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Great Bay Estuary Tidal Tributary Monitoring Program: Quality Assurance Project Plan, 2018

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Great Bay Estuary Tidal Tributary Monitoring Program (GBETTMP) 2018

Quality Assurance Project Plan

March 6, 2018

Prepared by

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Jody Potter, Water Quality Analysis Laboratory, University of New Hampshire

Project Manager and QA Officer:	3-14-2018 Signature / Date Kalle Matso, PREP
Laboratory Program Manager:	3-14-2018 Signature / Date Bill McDowell, UNH-WQAL
Field Operations Manager:	3-14-2018 Signature / Date Michelle Daley Shattuck, UNH-WQAL
Laboratory Manager:	3-14-2018 Signature / Date Jody Potter, UNH-WQAL
USEPA Project Officer:	3/21/18 Signature / Date Jean Brochi, US EPA
USEPA QA Officer:	3/20/2018 Signature / Date Nora Conlan, US EPA

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A3 – Distribution List

Table 1 presents a list of people who will receive the approved QAPP, any QAPP revisions, and any amendments.

Table 1: QAPP Distribution List

QAPP Recipient Name	Project Role	Organization	Telephone Number and E-mail Address
Rachel Rouillard	PREP Director	PREP	603-862-3948 rachel.rouillard@unh.edu
Kalle Matso	Project Manager/ & Project QA Officer	PREP	603-781-6591 kalle.matso@unh.edu
Bill McDowell	Laboratory Program Manager	UNH	603-862-2249 Bill.McDowell@unh.edu
Jody Potter	Laboratory Manager	UNH	603-862-2341 Jody.Potter@unh.edu
Michelle Shattuck	Field Operations Manager	UNH	603-862-2341 Michelle.shattuck@unh.edu
Ted Diers	Data User	NH DES	603-271-3289; ted.diers@des.nh.gov
Jean Brochi	EPA Project Officer	USEPA	617-918-1536 Brochi.Jean@epa.gov
Nora Conlon	EPA QA Officer	USEPA	617-918-8335; conlon.nora@epa.gov

A4 – Project/Task Organization

The Piscataqua Region Estuaries Partnership (PREP) is part of the U.S. Environmental Protection Agency's National Estuary Program, which is a joint local/state/federal program established under the Clean Water Act with the goal of protecting and enhancing nationally significant estuarine resources. The PREP receives its funding from the EPA and is administered by the University of New Hampshire.

The project will be conducted and managed by PREP. The Project Manager (Kalle Matso) will be responsible for coordinating all program activities.

The Field Operations Manager (Michelle Shattuck) will manage all field staff, be responsible for "stop/go" decisions for daily sampling runs during extreme events and will notify the Laboratory Manager when samples will be delivered. The Field Operations Manager will be responsible for resolving any logistical problems and communicating the results to the field staff.

Samples will be analyzed by the Water Quality Analysis Laboratory (WQAL) at the University of New Hampshire (UNH). Laboratory operations will be managed by the Laboratory Manager (Jody Potter) and overseen by the Laboratory Program Manager (Bill McDowell). The Laboratory Manager will be responsible for conducting analyses according to the procedures in this QA Project Plan, identifying any non-conformities or analytical problems, and reporting any problems to the Laboratory Program Manager, Project QA Officer, and the Project Manager. The Laboratory Program Manager will be responsible for resolving any problems and communicating the results to the laboratory staff.

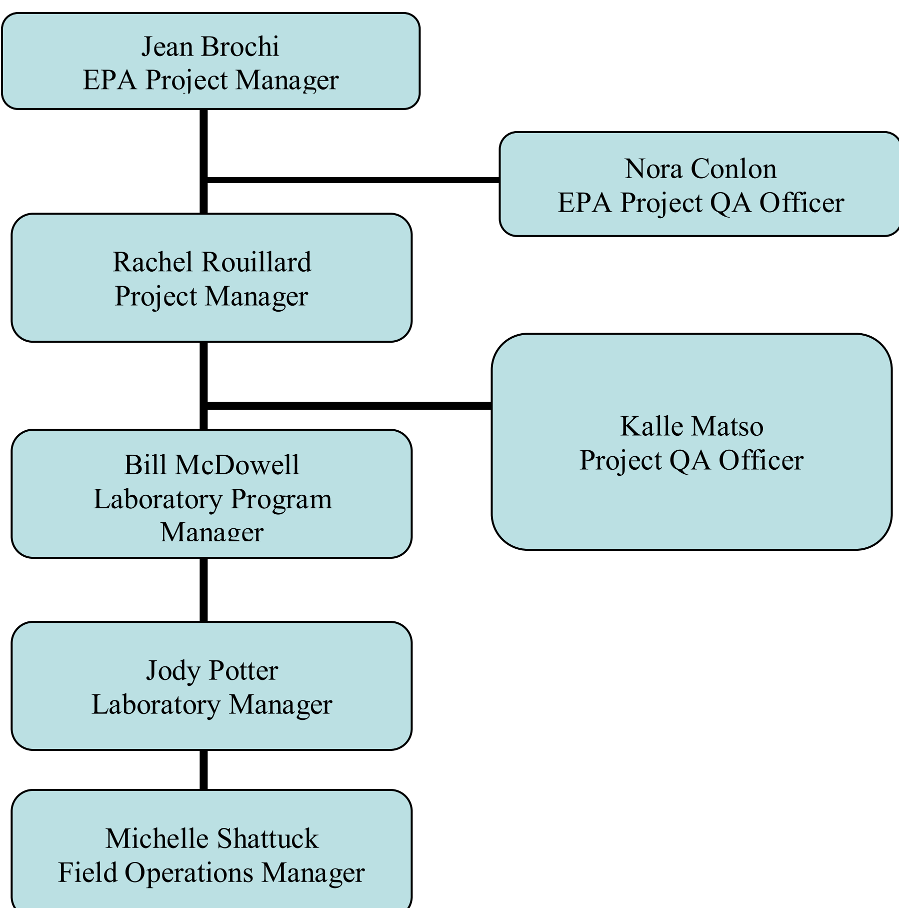
At the end of the project, the Project QA Officer (Kalle Matso) will review the results of QA/QC checks and verify that the procedures of this QA Project Plan were completed. The Project QA Officer will be responsible for a memorandum summarizing any deviations from the procedures in the QA Project Plan, the results of the QA/QC tests, and whether the reported data meets the data quality objectives of the project.

Funding for PREP is provided by the U.S. Environmental Protection Agency. Therefore, the Project Manager will be accountable to the EPA Project Manager (Jean Brochi) and the EPA Project QA Officer (Nora Conlon). The EPA Project Manager and EPA Project QA Officer will be responsible for approving the Quality Assurance Project Plan.

The principal user of the data from this project will be PREP for State of Our Estuaries Reports. The Project Manager will prepare a report at the end of the project with all the data and the QA summary report.

Figure 1 shows an organizational chart for this project.

Figure 1: Project Organization



A5 – Problem Definition/Background

Nitrogen enrichment continues to be a concern for the Great Bay Estuary. In the 2018 State of Our Estuaries report (PREP, 2017), PREP calculated the nitrogen load from tributaries to the Great Bay Estuary using data collected by UNH for the Great Bay Estuary Tidal Tributary Monitoring Program. PREP needs to update this indicator for future State of Our Estuaries reports. Therefore, the purpose of this study is to continue to collect representative data on the concentrations of total nitrogen and other parameters in ambient water in tributaries to the Great Bay Estuary in 2018. Nitrate+nitrite (NO_2/NO_3), ammonia (NH_4), total dissolved nitrogen (TDN), particulate nitrogen (PN), total phosphorus (TP), dissolved organic carbon (DOC), orthophosphate (PO_4) and total suspended solids (TSS) will be measured in the water samples and calculated measures include total nitrogen (TN; sum of TDN and PN) and dissolved organic nitrogen (DON; difference between TDN and sum of NO_2/NO_3 and NH_4).

The study design will follow the tributary sampling design which was implemented by the New Hampshire Department of Environmental Services (DES) between 2001 and 2007 and continued by PREP between 2008 and 2017. One significant change to that design was made in 2013 to TN analysis, which was changed from direct measurement of TN by the persulfate digestion method to the calculated method as suggested by USGS (Office of Water Quality Technical Memorandum 2013.01) and verified by split samples in this study for 2 years. The Sampling design is described in Section B of this QAPP. Grab samples will be collected from eight tributaries monthly from March to December of each year. One sample from each month will be replicated for QA purposes (>10% of samples). The samples will be analyzed by the Water Quality Analysis Laboratory at the University of New Hampshire.

The TN concentrations in each river will be matched with the daily average streamflow for that river. Stream flow data will be obtained from permanent USGS stream gages. The drainage area ratio method will be used to estimate stream flows for sampling locations that are not coincident with USGS stream gages. The USGS LOADEST statistical program will be used to estimate annual average TN loads from each tributary.

This QAPP will apply to the year 2018 and will be revised for 2019.

A6 – Project/Task Description

The tasks and schedule for the project in 2018 are summarized in Table 2.

Table 2: Project Schedule Timeline

Activity	Dates		Product	Due Date
	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion		
QAPP Preparation	11/01/17	01/24/18	QAPP Document	02/01/18
Training	03/11/18	03/15/18	Field crews trained on SOPs	03/28/18
Sample collection	03/28/18	12/18/18	Nutrient samples collected, delivered to laboratory, and stored	12/18/18
Sample analysis	03/28/18	12/18/18	Laboratory analyses for nutrient samples completed	02/28/19

Activity	Dates		Product	Due Date
	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion		
Laboratory Report	01/01/19	01/31/19	Report from the Laboratory Manager with the final, quality-assured results for tributary samples and QC samples	02/28/19
Data Quality Audit	03/01/19	03/15/19	Memo from QA Project Officer summarizing results of QC samples and QAPP nonconformances	03/15/19
Annual Report	02/16/19	03/31/19	Final project report	03/31/19

A7 – Quality Objectives and Criteria

Table 3 summarizes the performance criteria for the NO_2/NO_3, NH_4, TDN, PN, TP, DOC, PO_4 and TSS samples that will be collected for this project. More details on each data quality objective are provided in the paragraphs below the table.

Table 3: Measurement Performance Criteria for Laboratory Samples

Data Quality Indicators	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Measurement Performance
Precision-Overall	RPD < 30%	Field Duplicates
Precision-Lab	RPD < 15%	Lab Duplicates
Accuracy/Bias	>85% and <115% recovery	Certified Reference Material Samples Laboratory Fortified Matrix Samples
Comparability	Measurements should follow standard methods that are repeatable	NA
Sensitivity	Not expected to be an issue for this project (see discussion below)	NA
Data Completeness	Valid data for 90% of planned samples (9 samples at each tributary)	Data Completeness Check

Precision: Relative percent difference (RPD) of duplicate samples is used as one index of precision for nutrient analyses. This is defined as the absolute difference between the duplicates divided by the average of the duplicates. For laboratory duplicates, a difference greater than 10% requires further investigation of the sample run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in reanalysis of the entire sample queue, unless there is a reasonable and supported explanation for the inconsistency. For field duplicates, a difference greater than

30% will be flagged as a potential error. Duplicate precision will be analyzed by calculating the RPD using the equation:

$$RPD = \frac{|x_1 - x_2|}{\left(\frac{x_1 + x_2}{2}\right)} \times 100\%$$

where x_1 is the original sample concentration
x_2 is the duplicate sample concentration

Accuracy/Bias. For nutrient analyses, certified reference materials are analyzed periodically (approximately every 20 samples) in each sample queue to assure accuracy. Generally, a recovery <90% or >110% requires further investigation of the sample run. A recovery greater than or less than <85% or >115% is failure (unless the sample is less than 10X the MDL), and results in reanalysis of the entire sample queue, unless there is a reasonable and supported explanation for the inconsistency.

Percent recovery (R) for certified reference materials will be calculated using the following equation:

$$R = \frac{|x_1 - x_2|}{(x_2)} \times 100\%$$

where x_1 is the measured concentration
x_2 is the known concentration for the certified reference material

Laboratory Fortified Matrix samples are also used to assess accuracy of nutrient analyses. The difference of the spiked sample concentration (SA) minus the unspiked sample concentration (SU) divided by the known concentration added (A) (expressed as percent) gives percent recovery (R):

$$R = \frac{(SA - SU)}{A} \times 100\%$$

Representativeness: The samples will be taken at the same locations and using the same methods as used for the GBETTMP sampling in 2013-2017 if possible. (Samples collected prior to July 2016 have been moved to station 09-EXT-DAMMED in NHDES' Environmental Monitoring Database (EMD), to differentiate between samples that represent the impounded conditions verses those of the free flowing river following the dam removal.) Any necessary changes to sampling locations will be made with the Field Operations Manager's approval, with the goal of reproducing the original location as effectively as possible. Any such changes will be fully documented in project reports.

Comparability: Standardized field and analytical methods will be used. These methods will follow the current industry standard for the types of measurements being taken. Written SOPs will be followed for field and analytical measurements. Standardized field data sheets will be used.

Sensitivity: The laboratory methods used should be capable of detecting NO_2/NO_3, NH_4, TDN, PON, TN, TP, and TSS concentrations in ambient river water. Specifically, results must be greater than or equal to the method detection levels listed in Table 9.

Completeness: This study will be deemed successful if data meeting the data quality objectives is obtained for 90% of planned samples (not including field/laboratory duplicates). Therefore, at least nine valid results for each parameter should be obtained from each tributary.

A8 – Special Training/Certification

The Field Operations Manager will organize and implement a training session for field staff. The training session will cover SOPs for field instruments and field data sheets. The training will be based on the QAPP document. Field staff will sign an attendance sheet for the training, which will be retained by the Field Operations Manager. The training will be completed before sampling begins.

Table 4: Special Personnel Training Requirements

Project Function	Description of Training	Training Provided by	Training Provided to	Location of Training Records
Water quality sampling and field measurements	Sampling methods in Section B2 and field data sheets. This training will be conducted once at the beginning of the field season.	Field Operations Manager	All field team staff	With Field Operations Manager

A9 – Documents and Records

QA Project Plan

The Project Manager will be responsible for maintaining the approved QA Project Plan and for distributing the latest version to all parties on the distribution list in section A3. A copy of the approved plan will be posted to the PREP website (scholars.unh.edu/prep).

Field Data Sheets

The field data sheets for this project are attached as Appendix B. Field crews fill in these forms during the day and return them to the Field Operations Manager upon completion. The original forms, or scanned copies of the original forms will be retained on file by the Field Operations Manager.

Laboratory Data Sheets

Data packages from the Laboratory Manager to the Project QA Officer will be electronic laboratory data sheets containing the results of analyses plus the results of QC tests performed. See Appendix A (Section VI) for details of laboratory electronic and paper records maintained by the laboratory.

Reports to Management

The Project QA Officer will produce an annual report for PREP. The final work product will be an Excel spreadsheet containing quality assured results of the laboratory analyses for each station on each date and an annual report describing any deviations from the protocols established in the QA Project Plan. The annual report will be posted to the PREP website (scholars.unh.edu/prep).

Archiving

The QA Project Plan and final report will be kept on file at PREP for a minimum of 10 years after the publication date of the final report. The original field data sheets, or scanned copies of the original

field data sheets will be retained by the Field Operations Manager and laboratory data sheets will be retained by the Laboratory Manager for a minimum of 5 years.

B1 – Sampling Process Design

Eight tributaries to the Great Bay Estuary watershed will be sampled ten times for nitrate+nitrite (NO_2/NO_3), ammonia (NH_4), total dissolved nitrogen (TDN), particulate nitrogen (PN), total phosphorus (TP), dissolved organic carbon (DOC), orthophosphate (PO_4), and total suspended solids (TSS). One water sample will be collected as a grab from the head-of-tide station for each of the tributaries on each day of sampling. A total of ten field duplicate samples will be collected during the year for each parameter (one station per sampling date). Table 5 shows the number of samples that will be collected for each parameter. The critical parameters for this study are NO_2/NO_3, NH_4, TDN, PN, TN, TP, and TSS. Water temperature, dissolved oxygen, pH and specific conductance will be measured for information only.

The stations that will be sampled as part of this study are provided in Table 6. A map of the stations is provided in Figure 2.

The sampling dates for 2018 and station for the field duplicate sample are shown in Table 7.

Table 5: Sample Summary

Parameter	No. of Stations	Samples per Event per Site	Number of Sampling Events	Field Duplicate Samples	Total Number to Lab
NO_2/NO_3	8	1	10	10	90
NH_4	8	1	10	10	90
TDN	8	1	10	10	90
PN	8	1	10	10	90
TP	8	1	10	10	90
DOC	8	1	10	10	90
PO_4	8	1	10	10	90
TSS	8	1	10	10	90
Water Temperature	8	1	10	10*	0 (field measure)
Dissolved Oxygen Concentration	8	1	10	10*	0 (field measure)
Dissolved Oxygen Saturation	8	1	10	10*	0 (field measure)
pH	8	1	10	10*	0 (field measure)
Specific Conductance	8	1	10	10*	0 (field measure)
* See page 12 for description of how duplicate samples are achieved for parameters obtained with probes.					

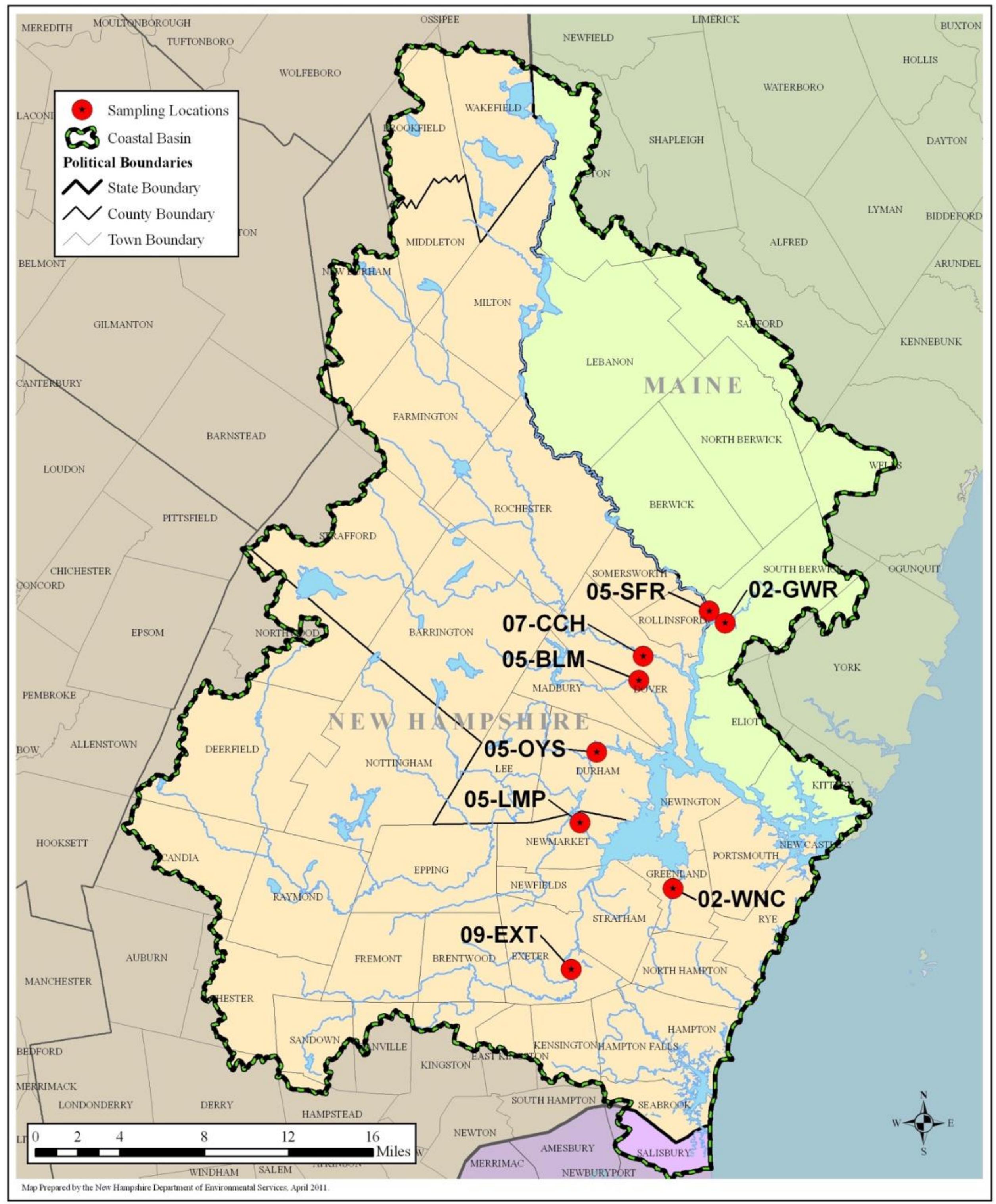
Table 6: Tributary Sample Locations

Station ID / Tributary	Town, State	Latitude	Longitude	Sample Location
02-GWR Great Works	South Berwick, ME	43.2189	-70.7967	Route 236 in south Berwick ME, turn right onto Brattle Street, sample on downstream side of Brattle street bridge.
05-SFR Salmon Falls	Rollinsford, NH	43.2272	-70.8115	Rte 4 bridge in Rollinsford NH, sample on upstream side of bridge
07-CCH Cocheco	Dover, NH	43.1965	-70.8741	Rte 9 bridge in Dover. Central Ave between Washington St and Portland Ave. sample on upstream side, midway on bridge.
05-OYS Oyster	Durham, NH	43.1309	-70.9186	Rte 108 bridge in Durham NH. Sample from top of fish ladder on river left side, upstream of dam.
05-LMP Lamprey	Newmarket, NH	43.0821	-70.9350	Rte 108 bridge in Newmarket NH. Sample on upstream side at midpoint of the bridge.
09-EXT Exeter	Exeter, NH	42.9820	-70.9455	High Street bridge in Exeter NH. Sample on downstream side at midpoint of bridge.
05-BLM Bellamy	Dover, NH	43.1799	-70.8782	Rte 108 bridge in Dover. Sample on downstream side at midpoint of bridge.
02-WNC Winnicut	Greenland, NH	43.0361	-70.8480	Route 33 bridge in Greenland NH. Sample on downstream side at midpoint of bridge.

Table 7: Sampling Schedule for 2018

Month	Day of Week	Date	Station for Duplicate Sample
March	4th Thursday	3/28/18	02-GWR
April	4th Wednesday	4/24/18	02-WNC
May	4th Wednesday	5/22/18	09-EXT
June	3rd Wednesday	6/19/18	05-LMP
July	3rd Wednesday	7/17/18	05-OYS
August	3rd Wednesday	8/21/18	07-CCH
September	3rd Wednesday	9/18/18	05-BLM
October	3rd Wednesday	10/16/18	05-SFR
November	4th Wednesday	11/27/18	05-LMP
December	3rd Wednesday	12/18/18	05-OYS

Figure 2: Sampling locations in the Great Bay Estuary Watershed, Coastal Basin



B2 – Sampling Methods

Sample Bottle Preparation

Two-liter Nalgene bottles are prepared before sampling by soaking bottles and caps in a 10% HCl solution for 10 minutes. Bottles and caps are subsequently rinsed with deionized water six times and air dried before being stored. During higher spring/fall flow, one bottle is prepared for each site. During low summer flow, two bottles are prepared for each site because the extra volume is required to get enough particulates for analysis. Before field sampling day, bottles are labeled with StationID, date, and program ("GBETTMP") and placed in a cooler for transfer and storage.

Water Sampling Field Procedures

All field measurements and samples collected for laboratory analyses are collected using a two-gallon bucket on a rope using the following procedure:

1. The bucket will be lowered from the middle of the bridge at the station down to the river. The bucket will be immersed three times in the river before it is filled and hauled up. The bucket will be filled to at least one-half of its capacity, which ensures sufficient volume for all field measurements and sample storage containers. This is considered a surface grab sample since the bucket sampling technique collects water from the top 1 foot of the water column.
2. The sample for laboratory analysis will be immediately filled by pouring water from the bucket into the individual sample storage container(s) (i.e., polyethylene bottles, prelabeled with the stationID, date, time, and program). The bucket should be shaken to fully mix the water before the water is poured off into the sample bottle.
3. If a field duplicate sample is needed at the station, the bucket will be emptied and then refilled from the river following Step 1 and all subsequent steps. Probe measurements are taken a second time when the bucket is re-filled in order to duplicate measurements for probe parameters.
4. The sample bottle(s) will be placed in a cooler with ice for transport to the laboratory.
5. The bucket will be emptied and then refilled from the river following Step 1 for field parameter measurements.
6. Field parameters will be measured in a new bucket of water using a YSI multiparameter meter by inserting the temperature/specific conductance probe in the bucket and moving the probe slowly for 15-30 seconds until the temperature and specific conductance values stabilize. Field parameters may also be measured directly form the river if accessible.
7. The results of the field parameters and any comments relevant to the sampling event (e.g., sampling and/or instrumentation problems) will be documented on field data sheets (Appendix B) prior to traveling to the next sampling location.

This procedure is repeated at all scheduled sampling locations for a particular day. Field teams are responsible for reporting sampling method problems to the Field Operations Manager who is responsible for taking corrective action.

Table 8: Sample Requirements

Analytical Parameter	Collection Method	Sampling SOP	Sample Volume*	Container Size and Type	Preservation Requirements	Max. Holding Time (Preparation and Analysis)
NO_2/NO_3	Grab	Section B2	10 mL	2000 ml HDPE bottle (same bottle for all analyses)	Filter a 60 mL subsample into a HDPE bottle and freeze within 8 hours of sample collection	Indefinite once frozen
NH_4	Grab	Section B2	10 mL	2000 ml HDPE bottle (same bottle for all analyses)	Filter a 60 mL subsample into a HDPE bottle and freeze within 8 hours of sample collection	Indefinite once frozen
TDN	Grab	Section B2	20 mL	2000 ml HDPE bottle (same bottle for all analyses)	Filter a 60 mL subsample into a HDPE bottle and freeze within 8 hours of sample collection	Indefinite once frozen
PN	Grab	Section B2	~1,600 mL	2000 ml HDPE bottle (same bottle for all analyses)	Dry filter (See Appendix D)	Indefinite once dried
DOC	Grab	Section B2	20 mL	2000 ml HDPE bottle (same bottle for all analyses)	Filter a 60 mL subsample into a HDPE bottle and freeze within 8 hours of sample collection	Indefinite once frozen
PO_4	Grab	Section B2	10 mL	2000 ml HDPE bottle (same bottle for all analyses)	Filter a 60 mL subsample into a HDPE bottle and freeze within 8 hours of sample collection	Indefinite once frozen
TP	Grab	Section B2	60 mL	2000 ml HDPE bottle (same bottle for all analyses)	Subsample 60 mL of unfiltered water and freeze within 8 hours of sample collection	Indefinite once frozen
TSS	Grab	Section B2	~1,600 mL	2000 ml HDPE bottle (same bottle for all analyses)	Dry filter (See Appendix D)	7 days
Field Parameters (measurements made in the field)						
Temperature	Surface Grab	YSI multiparameter meter manual	NA	NA	NA	NA
Specific Conductance	Surface Grab	YSI multiparameter meter manual	NA	NA	NA	NA
pH	Surface Grab	YSI multiparameter meter manual	NA	NA	NA	NA
Dissolved Oxygen	Surface Grab	YSI multiparameter meter manual	NA	NA	NA	NA

*One 60 ml filtered sample bottle satisfies the above requirements with the exception of TP, which accounts for the other 60 ml bottle; however, this bottle is unfiltered.

B3 – Sample Handling and Custody

Upon collection, nutrient samples will be transported on ice in a cooler until they arrive at WQAL. Samples will be delivered to WQAL by 15:00 on the sampling date. Sample login and handling procedures at WQAL are described in Section IV of Appendix A. Immediately after login, a portion of the sample will be filtered following the procedure below.

Filtration: Particulate material is separated from dissolved constituents via filtration in the laboratory immediately upon delivery to the laboratory (normally within 5 hours of collection). For total dissolved organic carbon and total dissolved nitrogen, a portion of the original sample (approx. 60 mL) is filtered through 47mm Whatman GF/F glass fiber filters (nominal pore size of 0.70µm) in the field, collected in a pre-washed HDPE bottle, and then immediately frozen. For total suspended sediments and particulate nitrogen, a portion of the original sample (generally 500-1900 mL) is processed using the filtration procedures in Appendix D with two pre-weighed glass fiber filters (25 mm Whatman GF/F). One of these filters is analyzed for both TSS and PN. The other filter is stored as backup for the PN analysis.

GF/F filters (nominal pore size of 0.70µm) are commonly used in nutrient studies for filtering particulates from water samples, for example, National Coastal Assessment uses 0.7 um filters for dissolved nutrient analysis, as does the Maryland Chesapeake Bay Water Quality Monitoring Program. GF/F filters will be used for this study because this type of filter is able to be combusted prior to use to remove traces of C and N to reduce contamination of samples. After filtration, the sample will be frozen at -20ºC.

B4 – Analytical Methods

Appendix A is the QA Plan for the UNH Water Quality Analysis Laboratory. This document describes the general SOPs for the laboratory. This QA plan has been included with other QAPPs that have been approved by EPA Region I.

Laboratory analytical methods for this study are described in detail in Appendices C, D, E, F, G, and H. Appendix C contains the SOP for DOC and TDN concentrations. Appendix D contains the protocol for filtering samples for total suspended solids. Appendix E contains the protocol for TP using alkaline persulfate digestion. Appendix F contains the SOP for ammonia concentrations. Appendix G contains the SOP for NO_2/NO_3 concentrations. Appendix H contains the protocol for PN using the EPA method. Appendix I contains the SOP for orthophosphate concentrations.

