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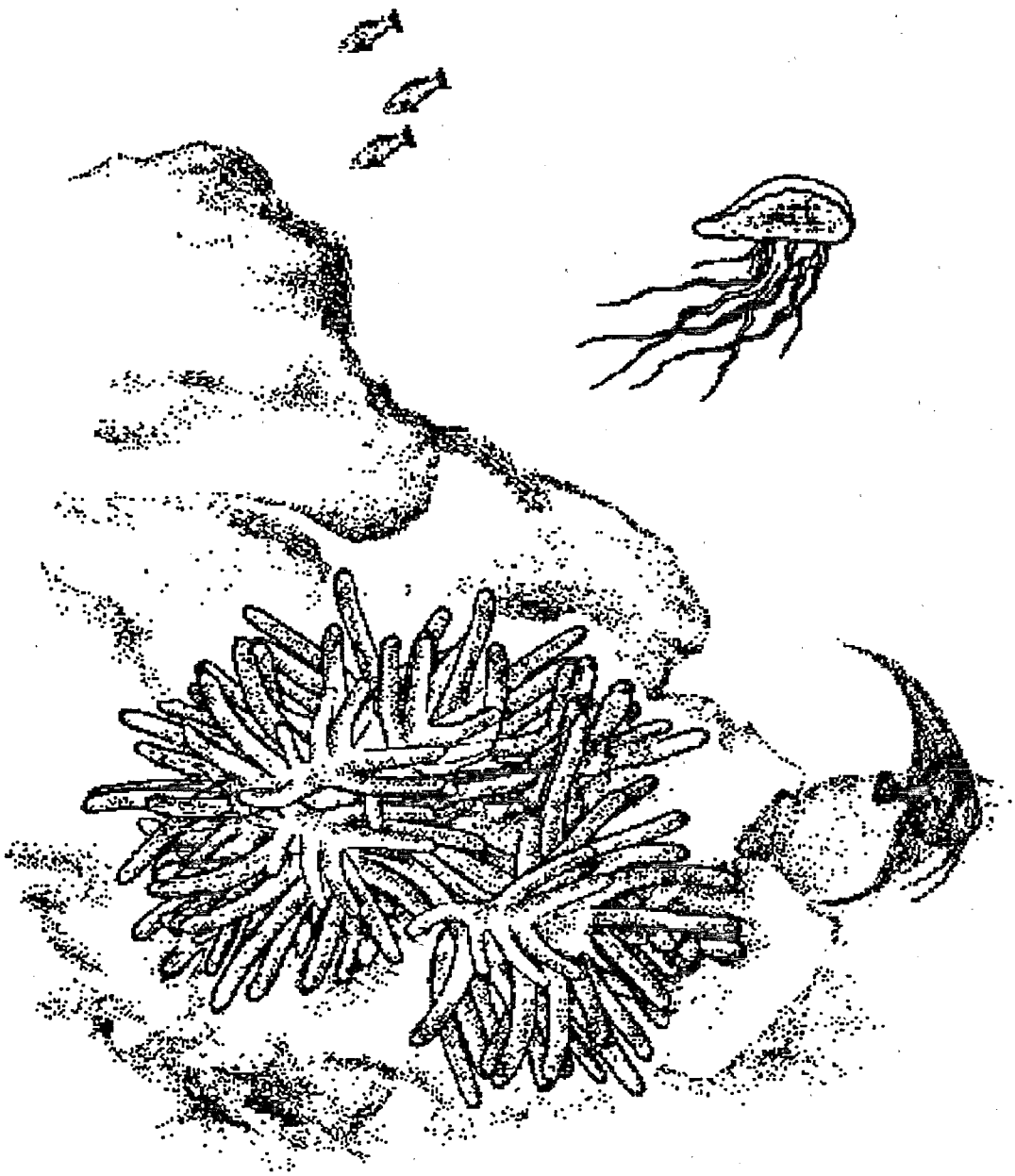
Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples



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Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples

Environmental Monitoring Systems Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Disclaimer

This 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, ground waters, 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 waters, surface waters, ground waters, wastewaters, sediments, and solid wastes.
- 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, "Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples" was prepared as the continuation of an initiative to gather together under a single cover a compendium of standardized laboratory analytical methods for the determination of nutrients, metals and organics in marine matrices. It is the goal of this initiative that the methods that appear in this manual will be multilaboratory validated. 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
Environmental Monitoring Systems
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Abstract

This manual contains seven methods for determination of nutrients, metals, and chlorophyll. Methods 353.4, revision 1.2, and 365.5, revision 1.3, for the measurement of nitrite + nitrate and orthophosphate, respectively, appeared in the 1991 interim manual. Since then they have undergone multilaboratory validation studies. Method 365.5 performed well in the study and multilaboratory data are presented in the revision of the method that appears here. The performance of Method 353.4 in the study indicated that the cadmium reduction column chemistry and maintenance require further investigation. The method has been retained in this manual so that further testing can continue using a standardized method description.

Method 440.0 for measurement of total particulate carbon and nitrogen is based upon a well established combustion technique. Procedures for partitioning the organic and inorganic fractions of carbon are also presented. A multilaboratory study is in progress, and the results will be included in a subsequent revision of the method.

The three metals methods represent current state-of-the-science in metals measurements. Two of the methods are graphite furnace atomic absorption techniques and the third uses inductively coupled plasma mass spectrometry. Single laboratory performance data are included in the methods. Although few laboratories currently have the instrumentation capabilities to perform all of these methods, it is extremely important to present them in order to stimulate the development of laboratory capability before multilaboratory studies can be conducted.

Method 445.0 is for the determination of chlorophyll *a* and the pheopigments using fluorescence detection. This method has been used for many years for low level measurement of chlorophyll. The method was evaluated using two natural water samples of primarily green and blue-green algae.

The numbering of methods was correlated with previous EMSL-Cincinnati methods whenever possible. The metals methods are 200 series, the nutrients nitrite + nitrate and orthophosphate are 300 series, and the particulate carbon and nitrogen, and chlorophyll methods are 400 series.

Contents

Acknowledgments

This methods manual was prepared by the Inorganic Chemistry Branch of the Chemistry Research Division, Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati). Individual manuscripts were prepared by the combined efforts of many people. The nutrient methods were prepared by Carl Zimmermann and Carolyn Keefe at the Chesapeake Biological Laboratory, University of Maryland, with the editorial assistance of Jerry Bashe and Stephen Long of Technology Applications, Inc. Additional contributions by James Longbottom, EMSL-Cincinnati, and Kenneth Edgell, The Bionetics Corporation, in the preparation and distribution of quality control samples and statistical evaluation of the data are very much appreciated.

The chlorophyll method evaluation was aided by the technical contributions of Gary Collins and Cornelius Weber both in EMSL-Cincinnati. Their expertise was and continues to be greatly appreciated. We would also like to thank John Macauley, Environmental Research Laboratory, Gulf Breeze, Florida, who provided 400 chlorophyll samples from Lake Pontchatrain, Louisiana. Those samples have allowed issues beyond chlorophyll measurement by fluorescence detection to be explored.

Diane Schirmann and Patricia Hurr provided invaluable assistance in manuscript production. Diane has no doubt read this manual more times than anyone else involved in its production. Their contributions were significant, and we thank them.

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. They provided insight from the regional perspective and served as technical advisors. Their reviews and comments to these methods were invaluable.

Introduction

During 1988, the coastal regions, led by Regions 2 and 3, began organizing and compiling a list of analytical methods research needs specific to the marine and estuarine monitoring community. In January 1990, after conducting an extensive survey, the 10 Regional Environmental Services Division Directors produced a document that contained seven marine and estuarine analytical methods research needs. This document was widely distributed and has been the basis for ordering research priorities. In May 1990, Regions 2 and 3, with support from the Office of Marine and Estuarine Protection (since renamed Office of Wetlands, Oceans, and Watersheds), held a workshop in Annapolis, Maryland, to bring together investigators from the marine and estuarine monitoring programs, representatives and experts from the private sector, and others with an interest in the marine environment. Their goals were to establish a network for technical exchange, restate analytical methods needs, and set a course of action. Toward that end, four workgroups were formed: (1) Nutrients, Demand, and Chlorophyll; (2) Metals; (3) Organics; and (4) Biologicals. Each workgroup was "charged with the collection, assembly, review, and evaluation of existing analytical methods and standard reference materials (SRMs) in saline water, sediments and biologicals." When methods or SRMs were identified, the workgroups were to present recommendations to the Office of Research and Development (ORD) for funding and further investigation. Nutrient methods and SRMs received the highest priority for immediate work.

In March 1991, William L. Budde (Director, Chemistry Research Division) and Larry Lobring (Chief, Inorganic Chemistry Branch) participated in a meeting at Region 2 with Barbara Metzger (Director, Environmental Services Division, Region 2), members of her staff, Claudia Walters (Chesapeake Bay Program, Region 3), Bettina Fletcher (Regional Operations, HQ), and Rich Pruell (Environmental Research Laboratory, Narragansett). The purpose of the meeting was to discuss priorities and planning for analytical methods research and development. The following immediate priorities were named from the seven priority items established by the regions in January 1990: (1) orthophosphate, nitrite + nitrate, particulate nutrients, and preservation studies; (2) nutrient reference materials; and (3) chlorophyll.

Larry Lobring, as Principal Investigator within ORD for the Marine Methods Initiative, subcontracted through Technology Applications, Inc., the Chesapeake Biological Laboratory (CBL) at the University of Maryland to evaluate the orthophosphate and nitrite + nitrate methods. CBL performed single-laboratory validation of the methods, wrote them in EMSL-Cincinnati format, and aided in the design and execution of the multilaboratory validation studies. In September 1991, an interim manual containing these two nutrient methods was delivered by EMSL-Cincinnati to all interested parties for review and comment.

During the last year, reviews of the nutrient methods have been duly noted, results of the two multilaboratory validation studies have been evaluated, key personnel have changed within EMSL-Cincinnati, and a subsequent meeting between EMSL-Cincinnati and the regions has reestablished priorities and renewed commitments by both parties to the mission of this initiative. William L. Budde, who replaced Larry Lobring as Principal Investigator, appointed Elizabeth J. Arar as the lead investigator for the nutrient methods, John T. Creed as lead investigator for the metals methods, and James W. Eichelberger as the lead investigator for the organics methods development effort. This team is responsible for current research in this area and the methods in this manual.

The principal aim of this manual is to bring together under one cover a suite of analytical methods specifically adapted or developed for the examination of marine and estuarine environmental samples. Three of the methods presented here are adaptations of analytical techniques that, for many years, have been used routinely by the marine community. Hallmarks of the methods that appear in this manual, however, are the integrated quality control/quality assurance requirements, the use of standardized terminology, and the use of the Environmental Monitoring Management Council (EMMC) methods format. The mandatory demonstration of laboratory capability and the continuing checks on method performance ensure the quality and comparability of data reported by different laboratories and programs. Another distinction of this manual is the eventual multilaboratory validation study of each method.

Multilaboratory validation studies test the ruggedness of methods, provide single-analyst and multilaboratory precision and accuracy statements and method detection limits that are "typical" of what most laboratories can achieve. Methods that reach this level of evaluation have been thoroughly investigated by a single laboratory and have usually been informally adopted as standard methods by the analytical community. Method 365.5, "Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis," a widely accepted method in the marine community, performed quite well in a multilaboratory study. A table has been added to the method to summarize single-analyst and multilaboratory precision and accuracy of the method for three water matrices. As a result of the study, pooled method detection limits for orthophosphate in a wide range of water salinities have also been added to the method.

On the other hand, Method 353.4, "Determination of Nitrite + Nitrate in Estuarine and Coastal Waters by Automated Colorimetric Analysis," did not give acceptable multilaboratory results, and it must return to the development phase. Method 353.4, despite its wide acceptance and routine use in the marine community, failed the ruggedness test when 50% of the participating laboratories in the multilaboratory study returned unacceptable data. Their data suggest that the cadmium reduction column chemistry and maintenance require further investigation. The method, nonetheless, appears in this manual with appropriate caveats for the user so that further testing can continue using a standardized method description.

Method 440.0 for particulate carbon and nitrogen uses a well established combustion technique and is currently undergoing multilaboratory validation. The results from that study will be incorporated into the next revision of this manual.

Method 445.0 for the *in vitro* determination of chlorophyll *a* and the pheopigments using fluorescence detection was evaluated using primarily freshwater phytoplankton samples. We do not believe this prohibits its inclusion in a marine methods manual since the analytical steps are the same regardless of algae classification. An effort was made to include a review of the current pertinent literature on chlorophyll measurement. A visible spectrophotometric method for chlorophyll *a, b,* and *c* and the carotenoids is not included in this edition of the manual because more research is required for a thorough evaluation of this method.

The three metals methods presented here represent current state-of-the-science in metals measurement and are suitable for low-level concentrations in high salinity waters. The two methods that use the chelation preconcentration chromatography system offer detection limits roughly an order of magnitude lower than their conventional counterpart methods. As the instrumentation for these techniques becomes more prevalent in analytical laboratories, the methods will undergo multilaboratory validation studies.

This manual should be viewed as a living document, with methods for organics, nutrients, and metals continually being added, updated, revised, and validated. There is also much

work to be done in assuring the provision of SRMs and quality control samples to the marine monitoring community. The energy to sustain this long-term effort comes from the commitment of personnel in Regions 2 and 3 and in EMSL-Cincinnati to the goals set by the coastal regions in 1990. We encourage users of the methods in this manual to share their experiences with us and to obtain new editions of the manual as they become available.

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 and metals in marine waters.

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Chemistry Research Division
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Method 200.10

Determination of Trace Elements in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry

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Revision 1.6
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Method 200.10

Determination of Trace Elements in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry

1.0 Scope and Application

1.1 This method describes procedures for preconcentration and determination of total recoverable trace elements in marine waters, including estuarine water, seawater, and brines.

1.2 Acid solubilization is required prior to the determination of total recoverable elements to facilitate breakdown of complexes or colloids that might influence trace element recoveries. This method should only be used for preconcentration and determination of trace elements in aqueous samples.

1.3 This method is applicable to the following elements:

Element		Chemical Abstracts Service Registry Numbers (CASRN)
Cadmium	(Cd)	7440-43-9
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Nickel	(Ni)	7440-02-0
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2

1.4 Method detection limits (MDLs) for these elements will be dependent on the specific instrumentation employed and the selected operating conditions. However, the MDLs should be essentially independent of the matrix because elimination of the matrix is a feature of the method. Reagent water MDLs, which were determined using the procedure described in Section 9.2.4, are listed in Table 1.

1.5 A minimum of 6-months experience in the use of commercial instrumentation for inductively coupled plasma mass spectrometry (ICP-MS) is recommended.

2.0 Summary of Method

2.1 This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin.[1,2] Following acid solubilization, the sample is buffered prior to the chelating column using an on-line system. Groups I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluted into a simplified matrix consisting of dilute nitric acid and are determined by ICP-MS using a directly coupled on-line configuration.

2.2 The determinative step in this method is ICP-MS.[3-5] Sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are registered by a continuous dynode electron multiplier or Faraday detector and the ion information is processed by a data handling system. Interferences relating to the technique (Section 4) must be recognized and corrected. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift must be corrected for by the use of internal standardization.

3.0 Definitions

3.1 ***Calibration Blank (CB)*** — A volume of reagent water fortified with the same matrix as the calibration standards but without the analytes, internal standards, or surrogate analytes.

3.2 ***Calibration Standard (CAL)*** — A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

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

3.4 ***Instrument Performance Check Solution (IPC)*** — A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.5 ***Internal Standard (IS)*** — A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

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

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

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

3.9 Linear Dynamic Range (LDR) — The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.10 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.

3.11 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.

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

3.13 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.

3.14 Total Recoverable Analyte (TRA) — The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid(s) as specified in the method.

3.15 Tuning Solution (TS)— A solution that is used to adjust instrument performance prior to calibration and sample analyses.

4.0 Interferences

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are:

4.1.1 Isobaric elemental interferences— Are caused by isotopes of different elements that form singly or doubly charged ions of the same nominal mass-to-charge ratio and that cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. The analytical isotopes recommended for use with this method are listed in Table 1.

4.1.2 Abundance sensitivity— Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

4.1.3 Isobaric polyatomic ion interferences— Are caused by ions consisting of more than one atom that have the same nominal mass-to-charge ratio as the isotope of interest and that cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions.

4.1.4 Physical interferences — Are associated with the physical processes that govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. Internal standardization may be effectively used to compensate for many physical interference effects.[6] Internal standards ideally should have similar analytical behavior to the elements being determined.

4.1.5 Memory interferences — Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones and from the buildup of sample material in the plasma torch and spray chamber. The site where these

effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. Memory interferences from the chelating system may be encountered especially after analyzing a sample containing high concentrations of the analytes. A thorough column rinsing sequence following elution of the analytes is necessary to minimize such interferences.

4.2 A principal advantage of this method is the selective elimination of species giving rise to polyatomic spectral interferences on certain transition metals (e.g., removal of the chloride interference on vanadium). As the majority of the sample matrix is removed, matrix induced physical interferences are also substantially reduced.

4.3 Low recoveries may be encountered in the preconcentration cycle if the trace elements are complexed by competing chelators in the sample or are present as colloidal material. Acid solubilization pretreatment is employed to improve analyte recovery and to minimize adsorption, hydrolysis, and precipitation effects.

5.0 Safety

5.1 Each chemical reagent used in this method should be regarded as a potential health hazard and exposure to these reagents should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[7,8] A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis.

5.2 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards.

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

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

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

6.0 Equipment and Supplies

6.1 ***Preconcentration System*** — System containing no metal parts in the analyte flow path, configured as shown in Figure 1.

6.1.1 Column — Macroporous iminodiacetate chelating resin (Dionex Metpac CC-1 or equivalent).

6.1.2 Sample loop — 10-mL loop constructed from narrow bore, high-pressure inert tubing, Tefzel ethylene tetra-fluoroethylene (ETFE) or equivalent.

6.1.3 Eluent pumping system (P1) — Programmable flow, high pressure pumping system, capable of delivering either one of two eluents at a pressure up to 2000 psi and a flow rate of 1-5 mL/min.

6.1.4 Auxiliary pumps — On line buffer pump (P2), piston pump (Dionex QIC pump or equivalent) for delivering 2M ammonium acetate buffer solution; *carrier pump (P3),* peristaltic pump (Gilson Minipuls or equivalent) for delivering 1% nitric acid carrier solution; *sample pump (P4),* peristaltic pump for loading sample loop.

6.1.5 Control valves — Inert double stack, pneumatically operated four-way slider valves with connectors.

6.1.5.1 Argon gas supply regulated at 80-100 psi.

6.1.6 Solution reservoirs — Inert containers, e.g., high density polyethylene (HDPE), for holding eluent and carrier reagents.

6.1.7 Tubing — High pressure, narrow bore, inert tubing (e.g., Tefzel ETFE or equivalent) for interconnection of pumps/valve assemblies and a minimum length for connection of the preconcentration system to the ICP-MS instrument.

6.2 Inductively Coupled Plasma - Mass Spectrometer

6.2.1 Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system.

6.2.2 Argon gas supply (high-purity grade, 99.99%).

6.2.3 A mass-flow controller on the nebulizer gas supply is recommended. A water-cooled spray chamber may be of benefit in reducing some types of interferences (e.g., polyatomic oxide species).

6.2.4 Operating conditions — Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer.

6.2.5 If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.

6.3 ***Labware*** — For the determination of trace elements, contamination and loss are of **critical** concern. Potential contamination sources include improperly

cleaned laboratory apparatus and general contamination within the laboratory environment. A clean laboratory work area, designated for trace element sample handling, must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching or (2) depleting element concentrations through adsorption processes. For these reasons, borosilicate glass is *not* recommended for use with this method. All labware in contact with the sample should be cleaned prior to use. Labware may be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for 4 hr in a mixture of dilute nitric and hydrochloric acids, followed by rinsing with ASTM type I water and oven drying.

6.3.1 Griffin beakers, 250-mL, polytetrafluoroethylene (PTFE) or quartz.

6.3.2 Storage bottles — Narrow mouth bottles, Teflon FEP (fluorinated ethylene propylene), or HDPE, 125-mL and 250-mL capacities.

6.4 Sample Processing Equipment

6.4.1 Air displacement pipetter — Digital pipet system capable of delivering volumes from 10 to 2500 µL with an assortment of metal-free, disposable pipet tips.

6.4.2 Balances — Analytical balance, capable of accurately weighing to ± 0.1 mg; top pan balance, accurate to ± 0.01 g.

6.4.3 Hot plate — Corning PC100 or equivalent.

6.4.4 Centrifuge — Steel cabinet with guard bowl, electric timer and brake.

6.4.5 Drying oven — Gravity convection oven with thermostatic control capable of maintaining 105°C ± 5°C.

6.4.6 pH meter — Bench mounted or hand-held electrode system with a resolution of ± 0.1 pH units.

7.0 Reagents and Standards

7.1 Water — For all sample preparation and dilutions, ASTM type I water (ASTM D1193) is required.

7.2 Reagents may contain elemental impurities that might affect the integrity of analytical data. Because of the high sensitivity of this method, ultra high-purity reagents must be used unless otherwise specified. To minimize contamination, reagents should be prepared directly in their designated containers where possible.

7.2.1 Acetic acid, glacial (sp. gr. 1.05).

7.2.2 Ammonium hydroxide (20%).

7.2.3 Ammonium acetate buffer 1M, pH 5.5 — Add 58 mL (60.5 g) of glacial acetic acid to 600 mL of ASTM type water. Add 65 mL (60 g) of 20% ammonium hydroxide and mix. Check the pH of the resulting solution by withdrawing a small aliquot and testing with a calibrated pH meter, adjusting the solution to pH 5.5 ± 0.1 with small volumes of acetic acid or ammonium hydroxide as necessary. Cool and dilute to 1 L with ASTM type I water.

7.2.4 Ammonium acetate buffer 2M, pH 5.5 — Prepare as for Section 7.2.3 using 116 mL (121 g) glacial acetic acid and 130 mL (120 g) 20% ammonium hydroxide, diluted to 1000 mL with ASTM type I water.

Note: The ammonium acetate buffer solutions may be further purified by passing them through the chelating column at a flow rate of 5.0 mL/min. With reference to Figure 1, pump the buffer solution through the column using pump P1, with valves A and B off and valve C on. Collect the purified solution in a container at the waste outlet. Following this, elute the collected contaminants from the column using 1.25M nitric acid for 5 min at a flow rate of 4.0 mL/min.

7.2.5 Nitric acid, concentrated (sp.gr. 1.41).

7.2.5.1 Nitric acid 1.25M — Dilute 79 mL (112 g) conc. nitric acid to 1000 mL with ASTM type I water.

7.2.5.2 Nitric acid 1% — Dilute 10 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.2.5.3 Nitric acid (1+1) — Dilute 500 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.2.5.4 Nitric acid (1+9) — Dilute 100 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.2.6 Oxalic acid dihydrate (CASRN 6153-56-6), 0.2M — Dissolve 25.2 g reagent grade $C_2H_2O_4 \cdot 2H_2O$ in 250 mL ASTM type I water and dilute to 1000 mL with ASTM type I water. **Caution** - Oxalic acid is toxic; handle with care.

7.3 Standard Stock Solutions — May be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure). All salts should be dried for 1 h at 105°C, unless otherwise specified. (**Caution**- Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.) Stock solutions should be stored in plastic bottles. The following procedures may be used for preparing standard stock solutions:

Note: Some metals, particularly those that form surface oxides require cleaning prior to being weighed. This may be achieved by pickling the surface of the metal in acid. An amount in excess of the desired weight should be pickled repeatedly, rinsed with water, dried, and weighed until the desired weight is achieved.

7.3.1 Cadmium solution, stock 1 mL = 1000 µg Cd: Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid,

heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.2 Cobalt solution, stock 1 mL = 1000 µg Co: Pickle cobalt metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.3 Copper solution, stock 1 mL = 1000 µg Cu: Pickle copper metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.4 Indium solution, stock 1 mL = 1000 µg In: Pickle indium metal in (1+1) nitric acid to an exact weight of 0.100 g. Dissolve in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.5 Lead solution, stock 1 mL = 1000 µg Pb: Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with ASTM type I water.

7.3.6 Nickel solution, stock 1 mL = 1000 µg Ni: Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.7 Scandium solution, stock 1 mL = 1000 µg Sc: Dissolve 0.1534 g Sc_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.8 Terbium solution, stock 1 mL = 1000 µg Tb: Dissolve 0.1176 g Tb_4O_7 in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.9 Uranium solution, stock 1 mL = 1000 µg U: Dissolve 0.2110 g $UO_2(NO_3)_2 \cdot 6H_2O$ (Do Not Dry) in 20 mL ASTM type I water. Add 2 mL (1+1) nitric acid and dilute to 100 mL with ASTM type I water.

7.3.10 Vanadium solution, stock 1 mL = 1000 µg V: Pickle vanadium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.11 Yttrium solution, stock 1 mL = 1000 µg Y: Dissolve 0.1270 g Y_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.4 Multielement Stock Standard Solution — Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for impurities that might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, new FEP or HDPE bottles for storage and monitored periodically for stability. A multielement stock standard solution containing the elements, cadmium, cobalt, copper, lead, nickel, uranium, and vanadium (1 mL = 10 µg) may be prepared by diluting 1 mL of each single element stock in the list to 100 mL with ASTM type I water containing 1% (v/v) nitric acid.

7.4.1 Preparation of calibration standards — Fresh multielement calibration standards should be prepared weekly. Dilute the stock multielement standard solution in 1% (v/v) nitric acid to levels appropriate to the required operating range. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. A suggested mid-range concentration is 10 µg/L.

7.5 Blanks— Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, and the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure. The laboratory fortified blank is used to assess the recovery of the method analytes and the rinse blank is used between samples to minimize memory from the nebulizer/spray chamber surfaces.

7.5.1 Calibration blank— Consists of 1% (v/v) nitric acid in ASTM type I water (Section 7.2.5.2).

7.5.2 Laboratory reagent blank (LRB)— Must contain all the reagents in the same volumes as used in processing the samples. The LRB must be carried through the entire sample digestion and preparation scheme.

