O·I·Analytical **Methodology**

Post-Distillation Phenol by Flow Injection Analysis (FIA)

(Cartridge Part #A002164)

1.0 Scope and Application

1.1 This method is used for the determination of phenolic compounds in drinking water, surface water and saline water, and domestic and industrial wastes (Reference 15.2).

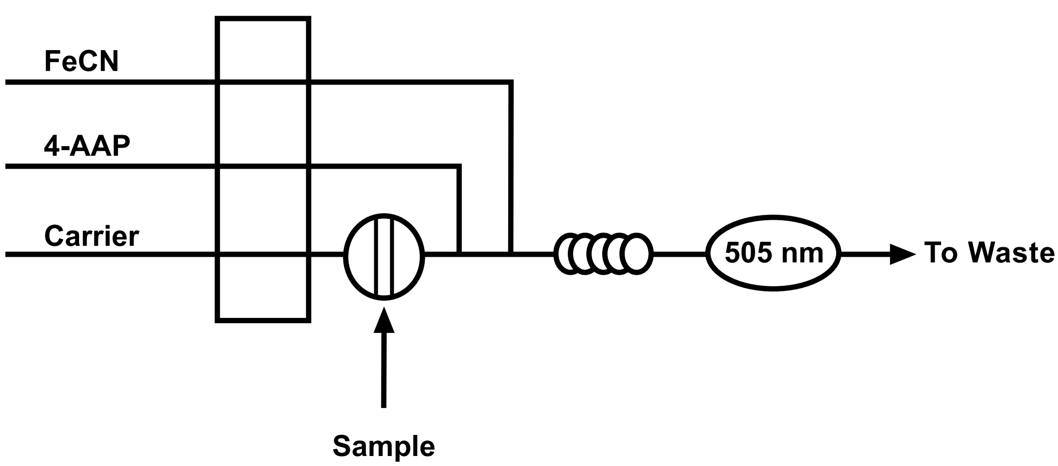
1.2 The Method Detection Limit (MDL) of this method is 5.0 µg/L phenol. The applicable range of the method is 10.0–2,000 µg/L phenol. The range may be extended to analyze higher concentrations by sample dilution.

2.0 Summary of Method

2.1 Prior to analysis, the phenol is distilled off-line from an acidic solution at 160°C. Phenol reacts with 4-aminoantipyrine (4-AAP) and alkaline ferricyanide (FeCN) to form a red complex. The absorbance is measured at 505 nm (Reference 15.2).

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 from sulfur compounds are eliminated by acidifying the sample to a pH of less than 4.0 with phosphoric acid, aerating briefly by stirring, and adding copper sulfate.

4.2 Oxidizing agents such as chlorine are removed immediately after sampling by adding an excess of ferrous ammonium sulfate. Oxidizing agents can be detected by the liberation of iodine upon acidification in the presence of potassium iodide. If chlorine is not removed, the phenolic compounds may be partially oxidized, and the results may be low.

4.3 Background contamination from plastic tubing and sample containers can be eliminated by using glass tubes or acid-washed plastic cups for the samples and calibrants.

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 4-Aminoantipyrine, $C_{11}H_{13}N_3O$ (FW 203.25)

5.3.2 Boric Acid, H_3BO_4 (FW 61.84)

5.3.3 Ferrous Ammonium Sulfate, $(NH_4)_2SO_4FeSO_4 \cdot 6H_2O$ (FW 392.13)

5.3.4 Phenol, C_6H_5OH (FW 94.11)

5.3.5 Phosphoric Acid, concentrated, 85%, H_3PO_4 (FW 98.00)

5.3.6 Potassium Chloride, KCl (FW 74.55)

5.3.7 Potassium Ferricyanide, $K_3Fe(CN)_6$ (FW 329.25)

5.3.8 Sodium Hydroxide, NaOH (FW 40.00)

5.3.9 Sulfuric Acid, concentrated, H_2SO_4 (FW 98.08)

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 505-nm optical filter

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

6.1.4 Post-Distillation Phenol Cartridge (Part #A002164)

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 4-Aminoantipyrine, $C_{11}H_{13}N_3O$ (FW 203.25)

7.1.2 Boric Acid, H_3BO_4 (FW 61.84)

7.1.3 DOWFAX® 2A1 (Part #A000080)

7.1.4 Deionized Water (ASTM Type I or II)

7.1.5 Ferrous Ammonium Sulfate, $(NH_4)_2SO_4FeSO_4 \cdot 6H_2O$ (FW 392.13)

7.1.6 Phenol, C_6H_5OH (FW 94.11)

7.1.7 Phosphoric Acid, concentrated, 85%, H_3PO_4 (FW 98.00)

7.1.8 Potassium Chloride, KCl (FW 74.55)

7.1.9 Potassium Ferricyanide, $K_3Fe(CN)_6$ (FW 329.25)

7.1.10 Sodium Hydroxide, NaOH (FW 40.00)

7.1.11 Sulfuric Acid, concentrated, H_2SO_4 (FW 98.08)

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 (1 L)

7.2.2.1 Add 4 mL of DOWFAX 2A1 to 800 mL of reagent water (Section 7.2.1) in a 1-L volumetric flask.

7.2.2.2 Dilute to 1,000 mL with reagent water and mix gently.

7.2.3 Distillation Reagent (1 L)

7.2.3.1 While stirring, carefully add 100 mL of concentrated phosphoric acid to approximately 800 mL of reagent water in a 1-L volumetric flask.

