O·I·Analytical

Methodology

Post-Distillation Cyanide by Flow Injection Analysis (FIA)

(Cartridge Part #A001083)

1.0 Scope and Application

1.1 This method is used for the determination of cyanide (CN) in distilled samples.

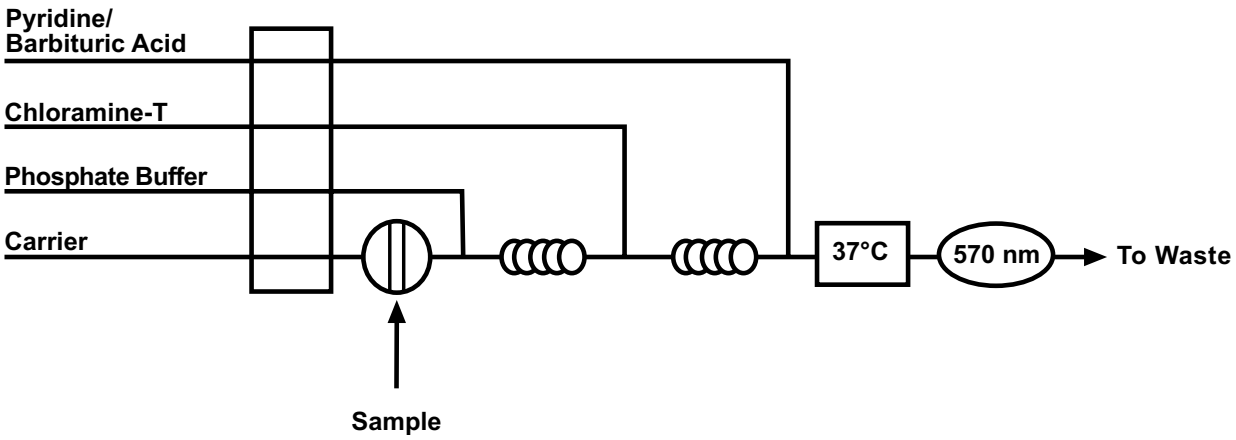
1.2 The Method Detection Limit (MDL) of this method is 0.11 µg/L cyanide. The applicable range of the method is 2.0–500 µg/L cyanide. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

2.1 Prior to analysis, cyanide is released from cyanide complexes by off-line manual distillation and collected in a sodium hydroxide receiver solution (Reference 15.2). Sodium cyanide is converted to cyanogen chloride by a reaction with chloramine-T trihydrate at a pH of less than eight. The cyanogen chloride then reacts with pyridene-barbituric acid to form a red-colored complex, and the absorbance is measured at 570 nm (References 15.2 and 15.4).

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 Interferences are eliminated or reduced by distillation prior to the analysis.

4.2 Treat samples containing sulfide by adding powdered lead carbonate (Section 8.6).

4.3 Treat samples containing residual chlorine by adding ascorbic acid (Section 8.7).

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 Barbituric Acid, $C_4H_4N_2O_3$ (FW 128.09)

5.3.2 Chloramine-T Trihydrate, $C_7H_7ClNO_2SNa \cdot 3H_2O$ (FW 281.70)

5.3.3 Hydrochloric Acid, concentrated, HCl (FW 36.46)

5.3.4 Potassium Cyanide, KCN (FW 65.12)

5.3.5 Pyridine, C_5H_5N (FW 79.10)

5.3.6 Sodium Hydroxide, NaOH (FW 40.00)

5.3.7 Sodium Phosphate Monobasic Monohydrate, $NaH_2PO_4 \cdot H_2O$ (FW 137.99)

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 Flow Solution® 3000) consisting of the following:

6.1.1 120-Place Autosampler

6.1.2 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 570-nm optical filter

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

6.1.4 Post-Distillation Cyanide Cartridge (Part #A001083)

6.2 Sampling equipment—Sample bottle, amber glass, 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 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 Barbituric Acid, $C_4H_4N_2O_3$ (FW 128.09)

7.1.2 Chloramine-T Trihydrate, $C_7H_7ClNO_2SNa \cdot 3H_2O$ (FW 281.70)

7.1.3 Deionized Water (ASTM Type I or II)

7.1.4 Hydrochloric Acid, concentrated, HCl (FW 36.46)

7.1.5 Potassium Cyanide, KCN (FW 65.12)

7.1.6 Pyridine, C_5H_5N (FW 79.10)

7.1.7 Sodium Hydroxide, NaOH (FW 40.00)

7.1.8 Sodium Phosphate Monobasic Monohydrate, $NaH_2PO_4 \cdot H_2O$ (FW 137.99)

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 Start-up Solution—Reagent water (Section 7.2.1)

7.2.3 Phosphate Buffer (1 L)

7.2.3.1 Dissolve 138 g of sodium phosphate monobasic monohydrate in approximately 800 mL of reagent water in a 1-L volumetric flask.

