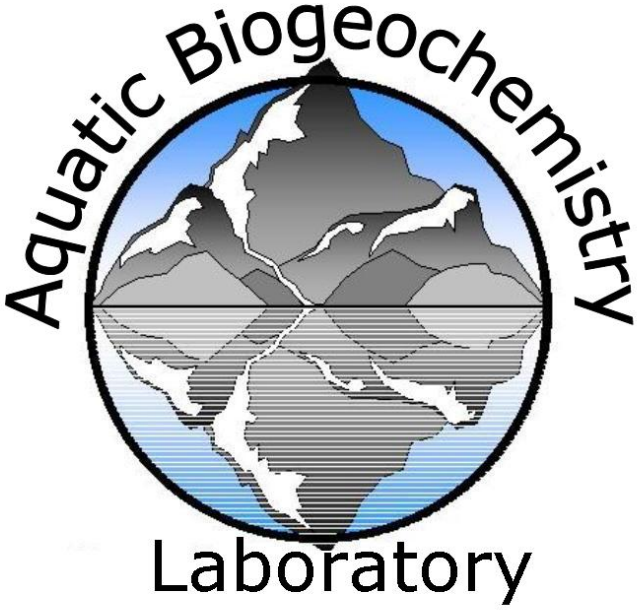
ANALYTICAL PROCEDURES



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Aquatic Biogeochemistry Lab at Utah State University

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Analytical Chemistry Quality Assurance/Quality Control (QA/QC) Process

I. Background

This protocol describes general procedures in documenting and assuring quality of analytical chemistry results that are produced at the USU Aquatic Biogeochemistry Laboratory. Please see protocols for specific analyses for additional information on analytical procedures, expected detection limits, sources for certified reference materials, etc. Strict adherence to this and analysis-specific protocols will be compensated by increased confidence in the data we produce. Stakeholders and manuscript reviewers are increasingly attentive to information on laboratory detection limits and QA/QC procedures.

II. Potential Hazards

a. Laboratory Safety

Analytical chemistry procedures are conducted in a chemical laboratory. Exposure to reactive, toxic, flammable, and/or corrosive chemicals is possible, as are chemical spills. All laboratory personnel must have up-to-date Chemical Laboratory Safety Training as mandated by OSHA and implemented by the USU Environmental Health and Safety Office. Please review the ABL Chemical Hygiene Plan for specific laboratory emergency plans, details on chemical spill clean-up, chemical inventory lists and MSDS for specific chemicals. Chemical laboratory personnel must wear appropriate attire including closed-toe shoes, lab coats, gloves, and safety glasses.

III. Quality Assurance

a. Policy Statement

The Utah State University Aquatic Biogeochemistry Laboratory (USU-ABL) is committed to provide scientifically valid analytical data for university-sponsored research projects as well as for outside customers, including government agencies and local stakeholders. We aim to produce data in a timely manner following established protocols for analyses of chemical constituents in fresh and saline waters, such as those approved by the Environmental Protection Agency (EPA). Aside from EPA-approved methods, and depending on user needs, we use more state-of-the-art protocols that have been vetted through the scientific peer review process and are in published literature.

Although the USU-ABL is not formally certified by any accreditation program, the lab does follow process used by most certified labs including the following: Lab director and manager have achieved advanced educational degree with background in analytical chemistry, technicians are typically students in a science field with some lab experience before they receive specific training on an analytical instrument, the lab has an up-to-date Chemical Hygiene Plan as well as a lab manual that contains documents

describing QA/QC process and specific standard operating procedures (SOPs) for each analysis/measurement. Instruments are calibrated using these SOPs and analyses are verified using certified reference materials appropriate for each analysis. Method detection limits are calculated for each analysis and updated biannually. Data below the method detection limit are reported with a flag. The lab conducts self-audits to ensure compliance with OSHA and university regulations and is inspected annually by USU's safety office and irregularly by EPA.

b. Organizational Structure

The USU-ABL is staffed by a Director (Dr. Michelle Baker) who has nearly 20 years of experience in aquatic biogeochemistry research. A lab manager (position currently vacant) coordinates laboratory analyses, oversees QA/QC process, ensures compliance with OSHA and university rules and regulations, trains and supervises graduate and undergraduate student technicians. During any given semester the lab is staffed by 2-3 part-time undergraduate student technicians, as well as 2-3 graduate students.

c. Staff Responsibilities

All personnel employed by the USU-ABL are required to be familiar with policies and procedures outlined in this document, as well as individual standard operating procedures (SOPs) used for specific analyses.

All USU-ABL employees must abide by USU's Personnel Policies. These are available in detail from the Human Resources website (http://www.usu.edu/hr/htm/policies). USU conducts background checks on all new employees. USU operates a strict drug- and alcohol-free workplace.

All USU-ABL employees must complete the following training upon appointment, with renewal at the specified rates:

Training	Administrator	Renewal Period
Laboratory Safety	Environmental Health and Safety Office	Annual
Specific SOPs	USU-ABL	Annual
Drivers Training	Motor Pool	Every other year
Purchasing Card	Purchasing Office	Every three years

Students, post-docs, and classified staff sponsored in whole or in part by funds from the National Science Foundation must complete training in the Responsible Conduct of Research. This training is offered annually by the Office of Compliance Assistance, and satisfies requirements of the America COMPETES Act.

All USU-ABL employees must support scientific integrity as outlined in USU Policy #306. Scientific misconduct defined in USU policy as "any incident of

fabrication, falsification, or plagiarism in proposing, conducting, or reporting research. It does not include honest error or honest differences in interpretations or judgments of data. Fabrication refers to the making up of data that were not observed as purported. Falsification includes the changing of data or the way in which observations are reported, and spans a broad spectrum, from omitting observed data points from reported data sets to wholesale changing of data to fit the investigator's hypothesis. Plagiarism is the claiming as one's own material that is the product of someone else's work." Scientific misconduct by USU-ABL employees will not be tolerated and will be grounds for immediate dismissal.

d. Training and Performance Requirements

Employees of USU-ABL must maintain training records as outlined in the table above. Performance will be documented initially and on an on-going basis as outlined below.

1) Initial Demonstration of Capability
   Before performing any chemical analysis, USU-ABL employees will conduct an initial demonstration of capability (IDC) to demonstrate that the employee is proficient in the analysis. The IDC includes comparing the calibration standard curve of the new analyst (or method if modified) to that of a previous analyst. Reagent blanks and check standards are also compared within a sample run. Calibration curves should differ by no more than 10%. Reagent blanks should contain no contamination at concentrations ½ of the method detection limit. Percent recovery for check standards should be within acceptance criteria for the given method, typically within $\pm$10%. IDC information should be recorded in the USU-ABL Laboratory manual at the end of the appropriate SOP.

2) Ongoing Demonstration of Capability
   Ongoing capability for a given SOP is demonstrated using reagent blanks, check standards, and quality-control standards prepared from certified reference materials. Check standards made from calibration stock solutions and reagent blanks must be included every 20 samples in a run. Certified reference materials should be analyzed quarterly (or about every 500 samples). Check standards and certified reference materials are analyzed semi-annually for each analyst and method. Each analyst must update signatures on specific SOPs annually as part of their annual performance evaluation.

e. Analytical Capabilities of the ABL

1) Analytical capability includes ion chromatography, total organic carbon and total nitrogen analyses, inorganic and total nitrogen and phosphorus analyses, chlorophyll, biochemical oxygen demand, and gas chromatography with electron capture detection (GC-ECD),

flame ionization detection (GC-FID), and thermal conductivity detection (GC-TCD).

2) Additional services include method development, sample processing for stable isotope analysis, dry mass and ash-free dry mass, total suspended solids, volatile suspended solids.

f. Receiving and Handling Samples, and Chain-of-Custody Procedure

1) Receiving samples and storage
Samples are typically received in a cooler. Samples usually should be transported to the lab on ice; please see individual SOPs for specific details. Each user must fill out an Analysis Order Form (available online or in BNR 142) with name and contact information, sample matrix, number of desired analyses, estimated concentration range, data deadline, and billing information. Samples for nutrient analysis are stored in the freezer in boxes labeled with identification tags (available on each freezer; be sure to note freezer name on Analysis Order Form) that indicate desired analyses. We have found that for most nutrient analyses, samples are stable if stored frozen for several months. Samples for dissolved organic carbon analysis typically are received in amber glass bottles and are acidified to pH<2. These samples should be stored in the dark at room temperature.

2) Handling samples
Frozen samples should be thawed in a hot water bath prior to analysis. Samples to be analyzed for multiple parameters should be processed for ammonium first since this analyte is most sensitive to repeated freezing and thawing of samples. Total N and P should be done last. Analyst should cross off analyses on the box identification tag after each one is done. When all analyses are complete, and data have been checked and sent to the end-user, samples should be removed from the freezer and returned to the user, or disposed of.

