Table should also be used to analyze all necessary QC samples as well as the analytical batch of samples to be analyzed. For help on creating a Sample Table, refer to the WinFLOW Operator's Manual (Reference 15.12).

10.3.3 Select **Collect Data** in the WinFLOW main window, enter the user's identification, select the appropriate Method and Sample Table, and begin to collect baseline data. Very sharp fluctuations in the baseline and/or consistent drifting are typically signs of bubbles in the flowcell. The flowcell must be free of bubbles prior to beginning analysis.

10.4 Calibration and Standardization

10.4.1 Prepare a series of at least three working calibrants using the stock solutions (Section 7.3) according to Equation 2, covering the desired analysis range.

10.4.2 Place the calibrants in the autosampler in order of increasing concentration. Each calibrant should be analyzed according to the analytical procedures in Section 11.0. A calibration curve will be calculated by the WinFLOW software.

10.4.3 Acceptance or control limits for the calibration results should be established using the difference between the measured value of each calibrant and the corresponding "true" concentration.

10.4.4 Each calibration curve should be verified by analysis of a Laboratory Control Sample (LCS, Section 9.5). Using WinFLOW software, calibration, verification, and sample analysis may be performed in one continuous analysis.

11.0 Procedure

11.1 Ligand Exchange Reagent Treatment

Note: When the ligand exchange reagents are added to the cyanide containing solution in the specified proportions, up to 5 mg/L cyanide ion will be liberated from metal complexes of intermediate stability. If higher concentrations of cyanide are present, increase the amount of ligand exchange reagent added, or dilute the cyanide containing solution appropriately .

11.1.1 Adjust the pH of the sample, standard, or blank to approximately 12.

11.1.2 Add 50 µL of WAD Reagent A (Section 7.2.3.4) and 100 µL of WAD Reagent B (Section 7.2.3.5) to 100 mL of sample, standard, or blank. Mix well.

11.1.3 Analyze the treated samples within 2 hours of adding ligand exchange reagents.

11.2 Analysis

11.2.1 Place all reagents on-line and allow to pump at least 10–15 minutes. Verify that there are no bubbles in the flowcell. Obtain a stable baseline and autozero the baseline before beginning the analysis.

11.2.2 Load the sampler tray with calibrants, blanks, samples, and QC samples.

Note: The matrix of the working standards, blanks, and QC samples should match that of the samples being analyzed.

11.2.3 Using the Method and Sample Table created for the analytical batch to be analyzed and with the baseline verified to be stable, begin the analysis by selecting the “Fast Forward” button on the left side of the Data Analysis window in WinFLOW. This will initiate the sequential analysis of samples as defined in the Sample Table.

11.2.4 When analysis is complete, pump reagent water through the system for at least 10–15 minutes. Stop the pump, release the tension on all pump tubes, and power off the system.

12.0 Data Analysis and Calculations

12.1 The calibration curve allows for accurate quantitation of the concentration in each sample.

12.2 WinFLOW software reports the concentration of each sample relative to the calibration curve.

13.0 Method Performance

Range:	2.0 µg/L–5.0 mg/L
Throughput:	48 samples/hour
Precision:	
2.0 µg/L	<2% RSD
5.0 mg/L	<1% RSD
Method Detection Limit (MDL):	0.5 µg/L

14.0 Pollution Prevention and Waste Management

14.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land-disposal restrictions. In addition, it is the laboratory's responsibility to protect air, water, and land resources by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations.

14.2 For further information on waste management, consult Section 13.6 of *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 15.7).

15.0 References

15.1 Wilmont, J.C.; Solujic, L.; Milosavljevic, E. B.; Hendrix, J.L.; Rader, W.S. Formation of Thiocyanate During Removal of Sulfide as Lead Sulfide Prior to Cyanide Determination. *Analyst* **1996**, *121*, 799–801.

15.2 *Code of Federal Regulations*, Part 136, Appendix B, Title 40,1994.

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*: 1983; EPA-600/4-83-054; Environmental Protection Agency, Environmental Monitoring Systems Laboratory, U.S. Government Printing Office: Washington, DC, 1983.

15.4 Milosavljevic, E.B.; Solujic, L.; Hendrix, J.L. *Environ. Sci. Technol.* **1995**, *29* (No. 2), 426–430.

15.5 *Guide to Method Flexibility and Approval of EPA Water Methods*. Available from the National Technical Information Service (PB97-1 17766).

15.6 *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*: 1979; EPA-600/4-79-019; Environmental Protection Agency, Environmental Monitoring Systems Laboratory, U.S. Government Printing Office: Washington, DC, 1979.

15.7 *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.

15.8 Sample Preparation. *Methods for the Chemical Analysis of Water and Wastes*; EPA/600/4-79-020; Environmental Protection Agency, Environmental Monitoring Systems Laboratory , U.S. Government Printing Office: Washington, DC, 1983.

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

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

15.11 *Standard Methods for the Examination of Water and Wastewater*, 20th ed.; American Public Health Association: Washington, D.C., 1998.

15.12 WinFLOW Software and Operator 's Manual (Part #A002877). Available from OI Analytical, P.O. Box 9010, College Station, TX, 77842-9010.

16.0 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.

16.1 Units of weights and measures and their abbreviations

16.1.1 Symbols

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

16.1.2 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
N	normal solution

16.2 Definitions

16.2.1 Available cyanide (used interchangeably with weak acid dissociable (WAD) cyanide) consists of cyanide ion (CN^-), hydrogen cyanide in water (HCN(aq)), and the cayno-complexes of zinc, copper, cadmium, mercury, nickel, and silver.

16.2.2 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.

16.2.3 Laboratory Control Sample (LCS)—An aliquot of LRB to which a quantity of the analyte of interest 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.

16.2.4 Laboratory Reagent Blank (LRB)—An aliquot of reagent water and other blank matrix 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.