The Laboratory Manager is responsible for corrective actions if any problems with the analytical methods arise. Laboratory data reports are expected annually. All data for the project must be delivered from the laboratory to the Project Manager according to the schedule in Table 2.

Table 9: Surface Water Target Analytes and Reference Limits

Analyte	Analytical method (See Appendices for SOP details)	Project Action Level	Analytical/Achievable Method Detection Limit	Project Quantitation Limit
NO_2/NO_3	USEPA 353.2 Revision 2.0, August, 1993 (App. G)	NA-data will be used for trend analysis	0.005 mg N/L	0.005 mg N/L

Analyte	Analytical method (See Appendices for SOP details)	Project Action Level	Analytical/Achievable Method Detection Limit	Project Quantitation Limit
NH_4	USEPA method 350.1, 1971, modified March 1983 (App. F)	NA-data will be used for trend analysis	0.005 mg N/L	0.005 mg N/L
TDN	High temperature catalytic oxidation (App. C)	NA-data will be used for trend analysis	0.05 mg/L	0.05 mg/L
PN	USEPA Method 440.0 (App. H)	NA-data will be used for trend analysis	0.01 mg/L	0.01 mg/L
DOC	USEPA Method 415.3 (App. C)	NA-data will be used for trend analysis	0.1 mg/L	0.1 mg/L
PO_4	USEPA Method 365.3 (App. I)	NA-data will be used for trend analysis	0.001 mg P/L	0.001 mg P/L
TN - calculated	Calculated (TDN + PN)	NA-data will be used for trend analysis	0.05 mg/L*	0.05 mg/L*
DON	Calculated (TDN - DIN)	NA-data will be used for trend analysis	0.05 mg/L*	0.05 mg/L*
TP	USGS Method I-4650-03 Alkaline persulfate digestion (App. E)	NA-data will be used for trend analysis	0.007 mg/L	0.007 mg/L
TSS	APHA Method 2540-D (App. D)	NA-data will be used for trend analysis	1 mg/L	1 mg/L

*TDN limits are used, because TN is a calculated value.

B5 – Quality Control

Section VII of Appendix A describes the quality control measures that will be used for nutrient analyses by the UNH Water Quality Analysis Laboratory. Section A7 describes how the data quality objectives will be evaluated.

The Field Operations Manager will verify that the field crews are following the protocols correctly during the field sampling audit (see Section C1).

Databases of results will be checked for transcription errors and bad data using two methods. First, the entire data set will be printed and checked against the entries in each field or laboratory data sheet by the Laboratory Manager. Second, the Project QA Officer will construct box-plots and other graphical tools (such as scatter and timeseries plots) to determine if there are outliers in the data set. The Project QA Officer/Project Manager will determine whether these data should remain in the dataset.

B6/B7 – Instrument/Equipment Testing, Inspection, Maintenance, Calibration and Frequency

Equipment inspections and maintenance schedules for the laboratory are described in Section IX of Appendix A. Equipment calibration procedures for the laboratory are listed in Section V of Appendix A. Calibration runs are stored in the laboratory database along with the run sheets for environmental samples. Calibration records will be retained by the Laboratory Manager for a minimum of 10 years. For field measurements of specific conductance, the YSI multiparameter meter is checked in the morning before each sampling date to determine if the calibration is still accurate. The sensor is immersed in a standard of 500 uS/cm. The meter is considered to be in control if the reading is between 475 and 525 uS/cm. For field measurements of pH, the YSI multiparameter meter is calibrated using three pH buffer solutions (4.0, 7.0, and 10.0). For field measurements of DO, the YSI multiparameter meter is put inside the calibration cup with a small amount of tap water, ensured that the DO probe is not touching water and that the cup air is saturated with water, and calibrated to 100% saturation based on the barometric pressure in the lab. The temperature probe readings will be compared to a NIST calibrated thermometer in tap water as part of the field meter calibration procedure annually.

B8 – Inspection/Acceptance Requirements for Supplies and Consumables

Quality control procedures for consumables are listed in Section VII of Appendix A.

B9 – Non-Direct Measurements

The project will include use of USGS daily average stream flow measurements from stream gages in the Great Bay Estuary Watershed to help estimate annual loading of nitrogen. The data will be downloaded from the USGS website.

B10 – Data Management

Field data will be recorded on standard field data sheets. Laboratory data will be transferred from laboratory data sheets to Excel spreadsheets. All laboratory data will be stored electronically in Excel spreadsheets which will be transferred to the Project QA Officer as part of the laboratory report. The Project QA Officer will be responsible for uploading the data to the DES Environmental Monitoring Database (which is compatible with EPA's Water Quality Exchange). The ProjectID for the data will be "GBETTMP" (Great Bay Estuary Tidal Tributary Monitoring Program). Management of hardcopy data and documents is described in Section A9.

C1 – Assessments and Response Actions

In order to confirm that field sampling, field analysis and laboratory activities are occurring as planned, the Project QA Officer, Field Operations Manager, and Laboratory Manager shall confer, after the first sampling event each year, to discuss the methods being employed and to review the quality assurance samples. At this time all concerns regarding the sampling protocols and analysis techniques shall be addressed and any changes deemed necessary shall be made to ensure consistency and quality of subsequent sampling. The Project Manager will have the authority to resolve any problems encountered. Assessment frequencies and responsible personnel are shown in the following table.

Table 10: Project Assessment Table

Assessment Type	Frequency (Annual Basis)	Person Responsible for Performing Assessment	Person Responsible for Responding to Assessment Findings	Person Responsible for Monitoring Effectiveness of Corrective Actions
Field sampling audit	Once after first sampling day	Field Operations Manager	Field Operations Manager	Field Operations Manager
Field analytical audit	Once after first sampling day	Field Operations Manager	Field Operations Manager	Field Operations Manager
UNH laboratory audit	Quarterly (see Section VIII of Appendix A)	Laboratory Manager	Laboratory Manager	Laboratory Manager
Data Quality Audit	Annually	Project QA Officer	Project QA Officer	Project QA Officer

C2 – Reports to Management

The Project QA Officer will produce an annual report. The final work product will be a table containing quality assured laboratory and field results for each station on each date and an annual report describing any deviations from the protocols established in the QA Project Plan. Data from the annual reports will be published in PREP's State of Our Estuaries Reports and will also be sent to the distribution list and added to the PREP Publications website at: scholars.unh.edu

D1 – Data Review, Verification and Validation

The Project QA Officer will be responsible for a memorandum to PREP summarizing any deviations from the procedures in the QA Project Plan and the results of the QA/QC tests. The Project QA Officer will review all field data sheets and/or final computer data files for completeness and quality based on the criteria described in Section A7. The Project QA Officer will also *affirmatively* verify that the methods used for the study followed the procedures outlined in this QA Project Plan. If questionable entries or data are encountered during the review process (see methods in Section B5), the Project QA Officer will contact the appropriate personnel to determine their validity.

D2 – Verification and Validation Procedures

The Project Manager will compare the QA memorandum against the QA Project Plan. Any decisions made regarding the usability of the data will be left to the Project Manager; however, the Project Manager may consult with project personnel or with personnel from EPA, if necessary.

D3 – Reconciliation with User Requirements

The Project Manager will be responsible for reconciling the results from this study with the ultimate use of the data. Results that are qualified through the QA process may still be used if the limitations of the data are clearly reported to decision-makers. Data for this project are being collected as

part of a long-term monitoring program. It is not possible to repeat sampling events without disrupting the time series. Therefore, the Project Manager will:

1. Review data with respect to sampling design.

2. Compare the QA memorandum with the QA Project Plan.

3. If the data quality objectives from Section A7 are met, the user requirements have been met. If the data quality objectives have not been met, corrective action as discussed in D2 will be established by the Project Manager.

References

PREP. 2017. State of Our Estuaries 2018. Piscataqua Region Estuaries Partnership, University of New Hampshire, Durham, NH. Published online: www.stateofourestuaries.org.

New Hampshire Department of Environmental Services. 2013. Great Bay Estuary Tidal Tributary Monitoring Program (GBETTMP) 2013-2017 Quality Assurance Project Plan. *Quality Assurance Project Plans*. 1. https://scholars.unh.edu/qapp/1

USGS. 2013. Office of Water Quality Technical Memorandum 2013.01 Guidance on Methods for Determining the Concentration of Total Nitrogen in Whole-Water Samples. https://water.usgs.gov/admin/memo/QW/qw2013.01.pdf

Appendix A

QAPP for the Water Quality Analysis Lab at the University of New Hampshire, Department of Natural Resources, Durham, NH

Prepared by: Jeff Merriam
Date of Last Revision: 1/10/2018
Revised by: Jody Potter

I. Laboratory Organization and Responsibility

Dr. William H. McDowell - Director

Jody Potter – Lab Manager/QA manager. Mr. Potter supervises all activities in the lab. His responsibilities include data processing and review (QA review), database management, protocol development and upkeep, training of new users, instrument maintenance and repair, and sample analysis.

Katie Swan, James Casey, & Lisle Snyder – Lab Technicians. Ms Swan, Mr Casey, and Mr Snyder's responsibilities, with the help of undergraduate employees, include sample analysis, logging of incoming samples, sample preparation (filtering when appropriate), daily instrument inspection and minor maintenance.

All analyses are completed by Katie Swan, Lisle Snyder, James Casey or Jody Potter, and all data from each sample analysis batch (generally 40-55 samples) is reviewed by Jody Potter for QC compliance. All users are trained by the lab manager and must demonstrate (through close supervision and inspection) proficiency with the analytical instrumentation used and required laboratory procedures.

II. Standard Operating Procedures

Standard Operating Procedures for all instruments and methods are kept in a 3-ring binder in the laboratory, and are stored electronically on the Lab manager's computer. The electronic versions are password protected. SOPs are reviewed annually, or as changes are required due to new instrumentation or method development.

III. Field Sampling Protocols

Sample collection procedures are generally left up to the sample originators, however we recommend the guidelines described below, and provide our field filtering protocol on request.

All samples are filtered in the field through 0.7 um precombusted (5+ hours at 450 C) glass fiber filters (e.g. Whatman GF/F). Samples are collected in acid-washed 60-mL HDPE bottles. We prefer plastic to glass as our preservative technique is to freeze. Sample containers are rinsed 3 times with filtered sample, and the bottle is filled with filtered sample. Samples are stored in the dark and as cool as possible until they can be frozen. Samples must be frozen or refrigerated (SiO_2) within 8 hours of sample collection. Once frozen, samples can be stored indefinitely (Avanzino and Kennedy, 1993), although they are typically analyzed within a few months.

After collection and freezing, samples are either hand delivered to the lab, or are shipped via an over-night carrier. Samples arriving in the lab are inspected for frozen contents, broken caps, cracked bottles, illegible labels, etc. Any pertinent information is entered into a password protected database (MS Access).

We provide an electronic sample submission form that also serves as a chain of custody form. Submitters should indicate all analyses required for the samples,

preservation (if any), and sample information (name, date, etc …). They should also indicate project name and a description of the project.

IV. Laboratory Sample Handling Procedures

Samples are given a unique 5-digit code. This code and sample information including name, collection date, time (if applicable), project name, collector, logger, the date received at the WQAL, sample type (e.g. groundwater, surface water, soil solution) and any other miscellaneous information, are entered into a password protected database. From this point through the completion of all analyses, we use the log number to track samples. Log numbers are used on sample run queues, spreadsheets, and when importing concentrations and run information into the database

After samples are logged into the WQAL, they are stored frozen in dedicated sample walk-in freezer or refrigerator located next to the lab. These units log temperature and alarms indicate when they are out of range. The paper print-outs are replaced quarterly and kept on file. Samples from different projects are kept separated in cardboard box-tops, or in plastic bags. Samples that may pose a contamination threat (based on the source or presumed concentration range) are further isolated by multiple plastic bags, or isolation in separate freezer space. This is typically not an issue as we primarily deal with uncontaminated samples.

We do not pay special attention to holding time of samples, as frozen samples are stable indefinitely (Avanzino and Kennedy, 1993). However, we do keep track of the date samples arrive at the WQAL, and can report holding times if necessary. After samples are analyzed they are returned to the project's manager for safe keeping or they

are held for a period of time at the WQAL to allow necessary review and analysis of the data by the interested parties (not from a laboratory QC sense, but from a project specific viewpoint). Once the data is analyzed by the project's manager(s), the samples are returned or disposed of, based on the preference of the project's manager.

Samples that arrive unfrozen, with cracked bottles/caps, or with loose caps, are noted in the database and are not analyzed. These samples are disposed of to prevent accidental analysis. The sample originator is notified (generally via e-mail) of which samples were removed from the sample analysis stream. Similarly, if while in the possession of the WQAL, a sample bottle is broken or improperly stored (e.g. not frozen), the sample is removed and the sample originator is notified.

V. Calibration procedures for chemistry

Calibration curves are generally linear, and are made up of 4-7 points. A full calibration is performed at the beginning of each run (a run is generally 40-60 samples) with a reduced calibration (3-5 points) performed at the end of the run. Occasionally calibration data is best fit with a quadratic equation, and this is used if it best describes the data within a specific run.

Standards are made from reagent grade chemicals (typically Fisher Scientific or ACROS) that have been dried and are stored in a dessicator when required. Working stock solutions are labeled with the content description, concentration, initials of the maker, and the date the stock solution was made. Generally stock solutions are kept less than one week; however some stocks (Br, Na, Cl, C for DOC) can be stored for several

months. Standard solutions are kept for less than one week from the date they were made. Stocks and standards are stored tightly covered, in a dark refrigerator in the lab.

Control charts are prepared and evaluated by the lab manager frequently. However data from each run are looked at within days of analyses. Calibration curves, Laboratory Duplicates, Lab Fortified Blanks (LFB), Lab Fortified Sample Matrices (LFM) and Lab Reagent Blanks (LRB) are reviewed and are checked against known concentrations (where applicable) to ensure QC criteria are met for each run of samples.

VI. Data Reduction, validation, reporting and verification

Data reduction and validation are performed in a spreadsheet (MS Excel). The Raw data page of the spreadsheet lists the date of analysis, user, analysis performed, project, any issues or problems noted with the instrument on that date, and the sample queue and the raw data exported from the instruments. Most raw data is exported as an area or an absorbance value. This data is entered into an Excel QC template to guide the user on how to calculate data and QC summary. A second page (typically named "Calculations") is added to the spreadsheet where known concentrations of standards, check standards and reference solutions are added. The calibration curve(s) is calculated and the concentrations are calculated on this page. Calculated concentrations for all standards, LFB, LFM and IPC are compared to the "known" or prepared values. If these are acceptably close (+/- 10% of the "known") no further changes to the calculated concentrations are made. If there is evidence of drift in the response of the instrument during a run, we try to correct for the drift using the responses from the front end calibration curve and the set of standards analyzed at the end of the run. All reference

solutions and replicates must meet certain QC criteria (described below) for a run to be accepted.

Data are then exported to the WQAL database. Exported information includes the unique 5-digit code, calculated concentration, the analysis date, the user, the filename the raw data and calculations are saved in, and any notes from the run regarding the specific sample. Data are sent to sample originators upon completion of all requested sample analyses and following review by the WQAL lab manager. Generally the data include the 5-digit code, the sample name, collection date, and concentrations, in row-column format. Any information entered into the database can be included upon request. Data transfer is typically via e-mail or electronic medium (CD or floppy disk).

All data corrections are handled by the lab manager. Corrections to data already entered into the database are very infrequent. Typically they involve reanalysis of a sample. In this case, the old data is deleted from the database, and the new value is imported, along with a note indicating that it was re-analyzed, the dates of initial and secondary analysis and the reason for the correction.

Hand written or computer printed run sheets are saved for each run and filed, based on the project and the analysis. Spreadsheet files with raw data and calculations are stored electronically by analysis and date. Information in the database allows easy cross-reference and access from individual samples to the raw data and the runsheets. This provides a complete data trail from sample log-in to completion of analysis.

VII. Quality Control

All analyses conducted at the WQAL follow approved or widely accepted methods (Table 1).

Quality Control Samples (QCS) (from Ultra Scientific or SPEC Certiprep) are analyzed periodically (approximately every 10-15 samples) in each sample analysis batch to assure accuracy. The response/unit concentration is also used to monitor day-to-day variation in instrument performance. A difference from the certified concentration of more than 10% requires further investigation of that run. A difference greater than 15% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Table 2 lists historical average % recoveries. At least 2 QCS are analyzed on each run.

Standards and reagents are prepared from reagent grade chemicals (typically JT Baker) or from pre-made stock solutions. All glassware is acid washed (10% HCl) and rinsed 6 times with ultra pure-low DOC water (18.2 mega-ohm). All analyses (except CHN) use multi-point calibration curves (4-7) points, which are analyzed at the beginning and the end of each run. A Laboratory Reagent Blank (LRB), Laboratory Fortified Blank (LFB) (a standard run as a sample) and Laboratory Duplicate are analyzed every 10 to 15 samples during each run. At least one Laboratory Fortified Sample Matrix (LFM) is analyzed during each run to insure that sample matrices do not affect method analysis efficiency. Field Duplicates are not required by our lab, and are the responsibility of the specific project's manager.

Laboratory Duplicates must fall within 10% relative percent difference (RPD = abs(dup1-dup2)/average of dup1 and dup 2). A difference greater than 5% requires

further investigation of the sample run. A difference greater than 10% is failure (unless the average of the two samples is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for relative % difference are included in Table 2.

LFM must show 85% to 115% recovery. A recovery <90% or > 110% requires further investigation of the sample run. A recovery <85% or >115% is failure (unless the sample is less than 10X the MDL), and results in re-analysis of the entire sample queue, unless there is a very reasonable and supported explanation for the inconsistency. Long-term averages for % recovery are included in Table 2.

All QC information from each run is stored in a separate Access database. This includes calibration r^2, error, slope and intercept. The prepared concentration and measured concentration of LFM and calibration standards analyzed throughout the run are also entered. Finally, the lab duplicate measured concentrations are included. All this information can be queried for the project manager. Control charts (PDF) are generated from this database in R and reviewed weekly by the lab manager.

Method Detection Limits are calculated regularly, and whenever major changes to instrumentation or methods occur. Table 2 lists most recently measured MDL values.

VIII. Schedule of Internal/External Audits

Internal audits are not routinely performed, however, QC for each run is thoroughly reviewed by the lab manager before entering data into the database and a review of QC charts, and tables is done at least annually by the lab manager.

External audit samples are analyzed routinely throughout the year. The WQAL takes part in the USGS Round Robin inter-laboratory comparison study twice per year and the Environment Canada Proficiency Testing Program three times per year. The USGS and Environment Canada provide Standard Reference Samples and provide compliance results after analytical testing at the WQAL. Environment Canada is accredited by the American Association for Laboratory Accreditation. These audits are designed to quantify and improve the lab's performance. Poor results are identified and backtracked through the lab to the sources of the issue.

IX. Preventive maintenance procedures and schedules

The laboratory manager, Jody Potter, has 15 years of experience and is highly experienced with all laboratory equipment used within the WQAL. The laboratory manager conducts all maintenance and inspection of equipment based on manufacturer requirements and specifications.

Each day an instrument is used, it receives a general inspection for obvious problems (e.g. worn tubing, syringe plunger tips, leaks). The instruments are used frequently and data is inspected within a few days of sample analysis. This allows instrument (or user) malfunctions to be caught quickly, and corrected as needed.

Each day's run is recorded in the instrument's run log, with the date, the user, the number of injections (standards, samples, and QC samples), the project, and other notes of interests. Maintenance, routine or otherwise, is recorded in the instrument run log, and includes the date, the person doing the maintenance, what was fixed, and any other notes of interest.

X. Corrective Action Contingencies

Jody Potter is responsible for all QC checks and performs or supervises all maintenance and troubleshooting. When unacceptable results are obtained (based on within sample analysis batch QC checks) the data from the run are NOT imported into the database. The cause of the problem is determined and corrected, and the samples are re-analyzed. Problems are recorded in the sample queue's data spreadsheet, or on the handwritten runsheet associated with the run. Corrective actions (instrument maintenance and troubleshooting) are documented in each instrument's run log.

XI. Record Keeping Procedures

Protocols, Instrument Logs, QC charts, databases and all raw data files are kept on the lab manager's computer. These are backed up continuously, with the back up stored off site. The computer is password protected, and is only used by the lab manager. Protocols and the sample database are also password protected. Handwritten run sheets are stored in a filing cabinet in the lab. Instrument run and maintenance logs are combined with the QC data in an access database where instrument performance can easily be compared to instrument repair and the number of analyses, etc. This file is also stored on the lab manager's computer and is password protected.

All information pertinent to a sample is stored in the sample database. From this database we can easily determine the date of analysis and the location of the raw data file if further review is necessary. The amount of information provided to sample originators is dependent on what is required by the project or funding agencies.

Table 1. List of standard operating procedures and description of analyses done at the Water Quality Analysis Laboratory.

Standard Operating Procedure	Analysis	Instrument Used	Description	Protocol Latest Revision	EPA method or other reference
Ion Chromatography Protocol for Anions and Cations Protocol	Anions and Cations	Dionex ICS-1000; IonPac AS22 column Dionex ICS-1000 and ICS 1100; IonPac CS12 column	Anions via ion chromatography w/ suppressed conductivity. Cations via ion chromatography w/ suppressed conductivity	February 7, 2012	Anions EPA #300.0 Cations ASTN D6919-09
Dissolved Organic Carbon Protocol	DOC	Shimadzu TOC-V or TOC-L	High Temperature Catalytic Oxidation (HTCO)	April 4, 2016	EPA 415.3
Total Dissolved Nitrogen Protocol	TDN	Shimadzu TOC-V or TOC-L with TN module	HTCO with chemiluminescent N detection	April 4, 2016	Merriam et al, 1996; ASTM D5176
DOC and TDN combined Protocol	DOC and TDN	Shimadzu TOC-V or TOC-L with TN nitrogen module	HTCO with chemiluminescent N detection	April 4, 2016	EPA 415.3 and Merriam et al, 1996
Seal AQ2 discrete colorimetric analysis Protocol	Nitrate/Nitrite colorimetric (NO_3/NO_2)	Seal Analytical AQ2 discrete analyzer	Automated Cd-Cu reduction	April 25, 2016	EPA 353.2
SmartChem discrete colorimetric analysis Protocol	Ammonium colorimetric (NH_4)	SmartChem discrete analyzer	Automated Phenate	August 27, 2010	EPA 350.1
Seal AQ2 discrete	Soluble reactive	Seal Analytical	Automated Ascorbic acid	April 20, 2017	EPA 365.3

colorimetric analysis Protocol	Phosphorous colorimetric (SRP or PO_4)	AQ2 discrete analyzer			
SmartChem discrete colorimetric analysis Protocol	Silica (SiO_2)	SmartChem discrete analyzer		November, 10, 2005	EPA 370.1
Seal AQ2 discrete colorimetric analysis Protocol	Total Dissolved Phosphorus (TDP) (Filtered sample)	Seal Analytical AQ2 discrete analyzer	Persulfate Oxidation of filtered sample, followed by colorimetric SRP analysis.	April 25, 2016	USGS Test Method 1-4560-03
Seal AQ2 discrete colorimetric analysis Protocol	Total Phosphorus (TP) and Total Nitrogen (TN) (Unfiltered sample)	Seal Analytical AQ2 discrete analyzer	Persulfate Oxidation of unfiltered sample, followed by colorimetric SRP analysis.	April 25, 2016	Resources Investigations Report 03-4174
CHN Protocol	Particulate Carbon (PC) and Nitrogen (PN)	Perkin Elmer 2400 Series II CHN	Filtration of sample followed by Elemental Analysis of the filter and particulates	February 14, 2013	EPA 440.0
Particulate Carbon and Nitrogen filtration	Laboratory Sample Filtration		Filtration of samples for water chemical analysis and particulate analysis	February 14, 2013	EPA 440.0
Acid Washing Protocol	Glass and plastic-ware cleaning		10% HCl rinse and 6 rinses with DDW	July 19, 2012	
Field Filtering Protocol	Sample prep		3-times rinse with filtered sample	July 13, 2015	
Fluorescence	EEMs	Horiba Jobin Yvon Fluoromax 3	Scanning Fluorescence Excitation & Emission on whole water	June 26, 2013	
Absorbance	Abs 254 & SUVA	Shimadzu TOC-V & Shimadzu	Scanning absorbance	June 26, 2013	EPA 415.3

		PDA SPD-M20A	spectra on whole water		
pH, Closed cell	pH, Closed cell	Electrode & Thermo Orion 525A	pH in a closed environment under atmospheric CO_2 conditions	August 27, 2015	EPA 150.1
pH, aerated	pH, aerated	Electrode and Radiometer ION450	pH equilibrated with atmosphere	January 4, 2013	EPA 150.1
Specific conductance	Specific conductance	Electrode	Specific conductance	May 15, 2017	EPA 120.1
ANC protocol	ANC	Electrode & Radiometer ION450	Gran titration	May 15, 2017	EPA 310.1
Greenhouse Gases	Greenhouse Gases extracted from water	Shimadzu GC-2014	CH_4, N_2O, & CO_2 on GC with FID, ECD, & TCD	December 6, 2012	
Alkalinity protocol	Alkalinity	Electrode & Radiometer ION450	Inflection Point		EPA 310.1

Table 2. Detection limits, acceptable ranges, and recent historical averages for QC samples at the Water Quality Analysis Lab.

[1] Detection limit based on user experience and previous analysis (not statistically calculated). [2] Method Detection Limit (MDL) is the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero.

Analyte	Units	Typical Range	Regression Type	# of Cal. Points	MDL [2]	Lab Duplicate % Relative Difference	Limit	LFM % recovery	Limit +/-	IPC % recovery	Limit +/-
SiO_2	mg SiO2/L	0 – 40	Linear	4-7	.01	3.5	15.0	92.8	15.0		
PO_4	µg P/L	0 – 200	Linear	4-7	5	7.8	15.0	95.5	15.0	93.7	15.0
NH_4	µg N/L	0 – 200	Linear	4-7	5	7.1	15.0	103.9	15.0	95.0	15.0
NO_3 FIA	mg N/L	0 – 10	Linear	4-7	0.005	4.6	15.0	100.9	15.0	102.6	15.0
Na^+	mg Na/L	0 – 15	Quadratic	4-7	0.02	0.9	15.0			112.7	
K^+	mg K/L	0 – 7	Quadratic	4-7	0.02	10.4	15.0			97.8	
Mg^{2+}	mg Mg/L	0 – 7	Quadratic	4-7	0.02	4.5	15.0			89.7	
Ca^{2+}	mg Ca/L	0 – 10	Quadratic	4-7	0.1	4.0	15.0			98.2	
Cl^-	mg Cl/L	0 – 15	Quadratic	4-7	0.02	1.6	15.0			92.7	
NO_3^-	mg N/L	0 – 3	Quadratic	4-7	0.004	0.3	15.0			96.3	
SO_4^{2-}	mg S/L	0 – 8	Quadratic	4-7	0.04	2.2	15.0			86.5	
TDN	mg N/L	0 – 10	Linear	4-7	0.035	7.8	15.0	100.3	15.0	102.1	15.0
DOC	mg C/L	0 – 20	Linear	4-7	0.05	4.9	15.0	100.5	15.0	97.0	15.0

References

Avanzino R.J. and V.C. Kennedy, 1993. Long-term frozen storage of stream water samples for dissolved orthophosphate, nitrate plus nitrite, and ammonia analysis. *Water Resources Research*, 29(10) 3357-3362.

Merriam, J.L, W.H. McDowell, W.S. Currie, 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. *Soil Science Society of America Journal*, 60(4) 1050-1055.

Appendix B

New Hampshire Estuaries Project
Tributary Sampling Field Data Sheet

Collected By: ________________ Date/Time Samples Delivered to WQAL: ______________

Specific Conductance Meter Check (500 uS/cm standard): __________uS/cm (OK if 475 – 525 uS/cm)

Station ID	UNH ID	Sample Date	Sample Time	Water Temp (°C)	Conductance (uS/cm)	Specific Conductance (uS/cm at 25 °C)	pH	Dissolved Oxygen mg/L	Dissolved Oxygen %	Comments
05-OYS Oyster										
05-LMP Lamprey										
09-EXT Exeter										
02-WNC Winnicut										
05-BLM Bellamy										
02-GWR Great Works										
05-SFR Salmon Falls										
07-CCH Cocheco										
DUP										

For field duplicate samples, the StationID should be the station where the sample was collected followed by "DUP".
If you have any questions, contact Phil Trowbridge at 603-271-8872 or 603-340-5220 (cell) or Michelle Daley at 603-862-2341 or 603-767-2757 (cell)
WQAL Project: NH DES sub-project: Tidal Tributaries

Appendix C

Dissolved Organic Carbon (DOC) and Total Dissolved Nitrogen (TDN) Standard Operating Procedure Shimadzu TOCL and TOCV CPH

Water Quality Analysis Laboratory at the University of New Hampshire

Prepared by: Jody Potter
Date of Last Revision: 4/12/2016

Signature of Reviewer/Reviser:

____

Method is based on:

EPA Method 415.1 Organic Carbon, Total (Combustion or Oxidation).

And

TDN Method: Method Reference: Shimadzu Scientific Instruments Inc., TOC-V with TNM-1 Nitrogen Module. High Temperature Catalytic Oxidation with chemiluminescent detection. Merriam, J.L., W.H. McDowell, W.S. Currie, 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. Soil Science Society of America Journal, 60(4) 1050-1055.

Protocol for TOC-V CPH and TOC-L CPH

There are one of each of these machines. The TOC-V CSH can analyze NPOC and TDN in the same run.

NPOC Method: Official Name: **EPA Method 415.1** Organic Carbon, Total (Combustion or Oxidation). Organic carbon in a sample is converted to carbon dioxide by catalytic combustion or wet chemical oxidations. The carbon dioxide formed can be measured directly by an infrared detector or converted to methane and measured by a flame ionization detector. The amount of carbon dioxide or methane is directly proportional to the concentration of carbonaceous material in the sample.