3) Chain-of-custody
Some users require specific chain-of-custody procedures to verify sample integrity. USU-ABL will work with such clients on an individual basis to ensure proper paperwork is completed. In some cases chain-of-custody is indicated on the sample bottle, so each analyst should sign and date the appropriate space on the bottle on receipt, and after each analysis is completed. If samples are received with specific chain-of-custody forms, these are to be attached to the Analysis Order Form and signed as appropriate when specific analyses are conducted.

IV. Quality Control

a. Calibration, Verification, and Maintenance of Analytical Instrumentation

1) Calibration

Instrument calibration is conducted according to instrument manufacturer's instruction manual and SOP instructions. Initial calibrations are conducted with a minimum of 5 concentrations for linear curves, and 7 concentrations for non-linear curves, each time the instrument is started up for analysis. The calibration range varies by analysis but includes a low standard at or below the method detection limit, and a high concentration at the maximum expected by the end-user as reported on the Analysis Order Form. Calibration is acceptable by linear or nonlinear regression when a minimum correlation coefficient of 0.995 is achieved. For most analyses the correlation coefficient is 0.999. Initial calibration procedure is repeated if calibration criteria are not met within a run or if new reagents are added in the middle of a run.

2) Verification

Calibration verification within a run is assessed using laboratory-fortified blanks (check standards) prepared in a mid-range concentration. Duplicate check standards should be conducted on a 5% basis, or after every 20 samples. Reagent blanks are run at a minimum at the beginning and end of the run, and more often for certain analyses (see specific SOPs). Calibration verification is calculated as % difference in response factor for check standard compared to initial standard, and as % recovery of check standard. Deviations between 2-5% are flagged in the analysis, deviations greater than 5% require re-run. If contamination is present in reagent blanks (greater than detection limit), source of contamination should be identified and samples re-run.

3) Maintenance/Troubleshooting

Analysts must follow instrument users manual for maintenance schedules. Typically if initial calibration is not good, try the following: remake reagents, remake calibration standards, check tubing/plumbing integrity and replace as needed. Follow more detailed troubleshooting as recommended by specific instrument manual. Record maintenance conducted in instrument log book.

b. Quality Control Parameters

1) Method Detection Limit

The method detection limit (MDL) is determined at least annually for each method from a minimum of seven replicates of low-level

check standard that have been processed through the entire method, across three or more runs. The MDL is calculated as the standard deviation times the t-value from a one-sided t-distribution at the 99% level. For example for 7 replicates, the t-value is 7-1= 6 degrees of freedom = 3.14. The MDL is reported to the end user with each data set.

2) Repeatability
Sources of variation and bias in analytical measurement include but are not limited to sampling error and preparation, matrix effects, calibration errors, differences among analysts, reagent impurities, and instrument errors (hardware and software). It is usually not possible to minimize all of these errors simultaneously. We have found that sampling error – particularly field filtering of samples can be very large. Contamination of samples in the field, sample bottle, and from the atmosphere can be a large source of error. USU-ABL reports repeatability of measurements from analytical duplicates. End-users are advised to include field duplicates and field blanks with their samples. Analytical replicates that deviate more than 5% are re-run.

c. Data Reporting

1) Data are reported in an excel spreadsheet with these minimum paramaters: analysis date, analyst name, sample identification, concentration, calibration information, reagent blanks, check standards. USU-ABL will work with end-users to provide data in optimal format for their needs.
2) Data flags
In some instances reported data are qualified by flags indicating that quality control was not achieved. Flags include the following:
BDL = below method detection limit
CON = possible contamination because analyte found in reagent blank or % recovery exceeds expected value by more than 5%.
E = value exceeds high concentration calibrant. Sample is usually diluted and re-run.
RERUN = sample concentration rejected because of contaminant, method performance, etc.

d. Records Archiving

1) Each analyst is responsible for maintaining electronic copies of each analytical run in the appropriate instrument folder. These are duplicated and archived on USU's server, and stored for a minimum of 3 years. Electronic copies of excel spreadsheets should be made quarterly, saved to CD or DVD and stored in BNR 145.

2) Hard copies of Analytical Order Forms and other data sheets are stored in BNR 145 for a minimum of 3 years.
3) Lab notebooks should be scanned quarterly and the images stored on USU's server. Hard copies of lab notebooks should be stored indefinitely in BNR 140. Electronic copies of scanned lab notebooks should be saved to CD or DVD and stored in BNR 145.

V. References

1) American Public Health Association (APHA). 1998. Standard methods for the examination of water and wastewater 20th edition. APHA, Washington DC.

Sample Filtration Procedure

I. Principle/Background

The purpose of filtration is essentially remove all particles that are not dissolved in the water. It will be analyzed for dissolved NO_3, PO_4, Br, DOC, Cl, and the total phosphorous and nitrogen that are in dissolved form. Sample filtration also may retard some biological processing. Samples are filtered using Whatman GF/F filters that are ashed prior to use (see ashing protocol). Ashing removes any organic binders present in the glass that may contaminate samples.

II. Safety

Sample filtering in the field is generally safe. DOM samples are preserved using 12N HCl. Care should be avoid splashing on clothes or in eyes, and HCl may eat away clothing. Avoid inhalation of any fumes that may be present. Refer to Material Safety Data Sheets for treatment in case of exposure

III. Equipment

60 ml syringes
25 mm syringe filter holders
Ashed 25 mm Glass Microfibre Filters
Numbered acid washed nalgene sample bottles
Forceps
DI water

IV. Procedure

1. Assemble filter holders (make sure all plastic parts have been acid washed initially and keep them clean by thoroughly rinsing with DI water between each use). Use forceps to carefully place the 25 mm microfibre filter within the filtration manifold

2. Standing in the thalweg, face upstream and collect streamwater using the 60 ml syringe. Rinse the syringe three times with stream water before collecting final sample.

3. Filter a few milliliters of the sample into the stream to rinse the filter holder and then filter a few milliliters into sample bottle. Cap and shake thoroughly to rinse.

Discard the water. Fill the bottle with remaining water and repeat as needed to fill the bottle. To cap without headspace, gently squeeze the bottle as you close it.

4. Record the appropriate bottle number along with sample location, date, time, etc. in field book or on data sheet.

5. Change filter as needed or per site. If sampling plateau during an injection always work downstream to upstream to avoid contamination

When finished filtering, place any acid preserved samples in separate boxes from unpreserved samples. Label the boxes with the date, user, project, and field book number. Note the analyses to be done. For dissolved samples the analyses to be done are NO_3, PO_4, Br, DOC, Cl, and TN/TP. Place unpreserved samples in the refrigerator if analyses will be occurring within a few days, otherwise place in the freezer (always opt for the freezer if you are unsure). Place acid preserved samples in a cool dark place.

Inorganic Nutrients by Colorimetry

I. Background

USU-ABL uses EPA-approved methods on an Astoria-Pacific Autoanalyzer to measure nitrate+nitrite-N, ammonia+ammonium-N, and ortho-phosphate-P in water. The autoanalyzer uses segmented continuous flow to add, mix and react reagents in order to produce a color change that is detected by spectrophotometry.

II. Potential Hazards

The hazards of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be minimized. Adequate gloves are required for the preparation of the mobile phase, the samples and the standards. A reference file of Material Safety Data Sheets is available and a Laboratory Chemical Hygiene Plan is in place.

III. Certified Reference Materials

USU-ABL uses certified reference materials as part of analytical QA/QC. For nitrate+nitrite-N and ortho-P, we use the certified 7-ion reference material produced by DIONEX corporation. For ammonium-N we use the NH4Cl CRM prepared by Fluka.

IV. Analytical Procedures

USU-ABL uses the Astoria-Pacific instruction manual and SOPs, which are approved by EPA. These are attached unedited.

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.[1]

E. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Ammonium Sulfate $(NH_4)_2SO_4$ (FW 132.13)
Brij®-35, 30% w/v (API p/n 90-0710-04)
Chloroform, $CHCl_3$ (FW 119.38)
Phenol, liquefied 88% C_6H_5OH (FW 94.11)
Potassium Sodium Tartrate $KNaC_4H_4O_6 \cdot 4H_2O$ (FW 282.23)
Sodium Citrate, Dihydrate $Na_3C_6H_5O_7 \cdot 2H_2O$ (FW 294.11)
Sodium Hydroxide NaOH (FW 40.00)
Sodium Hypochlorite NaOCl 5.25% (household bleach)
Sulfuric Acid H_2SO_4, concentrated (FW 98.07)
Sodium Nitroferricyanide $Na_2Fe(CN)_5NO \cdot 2H_2O$ (FW 297.95)

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
$KNaC_4H_4O_6 \cdot 4H_2O$ (FW 282.23)
Sodium Citrate, Dihydrate ..... 24 g
$Na_3C_6H_5O_7 \cdot 2H_2O$ (FW 294.11)
Sulfuric Acid..... as required
H_2SO_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 µm.