16.2.5 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. The MS/MSD is analyzed like a sample. Its 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 must be corrected for the background concentration.

16.2.6 Method Detection Limit (MDL)—The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

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

16.2.8 Ongoing Precision and Recovery (OPR)—See Section 16.2.2, “Laboratory Control Sample.”

17.0 Figures

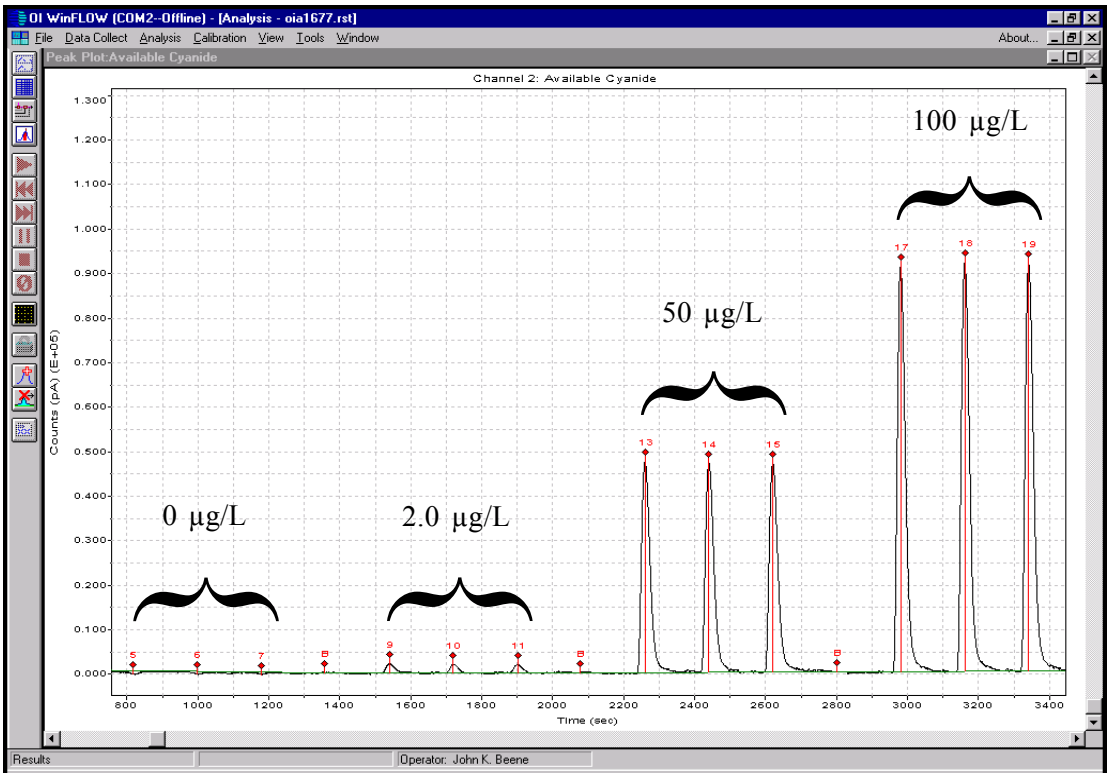

Figure 1. Calibration (2.0–100 µg/L)

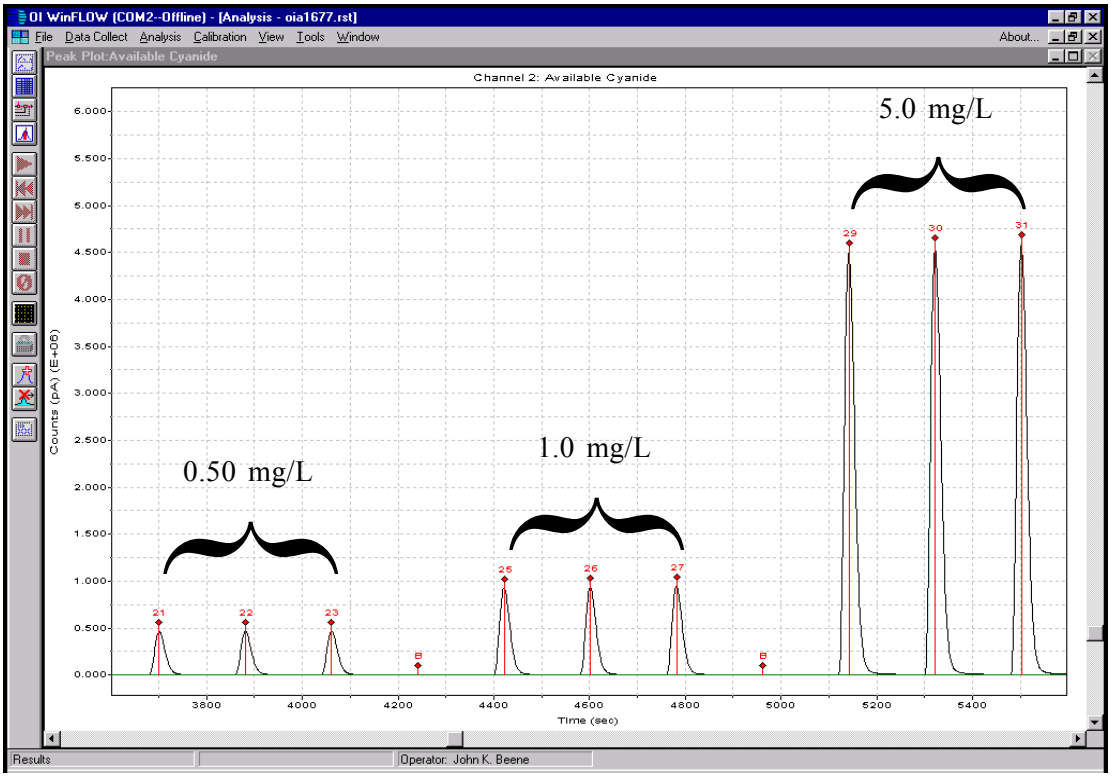

Figure 2. Calibration (0.50–5.0 mg/L)

