Mamaroneck Harbor and Little Neck Bay, NY
UWS Water Quality Monitoring QAPP

For monitoring activities conducted as part of the Long Island Sound Tier 2 Unified Water Study (UWS).

Monitoring Organization
Connecticut Fund for the Environment/Save the Sound

Funded By
National Fish and Wildlife Foundation

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2017.08.03

A. Project Management

A.1. Approval Page

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A.4. PROJECT / TASK ORGANIZATION

Table 1: Project Organization.
Key project personnel and their corresponding responsibilities.

Name(s)	***Project Title*** - Responsibility
Tracy Brown	***Monitoring Program Project Manager*** – Oversees all aspects of project that incorporate the monitoring program including: fiscal management, project objectives, data uses, program changes, etc.
Peter Linderoth	***Monitoring Program Coordinator*** (a.k.a. Monitoring Coordinator) – Volunteer recruitment and training, coordination with TAC. Develops *General QAPP Adoption Form*. Produces monitoring report. Produces or oversees outreach efforts, in coordination with project manager.
Peter Linderoth	***Monitoring Program Field Coordinator*** – Responsible for training and supervising volunteers in field work. Ensures field forms are properly filled out, samples and forms are transported to laboratories as needed; and performs QC checks to make sure procedures are followed or corrected as needed (in collaboration with project QC officer).
Peter Linderoth	***Monitoring Program Lab Coordinator*** – Makes arrangements with any lab(s) used to perform analyses according to QAPP. Ensures correct procedures are used, holding times are met, and adequate documentation is provided.
Peter Linderoth	***Monitoring Program Data Management Coordinator*** – Maintains the data systems for the program. Performs/oversees data entry and checks entries for accuracy against field and lab forms.
Tracy Brown	***Monitoring Program Quality Assurance Officer*** – Runs Quality Assurance (QA) program. This person cannot be directly involved with field sampling or sample analysis.
Lynn Dwyer	***Funding Agency Project Contact*** – Oversees grant administration and ensures reporting requirements are met.
Mark Tedesco	***USEPA Project Manager*** – Oversees QAPP submission to EPA.
Click here to enter name.	***Funding Agency Technical Reviewer*** – Reviews QAPP.
Kathryn Drisco	***USEPA Quality Assurance Officer*** – Reviews and approves the QAPP
Changes by year. Individual names are not listed.	***Monitoring Program Volunteers*** – Sample, perform field analyses, assist in laboratory analyses and/or data entry.
Dr. Jamie Vaudrey, Dr. Jason Krumholz	***Technical Advisory Committee*** (TAC) – Program oversight and advice.

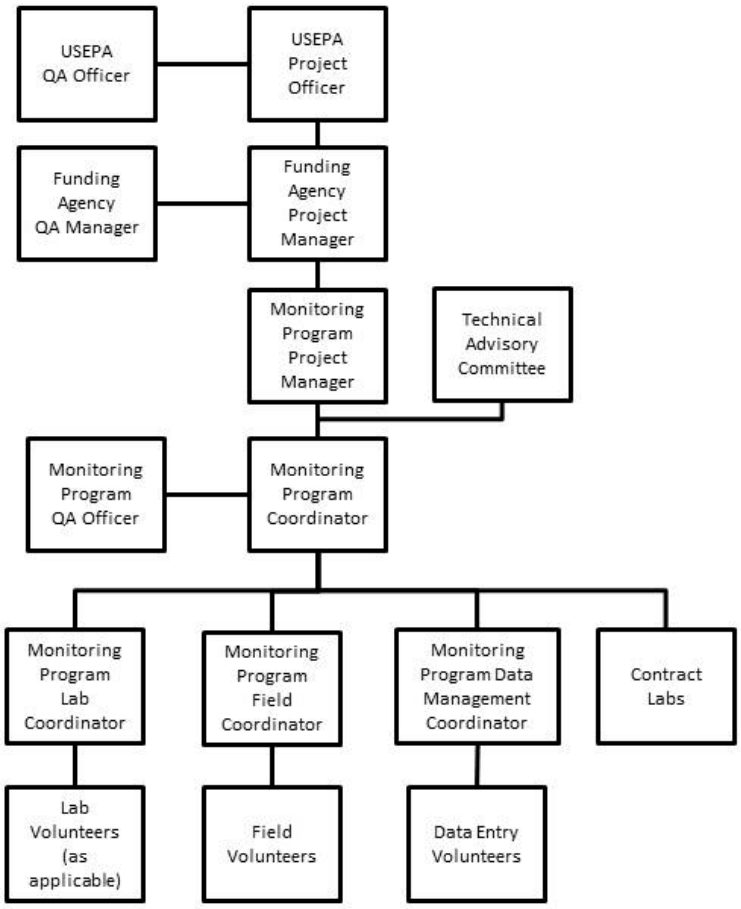

Figure 1: Organizational Chart.
Lines between boxes indicate direct communication.

A.5. PROBLEM DEFINITION / BACKGROUND

Despite three decades of effort to improve water quality, Long Island Sound remains a severely stressed environment. In the western Sound, from Greenwich to Nassau County, dissolved oxygen concentrations—a key measure of the Sound's health—consistently fall to levels too low to sustain aquatic wildlife.Extremely low levels of dissolved oxygen, or hypoxia, are worsened by excess nitrogen from outdated sewage collection systems, failing septic systems, contaminated stormwater runoff, and fertilizers. Moreover, there are serious eutrophication-

related impacts in embayments throughout the Sound[1]. According to the EPA's Nitrogen Reduction Strategy, "Impairments linked to excess discharges of nitrogen (N) include harmful algal blooms, low DO, poor water clarity, loss of submerged aquatic vegetation and tidal wetlands, and coastal acidification."

Mamaroneck Harbor and Little Neck Bay are embaytments located in the western portion of Long Island Sound. Mamaroneck Harbor's main tributary is the Mamaroneck River, NY which extends through multiple municipalities and urban areas contained in Westchester County, NY. It is likely that stormwater runoff and other factors contribute to eutrophic conditions in this embayment.

Little Neck Bay, NY is a combined sewer overflow receiving embayment in the western Long Island Sound. It is also surrounded by impervious surfaces that contribute to high stormwater runoff volumes. These factors could contribute to eutrophic conditions.

Organizational History and Mission

The mission of Connecticut Fund for the Environment (CFE) and its bi-state program Save the Sound is to protect and improve the land, air and water of Connecticut and Long Island Sound. Founded in 1978, CFE merged in 2004 with Save the Sound, a respected voice for the protection of Long Island Sound's shoreline, marine habitat and water quality with a track record of more than 40 years. The proposed project is in line with one of Save the Sound's strategic goals: "Our Long Island Sound, rivers and lakes are safe for people and wildlife."

Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the Unified Water Study (UWS). The UWS is a coordinated effort among groups monitoring Long Island Sound embayments with the goal of comparing water quality parameters associated with eutrophication within and among embayments using a report card format. The UWS will be divided into two tiers, Tier I and Tier II. Tier I data is required for entry into the study and is not covered in this QAPP or the respective project proposal to the National Fish and Wildlife Foundation. The parameters compared as part of the Tier I section of the study are dissolved oxygen, salinity, water clarity, chlorophyll-a, turbidity, and qualitative macrophyte assessments. Tier II parameters, covered under this QAPP and being piloted in 2017, will compare nutrient concentrations, logged dissolved oxygen and salinity data, and underwater camera assessments of macrophytes.

The UWS is divided into two Tiers of parameters and procedures. This QAPP covers the piloting of the Tier II parameters: Nutrients, Macrophyte Abundance via Camera, and Continuous

[1] Vaudrey, J. M., Yarish, C., Kim, J. K., Pickerell, C., Brousseau, L., Eddings, J., & Sautkulis, M. (2016). Comparative analysis and model development for determining the susceptibility to eutrophication of Long Island Sound embayments. Connecticut Sea Grant Final Project Report, 38.

Dissolved Oxygen and Salinity Data Logging. The piloting of these parameters and procedures will determine their usability, or exclusion, in future UWS Tier II efforts. Meetings with the Long Island Sound community will be held after the pilot season to discuss the future of UWS Tier II monitoring. New York DEC, Connecticut DEEP, and the Long Island Sound Study attended multiple meetings to discuss the data usability in respect to management. The management decisions to be made from these data are not finalized as this is a piloting of procedures for the Tier II portion of the UWS; however, verbal consensus was achieved around the parameters selected for this pilot season.

Monitoring History and Status

The Western Long Island Sound Office of Save the Sound initiated a pathogen-indicator and water monitoring program in 2013 and has since expanded the spatial and temporal scale of the program. The monitoring efforts of Save the Sound also include analyses of physical and chemical water quality parameters. Save the Sound was the lead facilitator of the development of the Unified Water Study (UWS) and participated in the 2016 UWS pilot season. Save the Sound continues to participate in the UWS. Measuring the eutrophic conditions in the bays and harbors of Long Island Sound directly relates to Save the Sound's overarching goal of reducing Nitrogen and other pollutants in the Sound.

The Unified Water Study conducted a pilot season with a few existing monitoring groups in 2016. The goal of the 2016 season was to develop protocols that followed standard methods for embayment monitoring and were achievable by the monitoring groups. All groups involved with the 2016 season had previous experience monitoring their embayments and were involved in ongoing monitoring programs. Groups involved with the 2016 pilot season assisted with revisions to the SOPs, to make these documents more complete and useful to new UWS participants.

Save the Sound will pilot the Tier II procedures during the summer of 2017 with a goal of developing an advanced layer of water quality monitoring for groups that have the resources.

Moving forward, monitoring group activities will be conducted under an EPA approved QAPP (this document), with each monitoring organization submitting and maintaining their own organization-specific QAPP. The organizational host of the Unified Water Study will update and provide web-based access to the SOPs for groups. Groups will be responsible for obtaining the latest version of the relevant SOPs when submitting a new QAPP.

Monitoring and Data Use Objectives

Coastal Water Quality Monitoring: Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the Unified Water Study (UWS). The UWS is designed to facilitate equitable water quality comparisons across Long Island Sound embayments. One of the core means of reaching this goal is that all groups that participating in the study will conduct sampling from a shared set of standard operating procedures (SOPs). The Tier I SOPs, not relevant to this QAPP, are established and are being followed by participating UWS groups.

The Tier II SOPs, contained in this QAPP, are being piloted this season before being distributed to the Long Island Sound water quality monitoring community. Data collected under the UWS will be shared with appropriate management agencies and have potential to be included in future Long Island Sound Report Cards. Participating monitoring groups will have the option to share data on a more local level as well to potentially elicit changes that could improve water quality in their respective embayments.

Additionally, the activities covered under this QAPP will provide quality-controlled data that support the assessment and restoration of coastal watersheds and critical habitats through the implementation of programs such as:

- EPA's 305(b) water body health assessments and 303(d) TMDL development for impaired waters
- Clean Water Act Section 319 projects
- CT DEEP and NY DEC Watershed Management Plans
- Long Island Sound Study's Comprehensive Conservation and Management Plan
- Connecticut's and New York's Nonpoint Source Management Program

A.6. PROJECT / TASK DESCRIPTION

Three types of stations are included:

1) A minimum of four water quality stations per embayment are required. If multiple sections of the embayment are to be evaluated, a minimum of three stations are required per section. Water quality stations are sampled from August to October 31, at least twice a month. Tributary stations will be sampled for salinity and nutrients on the same day as the embayment they flow into is sampled. These stations are chosen with respect to access and salinity value <1 ppt.

2) Continuous dissolved oxygen stations will be selected with consideration to representativeness and where access is granted for maintenance of the equipment arrays. A minimum of one continuous dissolved oxygen station is required per embayment section. Logging will commence from August to October 31.

3) Macrophyte stations are boat-based. They are sampled only mid to latesummer and will typically be sampled on different days from the *water quality* stations. Sampling occurs between August to September 21.

Water quality parameters are selected to facilitate comparisons between embayments and have established thresholds. The macrophyte abundance video survey is a relatively new method which could aid in identifying the dominant primary producer group in an embayment. Save the Sound will collect the following data to be included in the Tier II development process of the UWS. :

- for each water quality station
    - GPS coordinates of stations, recorded each sample date
    - date and time
    - 0.5 m below the surface
        - Total Nitrogen
        - Total Dissolved Nitrogen
        - Dissolved Inorganic Phosphorous
        - Dissolved Inorganic Nitrogen Species (nitrate, nitrite, ammonium, phosphate)
        - Dissolved Organic Nitrogen
        - Total Phosphorous
- for each continuous dissolved oxygen station
    - GPS coordinates of stations
    - biweekly data collection from instruments
- for each macrophyte station
    - GPS coordinates of stations, recorded each sample date
    - date and time interval
    - video of macrophyte abundance
- within 2 days of the field sampling day, read the GPS of a land-based reference station

How the proposed sampling plan supports the Monitoring Program objectives:

Data collected under this QAPP will be collected in a manner to allow the data to be used as part of the Unified Water Study (UWS). The UWS is a coordinated effort among groups monitoring Long Island Sound embayments with the goal of comparing water quality within and among embayments using a report card format. The Embayment Report Card is a vehicle to share data that is clear and visual, and to analyze that data and assign grades using a previously established scale based on objective scientific and regulatory criteria. The Report Card is a place for the data to do the talking and for the narrative to follow clear, observable data trends. When the data drives grades at specific locations that are outside the norm – whether high or low – this can create opportunities for local and regional press stories that focus on these data trends.

The 2017 Tier II monitoring undertaken by Save the Sound will serve as a pilot season for the Tier II element of the Unified Water Study. Standard Operating Procedures will be finalized and potentially adjusted after the completion of the monitoring season.

Overview of data handling processes.

Data will be collected on standardized field sheets. Examples are provided in the Appendix.

If a field team is delivering a sample for analysis by a lab external to the monitoring group, a Chain of Custody form is required. This is the case with this project. Example of the Chain of Custody Form is provided in the Appendix.

A.6.a. Sampling Types Covered by this QAPP

The types of sample information that are collected under this QAPP includes:

- Water depth the nutrient samples are collected
- Salinity concentration
- Dissolved oxygen concentration and percent saturation to determine the amount of oxygen available for aquatic life
- Nitrogen forms to measure nutrient levels
- Phosphorus forms to measure nutrient levels
- Biological monitoring to determine the nature of plant and/or animal communities

A.6.b. Maps of Study Area

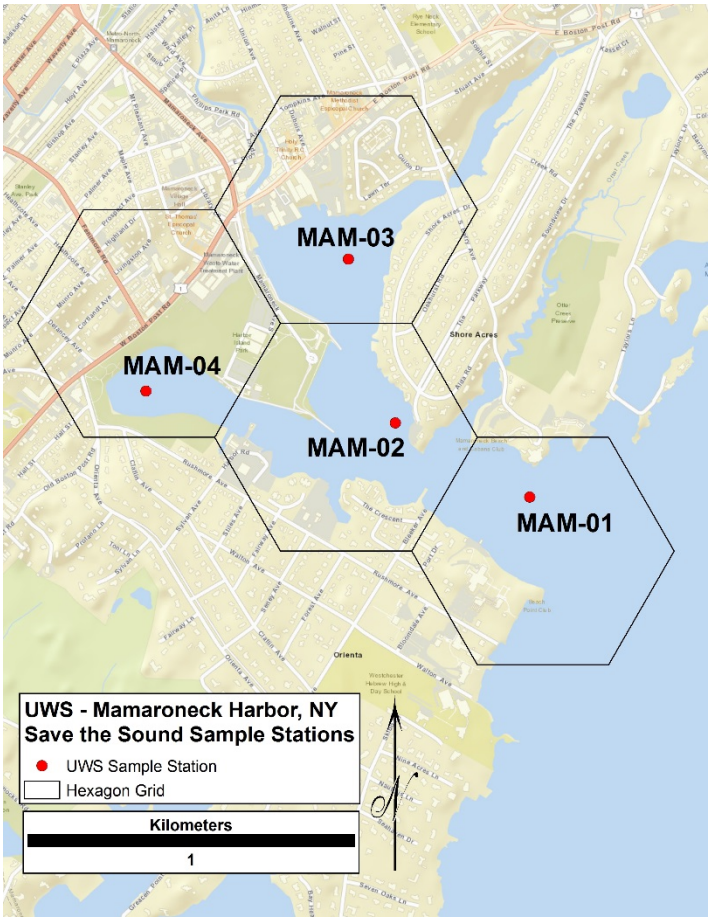

Figure 2: Map of the stations included in Mamaroneck Harbor, NY.

Red dots are station locations for estuary water quality monitoring. Continuous dissolved oxygen station will be in West Basin of the Harbor. Tributary stations will be sampled in Beaver Swamp Brook and Mamaroneck River. Station Coordinates provided below:

Station_ID	Longitude	Latitude
MAM-01	-73.72225	40.94088
MAM-03	-73.72894	40.94737
MAM-04	-73.73625	40.94367
MAM-02	-73.72717	40.94288

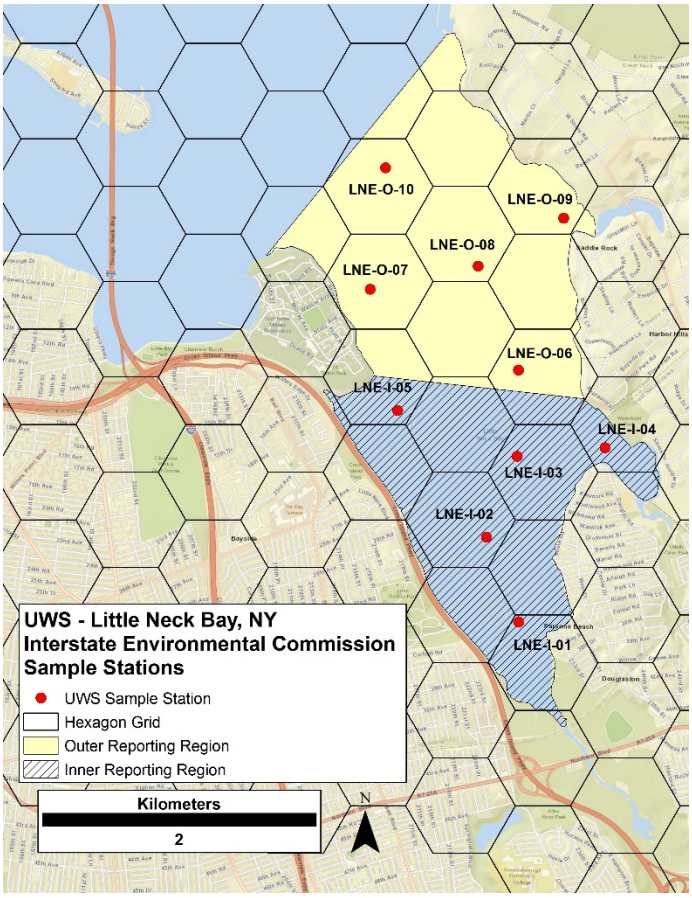

Figure 3: Map of the stations included in Little Neck Bay, NY.

Red dots are station locations for estuary water quality monitoring. Continuous dissolved oxygen station will be in both reporting regions. Reporting regions clearly delineated in figure. Tributary stations will be sampled in Alley Creek and Gabblers Creek. Station Coordinates provided below:

STATION_ID	Longitude	Latitude
LNE-O-06	-73.7582	40.7888
LNE-I-02	-73.7608	40.7778
LNE-I-01	-73.75791	40.77224
LNE-I-03	-73.75823	40.78314
LNE-I-05	-73.76862	40.78606
LNE-I-04	-73.75061	40.78377
LNE-O-09	-73.75442	40.79884
LNE-O-10	-73.76992	40.80202
LNE-O-07	-73.77112	40.794
LNE-O-08	-73.76179	40.79561

A.6.c. Annual Task Calendar

Table 2: Annual Task Calendar

Activity	J	F	M	A	M	J	J	A	S	O	N	D
Kickoff meeting with project team			x									
Develop draft QAPP				x	x	x						
Finalize QAPP						x	x	x				
Meeting with funding agency representatives												
Equipment inventory, purchase, inspection, and testing					x	x						
Field training and database-related training session(s)							x	x				
Meeting with analytical laboratory					x	x						
Lab training sessions (in-house analyses)												
Sampling surveys								x	x	x		
Data entry									x	x	x	

Activity	J	F	M	A	M	J	J	A	S	O	N	D
Data review and validation										x	x	
Field audit(s)												
Lab audit(s)												
Draft report											x	
Final report												x
Data uploads to website												x

A.7. DATA QUALITY OBJECTIVES

Taken together, precision, accuracy and bias, representativeness, comparability, completeness, and sensitivity comprise the major data quality indicators used to assess the quality of the program's data. A summary of criteria are provided in Table 3.

Definitions of these data quality indicator terms:

- **Precision** is the degree of agreement among repeated field measurements of the same indicator and gives information about the consistency of your methods. It is typically defined as relative percent difference, or RPD.

- **Accuracy** is a measure of confidence that describes how close a measurement is to its "true" or expected value; it includes a combination of random error (precision) and systematic error (bias) components of both sampling and analytical operations.

- **Bias** is the systematic or persistent distortion of a measurement process that causes errors in one direction.

- **Representativeness** is the extent to which measurements actually represent the true environmental condition. Parameters, site selection (including location of sampling point within the water column), time, and frequency of sample collection can all play a role in determining how representative a sample is.

- **Comparability** is the extent to which data can be compared between sample locations or periods of time within a project, or between different projects.

- **Completeness** is the comparison between the amount of valid or usable data the program originally intended to collect versus how much was actually collected. EPA Region 1 also expects an evaluation of critical samples that may require re-sampling even if the 80% goal has been achieved.

- **Sensitivity** is the capability of a method or instrument to discriminate between measurement responses representing different levels of the variable of interest.

Table 3: Example Measurement Performance Criteria

Data Quality Indicators	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Criteria
Precision – overall	RPD ≤ value indicated in Table 4.	field duplicates
Precision – analytical	RPD ≤ value indicated in Table 4.	analytical duplicates
Accuracy / Bias	85% ≤ recovery ≤ 115%	certified reference material lab fortified matrix (spikes)
Comparability	standard methods followed	NA
Completeness	data from surface and bottom at each station meet data quality objectives	data completeness check
Sensitivity	value ≥ IDL*	sample value check

* IDL = instrument detection limit. This is a reporting limit based on the lowest standard accurately analyzed in the analysis.

Precision - Typical precision objectives are listed in Table 4. Precision is evaluated in the field by taking duplicate measurements for at least 5% of samples, where applicable.

For UWS samples, estuary stations sampled for nutrients will have a field replicate sampled at each station.

When a multiparameter sonde is used, standards will be read before and following a trip, within one day of the field day. Multiparameter sondes can hold their calibrations for weeks. The pre and post sampling event readings can identify any potential drift outside of manufacturer recommendations for calibration. These values will be kept with all other data for review at the end of the project.

The Onset HOBO loggers (temperature, oxygen) and Star-Oddi loggers (temperature, salinity, depth) will be deployed in a common water bath before deployment and following deployment. Salinity, temperature and oxygen will be varied in the bath, allowing for multiple values for intercomparison. The temperature, salinity, and oxygen of the bath will be determined with the instruments being used by Save the Sound for conducting water quality profiles. These pre- and post-baths will serve to cross-calibrate all instruments and to determine if the deployed loggers exhibited any drift over the course of the deployment. The deployed loggers will be intercalibrated by applying a multiplicative correction if initial values differ by more than 10% from the reference value (as determined from the YSI EXO1 sonde or YSI 556).The frequency of field duplicate measurements for each parameter are described as in Table 8 (Section B.5., page 32).

Relative percent difference (RPD) of duplicate samples is used as one index of precision (Table 4) This is defined as the absolute difference between the duplicates divided by the average of

the duplicates. The allowable RPDs for each parameter are provided in Table 4. A difference greater than the designated RPD requires further investigation of the sample run. If the difference is large enough, it indicates failure (unless the average of the two samples is less than 10 times the method detection limit), and results in reanalysis of the entire set of replicates from that station depth, unless there is a reasonable and supported explanation for the inconsistency. Duplicate precision will be analyzed by calculating the RPD using the equation:

$$RPD\ (\%) = |x1 - x2| \ / \ ((x1 + x2)/2) * 100$$

where x1 is the original sample concentration and x2 is the duplicate sample concentration.

The Microsoft Excel formula for calculating the RPD is:

$$= ABS(X1-X2) \ / \ ((X1+X2) \ / \ 2) * 100$$

where X1 is the original sample concentration and X2 is the duplicate sample concentration. The RPD is automatically calculated in the UWS data entry template for duplicate profiles and duplicate field samples.

Accuracy and Bias - Accuracy objectives are listed in Table 4. Procedures used to test or ensure accuracy are described in Table 8. While training helps to ensure measurement accuracy and precision, quantitative measures of accuracy for laboratory analyses are estimated using laboratory QC data (blank results, fortified matrix results, and lab- fortified blanks). When a multiparameter sonde is used, standards will be read before and following a trip, within one day of the field day. Nutrient analysis will include a laboratory blank and reference standards. Data loggers will be calibrated prior to and after deployment. Biweekly comparative readings between loggers and sonde will be recorded to keep a log of any drift occurring with loggers. These data will be evaluated with the log data in the final report and during the season.

Representativeness – Estuary sampling sites are selected to be representative of the waterbody using EPA methods for representative station selection[2]. This approach allows for the use of probability-based statistics in data analysis, without bias introduced by non-random station choice. In this approach, a 0.42 km^2 hexagonal grid is overlain on the site map. Three random stations are generated in ArcGIS in each section of the embayment within a hexagon. Large embayments will have hexagons for random station generation selected with the UWS Science Advisors. A minimum of four stations will be sampled in each embayment, with larger sites having up to twelve stations. The location of the station in each hexagon will be randomly generated, with two alternate locations chosen ahead of time, in case the original location is

[2] EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

deemed unusable (e.g. too shallow or in the middle of a navigation channel). If none of the three random stations are accessible, a station will be determined as close as possible to a randomly generated station. The exception to this approach is the determination of station locations in the tidal fresh zone. These stations are sampled from land and are thus determined by accessibility to the water and the location of the tidal fresh zone. These stations will be sampled at a minimum of once during each tidal cycle (Flooding and Ebbing). Sample collection timing and frequency is selected to capture data that are representative of embayment conditions. Time of high and low tide are noted and will be considered in the analysis of results. Any abnormal or episodic conditions that may affect the representativeness of sample data are noted and maintained as metadata.

Data logging stations are selected with at least one station per reporting region. Access to station is a strong consideration in the selection process. The final station locations will be conferred with UWS Science Advisors before deployment.

Macrophyte surveys will occur along multiple transects in each embayment and in all reporting regions.

Comparability - The comparability of the data collected is assured by using known protocols and documenting methods, analysis, sampling sites, times and dates, sample storage and transfer, as well as laboratories and identification specialists; so that future surveys can produce comparable data by following similar procedures. Examples of project procedures are available in the collection of Standard Operating Procedures (SOPs) provided as an appendix to this document.

Completeness – We will attempt to maximize the completeness of the dataset, achieving a minimum of 80% of samples successfully collected and approved for use. In the end, however, any quality-controlled data are considered useful in some way.

Sensitivity – Sensitivity objectives are listed in Table 4. Sensitivity is the lowest detection limit of the method or instrument for each of the measurement parameters of interest. For analytical methods, these are the method detection limits (MDLs). For instruments (probes and loggers) these are usually listed in the instrument manual as sensitivity or resolution.

Table 4: Data Quality Objectives
Examples of standards used for these methods is included in Table 10.

Parameter	Units	Accuracy [a]	Precision [b] (RPD)	Approx. Expected Range	Sensitivity (Resolution or IDL)
location by coordinates (GPS) -use NAD 83 coordinate system or note alternative at each data entry	degrees and decimal minutes (Xo X.XX') -or- decimal degrees (X.XXXXX)	+/- 20 feet with Wide Area Augmentation System (WAAS) enabled	Repeated readings to verify coordinates essentially the same	NA	*
salinity (meter, e.g. YSI, In-Situ, Hobo, Hach, Star-Oddi)	psu (= ppt) (psu: practical salinity units)	± 0.1ppt or 1% of reading, whichever is greater	20%	0-32 psu	*
conductivity (meter, e.g. YSI, In-Situ, Hobo, Hach, Star-Oddi)	µS/cm (freshwater) mS/cm (salt water) (S: Siemens)	+/- 1% of reading or 0.001 mS/cm, whichever is greater	20%	10–1000 µS/cm in fresh 0.8–55 mS/cm in salt water	*
dissolved oxygen concentration / saturation (meter, e.g. YSI, In-Situ, Hobo, Hach, Star-Oddi)	mg/L O2 (= ppm = g/m3) % saturation O2	0 to 20 mg/L: ± 0.2 mg/L or 2% of reading, whichever is greater ± 2% sat or 2% of reading, whichever is greater	20%	0-14 mg/L 0-120%	*
(organic + inorganic, all forms; unfiltered sample)	mg/L TN (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	30%	0-5	0.03 mg/L
total dissolved nitrogen - TDN (organic + inorganic, all forms; filtered sample)	mg/L TDN (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	30%	0-4.5	0.03 mg/L
ammonia - NH3 (sum of NH_3, NH_{4+})	mg/L NH3 (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	30%	0-1	0.02 mg/L
nitrate-nitrite – NO_X or NO_{3-} + NO_{2-} (sum of NO3-, NO2-)	mg/L NOx (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	30%	0-2.5	0.02 mg/L
nitrite - NO2- (only NO2-)	mg/L NO2 (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	30%	0-0.7	0.02 mg/L
dissolved inorganic nitrogen – DIN (sum of ammonia, nitrate, nitrite)	mg/L DIN (= ppm = g/m3)	value calculated from multiple N analyses – see above from a filtered water sample	30%	0-4	0.02 mg/L
total inorganic nitrogen – TIN (sum of ammonia, nitrate, nitrite)	mg/L TIN (= ppm = g/m3)	value calculated from multiple N analyses – see above from an unfiltered water sample	30%	0-4.5	0.02 mg/L

Parameter	Units	Accuracy [a]	Precision [b] (RPD)	Approx. Expected Range	Sensitivity (Resolution or IDL)
total organic nitrogen – TON (TN - TIN)	mg/L TON (= ppm = g/m3)	value calculated from multiple N analyses – see above from an unfiltered water sample	30%	0-5	0.03 mg/L
dissolved organic nitrogen - DON (TDN - DIN)	mg/L DON (= ppm = g/m3)	value calculated from multiple N analyses – see above from a filtered water sample	30%	0-4.5	0.03 mg/L
particulate nitrogen – PN (organic and inorganic N on particles)	mg/L PN (= ppm = g/m3)	value calculated from multiple N analyses – see above -or- by analyzing the filter pad	30%	0-0.5	0.01 mg/L
total phosphorus – TP (sum of organic P, ortho-P, condensed P; unfiltered sample)	mg/L TP (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	20%	0-0.5	0.01 mg/L
dissolved organic nitrogen - DON (TDN - DIN)	mg/L DON (= ppm = g/m3)	value calculated from multiple N analyses – see above from a filtered water sample	30%	0-4.5	0.03 mg/L
total dissolved phosphorus – TP (sum of organic P, ortho-P, condensed P; filtered sample)	mg/L TDP (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	20%	0-0.5	0.01 mg/L
dissolved inorganic phosphate – PO43- or DIP (primarily orthophosphate)	mg/L PO4 mg/L DIP (= ppm = g/m3)	80% - 120% recovery of lab fortified matrix (LFM)	20%	0-0.3	0.01 mg/L
vegetation abundance (grasses, sedges, eelgrass, macroalgae, etc.)	percent cover (%) / quadrat (0.25 meter$_2$)	30% agreement among two separate evaluations; evaluate 10% of samples	30%	0-100	undefined

a) "General" accuracy objectives are estimates assuming a true value were known and could be tested; all analytical accuracy objectives (i.e., for samples) include non-detectable concentrations in ambient field blanks.

b) For analytical samples, the objective for overall precision is typically based on the relative percent difference (RPD) of co-located, simultaneous field duplicates

A.8. SPECIAL TRAINING / CERTIFICATION

Project training shall take place as specified in Table 5.

All members of the project team are required to review the SOPs and be trained by the Monitoring Coordinator on the following: filed safety, water sample collection, use of instruments, filling out field sheets, operation of underwater camera and associated equipment.