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
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.

4. Alkaline Phenol (1 L)

10 N Sodium Hydroxide ..... 85 ml
Phenol, liquefied ..... 12 ml
Phenol C_6H_5OH (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.

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
$Na_2Fe(CN)_5NO•2H_2O$ (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.

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 µl) of the stock calibrant in 100 ml final volume.

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

V_2 = 0.1 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.

I. Operating Notes

1. Prepare ammonia free water by passing distilled water through a mixture of strongly acidic cation and strongly basic anion exchange resins.[2]

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 ≤ 226 µl/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.

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

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Contrad®NF and Neutrad® are registered trademarks of Decon Labs., Inc., Bryn Mawr, Pennsylvania

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.[1] These reactions take place in acidic solution. Nydahl[2] provides a good discussion of nitrate reduction by cadmium metal, while the specific details of OTCR's are given by Patton.[5] 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_4OH. Samples containing large concentrations of oil and grease must be extracted with an organic solvent.[7] Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts.[8] Norwitz and Keliher have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrite.[10,11] Residual chlorine can interfere by oxidizing the cadmium coil, reducing its efficiency. Test for residual chlorine and treat if necessary.[12]

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.[3] 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.

E. Raw Materials Required

NOTE: Chemicals should be of ACS grade or equivalent.

Ammonium Chloride NH_4Cl (FW 53.50)
Ammonium Hydroxide NH_4OH (FW 35.05)
Brij-35®, 30% w/v (p/n 90-0710-04)
Chloroform $CHCl_3$ (FW 119.38)
Cupric Sulfate, Pentahydrate $CuSO_4 \cdot 5H_2O$ (FW 249.69)
Deionized Water (ASTM type I or II)
Disodium Ethylenediamine Tetraacetate $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)
Hydrochloric Acid, Concentrated HCl (FW 36.46)
N-1-naphthylethylenediamine Dihydrochloride $C_{12}H_{14}N_2 \cdot 2HCl$ (FW 259.18)
Phosphoric Acid, Concentrated H_3PO_4 (FW 98.00)
Potassium Nitrate KNO_3 (FW 101.11)
Potassium Nitrite KNO_2 (FW 85.11)
Sulfanilamide $C_6H_8N_2O_2S$ (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
NH_4Cl (FW 53.50)
Disodium Ethylenediamine Tetraacetate (disodium EDTA) ..... 0.1 g
$C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)
Ammonium Hydroxide, concentrated
NH_4OH (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 μm.

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)

Phosphoric Acid, concentrated ..... 50 ml
H_3PO_4 (FW 98.00)
Sulfanilamide ..... 20 g
$C_6H_8N_2O_2S$ (FW 172.21)
N-1-naphthylethylenediamine Dihydrochloride ..... 1 g
$C_{12}H_{14}N_2$•2HCl (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-napthylethylenediamine dihydrochloride in the phosphoric acid solution. Dilute to the mark with deionized water. Filter to 0.45 μm. 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)[4]

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
CuSO4•5H2O (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.

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.

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.

$$NO_3^- + 2H^+ + 2e^- = NO^-_2 + H_2O \quad (1)$$
$$NO_2^- + 6H^+ + 4e^- = H_3NOH^+ + H_2O \quad (2)$$
$$NO_2^- + 8H^+ + 6e^- = NH_4^+ + 2H_2O \quad (3)$$

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.[5] 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:

$$\frac{\text{Peak Height } (NO_3^-) \times 100}{\text{Peak Height } (NO_2^-)}$$

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.

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 µl) of the stock calibrant in 100 ml final volume.

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

$$V_2 = 0.1\ ml$$

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 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.

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 ≤ 226 µl/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.

J. References

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ACKNOWLEDGMENTS

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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 phosphoantimonylmolybdenum complex. This complex is subsequently reduced by ascorbic acid to a heteropolyblue with an absorbance maximum at 660 nm or 880 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.[1]

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)

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_2SO_4 (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
$K(SbO)C_4H_4O_6 \bullet 1/2H_2O$ (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
$(NH_4)_6Mo_7O_{24} \bullet 4H_2O$ (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
$C_6H_8O_6$ (FW176.13)
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.

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:

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

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.

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 ≤ 226 µl/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
$CH_3 (CH_2)_{10}CH_2OSO_3Na$ (FW 288.38)
Deionized Water ..... 85 ml

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

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 KI (FW 166.00)	1 g
5 N Sulfuric Acid (See Reagent Preparation) H_2SO_4 (FW 98.08)	25 ml
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 $C_8H_4K_2O_{12}Sb_2 \bullet 3H_2O$ (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.[5]

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Organic Phosphorus and Organic Nitrogen Digestion procedure.

Background.

Reacting nitrogen under basic conditions with potassium persulfate causes digestion of organic N in compounds such as proteins into inorganic nitrogen in the form of Nitrate. Reacting phosphorus under acidic conditions with potassium persulfate causes digestion of organic P in compounds such as ATP into inorganic phosphorus in the form of phosphates.

The following procedure (based on the experiments of Valderama, 1981) explains the process of accomplishing successful completion of both of these reactions at the same time in the same sample vial using a boric acid – sodium hydroxide system (with digestion efficiencies above 80%). To be run on the Astoria autoanalyser.

Methodological considerations.

• It is important to obtain a low and consistent set of blanks (digested persulfate in di H2O sample – 5 tubes minimum) in order to obtain reliable data at a low level of detection. These blanks will be used to fill the wash cups and the calibration blank and hence, they will set the baseline for the calibration and sample run.
The standards used should also be digested. Allow a couple of extra tubes for a mid range standard to be used as a check that will be sampled at the end of the run. About 10% of the samples should be duplicated throughout the whole process.

• In order to reduce contamination to a minimum and hence produce low, consistent blanks and reproducible results it is necessary to purify the potassium persulfate solid, using a re-crystallization procedure, before use. This re-crystallization works by dissolving the persulfate solid in as little warm water as possible, (so that it is close to the point of a super saturated solution), this also dissolves all of the impurities, but in a much lower concentration. Rapid cooling then forces the persulfate to crystallize out of solution, (a precipitous reaction, usually started with a 'seed' or point of 'irritation' on the wall of the beaker), due to the solute becoming insoluble at lower temperature. Most of the contaminants stay in solution as they were not near super saturation. Once the re-crystallization is complete, the persulfate crystals can be filtered off (the solvent + contaminants are removed!) and dried over vacuum and then stored in a desiccator.

• If saline samples are to be run then the auxiliary wash should match this matrix as close as possible. For sea water samples, (or certain GSL samples), Artificial Sea Water (ASW) would be used for the wash solution and can be made by dissolving 30g/L $NaCl_{(s)}$ into deionised water. The standards and blanks for this hypothetical run should also be made with the ASW.

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• When running Nitrates it is important to run a column efficiency check in order to quantify the percentage of NO_3 in your samples that actually got reduced to NO_2. Don't forget – the color reagent for this analysis reacts with NO_2 not NO_3! To do this, run a 1.0 mg/L NO_3 against a 1.0 mg/L NO_2 and calculate percentage column efficiency. It should be above 90% initially and ideally change by less than 10% over the whole run.

• The autoanalyser calibrates as it goes along. It will not give a peak reading ('peak height in absorbance' – 'the absorbance of the blank, C1') until after the blank and first three standards are run. After this it will 'refresh' the values as it updates the calibration with each new standard peak. Once the final standard and the following wash (W) have run, the calibration curve will remain constant. If you wish to drop a bad point on the calibration graph select the "Show calibration charts" button and select the point you wish to drop with the left mouse button. It will turn gray and the point will be indicated as not used.

• Acid wash, triple rinse, (with Di H2O), and dry all glassware prior to use. Re-use the same volumetric flasks for the same concentrations as they were used before. These need not be acid washed, just triple rinsed! Make sure the primary stock solution is at room temperature before use so as not to introduce an error due to the reduced volume of cooled liquid. Make sure the standard curve covers the expected range of the samples to be run.

• All standards are made to atomic concentrations of the appropriate element, i.e, mg/L NO_3-N, PO_4-P and NH_4-N. All sample concentrations are also reported in these units. Hence, a 500 mg/L NO_3-N stock contains 500 mg/L of N in the form of NO_3 not 500 mg/L NO_3.

• After digestion check the sample tubes to make sure none have lost volume. If volume loss has occurred, discard this sample and re-run when convenient.

