

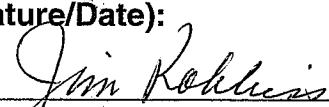
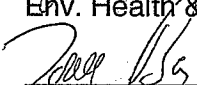


**Title: MEASUREMENT OF VARIOUS ANALYTES USING THE  
ASTORIA® ANALYZER  
(Methods 350.1, 351.2, 353.2, 365.1, 365.2/SM4500-P E)**

Approvals (Signature/Date):	
 Chris Amason Technical Manager	<u>4.27.09</u> Date
 Lou Schneider Quality Assurance Manager	<u>4/27/09</u> Date
 Jim Robbins Env. Health & Safety Coordinator	<u>4-28-09</u> Date
 Todd Baumgartner Laboratory Director	<u>4/27/09</u> Date

This SOP was previously identified as SOP No. GE01.

**Copyright Information:**

This documentation has been prepared by TestAmerica Laboratories, Inc. and its affiliates ("TestAmerica"), solely for their own use and the use of their customers in evaluating their qualifications and capabilities in connection with a particular project. The user of this document agrees by its acceptance to return it to TestAmerica upon request and not to reproduce, copy, lend, or otherwise disclose its contents, directly or indirectly, and not to use it for any other purpose other than that for which it was specifically provided. The user also agrees that where consultants or other outside parties are involved in the evaluation process, access to these documents shall not be given to said parties unless those parties also specifically agree to these conditions.

**THIS DOCUMENT CONTAINS VALUABLE CONFIDENTIAL AND PROPRIETARY INFORMATION. DISCLOSURE, USE OR REPRODUCTION OF THESE MATERIALS WITHOUT THE WRITTEN AUTHORIZATION OF TESTAMERICA IS STRICTLY PROHIBITED. THIS UNPUBLISHED WORK BY TESTAMERICA IS PROTECTED BY STATE AND FEDERAL LAW OF THE UNITED STATES. IF PUBLICATION OF THIS WORK SHOULD OCCUR THE FOLLOWING NOTICE SHALL APPLY:**

**©COPYRIGHT 2009 TESTAMERICA LABORATORIES, INC. ALL RIGHTS RESERVED.**

## 1.0 SCOPE AND APPLICATION

The purpose of this procedure is to provide guidance for the analysis of various analytes using the Astoria® Analyzer. The following analytes may be determined:

ANALAYTE	REFERENCE METHOD	ASTORIA PROCEDURE
Ammonia	EPA 350.1	Appendix A1
Nitrate+Nitrite	EPA 353.2	Appendix B1
Ortho-Phosphate	EPA 365.2/SM4500-P E	Appendix C1
Total Phosphorus*	EPA 365.1	Appendix D
Total Kjeldahl Nitrogen	EPA 351.2	Appendix E

\* total and dissolved (dissolved = field filtered)

## 2.0 SUMMARY OF METHOD AND DEFINITIONS

2.1 The SOP Appendices contain the method parameters required to perform the analysis. The appendices also contain a summary of the "chemistries" (reagents, procedures, wavelengths, etc.) used to measure the target analyte.

### 2.1.1 Instrument Design

The instrument system is composed of four parts, the X,Y,Z autosampler with an external pump, the main pump which is divided into three sections, each for a different chemistry, the cartridge housing which also contains heated water baths, and the digital UV/VIS detector. The instrument and software are computer controlled. The water baths are set for 37 °C for ortho-phosphate, total phosphorus, and ammonia, 50 °C for TKN, and are not used for nitrate+nitrite. Three Astoria units are currently in use.

The samples are poured into disposable 10 mL test tubes and loaded into the autosampler racks. Standards and blanks are loaded into a separate rack labeled "SR". The reagents are pumped from labeled bottles and added incrementally to the sample as it is pumped through the system.

The system is based on segmented continuous flow theory, which allows for complete automation of the reagent addition, incubation, and detection of the analyte.

### 2.1.2 Operation

After the specific analyte cartridge is ready for the specific chemistry, samples are aspirated sequentially and transported to the analytical cartridge via a peristaltic pump. There, the sample is segmented with air or nitrogen to minimize sample-to-sample interaction, then combined with specific reagents and diluents that continuously flow through the cartridge. As the flow passes through a flow-through cuvette (flowcell), the detector takes absorbance measurements and converts them to electronic signals. The resultant data appears as a peak trace with high points, where analyte is present. The analyte concentration is directly proportional to the peak height.

2.2 The reporting limit (RL) and method detection limit (MDL) are found in TL-QA-001: *TestAmerica Tallahassee Method/Analyte List* and the TALS LIMS. The accuracy and precision control limits are found in the TALS LIMS.

## 2.3 Definitions:

Most relevant definitions can be found in the 2003 NELAC Standard, Program Policy and Structure (Chapter 1).

Sampler wash solution – deionized water or acidified deionized water

Startup/shutdown solution – deionized water plus appropriate surfactant

Chemwash – cleaning solution purchased ready-to-use from Astoria-Pacific

OTCR – open tubular cadmium reactor

EDTA – ethylenediamine tetra-acetic acid

Diluent – solution used to dilute reagents and samples

## 2.4 Method Deviations

2.4.1 Method 353.2: The method requires that samples be pH adjusted to pH 5-9. Per the Astoria technical support staff, the ammonium chloride-EDTA buffer accomplishes this step in-line; therefore, samples do not need to be adjusted prior to analysis. Additionally, the buffer pH should be 9.1 instead of 8.5 to account for the extra acidity caused by analyzing preserved samples.

2.4.2 Method 365.2: The method requires that samples be pH adjusted to  $7 \pm 0.2$ . Per the Astoria technical support staff, this pH is not critical as long as the samples are between 5 and 9, as most incoming unpreserved samples are. Therefore, samples do not need to be pH adjusted prior to analysis unless they are outside this range at the initial pH check.

## 3.0 SAFETY

Employees must abide by the policies and procedures in the Corporate Environmental Health and Safety Manual and this document.

### 3.1 SPECIFIC SAFETY CONCERNS OR REQUIREMENTS

3.1.1 Each reagent, standard and sample should be treated as a potential health hazard. Exposure to these materials should be reduced to the lowest level possible. Lab coats, gloves, and other protective equipment should be used. Standards and highly contaminated samples should be handled in a hood. When working with pure acids and bases, extreme care should be taken. Lab coats, gloves, and full-face shields should be employed.

3.1.2 Sodium nitroferricyanide (sodium nitroprusside) will give off hydrogen cyanide gas (HCN) if combined with strong acids. Inhalation of HCN can cause irritation, dizziness, nausea, unconsciousness, and potentially death!

### 3.2 PRIMARY MATERIALS USED

The following is a list of the materials used in this method, which have a serious or significant hazard rating. NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the MSDS for each of the materials listed in the table. A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the MSDS for each material before using it for the first time or when there are major changes to the MSDS.

Material (1)	Hazards	Exposure Limit (2)	Signs and symptoms of exposure
Ammonium Hydroxide	Corrosive Poison	50 ppm-TWA	Vapors and mists cause irritation to the respiratory tract. Causes irritation and burns to the skin and eyes.
Antimony potassium tartrate	Corrosive Poison	0.5 mg (Sb)/m3-TWA	May cause irritation to the respiratory tract. Symptoms include sore throat, coughing, and shortness of breath. May cause irritation with redness and pain to the skin and eyes.
Chloroform	Carcinogen Irritant	50 ppm Ceiling	Acts as a relatively potent anesthetic. Irritates respiratory tract and may cause central nervous system effects, including headache, drowsiness, and dizziness. Causes skin irritation resulting in redness and pain. Removes natural oils from skin. Vapors may cause pain and irritation to eyes. Eye contact may cause severe irritation and possible eye damage.
Hydrochloric Acid	Corrosive Poison	5 ppm-Ceiling	Inhalation of vapors can cause coughing, choking, inflammation of the nose, throat, and upper respiratory tract, and in severe cases, pulmonary edema, circulatory failure, and death. Can cause redness, pain, and severe skin burns. Vapors are irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.
Methanol	Flammable Poison Irritant	200 ppm-TWA	A slight irritant to mucous membranes. Toxic effects exerted on the central nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness, and dizziness. Methanol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms parallel inhalation exposure. Irritant to the eyes.
Nitric Acid	Corrosive Oxidizer Poison	2 ppm-TWA 4 ppm-STEL	Nitric acid is extremely hazardous; it is corrosive, reactive, an oxidizer, and a poison. Inhalation of vapors can cause breathing difficulties and lead to pneumonia and pulmonary edema, which may be fatal. Other symptoms may include coughing, choking, and irritation of the nose, throat, and respiratory tract. Can cause redness, pain, and severe skin burns. Concentrated solutions cause deep ulcers and stain skin a yellow or yellow-brown color. Vapors are irritating and may cause damage to the eyes. Contact may cause severe burns and permanent eye damage.
Phenol	Corrosive	5 ppm-TWA	Breathing vapor, dust or mist results in digestive disturbances. Will irritate, possibly burn respiratory tract. Rapidly absorbed through the skin with systemic poisoning effects to follow. Discoloration and severe burns may occur, but may be disguised by a loss in pain sensation. Eye burns with redness, pain, blurred vision may occur. May cause severe damage and blindness.

Phosphoric Acid	Corrosive	1 Mg/M3 TWA	Inhalation is not an expected hazard unless misted or heated to high temperatures. May cause redness, pain, and severe skin burns. May cause redness, pain, blurred vision, eye burns, and permanent eye damage.
Potassium Ferricyanide	Irritant	None	This material will form Hydrogen Cyanide (HCN) gas when combined with strong acids. Breathing HCN gas may result in <b>death</b> . However it does not break down into cyanide compounds in the body. May cause irritation to the respiratory tract, skin and eyes
Potassium Nitrate	Oxidizer	None	Causes irritation to the respiratory tract, skin and eyes. Symptoms may include coughing, shortness of breath. Symptoms include redness, itching, and pain.
Potassium Nitrite	Oxidizer	None	Causes irritation to the respiratory tract, skin and eyes. Symptoms may include coughing, shortness of breath. Symptoms include redness, itching, pain and burns.
Potassium Persulfate	Oxidizer	None	Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. Causes irritation to skin and eyes. Symptoms include redness, itching, and pain. May cause dermatitis, burns, and moderate skin necrosis.
Sulfuric Acid	Corrosive Oxidizer Dehydrator	1 mg/m <sup>3</sup>	This material will cause burns if comes into contact with the skin or eyes. Inhalation of vapors will cause irritation of the nasal and respiratory system.
Sodium Hydroxide	Corrosive Poison	2 ppm, 5 mg/m <sup>3</sup>	This material will cause burns if comes into contact with the skin or eyes. Inhalation of Sodium Hydroxide dust will cause irritation of the nasal and respiratory system.
Sodium Hypochlorite	Irritant	NA	Inhalation of vapors may irritate respiratory system. Contact with skin or eyes may cause irritation. Contact with acids may release hydrogen chloride gas.
Sodium Nitroferrocyanide (Sodium Nitroprusside)	Poison	LD50= 34 mg/kg	May cause eye and skin irritation. May be metabolized to cyanide. May affect respiratory systems, blood, and cardiovascular system. May generate hydrogen cyanide gas when combined with strong acids. Inhalation of HCN may cause SUDDEN DEATH.
Sodium Phosphate	Irritant	NA	Inhalation may irritate respiratory system. Contact with skin or eyes may cause irritation.

1 – Always add acid to water to prevent violent reactions.

2 – Exposure limit refers to the OSHA (Occupational Safety and Health Administration) regulatory exposure limit.

## 4.0 INTERFERENCES

### 4.1 Ammonia

Precipitation of calcium and magnesium hydroxides is eliminated by the addition of a combined sodium tartrate-sodium citrate complexing reagent. Turbid samples must be filtered before analysis. Samples with background absorbance at the analytical wavelength may cause interference.

## 4.2 Nitrate+Nitrite

Turbid samples can cause interference, and should be filtered with a 0.45u syringe filter. Adjusting EDTA during analysis will eliminate interference from iron, copper, or other metals. Adjusting the sample pH will eliminate or minimize negative dips caused by a mis-match in pH between the sample and reagents. Samples containing large amounts of oil and grease must be pre-extracted with a solvent. Samples containing sulfide cannot be analyzed by this method without first removing the sulfide by precipitation with cadmium salts. Residual chlorine can interfere by oxidizing the cadmium coil, reducing its efficiency.

## 4.3 Ortho-Phosphate and Total Phosphorus

Blue colored components may interfere. High concentrations of arsenic and iron may interfere. Ferric iron up to 70 mg/L, copper up to 10 mg/L, and silica up to 10 mg/L do not interfere. Commercial detergents containing phosphates should not be used to clean glassware.

## 4.4 TKN

Precipitation of calcium and magnesium hydroxides is eliminated by potassium sodium tartrate in the working buffer. Turbid samples must be filtered or centrifuged. Samples with background absorbance at the analytical wavelength may interfere. Ammonia contamination in the DI water, glassware, or air may interfere.

## 5.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

### 5.1 Nitrate+Nitrite, Ammonia, Total Phosphorus, and TKN

Aqueous samples are routinely collected in plastic 125-mL or 250-mL containers. Unpreserved samples must be stored at less than 6 °C with no frozen samples and analyzed within 48 hours. Samples may be preserved with sulfuric acid to pH <2, and must be stored at less than 6 °C with no frozen samples. The hold time for preserved samples is 28 days.

### 5.2 Ortho-Phosphate

Aqueous samples are routinely collected in 125-mL plastic bottles and are unpreserved. The samples are iced at the time of collection and stored at less than 6 °C with no frozen samples until the time of analysis. The holding time for orthophosphate in aqueous samples is 48 hours.

## 6.0 MATERIALS AND APPARATUS

### 6.1 Astoria® Analyzer

### 6.2 Analytical Balance

### 6.3 Drying oven

### 6.4 Class A volumetric glassware for preparation of reagents and standards

### 6.5 Disposable 10 mL test tubes for autosampler

### 6.6 Disposable 60mL test tubes and caps for digestion of total phosphorus

### 6.7 Autoclave

6.8 Autoclave racks, test tube racks of appropriate size

6.9 Nitrogen gas supply and appropriate fittings

6.10 Parafilm

## 7.0 REAGENTS

The preparation of reagents must be documented in accordance with SOP TL-AN-041: *Standard Materials and Reagent Traceability*. See the appropriate SOP Appendix for the preparation of the reagents used in each analysis:

Appendix A1 – Ammonia

Appendix B1 – Nitrate+Nitrite

Appendix C1 – Orthophosphate

Appendix D – Total Phosphorus

Appendix E – Total Kjeldahl Nitrogen (TKN)

## 8.0 STANDARDS

The preparation of standards must be documented in accordance with SOP TL-AN-041: *Standard Materials and Reagent Traceability*. See the appropriate SOP Appendix for the preparation of the standards used in each analysis:

Appendix A – Ammonia

Appendix B – Orthophosphate and Total Phosphorus

Appendix C - Nitrate+Nitrite

Appendix E - Total Kjeldahl Nitrogen (TKN)

## 9.0 SAMPLE PREPARATION

9.1 Nitrate+Nitrite and Ammonia

No sample preparation is necessary, unless the sample is turbid. If turbid, filter an aliquot using a 0.45um syringe filter .

9.2 Ortho-phosphate

No sample preparation is necessary, unless dissolved PO<sub>4</sub> is required. If the sample was not field-filtered, filter an aliquot using a 0.45um syringe filter. pH 7 ideal but not required; if samples are outside the range of pH 5-9, adjust to near 7.

9.3 Total Phosphorus

If dissolved phosphorus is required, it is preferred that the sample be field-filtered and then preserved with sulfuric acid. In the event the lab must filter the samples, it must be on an unpreserved aliquot of sample. All samples must undergo acid digestion.

- 9.3.1 To individual, labeled digestion tubes, add 25 mL of each sample, calibration standard, and batch QC sample.
- 9.3.2 Add 0.5 mL of 5.6M H<sub>2</sub>SO<sub>4</sub> to each digestion tube.
- 9.3.3 Add 1 scoop (approximately 0.2 g) of ammonium persulfate to each digestion tube.
- 9.3.4 For sample wash solution, add 5 mL of 5.6M H<sub>2</sub>SO<sub>4</sub> and 10 scoops of ammonium persulfate to 250 mL of DI water in a 250 mL plastic bottle.
- 9.3.5 Heat all samples, QC, and wash water in an autoclave at 121 °C ± 2 °C at 15-20 psi for 30 minutes after the autoclave comes to temperature.
- 9.3.6 Cool all samples to room temperature before analysis.
- 9.4 Total Kjeldahl Nitrogen
- 9.4.1 See SOP TL-GE-021 for the TKN digestion procedure.