The Monitoring Program Coordinator enters training into an Excel spreadsheet recording: subject matter (monitoring and procedures covered), date, name and qualification of trainers, and names of participants trained. The Monitoring Program Coordinator worked closely with the Science Advisor signatories on this QAPP to confirm procedures are appropriate. He was part of the three person team leading UWS Tier I trainings around Long Island Sound. The Coordinator oversees Save the Sound's Water Quality Program which samples water for pathogen-indicator bacteria and participates in the UWS Tier I monitoring. The Coordinator holds a Bachelor of Science Degree in Environmental Studies from University California Santa Barbara and a Master's of Science degree in Environmental Science and Management from Sacred Heart University.

Field and lab safety will be covered as part of every training. The specific information covered will vary depending on the location of sampling (e.g. from a boat, from a roadway) and the type of sampling. Issues covered may include, but are not limited to: personal safety, buddy system / informing others of plans to work in the field, situational awareness, safe handling of equipment, chemical safety, and planned response in the event of an emergency or chemical spill. Safety considerations are covered more fully in section B.1.

Table 5: Project-Specific Training

Training: Type & Description	Trainer(s)	Training Date(s)	Trainees	Location of Training Records
Nutrient sampling. Collection of samples, filtering, and preservation of samples.	Monitoring Coordinator	July 2017	Seasonal Save the Sound Staff and Volunteers (TBD)	Monitoring Coordinator Computer
Macrophyte surveys with camera. Operation of camera and associated equipment.	Monitoring Coordinator	July 2017	Seasonal Save the Sound Staff and Volunteers (TBD)	Monitoring Coordinator Computer
Data logging. Cleaning of loggers, data retrieval, and intercomparison field readings between sonde and loggers.	Monitoring Coordinator	July 2017	Seasonal Save the Sound Staff and Volunteers (TBD)	Monitoring Coordinator Computer

A.9. DOCUMENTS AND RECORDS

Field data sheets or logbook will be completed on site at the time of sampling. They will include the sample collection date and times, the site, number and/or location, and samplers' names.

Sample Labels will be put on all sample containers. Labels will include the site name, date, time, location, and type of sample. Nutrient samples are the only samples that will require labels.

Chain of Custody (COC) forms will accompany samples from collection sites to laboratories. COC forms will be signed by collectors and all individuals who gain custody of the samples until they arrive at a lab. Information will agree with the label information on the sample bottles. Information such as the ID number, date,, sample container volume, and people exchanging sample will be included on the Chain of Custody form.

Miscellaneous records for **instrument checks, calibrations, and maintenance** will be kept in a logbook. The logbook may be a hand-written bound book or a three-ring binder.

Training records for all volunteers involved in the project must be kept.

The electronic project **database** shall be organized and protected from loss and damage through proper back-up of digital data.

No **scientific collecting permits** or **Certificates of Permission** are required. If a form is required at any time, an update of this QAPP will be sent to all signatories.

The specific forms to be used for this project are provided in the Appendix.

B. Data Generation and Acquisition

B.1. Sampling Process Design (Experimental Design)

Estuary water quality sample stations were selected to represent the water quality of the entire embayment using a probability-based sampling design[3], as in the EPA National Coastal Assessment[4]. A scientific advisor affiliated with the Unified Water Study advised on the choice of station locations. The Unified Water Study (UWS) assigned unique ID codes for the embayments, reporting sections of the embayment, and stations.

Tributary water quality sample stations will be selected in tributary streams flowing into the embayment. These stations are selected at an accessible location outside of the section of the waterway in which salinity values are < 1 ppt. These stations will have their salinity sampled and recorded. They will be as close to the embayment as possible with salinity concentration and access being the key factors in their selection.

Continuous dissolved oxygen stations are selected with consideration to access and representation of the sampled waterway. These stations are located on docks or secured in open water.Parameters, number and location of sampling sites, sampling time of day, frequency, and season are selected to meet the monitoring objectives referred to in Section A.6.. Sampling design components are described below.

Sampling Safety. Personal safety shall be a primary consideration in all activities, including selection of sampling sites and dates, and training programs. No sampling shall occur when personal safety is thought to be compromised. The Monitoring Coordinator and Project Manager shall confer before each sampling event to decide whether adverse weather or other conditions pose a threat to safety of field volunteers, and will cancel/postpone sampling when necessary. Sampling shall take place in teams of two or more. Samplers shall wear life vests when sampling from boats or wading in waters under difficult conditions. Samplers shall wear proper clothing to protect against the elements as applicable, especially footwear and raingear. When sampling in rivers, samplers shall estimate flow and avoid sampling when river depth (in feet) times velocity (feet per second) appear to equal 5 or greater, e.g. 1.5 foot depth * 4 feet/second velocity = 6 = unsafe conditions!

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[3] Paul, J.F., J.L. Copeland, M. Charpentier, P.V. August, and J.W. Hollister. 2003, Overview of GIS applications in estuarine monitoring and assessment research. Marine Geodesy Journal 26: 63-72.

[4] EPA, U.S. 2001. National Coastal Assessment: Field Operations Manual. U. S. Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Gulf Ecology Division, Gulf Breeze, FL. EPA 620/R-01/003. 72 p.

Design Considerations. Specific design considerations incorporated into this project are included in Table 6. A summary of general design approaches to the number of stations, depth of sampling, frequency of sampling and time of day of sampling are included here:

Approximately 17 water quality stations will be monitored across the two study sites. Macrophyte surveys will be undertaken on transects in each site. Nutrients will be sampled 0.5 m from surface at estuary stations and at least 6 inches under the surface in tributaries. 6 60 mL sample bottles will be filled at each station for nutrient analysis. Three bottles for the inorganic dissolved fractions and three bottles for the filtered total nutrient analyses. A field duplicate will occur once per sampling event. This will consist of an addition 6 60 mL bottles being filled for nutrients at a station. Logging parameters will be recorded 0.5 m from the bottom and will log at 15 minute intervals through the course of the study. 6 transects will be navigated by boat for the benthic macrophyte surveys per embayment.

Table 6: Sampling Approaches.
Assessment Type: Water Quality Parameters

Indicators	Number of sample locations	Site location rationale	Frequency, duration, special conditions	Field survey QC
GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	each sampling site	NA	once per year to mark site; each visit to sampling site if site is not easily marked (e.g. center of estuary or longitudinal river profile)	repeat readings every time the station is sampled to verify coordinates . Coordinates indicating a 100 m or greater discrepancy from documented coordinates will be assessed and documented in data notes.
salinity (coastal), conductivity (freshwater, coastal)	at least one each for selected reach or tributary	representative or targeted, define clearly in the SOP; depth(s) of sampling should be consistent with Program goals and defined in SOPs*	at least monthly (August - October), minimum of three “dry” weather surveys	collect a field replicate for ~10% of samples, at least one field duplicate per survey; probe calibration prior to survey (as applicable)

Indicators	Number of sample locations	Site location rationale	Frequency, duration, special conditions	Field survey QC
dissolved oxygen	at least one each for selected reach or tributary	representative or targeted, define clearly in the SOP; depth(s) of sampling should be consistent with Program goals and defined in SOPs*	at least monthly (August - October), minimum of three "dry" weather surveys	collect a field replicate for ~10% of samples, at least one field duplicate per survey; probe calibration prior to survey (as applicable)
nutrients	at least one each for selected reach or tributary	representative or targeted, define clearly in the SOP; depth(s) of sampling should be consistent with Program goals and defined in SOPs*	at least monthly (August-October), minimum of three "dry" weather surveys	collect a field replicate for ~10% of samples, at least one field duplicate per survey; probe calibration prior to survey (as applicable)

Assessment Type: Wetland and Estuarine Biomonitoring

Indicators	Number of sample locations	Site location rationale	Frequency, duration, special conditions	Field survey QC
vegetation – presence, identification, abundance, canopy height, density, biomass	6 transects, stratified with consideration to navigation	representative of condition at study & reference site	once / year, late summer orfall; be consistent with previous studies;	qualified supervisor, multiple samplers, photos

B.2. SAMPLING METHODS

Pre-coordination shall occur with external lab(s) to ensure that sample collection procedures meet lab needs. The lab selected for this study is:

University of Rhode Island Graduate School of Oceanography
Marine Ecosystem Research Laboratory
11 Aquarium Road
Narragansett, RI 02882

All sample collections for this project shall follow detailed methods on how samples will be collected and preserved as stated in the standard operating procedures (SOPs) contained in Appendix. The lab has reviewed the SOPs for nutrient collection and confirms they are appropriate for the select analyses.

A summary overview of sample collection methods is provided in Table 5. A summary of field sampling considerations is provided in Table 6.

Any water collected for nutrient analysis will be stored in a cooler, on ice during the sampling trip. The cooler designated for nutrient samples will not be used for the storage of macrophytes, sediment, or food. The original EPA standard methods require that samples for nutrient analyses be acidified with H_2SO_4 to a pH < 2 and stored at 4°C until analysis[5,6,7]. More recent methods acknowledge that no method for preservation is truly satisfactory. In addition, preservation with H_2SO_4 precludes the analysis of nitrate and nitrite separately, yielding only the sum of these two constituents. Freezing of samples at -20° C is a suggested preservation technique in more recent EPA methods[8] and is widely used as a preservation technique. The storage and preservation techniques detailed here match the techniques presented in other QAPPs approved by the EPA and used as examples for QAPP preparation on the EPA website (http://www.epa.gov/ NE/measure/qapp_examples/)[9,10]. If the analytical lab requires freezing versus acidification, samples will be delivered to a freezer within 8 hours of collection and stored at -20°C. Frozen samples will be analyzed within 28 days of collection, but delays in analysis will not affect the validity or usability of results[11].

All deviations from the Standard Operating Procedures of this QAPP will be documented and subsequently reviewed by the Monitoring Coordinator and the project Science Advisors. This information will be available to all signatories at the completion of this project at which time acceptability of data will be determined.

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[5] Ammonium: Standard Methods 4500-NH3-G [19th ,20th, and 21st Edition] and 4500-NH3-H [18th Edition]

[6] Nitrate and Nitrite: USEPA 353.2. Revision 2.0 (1993)

[7] Phosphorus (Ortho-phosphate): EPA 365.1 Rev. 2.0 (1993)

[8] Nitrate and Nitrite: USEPA 353.4. Revision 2.0 (1997)

[9] Leo WS, Prasse J, Delaney MF, Epelman P, Rhode S, Lao Y. 2010. Combined Work/Quality Assurance Project Plan (QAPP) for Nutrient and Chlorophyll Analyses for Outfall Monitoring. Boston: Massachusetts Water Resources Authority. Report 2010-09. 40 p.

[10] Pennock J, Trowbridge P. 2003. UNH Nutrient and Light Extinction Monitoring Program Quality Assurance Project Plan. University of New Hampshire and New Hampshire Department of Environmental Services. 71p.

[11] 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.

Table 4: Overview of Sample Collection Methods

Assessment Type: Water Quality Parameters

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity	Sample Preservation	Maximum Holding Time
[4] GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	field data sheets -or- log to GPS	NA	transfer to digital format; maintain back-up copies of digital data	NA
multi-parameter or single parameter meter (e.g. YSI, In-Situ, HoBo, Hach) – temperature salinity conductivity dissolved oxygen	field data sheets -or- log to internal memory	NA	transfer to digital format; maintain back-up copies of digital data	NA
inorganic nutrients	high density polyethylene (HDPE) polypropylene (PP) borosilicate glass (containers pre-acid-washed with 10% hydrochloric acid)	120 mL per station	ice or refrigerate filtered water samples at a temperature of <4$_o$C while in the field, store at <-20$_o$C	holding time of ~1 year once frozen
organic and total nutrients	high density polyethylene (HDPE) polypropylene (PP) borosilicate glass (containers pre-acid-washed with 10% hydrochloric acid)	120 mL per station	ice or refrigerate water samples at a temperature of <4$_o$C while in the field, freeze at <-20$_o$C	max holding time of 3 months

Assessment Type: Wetland and Estuarine Biomonitoring

Parameter(s)	Container Type(s) and Preparation	Minimum Sample Quantity	Sample Preservation	Maximum Holding Time
vegetation, (grasses, sedges, eelgrass, macroalgae, etc.) – video surveys	Field data sheets and computer storage	6 transects per embayment with a minimum of 20 images from video analyzed for each embayment. Stations are distributed at even intervals across the transects	maintain back-up copies of digital data	NA

Table 5: Overview of Field Sampling Considerations

Sample Type	Parameter(s)	Sampling Considerations
in-situ sampling GPS unit	GPS: latitude & longitude in decimal degrees; NAD83 coordinate system or record system used	NAD83 coordinate system or record system used; check GPS accuracy relative to a known, fixed location
in-situ sampling multiparameter sonde and data loggers	Temperature, salinity, conductivity, dissolved oxygen	Inspection, maintenance as specified by manufacturer, pre-calibration and post-checking of probes and instruments are critical to achieving accurate and precise measurements, especially for DO. Probes and loggers are rinsed and cleaned with freshwater after use.
grab samples - i.e. collection of a water sample in bottle	Inorganic, organic, and total nutrients	Triple-rinse sample container in ambient water immediately prior to sample collection. Care must be taken to avoid contact between fingers and inside surfaces of containers, including bottle caps. New, pre-washed bottles preferred; if not, containers for nutrient samples should be acid-washed and rinsed with deionized water. This process is overseen by the Monitoring Coordinator. These bottles will be obtained by appropriate suppliers such as Fischer Scientific. Field filtration preferred for dissolved fractions. If filtering water, triple-rinse container with *filtered* water immediately prior to sample collection, not ambient water.
in-situ sampling video surveys	vegetation	Maintain low speed to minimize potential damage to camera. Monitoring Coordinator and two additional members of the sampling team, under Monitoring Coordinator supervision, will analyze the macrophyte videos as described in SOP

B.3. SAMPLE HANDLING AND CUSTODY

Sample handling shall be in compliance with project Standard Operating Procedures (SOPs). Samples will be driven to lab in a color filled with ice, with no more than 24 hours spent out of the freezer to avoid thawing.

Sample labels will be associated with: Site ID number or name, sample type, date and time. These details will be written on the label. The field data sheet will also have this information recorded.

Chain of Custody shall be tracked with a Chain of Custody form provided in the Appendix of this document.**The following steps shall be taken to avoid sample mislabeling:**

Field team will check data sheet versus sample bottle labels before storing in the cooler.

Labels will always include the unique UWS sample ID and the sample date. The UWS sample ID provides information on the reporting region and station location.

The following steps shall be taken to avoid sample mislabeling:
Nutrient bottle labels will be cross checked with the day's field data sheet prior to undertaking a sampling event. A white board with name of the embayment, site and station id, and date will be filmed prior to recording every station in the macrophyte video surveys.

B.4. ANALYTICAL METHODS

All analytical methods used in the Monitoring Program, including methods used by laboratories performing analyses for the project, shall be based on standardized laboratory methods.

All analytical methods used for this project are be provided in the Appendix.

Table 7 provides an overview of the analytical methods utilized in this Monitoring Program. The SOPs associated with these methods are included in Appendix.

Table 6: Overview of Analytical Methods

Parameter	Method #	Source of Method	MDL & RL	Alternative Applications Special Provisions "Kit" availability
total nitrogen (TN)	SM 4500-N B SM 4500-N C	Standard Methods, 21st	0.03 & 0.09 mg/L	
total nitrogen (TN)	WRIR 03-4174	USGS	0.03 mg/L & 0.09 mg/L	
ammonia (NH3)	EPA 350.1, 350.2, 350.3	EPA	0.02 mg/L & 0.06 mg/L	When the samples to be analyzed are saline waters, Synthetic Ocean Water (SOW) should be used for preparing the standards; otherwise, distilled water is used. Field filtration
ammonia (NH3)	SM 4500-NH3	Standard Methods, 21st	0.02 mg/L & 0.06 mg/L	When the samples to be analyzed are saline waters, Synthetic Ocean Water (SOW) should be used for preparing the standards; otherwise, distilled water is used. Field filtration
nitrate-nitrite (NO3-NO2)	SM 4500-NO3	Standard Methods, 21st	0.02 mg/L & 0.06 mg/L	Field filtration
nitrate-nitrite (NO3-NO2)	SM 4500-NO3	Standard Methods, 21st	0.02 mg/L & 0.06 mg/L	Field filtration
nitrate-nitrite (NO3-NO2)	EPA 353.1, 353.2, 353.3	EPA	0.02 mg/L & 0.06 mg/L	When the samples to be analyzed are saline waters, Synthetic Ocean Water (SOW) should be used for preparing the standards; otherwise,

Parameter	Method #	Source of Method	MDL & RL	Alternative Applications Special Provisions "Kit" availability
				distilled water is used. Field filtration
total phosphorus (TP)	SM 4500-P	Standard Methods, 21st	0.01 mg/L & 0.03 mg/L	
total phosphorus (TP)	EPA 365.1, 365.2, 365.3	EPA	0.01 mg/L & 0.03 mg/L	
orthophosphate (PO_{43-})	SM 4500-P	Standard Methods, 21st	0.01 mg/L & 0.03 mg/L	field filtration
orthophosphate (PO_{43-})	EPA 365.1, 365.2, 365.3	EPA	0.01 mg/L & 0.03 mg/L	field filtration

B.5. QUALITY CONTROL

Lab Quality Control (QC) protocols shall be discussed with the external lab facility or contractor analyzing nutrient samples prior to sampling to ensure acceptability.

Quality control shall be discussed and defined prior to sampling (e.g., during training).

Intercomparison of instruments used for this study will be conducted by the Monitoring Coordinator.

Details on quality control procedures are provided in Table 8.

Table 7: Quality Control Measures

Sample Type	Instrument/ Parameter	Accuracy Checks	Precision Checks	% Field QC Samples (blanks and field duplicates)
Multiprobe instruments	salinity, conductivity, temperature, oxygen, pH, turbidity, chlorophyll, light	Pre-survey calibration and post-survey checks, including "zero" DO standard check	field duplicates -or- 3-5 minutes of stable readings recorded	verify repeatability in the field
Single probe instruments	Conductivity & Salinity	Field blanks, QC standard	field duplicates -or- 3-5 minutes of stable readings recorded	5%
Single probe instruments	Dissolved Oxygen	Compare with audit samples or Winkler titration method	field duplicates -or- 3-5 minutes of stable readings recorded	5%
Water samples – grab	TP, P fractions TN NH3 NO3-NO2	Field: blanks Lab: analysis of lab-fortified matrix (spiked samples) and/or lab QC standard	Field duplicates Lab duplicates	Minimum 5%

For field measurements, if QC target is not met, a third sample is taken to determine if one of the samples was bad. If 2 of 3 samples still do not pass, continue this process until N-1 of N samples can pass the QA.

For lab samples, if QC is not met, the field duplicate will be run.

B.6. INSTRUMENT / EQUIPMENT TESTING, INSPECTION AND MAINTENANCE

Maintenance of instruments and equipment shall occur as needed during the field season.

Records of equipment inspection, maintenance, repair and replacement shall be kept in a logbook. A backup of the logbook will be kept in a separate location. If the logbook is digital, appropriate backups of the computer files will be maintained by the Monitoring Coordinator on the Save the Sound S-Drive. Laboratory files, LACHAT instrument, will be maintained by University of Rhode Island EPSCOR Marine Science Research Facility.

Table 8: Instrument / Equipment Inspection and Testing Procedures

Equipment Type	Inspection Frequency	Type Inspection	Maintenance, Corrective Action
water quality meters	before each sampling date	battery life, electrical connections, membrane condition	spare membranes, batteries
gps unit	before each sampling date	battery life	charge battery
nutrient sample bottles	before each use	visual for integrity, cleanliness.	acid washed when reused or rinsed with deionized water prior to sampling event
filtering apparatus (nutrients, esp. P)	before each use	proper functioning, clean storage	spare syringe, spare filters
logging sensors	Every 7-10 days or as needed	biofouling and battery check	Clean off fouling organisms, check battery life from data log
underwater camera and equipment	before each use	battery life, test video	Recharge/replace batteries and clean lens if required

B.7. INSTRUMENT / EQUIPMENT CALIBRATION AND FREQUENCY

Calibration shall occur within a day prior to a sampling trip. The calibrations will be overseen by the Monitoring Coordinator.

Records of calibration shall be kept in a logbook (hard copy or digital, with back-ups). Calibration records shall be maintained for a minimum of four years, ideally longer.

A summary of calibration procedures for instruments and equipment is provided in Table 10.

Detailed calibration procedures are described in applicable SOP.

Table 9: Instrument / Equipment Calibration Procedures

Instrument	Inspection and Calibration Frequency	Standard of Calibration Instrument Used	Calibration Acceptance Criteria	Corrective Action
multi-probe meter	before each sampling run	standard solutions, according to manufacturer's recommendations	according to manufacturer's instruction or when not provided a maximum difference of %10 of the calibration standard value	according to manufacturer's instruction
logging sensors	before and after deployment	standard solutions, according to manufacturer's recommendations	according to manufacturer's instruction or when not provided a maximum difference of %10 of the calibration standard value	according to manufacturer's instruction

B.8. INSPECTION / ACCEPTANCE OF SUPPLIES AND CONSUMABLES

The procedures for inspection and acceptance of supplies and consumables listed in Table 11 shall be followed by the Monitoring Program.

Table 10: Supplies Inspection and Acceptance Procedures

Supplies	Inspection Frequency	Type of Inspection	Available Parts	Maintenance
calibration standards	before each sampling date	visual inspection of quantity and expiration date	spare, fresh solutions	storage according to manufacturer's recommendations, annual replacement at beginning of sampling season
membranes, filters	before each sampling date	visual inspection of quantity, integrity	spares	storage according to manufacturer's recommendations
field and lab sample sheets	before each sampling date	visual	additional copies	
waders or life preservers	before each sampling date	visual inspection for damage	patch kit	as needed
sample bottles	before each sampling date	integrity, cleanness and seal for nutrient bottles, verified sterility of bacterial sample bottles	one set of spare bottles	clean after use (note that nutrient bottles require acid washing before reuse)
cooler	before each sampling date	cleanness, ice packs		annually or as needed

B.9. NON-DIRECT MEASUREMENTS

To provide high-quality data to enhance the interpretation of data collected as part of this Monitoring Program, data may be acquired from a variety of sources. NOAA tide gauges will be

used for tide information. Precipitation will be acquired from local weather stations that log reasonable (in respect to northeastern USA conditions) volumes. Little Neck Bay site will include precipitation data from LaGuardia Airport and Mamaroneck Harbor will include precipitation data from the Larchmont Fire Department. And precipitation data out of the expected annual volumes and the observed conditions will be flagged by the Monitoring Coordinator in the data sheet and shared with the project team for review. External data sources are described in Table 12.

Table 11: Non-Project Data Validity
The following data will be used as part of the Monitoring Program.

Title or descriptive name of data document.	Source of data.	QAPP written? Y/N	Notes on quality of data.	Planned restrictions in use of the data due to questions about data quality.
time of low and high tide	NOAA tide gauges	N	NOAA has internal requirements for data suitability, on par with EPA QAPP requirements.	Data quality is acceptable. However, local tidal stage will differ from the nearest NOAA gauge even when corrected for difference in location. These data are rough predictors only.
high and low temperature and precipitation within the 24 hours prior to the field trip	local weather station	N	Data from nearby weather stations are acceptable; data are used in broad scale, to determine the potential impact of weather on the day of sampling.	Data will be used in comparing among embayments or among dates, as a general indication of weather during the day prior to sampling.

B.10. DATA MANAGEMENT

Field teams shall record data on field sheets, review them, and turn over to Field Coordinator.

Field Coordinator shall review sheets and confer with field teams on any needed corrective action.

Each person who handles or transports samples shall also sign the custody form upon receipt of the samples. Chain of custody forms will follow samples to the lab and back to Monitoring Coordinator by mail or pickup after each analysis run is completed. Alternatively, scanned copies may be emailed or faxed.

Once laboratory analyses are complete, the laboratory personnel shall deliver (digital or hard copy) lab results to the Monitoring Coordinator or arrange for pickup.

The Monitoring Coordinator will enter raw field and lab data into the project computer system.

Computer-entered data shall be compared with field sheets for accuracy. This task will be conducted by someone on the sampling team other than the individual that entered the data. Data sheets will be signed and dated after this QA process is complete. This task will be supervised by the Monitoring Coordinator.

Original data sheets will be stored by the Monitoring Coordinator.

Digital back-ups and copies of the non-digitized data will be made and stored in a separate location designated by the Monitoring Coordinator.

Documentation of data recording and handling, including all problems and corrective actions, shall be included in all preliminary and final reports.

Examples of data forms are provided in the Appendix.

Table 13 in this document accurately represents the procedures utilized by this Monitoring Program for data management, review, validation, and verification.

Data Management Systems - spreadsheets, databases, statistical or graphical software packages, location of data records (paper and electronic), are described here:

All data will be entered from field data sheets to an Excel spreadsheet for storage and retrieval. Field data sheets will be kept on file in Save the Sound office for at least 4 years. Digital scans of paper records will be generated from scans and stored on the Save the Sound S-Drive server. The S-Drive is backed up weekly.

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Table 12: Data Management, Review, Validation, Verification Process Summary

Activity	By whom	Corrective action, if needed
Check labels just prior to sampling, to ensure correct labeling of container.	Field sampler	Correct label or change container.
At time of sampling, record data, sign field sheets.	Field sampler	Remind samplers of proper procedures; retrain if needed.
Fill out, sign chain of custody (CoC) forms for any samples going to lab.	Field sampler or designated person	Remind person of proper procedures; retrain if needed.
Before turning field sheets over to field/monitoring coordinator, check for reasonableness to expected range, compared to previous sampling events, completeness.	Field sampler	Resample if feasible; otherwise, flag suspect data.
Upon receipt of field sheets, recheck for reasonableness to expected range, completeness, accuracy, and legibility. Sign CoC form.	Field Coordinator or Monitoring Coordinator	Confer with field sampler(s) immediately or within 24 hours. Resample if feasible; otherwise, flag suspect data.
Upon receipt of samples, field sheets and CoC forms, check to see that sheets and forms correspond to number of samples, condition of samples as stated on CoC forms. Sign CoC forms. Copies of field sheets and CoC forms are made, given to field/monitoring coordinator.	Lab Coordinator or Field Coordinator or Monitoring Coordinator	Confer with field/monitoring coordinator. Contact field samplers as needed to locate missing samples, data records. In case of missing/spoiled samples or data records, authorize resampling as needed and feasible. If resampling is not feasible, flag all suspect data.
Upon completion of laboratory analyses, fill out lab sheets, including data on QC tests. Review for reasonableness to expected range, completeness. Make copies of lab sheets.	Lab Coordinator	Re-analyze if possible. If not, confer with monitoring coordinator. Flag all suspect data.
Upon receipt of lab sheets, review for completeness and legibility.	Monitoring Coordinator	Confer with Lab Coordinator.
Upon completion of data entry, print out raw data. Compare with field/lab sheets for accuracy.	Data Entry Coordinator or other volunteer. Data entry personnel may review their own work, but a different person than data entry person shall perform the final accuracy comparison.	Re-enter data.
Translate raw data printouts into preliminary data reports: run statistical analyses and/or prepare graphical summaries of data. Check for agreement with QC objectives for completeness.	Monitoring Coordinator or Data Entry Coordinator	Confer with QA Officer. Flag or discard suspect data.

Activity	By whom	Corrective action, if needed
In-season (at least once) and end of season review of collected data sets (individual sample runs and season-total compilations); review for completeness and agreement with QC objectives and DQOs.	Monitoring Coordinator TAC if applicable Share with QA Officer	Flag or discard suspect data. Decide upon any restrictions in use of data with respect to original data use goals.

C. Assessment and Oversight

The Monitoring Coordinator, QA Officer and TAC (as applicable) will identify and effectively address any issues that affect data quality, personal safety, and other important project components. The progress and quality of the monitoring program shall be assessed to ensure the objectives are being accomplished. The Monitoring Coordinator will check at the beginning of the project, mid project, at the end of project, and on a continual basis when needed to confirm the following:

a. Monitoring is occurring as planned.
b. Sufficient written commentary and supporting photographs exist.
c. Sufficient field members are available.
d. Samplers are collecting in accordance with project schedules.
e. Data sheets and custody control sheets are being properly completed and signed.
f. Data are properly interpreted.
g. Plans for dealing with adverse weather are in place.
h. Retraining or other corrective action is implemented at the first hint of non-compliance with the QAPP or SOPs. These actions will be documented in a Word document with the final report and field data sheet for the sampling event.
i. Labs are adhering to the requirements of their QAPP, in terms of work performed, accuracy, acceptable holding times, timely and understandable results and delivery process.
j. Data management is being handled properly, i.e. data are entered on a timely basis, is properly backed up, is easily accessed, and raw data are properly stored in a safe place.
k. Procedure for developing and reporting the results exists.

The Monitoring Coordinator shall confer with the QA Officer as necessary to discuss any problems that occur and what corrective actions are needed to maintain program integrity. In addition, the Monitoring Coordinator and QA Officer shall meet at the end of the sampling season, to review the draft report and discuss all aspects of the program and identify necessary program modifications for future sampling activities. If the program includes a technical advisory committee, the TAC shall be included in these discussions. All problems discovered and program modifications made shall be documented in the final version of the project report.

If modifications require changes in the Quality Assurance Project Plan, these changes shall be submitted to the QAPP distribution list for review.

If data are found to be consistently outside the Data Quality Objectives as defined in section A.7. of this document ,the Monitoring Coordinator and the TAC (as applicable) shall review the program and correct problems as needed. Corrections may include retraining staff; rewriting sampling instructions; replacement of staff; alteration of sampling schedules, sites or methods; or other actions deemed necessary.

C.2. REPORTS TO MANAGEMENT

Data that have passed preliminary QC analysis as described in Table 13 may be posted on the organization's web site, shared with the local media or at other venues (e.g. kiosks at recreation access sites), and submitted to the Long Island Sound Study, US Environmental Protection Agency, New England Interstate Water Pollution Control Commission, Interstate Environmental Commission, New York State Department of Environmental Conservation, and/or Connecticut Department of Energy and Environmental Protection. A caveat will accompany these or any data released on a preliminary basis, explaining that they are for review purposes only and subject to correction after completion of a full data review occurring at the end of the sampling season.

The Monitoring Coordinator will write a final report. This will be sent to the distribution list. The final report will include (updated as necessary) any tables and graphs that were developed for initial data distribution efforts (i.e. the web site and media), and it will describe the program's goals, methods, quality control results, data interpretation, and recommendations. This report may also be used in public presentations.

All reports, preliminary or final, will include discussion of steps taken to assure data quality, findings on data quality, and decisions made on use, censorship, or flagging of questionable data. Any data that are censored in reports will be either referred to in this discussion, or presented but noted as censored.

In short, the final report will include:

- Raw data
- QC data
- Associated metadata
- Questionable data, flagged
- Identification of status as "preliminary" or "final" report

Table 13: Report Mechanisms, Responsibilities, and Distribution

Reporting Mechanism	Person Responsible for writing report.	Distribution list.
Master Data Entry Template	Monitoring Coordinator	All signatories of this QAPP
Monitoring Data	Monitoring Coordinator	EPA Store, NYC DEC and other Management Groups
Website and other Public Communications	Monitoring Coordinator, Project Manager, and other Save the Sound staff	All signatories of this QAPP and Public

D. Data Validation and Usability

D.1. Data Review, Verification, and Validation

All project data, metadata, and quality control data shall be critically reviewed to look for problems that may compromise data usability.

The Monitoring Coordinator will review field and laboratory data after each sampling run and take corrective actions as described in Table 13 of this document. At least once during the season, at the end of the season and if questions arise, the Monitoring Coordinator will share the data with the UWS Quality Assurance Officer to determine if the data appear to meet the objectives of the QAPP. Together, they will decide on any actions to take if problems are found.

D.2. Verification and Validation Methods

All project data and metadata are reviewed and approved as usable data, or as un-usable when the data are questionable for any reason.

Data verification and validation will occur as described in Table 13, and will include checks on:

- Completion of all fields on data sheets; missing data sheets
- Completeness of sampling runs (e.g. number of sites visited / samples taken vs. number proposed, were all parameters sampled / analyzed)
- Completeness of QC checks (e.g. number and type of QC checks performed vs. number or type proposed)
- Number of samples exceeding QC limits for accuracy and precision and how far limits were exceeded.