Procedure.

Initially, the persulfate solid was re-crystallized. About 50g was dissolved in ~350ml Di H_2O with heating (mild – do not boil or it will auto-decompose). Once dissolved, the solution was filtered hot and then rapidly cooled in ice to 4°C while stirring. After a couple of hours, the crystals were poured onto a scintered glass filter and then washed with a small amount of ice cold water. The crystals were then dried over vacuum for about ten minutes (covered) and stored in a vacuum desiccator.

Using the recrystalized persulfate, a digestion reagent was made. NaOH (35ml, 1M) was added to a 250ml glass beaker with a magnetic stir bar. To this solution was added, Potassium persulfate (6.25 g) and then Boric acid (3 g) with stirring. The mixture was heated mildly until dissolved to form a clear, colorless solution. A few ml of di H_2O were added to aid this process. After the dissolution was complete, the solution was added to a

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volumetric flask (100 ml) and, after cooling, was made up to the mark with di H_2O and shaken well.

Please note, this is the most efficient digestion reagent out of the recipes I have tested, however, it can be altered to a new stoichiometry to potentially find a better reagent for certain conditions (i.e. different matrix, different pH etc….).

The samples / standards were added (15ml) to each autoclavable vial (Pyrex / Kimax with Teflon lined lids) and then the persulfate reagent (2ml) was added to each. The vials were then sealed tight and shaken for a few seconds before being autoclaved for 90 minutes at 100°C (however, the samples will not have boiled because the pressure was elevated so this should have prevent auto-decomposition!)

After cooling the samples were then ready to be run on the Astoria autoanalyser using the NO_3 reduction and SRP methods.

Standards.

A 500 mg/L NO_3-N and PO_4-P primary stock solution was originally made by adding vacuum desiccated KNO_3 (3.60 g) and KH_2PO_4 (2.19 g) to a volumetric flask (1 liter), dissolving with and making up to the mark with, Di H_2O.

A 1 mg/L secondary stock solution was made from this by adding primary stock solution (0.2 ml / 100 ml) to a volumetric flask and diluting with Di H_2O.

From the secondary stock solution appropriate standards for the expected sample concentration range were made. For example 10, 25, 50, 75, 100 and 200 µg/L (see table).

Standard compositions, made from 1mg/L NO_3-N + PO_4-P stock.

Standard.	Volume of 1 mg/L stock to be added to 100 ml Di H_2O.
10 µg/L	1.0 ml
25 µg/L	2.5 ml
50 µg/L	5.0 ml
75 µg/L	7.5 ml
100 µg/L	10.0 ml
200 µg/L	20.0 ml

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Alternative procedures.

Sample runs to detect for TP only (and not TN) were the same as above but with the following changes;

The Persulfate reagent was made by adding Potassium persulfate (50 g/L, recrystalized) to a volumetric flask (100ml – enough for 50 vials) and dissolving in and making up to the mark with DiH_2O.
Each sample was acidified to pH 2-3 with $HCl_{(aq)}$ (60µL, 1M). Then they were sealed and shaken. After this the persulfate reagent could be added and once tightly re-sealed and shaken, the samples could be autoclaved.

Sample runs to detect for TN only (and not TP) were the same as initial procedure but with the following changes;
The Persulfate reagent was made by adding Potassium persulfate (50 g/L, recrystalized) to a volumetric flask (100ml – enough for 50 vials) and dissolving in and making up to the mark with DiH_2O.
Each samples pH was adjusted to 10-11 with NaOH (10µL, 10M). Then they were sealed and shaken. After this the persulfate reagent could be added and once tightly re-sealed and shaken, the samples could be autoclaved.

The different persulfate reagents.

Reagent # 7; 6.25g $K_2S_2O_8$ + 3g $B(OH)_3$ dissolved in 35ml NaOH (1M) and made up to 100ml with deionised H_2O. This is the general reagent I use for both TP and TN fresh water or saline samples that are close to neutral.

Reagent # 10; 6.25g $K_2S_2O_8$ + 3g $B(OH)_3$ dissolved in 47ml NaOH (1M) and made up to 100ml with deionised H_2O. This reagent works well for acidified (pH 1.5) fresh water samples. However, make sure the standard curve and blanks are acidified to the same pH as the samples!

Pure Persulfate Reagent; 5g $K_2S_2O_8$ dissolved with and made up to 100ml with diH2O. This reagent is used if just TP or just TN is to be digested.

Note: The other reagents, from #1 - #9, were variations on #7 but not as efficient at duel digestion. Reagent #0 is the original as used by Valderrama in his experiments.

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Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Marine Chemistry 10: 109-122.

Nobuhiro Suzuki and Rokuro Kuroda. 1987. Direct simultaneous determination of nitrate and nitrite by ultraviolet second-derivative spectrophotometry. Analyst 112:1077-1079.

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TOC Analyzer Protocol

I. Machine: OI Corporation Model 700 TOC Analyzer

II. Reference:
Model 700 TOC, Total Organic Carbon Analyzer Users' Manual. 1989. Page numbers in this protocol refer to this manual.

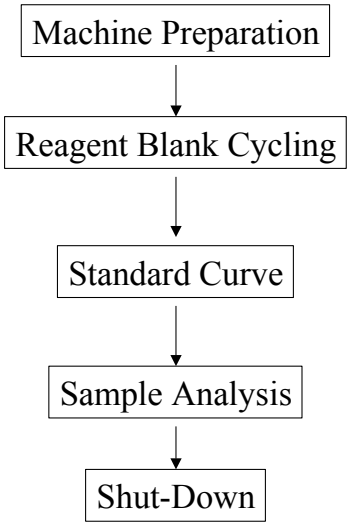
III. Overview:
The Model 700 TOC analyzer simultaneously measures inorganic (IC) and organic (OC) carbon in water samples. Phosphoric acid is first used to purge IC from the sample as CO_2. In a second step sodium persulfate is used to oxidize OC in the water to CO_2. After each step the CO_2 is collected and directed through a non-dispersive infrared analyzer to measure CO_2 produced. The mV response from the detector is used in conjunction with a scaling factor (µgC/mV) determined from a standard curve to calculate the amount of IC and OC in the sample.

V. Reagents and Materials:
Sodium Persulfate (100g/L)
Phosphoric Acid (5% vol/vol)
Potassium Biphthalate Stock (1000 ppm OC)
Sodium Carbonate Stock (1000 ppm IC)
Type I H_2O
glass test tubes (acid washed and pre-ashed

Reagent Preparation

1. **Sodium Persulfate (100 g/L):** Use the appropriately marked 2 L glass bottle. Fill this bottle halfway with DI water. Dissolve 200 g of $Na_2S_2O_8$ into the water. Fill the bottle to the top of the tape for 2 L of reagent.
2. **Phosphoric Acid (5% vol/vol):** Add 59 ml of 85% H_3PO_4 to DI water and bring the total volume to 1000 ml by adding more DI water. Use the appropriately marked glass bottle to store the solution.
3. **Sodium Carbonate Stock Solution (1000 mg-C/L):** Ash Na_2CO_3 for 2 hours at 110°C and then place in a dessicator for an hour (until cooled). Dissolve 8.826 g of Na_2CO_3 in DI water in a 1000 mL volumetric flask. Fill to the 1000 mL mark. Transfer the solution to the glass bottle marked 1000 mg-C/L Inorganic C, Organic C stock solution. When diluting this solution for calibration standards, don't take the solution right out of the stock bottle. A smaller working reagent bottle is used.
4. **Potassium Hydrogen Phthalate (KHP) Stock Solution (1000mg-C/L).** Weigh 2.12g of KHP and add to the inorganic solution previously prepared.

VI. Procedure:



***Step 1*: Machine Preparation**

Note: The machine requires ~8 minutes per sample AFTER initial setup and standard calculations!