## 10.0 ANALYTICAL PROCEDURES

See the appropriate SOP Appendix for application notes, QC summary, standards, and method setup for each analyte:

Appendix A1 – Ammonia

Appendix B1 – Nitrate+Nitrite

Appendix C1 – Orthophosphate

Appendix D – Total Phosphorus

Appendix E - Total Kjeldahl Nitrogen (TKN)

- 10.1 Astoria operation:
- Turn **on** main power switch on power strip.
  - Prepare fresh startup/shutdown solution.
  - Latch the pump platens and start the main and auxiliary pumps.
  - From the computer, open FASPacl. Verify PC is connected to detector (handshake symbol).
  - Run startup solution for approximately 20 minutes.
  - In FASPacl, select the appropriate **configuration** from the list box on the toolbar at the top of the main window. Enter a name for the run, and click **OK**.
  - Verify that all reagents have sufficient volume for the sample load.
  - After 20 minutes of rinsing, visually inspect for good flow. The pattern of bubbles in the mixing coils should be uniform and should move smoothly through the cartridge.
  - Place the reagent lines into the appropriate reagents and cover the bottle tops with Parafilm.
  - If running ammonia or any form of phosphorus, turn on the heat bath.
  - If running ammonia or nitrate+nitrite, connect the nitrogen bag.
  - If running nitrate+nitrite, connect the OTCR and re-verify good flow.
  - Check the reagent baseline by selecting **Options-Display Signal All**. The baseline should be flat and smooth. Click on **Zero-Signal All** if necessary. Be sure the proper range has been chosen.
  - Enter the sample sequence into the sample table Enter dilution factors in the dilution column.
  - Remove the working calibration standards from the refrigerator Pour into the appropriate calibration tubes in the SR rack of the autosampler. Note: Calibrants and samples may be loaded into the autosampler rack during the startup procedures to maximize efficiency.
  - Make sure the number of samples in the sample table matches the number of samples physically present in the autosampler rack.



- Once the sample table is complete and the samples and calibrants are loaded into the rack, select **Run-Begin** in FASpac.
- Once the run has completed, remove the OTCR (if applicable), turn off the heat bath (if applicable), and return all reagent lines to the Startup/Shutdown solution for 5 minutes. Place all lines in 1N HCl solution for 5 minutes, then return all lines to the Startup/Shutdown solution and run for 1 hour. Immediately after removing the OTCR, slowly flush with 10mL ammonium chloride/EDTA buffer and connect the tubing ends to each other to form a closed loop.
- Cover or cap all reagent bottles. Disconnect nitrogen bag (if applicable).
- Click on the **report** button to see the final tabular results. Select **File-Print** to print the tabular report.
- Click on "file – print set-up" and choose the correct **channel**. Select **File-Print** to print the graphics raw data report.
- Click on the **calibration charts** button to see the calibration curve. Select **File-Print** to print the calibration curve.
- After at least 20 minutes, with the heat bath cool, turn off the main pump. RELEASE THE PLATENS. Release the auxiliary pump platen.
- Close FASpacII. Turn **off** the main switch on the power strip.
- Empty the waste container if necessary.

10.2 Initial calibration requirements and criteria vary between the EPA methods and the Standard Methods method. The EPA 300-series methods only require a blank and two or three standards. In order to adequately represent a larger range of concentrations, TestAmerica Tallahassee employs a blank and five to seven standards in the ICAL. Methods 350.1, 351.2, 353.2, and 365.1 also require a semi-annual determination of the Linear Calibration Range (LCR). Since the ICAL meets the LCR criteria and is analyzed with nearly every run, the ICAL is considered to have met the LCR requirement. Method SM4500-P E requires a blank and six standards for the ICAL.

10.3 Analytical sequence:

Initial calibration curve
Initial calibration verification standard (ICV)
Initial calibration blank (ICB) – (may use as MB)
MB (digestion blank for TP and TKN)
LCS/LCSD
Up to 10 samples, including field samples and QC
Rinse blank (optional)
Continuing calibration verification standard (CCV)
Continuing calibration blank (CCB)
Up to 10 samples, including field samples and QC
Continuing calibration verification standard (CCV)
Continuing calibration blank (CCB)
MB (digestion blank for TP)
LCS/LCSD
Up to 10 samples, including field samples and QC
Rinse blank (optional)
Continuing calibration verification standard (CCV)
Continuing calibration blank (CCB)
Up to 10 samples, including field samples and QC
Continuing calibration verification standard (CCV)
Continuing calibration blank (CCB)

The ICV is always analyzed immediately after the curve. The ICB is analyzed immediately after the ICV. The prep blank (if applicable), LCS, and LCSD will follow in the exact sequence. The samples, MS, and MSD will

then follow with a **maximum of 10 analyses between CCVs/CCBs. All batch QC samples (MB, LCS, LCSD) must be identified by their respective batch IDs.**

Autowashes may be inserted at the analyst's discretion. Frequent autowashes help maintain a stable baseline and/or account for baseline drift. A common practice is to insert one or two autowashes after every five samples.

- 10.4 Dilutions: If the response of the sample exceeds that of the highest standard in the calibration curve, the sample must be diluted and reanalyzed. Make sure the correct dilution factor is entered in the sequence and/or LIMS.

## 11.0 DATA ANALYSIS AND CALCULATIONS

### 11.1 Sample concentration

$$\text{Concentration}(\text{mg} / \text{L}) = C_{\text{curve}} \otimes DF$$

C<sub>curve</sub> = concentration of sample or sample dilution from the calibration curve (mg/L)

DF = dilution factor

The sample result is generated by the data system from the calibration curve. If dilutions are made, the dilution factor must be entered into the sample sequence for proper calculation.

## 12.0 QUALITY CONTROL AND QUALITY ASSURANCE

### 12.1 Analytical batch

SOP TL-AN-002: *Analytical Batching and Evaluation of QC Data* provides guidance for establishing and evaluating QC items to be included in an analytical batch. The batch consists of up to 20 field samples and the associated QC items. The QC items are the method blank, LCS, LCSD, and matrix spikes as specified in the analytical methods. Methods 350.1, 351.2, 353.2, and 365.1 require one MS for every 10 samples. Client or program requirements may specify an MS/MSD pair; in this case, the batch (if greater than 10 samples) will contain one MS and an MS/MSD pair on a different sample. Method 365.2 does not specify a matrix spike frequency; the default is an MS/MSD pair per batch. Standard Methods 4500-P E specifies an MS/MSD pair per batch. These requirements are summarized in the SOP summaries in the appendices for each analyte.

- 12.2 A digestion blank must be analyzed for total phosphorus and TKN. The ICB/CCB may be used as the method blank for analytes requiring no preparation.

### 12.3 Initial and On-Going Demonstration of Capability

Initial and on-going demonstration of capability must be performed in accordance with SOP TL-CA-092: *Evaluation of DOCs*.

### 12.4 Method Detection Limit

The method detection limit must be determined initially, every 6 months, when a new operator begins, and when conditions change that might affect instrument sensitivity, for each analyte in accordance with SOP TL-CA-090: *Determination of the Method Detection Limit (MDL)*. The MDL must be verified by analysis of an MDL verification standard (MDLV) at a concentration of 1-2 times the calculated MDL. The MDLV must undergo all the same preparation steps as the samples, standards, and QC.

- 12.5 Corrective actions for out-of-control data are summarized in Table 1. This table describes checks, acceptance criteria, and the recommended corrective action for various QC events.
- 12.6 Data that do not meet acceptance criteria may be conditionally reported using data flags or qualifiers and/or a case narrative attached to the final report. Non-conformance memos (NCMs) must be generated for all out-of-control events.
- 12.7 The Astoria computers have a backup system for data archival. The data are transferred to a server monthly. The analyst should periodically check that the backup is functioning, and, if desired, manually back up all data files to a floppy disk or CD. If data are backed up to a disk, the disk must be labeled with the instrument ID, start date, and end date, and stored in the laboratory until full. Full disks must be given to the QA department for long-term storage.

## 13.0 PREVENTATIVE MAINTENANCE AND TROUBLESHOOTING

- 13.1 Preventative maintenance for **ammonia, nitrate+nitrite, and TKN**: The following procedure should be performed monthly or as needed, with the heat baths cooled and the OTCR removed.
- Place all lines in DI water and pump for 10 minutes.
  - Place all lines in Chemwash and pump for 10-15 minutes.
  - Place all lines in DI water and pump for 10 minutes.
  - Place all lines in 1N HCl and pump for 10 minutes.
  - Place all lines in a clean beaker of DI water for 10 minutes.
  - Change all pump tubes and if applicable, all polyflow tubing.
  - Check the injection fittings on the cartridge and the sample splitter(s), sample probe, and reagent lines and straws on the cartridge for debris. If necessary clean with wire stylus or replace with clean parts.
  - Clean the tracks on the cartridge block with a soft-bristle toothbrush and DI water; dry with a lint-free wipe (Kimwipe).
  - Clean all reagent bottles with dilute HCl and rinse thoroughly with DI water.
  - Clean all platens using a Kimwipe moistened with isopropanol or methanol.
  - Wipe the pump rollers using a Kimwipe moistened with isopropanol or methanol. Try to remove any debris or particulates around the pump rollers and bushings.
  - Pump Startup/Shutdown solution for 10 minutes until good flow is observed.
  - Replace and activate a new OTCR for nitrate+nitrite analysis (see 13.3 below).
- 13.2 Preventative maintenance for **orthophosphate and total phosphorus**: The following procedure should be performed monthly or as needed, with the heat baths cool.
- Place all lines in DI water and pump for 10 minutes.
  - Place all lines in Chemwash and pump for 10-15 minutes.
  - Place all lines in DI water and pump for 10 minutes.
  - Place all lines in 1N NaOH and pump for 10 minutes.
  - Place all lines in a clean beaker of DI water for 10 minutes.
  - Change all pump tubes and if applicable, all polyflow tubing.
  - Check the injection fittings on the cartridge and the sample splitter(s), sample probe, and reagent lines and straws on the cartridge for debris. If necessary clean with wire stylus or replace with clean parts.
  - Clean the tracks on the cartridge block with a soft-bristle toothbrush and DI water; dry with a lint-free wipe (Kimwipe).
  - Clean all reagent bottles with 10 % bleach and rinse with DI water. Rinse with dilute HCl and rinse thoroughly with DI water.

- Clean all platens using a Kimwipe moistened with isopropanol or methanol.
- Wipe the pump rollers using a Kimwipe moistened with isopropanol or methanol. Try to remove any debris or particulates around the pump rollers and bushings.
- Pump Startup/Shutdown solution for 10 minutes until good flow is observed.

- 13.3 OTCR activation: Using a 10mL plastic syringe fitted with a piece of 0.040 PVC tubing and a short 0.034" ID polyethylene extension, slowly flush the OTCR with the following solutions in order: DI water, 1N HCl, DI water, 2% copper sulfate solution (10mL twice). Then flush forcefully with several portions of DI water, until no more black particles are seen exiting the OTCR. Flush with ammonium chloride/EDTA (nitrate+nitrite) buffer. Do not allow air to enter the OTCR. Store filled with buffer.
- 13.4 OTCR maintenance: Following each nitrate+nitrite run remove the OTCR and slowly flush the OTCR with about 10 mL of ammonium chloride/EDTA (nitrate+nitrite) buffer. Do not allow air to enter the OTCR. Store filled with buffer.
- 13.5 Troubleshooting: The Astoria manual provides a good troubleshooting section. Obvious "blunder opportunities" include reagent lines in the wrong bottles, old or contaminated reagents, heat bath not on, pump tubing worn out, auxiliary pump not on or platen not latched, calibrants in wrong positions, bubbles in detector flow cell not eliminated, and sample wash acid content different from samples' acid content.

## 14.0 WASTE MANAGEMENT AND POLLUTION PREVENTION

All waste will be disposed of in accordance with Federal, State and Local regulations. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this method and the policies in section 13 of the Corporate Environmental Health and Safety Manual for "Waste Management and Pollution Prevention."

### 14.2 Waste Streams Produced by the Method

Excess samples, reagents, and standards must be disposed in accordance with SOP TL-CA-070: *Waste Management*

Waste Stream	Category (Preferred Treatment)
Acid waste from excess acidified samples, sample preparation, and sample analysis.	RCRA Hazardous Waste (Waste is collected, neutralized, and submitted to the waste disposal team.)
Contaminated disposable glass or plastic materials utilized in the analysis.	Non-RCRA Hazardous Waste (Glass and plastic materials are emptied into the acidic waste stream container and then disposed of in the lab trash.)
Unused reagents	RCRA Hazardous Waste (Depending on the reagent, these are either segregated or combined with other acidic waste, neutralized, and submitted to the waste disposal team.)
Excess unpreserved samples	RCRA Hazardous Waste (Excess unpreserved samples are discharged to the sewer system (down the sink with water running.))

## 15.0 REFERENCES

*Standard Methods for the Examination of Water and Wastewater*, 21st Edition; American Public Health Association: Washington, DC, 2005.

*Methods for Chemical Analysis of Water and Wastes*; U.S. EPA Office of Research and Development: Cincinnati, OH, March, 1993.

TL-QAM: *TestAmerica Tallahassee Quality Assurance Manual (QAM)*, current revision.

*Astoria® Analyzer Instrument Manual*, Astoria-Pacific International.

## 16.0 TABLES, DIAGRAMS, AND FLOWCHARTS

(Tables and Appendices follow Section 17.0)

## 17.0 REVISION HISTORY

Revision 3: 05/13/2008

- Changed all STL references to TestAmerica
- Incorporated new logo, cover page, and naming convention
- Title: Added methods
- Added Section 17.0, REVISION HISTORY
- Revised SOP references to reflect new naming convention
- Updated CSM name: added "Environmental Health and..."
- Deleted all references to LQM, added QAM, TL-QA-001, and TL-QA-002
- Updated Standard Methods Reference to 21<sup>st</sup> Edition
- 10.2: Added section describing method differences for ICAL and defining LCR criteria (ICAL may be used as LCR)
- 10.2, 10.3 (old): Renumbered 10.3, 10.4
- 12.4, Table 1: Revised MDL frequency, added MDLV requirement

Revision 4: 08/13/2008

- 2.4: Added section for method deviations
- 2.4.1: Method deviation for 353.2 – pH does not need to be adjusted to 5-9 prior to analysis, buffer does it in-line; buffer pH should be 9.1, not 8.5
- 2.4.2: Method deviation for 365.2 – pH does not need to be adjusted to  $7 \pm 0.2$  prior to analysis, pH not critical if within 5-9
- 9.2: Added pH adjustment to ortho-phosphate sample preparation if outside 5-9
- 12.1: Redefined MS frequency; MS per 10 samples, MS/MSD if client requires and for SM methods
- 12.4: Changed MDLV spike level from 1-3 times to 1-2 times calculated MDL
- Table 1, Appendix A, B, C: Corrected MS, MS/MSD frequency requirements
- Appendix B1: Added hand-correction to Stock Ammonium Chloride-EDTA Buffer – pH 9.1 instead of 8.5 due to analyzing preserved samples

Revision 5: 04/28/2009

- Title, 10.2, 12.1, Table 1: Added 351.2
- 1.0: Added TKN, 351.2, reference to Appendix E
- 2.1.1: Added "50 °C for TKN" and "Three Astoria units are currently in use."
- 2.1.2: Added last sentence
- 2.2: Deleted reference to TL-QA-002
- 3.2: Added Sodium Hypochlorite and Sodium Phosphate to table
- 4.4: Added TKN interferences
- 5.1, 13.1: Added TKN
- 7.0, 8.0, 10.0: Added reference to Appendix E for TKN
- 9.4: Added SOP reference for TKN digestion
- 12.7: Added disk backup for data
- Appendix E: Added (TKN)

