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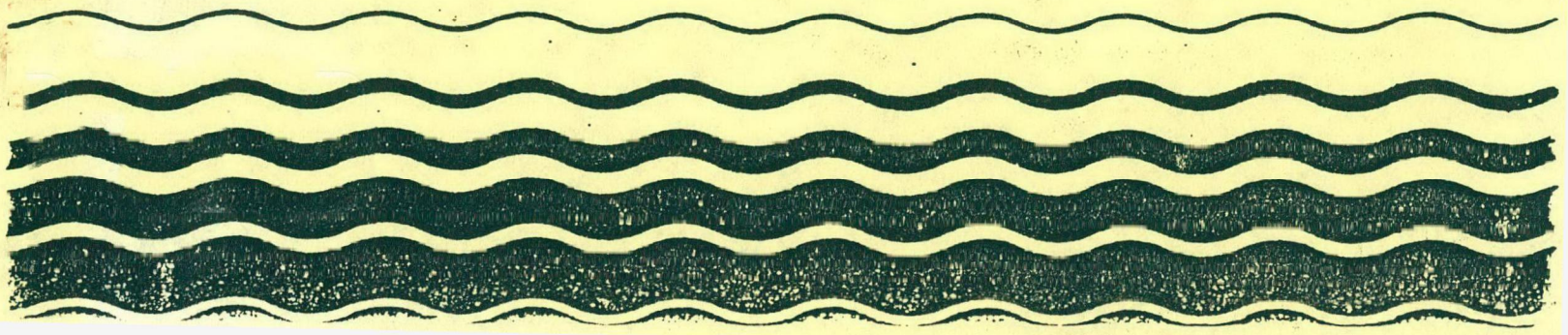
Research and Development

AN INTERIM MANUAL OF METHODS FOR THE DETERMINATION OF NUTRIENTS IN ESTUARINE AND COASTAL WATERS



September, 1991

AN INTERIM MANUAL OF METHODS FOR THE DETERMINATION OF NUTRIENTS IN ESTUARINE AND COASTAL WATERS

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DISCLAIMER

This interim manual has been reviewed by the Environmental Monitoring Systems Laboratory - Cincinnati, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in marine and estuarine waters, drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.

- Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.

- Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in marine and estuarine waters, drinking water, surface water, groundwater, wastewater, sediment and solid waste.

- Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

This EMSL-Cincinnati publication, "Interim Manual of Methods for the Determination of Nutrients in Estuarine and Coastal Waters" was prepared as a new initiative to gather together under a single cover a compendium of validated laboratory analytical methods for the determination of nutrients in the marine environment. We are pleased to provide this manual and believe that it will be of considerable value to many public and private laboratories involved in marine studies for regulatory or other reasons.

Thomas A. Clark, Director
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ABSTRACT

This interim manual currently consists of two analytical methods for the determination of nutrient analytes in marine water samples. The method for low level ortho-phosphate is based on conventional antimony-phospho-molybdate complex colorimetry. The applicable concentration range is approximately 0.0006 to 0.05 mg P/L. The nitrate method is based on cadmium reduction of the nitrate to nitrite. The nitrite originally present and the reduced nitrate are then determined colorimetrically by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride. The applicable concentration range is approximately 0.0002 to 0.07 mg N/L. These methods have been multilaboratory tested and contain a statistical summary of the results obtained in those studies. Methods for additional nutrient analytes are currently being validated in a similar fashion and will be added to this manual when completed. This interim manual is considered a growing document that will eventually expand in scope to include organic and additional inorganic analytes.

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ACKNOWLEDGMENT

This methods manual was prepared by the Inorganic Chemistry Branch of the Chemistry Research Division, Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati). Preparation of the individual manuscripts and coordination of the multilaboratory studies was performed by the staff of the University of Maryland System, Chesapeake Biological Laboratory with special thanks going to Carl Zimmermann and Carolyn Keefe. Additional efforts by Jim Longbottom EMSL-Cincinnati and Ken Edgell, The Bionetics Corporation in the preparation and distribution of quality control samples and statistical evaluation of the data are very much appreciated.

The overall USEPA effort to standardize analytical methods for use in the marine environment was identified as a need and championed by the USEPA Regions. The staff at Region 2 and Region 3 were, and continue to be, instrumental in identifying resources for this project and providing insight from the regional perspective. We appreciate the regional efforts and expect to call upon them on a continuing basis.

INTRODUCTION

An integral component of the role of the U.S Environmental Protection Agency (USEPA) in assessing and protecting the quality of the environment is the provision of means for monitoring environmental quality. In keeping with this role, USEPA develops and disseminates analytical methods for measuring chemical and physical parameters affecting this most important resource, including contaminants which may have potential adverse effects upon the health of our environment. This interim manual provides initially two analytical methods for nutrient analytes which have been identified by the USEPA Regions as the highest priority. Additional methods and revisions of these methods will be made available in regular updates of this manual. Additional methods for the quantitation of metals in the marine environment may be found in EPA/600/4-91/010 "Methods for the Determination of Metals in Environmental Samples", June, 1991.

GENERAL COMMENTS

The methods in this manual are not intended to be specific for any single USEPA regulation, compliance monitoring program, or specific study. In the past, manuals have been developed and published that respond to specific regulations, such as the Safe Drinking Water Act (SDWA) or to special studies such as the Environmental Monitoring and Assessment Program (EMAP) Near Coastal Demonstration Project. These methods are, however, available for incorporation into regulatory programs that require the measurement of nutrients in marine waters.

The quality assurance sections are uniform and contain minimum requirements for operating a reliable monitoring program: initial demonstration of performance, routine analyses of reagent blanks, analyses of fortified reagent blanks and fortified matrix samples, and analyses of quality control (QC) samples. Other QC practices are recommended and may be adopted to meet the particular needs of monitoring programs e.g., analyses of field reagent blanks, instrument control samples and performance evaluation samples.

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METHOD 353.4

DETERMINATION OF NITRITE+NITRATE IN ESTUARINE AND COASTAL WATERS BY AUTOMATED COLORIMETRIC ANALYSIS

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METHOD 353.4

DETERMINATION OF NITRITE+NITRATE IN ESTUARINE AND COASTAL WATERS BY AUTOMATED COLORIMETRIC ANALYSIS

1. SCOPE AND APPLICATION

1.1 This method provides a procedure for the determination of low level nitrite+nitrate concentrations normally found in estuarine and/or coastal waters using the cadmium reduction technique.[1] Nitrate concentrations are obtained by subtracting nitrite values, which have been previously analyzed, from the nitrite+nitrate values.

1.2 A statistically determined method detection limit (MDL) of 0.0002 mg N/L has been determined by one laboratory.[2] The method is linear to 0.070 mg N/L at a standard calibration setting of 9.0 on an AutoAnalyzer II system. Where higher concentrations are encountered, the method is also linear at less sensitive standard calibration settings.

1.3 Approximately 40 samples per hour can be analyzed.

1.4 This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. A minimum of six months experience under experienced supervision is recommended.