1. Select and install appropriate sample loop (see chart on page 63). We use a 5.04 mL loop for 5-10 ppm OC.
2. Prepare sodium persulfate and phosophoric acid solution.
3. Fill the reagent bottles on the side of the machine
4. Turn on Nitrogen and Compressed Air cylinders (The compressed air usually stays on)
5. Adjust the secondary gauge pressure on each cylinder to 40 psi. Verify the gas flow.
6. Turn on the printer
7. Turn on TOC analyzer and autosampler
   The following message will appear on the screen:
   TIC/TOC Analysis
   00:00:00 Standby <
   The screen will continue to indicate that the standby option is disengaged while the digestion vessel is heated to proper temperature. Once temperature is obtained the following message will appear on the screen:
   TIC/TOC Analysis
   00:00:00 *Ready*

8. Check reagent bottles to ensure the contents are being purged.

9. Check instrument flow meters (to left of power switch). Detector rotameter should read 5.0. Purge rotameter should read 6.0.
10. THIS STEP IS ALREADY DONE. Do this step only when the loop changes. Adjust the sample pump time such that the sample loop is rinsed and filled by the sample. Insert the auto-sample sipper into a sample tube full of water and manually turn the pump on. Use a timer to determine the time necessary to fill the sample loop (allow as much sample as possible to flow through the loop to rinse it). Press the SET TIMES button then the ENTER button to cycle to the sample pump time menu. Enter the pump time (10 ml loop - ~13 sec; 5 ml loop ~7 sec). The pump time is currently set for 15 sec.
11. THIS STEP IS ALREADY DONE. Set desired acid and oxidant volumes by pressing the SET OXIDANT VOLUME and SET ACID VOLUME keys and entering the appropriate values. The acid is currently set for 200 µL and the oxidant is set for 1000 µL.
12. THIS STEP IS ALREADY DONE. Set sample volume by pressing the SET SAMPLE VOLUME key and entering the appropriate value. The sample volume is currently set for 5.04 mL
13. Prime acid and oxidant pumps (press PRIME ACID and PRIME OXIDANT buttons on each keypad ~30 times or until the click goes away and there are no more bubbles).

***Step 2*: Reagent Blank Cycling**

Note: the machine must "warm-up" for at least several hours (or much longer depending on the frequency of use) by cycling reagent blanks. This procedure removes built up carbon from within the machine and is essential to successful operation.

1. Read the "Running Reagent Blanks" section of the manual (p. 66 in manual) and refer to the detailed instructions there
2. Clear previous reagent blank values
    - Press the SELECT DISPLAY MODE key to advance the red light on the display board to "Calibration Constants"
    - Press SELECT NEXT DISPLAY key repeatedly and read display. Set IC and OC blanks to zero by keying in zero and pressing the ENTER key
    - Press SELECT DISPLAY MODE key and reset mode to advance the red light back to Normal on the display board
3. Set system configuration to run reagent blanks. Press the SET SYSTEM CONFIG key, use the ENTER key to cycle through the menu, using the 1 or 0 keys to set the following:
    Acid pump: enabled
    Oxidant pump: enabled
    Sample pump: disabled
    Sample loop valve: disabled
    Auto-run: enabled
    Autoprint: enabled
    Autosampler: disabled

Ready/Standby Status Override: disabled
Sample ID number: 01
Sample Stop number: 00
Number of Reps per sample: 1
TIC alarm Hi: 0
TIC alarm Lo: 0
TOC alarm Hi: 0
TOC alarm Lo: 0
TIC only: disabled
TC only: disabled
Ampule analysis: disabled
Wafertoc analysis: disabled
POC only: disabled
POX option: disabled

4. Press the RUN/STOP button. Run reagent blanks until values are consistent: IC blanks should be ~1-2 mV OC blanks should be ~4-10 mV.
5. Enter reagent blanks into the machine. Average about 4 or more stable reagent blank mV values for both OC and IC and enter these values under "calibration constants" following step #2 above. This subtracts the mV reading for carbon in the reagents that are added to each sample. Fill out the form shown in appendix I for the IC and OC blank.

***Step 3*: Standard Curve**

1. Set the system configuration for running samples:
   Acid pump: enabled
   Oxidant pump: enabled
   Sample pump: enabled
   Sample loop valve: enabled
   Auto-run: enabled
   Autoprint: enabled
   Autosampler: enabled
   Ready/Standby Status Override: disabled
   Sample ID number: 01
   Sample Stop number: 00
   All others leave the same...
2. Load 8 DI Water samples onto the auto-sampler. These samples will be used to subtract the additional carbon added with the Type I H_2O used in making the standards.
3. Advance the auto-sampler so that the first tube lies directly beneath the sipper (a button on the back of the autosampler advances the rack).
4. Set the sample stop number to 8 so the sampler stops sampling after the last sample.
5. Press the red RUN/STOP key to start the analysis.

6. Prepare standards within your desired range from the Potassium Biphthalate Stock. In the lab, we generally prepare the following 4 standards as follows:

Standard Concentration	**Volume of Stock Solution to Add**	**Volume of Flask**
0.5 mg-C/L	0.5 mL	1000 mL
1 mg-C/L	1 mL	1000 mL
5 mg-C/L	5 mL	1000 mL
10 mg-C/L	10 mL	1000 mL

7. After all the DI water samples are run, load the standards into the autosampler (use 4 replicates of each). Set the following system configuration values:
   Sample ID number: 01
   Sample Stop number: 16
8. Average 4 or more DI water blank mV values for TOC and TIC. Add these values to the reagent blank values and enter these into the "calibration constants" for IC and OC and write these values under Total Blank on the standard curve processing data sheet (Appendix II).
9. Press the RUN/STOP button to run the standard.
10. Average the mV values for the standards and write this under "Mean mV" under "Standards" on the standard curve processing data sheet (Appendix II).
11. Determine the global scaling factor. Do this by dividing ugC/Mean mV for each standard. Below is an example:

PPM	Ug C	Mean mV	Ug/mV
0.5	2..52	25.1282	0.1003
1	5.04	50.0377	0.1004
5	25.2	254.377	0.09906
10	50.04	512.3975	.09836

Scaling factor (average ug/mV)= 0.09826

12. Enter the global scaling factor into the machine:
    - Follow the directions in #2 of Step 2 above to enter and exit the appropriate menu
    - Change the IC and OC values under "calibration constants" back to the reagent blank values.
    - Enter the global scaling factor that you calculated above into "scaling factor".
    - Return to the "Normal" mode.

Step 4: Sample Analysis

1. Using the template shown in Appendix I, load the samples and record the sample bottle number on the data sheet. Include a standard (usually 1 mg-C/L) every 20 samples. **Make sure you rinse each test tube once with the sample water**.
2. Load the samples into the auto-sampler.
3. Advance auto-sampler to the first sample.
4. Enter system configuration menu and reset the following:
   Sample ID number: 01
   Sample Stop Number: the last number of samples
   leave all other settings the same
5. Press RUN/STOP key to begin analysis.
6. It takes 8 minutes for each sample. Be sure to account for this time.
7. If the analyzer needs to be stopped suddenly, press the RUN/STOP key and CLEAR.

Step 5: Shut-Down

1. Turn off the machine
2. Turn off the autosampler
3. Turn off the nitrogen and compressed air tanks

VII. Data Management

1. Use the sample data sheet to record sample loading order etc. See Appendix I.
2. Use the standard curve processing data sheet to calculate the global scaling factor, etc. See Appendix II.
3. Place a copy of your standard curve processing sheet, sample data sheet, and all printout data from the reagent blank, DI water, standards, and samples in a manila file folder. Write the date, DOC analysis, and who the samples were run for on the folder and file in the appropriate file cabinet.

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Adapted from L. Hedin (Princeton University)

Determination of Inorganic Anions in Water by Ion Chromatography

I. Principle of the method.

The purpose of this method is the determination of inorganic anions such as Fluoride, (F^-) Chloride (Cl^-), Bromide (Br^-), Nitrate (NO_3^-), phosphate (PO_4^{3-}) and sulfate (SO_4^{2-}) at low level of concentration, µg/L or ppb. Nitrate and Phosphate ions are commonly reported in ecology as Nitrogen and Phosphorus species. After preconcentration, anions of interests are separated on an ion exchange column connected to a guard column. Then, the anions are converted to their acid form in the suppressor (SRS) and detected using a conductivity detector.

II. Safety

The hazards of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be minimized. Adequate gloves are required for the preparation of the mobile phase, the samples and the standards. A reference file of Material Safety Data Sheets is available and a Laboratory Chemical Hygiene Plan is in place.

III. Reagents and Standards

✓ **Deionized water**: 18MΩ-cm resistance or better.

✓ **Eluent**: 3.0 mM Sodium Carbonate, Na_2CO_3 / 1.0 mM Sodium bicarbonate, $NaHCO_3$

Prepare the eluent by pipeting 12.0 mL of 0.5 M Na_2CO_3 plus 4 mL of 0.5M $NaHCO_3$ into a 2L volumetric flask Use deionized water to dilute to a final volume of 2000 mL.

✓ **Sodium Carbonate Stock, Na_2CO_3, 0.5M**.

Dionex, P/N 037162 or thoroughly dissolve 26.49g of Na_2CO_3 in 400 mL of deionized water. Dilute to a final volume of 500 mL.

✓ **Sodium Bicarbonate Stock, $NaHCO_3$, 0.5 M.**

Dionex P/N 037163 or thoroughly dissolve 21.00 g in 400 mL of deionized water. Dilute to a final volume of 500 mL.