**TABLE 1 QC SUMMARY**

QC CHECK	MINIMUM FREQUENCY	ACCEPTANCE CRITERIA	CORRECTIVE ACTION
Initial Calibration -6 points with lowest point at RL; include zero as point	Before sample analysis, every six months, and when continuing calibration verification fails.	Regression curve with correlation coefficient $\geq 0.995$	-Evaluate curve and check calculations -Reanalyze standard(s) -Remake and reanalyze standard(s) -Inspect instrument for proper operation
Initial Calibration Verification (ICV) – independent source	After initial calibration	$\pm 10\%$ of expected value	-Check calculation -Reanalyze ICV -Remake and reanalyze ICV -Recalibrate -Inspect instrument for proper operation
Calibration Verification (CCV)-mid range standard	After every 10 sample measurements	$\pm 10\%$ of expected value	-Check calculation -Reanalyze CCV -Remake and reanalyze CCV -Recalibrate -Inspect instrument for proper operation
Initial Calibration Blank (ICB) and Continuing Calibration Blank (CCB)	After ICV and CCV	$< RL$ ; $< MDL$ for some applications	-Check calculations -Reanalyze ICB/CCB -Determine source of contamination and reanalyze samples if necessary
Linear Calibration Range (ICAL fulfills requirement)	Every 6 months	$\pm 10\%$ of expected value at each level	-Check calculations -Inspect instrument for proper operation -Reanalyze
Method Blank (MB) -for analyses that require no preparation, the ICB/CCB may be reported as the method blank	Per batch of 20 or fewer samples	$< RL$ ; $< MDL$ for some applications	-Check calculations -Reanalyze method blank -Determine source/cause of contamination and redigest/reanalyze associated samples if necessary
LCS/LCSD	Per batch of 20 or fewer samples	$\pm 10\%$ of the expected value ( $\pm 15\%$ or $\pm 20\%$ for some applications) RSD $\leq 20\%$	-Check calculations -Reanalyze -AN02 Decision Matrix
MS or MS/MSD	One MS per 10 samples (350.1, 351.2, 353.2, 365.1); MS/MSD per 20 samples (365.2, SM4500-P E and if client requires)	$\pm 10\%$ of the expected value ( $\pm 15\%$ or $\pm 20\%$ for some applications) RSD $\leq 30\%$	-Check calculations -Reanalyze -AN02 Decision Matrix -Flag Data
Initial Demonstration of Capability	Per analyst prior to initial independent analysis	-Evaluate per TL-CA-092 -Recovery of QCS within 90-110%	-Reanalyze IDOC
Continuing Demonstration of Capability	Per analyst, annually	-Evaluate per TL-CA-092 -Recovery of QCS within 90-110% OR -Result within acceptance limits for PT sample	-Reanalyze CDOC
MDL Study	Initially, every 6 months, new operator, and when conditions change that might affect instrument sensitivity	-SOP TL-CA-090	-Evaluate according to TL-CA-090
MDL Verification	Immediately following MDL	-SOP TL-CA-090	-Evaluate according to TL-CA-090

## Appendix A: Ammonia

### Stock Standards:

1000 mg/L Ammonia Nitrogen Stock Standard

- In a 1L volumetric flask, dissolve 4.7168 g of **ammonium sulfate** ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> - FW132.15), dried at 110 °C, in 900 mL DI water
- Dilute to 1 L with DI water
- Add 2 drops of **chloroform** (CHCl<sub>3</sub> – FW 119.38) and mix well.
- Store at 4 °C. Good for 1 year.

OR use purchased 1000 mg/L Ammonia Nitrogen Stock

10 mg/L Ammonia Nitrogen Stock Standard

- In a 100 mL volumetric flask, dilute 1.0 mL of 1000 mg/L stock standard to 100 mL with **sampler wash solution**.
- Store at 4 °C. Good for 3 months.

0.40 mg/L Ammonia Nitrogen Stock Standard

- In a 100 mL volumetric flask, dilute 4.0 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Make fresh daily.

Calibration Standards (prepare in **sampler wash solution** and make fresh daily)

Calibration Level	Volume of Stock, mL	Stock Concentration, mg/L	Final Volume, mL	Final Concentration, ug/L
0	NA	NA	NA	0
1	1.25	0.40	50	1.0
2	2.50	0.40	50	2.0
3	6.25	0.40	50	5.0
4	0.5	1.0	50	10
5	1.0	1.0	50	20
6	4.0	1.0	50	40

### Initial Calibration Verification Standard (ICV) at 10 ug/L

- Prepare stock at 100 mg/L using a **second source** of ammonia nitrogen: dissolve 0.0382 g ammonium chloride (NH<sub>4</sub>Cl) in 100 mL sampler wash solution. Make fresh daily.
- In a 10 mL volumetric flask, dilute 0.050 mL of 100 mg/L stock NH<sub>4</sub>Cl standard to 50 mL with **sampler wash solution** for a final concentration of 10 ug/L. Make fresh daily.

### Continuing Calibration Verification Standard (CCV) at 10 ug/L

- Use the level 4 calibration standard.

### LCS/LCSD at 40 ug/L (may use either source)

- In a 100 mL volumetric flask, dilute 0.40 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Make fresh daily.

### MS; MS/MSD at 40 ug/L (may use either source; ICAL source preferred))

- Add 0.080 mL of the 10 mg/L stock to 20 mL sample.



## Appendix B: Ortho-phosphate and Total Phosphorus

### Stock Standards:

#### 1000 mg/L Phosphate Stock Standard

- In a 1L volumetric flask, dissolve 4.3937 g of **potassium dihydrogen phosphate** ( $\text{KH}_2\text{PO}_4$  - FW136.09), dried at 110 °C, in 900 mL DI water
- Dilute to 1 L with DI water
- Add 2 drops of **chloroform** ( $\text{CHCl}_3$  – FW 119.38) and mix well.
- Store at 4 °C. Good for 2 years.

OR use purchased 1000 mg/L Phosphate Stock

#### 10 mg/L Phosphate Stock Standard

- In a 100 mL volumetric flask, dilute 1.0 mL of 1000 mg/L stock standard to 100 mL with **sampler wash solution**. Good for 1 year.

### Calibration Standards (prepare in **DI water**; digest for Total Phosphorus; good for 1 month)

Calibration Level	Volume of Stock, mL	Stock Concentration, mg/L	Final Volume, mL	Final Concentration, ug/L (mg/L)
0	NA	NA	NA	0 (0)
1	0.010	10	100	1.0 (0.0010)
2	0.050	10	100	5.0 (0.0050)
3	0.10	10	100	10 (0.010)
4	0.50	10	100	50 (0.050)
5	1.0	10	100	100 (0.10)
6	5.0	10	100	500 (0.50)
7	10	10	100	1000 (1.0)

### Initial/Continuing Calibration Verification Standards (ICV/CCV)

Prepare stocks at 1000 mg/L and 10 mg/l as above, using a **second source** of **potassium dihydrogen phosphate**.

#### ICV/CCV at 50 ug/L

- In a 100 mL volumetric flask, dilute 0.50 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Digest for Total Phosphorus.

#### LCS/LCSD at 20 ug/L (may use either source)

- In a 100 mL volumetric flask, dilute 0.20 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Store at 4°C. Good for 1 month.

#### LCS/LCSD at 100 ug/L (0.10 mg/L) (may use either source)

- In a 100 mL volumetric flask, dilute 1.0 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Store at 4°C. Good for 1 month.

### MS (365.1 one per 10 samples); MS/MSD (365.2 and SM4500-P E) at 20 ug/L (may use either source; ICAL source preferred))

- Add 0.040 mL of the 10 mg/L stock to 20 mL sample.

## Appendix C: Nitrate+Nitrite

### Stock Standards:

1000 mg/L Nitrate Stock Standard

- In a 1L volumetric flask, dissolve 7.2180 g of **potassium nitrate** (KNO<sub>3</sub> - FW101.11), dried at 110 °C, in 900 mL DI water
- Dilute to 1 L with DI water
- Add 2 drops of **chloroform** (CHCl<sub>3</sub> – FW 119.38) and mix well.
- Store at 4 °C. Good for 1 year.

OR use purchased 1000 mg/L Phosphate Stock

10 mg/L Nitrate Stock Standard

- In a 100 mL volumetric flask, dilute 1.0 mL of 1000 mg/L stock standard to 100 mL with **sampler wash solution**. Good for 3 months.

0.40 mg/L Nitrate Stock Standard

- In a 100 mL volumetric flask, dilute 4.0 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**. Make fresh daily.

Calibration Standards (prepare in **sampler wash solution** and make fresh daily)

Calibration Level	Volume of Stock, mL	Stock Concentration, mg/L	Final Volume, mL	Final Concentration, ug/L (mg/L)
0	NA	NA	NA	0 (0)
1	1.25	0.40	50	10 (0.010)
2	2.50	0.40	50	20 (0.020)
3	6.25	0.40	50	50 (0.050)
4	0.50	10	50	100 (0.100)
5	1.0	10	50	200 (0.200)
6	4.0	10	100	400 (0.400)

### Initial Calibration Verification Standard (ICV) at 10 ug/L

- Prepare stocks at 1000 mg/L and 10 mg/L as above, using a **second source** of **potassium nitrate**.
- In a 50 mL volumetric flask, dilute 0.50 mL of 10 mg/L stock standard to 50 mL with **sampler wash solution**.
- Make fresh daily.

### Continuing Calibration Verification Standards (CCV) at 10 ug/L

- Use the Level 4 calibration standard.


### LCS/LCSD at 20 ug/L (may use either source)

- In a 100 mL volumetric flask, dilute 0.20 mL of 10 mg/L stock standard to 100 mL with **sampler wash solution**.
- Make fresh daily.

### MS; MS/MSD at 20 ug/L (may use either source; ICAL source preferred))

- Add 0.040 mL of the 10 mg/L stock to 20 mL sample.

## Appendix A1: Astoria Ammonia Procedure



**AMMONIA NITROGEN**  
**A023**

---

### A. Scope and Application

This method is used for the determination of Ammonia Nitrogen as N in drinking, surface and saline waters, domestic and industrial wastes, plants and soils. The EPA range of this method is 0.01 to 2.0 mg/L. However, this method is also applicable to other ranges.

### B. Summary of Method

Ammonia reacts with alkaline phenol and hypochlorite to form indophenol blue. Sodium nitroferricyanide intensifies the blue color formed which is measured at 660 nm.

### C. Interferences

Precipitation of calcium and magnesium hydroxides is eliminated by the addition of a combined potassium sodium tartrate/sodium citrate complexing reagent. Turbid samples must be filtered or centrifuged prior to determination. Samples with background absorbance at the analytical wavelength may interfere.


### D. Sample Handling and Preservation

Determine unpreserved samples immediately upon collection. Samples may be preserved with 2 ml of concentrated sulfuric acid per liter of sample and refrigerated at 2-8° C. The holding time for preserved samples is 28 days.<sup>(1)</sup>

### E. Raw Materials Required

**NOTE: Chemicals should be of ACS grade or equivalent.**

- Ammonium Sulfate  $(\text{NH}_4)_2\text{SO}_4$  (FW 132.13)
- Brij®-35, 30% w/v (API p/n 90-0710-04)
- Chloroform,  $\text{CHCl}_3$  (FW 119.38)
- Phenol, liquefied 88%  $\text{C}_6\text{H}_5\text{OH}$  (FW 94.11)
- Potassium Sodium Tartrate  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  (FW 282.23)
- Sodium Citrate, Dihydrate  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$  (FW 294.11)
- Sodium Hydroxide  $\text{NaOH}$  (FW 40.00)
- Sodium Hypochlorite  $\text{NaOCl}$  5.25% (household bleach)
- Sulfuric Acid  $\text{H}_2\text{SO}_4$  concentrated (FW 98.07)
- Sodium Nitroferricyanide  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$  (FW 297.95)



Clackamas, Oregon 97015-0880, USA  
 503-657-9010 • 1-800-536-3111 • FAX 503-657-7367  
 e-mail: sales@astoria-pacific.com  
 www.astoria-pacific.com

Ammonia-Nitrogen

A023

## F. Reagent Preparation

All reagents and calibrants are prepared with ammonia-free deionized or distilled water. See Operating Notes for preparation of ammonia-free deionized water.

### 1. Stock Complexing Reagent (1L)

Potassium Sodium Tartrate .....	33 g
$\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$ (FW 282.23)	
Sodium Citrate, Dihydrate.....	24 g
$\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ (FW 294.11)	
Sulfuric Acid.....	as required
$\text{H}_2\text{SO}_4$ concentrated (FW 98.07)	
Deionized Water	

Dissolve 33 g of potassium sodium tartrate and 24 g of sodium citrate in approximately 800 ml of deionized water contained in a 1 L beaker. Place the beaker on top of a magnetic stirrer. Insert a magnetic stirring bar and a pH electrode into the solution. Adjust the pH of the solution to pH 5.0 with the sulfuric acid. Transfer the complexing reagent to a 1 L volumetric flask and dilute it to the mark with deionized water. Filter to 0.45  $\mu\text{m}$ .

A formulation that has been found to work well for seawater or acidic samples contains 140 g of sodium citrate, 5 g of sodium hydroxide and 24 g of potassium sodium tartrate per liter. The pH of this reagent should not be adjusted.

### 2. Working Complex Reagent (100 ml)

Stock Complexing Reagent .....	100 ml
Brij-35 (30% w/v).....	0.1 ml (4 drops)

Add 4 drops of Brij-35 for each 100 ml of complexing reagent required for the day's run.

### 3. Stock 10 N Sodium Hydroxide (1 L)

**CAUTION:** The dissolution of sodium hydroxide in water releases a great amount of heat.

Sodium Hydroxide.....	400 g
$\text{NaOH}$ (FW 40.00)	
Deionized Water	

Cautiously and with continuous stirring, add 400 g of sodium hydroxide to approximately 700 ml of deionized water contained in a 1 L volumetric flask. Cool the solution in an ice bath when adding the sodium hydroxide. When the solution is cool, dilute it to the mark with deionized water and mix well. Store in a tightly capped, plastic container.

Ammonia-Nitrogen

A023

#### 4. Alkaline Phenol (1 L)

10 N Sodium Hydroxide ..... 85 ml  
 Phenol, liquefied ..... 12 ml  
     Phenol C<sub>6</sub>H<sub>5</sub>OH (FW 94.11) liquefied 88%  
 Deionized Water

Place a 1 L volumetric flask that contains approximately 700 ml of deionized water and a magnetic stirring bar into an ice bath positioned on top of a magnetic stirrer. While stirring, add 85 ml of 10 N sodium hydroxide. When the solution is cold, slowly add 12 ml of liquefied phenol in small quantities, cooling after each addition. Dilute the solution to the mark with deionized water and mix it well. Filter to 0.45 µm. The resulting solution should be a light straw color. Store the reagent in a brown bottle and refrigerate it at 2-8°C. Stability is approximately 1 month. Discard the reagent if it becomes dark amber in color.

*OR: 85 ml 5N NaOH + 6 ml phenol ⇒ 500 ml*

#### 5. Sodium Hypochlorite (100 ml)

Sodium Hypochlorite Solution ..... 2.5 ml  
     NaOCl, 5.25% solution, household bleach  
 Deionized Water

Add 2.5 ml of sodium hypochlorite solution to approximately 75 ml of deionized water contained in a 100 ml volumetric flask. Dilute the solution to the mark with deionized water. This reagent is not stable; prepare it daily.

#### 6. Sodium Nitroferricyanide (1 L)

Sodium Nitroferricyanide ..... 0.5 g  
     Na<sub>2</sub>Fe(CN)<sub>5</sub>NO•2H<sub>2</sub>O (FW 297.95)  
 Deionized Water

Add 0.5 g of sodium nitroferricyanide to approximately 800 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water. Filter to 0.45 µm. Store this solution in an amber bottle at room temperature where it is stable for at least 1 month.

#### 7. Diluent and Startup/Shutdown Solution (1 L)

Brij-35 (30% w/v) ..... 1-2 ml  
 Deionized Water

Add 1 to 2 ml of Brij-35 to 1000 ml of deionized water.

Ammonia-Nitrogen

A023

## 8. Sampler Wash Solution for Ammonia Analysis

Deionized Water

**NOTE:** If the samples are preserved with sulfuric acid, add acid to the deionized water in the same proportion. The sampler wash solution should have the same acid content as the samples.

## G. Calibrants

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution. Dilute the solution to 100 ml with sampler wash solution and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

$C_1$  = desired concentration (in mg/L) of working calibrant to be prepared

$V_1$  = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

$C_2$  = concentration (in mg/L) of stock (or intermediate) calibrant

$V_2$  = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for  $V_2$  yields:

$$V_2 = \frac{C_1V_1}{C_2}$$

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100  $\mu$ l) of the stock calibrant in 100 ml final volume:

$$V_2 = \frac{(1.0 \text{ mg/L}) (100 \text{ ml})}{1000 \text{ mg/L}}$$

Ammonia-Nitrogen

A023

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

## H. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Brij-35 to the startup solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
7. If data collection software is being used, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
10. Select the appropriate parameters for the detector and sampler. (See Flow Diagram.)
11. Begin analysis.
12. At the end of analysis place all reagent lines in shutdown solution and turn off the heat bath. Pump shutdown solution for 20 to 30 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
13. Turn off the power to all units and release pump platens.