D.3. Reconciliation with User Requirements

At the conclusion of the sampling season, after all in-season quality control checks, assessment actions, validation and verification checks and corrective actions have been taken, the resulting

data set will be compared with the program's data quality objectives (DQOs) as defined in section A.7. This review will include, for each parameter, calculation of the following:

- Completeness goals: overall % of samples passing QC tests vs. number proposed.
- Percent of samples exceeding accuracy and precision limits.
- Average departure from accuracy and precision targets.

After reviewing these calculations, and taking into consideration such factors as clusters of unacceptable data (e.g. whether certain parameters, sites, dates, volunteer teams, etc. produced poor results), the Monitoring Coordinator, QA Officer, and TAC members (as applicable) will evaluate overall program attainment of DQOs and determine what limitations to place on the use of the data, or if a revision of the DQOs is allowable.

Appendix A. Sampling Methods and In-House Analysis

SOP Inorganic Nutrients, pg. 43

SOP Total Nitrogen and Total Phosphorous, pg. 47

SOP Macrophyte Percent Coverage Via Camera, pg. 52

SOP Continuous Dissolved Oxygen and Salinity, pg. 58

Appendix B. Data Forms, Checklists, and Chain of Custody Forms

Field Data Sheets

Chain of Custody Form

Appendix C. External Labs - Analytical Methods

MERL External Labs, Analytical Methods

Appendix A. Sampling Methods and In-House Analysis

SOP Inorganic Nutrients

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NAME: Peter Linderoth
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I. OBJECTIVE: Determine the inorganic nutrient concentrations in the water column.

II. OVERVIEW: Water samples are collected from the surface (0.5 m) of the water column. This water is filtered through GF/F filters and delivered into clean HDPE bottles. The samples are analyzed on a Lachat QC8500Quickchem Autoanalyzer, or similar instrument, following EPA approved colorimetric methods.

III. SOURCE:

Ammonium: SmartChem 200 Method 210-201B based on Standard Methods 4500-NH3-G [19th ,20th, and 21st Edition] and 4500-NH3-H [18th Edition]
Nitrate and Nitrite: SmartChem 200 Method 375-100E-1 based on USEPA 353.2. Revision 2.0, (1993) and Standard Methods Method 4500 NO3 F. 18th and 19th Editions.
Phosphorus (Ortho-phosphate): SmartChem 200 Method 410-3651 based on EPA 365.1 Rev. 2.0 (1993), and Standard methods 4500-P-F 18th, 19th Editions.

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

Plastic bottles, 60 mL, HDPE, (Fisher Scientific Catalog Number: 03-313-11B)

Plastic bottles, 1 L, HDPE, (Fischer Scientific Catalog Number: 02-925-3E)

Glass Fiber Filters (GF/F), 2.5 cm diameter, retains particles down to 0.7 μm, (Fisher Scientific Catalog Number: 09-874-64; Whatman Number:1825-025)

Filter holders, 2.5 cm (*preferred*: Pall brand, 25 mm Easy Pressure Syringe Filter Holder, Delrin Plastic; *second choice*: Millipore Swinnex Filter Holder, 25 mm polypropylene with silicon gasket, Fisher Scientific Catalog Number: SX00 025 00; Millipore Number:SX0002500)

Acid Bath: 1 N (Certified) hydrochloric acid (Fisher Scientific Catalog Number: SA48-4)

Forceps for handling filters

2 L of artificial seawater for field blank

Equipment for getting water samples and delivering through the filter:

sampling poles with 1 L Bottle

60 mL syringe

B. LABORATORY ANALYSIS

Materials include chemicals necessary for the analysis. Details are provided in the Lachat Autoanalyzer, or similar instrument, Operating Instructions.

V. METHODS

A. PREPARATION

- check that the sampling equipment are operational
- prepare the sample bottles:

  Wear gloves.

  If HDPE bottles are new and un-used, rinse the vials three times with ultrapure, ASTM type I water. Allow to dry and store with caps on.

  If HDPE bottles have been used, acid wash the vials. Acid washing entails soaking the vials in a 1 N hydrochloric acid bath for 60 minutes followed by rinsing three times with ultrapure, ASTM type I water. Allow to dry and store with caps on.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

Sampling Poles with 1 L Bottle

This method involves reaching into the water with the sample bottle inverted and full of air, then righting the bottle and allowing it to fill at the appropriate depth. The pole is marked at a 0.5 m location to confirm sampling is occurring where planned.

- Rinse the sample bottle three times with surface water.
- Remove the cap from the bottle.
- Holding the bottle with the mouth down, lower it to 0.5 m below the surface.

- Invert the bottle so that air empties out of the bottle and the bottle fills with water.
- Bring to the surface.

Below are steps after sample is collected from 0.5 m depth:

1. Rinse the sampling equipment with sample water. This involves rinsing with three times the volume of the sampling device.
2. Assemble the filtering mechanism. Rinse the 60 mL syringe with 5 mL of sample water. Repeat for a total of three rinses. Fill the 60 mL syringe with sample water. Connect the filter holder (with filter loaded into the holder).
    a. Filter mechanisms can be assembled in advance.
3. Rinse the HDPE bottles. Deliver a small volume of the filtered sample into the HDPE bottles. Cap the bottles and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per bottle.
4. Deliver between 45 mL and 50 mL of sample (through the filter) to the bottles. Be sure to keep the volume below 50 mL, this head space will allow for expansion during freezing. Change the filter as needed.
5. Store the bottles with sample in a dark cooler with ice.
6. Upon return to the lab, store the bottles in the freezer (- 20^{o} C).

Note – About mid-way through the sampling day, perform a field blank. Follow the procedures for sampling above, but use artificial seawater brought out on the boat in place of the field water.

A sample, duplicate, and sample in reserve are collected at each station.

C. LABORATORY ANALYSIS

- The nutrients are analyzed on a Lachat Quickchem Autoanalyzer, or similar instrument, at the University of Rhode Island. Instrument operations follow the standard methods.

VI. TROUBLE SHOOTING / HINTS

- Avoid contaminating the samples – do not touch: the insides of the bottle caps, the mouth of the bottles, the insides of the graduated cylinders, the filter pads
- Two people working in tandem will speed the process. One person focuses on filtering while the other handles the sample bottles and filters. The person filtering the sample can lend a hand when s/he gets ahead of the sample handler.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!
- Enter field data into the Excel template.
- From the Autoanalyzer, extract the data from the output and enter into the template.
- Check the QAQC samples for agreement of samples and operation of the instrument over time.

IX. QUICK SHEET

Refer to the SOP for details, this list is only a reminder!!

FIELD

Rinse everything three times before collecting sample (pump / sample collection vessel; vials)
store in cooler on ice while in the field

STORAGE

store samples *upright* in the freezer
separate replicates a and b (samples to be analyzed) from c (sample held in reserve)

ANALYSIS

follow any potential guidelines established for analytical lab

SOP Total Nitrogen and Total Phosphorous

POINT OF CONTACT

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I. OBJECTIVE: Determine the total nitrogen and total phosphorous concentrations in the water column.

II. OVERVIEW: Water samples are collected from the surface (0.5 m) in the water column. The samples are analyzed on a Lachat Quickchem QC8500 Analyzer, or similar instrument, using the EPA approved Alkaline Persulfate extraction methodology (Solorazano and Sharp 1980). Dissolved organic nitrogen is calculated by difference as TN – DIN.

III. SOURCE:

Sharp, J. H., A. Y. Beauregard, D. Burdige, G. Cauwet, S. E. Curless, R. Lauck, K. Nagel, H. Ogawa, A. E. Parker, O. Primm, M. Pujo-Pay, W. B. Savidge, S. Seitzinger, G. Spyres, and R. Styles. 2004. A direct instrument comparison for measurement of total dissolved nitrogen in seawater. Mar. Chem. 84: 181-193.

Solorzano, Lucia, and Jonathan H. Sharp. "Determination of total dissolved phosphorus and particulate phosphorus in natural waters." *Limnology and Oceanography* 25.4 (1980): 754-758.

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

Plastic bottles, 60 mL, HDPE, (Fisher Scientific Catalog Number: 03-313-11B)

Plastic bottles, 1 L, HDPE, (Fischer Scientific Catalog Number: 02-925-3E)

Acid Bath: 1 N (Certified) hydrochloric acid (Fisher Scientific Catalog Number: SA48-4)

2 L of artificial seawater for field blank

equipment for getting water samples:

sampling poles with 1 L Bottle

B. LABORATORY ANALYSIS

Materials include chemicals necessary for the analysis. Lachat Autoanalyzer, or similar instrument, Operating Instructions.

V. METHODS

A. PREPARATION

- check that the sampling equipment are operational
- prepare the sample bottles:

  Wear gloves.

  Use only ultrapure, ASTM type I water

  If HDPE bottles are new and un-used, rinse the vials three times with ultrapure, ASTM type I water. Allow to dry and store with caps on.

If HDPE bottles have been used, acid wash the vials. Acid washing entails soaking the vials in a 1 N hydrochloric acid bath for 60 minutes followed by rinsing three times with ultrapure, ASTM type I water. Allow to dry and store filled with deionized water with caps on.

B. FIELD COLLECTION AND STORAGE OF SAMPLES

Sampling Poles with 1 L Bottle

This method involves reaching into the water with the sample bottle inverted and full of air, then righting the bottle and allowing it to fill at the appropriate depth. The pole is marked at a 0.5 m location to confirm sampling is occurring where planned.

- Rinse the sample bottle three times with surface water.
- Remove the cap from the bottle.
- Holding the bottle with the mouth down, lower it to 0.5 m below the surface.
- Invert the bottle so that air empties out of the bottle and the bottle fills with water.
- Bring to the surface.

Below are steps after sample is collected from 0.5 m depth:

7. Rinse the sampling equipment with sample water. This involves rinsing with three times the volume of the sampling device.
8. Rinse the HDPE bottles. Deliver a small volume of the sample into the HDPE bottles. Cap the bottles and shake to rinse. Discard the sample used as a rinse. Repeat for a total of three small volume rinses per bottle.
9. Deliver between 45 mL and 50 mL of sample to the bottles. Be sure to keep the volume below 50 mL, this head space will allow for expansion during freezing.
10. Store the bottles with sample in a dark cooler with ice or ice packs.
11. Upon return to the lab, store the bottles in the freezer (- 20^{o} C).

Note – About mid-way through the sampling day, perform a field blank. Follow the procedures for sampling above, but use artificial seawater brought out on the boat in place of the field water.
A sample, replicate, and sample in reserve are collected at each station

C. LABORATORY ANALYSIS

- Total nitrogen and total phosphorous are analyzed on a Lachat Quickchem Autoanalyzer, or similar instrument. Samples will be pre-extracted using the alkaline persulfate technique (with recrystallized ACS+ grade potassium persulfate to minimize background contamination). Pre-extracted samples are run using standard colorimetric methodology for nitrate+nitrite (Open Tubular Cadmium Reaction) and phosphate (heteropoly blue)..

VI. TROUBLE SHOOTING / HINTS

- Avoid contaminating the samples – do not touch: the insides of the vial caps, the mouth of the vials, the insides of the graduated cylinders.
- Two people working in tandem will speed the process.

VII. DATA PROCESSING AND STORAGE

- Enter the data on the field sheet. Be sure to fill out the data sheet completely!!
- Enter field data into the Excel template.
- From the analyzer, extract the data from the output and enter into the template.
- Check the QAQC samples for agreement of samples and operation of the instrument over time.
- DON is calculated as TN – DIN.

VIII. REFERENCES:

Sharp, J. H., A. Y. Beauregard, D. Burdige, G. Cauwet, S. E. Curless, R. Lauck, K. Nagel, H. Ogawa, A. E. Parker, O. Primm, M. Pujo-Pay, W. B. Savidge, S. Seitzinger, G. Spyres, and R. Styles. 2004. A direct instrument comparison for measurement of total dissolved nitrogen in seawater. Mar. Chem. 84: 181-193.

IX. QUICK SHEET

Refer to the SOP for details, this list is only a reminder!!

FIELD

Rinse everything three times before collecting sample (pump / sample collection vessel; vials)

store in cooler on ice while in the field

STORAGE

store samples *upright* in the freezer

separate replicates a and b (samples to be analyzed) from c (sample held in reserve)

ANALYSIS

follow guidelines established for use of URI analyzer

SOP Macrophyte Percentage Coverage via Camera

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I. OBJECTIVE: To quantify the percent coverage of macrophytes and determine bottom type at select locations.

II. OVERVIEW: An underwater camera (video or still) is used to capture images of the bottom in the area of a water quality station. Bottom type is identified and the percent cover of macrophytes is calculated.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. FIELD COLLECTION AND STORAGE OF SAMPLES

- camera (e.g. SeaViewer video system, still camera)
- method for keeping camera a set distance from the bottom (to maintain consistent areal size in the field of vision)
- GPS unit
- whitboard and markers

B. LABORATORY ANALYSIS

- not applicable

V. METHODS

A. PREPARATION

- check the charge on the GPS battery
- check the charge on the camera battery
- check the camera system for proper functioning

- check the deployment rig for the camera system (must have a size reference in the field of vision)

B. FIELD COLLECTION AND STORAGE OF SAMPLES

- samples stored on the SD card of the camera
- write name of the embayment, site and station id, and date on white board and film before each station survey commences.
- collect between 20 and 40 bottom images – in areas with greater heterogeneity, collect more samples (~40), if an area is fairly homogenous (e.g. sand, oyster, cobble) you may collect fewer (~20)

C. LABORATORY ANALYSIS

- not applicable

VI. TROUBLE SHOOTING / HINTS

- for the video system – keep the speed of the boat low, so as not to put too much tension on the pole (the pole will snap at high speeds)

VII. DATA PROCESSING AND STORAGE

- Video (or still photos) will be downloaded the following day and stored in the Dropbox cloud file service (providing back-up on multiple computers and the remote server).
- For video - A trained analyst will watch the video and do a screen-capture for all instances where the base of the pole is resting on the bottom (Figure 1).
- The bottom images will be pasted into the Excel camera work template (Figure 2).
- A 100 cell grid is overlaid on the image in cases where the coverage is not 0 % or 100%. The first analyst reviews the image and enters the % cover in three categories: eelgrass, macroalgae, bare sediment. The first analyst also enters the GPS coordinates, when available. The first analyst hides their columns of data, so that other analysts cannot see the first set of estimates.
- A second analyst reviews the images and makes an independent assessment of percent cover. The second analyst also checks the GPS coordinate data entry. The second analyst hides their columns of data, so that the other analysts cannot see their estimates.

- A third analyst performs the same tasks as the second analyst.

- The lab manager reviews a minimum of 10% of the images for accuracy of percent coverage estimates.
- INSERT ANY QUESTIONS INTO THE “COMMENTS” COLUMN
- In the Excel template, data are condensed into a table. Estimates from the three analysts are compared. If the relative percent difference among the three estimates is greater than 5%, the lab manager examines the image and the three estimates, choosing the appropriate value. The three estimates will not be changed, values are retained to show the inconsistency. The lab manager decides on the final value for the estimate. If a specific analyst’s estimate consistently differs, the analyst will receive further training or may be removed from conducting future estimates.
- See Figure 3 for examples of coverage estimates and how to handle sparse coverage.

Figure 1: Image of the bottom captured using the video camera system. Note that the black circular disc on the end of the pole keeps the pole from sinking into the sediment and provides a size reference (diameter of disc = 10 cm).

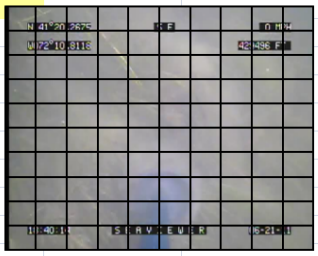
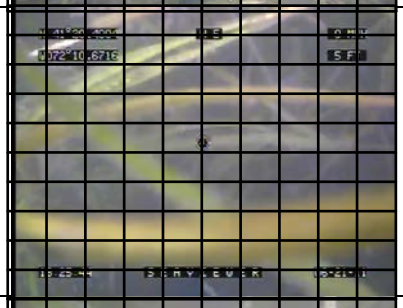
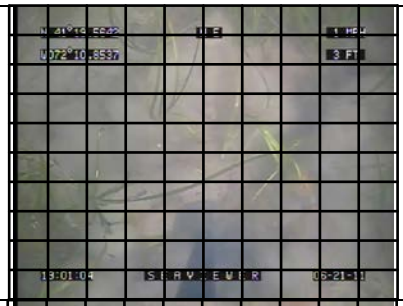
rmat > column > hide)	B								
	NR 062120011 stn 6	41	72	MM	MM	MM	Christophe	Christopher	Christophe
Photo		N	W	eelgrass	macroalgae	bare	eelgrass	macroalgae	bare
		20.26750	10.81180	15	0	85	15	0	85

Figure 2: The image from Figure 1 has been entered into the data template and overlaid with the 100 cell grid.

Figure 3: Examples and tips for estimating coverage.

 	eelgrass = 100 % macroalgae = 0 % bare = 0 % bare sediment is not visible, do not count the animal (orange)
 	eelgrass = 100 % macroalgae = 0 % bare = 0 % while leaves block some field of vision, this appears to be dense coverage
 	eelgrass = 76 % macroalgae = 0 % bare = 24 % the turbidity of the water makes this one tricky to analyze, zoom in and look closely

		eelgrass = 16 % macroalgae = 1 % bare = 83 % in a sparse eelgrass area, do not count the leaves that extend across the frame as area – look for the base of the plants
		eelgrass = 0 % macroalgae = 13 % bare = 87 % count shells as bare area, unless colonized by macroalgae

IX. QUICK SHEET

Refer to the SOP for details, this list is only a reminder!!

FIELD

- collect 20 – 40 images
- **Camera How-To**
    - Connect camera and GPS cables
    - Turn power switch to “BAT”
    - Push power button on Sea-Trak
    - Press and hold power button on remote until first green light is steady and second green light is blinking on video recorder.
    - To record press and hold record button until last yellow light is steady and red light is blinking.
    - To stop recording press the stop button, message will appear on screen to confirm, press OK button.
    - Switch power button to “OFF”

STORAGE

- download images ASAP, at least by the day following field work

ANALYSIS

- three analysts check
- record to the nearest 1% of coverage
- Any questions go in the comments column.

SOP Continuous Dissolved Oxygen and Salinity

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I. OBJECTIVE: Determine the dissolved oxygen concentration and saturation over a period of time at select intervals.

II. OVERVIEW: Continuous dissolved oxygen sampling array of instruments – dissolved oxygen, conductivity, and barometer loggers – are deployed in the field to log dissolved oxygen and saturation. Dissolved oxygen and conductivity are logged at approximately 0.5 meters off the bottom and barometric pressured is logged in a secure location in close proximity to the other loggers; above water surface.

III. SOURCE:

IV. MATERIALS AND EQUIPMENT:

A. Continuous logging stations

Dissolved oxygen logger (such as HOBO U26 or YSI EXO 2)

Barometric pressure logger (such as HOBO Onset Water Level or Extech SD700)

Conductivity Logger (such as Star-Oddi DST-CT)

NOTE: Conductivity logger must have appropriate range for Long Island Sound salinity fluctuations

Aquarium water pump for inter comparison of DO and Conductivity loggers and sonde

Aquarium air stone for inter comparison of DO and Conductivity loggers and sonde

Sodium sulfite DO calibration solution (HOBO U26-CAL-SOL)

Ice/tap water for adjusting conductivity in water bath

Multiparameter sonde with DO and conductivity attachments

salinity standard between 20 and 32 ppt; or a conductivity standard between 30 and 50 mS/cm (30,000 to 50,000 µS/cm) for calibrating sonde

B. LABORATORY ANALYSIS

No laboratory analysis.

V. METHODS

A. Logger Inter Comparison

- check that all sampling equipment are operational and ready for deployment
- calibrate conductivity and DO on multiparameter sonde to manufacturer recommendations the day before or morning of this logger comparison
- calibrate all loggers according to manufacturer recommendations
- set DO logger internal clock and multiparameter sonde to same time (i.e. set both at noon at same time)
- prepare a water bath before and after deployment with water similar to deployment stations; enough volume to fit all loggers for comparison and sonde
- place all loggers ready for deployment in the water bath and note the time the loggers will be taking readings
- run the aquarium pump to keep water flowing in water bath
- record readings with the multiparameter sonde at the same time the loggers are recording data
- Add sodium sulfite solution to lower the DO level in the water bath
- record readings with the multiparameter sonde at the same time the loggers are recording data
    - NOTE: Do not leave equipment in this solution for too long. Two readings will suffice
- place aquarium stone in water bath to raise the DO levels
- add ice or tap water to adjust the salinity concentration in the water bath
- record readings with the multiparameter sonde at the same time the loggers are recording data

B. FIELD DATA COLLECTION

1. Logger Deployment and Data Collection

- Continuous DO and Conductivity loggers are deployed at approximately 0.5 m off bottom
- Barometric pressure logger is deployed above water at a secure and close location
- All loggers are set to record at 15 minute intervals
- Station visits are required every 7-10 days for cleaning and comparison with multiparameter sonde
    - Conductivity, dissolved oxygen, and barometric pressure are all recorded at the station at 0.5 m off bottom using sonde and handheld (or other barometer)
    - Readings must coincide with a known time the loggers are recording data
    - Record readings on field data sheet
- Retrieve submerged loggers and clean all fouling organisms
- Couple loggers with data retrieval method of choice, either the manufacturer LED coupler or direction connection to a computer, and collect data for storage
- Repeat the retrieval and inter comparison sampling procedure after the loggers are cleaned and redeployed
- Bring data retrieval instrument to the laboratory/office and upload data to analysis software provided by manufacturer

IX. QUICK SHEET

Refer to the SOP for details, this list is only a reminder!!

FIELD

Secure all loggers with backup ropes and security measures. Inter comparison readings are taken before and after cleaning loggers of fouling organisms.

STORAGE

N/A

ANALYSIS

Upload data from field onto manufacturer software for analysis.

Appendix B. Data Forms, Checklists, and Chain of Custody Forms

Field Data Sheets

Surface Nutrients Date _____ Site Code

People: Location _____

0.5 m below surface	surface	surface	surface	MeQ - field blank
station (number, letter)				MeQ - field blank
time				
depth of sample (m)				
salinity (ppt)				0
60mL bottle ID	(a-d)	(a-d)	(a-d)	(a-d)

0.5 m below surface	surface	surface	surface	surface
station (number, letter)				
time				
depth of sample (m)				
salinity (ppt)				
60mL bottle ID	(a-d)	(a-d)	(a-d)	(a-d)

0.5 m below surface	surface	surface	surface	surface
station (number, letter)				
time				
depth of sample (m)				
salinity (ppt)				
60mL bottle ID	(a-d)	(a-d)	(a-d)	(a-d)

** Be sure to collect an additional set of 4 bottles once per day or 1 / every 10 stations

Chain of Custody - Nutrients

Sample ID	Size (60mL or 1L)	Salinity (ppt)	Sent by	Received by	Date of Transfer

UWS Tier 2, Chain of Custody for Nutrient Samples

Camera Sampling

People:

Date ____________________

Location ____________________

Site Code ____________________

Sta	video time	ID on White Board	Notes
	start		
	stop		
Sta	video time	ID on White Board	Notes
	start		
	stop		
Sta	video time	ID on White Board	Notes
	start		
	stop		
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Sta	video time	ID on White Board	Notes
	start		
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d data entry in spreadsheet ________ PI checked this sheet ________ Entered into spreadsheet ________

initial when complete

sampling date ____________
location ____________
site code ____________

Video Analysis Checklist

Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
Station Number:	
	Images Captured
	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete
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	Images Imported to Excel
	Reviewer Initials
	Reviewer Initials
	Reviewer Initials
	Final Review - Stn Complete

Appendix C. External Labs - Analytical Methods

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QuikChem® Method 31-107-04-1-A

DETERMINATION OF NITRATE/NITRITE IN BRACKISH OR SEAWATER BY FLOW INJECTION ANALYSIS

Written by David Diamond

Applications Group

Revision Date: 02 May 2008

LACHAT INSTRUMENTS 5600 LINDBURGH DRIVE LOVELAND, CO 80539 USA

LACHAT INSTRUMENTS
A Hach Company Brand

QuikChem® Method 31-107-04-1-A

Nitrate/Nitrite in Brackish Waters or Seawaters

1.25 to 5.0 µM N/L

17.5 to 70 µg N/L

– Principle –

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs light at 520 nm. Nitrate concentrations are obtained by subtracting nitrite values, which have been previously analyzed, from the nitrite + nitrate values.

Though the method is designed for seawater and brackish water, it is also applicable to non-saline sample matrixes.

The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinites (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 520 nm. The salt effect is less than 2%.

– Special Apparatus –

Please see Parts and Price list for Ordering Information

1. 60 position racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position rack (Lachat Part No. A81122 [110V]/A81222 [220V]); RAS A81136 [110V]/A81236 [220V]).
2. Sample tubes are needed for 60 Position Samplers (Lachat Part No. 21042).

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QuikChem® Method 31-107-04-1-A

DETERMINATION OF NITRATE/ NITRITE BY FLOW INJECTION ANALYSIS COLROIMETRY

1.SCOPE AND APPLICATION

1.1. This method covers the determination of nitrate/nitrite in brackish, seawater, or non-saline sample matrices.

1.2. The method is based on reactions that are specific for the nitrite (NO_2-) ion.

1.3. The applicable range is 1.25 to 5.0 uM. The method detection limit is 0.009 μM N (0.126 μg). The method throughput is 48 injections per hour.

2.SUMMARY OF METHOD

2.1. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The resulting diazonium ion is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs light at 520 nm. Nitrate concentrations are obtained by subtracting nitrite values, which have been previously analyzed, from the nitrite + nitrate values.

2.2. Though the method is designed for seawater and brackish water, it is also applicable to non-saline sample matrixes.

2.3. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinites (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 520 nm. The salt effect is less than 2%.

3.DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. ANALYTICAL BATCH -- The set of samples extracted/distilled/or digested at the same time to a maximum of 10 samples.

3.2. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.3. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.4. FIELD BLANK (FMB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all pre-analysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

3.5. FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.6. LABORATORY BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. It's purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

3.8. LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

3.9. QUALITY CONTROL CHECK SAMPLE (QCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

3.10. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

4.**INTERFERENCES**

4.1. Sample turbidity may interfere. Remove turbidity by filtration with a 0.45 um pore diameter membrane filter prior to analysis.

4.2. A positive error would be obtained for samples that contain high concentrations of iron, copper, or other metals. EDTA in the buffer helps eliminate this interference.

4.3. Surfactant concentrations >10 mg/L may interfere with the determination; this interference can be prevented by a dialysis of the sample, if need be in the online process.

4.4. Samples that contain large concentrations of oil and grease will coat the surface of the cadmium. This interference can be eliminated by pre-extracting the sample with an organic solvent.

4.5. Sample color may be subtracted by analyzing the samples with a substitute color reagent with does not contain the diazotizing agent. This is done by replacing the sulfanilamide-NED-phosphoric acid reagent with a solution containing 100 mL of phosphoric acid per liter.

5.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 should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Hydrochloric acid

5.3.2. Ammonium hydroxide

5.3.3. Sodium hydroxide

5.3.4. Phosphoric acid

5.3.5. Sulfanilamide

5.3.6. N-(1-naphthyl)-ethylenediamine (NED)

5.3.7. Cadmium

6.EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special Apparatus

6.4.1. 60 position racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position rack (Lachat Part No. A81122 [110V]/A81222 [220V]); RAS A81136 [110V]/A81236 [220V]).

6.4.2. Sample tubes are needed for 60 Position Samplers (Lachat Part No. 21042).

7.REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

9 **Degassing with helium:**

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

10 **Reagent 1. Buffer**

Note: An ammonium chloride solution, pH 8.5, is typically used as a buffer prior to the reduction of NO_3- to NO_2-. At pH 8.5, ammonia gas is evolved and may cause contamination of reagents, standards, and samples in nearby ammonia determination. If ammonia is also being analyzed, it is important to keep this reagent and the waste container covered with parafilm.

11 **Ammonium Chloride Buffer, CAUTION: Fumes!**

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **4.0 g disodium ethylenediamine tetra-acetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) in about **800 mL DI water.** Dilute to the mark and invert to mix. Adjust the **pH to 8.5** with **13 N sodium hydroxide solution.**

By Weight: To a tared **1 L** container, add **85.0 g ammonium chloride** (NH_4Cl**), 4.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) and **938 g DI water**. Shake or stir until dissolved. Then adjust the **pH to 8.5** with **13 N sodium**

12 **hydroxide solution.**

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. (The main symptom of this type of contamination would be a larger than normal increase in baseline when the cadmium column is placed in line). An alternative recipe for the ammonium chloride buffer is:

By Volume: In a fume hood, to a **1 L** volumetric flask add **500 mL DI water, 105 mL concentrated hydrochloric acid** (HCl), and **95 mL ammonium hydroxide** (NH_4OH). Add **1.0 g disodium EDTA**, dissolve and dilute to the mark. Invert to mix. Adjust to pH
8.5 with HCl or NaOH solution.

13 **Reagent 2. Sulfanilamide Color Reagent**

By Volume: To a **1 L** volumetric flask add about **600 mL DI water**. Then add **100 mL 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide** and **1.0 g N-(1-naphthyl)- ethylenediamine dihydrochloride** (NED). Shake to wet, and stir to dissolve for 30 min. Dilute to the mark, and invert to mix. Store in a dark bottle and discard when the solution turns pink.

By Weight: To a tared, dark **1 L** container add **876 g DI water, 170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide,** and **1.0 g N-(1-naphthyl)ethylene- diamine dihydrochloride** (NED). Shake until wetted and stir with a stir bar for 30 min. until dissolved. Store in a dark bottle and discard when the solution turns pink.

7.2. PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be required:

14 **Standard 1. Stock Standard 5.00 mM**

By Volume: In a **1 L** volumetric flask dissolve **0.5055 g potassium nitrate** (KNO_3), dried at 60°C for 1 hour or **0.3460 g of sodium nitrite** ($NaNO_2$) in about **800 mL DI water**. Dilute to the mark and invert to mix. When refrigerated this standard may be stored in glass for up to three months.

15 **Standard 2. Working Stock Standard 50 uM**

By Volume: In a **1 L** volumetric flask dilute **10.0 mL Stock Standard** (Standard 1) to the mark with **DI water**. Invert to mix. Refrigerate and store no longer than three days.

By Weight: To a tared **1 L** container add about **8 to 10 g Stock Standard** (Standard 1). multiply the actual weight of the solution added by 100 and make up to this resulting total weight with **DI water**, using a wash bottle for the last 10 g or so. Shake to mix. Store in glass. Refrigerate and store no longer than three days.

By Volume

Volume (mL) of **working stock standard 2** diluted to **250 mL with DI water**	25	12.5	6.25	0.00

By Weight

Weight (g) of **working stock standard 2** diluted to final weight (~250 g) multiplied by factor below with **DI water**	25	12.5	6.25	0.00
Division Factor Divide exact weight of the standard by this factor to give final weight	0.1	0.05	0.025	---

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Since there is no single preservation method that may be recommended for all types of samples the analyst must examine each situation critically.

8.2 Ideally, analysis should be commenced within 24 hours of sample collection. Plastic or glass bottles can be used. If longer term storage is necessary, samples should be filtered on-site with Nucleopore 0.45 uM membrane filters (washed with greater than 200 mL of sample), and frozen at -20°C. Samples should be frozen in plastic bottles, leaving about 30% headspace for expansion. Frozen samples may be stored for up to 10 days.

8.3 Thaw samples by immersion in warm water, with occasional mixing to ensure uniform sample temperature. Do not warm samples above ambient temperature. Since the

analyte is in the liquid portion of the thawing sample, care should be taken to ensure complete thawing.

8.4 If samples must be chemically preserved add 2 mL of 8N H_2SO_4 per liter of sample. Store in glass or polyethylene at 4°C. Analyze within two months.

8.5 It should be mentioned that some researchers have found serious errors when investigating the effects of filtration. It is imperative that the analyst examine sample preparation and preservation techniques before routine testing.

9. 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 periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. An analytical batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

9.1.1. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in section 9.3.

9.1.2. Analyses of laboratory blanks are required to demonstrate freedom from contamination.

9.1.3. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

9.1.4. The laboratory should maintain records to define the quality of data that is generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. Method Detection Limit (MDL) –To establish the ability to detect the analyte, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards, that will be used in the practice of this method. An MDL less than or equal to the MDL in section 1.2 must be achieved prior to the practice of this method.