✓ **Calibration Standards: Seven Anion Standard, Dionex P/N 56933 (see Appendix A)**

Components	Concentration	
	Labeled (mg/L)	Measured (mg/L)
Fluoride	20	20.1
Chloride	30	29.8
Nitrite	100	100.0
Bromide	100	101.0
Nitrate	100	102.0
Phosphate	150	151.0
Sulfate	150	150.0

✓ **Check Standards**: A 10 ppb Bromide and Nitrate (2.2 ppb NO_3-N) and 15 ppb Phosphate (4.9 ppb PO_4^{3-}-P) and Sulfate (5ppb SO_4^{2-}S) solution is used as a check standard. This solution is made daily. Place 1mL of the Dionex seven-anion standard into 100mL volumetric flask. Complete to 100 mL with DI water. Take 1mL of the previous solution; place it into a 100 mL volumetric flask, complete to 100 mL with DI water. This solution constitutes your check standard.

IV. Equipment

Dionex DX500 ion chromatography system is used for this work; DX-500 is a modular system, which consists of:
GP50 Gradient pump
CD 20 Conductivity detector
ED40 electrochemical detector
AS40 automated sampler (5mL vials)
PeakNet Chromatography Workstation, software version 5.2
Ion Pac AS14 analytical column (4× 250 mm) (P/N 46124), Ion Pac AG14 guard column (4 × 50 mm) (P/N 46134), Ion Pac AG14 guard column as concentrator (plumbed in reverse in place of sample loop)

Anion Self-regenerating suppressor, (SRS)(P/N 53946)

V. Procedure

V.1. Sample Preparation

On the automated sampler, make sure that the following selection is made:

- **Vial Type**: 5mL
- **Injtype**: Conc
- **Bleed**:on
- **Inj mode**: prop
- **Inj/vial**: 1
- **Sampler**: search
- **Injection**: first
- **Operation**: lcl, and hold.

Samples are placed into 5mL disposable plastic Poly Vials (P/N 038141) and a **cap (P/N) must be installed in each vial for the auto sampler to operate correctly.**

Do not forget to place first a vial with DI water to run a baseline. In addition every ten samples, insert a vial with a duplicate or spiked sample followed by a vial with a standard check. Standard checks are prepared according **to section III (Reagents and Standards). For spiked samples see quality control section.**

1. Thaw out samples in a warm water bath. Allow them to warm-up to room temperature.

2. Use forceps to handle the vials and avoid touching any surface that will be wetted with sample.

.

3. Shake well the sample. Rinse the vial with sample. Swish and dump the solution. Do the same if you are filling the vials with DI water or a check standard.

4. Fill the vials with sample to the fill line marked on the vial body. **Do not under fill the vials. This may result in insufficient sample being loaded.** After filling, inspect the vials to make sure no air bubbles are trapped.

5. Place a cap on each vial. Use forceps when handling the caps to prevent contamination and avoid touching any surface that will be wetted by sample.

6. Rinse the cap with the sample. Rinse the cap with DI water or a check standard if the vial contains DI water or a check standard.

7. An insertion tool helps prevent contamination of the cap socket and ensures that the cap is inserted to the proper depth. One end of the tool inserts the cap to the proper depth for a sample. The top of the cap is flush with the lip of the vial. Place the vial into the cassette. The first vial **must be placed near the black dot**.

8. When the cassettes are full, load the auto sampler tray. Slide the spring-loaded cassette pusher back and hold it. Placed the filled cassettes into the tray, **with the black dots to the right.**

9. After all the cassettes are in place, allow the pusher to slide forward into place against the last cassette

10. On the auto sampler, press the **Hold/Run** switch to set the sampler to **Run.** The first cassette will automatically feed into the sampling mechanism, positioning the first vial under the sampling head.

When the auto sampler is correctly loaded, the following selection is changed to:
- **Sampler**: ready
- **Operation**: lcl and run

11. Record the bottle number and the position of the bottle in the cassette on the Dionex data Sheet. Place the Dionex data Sheet in the IC data binder with your raw data.

V.2. Getting reading

1. Eluent

Make sure there is enough eluent for the day's run. Prepare eluent according to **section**. A full load of samples (66) needs between 1.5 and 2.0 liters of eluent to be completed. For other numbers of samples:

a. Determine how many samples are to be run. Multiply by 20 minutes per sample. Add an extra 60 minutes for baseline establishment. Multiply by eluent flow rates -- 1.0 ml/min.

Ex: 15 samples will take 15×20min+60 min= 360 min or 6 hours
The flow rate is $1mLmin^{-1}$, so you need 360 min $\times 1mLmin^{-1}$= 360 mL at least

2. Priming the Pump:

Before pumping the eluent through the column, prime the pump.

- **a.** Press **Menu** and **Enter** to enter the main screen. Move the cursor to the **Remote Field** and press **Select** button to toggle to **Local** mode
- **b.** Move cursor to the desired eluent. Enter 100 to select 100% of that eluent and press **Enter.** This automatically sets other eluents to 0%.
- **c.** Turn the priming block valve counterclockwise about two turns. A 10 mL syringe is connected to the luer port in the priming block
- **d.** Turn on the pump
- **e.** Draw the the syringe back to pull eluent through the flow path. When the manifold has been primed, turn the priming block valve counterclock wise until close. Do not overtight.

3. Waste Container.

Make sure that the waste container is not full. Disposal of the waste can be done in the sink after neutralization.

4. Gas

Turn on the nitrogen gas tank. The pressure should be 80 psi at the tank regulator, 3 psi on regulator immediately in front of eluent reservoirs on the Dionex ‘s machine.

5.Software

On the computer desktop, start the PeakNet software. On the PeakNet main menu, select **Schedule.** You will open the “schedule Editor”

V.3. Creating a Schedule

The sample schedule lists the contents of each sample vial in the same order you load them into the auto sampler. It is **essential** that this table is completely accurate.

The columns to be filled out in the sample schedule are; **sample, sample type, level, method, data file, volume dilution, weight, int. std. and comment.**

1. **Sample:** The sample names are most often bottle numbers, but can be anything unique and meaningful to you.

2. **Sample Type:** The sample type is almost always "Sample". When running a check standard , the sample type becomes **Check Std**. This option is chosen from the drop down list.

3. **Level:** The Level column is only used for Calibration Standards or Check Standard . For routine samples, leave it blank. For **Check Std**, type **4.**

4. **Method**: will be **totanions.met** except for the last line of the file. Double click on the "method" line to open a window containing all the methods stored on the computer.

5. **Data File**: specifies the name you wish your chromatograms to be stored under. . To organize better the data, create a folder where your files will be stored. Click on the "data file" line. A pop-up window will appear. Create a new folder by clicking on the new folder icon. Give a name to the new folder such as the date of the day, ex: 03Oct02. Find the folder you have just created. Click on "**open**", and in the file name type: **Day Month (3letters) year_AAA_**

Ex: 03Oct02_AAA_
The program will automatically increment this name

6. **Volume**, **Dilution**, and **Weight** will almost always be 1. **Int. Std**. Does not apply. We have previously run an external calibration.

7. **Comment:** You can use the Comment column at your own discretion.

- ✓ **Last Line IMPORTANT!** The sample schedule must end with a line specifying the pump to stop. On the method line, double click to open the methods' window, and select the method **"end"** This line stops the pump and keeps the eluent from running dry after the end of your run. Allowing the eluent to run dry will damage the columns.

- ✓ Save Schedule using the same code: Day Month (3letters) Year Letter.

Ex03Oct02A. The letter "A" identifies the first schedule of the day. The PeakNet software automatically gives the schedule file a unique extension.

V.4. Collecting Data.

1. Click on the "**Run**" icon in the PeakNet main menu. On the PeakNet run menu; click on "**file**" and on "**Load Schedule**". Choose the schedule you want to run from the list that appears.
2. Select **Open.** The load Schedule dialog box will appear.
3. Change parameters and options from the tab pages
    - Data Tab Page
        - Number of loops for the schedule: enter **–1.**
        - Starting Line for the schedule: make sure it's **1**
        - Ending line for the schedule: make sure it's **end**
        - Operator: Enter your name
    - Batch Tab page. Do not select anything
    - Modes Tab page
        - Automatically: Select this option. Starts the next run immediately after the preceding run.
        - When you are finished, select **Ok**

4. On the main screen, the run program will load the first line of the Schedule.
5. The pump is now on. Wait 60 minutes for full equilibration of the column
6. Click on **start.** A confirmation message box appears.
7. Select **Ok**

The auto sampler should load immediately the first sample and the status of the instrument should switch to "Running".

- ✓ Monitor your progress through the first two samples. The first sample should be DI water. This sample will give you an idea regarding the noise. Besides the void volume, no other peaks should be observed. The second sample should be a check standard. Make sure your peaks are coming out where they should. To do so compare with the standard chromatogram given in **Appendix B** and with the retention times given in the calibration table. Moreover, when the method is run, the run program reports whether the check standard response falls within the confidence intervals for the calibration plot.