7.5.3 Laboratory Fortified Blank (LFB)— To an aliquot of LRB, add aliquots from the multielement stock standard (Section 7.4) to produce a final concentration of 10 µg/L for each analyte. The fortified blank must be carried through the entire sample pretreatment and analytical scheme.

7.5.4 Rinse Blank (RB) — Is a 1% (v/v) nitric acid solution that is delivered to the ICP-MS between samples (Section 7.2.5.2).

7.6 Tuning Solution — This solution is used for instrument tuning and mass calibration prior to analysis (Section 10.2). The solution is prepared by mixing nickel, yttrium, indium, terbium, and lead stock solutions (Section 7.3) in 1% (v/v) nitric acid to produce a concentration of 100 µg/L of each element.

7.7 Quality Control Sample (QCS) — A quality control sample having certified concentrations of the analytes of interest should be obtained from a source outside the laboratory. Dilute the QCS if necessary with 1% nitric acid, such that the analyte concentrations fall within the proposed instrument calibration range.

7.8 Instrument Performance Check (IPC) Solution — The IPC solution is used to periodically verify instrument performance during analysis. It should be prepared by combining method analytes at appropriate concentra-

tions to approximate the midpoint of the calibration curve. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.9 Internal Standards Stock Solution, 1 mL = 100 µg. — Dilute 10 mL of scandium, yttrium, indium, terbium, and bismuth stock standards (Section 7.3) to 100 mL with ASTM type I water, and store in a Teflon bottle. Use this solution concentrate for addition to blanks, calibration standards and samples (Method A, Section 10.5), or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump (Method B, Section 10.5).

Note: Bismuth should not be used as an internal standard using the direct addition method (Method A, Section 10.5) as it is not efficiently concentrated on the iminodiacetate column.

8.0 Sample Collection, Preservation, and Storage

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, sc that appropriate preservation and pretreatment steps can be taken. Acid preservation should be performed at the time of sample collection or as soon thereafter as practically possible. The pH of all aqueous samples must be tested immediately prior to aliquoting for analysis to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

8.2 For the determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid (high purity) at the time of collection to pH < 2; normally, 3 mL of (1+1) acid per liter of sample is sufficient for most samples. The sample should not be filtered prior to analysis.

Note: Samples that cannot be acid preserved at the time of collection because of sampling limitations or transport restrictions, or are > pH 2 because of high alkalinity should be acidified with nitric acid to pH < 2 upon receipt in the laboratory. Following acidification, the sample should be held for 16 h and the pH verified to be < 2 before withdrawing an aliquot for sample processing.

8.3 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and the 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 generated.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to samples being analyzed by this method.

9.2.2 Linear calibration ranges — The upper limit of the linear calibration range should be established for each analyte. Linear calibration ranges should be determined every six months or whenever a significant change in instrument response is expected.

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 (Section 7.7). 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 method detection limits or continuing with ongoing 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.[9] 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].

S = standard deviation of the replicate analyses.

Note: If the relative standard deviation (RSD) from the analyses of the seven aliquots is < 15%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. If additional confirmation of the MDL is desired, reanalyze the seven replicate aliquots on two more

nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 1.

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 (Mandatory)

9.3.1 Laboratory reagent blank (LRB) — The laboratory must analyze at least one LRB (Section 7.5.2) with each batch of 20 or fewer samples. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. Any determined source of contamination must be corrected and the samples reanalyzed for the affected analytes after acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) — The laboratory must analyze at least one LFB (Section 7.5.3) with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.3). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

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

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

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

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

9.3.4 Instrument performance check (IPC) solution — For all determinations the laboratory must analyze the IPC solution (Section 7.8) 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 within ± 15%. 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 the sample analyses data.

9.3.5 The overall sensitivity and precision of this method are strongly influenced by a laboratory's ability to control the method blank. Therefore, it is recommended that the calibration blank response be recorded for each set of samples. This record will aid the laboratory in assessing both its long- and short-term ability to control the method blank.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess these effects. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Section 9.4.2) is required.

9.4.2 The laboratory must add a known amount of each analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 9.3.2).

9.4.3 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 of 75-125%. Recovery calculations are not required if the concentration added is less than 25% of the unfortified sample concentration. Percent recovery may be calculated in units appropriate to the matrix, 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.

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

9.4.5 If analysis of LFM sample(s) and the test routines above indicate an operative interference and the LFMs are typical of the other samples in the batch, those samples that are similar must be analyzed in the same manner as the LFMs. Also, the data user must be informed when a matrix interference is so severe that it prevents the successful analysis of the analyte or when the heterogeneous nature of the sample precludes the use of duplicate analyses.

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.

10.0 Calibration and Standardization

10.1 Initiate proper operating configuration of ICP-MS instrument and data system. Allow a period of not less than 30 min for the instrument to warm up. During this process conduct mass calibration and resolution checks using the tuning solution. Resolution at low mass is indicated by nickel isotopes 60, 61, 62. Resolution at high mass is indicated by lead isotopes 206, 207, 208. For good performance adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass.

10.2 Instrument stability must be demonstrated by analyzing the tuning solution (Section 7.6) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.

10.3 Prior to initial calibration, set up proper instrument software routines for quantitative analysis and connect the ICP-MS instrument to the preconcentration apparatus. The instrument must be calibrated for the analytes of interest using the calibration blank (Section 7.5.1) and calibration standard (Section 7.4.1) prepared at one or more concentration levels. The calibration solutions should be processed through the preconcentration system using the procedures described in Section 11.

10.4 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After initial calibration is successful, a calibration check is required at the beginning and end of each period during which analyses are performed and at requisite intervals.

10.4.1 After the calibration has been established, it must be initially verified for all analytes by analyzing the IPC (Section 7.8). If the initial calibration verification exceeds ± 10% of the established IPC value, the analysis should be terminated, the source of the problem identified and corrected, the instrument recalibrated, and the new calibration verified before continuing analyses.

10.4.2 To verify that the instrument is properly calibrated on a continuing basis, analyze the calibration blank (Section 7.5.1) and IPC (Section 7.8) after every 10 analyses. The results of the analyses of the standards will indicate whether the calibration remains valid. If the indicated concentration of any analyte deviates from the true concentration by more than 15%, reanalyze the standard. If the analyte is again outside the 15% limit, the instrument must be recalibrated and the previous 10 samples reanalyzed. The instrument responses from the calibration check may be used for recalibration purposes.

10.5 Internal Standardization - Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. For full mass range scans, a minimum of three internal standards must be used. Internal standards must be present in all samples, standards and blanks at identical levels. This may be achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A), or alternatively by mixing with the solution prior to nebulization using a second channel of the peristaltic pump and a mixing coil (Method B). The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. Internal standards should be added to blanks, samples and standards in a like manner, so that dilution effects resulting from the addition may be disregarded.

Note: Bismuth should not be used as an internal standard using the direct addition method (Method A, Section 10.5) because it is not efficiently concentrated on the iminodiacetate column.

11.0 Procedure

11.1 Sample Preparation - Total Recoverable Elements

11.1.1 Add 2-mL (1+1) nitric acid to the beaker containing 100 mL of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating, adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the

center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.1.2 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. **Do Not Boil.** This step takes about 2 h for a 100-mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.1.3 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 min. (Slight boiling may occur, but vigorous boiling must be avoided.)

11.1.4 Allow the beaker to cool. Quantitatively transfer the sample solution to a 100-mL volumetric flask, dilute to volume with reagent water, stopper and mix.

11.1.5 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight, the sample contains suspended solids, a portion of the sample may be filtered prior to analysis. However, care should be exercised to avoid potential contamination from filtration.) The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.2 Prior to first use, the preconcentration system should be thoroughly cleaned and decontaminated using 0.2M oxalic acid.

11.2.1 Place approximately 500-mL 0.2M oxalic acid in all the eluent/solution containers and fill the sample loop with 0.2M oxalic acid using the sample pump (P4) at a flow rate of 3-5 mL/min. With the preconcentration system disconnected from the ICP-MS instrument, use the pump program sequence listed in Table 2 to flush the complete system with oxalic acid. Repeat the flush sequence three times.

11.2.2 Repeat the sequence described in Section 11.2.1 using 1.25M nitric acid and again using ASTM type I water in place of the 0.2M oxalic acid.

11.2.3 Rinse the containers thoroughly with ASTM type I water, fill them with their designated reagents (see Figure 1) and run through the sequence in Table 2 once to prime the pump and all eluent lines with the correct reagents.

11.3 Initiate ICP-MS instrument operating configuration. Tune the instrument for the analytes of interest (Section 10).

11.4 Establish instrument software run procedures for quantitative analysis. Because the analytes are eluted from the preconcentration column in a transient manner, it is recommended that the instrument software is configured in a rapid scan/peak hopping mode. The instrument is now ready to be calibrated.

11.5 Reconnect the preconcentration system to the ICP-MS instrument. With valves A and B in the off position and valve C in the on position, load sample through the sample loop to waste using pump P4 for 4 min at 4 mL/min. Switch on the carrier pump (P3) and pump 1% nitric acid to the nebulizer of the ICP-MS instrument at a flow rate of 0.8-1.0 mL/min.

11.6 Switch on the buffer pump (P2), and pump 2M ammonium acetate at a flow rate of 1.0 mL/min.

11.7 Preconcentration of the sample may be achieved by running through an eluent pump program (P1) sequence similar to that illustrated in Table 2. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.7.1 Inject sample—With valves A, B, and C on, load sample from the loop onto the column using 1M ammonium acetate for 4.5 min at 4.0 mL/min. The analytes are retained on the column, while the majority of the matrix is passed through to waste.

11.7.2 Elute analytes — Turn off valves A and B and begin eluting the analytes by pumping 1.25M nitric acid through the column at 4.0 mL/min, then turn off valve C and pump the eluted analytes into the ICP-MS instrument at 1.0 mL/min. Initiate ICP-MS software data acquisition and integrate the eluted analyte profiles.

11.7.3 Column Reconditioning — Turn on valve C to direct column effluent to waste, and pump 1.25M nitric acid, 1M ammonium acetate, 1.25M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min. During this process, the next sample can be loaded into the sample loop using the sample pump (P4).

11.8 Repeat the sequence described in Section 11.7 for each sample to be analyzed. At the end of the analytical run leave the column filled with 1M ammonium acetate buffer until it is next used.

11.9 Samples having concentrations higher than the established linear dynamic range should be diluted into range with 1% HNO_3 (v/v) and reanalyzed.

12.0 Data Analysis and Calculations

12.1 Analytical isotopes and elemental equations recommended for sample data calculations are listed in Table 3. Sample data should be reported in units of µg/L. Do not report element concentrations below the determined MDL.

12.2 For data values less than 10, two significant figures should be used for reporting element concentrations. For data values greater than or equal to 10, three significant figures should be used.

12.3 Reported values should be calibration blank subtracted. If additional dilutions were made to any samples, the appropriate factor should be applied to the calculated sample concentrations.

12.4 Data values should be corrected for instrument drift by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data.

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

13.0 Method Performance

13.1 Experimental conditions used for single laboratory testing of the method are summarized in Table 4.

13.2 Data obtained from single laboratory testing of the method are summarized in Tables 5 and 6 for two reference water samples consisting of National Research Council Canada (NRCC) Estuarine Water (SLEW-1) and Seawater (NASS-2). The samples were prepared using the procedure described in Section 11.1.1. For each matrix, three replicates were analyzed and the average of the replicates was used to determine the sample concentration for each analyte. Two further sets of three replicates were fortified at different concentration levels, one set at 0.5 µg/L, the other at 10 µg/L. The sample concentration, mean percent recovery, and the relative standard deviation of the fortified replicates are listed for each method analyte. The reference material certificate values are also listed for comparison.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that place pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

15.0 Waste Management

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

16.0 References

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9. Code of Federal Regulations *40*, Ch. 1, Pt. 136 Appendix B.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Total Recoverable Method Detection Limits for Reagent Water

Element	Recommended Analytical Mass	MDL[1] µg/L
Cadmium	111	0.041
Cobalt	59	0.021
Copper	63	0.023
Lead	206,207,208	0.074
Nickel	60	0.081
Uranium	238	0.031
Vanadium	51	0.014

[1]Determined using 10-mL sample loop.

Table 2. Eluent Pump Programming Sequence for Preconcentration of Trace Elements

Time (min)	Flow (mL/min)	Eluent	Valve A,B	Valve C
0.0	4.0	1M ammonium acetate	ON	ON
4.5	4.0	1.25M nitric acid	ON	ON
5.1	1.0	1.25M nitric acid	OFF	ON
5.5	1.0	1.25M nitric acid	OFF	OFF
7.5	4.0	1.25M nitric acid	OFF	ON
8.0	4.0	1M ammonium acetate	OFF	ON
10.0	4.0	1.25M nitric acid	OFF	ON
11.0	4.0	1M ammonium acetate	OFF	ON
12.5	0.0		OFF	ON

Table 3. Recommended Analytical Isotopes and Elemental Equations for Data Calculations

Element	Isotope	Elemental Equation	Note
Cd	106,108,*111*,114	$(1.000)(^{111}C)-(1.073)[(^{108}C)-(0.712)(^{106}C)]$	(1)
Co	59	$(1.000)(^{59}C)$	
Cu	*63*,65	$(1.000)(^{63}C)$	
Pb	*206,207,208*	$(1.000)(^{206}C)+(1.000)(^{207}C)+(1.000)(^{208}C)$	(2)
Ni	60	$(1.000)(^{60}C)$	
U	238	$(1.000)(^{238}C)$	
V	51	$(1.000)(^{51}C)$	

C - calibration blank subtracted counts at specified mass.
(1) - correction for MoO interference. An additional isobaric elemental correction should be made if palladium is present.
(2) - allowance for isotopic variability of lead isotopes.
NOTE: As a minimum, all isotopes listed should be monitored. Isotopes recommended for analytical determination are italicized.

Table 4. Experimental Conditions for Single Laboratory Validation

Chromatography	
Instrument	Dionex chelation system
Preconcentration column	Dionex MetPac CC-1
ICP-MS Instrument Conditions	
Instrument	VG PlasmaQuad Type I
Plasma forward power	1.35 kW
Coolant flow rate	13.5 L/min
Auxiliary flow rate	0.6 L/min
Nebulizer flow rate	0.78 L/min
Internal standards	Sc, Y, In, Tb
Data Acquisition	
Detector mode	Pulse counting
Mass range	45-240 amu
Dwell time	160 µs
Number of MCA channels	2048
Number of scan sweeps	250

Table 5. Precision and Recovery Data for Estuarine Water (SLEW-1)

Analyte	Certificate (µg/L)	Sample Conc. (µg/L)	Spike Addition (µg/L)	Average Recovery (%)	RSD (%)	Spike Addition (µg/L)	Average Recovery (%)	RSD (%)
Cd	0.018	<0.041	0.5	94.8	9.8	10	99.6	1.1
Co	0.046	0.078	0.5	102.8	4.0	10	96.6	1.4
Cu	1.76	1.6	0.5	106.0	2.7	10	96.0	4.8
Pb	0.028	<0.074	0.5	100.2	4.0	10	106.9	5.8
Ni	0.743	0.83	0.5	100.0	1.5	10	102.0	2.1
U	—	1.1	0.5	96.7	7.4	10	98.1	3.6
V	—	1.4	0.5	100.0	3.2	10	97.0	4.5

— No certificate value

Table 6. Precision and Recovery Data for Seawater (NASS-2)

Analyte	Certificate (µg/L)	Sample Conc. (µg/L)	Spike Addition (µg/L)	Average Recovery (%)	RSD (%)	Spike Addition (µg/L)	Average Recovery (%)	RSD (%)
Cd	0.029	<0.041	0.5	101.8	1.0	10	96.4	3.7
Co	0.004	<0.021	0.5	98.9	3.0	10	99.2	1.7
Cu	0.109	0.12	0.5	95.8	2.3	10	93.1	0.9
Pb	0.039	<0.074	0.5	100.6	8.5	10	92.1	2.6
Ni	0.257	0.23	0.5	102.2	2.3	10	98.2	1.2
U	3.00	3.0	0.5	94.0	0.7	10	98.4	1.7
V	—	1.7	0.5	104.0	3.4	10	109.2	3.7

— No certificate value

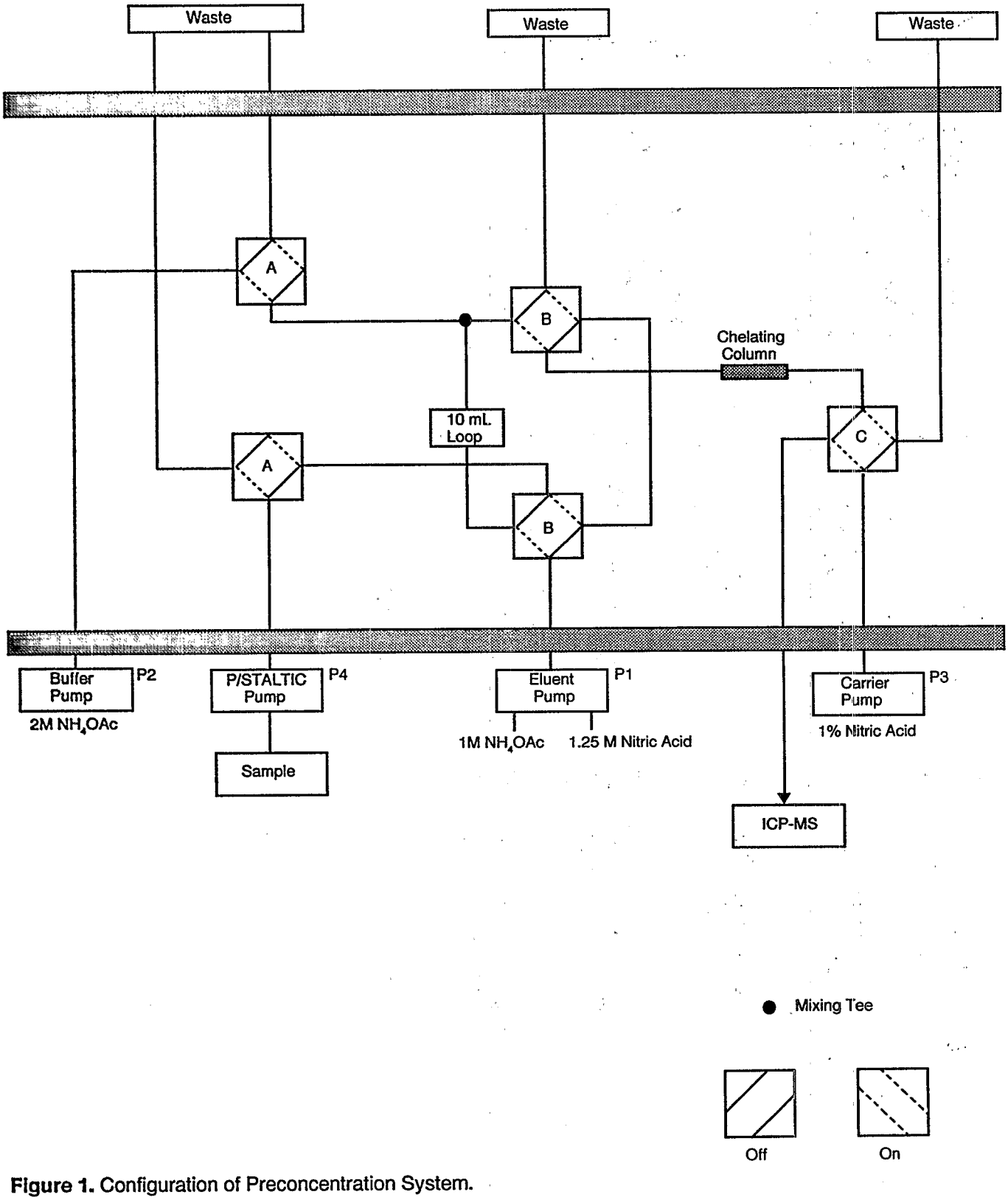

Figure 1. Configuration of Preconcentration System.

Method 200.12

Determination of Trace Elements in Marine Waters by Stabilized Temperature Graphite Furnace Atomic Absorption

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Method 200.12

Determination of Trace Elements in Marine Waters by Stabilized Temperature Graphite Furnace Atomic Absorption

1.0 Scope and Application

1.1 This method provides procedures for the determination of total recoverable elements by graphite furnace atomic absorption (GFAA) in marine waters, including estuarine, ocean and brines with salinities of up to 35 ppt. This method is applicable to the following analytes:

Analyte		Chemical Abstracts Service Registry Numbers (CASRN)
Arsenic	(As)	7440-38-2
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2

1.2 For determination of total recoverable analytes in marine waters, a digestion/extraction is required prior to analysis.

1.3 Method detection limit and instrumental operating conditions for the applicable elements are listed in Tables 1 and 2. These are intended as a guide and are typical of a commercial instrument optimized for the element. However, actual method detection limits and linear working ranges will be dependent on the sample matrix, instrumentation and selected operating conditions.

1.4 Users of the method data should state the data quality objectives prior to analysis. The ultra-trace metal concentrations typically associated with marine water may preclude the use of this method based on its sensitivity. Users of the method must document and have on file the required initial demonstration performance data described in Section 9.2 prior to using the method for analysis.

2.0 Summary of Method

2.1 Nitric acid is dispensed into a beaker containing an accurately weighed or measured, well-mixed, homogeneous aqueous sample. Then, for samples with undissolved material, the beaker is covered with a watch glass and heated, made up to volume, centrifuged or allowed to settle, and the sample is then analyzed.

2.2 The analytes listed in this method are determined by stabilized temperature platform graphite furnace atomic absorption (STPGFAA). In STPGFAA, the sample and the matrix modifier are first pipetted onto the platform or a device which provides delayed atomization. The furnace chamber is then purged with a continuous flow of a premixed gas (95% argon - 5% hydrogen) and the sample is dried at a relatively low temperature (about 120°C) to avoid spattering. Once dried, the sample is pretreated in a char or ashing step which is designed to minimize the interference effects caused by the concomitant sample matrix. After the char step, the furnace is allowed to cool prior to atomization. The atomization cycle is characterized by rapid heating of the furnace to a temperature where the metal (analyte) is atomized from the pyrolytic graphite surface into a stopped gas flow atmosphere of argon containing 5% hydrogen. (Only selenium is determined in an atmosphere of high purity argon.) The resulting atomic cloud absorbs the element-specific atomic emission produced by a hollow cathode lamp (HCL) or an electrodeless discharge lamp (EDL). Following analysis, the furnace is subjected to a cleanout period of high temperature and continuous argon flow. Because the resulting absorbance usually has a nonspecific component associated with the actual analyte absorbance, Zeeman background correction is required to subtract from the total signal the component which is nonspecific to the analyte. In the absence of interferences, the background-corrected absorbance is directly related to the concentration of the analyte. Interferences relating to STPGFAA (Section 4.0) must be recognized and corrected. Suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the method of standard addition (Section 11.3).

3.0 Definitions

3.1 ***Calibration Blank (CB)*** — A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 ***Calibration Standard (CAL)*** — A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

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

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

3.5 Instrument Performance Check Solution (IPC) — A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

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

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

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

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

3.10 Linear Dynamic Range (LDR) — The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.11 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.

3.12 Matrix Modifier (MM) — A substance added to the instrument along with the sample in order to minimize the interference effects by selective volatilization of either analyte or matrix components.

3.13 Matrix Performance Check (MPC) — A solution of method analytes used to evaluate the laboratory's ongoing capabilities in analyzing high salinity samples. The reference material NASS-3 or its equivalent is fortified with the same analytes at the same concentration as the LFB. This provides an ongoing check of furnace operating conditions to assure the analyte false positives are not being introduced via elevated backgrounds.

3.14 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.

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

3.16 Standard Addition — The addition of a known amount of analyte to the sample in order to determine the relative response of the detector to an analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration.

3.17 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.

3.18 Total Recoverable Analyte (TRA) — The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid(s) as specified in the method.

4.0 Interferences

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by GFAA. These interferences can be classified into three major subdivisions: spectral, matrix, and memory.

4.2 Spectral interferences are caused by absorbance of light by a molecule or atom which is not the analyte of interest or emission from black body radiation.

4.2.1 Spectral interferences caused by an element only occur if there is a spectral overlap between the wavelength of the interfering element and the analyte of interest. Fortunately, this type of interference is relatively uncommon in STPGFAA because of the narrow atomic line widths associated with STPGFAA. In addition, the use of appropriate furnace temperature programs and high spectral purity lamps as light sources can minimize the possibility of this type of interference. However, molecular absorbances can span several hundred nanometers producing broadband spectral interferences. This type of interference is far more common in STPGFAA. The use of matrix modifiers, selective volatilization, and background correctors are all attempts to eliminate unwanted nonspecific absorbance. Table 2 contains typical background absorbances associated with the analysis of

the MPC solution (NASS-3) which has a salinity of 35 ppt. These background absorbances were obtained using the suggested matrix modifiers and the appropriate furnace charring conditions. Figure 1 is a plot of integrated background absorbance vs. char temperature for Ni, Cd, Pb, and Se. Figure 1 indicates that the background absorbance in a saline matrix is strongly affected by the char temperature. Therefore, char temperature optimization is a critical part of the successful analysis of metals in saline water by GFAA. The elevated backgrounds associated with ocean water can produce false positives. For this reason, the char temperature profiles shown in Figure 1 should be constructed for each analyte prior to using this method for saline water analysis.

Note: False analyte positives can be generated by large backgrounds. Figure 2 is an atomization profile for Pb using a 800°C char temperature. The background shown in the figure has exceeded the capabilities of the Zeeman corrector. This profile can be used as a guide in screening other analyses which may have background absorbances which exceed the Zeeman capability. The background profile is characterized by a smooth baseline in the beginning of the atomization cycle followed by a sharp increase. During this sharp increase the background peak profile may remain relatively smooth, but when the background exceeds the Zeeman correction capability, the background profile will appear extremely erratic. The atomic profile is also erratic during this part of the atomization cycle. These types of background/atomic profiles obtained during atomization result in false positives.