9.2.2. Initial Precision and Recovery – To establish the ability to generate acceptable precision results, the operator shall perform 10 replicates of a mid-range standard, according to the procedure beginning in Section 11.

9.2.2.1. Using the results of the replicates compute the average percent recovery (X) and the standard deviation (s) for the analyte. Use the following equation for the calculation of the standard deviation.

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

Where, n = Number of samples, x = concentration in each sample

9.2.2.2. Compare s and x results with the corresponding data in Section 17. If the results meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If however, s and x do not match the data in Section 17, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3. Matrix spikes- The laboratory must spike, in duplicate, a minimum of 5 percent of all samples (one sample in each batch of no more than twenty samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

9.3.1.1. If, as in compliance monitoring, the concentration of the analyte in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), which ever is higher.

9.3.1.2. If the concentration of the analyte in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard used in Section 9.2.5 or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2. Analyze one sample aliquot out of each set of no more than twenty samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A)

9.3.3. Calculate the percent recovery (P) of the analyte in each aliquot using the following equation.

$$P = \frac{(A - B)100}{T}$$

Where, A = Measured concentration of analyte after spiking, B = measured background concentration of analyte, T = True concentration of the spike

9.3.4. The percent recovery of the analyte should meet current laboratory acceptance criteria.

9.3.4.1. If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test of the analytical batch is within the current laboratory acceptance criteria, an interference is present. In this case, the results may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2 and repeat the analysis of the sample and the matrix spike.

9.3.4.2. If the results of both the spike and ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample reanalyzed.

9.3.5. Compute the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \text{ x } 100$$

Where, D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

9.3.6. The RPD for duplicates shall meet the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.4 Laboratory blanks – Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1. Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch of no more than twenty samples. The blank must be subjected to the same procedural steps as a sample.

9.4.2. If analyte is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

9.5. Calibration Verification – Verify calibration using the procedure described in Section 10

9.6. On-going Precision and Recovery (OPR) – With every analytical batch of no more than twenty samples, a midrange standard must be prepared using the procedure described in Section 11.

9.6.1. Compare the results with the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.7. Quality Control Samples (QCS) – It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 9.7.1. The QCS is used to verify the concentrations of the calibration standards.

9.8. Depending on the specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare reagents and standards as described in Section 7.

10.2. Set up manifold as shown in Section 17.

10.3. Input data system parameters as shown in Section 17.

10.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

10.4. Once reagents are flowing, to analyze for nitrate-nitrite, put the cadmium column in-line.

10.5. Place standards in the sampler. Input the information required by the data system.

10.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7. Verify calibration using a midrange calibration standard every ten samples or every analytical batch. Compute the percent recovery using the following equation:

$$\%recovery = \frac{D}{K} \text{x } 100$$

Where, D = Determined concentration of analyte in the calibration standard, K = Actual concentration of the analyte in the calibration standard

10.8. If % recovery exceeds +/-10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed

11. PROCEDURE

11.1. Prepare reagents and standards as described in Section 7.

11.2. Set up manifold as shown in Section 17.

11.3. Input data system parameters as in Section 17.

11.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.5. Adjust samples to pH between 5 and 9 before analysis with either concentrated HCl or NaOH for preserved samples.

11.6. Place samples in the autosampler. Input the sample identification required by the data system.

11.7. Troubleshooting Guide in the System Operation Manual. This guide is also available on request from Lachat.

11.8. Instructions for repacking a cadmium column are available at customer request. Please request a copy from the Technical Services Department.

11.2. SYSTEM NOTES

11.2.1. When running this chemistry as part of a multichannel method, the sequential filling of large volume sample loops may limit the cycle time and consume extra sample. To prevent these effects, the sample stream is split prior to the pump by adding a tee at the pump inlet and using two green/green sample pump tubes.

11.2.2. Reagent recipes from other automated wet chemistry analyzers should not be substituted.

11.2.3. It is important to check column efficiency each time the column is replaced. Once the efficiency is known, a nitrite standard can be inserted in the sample tray to verify that the column remains efficient.

11.2.4. Poor correlation coefficients are sometimes the result of substandard column performance. If the standards are freshly prepared and the calibration fails consistently, replace the column.

11.2.5. If sample tube or standard container materials other than polystyrene are used, standards and samples in these containers should be analyzed to investigate absorption or contamination.

11.2.6. The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.2.7. Samples determined with this method should not give results less than -0.05 uM. If sample results are negative a blank of HPLC water or water from another source should be determined. Contamination from the DI water reservoir is also possible.

11.2.8. If samples are colored, this interference can be determined and subtracted. First, calibrate in the standard fashion. Next, replace the color reagent with a solution containing 100 mL H_3PO_4/L. Finally, reanalyze the samples. The color interference concentration can then be subtracted from the original determined concentration.

11.2.9. It is critical that the peak be detected on the center “flat portion” of the seawater peaks. This is done by injecting a seawater blank. If the window is not on the “flat portion”, the peak start time should be adjusted.

11.2.10. To ascertain that the standard peak is properly positioned in the window, observe the peaks on screen. The baseline trace should be flat where the baseline times are set. If the peak is not properly positioned, change the timing.

11.2.11. For low level analysis it is recommended that samples be analyzed in duplicate from each sample cup. This is done be entering Replicates = 2 when entering sample information.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting peak area versus standard concentration. Sample concentration is calculated from the regression equation.

12.2. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3 Report sample results for nitrate/nitrite in μM N/L as NO_3^- or NO_2^- to two significant figures for samples above the MDL. Report results below the MDL as less than the detection limit.

13. METHOD PERFORMANCE

13.1. The method support data are presented in section 17. This data was generated according to a Lachat Work Instruction during development of the method.

13.2. Although Lachat Instrument publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance. Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it.

14. 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 USEPA 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 United States Environmental Agency (USEPA) recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. 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 Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and

controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.

15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

16.**REFERENCES 10. REFERENCES**

16.1. Grasshoff, K. Methods of Seawater Analysis, Verlag Chemie, Second Edition, 1976.

16.2 Zimmerman, Carl. F. and Keefe, Carolyn W., EPA Method 353.4, Determination of Nitrate + Nitrite in Estuarine and Coastal Waters by Automated Colormetric Analysis in An Interim Manual of Methods for the Determination of Nutrients in Estuarine and Coastal Waters., Revision 1.1, June 1991.

16.3. Johnson, K S. and Petty, R. L., Determination of Nitrate and Nitrite in Seawater by Flow Injection Analysis, Limnol. Oceanogr., 28(6) p.1260-1266.

16.4. Anderson, Leif, Simultaneous Spectrophotometric Determination of Nitrite and Nitrate by Flow Injection Analysis, Analytica Chemica Acta, Vol 110, 1979 p.123-128.

16.5. Yamane, T. and Asito, M., Simple Approach for Elimination of Blank Peak Effects in Flow Injection Analysis of Samples Containing Trace Analyte and Excess of Another Solute., Talanta, Vol. 39, No. 3, 1992 p. 215-219.

16.6. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

16.7. USEPA method number 353.4. Determination of Nitrate and Nitrite in Estuarine and Costal Waters by Gas Segmented Continuous Flow Colorimetric Analysis. Rev. 2.0; Sept 1997.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	48 samples/h, 75 s/sample
Pump Speed:	35
Cycle Period:	75

16 Analyte Data:

Concentration Units:	µM
Chemistry:	Brackish
Inject to BW Baseline Start	12.7 s
Inject to BW Baseline End	70.1 s
Inject to BW Integ Start	33.5 s
Inject to BW Integ End	40.5

17 Calibration Data:

Level	1	2	3	4
Concentration µM	5.00	2.50	1.25	0.00

Calibration Rep Handling:	Average
Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None
Force through zero:	No

18 Sampler Timing:

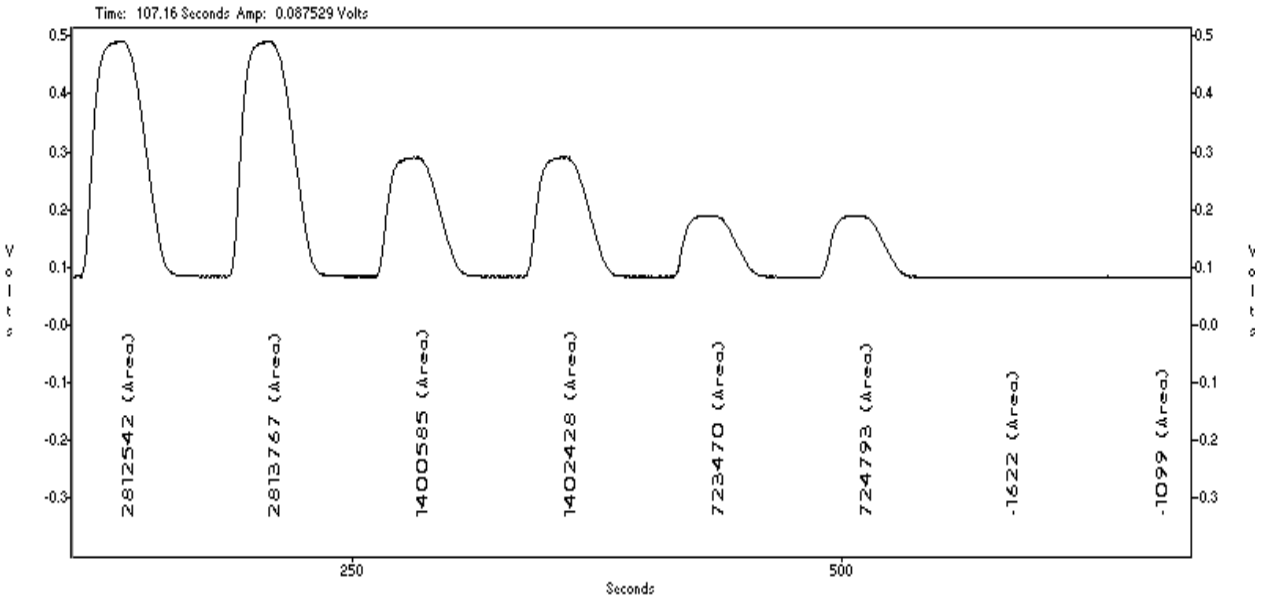
Min. Probe in Wash Period:	5 s
Probe in Sample Period:	59 s

19 Valve Timing:

Load Time:	0 s
Load Period:	40 s
Inject Period:	35 s

17.2. SUPPORT DATA FOR QUIKCHEM 8000

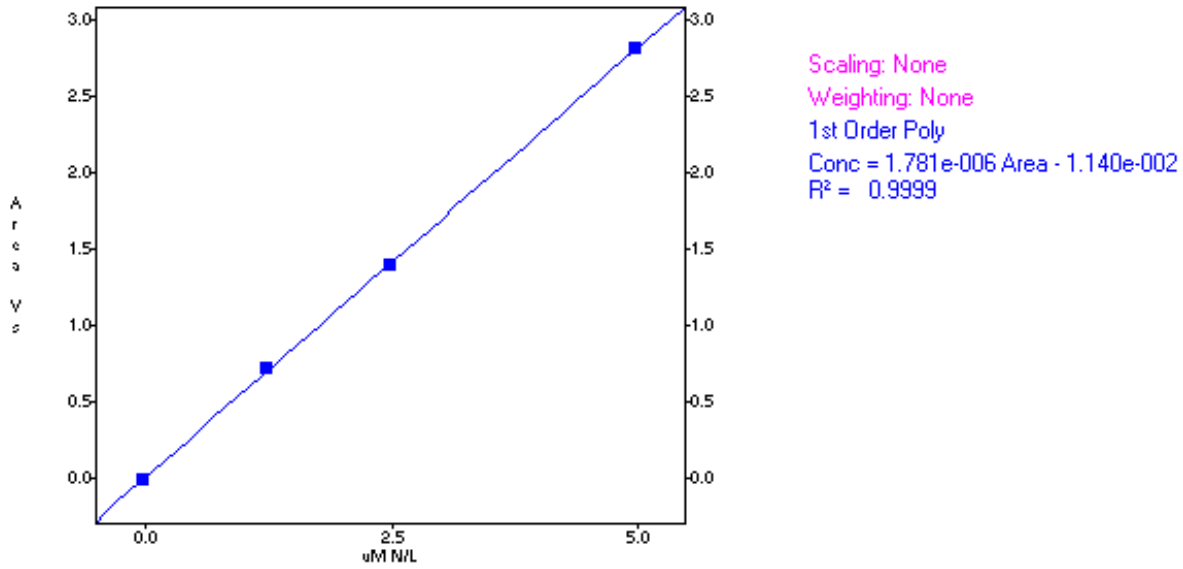
Calibration Data for Nitrate/Nitrite

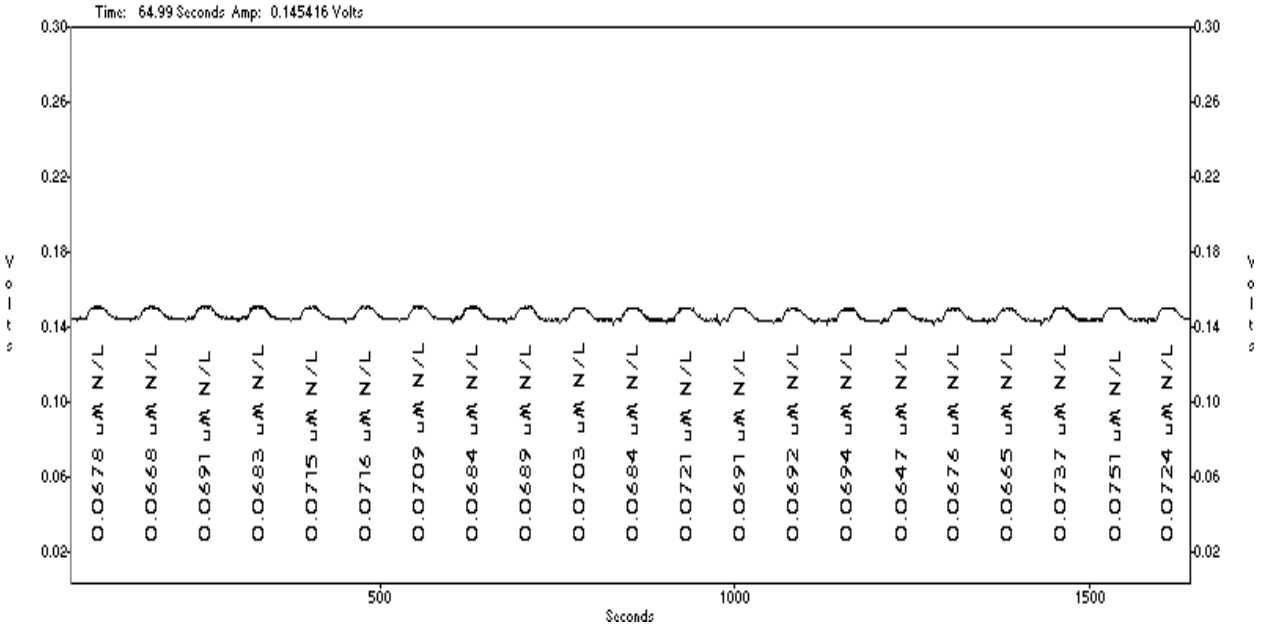


File Name: 960624c5.fdt
Acq. Time: 24 June 1996, 13:44:56

20 Calibration Graph and Statistics

Level	Area (V-s)	µM	Determined	Replicate %RSD	% residual
1	2813154	5.00	5.00	0.0	0.0
2	1401506	2.50	2.49	0.1	0.6
3	724132	1.25	1.29	0.1	-2.3
4	-1361	0.00	-0.01	-27.2	---



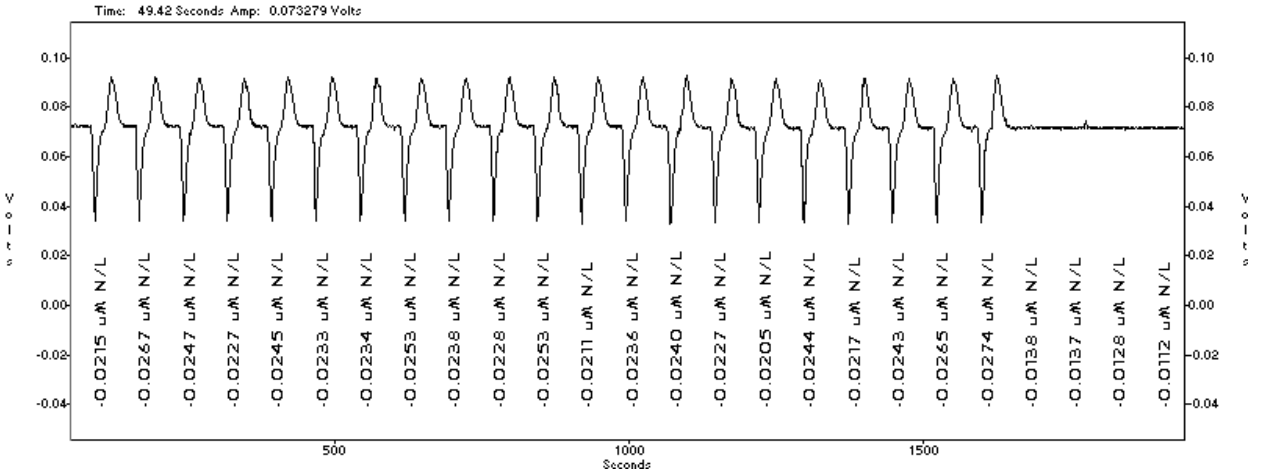


Method Detection Limit for nitrate using a 0.05 µM standard

MDL= 0.006 µM

Standard Deviation (s) = 0.002, Mean (x) = 0.07 µM, Known Value = 0.05 µM FileName: 960701m1.fdt

Acq. Time: 01 July 1996, 12:49:16

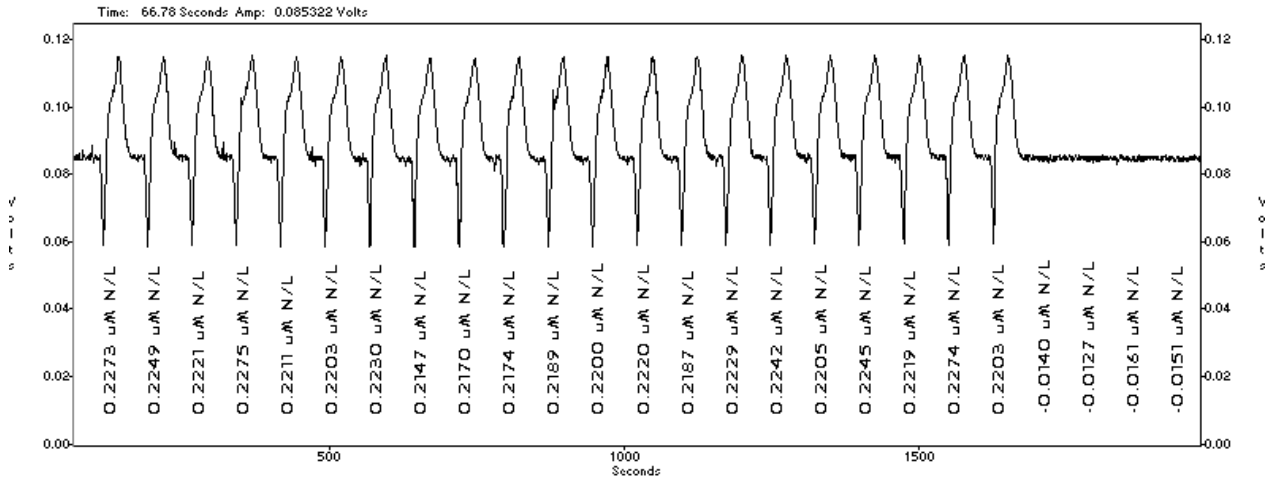


Method Detection Limit for nitrate (Background MDL with Sargasso Seawater)

MDL= 0.005 µM

Standard Deviation (s) = 0.002, Mean (x) = 0.02 µM, Data Filename: 96624bm1.fdt

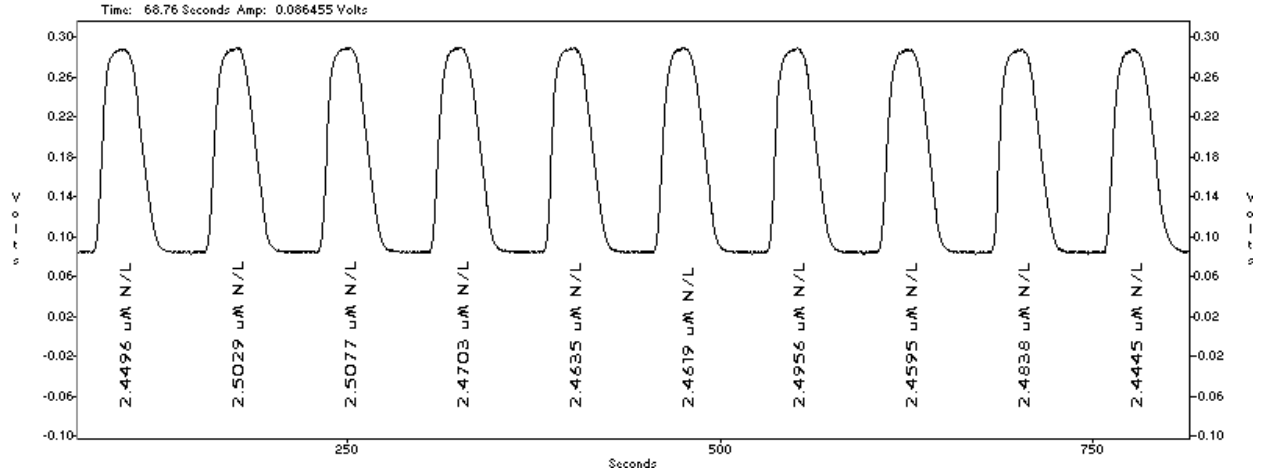
Acq. Time: 24 June 1996, 13:58:21



Method Detection Limit for nitrate (Color forming with Sargasso Seawater)

MDL= 0.009 µM

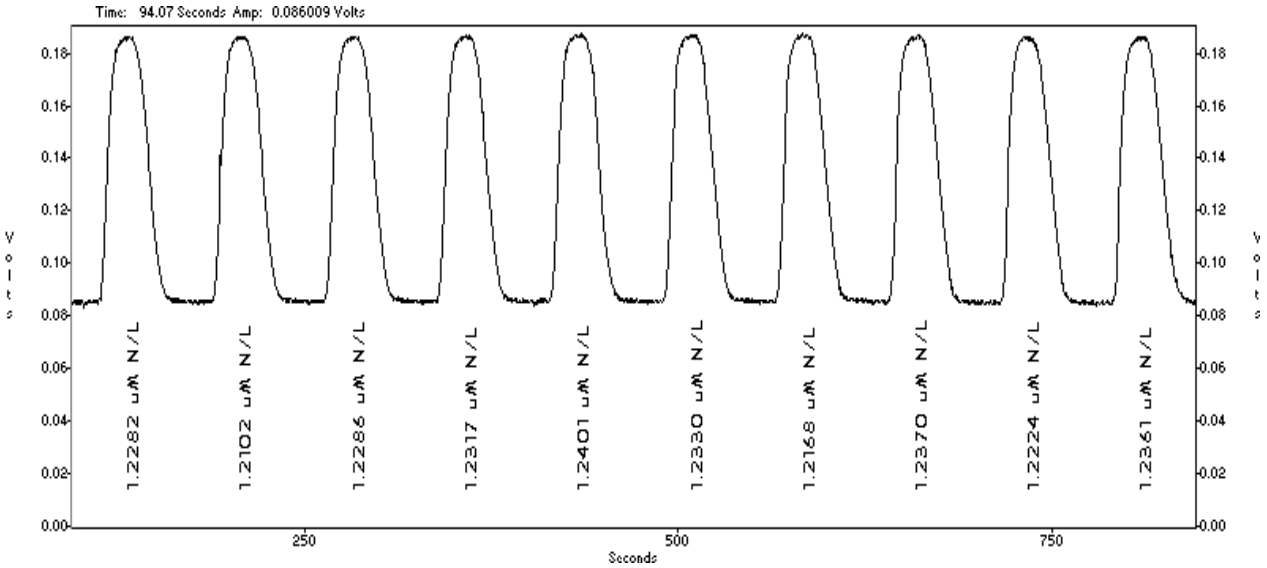
Standard Deviation (s) = 0.003, Mean (x) = 0.22 µM Data
Filename: 960624m2.fdt
Acq. Time: 24 June 1996, 15:09:29



Precision Data for nitrate using a 2.50 µM standard

% RSD =0.90

Standard Deviation (s) =0.022, Mean (x) = 2.47 µM, Known Value = 2.50 µM Data
Filename: 960624p1.fdt
Acq. Time: 24 June 1996, 14:37:14

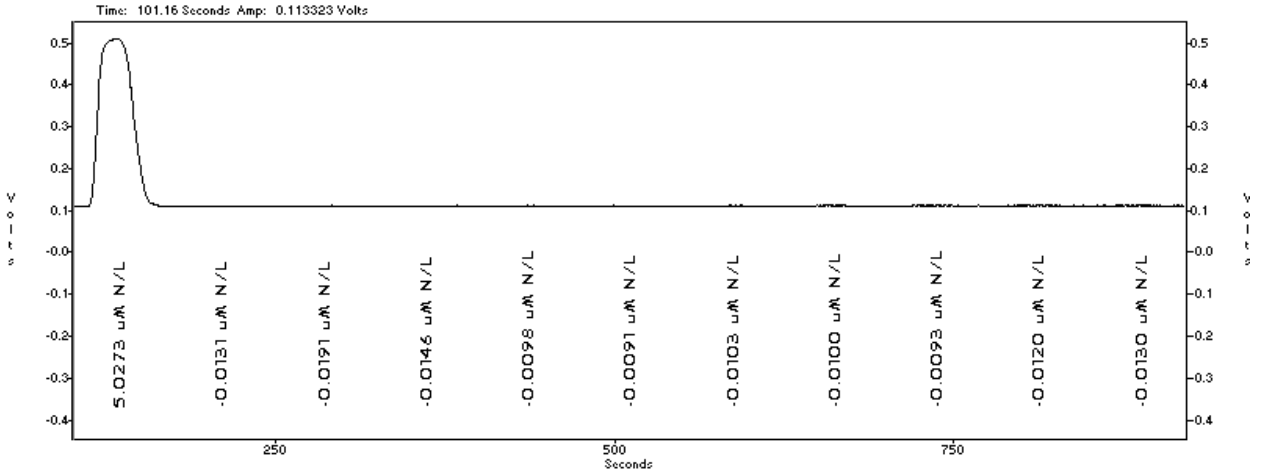


Precision Data for nitrate using a 1.25 µM/L standard

% RSD =0.77

Standard Deviation (s) =0.009, Mean (x) = 1.23 µM/L, Known Value = 1.25 µM/L Data Filename: 960624p2.fdt

Acq. Time: 24 June 1996, 14:53:31

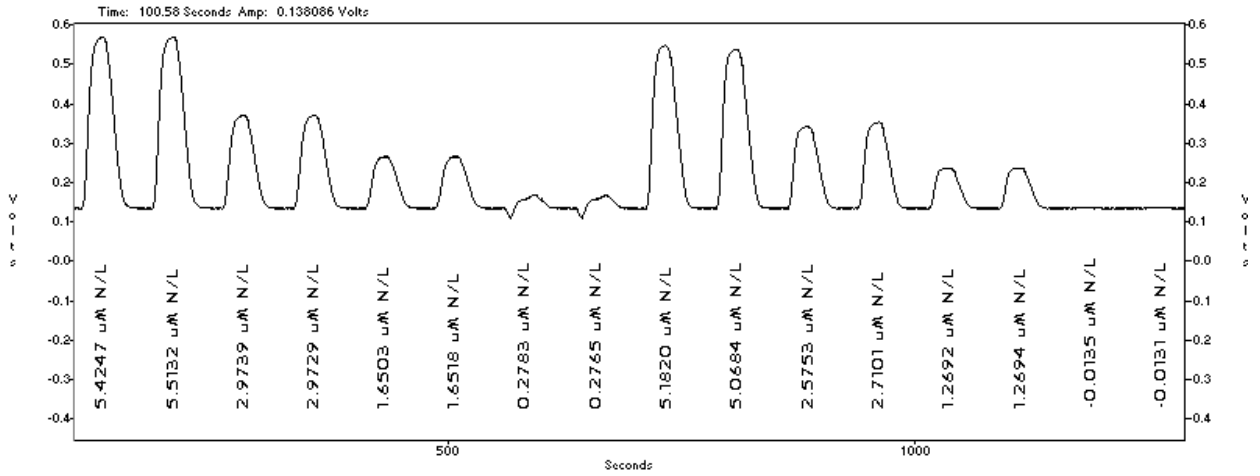


Carryover Study: 5.00 µM nitrate standard followed by 10 blanks

Carryover Passed

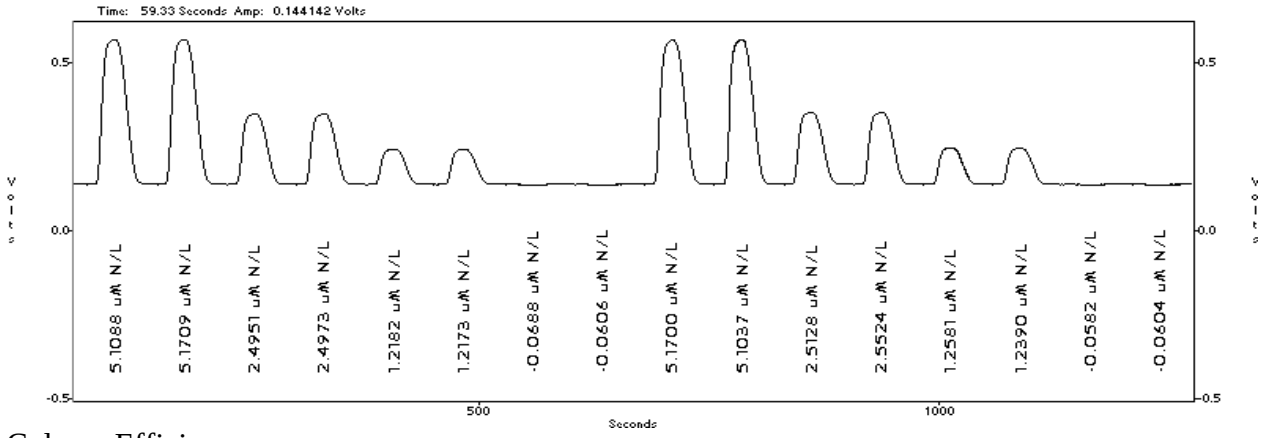
Data Filename: 960625r1.fdt

Acq. Time: 25 June 1996, 12:36:40



Salt Effect

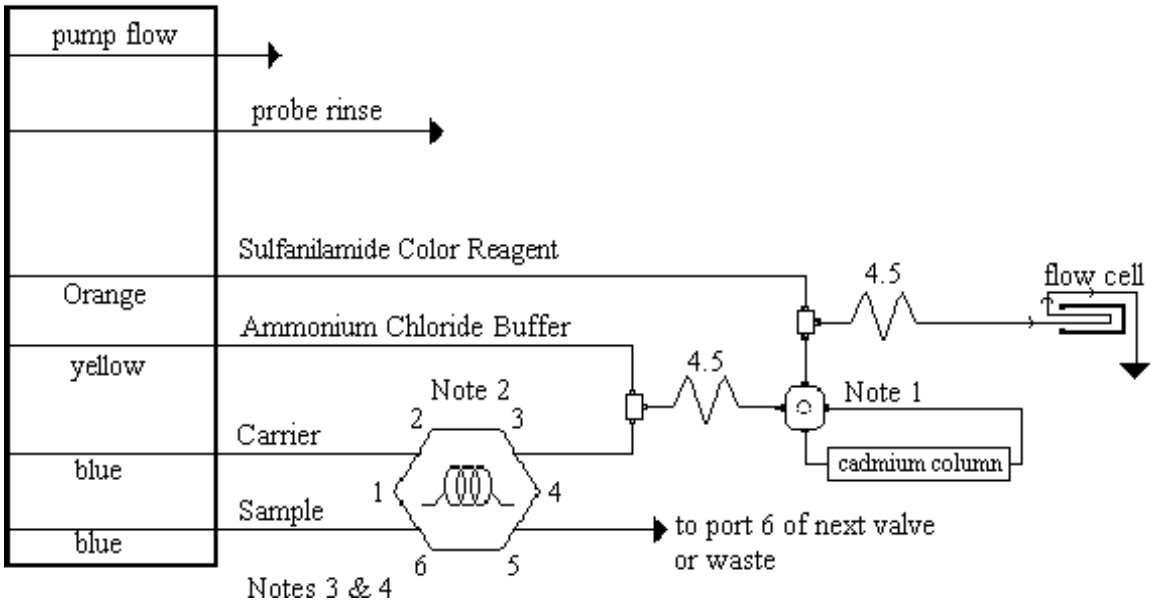
Seawater Spike Level (µM)	Determined Seawater plus spike (µM)	Calculated Seawater spike minus unspiked (µM)	Recovery (%)
5.00	5.47	5.19	103.8
2.50	2.97	2.69	107.6
1.25	1.65	1.36	108.8
0.00	0.28	---	---



Column Efficiency

Concentration (µM)	Nitrate (NO_3)	Nitrite (NO_2)	Column Efficiency (%) (NO_3/NO_2 x 100)
5.00	5.14	5.14	100.0%
2.50	2.49	2.53	98.6%
1.25	1.22	1.25	97.5%

17.3. NITRATE MANIFOLD DIAGRAM

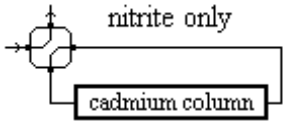


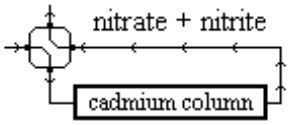
Carrier: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 150 cm x 0.042 in i.d.
QC8000 Sample Loop: 150 cm x 0.042 in i.d.
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell , and a colorimetric detector module is required.