2. SUMMARY OF METHOD

2.1 An automated colorimetric method for the analysis of low level nitrite+nitrate concentrations will be described. Filtered samples are passed through a granulated copper cadmium column to reduce nitrate to nitrite. The nitrite originally present and the reduced nitrate are then determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a colored azo dye. The color produced is proportional to the nitrite+nitrate concentration present in the sample. Nitrate is obtained by subtracting nitrite values which have been previously analyzed without the cadmium reduction column from the nitrite+nitrate values.

3. DEFINITIONS

3.1 Calibration Standard - A solution prepared from the stock standard solution which is used to calibrate the instrument response with respect to analyte concentration. One of the standards in the standard curve.

3.2 Dissolved - Material that will pass through a 0.45 µm membrane filter assembly.

3.3 Laboratory Fortified Blank (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the method is within accepted control limits. This is basically a standard prepared in reagent water which is analyzed as a sample.

3.4 Laboratory Reagent Blank (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus.

3.5 Linear Calibration Range - The concentration range over which the analytical working curve remains linear.

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

3.7 Refractive Index - The difference in light intensity due to the differences between the index of refraction of light in seawater/estuarine water and deionized distilled water.

3.8 Stock Standard Solution - A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 Metal ions may produce a positive error if present in sufficient concentrations. The presence of large concentrations of sulfide and/or sulfate will cause a loss of sensitivity to the copper-cadmium column.[5,6]

4.2 Sample turbidity should be removed by filtration prior to analysis.

4.3 Refractive Index and "Salt Error" interferences should be corrected for when analyzing estuarine/coastal samples (Sects. 12.2 and 12.3).

5. SAFETY

5.1 Water samples collected from the estuarine and/or ocean environments are most often not at all hazardous. The individual who collects samples should use proper technique, however.

5.2 Good laboratory technique should be used when preparing reagents. A lab coat, safety goggles and gloves should be worn when preparing the reagents; particularly the copper sulfate, and color reagent.

5.3 Proper care needs to be demonstrated when operating any scientific instrument.

6. APPARATUS AND EQUIPMENT

6.1 Continuous flow automated analytical system consisting of:

6.1.1 Sampler.

6.1.2 Manifold or Analytical Cartridge.

6.1.3 Proportioning pump.

6.1.4 Colorimeter equipped with 1.5 X 50 mm tubular flow cell and 550 nm filter.

6.1.5 Phototube: which can be used for 500-550 nm range.

6.1.6 Recorder or computer based data system.

6.2 Nitrogen-free glassware: All glassware used in the determination must be low in residual nitrate to avoid sample/reagent contamination. Washing with 10% HCl and thoroughly rinsing with reagent water has been found to be effective.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 Stock Reagent Solutions

7.1.1 Ammonium Chloride Reagent--Dissolve 10.0 g of ammonium chloride (NH_4CL, CAS RN 12125-02-9) in one liter of reagent water. Adjust to pH 8.5 by adding three to four NaOH (CAS RN 1310-73-2) pellets as necessary. Add 5 drops of 2% copper sulfate solution (Sect. 7.1.3). No addition of EDTA is necessary. This reagent is stable for one week if kept refrigerated.

7.1.2 Color Reagent--Combine 1500 mL reagent water, 200.0 mL concentrated phosphoric acid (H_3PO_4, CAS RN 7664-38-2), 20.0 g sulfanilamide (CAS RN 63-74-1), and 1.0 g N-1 napthylethylenediamine dihydrochloride (CAS RN 1465-25-4). Dilute to 2000 mL with reagent water. Add 2.0 mL BRIJ-35 (Bran & Luebbe, Elmsford, N.Y.). Store at 4°C in the dark. This should be prepared every six weeks.

7.1.3 Copper Sulfate--Dissolve 2.0 g of copper sulfate ($CuSO_4.5H_2O$, CAS RN 7758-98-7) in 90.0 mL of reagent water. Bring up to 100 mL with reagent water.

7.1.4 Refractive Reagent--Combine 100 mL of concentrated phosphoric acid (H_3PO_4) to 800 mL reagent water. Dilute to 1000 mL with reagent water. Add 1.0 mL BRIJ-35.

7.1.5 Stock Nitrate Solution--Dissolve 0.721 g of pre-dried (60°C for 1 hour) potassium nitrate (KNO_3, CAS RN 7758-09-0) in reagent water and dilute to 1000 mL. 1.0 mL = 0.100 mg N. The stability of this stock standard is approximately 3 months, if kept refrigerated.

7.1.6 Stock Nitrite Solution--Dissolve 0.493 g pre-dried (60°C for 1 hour) sodium nitrite ($NaNO_2$, CAS RN 7632-00-0) in reagent water and dilute to 1000 mL. 1.0 mL = 0.100 mg N. The stability of this stock standard is approximately 3 months, if kept refrigerated.

7.1.7 Reagent Water--Type 1 reagent grade water equal to or exceeding standards established by American Society of Testing Materials (ASTM) should be used in the preparation of reagents and standards. Reverse osmosis systems or distilling units which produce 18 megohm water are two examples of acceptable water sources.

7.1.8 Low Nutrient Seawater--Obtain natural low nutrient seawater (36 ppt salinity; <0.0002 mg N/L) or dissolve 31 g analytical reagent grade sodium chloride, NaCl (CAS 7647-14-5); 10 g analytical reagent grade magnesium sulfate, $MgSO_4$ (CAS 10034-99-8); and 0.05 g analytical reagent grade sodium bicarbonate, $NaHCO_3$ (CAS 144-55-8), in 1 liter of reagent water.

7.2 Cadmium Preparation--The following description of cadmium and the column preparation is to be used as a general guideline. It is recognized that between institution differences in the preparation, shape, size and configuration of cadmium columns exist. All have the capability of obtaining excellent results. The ultimate goal is to obtain 100% reduction of nitrate to nitrite.

Use good quality cadmium (CAS RN 7440-43-9) filings. Depending on the reductor column shape and size, cadmium filings should generally be less than 0.5 mm but greater than 0.3 mm for glass columns and in the 25-60 mesh size range for columns prepared by using flexible tubing.

New cadmium filings should be rinsed with diethyl ether to remove dirt and grease.

Approximately 10 grams of this cadmium is then treated with 50 mL of 6N HCl in a 150 mL beaker. Swirl VERY CAREFULLY for one minute. Carefully decant the HCl and thoroughly rinse (at least 10 times) with reagent water. Decant the reagent water and add a 50 mL portion of 2% (w/v) copper sulfate solution (Sect. 7.1.3). While swirling, brown flakes of colloidal copper will appear and the blue color of the solution will fade. Decant and repeat this sequence until the blue color does not fade and a brown colloidal precipitate

forms. From this point on the Cu-Cd filings should not come in contact with air.

Wash the filings thoroughly with reagent water until all blue color is gone and the supernatant is free of particulate matter (usually a minimum of 10 rinses is necessary). The filings are now ready to be packed into the column.

7.2.1 Column Preparation--Fill the reductor column with ammonium chloride reagent (Sect. 7.1.1) and transfer the prepared cadmium filings to the column using a Pasteur pipette or employ some other method which avoids contact of the Cd particles with air. One end of the reductor column should be plugged with glass wool. Column shape and size varies with users. Some examples include a 22 cm length of 0.110" ID tubing, a 35 cm length of 0.090" ID tubing or a 3.5" length of glass tubing.