TDN Method: Method Reference: Shimadzu Scientific Instruments Inc., TOC-V with TNM-1 Nitrogen Module. High Temperature Catalytic Oxidation with chemiluminescent detection. Merriam, J.L., W.H. McDowell, W.S. Currie, 1996. A high-temperature catalytic oxidation technique for determining total dissolved nitrogen. Soil Science Society of America Journal, 60(4) 1050-1055.

A precisely measured aliquot of filtered sample is injected and combusted on a catalyst at 720 C. All fixed N is converted to Nitric Oxide (NO) and then coupled with ozone (O_3) producing Nitrogen Dioxide* (NO_2^*) which is measured chemiluminescently.

1. Preparation of Standard Solutions

A. **NPOC**. Weigh out 2.125 g dried potassium acid phthalate (KHP). Dissolve it in 500 mL of Milli-Q water (DDW) in a 1 L volumetric flask. Bring the solution to volume. This makes a 1000 mg C L^{-1} TC stock (1000 ppm). **TDN.** Weigh out 0.60677 g dried sodium nitrate. Dissolve it in a 100 mL volumetric flask and fill to volume. This makes a 1000 mg N L^{-1} NO3 stock solution.

B. If the samples to be analyzed are at the lower end of the concentration range, it may be necessary to make an intermediate standard (100 mg L^{-1}).

C. When doing more than one analysis, NPOC and TDN standards should be combined in the same volumetric flask to reduce the amount of standard vials taking up space on a run. The lowest NPOC standard should be combined with the lowest TDN standard and so on.

D. Make working standards by pipetting the appropriate amount of stock (or intermediate standard) into 250 mL volumetric flasks, and bring them to volume. You can put the 250 mL volumetric flasks directly on the analytical balance, allowing you to know exactly how much stock you are adding. This eliminates the necessity of weighing water (to determine the volume dispensed) before using the adjustable pipettes.

E. Store stock solution in clean, airtight, glass container in the refrigerator. TOC stock will keep for two (2) months. The NO3 and IC stock will keep for about one (1) month. Working standards can be stored in the volumetric flasks that they were made in. Be sure to cover them tightly with Parafilm. An airtight seal is especially important for the IC stock and standards due to absorption of CO_2 from the atmosphere. TOC and TDN standards are good for a week or so. IC standards should be remade every 2 or 3 days. Standards should be made weekly, or more frequently if dealing with low concentrations (< 0.3 mg/L). Refer to Acid Washing protocol for details.

2. Sample Preparation

A. Sample vials (9 mL or 22 mL) are prepared by rinsing them at least 2 times with DDW and then combusting them in the muffle furnace at 450 - 500°C for 6 hours. It takes the muffle furnace one hour to get up to temperature.

B. Fill each vial about ½ full for 22 mL vials. Fill the 9 mL vials completely full.

C. Cover the 22 mL vials with the caps that are provided. The septa should be removed and new ones should be put in the caps every 3-4 runs. You should be able to tell how many times the caps have been pierced. The dark side of the septa should face up.

D. Put the vials in the sample tray. The sample tray can be removed from the autosampler by lifting the hood and releasing the magnet that holds the tray down. You can then simply lift the sample tray off the autosampler.

E. Please refer to the **Quality Assurance and Control Section** for information on replicates, certified reference standards and check standards. A copy of the NPOC/TDN/POC runsheet is attached.

3. System Inspection

A. Confirm gas pressure on the TOC gas generator. Carrier Flow meter (on TOC-V CSH) should read about 150 mL min^{-1}.

B. Inspect the dehumidifier drain vessel water level. The water in the drain vessel should reach the outflow port on the drain vessel sidewall. Add DDW to get it to that level, if necessary. Make sure there is no bubbling in the drain vessel. If there is, inspect the halogen scrubber and membrane filter for plug.

C. Inspect humidifier water level. Confirm that the water level is between the two line markings. Add DDW through the supply port if necessary.

D. Inspect the IC reagent reservoir on outside of instrument. This reservoir should have some solution. If it is empty, you must fill it with H_3PO_4 according to recipe.

E. Inspect needle rinse bottle to the left of the autosampler. It should be filled with DDW.

F. Inspect the HCl bottle on the outside of the instrument. It should be filled with 2N HCl for NPOC and POC analysis.

G. Inspect the dilution water bottle to the left of the instrument and make sure it is filled with DDW (only necessary if going to do auto dilution with the instrument).

H. Perform a leak check. The IC vessel inside the instrument should be bubbling.

4. Preparation for Analysis

A. Check to see that waste vessel for the TOC-V (TOC-L drains to sink) is relatively empty, and that the waste tube is in the waste vessel and has no kinks.

B. The TOCs are normally left on. If it is off, then turn it on and allow the furnace to heat up.

C. The system setup for the instrument is usually set to be done from the computer. Ask the lab manager for help if the instrument screen is on. **Start up the software**, which is labeled TOC Control L or V. Then click on the **Sample Table Editor** icon. It will ask you for user and password, but just click ok with nothing filled in.

D. Open a new sample table by selecting **New** from the **File menu**. Click on the **sample run** icon and then click **OK**.

E. To establish communication between the software and the instrument, select the **connect** icon on the toolbar. The Parameter Configuration dialog box is displayed. Click the **Use Settings on PC button** for TOC-V.

F. Insert the samples by first placing the cursor in the first line of the sample table. From the insert menu, select **sample**. The Insert **multiple samples** for TOC-L and **Auto Generate** for TOC-V option may also be selected if you have several values of the same type (i.e. standards or samples) in a row (manual section 4.4.5.1 “Auto Generate).

For single samples (sample):

1. Click on the **Method** radio button. Select one of the previously created method files depending on which method you need (i.e. NPOC-TN method) to perform the type of analysis you are doing. Then click **next**.
2. Type in the **name of the sample** in sample name and sample ID. Change the **number of determinations** if you want it to be sampled more than once.
3. Click on **next** until you click on **finish**.
4. Continue as needed.

For several samples in a row (Auto Generate):

1. Click on the method radio button. Select one of the previously created method files depending on which method you need (i.e. NPOC-TN method) to perform the type of analysis you are doing. Then click **next**.
2. Type in the **number of vials**, the **start vial**, and the **name of the samples**. If entering ID numbers, select **Index Start** instead of entering the name of the samples. Type in the ID number of the beginning vial for the string of samples.
3. Click **next** until reach finish and then click **finish**.

G. **Save** the Sample Table by selecting Save from the File menu.

H. Check the status of the instrument detectors before starting analysis. From the Instrument menu, select **Background Monitor**. On the TOC tab, the status of the baseline should be OK for each parameter (position, fluctuation, and noise). Do the same for the TN tab. Then close the window.

I. Place the cursor in the first row of the Sample Table. From the Instrument menu, **select Start**, or click on the Start button on the toolbar. The Standby window is displayed.

J. Press **standby**. The Sparging/Acid Addition window is displayed.

K. Verify the vial positions, and then click **OK**.

L. The Start ASI measurement window is displayed. Click on **Start**.

5. Data Export

A. Click on File and select **ASCII Export options**.

B. Click on the **data** tab and select sample ID, dilution (if needed), inj no, analysis (inj.), and mean area. Click **OK**.

C. Click on File and select **ASCII Export.** Choose a file name and **save** it under the data directory for the TOCs. The data file is now ready to be used in Excel.

6. Quality Assurance and Control

A. **Blank Stabilization.** At least three blanks should be run at the start of your run to allow for blank stabilization.

B. **Standard Replicates, Sample Replicates, Certified Reference Standards**

1. A blank, two standard replicates, a known stream sample (CCV) and two certified reference standards (“QC”; one for NPOC/POC and one for TDN) will be run about every 12 samples as identified on the run sheets. The date for the QC standards and CCVs should be written down on the run sheet. This will allow you to track the run to run variability of your analysis, as well as to confirm the accuracy of your standards.
2. At the end of your run, a standard curve consisting of four standards and a blank will be run. This will help to detect and account for any drift in the calibration during the run.

D. **Quality Control Table.**

1. The data is to be copied and pasted into the appropriate lab Excel Report Template on the TOC-V computer and the file should be named by date of analysis (described in worksheet). This template will guide you to report the QC results for the run. This includes % recovery of QC standards (CRM), run time check standards, and lab duplicates. Lab % recovery of sample duplicates, run time check standards, and QC standards should be between 85 and 115 % (see WQAL QAPP for more information).
2. When completed copy the Excel file into the lab manager’s directory on the main lab computer. This information, along with the data, will be entered into the WQAL database by the lab manager to create control charts.

Appendix D

Total Suspended Sediments Protocol

Water Quality Analysis Laboratory at the University of New Hampshire

Prepared by: Jeffrey Merriam
Date of Last Revision: 3/18/2008

Signature of Reviewer/Reviser: ______________________________

Total Suspended Sediments Protocol

(can also be used for determination of Particulate Carbon (PC) and Particulate Nitrogen (PN)

The filters we are using are prepared and pre-weighed for suspended sediment analysis. They are in numbered pans. **It is very important that the filter be kept in its respective pan to assure correct identification.**

Overview

A known amount of a well mixed water sample is filtered thru a preweighed filter. The filter is then dried and weighed. The initial filter weight is subtracted from the filter+sediment weight and divided by the volume filtered to give Total Suspended Sediment concentration (mg/L).

Sample Filtration

1. Take the pan and filter from the **bottom** of the stack.
2. Record the pan ID number and filter weight (written on the side of the pan) on your log sheet.
3. Using forceps, place the filter on the base of the filter tower.
4. Gently place the top of the filter tower on the base, and secure as necessary.
5. Record the sample name and collection date (if available) of the sample on the data sheet. You may also want to indent the filter pan with the sample ID, though this isn't completely necessary as you've also recorded the pan ID number.
6. Shake the sample bottle vigorously several times to produce a homogenous solution.
7. Pour known volume into filter tower, and apply a vacuum. The amount of sample you'll filter depends on how much sediment is in the sample. Rivers and streams at low flow may require several liters to clog the filter. Samples collected at high flow or following major disturbance may require 100 mL or less
8. Continue to add known aliquots until the filter is nearly plugged.
9. Record how many mL of sample you filtered on the data sheet.
10. Rinse sides of filter tower with a minimal amount of DDW to wash any particulates off the tower onto the filter.
11. Continue to apply vacuum until the filter appears dry.
12. Remove the top of the filter tower.
13. Carefully remove the filter using forceps, and place it in its identified pan. Be sure to get every piece of the filter.
14. Place pan/filters in the drying oven at 103-105 C for at least several hours.
15. Record the date and time you put the samples in the oven on the data sheet.
16. Record any notes, problems, observations, difficulties, etc. on the data sheet.
17. Perform a replicate filtration every 20 samples if you have sufficient volume.

Weighing Filters

1. Follow appropriate Analytical Balance protocols (see **Analytical Balance 24Jan2008.doc**).
2. Remove the pan/filters from the drying oven and cool in a desiccator to until at room temperature.
3. Record the time and date on the data sheet.
4. Limit the time the filters are out of the desiccator prior to weighing as they may absorb moisture from the air.
5. Zero the balance.
6. Using forceps, place the filter on the balance. Weigh only the filter!
7. Allow the balance to stabilize and record the weight on the data sheet.
8. Record any notes, problems, or observations on the data sheet.
9. Put the weighed filter back into its respective pan, and put the pan/filter back into a desiccator until the data can be calculated and checked.

Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water

Water-Resources Investigations Report 03–4174

U.S. Department of the Interior
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CONVERSION FACTORS AND ABBREVIATED WATER-QUALITY UNITS

Multiply	By	To obtain
	Length	
centimeter (cm)	3.94×10^{-1}	inch
micrometer (µm)	3.94×10^{-5}	inch
millimeter (mm)	3.94×10^{-2}	inch
nanometer (nm)	3.94×10^{-8}	inch
	Volume	
liter (L)	2.64×10^{-1}	gallon
liter (L)	33.81	ounce, fluid
microliter (µL)	2.64×10^{-7}	gallon
milliliter (mL)	2.64×10^{-4}	gallon
	Mass	
gram (g)	3.53×10^{-2}	ounce, avoirdupois
milligram (mg)	3.53×10^{-5}	ounce, avoirdupois
	Pressure	
kilopascal (kPa)	1.45×10^{-1}	pounds per square inch

Degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) by using the following equation:

$$°F = (1.8 \times °C) + 32.$$

ABBREVIATIONS AND ACRONYMS

A/D analog-to-digital converter
ASTM American Society for Testing and Materials
CCV continuing calibration verification
DI deionized water
DN_{AlkP} alkaline persulfate dissolved nitrogen
DP_{AlkP} alkaline persulfate dissolved phosphorus
FCA filtered, chilled, acidified (bottle type for USGS dissolved nutrient samples)
FCC filtered, chilled (bottle type for USGS dissolved nutrient samples)
FW formula weight
h hour
Hz hertz
i.d. inside diameter
KDN Kjeldahl dissolved nitrogen
KDP Kjeldahl dissolved phosphorus
KN used collectively for KDN and KTN
KTN Kjeldahl total nitrogen
KTP Kjeldahl total phosphorus
lb/in^2 pounds per square inch
LRL laboratory reporting level

Abbreviation	Definition
mg-As/L	milligrams arsenic per liter
mg-C/L	milligrams carbon per liter
mg-N/L	milligrams nitrogen per liter
mg/L	milligrams per liter
mg-P/L	milligrams phosphorus per liter
M	molarity (moles per liter)
max	maximum
MDL	method detection limit
MPV	most probable value
N	normality (equivalents per liter)
N_{AlkP}	used collectively for DN_{AlkP} and TN_{AlkP}
NED	N-(1-Naphthyl)ethylenediamine dihydrochloride reagent
NOM	natural organic matter
NWQL	National Water Quality Laboratory
OC	organic carbon
OWQ	Office of Water Quality
o.d.	outside diameter
PC	personal computer
PBCdR	packed-bed cadmium reactor
P/N	part number
PTFE	polytetrafluoroethylene
TN_{AlkP}	alkaline persulfate total nitrogen
TP_{AlkP}	alkaline persulfate total phosphorus
QC	quality control
s	second
sp. gr.	specific gravity
SAN	sulfanilamide reagent
SLS	sodium lauryl sulfate
SOP	standard operating procedure
SRWS	U.S. Geological Survey Standard Reference Water Sample
STD CAL	standard calibration control; adjusts absorbance range of photometric detectors used in this study
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
v/v	volume per volume
w/v	weight per volume
WCA	whole water, chilled, acidified (bottle type for USGS whole-water nutrient samples)
$=$	equivalent to
$>$	greater than
$<$	less than
$\leq$	less than or equal to
$\approx$	nearly equal to
$\pm$	plus or minus

Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Evaluation of Alkaline Persulfate Digestion as an Alternative to Kjeldahl Digestion for Determination of Total and Dissolved Nitrogen and Phosphorus in Water

By Charles J. Patton and Jennifer R. Kryskalla

Abstract

Alkaline persulfate digestion was evaluated and validated as a more sensitive, accurate, and less toxic alternative to Kjeldahl digestion for routine determination of nitrogen and phosphorus in surface- and ground-water samples in a large-scale and geographically diverse study conducted by U.S. Geological Survey (USGS) between October 1, 2001, and September 30, 2002. Data for this study were obtained from about 2,100 surface- and ground-water samples that were analyzed for Kjeldahl nitrogen and Kjeldahl phosphorus in the course of routine operations at the USGS National Water Quality Laboratory (NWQL). These samples were analyzed independently for total nitrogen and total phosphorus using an alkaline persulfate digestion method developed by the NWQL Methods Research and Development Program. About half of these samples were collected during nominally high-flow (April-June) conditions and the other half were collected during nominally low-flow (August-September) conditions. The number of filtered and whole-water samples analyzed from each flow regime was about equal.

By operational definition, Kjeldahl nitrogen (ammonium + organic nitrogen) and alkaline persulfate digestion total nitrogen (ammonium + nitrite + nitrate + organic nitrogen) are not equivalent. It was necessary, therefore, to reconcile this operational difference by subtracting nitrate + nitrite concentrations from alkaline persulfate dissolved and total nitrogen concentrations prior to graphical and statistical comparisons with dissolved and total Kjeldahl nitrogen concentrations. On the basis of two-population paired *t*-test statistics, the means of all nitrate-corrected alkaline persulfate nitrogen and Kjeldahl nitrogen concentrations (2,066 paired results) were significantly different from zero at the $p = 0.05$ level. Statistically, the means of Kjeldahl nitrogen concentrations were greater than those of nitrate-corrected alkaline persulfate nitrogen concentrations. Experimental evidence strongly suggests, however, that this apparent low bias resulted from nitrate interference in the Kjeldahl digestion method rather than low nitrogen recovery by the alkaline persulfate digestion method. Typically, differences between means of Kjeldahl nitrogen and nitrate-corrected alkaline persulfate nitrogen in low-nitrate concentration (≤ 0.1 milligram nitrate nitrogen per liter) subsets of filtered surface- and ground-water samples were statistically equivalent to zero at the $p = 0.05$ level.

Paired analytical results for dissolved and total phosphorus in Kjeldahl and alkaline persulfate digests were directly comparable because both digestion methods convert all forms of phosphorus in water samples to orthophosphate. On the basis of two-population paired *t*-test statistics, the means of all Kjeldahl phosphorus and alkaline persulfate phosphorus concentrations (2,093 paired results) were not significantly different from zero at the $p = 0.05$ level. For some subsets of these data, which were grouped according to water type and flow conditions at the time of sample collection, differences between means of Kjeldahl phosphorus and alkaline persulfate phosphorus concentrations were not equivalent to zero

at the p = 0.05 level. Differences between means of these subsets, however, were less than the method detection limit for phosphorus (0.007 milligram phosphorus per liter) by the alkaline persulfate digestion method, and were therefore analytically insignificant.

This report provides details of the alkaline persulfate digestion procedure, interference studies, recovery of various nitrogen- and phosphorus-containing compounds, and other analytical figures of merit. The automated air-segmented continuous flow methods developed to determine nitrate and orthophosphate in the alkaline persulfate digests also are described. About 125 microliters of digested sample are required to determine nitrogen and phosphorus in parallel at a rate of about 100 samples per hour with less than 1-percent sample interaction. Method detection limits for nitrogen and phosphorus are 0.015 milligram nitrogen per liter and 0.007 milligram phosphorus per liter, respectively.

INTRODUCTION

Semiautomated, batch Kjeldahl digestion methods used at the U.S. Geological Survey (USGS) National Water Quality Laboratory (NWQL) for simultaneous nitrogen and phosphorus determinations in filtered- and whole-water samples (Patton and Truitt, 1992, 2000) are rapid and robust, but they suffer from several drawbacks, including:

- health and safety risks posed by concentrated acids, toxic reagents (mercury), and high temperatures (370°C);
- environmental effects and cost associated with processing and disposing of the mercury-containing waste stream;
- propensity of acidic digests to trap and become contaminated by ammonia vapors in ambient laboratory air; and
- laboratory reporting limits (0.1 mg-N/L; 0.04 mg-P/L) higher than those of other inorganic nitrogen- and phosphorus-containing species, which limit the precision of mass balance estimates.

Alkaline persulfate digestion (Valderrama, 1981; Hosomi and Sudo, 1986; D'Elia and others, 1987; Ameel and others, 1993; D'Elia and others, 1997) provides a safer and more environmentally benign alternative to Kjeldahl digestion for routine, single-digest nitrogen and phosphorus determinations in water. Desirable characteristics of alkaline persulfate digestion compared to Kjeldahl digestion include:

- reagents that contain no mercury;
- fume hoods and acid scrubbers are not needed because digestion occurs in sealed tubes inside an autoclave;
- post-digestion contamination by ambient ammonia vapors is not a problem because all nitrogen-containing compounds are oxidized to and determined as nitrate;
- laboratory reporting limits (0.03 mg-N/L; 0.01 mg-P/L) are similar to those of inorganic nitrogen- and phosphorus-containing nutrients; and
- waste-stream processing and disposal are straightforward.

During the past 15 years, alkaline persulfate digestion methods have been widely applied for estuarine and marine water analysis in preference to Kjeldahl digestion methods. Kjeldahl digestion methods continue to be widely applied for freshwater analysis, possibly because alkaline persulfate digestion methods are not approved for National Pollution Discharge Elimination System (NPDES) and Safe Drinking Water Act (SDWA) compliance monitoring. Nonetheless, an alkaline persulfate digestion method for total nitrogen determination (method 4500-N C, which does not include determination of phosphorus) is included in the 20th Edition of *Standard Methods* (American Public Health Association, 1998b, p. 4-102 and 4-103). Note, however, that the method described in this report differs in two important respects from method 4500-N C. First, method 4500-N C states "samples preserved with acid cannot be analyzed […]." The method described in this report is applicable to acidified nutrient samples—USGS FCA (filtered, chilled, acidified) and WCA (whole water, chilled, acidified) bottle types—provided that they have been processed according to USGS field manual protocols (Wilde and others, 1998). Second, nitrogen and phosphorus are recovered quantitatively from digests prepared by the method described in this report as explained in section 2.2. Furthermore, manual post-digestion pH adjustment prior to colorimetric determinations required by other previously published alkaline persulfate digestion methods (Valderrama, 1981; Hosomi and Sudo, 1986; D'Elia and others, 1987; Ameel and others, 1993; D'Elia and others, 1997) is not necessary in the method described in this report. This modification reduces digest preparation

time substantially. Hopefully, methodological improvements and comparative data in this report in concert with publication of *Standard Methods* method 4500-N C will encourage analysts and regulators to consider potential benefits of more widespread application of alkaline persulfate digestion as an alternative to Kjeldahl digestion for nitrogen and phosphorus determinations in freshwater regimes.

This report provides complete details of the large-scale and geographically diverse study conducted by the USGS between October 1, 2001, and September 30, 2002, to evaluate and validate alkaline persulfate digestion as a more sensitive, accurate, and less toxic alternative to Kjeldahl digestion for routine determination of nitrogen and phosphorus in surface- and ground-water samples.

Purpose and Scope

This report describes USGS methods I-2650-03 and I-4650-03 for determining total nitrogen and total phosphorus in filtered and whole-water alkaline persulfate digests, respectively. All aspects of the methods are described, including sample preparation and digestion, colorimetric determinations of nitrate and orthophosphate in alkaline persulfate digests, calculation of results, bias, precision, and repeatability of results, and conventions for reporting results. These methods supplement other methods of the USGS for determination of inorganic substances in water that are described by Fishman and Friedman (1989) and Fishman (1993). Primary objectives of this study were as follows:

1. To eliminate hazards and toxic wastes associated with Kjeldahl nitrogen and Kjeldahl phosphorus determinations.
2. To ascertain if and under what conditions alkaline persulfate digestion methods can be applied to samples preserved by acidification.
3. To develop an alkaline persulfate digestion procedure that is amenable to automation and less labor intensive than existing Kjeldahl digestion procedures.
4. To achieve lower detection limits for total and dissolved nitrogen than can be achieved by typical Kjeldahl digestion methods.
5. To evaluate statistical equivalence of dissolved and total nitrogen concentrations determined by Kjeldahl and alkaline persulfate digestion methods
6. To evaluate statistical equivalence of dissolved and total phosphorus concentrations determined by Kjeldahl and alkaline persulfate digestion methods.
7. To establish guidelines for interpreting dissolved and total nitrogen and phosphorus concentrations that result from alkaline persulfate digestion in relation to those that result from Kjeldahl digestion.
8. To verify that alkaline persulfate digestion is a more sensitive, accurate, and environmentally responsible alternative to Kjeldahl digestion for routine, simultaneous determination of nitrogen and phosphorus in surface and ground water—the conclusion of several previously published, smaller scale studies—on the basis of a large, geographically and seasonally diverse data set and to demonstrate the method's applicability for compliance monitoring and water-quality assessment studies.

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ANALYTICAL METHOD

Inorganic Constituents and Parameter Codes (see table 1): Nitrogen and phosphorus, total dissolved, I-2650-03 (mg/L as N or P); nitrogen and phosphorus, total whole water, I-4650-03 (mg/L as N or P)

1. Application

These methods are intended for determination of total nitrogen (organic nitrogen + ammonium + nitrate + nitrite) and phosphorus (all forms) in filtered and whole-water samples by alkaline persulfate digestion. They were validated for determination of total nitrogen and total phosphorus in drinking water, wastewater, and water-suspended sediment. Their applicability to bottom materials was not investigated. Analytical ranges are 0.03 to 5.00 mg-N/L for dissolved and total nitrogen and 0.01 to 2.00 mg-P/L for dissolved and total phosphorus.

2. Method Summary and Analytical Considerations

2.1 Filtered and whole-water samples are dispensed into glass culture tubes, dosed with alkaline persulfate reagent, capped tightly, and digested in an autoclave at 250ºF (121ºC) and 17 lb/in^2 (117.2 kPa) for 1 hour. The alkaline persulfate digestion procedure oxidizes all forms of inorganic and organic nitrogen to nitrate and hydrolyzes all forms of inorganic and organic phosphorus to orthophosphate. Nitrate and orthophosphate in alkaline persulfate digests are determined in parallel with a 2-channel photometric, air-segmented continuous flow analyzer.

2.2 Digest preparation protocols and reagent formulations were adapted from previously published procedures (Valderrama, 1981; Hosomi and Sudo, 1986; Ameel and others, 1993; D'Elia and others, 1997; American Public Health Association, 1998b). Two other reports (Nydahl, 1978; Cabrera and Beare, 1993) provided insight into the potential for low nitrogen recovery in samples containing high concentrations of dissolved and particulate organic carbon.

Quantitative recovery of nitrogen and phosphorus by alkaline persulfate digestion depends critically on a

Table 1. Laboratory, parameter, and method codes for U.S. Geological Survey alkaline persulfate digestion total nitrogen and total phosphorus methods I-2650-03 and I-4650-03

[Lab, laboratory; FCC, filtered chilled container; FCA, filtered, chilled, acidified; WCA, whole water, chilled, acidified; µm, micrometer; mL, milliliter; USGS, U.S. Geological Survey]

Description	Codes			Bottle type
	Lab	Parameter	Method	
Nitrogen, total dissolved, alkaline persulfate digestion	2754	62854	A	FCC[1]
Nitrogen, total dissolved, alkaline persulfate digestion, acidified	2755	62854	B	FCA[2]
Nitrogen, total whole-water, alkaline persulfate digestion, acidified	2756	62855	A	WCA[2]
Phosphorus, total dissolved, alkaline persulfate digestion	2757	00666	I	FCC[1]
Phosphorus, total dissolved, alkaline persulfate digestion, acidified	2758	00666	J	FCA[2]
Phosphorus, total whole-water, alkaline persulfate digestion, acidified	2759	00665	H	WCA[2]

[1]FCC samples must be processed through 0.45-µm filters at collection sites.

[2]FCA and WCA samples must be amended with 1 mL of 4.5 *N* H_2SO_4 solution (USGS water-quality field supply number Q438FLD) per 120 mL of sample at collection sites.

progressive decrease in pH (initial pH >12, final pH ≤ 2.2) during the 1-hour course of the digestion (Hosomi and Sudo, 1986). These dynamic reaction conditions are achieved by formulating the digestion reagent with approximately equimolar concentrations of persulfate and hydroxide ions—0.05 ***M***, initial pH >12 after 1 + 2 dilution by samples in this method. Under these initially alkaline conditions, dissolved and suspended nitrogen in samples oxidize to nitrate. As the digestion proceeds, bisulfate ions resulting from thermal decomposition of persulfate first neutralize and then acidify the reaction mixture by the following chemical reaction:

$$S_2O_8^{2-} + H_2O \xrightarrow{\Delta} 2\ HSO_4^{-} + \tfrac{1}{2}O_2$$

After all of the persulfate has decomposed, the digest mixture pH approaches 2, and under these acidic conditions, dissolved and suspended phosphorus hydrolyze to orthophosphate. The foregoing discussion indicates that analysis of samples with variable and unknown acidity or alkalinity by alkaline persulfate digestion methods will be problematic. Users of this method are cautioned that amending FCA and WCA samples with concentrations of sulfuric acid other than those specified in USGS field manual protocols (Wilde and others, 1998) likely will result in undetected method failure and possible reporting of erroneous results. See section 3.1.4 of this report for additional details.

As is the case for Kjeldahl digestion, alkaline persulfate digestion converts all forms of phosphorus to orthophosphate. Thus alkaline persulfate digestion dissolved and total phosphorus (DP_{AlkP} and TP_{AlkP}) concentrations can be compared directly with Kjeldahl digestion dissolved and total phosphorus (KDP and KTP) concentrations by graphical and statistical analysis. This is not the case, however, for Kjeldahl dissolved and total nitrogen (KDN and KTN) concentrations and alkaline persulfate digestion dissolved and total nitrogen (DN_{AlkP} and TN_{AlkP}) concentrations. In principle, organic nitrogen, but not nitrate or nitrite, is reduced to ammonium during Kjeldahl digestion. Determining ammonium in Kjeldahl digests, therefore, measures organic nitrogen + ammonium. Alkaline persulfate digestion oxidizes all forms of nitrogen to nitrate. Determining nitrate + nitrite in alkaline persulfate digests, therefore, measures total nitrogen (organic nitrogen + ammonium + nitrite + nitrate). To reconcile this difference between the two methods, nitrate + nitrite concentrations were subtracted from DN_{AlkP} and TN_{AlkP} concentrations prior to graphical and statistical comparisons with KDN and KTN concentrations throughout this report. For this purpose and as a quality-control (QC) check, all filtered and whole-water samples selected for alkaline persulfate digestion also were analyzed for dissolved nitrate + nitrite, ammonium, and orthophosphate on the same day that digests were prepared. Particulates were removed from acidified, whole-water samples (WCA bottle type) by 0.45-µm filtration prior to dissolved nutrient determinations, as described in section 4.6 of this report.