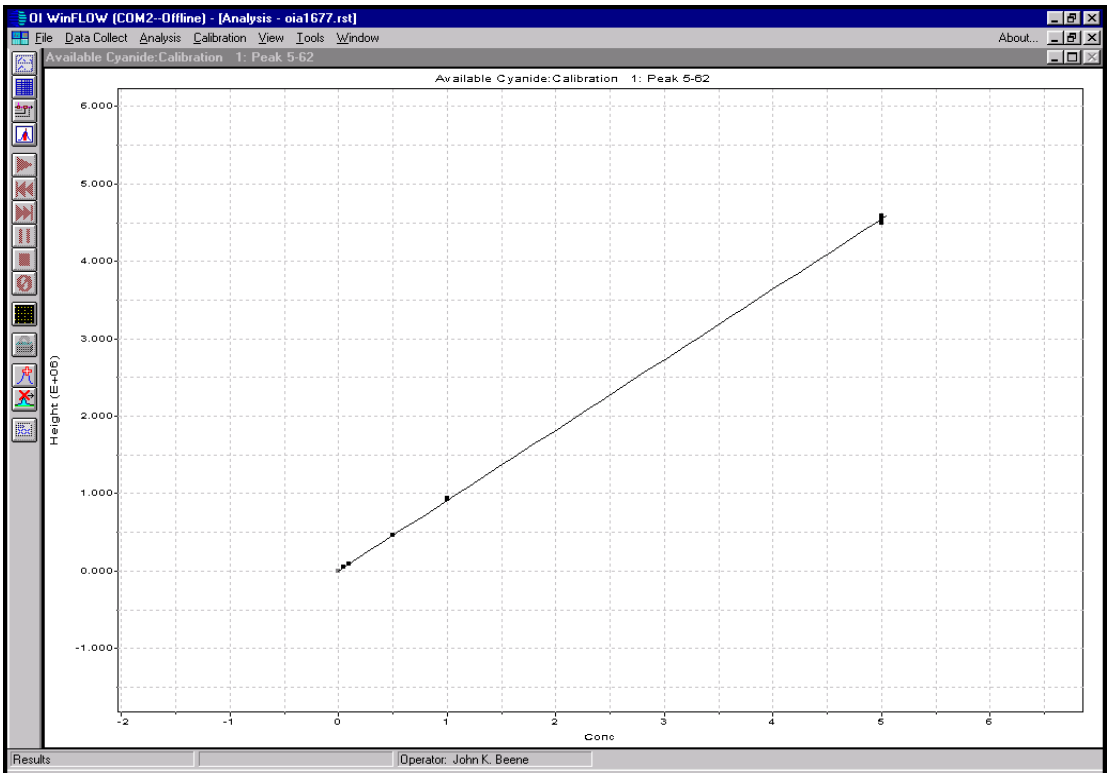

Figure 3. Calibration Curve (2.0 µg/L–5.0 mg/L)

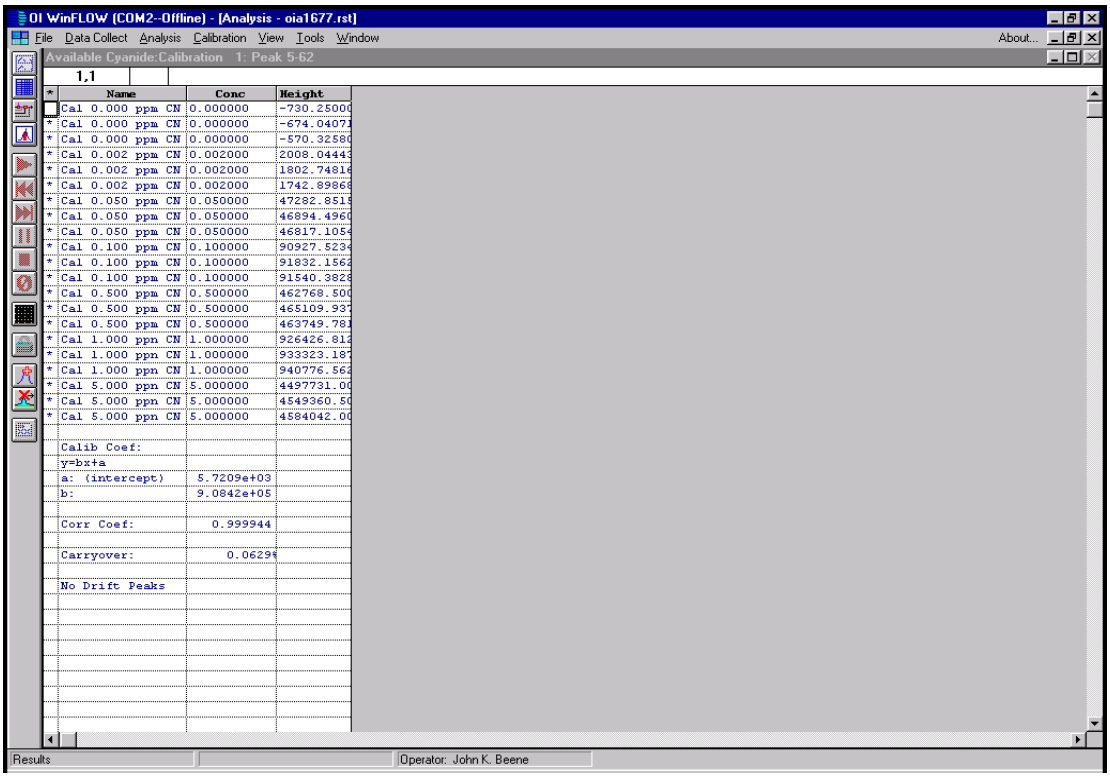

Figure 4. Calibration Results (2.0 µg/L–5.0 mg/L)