When the entire column is fairly well packed with granules, insert another glass wool plug at the top of the column and with reagents pumping through the system, attach the column. Remember to have no air bubbles in the valve (Figure 1) and to attach the column to the intake side of the valve first.

Check for good flow characteristics (regular bubble pattern) after the addition of air bubbles beyond the column. If the column is packed too tightly, an inconsistent flow pattern will be evident.

Prior to sample analysis, condition the column by pumping through the sample line approximately 1 mg N (nitrate)/L for five minutes followed by 1 mg N (nitrite)/L for 10 minutes.

7.2.2 Secondary Nitrate Solution--Dilute 1.0 mL of stock nitrate solution (Sect. 7.1.5) to 100 mL with reagent water. 1.0 mL of this solution = 0.001 mg N. Refrigerate and store no longer than a few days.

7.2.3 Prepare a series of standards by diluting suitable volumes of Secondary Nitrate Solution (Sect. 7.2.2) to 100 mL with reagent water. Prepare these standards daily. When working with samples of known salinity it is recommended that the standard curve concentrations be prepared in Low Nutrient Seawater (Sect. 7.1.8) diluted to that salinity and that the Sampler Wash Solution also be Low Nutrient Seawater (Sect. 7.1.8) diluted to that salinity. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in reagent water and refractive index corrections be made to the sample concentrations (Sect. 12.2). The following dilutions, brought up to 100 mL with reagent water, are suggested.

mL of Standard Nitrate Solution (7.2.2) up to 100 mL reagent water	Conc., ng N/L
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
6.0	0.060

7.2.4 Saline Nitrate Standards--When analyzing samples of varying salinities, it is also recommended that standards be prepared in a series of salinities in order to quantify the "salt error," the shift in the colorimetric response of nitrate due to the change in the ionic strength of the solution. The following dilutions prepared in 100 mL volumetric flasks, brought up to volume with reagent water, are suggested.

Salinity (ppt)	mL of Low Nutrient Seawater (7.1.8)	mL of Standard Nitrate Solution (7.2.2)	Conc., mg N/L
0	0	6.0	0.060
9	25	6.0	0.060
18	50	6.0	0.060
27	75	6.0	0.060
34	94	6.0	0.060

7.2.5 Secondary Nitrite Solution--Dilute 1.0 mL of stock nitrite solution (Sect. 7.1.6) to 100 mL with reagent water. 1.0 mL of this solution = 0.001 mg N. Refrigerate and store no longer than a few days.

7.2.6 Working Nitrite Solution--One working standard needs to be prepared which will act as a check on the reduction capability of the cadmium column. Therefore, 6.0 mL of (Sect. 7.2.5) up to 100 mL will yield a concentration of 0.060 mg N/L.

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Sample Collection: Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods; hydrocast and submersible pump systems. The dissolved fraction is defined as that fraction which passes through a 0.45 μm pore size filter.

8.1.1 A hydrocast encompasses a series of sampling bottles (Niskin, Nansen, Go-Flo or equivalent) which are attached at fixed intervals to a hydro wire. These bottles are sent through the water column "open" and are then closed either

electronically or via a "messenger" when the bottles have reached the desired depth.

8.1.2 The submersible pump system requires a weighted hose being sent to the desired depth in the water column and water then being pumped from that depth to the deck of the ship for processing.

8.1.3 Another method used to collect surface samples involves the use of a plastic bucket or large plastic bottle. While not the most ideal method, it is commonly used in citizen monitoring programs.

8.2 Sample Preservation: Samples should be analyzed as quickly as possible. If the samples are to be analyzed within 24 hours of collection, then refrigeration at 4°C is acceptable. A widely accepted method of preservation within the oceanographic/estuarine community for samples requiring longer storage is freezing at -20°C. A maximum two month limit of storing these frozen estuarine and coastal samples has been recommended. Differences between nitrate samples analyzed immediately on board ship and analyses of frozen samples were not significant after several days.[5] Studies[6,7] recommended freezing inorganic nitrogen samples for a maximum ten days and that the observed differences between immediate analysis and that of frozen samples had no practical effect. Freezing estuarine water samples for nutrient analyses is now an accepted form of preservation in the Chesapeake Bay Monitoring Program and the Long Island Sound Study, two National Estuary Program efforts.

8.3 Sample Storage: Long-term storage of frozen samples should be in clearly labelled polyethylene bottles or polystyrene cups compatible with the analytical system's automatic sampler (Sect. 6.1.1).

9. CALIBRATION AND STANDARDIZATION

9.1 Calibration (Refer to Sect. 10.2.3).

9.2 Internal standardization (Refer to Sects. 1.2, 6.1.2, 6.1.4, and 6.1.5)

10. QUALITY CONTROL

10.1 A formal quality control (QC) program is strongly recommended. The minimum requirements of this program should consist of an initial demonstration of laboratory capability, the continued analysis of unknowns on an irregular basis as a continuing check on performance, and an internal program of laboratory duplicates, spikes and fortified samples which are used as a check of precision and recovery.

10.2 Initial Demonstration of Performance

10.2.1 The initial demonstration of performance is used to characterize instrument performance (method detection limits and linear calibration ranges).

10.2.2 MDLs should be established for all analytes, using a low level estuarine water sample. To determine MDL values, analyze seven replicate aliquots of 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:

$$MDL = 3(S)$$

where S = the standard deviation of the replicate analyses.

MDLs should be determined every six months or whenever a significant change in background or instrument response occurs.

10.2.3 Linear calibration ranges - Standard curves should be analyzed through several standard calibration settings (calibrated sensitivity settings). In so doing, where higher concentrations are encountered, the method will also have been checked for linearity using sets of standards of higher concentrations.

10.3 Assessing Laboratory Performance

10.3.1 Laboratory Reagent Blank (LRB) - A laboratory should analyze at least one reagent blank (Sect. 3.5) with each set of samples. Reagent blank data are used to assess contamination from the laboratory environment and should an analyte value in the reagent blank exceed the MDL, then laboratory or reagent contamination should be suspected.

10.3.2 Laboratory Fortified Blank (LFB) - A laboratory should analyze at least one fortified blank (Sect. 3.4) with each batch of samples. Calculate accuracy as percent recovery. If the recovery of an analyte falls or rises in a consistent pattern, then the source of the problem should be identified and resolved before continuing the analyses. This is basically analyzing standards as sample and is an excellent check on the overall performance of the entire analytical system.

10.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

10.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the routine samples or one sample per sample set, whichever is greater. The analyte concentration

should be high enough to be seen above the original concentration of the sample and should be at least four times greater than the MDL.

10.4.2 Calculate the percent recovery of the analyte, corrected for background concentrations measured in the unfortified sample, and compare these values with the values obtained from the LFB's. Percent recoveries may be calculated using the following equation:

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

where, R = percent recovery
Cs = actual fortified sample concentration (background + concentrated addition)
C = Sample background concentration
s = Concentrated addition to the sample

10.4.3 If the recovery of the analyte falls outside the designated range, but the laboratory performance for that analyte is in control, the fortified sample should be prepared again and reanalyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related, not system related.