2.3 A 2-channel, air-segmented continuous flow analyzer was configured for simultaneous photometric determination of nitrate + nitrite and orthophosphate in alkaline persulfate digests. Nitrate + nitrite was determined by a cadmium-reduction, Griess-reaction method (Wood and others, 1967) equivalent to U.S. Environmental Protection Agency (USEPA) method 353.2 (U.S. Environmental Protection Agency, 1993) and U.S. Geological Survey (USGS) method I-2545-90 (Fishman, 1993, p. 157) except that sulfanilamide and N-(1-naphthy)ethylenediamine reagents were separate rather than combined. The analytical cartridge diagram is shown in figure 1. Orthophosphate was determined by a phosphoantimonylmolybdenum blue method (Murphy and Riley, 1962; Pai and others, 1990), which is equivalent to the 2-reagent variants (separate molybdate and ascorbic acid reagents) of USEPA method 365.1 (U.S. Environmental Protection Agency, 1993) and USGS method I-2601-90 (Fishman, 1993). The analytical cartridge diagram is shown in figure 2.

3. Interferences

3.1 Alkaline Persulfate Digestion

3.1.1 Chloride concentrations up to 1,000 mg/L (the highest tested for this report) do not interfere. Furthermore, because good results are obtained for seawater in 2 + 1 mixture with digestion reagent (D'Elia and others, 1997), chloride concentrations of about 10,000 mg/L apparently are tolerated provided that calibrants are matrix matched. Higher chloride concentrations, however, are likely to interfere because of reaction with persulfate to form oxychlorides or chlorine that might deplete persulfate required to oxidize inorganic and organic nitrogen

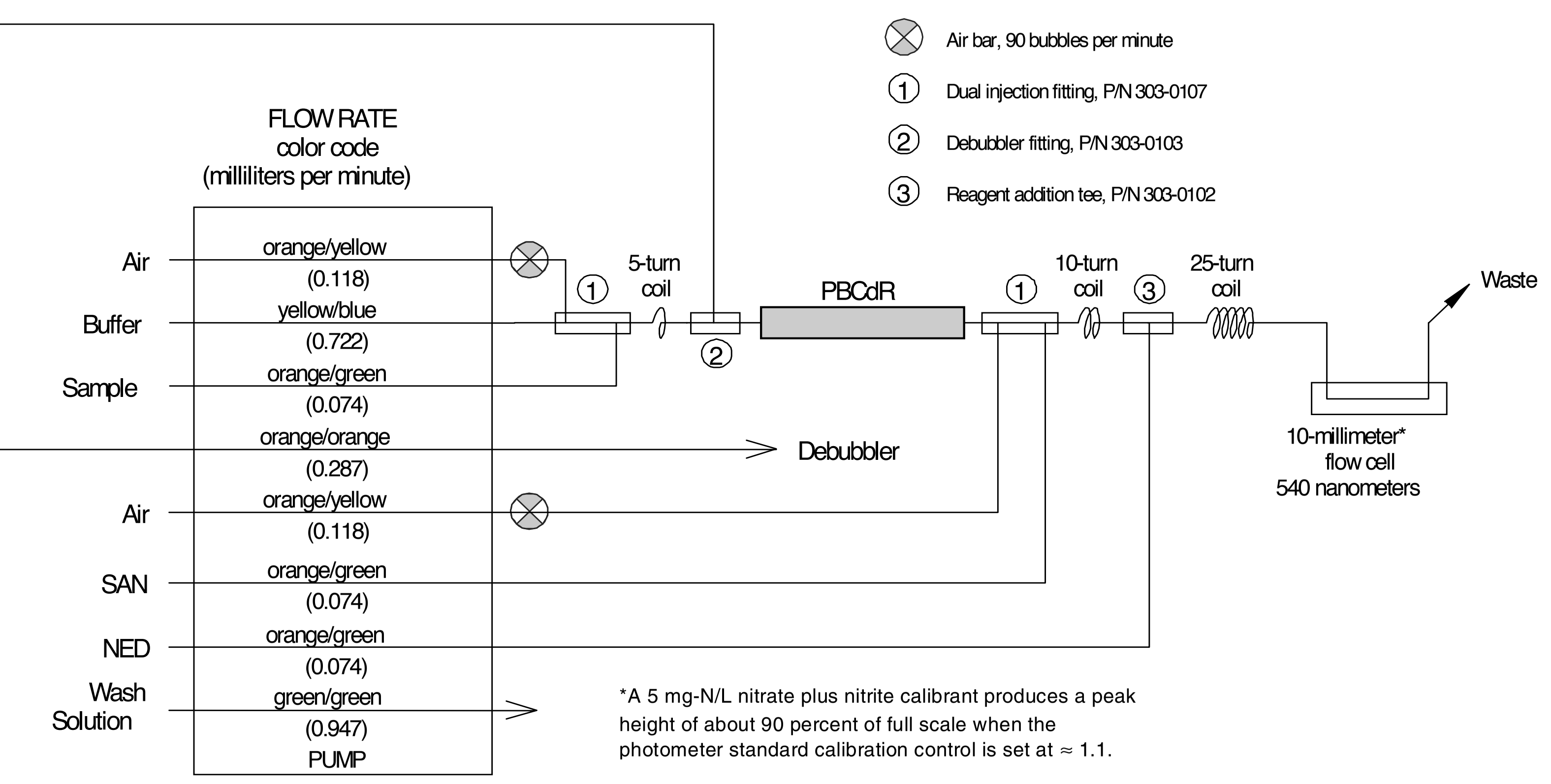

Figure 1. Analytical cartridge diagram for the air-segmented continuous flow analyzer (Alpkem RFA-300) used to automate photometric determination of nitrate + nitrite in alkaline persulfate digests with a cadmium-reduction, Griess reaction method.

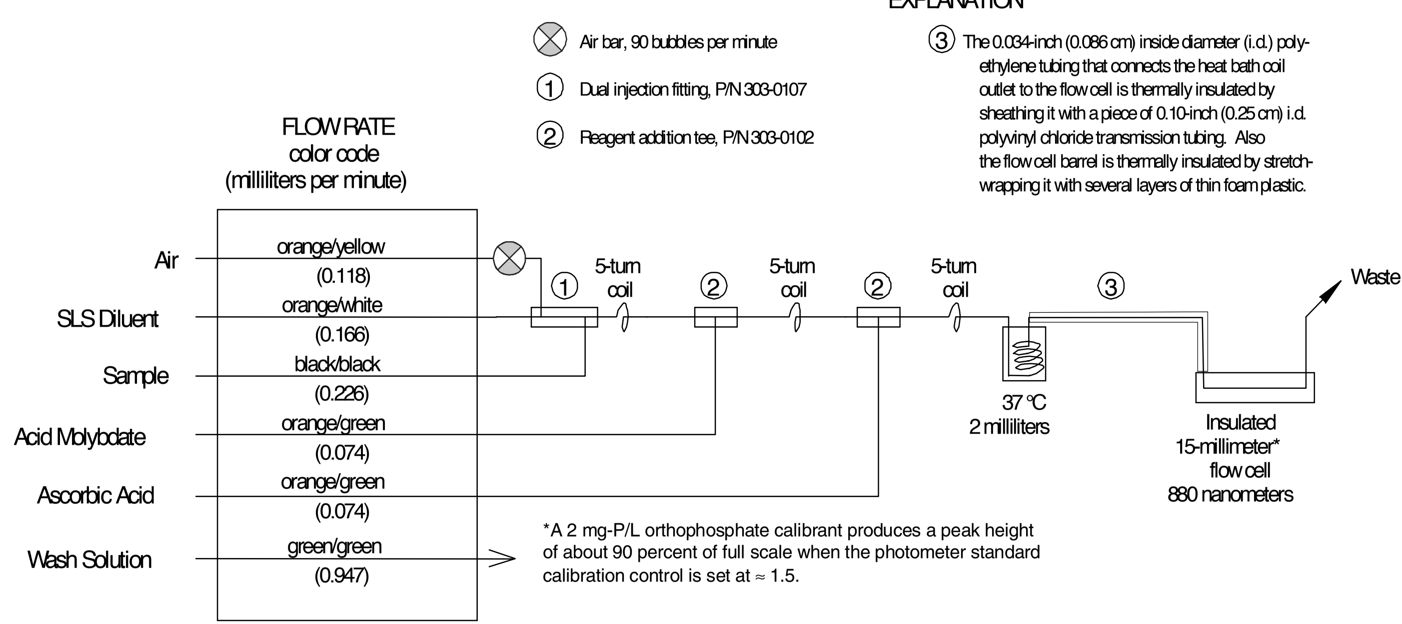

Figure 2. Analytical cartridge diagram for the air-segmented continuous flow analyzer (Alpkem RFA-300) used to automate photometric determination of orthophosphate in alkaline persulfate digests with a reduced phosphoantimonylmolybdenum blue reaction method.

species to nitrate. Resulting active chlorine species also can interfere in colorimetric reactions used to determine nitrate and orthophosphate in digests.

3.1.2 Sulfate concentrations up to 1,000 mg/L (the highest tested for this report) do not interfere.

3.1.3 Organic carbon concentrations greater than 150 mg/L interfere because of reaction with persulfate to form carbon dioxide, thus depleting persulfate required to oxidize inorganic and organic nitrogen species to nitrate.

3.1.4 Overacidification of FCA and WCA samples at collection sites can result in low recovery of inorganic and organic nitrogen at the NWQL. The possibility of overacidification can be avoided by exclusive use of the sulfuric acid field-amendment solution—one vial containing 1 mL of 4.5 N H_2SO_4 (One Stop Shopping number FLD-438) per 120 mL of sample—which is specified in the USGS National Field Manual (Wilde and others, 1998). See the first note in section 6.1 of this report for additional details.

3.1.5 Nitrate and nitrite do not contribute to KDN and KTN concentrations in principle, but in practice, positive and negative interferences by these ions are well known—see, for example, American Public Health Association, 1998c; Patton and Truitt, 2000. This interference can confound comparison of KN and N_{AlkP} concentrations when dissolved nitrate concentrations are greater than about 0.1 mg NO_3^--N/L.

3.1.6 Suspended particles remaining in digests must be removed by sedimentation and decantation or filtration prior to colorimetric analyses.

3.2 Colorimetric Nitrate + Nitrite Determination

3.2.1 Typically, concentrations of substances with potential to interfere in cadmium-reduction, Griess-reaction nitrate + nitrite methods are negligible in ambient surface- and ground-water samples. For specific details of inorganic and organic compounds that might interfere in the color reaction, see Norwitz and Keliher (1985, 1986), as well as more general information by the American Public Health Association (1998a).

3.2.2 Sulfides, which are often present in anoxic water and well known to deactivate cadmium reduction reactors, are oxidized during the alkaline persulfate digestion and are unlikely to interfere.

3.3 Colorimetric Orthophosphate Determination

3.3.1 Barium, lead, and silver can interfere by forming insoluble phosphates, but their concentrations in natural-water samples usually are less than the interference threshold (Fishman, 1993)

3.3.2 Interference from silicate, which also can form reduced heteropoly acids with molybdenum (Zhang and others, 1999), is negligible under reaction conditions used for this report.

3.3.3 Arsenate, AsO_4^{3-}—but not arsenite, AsO_3^{3-}—can interfere by forming reduced heteropoly acids analogous to those formed by orthophosphate (Johnson, 1971). Because of the possibility that arsenite might be oxidized to arsenate by persulfate, both species at concentrations up to 20 mg-As/L in deionized water were digested and analyzed. With reference to table 2, it is apparent that a major fraction of arsenite is oxidized to arsenate during alkaline persulfate digestion and that interference by either species up to 1 mg-As/L is negligible.

Table 2. Data from a study of arsenate and arsenite interference in alkaline persulfate total phosphorus determinations

[mg-As/L, milligrams of arsenic per liter; mg-P/L, milligrams of phosphorus per liter; nd, not detected; ≈, nearly equal to; ±, plus or minus]

AsO_4^{3-} added mg-As/L	PO_4^{3-} found mg-P/L	AsO_3^{3-} added mg-As/L	PO_4^{3-} found mg-P/L
0.5	nd	0.5	nd
1.0	nd	1.0	nd
2.0	≈ 0.05	2.0	nd
5.0	0.32 ± 0.01	5.0	0.29 ± 0.04
10.0	1.14 ± 0.13	10.0	0.91 ± 0.06
20.0	off scale	20.0	off scale

4. Instrumentation and Auxiliary Analyses

4.1 RFA-300™, third-generation, air-segmented continuous flow analyzers (Alpkem) were used to automate photometric determination of nitrate + nitrite and orthophosphate in alkaline persulfate digests and dissolved ammonium, nitrate + nitrite, and orthophosphate in filtered- and whole-water samples prior to digestion. Modules in these systems include 301 samplers, 302 peristaltic pumps, 313 analytical cartridge bases, 314 power modules, 305A photometers, and a personal computer (PC)-based data acquisition and processing system. Alternative instrumentation—flow injection analyzers, sequential injection analyzers, other second- or third-generation continuous flow analyzers, or automated batch analyzers—also could be used to automate photometric finishes.

4.2 Photometric data were acquired and processed automatically using FASPac™ version 1.34 software (Astoria-Pacific, Clackamas, Ore.). This software operates under Microsoft Windows on a PC platform and includes a model 350 interface box that controls the sampler and digitizes analog photometer outputs with 16-bit resolution. Other data acquisition systems could be used provided that the A/D converter has 16-bit resolution and is capable of acquiring data at frequencies ranging from 0.5 to 2 Hz, that is, from 30 points/min to 120 points/min. As a general rule, data acquisition frequencies for air-segmented continuous flow analyzers should match the roller lift-off frequency of the peristaltic pump (Patton and Wade, 1997), that is, 0.5 Hz for Technicon AutoAnalyzer II ™ and 1.5 Hz for Alpkem RFA-300 equipment. Data acquisition frequencies in the range of 2 to 5 Hz are suitable for photometric flow-injection analyzers.

4.3 Operating characteristics for this equipment are listed in table 3.

4.4 Dissolved ammonium, nitrate + nitrite, and orthophosphate in undigested samples were determined photometrically by USGS automated continuous flow methods I-2522-90, I-2545-90 (2-reagent variant), and I-2601-90 (2-reagent variant), respectively. These methods are described in Fishman (1993).

4.5 The pH of WCA samples was estimated with narrow range (0–2.5) colorimetric pH-indicating test strips to detect improperly acidified samples that had pH values outside the expected range of 1.6 to 1.9.

4.6 WCA samples were processed through 5-mL capacity UniPrep™ syringeless filters equipped with 0.45-μm nylon membranes (Whatman, Clifton, N.J.) to remove suspended solids prior to determination of dissolved ammonium, nitrate + nitrite, and orthophosphate. These syringeless filters also were used to remove suspended solids from WCA-sample digests prior to photometric analysis when simple sedimentation and decantation into analyzer cups failed to do so.

5. Apparatus

5.1 Samples were digested in an autoclave (model number STME, Market Forge Industries, Inc., Everett, Mass.) operated at 250ºF (121ºC) and 17 lb/in^2 (117.2 kPa) for 1 hour.

5.2 Filtered and chilled sample (FCC bottle type) digests were prepared robotically using a large-scale, syringe-pump-based *x-y-z* sample dispenser/diluter module (model number ML-4200, Hamilton Company, Reno, Nev.). This system is equipped with four probes and four 10-mL syringe pumps that operate in tandem under control of DOS-based *Eclipse*™ software (Hamilton Company, Reno, Nev.). Custom modifications to the ML-4200 system, including a pneumatically actuated probe expander, fixtures, and a variety of bottle and test-tube racks, were obtained from another vendor (Robotics Plus, Houston, Tex.).

5.3 Whole-water (WCA bottle type) sample digests were prepared manually using EDP Plus™

Table 3. Settings and operational details of Alpkem RFA-300 continuous flow analyzers used for this study

[nm, nanometer; mm, millimeter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ≈, nearly equal to; min, minute; mL, milliliter; –, not applicable; °C, degrees Celsius; s, second; h, hour]

Instrumental conditions	Nitrate + nitrite	Orthophosphate
Analytical wavelength	540 nm	880 nm
Flow cell path length	10 mm	15 mm
Calibration range	0.05 to 5.0 mg-N/L	0.01 to 2.0 mg-P/L
Standard calibration control setting	≈1.1	≈1.5
Segmentation rate (bubbles min^{-1})	90	90
Heated reaction coil volume	None used	2 mL
Heated reaction coil temperature	–	37°C
Dwell time (seconds)	140	260
Sample time (volume)	25 s (95 μL)	25 s (31 μL)
Wash time (volume)	10 s (38 μL)	10 s (12 μL)
Analysis rate, sample-to-wash ratio	≈103/h, 5:2	≈103/h, 5:2

electronic, digital pipets (Rainin Instruments, Emeryville, Calif.) equipped with a 10-mL liquid end.

5.4 Digestion vessels were 20 x 150 mm Pyrex®, screw-cap culture tubes (VWR 53283-810; Fisher 14-957-76E or 14-959-37C; or equivalent), and 18-415 linerless polypropylene caps (Comair Glass, Inc., Vineland, N.J.—Part number 14-0441-004).

6. Reagents

This section provides detailed instructions for preparing digestion and colorimetric reagents. All references to deionized water (DI) refer to NWQL in-house DI water, which is equivalent to ASTM type I DI water (American Society for Testing and Materials, 2001, p. 107–109) for nutrient analysis. All volumetric glassware and reagent and calibrant storage containers should be triple rinsed with dilute (≈5 percent v/v) hydrochloric acid and DI water just prior to use. Additionally storage containers for reagents and calibrants should be triple rinsed with small portions of the solutions before they are filled.

6.1 Digestion Reagents

NOTE: The alkaline persulfate digestion reagent for FCA and WCA samples (section 6.1.4) contains an additional amount of sodium hydroxide that is calculated to neutralize the sulfuric acid added to these samples at collection sites.

6.1.1 *Sodium hydroxide, 1.5* ***M*** *(for FCC samples)*: Dissolve 60 g of sodium hydroxide (NaOH, FW=40.0) in about 800 mL of DI water in a 1-L volumetric flask. [**Caution:** When NaOH dissolves in water, heat is released.] After dissolution is complete, allow the resulting solution to cool and dilute it to the mark with DI water. Transfer this reagent to a plastic bottle in which it is stable at room temperature for 6 months.

6.1.2 *Sodium hydroxide, 2.3* ***M*** *(for FCA and WCA samples)*: Dissolve 92 g of sodium hydroxide (NaOH, FW=40.0) in about 800 mL of DI water in a 1-L volumetric flask. [**See caution in 6.1.1.**] After dissolution is complete, allow the resulting solution to cool and dilute it to the mark with DI water. Transfer this reagent to a plastic bottle in which it is stable at room temperature for 6 months.

6.1.3 *Alkaline persulfate digestion reagent (for FCC samples)*: Add 18.0 g of potassium persulfate ($K_2S_2O_8$, FW=270.33) and 45 mL of 1.5 ***M*** sodium hydroxide solution to about 350 mL of DI water in a graduated 500-mL Pyrex™ media bottle (Corning number 1395-500 or equivalent). Cap the bottle, swirl its contents, and place it in an ultrasonic bath until potassium persulfate dissolution is complete (about 10 minutes). Remove the bottle from the ultrasonic bath, dry its outer surfaces, and then add enough DI water to bring the volume to 450 mL. (Make a line on the side of the bottle that indicates this volume to within ±5 mL.) Swirl the bottle to mix its contents and then divide the resulting solution among four, 125-mL clear plastic bottles used with the robotic digest preparation system. Prepare this reagent daily.

6.1.4 *Alkaline persulfate digestion reagent (for FCA and WCA samples)*: Add 18.0 g of potassium persulfate ($K_2S_2O_8$, FW=270.33) and 45 mL of 2.3 ***M*** sodium hydroxide solution to about 350 mL of DI water in a graduated 500-mL Pyrex™ media bottle (Corning number 1395-500 or equivalent). Then complete preparation of this reagent exactly as described in 6.1.3. Prepare this reagent daily.

NOTE: Reagent volumes in 6.1.3 and 6.1.4 (450 mL) are sufficient to prepare 80 digests plus a 15-percent excess for rinsing and providing a liquid level in the 125-mL bottles necessary to prevent air aspiration during robotic dispensing operations. For manual digest preparation, a 400-mL volume of digestion reagent should be sufficient.

6.2 Colorimetric Reagents

6.2.1 *Sampler wash reservoir solution (0.05* ***M*** *sodium bisulfate)*: Dissolve 6.9 g of sodium bisulfate ($NaHSO_4 \bullet H_2O$, FW=138.08) in about 800 mL of DI water in a graduated 1-L Pyrex™ media bottle. Dilute this solution to the mark with DI water, mix it well, and store it tightly capped at room temperature.

NOTE: This solution matches the matrix of sample digests. Use it as the matrix for continuing calibration verification (CCV) solutions and any other undigested check samples.

6.3 Orthophosphate Determination

6.3.1 *Stock potassium antimony tartrate reagent*: Dissolve 3.0 g of antimony potassium tartrate [$K(SbO)C_4H_4O_7 \bullet \frac{1}{2} H_2O$, FW=333.93] in about 800 mL of DI water in a 1-L volumetric flask. Dilute this solution to the mark with DI water and mix it well.

Transfer this reagent to a plastic bottle in which it is stable for 6 months at room temperature.

6.3.2 *Stock ascorbic acid reagent*: Dissolve 4.5 g of ascorbic acid ($C_6H_8O_6$, FW=176.1) in about 200 mL of DI water in a 250-mL volumetric flask. Dilute this solution to the mark with DI water, mix it well, and transfer to a 250-mL glass bottle that has been previously rinsed with 5 percent (v/v) hydrochloric acid solution and DI water. This reagent is stable for 2 weeks at 4°C.

6.3.3 *Stock sodium lauryl sulfate reagent (15 percent w/w)*: Add 340 mL of DI water to 60 g of sodium lauryl sulfate [SLS, $CH_3(CH_2)_{11}OSO_3Na$, FW=288.38] in a 500-mL Pyrex™ media bottle. Cap the bottle and place it in an ultrasonic bath until the SLS dissolves completely (about 30 minutes). Manual inversion of the bottle at 5-minute intervals speeds dissolution. Transfer this solution to a plastic bottle in which it is stable indefinitely at room temperature.

6.3.4 *Acidic molybdate-antimony reagent*: Using a graduated cylinder, cautiously add 72 mL of concentrated sulfuric acid (H_2SO_4, sp. gr. 1.84) to about 700 mL of DI water in a 1-L volumetric flask. Work in a hood and manually swirl or magnetically stir the flask during each addition of sulfuric acid. Next add 7.7 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}$•$4H_2O$, FW=1235.86] to the hot sulfuric acid solution. Manually swirl or magnetically stir the contents of the flask until the ammonium molybdate dissolves. Then add 50 mL of stock antimony potassium tartrate solution (6.3.1) and again mix the contents of the flask thoroughly. After the resulting solution has cooled, dilute it to the mark with DI water, mix it well, and transfer it to a clean 1-L plastic bottle in which it is stable for 1 year at room temperature.

6.3.5 *Sodium lauryl sulfate diluent reagent*: Use a 100-mL graduated cylinder to dispense 10 mL of stock SLS (6.3.3) and 90 mL of DI water into a small plastic bottle. Manually swirl the bottle to mix its contents. Prepare this reagent daily.

6.3.6 *Ascorbic acid reagent*: Use a 50-mL graduated cylinder to dispense 5 mL of the stock ascorbic acid reagent (6.3.2) and 25 mL of DI water into an amber glass reagent bottle. Manually swirl the bottle to mix its contents. Prepare this solution daily.

6.3.7 *Startup/shutdown solution*: Add 1 mL of stock SLS reagent to 100 mL of DI water in a small plastic bottle. Thoroughly rinse the bottle and prepare a fresh solution every few days or as needed.

6.4 Nitrate Determination

6.4.1 *Copper (II) sulfate reagent (2 percent w/v)*: Dissolve 20 g of copper sulfate pentahydrate ($CuSO_4$•$5H_2O$, FW=249.7) in about 800 mL of DI water in a 1-L volumetric flask. Dilute this solution to the mark with DI water, mix it well, and transfer it to a 1-L plastic bottle. This reagent is stable for several years at room temperature.

6.4.2 *Imidazole buffer, 0.1* ***M****, (pH 7.5)*: In a hood, cautiously add 5.0 mL of concentrated hydrochloric acid (HCl, ~12 ***M***) and 1.0 mL of 2 percent copper sulfate solution to 1,600 mL of DI water in a 2-L volumetric flask. Mix the contents of the flask thoroughly and then add 13.6 g of imidazole ($C_3H_4N_2$, FW=68.08). Again swirl or shake the flask until the imidazole dissolves. Dilute the resulting solution to the mark with DI water, mix it well, and transfer it into two 1-L plastic bottles. This reagent is stable for 6 months at room temperature.

NOTE: Add 250 µL of Brij-35 surfactant to 250 mL of imidazole buffer each time its container is refilled on the continuous flow analyzer. Do not add Brij-35 to the bulk buffer solution.

6.4.3 *Packed bed cadmium reactor*: Cadmium reactors are prepared by slurry packing 40- to 60-mesh, copperized cadmium granules into 6-cm lengths of PTFE Teflon™ tubing (1.6 mm i.d. × 3.2 mm o.d.). Cadmium granules are retained in the column with hydrophilic plastic frits (40-µm nominal pore size). Detailed instructions for preparing copperized cadmium granules and packing them into columns can be found in NWQL standard operating procedure (SOP) IM0384.0 (or subsequent revisions; available on request).

6.4.4 *Sulfanilamide reagent ("SAN")*: Use a graduated cylinder to dispense 100 mL of concentrated hydrochloric acid (HCl, 36.5–38.0 percent, ≈12 ***M***) into about 700 mL of DI water in a 1-L volumetric flask. Work in a hood and manually swirl or magnetically stir the flask during each addition of HCl. Add 10.0 g of SAN ($C_6H_8N_2O_2S$, FW=172.20) to the warm hydrochloric acid solution. Manually shake, sonicate, or magnetically stir the contents of the flask until the SAN dissolves. After the resulting solution has cooled, dilute it to the mark with DI water, mix it well, and transfer it to a clean 1-L plastic bottle in which it is stable for 1 year at room temperature.

6.4.5 *N-(1-Naphthyl)ethylenediamine dihydrochloride reagent ("NED")*: Dissolve 1.0 g

NED ($C_{12}H_{14}N_2$•2HCl, FW=259.2) in about 800 mL of DI water in a 1-L volumetric flask. Dilute the resulting solution to the mark with DI water and mix well by manually shaking the flask. Transfer this reagent to a 1-L amber glass bottle in which it is stable for 6 months at room temperature.

6.4.6 *Startup/shutdown solution*: Add 250 µL of Brij-35 surfactant to 250 mL of DI water in a plastic bottle. Thoroughly rinse the bottle and prepare a fresh solution every few days or as needed.

7. Calibrants and Quality-Control Solutions

This section provides detailed instructions for preparing calibrants, matrix spike solution, quality-control check solutions, and digestion check solution.

7.1 *Potassium nitrate stock calibrant solution, 1 mL=2.5 mg-N*: Dissolve 1.805 g of potassium nitrate (KNO_3, FW=101.1) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock calibrant to a 100-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.2 *Potassium di-hydrogen phosphate stock calibrant solution, 1 mL=1.0 mg-P*: Dissolve 0.4394 g potassium di-hydrogen phosphate (KH_2PO_4, FW=136.09) in about 80 mL of DI water in a 100-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock calibrant to a 100-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.3 *Sulfuric acid* ≈1.8 ***M***: Use a 25-mL graduated cylinder to dispense 10 mL of concentrated sulfuric acid (H_2SO_4, sp. gr. 1.84) into about 75 mL of DI water in a 100-mL volumetric flask. After the solution cools, dilute it to the mark with DI water, mix it well, and transfer it to a 125-mL plastic bottle. Make a new batch of this acid each time acidified working calibrants and blanks are prepared and use the remainder to prepare acidified blank solution as needed.

7.4 *Mixed stock calibrant solution, 1 mL = 1.25 mg-N and 0.5 mg-P*: Dispense equal volumes (minimum of 2 mL each) of nitrate (7.1) and phosphate (7.2) stock calibrants into a small beaker and mix them thoroughly. Prepare this solution each time working calibrants are prepared.

7.5 *Working calibrant solutions (for FCC samples)*: Use two adjustable, digital pipets (ranges 10 to 100 µL and 100 to 1,000 µL) to dispense the volumes of mixed stock calibrant (7.4) listed in table 4 into 250-mL volumetric flasks that each contain about 200 mL of DI water. Dilute the working calibrants to the mark with DI water and mix them thoroughly by manual inversion and shaking. Transfer the working calibrants to 250-mL Pyrex™ media bottles in which they are stable for 4 weeks at 4°C.

7.6 *Acidified working calibrant solutions (for FCA and WCA samples)*: Prepare these calibrants

Table 4. Volumes of mixed calibrant and amendment solution required to prepare working calibrants and blanks for determination of total nitrogen and phosphorus by the alkaline persulfate digestion method. Final volumes are 250 mL

[µL, microliter; mL, milliliter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ***M***, molarity (moles per liter); FCA, filtered, chilled, acidified (bottle type); WCA, whole water, chilled, acidified (bottle type)]

Calibrant identity	Mixed calibrant volume (µL)	Volume 1.8 *M* H_2SO_4 [1] (mL)	Nominal concentration (mg-N/L)	Nominal concentration (mg-P/L)
C1	1,000	2.5	5.00	2.00
C2	750	2.5	3.75	1.50
C3	500	2.5	2.50	1.00
C4	250	2.5	1.25	0.50
C5	100	2.5	0.50	0.20
C6	[2] 6	[2] 2.5	0.03	0.012
C7	0	2.5	0	0

[1]Add H_2SO_4 only to acidified calibrants as described in section 7.6.