4.5: **70** cm of tubing on a 4.5 cm coil support

Note 1: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.





It is important to check column efficiency each time the column is replaced. Once the efficiency is known, a nitrite standard can be inserted in the sample tray to verify that the column remains efficient.

Note 2: The sample loop should be cut on a 30° - 45° angle for best fit.

Note 3: For the Cetac sampler: From the probe to the sample pump tube 190 cm x 0.022 i.d. From sample pump tube to port 6 = 25 cm x 0.022 i.d.

Note 4: For the AI/Gilson samplers: From probe to sample pump tube: 130 cm x 0.022 i.d. From sample pump tube to port 6 =20 cm x 0.022 in. i.d.

Note5: The sample loop is 150cm of 0.042" i.d. Teflon tubing. For the AE, the tubing is connected to unions connected to valve flares. For the QC8000, the 150 cm loop is connected directly to the valve.

QuikChem® Method 31-107-05-1-A

DETERMINATION OF NITRITE IN BRACKISH OR SEAWATER BY FLOW INJECTION ANALYSIS

21 **Written by Scott Schroeder**

Applications Group

Revision Date: 13 May 2008

LACHAT INSTRUMENTS 5600 LINDBURGH DRIVE LOVELAND, CO 80539 USA

LACHAT INSTRUMENTS
A Hach Company Brand

QuikChem® Method 31-107-05-1-A

Nitrite in Brackish Waters or Seawater

1.25 to 5.0 μM N/L as NO_2^-

– Principle –

In this method, nitrite is determined by diazotization with sulfanilamide under acidic conditions to form a diazonium ion. The diazonium ion is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting pink dye absorbs at 520 nm. Nitrate is determined using a different method incorporating a cadmium reductor column to convert nitrate to nitrite.

Though the method is written for seawater and brackish water, it is also applicable to non-saline sample matrices.

The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinity (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 520 nm. The salt effect is less than 2%.

--Interferences –

1. Sample turbidity may interfere. Remove turbidity by filtration with a 0.45 μm membrane filter prior to analysis.
2. Sample color may be subtracted by analyzing the samples with a substitute color reagent with does not contain the diazotizing agent. This is done by replacing the sulfanilamide-NED-phosphoric acid reagent with a solution containing 100 mL of phosphoric acid per liter.

--Special Apparatus –

3. 60 Position Racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position Rack (Lachat Part No. A81122 [110V]/A81222 [220V]).
4. Sample tubes are needed for 60 Position Samplers (Lachat Part No. 21042).

Instruments, 5600 Lindburgh Drive, Loveland, CO 80539 USA. Phone: 970-663-1377 FAX: 970-962-6710.
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QuikChem® Method 31-107-05-1-A

DETERMINATION OF NIRITE BY FLOW INJECTION ANALYSIS COLORIMETRY

1.SCOPE AND APPLICATION

1.1. This method covers the determination of nitrite in brackish, seawater, or non-saline sample matrices.

1.2. The method is based on reactions that are specific for the nitrite (NO_2^-) ion.

1.3. The applicable range is 1.25 to 5.0 μM N as NO_2^-. The method detection limit is 0.01 μM N as NO_2^-.. The method throughput is 48 injections per hour.

2.SUMMARY OF METHOD

2.1. The nitrite is determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm

3.DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. ANALYTICAL BATCH -- The set of samples extracted/distilled/or digested at the same time to a maximum of 10 samples.

3.2. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.3. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.4. FIELD BLANK (FMB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all preanalysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

3.5. FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.6. LABORATORY BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known

amount of reagent water. It's purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

3.8. LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

3.9. QUALITY CONTROL CHECK SAMPLE (QCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

3.10. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

4.**INTERFERENCES**

4.1. Sample turbidity may interfere. Remove turbidity by filtration with a 0.45 μm membrane filter prior to analysis.

4.2. Sample color may be subtracted by analyzing the samples with a substitute color reagent with does not contain the diazotizing agent. This is done by replacing the sulfanilamide-NED-phosphoric acid reagent with a solution containing 100 mL of phosphoric acid per liter.

5.**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 should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Hydrochloric Acid

5.3.2. Sodium Hydroxide

5.3.3. Phosphoric Acid

5.3.4. Sulfanilamide

5.3.5. N-(1-naphthyl)-ethylenediamine (NED)

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special Apparatus

6.4.1. 60 Position Racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position Rack (Lachat Part No. A81122 [110V]/A81222 [220V]).

6.4.2. Sample tubes are needed for 60 Position Samplers (Lachat Part No. 21042).

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

22 **Degassing with helium:**

To prevent bubble formation, degas all solutions except the standards with helium. Use He at 140kPa (20 lb/in^2) through a helium degassing tube (Lachat Part No. 50100.) Bubble He through the solution for one minute.

23 **Reagent 1. Buffer**

Ammonium Chloride Buffer, CAUTION: Fumes!

By Volume: In a **1 L** volumetric flask, dissolve **85.0 g ammonium chloride** (NH_4Cl) and **1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) in about **800 mL DI water.** Dilute to the mark and invert to mix. Adjust the **pH to 8.5** with **15 N sodium hydroxide solution.**

By Weight: To a tared **1 L** container, add **85.0 g ammonium chloride** (NH_4Cl**), 1.0 g disodium ethylenediamine tetraacetic acid dihydrate** ($Na_2EDTA{\cdot}2H_2O$) and **938 g DI water**. Shake or stir until dissolved. Then adjust the **pH to 8.5** with **15 N sodium hydroxide solution.**

ACS grade ammonium chloride has been found occasionally to contain significant nitrate contamination. An alternative recipe for the ammonium chloride buffer is:

By Volume: In a fume hood, to a **1 L** volumetric flask add **500 mL DI water, 105 mL concentrated hydrochloric acid** (HCl), and **95 mL ammonium hydroxide** (NH_4OH). Add **3.0 g disodium EDTA**, dissolve and dilute to the mark. Invert to mix. Adjust to pH

24 **8.5** with **HCl or NaOH solution**.

Reagent 2. Sulfanilamide Color Reagent

By Volume: To a **1 L** volumetric flask add about **600 mL DI water**. Then add **100 mL 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide** and **1.0 g N-(1-naphthyl)- ethylenediamine dihydrochloride** (NED). Shake to wet, and stir to dissolve for 30 min. Dilute to the mark, and invert to mix. Store in a dark bottle and discard when the solution turns pink.

By Weight: To a tared, dark **1 L** container add **876 g DI water, 170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide,** and **1.0 g N-(1-naphthyl)ethylene- diamine dihydrochloride** (NED). Shake until wetted and stir with a stir bar for 30 min. until dissolved. Store in a dark bottle and discard when the solution turns pink.

7.2. PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be requires:

By Volume: Two 1 L and four 250 mL volumetric flasks.

By Weight: Two 1 L and four 250 mL containers.

25 **Standard 1. Stock Standard 5.00 mM N as NO_2^-**

By Volume: In a **1 L** volumetric flask **0.3460 g of sodium nitrite** ($NaNO_2$) in about **800 mL DI water**. Dilute to the mark and invert to mix. When refrigerated this standard may be stored in glass for up to one month.

26 **Standard 2. Working Stock Standard 25 µM N as NO_2^-**

By Volume: In a **1 L** volumetric flask dilute **5.0 mL Stock Standard** (Standard 1) to the mark with **DI water**. Invert to mix. Refrigerate and store no longer than three days.

By Weight: To a tared **1 L** container add about **4 to 5 g Stock Standard** (Standard 1). multiply the actual weight of the solution added by 50 and make up to this resulting total weight with **DI water**, using a wash bottle for the last 10 g or so. Shake to mix. Store in glass. Refrigerate and store no longer than three days.

Working Standards (Prepare Daily)	**A**	**B**	**C**	**D**
Concentration µM N as NO_2^-	5.00	2.50	1.25	0.00

By Volume

Volume (mL) of **working stock standard 2** diluted to **250 mL with DI water**	50	25	12.5	0.00

By Weight

Weight (g) of **working stock standard 2** diluted to final weight (~250 g) multiplied by factor below with **DI water**	50	25	12.5	0.00
Division Factor Divide exact weight of the standard by this factor to give final weight	0.20	0.10	0.05	---

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Since there is no single preservation method that may be recommended for all types of samples the analyst must examine each situation critically.

8.2. For NPDES Monitoring: Nitrite will be oxidized by air (O2) to nitrate in a few days. If the nitrite samples are not immediately analyzed for nitrite, the samples should be stored in a plastic or glass container with refrigeration at <6°C. The maximum holding time is 48 hours.

8.3 Ideally, analysis should be commenced within 24 hours of sample collection. Plastic or glass bottles can be used. If longer term storage is necessary, samples should be filtered on-site with Nucleopore 0.45 μM membrane filters (washed with greater than 200 mL of sample), and frozen at -20°C. Samples should be frozen in plastic bottles, leaving about 30% headspace for expansion. Frozen samples may be stored for up to 10 days.

8.4 Thaw samples by immersion in warm water, with occasional mixing to ensure uniform sample temperature. Do not warm samples above ambient temperature. Since the analyte is in the liquid portion of the thawing sample, care should be taken to ensure complete thawing.

8.5 It should be mentioned that some researchers have found serious errors when investigating the effects of filtration. It is imperative that the analyst examine sample preparation and preservation techniques before routine testing.

9. 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 periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. An analytical batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

9.1.1. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in section 9.3.

9.1.2. Analyses of laboratory blanks are required to demonstrate freedom from contamination.

9.1.3. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

9.1.4. The laboratory should maintain records to define the quality of data that is generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. Method Detection Limit (MDL) –To establish the ability to detect the analyte, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards, that will be used in the practice of this method. An MDL less than or equal to the MDL in section 1.2 must be achieved prior to the practice of this method.

9.2.2. Initial Precision and Recovery – To establish the ability to generate acceptable precision results, the operator shall perform 10 replicates of a mid-range standard, according to the procedure beginning in Section 11.

9.2.2.1. Using the results of the replicates compute the average percent recovery (X) and the standard deviation (s) for the analyte. Use the following equation for the calculation of the standard deviation.

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

Where, n = Number of samples, x = concentration in each sample

9.2.2.2. Compare s and x results with the corresponding data in Section 17. If the results meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If however, s and x do not match the data in Section 17, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3. Matrix spikes- The laboratory must spike, in duplicate, a minimum of 5 percent of all samples (one sample in each batch of no more than twenty samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

9.3.1.1. If, as in compliance monitoring, the concentration of the analyte in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), which ever is higher.

9.3.1.2. If the concentration of the analyte in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard used in Section 9.2.5 or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2. Analyze one sample aliquot out of each set of no more than twenty samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A)

9.3.3. Calculate the percent recovery (P) of the analyte in each aliquot using the following equation.

$$P = \frac{(A - B)100}{T}$$

Where, A = Measured concentration of analyte after spiking, B = measured background concentration of analyte, T = True concentration of the spike

9.3.4. The percent recovery of the analyte should meet current laboratory acceptance criteria.

9.3.4.1. If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test of the analytical batch is within the current laboratory acceptance criteria, an interference is present. In this case, the results may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2 and repeat the analysis of the sample and the matrix spike.

9.3.4.2. If the results of both the spike and ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample reanalyzed.

9.3.5. Compute the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \text{ x } 100$$

Where, D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

9.3.6. The RPD for duplicates shall meet the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.4 Laboratory blanks – Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1. Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch of no more than twenty samples. The blank must be subjected to the same procedural steps as a sample.

9.4.2. If analyte is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

9.5. Calibration Verification – Verify calibration using the procedure described in Section 10

9.6. On-going Precision and Recovery (OPR) – With every analytical batch of no more than twenty samples, a midrange standard must be prepared using the procedure described in Section 11.

9.6.1. Compare the results with the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.7. Quality Control Samples (QCS) – It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 9.7.1. The QCS is used to verify the concentrations of the calibration standards.

9.8. Depending on the specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare reagents and standards as described in Section 7.

10.2. Set up manifold as shown in Section 17.

10.3. Input data system parameters as shown in Section 17.

10.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

10.5. Place standards in the sampler. Input the information required by the data system.

10.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7. Verify calibration using a midrange calibration standard every ten samples or every analytical batch. Compute the percent recovery using the following equation:

$$\%recovery = \frac{D}{K} \text{x } 100$$

Where, D = Determined concentration of analyte in the calibration standard, K = Actual concentration of the analyte in the calibration standard

10.8. If % recovery exceeds +/-10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed

11. PROCEDURE

11.1. CALIBRATION PROCEDURE

11.1.1. Prepare reagent and standards as described in Section 7.

11.1.2. Set up manifold as shown in Section 17.

11.1.3. Input data system parameters as in Section 17.

11.1.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.1.5. Place samples and/or standards in the autosampler. Input the information required by the data system, such as concentration, replicates and QC scheme.

11.1.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.2. SYSTEM NOTES

11.2.1. When running this chemistry as part of a multichannel method, the sequential filling of large volume sample loops may limit the cycle time and consume extra sample. To prevent these effects, the sample stream is split prior to the pump by adding a tee at the pump inlet and using two green/green sample pump tubes.

11.2.2. Reagent recipes from other automated wet chemistry analyzers should not be substituted.

11.2.3. If sample tube or standard container materials other than polystyrene are used, standards and samples in these containers should be analyzed to investigate absorption or contamination.

11.2.4. The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.2.5. Samples determined with this method should not give results less than -0.05 uM N as NO_2^-. If sample results are negative a blank of HPLC water or water from another source should be determined. Contamination from the DI water reservoir is also possible.

11.2.6. If samples are colored, this interference can be determined and subtracted. First, calibrate in the standard fashion. Next, replace the color reagent with a solution containing 100 mL H_3PO_4/L. Finally, reanalyze the samples. The color interference concentration can then be subtracted from the original determined concentration.

11.2.7. It is critical that the peak be detected on the center “flat portion” of the seawater peaks. This is done by injecting a seawater blank. If the window is not on the “flat portion”, the peak start time should be adjusted.

11.2.8. To ascertain that the standard peak is properly positioned in the window, observe the peaks on screen. The baseline trace should be flat where the baseline times are set. If the peak is not properly positioned, change the timing.

11.2.9. For low level analysis it is recommended that samples be analyzed in duplicate from each sample cup. This is done be entering Replicates = 2 when entering sample information.

11.2.10. Carrier should be degassed for at least 1 minute per liter.

11.2.11. If after degassing and adding the backpressure coil, air spikes are not eliminated a tubular membrane debubbler may be added in line before the flow cell. This should be mounted in a vertical position to be most effective.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.2. Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in $\mu g\ NO_2/L$ $\mu g\ NO_2 / 1000 = mg\ NO_2$

$\mu g\ NO_2/L \times 0.304 = \mu g\ N/L$

13. METHOD PERFORMANCE

13.1. The method support data are presented in section 17. This data was generated according to a Lachat Work Instruction during development of the method.

13.2. Although Lachat Instrument publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance. Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it.

14. 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 USEPA 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 United States Environmental Agency (USEPA) recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. 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 Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and

controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.

15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

16.**REFERENCES**

16.1. Grasshoff, K. Methods of Seawater Analysis, Verlag Chemie, Second Edition, 1976.

16.2 Zimmerman, Carl. F. and Keefe, Carolyn W., EPA Method 353.4, Determination of Nitrate + Nitrite in Estuarine and Coastal Waters by Automated Colormetric Analysis in An Interim Manual of Methods for the Determination of Nutrients in Estuarine and Coastal Waters., Revision 1.1, June 1991.

16.3. Johnson, K S. and Petty, R. L., Determination of Nitrate and Nitrite in Seawater by Flow Injection Analysis, Limnol. Oceanogr., 28(6) p.1260-1266.

16.4. Anderson, Leif, Simultaneous Spectrophotometric Determination of Nitrite and Nitrate by Flow Injection Analysis, Analytica Chemica Acta, Vol 110, 1979 p.123-128.

16.5. Yamane, T. and Asito, M., Simple Approach for Elimination of Blank Peak Effects in Flow Injection Analysis of Samples Containing Trace Analyte and Excess of Another Solute., Talanta, Vol. 39, No. 3, 1992 p. 215-219.

16.6. U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, revised March 1983, method 353.2. and 354.1

16.7. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput:	48 samples/h, 75 s/sample
Pump Speed:	35
Cycle Period:	75

27 Analyte Data:

Concentration Units:	µM
Peak Base Width:	26 s
% Width Tolerance:	100
Threshold:	10000
Inject to Peak Start:	16 s
Chemistry:	Brackish
Inject to BW Baseline Start	4.7 s
Inject to BW Baseline End	46.5 s
Inject to BW Integ Start	12.4 s
Inject to BW Integ End	26.0 s

28 Calibration Data:

Level	1	2	3	4
Concentration µM N as NO_2^-	5.00	2.50	1.25	0.00

Calibration Rep Handling:	Average
Calibration Fit Type:	1st Order Polynomial
Weighting Method:	None
Force through zero:	No

29 Sampler Timing:

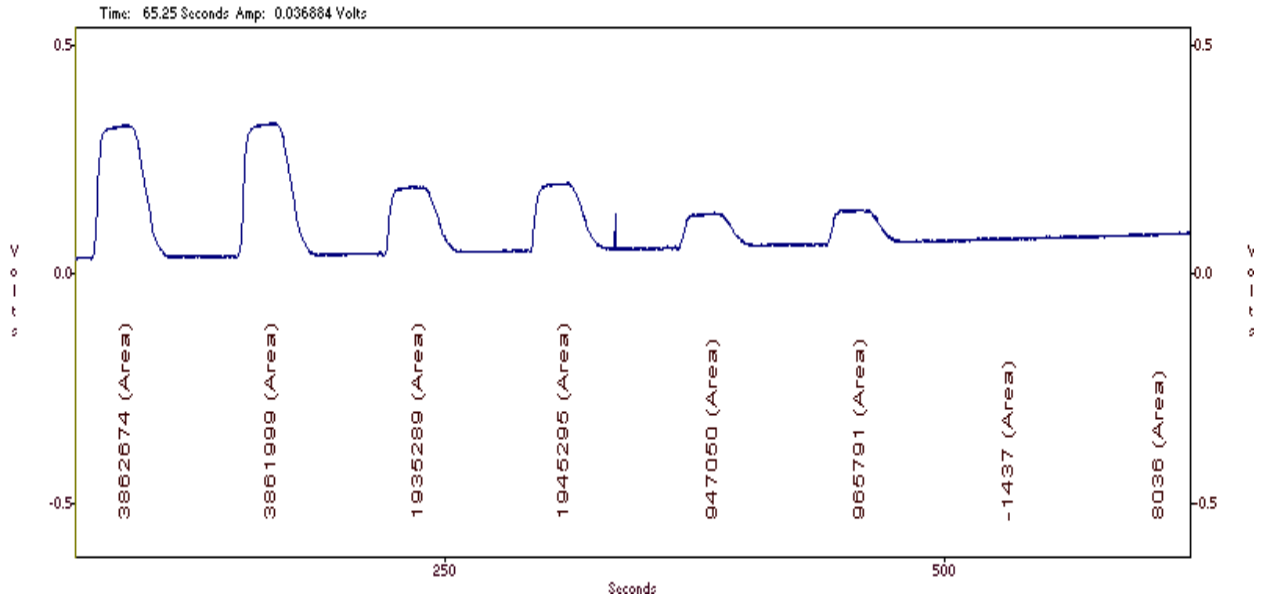
Min. Probe in Wash Period:	5 s
Probe in Sample Period:	59 s

30 Valve Timing:

Load Time:	0 s
Load Period:	40 s
Inject Period:	35 s

17.2. SUPPORT DATA FOR QUIKCHEM 8000

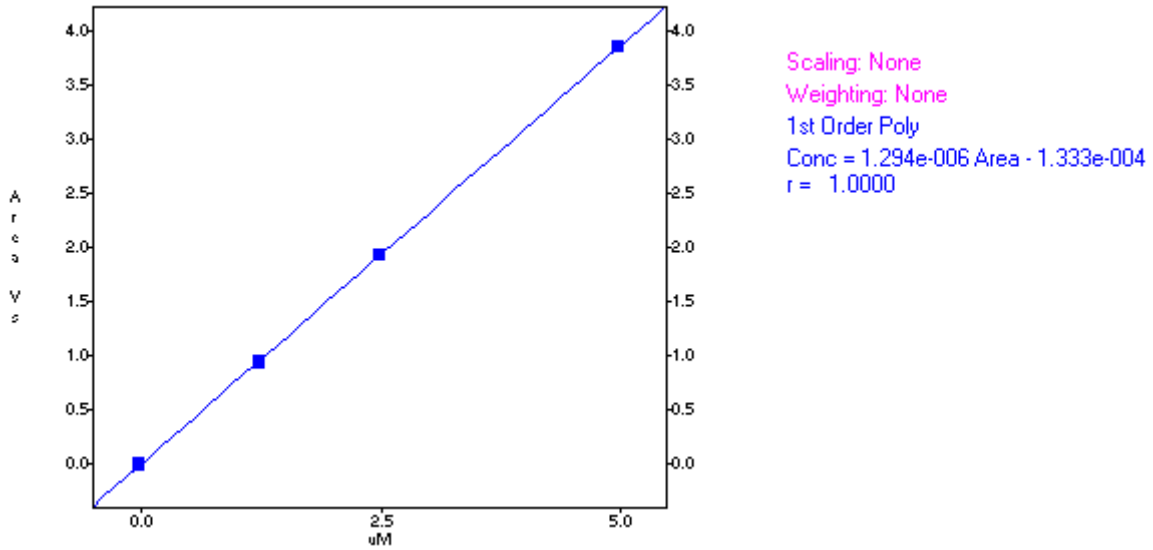
Calibration Data for Nitrite

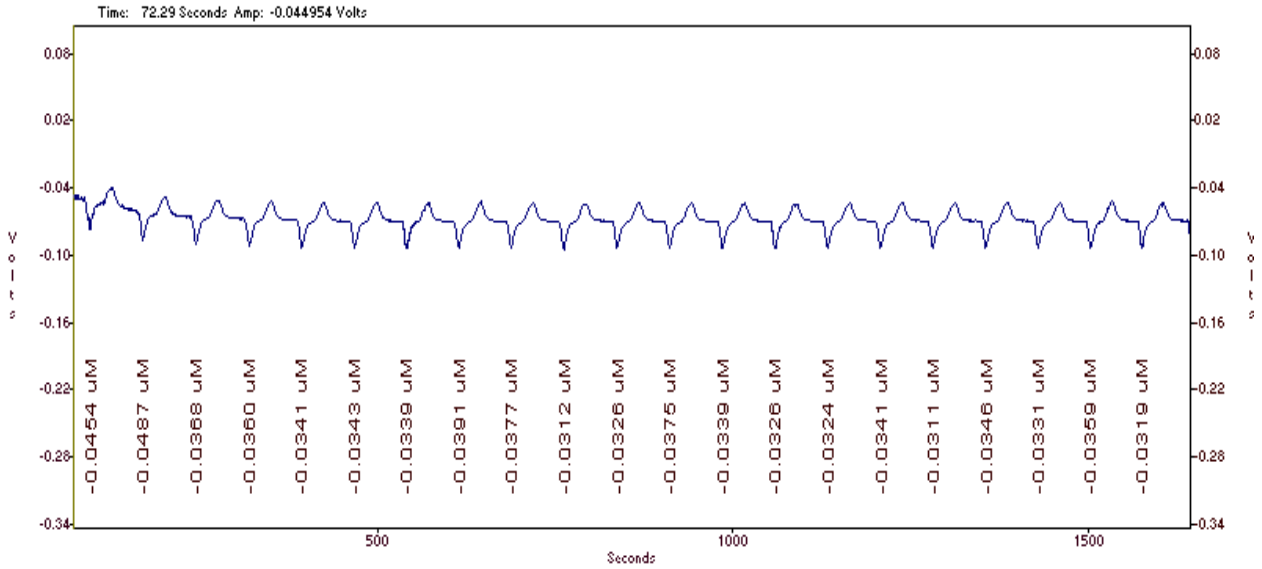


Filename: 970404c.fdt Acq.
Date: 4 April 1997

31 Calibration Graph and Statistics

Level	Area (V-s)	µM	Determined	Replicate %RSD	% residual
1	3862337	5.00	5.00	0.0	0.0
2	1940292	2.50	2.51	0.4	-0.4
3	956421	1.25	1.24	1.4	1.0
4	3300	0.00	0.00	---	---

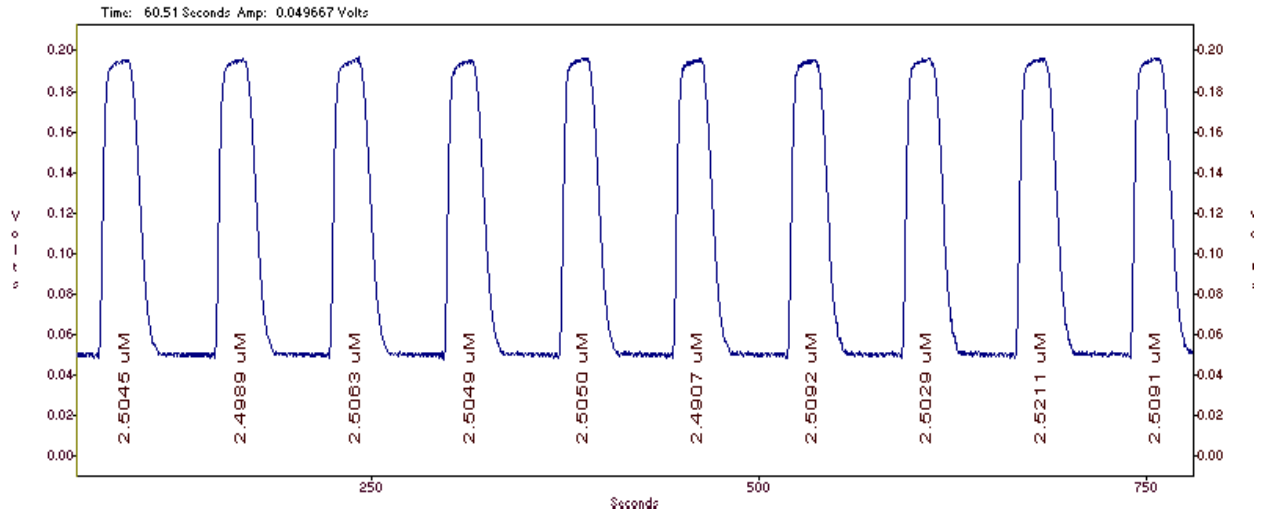




Method Detection Limit for nitrite using a seawater blank, no color forming

MDL= 0.01 µM

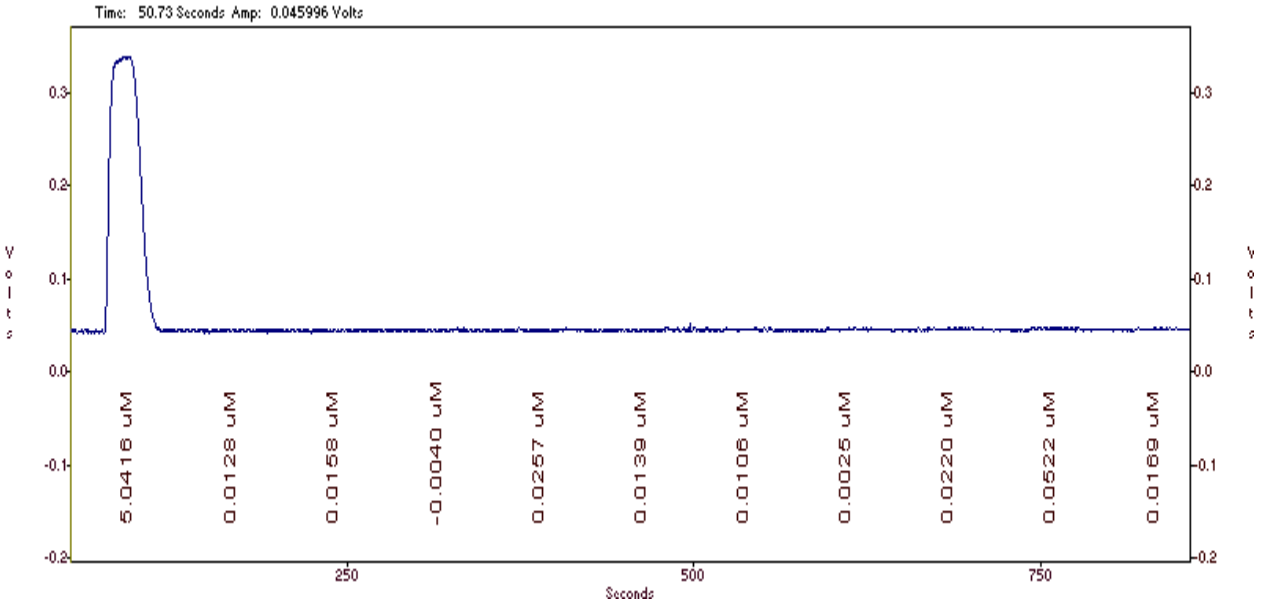
Standard Deviation (s) = 0.0044 µM, Mean (x) = 0.035 µM, Known Value =blank Acq.
Date: 15 April 1997