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

Warning: The mixing of phosphoric acid with water releases a great amount of heat. Take appropriate precautions.

Note: Prepare this solution weekly.

7.2.4 4-Aminoantipyrene (4-AAP) (100 mL)

7.2.4.1 Dissolve 0.065 g of 4-aminoantipyrene in approximately 80 mL of reagent water in a 100-mL volumetric flask.

7.2.4.2 Add 1.0 mL of DOWFAX 2A1. Dilute to 100 mL with reagent water. Mix well.

Note: Prepare this reagent daily.

7.2.5 1 N Sodium Hydroxide (1 L)

7.2.5.1 While stirring, carefully add 40.0 g of sodium hydroxide to approximately 700 mL of reagent water in a 1-L volumetric flask.

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

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

Note: Prepare this solution weekly.

7.2.6 Buffered Potassium Ferricyanide (500 mL)

7.2.6.1 Dissolve 1.0 g of potassium ferricyanide, 1.6 g of boric acid, and 1.9 g of potassium chloride in approximately 400 mL of reagent water in a 500-mL beaker.

7.2.6.2 Adjust the pH of the solution to pH 10.3 with 1 N sodium hydroxide (Section 7.2.5).

7.2.6.3 Quantitatively transfer the solution to a 500-mL volumetric flask. Dilute the solution to 500 mL with reagent water.

7.2.6.4 Add 1.0 mL of DOWFAX 2A1, and mix well.

Note: Prepare this solution weekly.

7.2.7 Sampler Wash—Reagent water

7.2.8 Carrier—Reagent water

7.2.9 Ferrous Ammonium Sulfate (1 L)

7.2.9.1 Dissolve 1.1 g of ferrous ammonium sulfate in approximately 500 mL of reagent water containing 1 mL of concentrated sulfuric acid in a 1-L volumetric flask.

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

7.3 Calibrant Preparation

7.3.1 Stock Calibrant, 100 mg/L Phenol (1 L)

7.3.1.1 Dissolve 0.100 g of phenol in approximately 900 mL of reagent water in a 1-L volumetric flask.

7.3.1.2 Dilute to 1,000 mL with reagent water. Preserve the solution by adding 1.0 mL of concentrated sulfuric acid. Mix well.

Note: Store in an amber glass bottle at 4°–6°C.

7.3.2 Intermediate Calibrant, 10 mg/L Phenol (100 mL)

7.3.2.1 Using a volumetric pipette, add 10 mL of Stock Calibrant 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: Store in an amber glass bottle at 4°–6°C.

7.3.3 Working Calibrants (100 mL)

7.3.3.1 Add the designated volumes of stock or intermediate calibrant (see Equation 1) to the required number of 100-mL volumetric flasks that each contain approximately 80 mL of reagent water. Preserve the solutions by adding 100 µL of concentrated sulfuric acid.

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

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 Calibrant (mg/L)	Final Volume (mL)
10	100	10	100

Final Concentration (µg/L)	Vol. of Stock Cal. (µL)	Conc. of Calibrant (mg/L)	Final Volume (mL)
50	50	100	100
100	100	100	100
500	500	100	100
1,000	1,000	100	100
2,000	2,000	100	100

8.0 Sample Collection, Preservation and Storage

8.1 Samples should be collected in 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 Sample analysis should be performed as soon as possible to eliminate loss of analyte.

8.4 To preserve the sample, acidify with 1 mL of concentrated sulfuric acid per liter of sample, and cool to 4°C.

8.5 Holding time for preserved samples is 28 days from the time of collection (Reference 15.3).

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 phenol 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 ten 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 phenol 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 phenol 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 Phenol Cartridge (Part #A002164) 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 at low speed, 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 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 15.4) 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 15.4).

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 decreasing 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) and verify a stable baseline (Section 10.3).

11.1.2 After the baseline has been verified, place all reagents on-line and allow to pump at least 10–15 minutes and verify there are no bubbles in the flowcell. Obtain a stable baseline at 505 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 For determining very low levels of phenol, it may be necessary to use glass sample cups. Thoroughly clean and rinse all glassware with reagent water (Section 6.2).

11.2.2 Samples and calibrants must be at a neutral pH. Measure the sample as soon as possible after manual distillation. Prepare calibrants in reagent water.