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

Note: Store at 4°C.

7.2.4 Chloramine-T Solution (500 mL)

7.2.4.1 Dissolve 2 g of chloramine-T trihydrate in approximately 400 mL of reagent water in a 500-mL volumetric flask.

7.2.4.2 Dilute to 500 mL with reagent water and mix well.

7.2.5 Pyridine-Barbituric Acid Solution (500 mL)

7.2.5.1 In a hood, place 7.5 g of barbituric acid in a 500-mL beaker. Add 50 mL of reagent water, rinsing down the sides of the beaker.

7.2.5.2 While stirring, add 37.5 mL of pyridine and 7.5 mL of concentrated hydrochloric acid.

7.2.5.3 Add 300 mL of reagent water. Continue stirring until the barbituric acid is completely dissolved.

7.2.5.4 Quantitatively transfer the solution to a 500-mL volumetric flask. Dilute to 500 mL with reagent water and mix well.

7.2.5.5 Filter the solution through a 0.45-µm filter.

Warning: This reagent must be prepared in a hood. Barbituric acid and pyridine are irritants and are toxic if inhaled or ingested.

Note: Prepare this solution weekly.

7.2.6 Carrier, 0.25 N Sodium Hydroxide (1 L)

7.2.6.1 While stirring, add 10 g of sodium hydroxide to approximately 800 mL of reagent water in a 1-L volumetric flask.

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

Warning: Mixing sodium hydroxide and water releases a great amount of heat. Take appropriate precautions.

7.3 Calibrant Preparation

Warning: Potassium cyanide is a contact poison. It does not need to be ingested to produce toxicity. Also, cyanide solutions produce fatally toxic hydrogen cyanide gas when acidified. For these reasons, work with cyanide must be carried out in a well-ventilated hood by properly trained personnel wearing adequate protective equipment.

7.3.1 Stock 100 mg/L Cyanide Solution (1 L)

7.3.1.1 Dissolve 2 g of sodium hydroxide in approximately 900 mL of reagent water in a 1-L volumetric flask.

7.3.1.2 Add 0.2505 g of potassium cyanide and stir until dissolved.

7.3.1.3 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 Intermediate 10 mg/L Cyanide Solution (100 mL)

7.3.2.1 Use a volumetric pipet to add 10 mL of stock 100 mg/L cyanide solution (Section 7.3.1) to approximately 80 mL of reagent water in a 100-mL volumetric flask.

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

Note: Prepare the intermediate calibrant fresh daily.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Add the designated volumes of stock or intermediate calibrant solution (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of 0.25 N sodium hydroxide (Section 7.2.6).

7.3.3.2 Dilute each solution to 100 mL with 0.25 N sodium hydroxide and mix well.

Note: Prepare working calibrants fresh daily.

EQUATION 1

$$C_1V_1 = C_2V_2$$

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 tables.

Final Concentration (µg/L)	Vol. of Inter. Cal. (µL)	Conc. of Inter. Cal. (mg/L)	Final Volume (mL)
5.0	50	10	100
10	100	10	100

Final Concentration (µg/L)	Vol. of Stock Cal. (µL)	Conc. of Stock Cal. (mg/L)	Final Volume (mL)
50	50	100	100
100	100	100	100
200	200	100	100
300	300	100	100
400	400	100	100
500	500	100	100

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 as soon as possible after collection to eliminate loss of analyte (Reference 15.3).

8.4 Preserve samples by adding 2 mL of 10 N sodium hydroxide per liter of sample to obtain a pH of greater than 12. Store at 4°C.

8.5 The holding time for preserved samples without the presence of sulfide is 14 days from the time of collection. The maximum holding time for samples with sulfide present is 24 hours.

8.6 Samples may be tested for sulfide with lead acetate paper before adding sodium hydroxide (Reference 15.4).

8.6.1 If sulfide is present, remove it by adding powdered lead carbonate ($Pb(CO_3)_2$) until the lead acetate paper test is negative.