10.5 Precision

10.5.1 A single laboratory analyzed four filtered samples collected from the Chesapeake Bay. Seven replicates of each sample were processed and analyzed. The results are as follows:

Sample	Concentration (mg N/L)	Standard Deviation (mg N/L)
1	0.1491	0.0009
2	0.0053	0.0012
3	1.3543	0.0127
4	0.0064	0.0004

11. PROCEDURE

11.1 If samples have not been freshly collected and are frozen, thaw the samples to room temperature.

11.2 Set up manifold as shown in Figure 2.

11.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a steady baseline with reagent water pumping through the system, add reagents to the sample stream and after the reagent

baseline is steady; note that rise (reagent baseline), and adjust baseline.

For analysis of samples with a narrow salinity range, it is advisable to use low nutrient seawater as wash water in the sampler in place of deionized water. For samples with a large salinity range, it is suggested that deionized water and procedures (Sects. 12.2 and 12.3) be employed.

11.4 A good sampling rate is approximately 40 samples/hr. with a 9:1 sample:wash ratio.

11.5 Place standards (prepared in reagent water--[Sect. 7.2.3] and saline water--[Sect. 7.2.4], and the working nitrite standard--[Sect. 7.2.6]) in Sampler in order of decreasing concentration. Complete filling the sampler tray with samples, blanks, internal standards and other quality control samples.

11.6 Commence analysis.

11.6.1 If the peak height of the 0.060 mg N/L nitrate standard prepared in deionized water (Sect. 7.2.3) is less than 90% of the peak height of the 0.060 mg N/L nitrite standard (Sect. 7.2.6), halt analyses and prepare a new cadmium reduction column (Sect. 7.2.1).

11.6.2 If a low concentration sample peak follows a high concentration sample peak, a certain amount of carry over can be expected. It is recommended that if there is not a clearly defined low concentration peak, that the sample be reanalyzed at the end of the sample run.

12. CALCULATIONS

12.1 Concentrations of nitrite+nitrate are calculated from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variable and their corresponding peak heights are the dependent variable.

12.2 Refractive Index Correction For Estuarine/Coastal Systems

12.2.1 The absorbance peak obtained by an automated system for nitrate in a seawater sample (when compared to a reagent [deionized] water baseline) represents the sum of absorbances from at least four sources: (1) the light changes due to the differences in the index of refraction of the seawater and reagent water; (2) reaction products (e.g., precipitates) of BRIJ-35 and the seawater; (3) the absorbance of colored substances dissolved in the sample; and (4) reaction products of the nitrite and the nitrate (reduced to nitrite by the cadmium column) in the sample

with the color reagent.[8] The first three sources of color are corrected for by the refractive index correction described here.

12.2.2 Obtain a second set of peak heights for all samples and standards with Refractive Reagent (Sect. 7.1.4) being pumped through the system in place of Color Reagent (Sect. 7.1.2). All other reagents remain the same. Peak heights for the refractive index correction must be obtained at the same Standard Calibration Setting and on the same colorimeter as the corresponding samples and standards.[9]

12.2.3 Subtract the refractive index peak heights from the heights obtained for the nitrate determination.

12.2.4 When a large data set has been amassed in which each sample's salinity is known, a regression for the refractive index correction on a particular colorimeter can be calculated. First analyze a set of nitrate standards (Sect. 7.2.3) with Color Reagent (Sect. 7.1.2) and obtain a linear regression from the standard curve (Sect. 12.1). For each sample, the apparent nitrate concentration due to refractive index is then calculated from its peak height obtained with Refractive Reagent (Sect. 7.1.4) and the regression of nitrate standards obtained with Color Reagent (Sect. 7.1.2) for each sample. Its salinity is entered as the independent variable (X variable) and its apparent nitrate due to its refractive index in that colorimeter is entered as the dependent variable (Y variable). The resulting regression allows the operator to subtract an apparent nitrate concentration when the salinity is known, as long as other matrix effects (Sects. 12.2.2-2 and 12.2.2-3) remain unchanged. Thus, the operator would not be required to obtain refractive index peak heights for all samples after a large data set has been found to yield consistent apparent nitrate concentrations due to salinity. An example of typical results from one laboratory follows:

Salinity (ppt)	Apparent nitrate conc. due to refractive index (mg N/L)
1	0.0001
6	0.0004
10	0.0007
22	0.0015

12.2.5 An example of a typical equation is:

mg N/L apparent NO_3 = 0.000069 X Salinity (ppt)

where 0.000069 is the slope of the line

12.3 Correction for Salt Error in Estuarine/Coastal Samples

12.3.1 When calculating concentrations of samples of varying salinities from standards prepared in reagent water, it is necessary to first correct for Refractive Index errors (Sect. 12.2), then correct for the "Salt Error" alteration in color development due to the ionic strength of the samples.

12.3.2 Plot the salinity of the saline standards as the independent variable (X variable) and the apparent concentration of nitrate (mg N/L) from the peak height corrected for refractive index (Sect. 12.2) calculated from the regression of standards in reagent water (Sects. 7.2.3 and 12.1) as the dependent variable (Y variable) for all 0.060 mg N/L standards. The resulting regression allows the operator to correct the concentrations of the samples of known salinity for the color enhancement due to Salt Error. An example of typical results from one laboratory follows:

Salinity (ppt)	Peak height of 0.060 mg N/L standard after correction for refractive index	Uncorrected mg N/L calculated from regression of standards in reagent water
0	85	0.0600
9	87	0.0614
18	89	0.0628
27	92	0.0649
34	94	0.0663

12.3.3 An example of a typical equation to correct for "Salt Error" is:

$$\text{Corrected mg N/L} = \frac{\text{Uncorrected mg N/L X } 0.0600}{(\text{Salinity X } 0.000187) + 0.060}$$

where 0.0600 is the concentration of nitrate standard (Sect. 7.2.4) present in each saline standard; salinity of the sample is in ppt; 0.000187 is the slope of the regression equation (Sect. 12.3.1); and 0.060 is the y intercept of the regression equation (Sect. 12.3.1).

12.4 Results should be reported in mg N/L, µg N/L or µg at N/L.

Table 1. describes these various units and conversions normally used in estuarine/coastal nutrient analyses.

13. PRECISION AND ACCURACY

Not Yet Determined - See Attachment 1.

14. REFERENCES

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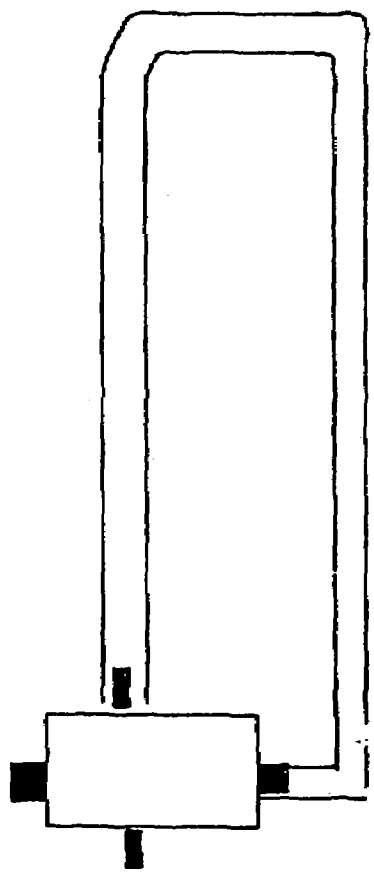
Table 1. Commonly used terminology to describe concentration.