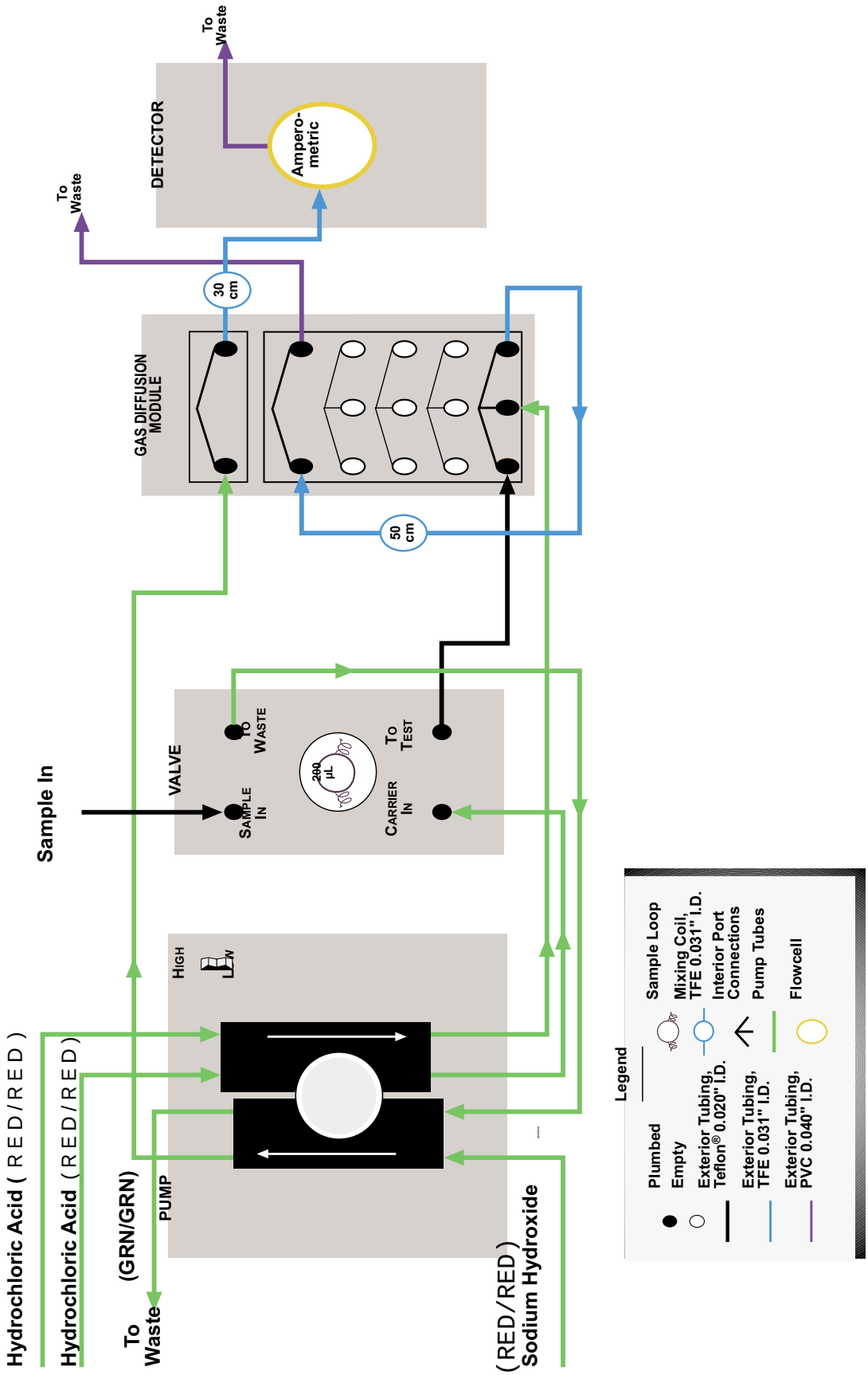

Figure 5. Detailed Flow Diagram for Available Cyanide, Method OIA-1677 by SFA on a CNSolution 3000, Cartridge Part #A001982 or A002654