8.6.2 Filter the sample immediately. Add 2 mL of 10 N sodium hydroxide per liter of sample.

8.6.3 Samples can be held for 14 days following removal of sulfide.

8.7 Treat samples with residual chlorine by adding 1.2 g ascorbic acid per liter of sample.

9.0 Quality Control

Note: The following QC procedures are provided for reference purposes only and are not a substitute for any QC procedures that may be required for regulatory compliance.

9.1 It is recommended that each laboratory that uses this method operate a formal quality control program. The minimum requirements of such a program should 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 should be compared to established performance criteria to determine if the results of the analyses meet the performance characteristics of the method.

9.2 Method Detection Limit (MDL)—To establish the ability to detect cyanide at low levels, the analyst should determine the MDL using the apparatus, reagents, and calibrants that will be used in the practice of this method. An MDL less than or equal to the MDL listed in Section 1.2 should be achieved prior to practice of this method.

9.2.1 An MDL is calculated by analyzing a matrix spike at a concentration of two to three times the expected detection limit of the analyzer. Seven consecutive replicate analyses of this matrix spike should be analyzed, and the MDL should be calculated using Equation 2.

EQUATION 2

$$MDL = (t) \times (S)$$

Where:
t = Student's t value for a 99% confidence level and a standard deviation estimate with n–1 degrees of freedom (t = 3.14 for seven replicates)
S = Standard deviation of the replicate analyses

9.2.2 It is recommended that the MDL be calculated after every six months of operation, when a new operator begins work, or whenever there is any significant change in the instrument response.

9.3 Analyses of MS/MSD samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix).

9.3.1 Matrix Spike/Matrix Spike Duplicate (MS/MSD)—The laboratory should 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.

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

9.3.2.1 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.

9.3.2.2 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.

9.4 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.

9.5 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records should be maintained.

9.5.1 An LCS should be analyzed with every sample batch, and the mean (m) and the standard deviation (S) should be recorded. After multiple analyses, the mean should be plotted with limits of $m+2S$ and $m–2S$. The mean and the limits should be recalculated after every 5–10 new measurements.

9.5.2 If the LCS measurement falls outside the range calculated in Section 9.5.1, then the problem should be addressed, and that sample batch should be reanalyzed if necessary.

9.6 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 Flow Solution 3000 Analyzer according to the Operator's Manual and verify that each module is properly powered on.

10.1.2 Verify that the Post-Distillation Cyanide Cartridge (Part #A001083) 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 Connect the reagent pump tubes to a reagent bottle containing the start-up solution (Section 7.2.2). Start the pump, allowing the start-up solution 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 before proceeding.

10.3 Baseline Verification

10.3.1 Create and save a Method in WinFLOW. Refer to the WinFLOW Operator's Manual (Reference 15.5) 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 15.5).

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 1, 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 Analysis

11.1.1 Begin pump flow with the start-up solution (Section 7.2.2). Once the heater unit has reached 37°C, verify a stable baseline (Section 10.3).

11.1.2 Place all reagents on-line and allow to pump at least 10–15 minutes. Verify there are no bubbles in the flowcell. Obtain a stable baseline at 570 nm and autozero the baseline before beginning the analysis.

11.1.3 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.

11.1.4 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.

11.1.5 When analysis is complete, pump start-up solution through the system for at least 10–15 minutes. Stop the pump, release the tension on all pump tubes, and power off the system.

11.2 Operating Notes

11.2.1 Operate the system in a hood or in a well-ventilated area.

Warning: Cyanogen chloride is a very toxic gas. Use care in operating the system to ensure complete color formation.

11.2.2 Add sodium hydroxide to the waste container so that the wastes do not become acidic and emit hydrogen cyanide gas.

11.2.3 If the normality of the distillates varies from 0.25 N sodium hydroxide, adjustment of the buffer may be necessary. After adding buffer to the distillate, the resulting pH must be less than eight.

11.2.4 If the standards are not distilled, check the efficiency of the distillation process by taking at least one standard through the distillation procedure.

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:	2.0–500 µg/L CN
Throughput:	50 samples/hour
Precision:	
100 µg/L	<0.5% RSD
400 µg/L	<0.5% RSD
Method Detection Limit (MDL):	0.11 µg/L CN

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 15.1).

15.0 References

15.1 *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.

15.2 Cyanide (Colorimetric, Automated, UV). *Methods for Chemical Analysis of Water and Wastewater;* EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; Method 355.3.