Since the nonspecific component of the total absorbance can vary considerably from sample type to sample type, to provide effective background correction and eliminate the elemental spectral interference of palladium on copper and iron on selenium, the exclusive use of Zeeman background correction is specified in this method.

4.2.2 Spectral interferences are also caused by black body radiation produced during the atomization furnace cycle. This black body emission reaches the photomultiplier tube, producing erroneous results. The magnitude of this interference can be minimized by proper furnace tube alignment and monochromator design. In addition, atomization temperatures which adequately volatilize the analyte of interest without producing unnecessary black body radiation can help reduce unwanted background emission produced during atomization.

4.3 Matrix interferences are caused by sample components which inhibit the formation of free atomic analyte atoms during atomization. In this method the use of a delayed atomization device which provides a warmer gas phase environment during atomization is required. These devices provide an environment which is more conducive to the formation of free analyte atoms and thereby minimize this type of interference. This type of interference can be detected by analyzing the sample plus a sample aliquot fortified with a known concentration of the analyte. If the determined concentration of the analyte addition is outside a designated range (Section 9.4.3), a possible matrix effect should be suspected. In addition, the matrix can produce analyte complexes which are lost via volatilization during the char. These losses will result in poor recovery of the analyte within the matrix and should be corrected by adjusting the char temperature.

4.4 Memory interferences result from analyzing a sample containing a high concentration of an element (typically a high atomization temperature element) which cannot be removed quantitatively in one complete set of furnace steps. The analyte which remains in the furnace can produce false positive signals on subsequent sample(s). Therefore, the analyst should establish the analyte concentration which can be injected into the furnace and adequately removed in one complete set of furnace cycles. If this concentration is exceeded, the sample should be diluted and a blank analyzed to assure the memory effect has been eliminated before reanalyzing the diluted sample.

4.5 ***Specific Element Interferences.*** The matrix effects caused by the saline water can be severe. In order to evaluate the extent of the matrix suppression as a function of increasing salinity a plot of normalized integrated absorbance vs. microliters NASS-3 (Reference Material from the National Research Council of Canada) is constructed. Figure 3 is a plot of relative response of As, Se, Cd, Ni, Cu, and Pb in waters containing salinity of 3.5 ppt (1 µL NASS-3) to 35 ppt (10 µL NASS-3). Figure 3 indicates that the matrix effects caused by the increasing salinity are minor for Pb, Cu, and Ni. The relative responses of Pb, Ni, and Cu shown in Figure 3 are within ± 5% of the 1% HNO_3 standard or zero µL of matrix. Figure 3 indicates that the increasing salinity does cause a substantial matrix interference for Se and Cd. This suppression must be compensated for by methods of standard addition or the use of matrix matched standards where applicable.

4.5.1 Cadmium: The background level associated with the direct determination of Cd in NASS-3 exceeds the Zeeman background correction. Therefore, NH_4NO_3 is used as a matrix removing modifier in addition to the Pd/$Mg(NO_3)_2$.[1] Figure 4 is a plot of the relative Cd response vs. the amount of seawater on the platform. A similar response profile is observed in a solution containing 10,000 ppm NaCl. Therefore, in well-characterized samples of known salinity it is possible to effectively matrix match the standards with NaCl and perform the analysis directly using matrix matched standards, thereby avoiding the time consuming method of standard additions. If the matrix matched standards are going to be used, it is necessary to document that the use of NaCl is indeed compensating for the suppression. This documentation should include a response plot of increasing matrix vs. relative response similar to Figure 4.

4.5.2 Selenium: The background level associated with the direct determination of Se in NASS-3 exceeds the Zeeman correction capability. Therefore, HNO_3 is used as a matrix removing modifier in addition to the Pd/$Mg(NO_3)_2$ for the determination of Se in saline waters. Figure 5 is a plot of relative response vs. the amount of seawater on the platform. A similar suppression is ob-

served in a solution containing 10,000 ppm NaCl. Therefore, in well-characterized samples of known salinity it is possible to effectively matrix match the standards with NaCl and perform the analysis directly using matrix matched standards, thereby avoiding the time consuming method of standard additions. If the matrix matched standards are going to be used, it is necessary to document that the use of NaCl is indeed compensating for the suppression. This documentation should include a response plot of increasing matrix vs. relative response similar to Figure 5.

4.5.3 Arsenic: The elevated char temperatures possible with the determination of As minimize the interferences produced by the marine water background levels. Figure 3 is a plot of relative response vs. the amount of seawater on the platform. Figure 3 indicates a matrix suppression on As caused by the seawater. Although this suppression does cause a slight bias as shown in the recovery data in Table 3, the suppression does not warrant the method of standard additions (MSA) given the recovery criteria of 75-125% for LFMs.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[2-5] A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing, and observe proper mixing when working with these reagents.

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

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

5.4 The graphite tube during atomization emits intense UV radiation. Suitable precautions should be taken to protect personnel from such a hazard.

5.5 The use of the argon/hydrogen gas mixture during the dry and char steps may evolve a considerable amount of HCl gas. Therefore, adequate ventilation is required.

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

6.0 Equipment and Supplies

6.1 Graphite Furnace Atomic Absorption Spectrometer

6.1.1 The GFAA spectrometer must be capable of programmed heating of the graphite tube and the associated delayed atomization device. The instrument must be equipped with Zeeman background correction and the furnace device must be capable of utilizing an alternate gas supply during specified cycles of the analysis. The capability to record relatively fast (< 1 s) transient signals and evaluate data on a peak area basis is preferred. In addition, a recirculating refrigeration unit is recommended for improved reproducibility of furnace temperatures.

6.1.2 Single element hollow cathode lamps or single element electrodeless discharge lamps along with the associated power supplies.

6.1.3 Argon gas supply (high-purity grade, 99.99%) for use during the atomization of selenium, for sheathing the furnace tube when in operation, and during furnace cleanout.

6.1.4 Alternate gas mixture (hydrogen 5% - argon 95%) for use as a continuous gas flow environment during the dry and char furnace cycles.

6.1.5 Autosampler capable of adding matrix modifier solutions to the furnace, a single addition of analyte, and completing methods of standard additions when required.

6.2 Analytical balance, with capability to measure to 0.1 mg, for preparing standards, and for determining dissolved solids in digests or extracts.

6.3 A temperature adjustable hot plate capable of maintaining a temperature of 95°C.

6.4 An air displacement pipetter capable of delivering volumes ranging from 100 to 2500 µL with an assortment of high quality disposable pipet tips.

6.5 Labware — All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) should be sufficiently clean for the task objectives. Several procedures found to provide clean labware include washing with a detergent solution, rinsing with tap water, soaking for 4 h or more in 20% (v/v) nitric acid or a mixture of HCl and HNO_3, rinsing with reagent water and storing clean. Chromic acid cleaning solutions must be avoided because chromium is an analyte.

Note: Glassware having ground glass stoppers, etc. should be avoided because the ground glass surface is difficult to clean properly and can contain active sites which adsorb metals.

6.5.1 Glassware — Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (glass and/or metal-free plastic).

6.5.2 Assorted calibrated pipettes.

6.5.3 Griffin beakers, 250-mL with 75-mm watch glasses

and (optional) 75-mm ribbed watch glasses.

6.5.4 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with screw closure, 125-mL to 1-L capacities.

6.5.5 One-piece stem FEP wash bottle with screw closure, 125-mL capacity.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications[6] should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. All acids used for this method must be of ultra high-purity grade or equivalent. Suitable acids are available from a number of manufacturers. Redistilled acids prepared by sub-boiling distillation are acceptable.

7.2 Nitric acid, concentrated (sp.gr. 1.41) - HNO_3.

7.2.1 Nitric acid (1+1) — Add 500 mL concentrated HNO_3 to 400 mL reagent water and dilute to 1 L.

7.2.2 Nitric acid (1+5) — Add 50 mL concentrated HNO_3 to 250 mL reagent water.

7.2.3 Nitric acid (1+9) — Add 10 mL concentrated HNO_3 to 90 mL reagent water.

7.3 Reagent water. All references to water in this method refer to ASTM Type I grade water.[7]

7.4 Ammonium hydroxide, concentrated (sp. gr. 0.902).

7.5 Matrix Modifier, dissolve 300 mg palladium (Pd) powder in concentrated HNO_3 (1 mL of HNO_3, adding 10 µL of concentrated HCl if necessary). Dissolve 200 mg of $Mg(NO_3)_2 \cdot 6H_2O$ in ASTM Type I water. Pour the two solutions together and dilute to 100 mL with ASTM Type I water.

Note: It is recommended that the matrix modifier be analyzed separately in order to assess the contribution of the modifier to the absorbance of calibration and reagent blank solutions.

7.6 Standard stock solutions may be purchased or prepared from ultra-high purity grade chemicals (99.99 to 99.999% pure). All compounds must be dried for 1 h at 105°C, unless otherwise specified. It is recommended that stock solutions be stored in FEP bottles. Replace stock standards when succeeding dilutions for preparation of calibration standards cannot be verified.

Caution: Many of these chemicals are extremely toxic if inhaled or swallowed (Section 5.1). Wash hands thoroughly after handling.

Typical stock solution preparation procedures follow for 1-L quantities, but for the purpose of pollution prevention, the analyst is encouraged to prepare smaller quantities when possible. Concentrations are calculated based upon the weight of the pure element or upon the weight of the compound multiplied by the fraction of the analyte in the compound.

From pure element,

$$\text{Concentration} = \frac{\text{weight (mg)}}{\text{volume (L)}}$$

From pure compound,

$$\text{Concentration} = \frac{\text{weight (mg)} \times \text{gravimetric factor}}{\text{volume (L)}}$$

where:

gravimetric factor = the weight fraction of the analyte in the compound.

7.6.1 Arsenic solution, stock, 1 mL = 1000 µg As: Dissolve 1.320 g of As_2O_3 (As fraction = 0.7574), weighed accurately to at least four significant figures, in 100 mL of reagent water containing 10.0 mL concentrated NH_4OH. Warm in solution gently to effect dissolution. Acidify the solution with 20.0 mL concentrated HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.6.2 Cadmium solution, stock, 1 mL = 1000 µg Cd: Dissolve 1.000 g Cd metal, acid cleaned with (1+9) HNO_3, weighed accurately to at least four significant figures, in 50 mL (1+1) HNO_3 with heating to effect dissolution. Let solution cool and dilute with reagent water in a 1-L volumetric flask.

7.6.3 Chromium solution, stock, 1 mL = 1000 µg Cr: Dissolve 1.923 g CrO_3 (Cr fraction = 0.5200), weighed accurately to at least four significant figures, in 120 mL (1+5) HNO_3. When solution is complete, dilute to volume in a 1-L volumetric flask with reagent water.

7.6.4 Copper solution, stock, 1 mL = 1000 µg Cu: Dissolve 1.000 g Cu metal, acid cleaned with (1+9) HNO_3, weighed accurately to at least four significant figures, in 50.0 mL (1+1) HNO_3 with heating to effect dissolution. Let solution cool and dilute in a 1-L volumetric flask with reagent water.

7.6.5 Lead solution, stock, 1 mL = 1000 µg Pb: Dissolve 1.599 g $Pb(NO_3)_2$ (Pb fraction = 0.6256), weighed accurately to at least four significant figures, in a minimum amount of (1+1) HNO_3. Add 20.0 mL (1+1) HNO_3 and dilute to volume in a 1-L volumetric flask with reagent water.

7.6.6 Nickel solution, stock, 1 mL = 1000 µg Ni: Dissolve 1.000 g of nickel metal, weighed accurately to at least four significant figures, in 20.0 mL hot concentrated HNO_3, cool, and dilute to volume in a 1-L volumetric flask with reagent water.

7.6.7 Selenium solution, stock, 1 mL = 1000 µg Se: Dissolve 1.405 g SeO_2 (Se fraction = 0.7116), weighed accurately to at least four significant figures, in 200 mL reagent water and dilute to volume in a 1-L volumetric flask with reagent water.

7.7 ***Preparation of Calibration Standards*** — Fresh calibration standards (CAL Solution) should be prepared

weekly, or as needed. Dilute each of the stock standard solutions to levels appropriate to the operating range of the instrument using the appropriate acid diluent. The element concentrations in each CAL solution should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. The instrument calibration should be initially verified using a IPC sample (Section 7.9).

7.8 Blanks—Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank (LRB) is used to assess possible contamination from the sample preparation procedure and to assess spectral background, the laboratory fortified blank is used to assess routine laboratory performance, and a rinse blank is used to flush the instrument autosampler uptake system. All diluent acids should be made from concentrated acids (Section 7.2) and ASTM Type I water.

7.8.1 The calibration blank consists of the appropriate acid diluent in ASTM Type I water. The calibration blank should be stored in a FEP bottle.

7.8.2 The laboratory reagent blanks must contain all the reagents in the same volumes as used in processing the samples. The preparation blank must be carried through the entire sample digestion and preparation scheme.

7.8.3 The laboratory fortified blank (LFB) is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to provide a final concentration which will produce an absorbance of approximately 0.1 for each analyte. The LFB must be carried through the complete procedure as used for the samples.

7.8.4 The rinse blank is a 0.1% HCl and 0.1% HNO_3 solution used to flush the autosampler tip and is stored in the appropriate plastic containers.

7.9 Instrument Performance Check (IPC) Solution—The IPC solution is used to periodically verify instrument performance during analysis. It should be prepared in the same acid mixture as the calibration standards by combining method analytes at appropriate concentrations to approximate the midpoint of the calibration curve. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.10 Quality Control Sample (QCS) — For initial and periodic verification of calibration standards and instrument performance, analysis of a QCS is required. The QCS must be obtained from an outside source different from the standard stock solutions and prepared in the same acid mixture as the calibration standards. The concentration of the analytes in the QCS solution should be such that the resulting solution will provide an absorbance reading of approximately 0.1. The QCS solution should be stored in a FEP bottle and analyzed as needed to meet data-quality needs. A fresh solution should be prepared quarterly or as needed.

7.11 Matrix Performance Check (MPC) — The MPC solution is used to periodically evaluate the laboratory/instrument performance in saline samples. It should be prepared in the same acid mixture as the calibration standards by combining method analytes at appropriate concentrations in a seawater matrix (NASS-3, or its equivalent) to produce an absorbance of 0.1. The MPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. The MPC sample should be analyzed after every 10 samples to assure saline matrix is not producing false positives.

8.0 Sample Collection, Preservation and Storage

8.1 Prior to collection of an aqueous sample, consideration should be given to the type of data required. Acid preservation should be performed at the time of sample collection or as soon thereafter as practically possible. The pH of all aqueous samples must be tested immediately prior to aliquoting for analysis to ensure the sample has been properly preserved. If properly acid-preserved, the sample can be held up to 6 months before analysis.

8.2 For determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid at the time of collection to pH<2. Normally, 3 mL of (1+1) nitric acid (ultra high purity) per liter of sample is sufficient for most ambient water samples. The sample should not be filtered prior to analysis.

Note: Samples that cannot be acid-preserved at the time of collection because of sampling limitations or transport restrictions, or are > pH 2 because of high alkalinity should be acidified with nitric acid to pH<2 upon receipt in the laboratory. Following acidification, the sample should be held for 16 h and the pH verified to be<2 before withdrawing an aliquot for sample processing.

8.3 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the 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 thus generated.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to samples being analyzed by this method.

9.2.2 Linear dynamic range (LDR) — The upper limit of the LDR must be established for the wavelength utilized for each analyte by determining the signal responses from a minimum of six different concentration standards across the range, two of which are close to the upper limit of the LDR. Determined LDRs must be documented and kept on file. The linear calibration range which may be used for the analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from the four lower standards. New LDRs should be determined whenever there is a significant change in instrument response, a change in instrument analytical hardware or operating conditions.

Note: Multiple cleanout furnace cycles may be necessary in order to fully define or utilize the LDR for certain elements such as chromium. For this reason, the upper limit of the linear calibration range may not correspond to the upper operational LDR limit.

Measured sample analyte concentrations that exceed the upper limit of the linear calibration range must either be diluted and reanalyzed (with concern for memory effects Section 4.4) or analyzed by another approved method.

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 (Section 7.10). 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 on with the initial determination of method detection limits or continuing with ongoing 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.[8] 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].

S = standard deviation of the replicate analyses.

Note: If the percent relative standard deviation (% RSD) from the analyses of the seven aliquots is < 15%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in calculation of an unrealistically low MDL. If additional confirmation of the MDL is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide a more appropriate MDL estimate. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) and the analyte addition test described in Section 9.5.1 can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 2.

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.

The MDLs reported in Table 2 were determined in fortified NASS-3 samples. It is recommended that a certified saline matrix such as NASS-3 be used to determine MDLs.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory reagent blank (LRB) — The laboratory must analyze at least one LRB (Section 7.8.2) with each batch of 20 or fewer samples. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. Any determined source of contamination must be corrected and the samples reanalyzed for the affected analytes after acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) — The laboratory must analyze at least one LFB (Section 7.8.3) with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.3). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

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

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

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

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

9.3.4 Instrument performance check (IPC) solution — For all determinations the laboratory must analyze the IPC solution (Section 7.9) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and after the last sample in the batch is analyzed. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ± 5% of calibration. Subsequent analyses of the IPC solution must verify the calibration 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. Data for the calibration blank and IPC solution must be kept on file with associated sample data.

9.3.5 Matrix performance check (MPC) solution — For all determinations, the laboratory must analyze the MPC solution (Section 7.11) immediately following daily calibration, after every tenth sample (or more frequently, if required) and after the last sample in the batch is analyzed. Analysis of the MPC must verify that the instrument is within ± 15% of calibration and confirm that the matrix is not causing matrix/background interferences. If the MPC is not within ± 15%, reanalyze the MPC solution. If the second analysis of the MPC solution is 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 MPC solution must be reanalyzed. The analysis data for the calibration blank and MPC solution must be kept on file with the sample analyses data.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the data quality. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess these effects. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Section 9.4.2) is required. Also, the analyte addition test (Section 9.5.1) can aid in identifying matrix interferences. However, all samples must have a background absorbance < 1.0 before the test results obtained can be considered reliable.

9.4.2 The laboratory must add a known amount of each analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 9.3.2).

9.4.3 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 of 75-125%. Recovery calculations are not required if the concentration added is less than 25% of the unfortified sample concentration. Percent recovery may be calculated in units appropriate to the matrix, 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.

9.4.4 If the recovery of any analyte falls outside the designated LFM recovery range (but is still within the range of calibration and the background absorbance is < 1.0 abs.) and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related. A flowchart of the remainder of this section can be found in Figure 6. This flowchart may clarify the verbal discussion given below.

If the background absorbance is > 1 abs., the sample and the LFM should be diluted 1:3 and reanalyzed until the background absorbance is < 1, at which point a percent recovery of the LFM should be calculated. If the fortified analyte in the diluted LFM is found to be < 25% of the sample concentration or the diluted LFM produces an atomic signal of < 10 times the MDL, the diluted sample should be analyzed by methods of standard addition. If the calculated recovery of the diluted sample is within the designated range, the sample concentration should be calculated from the diluted sample. If the calculated recovery of the diluted sample is outside the designated range, follow the directions given below. If the background is reduced and/or the matrix effect is reduced by dilution, all samples of a similar matrix should be diluted and analyzed in a similar fashion. The result should be flagged indicating the methods sensitivity has been reduced by the dilution. If dilution is unacceptable because of data quality objectives the sample should be flagged indicating the analysis is not possible via this analytical procedure.

If the analyte recovery on the LFM is < 75% and the background absorbance is < 1, complete the analyte addition test (Section 9.5.1) on the original sample (or its dilution). The results of the test should be evaluated as follows:

1. If recovery of the analyte addition test (≤ 85%) confirms a low recovery for the LFM, a suppressive matrix interference is indicated and the unfortified sample aliquot must be analyzed by method of standard additions (Section 11.3).

2. If the recovery of the analyte addition test is between 85% to 115%, a low recovery of the analyte

in the LFM (<75%) may be related to the heterogeneity of the sample, sample preparation or a poor transfer, etc. Report the sample concentration based on the unfortified sample aliquot.

3. If the recovery of the analyte addition test is less than recovery calculated for the LFM, matrix suppression is confirmed. The unfortified sample should be analyzed by MSA (Section 11.3). Significantly lower recoveries (relative to the LFM) associated with the analyte addition test are unlikely unless the sample is heterogeneous.

4. If the recovery of the analyte addition test is >115%, the dramatic change in analyte response should be verified by fortifying the LFM. The recovery in the sample and the recovery in the LFM should be compared. If the recoveries verify the dramatic response difference, the sample results should be flagged indicating the sample matrix is not homogeneous.

If the analyte recovery in the LFM is > 125% and the background absorbance is < 1, complete the analyte addition test (Section 9.5.1) on the unfortified sample (or its dilution) aliquot.

1. If the percent recovery of the analyte addition test is > 115% and the LFB does not indicate laboratory contamination, an enhancing matrix interference (albeit rare) is indicated, and the unfortified sample aliquot must be analyzed by method of standard additions (Section 11.3).

2. If the percent recovery of the analyte addition test is between 85% to 115%, either random sample contamination of the LFM, an incorrect analyte concentration was added to the LFM prior to sample preparation, or sample heterogeneity should be suspected. Report analyte data determined from the analysis of the unfortified sample aliquot.

3. If the percent recovery of the analyte addition test is < 85%, a heterogeneous sample with matrix interference is suspected. This dramatic change in response should be verified by performing the analyte addition test to the LFM. The recovery in the sample and the recovery in the LFM should be compared. If the recoveries verify the dramatic response difference the sample results should be flagged indicating the sample matrix is not homogeneous.

9.4.5 If the analysis of a LFM sample(s) and the test routines above indicate an operative interference and the LFMs are typical of the other samples in the batch, those samples that are similar must be analyzed in the same manner as the LFMs. Also, the data user must be informed when a matrix interference is so severe that it prevents successful determination of the analyte or when the heterogeneous nature of the sample precludes the use of duplicate analyses.

9.4.6 Where reference materials are available, they should be analyzed to provide additional performance data. Analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably. It is recommended that NASS-3 or its equivalent be fortified and used as an MPC.

9.5 Matrix interference effects and the need for MSA can be assessed by the following test. Directions for using MSA are given in Section 11.3.

9.5.1 Analyte addition test: An analyte standard added to a portion of a prepared sample or its dilution should be recovered to within 85-115% of the known value. The analyte addition should occur directly to sample in the furnace and should produce a minimum absorbance of 0.1. The concentration of the analyte addition plus that in the sample should not exceed the linear calibration range of the analyte. If the analyte is not recovered within the specified limits, a matrix effect should be suspected and the sample must be analyzed by MSA.

10.0 Calibration and Standardization

10.1 Specific wavelengths and instrument operating conditions are listed in Table 1. However, because of differences among makes and models of spectrophotometers and electrothermal furnace devices, the actual instrument conditions selected may vary from those listed.

10.2 Prior to the use of this method, the instrument operating conditions must be optimized. The analyst should follow the instructions provided by the manufacturer while using the conditions listed in Table 1 as a guide. The appropriate charring condition for each of the analytes is a critical part of the metal analysis in saline waters; therefore, the char temperature profiles should be determined in a saline water matrix. The appropriate charring temperature should be chosen so as to minimize background absorbance while providing some furnace temperature variation without the loss of analyte. For analytical operation, the charring temperature is usually set at least 100°C below the point at which analyte begins to be lost during the char. Because the background absorbance can be affected by the atomization temperature, care should be taken in the choice of an appropriate atomization temperature. The optimum conditions selected should provide the lowest reliable MDLs and be similar to those listed in Table 2. Once the optimum operating conditions are determined, they should be recorded and available for daily reference. The effectiveness of these operating conditions are continually evaluated by analyzing the MPC.

10.3 Prior to an initial calibration the linear dynamic range of the analyte must be determined (Sect 9.2.2) using the optimized instrument operating conditions. For all determinations allow an instrument and hollow cathode lamp warm-up period of not less than 15 min. If an EDL is to be used, allow 30 min for warm-up.

10.4 Before using the procedure (Section 11.0) to analyze samples, there must be data available documenting initial demonstration of performance. The required data and procedure are described in Section 9.2. This data must be generated using the same instrument operating conditions and calibration routine to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.

11.0 Procedure

11.1 Aqueous Sample Preparation – Total Recoverable Analytes

11.1.1 Add 2 mL (1+1) nitric acid to the beaker containing 100 mL of sample. Place the beaker on a hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.1.2 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. DO NOT BOIL. This step takes about 2 h for a 100-mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.1.3 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 min.

11.1.4 Allow the beaker to cool. Quantitatively transfer the sample solution to a 100-mL volumetric flask, dilute to volume with reagent water, stopper and mix.

11.1.5 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids, a portion of the sample may be filtered prior to analysis. However, care should be exercised to avoid potential contamination from filtration.) The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.2 Sample Analysis

11.2.1 Prior to daily calibration of the instrument, inspect the graphite tube and contact rings for salt buildup, etc. Generally, it will be necessary to clean the contact rings and replace the graphite tube daily. The contact rings are a cooler environment in which salts can deposit after atomization. A cotton swab dipped in a 50/50 mixture of isopropyl alcohol (IPA) and H_2O (such that it is damp but not dripping) can be used to remove the majority of the salt buildup. A second cotton swab is dipped in IPA and the contact rings are wiped down to assure they are clean. The rings are then allowed to thoroughly dry and then a new tube is placed in the furnace and conditioned according to instrument manufacturer's specifications.

11.2.2 Configure the instrument system to the selected optimized operating conditions as determined in Sections 10.1 and 10.2.

11.2.3 Before beginning daily calibration the instrument should be reconfigured to the optimized conditions. Initiate the data system and allow a period of not less than 15 min for instrument and hollow cathode lamp warm up. If an EDL is to be used, allow 30 min for warm up.

11.2.4 After the warm up period but before calibration, instrument stability must be demonstrated by analyzing a standard solution with a concentration 20 times the IDL a minimum of five times. The resulting relative standard deviation of absorbance signals must be $\leq$ 5%. If the relative standard deviation is > 5%, determine and correct the cause before calibrating the instrument.