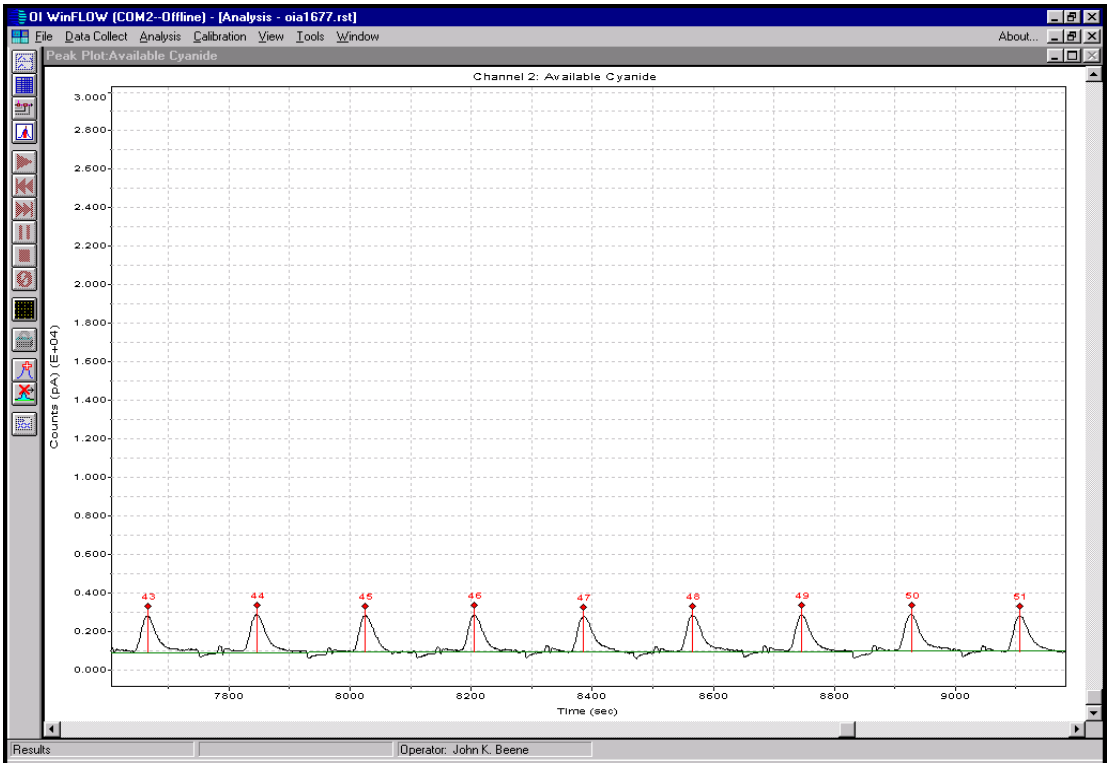

Figure 6. Precision at 2.0 µg/L (<2% RSD)

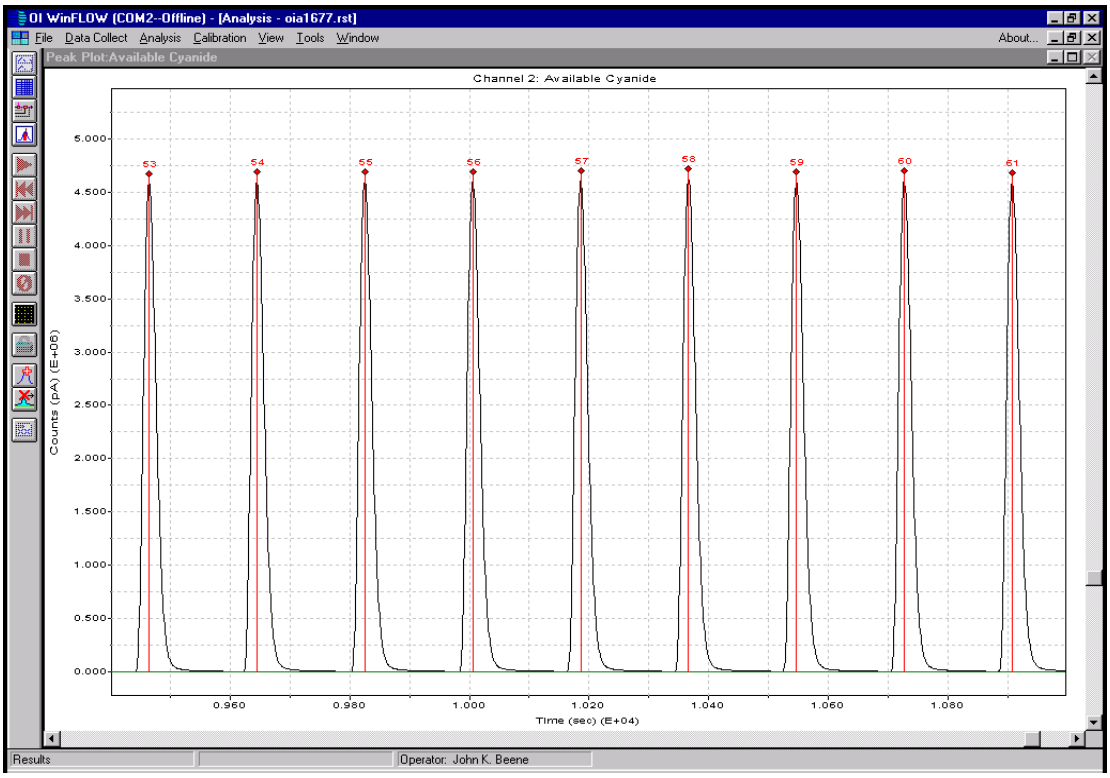

Figure 7. Precision at 5.0 mg/L (<1% RSD)