## Ammonia-Nitrogen

A023

### 1. Operating Notes

1. Prepare ammonia free water by passing distilled water through a mixture of strongly acidic cation and strongly basic anion exchange resins.<sup>(2)</sup>
2. To prevent ammonia contamination from the air, segment the analytical stream with nitrogen or draw air through a 5 N sulfuric acid solution.
3. In some cases, samples have been found to absorb ammonia from the air. If you suspect that this is occurring try pouring each sample just prior to aspiration by the system. This should help to minimize the contamination from the air.
4. When analyzing ammonia nitrogen, precipitation following the addition of alkaline phenol may indicate poor reagent quality. Change the source of potassium sodium tartrate and sodium citrate.
5. Precipitation following the addition of alkaline phenol may also occur if the samples being determined contain calcium and/or magnesium in amounts that exceed the capacity of the complexing reagent. In such cases, increasing the amount of sodium citrate in the complexing reagent should alleviate the problem.
6. Clean precipitates from the system by pumping 10% v/v HCl, with Brij-35 added, through the sample line and all reagent lines. Wash the system thoroughly with startup solution before proceeding with analyses.
7. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad®NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
8. If the flowrate of the sample pump tube is  $\leq 226 \mu\text{l/minute}$  (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

**NOTE:** If the sample line is debubbled, a helper line is not necessary.

9. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.



Ammonia-Nitrogen

A023

## J. References

1. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Sample Preservation", Page XVII, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45286.
2. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen, Ammonia", Method 350.1 (Colorimetric, Automated Phenate) STORET NO. Total 00610, Dissolved 00608.

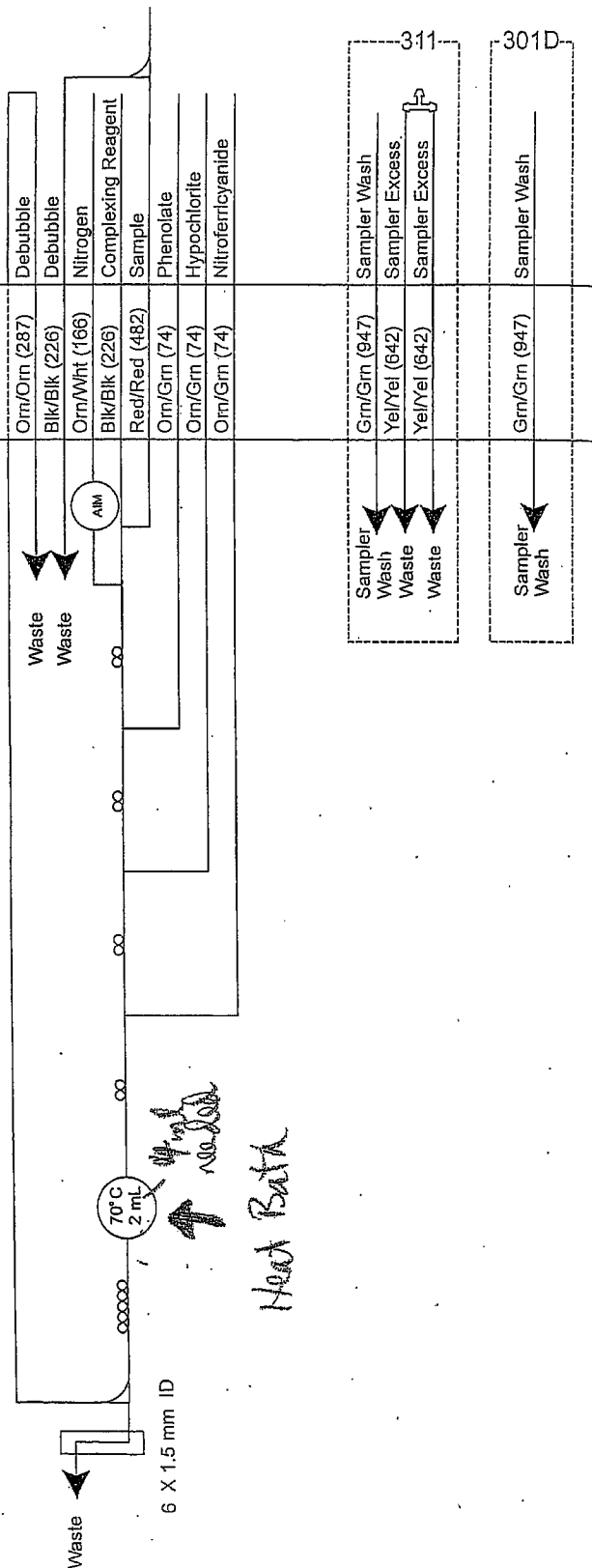
## ACKNOWLEDGMENTS

Astoria® and FASPac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon  
Brij®-35 is a registered trademark of ICI Americas, Wilmington, Delaware  
Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

## Astor - Pacific International

AMMONIA  
A023-A01  
3/2006 Rev. A

RANGE: 1 - 50 µg/L as N  
0.05 - 2 mg/L as N  
Water/Wastewater



**SAMPLER PARAMETERS**  
Analysis Rate: 45/hr  
Sample Time: 35 s  
Wash Time: 45 s  
Pecking: OFF  
Pump Speed: 42

**DETECTOR PARAMETERS**  
Interference Filter: 660 nm  
Neutral Density Filter: 30 or 60  
Smoothing: Poly 20 - 40  
Smoothing: MAF 20 - 40

**CURVE PARAMETERS**  
Curve 1: 1 - 50 µg/L  
Curve 2: 0.05 - 2 mg/L

**2 nd Order**  
1 st Order

**CLEANING SOLUTIONS**  
CHEMWASH (API p/n 80-0005-250)

**STARTUP/SHUTDOWN SOLUTION**  
Deionized Water with Brj@-35 (1-2 ml/L) (API p/n 90-0710-04)

Symbol Key, Copyright Information

o = 5 turns

Debubblle (API p/n 303-0108-00)

Stream Splitter (API p/n 303-0109-00)

311

301D

6 X 1.5 Flowcell (API p/n 315-0108-00)

Brj@-35 is a registered trademark of ICI Americas, Wilmington, DE

## Calibrant Preparation

### AMMONIA

A023-A01

1 – 50 µg/L as N | 0.05 – 2 mg/L as N

#### 1,000 mg/L Ammonia Nitrogen Stock Standard (1.0 L)

- Dissolve 4.7168 g Ammonium Sulfate,  $(\text{NH}_4)_2\text{SO}_4$  (FW 132.15), dried at 110°C in 900 ml deionized water.
- Dilute to 1.0 L with deionized water.
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### 100 mg/L Ammonia Nitrogen Intermediate Standard (100 ml)

- 10.0 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well

#### 10 mg/L Ammonia Nitrogen Intermediate Standard (100 ml)

- 1.00 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well

#### Working Standard (100 ml)

Use adjustable, microliter pipettes to add the designated microliter volumes of stock, intermediate or working standard to 100 ml volumetric flasks containing approximately 80 ml of sampler wash solution. Dilute each solution to the mark with the sampler wash solution and mix well. Make standards covering the range being run.

Range	Working Standard Concentration	Standard to Pipet (Stock, Intermediate or Working)	Volume to Pipet (µl)
0.05 – 2 mg/L	2 mg/L	100 mg/L	2,000
	1 mg/L	100 mg/L	1,000
	0.5 mg/L	100 mg/L	500
	0.1 mg/L	10 mg/L	1,000
↓ 1 – 50 µg/L	0.05 mg/L or 50 µg/L	10 mg/L	500
	20 µg/L	1 mg/L	2,000
	10 µg/L	1 mg/L	1,000
	5 µg/L	1 mg/L	500
	2 µg/L	0.1 mg/L	2,000
↓	1 µg/L	0.1 mg/L	1,000
All Ranges	0.0 mg/L	N/A	N/A

NOTE: To prepare alternate calibrant concentrations consult the methodology.

## Appendix B1: Astoria Nitrate+Nitrite Procedure

### Astoria Analyzer

### NITRATE+NITRITE A173

#### A. Scope and Application

This method is used for the determination of nitrite or nitrate plus nitrite in drinking, surface and saline waters, domestic and industrial wastes, plants and soils. The EPA range of this method is 0.05 to 10.0 mg/L nitrate+nitrite and nitrite nitrogen. However, this method is also applicable to other ranges.

#### B. Summary of Method

Nitrate is reduced quantitatively to nitrite by cadmium metal in the form of an open tubular cadmium reactor (OTCR). The nitrite thus formed plus any originally present in the sample is determined as an azo dye at 520 nm following its diazotization with sulfanilamide and subsequent coupling with N-1-naphthylethylenediamine.<sup>(1)</sup> These reactions take place in acidic solution. Nydahl<sup>(2)</sup> provides a good discussion of nitrate reduction by cadmium metal, while the specific details of OTCR's are given by Patton.<sup>(5)</sup> The information concerning mechanisms and kinetics of the color forming reactions can be found in References 4 and 5.

#### C. Interferences

Pre-filter turbid samples prior to analysis. EDTA is added during analysis to eliminate interference from iron, copper or other metals. Adjust samples to pH 5 to 9 with either concentrated HCl or NH<sub>4</sub>OH. Samples containing large concentrations of oil and grease must be extracted with an organic solvent.<sup>(7)</sup> Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts.<sup>(8)</sup> Norwitz and Keliher have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrite.<sup>(10,11)</sup> Residual chlorine can interfere by oxidizing the cadmium coil, reducing its efficiency. Test for residual chlorine and treat if necessary.<sup>(12)</sup>

#### D. Sample Handling and Preservation

When determining Nitrate + Nitrite, samples should be analyzed as soon as possible if unpreserved. Samples may be preserved with sulfuric acid to pH 2.0. Holding time for preserved samples is 28 days.<sup>(9)</sup> Refrigerate all samples at 2-8°C. Do not preserve samples with mercuric chloride.

If values for nitrite and nitrate are required separately, acid preservation should not be used. Samples should be analyzed as soon as they are collected. If this is not possible, samples may be stored (unpreserved) in the dark at 2-8°C for up to 48 hours.

Astoria-Pacific  
INTERNATIONAL

Clackamas, Oregon 97015-0830 USA  
1-503-657-3010 • 1-800-536-3111 • FAX 1-503-655-7367  
e-mail: sales@astoria-pacific.com  
www.astoria-pacific.com

Nitrate+Nitrite

A173

## E. Raw Materials Required

**NOTE:** Chemicals should be of ACS grade or equivalent.

Ammonium Chloride  $\text{NH}_4\text{Cl}$  (FW 53.50)  
 Ammonium Hydroxide  $\text{NH}_4\text{OH}$  (FW 35.05)  
 Brij-35®, 30% w/v (p/n 90-0710-04)  
 Chloroform  $\text{CHCl}_3$  (FW 119.38)  
 Cupric Sulfate, Pentahydrate  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (FW 249.69)  
 Deionized Water (ASTM type I or II)  
 Disodium Ethylenediamine Tetraacetate  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  (FW 372.24)  
 Hydrochloric Acid, Concentrated  $\text{HCl}$  (FW 36.46)  
 N-1-naphthylethylenediamine Dihydrochloride  $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$  (FW 259.18)  
 Phosphoric Acid, Concentrated  $\text{H}_3\text{PO}_4$  (FW 98.00)  
 Potassium Nitrate  $\text{KNO}_3$  (FW 101.11)  
 Potassium Nitrite  $\text{KNO}_2$  (FW 85.11)  
 Sulfanilamide  $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$  (FW 172.21)

## F. Reagent Preparation

### 1. Stock Ammonium Chloride-EDTA Buffer, pH 8.5 (1 L)

**CAUTION:** Work with ammonium hydroxide in a fume hood. Avoid breathing fumes. Wear protective clothing.

Ammonium Chloride ..... 85 g  
 $\text{NH}_4\text{Cl}$  (FW 53.50)  
 Disodium Ethylenediamine Tetraacetate (disodium EDTA) ..... 0.1 g  
 $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  (FW 372.24)  
 Ammonium Hydroxide, concentrated ..... or use 1:1 NaOH ; ~7.5 ml  $\Rightarrow$  pH 8.5  
 $\text{NH}_4\text{OH}$  (FW 35.05)  
 Deionized Water

Dissolve 85 g of ammonium chloride and 0.1 g of disodium EDTA in 900 ml of deionized water contained in a 1 L beaker. Adjust the pH to 8.5 with concentrated ammonium hydroxide. Transfer the solution to a 1 L volumetric flask and dilute to the mark with deionized water. Filter to 0.45  $\mu\text{m}$ .

### 2. Working Ammonium Chloride-EDTA Buffer (200 ml)

Stock Buffer ..... 200 ml  
 Brij-35, 30% ..... (8 drops) 0.2 ml

Add 8 drops Brij-35 to each 200 ml of Stock Buffer required. Mix well.

### 3. Color Reagent (500 ml)

Nitrate+Nitrite

A173

Phosphoric Acid, concentrated ..... 50 ml  
 $\text{H}_3\text{PO}_4$  (FW 98.00)  
 Sulfanilamide ..... 20 g  
 $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$  (FW 172.21)  
 N-1-naphthylethylenediamine Dihydrochloride ..... 1 g  
 $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$  (FW 259.18)  
 Deionized Water

Cautiously add 50 ml of concentrated phosphoric acid to 400 ml of deionized water (while stirring) contained in a 500 ml volumetric flask. Dissolve 20 g of sulfanilamide and 1 g N-1-naphthylethylenediamine dihydrochloride in the phosphoric acid solution. Dilute to the mark with deionized water. Filter to 0.45  $\mu\text{m}$ . Store in a brown bottle and refrigerate at 2-8° C when not in use. Reagent is stable for several months. Discard if it turns dark pink.

#### 4. Startup/Shutdown Solution

Add 1 to 2 ml of Brij-35, 30% to each liter of deionized water and mix.

#### 5. Sampler Wash Solution

Deionized Water

#### 6. Open Tubular Cadmium Reactor (OTCR)<sup>(4)</sup>

The Astoria analytical cartridge uses an Open Tubular Cadmium Reactor coil to reduce nitrate to nitrite. Nitrogen is used to segment the analytical stream to prevent a pH increase due to reaction between oxygen in ambient air and cadmium.

##### A. OTCR Activation

The OTCR (API p/n 303-0500-12) is a coiled cadmium tube (12") that has been cleaned of manufacturing oils inside and coated outside with plastic. The outside diameter is 0.090 inches, with an inside diameter of 0.050 inches, and a wall thickness of 0.020 inches. Short lengths of 0.034" ID polyethylene are sleeved to the reactor coil to allow installation of the reactor in the manifold. These sleeves are joined by a N-13 (N-2) nipple.

##### B. Reagents for OTCR Activation

1. Stock Ammonium Chloride/EDTA Buffer (previously prepared)
2. Cupric Sulfate Solution (1000 ml)

Cupric Sulfate ..... 20 g  
 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (FW 249.69)  
 Deionized Water

Dissolve 20 g of cupric sulfate in approximately 900 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix well.

Nitrate+Nitrite

A173

3. 1.0 N Hydrochloric Acid (100 ml)

Hydrochloric Acid, concentrated ..... 8.3 ml  
HCl (FW 36.46)  
Deionized Water

Add 8.3 ml of concentrated hydrochloric acid to about 80 ml of deionized water contained in a 100 ml volumetric flask. Dilute to the mark with deionized water and mix well.

C. Procedure

1. Detach one end of the polyethylene tubing from the N-13 (N-2) nipple.
2. Using a 10 cc plastic syringe fitted with 0.040 PVC tubing and a short 0.034" ID polyethylene extension, flush the OTCR with the described solutions using the following procedure:
  - a) Deionized Water
  - b) 1.0 N Hydrochloric Acid

**NOTE: The hydrochloric acid may cause pitting of the cadmium reactor interior surface if left in the OTCR for longer than a few seconds. After the HCl flush, proceed quickly to Step C.**

- c) Deionized Water
- d) 2% Copper Sulfate

Slowly flush the OTCR with 10 cc of 2% copper sulfate. Repeat. Precipitated copper may be observed exiting the reactor (black particles).

- e) Deionized Water

Flush with deionized water until no more precipitated copper is flushed from the reactor. This requires a forceful flush. Repeat 2-3 times.