11.2.5 For initial and daily operation, calibrate the instrument according to the instrument manufacturer's recommended procedures using the calibration blank (Section 7.8.1) and calibration standards (Section 7.7) prepared at three or more concentrations within the usable linear dynamic range of the analyte (Sections 4.4 and 9.2.2).

11.2.6 An autosampler must be used to introduce all solutions into the graphite furnace. Once the sample and the matrix modifier are injected, the furnace controller completes a set of furnace cycles and a cleanout period as programmed. Analyte signals must be reported on an integrated absorbance basis. Background absorbances, background heights and the corresponding peak profiles should be displayed to the CRT for review by the analyst and be available as hard copy for documentation to be kept on file. Flush the autosampler solution uptake system with the rinse blank (Section 7.8.4) between each solution injected.

11.2.7 After completion of the initial requirements of this method (Section 9.2), samples should be analyzed in the same operational manner used in the calibration routine.

11.2.8 During sample analyses, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4.

11.2.9 For every new or unusual matrix, when practical, it is highly recommended that an inductively coupled plasma atomic emission spectrometer be used to screen for high element concentration. Information gained from this may be used to prevent potential damage to the instrument and to better estimate which elements may require analysis by graphite furnace.

11.2.10 Determined sample analyte concentrations that are ≥ 90% of the upper limit of calibration must either be diluted with acidified reagent water and reanalyzed with concern for memory effects (Section 4.4), or determined by another approved but less sensitive procedure. Samples with background absorbances > 1 must be diluted with appropriate acidified reagent water such that the background absorbance is < 1 (Section 9.4.4). If the method of standard additions is required, follow the instructions described in Section 11.3.

11.2.11 When it is necessary to assess an operative matrix interference (e.g., signal reduction due to high dissolved solids), the test described in Section 9.5 is recommended.

11.2.12 Report data as directed in Section 12.

11.3 Standard Additions— If the method of standard addition is required, the following procedure is recommended:

11.3.1 The standard addition technique[9] involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interference, which causes a baseline shift. The simplest version of this technique is the single-addition method. The procedure is as follows: Two identical aliquots of the sample solution, each of volume V_X, are taken. To the first (labeled A) is added a small volume V_S of a standard analyte solution of concentration C_S. To the second (labeled B) is added the same volume V_S of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration C_X is calculated:

$$C_X = \frac{S_B V_S C_S}{(S_A - S_B) V_X}$$

where, S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_S and C_S should be chosen so that S_A is roughly twice S_B on the average. It is best if V_S is made much less than V_X, and thus C_S is much greater than C_X, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond in the same manner as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

12.0 Data Analysis and Calculations

12.1 Sample data should be reported in units of µg/L for aqueous samples.

12.2 For total recoverable aqueous analytes (Section 11.1), when 100-mL aliquot is used to produce the 100 mL final solution, round the data to the tenths place and report the data in µg/L up to three significant figures. If a different aliquot volume other than 100 mL is used for sample preparation, adjust the dilution factor accordingly. Also, account for any additional dilution of the prepared sample solution needed to complete the determination of analytes exceeding the upper limit of the calibration curve. Do not report data below the determined analyte MDL concentration or below an adjusted detection limit reflecting smaller sample aliquots used in processing or additional dilutions required to complete the analysis.

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

13.0 Method Performance

13.1 Instrument operating conditions used for single laboratory testing of the method and MDLs are listed in Tables 1 & 2.

13.2 Table 3 contains precision and recovery data obtained from a single laboratory analysis of four fortified sample replicates of NASS-3. Five unfortified replicates were analyzed, and their average concentration was used to determine the sample concentration. Samples were prepared using the procedure described in Section 11.1. Four samples were fortified at the levels reported in Table 3. Average percent recovery and percent relative standard deviation are reported in Table 3 for the fortified samples.

14.0 Pollution Prevention

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

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult Less is Better: Laboratory Chemical Management for Waste Reduction, available from the American Chemical Society's Department of Government Re-

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

15.0 Waste Management

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

16.0 References

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9. Winefordner, J.D., Trace Analysis: Spectroscopic Methods for Elements, *Chemical Analysis*, Vol. 46, pp. 41-42, 1976.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Furnace Conditions for Determination of Metals in Seawater [1]

Element	Wavelength (nm) / Slit Width (nm)	Method of Analysis	Modifier[2,3]	Furnaces[5] Cycle	Temp °C	Temp Ramp	Hold Time (sec)
As	193.7 / 0.7	Direct	Pd/Mg	Dry	130	1	60
				Char	1400[4]	10	60
				Atomization	2200	0	5
Cd	228.8 / 0.7	Matrix Match Standard or Std. Addition	Pd/Mg + 600 µg NH_4NO_3	Dry	130	1	60
				Char 1	350	45	30
				Char 2	850	1	30
				Atomization	1500	0	5
Cr	357.9 / 0.7	Direct	Pd/Mg	Dry	130	1	60
				Char	1500	5	30
				Atomization	2600	0	5
Cu	324.8 / 0.7	Direct	Pd/Mg	Dry	130	1	60
				Char	1300	10	30
				Atomization	2600	0	5
Ni	232.4 / 0.2	Direct	Pd/Mg	Dry	130	1	60
				Char	1400[4]	10	30
				Atomization	2600	0	7
Pb	283.3 / 0.7	Direct	Pd/Mg	Dry	130	1	60
				Char	1200	10	45
				Atomization	2200	0	5
Se	196.0 / 2.0	Matrix Match Standard or Std. Addition	Pd/Mg 9% HNO_3 on Platform	Dry	130	1	60
				Char	1000	5	60
				Atomization	2100	0	5

[1] 10-µL sample size.
[2] 5 µL of (30 mg Pd Powder and 20 mg $Mg(NO_3)_2 \cdot 6H_2O$ to 10 mL).
[3] A gas mixture of 5% H_2 in 95% Ar is used during the dry and char.
[4] Sodium emission is visibly exiting from the sample inlet port.
[5] The furnace program has a cool down step of 20° between char and atomization and a clean out step of 2600°C after atomization.

Table 2. MDLs and Background Absorbances Associated with a Fortified NASS-3[1-3]

Element	MDL[5] µg/L	Typical Integrated Background Absorbances[6]
Cd	0.1	1.2
Cr	-	0.2
Cu	2.8	0.2
Ni	1.8	0.1
Pb	2.4	0.4
Se[4]	9.5	1.4
As[4]	2.6	0.3

[1] Matrix Modifier = 0.015 mg Pd + 0.01 mg $Mg(NO_3)_2$.
[2] A 5% H_2 in Ar gas mix is used during the dry and char steps at 300 mL/min for all elements.
[3] 10-µL sample size.
[4] An electrodeless discharge lamp was used for this element.
[5] MDL calculated based on fortifying NASS-3 with metal analytes.
[6] Background absorbances are affected by the atomization temperature for analysis, therefore, lowering atomization temperatures may be advantageous if large backgrounds are observed.
- Not Determined.

Table 3. Precision and Recovery Data for Fortified NASS-3

Element	Certified Value µg/L	Observed Value µg/L	Fortified Conc. µg/L [2]	Avg. Recovery, %	% RSD	Fortified Conc. µg/L	Avg. Recovery, %	% RSD
As	1.65 ± 0.19	< MDL	15	89	3.6	37.5	85	1.6
Cd[1]	0.029 ± 0.004	< MDL	1.0	107	4.5	2.5	104	3.8
Cr	0.175 ± 0.010	< MDL	5	88	0.7	12.5	85	1.6
Cu	0.109 ± 0.011	< MDL	15	95	4.4	37.5	91	0.9
Pb	0.039 ± 0.006	< MDL	15	103	2.3	37.5	99	3.4
Ni	0.257 ± 0.027	< MDL	15	92	10.1	37.5	93	7.1
Se[1]	0.024 ± 0.004	< MDL	25	101	2.9	62.5	99	3.9

[1] Standards were made in 10,000 ppm NaCl for this analysis.

[2] Determined from four sample replicates.

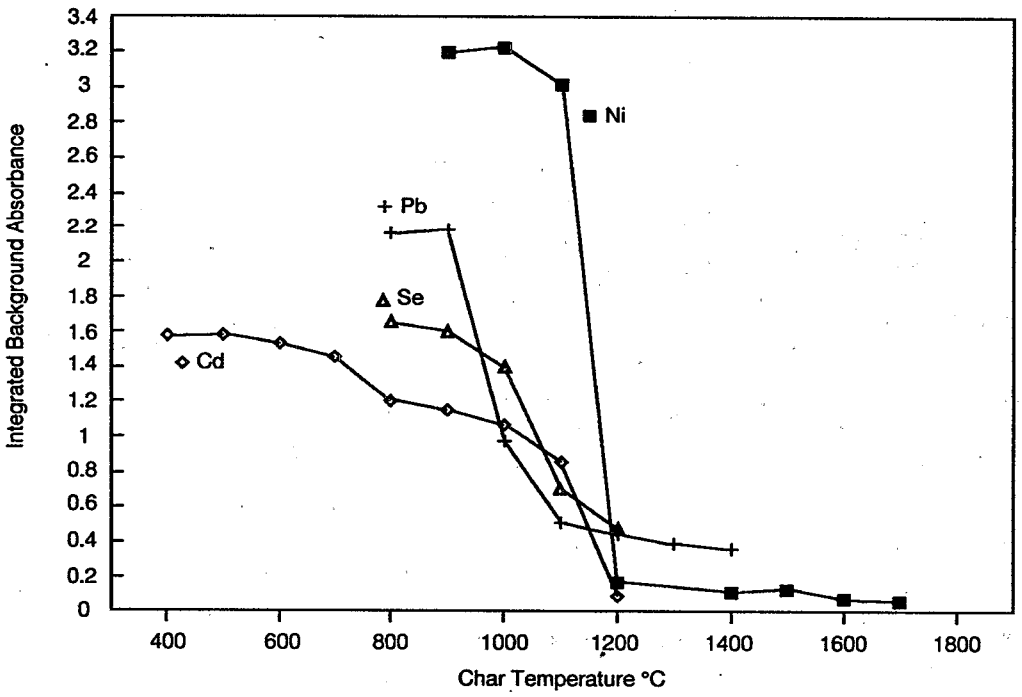

Figure 1. Integrated Background Absorbance vs. Char Temperature.

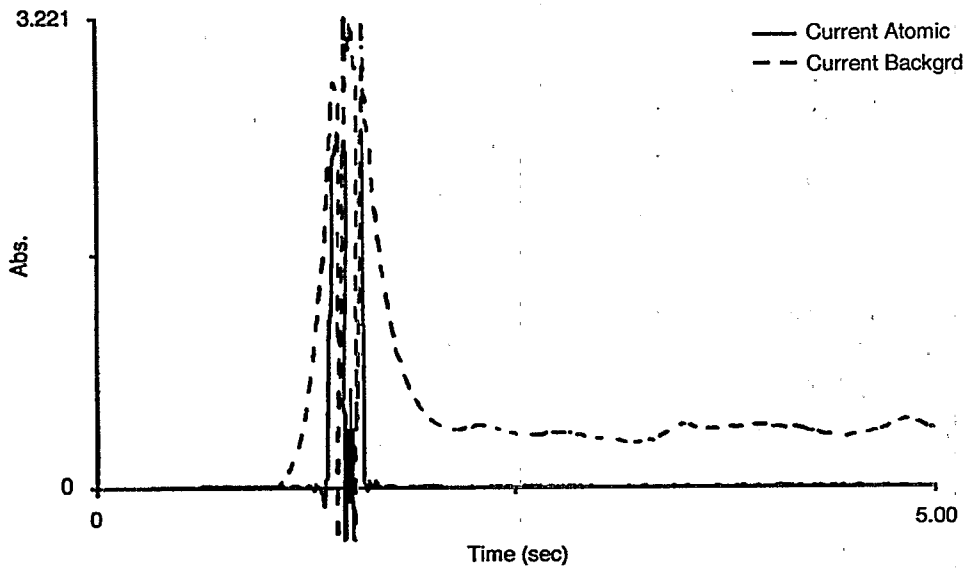

Figure 2. Pb Atomization Profile Utilizing a 800°C Char.

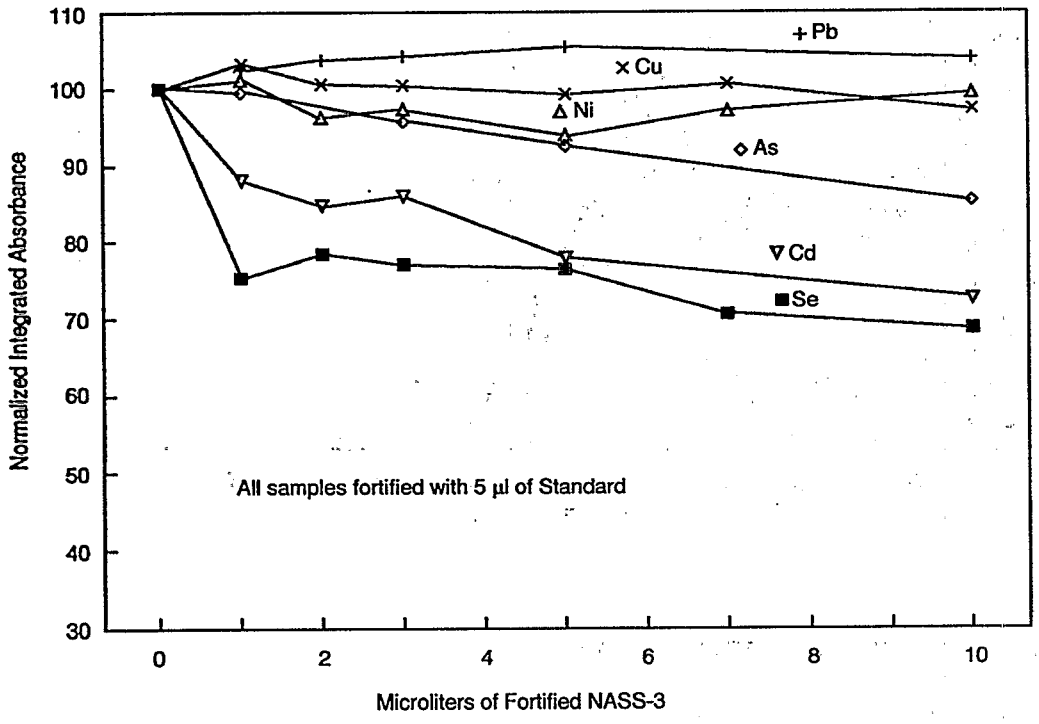

Figure 3. Normalized Integrated Absorbance vs. Microliters of Fortified NASS-3.

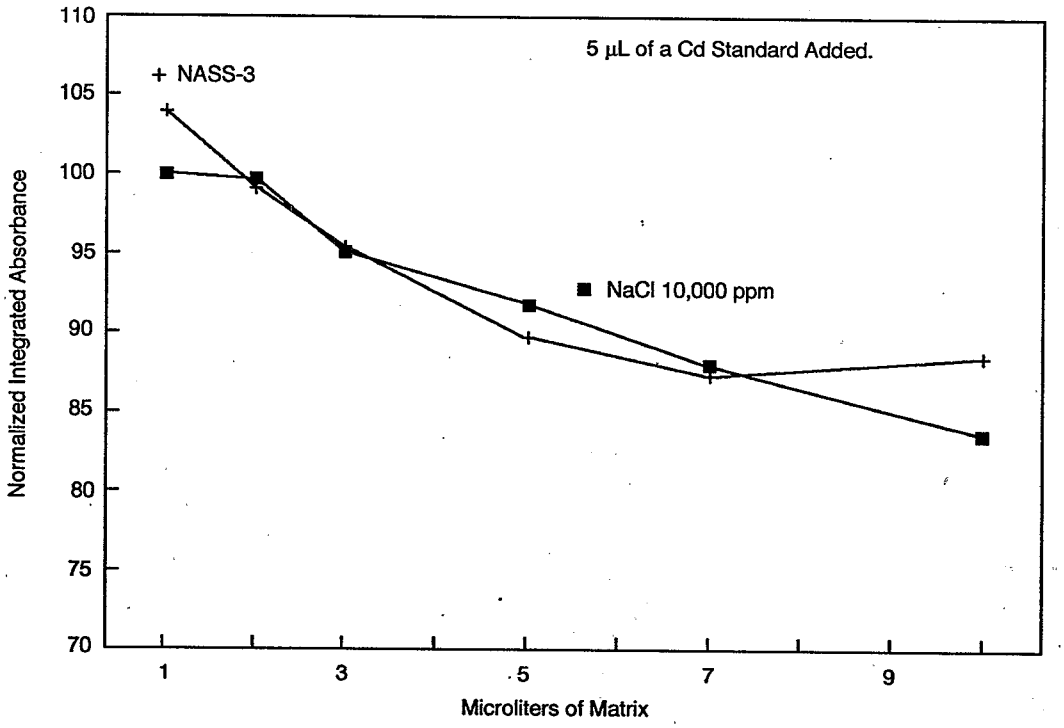

Figure 4. Cd Response in NASS-3 and 10,000 ppm NaCl.

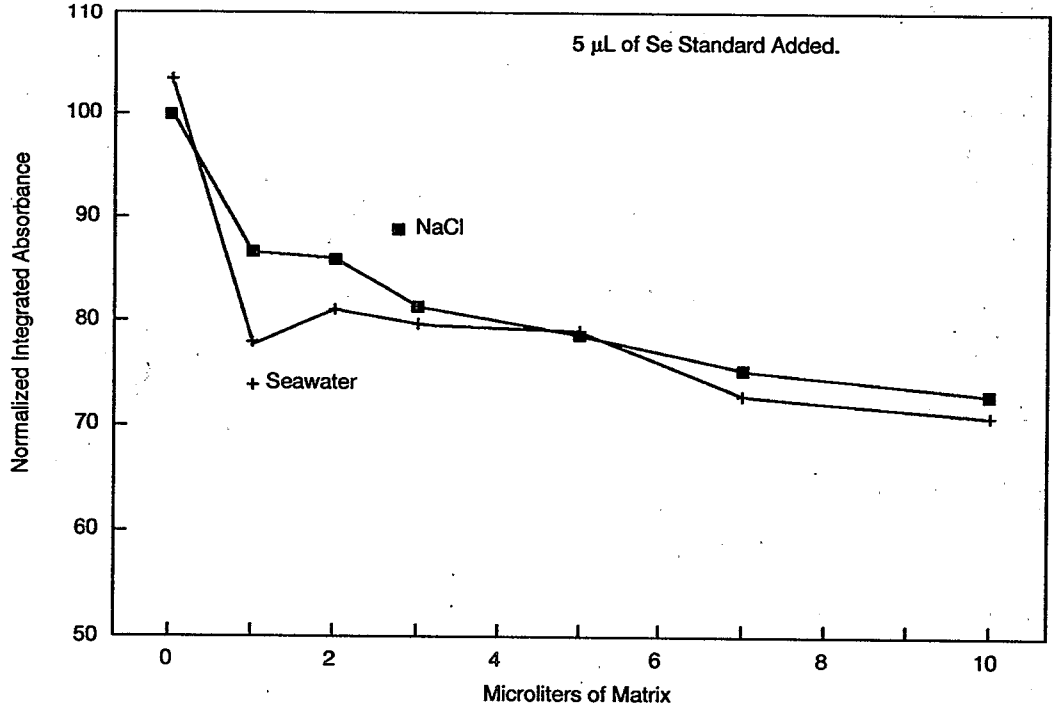

Figure 5. Se Response in Seawater vs. 10,000 ppm NaCl.

(1) Poor Transfer
(2) Sample Heterogeneity
(3) Digestion/Precipitation
(4) Matrix Suppression/Enhancements
(5) Contamination

IFA = In Furnace Analyte Addition

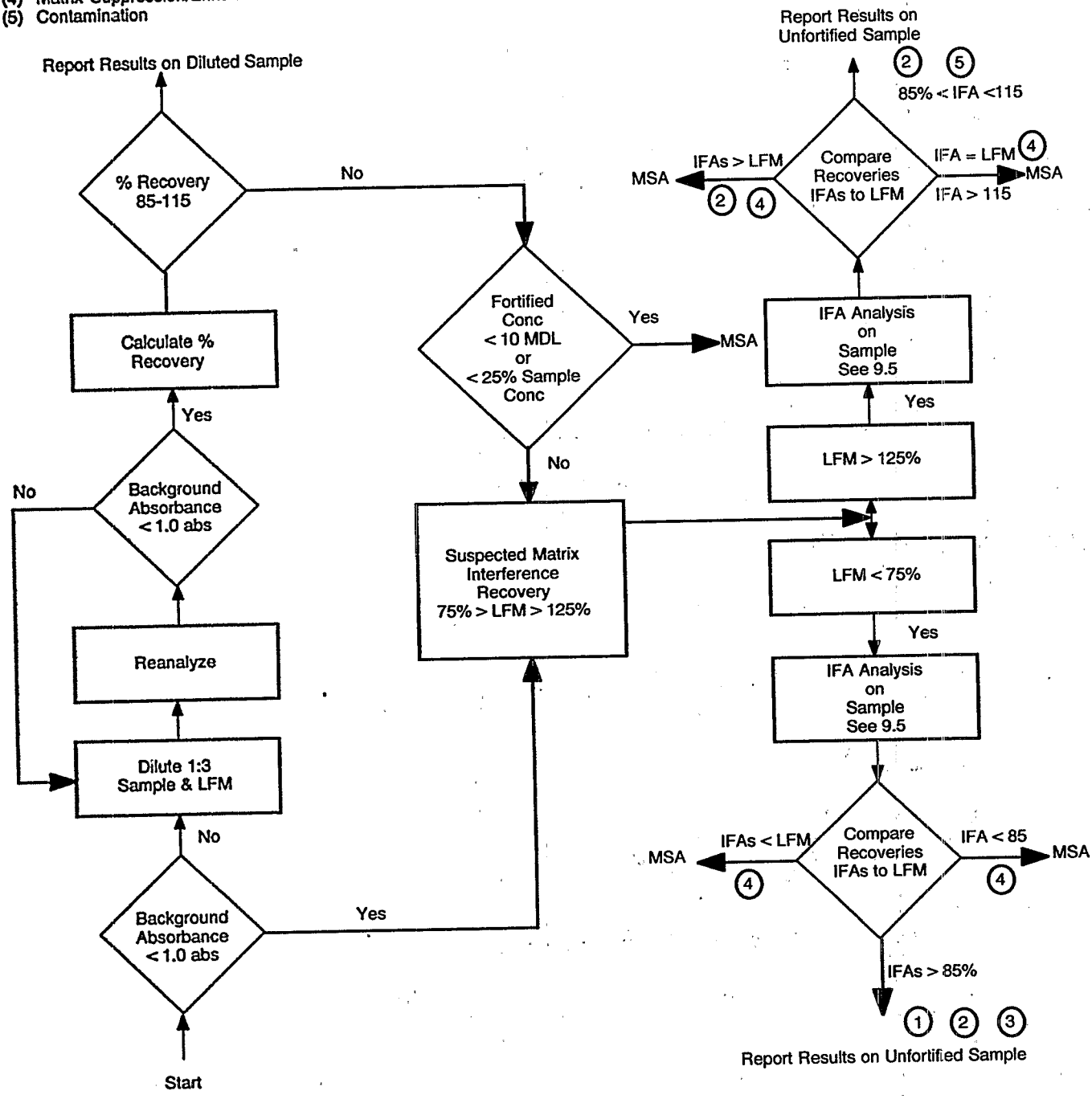

Figure 6. Matrix Interference Flowchart.

Method 200.13

Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption

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Inorganic Chemistry Branch
Chemistry Research Division

Revision 1.0
November 1992

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Method 200.13

Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption

1.0 Scope and Application

1.1 This method describes procedures for preconcentration and determination of total recoverable trace elements in marine waters, including estuarine water, seawater and brines.

1.2 Acid solubilization is required prior to determination of total recoverable elements to facilitate breakdown of complexes or colloids which might influence trace element recoveries. This method should only be used for preconcentration and determination of trace elements in aqueous samples.

1.3 This method is applicable to the following elements:

Element		Chemical Abstracts Service Registry Numbers (CASRN)
Cadmium	(Cd)	7440-43-9
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Nickel	(Ni)	7440-02-0

1.4 Method detection limits (MDLs) for these elements will be dependent on the specific instrumentation employed and the selected operating conditions. MDLs in NASS-3 (Reference Material, National Research Council of Canada) were determined using the procedure described in Section 9.2.4 and are listed in Table 1.

1.5 A minimum of 6 months experience in graphite furnace atomic absorption (GFAA) is recommended.

2.0 Summary of Method

2.1 Nitric acid is dispensed into a beaker containing an accurately weighed or measured, well-mixed, homogeneous aqueous sample. The sample volume is reduced to approximately 20 mL and then covered and allowed to reflux. The resulting solution is diluted to volume and is ready for analysis.

2.2 This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin.[1,2] Following acid solubilization, the sample is buffered using an on-line system prior to entering the chelating column. Group I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluted into a simplified matrix consisting of 0.75 M nitric acid and are determined by GFAA.

3.0 Definitions

3.1 ***Calibration Blank (CB)*** — A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 ***Calibration Standard (CAL)*** — A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

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

3.4 ***Instrument Performance Check Solution (IPC)*** — A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

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

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

3.7 ***Laboratory Reagent Blank (LRB)*** — An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interfer-

ences are present in the laboratory environment, the reagents, or the apparatus.

3.8 Linear Dynamic Range (LDR) — The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.9 Matrix Modifier (MM) — A substance added to the instrument along with the sample in order to minimize the interference effects by selective volatilization of either analyte or matrix components.

3.10 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.

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

3.12 Standard Addition — The addition of a known amount of analyte to the sample in order to determine the relative response of the detector to an analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration.

3.13 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.

3.14 Total Recoverable Analyte (TRA) — The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid(s) as specified in the method.

4.0 Interferences

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by GFAA. These interferences can be classified into three major subdivisions: spectral, matrix, and memory. Some of these interferences can be minimized via the preconcentration step, which reduces the Ca, Mg, Na and Cl concentration in the sample prior to GFAA analysis.