[2]Prepare 1 L of C6 (24 µL of mixed calibrant and 10 mL of 1.8 ***M*** H_2SO_4, if appropriate, diluted to 1 L with DI water) to minimize dispensing error.

identically to those described in section 7.5, except add 2.5 mL of 1.8 ***M*** H_2SO_4 to each flask before diluting it to the mark with DI water.

7.7 *Check standards (for FCC samples)*: Check standards in three concentration ranges, which were designated *Low*, *High*, and *Very high*, were prepared from a concentrated commercial nutrient QC mixture (*Demand*™, Environmental Resource Associates, Arvada, Colo.), as listed in table 5. Transfer check standards to 1-L Pyrex™ media bottles in which they are stable for 2 months at 4ºC. Each of these check standards was dispensed, digested, and analyzed along with every batch of filtered and whole-water samples analyzed for this study.

7.8 *Acidified check standards (for FCA and WCA samples)*: Prepare these check standards identically to those described in section 7.7, except add 10.0 mL of 1.8 ***M*** H_2SO_4 to the flasks before diluting them to the mark with DI water.

7.9 Spike Solutions

7.9.1 *Nitrogen stock spike solution (1 mL = 0.50 mg-N)*: Dissolve 0.955 g ammonium chloride (NH_4Cl, FW=53.49) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock spike solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.9.2 *Phosphorus stock spike solution (1 mL = 0.20 mg-P)*: Dissolve 0.439 g potassium dihydrogen phosphate (KH_2PO_4, FW=136.1) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock spike solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.9.3 *Mixed spike solution (100 µL = 0.005 mg-N and 0.002 mg-P)*: Dispense 1 mL each of ammonium chloride and orthophosphate stock spike solutions into a 10-mL volumetric flask and dilute to the mark with DI water. Transfer the mixed spike solution to a 15-mL, screw-cap polyethylene centrifuge tube in which it is stable for 2 weeks at 4°C.

NOTE: An equivalent mixed spike solution can be prepared more conveniently from stock calibrants (sections 7.1 and 7.2) by diluting 500 µL of each to 25 mL in a volumetric flask.

7.10 Digest-Check Stock Solutions

7.10.1 *Glycine digest-check stock solution (1 mL = 1.0 mg-N)*: Dissolve 3.98 g glycine ($C_2H_5NO_2$•HCl, FW=111.5) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.10.2 *Glycerophosphate digest-check stock solution (1 mL = 0.4 mg-P)*: Dissolve 1.976 g glycerophosphate ($C_3H_7O_6PNa_2$•$5H_2O$, FW=306.1) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

7.10.3 *Glucose digest-check stock solution (1 mL = 1.25 mg-C)*: Dissolve 1.564 g glucose ($C_6H_{12}O_6$, FW=180.2) in about 400 mL of DI water in a 500-mL volumetric flask. Dilute this solution to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 500-mL Pyrex™ media bottle in which it is stable for 6 months at 4°C.

Table 5. Volumes of Environmental Resource Associates (ERA) *Demand*™ nutrient concentrate used to prepare 1-liter volumes of check standards used in this study

[µL, microliter; mL, milliliter; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter]

Check standard identity	ERA *Demand*™ volume (µL)	Volume 1.8 *M* H_2SO_4 [1] (mL)	Nominal concentration (mg–N/L)	Nominal concentration (mg–P/L)
Low	100	10.0	0.22	0.11
High	500	10.0	1.09	0.54
Very high	1,000	10.0	2.20	1.08

[1] Add H_2SO_4 only to acidified check standards as described in section 7.8.

7.10.4 *Mixed digest-check solution (for FCC samples—nominal concentration 4 mg-N/L, 1.6 mg-P/L, and 50 mg-C/L)*: Dispense 1 mL each of glycine and glycerophosphate stock digest-check solutions and 10 mL of the glucose digest-check stock solution into a 250-mL volumetric flask that contains about 200 mL of DI water. Dilute the contents of the flask to the mark with DI water and mix it thoroughly by manual inversion and shaking. Transfer the stock digest-check solution to a 250-mL Pyrex™ media bottle in which it is stable for 1 month at 4°C.

7.10.5 *Acidified mixed digest-check solution (for FCA and WCA samples)*: Prepare this digest-check solution identically to the one described in section 7.10.4, except add 2.5 mL of 1.8 ***M*** H_2SO_4 to the flask before diluting its contents to the mark with DI water. Transfer the acidified mixed digest-check solution to a 250-mL Pyrex™ media bottle in which it is stable at 4°C for 1 month.

8. Sample Preparation

8.1 Alkaline persulfate digests are prepared by dispensing samples and digestion reagent into 30-mL, screw-cap, Pyrex™ culture tubes in the volume ratio of 2 + 1. For filtered samples (FCC bottle types) that were prepared robotically, 9.5-mL volumes of samples, blanks, calibrants, and reference materials were dosed with 4.75-mL volumes of alkaline persulfate digestion reagent (see section 6.1.3). This is the maximum sample volume that could be delivered by the robotic dispenser/diluter system's 10.000-mL syringes because 0.500 mL of their capacity is expended in the creation of air gaps that minimize interaction between samples and the DI water carrier fluid. Whole-water samples (WCA bottle types) that require vigorous shaking (and in a few cases, continuous magnetic stirring) just prior to dispensing operations were prepared manually with conventional, high-precision, hand-held electronic pipets (Rainin EDP *Plus*™). Here dispensed volumes of sample and digestion reagent (see section 6.1.4) were 10.000 and 5.000 mL, respectively. After robotic or manual sample and reagent-dispensing operations are complete, 100 µL of mixed spike solution (see section 7.9.3) is added manually to the designated tube. Then all tubes are capped tightly and mixed thoroughly either by manual inversion (three times) or with a vortex mixer (3, 5-second cycles). The capped tubes positioned in a purpose-built, 80-position stainless-steel rack then are placed in an autoclave where they are digested at 121ºC and 117.2 kPa for 1 hour. Table 6 lists the rack protocol suggested for a batch of 80 tubes consisting of up to 64 samples plus six calibrants, four blanks, three quality-control (QC) check solutions, one digest-check solution, one duplicate sample, and one spiked sample. A step-by-step procedure for

Table 6. Suggested rack protocol for alkaline persulfate digest preparation

[ID, identification; QC, quality control; yyyy, year; ddd, Julian day]

Tube number	ID	Tube number	ID	Tube number	ID	Tube number	ID
1	C1	21	yyyyddd0007	41	yyyyddd0027	61	yyyyddd0047
2	C2	22	yyyyddd0008	42	yyyyddd0028	62	yyyyddd0048
3	C3	23	yyyyddd0009	43	yyyyddd0029	63	yyyyddd0049
4	C4	24	yyyyddd0010	44	yyyyddd0030	64	yyyyddd0050
5	C5	25	yyyyddd0011	45	yyyyddd0031	65	yyyyddd0051
6	C6	26	yyyyddd0012	46	yyyyddd0032	66	yyyyddd0052
7	C7 (blank)	27	yyyyddd0013	47	yyyyddd0033	67	yyyyddd0053
8	blank	28	yyyyddd0014	48	yyyyddd0034	68	yyyyddd0054
9	blank	29	yyyyddd0015	49	yyyyddd0035	69	yyyyddd0055
10	blank	30	yyyyddd0016	50	yyyyddd0036	70	yyyyddd0056
11	QC low	31	yyyyddd0017	51	yyyyddd0037	71	yyyyddd0057
12	Digest check	32	yyyyddd0018	52	yyyyddd0038	72	yyyyddd0058
13	QC high	33	yyyyddd0019	53	yyyyddd0039	73	yyyyddd0059
14	QC very high	34	yyyyddd0020	54	yyyyddd0040	74	yyyyddd0060
15	yyyyddd0001	35	yyyyddd0021	55	yyyyddd0041	75	yyyyddd0061
16	yyyyddd0002	36	yyyyddd0022	56	yyyyddd0042	76	yyyyddd0062
17	yyyyddd0003	37	yyyyddd0023	57	yyyyddd0043	77	yyyyddd0063
18	yyyyddd0004	38	yyyyddd0024	58	yyyyddd0044	78	yyyyddd0064
19	yyyyddd0005	39	yyyyddd0025	59	yyyyddd0045	79	Duplicate
20	yyyyddd0006	40	yyyyddd0026	60	yyyyddd0046	80	Spike

alkaline persulfate digest preparation is provided in NWQL SOP IM0384.0 (available on request).

NOTE: When samples contain large quantities of suspended solids, continuous stirring during sample aspiration might provide the only means of obtaining representative aliquots.

8.2 When the digestion cycle is complete and pressure and temperature gages on the autoclave indicate 0 kPa and less than 80°C, remove the alkaline persulfate digests from the autoclave and allow them to cool sufficiently to be handled comfortably. Then mix the contents of each capped digestion tube by manual inversion (three times) or with a vortex mixer (three, 5-second cycles). FCC and FCA digests can be poured into analyzer cups immediately after mixing. Wait about 1 hour after mixing WCA digests to allow suspended solids to settle. If it is not possible to decant or pipet a clear supernatant solution from digest tubes into analyzer cups, then suspended solids must be removed by 0.45-µm filtration prior to colorimetric analysis. Note that tightly capped digests can be stored at room temperature for several days (4 days was the maximum delay tested) before their nitrogen and phosphorus concentrations are determined by automated colorimetry.

9. Instrument Performance

An 80-tube batch of samples, calibrants, and reference materials can be prepared robotically and made ready for digestion in about 1 hour. Digestion time—including warm up, cool down, and postdigestion mixing—is about 2 hours. The NWQL Nutrients Unit has two autoclaves, each of which can hold two, 80-tube racks of alkaline persulfate digests. Nitrate and orthophosphate in alkaline persulfate digests can be determined simultaneously with the 2-channel air-segmented continuous flow analyzer at a rate of about 100 samples per hour with less than 1 percent interaction. Thus, using a combination of robotic and manual sample preparation, up to six racks (384 actual samples out of 480 total tubes) of alkaline persulfate digests can be prepared in an 8-hour day. This estimate assumes the use of both NWQL autoclaves and a combination of robotic (FCC samples) and manual (WCA samples) sample preparation. Likewise, up to six racks of previously digested samples can be analyzed for nitrate and orthophosphate in an 8-hour day. This production rate assumes that digest analysis can lag sample digestion by 1 to 3 days.

10. Calibration

With a second-order polynomial least-squares curve-fitting function ($y = a+bx+cx^2$, where y is the baseline and blank-corrected peak height and x is the nominal concentration), calibration plots with correlation coefficients (r^2) greater than 0.999 are achieved routinely. Typical calibration plots for nitrate and orthophosphate in alkaline persulfate digests are shown in figures 3 and 4.

NOTE: In addition to baseline drift correction, a digestion blank correction must be applied to calibrants, check standards, and samples prior to calculation of final results, as described in sections 12.3 and 12.4.

11. Procedure and Data Evaluation

Set up the continuous flow analyzer analytical cartridges as shown in figures 1 and 2. Turn on electrical power to all system modules and put fresh sampler wash reservoir solution and reagents on-line. After about 10 minutes, verify that the sample and reference outputs of both photometers are set at about 5 volts. A suggested sampler tray protocol for automated determination of nitrate and orthophosphate in alkaline persulfate digests is listed in table 7.

NOTE: To minimize errors that result from contaminated analyzer cups, rinse them several times with the solution they are to contain before placing them on the analyzer sampler tray.

NOTE: The full-scale absorbance range control (STD CAL) of photometers should not require daily adjustment. Between-analysis/between-day variations in baseline-absorbance level and calibration curve slope of about ±5 percent are acceptable. Adjustment of the STD CAL control to compensate for larger variations in sensitivity or baseline (reagent blank) levels will only mask underlying problems, such as incipient light source failure, partially clogged flow cells, or contaminated or improperly prepared reagents, any of which could compromise analytical results.

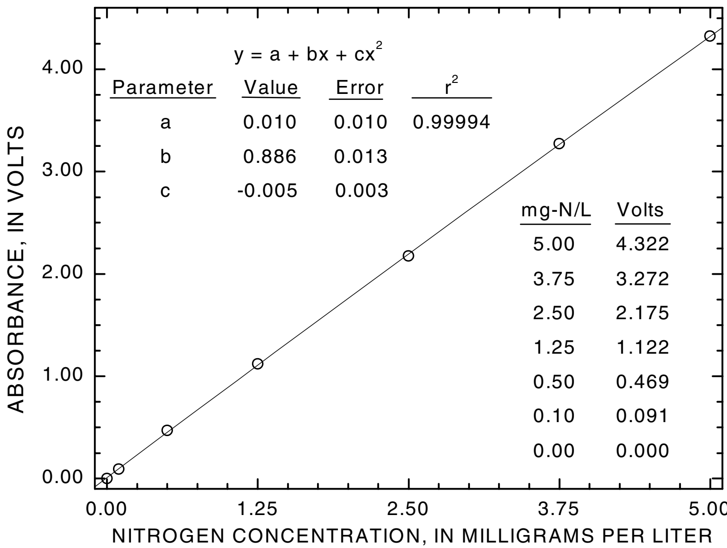

Figure 3. Typical calibration graph for total nitrogen determined as nitrate in alkaline persulfate digests.

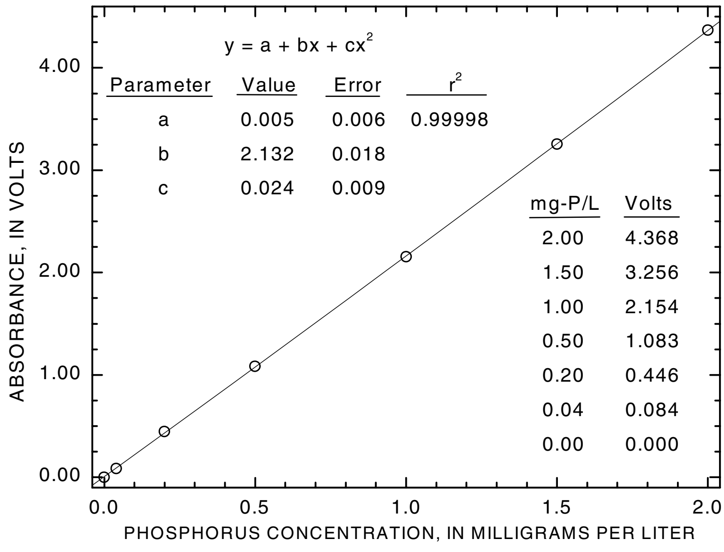

Figure 4. Typical calibration graph for total phosphorus determined as orthophosphate in alkaline persulfate digests.

12. Calculations

12.1 Instrument calibration requires preparing a set of solutions (calibrants) in which the analyte concentration is known. These calibrants are digested along with samples and used to establish a calibration function that is estimated from a least-squares fit of nominal calibrant concentrations (x) in relation to peak absorbance (y). A second-order polynomial function ($y = a+bx+cx^2$) usually provides improved concentration estimates at the upper end of the calibration range than a more conventional linear function ($y = a+bx$). Accuracy is not lost when a second-order fit is used, even if the calibration function is strictly linear, because, in this case, the value estimated for the quadratic parameter c will approach zero.

12.2 Before the calibration function can be estimated, the baseline absorbance component of measured peak heights, including drift (continuous increase or decrease in the baseline absorbance during the course of an analysis), if present, needs to be removed. Baseline absorbance in continuous flow analysis is analogous to the reagent blank absorbance in batch analysis. Correction for baseline absorbance is an automatic function of most data acquisition and processing software sold by vendors of continuous flow analyzers.

NOTE: These correction algorithms are based on linear interpolation between initial and intermediate or final baseline measurements, and so they do not accurately correct for abrupt, step-changes in baseline absorbance that usually indicate partial flow-cell blockage. It is prudent, therefore, to reestablish baseline absorbance at intervals of 20 samples or so.

Table 7. Suggested analyzer sample tray protocol for automated determination of nitrate and orthophosphate in alkaline persulfate digests

[#, number; ID, identification; SYNC, synchronization peak; CO, carry-over peak; W, wash; UB, undigested blank; DB, digested blank; CCV, continuing calibration verification; QC, quality control; yyyy, year; ddd, Julian day]

Cup #	ID	Cup #	ID	Cup #	ID	Cup #	ID
1	SYNC	24	yyyyddd0006	47	yyyyddd0029	70	yyyyddd0050
2	CO (C6)	25	yyyyddd0007	48	yyyyddd0030	71	yyyyddd0051
3	(C6)	26	yyyyddd0008	49	yyyyddd0031	72	yyyyddd0052
4	W	27	yyyyddd0009	50	yyyyddd0032	73	yyyyddd0053
5	C1	28	yyyyddd0010	51	UB	74	yyyyddd0054
6	C2	29	yyyyddd0011	52	W (DB)	75	yyyyddd0055
7	C3	30	yyyyddd0012	53	yyyyddd0033	76	yyyyddd0056
8	C4	31	yyyyddd0013	54	yyyyddd0034	77	yyyyddd0057
9	C5	32	yyyyddd0014	55	yyyyddd0035	78	yyyyddd0058
10	C6	33	yyyyddd0015	56	yyyyddd0036	79	yyyyddd0059
11	C7	34	yyyyddd0016	57	yyyyddd0037	80	yyyyddd0060
12	W	35	yyyyddd0017	58	yyyyddd0038	81	yyyyddd0061
13	CCV	36	yyyyddd0018	59	yyyyddd0039	82	yyyyddd0062
14	UB[1]	37	yyyyddd0019	60	yyyyddd0040	83	yyyyddd0063
15	QC low[2]	38	yyyyddd0020	61	yyyyddd0041	84	yyyyddd0064
16	Digest check[3]	39	yyyyddd0021	62	yyyyddd0042	85	duplicate
17	QC high[2]	40	yyyyddd0022	63	yyyyddd0043	86	Spike
18	QC very high[2]	41	yyyyddd0023	64	yyyyddd0044	87	UB
19	yyyyddd0001	42	yyyyddd0024	65	yyyyddd0045	88	CCV
20	yyyyddd0002	43	yyyyddd0025	66	yyyyddd0046	89	UB
21	yyyyddd0003	44	yyyyddd0026	67	yyyyddd0047	90	W (DB)
22	yyyyddd0004	45	yyyyddd0027	68	yyyyddd0048		
23	yyyyddd0005	46	yyyyddd0028	69	yyyyddd0049		

[1]Undigested blank (sampler wash reservoir solution, see section 6.2.1).
[2]NWQL Check Standard, see sections 7.7 and 7.8.
[3]Digest-check sample; see sections 7.10.4 and 7.10.5.

12.3 After peaks are baseline corrected, they need to be digestion-blank corrected.

This correction can be applied in several ways:

1. Subtract the baseline-corrected absorbance of the digestion blank—compute an average concentration if multiple digested blanks are included in each block—from the baseline-corrected absorbance of all calibrants, check standard, and samples in the block. Then estimate regression parameters (a, b, and c terms) for the calibration function by using a second-order polynomial least-squares algorithm. For second and higher order calibration functions, use the Newton-Raphson successive approximations algorithm (Draper and Smith, 1966; Swartz, 1976, 1977, 1979) to convert corrected peak heights into concentrations.
2. Designate digestion blanks as a calibrant with a nominal concentration of zero. In this case the resulting calibration function will have a positive *y*-intercept that approximates the baseline-corrected absorbance of the digestion blank. If this method is used, be sure that the curve-fitting algorithm does not force a zero *y*-intercept by including one or more "dummy" (0,0) points in the data set used for calibration.
3. Designate digested blanks as baseline correction samples—that is, "W" in the FasPac™ software used to acquire and process data at the NWQL. In this case initial, intermediate (if included), and final baselines are interpolated between digested blank peak maxima. Thus, baseline and digestion blanks are corrected in a single operation.

NOTE: Digestion blanks were corrected for data in this report by using method 3. However, analytical results calculated by the other two methods should be equivalent. Regardless of the blank correction algorithm chosen, make sure that it is documented in the SOP and that analysts understand it. The SOP for these methods must be updated whenever any changes in data acquisition and processing software or in calculation algorithms are implemented.

12.4 Most software packages provide a data base for entering appropriate dilution factors. Usually these factors can be entered before or after samples are analyzed. If dilution factors are entered, reported concentrations will be compensated automatically for the extent of dilution. The dilution factor is the number by which a measured concentration must be multiplied to obtain the analyte concentration in the sample prior to dilution. For example, dilution factors of 2, 5, and 10 indicate that sample and diluent were combined in proportions of 1+1, 1+4, and 1+9, respectively.

13. Reporting Results

Total nitrogen (lab codes 2754, 2755, 2756)

- 2 decimal places for concentrations up to 5.00 mg-N/L
- 2 significant figures for concentrations greater than 5.00 mg-N/L

Total phosphorus (lab codes 2757, 2758, 2759)

- 2 decimal places for concentrations up to 2.00 mg-P/L
- 2 significant figures for concentrations greater than 2.00 mg-P/L

14. Detection Levels, Bias, and Precision

14.1 Method detection limits (MDL) for composited, low-concentration FCC and WCA samples (five of each) were estimated using the U.S Environmental Protection Agency (1997) protocol—see table 8. Target concentrations for nitrogen and phosphorus in FCC and WCA composite samples were 0.05 mg-N/L and 0.02 mg-P/L, respectively. The MDL for nitrogen was 0.015 mg-N/L and for phosphorus was 0.007 mg-P/L. Laboratory reporting levels (LRL) will be about twice the MDL concentrations.

14.2 Table 9 lists the average and standard deviation of 9987L, 9987H, and 9987VH QC check solutions that were included in every rack of alkaline persulfate digests. Most probable values (MPVs) and standard deviations in table 9 were published by the USGS Branch of Quality Systems for the 2002 water year (12-month period ending September 30 each year is called the "water year"). In all cases, total nitrogen and total phosphorus concentrations determined for these reference materials by the alkaline persulfate digestion method were tightly centered around published MPVs and well within published control limits.

Table 8. Data and calculations used to estimate method detection limits (MDL) for nitrogen and phosphorus in unacidified (FCC) and acidified (WCA) samples following alkaline persulfate digestion. Low-concentration FCC and WCA samples (five of each) were composited for these determinations

[mg-N (-P)/L, milligrams nitrogen (or phosphorus) per liter; %, percent; MDL, method detection limit]

Target concentration [mg-N (-P)/L]	Concentration found (mg-N/L or mg-P/L)			
	Dissolved nitrogen (unacidified)	Total nitrogen (acidified)	Dissolved phosphorus (unacidified)	Total phosphorus (acidified)
0.05 (0.02)	0.064	0.041	0.026	0.033
0.05 (0.02)	.078	.042	.024	.029
0.05 (0.02)	.072	.035	.026	.029
0.05 (0.02)	.066	.035	.029	.027
0.05 (0.02)	.067	.032	.026	.029
0.05 (0.02)	.066	.039	.023	.027
0.05 (0.02)	.071	.026	.022	.026
0.05 (0.02)	.063	.035	.026	.026
Average	**.068**	**.035**	**.025**	**.028**
Standard deviation	.005	.005	.002	.002
Number of values	8	8	8	8
Degrees of freedom	7	7	7	7
t-value (1-sided, 99%)	2.998	2.998	2.998	2.998
MDL	.015	.015	.007	.007

Table 9. Most probable values and standard deviations for reference samples 9987L, 9987H, and 9987VH along with averages and standard deviations of these reference materials that were included in every rack of alkaline persulfate digests

[ID, identification of reference sample; MPV, most probable value; FCC, filtered, chilled (bottle type); WCA, whole water, chilled, acidified (bottle type); mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; ±, plus or minus]

ID	MPV	High-flow samples		Low-flow samples	
		FCC[1]	WCA[2]	FCC[3]	WCA[4]
		Alkaline persulfate dissolved and total nitrogen concentration (mg-N/L)			
9987L	0.22 ± 0.08	0.21 ± 0.03	0.21 ± 0.03	0.19 ± 0.03	0.20 ± 0.02
9987H	1.09 ± 0.15	1.09 ± 0.03	1.09 ± 0.03	1.06 ± 0.08	1.04 ± 0.04
9987VH	2.20 ± 0.24	2.27 ± 0.05	2.18 ± 0.06	2.16 ± 0.07	2.13 ± 0.06
		Alkaline persulfate dissolved and total phosphorus concentration (mg-P/L)			
9987L	0.108 ± 0.008	0.105 ± 0.004	0.104 ± 0.004	0.107 ± 0.006	0.105 ± 0.004
9987H	0.54 ± 0.02	0.54 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.54 ± 0.01
9987VH	1.08 ± 0.05	1.13 ± 0.02	1.10 ± 0.03	1.13 ± 0.03	1.09 ± 0.02

[1]Number of points: *n* = 19; [2]*n* = 21; [3]*n* = 21; [4]*n* = 18.

14.3 Spike Recoveries

Median, 90th and 10th percentiles of percent spike recoveries measured in samples collected during high-flow and low-flow conditions are listed in table 10. Median spike recoveries for nitrogen (0.5 mg-N/L as glycine) ranged from about 92 to100 percent and for phosphorus (0.2 mg-P/L as glycerophosphate) from about 86 to 108 percent.

14.4 Duplication of Results

Median, tenth percentiles, and ninetieth percentiles for concentration differences for duplicate samples collected during the nominally high- and low-flow conditions are listed in table 11. Median concentration differences between duplicate analyses are about the same as the MDLs. Larger tenth-percentile differences for whole-water samples that were collected during nominally high-flow conditions in relation to those of filtered water samples likely reflect the difficulty of obtaining reproducible aliquots from samples that contain large amounts of suspended solids. Such samples were purposely chosen as duplicates to assess "worst-case" digest-preparation sampling precision.

ANALYTICAL PERFORMANCE AND COMPARATIVE RESULTS

This section documents analytical performance of the alkaline persulfate digestion method (I-2650-03/4650-03) developed and adapted for use at the NWQL as an alternative to USGS Kjeldahl digestion methods for nitrogen (I-2515-91/4515-91) and phosphorus (I-2610-91/4610-91). It also provides statistical and graphical analysis of data and interpretation of results for about 2,100 dissolved and whole-water samples that were collected during nominally high- and low-flow conditions and analyzed by alkaline persulfate and Kjeldahl digestion methods.

Table 10. Spike recoveries of glycine and glycerophosphate in randomly selected high-flow and low-flow samples that were included in every rack of alkaline persulfate digests

[*n*, number of samples; DN_{AlkP}, alkaline persulfate dissolved nitrogen; TN_{AlkP}, alkaline persulfate total nitrogen; DP_{AlkP}, alkaline persulfate dissolved phosphorus; TP_{AlkP}, alkaline persulfate total phosphorus]

	High-flow samples				Low-flow samples			
	n	Percent recovery			*n*	Percent recovery		
		Median	90th	10th		Median	90th	10th
DN_{AlkP}	18	100.3	108.6	90.1	18	95.0	103.2	88.7
TN_{AlkP}	22	95.1	103.1	84.0	18	92.1	101.7	83.0
DP_{AlkP}	18	97.9	112.9	86.5	17	108.3	119.2	93.4
TP_{AlkP}	22	85.8	93.3	69.5	18	99.6	107.5	91.4

Table 11. Concentration differences between selected samples prepared and analyzed in duplicate in each block of alkaline persulfate digests

[*n*, number of samples; mg-N/L, milligrams nitrogen per liter; mg-P/L, milligrams phosphorus per liter; DN_{AlkP}, alkaline persulfate dissolved nitrogen; TN_{AlkP}, alkaline persulfate total nitrogen; DP_{AlkP}, alkaline persulfate dissolved phosphorus; TP_{AlkP}, alkaline persulfate total phosphorus]

	High-flow samples				Low-flow samples			
	n	Concentration difference for mg-N/L or mg-P/L (percentile)			*n*	Concentration difference for mg-N/L or mg-P/L (percentile)		
		Median	90th	10th		Median	90th	10th
DN_{AlkP}	20	0.011	0.050	-0.023	20	-0.023	0.008	-0.109
TN_{AlkP}	20	-0.007	0.052	-0.296	20	-0.024	0.027	-0.093
DP_{AlkP}	20	0.000	0.009	-0.024	20	-0.002	0.010	-0.034
TP_{AlkP}	20	0.000	0.015	-0.040	20	-0.004	0.006	-0.023

Analytical Performance

Prior to beginning the large-scale evaluation and validation study with samples collected during nominally high- and low-flow conditions, preliminary experiments were performed to establish performance of the alkaline persulfate digestion method. Recoveries of nitrogen and phosphorus from individual nitrogen- and phosphorus-containing compounds that were prepared in deionized water are listed in tables 12 and 13. Inspection of table 12 reveals greater than 95-percent recovery of nitrogen for compounds tested. The somewhat lower recoveries obtained for phosphorus compounds listed in table 13 result from the lower purity of test compounds (phenyl phosphate and phytic acid ≤95 percent according to vendor labels; ATP was purchased and first opened in 1991). Comparable, though slightly higher, phosphorus recoveries for these compounds by acid persulfate digestion (USEPA method 365.1—the generally accepted reference method for total phosphorus determinations), which also are shown in table 13, substantiate this assertion. Other researchers (Ebina and others, 1983; Hosomi and Sudo, 1986; Ameel and others, 1993), who used alkaline persulfate digestion methods similar to the one developed at the NWQL, reported phosphorus recoveries greater than 95 percent for a variety of phosphorus-containing compounds, including ATP.