- f) Stock Ammonium Chloride/EDTA Buffer

Fill the OTCR with Stock Buffer. The reactor should be stored with Stock Buffer when not in use.

**NOTE: Do not introduce air into the OTCR during this process.**

D. Installation of the OTCR

The analytical cartridge is provided with a jumper of 0.034"ID polyethylene sleeved at both ends in the position where the OTCR is to be installed.

1. With the N-13 (N-2) nipple in place, pump reagents segmented with nitrogen until a stable flow is established.

**NOTE: The working buffer must be in the cartridge before the OTCR is installed.**

## Nitrate+Nitrite

A173

2. Turn the pump off and disconnect the N-13 (N-2) in the jumper connection.
3. Install the OTCR in the jumper, attaching each free end with one N-13 (N-2) nipple.
4. Resume pumping and wait until a stable bubble pattern is established before proceeding with the determinations.

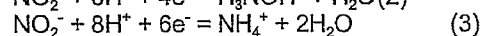
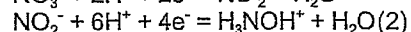
### E. Removal of the OTCR

1. Before the reagent lines are removed from the reagents, stop the pump, remove the OTCR and reconnect the N-13 (N-2) nipple in the jumper connection.
2. Resume pumping. Place the reagent lines in startup solution and pump until the cartridge has been thoroughly rinsed.
3. Attach the syringe to the N-13 (N-2) nipple on the OTCR. Draw 10 to 15 ml of Stock Buffer through the OTCR. Leaving buffer in the OTCR, remove the syringe and join the tubing ends with the N-13 (N-2) nipple.

**NOTE:** Do not leave any air in the OTCR. It must be stored filled with Stock Buffer.

### F. Reduction Efficiency and Stabilization of the OTCR

In the OTCR, nitrate is reduced to nitrite. However, under some conditions reduction may proceed further with nitrite being reduced to hydroxylamine and ammonia. These reactions are pH dependent.



At the buffered pH of the reactions, equation 1 predominates. However, if the cadmium surface is overly active, equation 2 will proceed sufficiently to give low results. If the cadmium surface is insufficiently active, there will be a low recovery of nitrate as nitrite.<sup>(5)</sup> This latter is defined as poor reduction efficiency.

To determine the reduction efficiency, run a high level nitrite calibrant followed by a nitrate calibrant of the same nominal concentration. A range of 90%-110% gives reasonable accuracy. The reduction efficiency is calculated as follows:

$$\frac{\text{Peak Height (NO}_3^-) \times 100}{\text{Peak Height (NO}_2^-)} = \% \text{ efficiency}$$

If the response of the nitrite is as expected but the reactor efficiency is poor, it may be necessary to repeat the activation procedure. However, if the nitrite response is much less than expected, it is an indication that the nitrite is being further reduced and stabilization of the OTCR is necessary.

With some types of samples, notably those of high chloride content such as potassium chloride soil extracts or seawater samples, a longer OTCR may be necessary. A 24" OTCR, API p/n 303-0500-24, is available.



Nitrate+Nitrite

A173

## G. Stabilization

When an OTCR is first activated, it may be necessary to stabilize the activity of the reactor. In order to stabilize the OTCR, pump a mid or high calibrant continuously and record the steady state signal. Continue the steady state until a drift is no longer observed. Alternatively, pump a 5 mg/L nitrate solution for 5 minutes (but do not attempt to monitor the signal). Return the sampler probe to wash and proceed with determinations when the baseline has stabilized.

## G. Calibrants

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution. Dilute the solution to 100 ml with sampler wash solution and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

$C_1$  = desired concentration (in mg/L) of working calibrant to be prepared

$V_1$  = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

$C_2$  = concentration (in mg/L) of stock (or intermediate) calibrant

$V_2$  = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for  $V_2$  yields:

$$V_2 = \frac{C_1V_1}{C_2}$$

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100  $\mu$ l) of the stock calibrant in 100 ml final volume.

$$V_2 = \frac{(1.0 \text{ mg/L})(100 \text{ ml})}{1000 \text{ mg/L}}$$

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

Nitrate+Nitrite

A173

## H. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units and latch platens to begin liquid flow.
4. Open valve on nitrogen pillow.
5. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Brij-35 to the startup solution.
6. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered before use. Be sure all containers are properly labeled and filled before pumping reagents.
7. After a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
8. If using data collection software, set up the appropriate sample table.
9. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
10. Once the reagent baseline is satisfactory, add the OTCR into the cartridge flow. Always connect the inlet first and the outlet second. It is important to avoid the introduction of air into the coil during this procedure.
11. Once the OTCR is on-line, run for 5-10 minutes then re-verify the bubble pattern and baseline stability. Make any necessary adjustments.
12. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
13. Select the appropriate parameters for the detector and sampler. (See Flow Diagram at the end of methodology.)
14. Begin analysis.
15. At the end of analysis remove the OTCR from the cartridge. Disconnect the outlet first, then the inlet. Flush the OTCR with buffer which contains no surfactant (Brij).
16. Place all reagent lines in startup solution. Pump for 5 to 10 minutes to flush all of the reagents out of the cartridge.
17. Close valve on nitrogen pillow.
18. Turn off the power to all units and release pump platens.

Nitrate+Nitrite

A173

## I. Operating Notes

1. The OTCR may be conditioned by running a mid scale standard through the manifold for 10-15 minutes.
2. Life expectancy of the OTCR varies and is difficult to predict. It is recommended that a nitrite standard of the same nominal concentration as the high scale standard be run as a check on column reduction efficiency.
3. The color reagent will turn pink as it is exposed to air. To extend its life, pour only the amount needed into a small dark bottle and keep the remainder refrigerated. Cover the mouth of the small bottle with Parafilm® (or similar) during use.
4. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad®NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
5. If the flowrate of the sample pump tube is  $\leq 226 \mu\text{l}/\text{minute}$  (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

**NOTE:** If the sample line is debubbled, a helper line is not necessary.

6. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.
7. The bubble pattern coming out of the OTCR after running several samples may become erratic when the samples have a high salt content, such as soil samples extracted in KCl or seawater. The following steps may correct the problem. Slowly push one 10 ml syringe full of copper sulfate through the OTCR and let it sit about one minute. Follow by quickly pushing one syringe full of buffer through the OTCR. It may be helpful to limit the length of runs to avoid this symptom. If this is a recurring symptom, performing this operation at shutdown may also help to obtain a smooth startup the next day.
8. When running a very wide range of analysis, it may be necessary to increase the wash time or use extra blanks to minimize carryover effects at the low end. It is also helpful to group high samples and low samples separately if possible.

Nitrate+Nitrite

A173

## J. References

1. Standard Methods for the Examination of Water and Wastewater, Fifteenth Edition, 1981, American Public Health Association, Washington, D.C., Pages 370-373, 380-383.
2. F. Nydahl, Talanta, 23, Pages 349-357 (1976).
3. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Sample Preservation", Page xvii, Environmental Monitoring and Support
4. Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45286.
5. Patton, C.J., Doctoral Dissertation, Michigan State University, 1982, Page 87-121.
6. Fox, J.B., Anal. Chem., 51, 1493 (1979).
7. Norwitz, G., Keliher, P.N., Analyst, 109, 1281 (1984).
8. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020. "Nitrogen, Nitrate-Nitrite", Method 353.2 (Colorimetric, Automated, Cadmium Reduction) STORET No. Total 00630.
9. Standard Methods for the Examination of Water and Wastewater, Fourteenth Edition, 1975, American Public Health Association, Washington, D.C., Page 365.
10. Norwitz, G., Keliher, P.N., Analyst, June 1985. Volume 110, Page 689-694.
11. Norwitz, G., Keliher, P.N., Analyst, September 1986. Volume 111, Pages 1033-1037.
12. Standard Methods for the Examination of Water and Wastewater, Fifteenth Edition, 1981, American Public Health Association, Washington, D.C., Page 371.

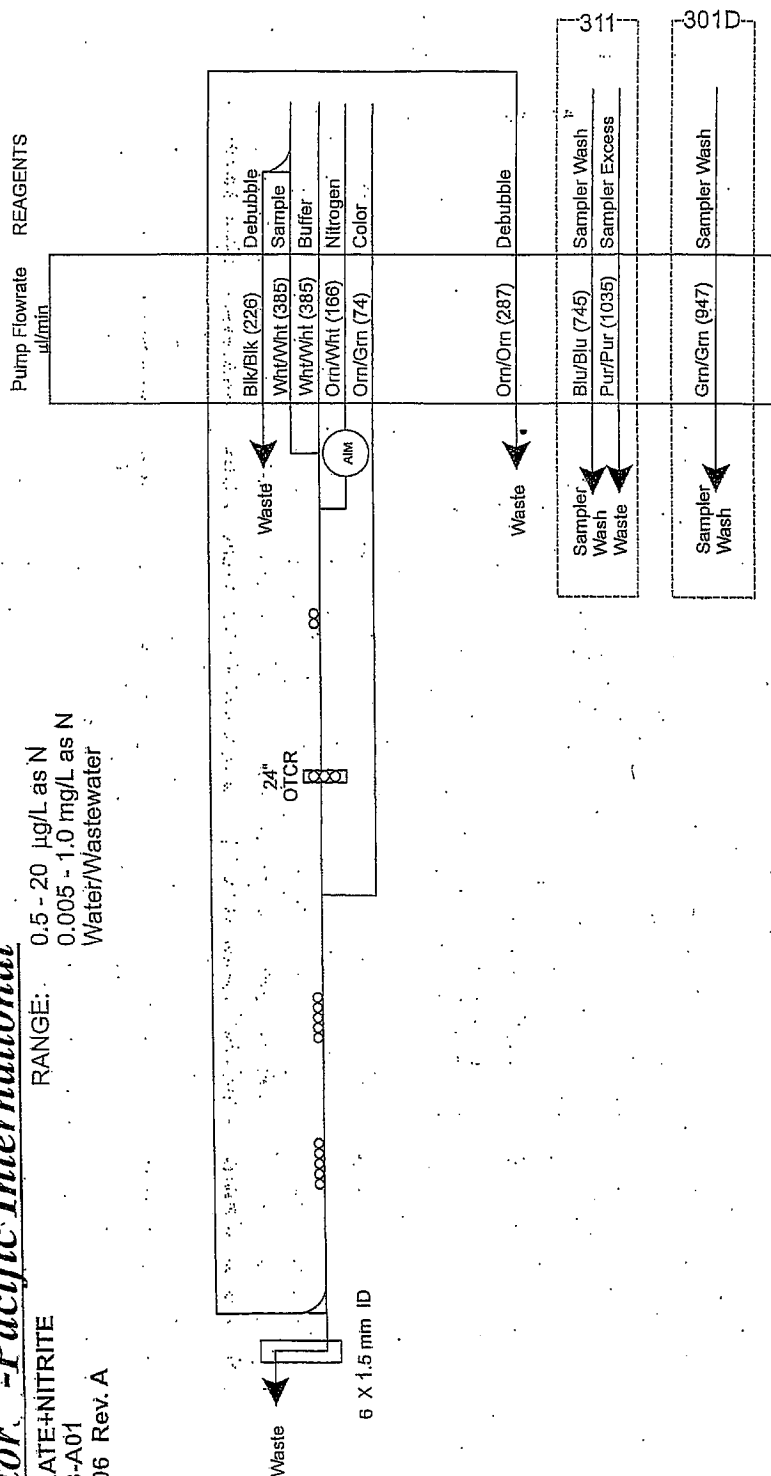
### ACKNOWLEDGMENTS

Astoria® and FASPac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon  
Brij-35 is a registered trademark of ICI Americas, Wilmington, Delaware  
Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania  
Parafilm® is a registered trademark of American National Can, Norwalk, CT.

## Astor -Pacific International

NITRATE+NITRITE  
A173-A01  
3/2006 Rev. A

RANGE: 0.5 - 20 µg/L as N  
0.005 - 1.0 mg/L as N  
Water/Wastewater



**SAMPLER PARAMETERS**  
Analysis Rate: 60/hr  
Sample Time: 20 s  
Wash Time: 40 s  
Packing: OFF  
Pump Speed: 42

**DETECTOR PARAMETERS**  
Interference Filter: 520 or 540 nm  
Neutral Density Filter: None  
Smoothing: Poly 20-40  
Smoothing: MAF 20-40

**CURVE PARAMETERS**  
Curve 1 0.5 - 20 µg/L 2<sup>nd</sup> Order  
Curve 2 0.005 - 1.0 mg/L 2<sup>nd</sup> Order

**CLEANING SOLUTIONS**  
CHEMWASH (API p/n 80-0005-250)

**STARTUP/SHUTDOWN SOLUTION**  
Deionized Water with Brij®-35 (1-2 ml/L) (API p/n 90-0710-04)

Symbol Key, Copyright Information

o = 5 turns

Debubbler (API p/n 303-0122-00  
or 303-0103-00)

24" OTCR  
(API p/n 303-0500-24)

Flowcell (API p/n 315-0106-00)

Brij®-35 is a trademark of ICI Americas, Wilmington, DE

## Calibrant Preparation

### NITRATE + NITRITE

A173-A01

0.5 – 20 µg/L as N | 0.005 – 1.0 mg/L as N

#### 1,000 mg/L Nitrate Nitrogen Stock Standard (1.0 L)

- Dissolve 7.218 g Potassium Nitrate,  $\text{KNO}_3$  (FW 101.11), dried at 110°C, in 500 ml deionized water.
- Dilute to 1.0 L with deionized water.
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### 100 mg/L Nitrate Nitrogen Intermediate Standard (100 ml)

- 10.0 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well.

#### 10 mg/L Nitrate Nitrogen Intermediate Standard (100 ml)

- 1.00 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well

#### Working Standard (100 ml)

Use adjustable, microliter pipettes to add the designated microliter volumes of stock, intermediate or working standard to 100 ml volumetric flasks containing approximately 80 ml of sampler wash solution. Dilute each solution to the mark with the sampler wash solution and mix well. Make standards covering the range being run.

Range	Working Standard Concentration	Standard to Pipet (Stock, Intermediate or Working)	Volume to Pipet (µl)
0.005 – 1.0 mg/L	1.0 mg/L	100 mg/L	1,000
	0.5 mg/L	100 mg/L	500
	0.1 mg/L	10 mg/L	1,000
	0.05 mg/L	10 mg/L	500
0.5 – 20 µg/L	20 µg/L or 0.02 mg/L	1 mg/L	2,000
	10 µg/L or 0.01 mg/L	1 mg/L	1,000
	5 µg/L or 0.005 mg/L	1 mg/L	500
	2.0 µg/L	0.1 mg/L	2,000
	1.0 µg/L	0.1 mg/L	1,000
	0.5 µg/L	0.1 mg/L	500
All Ranges	0.00 mg/L	N/A	N/A

NOTE: To prepare alternate calibrant concentrations consult the methodology.

### OTCR Reduction Efficiency Check Standard

#### 100 mg/L Nitrite Nitrogen Stock Standard (1.0 L)

- Dissolve 0.6076 g Potassium Nitrite,  $\text{KNO}_2$  (FW 85.11), dried at 110°C in 500 ml deionized water.
- Dilute to 1.0 L with deionized water.
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### Nitrite Nitrogen Reduction Efficiency Check Standard (100 ml)

- The reduction efficiency check standard should match the concentration the highest working standard being run.
- Consult the equations in Section G of the method to determine the volume of 100 mg/L stock Nitrite Nitrogen standard required to prepare 100 ml of an appropriate Reduction Efficiency Check Standard.

NOTE:  
See method section F.6.F for more information on calculating reduction efficiencies.

## Appendix C1: Astoria Ortho-phosphate Procedure

**Astoria**  
*Analyzer*

ORTHO-PHOSPHATE  
A203

### A. Scope and Application

This method is used for the determination of ortho-phosphate in water and wastewater. The EPA range of this method is 0.01 to 1.0 mg/L as Phosphorus. However, this method is also applicable to other ranges.

### B. Summary of Method

Ortho-phosphate reacts with molybdenum (VI) and antimony (III) in an acid medium to form a phosphoantimonymolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 660 nm.

### C. Interferences

Ferric iron up to 70 mg/L, copper up to 10 mg/L and silica up to 10 mg/L do not interfere. Filter turbid samples prior to analysis.<sup>(1)</sup>

### D. Sample Handling and Preservation

Analyze samples as soon as possible. Samples may be held for 48 hours if refrigerated at 2-8°C.