4.2 Spectral interferences are caused by absorbance of light by a molecule or atom which is not the analyte of interest or emission from black body radiation.

4.2.1 Spectral interferences caused by an element only occur if there is a spectral overlap between the wavelength of the interfering element and the analyte of interest. Fortunately, this type of interference is relatively uncommon in STPGFAA (Stabilized Temperature Platform Graphite Furnace Atomic Absorption) because of the narrow atomic line widths associated with STPGFAA. In addition, the use of appropriate furnace temperature programs and high spectral purity lamps as light sources can minimize the possibility of this type of interference. However, molecular absorbances can span several hundred nanometers, producing broadband spectral interferences. This type of interference is far more common in STPGFAA. The use of matrix modifiers, selective volatilization, and background correctors are all attempts to eliminate unwanted nonspecific absorbance. Because the nonspecific component of the total absorbance can vary considerably from sample type to sample type, to provide effective background correction and eliminate the elemental spectral interference of palladium on copper and iron on selenium, the exclusive use of Zeeman background correction is specified in this method.

4.2.2 Spectral interferences are also caused by emissions from black body radiation produced during the atomization furnace cycle. This black body emission reaches the photomultiplier tube, producing erroneous results. The magnitude of this interference can be minimized by proper furnace tube alignment and monochromator design. In addition, atomization temperatures which adequately volatilize the analyte of interest without producing unnecessary black body radiation can help reduce unwanted background emission produced during atomization.

4.3 Matrix interferences are caused by sample components which inhibit formation of free atomic analyte atoms during the atomization cycle. In this method the use of a delayed atomization device which provides warmer gas phase temperatures is required. These devices provide an environment which is more conducive to the formation of free analyte atoms and thereby minimize this type of interference. This type of interference can be detected by analyzing the sample plus a sample aliquot fortified with a known concentration of the analyte. If the determined concentration of the analyte addition is outside a designated range, a possible matrix effect should be suspected (Section 9.4).

4.4 Memory interferences result from analyzing a sample containing a high concentration of an element (typically a high atomization temperature element) which cannot be removed quantitatively in one complete set of furnace steps. The analyte which remains in the furnace can produce false positive signals on subsequent sample(s). Therefore, the analyst should establish the analyte concentration which can be injected into the furnace and adequately removed in one complete set of furnace cycles. If this concentration is exceeded, the sample should be diluted and a blank analyzed to assure the memory effect has been eliminated before reanalyzing the diluted sample.

4.5 Low recoveries may be encountered in the preconcentration cycle if the trace elements are complexed by competing chelators (humic/fulvic) in the sample or are present as colloidal material. Acid solubilization pretreatment is employed to improve analyte recovery and to minimize adsorption, hydrolysis and precipitation effects.

4.6 Memory interferences from the chelating system may be encountered, especially after analyzing a sample containing high analyte concentrations. A thorough column rinsing sequence following elution of the analytes is necessary to minimize such interferences.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[3-6] A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

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

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

5.4 The graphite tube during atomization emits intense UV radiation. Suitable precautions should be taken to protect personnel from such a hazard.

5.5 The use of the argon/hydrogen gas mixture during the dry and char steps may evolve a considerable amount of HCl gas. Therefore, adequate ventilation is required.

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

6.0 Equipment and Supplies

6.1 Graphite Furnace Atomic Absorption Spectrometer

6.1.1 The GFAA spectrometer must be capable of programmed heating of the graphite tube and the associated delayed atomization device. The instrument should be equipped with an adequate background correction device capable of removing undesirable non-specific absorbance over the spectral region of interest. The capability to record relatively fast (< 1 sec) transient signals and evaluate data on a peak area basis is preferred. In addition, a recirculating refrigeration unit is recommended for improved reproducibility of furnace temperatures. The data shown in the tables were obtained using the stabilized temperature platform and Zeeman background correction.

6.1.2 Single element hollow cathode lamps or single element electrodeless discharge lamps along with the associated power supplies.

6.1.3 Argon gas supply (high-purity grade, 99.99%).

6.1.4 A 5% hydrogen in argon gas mix and the necessary hardware to use this gas mixture during specific furnace cycles.

6.1.5 Autosampler— Although not specifically required, the use of an autosampler is highly recommended.

6.1.6 Graphite Furnace Operating Conditions—A guide to experimental conditions for the applicable elements is provided in Table 1

6.2 Preconcentration System - System containing no metal parts in the analyte flow path, configured as shown with a sample loop in Figure 1 and without a sample loop in Figure 2.

6.2.1 Column— Macroporous iminodiacetate chelating resin (Dionex Metpac CC-1 or equivalent).

6.2.2 Control valves — Inert double stack, pneumatically operated four-way slider valves with connectors.

6.2.2.1 Argon gas supply regulated at 80-100 psi.

6.2.3 Solution reservoirs — Inert containers, e.g., high density polyethylene (HDPE), for holding eluent and carrier reagents.

6.2.4 Tubing— High pressure, narrow bore, inert tubing such as Tefzel ETFE (ethylene tetra-fluoroethylene) or equivalent for interconnection of pumps/valve assemblies and a minimum length for connection of the preconcentration system with the sample collection vessel.

6.2.5 Eluent pumping system (Gradient Pump) — Programmable flow, high-pressure pumping system, capable of delivering either one of three eluents at a pressure up to 2000 psi and a flow rate of 1-5 mL/min.

6.2.6 System setup, including sample loop (See Figure 1).

6.2.6.1 Sample loop — 10-mL loop constructed from narrow bore, high-pressure inert tubing, Tefzel ETFE or equivalent.

6.2.6.2 Auxiliary pumps — On-line buffer pump, piston pump (Dionex QIC pump or equivalent) for delivering 2M ammonium acetate buffer solution; carrier pump, peristaltic pump (Gilson Minipuls or equivalent) for delivering 1% nitric acid carrier solution; sample pump, peristaltic pump for loading sample loop.

6.2.7 System setup without sample loop (See Figure 2).

6.2.7.1 Auxiliary Pumps — Sample pump (Dionex QIC Pump or equivalent) for loading sample on the column.

Carrier pump (Dionex QIC Pump or equivalent) used to flush collection line between samples.

6.3 Labware — For determination of trace elements, contamination and loss are of **critical** consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment. A clean laboratory work area, designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in determination of trace elements by (1) contributing contaminants through surface desorption or leaching and (2) depleting element concentrations through adsorption processes. For these reasons, borosilicate glass is not recommended for use with this method. All labware in contact with the sample should be cleaned prior to use. Labware may be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for 4 h in a mixture of dilute nitric and hydrochloric acids, followed by rinsing with ASTM type I water and oven drying.

6.3.1 Griffin beakers, 250 mL, polytetrafluoroethylene (PTFE) or quartz.

6.3.2 Storage bottles — Narrow mouth bottles, Teflon FEP (fluorinated ethylene propylene), or HDPE, 125-mL and 250-mL capacities.

6.4 Sample Processing Equipment

6.4.1 Air displacement pipetter — Digital pipet system capable of delivering volumes from 100 to 2500 µL with an assortment of metal-free, disposable pipet tips.

6.4.2 Balances — Analytical balance, capable of accurately weighing to ± 0.1 mg; top pan balance, accurate to ± 0.01 g.

6.4.3 Hot plate — Corning PC100 or equivalent.

6.4.4 Centrifuge — Steel cabinet with guard bowl, electric timer and brake.

6.4.5 Drying oven — Gravity convection oven with thermostatic control capable of maintaining 105°C ± 5°C.

6.4.6 pH meter — Bench mounted or hand-held electrode system with a resolution of ± 0.1 pH units.

6.4.7 Class 100 hoods are recommended for all sample handling.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications[7] should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. All acids used for this method must be of ultra high-purity grade or equivalent. Suitable acids are available from a number of manufacturers. Redistilled acids prepared by sub-boiling distillation are acceptable.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.1.1 Nitric acid 0.75M — Dilute 47.7 mL (67.3 g) conc. nitric acid to 1000 mL with ASTM type I water.

7.1.1.2 Nitric acid (1+1) — Dilute 500 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.1.1.3 Nitric acid (1+9) — Dilute 100 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.1.2 Matrix Modifier, dissolve 300 mg Palladium (Pd) powder in a minimum amount of concentrated HNO_3 (1 mL of HNO_3, adding concentrated HCl only if necessary). Dissolve 200 mg of $Mg(NO_3)_2 \cdot 6H_2O$ in ASTM type 1 water. Pour the two solutions together and dilute to 100 mL with ASTM type I water.

Note: It is recommended that the matrix modifier be analyzed separately in order to assess the contribution of the modifier to the overall laboratory blank.

7.1.3 Acetic acid, glacial (sp.gr. 1.05). High purity acetic acid is recommended.

7.1.4 Ammonium hydroxide (20%). High purity ammonium hydroxide is recommended.

7.1.5 Ammonium acetate buffer 1M, pH 5.5 — Add 58 mL (60.5 g) of glacial acetic acid to 600 mL of ASTM type I water. Add 65 mL (60 g) of 20% ammonium hydroxide and mix. Check the pH of the resulting solution by withdrawing a small aliquot and testing with a calibrated pH meter, adjusting the solution to pH 5.5 ± 0.1 with small volumes of acetic acid or ammonium hydroxide as necessary. Cool and dilute to 1 L with ASTM type I water.

7.1.6 Ammonium acetate buffer 2M, pH 5.5 — Prepare as for Section 7.1.5 using 116 mL (121 g) glacial acetic acid and 130 mL (120 g) 20% ammonium hydroxide, diluted to 1000 mL with ASTM type I water.

Note: If the system is configured as shown in Figure 1, the ammonium acetate buffer solutions may be further purified by passing them through the chelating column at a flow rate of 5.0 mL/min. Collect the purified solution in a container. Following this, elute the collected contaminants from the column using 0.75M nitric acid for 5 min at a flow rate of 4.0 mL/min. If the system is configured as shown in Figure 2, the majority of the buffer is being purified in an on-line configuration via the clean-up column.

7.1.7 Oxalic acid dihydrate (CASRN 6153-56-6), 0.2M - Dissolve 25.2 g reagent grade $C_2H_2O_4 \cdot 2H_2O$ in 250 mL ASTM type I water and dilute to 1000 mL with ASTM type I water. **CAUTION** - Oxalic acid is toxic; handle with care.

7.2 Water — For all sample preparation and dilutions, ASTM type I water (ASTM D1193) is required.

7.3 Standard Stock Solutions — May be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure). All salts should be dried for one hour at 105°C, unless otherwise specified. (CAUTION - Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.) Stock solutions

should be stored in plastic bottles. The following procedures may be used for preparing standard stock solutions:

Note: Some metals, particularly those which form surface oxides require cleaning prior to being weighed. This may be achieved by pickling the surface of the metal in acid. An amount in excess of the desired weight should be pickled repeatedly, rinsed with water, dried and weighed until the desired weight is achieved.

7.3.1 Cadmium solution, stock 1 mL = 1000 µg Cd — Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.2 Cobalt solution, stock 1 mL = 1000 µg Co — Pickle cobalt metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.3 Copper solution, stock 1 mL = 1000 µg Cu — Pickle copper metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.4 Lead solution, stock 1 mL = 1000 µg Pb — Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with ASTM type I water.

7.3.5 Nickel solution, stock 1 mL = 1000 µg Ni — Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.4 Multielement Stock Standard Solution — Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, new FEP or HDPE bottles for storage and monitored periodically for stability. A multielement stock standard solution containing cadmium, cobalt, copper, lead, and nickel may be prepared by diluting an appropriate aliquot of each single element stock in the list to 100 mL with ASTM type I water containing 1% (v/v) nitric acid.

7.4.1 Preparation of calibration standards — Fresh multielement calibration standards should be prepared weekly. Dilute the stock multielement standard solution in 1% (v/v) nitric acid to levels appropriate to the required operating range. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve.

7.5 Blanks— Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank (LRB) is used to assess possible contamination from the sample preparation procedure and to assess spectral background. The laboratory fortified blank is used to assess routine laboratory performance, and a rinse blank is used to flush the instrument autosampler uptake system. All diluent acids should be made from concentrated acids (Section 7.1) and ASTM Type I water.

7.5.1 The calibration blank consists of the appropriate acid diluent in ASTM Type I water. The calibration blank should be stored in a FEP bottle.

7.5.2 The laboratory reagent blanks must contain all the reagents in the same volumes as used in processing the samples. The preparation blank must be carried through the entire sample digestion and preparation scheme.

7.5.3 The laboratory fortified blank (LFB) is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to provide a final concentration which will produce an absorbance of approximately 0.1 for each analyte. The LFB must be carried through the complete procedure as used for the samples.

7.5.4 The rinse blank is prepared as needed by adding 1.0 mL of conc. HNO_3 and 1.0 mL conc. HCl to 1 L of ASTM Type I water and stored in a convenient manner.

7.6 Instrument Performance Check (IPC) Solution – The IPC solution is used to periodically verify instrument performance during analysis. The IPC solution should be a fortified seawater prepared in the same acid mixture as the calibration standards and should contain method analytes such that the resulting absorbances are near the midpoint of the calibration curve. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.7 Quality Control Sample (QCS) — A quality control sample having certified concentrations of the analytes of interest should be obtained from a source outside the laboratory. Dilute the QCS if necessary with 1% nitric acid, such that the analyte concentrations fall within the proposed instrument calibration range.

8.0 Sample Collection, Preservation and Storage

8.1 Prior to collection of an aqueous sample, consideration should be given to the type of data required, so that appropriate preservation and pretreatment steps can be taken. Acid preservation, etc., should be performed at the time of sample collection or as soon thereafter as practically possible. The pH of all aqueous samples must be tested immediately prior to aliquoting for analysis to ensure the sample has been properly preserved. If properly acid-preserved, the sample can be held up to 6 months before analysis.

8.2 For determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid at the time of collection to pH < 2. Normally 3 mL of (1+1) acid per

liter of sample is sufficient. The sample should not be filtered prior to analysis.

Note: Samples that cannot be acid-preserved at the time of collection because of sampling limitations or transport restrictions, or have pH > 2 because of high alkalinity should be acidified with nitric acid to pH < 2 upon receipt in the laboratory. Following acidification, the sample should be held for 16 h and the pH verified to be < 2 before withdrawing an aliquot for sample processing.

8.3 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container type and acid as used in sample collection.

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability and 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 generated.

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to samples being analyzed by this method.

9.2.2 Linear dynamic range (LDR) — The upper limit of the LDR must be established for the wavelength utilized for each analyte by determining the signal responses from a minimum of 6 different concentration standards across the range, two of which are close to the upper limit of the LDR. Determined LDRs must be documented and kept on file. The linear calibration range which may be used for analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from the four lower standards. New LDRs should be determined whenever there is a significant change in instrument response, a change in instrument analytical hardware or operating conditions.

Note: Multiple cleanout furnace cycles may be necessary in order to fully define or utilize the LDR for certain elements such as nickel. For this reason, the upper limit of the linear calibration range may not correspond to the upper LDR limit.

Measured sample analyte concentrations that exceed the upper limit of the linear calibration range must either be diluted and reanalyzed with concern for memory effects (Section 4.4) or analyzed by another approved method.

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 (Section 7.7). 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 on with the initial determination of method detection limits or continuing with ongoing 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.[8] 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].

S = standard deviation of the replicate analyses.

Note: If the relative standard deviation (RSD) from the analyses of the seven aliquots is < 15%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. If additional confirmation of the MDL is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. Determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 1 MDLs should be determined every 6 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 (Mandatory)

9.3.1 Laboratory reagent blank (LRB) — the laboratory must analyze at least one LRB (Section 7.5.2) with each batch of 20 or fewer samples. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. Any determined source of contamination must be corrected and the samples reanalyzed for the affected analytes after acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) — the laboratory must analyze at least one LFB (Section 7.5.3) with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.3). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

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

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

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

The optional control limits must be equal to or better than the required control limits of 85-115%. After each 5-10 new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check (IPC) Solution — For all determinations the laboratory must analyze the IPC solution (Section 7.6) and a calibration blank immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. The IPC solution should be a fortified seawater matrix. 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 be within ± 10% of calibration. 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 the sample analyses data.

9.3.5 The overall sensitivity and precision of this method are strongly influenced by a laboratory's ability to control the method blank. Therefore, it is recommended that the calibration blank response be recorded for each set of samples. This record will aid the laboratory in assessing both its long and short term ability to control the method blank.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and data quality. Taking separate aliquots from the sample for replicate and fortified analyses can, in some cases, assess these effects. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Sect 9.4.2) is required.

9.4.2 The laboratory must add a known amount of each analyte to a minimum of 10% of routine samples. In each case, the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 7.5.3). Over time, samples from all routine sample sources should be fortified.

9.4.3 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 of 75-125%. Recovery calculations are not required if the concentration added is <25% of the unfortified sample concentration. Percent recovery may be calculated in units appropriate to the matrix, 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.

9.4.4 If the recovery of any analyte falls outside the designated LFM recovery range (but is still within the range of calibration and the background absorbance is < 1 abs.) and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related. This situation should be rare given the matrix elimination preconcentration step prior to analysis. If a low recovery is found, check the pH of the sample plus the buffer mixture. The resulting pH should be about 5.5. The pH of the sample strongly influences the column's ability to preconcentrate the metals; therefore, a low recovery may be caused by a low pH. If the pH for the LFM/buffer mixture is about 5.5, the analyst is advised to make an in furnace analyte addition to the LFM using the preconcentrated standard solution. If recovery of the in furnace analyte addition is shown to be out of control, a matrix interference is confirmed and the sample must be analyzed by MSA.

9.5 Utilizing Reference Materials

9.5.1 It is recommended that a reference material such as NASS-3 (from the Research Council of Canada) be fortified and used as an Instrument Performance Check Solution.

10.0 Calibration and Standardization

10.1 The preconcentration system can be configured utilizing a sample loop to define the sample volume (Figure 1) or the system can be configured such that a sample pump rate and a pumping time defines the sample volume (Figure 2). The system illustrated in Figure 1 is recommended for sample sizes of < 10 mL. A thorough rinsing of the sample loop between samples with HNO_3 is required. This rinsing will minimize the cross-contamination which may be caused by the sample loop. The system in Figure 2 should be used for sample volumes of > 10 mL. The sample pump used in Figure 2 must be calibrated to assure that a reproducible/defined volume is being delivered.

10.2 Specific wavelengths and instrument operating conditions are listed in Table 1. However, because of differences among makes and models of spectrophotometers and electrothermal furnace devices, the actual instrument conditions selected may vary from those listed.

10.3 Prior to the use of this method, instrument operating conditions must be optimized. The analyst should follow the instructions provided by the manufacturer while using the conditions listed in Table 1 as a guide. Of particular importance is the determination of the charring temperature limit for each analyte. This limit is the furnace temperature setting where a loss in analyte will occur prior to atomization. This limit should be determined by conducting char temperature profiles for each analyte and when necessary, in the matrix of question. The charring temperature selected should minimize background absorbance while providing some furnace temperature variation without loss of analyte. For routine analytical operation the charring temperature is usually set at least 100°C below this limit. The optimum conditions selected should provide the lowest reliable MDLs and be similar to those listed in Table 1. Once the optimum operating conditions are determined, they should be recorded and available for daily reference.

10.4 Prior to an initial calibration, the linear dynamic range of the analyte must be determined (Sect 9.2.2) using the optimized instrument operating conditions. For all determinations allow an instrument and hollow cathode lamp warm-up period of not less than 15 min. If an EDL is to be used, allow 30 min for warm-up.

10.5 Before using the procedure (Section 11.0) to analyze samples, data must be available to document initial demonstration of performance. The required data and procedure are described in Section 9.2. This data must be generated using the same instrument operating conditions and calibration routine (Section 11.4) to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.

11.0 Procedure

11.1 Sample Preparation - Total Recoverable Elements

11.1.1 Add 2 mL (1+1) nitric acid to the beaker containing 100 mL of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated (ribbed) watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.1.2 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. DO NOT BOIL. This step takes about 2 h for a 100-mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.1.3 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 min. Slight boiling may occur, but vigorous boiling must be avoided.

11.1.4 Allow the beaker to cool. Quantitatively transfer the sample solution to a 100-mL volumetric flask, dilute to volume with reagent water, stopper and mix.

11.1.5 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids that would clog or affect the sample introduction system, a portion of the sample may be filtered prior to analysis. However, care should be exercised to avoid potential contamination from filtration.) The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.2 Prior to first use, the preconcentration system should be thoroughly cleaned and decontaminated using 0.2M oxalic acid.

11.2.1 Precleaning the Preconcentration System

11.2.1.1 Place approximately 500 mL 0.2M oxalic acid in each of the sample/eluent containers. Flush the entire system by running the program used for sample analysis 3 times.

11.2.1.2 Rinse the containers with ASTM Type I water and repeat the sequence described in Section 11.2.1.1 using 0.75M nitric acid and again using ASTM type I water in place of the 0.2M oxalic acid.

11.2.1.3 Rinse the containers thoroughly with ASTM type I water, fill them with their designated reagents and run through the program used for sample analysis in order to prime the pump and all eluent lines with the correct reagents.

11.2.2 Peak Profile Determination

11.2.2.1 The peak elution time or the collection window should be determined using an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer) or Flame AA. Figure 3 is a plot of time vs. emission intensity for Cd, Pb, Ni, and Cu. The collection window is marked in Figure 3 and should provide about 30 sec buffer on either side of the peak. If an ICP-AES is not available, it is recommended that the peak profile be determined by collecting 200-µL samples during the elution part of the preconcentration cycle and then reconstructing the peak profile from the analysis of the 200-µL samples.

11.3 Sample Preconcentration

11.3.1 Preconcentration utilizing a sample loop.

11.3.1.1 Loading Sample Loop—With valve 1 in the off position and valve 2 in the on position, load sample through the sample loop to waste using the sample pump for 4 min at 4 mL/min. Switch on the carrier pump and pump 1% nitric acid to flush the sample collection line.

11.3.1.2 Column Loading — With valve 1 in the on position, load sample from the loop onto the column using 1M ammonium acetate for 4.5 min at 4.0 mL/min. Switch on the buffer pump, and pump 2M ammonium acetate at a flow rate of 1 mL/min. The analytes are retained on the column, while the majority of the matrix is passed through to waste.

11.3.1.3 Elution Matrix— With valve 1 in the on position the gradient pump is allowed to elute the matrix using the 1M ammonium acetate. During which time the carrier, buffer and the sample pumps are all off.

11.3.1.4 Elute Analytes — Turn off valve 1 and begin eluting the analytes by pumping 0.75M nitric acid through the column and turn off valve 2 and pump the eluted analytes into the collection flask. The analytes should be eluted into a 2-mL sample volume.

11.3.1.5 Column Reconditioning — Turn on valve 2 to direct column effluent to waste, and pump 0.75M nitric acid, 1M ammonium acetate, 0.75M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min. Each solvent should be pumped through the column for 2 min. During this process, the next sample can be loaded into the sample loop using the sample pump.

11.3.1.6 Preconcentration of the sample may be achieved by running through an eluent pump program. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.3.2 Preconcentration utilizing an auxiliary pump to determine sample volume.

11.3.2.1 Sample Loading—With the valves 1 and 2 on and the sample pump on, load the sample on the column buffering the sample utilizing the gradient pump and the 2M buffer. The actual sample volume is determined by knowing the sample pump rate and the time. While, the sample is being loaded the carrier pump can be used to flush the collection line.

11.3.2.2 Elution Matrix—With valve 1 in the off position the gradient pump is allowed to elute the matrix using the 1M ammonium acetate. During which time the carrier, buffer and the sample pumps are all off.

11.3.2.3 Elution of Analytes—with valves 1 and 2 in the off position the gradient pump is switched to 0.75M HNO_3 and the analytes are eluted into the collection vessel. The analytes should be eluted into a 2 mL sample volume.

11.3.2.4 Column Reconditioning — Turn on valve 2 to direct column effluent to waste, and pump 0.75M nitric acid, 1M ammonium acetate, 0.75M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min.

Note: When switching the gradient pump from nitric acid back to the ammonium acetate it is necessary to flush the line connecting the gradient pump to valve 2 with the ammonium acetate prior to switching the valve. If the line contains nitric acid it will elute the metals from the clean-up column.

11.3.2.5 Preconcentration of the sample may be achieved by running through an eluent pump program. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.4 Repeat the sequence described in Section 11.3.1 or 11.3.2 for each sample to be analyzed. At the end of the analytical run leave the column filled with 1M ammonium acetate buffer until it is next used.

11.5 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed.

11.6 Sample Analysis

11.6.1 Prior to daily instrument calibration, inspect the graphite furnace, the sample uptake system and autosampler injector for any change that would affect instrument performance. Clean the system and replace the graphite tube and/or platform when needed or on a daily basis. A cotton swab dipped in a 50/50 mixture of isopropyl alcohol (IPA) and H_2O (such that it is damp but not dripping) can be used to remove the majority of the salt buildup. A second cotton swab is dipped in IPA and the contact rings are wiped down to assure they are clean. The rings are then allowed to thoroughly dry and then a new tube is placed in the furnace and conditioned according to instrument manufacturers specifications.

11.6.2 Configure the instrument system to the selected optimized operating conditions as determined in Sections 10.1 and 10.2.

11.6.3 Before beginning daily calibration the instrument should be reconfigured to the optimized conditions. Initiate data system and allow a period of not less than 15 min for instrument and hollow cathode lamp warm-up. If an EDL is to be used, allow 30 min for warm-up.

11.6.4 After the warm-up period but before calibration, instrument stability must be demonstrated by analyzing a standard solution with a concentration 20 times the IDL a minimum of five times. The resulting relative standard deviation of absorbance signals must be < 5%. If the relative standard deviation is > 5%, determine and correct the cause before calibrating the instrument.

11.6.5 For initial and daily operation calibrate the instrument according to the instrument manufacturer's recommended procedures using the calibration blank (Section 7.5.1) and calibration standards (Section 7.4) prepared at three or more concentrations within the usable linear dynamic range of the analyte (Sections 4.4 & 9.2.2).