Caution: The reaction is very sensitive to pH and will not proceed properly if samples are acidic. Do not preserve samples or calibrants with acid ***after distillation***.

11.2.3 Periodically (once or twice per week), pump Kleenflow™ Basic (Part #A001252) through the manifold for 10–15 minutes. This will prevent an accumulation of deposits. Follow the Kleenflow with large amounts of reagent water.

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:	10.0–2,000 µg/L
Throughput:	33 samples/hour
Precision:	
100 µg/L	<2% RSD
500 µg/L	<3% RSD
Method Detection Limit (MDL):	5.0 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 15.1).

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 Phenolics, Total Recoverable (Colorimetric, Automated 4-AAP with Distillation). *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 420.2.

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 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 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.

16.2.2 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.

16.2.3 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.

16.2.4 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.

16.2.5 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.

17.0 Figures

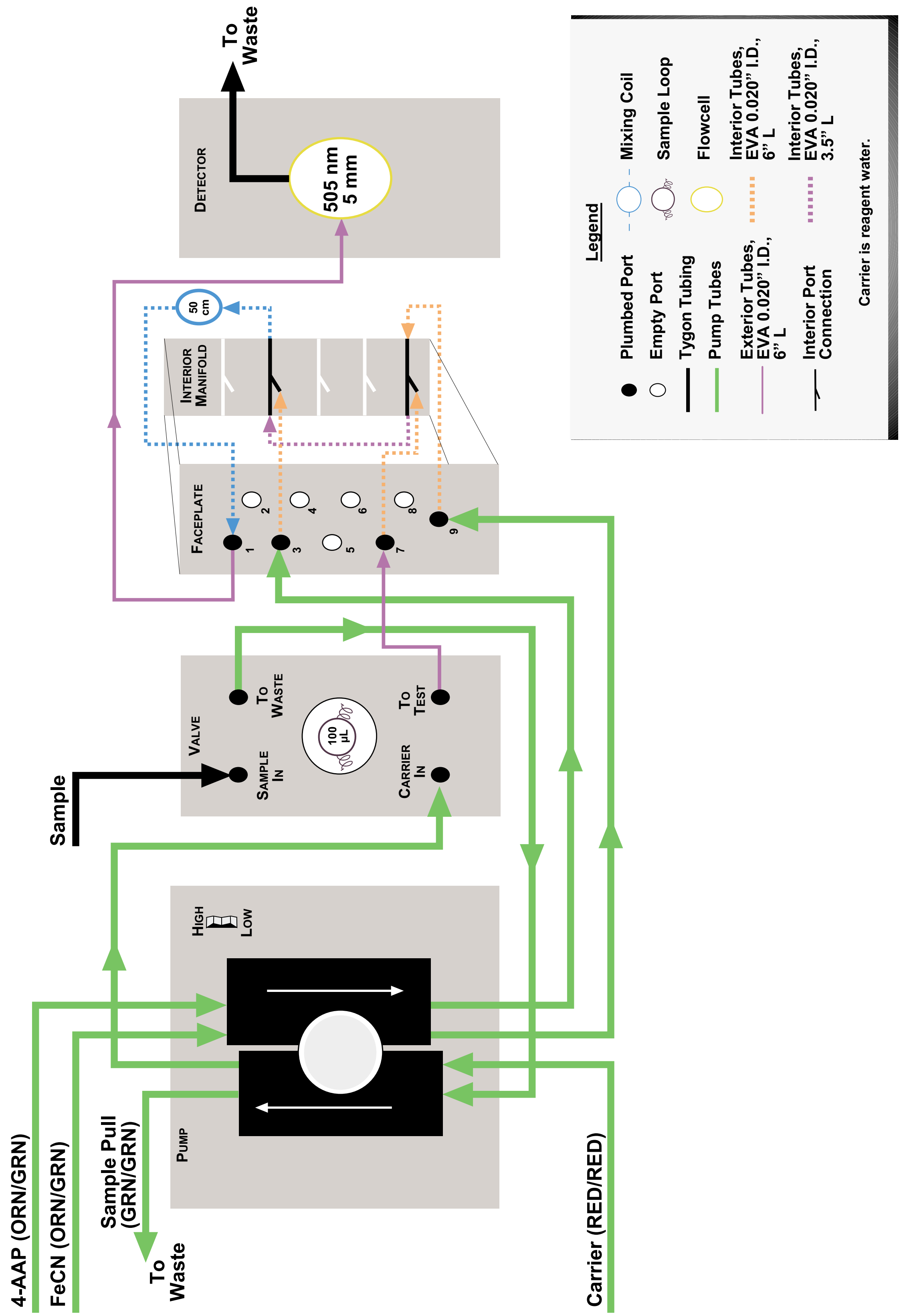


Figure 1. Detailed Flow Diagram for Post-Distillation Phenol by FIA on a Flow Solution 3000, Cartridge Part #A002164

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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