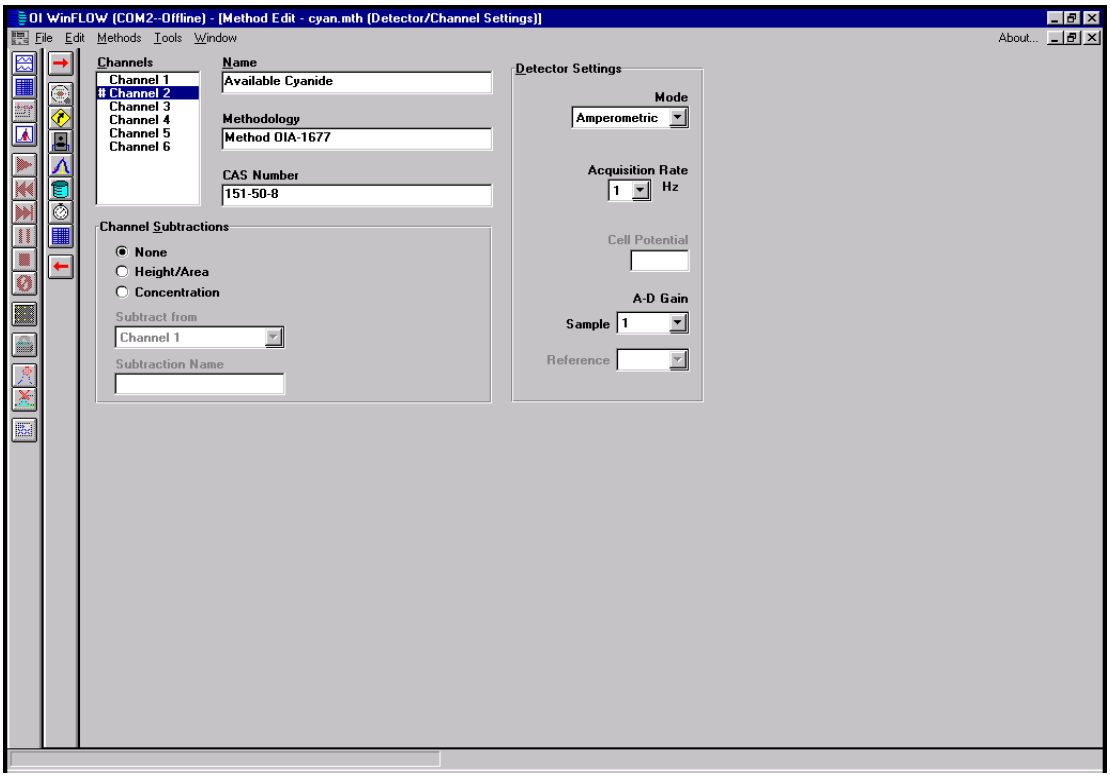

Figure 8. WinFLOW Method Editor—Detector Channel Settings

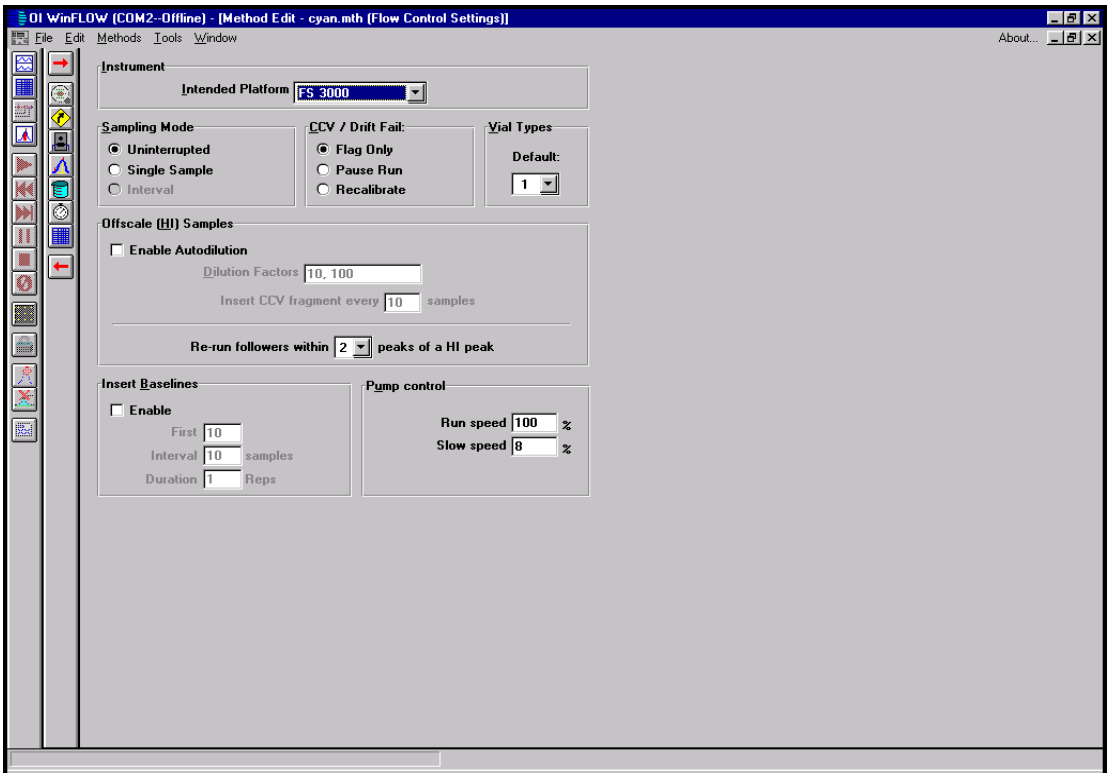

Figure 9. WinFLOW Method Editor—Flow Control Settings

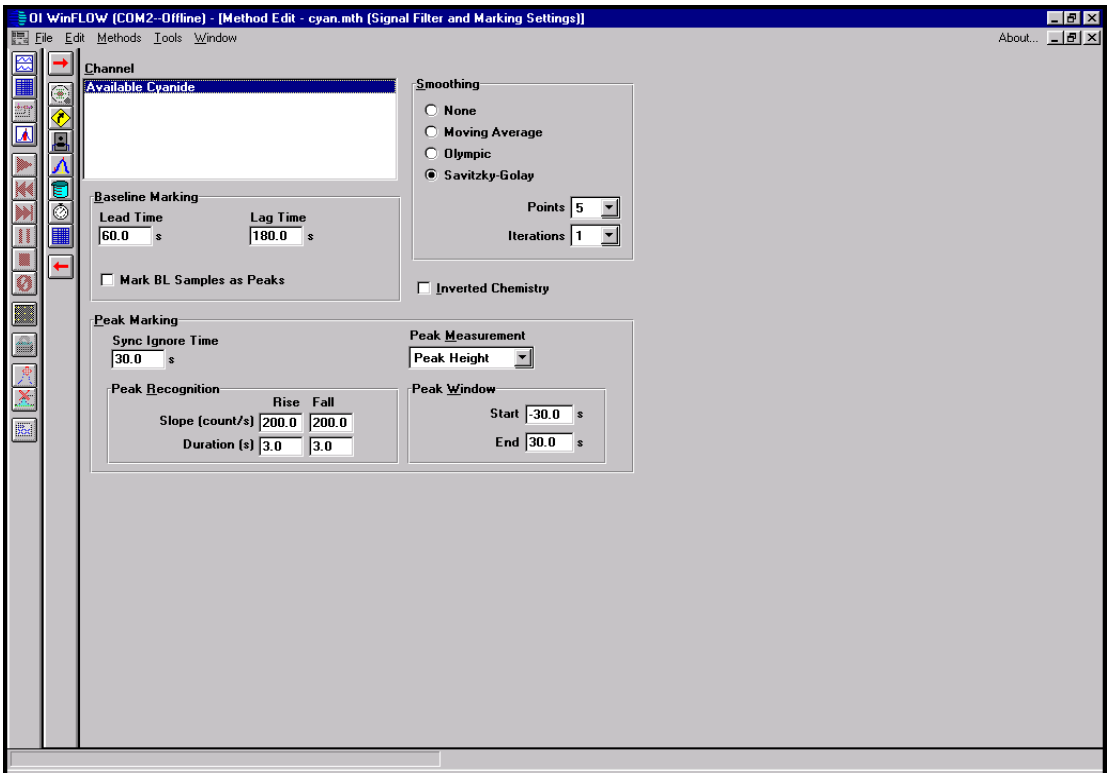

Figure 10. WinFLOW Method Editor—Signal Filter and Marking Settings

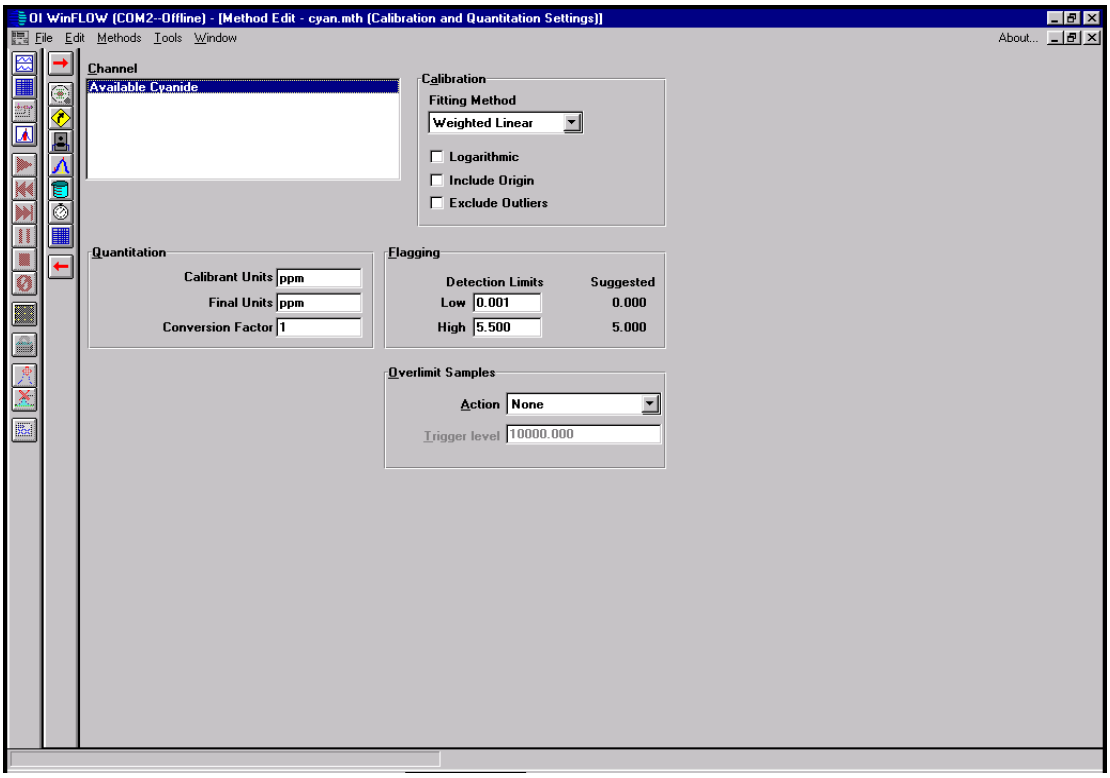

Figure 11. WinFLOW Method Editor—Calibration and Quantitation Settings

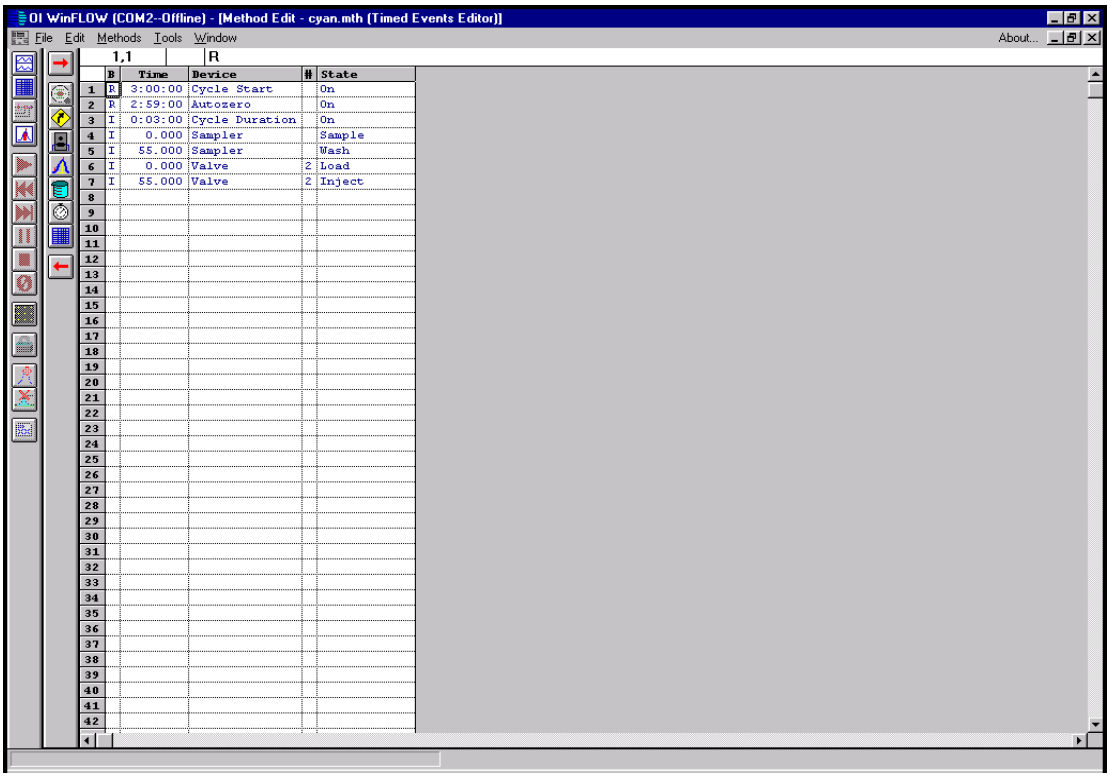

Figure 12. WinFLOW Method Editor—Timed Events Editor

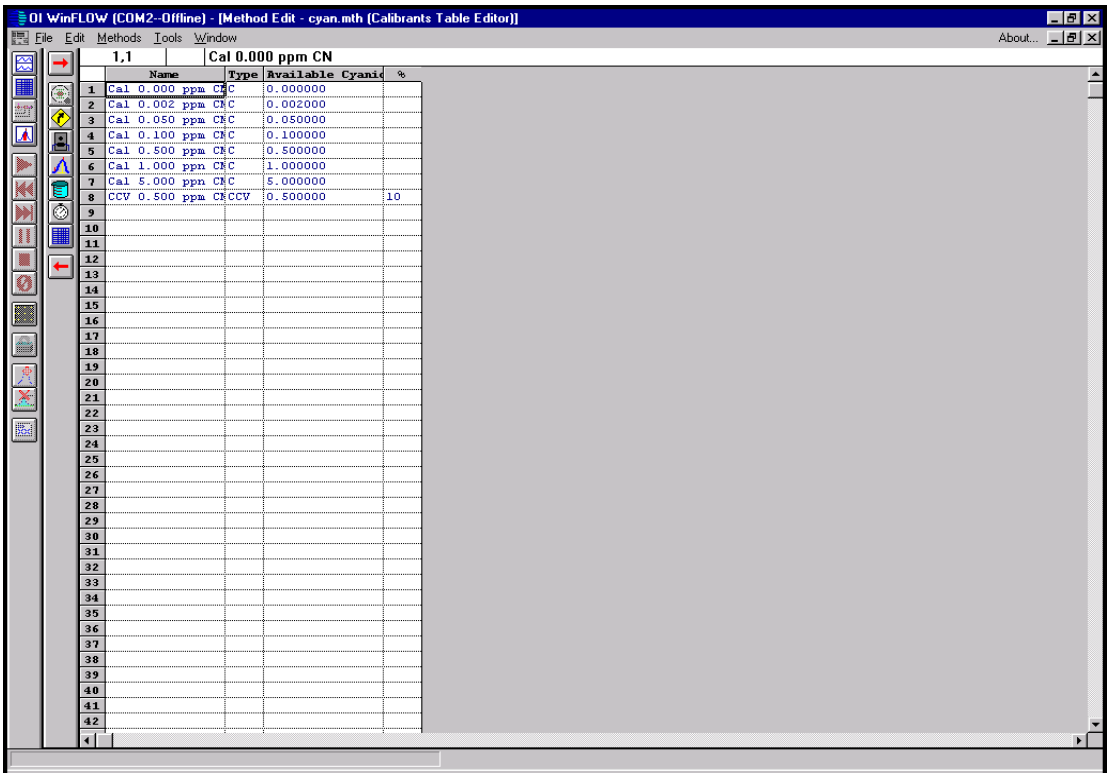

Figure 13. WinFLOW Method Editor—Calibrants Table Editor

18.0 Appendix

18.1 MDLs from nine laboratories were pooled to develop the MDL of 0.5 µg/L given in Section 1.3 (Reference 15.9).

18.2 Data obtained from single laboratory testing of the method are summarized in Table 1. Recoveries and reproducibility for "free" forms of cyanide are shown, including the recovery and reproducibility of silver, nickel, mercurous, and mercuric 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 Method OIA-1677 Single Laboratory Validation Study* (Reference 15.10).