11.6.6 An autosampler must be used to introduce all solutions into the graphite furnace. Once the standard, sample or QC solution plus the matrix modifier is injected, the furnace controller completes furnace cycles and cleanout period as programmed. Analyte signals must be integrated and collected as peak area measurements. Background absorbances, background corrected analyte signals, and determined analyte concentrations on all solutions must be able to be displayed on a CRT for immediate review by the analyst and be available as hard copy for documentation to be kept on file. Flush the autosampler solution uptake system with the rinse blank (Section 7.5.4) between each solution injected.

11.6.7 After completion of the initial requirements of this method (Section 9.2), samples should be analyzed in the same operational manner used in the calibration routine.

11.6.8 During sample analyses, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4.

11.6.9 Determined sample analyte concentrations that are ≥ 90% of the upper limit of calibration must either be diluted with acidified reagent water and reanalyzed with concern for memory effects (Section 4.4), or determined by another approved test procedure that is less sensitive. Samples with a background absorbance > 1.0 must be appropriately diluted with acidified reagent water and reanalyzed (Section 9.4.6). If the method of standard additions is required, follow the instructions described in Section 11.5.

11.6.10 Report data as directed in Section 12.

11.7 Standard Additions — If the method of standard addition is required, the following procedure is recommended:

11.7.1 The standard addition technique[9] involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interference, which causes a baseline shift. The simplest version of this technique is the single-addition method. The procedure is as follows: Two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a small volume V_s of a standard analyte solution of concentration C_s. To the second (labeled B) is added the same volume V_s of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration C_x is calculated:

$$C_x = \frac{S_B V_s C_s}{(S_A - S_B) V_x}$$

where, S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_s and C_s should be chosen so that S_A is roughly twice S_B on the average. It is best if V_s is made much less than V_x, and thus C_s is much greater than C_x, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.
2. The chemical form of the analyte added must respond in the same manner as the analyte in the sample.
3. The interference effect must be constant over the working range of concern.
4. The signal must be corrected for any additive interference.

12.0 Data Analysis and Calculations

12.1 Sample data should be reported in units of µg/L for aqueous samples.

12.2 For total recoverable aqueous analytes (Section 11.1), when 100-mL aliquot is used to produce the 100 mL final solution, round the data to the tenths place and report the data in µg/L up to three significant figures. If an aliquot volume other than 100 mL is used for sample preparation, adjust the dilution factor accordingly. Also, account for any additional dilution of the prepared sample solution needed to complete the determination of analytes exceeding the upper limit of the calibration curve. Do not report data below the determined analyte MDL concentration or below an adjusted detection limit reflecting smaller sample aliquots used in processing or additional dilutions required to complete the analysis.

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

13.0 Method Performance

13.1 Experimental conditions used for single laboratory testing of the method are summarized in Table 1.

13.2 Table 2 contains precision and recovery data obtained from a single laboratory analysis of a fortified and a non-fortified sample of NASS-3. The samples were prepared using the procedure described in Sect. 11.1. Four replicates of the non-fortified samples were ana-

lyzed and the average of the replicates was used for determining the sample analyte concentration. The fortified samples of NASS-3 were also analyzed and the average percent recovery and the percent relative standard deviation is reported. The reference material certified values are also listed for comparison.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult The Waste Management Manual for Laboratory Personnel, available from the American Chemical Society at the address listed in the Section14.2.

16.0 References

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8. *Code of Federal Regulations 40*, Ch. 1, Pt. 136 Appendix B.

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Method Detection Limits for Total Recoverable Analytes in Reagent Water [1]

Element	Slit, nm	Recommended Analytical Wavelengths, nm	Char Temp, °C	Atomization Temp, °C	MDL[2], µg/L
Cadmium	0.7	228.8	800	1600	0.016
Cobalt	0.2	242.5	1400	2500	-
Copper	0.7	324.8	1300	2600	0.36
Lead	0.7	283.3	1250	2000	0.28
Nickel	0.2	232.4	1400	2500	*

[1] MDLs were calculated using NASS-3 as the matrix.
[2] MDLs were calculated based on a 10-mL sample loop.
* MDL was not calculated because the concentration in the matrix exceeds the MDL spike level.
- Not Determined.

Table 2. Precision and Recovery Data for NASS-3 using System Illustrated in Figure 1 [1,2]

Analyte	Certified Value, µg/L[3]	Sample Conc., µg/L[3]	Fortified Conc., µg/L	Avg. Recovery, %	% RSD
Cd	0.029 ± 0.004	0.026 ± 0.012	0.25	93	3.3
Co	0.004 ± 0.001	-	-	-	-
Cu	0.109 ± 0.011	<0.36	5.0	87	1.4
Pb	0.039 ± 0.006	<0.28	5.0	90	3.7
Ni	0.257 ± 0.027	0.260 ± 0.04	5.0	117	8.3

[1] Data collected using 10-mL sample loop.
[2] Matrix modifier is $Pd/Mg(NO_3)_2/H_2$.
[3] Uncertainties based on 95% confidence limits.
- Not determined.

	Valves 1	Valves 2	Buffer Pump	Carrier Pump	Sample Pump
Sample Loop Loading	Off	On	Off	On	On
Column Loading	On	On	On	Off	Off
Elution of Matrix	On	On	Off	Off	Off
Elution of Analytes	Off	Off	Off	Off	Off
Column Recondition	Off	On	Off	Off	Off

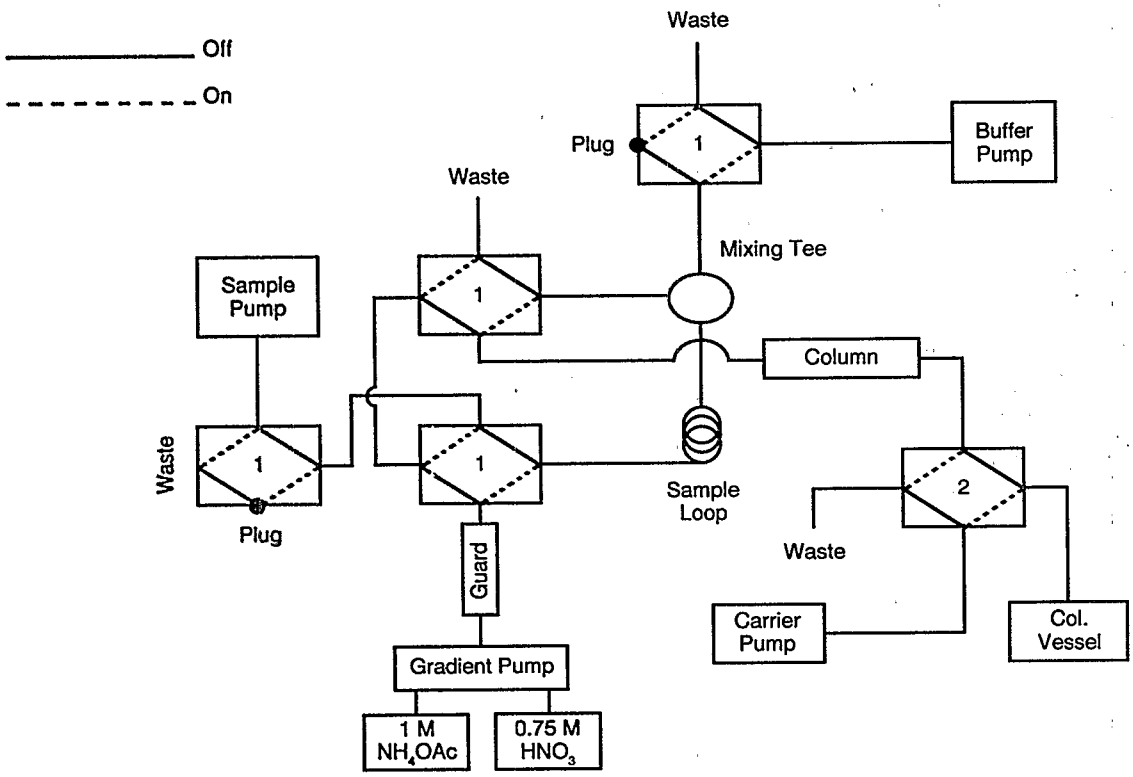

Figure 1. Sample Loop Configuration.

Event	Valves 1	Valves 2	Carrier Pump	Sample Pump
Sample Loading	On	On	On	On
Elution of Matrix	Off	On	Off	Off
Elution of Analytes	Off	Off	Off	Off
Column Recondition	Off	On	Off	On

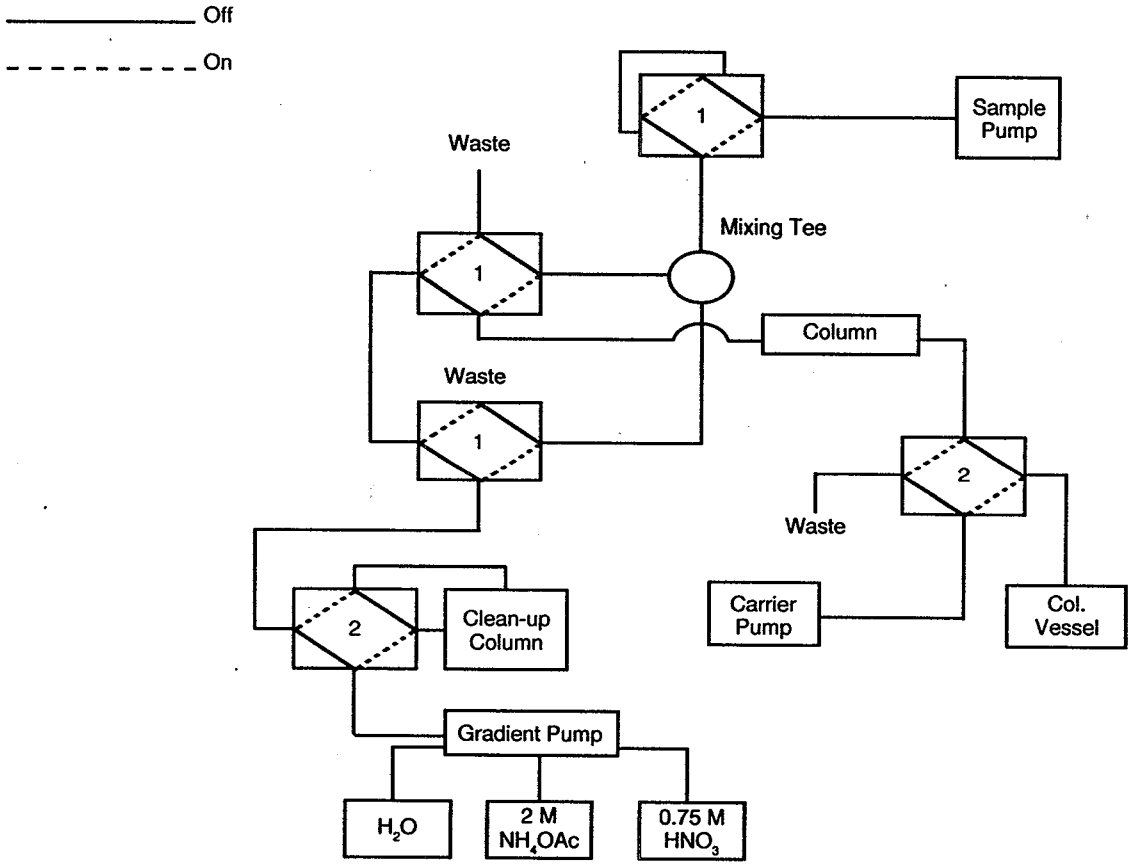

Figure 2. System Diagram without Sample Loop.

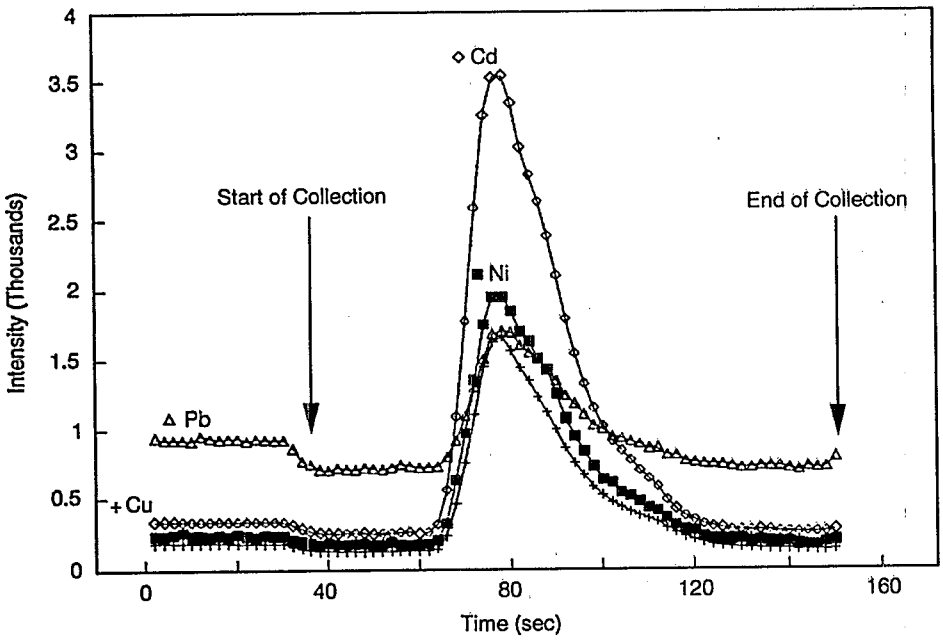

Figure 3. Peak Collection Window from ICP-AES.

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

1.0 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 (Cd) reduction technique.[1] Nitrate concentrations are obtained by subtracting nitrite values, which have been previously determined by this method without the Cd reduction procedure, from the nitrite + nitrate values.

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Nitrite	14797-65-0
Nitrate	14797-55-8

1.2 A statistically determined method detection limit (MDL) of 0.001 mg N/L in 3 parts per thousand (ppt) saline water has been determined by one laboratory.[2] The method is linear to 0.42 mg N/L using an AutoAnalyzer II System (Bran & Luebbe, Buffalo Grove, IL).

1.3 Approximately 40 samples can be analyzed in an hour.

1.4 This method should be used by analysts experienced in the use of automated colorimetric analyses and familiar with matrix interferences and procedures for their correction. A minimum of 6 months experience under experienced supervision is recommended.

1.5 This method was tested by 11 laboratories using deionized distilled water, high salinity sea water (36 ppt) and three estuarine waters of 8, 12, and 18 ppt salinity. When nitrite was determined (sample not passed through Cd reduction column), precision and accuracy were high and there were no statistically significant matrix effects. However, when nitrate was determined (sample passed through the Cd reduction column), 50% of the laboratories reported unacceptable data. Precision and accuracy decreased as salinity increased and nitrate concentration decreased. The user of this method is, therefore, cautioned as to its lack of ruggedness and accuracy when determining nitrate, and the user is admonished to carefully check and maintain the Cd reduction column required for the determination of nitrate.

2.0 Summary of Method

2.1 An automated colorimetric method for the analysis of low level nitrite + nitrate concentrations is described. Filtered samples are passed through a copperized 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 determined without the cadmium reduction step, from the nitrite + nitrate values.

3.0 Definitions

3.1 ***Calibration Standard (CAL)*** — A solution prepared from the primary dilution standard solution or stock standard solution containing the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.2 ***Dissolved Analyte (DA)*** — The concentration of analyte in an aqueous sample that passes through a 0.45 µm membrane filter assembly prior to sample acidification or other processing.

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

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

3.5 ***Linear Dynamic Range (LDR)*** — 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.[2]

3.7 ***Reagent Water*** — Type 1 reagent grade water equal to or exceeding standards established by American Society for Testing and Materials (ASTM). Reverse

osmosis systems or distilling units which produce 18 megohm water are two examples of acceptable water sources.

3.8 Refractive Index (RI) — The ratio of the velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as sea or estuarine water versus reagent water. The correction for this difference is referred to as the refractive index correction in this method.

3.9 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.

4.0 Interferences

4.1 Concentrations of iron, copper, or other metals above several mg/L alter reduction efficiency.[3] The presence of large concentrations of sulfide and/or sulfate will cause a loss of sensitivity of nitrate to the copperized cadmium column.[4,5]

4.2 Suspended solids restrict sample flow through the column. Sample turbidity should be removed by filtration prior to analysis.

4.3 This method corrects for refractive index and "salt error" interferences (Sections 12.2 and 12.3) which occur if calibration standards and samples are not matched in salinity.

5.0 Safety

5.1 Water samples collected from estuarine and/or ocean environments are generally not hazardous. However, the individual who collects samples should use proper techniques to insure safety.

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

6.0 Equipment and Supplies

6.1 Continuous Flow Automated Analytical System Consisting of:

6.1.1 Sampler.

6.1.2 Manifold or analytical aartridge equipped with copper/cadmium reduction column (prepared according to specifications in Section 7.2.1).

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 sensitive to 550 nm light.

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 have been found to be effective.

7.0 Reagents and Standards

7.1 Stock Reagent Solutions

7.1.1 Ammonium Chloride Reagent — Dissolve 10.0 g of ammonium chloride (NH_4CL)(CASRN 12125-02-9) in 1 L of reagent water. Adjust to pH 8.5 by adding 3-4 NaOH (CASRN 1310-73-2) pellets as necessary. Add 5 drops of 2% copper sulfate solution (Section 7.1.3). No addition of EDTA is necessary. This reagent is stable for 1 week when kept refrigerated.

7.1.2 Color Reagent — Combine 1500 mL reagent water, 200.0 mL concentrated phosphoric acid (H_3PO_4, CASRN 7664-38-2), 20.0 g sulfanilamide (CASRN 63-74-1), and 1.0 g N-1-napthylethylenediamine dihydrochloride (CASRN 1465-25-4). Dilute to 2000 mL with reagent water. Add 2.0 mL BRIJ-35 (Bran & Luebbe, Buffalo Grove, IL). Store at 4°C in the dark. This reagent should be prepared every 6 weeks.

7.1.3 Copper Sulfate Solution — Dissolve 2.0 g of copper sulfate ($CuSO_4.5H_2O$) (CASRN 7758-98-7) in 90 mL of reagent water. Dilute 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. Add 1.0 mL BRIJ-35. Dilute to 1000 mL with reagent water.

7.1.5 Stock Nitrate Solution — Dissolve 0.721 g of predried (60°C for 1 h) potassium nitrate (KNO_3)(CASRN 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 when kept refrigerated.

7.1.6 Stock Nitrite Solution — Dissolve 0.493 g of predried (60°C for 1 hr) sodium nitrite ($NaNO_2$) (CASRN 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, when kept refrigerated.

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

7.2 Cadmium Preparation — Use good quality cadmium (CASRN 7440-43-9) filings. Depending on the reductor column shape and size, cadmium filings should generally be <0.5 mm but >0.3 mm for glass columns and

in the 25-60 mesh size (0.25 mm to 0.71 mm) range for columns prepared by using flexible tubing.

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

Approximately 10 g of this cadmium is treated with 50 mL of 6N HCl in a 150-mL beaker. Swirl **very carefully** for 1 min. Carefully decant the HCl and thoroughly rinse the cadmium (at least 10 times) with reagent water. Decant the reagent water and add a 50-mL portion of 2% (w/v) copper sulfate solution (Section 7.1.3). While swirling, brown flakes of colloidal copper will appear and the blue color of the solution will fade. Decant and repeat sequential washing with reagent water and copper sulfate solution until blue color does not fade. Avoid exposure of treated copper-cadmium to 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 — The column should be prepared from flexible plastic tubing or glass. The following column dimensions are acceptable.

Glass tube: U-shaped, 35 cm (13.78 in.) in length with 2 mm (0.079 in.) ID.

Flexible plastic tube: 22 cm (8.66 in.) in length with 2.8 mm (0.11 in.) ID.

Plug one end of the column with glass wool. Fill the reductor column with ammonium chloride reagent (Section 7.1.1) and transfer the prepared cadmium filings to the column using a Pasteur pipette or some other method that prevents contact of the Cd filings with air. Pack the entire column uniformly with filings such that, visually, the packed filings have separation gaps no greater than approximately 1 mm. If the column is too densely packed, sample flow is restricted. Insert another glass wool plug at the top of the column and with reagents pumping through the system, attach the column to the valve assembly. 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 (Section 7.2.2) for 5 min.

7.2.2 Secondary Nitrate Solution — Dilute 1.0 mL of stock nitrate solution (Section 7.1.5) to 100 mL with reagent water. 1.0 mL of this solution = 0.001 mg N. Refrigerate and prepare fresh weekly.

7.2.3 Prepare a series of calibration standards (CAL) by diluting suitable volumes of Secondary Nitrate Solution (Section 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 CAL solutions be prepared in Low Nutrient Seawater (Section 7.1.7) diluted to the salinity of the samples, and the Sampler Wash Solution also be Low Nutrient Seawater (Section 7.1.7) diluted to that salinity. If this procedure is performed, it is unnecessary to perform the salt error and refractive index correction outlined in Sections 12.2 and 12.3. 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 (Section 12.2). The following solutions, diluted to 100 mL with reagent water, are suggested.

Volume (mL) of secondary nitrate solution (7.2.2) diluted to 100 mL	Conc., mg 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 — If CAL solutions will not be prepared to match salinity, then they must be prepared in a series of salinities in order to quantify the "salt error," the increase or decrease 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, diluted to volume with reagent water, are suggested.

Salinity (ppt)	Volume (mL) of low nutrient seawater (7.1.7)	Volume (mL) of secondary 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 (Section 7.1.6) to 100 mL with reagent water. 1.0 mL of this solution = 0.001 mg N. Refrigerate and prepare fresh weekly.

7.2.6 Working Nitrite Solution — Prepare one working standard to act as a check on the reduction capability of the cadmium column. Dilute 6.0 mL of (Section 7.2.5) to 100 mL to yield a concentration of 0.060 mg N/L. Store at 4°C and prepare fresh every 2 to 3 days.

8.0 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 or submersible pump systems. Filtration of the sample

through a 0.45-µm membrane or glass fiber filter immediately after collection is recommended.

8.1.1 A hydrocast uses 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 closed either electronically or via a mechanical "messenger" when the bottles have reached the desired depth.

8.1.2 When a submersible pump system is used, a weighted hose is sent to the desired depth in the water column and water is pumped 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 — After collection and filtration, samples should be analyzed as quickly as possible. If the samples will be analyzed within 24 h of collection, then refrigeration at 4°C is acceptable.

8.3 Sample Storage — Long-term storage of frozen samples should be in clearly labeled polyethylene bottles or polystyrene cups compatible with the analytical system's automatic sampler. If the samples cannot be analyzed within 24 h, then freezing at -20°C for a maximum period of 2 months is acceptable.[6-8]

9.0 Quality Control

9.1 A formal quality control (QC) program is required. The minimum requirements of this program consists of an initial demonstration of laboratory capability (Section 9.2), and the continued analysis of laboratory reagent blanks, laboratory duplicates, and laboratory fortified blanks with each set of samples as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

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

9.2.2 MDLs should be established for all analytes, using a low level estuarine water sample containing, or fortified at, approximately 5 times the estimated detection limit. To determine MDL values, analyze 7 replicate aliquots of water which have been processed through the entire analytical method. Perform all calculations defined in the method and report concentration in the appropriate units. Calculate the MDL as follows:

$$MDL = (t)(S)$$

where, S = Standard deviation of the replicate analyses.

t = Student's t value for n-1 degrees of freedom at the 99% confidence limit; t = 3.143 for 6 degrees of freedom.

MDLs should be determined every six months or whenever a significant change in background or instrument response occurs or when a new matrix is encountered.

9.2.3 Linear Dynamic Range (LDR) — The LDR should be determined by analyzing a minimum of 5 calibration standards ranging from 0.005 mg N/L to 0.30 mg N/L across all sensitivity settings of the autoanalyzer. Normalize responses by dividing the response by the sensitivity setting multiplier. Perform the linear regression of normalized response vs. concentration and obtain the constants **m** and **b**, where **m** is the slope and **b** is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, **R**, of a standard no longer yields a calculated concentration, $\mathbf{C_c}$, that is ± 10% of the known concentration, **C**, where $\mathbf{C_c}$= (R - b)/m. That concentration defines the upper limit of the LDR for your instrument. Should samples be encountered that have a concentration that is ≥90% of the upper limit of the LDR then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) — A laboratory should analyze at least one reagent blank (Section 3.4) with each set of samples. Reagent blank data are used to assess contamination from the laboratory environment. Should an analyte value in the reagent blank exceed the MDL, then laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing analyses.

9.3.2 Laboratory Fortified Blank (LFB) — A laboratory should analyze at least one fortified blank (Section 3.3) with each set of samples. Calculate accuracy as percent recovery. If the recovery of an analyte is not within 90-110%, then the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

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

The optional control limits must be equal to or better than the required control limits of 90-110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also the standard deviation (S) data should be

used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery — Laboratory Fortified Sample Matrix

9.4.1 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 2 to 4 times the ambient level and should be at least four times greater than the MDL.

9.4.2 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

C_s = determined fortified sample concentration (background + addition in mg N/L)

C = Sample background concentration (mg N/L)

S = Concentration in mg N/L added to the environmental sample.

9.4.3 If the recovery of an analyte falls outside the designated range of 85-115% 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 and the sample data should be flagged.

10.0 Calibration and Standardization

10.1 Calibration (Refer to Section 12.1).

10.2 Standardization (Refer to Sections 12.2, and 12.3).

11.0 Procedure

11.1 If samples are frozen, thaw the samples to room temperature.

11.2 Set up the manifold as shown in Figure 2. The tubing, flow rates, sample:wash ratio, sample rate, etc. are based on the Technicon AutoAnalyzer II System. Specifications for other segmented flow analyzers vary, so slight adjustments may be necessary.

11.3 Allow both colorimeter and recorder to warm up for 30 min. Obtain a steady baseline with reagent water pumping through the system. Add reagents to the sample stream and after the baseline has equilibrated; note the rise (reagent baseline), and adjust the 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 reagent water. For samples with a large salinity range, it is suggested that reagent water and procedures in Sections 12.2 and 12.3 be employed.