Table 12. Recovery of inorganic and organic nitrogen from representative compounds

[mg-N/L, milligrams nitrogen per liter; ±, plus or minus]

Nitrogen compound	Nominal concen-tration (mg-N/L)	Found (mg-N/L)	Recovery (percent)
Ammonia	2.5	2.51 ± 0.05	100.5
Urea	2.5	2.50 ± 0.06	100.0
Nicotinic acid	2.5	2.47 ± 0.04	98.7
Glycine	2.5	2.50 ± 0.07	97.9

Results from an experiment to assess nitrogen recovery in the presence of organic carbon (OC) are shown in figure 5. In these experiments a series of solutions containing 2.5 mg NH_4^+-N/L and increasing concentrations of OC (as glucose) were digested and analyzed for nitrogen. Data plotted in figure 5 indicate that complete oxidation of ammonium to nitrate was achieved for OC concentrations up to 150 mg/L. Similar results have been reported previously (Langer and Hendrix, 1982; Cabrera and Beare, 1993). OC in surface- and ground-water samples analyzed at the NWQL rarely exceeds 150 mg/L. Note, however, that nitrogen recovery in Kjeldahl digests is quantitative at OC concentrations 10 to 20 times greater than the 150-mg/L limit typical for alkaline persulfate digestion methods (Ebina and others, 1983).

During preliminary validation work, the cause of low nitrogen recovery in about 10 WCA samples was traced to overacidification at collection sites. When these samples were dosed with alkaline persulfate reagent, the resulting pH was less than 7. As discussed previously in section 2.2, an initial pH greater than 12 is necessary for complete oxidation of ammonium and organic nitrogen to nitrate. For this reason the pH of all WCA samples was checked with narrow range colorimetric test strips during the large-scale evaluation and validation study. Nitrogen

Table 13 Recovery of organic phosphorus from representative compounds by alkaline persulfate and low-level acid persulfate digestion methods

[mg-P/L, milligrams phosphorus per liter; ±, plus or minus]

Compound	Nominal concen-tration (mg-P/L)	Alkaline persulfate method		Acid persulfate method	
		Found (mg-P/L)	Recovery (percent)	Found (mg-P/L)	Recovery (percent)
Adenosine tri-phosphate (ATP)	0.200	0.166 ± 0.000	83.0	0.176 ± 0.001	88.0
	1.000	0.87 ± 0.03	86.8		
Glycerophosphate	0.200	0.196 ± 0.007	100.2	0.204 ± 0.002	102.1
	1.000	1.019 ± 0.008	101.9		
Phenyl Phosphate	0.200	0.168 ± 0.004	84.2	0.179 ± 0.000	89.5
	1.000	0.872 ± 0.002	87.2		
Phytic Acid	0.200	0.177 ± 0.002	88.4	0.180 ± 0.001	91.8
	1.000	0.906 ± 0.009	90.6		

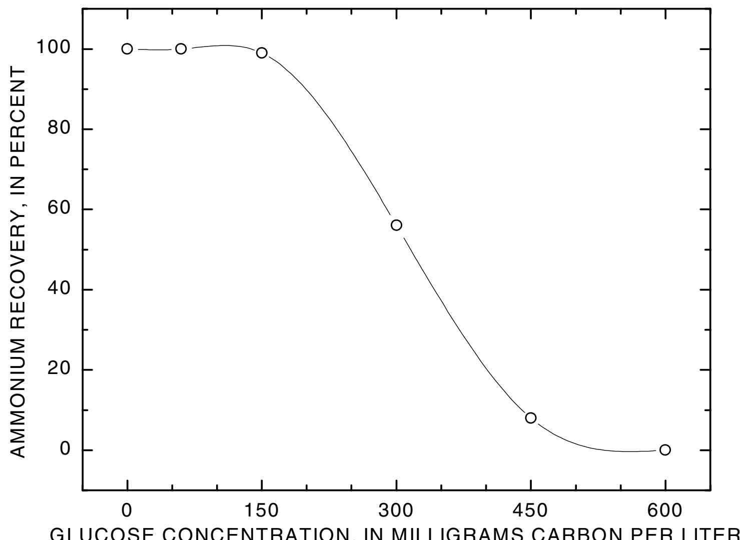

Figure 5. Percent recovery of nitrogen from a series of 2.5 mg NH_4^+-N solutions that contained increasing concentrations of organic carbon (OC) as glucose. See text for additional details.

concentration results for WCA samples with pH outside the expected range of 1.6 to 1.9 were disqualified. For more information, see section 3.1.4. About 150 results for samples with medium codes other than 6 (ground water) and 9 (surface water)—specifically, Q (quality-assurance sample, artificial), R (quality-assurance sample, surface water), S (quality-assurance sample, ground water), 2 (leachate), and 5 (elutriation)—also were not included in graphical and statistical analyses.

In Kjeldahl digestion procedures, digests are evaporated to near dryness and then resolvated with DI water prior to analytical determinations. Variation in the postdigestion volume of DI water added to each tube—and therefore in estimated mass-per-unit volume nitrogen and phosphorus concentrations in resolvated digests—is a function of the DI water dispenser precision, typically 2 to 3 percent. Alkaline persulfate digests, in contrast, are tightly capped and lose little water during digestion. After digestion, nitrogen and phosphorus are determined directly without volume adjustment. It was of interest, therefore, to assess the variation in liquid loss during alkaline persulfate digestion. To this end, pre- and postdigestion weights for one, 80-tube batch of prepared alkaline persulfate digests were measured to the nearest 0.01 g and recorded. A weight of 15 g (10 mL of sample + 5 mL of digestion reagent) was assumed in percent weight-loss calculations using equation 1 below. The results from this experiment, which indicate a weight loss of 3 percent or less for 85 percent of all tubes, are shown in figure 6. The maximum percent weight loss observed was 6 percent.

Comparative Results for Nitrogen

In discussions that follow, the designations KDN and KTN apply to Kjeldahl digestion dissolved nitrogen (ammonium + organic nitrogen determined in filtered-water digests) and Kjeldahl digestion total nitrogen (ammonium + organic nitrogen determined in acidified, whole-water digests), respectively. When filtered- and whole-water samples are considered

$$Percent\ weight\ loss = \frac{Digest\ weight_{initial} - Digest\ weight_{final}}{15g} \times 100 \qquad (1)$$

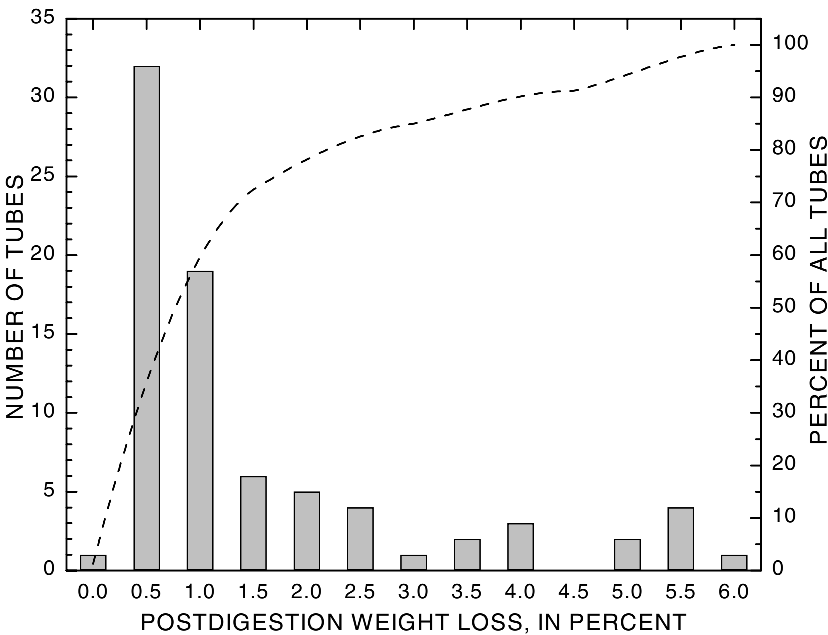

Figure 6. Histogram of postdigestion weight loss expressed as a percent of initial digest weight for one batch of 80 alkaline persulfate digests. Weights of capped digest tubes, each containing precisely dispensed volumes of sample and reagent, were weighed before and after digestion. Left and right *y*-axes relate to gray bars and the dashed line, respectively. Additional details can be found in supporting text.

together, the designation Kjeldahl nitrogen (KN) is applied. The designations alkaline persulfate digestion dissolved nitrogen (DN_{AlkP}), alkaline persulfate digestion total nitrogen (TN_{AlkP}), and alkaline persulfate nitrogen (N_{AlkP}) are applied analogously. Note also that *nitrate-corrected* DN_{AlkP} , TN_{AlkP} , and N_{AlkP} concentrations are those from which nitrate + nitrite concentrations have been subtracted to make them operationally equivalent to KDN, KTN, and KN concentrations.

A logarithmic scatter plot of nitrate-corrected N_{AlkP} concentrations and KN concentrations around a unity slope line—that is, the line of equal relation—for paired data combined from the large-scale validation experiments is shown in figure 7. Despite the large scatter between individual data pairs, linear regression analysis of these data indicate good correlation between nitrate-corrected N_{AlkP} and KN concentrations—KN = (1.023 ± 0.003) N_{AlkP} + 0.038 ± 0.004, $r^2 = 0.976$. The positive y-intercept and slightly greater than unity slope of the regression line indicate low bias for nitrate-corrected N_{AlkP} in relation to KN, which might be interpreted as low nitrogen recovery for the alkaline persulfate digestion method. An alternate interpretation—that KN concentrations are biased high because a small fraction of nitrate present in samples is reduced to ammonium during Kjeldahl digestion—also could account for observed concentration differences. Interference by nitrate during Kjeldahl digestion (American Public Health Association, 1998c, p. 4-123; Patton and Truitt, 2000) is well known.

To explore this alternative interpretation further, differences between nitrate-corrected N_{AlkP} and KN concentrations (*y*-axes) were plotted as a function of nitrate concentrations (*x*-axes) in the four panels of figure 8. In this figure panels A and B relate to data for filtered surface- and ground-water samples; panels C and D relate to data for whole-water acidified surface- and ground-water samples. Nitrate concentrations are plotted on logarithmic scales to provide equal linear spacing for each decade of nitrate concentration. Lines of zero concentration difference were added to facilitate visual interpretation of data. With the exception of some unexplained outliers, differences between nitrate-corrected DN_{AlkP} and KDN concentrations in filtered samples (fig. 8A and 8B) tend to scatter symmetrically about the lines of zero difference up to nitrate concentrations of about

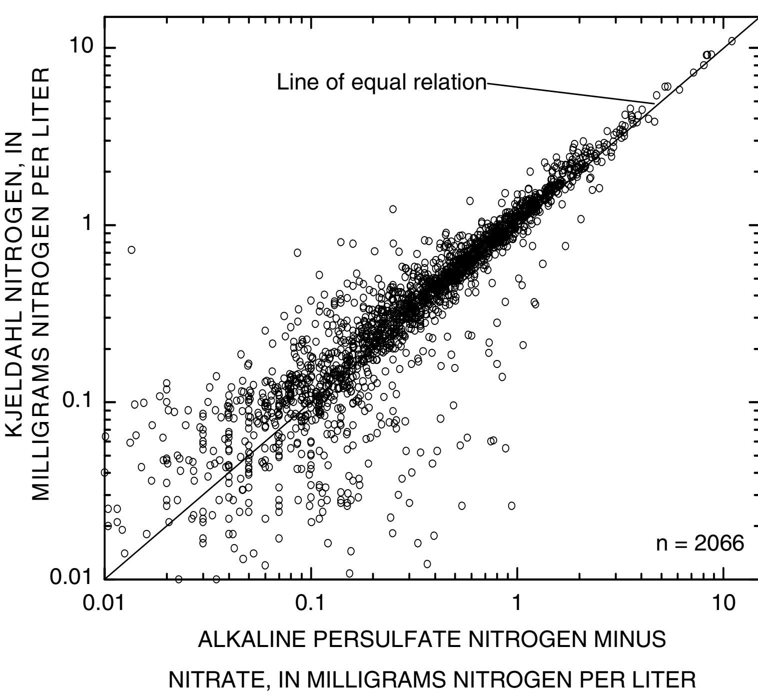

Figure 7. Logarithmic scatter plot of nitrate-corrected N_{AlkP} concentrations (x-axis) and KN concentrations (*y*-axis) around the line of equal relation for 2,066 data pairs combined from large-scale validation experiments. The linear regression equation for these data is $y = (1.023 \pm 0.003)\,x + 0.038 \pm 0.004$, with a correlation coefficient (r^2) of 0.976.

1 mg-N/L. At higher nitrate concentrations, differences between nitrate-corrected DN_{AlkP} and KDN concentrations increase about the line of zero difference—erratically for filtered ground water and negatively for filtered surface water. In contrast, concentration differences between nitrate-corrected TN_{AlkP} and KTN for unfiltered, acidified samples (fig. 8C and 8D) are predominately negative with differences becoming more negative as nitrate concentrations increase. This trend is particularly evident for unfiltered, acidified surface water.

The result of sorting data from each panel in figure 8 according to nominal flow conditions at the time of sample collection and recasting them as box plots is shown in figure 9. In this figure, concentration differences between nitrate-corrected N_{AlkP} and KN for samples collected during nominally high-flow (HF) and low-flow (LF) conditions are grouped into three nitrate concentration ranges: NO_3^--N ≤ 0.1 mg/L, 0.1 mg/L < NO_3^--N ≤ 1.0 mg/L, and NO_3^--N > 1.0 mg/L. Figure 9 further substantiates the hypothesis that concentration differences between nitrate-corrected N_{AlkP} and KN likely result from the well known, though poorly characterized, high-temperature reactions between nitrate and natural organic matter (NOM) that can produce positive (reduction of nitrate to ammonium) or negative (oxidation of ammonium to nitrous oxide) interference in Kjeldahl nitrogen determinations (see section 3.1.5 and Patton and Truitt, 2000). Positive nitrate interference in KN concentrations predominates for surface-water samples and is greater for whole-water samples than for filtered-water samples. This result is consistent with typically larger NOM concentrations in whole-water samples than in filtered-water samples. Nitrate appears to interfere positively and negatively in KN concentrations for ground-water samples, although the trends are less clear than for surface-water samples. In general, differences between nitrate-corrected N_{AlkP} and KN concentrations were least for samples with nitrate concentrations less than 0.1 mg NO_3^--N/L—a finding consistent with nitrate interference during Kjeldahl digestion. Complete two-population, paired *t*-test results for subsets of nitrate-corrected DN_{AlkP} and

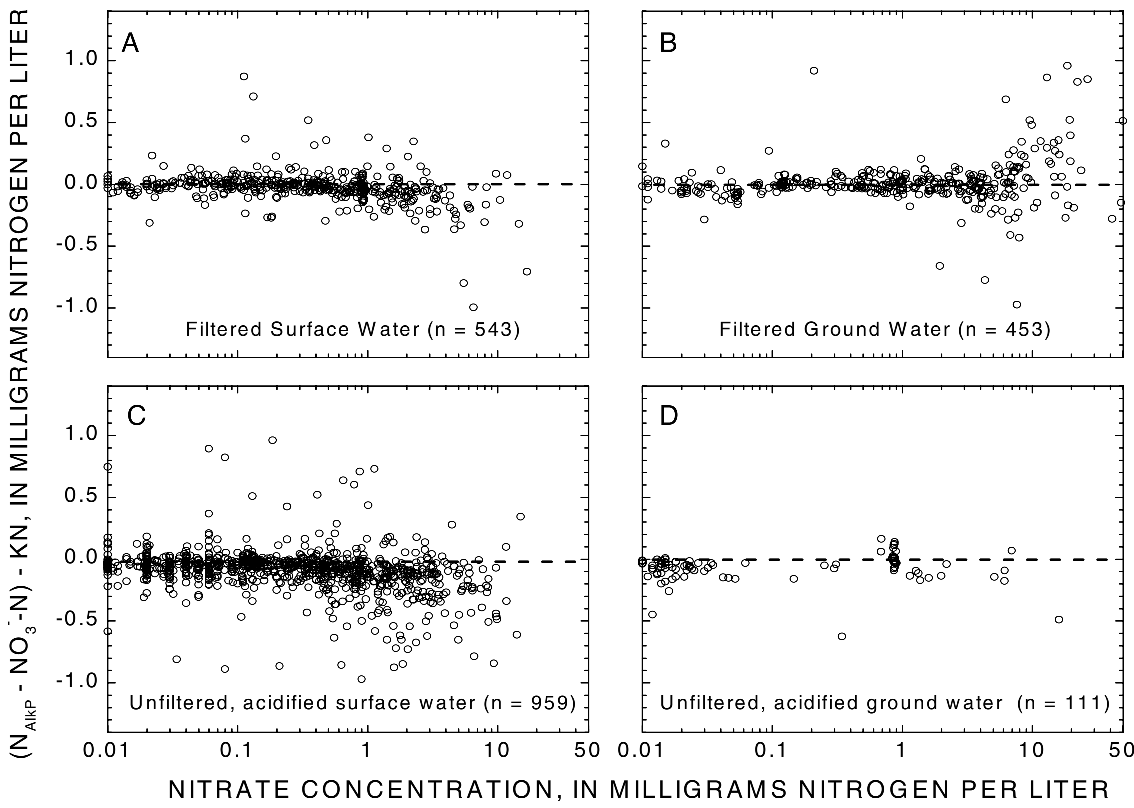

Figure 8. Concentration differences between nitrate-corrected alkaline persulfate digestion nitrogen (N_{AlkP}) and Kjeldahl digestion nitrogen (KN) plotted about the line of zero difference as a function of nitrate concentration.

KDN concentrations in filtered-water samples are listed in table 14. Nitrate-corrected TN_{AlkP} and KTN concentrations in acidified whole-water samples are listed in table 15.

Comparative Results for Phosphorus

A logarithmic scatter plot of P_{AlkP} concentrations (*x*-axis) and KP concentrations (*y*-axis) around a unity slope line for 2,093 data pairs combined from high- and low-flow phases of validation experiments is shown in figure 10. This plot reveals good correlation among phosphorus concentrations determined by the P_{AlkP} and KP digestion methods. The slope and *y*-intercept of the linear least squares regression of these data—KP = (0.994 ± 0.002) P_{AlkP} + 0.0003 ± 0.0005; correlation coefficient (r^2) = 0.994 closely approximate 1 and 0. A two-population, paired *t*-test confirmed the null hypothesis that the difference between means of phosphorus concentrations for these 2,093 paired results determined by the P_{AlkP} and KP digestion methods were not significantly different from zero at the p = 0.05 level. Differences between means of alkaline persulfate phosphorus and Kjeldahl phosphorus concentrations for some subsets of these data, which were grouped according to water type and flow conditions at the time of sample collection, were statistically different from zero at the p = 0.05 level. In all such cases, however, differences between means were less than method detection limits—0.007 mg-P/L for P_{AlkP} and 0.02 mg-P/L for KP—and therefore were not analytically significant. Complete results for these *t*-tests are listed in table 16.

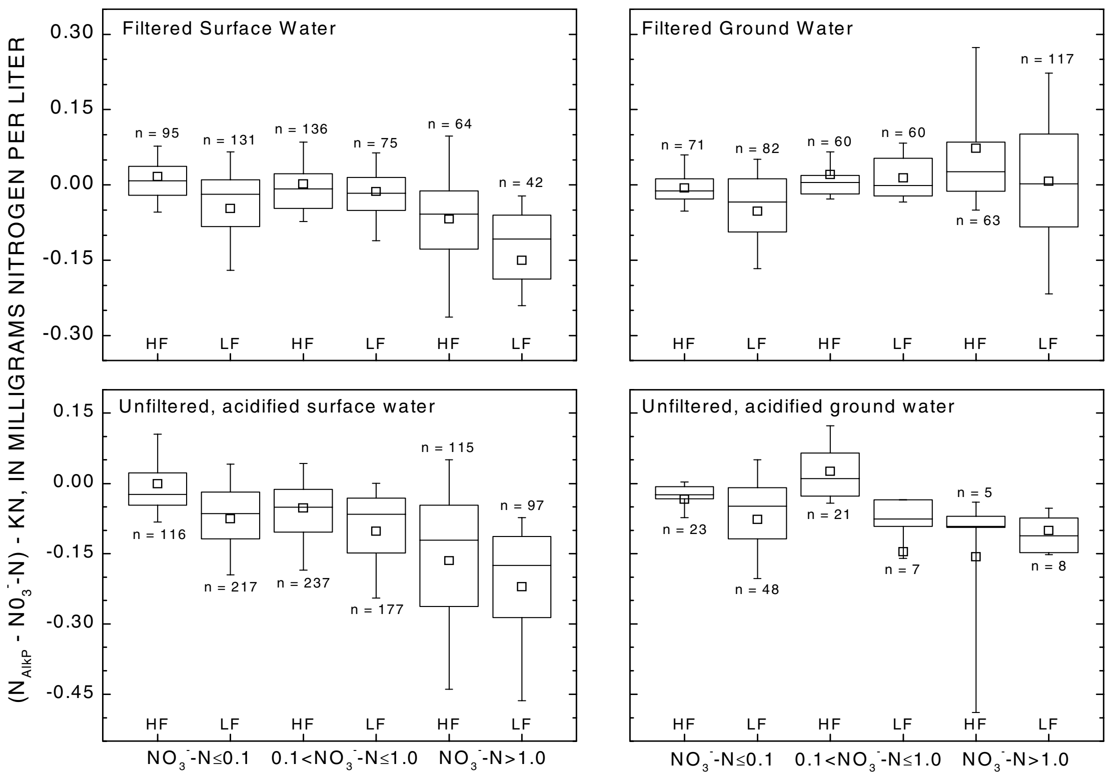

Figure 9. Boxplots of concentration differences between nitrate-corrected alkaline persulfate nitrogen (N_{AlkP}) and Kjeldahl nitrogen (KN) for surface- and ground-water samples collected during nominally high- and low-flow conditions. Data in each panel are grouped according to nominal flow conditions (HF = high-flow; LF = low-flow) at the time of sample collection and dissolved nitrate concentrations (milligram nitrogen per liter). In each boxplot, open squares, hinges, gates and whiskers indicate average, median, 75th and 25th percentiles, and 90th and 10th percentiles for differences between nitrate-corrected N_{AlkP} and KN.

SUMMARY AND CONCLUSIONS

An alkaline persulfate digestion method and automated colorimetric finishes for simultaneous nitrogen and phosphorus determinations in filtered and whole-water acidified water samples were developed and validated. This method is more sensitive, accurate, and uses less toxic reagents than Kjeldahl digestion methods, such as U.S. Geological Survey (USGS) I-2515/4515-91 and U.S. Environmental Protection Agency (USEPA) 351.2 for nitrogen and USGS I-2610/4610-91 and USEPA 365.4 for phosphorus. Data in this report result from about 2,100 filtered and whole-water samples that were analyzed for alkaline persulfate dissolved and total nitrogen (DN_{AlkP} and TN_{AlkP}), Kjeldahl dissolved and total nitrogen (KDN and KTN), alkaline persulfate dissolved and total phosphorus (DP_{AlkP} and TP_{AlkP}), and Kjeldahl dissolved and total phosphorus (KDP and KTP). All filtered and whole-water samples analyzed by the alkaline persulfate digestion method also were analyzed for dissolved nitrate + nitrite, ammonium, and orthophosphate on the same day that digests were prepared. Results of these analyses were compared by statistical and graphical methods. About half the data

Table 14. Results of paired *t*-tests for nitrate-corrected alkaline persulfate dissolved nitrogen and Kjeldahl dissolved nitrogen concentrations determined in filtered-water samples. Distributions of nitrate concentrations for each subgroup are shown in the rightmost three columns

[mg-N/L, milligrams nitrogen per liter; DN_{AlkP}, alkaline persulfate digestion dissolved nitrogen; KDN, Kjeldahl digestion dissolved nitrogen; *n*, number of samples; max, maximum; 75th, 75th percentile; <, less than; ≤, less than or equal to; >, greater than]

Filtered ground water	Mean (mg-N/L)		Difference (mg–N/L)	Variance (mg-N/L)		*n*	Significance[1]			Nitrate (mg-N/L)		
	DN_{AlkP}	KDN		DN_{AlkP}	KDN		p_{calc}	$p_{0.05}$	$p_{0.01}$	Median	Max	75th
All	0.340	0.333	0.007	2.387	2.397	453	0.386	no	no	0.513	50	3.289
High-flow												
All	0.415	0.390	0.025	5.153	5.179	194	0.012	yes	no	0.212	50	1.875
$NO_3^- < 0.1$	0.446	0.456	-0.010	0.526	0.535	71	0.341	no	no	0	0.094	0.007
$0.1 < NO_3^- \leq 1.0$	0.125	0.106	0.019	0.029	0.020	60	0.229	no	no	0.372	0.984	0.795
$NO_3^- > 1.0$	0.657	0.588	0.069	15.278	15.375	63	0.004	yes	yes	3.536	50	6.27
Low-flow												
All	0.283	0.290	-0.007	0.319	0.321	259	0.540	no	no	0.674	48	3.96
$NO_3^- < 0.1$	0.466	0.494	-0.027	0.708	0.698	82	0.018	yes	no	0.02	0.084	0.04
$0.1 < NO_3^- \leq 1.0$	0.107	0.100	0.007	0.096	0.099	60	0.303	no	no	0.467	0.988	0.628
$NO_3^- > 1.0$	0.245	0.244	0.000	0.124	0.127	117	0.993	no	no	4.222	48	8.6
Filtered surface water												
All	0.41	0.435	-0.025	0.191	0.226	543	<0.001	yes	yes	0.182	16.88	0.883
High-flow												
All	0.385	0.393	-0.008	0.078	0.088	295	0.21	no	no	0.358	16.88	0.922
$NO_3^- < 0.1$	0.365	0.34	0.025	0.082	0.088	95	0.041	yes	no	0.016	0.096	0.053
$0.1 < NO_3^- \leq 1.0$	0.403	0.401	0.002	0.073	0.081	136	0.836	no	no	0.329	1.547	0.541
$NO_3^- > 1.0$	0.388	0.456	-0.068	0.084	0.096	64	0.002	yes	yes	2.111	16.88	3.199
Low-flow												
All	0.44	0.484	-0.044	0.325	0.387	248	<0.001	yes	yes	0.084	9.76	0.475
$NO_3^- < 0.1$	0.436	0.462	-0.026	0.226	0.232	131	<0.001	yes	yes	0.009	0.098	0.027
$0.1 < NO_3^- \leq 1.0$	0.359	0.374	-0.015	0.14	0.128	75	0.342	no	no	0.236	0.967	0.468
$NO_3^- > 1.0$	0.599	0.748	-0.149	0.95	1.279	42	<0.001	yes	yes	2.238	9.757	3.819

[1] p_{calc} is the probability that population means of nitrate-corrected DN_{AlkP} and KDN concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

Table 15. Results of paired *t*-tests for nitrate-corrected alkaline persulfate total nitrogen and Kjeldahl total nitrogen concentrations determined in acidified whole-water samples. Distributions of nitrate concentrations for each subgroup are shown in the rightmost three columns

[mg-N/L, milligrams nitrogen per liter; TN_{AlkP}, alkaline persulfate digestion total nitrogen; KTN, Kjeldahl digestion total nitrogen; *n*, number of samples; max, maximum; 75th, 75th percentile; <, less than; ≤, less than or equal to; >, greater than]

Unfiltered ground water	Mean (mg-N/L)		Difference (mg-N/L)	Variance (mg-N/L)		*n*	Significance[1]			Nitrate (mg-N/L)		
	TN_{AlkP}	KTN		TN_{AlkP}	KTN		p_{calc}	$p_{0.05}$	$p_{0.01}$	Median	Max	75th
All	0.514	0.577	-0.063	0.319	0.362	111	<0.001	yes	yes	0.022	16.04	0.859
High-flow												
All	0.509	0.53	-0.021	0.178	0.179	48	0.135	no	no	0.498	16.04	0.868
$NO_3^- < 0.1$	0.242	0.277	-0.035	0.162	0.181	23	0.001	yes	yes	0.010	0.052	0.017
$0.1 < NO_3^- \leq 1.0$	0.821	0.795	0.026	0.034	0.032	21	0.096	no	no	0.864	0.904	0.872
$NO_3^- > 1.0$	0.414	0.593	-0.179	0.135	0.285	4	0.185	no	no	4.164	16.04	8.613
Low-flow												
All	0.518	0.613	-0.095	0.431	0.503	63	<0.001	yes	yes	0.017	6.962	0.052
$NO_3^- < 0.1$	0.596	0.679	-0.083	0.51	0.613	48	<0.001	yes	yes	0.015	0.062	0.022
$0.1 < NO_3^- \leq 1.0$	0.429	0.575	-0.146	0.114	0.097	7	0.127	no	no	0.344	0.885	0.866
$NO_3^- > 1.0$	0.092	0.211	-0.119	0.062	0.062	8	<0.001	yes	yes	1.797	6.962	5.376
Unfiltered surface water												
All	0.728	0.820	-0.092	0.911	1.007	959	<0.001	yes	yes	0.215	61.65	0.874
High-flow												
All	0.677	0.744	-0.067	0.608	0.694	468	<0.001	yes	yes	0.345	11.74	0.993
$NO_3^- < 0.1$	0.654	0.655	-0.001	0.560	0.517	116	<0.001	no	no	0.020	0.095	0.054
$0.1 < NO_3^- \leq 1.0$	0.573	0.625	-0.052	0.331	0.380	237	<0.001	yes	yes	0.349	0.999	0.585
$NO_3^- > 1.0$	0.914	1.080	-0.166	1.160	1.385	115	<0.001	yes	yes	2.119	11.74	3.125
Low-flow												
All	0.778	0.892	-0.114	1.197	1.297	491	<0.001	yes	yes	0.134	61.65	0.807
$NO_3^- < 0.1$	0.898	0.974	-0.076	1.303	1.374	217	<0.001	yes	yes	0.034	0.099	0.057
$0.1 < NO_3^- \leq 1.0$	0.588	0.689	-0.101	0.299	0.395	177	<0.001	yes	yes	0.330	0.974	0.557
$NO_3^- > 1.0$	0.855	1.076	-0.221	2.524	2.681	97	<0.001	yes	yes	2.380	61.65	4.648