mg N/L = ppm (parts per million)
μg N/L = ppb (parts per billion)

$$\mu g \text{ at } N/L = \frac{mg\ N/L}{0.014}$$

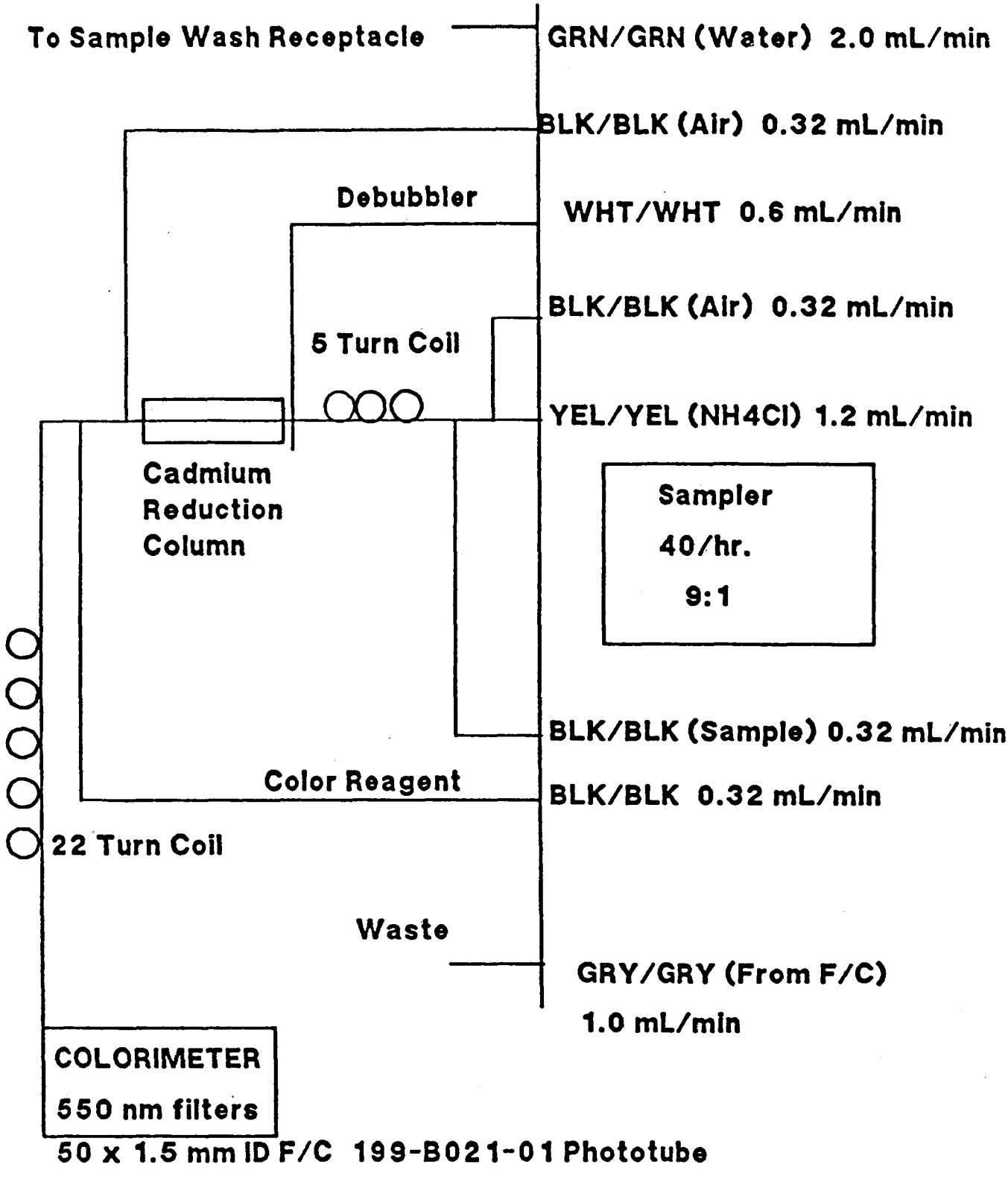
$$\mu g \text{ at } N/L = \frac{\mu g\ N/L}{14}$$

Figure 1. Reduction column valve assembly



Not drawn to scale

Figure 2. MANIFOLD CONFIGURATION FOR NITRITE+NITRATE



EMSL MARNITRATE JULY 1991
NITRITE+NITRATE IN ESTUARINE/COASTAL WATERS

LAB SAMPLE	0	1 A.A HPEL	3 UNH	4 AMRL	5 CBL	6 MERL	7 ECU	8 USEPA	10 H3 PO4 VIMS	11 MEL	12 MOSS LAND	13 HRSONSL
URN		0.0000	0.0000	0.0018	0.0000	-0.0001	0.0019	0.0000	0.0000	-0.0001	0.0000	0.0000
RWS 1		0.0095	0.0082	0.0136	0.0111	0.0106	0.0094	0.0123	0.0080	0.0110	0.0108	0.0137
RWS 2		0.0072	0.0068	0.0110	0.0090	0.0090	0.0083	0.0109	0.0055	0.0089	0.0094	0.0088
RWS 3		0.0210	0.0188	0.0253	0.0220	0.0216	0.0186	0.0253	0.0172	0.0223	0.0230	0.0249
RWS 4		0.0264	0.0245	0.0309	0.0277	0.0270	0.0244	0.0296	0.0224	0.0279	0.0284	0.0325
RWS 5		0.0024	0.0044	0.0051	0.0044	0.0042	0.0056	0.0054	0.0049	0.0045	0.0048	0.0038
RWS 6		0.0010	0.0020	0.0036	0.0032	0.0032	0.0053	0.0049	0.0025	0.0033	0.0036	0.0043
RWS 7		0.0429	0.0405	0.0452	0.0437	0.0434	0.0418	0.0474	0.0398	0.0460	0.0460	0.0489
RWS 8		0.0548	0.0531	0.0574	0.0552	0.0570	0.0537	0.0559	0.0483	0.0553	0.0577	0.0606
QC 1		0.0152	0.0160	0.0169	0.0171	0.0164	0.0156	0.0167	0.0135	0.0171	0.0175	0.0155
USS		0.0000	0.0079	0.0006	0.0050	0.0013	0.0076	0.0000	0.0031	0.0006	0.0070	0.0023
SSS 9		0.0063	0.0177	0.0249	0.0119	0.0195	0.0134	0.0617	0.0376	0.0265	0.0185	0.0153
SSS 10		0.0036	0.0158	0.0235	0.0098	0.0165	0.0126	0.0584	0.0370	0.0251	0.0156	0.0105
SSS 11		0.0183	0.0230	0.0210	0.0254	0.0300	0.0226	0.0693	0.0326	0.0342	0.0279	0.0176
SSS 12		0.0251	0.0306	0.0208	0.0300	0.0357	0.0291	0.0709	0.0232	0.0349	0.0331	0.0238
SSS 13		0.0006	0.0082	0.0052	0.0080	0.0119	0.0103	0.0305	0.0061	0.0106	0.0110	0.0096
SSS 14		0.0000	0.0040	0.0046	0.0062	0.0057	0.0096	0.0288	0.0039	0.0045	0.0103	0.0053
SSS 15		0.0408	0.0441	0.0318	0.0491	0.0463	0.0444	0.0326	0.0392	0.0316	0.0488	0.0288
SSS 16		0.0536	0.0586	0.0378	0.0573	0.0609	0.0560	0.0410	0.0530	0.0377	0.0589	0.0370
QC 2		*	0.0253	0.0179	0.0181	0.0167	0.0148	0.0087	0.0129	0.0190	0.0183	0.0153
UCB 1		0.0000	0.0031	0.0013	0.0010	0.0027	0.0014	0.0000	-0.0004	0.0008	0.0014	0.0021
CB1 17		0.0424	0.0455	0.0429	0.0445	0.0454	0.0450	0.0415	0.0402	0.0412	0.0498	0.0418
CB1 18		0.0542	0.0580	0.0525	0.0558	0.0613	0.0557	0.0510	0.0518	0.0513	0.0614	0.0490
QC 3		0.0151	0.0167	0.0165	0.0174	0.0168	0.0159	0.0180	0.0129	0.0184	0.0174	0.0153
UCB 2		0.0008	0.0018	0.0074	0.0016	0.0007	0.0018	0.0000	0.0052	0.0005	0.0015	0.0028
CB2 19		0.0116	0.0089	0.0165	0.0122	0.0113	0.0079	0.0096	0.0122	0.0089	0.0112	0.0099
CB2 20		0.0096	0.0089	0.0147	0.0106	0.0087	0.0064	0.0085	0.0116	0.0074	0.0094	0.0156
QC 4		0.0155	0.0139	0.0173	0.0171	0.0168	0.0159	0.0182	0.0141	0.0193	*	0.0153
UCB 3		0.0174	0.0203	0.0178	0.0209	0.0224	0.0116	0.0189	0.0160	0.0175	0.0182	0.0111
CB3 21		0.0397	0.0452	0.0373	0.0426	0.0682	0.0340	0.0400	0.0356	0.0355	0.0434	0.0294
CB3 22		0.0457	0.0525	0.0422	0.0481	0.0732	0.0395	0.0430	0.0432	0.0404	0.0485	0.0346
QC 5		0.0156	0.0192	0.0171	0.0176	0.0169	0.0150	0.0184	0.0138	0.0194	0.0172	0.0139