- ✓ Fill in the IC logbook. Note type of samples, number of samples; pump pressure, file names, any problems.

- ✓ After all your samples have been processed, and the PeakNet program has saved your data files. Rinse the system with DI water for 30 min Click on the Direct Control icon on the tool bar. The Direct Control dialog box appears, with a tab for each module in the system.
  Select the tab for the pump to be controlled, select 100 % for pump A and set a flow of 1mL min^{-1}. Make sure there is enough DI water in the bottle A.

- ✓ After changing the tab page controls, you may do one of the following:

Select **OK** to immediately send the commands to the module and close the current tab page or select **Apply** to immediately send the commands to the module but leave the current tab page on display.

VI. Data Analysis

A. To look at the data from a completed run, from the PeakNet main menu click the **Optimize** button. Click on **file** and select **Open Data File**. The optimize file window will appear. Select the directory where your data are stored and select the first file you want to review by double clicking on it.

B. When your file is on the screen, click on **View,** select **Start/Stop marker,** green and red ticks appear at the beginning and end of each peak. These markers tell you where the computer starts and stops the integration of the peak. Make sure these markers are correctly located. Make sure that the computer correctly assigns the peaks. Click on **Print** to get a hard copy of your file.

VII. Saving and Reporting Data

A. **Records**: The original PeakNet files should stay in place for 3 months on the computer then are transferred to a CDRom. A hard copy report, including raw data, should be printed out and put in the IC Data binder and the data should be exported to Excel or QuatroPro and stored in the project specific folder.

B. Both these reports can be generated using the Batch section of PeakNet. On the PeakNet main menu, select the **Batch** button. On the main menu, select processing. You will open a drop down box. Select **Input**. **Input** should be

your sample schedule. To access your schedule, click on **Select**. Select the schedule you want to reprocess. Click on the **Output** tab. Select **Print Summary report**. Then, select the **Export** tab. Export should be a file with the extension .CSV. You can select the fields to export and the Report type. For the Report type select, **Summary**. In the fields, at minimum you need **Sample name**, **Component amount**, **peak height**, **peak area** and peak retention time. Three holes punch the printed report and put it in the IC Data binder. Make sure your data file is in the project specific folder.

C. You can also use Batch to reprocess your data files using a different method or a different standard curve. This is helpful if your concentrations were higher or lower than you expected. From the Processing Menu, click **Input**. Then, use **Build** to edit your sample schedule to use the different method file. Save the schedule and go on with batching. You can also create a new sample schedule by editing the old one and saving it under a new name.

D. Report only those values that fall between the lowest detectable and highest calibration standards. Samples higher than the highest standard can be diluted and reanalyzed. Samples lower than the lowest detectable standard can be reported as less than that amount. In calculations, you generally include the below detection level results as 0.5 x the detection limit. Check the calibration section to determine the detection limits of each component.

F. Report results in ppb or μg /L of the ions as identified in the preparation of calibration standard. Take particular care in units for reporting nitrate, nitrite, and phosphate as they are commonly reported both as the entire ion and as only N or P. Note that form of phosphate detected by the IC is dissolved ortho-phosphate.

VIII. Quality control

1. To assess precision, calculate relative range values on duplicate analyses:
ABS (X1-X2)/MEAN (X1, X2)
These values should be less than 0.20.
2. To assess accuracy or bias, calculate percent recovery values on spiked samples:
(100*(V1*X1)-(V2*X2))/(Vs*Xs)
Where V1 is the volume of the spiked sample, X1 is its measured concentration, V2 is the volume of the unspiked sample, X2 is its measured concentration, Vs is the

volume of the spike, and Xs is its known concentration. These values should be between 80-120%.
In general, the final concentration of the spiked sample should be from 2 to 5 times the concentration of the unspiked sample. If sample concentration is below the detection limit, the spike should be at least five times the detection limit. The spiking solution should constitute less than 2% of the volume of the spiked sample.

IX. Emergency Shut Down.

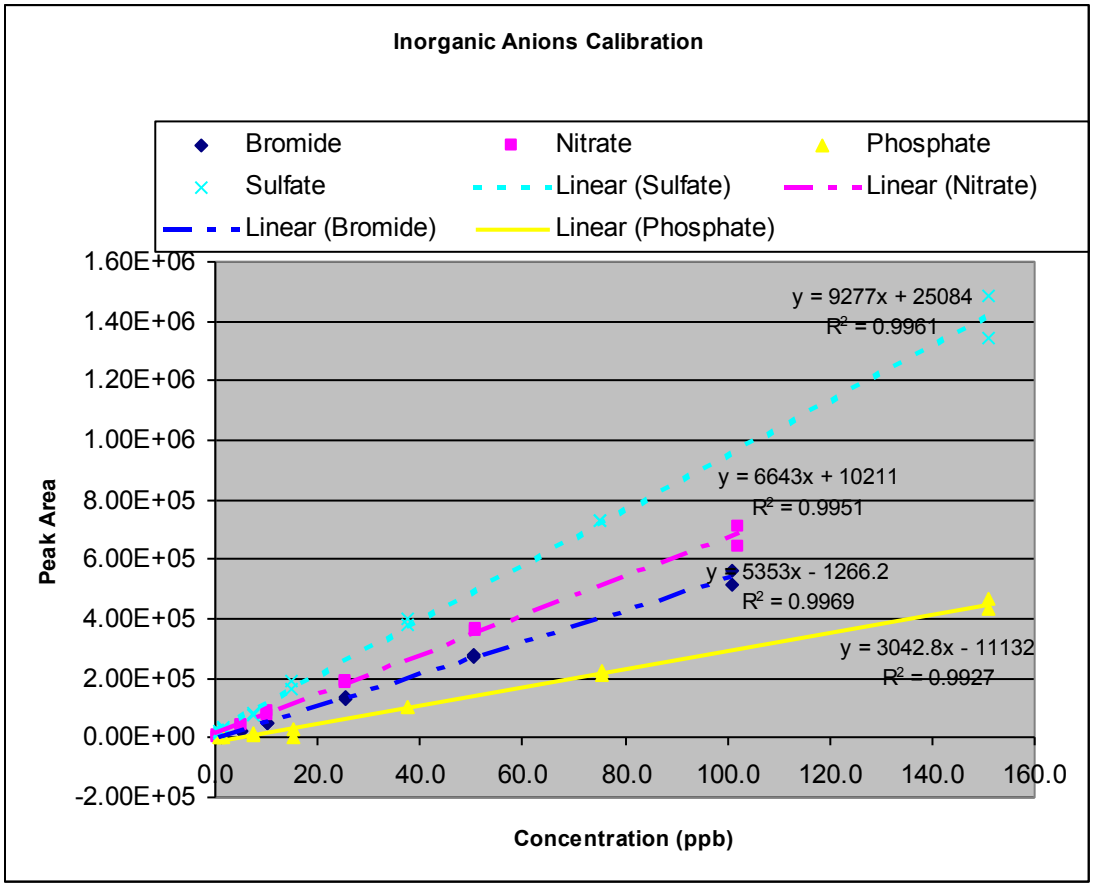
To shut-down the equipment, turn off the two main power switches located in the lower left corner of the front panel on the detector module and on the pump module, respectively. For the auto sampler, the main power switch is located on the right hand side of the back panel.

X. Calibration procedure

Because the IC response is extremely reproducible over time, it is not necessary to run a complete calibration curve on each day. Instead, the calibration is updated quarterly and check standards are included in each run, every 10 samples. If the check standards differ by more than 10% from the known concentration, then a complete standard curve should be run.

For the calibration, seven level calibration standards are made. Working standards in the appropriate concentration range should be prepared on the day they are run by diluting the stock standards. Make the highest standard by diluting the appropriate amount of stock, and then serially dilute the other standards.

Components	**Concentration (µg/L)**						
	Std.1	Std 2	Std. 3	Std. 4	Std. 5	Std. 6	Std. 7
Bromide	101	50.5	25.2	10	5	1.0	.50
Nitrate	102	51	25.5	10.1	5	1.0	.51
Phosphate	151	75.5	37.7	15.1	7.5	1.51	.75
Sulfate	150	75	37.5	15	7.5	1.5	.75



Limits of detection:

The limit of detection is defined considering a Signal to Noise equal to 3σ. The Noise is equal to 1.8×10^{-2} µS. The limit of detection for Bromide, Nitrate, Phosphate and Sulfate as ions is equal to 1.2ppb, 1.5ppb, 6 ppb and 1 ppb, respectively. The same detection limit for Br, Nitrate, Phosphate and Sulfate expressed in species is equal to 1.2ppb, 0.3ppb-N, 2ppb-P and 0.3ppb-S respectively.