### E. Raw Materials Required

**NOTE: Chemicals should be of ACS grade or equivalent.**

Antimony Potassium Tartrate  $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$  (FW 333.94)

Ammonium Molybdate  $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$  (FW 1235.86)

Ascorbic Acid  $C_6H_8O_6$  (FW 176.13)

Deionized Water (ASTM Type I or Type II)

Dowfax™ 2A1 (API p/n 90-0720-04)

Potassium Dihydrogen Phosphate  $KH_2PO_4$  (FW 136.09)

Sulfuric Acid, conc  $H_2SO_4$  (FW 98.08)

*Astoria-Pacific*  
INTERNATIONAL

Clackamas, Oregon 97015-0830 USA  
1-503-657-3010 • 1-800-536-3111 • FAX 1-503-655-7367  
e-mail: sales@astoria-pacific.com  
www.astoria-pacific.com

Ortho-Phosphate

A203

## F. Reagent Preparation

### 1. Diluent and Startup/Shutdown Solution (200 ml)

Deionized Water..... 200 ml  
Dowfax 2A1..... 0.5 to 1 ml

Add 0.5 to 1 ml Dowfax 2A1 to 200 ml of deionized water. Mix well.

### 2. Sulfuric Acid, 5 N (1000 ml)

**CAUTION: Mixing sulfuric acid with water generates a great amount of heat.**

Sulfuric Acid, concentrated..... 140 ml  
H<sub>2</sub>SO<sub>4</sub> (FW 98.08)  
Deionized Water

Cautiously add 140 ml of concentrated sulfuric acid to 600 ml of deionized water contained in a 1000 ml Erlenmeyer flask. Cool to room temperature and transfer to a 1000 ml volumetric flask. Dilute to the mark with deionized water.

### 3. Antimony Potassium Tartrate (50 ml)

Antimony Potassium Tartrate..... 0.15 g  
K(SbO)C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>•1/2H<sub>2</sub>O (FW 333.94)  
Deionized Water

Dissolve 0.15 g of antimony potassium tartrate in 40 ml of deionized water contained in a 50 ml volumetric flask. Dilute to the mark with deionized water. Store at 2-8° C in a dark bottle.

### 4. Ammonium Molybdate (150 ml)

Ammonium Molybdate..... 6 g  
(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>•4H<sub>2</sub>O (FW 1235.86)  
Deionized Water

Dissolve 6 g of ammonium molybdate in 75 ml of deionized water. Add deionized water to final volume of 150 ml and mix well. Store at 2-8° C in a polyethylene bottle.

### 5. Ascorbic Acid (300 ml)

Ascorbic Acid..... 5.4 g  
C<sub>6</sub>H<sub>8</sub>O<sub>6</sub> (FW 176.13)  
Deionized Water

Dissolve 5.4 g of ascorbic acid in 150 ml of deionized water. Add deionized water to a final volume of 300 ml and mix well. Stable for 10 days if stored at 2-8° C.



Ortho-Phosphate

A203

**6. Color Reagent (100 ml)**

Sulfuric Acid, 5 N..... 50 ml  
Antimony Potassium Tartrate..... 5 ml  
Ammonium Molybdate ..... 15 ml  
Ascorbic Acid ..... 30 ml

Add reagents in the order stated and mix after each addition. Filter to 0.45 µm.  
Prepare reagent daily.

**7. Sampler Wash Solution**

Deionized Water

## **G. Calibrants**

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution. Dilute the solution to 100 ml with sampler wash solution and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

$C_1$  = desired concentration (in mg/L) of working calibrant to be prepared

$V_1$  = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

$C_2$  = concentration (in mg/L) of stock (or intermediate) calibrant

$V_2$  = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for  $V_2$  yields:

$$V_2 = \frac{C_1V_1}{C_2}$$

## Ortho-Phosphate

A203

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100 µl) of the stock calibrant in 100 ml final volume.

$$V_2 = \frac{(1.0 \text{ mg/L}) (100 \text{ ml})}{1000 \text{ mg/L}}$$

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

## H. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch pump platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Dowfax to the startup solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered weekly. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
7. If using data collection software, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
10. Select the appropriate parameters for the detector and sampler. (See Flow Diagram.)
11. Begin analysis.

Ortho-Phosphate

A203

12. At the end of analysis place all reagent lines in startup/shutdown solution and turn off the heat bath. Pump startup/shutdown solution for 20 to 30 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
13. Turn off the power to all units and release pump platens.

## I. Operating Notes

1. If the flowrate of the sample pump tube is  $\leq 226 \mu\text{l/minute}$  (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

**NOTE:** If the sample line is debubbled, a helper line is not necessary.

2. A common cause of low sensitivity and noise in the baseline is debris in the flowcell. Particulate matter from the reagents and samples may become lodged in the flowcell restricting the amount of light that is passed through. Flushing the flowcell with approximately 10 ml of sampler wash solution with a syringe will dislodge any debris in the flowcell. Following proper filtration procedures for the reagents and samples will reduce the frequency of this occurring.
3. To prevent the accumulation of background contamination forming in the color reagent, keep the reagent bottle covered at all times. Baseline drift may also be reduced by placing the color reagent in an ice bath during analysis.
4. If increased carryover and drift are experienced, make sure the ascorbic acid and ammonium molybdate solutions are fresh.
5. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad®NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
6. Sodium Lauryl Sulfate can also be used as a wetting agent for this chemistry, replacing Dowfax. See recipe below. Use 1 to 2 ml of SLS (15% w/w) per 100 ml of deionized water or reagent.

**NOTE:** High quality SLS is important. Fisher catalog numbers 02674-25, BP166-100 or BP166-500 are acceptable.

### Sodium Lauryl Sulfate (SLS) 15% w/w

Dodecyl Sodium Sulfate.....	15 g
<chem>CH3(CH2)10CH2OSO3Na</chem> (FW 288.38)	
Deionized Water.....	85 ml

Dissolve 15 g of dodecyl sodium sulfate in 85 ml of deionized water.

Ortho-Phosphate

A203

7. For chronic carryover and drift problems, the following cleaning solution can be used to flush the analytical cartridge and flowcell.

**Potassium Iodide Cleaning Solution (55 ml)**

Potassium Iodide.....	1 g
KI (FW 166.00)	
5 N Sulfuric Acid (See Reagent Preparation).....	25 ml
H <sub>2</sub> SO <sub>4</sub> (FW 98.08)	
Deionized Water.....	30 ml

Add 1 g KI to about 25 ml 5 N sulfuric acid. Stir vigorously until a strong yellow-orange color has formed. This may take at least one hour. Add about 30 ml deionized water. The solution will darken over time, and is usable for one month. Pump the cleaning solution through all lines in the cartridge for 10 to 15 minutes, followed by startup/shutdown solution.

8. Antimony Potassium Tartrate C<sub>8</sub>H<sub>4</sub>K<sub>2</sub>O<sub>12</sub>Sb<sub>2</sub>•3H<sub>2</sub>O (FW 667.85) may also be used. Weigh the same amount.
9. Acid washed glassware should be used for all reagents and calibrants. Commercial detergents containing phosphorus should never be used to clean glassware used in phosphorus determination. Wash the glassware with 1:1 hydrochloric acid and rinse it thoroughly with deionized water. Store the glassware filled with deionized water. If the glassware is reserved for use only in phosphorus determination, treatment with hydrochloric acid is necessary only occasionally.<sup>(5)</sup>

## J. References

1. Standard Methods for the Examination of Water and Wastewater, 14th Ed. 1975, p. 624.
2. Methods for Chemical Analysis of Water and Wastewater, March 1984, EPA-600/4-79-020, "Sample Preservation", p. xvii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency Cincinnati, OH 45286.
3. Methods for Chemical Analysis of Water and Wastewater, March 1984, EPA-600/4-79-020, "Phosphorus, All Forms", Method 365.1 (Colorimetric, Automated Ascorbic Acid).

**Acknowledgments**

Astoria® and FASpac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon  
 Dowfax™ 2A1 is a trademark of Dow Chemical, USA  
 Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

See Methodology

## Calibrant Preparation

### ORTHO-PHOSPHATE

305-A203-A00

2 – 50 µg/L | 5 – 500 µg/L | 0.02 – 2 mg/L

#### 1,000 mg/L Phosphate as P Stock Standard (1.0 L)

- Dissolve 4.3937 g Potassium Dihydrogen Phosphate,  $\text{KH}_2\text{PO}_4$  (FW 136.09), dried at 110°C in 900 ml deionized water.
- Dilute to 1.0 L with deionized water and mix well.
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### 100 mg/L Phosphate Intermediate Standard (100 ml)

- 10.0 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well

#### 10 mg/L Phosphate Intermediate Standard (100 ml)

- 1.00 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution (See Methodology) to 100 ml.
- Mix Well

#### Working Standard (100 ml)

Use adjustable, microliter pipettes to add the designated microliter volumes of stock, intermediate or working standard to 100 ml volumetric flasks containing approximately 80 ml of sampler wash solution. Dilute each solution to the mark with the sampler wash solution and mix well. Make standards covering the range being run.

Range	Working Standard Concentration	Standard to Pipet (Stock, Intermediate or Working)	Volume to Pipet (µl)
0.02 – 2.0 mg/L	2.0 mg/L	100 mg/L	2,000
	1.0 mg/L	100 mg/L	1,000
5.0 – 500 µg/L	500 µg/L or 0.5 mg/L	100 mg/L	500
	200 µg/L or 0.2 mg/L	10 mg/L	2,000
	100 µg/L or 0.1 mg/L	10 mg/L	1,000
2.0 – 50 µg/L	50 µg/L or 0.050 mg/L	10 mg/L	500
	20 µg/L or 0.020 mg/L	1 mg/L	2,000
	10 µg/L	1 mg/L	1,000
	5 µg/L	1 mg/L	500
	2 µg/L	0.1 mg/L	2,000
All Ranges	0.0 mg/L	N/A	N/A

NOTE: To prepare alternate calibrant concentrations consult the methodology.

## Appendix D: Astoria Total Phosphorus Procedure

### Astoria Analyzer

### TOTAL PHOSPHORUS A050

#### A. Scope and Application

This method is used for the determination of total phosphorus in water and wastewater. The EPA range of this method is 0.01 to 20.0 mg/L as P. However, this method is also applicable to other ranges.

#### B. Summary of Method

Samples are digested to hydrolyze phosphorus to ortho-phosphate. The acidic digestate is neutralized and analyzed for ortho-phosphate. Ortho-phosphate reacts with molybdenum (VI) and antimony (III) in an acid medium to form a phospho-antimonylmolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 660 nm.<sup>(2)</sup>

#### C. Interferences

Ferric iron up to 70 mg/L, copper up to 10 mg/L and silica up to 10 mg/L do not interfere. Turbid samples must be filtered before determination. Samples with background absorbance at the analytical wavelength may interfere.

#### D. Sample Handling and Preservation

Determine the total phosphorus of unpreserved samples immediately after they are collected. Unpreserved samples may be held for 48 hours when cooled immediately and stored at 2-8° C. Samples may be preserved by the addition of 1 ml of concentrated sulfuric acid per liter of sample and cooled immediately. The holding time for acid preserved samples is 28 days.<sup>(3)</sup>

#### E. Raw Materials Required

**NOTE:** Chemicals should be of ACS grade or equivalent.

Acetone  $\text{CH}_3\text{COCH}_3$  (FW 58.08)  
 Ammonium Molybdate  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  (FW 1235.86)  
 Antimony Potassium Tartrate  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$  (FW 333.94)  
 Ascorbic Acid  $\text{C}_6\text{H}_8\text{O}_6$  (FW 176.13)  
 Chloroform  $\text{CHCl}_3$  (FW 119.38)  
 Deionized Water (ASTM Type I or Type II)  
 Dowfax 2A1 (API p/n 90-0720-04)  
 Potassium Dihydrogen Phosphate  $\text{KH}_2\text{PO}_4$  (FW 136.09)  
 Sodium Chloride  $\text{NaCl}$  (FW 58.45)  
 Sodium Hydroxide  $\text{NaOH}$  (FW 40.00)  
 Sulfuric Acid, concentrated  $\text{H}_2\text{SO}_4$  (FW 98.08)

**Astoria-Pacific**  
 INTERNATIONAL

Clackamas, Oregon 97015-0830 USA  
 1-503-657-3010 • 1-800-536-3111 • FAX 1-503-655-7367  
 e-mail: sales@astoria-pacific.com  
 www.astoria-pacific.com

Total Phosphorus

A050

## F. Reagent Preparation

### 1. Diluent (200 ml)

Deionized Water ..... 200 ml  
Dowfax 2A1 ..... 0.5 to 1 ml

Add 0.5 to 1 ml Dowfax 2A1 to 200 ml of deionized water. Mix well.

### 2. Stock Antimony Potassium Tartrate (1 L)

Antimony Potassium Tartrate ..... 3.0 g  
 $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$  (FW 333.94)  
Deionized Water

Dissolve 3.0 g of antimony potassium tartrate in approximately 900 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix well.

### 3. Stock Molybdate/Antimony Reagent (1 L)

**CAUTION: Mixing sulfuric acid with water releases a great amount of heat.**

Sulfuric Acid ..... 70 ml  
 $H_2SO_4$ , concentrated (FW 98.08)  
Ammonium Molybdate ..... 6.0 g  
 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$  (FW 1235.86)  
Stock Antimony Potassium Tartrate ..... 50 ml  
Deionized Water

Slowly, while stirring, add 70 ml of sulfuric acid to approximately 600 ml of deionized water contained in a 1 L volumetric flask and mix it well. Add 6.0 g of ammonium molybdate to the sulfuric acid solution and stir until the ammonium molybdate dissolves. Add 50 ml of stock antimony potassium tartrate and mix the solution well. Allow to cool. Dilute the solution to the mark with deionized water and mix it well. Filter to 0.45  $\mu m$ . Do not refrigerate this reagent. Discard the solution if it becomes blue.

### 4. Working Molybdate/Antimony Reagent (100 ml)

Stock Molybdate/Antimony ..... 100 ml  
Dowfax 2A1 ..... 0.2-0.3 ml (8 – 12 drops)

Mix together 100 ml of Stock molybdate/antimony and 0.2-0.3 ml (8 – 12 drops) of Dowfax. Prepare daily the quantity sufficient for the day's run.



## Total Phosphorus

A050

### 5. Stock Ascorbic Acid (400 ml)

Ascorbic Acid ..... 6.0 g  
 $C_6H_8O_6$  (FW 176.13)  
 Acetone ..... 200 ml  
 $CH_3COCH_3$  (FW 58.08)  
 Deionized Water ..... 200 ml

Dissolve 6.0 g of ascorbic acid in a mixture of 200 ml of acetone and 200 ml of deionized water. Filter to 0.45  $\mu$ m. Store the solution at 2-8°C.

### 6. Working Ascorbic Acid Reagent (60 ml)

Stock Ascorbic Acid ..... 10 ml  
 Deionized Water ..... 50 ml

Mix together 10 ml of stock ascorbic acid and 50 ml of deionized water. Prepare this reagent daily.

### 7. Sampler Wash Solution (1L)

**CAUTION: Mixing sulfuric acid with water releases a great amount of heat.**

Sulfuric Acid ..... as required  
 $H_2SO_4$ , concentrated  
 Deionized Water

Carefully add the required amount of sulfuric acid to 800 ml of deionized water contained in a 1 L volumetric flask. Cool the solution and then dilute it to the mark with deionized water. The concentration of acid in the wash should match that of your digested samples.

### 8. Startup/Shutdown Solution (200 ml)

Deionized Water ..... 200 ml  
 Dowfax 2A1 ..... 0.5 to 1 ml

Add 0.5 to 1 ml of Dowfax 2A1 to 200 ml of deionized water. Mix well.

## G. Optional Reagent Preparation: See Flow Diagram

### 1. Stock 10 N Sodium Hydroxide (250 ml)

Sodium Hydroxide ..... 100 g  
 $NaOH$  (FW 40.00)  
 Deionized Water

## Total Phosphorus

A050

This solution will get VERY hot. Add cautiously and with continuous stirring, 100 g of sodium hydroxide to approximately 150 ml of deionized water contained in a 250 ml volumetric flask. Cool the solution in an ice bath when adding the sodium hydroxide. When the solution is cool, dilute to the mark with deionized water and mix well.

### 2. Working Sodium Hydroxide (100 ml)

10 N Sodium Hydroxide ..... as required  
Dowfax 2A1 ..... 0.2-0.3 ml (8 - 12 drops)  
Deionized Water

Add the required amount of the 10 N sodium hydroxide to 50 ml of deionized water contained in a 100 ml volumetric flask. Dilute the solution to the mark with deionized water, add 0.2-0.3 ml (8 - 12 drops) of Dowfax 2A1 and mix well.