18.3 Listed in Table 2 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 USEPA Water Methods* (Reference 15.5). 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 percent 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., platinum and iron 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 Method OIA-1677 Interlaboratory Validation Study* (Reference 15.9).

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

Parameter	Required Recovery Range (%)	Precision
Initial Precision and Recovery	92–122	< 5.1% RSD
Ongoing Precision and Recovery	82–132	n/a
Calibration Verification	86–118	n/a

[1] Values are percent recoveries; numbers in parentheses are percent relative standard deviations.

Table 2. QC Acceptance Criteria

Species	0.20 mg/L CN^-		2.0 mg/L 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)
$[Fe(CN)_6]^{4-}$	0.0	(n/a)	0.0	(n/a)
$[Fe(CN)_6]^{3-}$	0.0	(n/a)	0.0	(n/a)

Table 3. Cyanide Recoveries From Various Aqueous Matrices

Sample Description	Sample CN^- Concentration	Added CN^- Concentration[1]	Average % Recovery	Relative Standard Deviation
Reagent Water w/0.01 M Sodium Hydroxide	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/0.01 M Sodium Hydroxide	0 µg/L	200 µg/L as KCN	101	8.0
Reagent Water w/0.01 M Sodium Hydroxide	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 platinum and iron were added to the POTW and petroleum refinery effluents, respectively. Thiocyanate was added to the metals finishing effluent to demonstrate that the FI/LE system does not determine these forms of cyanide.

Results were obtained under optimal operating conditions. Actual results may vary depending on sample introduction, cleanliness of sample containers, reagent purity , operator skill, and maintenance of instruments.

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