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

11.5 Place CAL solutions (Section 7.2.3) and saline standards (Section 7.2.4) (optional) and the working nitrite standard (Section 7.2.6) in sampler in order of decreasing concentration. Complete filling the sampler tray with samples, laboratory reagent blanks, laboratory fortified blanks, laboratory fortified matrices, and QC samples.

11.6 Commence Analysis

11.6.1 If the peak height of the 0.060 mg N/L nitrate standard prepared in reagent water (Section 7.2.3) is <90% of the peak height of the 0.060 mg N/L nitrite standard (Section 7.2.6), halt analyses and prepare a new cadmium reduction column (Section 7.2).

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

11.6.3 Obtain a second set of peak heights for all samples and standards with refractive reagent (Section 7.1.4) being pumped through the system in place of color reagent (Section 7.1.2). The peak heights obtained from these analyses must be subtracted from the peak heights of samples analyzed with color reagent to eliminate positive bias due to color of the water sample.

12.0 Data Analysis and 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.

Note: If the standards are prepared in low nutrient seawater of same salinity as the samples, there is no need to apply the correction factor for "salt error."

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 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.[9]

12.2.2 Obtain a second set of peak heights for all samples and standards with refractive reagent (Section 7.1.4) being pumped through the system in place of color reagent (Section 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.[10]

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 (Section 7.2.3) with color reagent (Section 7.1.2) and obtain a linear regression from the standard curve (Section 12.1). For each sample, the apparent nitrate concentration due to refractive index is then calculated from its peak height obtained with refractive reagent (Section 7.1.4) and the regression of nitrate standards obtained with color reagent (Section 7.1.2) for each sample. Salinity is entered as the independent variable and the apparent nitrate due to refractive index in that colorimeter is entered as the dependent variable. The resulting regression allows the operator to subtract an apparent nitrate concentration when the salinity is known, as long as other matrix effects (Sections 12.2.1-2) 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 (Section 12.2), then correct for the alteration in color development due to the ionic strength of the samples ("salt error").

12.3.2 Plot the salinity of the saline standards as the independent variable and the apparent concentration of nitrate (mg N/L) from the peak height corrected for refractive index (Section 12.2) calculated from the regression of standards in reagent water (Sections 7.2.3 and 12.1) as the dependent variable for all 0.060 mg N/L standards. The resulting regression equation 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 (Section 7.2.4) present in each saline standard; salinity of the sample is in parts per thousand; 0.000187 is the slope of the regression equation (Section 12.3.1); and 0.060 is the y-intercept of the regression equation (Section 12.3.1).

12.4 Results of sample analyses should be reported in mg N/L or in µg N/L.

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

13.0 Method Performance

13.1 Single-Analyst Precision

13.1.1 A single laboratory analyzed three samples collected from the Chesapeake Bay, MD, and East Bay, FL. Seven replicates of each sample were processed and analyzed, randomly throughout a group of 75 samples with salinities ranging from 3 to 36 ppt. The results were as follows:

Sample	Salinity (ppt)	Concentration (mg N/L)	% Relative standard deviation
1	36	0.0165	5.2
2	18	0.0251	0.7
3	3	0.0040	4

13.2 Pooled Precision and Accuracy

No data are available at this time. In a collaborative validation study of the method, precision and accuracy decreased as salinity increased and concentration decreased.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The 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 Agency recommends recycling as the next best option.

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

15.0 Waste Management

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

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

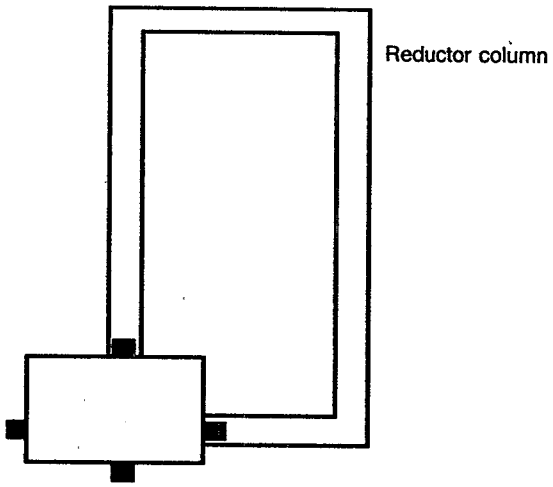

Figure 1. Reductor column valve assembly.

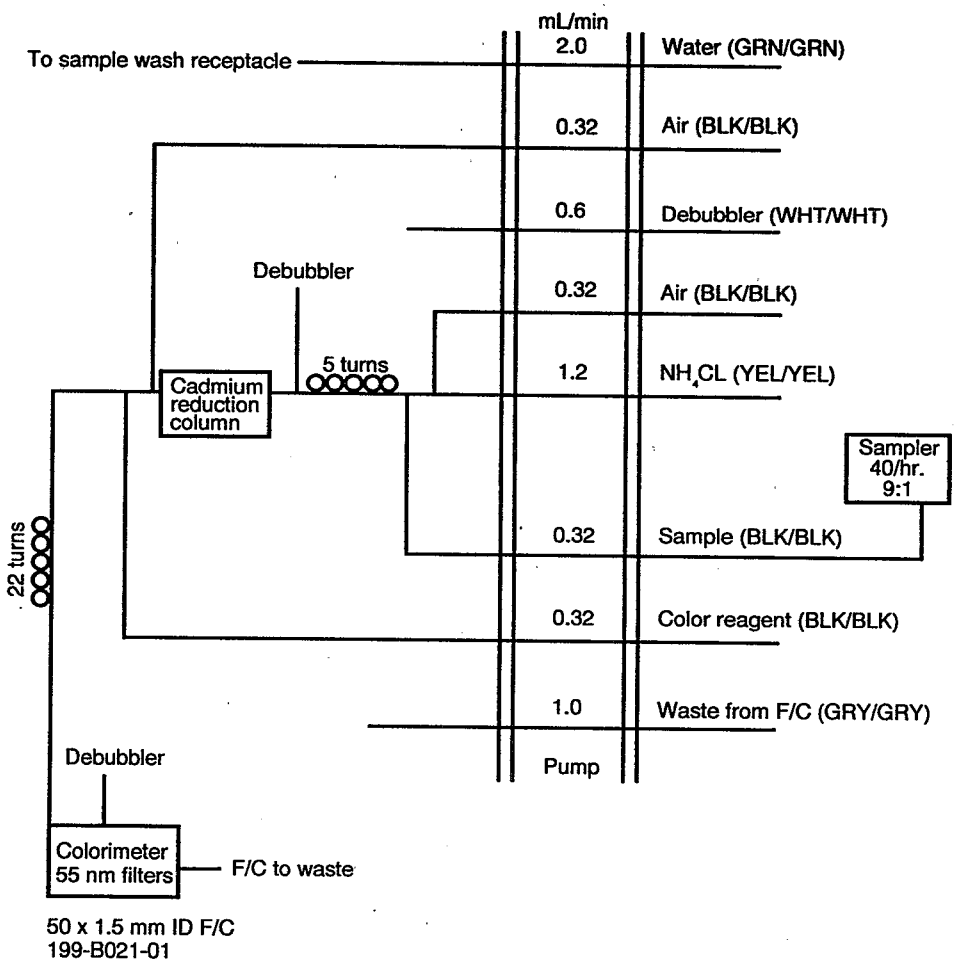

Figure 2. Manifold configuration for nitrite + nitrate.

Method 365.5

Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis

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Method 365.5

Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis

1.0 Scope and Application

1.1 This method provides a procedure for the determination of low-level orthophosphate concentrations normally found in estuarine and/or coastal waters. It is based upon 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.

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Phosphate	14265-44-2

1.2 A statistically determined method detection limit (MDL) of 0.0007 mg P/L has been determined by one laboratory in 3 parts per thousand (ppt) saline water.[3] The method is linear to 0.39 mg P/L using a Technicon AutoAnalyzer II system (Bran & Luebbe, Buffalo Grove, IL).

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, and familiar with matrix interferences and procedures for their correction. A minimum of 6-months experience under experienced supervision is recommended.

2.0 Summary of Method

2.1 An automated colorimetric method for the analysis of low-level orthophosphate concentrations is described. Ammonium molybdate and antimony potassium tartrate react in an acidic medium with dilute solutions of 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 present in the sample. Positive bias caused by differences in the refractive index of seawater and reagent water is corrected for prior to data reporting.

3.0 Definitions

3.1 ***Calibration Standard (CAL)*** — A solution prepared from the stock standard solution that is used to calibrate the instrument response with respect to analyte concentration. One of the standards in the standard curve.

3.2 ***Dissolved Analyte (DA)*** — The concentration of analyte in an aqueous sample that will pass through a 0.45-µm membrane filter assembly prior to sample acidification or other processing.

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 method performance is within acceptable control limits. This is basically a standard prepared in reagent water that is analyzed as a sample.

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

3.5 ***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.

3.6 ***Linear Dynamic Range (LDR)*** — The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.7 ***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.

3.8 ***Reagent Water (RW)*** — Type 1 reagent grade water equal to or exceeding standards established by American Society of Testing Materials (ASTM). Reverse osmosis systems or distilling units that produce 18 megohm water are two examples of acceptable water sources.

3.9 ***Refractive Index (RI)*** — The ratio of the velocity of light in a vacuum to that in a given medium. The relative refractive index is the ratio of the velocity of light in two different media, such as sea or estuarine water versus reagent water. The correction for this difference is referred to as the refractive index correction in this method.

3.10 **Stock Standard Solution (SSS)** — A concentrated solution of method analyte prepared in the labora-

tory using assayed reference compounds or purchased from a reputable commercial source.

4.0 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 or coastal waters. High iron concentrations can cause precipitation of and subsequent loss of phosphate from the dissolved phase. Hydrogen sulfide effects, such as occur in 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 Sample turbidity is removed by filtration prior to analysis.

4.3 Refractive Index interferences are corrected for estuarine/coastal samples (Section 12.2).

5.0 Safety

5.1 Water samples collected from the estuarine and/or ocean environment are generally not hazardous. However, the individual who collects samples should use proper technique.

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.

6.0 Equipment and Supplies

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 a 880 nm filter.

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

6.1.6 Strip chart recorder or computer based data system.

6.2 Phosphate-Free Glassware and Polyethylene Bottles

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

6.2.2 Membrane or glass fiber filters, 0.45 µm nominal pore size.

7.0 Reagents and Standards

7.1 Stock Reagent Solutions

7.1.1 Ammonium Molybdate Solution (40 g/L) — Dissolve 20.0 g of ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, CASRN 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 three months.

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

7.1.3 Ascorbic Acid Solution (18.0 g/L) — Dissolve 18.0 g of ascorbic acid ($C_6H_6O_6$, CASRN 50-81-7) in approximately 800 mL of reagent water and dilute to 1 L. Dispense approximately 75 mL into clean polyethylene bottles and freeze. The stability of the frozen ascorbic acid is approximately three 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 ($CH_3(CH_2)_{11}OSO_3Na$, CASRN 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 its stability is approximately three weeks.

7.1.5 Sulfuric Acid Solution (4.9 N) — Slowly add 136 mL of concentrated sulfuric acid (H_2SO_4, CASRN 7664-93-9) to approximately 800 mL of reagent water. After the solution is cooled, dilute to 1 L with reagent water.

7.1.6 Stock Phosphorus Solution — Dissolve 0.439 g of pre-dried (105°C for 1 h) monobasic potassium phosphate (KH_2PO_4, CASRN 7778-77-0) in reagent water and dilute to 1000 mL. (1.0 mL = 0.100 mg P.) The stability of this stock standard is approximately three months when kept refrigerated.

7.1.7 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, CASRN 7647-14-5); 10 g analytical grade magnesium sulfate, ($MgSO_4$, CASRN 10034-99-8); and 0.05 g analytical reagent grade sodium bicarbonate, ($NaHCO_3$, CASRN 144-55-8), in 1 L of reagent water.

7.2 Working Reagents

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

7.2.2 Reagent B — Add approximately 0.5 mL of the SLS solution (Section 7.1.4) to the 75 mL of ascorbic acid

solution (Section 7.1.3). Stability is approximately 10 days when kept refrigerated.

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

7.2.4 Secondary Phosphorus Solution— Take 1.0 mL of Stock Phosphorus Solution (Section 7.1.6) and dilute to 100 mL with reagent water. (1.0 mL = 0.0010 mg P.) Refrigerate and prepare fresh every 10 days.

7.2.5 Prepare a series of standards by diluting suitable volumes of standard solutions (Section 7.2.4) 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-level natural seawater (Section 7.1.7) diluted to match the salinity of the samples. Doing so obviates the need to perform the refractive index correction outlined in Section 12.2. 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 (Section 12.2). The following dilutions are suggested.

mL of Secondary 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

8.0 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 or submersible pump systems. Filtration of the sample through a 0.45-µm membrane or glass fiber filter immediately after collection is required.

8.1.1 A hydrocast uses a series of sampling bottles (Niskin, Nansen, Go-Flo or equivalent) that are attached at fixed intervals to a hydro 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.

8.1.2 When a submersible pump system 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.

8.1.3 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 — After collection and filtration, samples should be analyzed as quickly as possible. If the samples are to be analyzed within 24 h of collection, then refrigeration at 4°C is acceptable.

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 (Section 6.1.1). If samples cannot be analyzed within 24 h, then freezing at -20°C for a maximum period of two months is acceptable.[5-8]

9.0 Quality Control

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, the continued analysis of LRBs, laboratory duplicates, and LFBs as a continuing check on performance.

9.2 Initial Demonstration of Performance (Mandatory)

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

9.2.2 MDLs should be established using a low-level estuarine water sample fortified to approximately five times the estimated detection limit.[3] 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:

$$MDL = (t)(S)$$

where, S = the standard deviation of the replicate analyses.

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

MDLs should be determined every six months or whenever a significant change in background or instrument response occurs or when a new matrix is encountered.

9.2.3 Linear Dynamic Range (LDR) — The LDR should be determined by analyzing a minimum of five calibration standards ranging in concentration from 0.001 mg P/L to 0.20 mg P/L across all sensitivity settings of the autoanalyzer. Normalize responses by dividing the response by the sensitivity setting multiplier. Perform the linear regression of normalized response vs. concentra-

tion and obtain the constants *m* and *b*, where *m* is the slope and *b* is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration C_c, that is ± 10% of the known concentration, *C*, where $C_c = (R - b)/m$. That concentration defines the upper limit of the LDR for your instrument. Should samples be encountered that have a concentration that is ≥90% of the upper limit of the LDR, then these samples must be diluted and reanalyzed.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) — A laboratory should analyze at least one LRB (Section. 3.5) with each set of samples. LRB data are used to assess contamination from the laboratory environment. Should an analyte value in the LRB exceed the MDL, then laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates of the samples must be prepared and analyzed again after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory Fortified Blank (LFB) — A laboratory should analyze at least one LFB (Section 3.3) with each batch of samples. Calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90 - 110%, the analyte is judged out of control and the source of the problem should be identified and resolved before continuing the analyses.

9.3.3 The laboratory must use LFB data to assess laboratory performance against the required control limits of 90 - 110% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20 to 30 analyses), optional control limits can be developed from the percent mean recovery (x) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

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

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

The optional control limits must be equal to or better than the required control limits of 90 - 110%. After each 5 to 10 new recovery measurements, new control limits can be calculated using only the most recent 20 to 30 data points. Also, the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.4 Assessing Analyte Recovery - Laboratory Fortified Sample Matrix

9.4.1 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 two to four times the ambient concentration and should be at least four times the MDL.

9.4.2 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 LFBs.

Percent recoveries may be calculated using the following equation:

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

where, R = percent recovery

C_s = measured fortified sample concentration (background + concentrated addition in mg P/L)

C = sample background concentration (mg P/L)

S = concentration in mg P/L added to the environmental sample.

9.4.3 If the recovery of the analyte falls outside the designated range of 90-110% recovery, but the laboratory performance for that analyte is in control, the fortified sample should be prepared again and analyzed. 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.

10.0 Calibration and Standardization

10.1 Calibration (Refer to Sections 11.5 and 12.0).

10.2 Standardization (Refer to Section 12.2).

11.0 Procedure

11.1 If samples are frozen, thaw the samples to room temperature.

11.2 Set up manifold as shown in Figure 1. The tubing, flow rates, sample:wash ratio, sample rate, etc., are based on a Technicon AutoAnalyzer II system. Specifications for similar segmented flow analyzers vary, so slight adjustments may be necessary.

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

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

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

11.5 Place standards (Section 7.2.5) in sampler in order of decreasing concentration. Complete filling the sampler tray with samples, LRBs, LFBs, and LFMs.

11.6 Commence analysis.

11.7 Obtain a second set of peak heights for all samples and standards with Refractive Reagent A (Section 7.2.3) being pumped through the system in place of Reagent A (Section 7.2.1). This "apparent" concentration due to coloration of the water should be subtracted from concentrations obtained with Reagent A pumping through the system.

12.0 Data Analysis and Calculations

12.1 Concentrations of orthophosphate are calculated from the linear regression obtained from the standard curve in which the concentrations of the calibration standards are entered as the independent variable and the corresponding peak height is the dependent variable.

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 (Section 7.2.3) being pumped through the system in place of Reagent A (Section 7.2.1). Reagent B (Section 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.[9]

12.2.2 Subtract the refractive index peak heights from the heights obtained for the orthophosphate 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 orthophosphate concentration due to refractive index is calculated from its peak height obtained with Refractive Reagent A (Section 7.2.3) and Reagent B (Section 7.2.2) and the regression of orthophosphate standards obtained with orthophosphate Reagent A (Section 7.2.1) and Reagent B (Section 7.2.2) for each sample. Its salinity is entered as the independent variable and its apparent orthophosphate concentration due to its refractive index in that colorimeter is entered as the dependent variable. The resulting regression equation allows the operator to subtract an apparent orthophosphate 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 orthophosphate concentrations due to salinity. An example follows:

Salinity (ppt)	Apparent orthophosphate 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^{3-} = 0.000087 X Salinity (ppt)

where, 0.000087 is the slope of the line.

12.3 Results should be reported in mg PO_4^{3-}- P/L or µg PO_4^{3-}- P/L.

mg PO_4^{3-} - P/L = ppm (parts per million)

µg PO_4^{3-} - P/L = ppb (parts per billion)

13.0 Method Performance

13.1 Single Analyst Precision — A single laboratory analyzed three samples collected from Chesapeake Bay, Maryland, and East Bay, Florida. Seven replicates of each sample were processed and analyzed randomly throughout a group of 75 samples with salinities ranging from 3 to 36 ppt. The results were as follows:

Sample	Salinity (ppt)	Concentration (mg P/L)	Percent Relative Standard Deviation
1	36	0.0040	6.5
2	18	0.0024	10
3	3	0.0007	24

13.2 Pooled Precision and Accuracy

13.2.1 This method was tested by nine laboratories using reagent water, high salinity seawater from the Sargasso Sea (36 ppt) and three different salinity waters from Chesapeake Bay, Maryland (8.3 ppt, 12.6 ppt, and 19.5 ppt). The reagent water and the Sargasso Sea water were fortified at four Youden pair concentrations ranging from 0.0012 mg P/L to 0.1000 mg P/L.[10] The Chesapeake Bay waters were fortified at three Youden pair concentrations ranging from 0.0050 mg P/L to 0.0959 mg P/L with the highest salinity waters containing the lowest Youden pair and the lowest salinity waters containing the highest Youden pair. Analysis of variance (ANOVA) at the 95%

confidence level found no statistical differences between water types indicating that the refractive index correction for different salinity waters is an acceptable procedure. Table 1 contains the linear equations that describe the single-analyst standard deviation, overall standard deviation, and mean recovery of orthophosphate from each water type.

13.2.2 Pooled Method Detection Limit (p-MDL) — The p-MDL is derived from the pooled precision obtained by single laboratories for the lowest analyte concentration level used in the multilaboratory study. The p-MDLs using reagent water and Sargasso Sea water were 0.00128 and 0.00093 mg P/L, respectively.

14.0 Pollution Prevention

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

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

15.0 Waste Management

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

16.0 References

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Single-Analyst Precision, Overall Precision and Recovery from Multilaboratory Study

Reagent Water	
(0.0012 - 0.100 mg P/L)	
Mean Recovery	$X = 0.972C - 0.000018$
Overall Standard Deviation	$S_R = 0.033X + 0.000505$
Single-Analyst Standard Deviation	$S_r = 0.002X + 0.000448$
Sargasso Sea Water	
(0.0012 - 0.100 mg P/L)	
Mean Recovery	$X = 0.971C - 0.000002$
Overall Standard Deviation	$S_R = 0.021X + 0.000550$
Single-Analyst Standard Deviation	$S_r = 0.010X + 0.000249$
Chesapeake Bay Water	
(0.005 - 0.100 mg P/L)	
Mean Recovery	$X = 1.019C - 0.000871$
Overall Standard Deviation	$S_R = 0.066X + 0.000068$
Single-Analyst Standard Deviation	$S_r = 0.030X + 0.000165$

C True value or spike concentration, mg P/L.
X Mean concentration found, mg P/L, exclusive of outliers.
S_R Overall standard deviation, mg P/L, exclusive of outliers.
S_r Single-analyst standard deviation, mg P/L, exclusive of outliers.

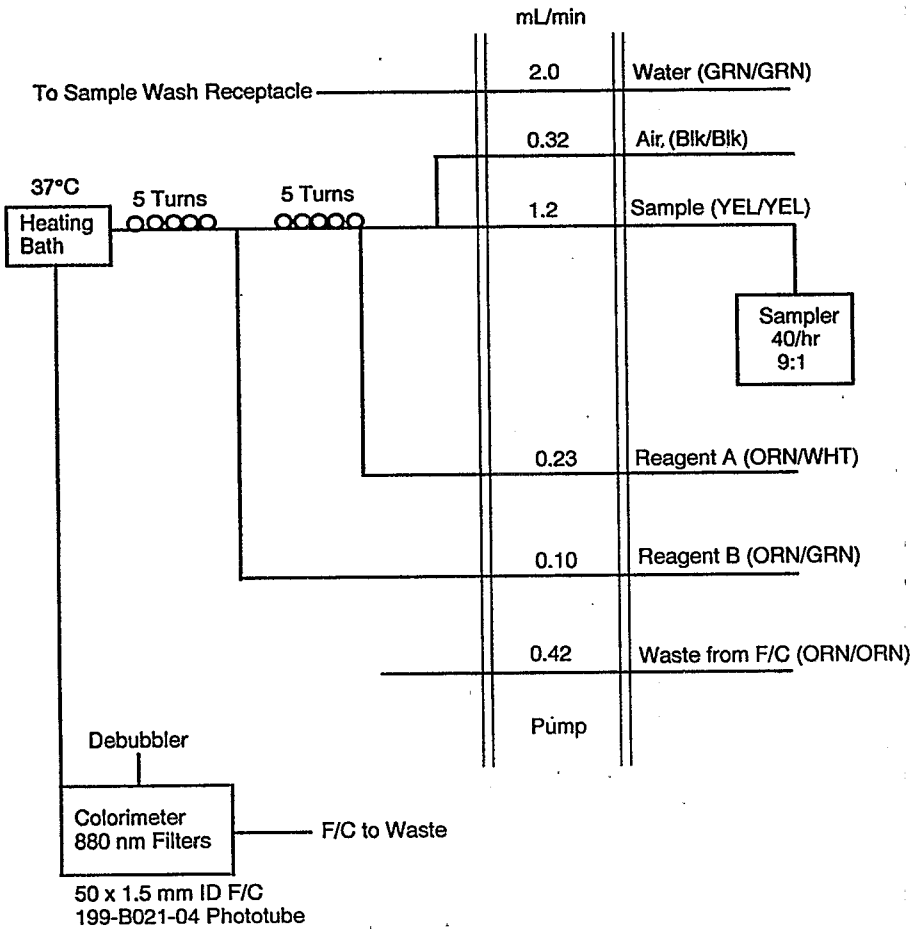

Figure 1. Manifold Configuration for Orthophosphate.

Method 440.0

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

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Method 440.0

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

1.0 Scope and Application

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

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

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

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

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

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

2.0 Summary of Method

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

3.0 Definitions

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

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

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

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

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

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

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

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

ment response is used to calculate the concentrations of the analyte(s) in the sample.

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

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

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

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

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

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

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

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

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

4.0 Interferences

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

5.0 Safety

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

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

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

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

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

6.0 Apparatus and Equipment

6.1 Elemental Analyzer

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

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

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

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

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

6.6 Mortar and pestle.

6.7 Desiccator, glass.

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

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

6.10 13-mm Swinlok filter holder.

6.11 Teflon-tipped, flat blade forceps.

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

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

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

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

6.12.4 Syringes, 60-mL, glass.

7.0 Reagents and Standards

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

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

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

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

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

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

8.0 Sample Collection, Preservation and Storage

*8.1 **Water Sample Collection*** — Samples collected for PC and PN analyses from estuarine/coastal waters are normally collected from a ship using one of two methods; hydrocast or submersible pump systems. Follow the recommended sampling protocols associated with the method used. Whenever possible, immediately filter the samples as described in Section 11.1.1. Store the filtered sample pads by freezing at -20°C or storing in a desiccator after drying at 103-105° C for 24 hr. No significant difference has been noted in comparing the two storage procedures for a time period of up to 100 days. If storage of the water sample is necessary, place the sample into a clean amber bottle and store at 4°C until filtration is done.

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

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

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

9.0 Quality Control

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

9.2 Initial Demonstration of Performance (Mandatory)

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

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

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

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

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

where, S = Standard deviation of the replicate analyses.