[1]p_{calc} is the probability that population means of nitrate-corrected TN_{AlkP} and KTN concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

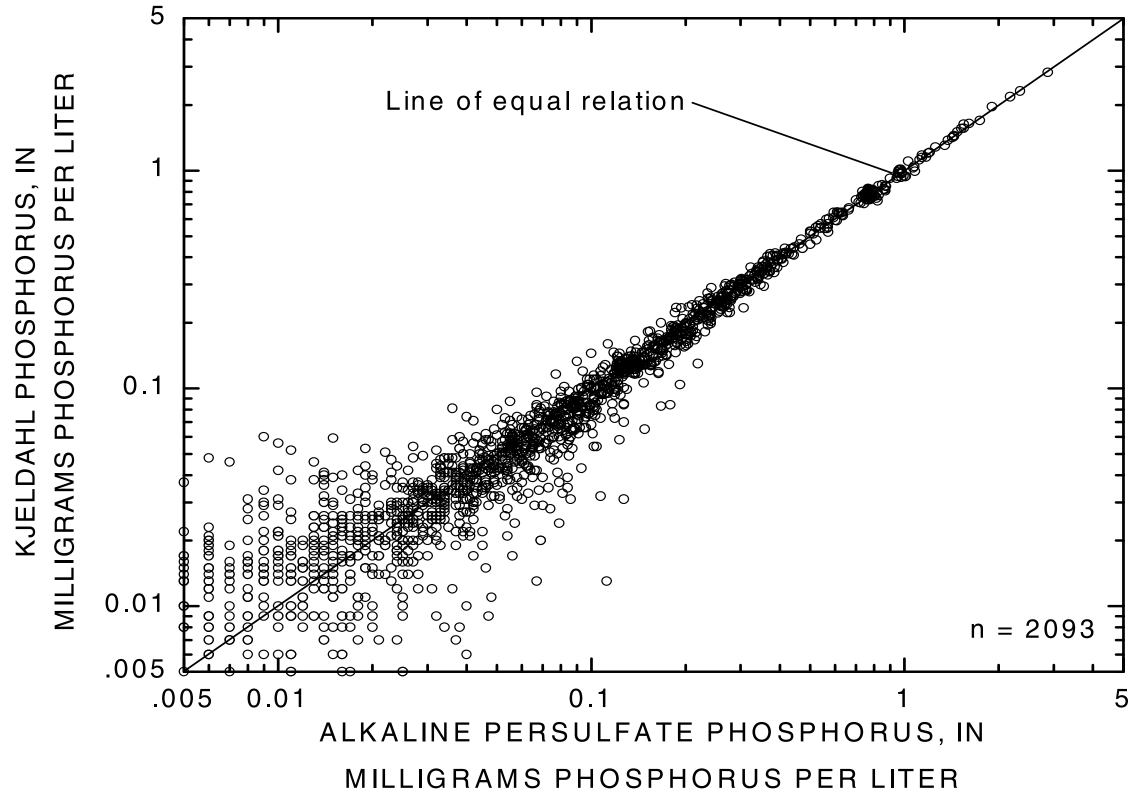

Figure 10. Logarithmic scatter plot of P_{AlkP} concentrations (*x*-axis) and KP concentrations (*y*-axis) around the line of equal relation for 2,093 data pairs combined from large-scale validation experiments. The linear regression equation for these data is KP = (0.994 ± 0.002) P_{AlkP} + 0.0003 ± 0.0005, with a correlation coefficient (r^2) of 0.994.

in this report were obtained from samples collected during nominally high-flow (April–June 2002) conditions, and the other half were collected during nominally low-flow (August–September 2002) conditions. Numbers of filtered and acidified whole-water samples were about equal. This report provides details of alkaline persulfate digest preparation as well as complete operational information, including interferences and analytical figures of merit for the automated colorimetric methods developed to determine nitrate and orthophosphate in alkaline persulfate digests. Primary conclusions of this report follow:

1. Hazards to analysts and toxic wastes are substantially less for alkaline persulfate digestion methods than for Kjeldahl digestion methods.
2. Alkaline persulfate digestion methods described in this report can be applied successfully to acidified samples (USGS FCA and WCA bottle types) provided that samples are acidified at collection sites using supplies and protocols specified in the USGS field manual (Wilde and others, 1998).
3. Alkaline persulfate digestion methods described in this report are amenable to automation and should prove substantially less labor intensive than the existing Kjeldahl digestion methods. For example, filtered-water sample digests can be prepared robotically, and the manual

Table 16. Results of paired *t*-tests for dissolved and total phosphorus concentrations determined in filtered and acidified whole-water samples by alkaline persulfate digestion (P_{AlkP}) and Kjeldahl digestion (KP) methods

[mg-P/L, milligrams phosphorus per liter; *n*, number of samples; FCC, filtered, chilled (bottle type for samples); WCA, whole water, chilled, acidified (bottle type for samples); MC 6, ground water medium code; MC 9, surface water medium code; HF, high flow; LF, low flow; <, less than]

Water type	Mean (mg-P/L)		Difference (mg-P/L)	Variance (mg-P/L)		*n*	Significance[1]		
	P_{AlkP}	KP		P_{AlkP}	KP		p_{calc}	$p_{0.05}$	$p_{0.01}$
All	0.134	0.133	0.001	0.061	0.060	2,093	0.242	no	no
All FCC	0.100	0.103	-0.003	0.063	0.062	1,115	<0.0001	yes	yes
All WCA	0.171	0.167	0.004	0.055	0.056	978	<0.0001	yes	yes
All MC 6	0.084	0.087	-0.003	0.045	0.045	645	<0.0001	yes	yes
All MC 9	0.155	0.154	0.002	0.066	0.066	1,448	<0.0005	yes	yes
High-flow									
All	0.123	0.123	0.000	0.045	0.046	990	0.640	no	no
FCC, MC 6	0.068	0.069	-0.001	0.026	0.028	204	0.557	no	no
FCC, MC 9	0.126	0.131	-0.006	0.059	0.061	320	<0.0001	yes	yes
WCA, MC 6	0.296	0.297	-0.001	0.124	0.125	49	0.787	no	no
WCA, MC 9	0.127	0.123	0.004	0.028	0.029	417	<0.0001	yes	yes
Low-flow									
All	0.143	0.142	0.001	0.075	0.073	1,103	0.043	yes	no
FCC, MC 6	0.046	0.050	-0.004	0.023	0.022	337	<0.0005	yes	yes
FCC, MC 9	0.166	0.166	0.000	0.141	0.135	254	0.833	no	no
WCA, MC 6	0.190	0.193	-0.003	0.116	0.119	55	0.140	no	no
WCA, MC 9	0.196	0.190	0.006	0.062	0.063	457	<0.0001	yes	yes

[1]p_{calc} is the probability that population means of P_{AlkP} and KP concentrations are the same—that is, difference between the population means is statistically equivalent to zero—on the basis of calculated paired *t*-tests. Difference between population means is significant at the 95-percent confidence level ($p_{0.05}$) when p_{calc} is less than 0.05 and at the 99-percent confidence level ($p_{0.01}$) when p_{calc} is less than 0.01.

post-digestion, pH adjustment step typical in previously reported alkaline persulfate digestion procedures (Ameel and others, 1993; D'Elia and others, 1997) has been eliminated.

4. Method detection limits (MDLs) for the alkaline persulfate digestion dissolved and total nitrogen (0.015 mg-N/L) and phosphorus (0.007 mg-P/L) are substantially less than those of USGS methods I-2515/4515/91 for dissolved and total Kjeldahl nitrogen (0.05 mg-N/L) and USGS methods I-2610/4610/91 for dissolved and total Kjeldahl phosphorus (0.02 mg-P/L) methods. The lower nitrogen and phosphorus MDLs of alkaline persulfate digestion methods described in this report improve the precision of nutrient-mass balance estimates.
5. On the basis of two-population, paired *t*-test statistics, the means of all nitrate-corrected alkaline persulfate digestion nitrogen (N_{AlkP}) and Kjeldahl digestion nitrogen (KN) concentrations (2,066 paired results) were significantly different from zero at the p = 0.05 level. Statistical and graphical analyses of experimental data indicate that concentration differences between nitrate-corrected N_{AlkP} and KN result from nitrate interference in the Kjeldahl digestion method rather than incomplete recovery of nitrogen by the alkaline persulfate digestion method. Alkaline persulfate digestion, therefore, provides more accurate estimates of total nitrogen concentrations in samples that contain nitrate concentrations greater than about 0.1 mg NO_3^--N/L. For some subsets of these data, the means were not different from zero at the p = 0.05 level, typically in ground-water samples or in surface-water samples with nitrate concentrations less than 0.1 mg-N/L.
6. On the basis of two-population, paired *t*-test statistics for 2,093 paired results, the means of all Kjeldahl digestion phosphorus concentrations determined by USGS method I-2610/4610-91 (similar to USEPA method 365.4) and those determined by the alkaline persulfate digestion method reported here were not significantly different from zero at the p = 0.05 level. For some subsets of these data, the means were different from zero at the p = 0.05 level, but in such cases differences were less than the method detection limit (0.007 mg-P/L) for the alkaline persulfate digestion method and were not analytically significant. Changing from Kjeldahl digestion to alkaline persulfate digestion, therefore, does not affect comparisons with historical dissolved and total phosphorus concentrations.
7. Data and analysis provided in this report establish guidelines necessary to interpret total and dissolved nitrogen and phosphorus concentrations that result from alkaline persulfate digestion methods in relation to those that result from Kjeldahl digestion methods. Specifically—
   a. Systematic differences between DN_{AlkP} /KDN and TN_{AlkP} /KTN concentrations are expected for samples with dissolved nitrate concentrations greater than or equal to 0.1 mg-N/L.
   b. Concentration differences between N_{AlkP} and historical KN data are likely to increase in proportion to dissolved nitrate concentrations in samples. Whether concentration differences are positive or negative depend on water type in ways that were not possible to describe fully.
      (1) Negative differences between KN and N_{AlkP} were found most often for surface-water samples and unfiltered ground-water samples.
      (2) Differences between KN and N_{AlkP} in filtered ground water are as likely to be negative as positive.
   c. Samples with organic carbon (OC) concentrations greater than about 150 mg/L are not amenable to N_{AlkP} determinations unless OC concentrations are diluted below this threshold prior to digestion.
   d. As nitrate concentrations increase, N_{AlkP} digestion provides better estimates of total and dissolved nitrogen than KN digestion, which suffers from positive and negative interference by nitrate. On the other hand, estimating organic nitrogen concentrations as the small difference between two large numbers when dissolved nitrate, and therefore N_{AlkP} , concentrations are large also can be problematic.
   e. Systematic concentration differences between P_{AlkP} and historical KP data are not expected.
8. One major conclusion of this report—that alkaline persulfate digestion is a more sensitive, accurate, and environmentally responsible alternative to

Kjeldahl digestion for routine, simultaneous determination of nitrogen and phosphorus in surface and ground water—is consistent with previously published studies that are cited throughout this report. In comparison to these earlier studies, however, conclusions of this report are based on a much larger and geographically diverse sample population collected during high-flow and low-flow conditions. Furthermore, samples were collected, preserved, stored, and analyzed by rigorously controlled protocols established and documented by the USGS. In these respects, this report describes the most comprehensive study to date supporting applicability of the alkaline persulfate digestion method as a superior alternative to the time honored, but operationally flawed, Kjeldahl digestion method.

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Appendix F
NH_4 Standard Operating Procedure using Smartchem Discrete Analyzer

Water Quality Analysis Laboratory at the University of New Hampshire

Prepared by: Jody Potter

Date of Last Revision: 8/27/10

Signature of Reviewer/Reviser: ____

Method is based on:

USEPA Method 350.1, 1971, modified March 1983. Determination of Ammonia Nitrogen by Semi-Automated Colorimetry.

Protocol NH_4

Introduction

The Smartchem discrete auto-analyzer performs the same analytical methods as manual colorimetric assays done on a lab bench. We analyze NO_3+NO_2, PO_4, NH_4, and SiO_2 on surface, ground, soil extracts and saline waters routinely with this instrument.

The NH4 method is based on the USEPA method 350.1, 1971, modified March 1983. The sample is buffered at a pH of 9.5 with a borate buffer to decrease the hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Ammonia reacts with alkaline phenol and then hypochlorite to form indophenol blue. The amount of color developed is proportional to the concentration of ammonia. The color is further intensified through the addition of sodium nitroprusside and measured at 630 nm.

Preparation of Standards and Reagents

1. Prepare 1000 mg N L^{-1} NH_4 stock by dissolving 3.819g ammonium chloride in a 1000 mL volumetric flask and fill to volume.
2. If the samples to be analyzed are at the lower end of the concentration range, it may be necessary to make an intermediate standard (100 mg N L^{-1}).
3. Make working standards by pipetting the appropriate amount of stock (or intermediate standard) into 100 mL volumetric flasks, and bring them to volume. You can put empty 100 mL volumetric flasks directly on the analytical balance, allowing you to know exactly how much stock you are adding. This eliminates the necessity of weighing water (to determine the volume dispensed) before using the adjustable pipettes. Write down how much standard was added & give to lab manager. We typically use 6 working standards ranging 0-200 μg NH_4-N/L for the NH_4 determination in surface waters.
4. Store stock solution in clean, airtight, glass container in the refrigerator. The NH_4 stock will keep for about two weeks. Working standards can be stored in the volumetric flasks that they were made in. Be sure to cover them tightly with Parafilm. Standards are good for a week or so. Standards should be made weekly, or more frequently if dealing with low concentrations (< 200 ug/L).
5. A QC standard reference sample is run along with samples in a run. They can be found in the freezer with its concentration on the bag label. Dilute as necessary to bring it within your working concentration range. Also run a Lamprey QC, which is a large batch sample from the weekly Lamprey site, as a reference. There should be a bag of them in the freezer as well.
6. Preparation of the working reagents for the method:
    a. Sodium phenolate: Using a 100 mL volumetric flask, dissolve 3.2g NaOH in 50 mL DI water. Cool the flask containing the solution to room temperature (I

usually put in the freezer for 10-15 minutes) and then add and dissolve 8.8 mL phenol. Keep away from light. Solution is stable for two weeks.

b. Sodium hypochlorite solution: Prepare fresh daily. Dilute 33 mL of bleach containing 5.25% NaCOl to 100 mL with DI water. Add 1.0 mL concentrated Probe Rinse Solution.
c. Disodium ethylenediamine-tetraacetate (EDTA): Dissolve 5g EDTA disodium salt dihydrate and 2.75 g of NaOH in appromixately 75 mL DI water. Add 0.6 mL Probe Rinse solution and dilute to 100 mL.
d. Sodium nitroprusside: Dissolve 0.3g sodium nitroprusside dihydrate (sodium nitroferricyanide dihydrate) in 100 mL of DI water. Add 0.5 mL Probe Rinse. Store solution in glass. Solution should be prepared fresh weekly.

Sample Preparation

1. Frozen samples should be completely thawed the day of analysis.

Preparation for Analysis

1. New reagents should be put into the reagent cups each day. If there is old reagent in the cups, dump them into the appropriate waste container and rinse the reagent cups several times with DI water and then add the refrigerated reagent.
2. The diluent cup should be dumped and replaced with fresh DI water (or extract) each day.
3. The reservoirs on the side of the machine should be full at the start of each day and may need to be refilled if more than one run is done in a day. To refill, rinse the reservoirs several times with DI water. The DI water reservoir needs DI water only. Fill the Probe Rinse reservoir with DI water to the top and then 1 mL of Smartchem Probe Rinse is added. Fill the Cleaning Solution reservoir to 1 L and then add 50 mL Smartchem Cleaning Solution.
4. The Smartchem may need to be turned on & will need to be reset (shut instrument off and restart software) if it is on. The power switch is on the back left side of the instrument. Start up the Smartchem software that is labeled "SmartchemNew". To log in the username is "Westco" and password is "joe".
5. When the software says "Standby" at the bottom of the window, click the "Diagnostic" button on the lower right. Click on the "Miscellaneous" tab and click on "Reset" in the "General" area of the window. After system is finished resetting, click on "Diagnostic" tab again to close. Allow system to go to "Standby" again before proceeding.
6. Wash cuvetts prior to every run and wait 15 minutes for cuvetts to dry before starting the run. This can be done while you are entering samples and preparing the sample racks.
7. If this is the first run with new working standards, then the calculated standard concentrations need to be entered into the Method. Click on "Method" and enter the standards into the appropriate spaces to the right of the window.
8. Click on "Sample Entry" and then start up the appropriate method by double clicking on it at the bottom of the window. In the upper left of the window enter the number of samples and standards that you are going to run and click on the check mark to accept. The method is set up to automatically enter blanks, QC standards, duplicates, and spikes

every 12 samples, so this does not to be included in the amount that you enter. On the right side of the window enter the UNH ID # and standards for your run.

9. In addition to the standards automatically entered, two standards should be run every 12 samples and the full range of working standards should be run at the end of the run. Standards are typically run after the Blanks and QC sample so that duplicates are performed on samples not standards. Names cannot be duplicated, so change names of standards slightly each time you enter them. When finished entering, click the “save” icon at the top right of the window.
10. Name the file as you wish to differentiate between runs. Click “Yes” to print and then click on the printer icon. This will print your run sheet. Attach the run sheet header provided and write in the information that it asks for. Staple the header to the top of the run sheets.
11. Rinse each vial once with sample or standard and then fill between the top two lines of the Smartchem vial.
12. Samples should be placed in the appropriate Smartchem rack and location number, which is indicated on the run sheet. Racks should be placed in the proper position & are keyed to ensure that they are.
13. START the run by clicking on the Play icon in the upper left of the window. Uncheck “RBL” and then check “WBL” to initiate Water Baseline at the beginning of the run. This measures the absorbance of water in the cuvette to account for changes in the cuvette over time and check the condition of the filters and lamp. WBL only needs to be run once a day.
14. After the run has started & the calibration curve window appears, check the “results” page to make sure the calibration curve is acceptable and that the first set of NH4 and QC standards are recovered appropriately.
15. When run is complete click on “Export” to the left of the window and export to an Excel file.

Quality Assurance and Control

1. Prior to running the Smartchem you must log-in on the Log-In Excel sheet on the Smartchem computer. Please fill-in all designated information. This information will aid in maintenance of the instrument and will be used in conjunction with the Quality Control data.
2. Following completion of your analysis you are responsible for checking the data. The data is to be copied and pasted into the appropriate lab Excel Report Template on the Smartchem computer and the file should be named by date of analysis (described in worksheet). This template will guide you to report the QC results for the run. This includes % recovery of QC standards (CRM), run time check standards, and lab duplicates. Lab % recovery of sample duplicates, run time check standards, and QC standards should be between 85 and 115 % (see WQAL QAPP for more information).
3. When completed copy the Excel file into the lab manager’s directory on the main lab computer. This information, along with the data, will be entered into the WQAL database by the lab manager to create control charts.

Appendix G
NO_3-NO_2 Standard Operating Procedure using Smartchem Discrete Analyzer

Water Quality Analysis Laboratory at the University of New Hampshire

Prepared by: Jody Potter

Date of Last Revision: 8/27/10

Signature of Reviewer/Reviser: ____

Method is based on:

USEPA 353.2 Revision 2.0, August, 1993. Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry.

Protocol NO_3+NO_2

Introduction

The Smartchem discrete auto-analyzer performs the same analytical methods as manual colorimetric assays done on a lab bench. We analyze NO_3+NO_2, PO_4, NH_4, and SiO_2 on surface, ground, soil extracts, and saline waters routinely with this instrument.

The NO_3+NO_2 method is based on USEPA 353.2 Revision 2.0, August, 1993. This method determines the combined nitrate (NO_3) + nitrite (NO_2) present in the sample. Nitrate is reduced to nitrite by passage of a filtered sample through an open tubular copperized cadmium redactor (OTCR). The nitrate reduced to nitrite plus any nitrite originally present in the sample is then determined as nitrite by diazotizing with sulfanilamide followed by coupling with N-(naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye, which is measured colorimetrically at 550 nm.

Preparation of Standards and Reagents

1. Prepare 1000 mg N L^{-1} NO_3 stock by dissolving 6.0667 g sodium nitrate a 1000 mL volumetric flask and fill to volume. Also, prepare 1000 mg N L^{-1} NO_2 stock by dissolving 4.926g sodium nitrite in a 1000 mL volumetric flask and fill to volume.
2. If the samples to be analyzed are at the lower end of the concentration range, it may be necessary to make an intermediate standard (100 mg N L^{-1}).
3. Make working standards for by pipetting the appropriate amount of stock (or intermediate standard) into 100 mL volumetric flasks, and bring them to volume. You can put empty 100 mL volumetric flasks directly on the analytical balance, allowing you to know exactly how much stock you are adding. This eliminates the necessity of weighing water (to determine the volume dispensed) before using the adjustable pipettes. Write down how much standard was added & give to lab manager.
4. We typically use 6 working standards ranging 0 to 1.0 mg NO_3-N/L for the NO_3 determination in surface waters. Two working NO_2 standards are also needed as a check to ensure that the cadmium column is reducing NO_3 fully to NO_2. Make the NO_2 standards within the working NO_3 range.
5. Store stock solution in clean, airtight, glass container in the refrigerator. The NO3 stock will keep for about one (1) month. Working standards can be stored in the volumetric flasks that they were made in. Be sure to cover them tightly with Parafilm. Standards are good for a week or so. Standards should be made weekly, or more frequently if dealing with low concentrations (< 0.3 mg/L).

6. A QC standard reference sample is run along with samples in a run. They can be found in the freezer with its concentration on the bag label. Dilute as necessary to bring it within your working concentration range. Also run a Lamprey QC, which is a large batch sample from the weekly Lamprey site, as a reference. There should be a bag of them in the freezer as well.
7. Preparation of the working reagents for the method:
    a. Ammonium Hydroxide-EDTA Buffer solution: In a hood, to the dedicated 1L plastic bottle add and dissolve 500 mL DI water, 105 mL hydrochloric acid (HCl), 95 mL ammonium hydroxide (NH_4OH), and 1.0 g disodium EDTA. Adjust the pH to 8.5 with HCl or NaOH. Dilute to 1 L and mix.
    b. Color Reagent: To approximately 75 mL of DI water in a dark 125 mL plastic bottle add 12.5 mL concentrated phosphoric acid. Cool to room temperature and then dissolve 5 g sulfanilamide. Add 0.25 g of N-(1-napthyl)ethylenediamine dihydrochloride and dissolve. Add 0.5 mL Probe Rinse and dilute to 125 mL with DI water. Solution is stable for several weeks.
    c. Nitrate Module Reservoir Buffer Solution: dilute 100 mL concentrated Ammonium Hydroxide-EDTA Buffer solution to 1 L in the dedicated bottle. This should be used as a carrier for the nitrate module & a line in the back right of the instrument should be placed into the solution.

Sample Preparation

1. Frozen samples should be completely thawed the day of analysis.

Preparation for Analysis

1. New reagents should be put into the reagent cups each day. If there is old reagent in the cups, dump them into the appropriate waste container and rinse the reagent cups several times with DI water and then add the refrigerated reagent.
2. The diluent cup should be dumped and replaced with fresh DI water (or extract) each day.
3. The reservoirs on the side of the machine should be full at the start of each day and may need to be refilled if more than one run is done in a day. To refill, rinse the reservoirs several times with DI water. The DI water reservoir needs DI water only. Fill the Probe Rinse reservoir with DI water to the top and then 1 mL of Smartchem Probe Rinse is added. Fill the Cleaning Solution reservoir to 1 L and then add 50 mL Smartchem Cleaning Solution.
4. The Smartchem may need to be turned on & will need to be reset (shut instrument off and restart software) if it is on. The power switch is on the back left side of the instrument. Start up the Smartchem software that is labeled “SmartchemNew”. To log in username is “Westco” and password is “joe”.
5. When the software says “Standby” at the bottom of the window, click the “Diagnostic” button on the lower right. Click on the “Miscellaneous” tab and click on “Reset” in the “General” area of the window. After system is finished resetting, click on “Diagnostic” tab again to close. Allow system to go to “Standby” again before proceeding.
6. Wash cuvetts at the start of each day (there is no drying time needed for NO_3 analysis). This can be done while you are entering samples and preparing the sample racks.

7. If this is the first run with new working standards, then the calculated standard concentrations need to be entered into the Method. Click on “Method” and enter the standards into the appropriate spaces to the right of the window.
8. Click on “Sample Entry” and then start up the appropriate method by double clicking on it at the bottom of the window. In the upper left of the window enter the number of samples and standards that you are going to run and click on the check mark to accept. The method is set up to automatically enter blanks, QCs, duplicates, and spikes every 12 samples, so this does not to be included in the amount that you enter. On the right side of the window enter the UNH ID # and standards for your run.
9. In addition to the standards automatically entered, two NO2 standards should be run at the beginning and end of each run to check cadmium reduction. Also, two NO3 standards should be run every 12 samples and the full range of working standards should be run at the end of the run. Standards are typically run after the Blanks and QC sample so that duplicates are performed on samples not standards. Names cannot be duplicated, so change names of standards slightly each time you enter them. When finished entering, click the “save” icon at the top right of the window.
10. Name the file as you wish to differentiate between runs. Click “Yes” to print and then click on the printer icon. This will print your run sheet. Attach the run sheet header provided and write in the information that it asks for. Staple the header to the top of the run sheets.
11. Rinse each vial once with sample or standard and then fill between the top two lines of the Smartchem vial.
12. Samples should be placed in the appropriate Smartchem rack and location number, which is indicated on the run sheet. Racks should be placed in the proper position & are keyed to ensure that they are.
13. START the run by clicking on the Play icon in the upper left of the window. Uncheck “RBL” and then check “WBL” to initiate Water Baseline at the beginning of the run. This measures the absorbance of water in the cuvette to account for changes in the cuvette over time and check the condition of the filters and lamp. WBL only needs to be run once a day.
14. After the run has started, check the “results” page to make sure the calibration curve is acceptable and that the first set of NO_2, NO_3 and QC standards are recovered appropriately. If NO_2 recovery is high, the OTCR may need to be repacked.
15. When the run is complete, click on “Export” to the left of the window and export to an Excel file.

Data Export

1. Prior to running the Smartchem you must log-in on the Log-In Excel sheet on the Smartchem computer. Please fill-in all designated information. This information will aid in maintenance of the instrument and will be used in conjunction with the Quality Control data.
2. Following completion of your analysis you are responsible for checking the data. The data is to be copied and pasted into the appropriate lab Excel Report Template on the Smartchem computer and the file should be named by date of analysis (described in worksheet). This template will guide you to report the QC results for the run. This includes % recovery of QC standards (CRM), run time check standards, and lab duplicates. Lab % recovery of sample duplicates, run time check standards, and QC standards should be between 85 and 115 % (see WQAL QAPP for more information).

3. When completed copy the Excel file into the lab manager’s directory on the main lab computer. This information, along with the data, will be entered into the WQAL database by the lab manager to create control charts.

Appendix H

Method 440.0

Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis

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Revision 1.4
September 1997

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Method 440.0

Determination of Carbon and Nitrogen in Sediments and Particulates of Estuarine/Coastal Waters Using Elemental Analysis

1.0 Scope and Application

1.1 Elemental analysis is used to determine particulate carbon (PC) and particulate nitrogen (PN) in estuarine and coastal waters and sediment. The method measures the total carbon and nitrogen irrespective of source (inorganic or organic).

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Carbon	7440-44-0
Nitrogen	1333-74-0

1.2 The need to qualitatively or quantitatively determine the particulate organic fraction from the total particulate carbon and nitrogen depends on the data-quality objectives of the study. Section 11.4 outlines procedures to ascertain the organic/inorganic particulate ratio. The method performance presented in the method was obtained on particulate samples with greater than 80% organic content. Performance on samples with a greater proportion of particulate inorganic versus organic carbon and nitrogen has not been investigated.

1.3 Method detection limits (MDLs)[1] of 10.5 µg/L and 62.3 µg/L for PN and PC, respectively, were obtained for a 200-mL sample volume. Sediment MDLs of PN and PC are 84 mg/kg and 1300 mg/kg, respectively, for a sediment sample weight of 10.00 mg. The method has been determined to be linear to 4800 µg of C and 700 µg of N in a sample. Multilaboratory study validation data are in Section 13.

1.4 This method should be used by analysts experienced in the theory and application of elemental analysis. A minimum of 6 months experience with an elemental analyzer is recommended.

1.5 Users of the method data should set the data-quality objectives prior to analysis. Users of the method must document and have on file the required initial demonstration of performance data described in Section 9.2 prior to using the method for analysis.

2.0 Summary of Method

2.1 An accurately measured amount of particulate matter from an estuarine water sample or an accurately weighed dried sediment sample is combusted at 975°C using an elemental analyzer. The combustion products are passed over a copper reduction tube to convert the oxides of N into molecular N. Carbon dioxide, water vapor and N are homogeneously mixed at a known volume, temperature and pressure. The mixture is released to a series of thermal conductivity detectors/traps, measuring in turn by difference, hydrogen (as water vapor), C (as carbon dioxide) and N (as N_2). Inorganic and organic C may be determined by two methods which are also presented.