To determine the required amount of sodium hydroxide, do the following:

For any cartridge the correct concentration of sodium hydroxide to pump in the NaOH (or Diluent) line can be calculated as follows:

$$C_1 V_1 = C_2 V_2$$

Where:

$C_1$  = desired concentration (in Normality) of NaOH to be prepared.

$V_1$  = flow rate of the NaOH (or Diluent) pump tube.

$C_2$  = concentration (in Normality) of the acid in the samples and wash solution.

$V_2$  = flow rate of the sample pump tube.

Rearranging the equation to solve for  $C_1$  yields:

$$C_1 = \frac{C_2 V_2}{V_1}$$

For example, on a cartridge that has a red/red (482  $\mu\text{l}/\text{min}$ ) Diluent line and an orn/orn (287  $\mu\text{l}/\text{min}$ ) sample line, and the samples are 0.224 N acid (0.62% sulfuric), then

$$C_1 = \frac{(0.224)(287)}{482}$$

$C_1 = 0.13 \text{ N sodium hydroxide}$

Prepare this concentration of sodium hydroxide by diluting 1.3 ml of 10 N sodium hydroxide to 100 ml as described above.

Total Phosphorus

A050

### 3. Stock Sodium Chloride, 0.5% (1 L)

Sodium Chloride ..... 5.0 g  
NaCl (FW 58.45)  
Deionized Water

Dissolve 5.0 g of sodium chloride in approximately 950 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix it well.

### 4. Working Sodium Chloride

Stock Sodium Chloride ..... 100 ml  
Dowfax 2A1 ..... 0.2 - 0.3 ml (8 - 12 drops)

Mix together 100 ml of stock sodium chloride and 0.2 - 0.3 ml (8 - 12 drops) of Dowfax.

## H. Calibrants

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution or deionized water (See Operating Notes 7 and 15). Dilute the solution to 100 ml with sampler wash solution or deionized water and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

$C_1$  = desired concentration (in mg/L) of working calibrant to be prepared

$V_1$  = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

$C_2$  = concentration (in mg/L) of stock (or intermediate) calibrant

$V_2$  = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for  $V_2$  yields:

$$V_2 = \frac{C_1V_1}{C_2}$$

## Total Phosphorus

A050

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100 µl) of the stock calibrant in 100 ml final volume.

$$V_2 = \frac{(1.0 \text{ mg/L}) (100 \text{ ml})}{1000 \text{ mg/L}}$$

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

### I. Operation Procedure

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Dowfax to the startup/shutdown solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered weekly. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place reagent lines in reagent bottles.
7. If using data collection software, set up the appropriate sample table.
8. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
9. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
10. Select the appropriate parameters for the detector and sampler. (See Flow Diagram at the end of methodology.)
11. Begin analysis.
12. At the end of analysis place all reagent lines in startup/shutdown solution and turn off the heat bath. Pump startup/shutdown solution for 20 to 30 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
13. Turn off the power to all units and release pump platens.

Total Phosphorus

A050

## J. Operating Notes

1. Matching the sample matrix in the wash solution is very important for successful analysis of total phosphorus. If necessary, titrate the digested samples to find the exact acid concentration and adjust the sampler wash recipe to match.
2. To prevent the accumulation of background contamination forming in the working reagents, keep the reagent bottles covered at all times. Baseline drift may also be reduced by placing the ascorbic acid reagent in an ice bath during analysis.
3. If used, the working NaOH concentration is based on the concentration of acid in the digestate. It should be made so that the acid concentration of the analytical stream is less than 0.5% prior to the addition of the color forming reagents.
4. If calibrants are not digested, they should be made up in sampler wash solution.
5. Acid washed glassware should be used for all reagents and calibrants. Commercial detergents containing phosphorus should never be used to clean glassware used in phosphorus determination. Wash the glassware with 1:1 hydrochloric acid and rinse it thoroughly with deionized water. Store the glassware filled with deionized water. If the glassware is reserved for use only in phosphorus determination, treatment with hydrochloric acid is necessary only occasionally.<sup>(5)</sup>
6. If increased carryover and drift are experienced, make sure the ascorbic acid and molybdate/antimony reagent solutions are fresh.
7. If refractive index effects are present, adding the same amount of digestion salts to the sampler wash as are present in the digested samples may help to alleviate the symptom.
8. A common cause of low sensitivity and noise in the baseline is debris in the flowcell. Particulate matter from the reagents and samples may become lodged in the flowcell restricting the amount of light that is passed through the flowcell. Flushing the flowcell with approximately 10 ml of sampler wash solution with a syringe will dislodge any debris in the flowcell. Follow proper filtration procedures for the reagents and samples to reduce the frequency of this occurring.
9. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad®NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
10. If the flowrate of the sample pump tube is  $\leq 226 \mu\text{l}/\text{minute}$  (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

**NOTE:** If the sample line is debubbled, a helper line is not necessary.

## Total Phosphorus

A050

11. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.
12. Sodium Lauryl Sulfate can also be used as a wetting agent for this chemistry, replacing Dowfax. See recipe below. Use 2 ml SLS, 15% per 100 ml of water or reagent.

**NOTE:** High quality SLS is important. Fisher catalog numbers 02674-25, BP166-100 or BP166-500 are acceptable.

### Sodium Lauryl Sulfate (SLS) 15% w/w

Dodecyl Sodium Sulfate ..... 15 g  
 $\text{CH}_3(\text{CH}_2)_{10}\text{CH}_2\text{OSO}_3\text{Na}$  (FW 288.38)  
 Deionized Water ..... 85 ml

Dissolve 15 g of dodecyl sodium sulfate in 85 ml of deionized water.

13. For chronic carryover and drift problems, the following cleaning solution can be used to flush the analytical cartridge and flowcell.

### Potassium Iodide Cleaning Solution (55 ml)

Potassium Iodide ..... 1 g  
 KI (FW 166.00)  
 5 N Sulfuric Acid ..... 25 ml  
 $\text{H}_2\text{SO}_4$  (FW 98.8)  
 Deionized Water ..... 30 ml

**CAUTION:** Mixing sulfuric acid with water releases a great amount of heat.

To prepare 5 N sulfuric acid, cautiously add 14 ml of concentrated sulfuric acid to 60 ml of deionized water contained in a 125 ml Erlenmeyer flask. Cool to room temperature and transfer to a 100 ml volumetric flask. Dilute to the mark with deionized water.

Add 1 g KI to about 25 ml 5 N sulfuric acid. Stir vigorously until a strong yellow-orange color has formed. This may take at least one hour. Add about 30 ml deionized water. The solution will darken over time, and is usable for one month. Pump the cleaning solution through all lines in the cartridge for 10 to 15 minutes, followed by startup/shutdown solution.

14. Antimony Potassium Tartrate,  $\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_2 \cdot 3\text{H}_2\text{O}$  (FW 667.85) may also be used. Weigh the same amount.
15. If experiencing poor recoveries for QC samples, digest the calibrants along with the samples. If the calibrants will be digested, do not prepare them in the sampler wash solution. Dilute with deionized water and handle them in the same manner as samples.

Total Phosphorus

A050

## K. References

1. Murphy, J. and J.P. Riley, "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters", Anal. Chim. Acta., Volume 27, Page 31-36.
2. Whittlege, T.E., S.C. Malloy, C.J. Patton and C.D. Wirick, Automated Nutrient Analyses in Seawater, Technical Report, Brookhaven National Laboratory, Upton, New York, 1981, Page 48.
3. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Sample Preservation", Page xviii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45286.
4. Automated Nutrient Analyses in Seawater, Technical Report, Brookhaven National Laboratory, Upton, New York, 1981, p. 48.
5. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Phosphorus, All Forms", Method 365.3 (Colorimetric, Ascorbic Acid, Two Reagent).

## ACKNOWLEDGMENTS

Astoria® and FASPac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon  
Dowfax 2A1 is a trademark of Dow Chemical, USA  
Conrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

## Astor - Pacific International

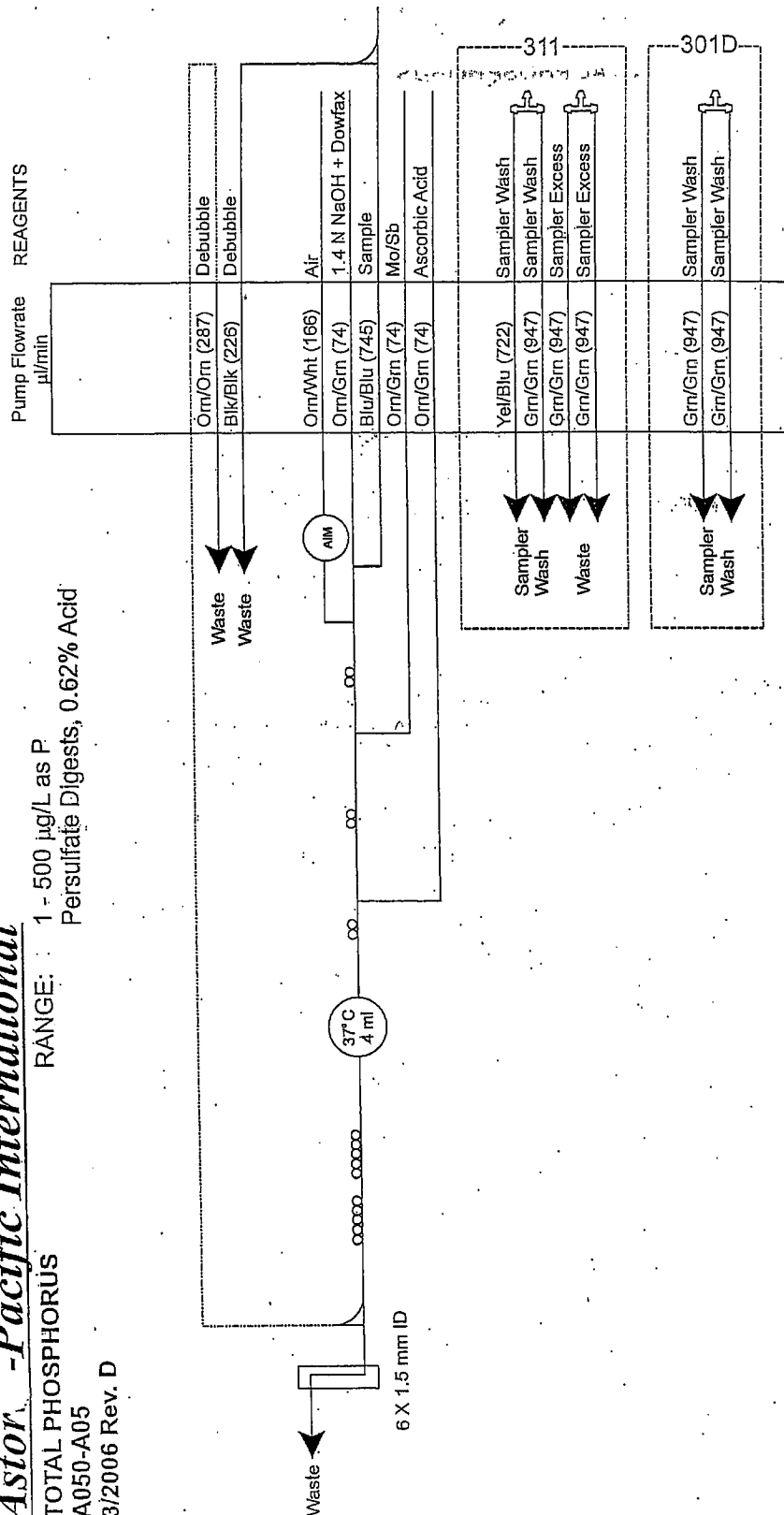
TOTAL PHOSPHORUS

A050-A05

3/2006 Rev. D

RANGE: 1 - 500 µg/L as P

Persulfate Digests, 0.62% Acid



### DETECTOR PARAMETERS

Interference Filter: 880 nm  
Neutral Density Filter: 90 or 130  
Smoothing: Poly  
Smoothing: MAF

### SPLIT CURVE PARAMETERS

Segment 1: 1 - 20 µg/L  
Segment 2: 20 - 500 µg/L

### SAMPLER PARAMETERS

Analysis Rate: 40/hr  
Sample Time: 40 s  
Wash Time: 50 s  
Pecking: OFF  
Pump Speed: 65 or 42

### CLEANING SOLUTIONS

CHEMWASH (API p/n 80-0005-250)

### STARTUP SOLUTION

Deionized Water with Surfactant\*

### Symbol Key, Copyright Information

o = 5 turns

Debubbler (API p/n 303-0122-00 or 303-0103-00)

Tee, Reagent Addition, Large (API p/n 303-0109-00)

Poly Flow Tubing (API p/n 303-2674-01)

Flowcell (API p/n 315-0106-00)

\*See Methodology



## Calibrant Preparation

### TOTAL PHOSPHORUS

A050-A05

1 – 500 µg/L as P

#### 100 mg/L Phosphorus Stock Standard (1.0 L)

- Dissolve .4394 g Potassium Dihydrogen Phosphate (FW 136.09), dried at 110°C in 800 ml deionized water.
- Dissolve and dilute to 1.0 L with deionized water.
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### 10 mg/L Phosphorus Intermediate Standard (100 ml)

- 10.0 ml of 100 mg/L Stock Standard
- Dilute with Sampler Wash Solution or deionized water. (See Methodology Operating Notes) to 100 ml.
- Mix Well

#### 1 mg/L Phosphorus Intermediate Standard (100 ml)

- 1.0 ml of 100 mg/L Stock Standard
- Dilute with Sampler Wash Solution or deionized water. (See Methodology Operating Notes) to 100 ml.
- Mix Well

#### Working Standard (100 ml)

Use adjustable, microliter pipettes to add the designated microliter volumes of stock, intermediate or working standard to 100 ml volumetric flasks containing approximately 80 ml of sampler wash solution or deionized water. Dilute each solution to the mark with the sampler wash solution or deionized water and mix well. Make standards covering the range being run.

Range	Working Standard Concentration	Standard to Pipet (Stock, Intermediate or Working)	Volume to Pipet (µl)
1 – 500 µg/L ↓	500 µg/L	100 mg/L	500
	200 µg/L	100 mg/L	200
	100 µg/L	10 mg/L	1,000
	50 µg/L	10 mg/L	500
	20 µg/L	10 mg/L	200
	10 µg/L	1 mg/L	1,000
	5 µg/L	1 mg/L	500
	2 µg/L	1 mg/L	200
	1 µg/L	0.1 mg/L	1,000
All Ranges	0.0 µg/L	N/A	N/A

NOTE: To prepare alternate calibrant concentrations consult the methodology.

## Appendix E: Astoria Total Kjeldahl Nitrogen Procedure

### TOTAL KJELDAHL NITROGEN A071

#### A. Scope and Application

This method is used for the determination of Total Kjeldahl Nitrogen in drinking, surface and saline waters, domestic and industrial wastes, plants and soils. The EPA range of this method is 0.1 to 20 mg/L as nitrogen. However, this method is also applicable to other ranges.

#### B. Summary of Method

Ammonia reacts with salicylate and hypochlorite in a buffered alkaline solution in the presence of sodium nitroferricyanide (pH 12.8-13) to form the salicylic acid analog of indophenol blue. The blue-green color produced is measured at 660 nm.

#### C. Interferences

Precipitation of calcium and magnesium hydroxides is eliminated by potassium sodium tartrate in the working buffer. Samples that are turbid must be filtered or centrifuged prior to determination. Samples with background absorbance at the analytical wavelength may interfere.