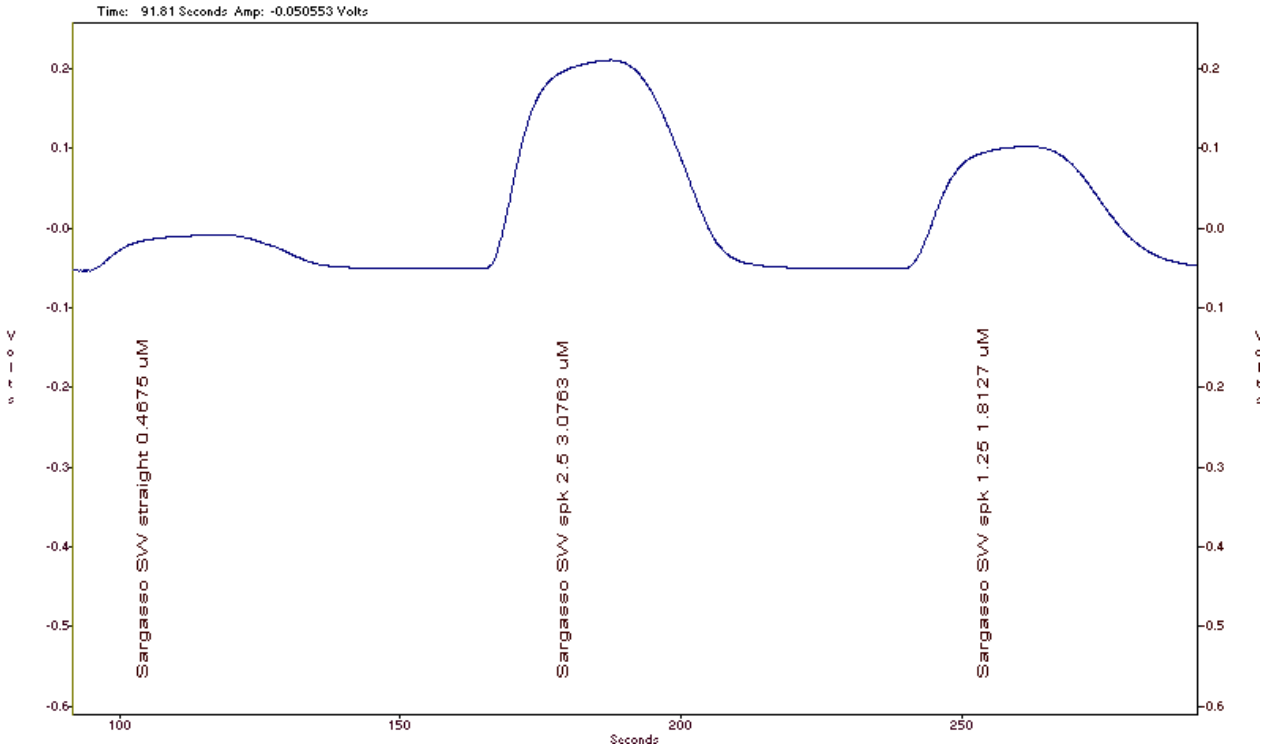
Precision Data for nitrite using a 2.50 µM standard

% RSD =0.32

Standard Deviation (s) =0.008 µM, Mean (x) = 2.50 µM, Known Value = 2.50 µM Acq.
Date: 4 April 1997

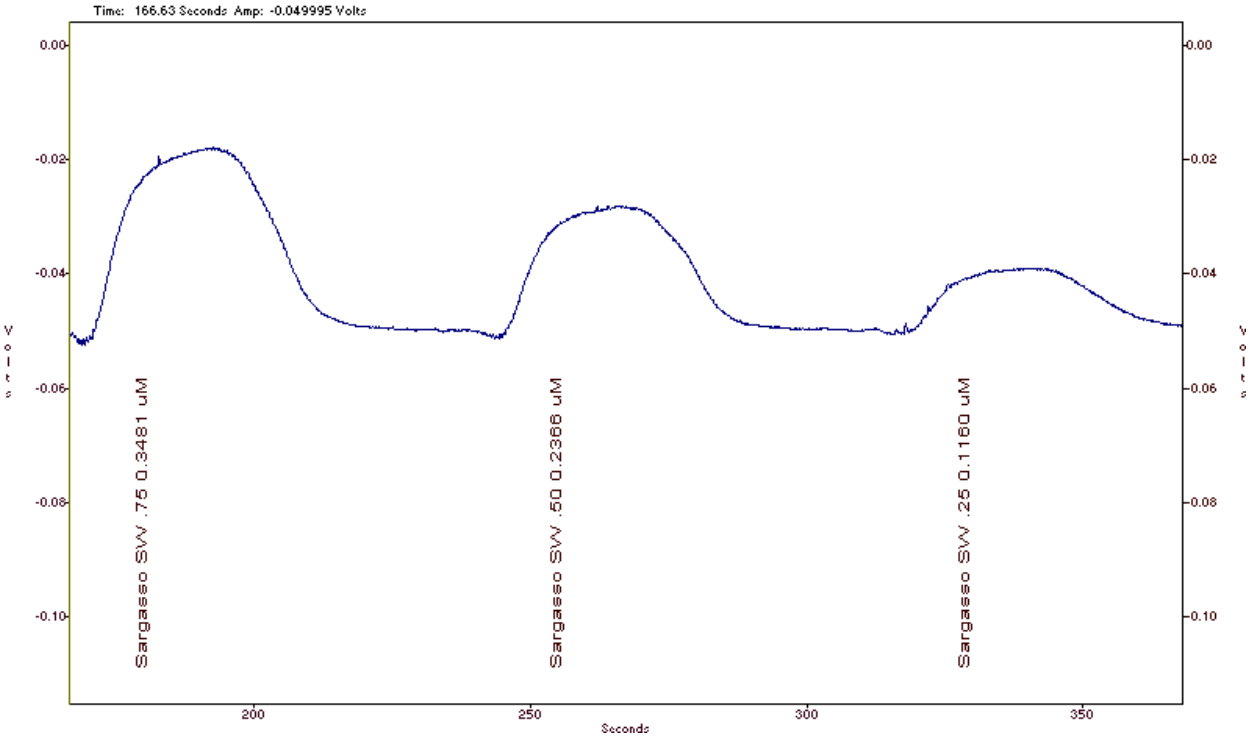


Carryover Passed, all blanks < MDL. Acq.
Date: 4 April 1997



32 Salt Effect (Spikes of seawater)

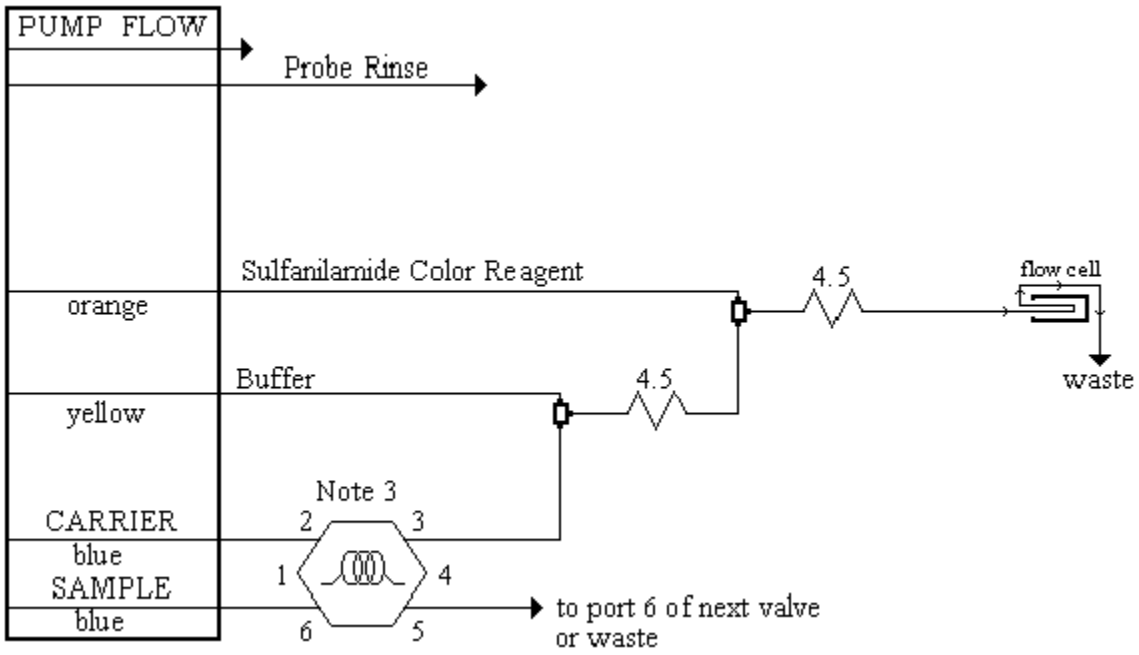
Spike Level (µM NO_2^-)	Concentration (µM N as NO_2^-)	%Recovery
---	0.47	---
1.25	1.81	107.2
2.50	3.08	104.4



33 **Brackish effect (Dilution of spiked seawater)**

Dilution Factor	Determined Concentration	Theoretical Concentration	Brackish Effect
	(µM N as NO_2^-)	(µM N as NO_2^-)	(%)
1.00	0.468	---	---
1.33	0.348	0.351	-0.8
2.00	0.237	0.234	+1.3
4.00	0.116	0.117	+0.9

17.3. NITRITE MANIFOLD DIAGRAM



Carrier: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 µL/cm**.
AE Sample Loop: 150 cm x 0.042" i.d.
QC8000 Sample Loop: 150 cm x 0.042" i.d.
Interference Filter: 520 nm

Apparatus: An injection valve, a 10 mm path length flow cell , and a colorimetric detector module is required.

4.5: **70** cm of tubing on a 4.5 cm coil support

Note 1: The sample loop is 150 cm of 0.042" i.d. Teflon tubing. For the AE, the tubing is connected to unions connected to valve flares. For the QC8000, the 150 cm loop is connected directly to the valve.

Note 2: From probe to sample pump tube: 130 cm x 0.022 i.d. From sample pump tube to port 6=20 cm x 0.022 in. i.d.

Note 3: The sample loop should be cut on a 30° - 45° angle for best fit.

QuikChem® Method 31-107-06-1-B

DETERMINATION OF AMMONIA IN BRACKISH OR SEAWATER BY FLOW INJECTION ANALYSIS

34 **Written by Ninglan Liao**

Applications Group

Revision Date: 18 Sept 2008

LACHAT INSTRUMENTS 5600 LINDBERGH DRIVE LOVELAND, CO 80539 USA

LACHAT INSTRUMENTS
A Hach Company Brand

QuikChem® Method 31-107-06-1-B

AMMONIA IN BRACKISH OR SEAWATER

5 to 600 μg NH_3-N/L

0.36 to 42.86 μM NH_3-N/L

– Principle –

This method is based on the Berthelot reaction. Ammonia reacts in alkaline solution with hypochlorite to form monochloramine, which, in the presence of phenol, catalytic amounts of nitroprusside (nitroferricyanide), and excess hypochlorite gives indophenol blue. The formation of monochloramine requires a pH between 8 and 11.5. At higher pH, ammonia may begin to oxidize to nitrate. At pH greater than 9.6, some precipitation of calcium and magnesium as hydroxides and carbonates occurs in seawater. EDTA added to the buffer prevents this from occurring. The indophenol blue measured at 630 nm is proportional to the original ammonia concentration.

Though the method is written for seawater and brackish water, it is also applicable to non-saline sample matrixes.

The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 630 nm.

– Interferences –

1. Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA is added to the sample in-line to prevent this problem.
2. Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation. See System Note 11 for specific instructions.
3. Sulfide may interfere at levels greater than 2 mg H_2S/L. Samples containing concentrations greater than this should be diluted.
4. Salinity does not normally interfere in this method. This may be verified by running the samples through the manifold with all reagents pumping, except hypochlorite, which is replaced by deionized water. The resulting concentrations are then compared to those obtained for samples determined with color formation.
5. The salt effect (salinity influence on absorbance) is less than 2 %.

– Special Apparatus –

Please see Parts and Price list for Ordering Information

1. Heating Unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)
2. Seawater Accessories Kit Lachat Part No. 50969 – RAS or 50970 – ASX510.
3. 60 Position Racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position Rack (Lachat Part No. A81122 [110V]/A81222 [220V]).
4. Sample tubes are needed for 60 Position Samplers Lachat Part No. 21042.
4. PVC PUMP TUBES MUST BE USED FOR THIS METHOD

Revised by L. Egan on Sept 18 2008. Written and copyrighted © by Ninglan Liao on 3 January 2001 by **Lachat Instruments 5600 Lindbergh Drive, Loveland, CO 80539 USA**. Phone: 970-663-1377 FAX: 970-962-6710.. This document is the property of Lachat Instruments. Unauthorized copying of this document is prohibited.

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QuikChem® Method 31-107-06-1-B

DETERMINATION OF AMMONIA IN BRACKISH OR SEAWATER BY FLOW INJECTION ANALYSIS

1. SCOPE AND APPLICATION

1.1. This method covers the determination of ammonia in drinking, ground, and surface waters, and domestic and industrial wastes.

1.2. The applicable range is 5 to 600 μg N/L as NH_4. The method detection limit is 0.7 μg N/L as NH_3. The method throughput is 45 injections per hour.

2. SUMMARY OF METHOD

2.1. This method is based on the Berthelot reaction. Ammonia reacts with alkaline phenol, and sodium hypochlorite to form indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. The absorbance of the reaction product is measured at 630 nm, and is directly proportional to the original ammonia concentration in the sample.

If distillation is required, the sample is buffered at a pH of 9.5 with a borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid.

3. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.2. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3. INSTRUMENT PERFORMANCE CHECK SOLUTION (ICP) -- A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.4. LABORATORY SPIKED BLANK (LSB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LSB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

3.5. LABORATORY SPIKED SAMPLE MATRIX (LSM) -- An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LSM is analyzed exactly like 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.

3.6. LABORATORY REAGENT BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LINEAR CALIBRATION RANGE (LCR) -- The concentration range over which the instrument response is linear.

3.8. 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.

3.9. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

3.10. PRACTICAL QUANTITATION LIMIT (PQL) -- The lower level where measurements become quantitatively useful is called the PQL. The PQL is defined as PQL = 10 x s, where s = the standard deviation of 21 replicates of a standard 2.5 – 5 times the MDL.

3.11. QUALITY CONTROL SAMPLE (QCS) -- A solution of method analytes of known concentrations that is used to spike an aliquot of LRB or sample matrix. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

3.12. STOCK STANDARD SOLUTION (SSS) -- A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.

3.13. USEPA -- United States Environmental Protection Agency.

4.INTERFERENCES

4.1. Calcium and magnesium ions may precipitate if present in sufficient concentration. EDTA is added to the sample in-line to prevent this problem.

4.2. Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation. See System Note 11 for specific instructions.

4.3. Sulfide may interfere at levels greater than 2 mg H_2S/L. Samples containing concentrations greater than this should be diluted.

4.4. Salinity does not normally interfere in this method. This may be verified by running the samples through the manifold with all reagents pumping, except hypochlorite, which is replaced by deionized water. The resulting concentrations are then compared to those obtained for samples determined with color formation.

4.5. The salt effect (salinity influence on absorbance) is less than 2 %.

5.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 should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Sodium hydroxide

5.3.2. Phenol

6.EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special Apparatus

6.4.1. Seawater Accessories Kit (Lachat Part No. 50969 – RAS or 50970 – ASX510).

6.4.2. 60 Position Racks for samples are required to allow replicate sample analyses from a single tube. XYZ with 60 Position Rack (Lachat Part No. A81122 [110V]/A81222 [220V]).

6.4.3. Sample tubes are needed for 60 Position Samplers (Lachat Part No. 21042).

6.4.4. PVC PUMP TUBES MUST BE USED FOR THIS METHOD

7.REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

35 **Degassing with helium:**

To prevent bubble formation, DO NOT DEGAS USING AN INVASIVE PROCDURE SUCH AS A WAND TO AVOID CONTAMINATION. Degas by vacuum or sonication. DO NOT DEGAS PHENATE, HYPOCHLORITE OR STANDARDS.

36 **Reagent 1. Buffer Chelating Agent**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetate** (Na_2EDTA) and **11.0 g sodium hydroxide** (NaOH) in about **900 mL DI water.** Stir to mix and dilute to the mark with **DI water**. Degas as above. Prepare fresh monthly.

By Weight: To a tared **1 L** container, add **966 g DI water**. Add **50.0 g disodium ethylenediamine tetraacetate** (Na_2EDTA) and **11.0 g sodium hydroxide** (NaOH). Stir to mix. Degas as above. Prepare fresh monthly.

37 **Reagent 2. Phenate Reagent**

CAUTION: Wear gloves. Phenol causes skin burns and is rapidly absorbed into the body through the skin. Do not degas this reagent.

By Volume: In a **1 L** volumetric flask, dissolve **83 g crystalline phenol** (C_6H_5OH) in approximately **500 mL DI water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark with **DI water**, and invert to mix. The color of this reagent darkens with age, increasing the baseline absorbance. Prepare fresh reagent after 72 hours. Prepare fresh every 3-5 days and discard when turns dark brown.

By Weight: To a tared **1 L** container, add **888 g DI water**. Add **83 g crystalline phenol** (C_6H_5OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and stir to mix. The color of this reagent darkens with age, increasing the baseline absorbance. Prepare fresh every 3-5 days and discard when turns dark brown.

38 **Reagent 3. Sodium Hypochlorite**

By Volume: Dilute **250 mL sodium hypochlorite** (SS290-1 hypochlorite solution, 4- 6% NaOCl from Fisher) to **500 mL** with **DI water.** Prepare fresh daily.

By Weight: Dilute **250 g sodium hypochlorite** (SS290-1 hypochlorite solution, 4-6% NaOCl from Fisher) to **500 g** with **DI water.** Prepare fresh daily.

39 **Reagent 4. Sodium Nitroprusside**

By Volume: Dissolve **1.75 g sodium nitroprusside** in **500 mL DI water.** Prepare fresh every 1-2 weeks.

By Weight: Dissolve **1.75 g sodium nitroprusside** in **500 g DI water.** Prepare fresh every 1-2 weeks.

7.2. PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be required:

By Volume: One 1 L and four 200 mL volumetric flasks.

By Weight: One 1 L and four 200 mL containers.

40 **Standard 1. Stock Standard 50.0 mg N/L**

By Volume: In a **1 L** volumetric flask dissolve **0.1909 g ammonium chloride** (NH_4Cl) in about **800 mL DI water**. Dilute to the mark with **DI water** and invert to mix.

41 **Standard 2. Working Standard 1.0 mg N/L**

By Volume: In a **500 mL** volumetric flask add **10.0 mL of Stock Standard 1** (Standard 1). Dilute to the mark with **DI water** and invert to mix.

Working Standards (Prepare Daily)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Concentration µg N/L	600	300	100	50.0	10.0	5.0	0.0

By Volume

Volume (mL) of **standard 2** diluted to 200 mL with DI water	120	60	20	10	2	1	---

By Weight

Weight (g) of **standard 2** diluted to final weight (~200 g) divided by **factor** below with DI water	120	60	20	10	2	1	---
Division Factor Divide exact weight of the standard by this **factor** to give the final weight	0.6	0.3	0.1	0.05	0.01	0.005	---

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. There is no single preservation method that may be recommended for all types of samples. The analyst must examine each situation critically and treat this information as a guide.

8.2. If samples must be chemically preserved, samples should be cooled, and adjusted to pH < 2 with H_sSO_4 and store at 4°C in glass or polyethylene. Analyze within 28 days. Samples in polyethylene bottles may be stored frozen. If samples are not run within 24 hours a storage stability study should be done.

8.3. Researchers have found serious errors when investigating the effects of filtration. The analyst should examine sample preparation and preservation techniques before routine testing.

9. 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 periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance (determination of MDLs) prior to performing analyses by this method.

9.2.2. Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data exceeds the initial values by +/- 10%, linearity must be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3. Quality Control Sample (QCS) -- 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 preparation and analyses of a QCS. If the determined concentrations are not within +/-10% 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 on-going analyses.

9.2.4. Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times S$$

Where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates, t= 2.528 for twenty-one replicates]. S = standard deviation of the replicate analyses.

MDLs should be determined every six months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3. ASSESSING LABORATORY PERFORMANCE

9.3.1. Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2. Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, 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 used LFB analyses data to assess laboratory performance against the required control limits of 90-110%. 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 and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = $\bar{X} + 3S$

LOWER CONTROL LIMIT = $\bar{X} - 3S$

The optional control limits must be equal to or better than the required control limits of 90-110%. 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4. Instruments Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within +/-10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is still within +/-10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with sample analyses data.

9.4. ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1. Laboratory Spiked Sample Matrix (LSM) -- The laboratory must spike, in duplicate, minimum of 10% of routine samples. In each case the LSM aliquots must be a duplicate of the aliquot used for sample analysis. The spiking level shall be at 1 to 5 times higher than the background concentration of the sample.

Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range 90-110%. Percent recovery may be calculate using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

Where, R = percent recovery, C_S = fortified sample concentration, C = sample background concentration, s = concentration equivalent of analyte added to sample.

9.4.3. If the recovery of any analyte falls outside the designated LSM recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.4. Compute the relative percent difference (RPD) between the two LSM results and compare the value to the designated RPD recovery range of 10%. The RPD may be calculated using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \text{ x } 100$$

Where: D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

9.4.5. If the RPD falls outside the designated recovery range and the laboratory performance for that analyte is shown to be in control (sect. 9.3), the recovery problem encountered with the LSM is judged to be either matrix or solution related, not system related.

9.4.6. Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare a series of standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (suggested range in Section 7.2.).

10.2. Calibrate the instrument as described in Section 11.

10.3. Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solution concentration/response data using the computer. Acceptance or control limits should be established using the difference between the measured value of the calibration solution and the "true value" concentration.

10.4. After the calibration has established, it must be verified by the analysis of a suitable quality control sample (QCS). If measurements exceed ± 10% of the established QCS value, the analysis should be terminated and the instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11. PROCEDURE

11.1. CALIBRATION PROCEDURE

11.1.1. Prepare reagent and standards as described in section 5.

11.1.2. Set up manifold as shown in section 11.

11.1.3. Input data system parameters as shown in section 11.

11.1.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.1.5. Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See section 11).

11.1.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.2. **SYSTEM NOTES**

11.2.1. For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual. This guide is also available on request from Lachat.

11.2.2. Allow 15 minutes for heating module to warm up to 60°C.

11.2.3. Allow 15 minutes with reagents pumping for the system to equilibrate.

11.2.4. To check for gross carrier contamination:

A. Monitor the baseline on the system unit screen.

B. Equilibrate the pumping system with DI water in all reagent lines.

C. Equilibrate the pumping system with reagents in the appropriate lines.

D. The increase in absorbance should be 0.02 V or less.

11.2.5. A backpressure coil (100 cm x 0.5 mm (0.022") i.d. Teflon tubing) is used to prevent air bubble formation.

11.2.6. All reagent containers should be covered with Parafilm after insertion of the transmission lines to prevent contamination from airborne ammonia.

11.2.7. Reagent recipes from other automated wet chemistry analyzers should not be substituted.

11.2.8. If sample tube or standard container materials other than polystyrene are used, standards and samples in these containers should be analyzed to investigate absorption or contamination.

11.2.9. The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.2.10. If the detection limit is greater than that specified in the method the following outline should be followed.

A. Verify standards preparation procedures.

B. Verify that a 630 nm filter is being used.

C. Verify that the sample loop is completely filled by running dye.

D. Verify that the reagents are being added in the correct order.

E. Verify that the reagents are flowing smoothly. If not, check for obstructions in the manifold and replace worn tubing.

F. Prepare fresh reagents. Take care to be sure that the EDTA is completely dissolved.

11.2.11. If the samples are colored or are suspected to show a background absorbance, this interference should be subtracted. This can be done by using the following procedure:

A. Calibrate the system in the normal manner.

B. Disable the check standards or DQM features and analyze the samples.

C. Place reagent and carrier lines in DI water and allow the baseline to stabilize.

D. Inject samples again without recalibrating.

E. Subtract the "background" concentration from the original concentration to give the corrected concentration.

Corrected Concentration = Original Concentration – Background Concentration.

11.2.12. It is critical that the peak be detected on the "flat top" of the standard peaks. If the window is not on the "flat top", the peak start time should be adjusted.

11.2.13. To ascertain that the standards peak is properly positioned in the window, observe the peaks on the system unit screen. The baseline trace should be flat for the duration of the baseline window. If the peak is not properly positioned, change the peak detection parameters. Low nutrient or artificial seawater, as is and spiked at 1 or 2 low levels, can be used to set timing parameters.

11.2.14. For low level analysis it is recommended that samples be analyzed in replicate from each sample cup. This is done by entering Replicates = 2 when entering sample information.

11.2.15. Use consumer bleaches with caution. Proprietary additives may contribute to staining of tubing and data quality.

11.2.16. Add reagents in the order that they appear on the manifold to reduce staining.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Calibration is done by injecting standards. The data system will then prepare a calibration curve by plotting response versus standard concentration. Sample concentration is calculated from the regression equation.

12.2. Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.

12.3. Report results in μg N/L or μM N.

13. METHOD PERFORMANCE

13.1. The method support data is presented in section 11. This data was generated according to a Lachat Work Instruction during development of the method.

13.2. Although Lachat Instruments publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance.

Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it.

14. 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 USEPA 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 United States Environmental Agency (USEPA) recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. 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 Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.

15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

16. REFERENCES

16.1. Grasshoff, K. **Methods of Seawater Analysis**, Verlag Chemie, Second Edition, 1976.

16.2. U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastewater**, EPA-600/4-79-020, Rev 2; August 1993, Method 350.1.

16.3. Spectrophotometric and Kinetics Investigation of the Berthelot Reaction for the Determination of Ammonia, **Analytical Chemistry**, 1977, Vol. 49, No.3, p. 464-469.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput: 45 samples/h, 80 s/sample
Pump Speed: 35
Cycle Period: 80

42 Analyte Data:

Concentration Units: µg N/L
Chemistry: Brackish
Inject to BW Baseline Start 2.1 s
Inject to BW Baseline End 64.7 s
Inject to BW Integ Start 25.6 s
Inject to BW Integ End 28.6

43 Calibration Data:

Level	1	2	3	4	5	6	7
Concentration µg N/L	600	300	100	50.0	10.0	5.0	0

Calibration Rep Handling: Average
Calibration Fit Type: 2nd Order Polynomial
Weighting Method: 1/X
Force through zero: no

44 Sampler Timing:

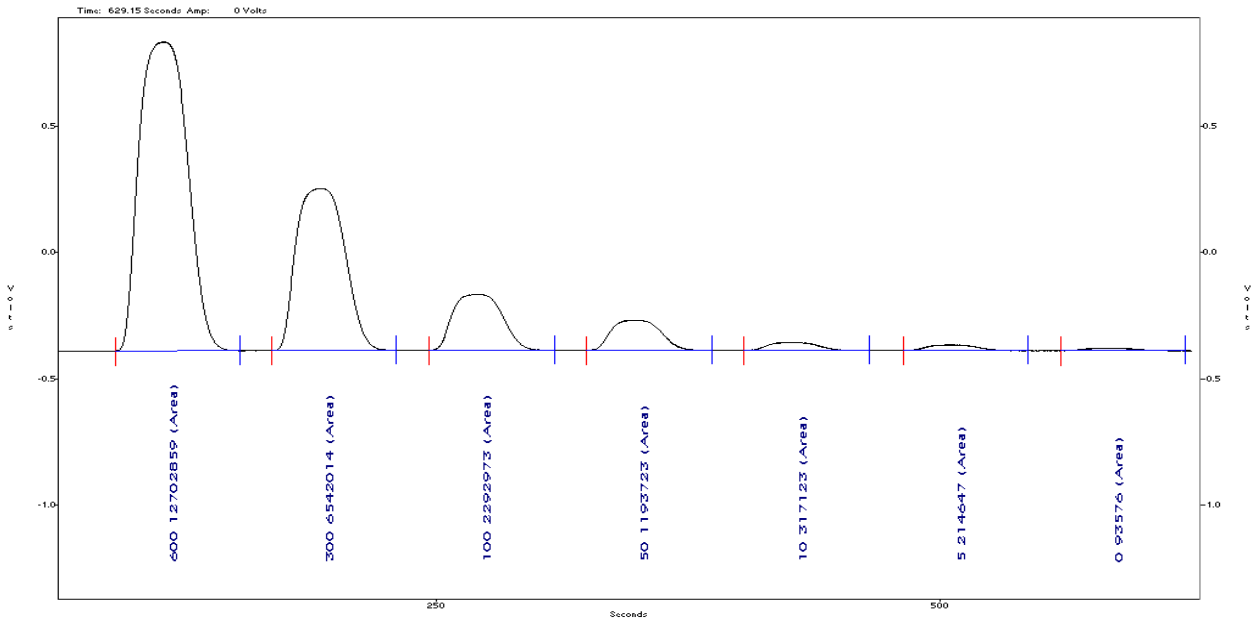
Min. Probe in Wash Period: 19 s
Probe in Sample Period: 50 s

45 Valve Timing:

Load Time: 0 s
Load Period: 30 s
Inject Period: 50 s

17.2. SUPPORT DATA FOR QUIKCHEM 8000

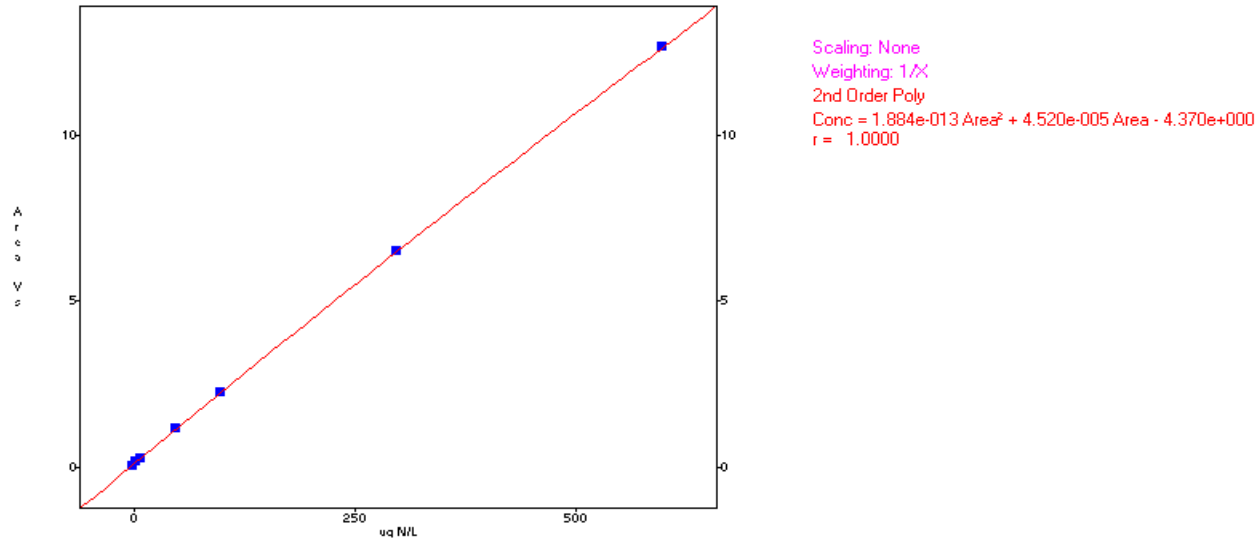
Calibration Data for Ammonia

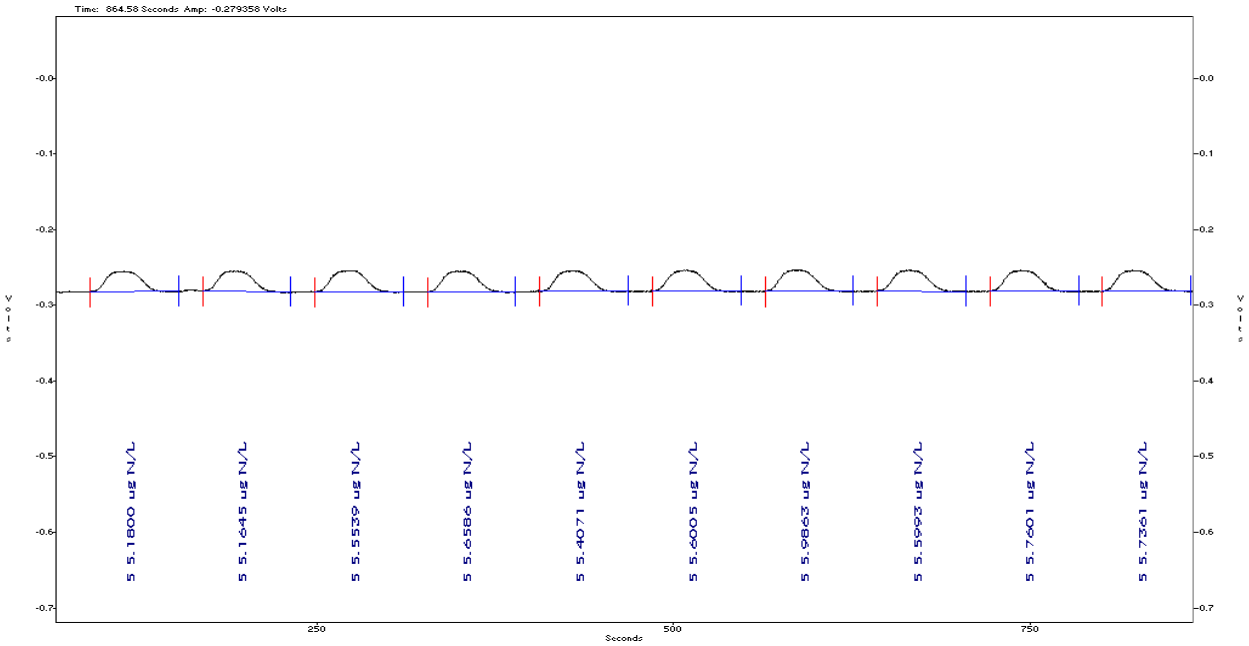


File Name: 001207c.fdt
Acq. Date: 7 December 2000

46 Calibration Graph and Statistics

Level	Area	µg N/L	Determined	% residual
1	12702859	600	600	-0.0
2	6542014	300	299.94	0.2
3	2292973	100	100.03	-0.3
4	1193723	50	49.99	0.3
5	317123	10	10.02	-0.2
6	214647	5	5.34	-6.8
7	93576	0	0	---