15.3 Sample Preservation. *Methods for Chemical Analysis of Water and Wastes*; EPA-600/4-79-020; U.S. Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory: Cincinnati, OH, 1984; xvii.

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

15.5 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 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.

16.2.2 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.

16.2.3 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.

16.2.4 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.

16.2.5 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.

16.2.6 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.

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

17.0 Figures

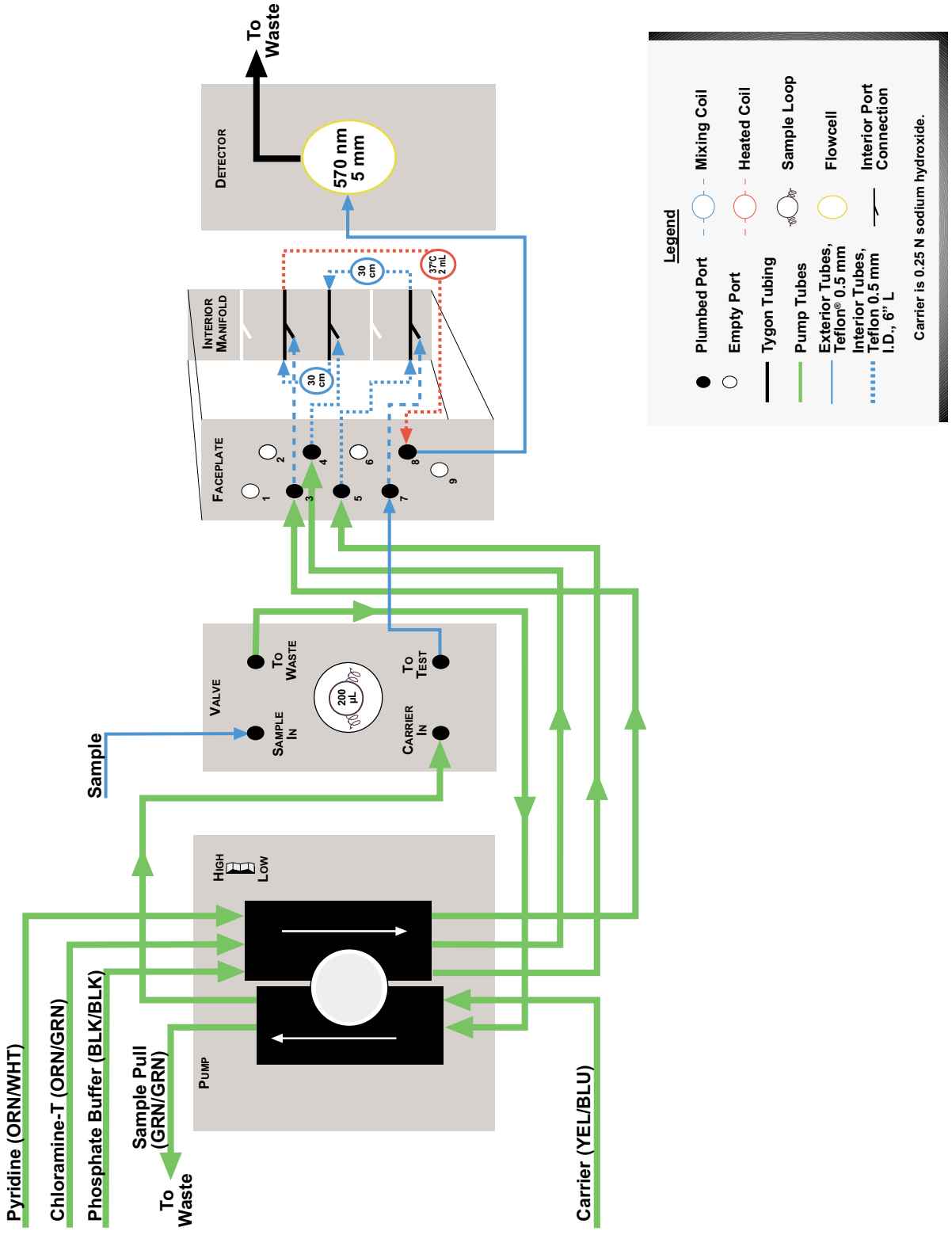


Figure 1. Detailed Flow Diagram for Post-Distillation Cyanide by FIA on a Flow Solution 3000, Cartridge Part #A001083

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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