XI. References

1. American Society for Testing and Materials. 2000. Test Method for Anions in Water by Ion Chromatography, D4327-91, Annual Book of ASTM Standards, Vol. 11.01, Philadelphia, PA.

2. American Public Health Association. American Water Works Association, Water Environment Federation. 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. CL. S. Clesceri, A. E. Greenberg, A. D. Eaton, eds., Method 4110. Determination of anions by ion chromatography. p. 4-2 to 4-8. American Public Health Association, Washington, DC.

3. Dionex. Determination of Inorganic Anions in Drinking Water by Ion Chromatography. Application Note 133. Dionex Corporation, Sunnyvale, CA.

4. Dionex. Determination of Nitrite and Nitrate in drinking water using ion chromatography with direct UV detection AU132. Application Note 132. Dionex Corporation, Sunnyvale, CA.

5. Dionex. Determination of Nitrite and Nitrate in drinking water using chemically suppressed ion chromatography AU131. Application Note 131. Dionex Corporation, Sunnyvale, CA.

6. USEPA. 1993. Method 300.0,Test Method for the determination of inorganic anions in water by ion chromatography. Environmental Monitoring Systems Laboratory, ORD, USEPA, Cincinnati, Ohio 45268

Appendix A

Stock Standards, 1000 mg/L, can also be prepared from ACS reagent grade salts dried at 105 C for 30 min and stored desicated. These Stock Standards are stable for at least 1 month when refrigerated. Prepare stock standards (1.0 ml = 1.0 mg) as follows:

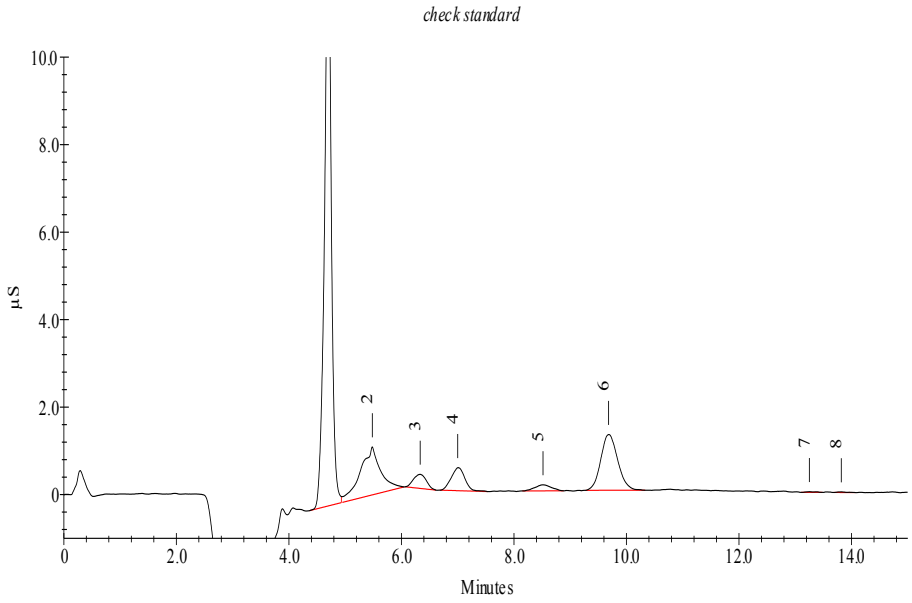
a. Fluoride. 2.2100 g NaF to 1 L
b. Chloride. 1.6485 g NaCl to 1 L
c. Nitrite as NO_2-N. 4.9257 g $NaNO_2$ to 1 L.
d. Bromide. 1.2876 g NaBr to 1 L
e. Nitrate as NO_3-N. 6.0679 g $NaNO_3$ to 1 L or 7.214 g KNO_3 to 1 L
f. Phosphate as ortho PO_4-P. 4.3937 g KH_2PO_4 to 1 L. NOTE: APHA et al. 1998 reports that phosphate curves below 1 mg/l are non-linear.
g. Sulfate. 1.8141 g K_2SO_4 to 1 L

Conversion table:
µg/L=ppb
mg/L=ppm
1ppm=1000ppb

Appendix B

Typical Chromatogram.

3- Bromide 10 ppb
4- Nitrate 10 ppb (2.2 ppb NO_3^--N)
5-Phosphate 15 ppb (4.9 ppb PO_4^{3-}-P)
6-Sulfate, 15 ppb (5ppb SO_4^{2-}-S)



Lab Methods for Estimation of Benthic Standing Stock as Chlorophyll *a*

I. Background

Benthic biomass and standing stocks are widely estimated as chlorophyll *a* (the pigment found in plants and algae). If the data are intended to be extrapolated to a whole reach, then a stream habitat and % cover survey is necessary and samples are collected using a stratified random sampling scheme is used to collect samples from different habitat units/cover type.

II. Laboratory Materials for Chlorophyll a Analysis

a. Biofilms on filters

1) film canisters or 15 ml falcon tubes (one per sample)
2) etching tool (diamond pencil)
3) forceps
4) data sheet with field information transferred to it
5) 95% ethanol
6) 25 ml pipette with bulb
7) 1 M HCl
8) micropipettor (0.1 ml and 5 ml) with tips
9) water bath heated to 78^{o}C
10) timer
11) aluminum foil
12) refrigerator
13) spectrophotometer or calibrated fluorometer (Aquafluor)
14) quartz cuvettes (1 cm) for spectrophotometer or glass cuvettes (5 ml) for fluorometer

III. Laboratory Methods for Chlorophyll a Analysis

a. Chlorophyll extraction

1) Use an etching tool such as a diamond pencil to label film canisters or 15 ml falcon tubes (one per sample) with an identifying number or letter that corresponds to each sample. (The solvent used to extract chlorophyll will remove permanent marker). Record this identifier on the data sheet in the appropriate place.
2) From this point work in the dark as extracted chlorophyll degrades when exposed to light.
3) Use forceps to transfer a sample on a filter to the correct canister or tube and add 25 ml of 95% ethanol. Cap to avoid evaporation of the ethanol. Record exact volume of ethanol used on the data sheet.
4) Loosen the caps and place samples to be extracted in the hot water bath (78^{o}C) for 5 minutes, remove, tighten caps and cover with aluminum foil. Place samples in refrigerator for 24 h before analysis.

b. Spectrophotometric analysis

1) Conduct all work in a dim/dark room to avoid chlorophyll degradation.
2) Turn on spectrophotometer and allow to warm up for 5-10 minutes.
3) Remove samples from refrigerator.
4) Pipette 5 ml of 95% ethanol in cuvette to be used as a blank.
5) Pipette 5 ml of sample into separate cuvette.
6) Blank the instrument at 665 nm
7) Record sample absorbance at 665 nm
8) Blank the instrument at 750 nm
9) Record sample absorbance at 750 nm
10) If either reading is greater than instrument's capacity, then the sample will need to be diluted with 95% ethanol, be sure to record any dilution on the data sheet.
11) Pipette 0.1 ml of 1 M HCl into sample, place cap on cuvette and invert to mix. Allow to sit for 90 seconds. Record absorbances at 665 nm and 750 nm.
12) Chlorophyll a concentration is calculated as:

[E665b-E665a]*28.66*sample volume*extraction volume/subsample volume (volumes in L, E is absorbance at 665nm-750nm). This gives units of mg. Divide this by sample area to get mg/cm^2.

c. Fluorometric analysis

1) Conduct all work in a dim/dark room to avoid chlorophyll degradation.
2) Turn on fluorometer and allow to warm up for 5-10 minutes. (make sure fluorometer has been calibrated to chlorophyll standards within the last year, noting tape on the instrument).
3) Use the solid standard to calibrate the instrument.
4) Pipette 5 ml of sample into a glass cuvette and record the reading on the data sheet. If the reading is greater than the instruments range, dilute it with 95% ethanol and repeat the reading. Make sure to record the dilution on the data sheet.
5) Add 0.1 ml of 1 M HCl to the sample, cover with parafilm, invert to mix, and let sit for 90 seconds. Put cuvette in fluorometer and record the reading on the data sheet.
6) Chlorophyll (mg/cm_2) is calculated as follows:

(Reading before acid-reading after acid mg/L)*extraction volume (ml)* sample volume (L)/subsample volume (ml) * area (cm^2)

IV. Helpful tips

1) Make sure sampling supplies are ready and organized the night before

2) Prepare the data sheets ahead of time so they can be filled in fast in the lab

V. References

1) Hauer F.R. and G.A. Lamberti. 2006. Methods in Stream Ecology. Academic Press, San Diego, CA.