3.0 Definitions

3.1 ***Sediment Sample --*** A fluvial, sand, or humic sample matrix exposed to a marine, brackish or fresh water environment. It is limited to that portion which may be passed through a number 10 sieve or a 2-mm mesh sieve.

3.2 ***Material Safety Data Sheet (MSDS) --*** Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.3 ***Instrument Detection Limit (IDL) --*** The minimum quantity of analyte or the concentration equivalent which gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

3.4 ***Method Detection Limit (MDL) --*** The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.

3.5 ***Linear Dynamic Range (LDR) --*** The absolute quantity over which the instrument response to an analyte is linear.

3.6 ***Calibration Standard (CAL) --*** An accurately weighed amount of a certified chemical used to calibrate the instrument response with respect to analyte mass.

3.7 ***Conditioner --*** A standard chemical which is not necessarily accurately weighed that is used to coat the surfaces of the instrument with the analytes (water vapor, carbon dioxide, and nitrogen).

3.8 ***External Standards (ES) --*** A pure analyte(s) that is measured in an experiment separate from the experiment used to measure the analyte(s) in the sample. The signal observed for a known quantity of the pure external standard(s) is used to calibrate the instrument

response for the corresponding analyte(s). The instrument response is used to calculate the concentrations of the analyte(s) in the sample.

3.9 Response Factor (RF) -- The ratio of the response of the instrument to a known amount of analyte.

3.10 Laboratory Reagent Blank (LRB) -- A blank matrix (i.e., a precombusted filter or sediment capsule) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

3.11 Field Reagent Blank (FRB) -- An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

3.12 Laboratory Duplicates (LD1 and LD2) -- Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicate precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.13 Field Duplicates (FD1 and FD2) -- Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

3.14 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.15 Laboratory Fortified Sample Matrix (LFM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations.

3.16 Standard Reference Material (SRM) -- Material which has been certified for specific analytes by a variety of analytical techniques and/or by numerous laboratories using similar analytical techniques. These may consist of pure chemicals, buffers or compositional standards. These materials are used as an indication of the accuracy of a specific analytical technique.

3.17 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

4.0 Interferences

4.1 There are no known interferences for estuarine/coastal water or sediment samples. The presence of C and N compounds on laboratory surfaces, on fingers, in detergents and in dust necessitates the utilization of careful techniques (i.e., the use of forceps and gloves) to avoid contamination in every portion of this procedure.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[2-5] A reference file of material safety data sheets (MSDS) should also be made available to all personnel involved in the chemical analysis.

5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

5.4 Although most instruments are adequately shielded, it should be remembered that the oven temperatures are extremely high and that care should be taken when working near the instrument to prevent possible burns.

5.5 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6.0 Apparatus and Equipment

6.1 Elemental Analyzer

6.1.1 An elemental analyzer capable of maintaining a combustion temperature of 975°C and analyzing particulate samples and sediment samples for elemental C and N. The Leeman Labs Model 240 XA Elemental Analyzer was used to produce the data presented in this method.

6.2 A gravity convection drying oven. Capable of maintaining 103-105°C for extended periods of time.

6.3 Muffle furnace. Capable of maintaining 875°C ± 15°C.

6.4 Ultra-micro balance. Capable of accurately weighing to 0.1 µg. Desiccant should be kept in the weighing chamber to prevent hygroscopic effects.

6.5 Vacuum pump or source capable of maintaining up to 10 in. Hg of vacuum.

6.6 Mortar and pestle.

6.7 Desiccator, glass.

6.8 Freezer, capable of maintaining -20°C ± 5°C.

6.9 47-mm or 25-mm vacuum filter apparatus made up of a glass filter tower, fritted glass disk base and 2-L vacuum flask.

6.10 13-mm Swinlok filter holder.

6.11 Teflon-tipped, flat blade forceps.

6.12 Labware -- All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) should be sufficiently clean for the task objectives. Several procedures found to provide clean labware include washing with a detergent solution, rinsing with tap water, soaking for 4 hr or more in 20% (v/v) HCl, rinsing with reagent water and storing clean. All traces of organic material must be removed to prevent C-N contamination.

6.12.1 Glassware -- Volumetric flasks, graduated cylinders, vials and beakers.

6.12.2 Vacuum filter flasks -- 250 mL and 2 L, glass.

6.12.3 Funnel, 6.4 mm i.d., polyethylene.

6.12.4 Syringes, 60-mL, glass.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which affect analytical data. High-purity reagents that conform to the American Chemical Society specifications[6] should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. The acid used for this method must be of reagent grade purity or equivalent. A suitable acid is available from a number of manufacturers.

7.2 Hydrochloric acid, concentrated (sp. gr. 1.19)-HCl.

7.3 Acetanilide, 99.9% + purity, $C_8H_{19}NO$ (CASRN 103-84-4).

7.4 Blanks -- Three blanks are used for the analysis. Two blanks are instrument related. The instrument zero response (ZN) is the background response of the instrument without sample holding devices such as capsules and sleeves. The instrument blank response (BN) is the response of the instrument when the sample capsule, sleeve and ladle are inserted for analysis without standard or sample. The BN is also the laboratory reagent blank (LRB) for sediment samples. The LRB for water samples includes the capsule, sleeve, ladle and a precombusted filter without standard or sample. These blanks are subtracted from the uncorrected instrument response used to calculate concentration in Sections 12.3 and 12.4.

7.4.1 Laboratory fortified blank (LFB) -- The third blank is the laboratory fortified blank. For sediment analysis, add a weighed amount of acetanilide in an aluminum capsule and analyze for PC and PN (Section 9.3.2). For aqueous samples, place a weighed amount of acetanilide on a glass fiber filter the same size as used for the sample filtration. Analyze the fortified filter for PC and PN (Section 9.3.2)

7.5 Quality Control Sample (QCS) -- For this method, the QCS can be any assayed and certified sediment or particulate sample which is obtained from an external source. The Canadian Reference Material, BCSS-1, is just such a material and was used in this capacity for the data presented in this method. The percent PC has been certified in this material but percent PN has not.

8.0 Sample Collection, Preservation and Storage

8.1 Water Sample Collection -- Samples collected for PC and PN analyses from estuarine/coastal waters are normally collected from a ship using one of two methods; hydrocast or submersible pump systems. Follow the recommended sampling protocols associated with the method used. Whenever possible, immediately filter the samples as described in Section 11.1.1. Store the filtered sample pads by freezing at -20°C or storing in a desiccator after drying at 103-105° C for 24 hr. No significant difference has been noted in comparing the two storage procedures for a time period of up to 100 days. If storage of the water sample is necessary, place

the sample into a clean amber bottle and store at 4°C until filtration is done.

8.1.1 The volume of water sample collected will vary with the type of sample being analyzed. Table 1 provides a guide for a number of matrices of interest. If the matrix cannot be classified by this guide, collect 2 x 1L of water from each site. A minimum filtration volume of 200 mL is recommended.

8.2 Sediment Sample Collection -- Estuarine/coastal sediment samples are collected with benthic samplers. The type of sampler used will depend on the type of sample needed by the data-quality objectives.[7] Store the wet sediment in a clean jar and freeze at -20°C until ready for analysis.

8.2.1 The amount of sediment collected will depend on the sample matrix and the elemental analyzer used. A minimum of 10 g is recommended.

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the continued analysis of laboratory reagent blanks, laboratory duplicates, field duplicates and calibration standards analyzed as samples as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of data thus generated.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (MDLs, linear dynamic range) and laboratory performance (analysis of QC samples) prior to the analyses conducted by this method.

9.2.2 Linear dynamic range (LDR) -- The upper limit of the LDR must be established by determining the signal responses from a minimum of three different concentration standards across the range, one of which is close to the upper limit of the LDR. Determined LDRs must be documented and kept on file. The LDR which may be used for the analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from the lower standards. Determined sample analyte concentrations that are 90% and above the upper LDR must be reduced in mass and reanalyzed. New LDRs should be determined whenever there is a significant change in instrument response and for those analytes that periodically approach the upper LDR limit, every 6 months or whenever there is a change in instrument analytical hardware or operating conditions.

9.2.3 Quality control sample (QCS) (Section 7.5) -- When beginning the use of this method, on a quarterly basis or as required to meet data quality needs, verify the calibration standards and acceptable instrument performance with the analyses of a QCS. If the determined concentrations are not within ± 5% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with analyses.

9.2.4 Method detection limits (MDLs) -- MDLs should be established for PC and PN using a low level estuarine water sample, typically three to five times higher than the estimated MDL. The same procedure should be followed for sediments. To determine MDL values, analyze seven replicate aliquots of water or sediment and process through the entire analytical procedure (Section 11). These replicates should be randomly distributed throughout a group of typical analyses. Perform all calculations defined in the method (Section 12) and report the concentration values in the appropriate units. Calculate the MDL as follows:[1]

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

where, S = Standard deviation of the replicate analyses.

t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; t = 3.143 for six degrees of freedom.

MDLs should be determined whenever a significant change in instrumental response, change of operator, or a new matrix is encountered.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory reagent blank (LRB) -- The laboratory must analyze at least one LRB (Section 3.10) with each batch of 20 or fewer samples of the same matrix. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates of the samples must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained. For aqueous samples the LRB is a precombusted filter of the same type and size used for samples.

9.3.2 Laboratory fortified blank (LFB) -- The laboratory must analyze at least one LFB (Section 7.4.1) with each batch of samples. Calculate accuracy as percent recovery. If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\text{Upper Control Limit} = x + 3S$$

$$\text{Lower Control Limit} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Percent recoveries cannot be readily obtained from particulate samples. Consequently, accuracy can only be assessed by analyzing check standards as samples and quality control samples (QCS). The use of laboratory fortified matrix samples has not been assessed.

10.0 Calibration and Standardization

10.1 Calibration -- After following manufacturer's installation and temperature stabilization procedures, daily calibration procedures must be performed and evaluated before sample analysis may begin. Single point or standard curve calibrations are possible, depending on instrumentation.

10.1.1 Establish single response factors (RF) for each element (C,H, and N) by analyzing three weighed portions of calibration standard (acetanilide). The mass of calibration standard should provide a response within 20% of the response expected for the samples being analyzed. Calculate the (RF) for each element using the following formula:

$$\text{Response factor } (\mu v/\mu g) = \frac{RN - ZN - BN}{WTN}$$

where,
- RN = Average instrument response to standard (µv)
- ZN = Instrument zero response (µv)
- BN = Instrument blank response (µv)

and, $WTN = (M)(N_a)(AW/MW)$

where,
- M = The mass of standard material in µg
- N_a = Number of atoms of C, N or H, in a molecule of standard material
- AW = Atomic weight of C (12.01), N (14.01) or H (1.01)
- MW = Molecular weight of standard material (135.2 for acetanilide)

If instrument response is in units other than µv, then change the formula accordingly.

10.1.2 For standard curve preparation, the range of calibration standard masses used should be such that the low concentration approaches but is above the MDL and the high concentration is above the level of the highest sample, but no more than 90% of the linear dynamic range. A minimum of three concentrations should be used in constructing the curve. Measure response versus mass of element in the standard and perform a regression on the data to obtain the calibration curve.

11.0 Procedure

11.1 Aqueous Sample Preparation

11.1.1 Water Sample Filtration -- Precombust GF/F glass fiber filters at 500°C for 1.5 hr. The diameter of filter used will depend on the sample composition and instrument capabilities (Section 8.1.1). Store filters covered if not immediately used. Place a precombusted filter on fritted filter base of the filtration apparatus and attach the filtration tower. Thoroughly shake the sample container to suspend the particulate matter. Measure and record the required sample volume using a graduated cylinder. Pour the measured sample into the filtration tower, no more than 50 mL at a time. Filter the sample using a vacuum no greater than 10 in. of Hg. Vacuum levels greater than 10 in. of Hg can cause filter rupture. If less than the measured volume of sample can be practically filtered due to clogging, measure and record the actual volume filtered. **Do not** rinse the filter following filtration. It has been demonstrated that sample loss occurs when the filter is rinsed with an isotonic solution or the filtrate.[8] Air dry the filter after the sample has passed through by continuing the vacuum for 30 sec. Using Teflon-coated flat-tipped forceps, fold the filters in half while still on the fritted glass base of the filter apparatus. Store filters as described in Section 8.

11.1.2 If the sample has been stored frozen, place the sample in a drying oven at 103-105° C for 24 hr before analysis and dry to a constant weight. Precombust one nickel sleeve at 875° C for 1 hr for each sample.

11.1.3 Remove the filter pads containing the particulate material from the drying oven and insert into a pre-combusted nickel sleeve using Teflon-coated flat-tipped forceps. Tap the filter pad using a stainless steel rod. The sample is ready for analysis.

11.2 Sediment Samples Preparation

11.2.1 Thaw the frozen sediment sample in a 102-105°C drying oven for at least 24 hr before analysis and dry to a constant weight. After drying, homogenize the dry sediment with a mortar and pestle. Store in a desiccator until analysis. Precombust aluminum capsules at 550°C in a muffle furnace for 1.5 hr for each sediment sample being analyzed. Precombust one nickel sleeve at 875°C for 1 hr for each sediment sample.

11.2.2 Weigh 10 mg of the homogenized sediment to the nearest 0.001 mg with an ultra-micro balance into a precombusted aluminum capsule. Crimp the top of the aluminum capsule with the Teflon-coated flat-tipped forceps and place into a precombusted nickel sleeve. The sample is ready for analysis.

11.3 Sample Analysis

11.3.1 Measure instrument zero response (Section 7.4) and instrument blank response (Section 7.4) and record values. Condition the instrument by analyzing a conditioner. Calibrate the instrument according to Section 10 and analyze all preliminary QC samples as required by Section 9. When satisfactory control has been established, analyze samples according to the instrument manufacturer's recommendations. Record all response data.

11.3.2 Report data as directed in Section 12.

11.4 Determination of Particulate Organic and Inorganic Carbon

11.4.1 Method 1: Thermal Partitioning -- The difference found between replicate samples, one of which has been analyzed for total PC and PN and the other which was muffled at 550°C and analyzed is the particulate organic component of that sample. This method of thermally partitioning organic and inorganic PC may underestimate slightly the carbonate minerals' contribution in the inorganic fraction since some carbonate minerals decompose below 500°C, although $CaCO_3$ does not.[9]

11.4.2 Method 2: Fuming HCl -- Allow samples to dry overnight at 103-105°C and then place in a desiccator containing concentrated HCl, cover and fume for 24 hr in a hood. The fuming HCl converts inorganic carbonate in the samples to water vapor, CO_2 and calcium chloride. Analyze the samples for particulate C. The resultant data are particulate organic carbon.[10]

12.0 Data Analysis and Calculations

12.1 Sample data should be reported in units of µg/L for aqueous samples and mg/kg dry weight for sediment samples.

12.2 Report analyte concentrations up to three significant figures for both aqueous and sediment samples.

12.3 For aqueous samples, calculate the sample concentration using the following formula:

$$\text{Concentration } (\mu g/L) = \frac{\text{Corrected sample response } (\mu v)}{\text{Sample volume (L) x RF } (\mu v/\mu g)}$$

where, RF = Response Factor (Section 10.1.1)
Corrected Sample Response (Section 7.4)

12.4 For sediment samples, calculate the sample concentration using the following formula:

$$\text{Concentration } (mg/kg) = \frac{\text{Corrected sample response } (\mu v)}{\text{Sample weight (g) x RF } (\mu v/\mu g)}$$

where, RF = Response Factor (Section 10.1.1)
Corrected Sample Response (Section 7.4)

Note: Units of µg/g = mg/kg

12.5 The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results.

13.0 Method Performance

***13.1* Single Laboratory Performance**

13.1.1 Single laboratory performance data for aqueous samples from the Chesapeake Bay are provided in Table 2.

13.1.2 Single-laboratory precision and accuracy data for the marine sediment reference material, BCSS-1, are listed in Table 3.

***13.2* Multilaboratory Performance**

13.2.1 In a multilab study, 13 participants analyzed sediment and filtered estuarine water samples for particulate carbon and nitrogen. The data were analyzed

using the statistical procedures recommended in ASTM D2777-86 for replicate designs. See Table 4 for summary statistics.

13.2.2 Accuracy as mean recovery was estimated from the analyses of the NRC of Canada Marine Sediment Reference Material, BCSS-1. Mean recovery was 98.2% of the certified reference carbon value and 100% of the noncertified nitrogen value.

13.2.3 Overall precision for analyses of carbon and nitrogen in sediments was 1-11% RSD, while the analyses of both particulate carbon and nitrogen in estuarine water samples was 9-14% RSD.

13.2.4 Single analyst precision for carbon and nitrogen in sediment samples was 1-8% RSD and 4-9% for water samples.

13.2.5 Pooled method detection limits (p-MDLs) were calculated using the pooled single analyst standard deviations. The p-MDLs for particulate nitrogen and carbon in estuarine waters were 0.014 mg N/L and 0.064 mg C/L , respectively. The p-MDLs for percent carbon and nitrogen in estuarine sediments were not estimated because the lowest concentration sediment used in the study was still 20 times higher than the estimated MDLs. Estimates of p-MDLs from these data would be unrealistically high.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction,* available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202) 872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel,* available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Filter Diameter Selection Guide

Sample matrix	Filter diameter 47mm	25mm	13mm
	Sample matrix volume		
Open ocean	2000 mL	500 mL	100 mL
Coastal	1000 mL	400-500 mL	100 mL
Estuarine (low particulate)	500-700 mL	250-400 mL	50 mL
Estuarine (high particulate)	100-400 mL	75-200 mL	25 mL

Table 2. Performance Data--Chesapeake Bay Aqueous Samples

Sample	Measured nitrogen concentration (µg/L)	S.D.[A] (µg/L)	Measured carbon concentration (µg/L)	S.D.[A] (µg/L)
1	147	± 4	1210	± 49
2	148	± 11	1240	± 179
3	379	± 51	3950	± 269
4	122	± 9	1010	± 63

[A] Standard deviation based on 7 replicates.

Table 3. Precision and Accuracy Data - Canadian Sediment Reference Material BCSS-1

Element	T.V.[A]	Mean measured value (%)	%RSD[B]	%Recovery[C]
Carbon	2.19%	2.18	± 3.3	99.5
Nitrogen	0.195%	0.194	± 3.9	99.5

[A] True value. Carbon value is certified; nitrogen value is listed but not certified

[B] Percent relative standard deviation based on 10 replicates.

[C] As calculated from T.V.

Table 4. Overall and Single Analyst Precision Estimates from Collaborative Study

Analyte	Sample	N[1]	Mean[2] Conc.	Overall Std. Dev.	Overall %RSD	Analyst Std. Dev.	Analyst %RSD
Particulate Nitrogen (as N) in Estuarine Waters	A	11	0.0655	0.0081	12.4%	0.0050	7.6%
	B	12	0.0730	0.0076	10.3%	0.0057	7.7%
	C	12	0.0849	0.0110	12.9%	0.0060	7.1%
	D	12	0.126	0.0138	11.0%	0.0071	5.6%
	E	11	0.182	0.0245	13.5%	0.0157	8.6%
Nitrogen (as %N) in Estuarine Water	1	10	0.178	0.0190	10.7%	0.0131	7.3%
	2	10	0.295	0.0114	3.9%	0.0046	1.6%
	3	10	0.436	0.0178	4.1%	0.0104	2.4%
	4	10	0.497	0.0183	3.7%	0.0082	1.6%
	5	10	0.580	0.0207	3.6%	0.0150	2.6%
Particulate Carbon (as C) in Estuarine Waters	B	12	0.369	0.0505	13.7%	0.0222	6.0%
	A	12	0.417	0.0490	11.8%	0.0230	5.5%
	D	12	0.619	0..0707	11.4%	0.0226	3.6%
	C	12	0.710	0.0633	8.9%	0.0367	5.2%
	E	12	0.896	0.1192	13.3%	0.0569	6.4%
Carbon (as %C) in Estuarine Sediments	1	13	1.78	0.1517	8.5%	0.1346	7.6%
	2	13	2.55	0.0372	1.5%	0.0204	0.8%
	3	13	3.18	0.0435	1.4%	0.0348	1.1%
	4	13	4.92	0.1201	2.4%	0.0779	1.6%
	5	13	5.92	0.0621	1.1%	0.0547	0.9%

(1) N = Number of participants whose data was used.

(2) Concentration in mg/L or percent, as indicated.

Appendix I

Ortho-phosphate (PO_4) Standard Operating Procedure SEAL Analytical Discrete Multi-Chemistry Analyzer (AQ2)

Water Quality Analysis Laboratory at the University of New Hampshire

Prepared by: Katie Swan

Date of Last Revision: 4/25/16

Signature of Reviewer/Reviser: ____

Method is based on:

USEPA method 365.3, 1971, modified March 1983. . Determination of Ortho-phosphate by Semi-Automated Colorimetry.

Protocol PO_4

Introduction

The SEAL analytical discrete multi-chemistry auto-analyzer performs the same analytical methods as manual colorimetric assays done on a lab bench. We analyze NO_3+NO_2, PO_4, and TN/TP on surface, ground, soil extracts, and saline waters routinely with this instrument.

The PO_4 method is based on the USEPA method 365.2, 1971, modified March 1983. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorous to form an antimony-phospho-moybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color measured at 880nm is proportional to the phosphorous concentration.

Preparation of Standards and Reagents

1. Prepare 1000 mg N L^{-1} PO_4 stock by dissolving 4.3937 g potassium phosphate in a 1000 mL volumetric flask and fill to volume.
2. If the samples to be analyzed are at the lower end of the concentration range, it may be necessary to make an intermediate standard (100 mg P L^{-1}).
3. Make working standards for by pipetting the appropriate amount of stock (or intermediate standard) into 100 mL volumetric flasks, and bring them to volume. You can put empty 100 mL volumetric flasks directly on the analytical balance, allowing you to know exactly how much stock you are adding. This eliminates the necessity of weighing water (to determine the volume dispensed) before using the adjustable pipettes. Write down how much standard was added & give to lab manager. We typically use 6 working standards ranging 0 to 200 µg PO_4-P/L for the PO_4 determination in surface waters.
4. Store stock solution in clean, airtight, glass container in the refrigerator. The PO_4 stock will keep for about one month. Working standards can be stored in the volumetric flasks that they were made in. Be sure to cover them tightly with Parafilm. Standards are good for a week or so. Standards should be made weekly, or more frequently if dealing with low concentrations (< 200 ug/L).
5. A QC standard reference sample is run along with samples in a run. The QC is made using pre-made SPEX standards that is pipetted for specified amount and weighed out on the analytical balance and diluted to final desired volume. Refer to PO_4 electronic file under McDowell Shared file in drobox. Also run a Lamprey QC, which is a large batch sample from the weekly Lamprey site, as a reference. There should be a bag of them in the freezer as well.
6. Preparation of the working reagents for the method:
    a. Sulfuric acid solution, 5N: Slowly add 70 mL of concentrated H2SO4 to 400 mL DI water. Cool to room temperature and dilute to 500 mL.

b. Antimony potassium tartrate solution (0.3%): Weight 0.30 g Antimony potassium tartrate in 75 mL of DI water, dilute to 100 mL in dedicated plastic bottle. Prepare fresh monthly.
c. Ammonium moybdate solution (4%): Dissolve 4 g Ammonium moybdate tetrahydrate in 75 mL DI water, dilute to 100 mL in dedicated plastic bottle. Discard reagent if becomes turbid or discolored.
d. Working Ascorbic Acid: Dissolve 1.5 g Ascorbic acid in 80 mL of DI water. Add 2 mL of 15% SDS solution, dilute to 100 mL of DI water. Prepare this solution fresh daily.
e. Color Reagent: To a clean 125 mL plastic bottle add 75 mL of prepared 5N sulfuric acid and then add 18.0 mL Ammonium molybdate solution and mix. Add 7.5 mL Antimony potassium tartrate solution and mix. Add 4 mL 15% SDS and dilute to 100 mL with DI water. Mix. This solution should be prepared every 3 weeks.

Sample Preparation

1. Frozen samples should be completely thawed the day of analysis.

Preparation for Analysis

1. Reagents are poured into the Seal wedges and the reagent name and its position in the wedge tray should be marked. If there are reagents in the wedges and they were kept cold (i.e. in the fridge or in the Seal with it left on in the refrigerated compartment), then they can be reused if it is valid for the reagent to do so. Some reagents might need to be made daily, so please check the method. If they were not refrigerated and left in the wedges, then please dump them into the appropriate waste container and rinse the wedges several times with DI water and replace the reagent.
2. The DI water reagent wedge should be dumped and replaced with fresh DI water (or extract) each day.
3. The DI water reservoir on the side of the SEAL should be full at the start of each day and may need to be refilled if more than one run is done in a day. To refill, rinse the reservoir several times with DI water. The DI water reservoir needs DI water only.
4. Change out the appropriate reaction segments (1-10) that need to be changed (i.e. have been used). This can be checked in the “Maintenance” of the Seal software, which will be described below.
5. The SEAL may need to be turned on. The power switch is on the back right side of the instrument. Start up the SEAL software that is labeled “SEAL AQ2”. To log in the username is “wrrc lab” and password is “waterlab”.
6. When the software opens on the “run screen”, select the “Maintenance and Daily Start Up” tab on the top right of the screen.
    a. Click on the “Maintenance” tab and the “main maintenance and setup” window will come up. Want to zero reaction segments each run, select “Zero Segments” and select yes. To select desired maintenance function is on the left side of the window.

b. Select the “Diluter” tab. The diluter needs to be primed each day to ensure that there are no air bubbles present. To prime the diluter, select the “diluter” tab and select total number of primes (10x) and start prime.
c. When finished priming the diluter, then select the “Cuvette functions” tab. The aspiration wash bath needs to be auto washed at the start of the day. Select the total number of washes (2x) and click on the “auto wash” button. Make sure that the aspiration bath is filling up and draining.
d. When finished with the auto wash, select the “test aspiration tab”. Take off the cover in the left corner in SEAL and will see an inlet and outlet tubing from the cuvette. The value that is used (e.g. 200) is to ensure that the headspace in the outlet and inlet tubing is about 1 inch from the cuvette. Click on “test aspiration” tab and watch where the headspace is when the test finishes. If need to make adjustments to increase or decrease the headspace in the tubing, increase or decrease the initial value and run the aspiration test again.
e. When finished with the test aspiration, select the “extra wash” tab. Make sure to have the cuvette cleaning solution wedge in the first position in the reagent tray on the right side of the seal. Run the extra wash and watch to see if the syringe is pulling up the solution properly (no dripping or beading at the tip). Make sure that the syringe is landing in the right well in the reaction segments.
f. When finished with the extra wash, take the cuvette cleaning solution wedge out. Exit the main maintenance and setup screen and select “daily start up.” Hit continue. The daily startup will measure and absorbance and a list eight absorbance readings will be reported on the far right side of the main screen. Record the absorbance values each week and make sure the absorbance values do not drift too much each day. This measures the absorbance of water in the cuvette to account for changes in the cuvette over time and check the condition of the filters and lamp.

7. If this is the first run with new working standards, then the calculated standard concentrations need to be entered into the Tests. Click on “Tests” under the maintenance and daily startup tab, select the appropriate method, select calibration, and enter the standards into the appropriate spaces under the manual standards (S1-S7; S1 is a blank).
8. In “Maintenance” make sure that the appropriate tray is selected for the tray that you are going to use.
9. Click on “Scheduling”, select tray number and select reagent set #1, and type in the run file (i.e. 160304NO301). In the upper left of the window select the sample type (standards and unknowns), select standards 1-7 (S1-S7). Then enter the UNH ID # in sample ID, which automatically will be entered as type “unknown”, and enter a rep after every 12 samples and Enter a subset or all of the standards at the end of your run setup without using type “standards”, so that they will be entered as unknowns. The method is set up to automatically enter blanks, QCs, and duplicates every 12 samples, so this does not to be included in the amount that you enter. On the right hand side in the “Requested Tests” column highlight all the cells that contain samples in that column and then select “op1” at the top. When finished entering, click the “save” icon at the top left of the window.
10. Double click“run” when run is set up and saved. Select the run file for the run and continue.

11. Rinse each vial once with sample or standard and then fill ¾ full with the SEAL sample cups (1.2 mL or 2mL sample cups).
12. Samples should be placed in the appropriate SEAL sample tray (57 samples or 100 samples trays). Sample trays should be placed in the proper position and screwed in tightly to ensure the tray is not moving around during the run.
13. START the run by clicking on the "Run" tab and select to continue.
14. After the run has started, check the "calibration" tab to make sure the calibration curve is analyzed and check that it is acceptable after it has run the calibration standards at the beginning. Select the "Data Review" tab and that the first set of QC standards are recovered appropriately.
15. When the run is complete, click on "Data Review" to the left of the window, select "Accept All" on the top tabs and export to a document file and save under export file.

Data Export

1. Following completion of your analysis you are responsible for checking the data. The data is to be copied and pasted into the appropriate lab Excel Report Template on the SEAL computer and the file should be named by date of analysis (described in worksheet). This template will guide you to report the QC results for the run. This includes % recovery of QC standards (CRM), run time check standards, and lab duplicates. Lab % recovery of sample duplicates, run time check standards, and QC standards should be between 85 and 115 % (see WQAL QAPP for more information).
2. When completed copy the Excel file into the lab manager's directory on the main lab computer. This information, along with the data, will be entered into the WQAL database by the lab manager to create control charts.

Shut Down Instrument

1. When finished exporting data, need to shut down the instrument.

a. Go to the main screen, click on the seal icon on the upper left corner and choose to exit the software. A window will come up and select both boxes "shutting down instrument overnight? and close program?"

2. Put the reagent tray back in the fridge with the reagents in the wedges.
3. Turn off the lamp on the instrument, leave the reagent cooling tray on.
4. Empty the sample tray (sample in the sink and sample vials in the trash).
5. Empty out the DI water reservoir.