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

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

9.3 Assessing Laboratory Performance (Mandatory)

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

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

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

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

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

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

9.4 Assessing Analyte Recovery and Data Quality

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

10.0 Calibration and Standardization

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

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

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

where, RN = Average instrument response to standard (μv)

ZN = Instrument zero response (μv)

BN = Instrument blank response (μv)

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

where, M = The mass of standard material in μg

N_a = Number of atoms of C, N or H, in a molecule of standard material

AW = Atomic weight of C (12.01), N (14.01) or H (1.01)

MW = Molecular weight of standard material (135.2 for acetanilide)

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

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

11.0 Procedure

11.1 Aqueous Sample Preparation

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

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

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

11.2 Sediment Samples Preparation

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

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

11.3 Sample Analysis

11.3.1 Measure instrument zero response (Section 7.4) and instrument blank response (Section 7.4) and record

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

11.3.2 Report data as directed in Section 12.

11.4 Determination of Particulate Organic and Inorganic Carbon

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

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

12.0 Data Analysis and Calculations

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

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

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

$$\text{Concentration (µg/L)} = \frac{\text{Corrected sample response (µv)}}{\text{Sample volume (L) x RF (µv/µg)}}$$

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

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

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

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

Note: Units of µg/g = mg/kg

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

13.0 Method Performance

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

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

14.0 Pollution Prevention

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

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

15.0 Waste Management

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

16.0 References

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Filter Diameter Selection Guide

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

Table 2. Performance Data—Chesapeake Bay Aqueous Samples

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

[A] Standard deviation based on 7 replicates.

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

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

[A] True value. Carbon value is certified; nitrogen value is listed but not certified.
[B] Percent relative standard deviation based on 10 replicates.
[C] As calculated from T.V.

Method 445.0

In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence

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Revision 1.1
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Method 445.0

In Vitro Determination of Chlorophyll *a* and Pheophytin *a* in Marine and Freshwater Phytoplankton by Fluorescence

1.0 Scope and Application

1.1 This method provides a procedure for the low level determination of chlorophyll *a* (chl *a*) and its magnesium-free derivative, pheophytin *a*, (pheo *a*) in marine and freshwater phytoplankton using fluorescence detection.[1,2] Phaeophorbides present in the sample are determined collectively as pheo *a*.

Analyte	Chemical Abstracts Service Registry Numbers (CASRN)
Chl *a*	479-61-8

1.2 Instrumental detection limits of 0.05 µg chl *a*/L and 0.06 µg pheo *a*/L in a solution of 90% acetone were determined by this laboratory. Method detection limits using mixed assemblages of algae provide little information because of interferences from other pigments in the fluorescence of chl *a* and pheo *a*.[3] An estimated detection limit for chl *a* was determined to be 0.11 µg/L in 10 mL of final extraction solution. The upper limit of the linear dynamic range for the instrumentation used in this method evaluation was 250 µg chl *a*/L.

1.3 This method uses 90% acetone as the extraction solvent because of its efficiency for most types of algae. There is evidence that certain chlorophylls and carotenoids are more thoroughly extracted with methanol[4-6] or dimethyl sulfoxide.[7] Bowles, et al.[6] found that for chl *a*, however, 90% acetone was an effective extractant when the extraction period was optimized for the dominant species present in the sample.

1.4 Depending on the type of algae under investigation, this method can have uncorrectable interferences (Section 4.0). In cases where taxomonic classification is unavailable, a spectrophotometric or high performance liquid chromatographic (HPLC) method may provide more accurate data for chl *a* and pheo *a*.

1.5 This method is for use by analysts experienced in the handling of photosynthetic pigments and in the operation of fluorescence detectors or by analysts under the close supervision of such qualified persons.

2.0 Summary of Method

2.1 Chlorophyll-containing phytoplankton in a measured volume of sample water are concentrated by filtering at low vacuum through a glass fiber filter. The pigments are extracted from the phytoplankton in 90% acetone with the aid of a mechanical tissue grinder and allowed to steep for a minimum of 2 h, but not to exceed 24 h, to ensure thorough extraction of the chl *a*. The filter slurry is centrifuged at 675 g for 15 min (or at 1000 g for 5 min) to clarify the solution. An aliquot of the supernatant is transferred to a glass cuvette and fluorescence is measured before and after acidification to 0.003 N HCl with 0.1 N HCl. Sensitivity calibration factors, which have been previously determined on solutions of pure chl *a* of known concentration, are used to calculate the concentration of chl *a* and pheo *a* in the sample extract. The concentration in the natural water sample is reported in µg/L.

3.0 Definitions

3.1 ***Estimated Detection Limit (EDL)*** — The minimum concentration of an analyte that yields a fluorescence 3X the fluorescence of blank filters which have been extracted according to this method.

3.2 ***Linear Dynamic Range (LDR)*** — The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.3 ***Instrument Detection Limit (IDL)*** — The minimum quantity of analyte or the concentration equivalent that is detectable by the fluorometer. For this method the background is a solution of 90% acetone.

3.4 ***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.

3.5 ***Primary Dilution Standard Solution (PDS)*** — A solution of the analytes prepared in the laboratory from stock standard solutions and diluted as needed to prepare calibration solutions and other needed analyte solutions.

3.6 ***Calibration Standard (CAL)*** — A solution prepared from the primary dilution standard solution or stock standard solutions containing the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.7 ***Response Factor (RF)*** — The ratio of the response of the instrument to a known amount of analyte.

3.8 ***Laboratory Reagent Blank (LRB)*** — An aliquot of reagent water or other blank matrix that is treated exactly as a sample including exposure to all glassware,

equipment, solvents, reagents, internal standards, and surrogates that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.

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

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

3.11 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.

4.0 Interferences

4.1 Any substance extracted from the filter or acquired from laboratory contamination that fluoresces in the red region of the spectrum may interfere in the accurate measurement of both chl *a* and pheo *a*.

4.2 The relative amounts of chl *a*, *b* , and *c* vary with the taxonomic composition of the phytoplankton. Chl *b* and *c* may significantly interfere with chl *a* measurements depending on the amount present. Due to the spectral overlap of chl *b* with pheo *a* and chl *a*, underestimation of chl *a* occurs accompanied by overestimation of pheo *a* when chl *b* is present in the sample. The degree of interference depends upon the ratio of *a*:*b*. This laboratory found that at a ratio of 5:1, using the acidification procedure to correct for pheo *a*, chl *a* was underestimated by approximately 5%. Loftis and Carpenter[8] reported an underestimation of 16% when the *a*:*b* ratio was 2.5:1. A ratio of 2:1 is the highest ratio likely to occur in nature. They also reported overestimation of chl *a* in the presence of chl *c* of as much as 10% when the *a*:*c* ratio was 1:1 (the theoretical maximum likely to occur in nature). The presence of chl *c* also causes the underestimation of pheo *a* . The effect of chl *c* is not as severe as the effect of chl *b* on the measurement of chl *a* and pheo *a*. Knowledge of the taxonomy of the algae under consideration will aid in determining if the spectrophotometric method using trichromatic equations to determine chl *a*, *b*, and *c* or an HPLC method would be more appropriate.[9-14]

4.3 Quenching effects are observed in highly concentrated solutions or in the presence of high concentrations of other chlorophylls or carotenoids. Minimum sensitivity settings on the fluorometer should be avoided; samples should be diluted instead.

4.4 Fluorescence is temperature dependent with higher sensitivity occurring at lower temperatures. Samples, standards, LRBs and QCSs must be at the same temperature to prevent errors and/or poor precision. Analyses of samples at ambient temperature is recommended in this method. Ambient temperature should not fluctuate more than ± 3°C between calibrations or recalibration of the fluorometer will be necessary.

4.5 Samples must be clarified by centrifugation prior to analysis.

4.6 All photosynthetic pigments are light and temperature sensitive. Work must be performed in subdued light and all standards, QC materials and filter samples must be stored in the dark at -20°C to prevent degradation.

5.0 Safety

5.1 The toxicity or carcinogenicity of the chemicals used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and handled with caution and respect. Each laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method.[15-18] A file of MSDS should also be made available to all personnel involved in the chemical analysis.

5.2 The grinding of filters during the extraction step of this method should be conducted in a fume hood due to the volatilization of acetone by the tissue grinder.

6.0 Apparatus and Equipment

6.1 Fluorometer — Equipped with a high intensity F4T5 blue lamp, red-sensitive photomultiplier, and filters for excitation (CS-5-60) and emission (CS-2-64). (The F4T5D daylight white lamp is an acceptable substitute for the F4T5 blue lamp.) A Turner Designs Model 10 Series fluorometer was used in the evaluation of this method.

6.2 Centrifuge, capable of 675 g.

6.3 Tissue grinder, Teflon pestle (50 mm X 20 mm) with grooves in the tip with 1/4" stainless steel rod long enough to chuck onto a suitable drive motor and 30-mL capacity glass grinding tube.

6.4 Precombusted filters, glass fiber, 47-mm, nominal pore size of 0.45 or 0.7 µm. Whatman GF/F filters were used in this work.

6.5 Petri dishes, plastic, 50 X 9-mm, or some other solid container for transporting and storing sampled filters.

6.6 Aluminum foil.

6.7 Laboratory tissues.

6.8 Tweezers or flat-tipped forceps.

6.9 Vacuum pump or source capable of maintaining a vacuum up to 6 in. Hg.

6.10 Room thermometer.

6.11 Labware — All reusable labware (glass, polyethylene, Teflon, etc.) that comes in contact with chlorophyll solutions should be clean and acid free. An acceptable cleaning procedure is soaking for 4 h in laboratory grade detergent and water, rinsing with tap water, distilled deionized water and acetone.

6.11.1 Assorted Class A calibrated pipets.

6.11.2 Graduated cylinders, 500-mL and 1-L.

6.11.3 Volumetric flasks, Class A calibrated, 25-mL, 50-mL, 100-mL and 1-L capacity.

6.11.4 Glass rods.

6.11.5 Pasteur Type pipet or medicine dropper.

6.11.6 Disposable glass cuvettes for the fluorometer.

6.11.7 Filtration apparatus consisting of 1 or 2-L filtration flask, 47-mm fritted glass disk base and a glass filter tower.

6.11.8 Centrifuge tubes, polypropylene or glass, 15-mL capacity with nonpigmented screw-caps.

6.11.9 Polyethylene squirt bottles.

7.0 Reagents and Standards

7.1 Acetone, HPLC grade, (CASRN 67-64-1).

7.2 Hydrochloric acid (HCl), concentrated (sp. gr. 1.19), (CASRN 7647-01-0).

7.3 Magnesium carbonate ($MgCO_3$), light powder (CASRN 39409-82-0).

7.4 Chl *a* free of chl *b*. May be obtained from a commercial supplier such as Sigma Chemical (St. Louis, MO).

7.5 Water — ASTM Type I water (ASTM D1193) is required. Suitable water may be obtained by passing distilled water through a mixed bed of anion and cation exchange resins.

7.6 0.1 N HCl Solution — Add 8.5 mL of concentrated HCl to approximately 500 mL water and dilute to 1L.

7.7 Saturated Magnesium Carbonate Solution — Add 10 g $MgCO_3$ powder to a 1-L flask and dilute to volume with water (Section 7.5). Cap the flask and invert it several times. Let the suspended powder settle before using the solution in subsequent work.

7.8 Aqueous Acetone Solution — 90% acetone/10% saturated magnesium carbonate solution. Carefully measure 100 mL of the saturated magnesium carbonate solution into the 1-L graduated cylinder. Transfer to a 1-L flask or storage bottle. Measure 900 mL of acetone into the graduated cylinder and transfer to the flask or bottle containing the saturated magnesium carbonate solution. Mix, label and store.

7.9 Chl Stock Standard Solution (SSS) — Chl *a* from a commercial supplier will be shipped in an amber glass ampoule which has been flame sealed. This dry standard should be stored at -20°C in the dark and the SSS prepared just prior to use. Tap the ampoule until all the dried chl is in the bottom of the ampoule. In subdued light, carefully break the tip off the ampoule. Weigh the ampoule and its contents to the nearest .1 mg. Transfer the entire contents of the ampoule into a 50-mL volumetric flask and reweigh the empty ampoule. Determine by difference the mass of chl *a* added to the flask. Dilute to volume with 90% acetone, determine the concentration in mg/L (1 mg in 50 mL = 20 mg/L), label the flask and wrap with aluminum foil to protect from light. The concentration of the solution must be confirmed spectrophotometrically using a multiwavelength spectrophotometer.[9] When stored at -20°C, the SSS is stable for months. However, confirmation of the chl *a* concentration spectrophotometrically is required each time dilutions are made from the SSS.

7.10 Laboratory Reagent Blank (LRB) — A blank filter which is extracted and analyzed just as a sample filter. The LRB should be the last filter extracted of a sample set. It is used to assess possible contamination of the reagents or apparatus.

7.11 Chl a Primary Dilution Standard Solution (PDS) — Add 1 mL of the SSS (Section 7.9) to a clean 100-mL flask and dilute to volume with the aqueous acetone solution (Section 7.8). If exactly 1 mg of pure chl *a* was used to prepare the SSS, the concentration of the PDS is 200 µg/L. Prepare fresh just prior to use.

7.12 Quality Control Sample (QCS) — Chl *a* QCSs can be obtained from the Quality Assurance Research Division, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH 45268. QCSs are supplied with a calibration solution.

8.0 Sample Collection, Preservation and Storage

8.1 Water Sample Collection — Water may be obtained by a pump or grab sampler. Data quality objectives will determine the depth at which samples are taken. Healthy phytoplankton, however, are generally obtained from the photic zone (depth at which the illumination level is 1% of surface illumination). Enough water should be collected to concentrate phytoplankton on at least three filters. Filtration volume size will depend on the particulate load of the water. Four liters may be required for open ocean water where phytoplankton density is usually low, whereas 1 L or less is generally sufficient for lake, bay or estuary water. All apparatus should be clean and acid-

free. Filtering should be performed in subdued light as soon as possible after sampling. Aboard-ship filtration is highly recommended.

Assemble the filtration apparatus and attach the vacuum source with vacuum gauge and regulator. Vacuum filtration should not exceed 6 in. Hg (20 kPa). Higher filtration pressures may damage cells and result in loss of chlorophyll.

Prior to drawing a subsample from the water sample container, thoroughly shake the container to suspend the particulates. Pour the subsample into a graduated cylinder and accurately measure the volume. Pour the subsample into the filter tower of the filtration apparatus and apply a vacuum (not to exceed 20 kPa). A sufficient volume has been filtered when a visible green or brown color is apparent on the filter. Do not suck the filter dry with the vacuum; instead slowly release the vacuum as the final volume approaches the level of the filter and completely release the vacuum as the last bit of water is pulled through the filter. Remove the filter from the fritted base with tweezers, fold once with the particulate matter inside, lightly blot the filter with a tissue to remove excess moisture and place it in the petri dish or other suitable container. If the filter will not be immediately extracted, then wrap the container with aluminum foil to protect the phytoplankton from light and store the filter at -20°C. Short term storage (2 to 4 h) on ice is acceptable, but samples should be stored at -20°C as soon as possible.

8.2 Preservation — Sampled filters should be stored frozen (-20°C or -70°C) in the dark until extraction.

8.3 Holding Time — Filters can be stored frozen for as long as 3-1/2 weeks without significant loss of chl *a*.[19]

9.0 Quality Control

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

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (instrumental detection limits, linear dynamic range and EDLs) and laboratory performance (analyses of QCSs) prior to sample analyses.

9.2.2 Linear Dynamic Range (LDR) — The LDR should be determined by analyzing a minimum of 5 calibration standards ranging in concentration from 0.2 µg/L to 200 µg chl *a*/L across all sensitivity settings of the fluorometer. Normalize responses by dividing the response by the sensitivity setting multiplier. Perform the linear regression of normalized response vs. concentration and obtain the constants *m* and *b*, where *m* is the slope and *b* is the y-intercept. Incrementally analyze standards of higher concentration until the measured fluorescence response, *R*, of a standard no longer yields a calculated concentration, C_c, that is ± 10% of the known concentration, *C*, where $C_c = (R - b)/m$. That concentration defines the upper limit of the LDR for your instrument. Should samples be encountered that have a concentration which is 90% of the upper limit of the LDR, these samples must be diluted and reanalyzed.

9.2.3 Instrumental Detection Limit (IDL) — Zero the fluorometer with a solution of 90% acetone on the maximum sensitivity setting. Pure chl *a* in 90% acetone should be serially diluted until it is no longer detected by the fluorometer on a maximum sensitivity setting.

9.2.4 Estimated Detection Limit (EDL) — Several blank filters should be extracted according to the procedure in Section 11, using clean glassware and apparatus, and the fluorescence measured. A solution of pure chl *a* in 90% acetone should be serially diluted until it yields a response which is 3X the average response of the blank filters.

9.2.5 Quality Control Sample (QCS) — When beginning to use this method, on a quarterly basis or as required to meet data quality needs, verify the calibration standards and acceptable instrument performance with the analysis of a QCS (Section 7.12). If the determined value is not within the confidence interval provided with the reference value, then the determinative step of this method is unacceptable. The source of the problem must be identified and corrected before continuing analyses.

9.2.6 Extraction Proficiency — Personnel performing this method for the first time should demonstrate proficiency in the extraction of sampled filters (Section 11.1). Twenty to thirty natural samples should be obtained using the procedure outlined in Section 8.1 of this method. Sets of 10 samples or more should be extracted and analyzed according to Section 11.2. The percent relative standard deviation (%RSD) of uncorrected values of chl *a* should not exceed 15% for samples that are approximately 10X the IDL. RSD for pheo *a* might typically range from 10 to 50%.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory Reagent Blank (LRB) — The laboratory must analyze at least one blank filter with each sample batch. The LRB should be the last filter extracted. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the IDL indicate contamination from the laboratory environment. When LRB values constitute 10% or more of the analyte level determined for a sample, fresh samples or field duplicates must be analyzed after the contamination has been corrected and acceptable LRB values have been obtained.

10.0 Calibration and Standardization

10.1 Calibration — Calibration should be performed bimonthly or when there has been an adjustment made to the instrument, such as replacement of lamp, filters or photomultiplier. Prepare 0.2, 2, 5, 20 and 200 µg chl *a*/L calibration standards from the PDS (Section 7.11). Alternately, a calibration solution can be obtained from the address listed in Section 7.12. Allow the instrument to warm up for at least 15 min. Measure the fluorescence of each standard at sensitivity settings that provide midscale readings. Obtain response factors for chl *a* for each sensitivity setting as follows:

$$F_s = C_a/R_s$$

where,

F_s = response factor for sensitivity setting, S.

R_s = fluorometer reading for sensitivity setting, S.

C_a = concentration of chl *a*.

Avoid using the minimum sensitivity setting due to quenching effects.

If pheo *a* determinations will be made then it will be necessary to obtain before-to-after acidification response ratios of the chl *a* calibration standards as follows: (1) measure the fluorescence of the standard, (2) remove the cuvette from the fluorometer, (3) acidify the solution to 0.003 N HCl[4] with the 0.1 N HCl solution, (4) wait 90 sec. and measure the fluorescence of the standard solution again. Addition of the acid may be made using a medicine dropper. It will be necessary to know how many drops are equal to 1 mL of acid. For a cuvette that holds 5 mL of extraction solution, it will be necessary to add 0.15 mL of 0.1 N HCl to reach a final acid concentration of 0.003N in the 5 mL. Calculate the ratio, r, as follows:

$$r = R_b/R_a$$

where,

R_b = fluorescence of pure chl *a* standard solution before acidification.

R_a = fluorescence of pure chl *a* standard solution after acidification.

11.0 Procedure

11.1 Extraction of Filter Samples

11.1.1 If sampled filters have been frozen, remove them from the freezer but keep them in the dark. Set up the tissue grinder and have on hand tissues and squirt bottles containing water and acetone. Workspace lighting should be the minimum that is necessary to read instructions and operate instrumentation. Remove a filter from its container and place it in the glass grinding tube. Push it to the bottom of the tube with a glass rod. With a volumetric pipet, add 4 mL of the aqueous acetone solution (Section 7.8) to the grinding tube. After the filter has been converted to a slurry, grind the filter for approximately 1 min at 500 rpm. Pour the slurry into a 15-mL screw-cap centrifuge tube and, using a 6-mL volumetric pipet, rinse the pestle and the grinding tube with 90% acetone. Add the rinse to the centrifuge tube containing the filter slurry. Cap the tube and shake it vigorously. Place it in the dark before proceeding to the next filter extraction. Before placing another filter in the grinding tube, use the acetone and water squirt bottles to thoroughly rinse the pestle, grinding tube and glass rod. The last rinse should be with acetone. Use a clean tissue to remove any filter residue that adheres to the pestle or to the steel rod of the pestle. Proceed to the next filter and repeat the steps above. The entire extraction with transferring and rinsing steps takes 5 min. Approximately 500 mL of acetone and water waste are generated per 20 samples from the rinsing of glassware and apparatus.

11.1.2 Shake each tube vigorously before placing them to steep in the dark at 4°C. Samples should be allowed to steep for a minimum of 2 h but not to exceed 24 h. Tubes should be shaken at least once during the steeping period or placed horizontally to allow the extraction solution to have maximum contact with the filter slurry.

11.1.3 After steeping is complete, centrifuge samples for 15 min. at 675 g or for 5 min. at 1000 g. Samples should be allowed to come to ambient temperature before analysis. This can be done by placing the tubes in a constant temperature water bath or by letting them stand at room temperature for 30 min. Recalibrate the fluorometer if the room temperature fluctuated ±3°C from the last calibration date.

11.2 Sample Analysis

11.2.1 After the fluorometer has warmed up for at least 15 min, use the 90% acetone solution to zero the instrument on the sensitivity setting that will be used for sample analysis.

11.2.2 Pour or pipet the supernatant of the extracted sample into a sample cuvette. The volume of sample required in your instrument's cuvette should be known so that the correct amount of acid can be added in the pheo *a* determinative step. For a cuvette that holds 5 mL of extraction solution, 0.15 mL of the 0.1 N HCl solution is required to achieve 0.003 N HCl. Choose a sensitivity setting that yields a midscale reading when possible and avoid the minimum sensitivity setting. If the concentration of chl *a* in the sample is ≥90% of the upper limit of the LDR, then dilute the sample with the 90% acetone solution and reanalyze. Record the fluorescence measurement and sensitivity setting used for the sample. Remove the cuvette from the fluorometer and acidify the extract to a final concentration of 0.003 N HCl using the 0.1 N HCl solution. Wait 90 sec. before measuring

fluorescence again. Twenty-five to thirty-five samples can be extracted and analyzed in one 8-h day.

12.0 Data Analysis and Calculations

12.1 "Uncorrected" chl *a* may be determined in a sample extract by multiplying the fluorescence response of the sample by the appropriate response factors determined in Section 10.1. Determine the "corrected" chl *a* concentration in the sample extract and the pheo *a* concentration in µg/L as follows:

$$\text{Chl } a, \mu g/L = F_s\,(r/r\text{-}1)\,(R_b - R_a)$$

$$\text{Pheo } a, \mu g/L = F_s(r/r\text{-}1)\,(rR_a - R_b)$$

where,

F_s = response factor for the sensitivity setting used.

R_b = fluorescence of sample extract before acidification.

R_a = fluorescence of sample extract after acidification.

r = the before-to-after acidification ratio of a pure chl *a* solution (Section 10.1).

12.2 The concentration of chl *a* and pheo *a* in the natural water sample is calculated by multiplying the results obtained in Section 12.1 by 10 mL (the extraction volume) and dividing by the volume (mL) of natural water sample that was filtered. Any other dilution or concentration factors should be incorporated accordingly.

12.3 LRB and QCS data should be reported with each sample data set.

13.0 Method Performance

13.1 The IDL for the instrument used in the evaluation of this method was 0.05 µg/L for chl *a* and 0.06 µg/L pheo *a*. An EDL of 0.11 µg chl *a*/L was determined.

13.2 The precision (%RSD) for chl *a* in mostly blue-green and green phytoplankton natural samples which were steeped for 2 h vs. 24 h is reported in Table 1. Although the means were the same, precision was better for samples which were allowed to steep for 24 h prior to analysis. Since pheo *a* was found in the samples, the chl *a* values are "corrected" (Section 12.1). Table 2 contains precision data for pheo *a*. A statistical analysis of the pheo *a* data indicated a significant difference at the 0.05 significance level in the mean values obtain. The cause of the lower pheo *a* values in samples extracted for 24 h is not known.

13.3 Three QCS ampoules obtained from the USEPA were analyzed and compared to the reported confidence limits in Table 3. The reference values for QCS obtained from the USEPA are periodically updated and new confidence limits established.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Section 11.1.1). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

15.0 Waste Management

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

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17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Comparison of Precision of Two Extraction Periods

	Corrected Chl *a*			
	Sample A[1]		Sample B[2]	
	2 h[3]	24 h[3]	2 h[3]	24 h[3]
Mean concentation (µg/L)	49.6	52.9	78.6	78.8
Standard deviation (µg/L)	4.89	2.64	6.21	2.77
Relative standard deviation (%)	9.9	5.0	7.9	3.5

[1] Values reported are the mean measured concentrations (n=6) of chl *a* in the natural water based on a 100 mL filtration volume.
[2] Values reported are the mean measured concentrations (n=9) in the extraction solution. Sample filtration volume was 300 mL.
[3] The length of time that the filters steeped after they were ground.

Table 2. Comparison of Precision of Two Extractions Periods for Pheo *a*

	Pheo *a*			
	Sample A[1]		Sample B[2]	
	2 h[3]	24 h[3]	2 h[3]	24 h[3]
Mean concentation (µg/L)	9.22	8.19	13.10	10.61
Standard deviation (µg/L)	2.36	3.55	3.86	2.29
Relative standard deviation (%)	25.6	43.2	29.5	21.6

[1] Values reported are the mean measured concentrations (n=6) of chl *a* in the natural water based on a 100 mL filtration volume.
[2] Values reported are the mean measured concentrations (n=9) in the extraction solution. Sample filtration volume was 300 mL.
[3] The length of time that the filters steeped after they were ground.

Table 3. Analyses of USEPA QC Samples

Analyte	*Reference value*	*Confidence limits*
Chl *a*	2.1 µg/L	0.5 to 3.7 µg/L
Pheo *a*	0.3 µg/L	-0.2 to 0.8 µg/L
Analyte	*Mean Measured Value*	*% Relative Standard deviation*
Chl *a*	2.8 µg/L	1.5
Pheo *a*	0.3 µg/L	33

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