* NOT RUN

Method 365.5

DETERMINATION OF ORTHO-PHOSPHATE IN ESTUARINE AND COASTAL WATERS BY AUTOMATED COLORIMETRIC ANALYSIS

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REVISION 1.2

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METHOD 365.5

DETERMINATION OF ORTHO-PHOSPHATE IN ESTUARINE AND COASTAL WATERS BY AUTOMATED COLORIMETRIC ANALYSIS

1. SCOPE AND APPLICATION

1.1 This method provides a procedure for the determination of low level ortho-phosphate concentrations normally found in estuarine and/or coastal waters. It is basically the method of Murphy and Riley[1] adapted for automated segmented flow analysis[2] in which the two reagent solutions are added separately for greater reagent stability and facility of sample separation.

1.2 A statistically determined method detection limit (MDL) of 0.0006 mg P/l has been determined by one laboratory.[3] The method is linear to 0.050 mg P/l at a standard calibration setting of 9.0 on an AutoAnalyzer II system. Where higher concentrations are encountered, the method is also linear at lower standard calibration settings.

1.3 Approximately 40 samples per hour can be analyzed.

1.4 This method should be used by analysts experienced in the use of automated colorimetric analyses, matrix interferences and procedures for their correction. A minimum of six months experience under experienced supervision is recommended.

2. SUMMARY OF METHOD

2.1 An automated colorimetric method for the analysis of low level ortho-phosphate concentrations will be described. Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus 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 present in the sample.

3. DEFINITIONS

3.1 Calibration Standard - A solution prepared from the stock standard solution which is used to calibrate the instrument response with respect to analyte concentration. One of the standards in the standard curve.

3.2 Dissolved - Material that will pass through a 0.45 μm membrane filter assembly.

3.3 Laboratory Fortified Blank (LFB) - An aliquot of reagent water to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its

purpose is to determine whether the method is within accepted control limits. This is basically a standard prepared in reagent water which is analyzed as a sample.

3.4 Laboratory Reagent Blank (LRB) - An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, and reagents that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents or apparatus.

3.5 Linear Calibration Range - The concentration range over which the analytical working curve remains linear.

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

3.7 Refractive Index - The difference in light intensity due to the differences between the index of refraction of light in seawater/estuarine water and deionized distilled water.

3.8 Stock Standard Solution - A concentrated solution of method analyte prepared in the laboratory using assayed reference compounds or purchased from a reputable commercial source.

4. INTERFERENCES

4.1 It is reported that the interference caused by copper, arsenate and/or silicate is minimal to the ortho-phosphate determination because of their extremely low concentrations normally found in estuarine or coastal waters. High iron concentrations can cause precipitation of and subsequent loss of phosphorus. Hydrogen sulfide effects (samples collected from deep anoxic basins) can be treated by simple dilution of the sample, since high sulfide concentrations are most often associated with high phosphate values.[4]

4.2 Mercuric chloride, used as a preservative, interferes.[5]

4.3 Sample turbidity should be removed by filtration prior to analysis.

4.4 Refractive Index interferences should be corrected for estuarine/coastal samples (Sect. 12.2).

5. SAFETY

5.1 Water samples collected from the estuarine and or ocean environment are most often not at all hazardous. The individual who collects samples should use proper technique, however.

5.2 Good laboratory technique should be used when preparing reagents. A lab coat, safety goggles and gloves should be worn when preparing the sulfuric acid reagent.

5.3 Proper care needs to be demonstrated when operating any scientific instrument.

6. APPARATUS AND EQUIPMENT

6.1 Continuous flow automated analytical system consisting of:

6.1.1 Sampler.

6.1.2 Manifold or Analytical Cartridge equipped with 37°C heating bath.

6.1.3 Proportioning pump.

6.1.4 Colorimeter equipped with 1.5 X 50 mm tubular flow cell and 880 nm filter.

6.1.5 Phototube: which can be used for 600-900 nm range.

6.1.6 Recorder or computer based data system.

6.2 Phosphate-free glassware: All glassware used in the determination must be low in residual phosphate to avoid sample/reagent contamination. Washing with 10% HCl and thoroughly rinsing with distilled/deionized water has been found to be effective.

7. REAGENTS AND CONSUMABLE MATERIALS

7.1 Stock Reagent Solutions

7.1.1 Ammonium Molybdate Solution (40 g/L)-- Dissolve 20.0 g of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}.4H_2O$, CAS RN 12027-67-7)) in approximately 400 mL of reagent water and dilute to 500 mL. Store in a plastic bottle out of direct sunlight. This reagent is stable for approximately 3 months.

7.1.2 Antimony Potassium Tartrate Solution (3.0 g/L) -- Dissolve 0.3 g of antimony potassium tartrate [$(K(SbO)C_4H_4O_6 * 1/2 H_2O$, CAS RN 11071-15-1] in approximately 90 mL of reagent water and dilute to 100 mL. This reagent is stable for approximately 3 months.