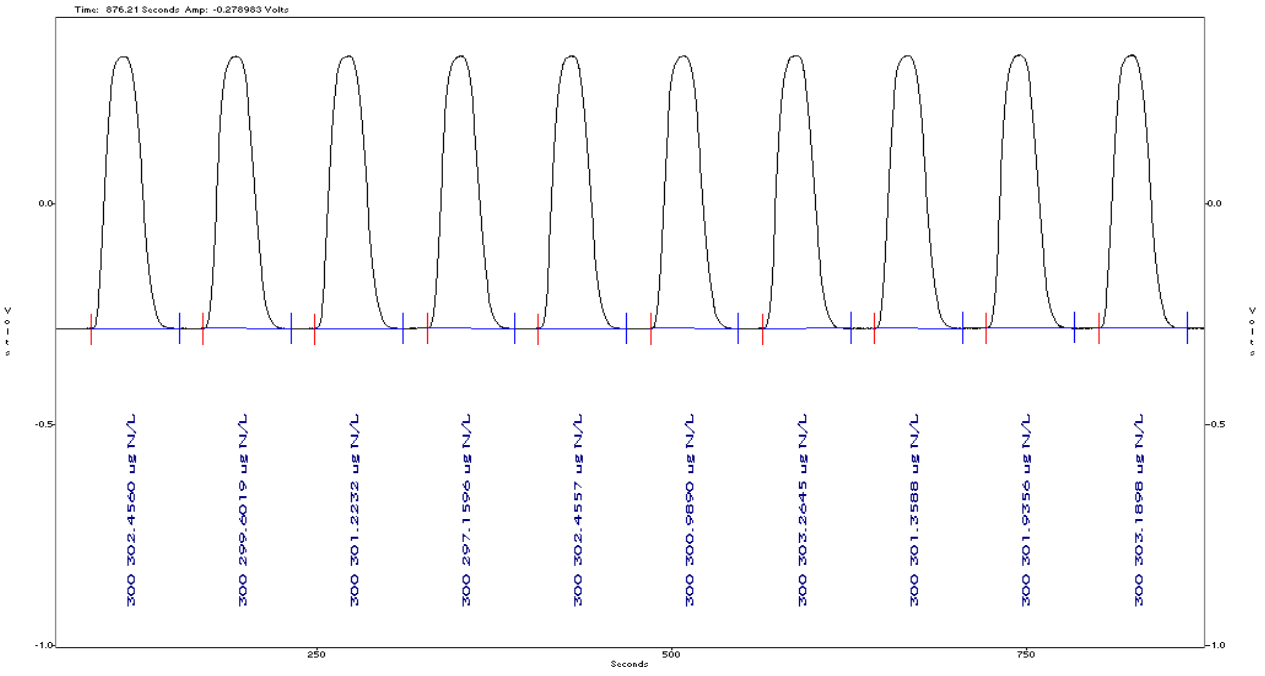




Method Detection Limit for ammonia using 5 µg N/L standard

MDL= 0.7 µg N/L

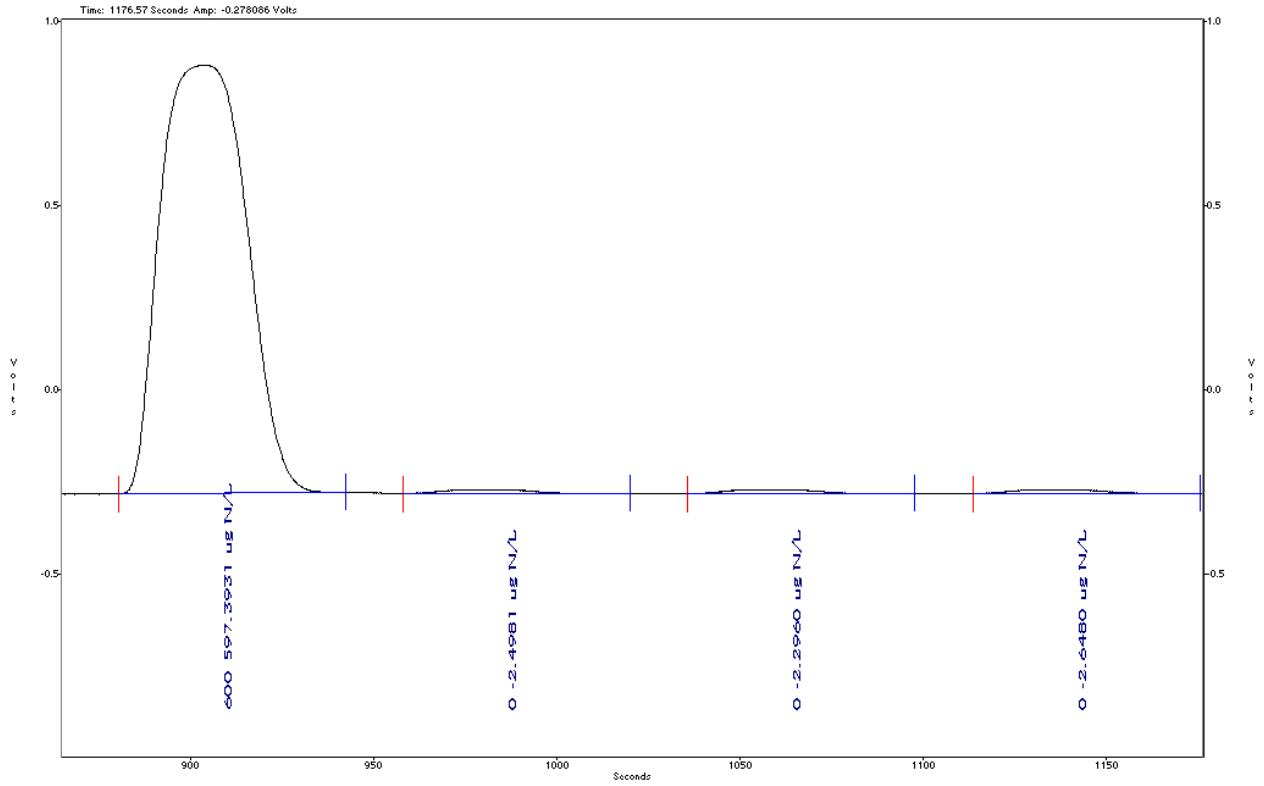
Standard Deviation (s) = 0.256 µg N/L, Mean (x) = 5.56 µg N/L, Known value = 5 µg N/L Acq. Date: 5 December 2000
File Name: 001205c1.fdt



Precision data for ammonia using 300 µg N/L standard

% RSD = 0.6

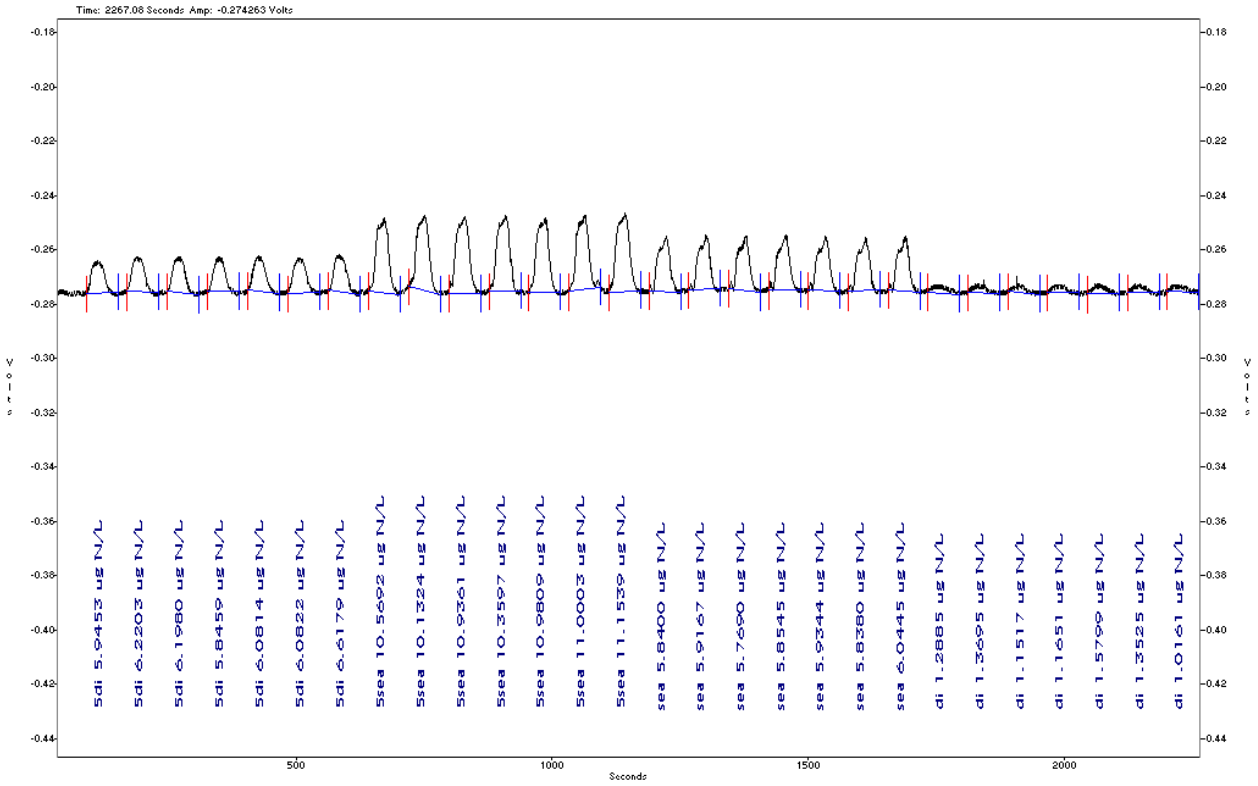
Standard Deviation (s) = 1.84 µg N/L, Mean (x) = 301.4 µg N/L, Known value = 300 µg N/L
Acq. Date: 5 December 2000
File Name: 001205p.fdt



Carryover Study: 600 µg N/L standard followed by 3 blanks

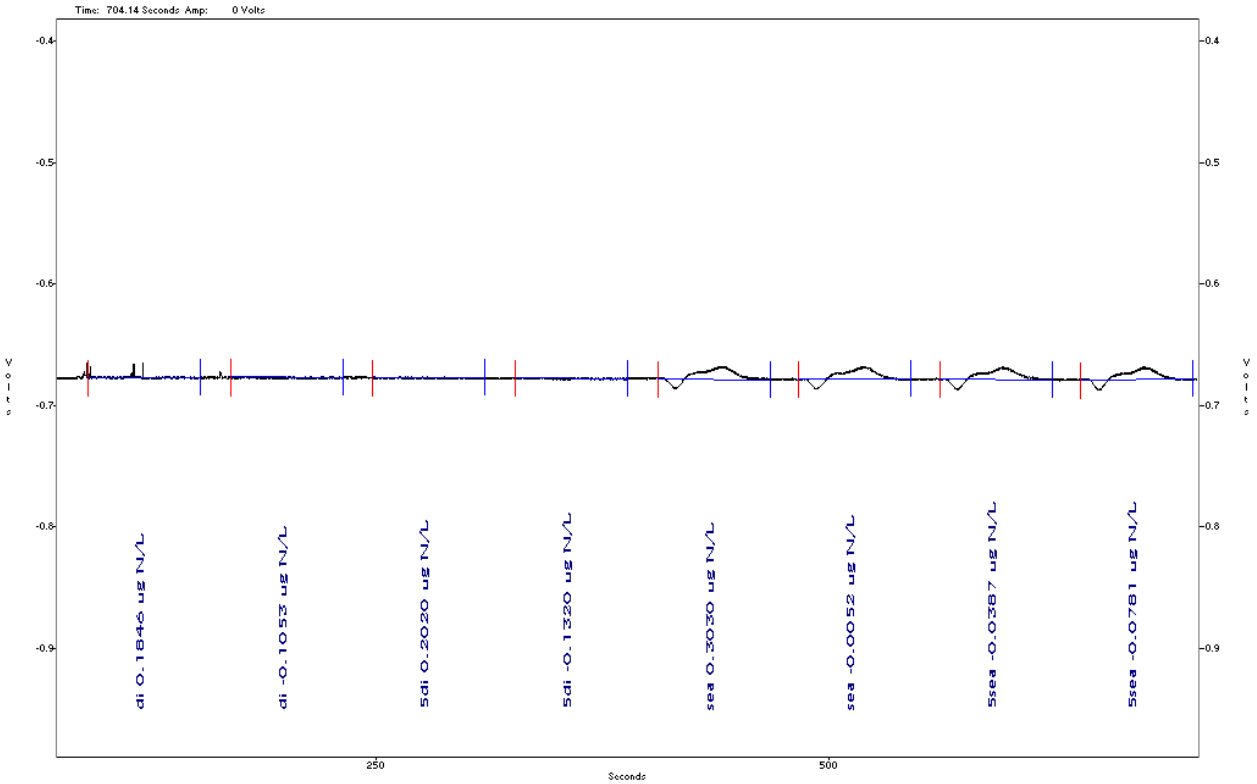
Carryover Passed

Acq. Date: 5 December 2000 File Name: 001205p.fdt



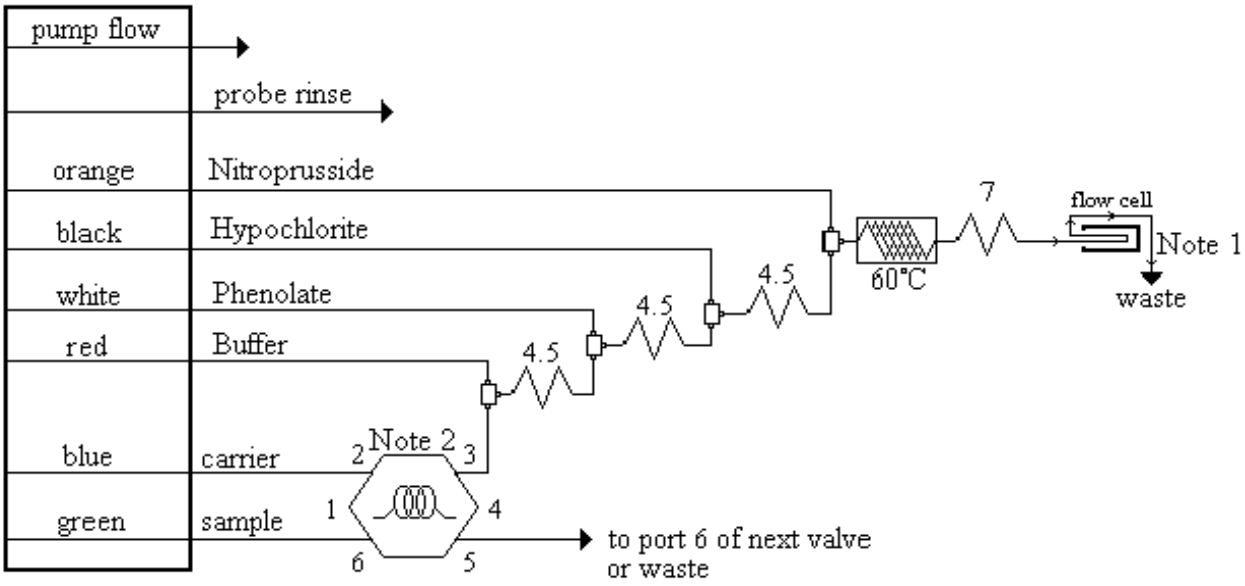
Sample ID	Sample	Average results (µg N/L)	Recovery (%)	MDL (µg N/L)
5di	Spike 5ppb in DI water	6.14	97.4	0.78
5sea	Spike 5ppb in sea water	10.73	96.8	1.20
sea	Sea water	5.89		0.28
di	DI water	1.27		0.58

Note: Low level spike recovery and data performance. The seawater was received from The Center for Great Lakes Research.

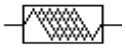


Test for RI index effect (Results were obtained by placing hypochlorite into DI water) Conclusion: the RI effect is smaller than the determined method detection limit.

17.3. AMMONIA MANIFOLD DIAGRAM



Carrier: DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
QC8000 Sample Loop: 150 cm x 0.040" i.d.
Interference Filter: 630 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 650 cm of tubing wrapped around the heater block at the specified temperature.

4.5: 70 cm of tubing on a 4.5 cm coil support
7: 135 cm of tubing on a 7 cm coil support

Note 1: 100 cm back pressure coil of 0.5 mm (0.022" i.d.)
Note 2: The sample loop should be cut at a 30-45° angle for the best fit.
47 **Note 3: PVC PUMP TUBES MUST BE USED FOR THIS METHOD.**

QuikChem® Method 31-115-01-1-H

DETERMINATION OF ORTHOPHOSPHATE BY FLOW INJECTION ANALYSIS

48 **Written by Krista Knepel and Karin Bogren**

Applications Group

Revision Date: 13 May 2008

LACHAT INSTRUMENTS 5600 LINDBURGH DRIVE LOVELAND, CO 80539 USA

LACHAT INSTRUMENTS
A Hach Company Brand

QuikChem® Method 31-115-01-1-H

Orthophosphorous in Seawaters

5 to 400 µg P/L

0.16 to 12.91 µM P/L

– Principle –

Ammonium molybdate and antimony potassium tartrate react in an acid medium with phosphate to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color produced is proportional to the phosphate concentration in the sample. Though there is a density difference between seawater and reagent water, the bias is less than 2%.

Though the method is designed for seawater and brackish water it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background absorbance is necessary only for samples which have color absorbing at 880 nm.

– Interferences –

1. Interferences caused by copper, arsenate and silicate are minimal relative to the orthophosphate determination because of the extremely low concentrations normally found in estuarine and coastal waters.
2. High iron can cause precipitation of and subsequent loss of phosphate from the dissolved phase.
3. By using ascorbic acid as the reductant, the color intensity is not influenced by variations in salinity. Stannous chloride reductant **does** show a significant salt effect.
4. Turbidity is removed by filtration.
5. Hydrogen sulfide effects, which may occur in samples from deep anoxic basins, can be treated by simple dilution since high sulfide concentrations are most often associated with high phosphate values.

– Special Apparatus –

Please see Parts and Price list for Ordering Information

1. Heating Unit, Lachat Part No. A85100 (A85X00, X=1 for 110V, X=2 for 220V)
2. Glass calibration vials must be used for this method, Lachat Part No. 21304

Revised by L. Egan on May 13 2008. Written and copyrighted ❤ by K. Knepel and K. Bogren on 4 January 2001 by **Lachat Instruments, 5600 Lindburgh Drive, Loveland, CO 80539 USA**. Phone: 970-663-1377 FAX: 970-962- 6710. This document is the property of Lachat Instruments. Unauthorized copying of this document is prohibited.

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QuikChem® Method 31-115-01-1-H

DETERMINATION OF ORTHOPHOSPHORUS BY FLOW INJECTION ANALYSIS

1. SCOPE AND APPLICATION

1.1. Though the method is written for seawater and brackish water, it is also applicable to non-saline sample matrixes. The method is calibrated using standards prepared in deionized water. Once calibrated, samples of varying salinities (0 to 35 ppt) may be analyzed. The determination of background is necessary only for samples, which have color absorbing at 880 nm.

1.2. The method is based on reactions that are specific for the orthophosphate ion.

1.3. The applicable range is 5 to 400 μg/L. The claimed method detection limit is 1.0 μg P/L. The method throughput is 48 injections per hour.

2. SUMMARY OF METHOD

2.1. The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex, which absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3. DEFINITIONS

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

3.1. ANALYTICAL BATCH: The set of samples extracted/distilled/or digested at the same time to a maximum of 10 samples.

3.2. CALIBRATION BLANK (CB) -- A volume of reagent water in the same matrix as the calibration standards, but without the analyte.

3.3. CALIBRATION STANDARD (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.4. FIELD BLANK (FMB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, including exposure to a sample bottle holding time, preservatives, and all preanalysis treatments. The purpose is to determine if the field or sample transporting procedures and environments have contaminated the sample.

3.5. FIELD DUPLICATE (FD) -- Two samples taken at the same time and place under identical circumstances which are treated identically throughout field and laboratory procedures. Analysis of field duplicates indicates the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

3.6. LABORATORY BLANK (LRB) -- An aliquot of reagent water or equivalent neutral reference material treated as a sample in all aspects, except that it is not taken to the

sampling site. The purpose is to determine if the if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

3.7. LABORATORY CONTROL STANDARD (LCS) -- A solution prepared in the laboratory by dissolving a known amount of one or more pure compounds in a known amount of reagent water. It's purpose is to assure that the results produced by the laboratory remain within the acceptable limits for precision and accuracy. (This should not be confused with a calibrating standard).

3.8. LABORATORY DUPLICATE (LD) -- Two aliquots of the same environmental sample treated identically throughout a laboratory analytical procedure. Analysis of laboratory duplicates indicates precision associated with laboratory procedures but not with sample collection, preservation, or storage procedures.

3.9. QUALITY CONTROL CHECK SAMPLE (QCS) -- A sample containing analytes of interest at known concentrations (true values) of analytes. The QCS is obtained for a source external to the laboratory or is prepared from standards obtained from a different source than the calibration standards. The purpose is to check laboratory performance using test materials that have been prepared independently from the normal preparation process.

3.10. METHOD DETECTION LIMIT (MDL) -- The lowest level at which an analyte can be detected with 99 percent confidence that the analyte concentration is greater than zero.

4.**INTERFERENCES**

4.1. Interferences caused by copper, arsenate and silicate are minimal relative to the orthophosphate determination because of the extremely low concentrations normally found in estuarine and coastal waters.

4.2. High iron can cause precipitation of and subsequent loss of phosphate from the dissolved phase.

4.3. Using ascorbic acid as the reductant, the color intensity is not influenced by variations in salinity. Stannous chloride reductant does show a significant salt effect.

4.4. Turbidity is removed by filtration.

4.5. Hydrogen sulfide effects, such as occur in samples from deep anoxic basins, can be treated by simple dilution since high sulfide concentrations are most often associated with high phosphate values.

5.**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 should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials.

5.2. Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

5.3. The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

5.3.1. Sulfuric acid

6. EQUIPMENT AND SUPPLIES

6.1. Balance -- analytical, capable of accurately weighing to the nearest 0.0001 g.

6.2. Glassware -- Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass.

6.3. Flow injection analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1. Sampler

6.3.2. Multichannel proportioning pump

6.3.3. Reaction unit or manifold

6.3.4. Colorimetric detector

6.3.5. Data system

6.4. Special Apparatus

6.4.1. Heating unit Lachat Part No. A85X05 (X=1 for 110V, X=2 for 220V)

6.4.2. Glass calibration vials must be used in this method, Lachat Part No. 21304

6.5. Phosphate-Free Glassware and Polyethylene Bottles

6.5.1. All labware used in the determination must be low in residual phosphate to avoid sample or reagent contamination. Washing with 10% (v/v) HCl and thoroughly rinsing with distilled, deionized water was found to be effective.

6.5.2. Use membrane or glass fiber filters, 0.45 μM nominal pore size and check for contamination by analyzing blanks.

6.5.3. When degassing reagents, DO NOT DEGAS USING AN INVASIVE PROCEDURE SUCH AS A WAND TO AVOID CONTAMINATION. Degas by vacuum or sonication.

7. REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

49 **Degassing:**

To prevent bubble formation, DO NOT DEGAS USING AN INVASIVE PROCEDURE SUCH AS A WAND TO AVOID CONTAMINATION. Degas by vacuum or sonication. **All reagents need to be thoroughly degassed.**

50 **Reagent 1. Stock Ammonium Molybdate Solution**

The molybdate solid may take up to four hours on a stir plate to dissolve. Store in plastic and refrigerate. Discard after six months.

By Volume: In a **1 L** volumetric flask, dissolve **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O)]$ in approximately **800 mL water**. Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

By Weight: To a tared **1 L** container add **40.0 g ammonium molybdate tetrahydrate** $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O)]$ and **983 g water**. Mix with a magnetic stirrer for a least four hours. Store in plastic and refrigerate.

51 **Reagent 2. Stock Antimony Potassium Tartrate Solution**

By Volume: In a **1 L** volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6\cdot 1/2\ H_2O$) or dissolve **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) in approximately **800 mL water**. Dilute to the mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

By Weight: To a **1 L** dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6\cdot 1/2\ H_2O$) or **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) and
995 g water. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

52 **Reagent 3. Molybdate Color Reagent**

This reagent may be stored at room temperature. Discard when the solution turns blue.

By Volume: To a **1 L** volumetric flask add **35.0 mL concentrated sulfuric acid** to about **500 mL water**, (CAUTION: The solution will get hot!) Swirl to mix. Add **213 mL Ammonium Molybdate Solution** (Reagent 1) and **72 mL Antimony Potassium Tartrate Solution** (Reagent 2). Dilute to the mark and invert to mix. Degas by vacuum or sonication.

By Weight: To a tared **1 L** container add **680 g DI water** and **64.4 g concentrated sulfuric acid** (CAUTION: The solution will get hot!) Swirl to mix. Add **213 mL Ammonium Molybdate Solution** (Reagent 1) and **72 mL Antimony Potassium Tartrate Solution** (Reagent 2). Dilute to the mark and invert to mix. Degas by vacuum or sonication.

53 **Reagent 4. Ascorbic Acid Reducing Solution**

By Volume: To a **1 L** volumetric flask, dissolve **60.0 g granular ascorbic acid** (Spectrum Chemicals, Catalogue # AS-102) in about **700 mL DI water**. Dilute to the mark and invert to mix. Degas by vacuum or sonication for at least 5 minutes. Add **1.0 g sodium dodecyl sulfate** (SDS $CH_3(CH_2)_{11}OSO_3Na$). Degas prior to the addition of SDS. Prepare fresh weekly.

By Weight: To a tared **1 L** container, add **60.0 g granular ascorbic acid** (Spectrum Chemicals, Catalogue # AS-102) and **975 g DI water**. Invert to mix. Degas by vacuum or sonication for at least 5 minutes. Add **1.0 g sodium dodecyl sulfate** (SDS $CH_3(CH_2)_{11}OSO_3Na$). Degas prior to the addition of SDS. Prepare fresh weekly.

54 **Reagent 5. Sodium Hydroxide – EDTA Rinse**

Dissolve **65 g sodium hydroxide** (NaOH) and **6 g tetrasodium ethylenediamine tetraacetic acid** (Na_4EDTA) in **1.0 L or l.0 kg DI water**. Use daily at the end of the day (~10 minutes, followed by a DI rinse) or if the baseline begins to drift upwards.

7.2. PREPARATION OF STANDARDS

To prepare the stock and working standards, the following containers will be requires:

By Volume: Three 1 L and five 250 mL volumetric flasks.

By Weight: Three 1 L and five 250 mL containers.

55 **Standard 1. Stock Standard 1000 mg P/L**

By Volume: In a **1 L** volumetric flask dissolve **4.39 g potassium dihydrogenphosphate** (KH_2PO_4) in approximately **500 mL DI water**. Dilute to the mark with DI water and invert to mix. This solution may be refrigerated and stored in glass for up to one month.

56 **Standard 2. Working Standard 1 - 1000 μg P/L**

By Volume: In a **1 L** volumetric flask add **1 mL of Standard 1** (1000 mg P/L). Dilute to the mark with **DI water** and invert to mix. Prepare fresh daily.

57 **Standard 3. Working Standard 2 – 100 μg P/L**

By Volume: In a **1 L** volumetric flask, add **100 mL** of **Working Standard 1** (1000 μg P/L). Dilute to the mark with **DI water** and invert to mix. Prepare fresh daily.

Working Standards (Prepare Daily)	**A**	**B**	**C**	**D**	**E**	**F**
Concentration μg P/L	400	200	100	25	5	0.0

By Volume

Volume (mL) of **Working standard 1** diluted to 250 mL with DI water	100	50	---	---	---	---
Volume (mL) of **Working standard 2** diluted to 250 mL with DI water	---	---	250	62.5	12.5	---

By Weight

Weight (g) of **Working standard 1** diluted to final weight (~250 g) divided by **factor** below with DI water	100	50	---	---	---	---
Weight (g) **Working standard 2** diluted to final weight (~250 g) divided by **factor** below with DI water	---	---	250	62.5	12.5	---
Division Factor Divide exact weight of the standard by this **factor** to give the final weight	0.4	0.2	---	0.25	0.05	---

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. The volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2. Samples should be stored at 4°C and determined as soon as possible after collection. If sulfuric acid preservation is used, hydrolysis of any polyphosphate species in the sample will occur.

8.3. For NPDES monitoring, The USEPA requires that samples be filtered immediately upon collection, with a maximum holding time of 48 hours.

8.4. Samples collected for nutrient analysis from estuarine and coastal waters are normally collected using one of two methods: hydrocast or submersible pump systems. Filtration of the sample through a 0.45 μM membrane or glass fiber filter immediately after collection is required.

8.4..1. A hydrocast uses a series of sampling bottles (Nisken, Nansen, Go-Flo or equivalent) that are attached at fixed intervals to a hydra wire. These bottles are sent through the water column open and are closed either electronically or via a mechanical "messenger" when the bottles have reached the desired depth.

8.4.2. When a submersible pump is used, a weighted hose is sent to the desired depth in the water column and water is pumped from that depth to the deck of the ship for processing.

8.4.3. Another method used to collect surface samples involves the use of a plastic bucket or large plastic bottle.

9. 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 periodic analysis of laboratory reagent blanks, fortified blanks and other laboratory solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data that are generated. An analytical batch shall be defined as environmental samples that are analyzed together with the same method and personnel, using the same lots of reagents, not to exceed the analysis of 20 environmental samples.

9.1.1. Analyses of matrix spike and matrix spike duplicate samples are required to demonstrate method accuracy and precision and to monitor matrix interferences (interferences caused by the sample matrix). The procedure and QC criteria for spiking are described in section 9.3.

9.1.2. Analyses of laboratory blanks are required to demonstrate freedom from contamination.

9.1.3. The laboratory shall, on an ongoing basis, demonstrate through calibration verification and analysis of the ongoing precision and recovery sample that the analysis system is in control.

9.1.4. The laboratory should maintain records to define the quality of data that is generated.

9.2. INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1. Method Detection Limit (MDL) –To establish the ability to detect the analyte, the analyst shall determine the MDL per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards, that will be used in the practice of this method. An MDL less than or equal to the MDL in section 1.2 must be achieved prior to the practice of this method.

9.2.2. Initial Precision and Recovery – To establish the ability to generate acceptable precision results, the operator shall perform 10 replicates of a mid-range standard, according to the procedure beginning in Section 11.

9.2.3. Using the results of the replicates compute the average percent recovery (X) and the standard deviation (s) for the analyte. Use the following equation for the calculation of the standard deviation.

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x\right)^2}{n}}{n-1}}$$

Where, n = Number of samples, x = concentration in each sample

9.2.4. Compare s and x results with the corresponding data in Section 17. If the results meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If however, s and x do not match the data in Section 17, system performance is unacceptable. In this event correct the problem, and repeat the test.

9.3. Matrix spikes- The laboratory must spike, in duplicate, a minimum of 5 percent of all samples (one sample in each batch of no more than twenty samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

9.3.1.1. If, as in compliance monitoring, the concentration of the analyte in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1 to 5 times higher than the background concentration of the sample (determined in Section 9.3.2), which ever is higher.

9.3.1.2. If the concentration of the analyte in a sample is not being checked against a limit, the spike shall be at the concentration of the precision and recovery standard used in Section 9.2.5 or at 1 to 5 times higher than the background concentration, whichever concentration is higher.

9.3.2. Analyze one sample aliquot out of each set of no more than twenty samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

9.3.2.1. If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1 to 5 times the background concentration (per Section 9.3.1).