#### D. Sample Handling and Preservation

Determine the Total Kjeldahl Nitrogen concentration in unpreserved samples immediately after they are collected. Samples may be preserved with 2 ml of concentrated sulfuric acid per liter of sample and refrigerated at 2-8°C. The holding time for preserved samples is 28 days.<sup>(1)</sup>

#### E. Raw Materials Required

**NOTE:** Chemicals should be of ACS grade or equivalent.

Ammonium Sulfate,  $(\text{NH}_4)_2\text{SO}_4$  (FW 132.14) or Ammonium Chloride,  $\text{NH}_4\text{Cl}$  (FW 53.49)  
Brij®-35, 30% w/v (API p/n 90-0710-04)  
Chloroform,  $\text{CHCl}_3$  (FW 119.35)  
Potassium Sodium Tartrate,  $\text{KNaC}_4\text{H}_4\text{O}_6 \cdot 4\text{H}_2\text{O}$  (FW 282.23)  
Sodium Chloride,  $\text{NaCl}$  (FW 58.44)  
Sodium Hydroxide,  $\text{NaOH}$  (FW 40.00)  
Sodium Hypochlorite Solution,  $\text{NaOCl}$  5.25% Solution (Household bleach)  
Sodium Nitroferricyanide,  $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO} \cdot 2\text{H}_2\text{O}$  (FW 297.95)  
Sodium Phosphate-Dibasic,  $\text{Na}_2\text{HPO}_4$  (FW 141.96)  
Sodium Salicylate,  $\text{NaC}_7\text{H}_5\text{O}_3$  (FW 160.10)  
Sulfuric Acid, concentrated  $\text{H}_2\text{SO}_4$  (FW 98.07)

Total Kjeldahl Nitrogen

A071

## **F. Reagent Preparation**

All reagents and calibrants are prepared with ammonia-free deionized or distilled water. See Operating Notes for details on the preparation of ammonia-free deionized water.

### **1. Stock 10 N Sodium Hydroxide (1 L)**

**CAUTION: The dissolution of sodium hydroxide in water releases a great amount of heat.**

Sodium Hydroxide .....400 g  
NaOH (FW 40.00)  
Deionized Water

Cautiously and with continuous stirring, add 400 g of sodium hydroxide to approximately 700 ml of deionized water contained in a 1 L volumetric flask. Cool the solution in an ice bath when adding the sodium hydroxide. When the solution is cool, dilute it to the mark with deionized water and mix well.

### **2. Stock Potassium Sodium Tartrate (1 L)**

Potassium Sodium Tartrate .....200 g  
KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub>·4H<sub>2</sub>O (FW 282.23)  
Deionized Water

Dissolve 200 g of potassium sodium tartrate in approximately 800 ml of deionized water contained in a 1 L volumetric flask. Dilute the solution to the mark with deionized water and mix it well.

### **3. Stock Sodium Phosphate Buffer (1 L)**

Sodium Phosphate-Dibasic .....134 g  
Na<sub>2</sub>HPO<sub>4</sub> (FW 141.96)  
10 N Sodium Hydroxide .....50 ml  
Deionized Water

Dissolve 134 g of sodium phosphate-dibasic in approximately 800 ml of deionized water contained in a 1 L volumetric flask. Add 50 ml of 10 N sodium hydroxide and dilute the solution to the mark with deionized water. Mix the solution well.

### **4. Working Buffer (250 ml)**

Stock Sodium Phosphate Buffer .....50 ml  
Stock Potassium Sodium Tartrate .....62.5 ml

Total Kjeldahl Nitrogen

A071

10 N Sodium Hydroxide.....22.0 ml  
 Deionized Water  
 Brij-35, 30%.....10 drops

While stirring, add 62.5 ml of stock potassium sodium tartrate to 50 ml of stock sodium phosphate buffer contained in a 250 ml volumetric flask. Continue stirring and slowly add 22.0 ml of 10 N sodium hydroxide. Dilute the solution to the mark with deionized water and mix well. Filter to 0.45  $\mu$ m. Add 10 drops of Brij-35 and mix gently to prevent foaming. Prepare as needed or once a week.

**5. Sodium Salicylate/Sodium Nitroferricyanide (500 ml)**

Sodium Salicylate .....75 g  
 $\text{NaC}_7\text{H}_5\text{O}_3$  (FW 160.10)  
 Sodium Nitroferricyanide .....0.15 g  
 $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$  (FW 297.95)  
 Deionized Water

Dissolve 75 g of sodium salicylate and 0.15 g of sodium nitroferricyanide in approximately 300 ml of deionized water contained in a 500 ml volumetric flask. Dilute the solution to the mark with deionized water and mix well. Filter to 0.45  $\mu$ m. Store the solution in a dark bottle.

**6. Sodium Hypochlorite (100 ml)**

Sodium Hypochlorite Solution .....6 ml  
 $\text{NaOCl}$ , 5.25% Solution (Household bleach)  
 Deionized Water

Add 6 ml of sodium hypochlorite solution to approximately 90 ml of deionized water contained in a 100 ml volumetric flask. Dilute the solution to the mark and mix it well. Prepare the sodium hypochlorite reagent daily.

**7. Sampler Wash 4%  $\text{H}_2\text{SO}_4$  (1 L)**

**CAUTION: The mixing of sulfuric acid with water releases a great amount of heat.**

Sulfuric Acid .....40 ml  
 $\text{H}_2\text{SO}_4$ , Concentrated (FW 98.07)  
 Deionized Water

Carefully add 40 ml of sulfuric acid to approximately 800 ml of deionized water contained in a 1 L volumetric flask and mix the solution well. Cool the solution and dilute it to the mark with deionized water and mix well.

Total Kjeldahl Nitrogen

A071

#### 8. Diluent and Startup/Shutdown Solution

Deionized Water ..... 1000 ml  
 Brij-35 ..... 1 to 2 ml

Add 1 to 2 ml of Brij-35 to 1000 ml of deionized water. Mix well.

### **G. Optional Reagent Preparation: See Flow Diagram**

#### 1. Sodium Chloride (Dialyzer Only) (1L)

Sodium Chloride.....5.0 g  
 NaCl (FW 58.44)  
 Brij-35 ..... 1 to 2 ml  
 Deionized Water

Dissolve 5.0 g sodium chloride into 800 ml deionized water contained in a 1 L volumetric flask. Dilute to 1 L with deionized water. Filter to 0.45 µm. Add 1 – 2 ml of Brij-35 and mix gently.

### **H. Calibrants**

Specific Stock and Working Calibrant preparation instructions can be found on the back of the flow diagram. Be sure to use the flow diagram which covers the concentration range you wish to analyze.

Working calibrants may be prepared to cover alternate ranges by adding the appropriate volumes of stock or intermediate calibrant to 100 ml volumetric flasks that contain approximately 80 ml of sampler wash solution. Dilute the solution to 100 ml with sampler wash solution and mix well.

The following formula can be used to calculate the amount of stock (or intermediate) calibrant to be used.

$$C_1V_1 = C_2V_2$$

Where:

$C_1$  = desired concentration (in mg/L) of working calibrant to be prepared

$V_1$  = final volume (in ml) of working calibrant to be prepared (generally 100 ml)

$C_2$  = concentration (in mg/L) of stock (or intermediate) calibrant

$V_2$  = volume (in ml) of stock (or intermediate) calibrant to be used

Rearranging the equation to solve for  $V_2$  yields:

Total Kjeldahl Nitrogen

A071

$$V_2 = \frac{C_1 V_1}{C_2}$$

For example, to prepare a 1.0 mg/L working calibrant from a 1000 mg/L stock calibrant, use 0.1 ml (100 µl) of the stock calibrant in 100 ml final volume.

$$V_2 = \frac{(1.0 \text{ mg/L}) (100 \text{ ml})}{1000 \text{ mg/L}}$$

$$V_2 = 0.1 \text{ ml}$$

Add this amount of stock calibrant to the volumetric flask and then dilute to volume with the sampler wash solution.

**I. Operation Procedure**

1. Set up the cartridge as shown in the flow diagram. Check all tubing and connections. Replace if necessary.
2. Place reagent lines in startup solution.
3. Turn on power to all units including heat bath and latch pump platens to begin liquid flow.
4. Verify that the bubble size and spacing is consistent throughout the cartridge. If bubbles are splitting up as they enter or exit a coil or heat bath, check and replace fittings if necessary. The bubbles should flow smoothly without dragging. If dragging occurs, add more Brij-35 to the startup/shutdown solution.
5. Check all reagent containers on the instrument for particulate matter. Reagents should be filtered. Be sure all containers are properly labeled and filled before pumping reagents.
6. After the heat bath has reached the desired temperature and a stable baseline has been verified on the startup solution, place all reagent lines, **EXCEPT** the salicylate/nitroferrocyanide line, in reagent bottles.
7. Wait a minimum of 5 minutes before transferring the salicylate/nitroferrocyanide line to its appropriate reagent bottle.

**CAUTION:** Premature addition of the salicylate/nitroferrocyanide or an acidic analytical stream may result in the formation of a white precipitate. It is possible to clog the cartridge completely requiring extensive cleaning. If this occurs, flush the system with startup solution and check all reagents for contamination.

## Total Kjeldahl Nitrogen

A071

8. If using data collection software, set up the appropriate sample table.
9. Allow reagents to run for 5 to 10 minutes and verify a stable baseline.
10. Load the sampler tray with calibrants, blanks, samples, and QC or monitor samples.
11. Select the appropriate parameters for the detector and sampler. (See Flow Diagram.)
12. Begin analysis.
13. At the end of analysis turn the heat bath off and place salicylate/nitroferricyanide line in startup solution. Pump for 5 minutes then place the remaining lines into startup solution. Pump startup solution for 10 to 15 minutes to flush all of the reagents out of the cartridge and to allow the heat bath to cool.
14. Turn off the power to all units and release pump platens.

## J. Operating Notes

1. When starting the manifold, pump all reagents for at least five minutes **EXCEPT** the salicylate/nitroferricyanide. Pump water through the salicylate/nitroferricyanide line. Then start the salicylate/nitroferricyanide reagent and pump for ten minutes or until the system stabilizes before starting determinations.
2. Reverse this procedure when the determinations are complete. Remove the salicylate/nitroferricyanide line and pump water through it and reagent through the other lines for at least five minutes before pumping water through all the reagent lines.

**CAUTION:** Salicylate precipitates if the analytical stream becomes acidic. It may clog the cartridge completely and require extensive cleaning.

3. Prepare ammonia-free water by passing distilled water through a mixture of strongly acidic cation and strongly basic anion exchange resins.<sup>(2)</sup>
4. To prevent ammonia contamination from the air, it may be necessary to segment the analytical stream with nitrogen or draw air through a 5 N sulfuric acid solution. (Add 35 ml of concentrated H<sub>2</sub>SO<sub>4</sub> to 200 ml of deionized water and dilute to 250 ml with deionized water.)

**CAUTION:** Heat is generated.

Total Kjeldahl Nitrogen

A071

5. If the acid concentration of the digested samples is not 4%, the sampler wash must be changed to match the sample matrix and the sodium hydroxide content of the working buffer changed to ensure a pH of 12.8 to 13 for the salicylate reaction.
6. In some cases, samples have been found to absorb ammonia from the air. If you suspect that this is occurring, try pouring each sample just prior to aspiration by the system. This should help to minimize the contamination from the air.
7. If bubbles are sticking in a debubbler, cleaning the debubbler will allow bubbles to escape smoothly out the debubble line. Bubbles sticking in the debubbler can cause a loss in the overall precision of the peak height. To clean, soak the debubbler for 2-3 hours in a mixture of 20-30% Contrad®NF (API p/n 80-0007-04) and hot tap water. Rinse thoroughly.
8. If the flowrate of the sample pump tube is  $\leq 226 \mu\text{l}/\text{minute}$  (a blk/blk pump tube) a helper line must be added when the cartridge is run alone. See Section 9 of the Astoria Analyzer Operation Manual for information on how to add a helper line.

**NOTE:** If the sample line is debubbled, a helper line is not necessary.

9. Cover all reagents and other solutions to avoid interference due to dust and other particulates. This will also help prevent contamination of the solutions from absorbance of analytes in the air.
10. If refractive index effects are present, adding the same amount of digestion salts to the sampler wash as are present in the digested samples may help to alleviate the symptom.
11. On systems with a 311 Sampler or with cartridges that use a dialyzer, the OUT line from the 311 Sampler wash and the waste line from the top of the dialyzer must be directed to a separate waste container. Otherwise the waste trough in the 303A will become acidic, causing precipitation of the salicylate and overflow of waste into the module.
12. If experiencing poor recoveries for QC samples, digest the calibrants along with the samples. If the calibrants will be digested, do not prepare them in the sampler wash solution. Dilute with deionized water and handle them in the same manner as samples.

**K. Digestion Notes**

1. When adding the digestion reagent to the digestion tubes, be sure to accurately measure the proper amount of reagent into each tube. This will help ensure a consistent acid content in the final solutions.
2. Standards and samples should be accurately measured into the digestion tubes.



## Total Kjeldahl Nitrogen

A071

3. If an exhaust system or scrubber is placed on top of the digestion tubes during the digestion, care must be taken not to draw off any significant amount of the fumes during the high temperature stage. Variable acid contents may result. The digestion can be carried out without an exhaust system if the digestion apparatus is placed in a properly operating fume hood.
4. After digestion, it is preferable not to allow the digest to crystallize while cooling. To prevent crystallization, cool the tubes for a few minutes then add and mix two or three milliliters of ammonia-free water to each tube.

**CAUTION:** If the water is added too soon and the acid is still too hot, the contents of the tube may react vigorously and some material may be lost from the tube.

5. The completed digests must be accurately diluted to a known volume in a calibrated container. This is important for the accuracy of the measurement as well as to help ensure a consistent acid content in the final solutions. If the contents of the tube are transferred to a separate container during this step, ensure that all of the digestate is transferred.
6. If problems are encountered in the analysis of the digests, titrate several aliquots from different tubes (after diluting to volume) to determine that a consistent acid content is being achieved.
7. The wash solution for the sampler must be of the same acid content as the digests. Also, if the titration of digests shows that the actual acid content is different from that shown on the flow diagram, the concentration of sodium hydroxide specified for the flow diagram (if applicable) will need to be adjusted proportionately.
8. If some digests give an off-scale response during the analysis, they should be diluted with blank solution having the same acid content before reanalysis.

## L. References

1. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020. "Sample Preservation", Page xvii, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45286.
2. Methods for Chemical Analysis of Water and Wastes, March 1984, EPA-600/4-79-020, "Nitrogen Kjeldahl, Total", Method 351.2 (Colorimetric, Semi-Automated Block Digestor, AAII), STORET NO. Total 00625.

### ACKNOWLEDGMENTS

Astoria® and FASPac™ are trademarks of Astoria-Pacific, Inc., Clackamas, Oregon  
Brj®-35 is a registered trademark of ICI Americas, Wilmington, Delaware  
Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

## Calibrant Preparation

### **TOTAL KJELDAHL NITROGEN in 4% ACID DIGESTS**

A071-A00

0.05 – 2 mg/L as Nitrogen | 0.1– 10 mg/L as Nitrogen

#### 1,000 mg/L Ammonia Nitrogen Stock Standard (1.0 L)

- Dissolve EITHER 4.7168 g Ammonium Sulfate ( $(\text{NH}_4)_2\text{SO}_4$  (FW 132.14), dried at 110°C, OR 3.8196 g Ammonium Chloride,  $\text{NH}_4\text{Cl}$  (FW 53.49), dried at 110°C in 900 ml deionized water.
- Dilute to 1.0 L with deionized water
- Add 2 drops Chloroform  $\text{CHCl}_3$  (FW 119.38) and mix well.
- Store @ 2 - 8°C

#### 100 mg/L Ammonia Nitrogen Intermediate Standard (100 ml)

- 10.0 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution or deionized water to 100 ml. (See Methodology-Operating Notes).
- Mix Well

#### 10 mg/L Ammonia Nitrogen Intermediate Standard (100 ml)

- 1.00 ml of 1,000 mg/L Stock Standard
- Dilute with Sampler Wash Solution or deionized water to 100 ml. (See Methodology-Operating Notes).
- Mix Well

#### Working Standard (100 ml)

Use adjustable, microliter pipettes to add the designated microliter volumes of stock, intermediate or working standard to 100 ml volumetric flasks containing approximately 80 ml of sampler wash solution or deionized water (See Operating Notes). Dilute each solution to the mark with the sampler wash solution or deionized water and mix well. Make standards covering the range being run.

Range	Working Standard Concentration	Standard to Pipet (Stock, Intermediate or Working)	Volume to Pipet (μl)
0.1 – 10 mg/L	10 mg/L	1,000 mg/L	1,000
	5 mg/L	1,000 mg/L	500
0.05 – 2 mg/L	2 mg/L	100 mg/L	2,000
	1 mg/L	100 mg/L	1,000
	0.5 mg/L	100 mg/L	500
	0.2 mg/L	10 mg/L	2,000
	0.1 mg/L	10 mg/L	1,000
	0.05 mg/L	10 mg/L	500
All Ranges	0.0 mg/L	N/A	N/A

**NOTE:** To prepare alternate calibrant concentrations consult the methodology.

Brij®-35 is a registered trademark of ICI Americas, Wilmington, DE