7.1.3 Ascorbic Acid Solution (18.0 g/L) -- Dissolve 18.0 g of ascorbic acid ($C_6H_6O_6$, CAS RN 50-81-7) in approximately 800 mL of reagent water and dilute to one liter. Dispense approximately 75 mL into clean poly bottles and freeze. The stability of the frozen ascorbic acid is approximately 3

months. Thaw overnight in the refrigerator before use. The stability of the thawed, refrigerated reagent is less than 10 days.

7.1.4 Sodium Lauryl Sulfate Solution (30.0 g/L) [Sodium Dodecyl sulfate, $CH_3(CH_2)11OSO_3Na$, CAS RN 151-21-3]-- Dissolve 3.0 g of sodium lauryl sulfate (SLS) in approximately 80 mL of reagent water and dilute to 100 mL. This solution is the wetting agent, and it's stability is approximately three weeks.

7.1.5 Sulfuric Acid Solution (4.9 N) -- Add 136 mL concentrated sulfuric acid (H_2SO_4, CAS RN 7664-93-9) to approximately 800 mL reagent water while cooling. After the solution is cooled, dilute to one liter with reagent water.

7.1.6 Stock Phosphorus Solution -- Dissolve 0.439 g of pre-dried (105 degree C for 1 hour) potassium phosphate, monobasic (KH_2PO_4, CAS RN 7778-77-0) in deionized water and dilute to 1000 mL. 1.0 mL = 0.100 mg P. The stability of this stock standard is approximately 3 months, if kept refrigerated.

7.1.7 Reagent Water -- Type I reagent grade water equal to or exceeding standards established by American Society of Testing Materials (ASTM) should be used in the preparation of reagents and standards. Reverse osmosis systems or distilling units which produce 18 megohm water are two examples of acceptable water sources.

7.1.8 Low Nutrient Seawater -- Obtain natural low nutrient seawater (36 ppt salinity; <0.0003 mg P/L) or dissolve 31 g analytical reagent grade sodium chloride, NaCl (CAS 7647-14-5); 10 g analytical grade magnesium sulfate, $MgSO_4$ (CAS 10034-99-8); and 0.05 g analytical reagent grade sodium bicarbonate, $NaHCO_3$ (CAS 144-55-8), in 1 liter of deionized water.

7.2 Working Reagents

7.2.1 Reagent A -- Mix the following reagents in these proportions for 142 mL of Reagent A: 100 mL of 4.9N H_2SO_4 (Sect. 7.1.5), 30 mL of ammonium molybdate solution (Sect. 7.1.1), 10 mL of antimony potassium tartrate solution (Secct. 7.1.2), and 2.0 mL of SLS solution (Sect. 7.1.4). Prepare daily.

7.2.2 Reagent B -- Add approximately 0.5 mL of the SLS solution (Sect. 7.1.4) to the 75 mL of ascorbic acid solution (Sect. 7.1.3). Stability is approximately 10 days if kept refrigerated.

7.2.3 Refractive Reagent A -- Add 50 mL of 4.9 N H_2SO_4 (Sect. 7.1.5) to 20 mL of reagent water. Add 1 mL of SLS (Sect. 7.1.4) to this solution. Prepare every few days.

7.2.4 Secondary Phosphorus Solution -- Take 1.0 mL of Stock Phosphorus Standard (Sect. 7.1.6) and dilute to 100 mL with deionized water. 1.0 mL = 0.0010 mg P. Refrigerate and keep no longer than 10 days.

7.2.5 Prepare a series of standards by diluting suitable volumes of standard solutions (Sect. 7.2.4) to 100 mL with deionized water. Prepare these standards daily. When working with samples of known salinity it is recommended that the standard curve concentrations be prepared in low level natural seawater. When analyzing samples of varying salinities, it is recommended that the standard curve be prepared in deionized water and refractive index corrections be made to the sample concentrations (Sect. 12.2). The following dilutions are suggested.

mL of Standard Phosphorus Solution (7.2.4)	Conc., mg P/L
0.1	0.0010
0.2	0.0020
0.5	0.0050
1.0	0.0100
2.0	0.0200
4.0	0.0400
5.0	0.0500

8. SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Sample Collection: Samples collected for nutrient analyses from estuarine and coastal waters are normally collected using one of two methods; hydrocast and submersible pump systems. The dissolved fraction is defined as that fraction which passes through a 0.45 μm pore size filter.

8.1.1 A hydrocast encompasses a series of sampling bottles (Niskin, Nansen, Go-Flo or equivalent) which are attached at fixed intervals to a hydro wire. These bottles are sent through the water column "open" and are then closed either electronically or via a "messenger" when the bottles have reached the desired depth.

8.1.2 The submersible pump system requires a weighted hose being sent to the desired depth in the water column and water then being pumped from that depth to the deck of the ship for processing.

8.1.3 Another method used to collect surface samples involves the use of a plastic bucket or large plastic bottle. While not the most ideal method, it is commonly used in citizen monitoring programs.

8.2 Sample Preservation: Samples should be analyzed as quickly as possible. If the samples are to be analyzed within 24 hours of collection, then refrigeration at 4°C is acceptable. A widely accepted method of preservation within the oceanographic/estuarine community for samples requiring longer storage is freezing at -20°C. No change in soluble inorganic phosphate concentration was found in river water samples after 12 weeks of freezing at -20°C.[6] A maximum two month limit of storing frozen estuarine and coastal samples has been recommended[2] while others[8] recommended freezing inorganic phosphorus samples for a maximum ten days and that the observed differences between immediate analysis and that of frozen samples had no practical effect.[9] Freezing estuarine water samples for nutrient analyses is now an acccepted form of preservation in the Chesapeake Bay Monitoring Program and the Long Island Sound Study, two National Estuary Program efforts.

8.3 Sample Storage: Long-term storage of frozen samples should be in clearly labelled polyethylene bottles or polystyrene cups compatible with the analytical system's automatic sampler (Sect. 6.1.1).

9. CALIBRATION AND STANDARDIZATION

9.1 Calibration (Refer to Sect. 10.2.3).

9.2 Internal standardization (Refer to Sects. 1.2, 6.1.2, 6.1.4, and 6.1.5)

10. QUALITY CONTROL

10.1 A formal quality control (QC) program is strongly recommended. The minimum requirements of this program should consist of an initial demonstration of laboratory capability, the continued analysis of unknowns on an irregular basis as a continuing check on performance, and an internal program of laboratory duplicates, spikes and fortified samples which are used as a check of precision and recovery.

10.2 Initial Demonstration of Performance

10.2.1 The initial demonstration of performance is used to characterize instrument performance (MDLs and linear calibration ranges).

10.2.2 MDLs should be established for all analytes, using a low level estuarine water sample. To determine MDL values, analyze seven replicate aliquots of 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:

$$MDL = 3(S)$$

where S = the standard deviation of the replicate analyses.

Method detection limits should be determined every six months or whenever a significant change in background or instrument response occurs.

10.2.3 Linear calibration ranges - Standard curves should be analyzed through a number of standard calibration settings. In so doing, where higher concentrations are encountered, the method will also be linear for different sets of standards at lower standard calibration settings.