9.3.2.2. Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A)

9.3.3. Calculate the percent recovery (P) of the analyte in each aliquot using the following equation.

$$P = \frac{(A - B)100}{T}$$

Where, A = Measured concentration of analyte after spiking, B = measured background concentration of analyte, T = True concentration of the spike

9.3.4. The percent recovery of the analyte should meet current laboratory acceptance criteria.

9.3.4.1. If the results of the spike fail the acceptance criteria, and the recovery of the QC standard in the ongoing precision and recovery test of the analytical batch is within the current laboratory acceptance criteria, an interference is present. In this case, the results may not be reported for regulatory compliance purposes and the analyst must assess the potential cause for the interference. If the interference is attributable to sampling, the site or discharge should be resampled. If the interference is attributable to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2 and repeat the analysis of the sample and the matrix spike.

9.3.4.2. If the results of both the spike and ongoing precision and recovery test fail the acceptance criteria, the analytical system is judged to be out of control, and the problem shall be identified and corrected, and the sample reanalyzed.

57.3.5. Compute the relative percent difference (RPD) between two sample results using the following equation:

$$RPD = \frac{(D_1 - D_2)}{(D_1 + D_2)/2} \text{ x } 100$$

Where, D1 = Concentration of analyte in the sample, D2 = Concentration of analyte in the second (duplicate) sample.

57.3.6. The RPD for duplicates shall meet the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.4 Laboratory blanks – Laboratory reagent water blanks are analyzed to demonstrate freedom from contamination.

9.4.1. Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch of no more than twenty samples. The blank must be subjected to the same procedural steps as a sample.

9.4.2. If analyte is detected in the blank at a concentration greater than the Minimum Level (Section 1.6), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

9.5. Calibration Verification – Verify calibration using the procedure described in Section 10

9.6. On-going Precision and Recovery (OPR) – With every analytical batch of no more than twenty samples, a midrange standard must be prepared using the procedure described in Section 11.

9.6.1. Compare the results with the current laboratory acceptance criteria. If the criteria are not met, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed.

9.7. Quality Control Samples (QCS) – It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 9.7.1. The QCS is used to verify the concentrations of the calibration standards.

9.8. Depending on the specific program requirements, field replicates and field spikes of the analytes of interest into samples may be required to assess the precision and accuracy of the sampling and sample transporting techniques.

10. CALIBRATION AND STANDARDIZATION

10.1. Prepare reagents and standards as described in Section 7.

10.2. Set up manifold as shown in Section 17.

10.3. Input data system parameters as shown in Section 17.

10.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

10.5. Place standards in the sampler. Input the information required by the data system.

10.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the peak area for each standard to determine the calibration curve.

10.7. Verify calibration using a midrange calibration standard every ten samples or every analytical batch. Compute the percent recovery using the following equation:

$$\%recovery = \frac{D}{K} \text{x } 100$$

Where, D = Determined concentration of analyte in the calibration standard, K = Actual concentration of the analyte in the calibration standard

10.8. If % recovery exceeds +/-10%, the analytical system is judged to be out of control, and the problem must be immediately identified and corrected and the analytical batch reanalyzed

11. PROCEDURE

11.1. CALIBRATION PROCEDURE

11.2.1. Prepare reagent and standards as described in section 7.

11.2.2. Set up manifold as shown in section 17.

11.2.3. Input data system parameters as shown in section 17.

11.2.4. Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate until a stable baseline is achieved.

11.2.5. Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See section 11).

11.2.6. Calibrate the instrument by injecting the standards. The data system will then associate the concentrations with the instrument responses for each standard.

11.2. SYSTEM NOTES

11.2.1. For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual. This guide is also available on request from Lachat.

11.2.2. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl solution and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

11.2.3. Allow 15 minutes for the heating unit to warm up to 45°C.

11.2.4. Reagent recipes from other automated wet chemistry analyzers should not be substituted.

11.2.5. **It is very important to use granular ascorbic acid as opposed to powder. If the ascorbic acid solution turns yellow upon preparation or storage it should be discarded.**

11.2.6. Over time a blue film may accumulate on the walls of the flowcell and in the manifold tubing. This may be removed by pumping the manifold rinse solution (reagent 5).

11.2.7. Care should be taken to keep the wash bottles used in standards preparation clean. Bottles should be autoclaved or rinsed with bleach monthly.

11.2.8. The blank in this method should not give a peak. If the blank peak is negative, the carrier is contaminated. If the blank peak is positive, the blank is contaminated.

11.2.9. Take extra care to verify the ammonium molybdate is completely dissolved when preparing **Reagent 1, Stock Molybdate Solution**. Stirring for four hours is recommended.

11.2.10. If samples are colored, this background can be subtracted. First calibrate in the normal manner. Next, replace the molybdate reagent with a solution containing 35 mL H_2SO_4/L. Finally, reanalyze the samples. The color interference concentration is then subtracted from the original determined concentration.

12. DATA ANALYSIS AND CALCULATIONS

12.1. Calibration is done by injecting standards. Prepare a calibration curve by plotting response versus standard concentration. Compute sample concentration by comparing sample response with the standard curve.

12.2. For NPDES testing report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Report three decimal places.

12.3. Report results in mg P/L.

13. METHOD PERFORMANCE

13.1. The method support data are presented in section 16. This data was generated according to a Lachat Work Instruction during development of the method.

13.2. Although Lachat Instrument publishes method performance data, including MDL, precision, accuracy and carryover studies, we cannot guarantee that each laboratory will be capable of meeting such performance. Individual laboratory and instrument conditions, as well as laboratory technique, play a major role in determining method performance. The support data serves as a guide of the potential method performance. Some labs may not be able to reach this level of performance for various reasons, while other labs may exceed it.

14. 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 USEPA 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 United States Environmental Agency (USEPA) recommends recycling as the next best option.

14.2. The quantity of chemicals purchased should be based on expected usage during their shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3. 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 Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

15. WASTE MANAGEMENT

15.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water and land by minimizing and controlling all releases from fume hoods and bench operation. Compliance with all sewage discharge permits and regulations is also required.

15.2. For further information on waste management consult the "Waste Management Manual for Laboratory Personnel", available from the American Chemical Society's Department of Government Regulations and Science Policy, 1155 16th Street N. W., Washington D. C. 20036, (202) 872-4477.

16. REFERENCES

16.1. U.S. Environmental Protection Agency, Methods for the Determination of Inorganic Substances in Environmental Samples, EPA-600/R-93/100, August 1993, Method 365.1

16.2. Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-85.

16.3. Standard Methods for the Examination of Water and Wastewater, 18th Edition, p. 4 - 116, Method 4500-P F (1992)

16.4. Guideline and Format for EMSL-Cincinnati Methods. EPA-600/8-83-020, August 1983.

16.5. 40 CFR, 136 Appendix B. Definition and Procedure for Determination of the Method Detection Limit. revision 1.1.

16.6. Grasshoff, K., Methods of Seawater Analysis, Verlag Chemie, Federal Republic of Germany, Second Edition, 1976, 419 pages.

16.7. Murphy J. and Riley, J.P., A Modified Single Solution Method for the Determination of Phosphate in Natural Waters, Anal. Chim. Acta, Vol 27, 1962, p. 31-36.

16.8. Murphy J. and Riley, J.P., The Storage of Seawater Samples for the Determination of Dissolved Inorganic Phosphate, Anal. Chim. Acta, Vol 14, 1956, p. 318-319.

16.9. MacDonald, R.W. and F.A. McLaughlin. The Effect of Storage by Freezing on Dissolved Inorganic Phosphate, Nitrate and Reactive Silicate for Samples from Coastal and Estuarine Waters, Water Research, Vol. 16, 1982 p. 95-104.

16.10. Johnson, K., and Petty, R., Determination of Phosphate in Seawater by Flow Injection Analysis with Injection of Reagent, Analytical Chemistry, Vol 54, 1982, p. 1185-1187.

16.11. Lachat Instruments Inc., QuikChem Method 31-115-01-3-C written by David Diamond on 06 July 1994.

17. TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1. DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

The timing values listed below are approximate and will need to be optimized using graphical events programming.

Sample throughput: 48 samples/h, 75 s/sample
Pump Speed: 35
Cycle Period: 75

58 Analyte Data:

Concentration Units: µg P/L
Chemistry: Brackish
Inject to BW Baseline Start 3.7 s
Inject to BW Baseline End 62.0 s
Inject to BW Integ Start 20.9 s
Inject to BW Integ End 27.3

59 Calibration Data:

Level	1	2	3	4	5	6
Concentration µg P/L	400	200	100	25	5	0

Calibration Rep Handling: Average
Calibration Fit Type: 2nd Order Polynomial
Weighting Method: None
Force through zero: No

60 Sampler Timing:

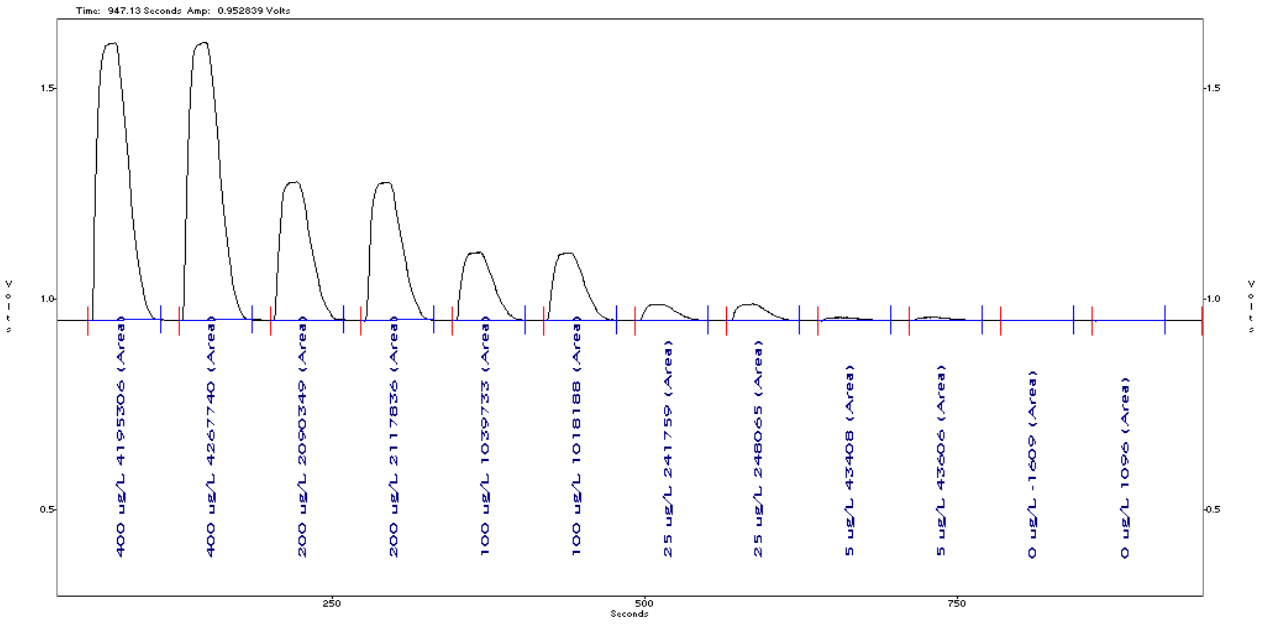
Min. Probe in Wash Period: 19 s
Probe in Sample Period: 45 s

61 Valve Timing:

Load Time: 0 s
Load Period: 30 s
Inject Period: 45 s

17.2. SUPPORT DATA FOR QUIKCHEM 8000

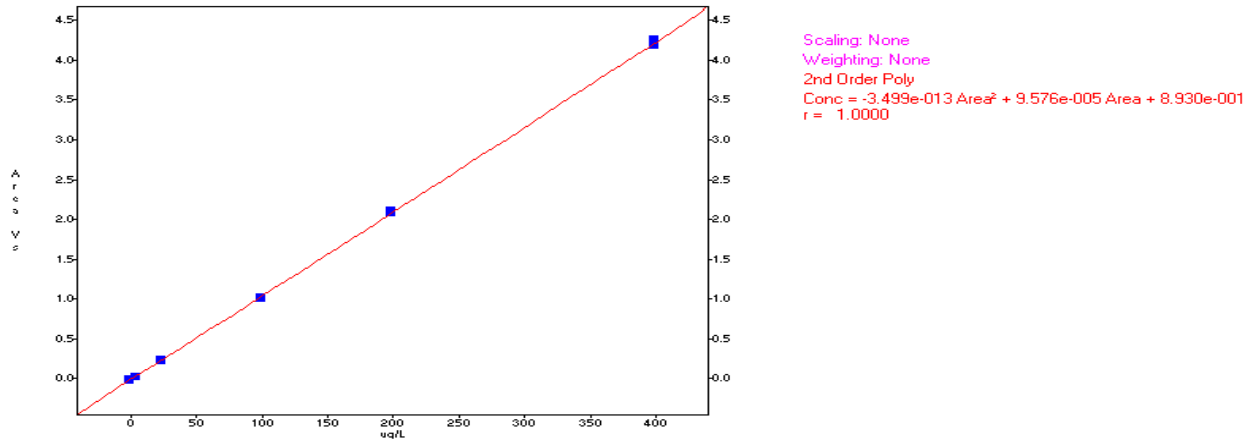
Calibration Data for Orthophosphorus

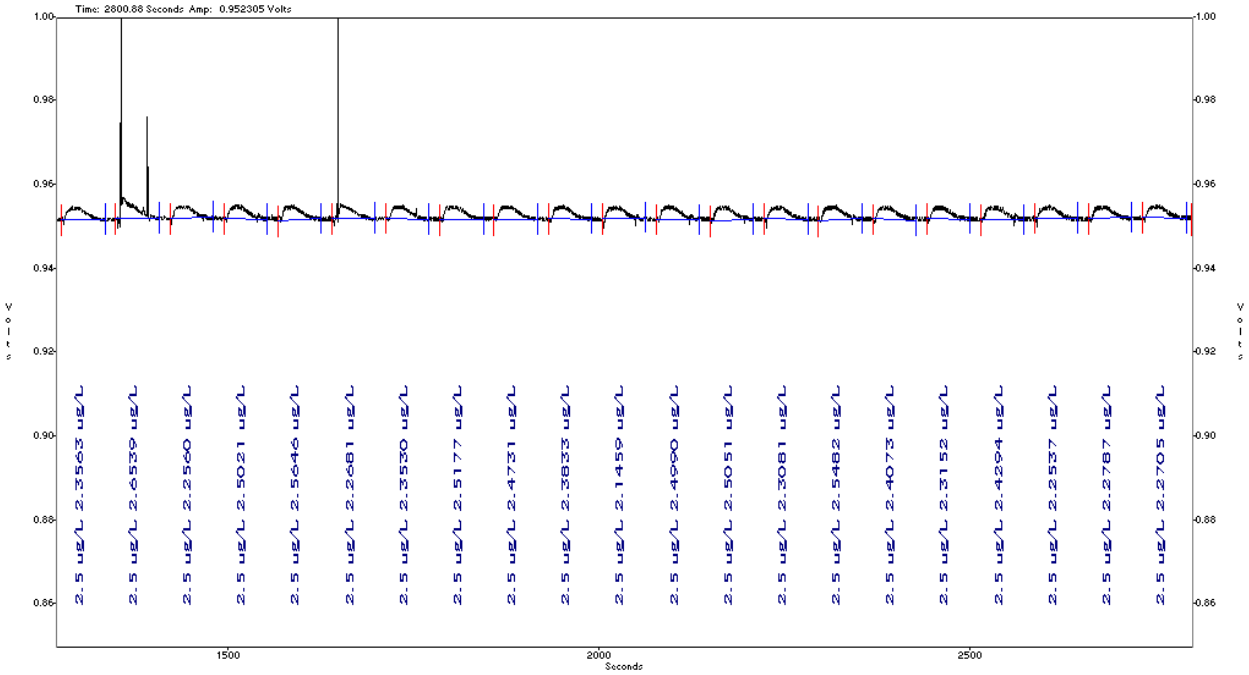


File Name: 201200c4.fdt Acq.
Date: 20 December 2000

62 Calibration Graph and Statistics

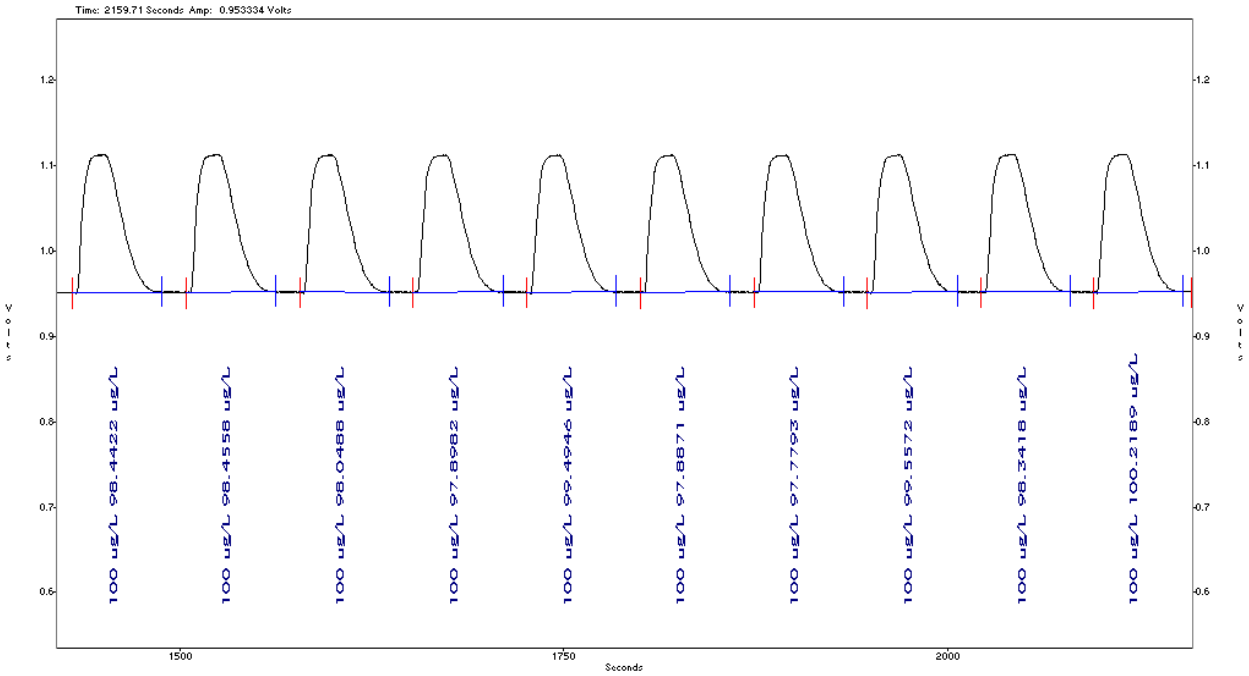
Level	Area	µg P/L	Determined	Replicate %RSD	% residual
1	4231523	400	395.2	1.2	0.0
2	2104093	200	199.82	0.9	-0.4
3	1028960	100	98.5	1.5	0.9
4	244912	25	24.55	1.8	2.7
5	43507	5	4.99	0.3	-1.1
6	-158	0	0	-1124.7	---





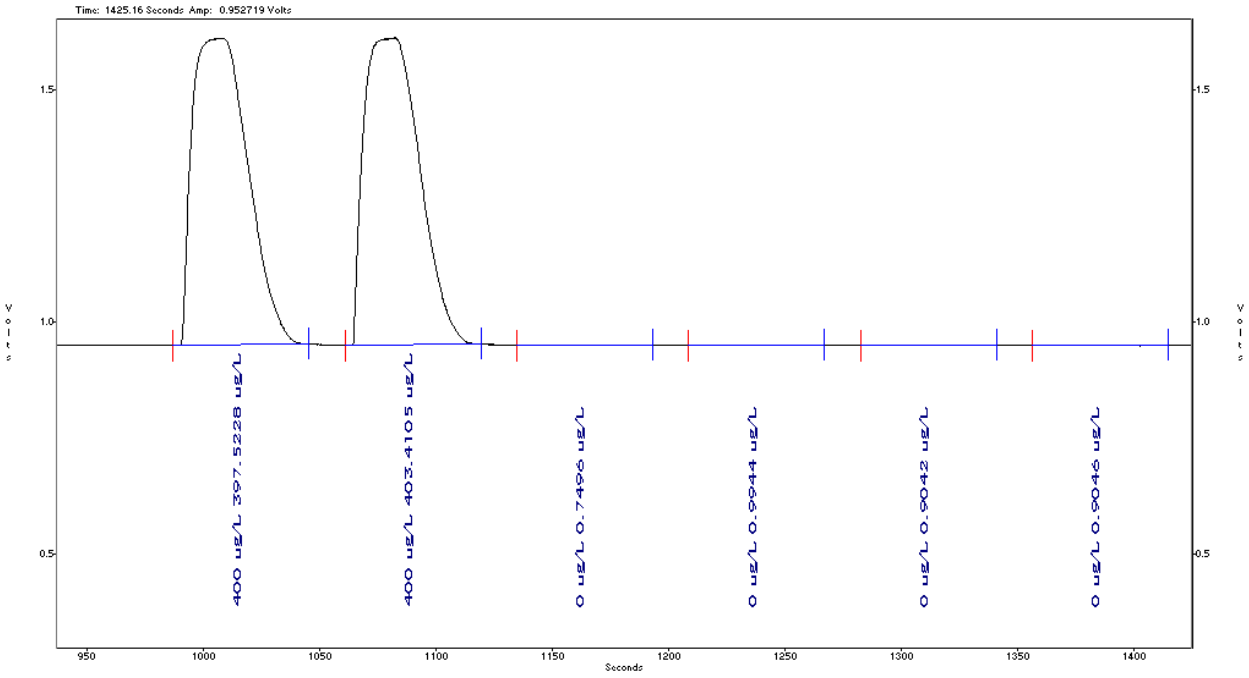
Method Detection Limit for Orthophosphorus using 2.5 µg P/L standard

MDL= 0.33 µg P/L * Claimed MDL is 1.0 µg P/L due to Y-intercept and carryover.
Standard Deviation (s) = 0.13 µg P/L, Mean (x) = 2.4 µg P/L, Known value = 2.5 µg/L Acq.
Date: 20 December 2000
File Name: 201200c3.fdt



Precision data for Orthophosphorus using 100 µg P/L standard

% RSD = 0.86 %
Standard Deviation (s) = 0.85 µg P/L, Mean (x) = 98.6 µg P/L, Known value = 100 µg P/L
Acq. Date: 20 December 2000
File Name: 201200c4.fdt



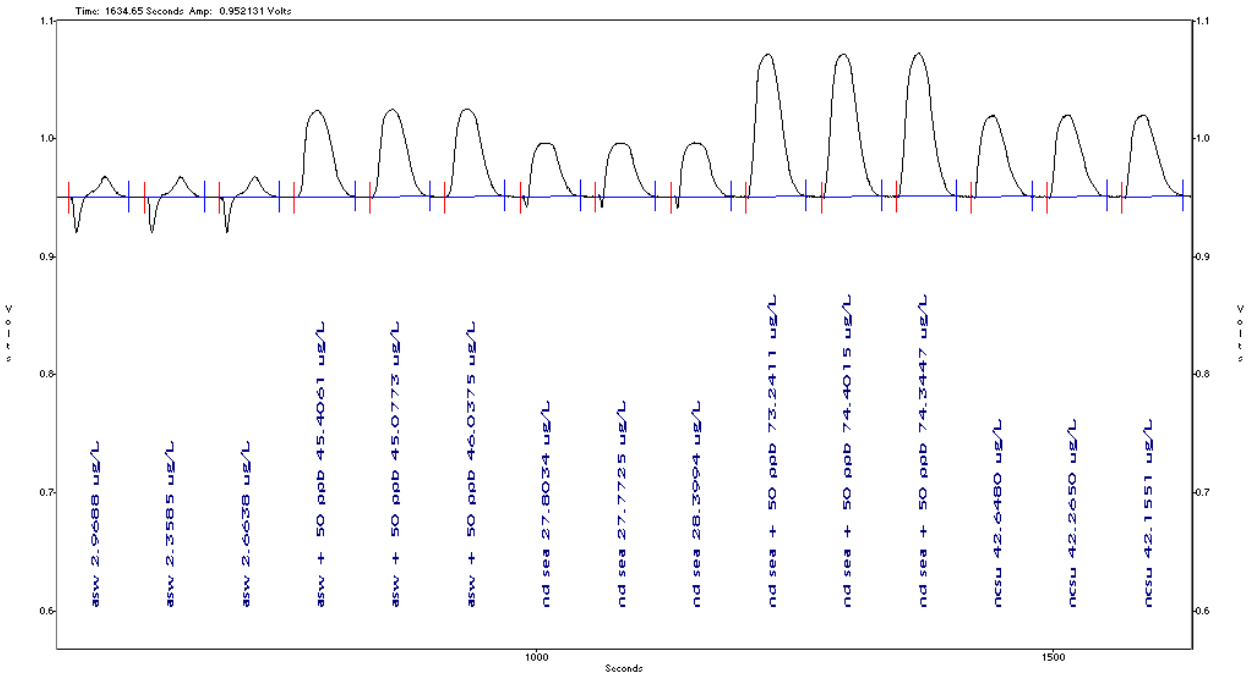
Carryover Study: 400 µg P/L standard followed by 4 blanks

Carryover Passed

Acq. Date: 20 December 2000

File Name: 201200c4.fdt

63 Various seawater samples showing different salinities and their spikes.

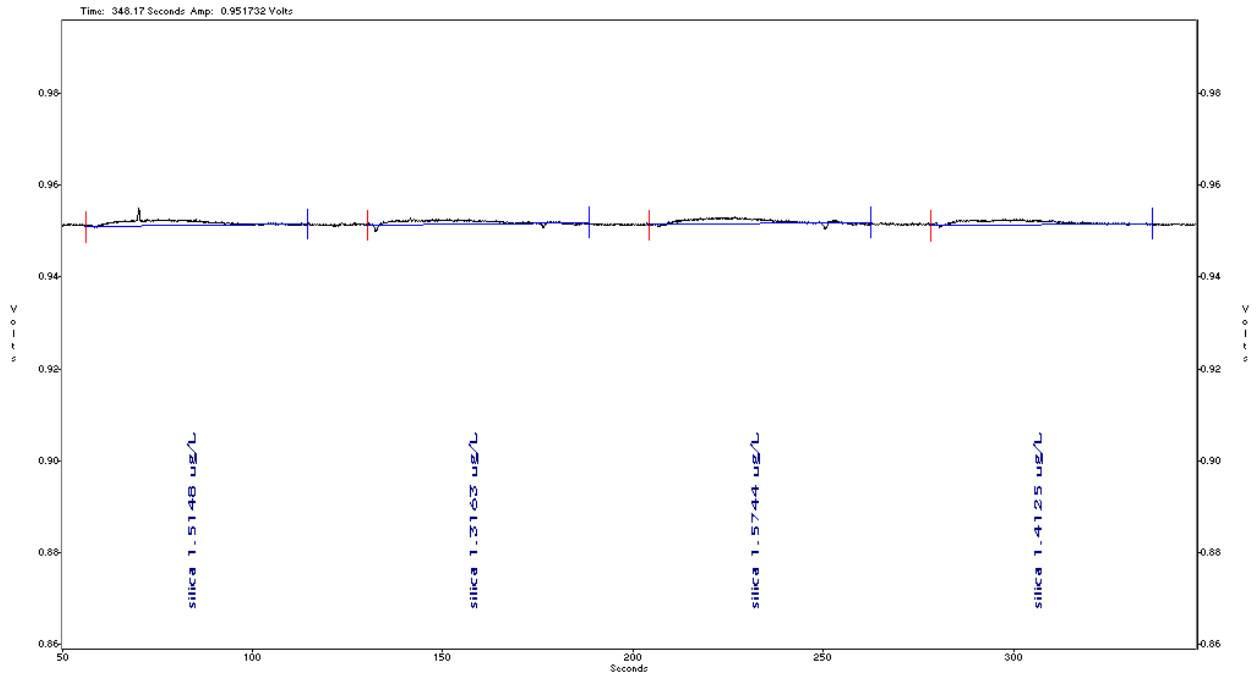


Acq. Date: 20 December 2000
File Name: 201200c6.fdt

Sample Type	Results µg P/L	Averages µg P/L	Spike Recoveries
Artificial Seawater	2.9688 2.3585 2.6638	2.6637	85.69%
Artificial Seawater plus 50 ppb spike	45.4061 45.0773 46.0375	45.5069	
*Nutrient Depleted Seawater	27.8034 27.7725 28.3994	27.992	92.01%
*Nutrient Depleted Seawater plus 50 ppb spike	73.2411 74.4015 74.3447	73.995	
*NCSU sample water	42.6480 42.2650 42.1551	42.356	---

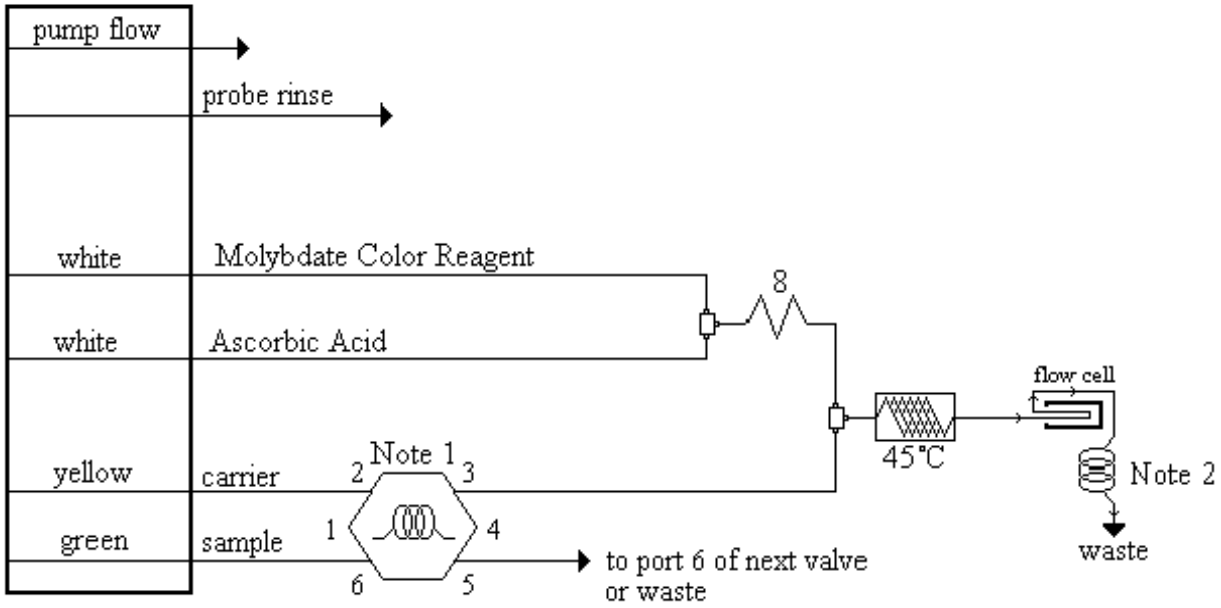
*These samples were overrange. They were diluted after the spike with a 1:2 dilution to bring them within range.

64 Interference Study using Silicate Standard



The selectivity of the method against silicate is 1172.4. For 1000 mg of silicate, the response would be 0.853 mg P/L. The amount of silicate in the above samples was 1700 µg Si/L. Conclusion: Silicate is not a significant interferent in this method.

17.3. **ORTHOPHOSPHATE MANIFOLD DIAGRAM**



Carrier: DI water
Manifold Tubing: 0.8 mm (0.032 in) i.d. This is 5.2 µL/cm.
AE Sample Loop: 150 cm x 0.042 in i.d.
QC8000 Sample Loop: 150 cm x 0.042 in i.d.
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

8: 168 cm of tubing on a 8 cm coil support

Note 1: The sample loop should be cut on a 30°-45° angle for the best fit. One o-ring can also be used instead of 2.
Note 2: Backpressure coil of 200 cm x 0.5 mm (0.022 in) i.d.
Note 3: For the Cetac sampler: From the probe to the sample pump tube is 190 cm x 0.032 in i.d. From the sample pump tube to port 6 is 20 cm x 0.032 in i.d.
For the AI/Gilson samplers: From probe to sample pump tube is 130 cm x 0.032 in i.d. From the sample pump tube to port 6 is 20 cm x 0.032 in i.d.
Note 4: Glass calibration vials must be used with this method (Lachat Part No. 21304).