10.3 Assessing Laboratory Performance

10.3.1 Laboratory Reagent Blank (LRB) - A laboratory should analyze at least one reagent blank (Sect. 3.5) with each set of samples. Reagent blank data are used to assess contamination from the laboratory environment and should an analyte value in the reagent blank exceed the MDL, then laboratory or reagent contamination should be suspected.

10.3.2 Laboratory Fortified Blank (LFB) - A laboratory should analyze at least one fortified blank (Sect. 3.4) with each batch of samples. Calculate accuracy as percent recovery. If the recovery of an analyte falls or rises in a consistent pattern, then the source of the problem should be identified and resolved before continuing the analyses. This is basically analyzing standards as sample and is an excellent check on the overall performance of the entire analytical system.

10.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

10.4.1 A laboratory should add a known amount of analyte to a minimum of 5% of the routine samples or one sample per sample set, whichever is greater. The analyte concentration should be high enough to be seen above the original concentration of the sample and should not be less than four times the MDL.

10.4.2 Calculate the percent recovery of the analyte, corrected for background concentrations measured in the unfortified sample, and compare these values with the values obtained from the LFB's.

Percent recoveries may be calculated using the following equation:

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

where, R = percent recovery
Cs = actual fortified sample concentration (background + concentrated addition)
C = Sample background concentration
s = Concentrated addition to the sample

10.4.3 If the recovery of the analyte falls outside the designated range, but the laboratory performance for that analyte is in control, the fortified sample should be prepared again and reanalyzed. If the result is the same after reanalysis, the recovery problem encountered with the fortified sample is judged to be matrix related, not system related.

10.5 Precision

10.5.1 A single laboratory analyzed four filtered samples collected from distinctly different areas of the Chesapeake Bay. Seven replicates of each sample were processed and analyzed. The results are as follows:

Sample	Concentration (mg P/L)	Standard Deviation (mg P/L)
1	0.0484	0.0005
2	0.0033	0.0007
3	0.0112	0.0002
4	0.0011	0.0004

11. PROCEDURE

11.1 If samples have not been freshly collected and are frozen, thaw the samples to room temperature.

11.2 Set up manifold as shown in Figure 1.

11.3 Allow both colorimeter and recorder to warm up for 30 minutes. Obtain a steady baseline with deionized water pumping through the system, add reagents to the sample stream and after the reagent baseline is steady; note that rise (reagent baseline), and adjust baseline.

For analysis of samples with a narrow salinity range, it is advisable to use low nutrient seawater as wash water in the sampler in place of deionized water. For samples with a large salinity range, it is suggested that deionized wash water and procedure Sect. 12.2 be employed.

11.4 A good sampling rate is approximately 40 samples/hr. with a 9:1 sample:wash ratio.

11.5 Place standards in Sampler in order of decreasing concentration. Complete filling the sampler tray with samples, blanks, internal standards and other quality control samples.

11.6 Commence analysis.

12. CALCULATIONS

12.1 Concentrations of ortho-phosphate are calculated from the linear regression obtained from the standard curve in which the concentrations of the standards are entered as the independent variables and their corresponding peak heights are the dependent variables.

12.2 Refractive Index Correction for Estuarine/Coastal Systems

12.2.1 Obtain a second set of peak heights for all samples and standards with Refractive Reagent A (Sect. 7.2.3) being pumped through the system in place of Reagent A (Sect. 7.2.1). Reagent B (7.2.2) remains the same and is also pumped through the system. Peak heights for the refractive index correction must be obtained at the same Standard Calibration Setting and on the same colorimeter as the corresponding samples and standards.[10]

12.2.2. Subtract the refractive index peak heights from the heights obtained for the ortho-phosphate determination. Calculate the regression equation using the corrected standard peak

heights. Calculate the concentration of samples from the regression equation using the corrected sample peak heights.

12.2.3 When a large data set has been amassed in which each sample's salinity is known, a regression for the refractive index correction on a particular colorimeter can be calculated. For each sample, the apparent ortho-phosphate concentration due to refractive index is calculated from its peak height obtained with Refractive Reagent A (Sect. 7.2.3) and Reagent B (Sect. 7.2.2) and the regression of ortho-phosphate standards obtained with ortho-phosphate Reagent A (Sect. 7.2.1) and Reagent B (Sect. 7.2.2) for each sample. Its salinity is entered as the independent variable (X variable) and its apparent ortho-phosphate concentration due to its refractive index in that colorimeter is entered as the dependent variable (Y variable). The resulting regression allows the operator to subtract an apparent ortho-phosphate concentration when the salinity is known, as long as other matrix effects are not present. Thus, the operator would not be required to obtain the refractive index peak heights for all samples after a large data set has been found to yield consistent apparent ortho-phosphate concentrations due to salinity. An example follows:

Salinity (ppt)	Apparent ortho-phosphate conc. due to refractive index (mg P/L)
1	0.0002
5	0.0006
10	0.0009
20	0.0017

12.2.4 An example of a typical equation is:

mg P/L apparent PO_4 = 0.000087 X Salinity (ppt)

where 0.000087 is the slope of the line

12.3 Results should be reported in mg P/L, µg P/L or µg at P/L.

Table 1. describes these various units and conversions normally used in estuarine/coastal nutrient analyses.

13. PRECISION AND ACCURACY

13.1 In a collaborative study involving ten laboratories, reagent water, mixed-salinity Chesapeake Bay water, and Sargasso Sea water were spiked and analyzed at three Youden pair concentrations in the range of 5.0-95.9 µg/L using this method. Data summaries and regression equations are provided in Table 2 that describe the mean recovery, single-operator standard deviation and overall standard deviation observed in this study.

13.2 In the same study, the laboratories also analyzed reagent water and Sargasso Sea water fortified at 1.2 and 1.6 µg/L. The interlaboratory estimate of the method detection limit (IMDL) was estimated from the reagent water results to be 2.0 µg/L.

14. REFERENCES

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Table 1. COMMONLY USED TERMINOLOGY TO DESCRIBE CONCENTRATION.

mg P/L = ppm (parts per million)
µg P/L = ppb (parts per billion)

$$\mu g \text{ at } P/L = \frac{mg\ P/L}{0.031}$$

$$\mu g \text{ at } P/L = \frac{\mu g\ P/L}{31}$$

TABLE 2. SINGLE-ANALYST PRECISION, OVERALL PRECISION AND RECOVERY

WATER TYPE	ORTHO-PHOSPHATE (µg/L)
APPLICABLE CONC. RANGE	(5.00 - 95.90)
REAGENT WATER	
SINGLE-ANALYST PRECISION	SR = 0.012X + 0.31
OVERALL PRECISION	S = 0.030X + 0.41
MEAN RECOVERY	X = 1.004C - 0.38
SARGASSO SEA WATER	
SINGLE-ANALYST PRECISION	SR = 0.026X + 0.29
OVERALL PRECISION	S = 0.097X + 0.38
MEAN RECOVERY	X = 1.068C - 0.74
CHESAPEAKE BAY WATER	
SINGLE-ANALYST PRECISION	SR = 0.030X + 0.16
OVERALL PRECISION	S = 0.066X + 0.07
MEAN RECOVERY	X = 1.019C - 0.87

X = MEAN RECOVERY
C = TRUE VALUE FOR THE CONCENTRATION

Figure 1. MANIFOLD CONFIGURATION FOR ORTHO-PHOSPHATE