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METHODS FOR THE DETERMINATION OF INORGANIC SUBSTANCES IN ENVIRONMENTAL SAMPLES

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
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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, groundwaters, wastewaters, sediments, sludges, and solid wastes.

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

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

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

This EMSL-Cincinnati publication, "Methods for the Determination of Inorganic Substances in Environmental Samples," was prepared as the continuation of an initiative to gather together a compendium of standardized laboratory analytical methods for the determination of inorganic substances in water and wastewater. We are pleased to provide this manual and believe that it will be of considerable value to many public and private laboratories involved in inorganic analyses for regulatory or other reasons.

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ABSTRACT

This manual contains ten updated and revised automated, semi-automated or methods amenable to automation for the determination of a variety of inorganic substances in water and wastewater.

These methods include and address, in an expanded form, information concerning safety, quality control, pollution prevention, and waste management. Methods were selected which minimize the amount of hazardous reagents required and maximize sample throughput to allow expanded quality control.

Automated methods are included for nitrate-nitrite, phosphorus, and sulfate. Semi-automated methods cover cyanide, ammonia, total kjeldahl nitrogen (TKN), chemical oxygen demand (COD) and generic phenolics. Methods amenable to automation include turbidity and inorganic anions by ion chromatography.

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ACKNOWLEDGMENTS

This methods manual was prepared and edited by the Inorganic Chemistry Branch (ICB) of the Chemistry Research Division, Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati).

Major contributors from the ICB include John D. Pfaff for anions by ion chromatography, Billy Potter for cyanide methodology, Theodore Martin and John Creed for quality control, and Diane Schirmann for manuscript production. James O'Dell selected the methods that are included from previous versions published in "Methods for the Chemical Analysis of Water and Wastes," EPA 600/4-79-020, Revised March 1983. He reorganized the previous versions into a format approved by the Environmental Monitoring Management Council and added new sections to address safety, quality control, pollution prevention, and waste management. Last but not least, a very special acknowledgement goes to former ICB member Morris E. Gales who started this project before his retirement and was responsible for most of the original versions of these methods. Thanks again Mo for your years of dedicated service to our environment, the USEPA, and your fellow employees.

INTRODUCTION

The original version of this manual was issued in November 1969 by the Federal Water Pollution Control Administration as "FWPCA Methods for Chemical Analysis of Water and Wastes." With the creation of the United States Environmental Protection Agency (USEPA) came "Methods for Chemical Analysis of Water and Wastes 1971" Publication No. 16020---07/71. The second edition was issued in 1974 as EPA 625/6-74-003, and the third edition in 1979 as EPA 600/4/79-020. The current version, an updated second printing of the third edition, was revised and issued in March 1983. The methods contained in the 1983 manual form the basis for most of the methodology approved for compliance monitoring of inorganic parameters specified under the Clean Water Act (NPDES) and contaminants regulated under the Safe Drinking Water Act.

In 1991, a number of new and revised metals methods were incorporated into a new publication entitled, "Methods for the Determination of Metals in Environmental Samples." Concurrently, the decision was made to revise and update selected non-metal methods to be issued under the name "Methods for the Determination of Inorganic Substances in Environmental Samples."

For both the metals and non-metals manuals, several important features were adopted:

- Consistent use of terminology, a feature especially helpful in the quality control sections where standardized terminology is not yet available. The terms were carefully selected to be meaningful without extensive definition, and therefore should be easy to understand and use.
- New sections are included with expanded useful coverage of safety, quality control, pollution prevention and waste management.
- All methods are presented in the new EPA standard Environmental Monitoring Management Council (EMMC) format.

Although a number of other methods included the 1983 edition of USEPA "Methods for Chemical Analysis of Water and Wastes", Standard Methods for the Examination of Water and Wastewater, and American Society for Testing and Materials Annual Book of Standards (ASTM) are acceptable for compliance monitoring, the revised methods contained in this publication are considered to be the most useful in terms of future regulatory requirements. They represent a selection of air segmented automated, semi-automated, or amenable to automation methodology that provides the following advantages over their manual counterparts.

- Higher sample throughput for faster analysis and improved precision.
- Faster analysis allows more time to perform the updated quality control required to insure valid results.
- Lower per analysis reagent consumption to reduce waste production and minimize disposal costs.

- The inclusion of multi-laboratory data generated from USEPA performance evaluation studies.

The following methods are included with specific features and improvements:

- A revised version of EPA turbidity Method 180.1 that minimizes the direct use of hydrazine sulfate.
- An updated version of EPA Method 300.0 for anions by ion chromatography.
- A new stand alone semi-automated revision of EPA cyanide Method 335.2 that specifies the use of the downsized midi-distillation procedure.
- A new semi-automated version of the EPA phenolics Method 420.2.
- The optional use of a non-mercury catalyst in EPA TKN Method 351.2.
- All methods allow the optional use of reduced reagent and distillation-digestion volumes.
- Most of the methods include the option of limited performance-based modifications or improvements.

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METHOD 180.1

DETERMINATION OF TURBIDITY BY NEPHELOMETRY

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Revision 2.0
August 1993

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METHOD 180.1

DETERMINATION OF TURBIDITY BY NEPHELOMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The applicable range is 0 to 40 nephelometric turbidity units (NTU). Higher values may be obtained with dilution of the sample.

2.0 SUMMARY OF METHOD

2.1 The method is based upon a comparison of the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension. The higher the intensity of scattered light, the higher the turbidity. Readings, in NTU's, are made in a nephelometer designed according to specifications given in sections 6.1 and 6.2. A primary standard suspension is used to calibrate the instrument. A secondary standard suspension is used as a daily calibration check and is monitored periodically for deterioration using one of the primary standards.

2.1.1 Formazin polymer is used as a primary turbidity suspension for water because it is more reproducible than other types of standards previously used for turbidity analysis.

2.1.2 A commercially available polymer primary standard is also approved for use for the National Interim Primary Drinking Water Regulations. This standard is identified as AMCO-AEPA-1, available from Advanced Polymer Systems.

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 surrogates analytes.

3.2 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.

3.3 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.

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

3.5 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.

3.6 PRIMARY CALIBRATION STANDARD (PCAL) -- A suspension prepared from the primary dilution stock standard suspension. The PCAL suspensions are used to calibrate the instrument response with respect to analyte concentration.

3.7 QUALITY CONTROL SAMPLE (QCS) -- A solution of the method analyte of known concentrations that is used to fortify an aliquot of LRB matrix. The QCS is obtained from a source external to the laboratory, and is used to check laboratory performance.

3.8 SECONDARY CALIBRATION STANDARDS (SCAL) -- Commercially prepared, stabilized sealed liquid or gel turbidity standards calibrated against properly prepared and diluted formazin or styrene divinylbenzene polymers.

3.9 STOCK STANDARD SUSPENSION (SSS) -- A concentrated suspension containing the analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source. Stock standard suspension is used to prepare calibration suspensions and other needed suspensions.

4.0 INTERFERENCES

4.1 The presence of floating debris and coarse sediments which settle out rapidly will give low readings. Finely divided air bubbles can cause high readings.

4.2 The presence of true color, that is the color of water which is due to dissolved substances that absorb light, will cause turbidities to be low, although this effect is generally not significant with drinking waters.

4.3 Light absorbing materials such as activated carbon in significant concentrations can cause low readings.

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 should be as low as reasonably achievable.

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

5.3 Hydrazine Sulfate (7.2.1) is a carcinogen. It is highly toxic and may be fatal if inhaled, swallowed, or absorbed through the skin. Formazin can contain residual hydrazine sulfate. Proper protection should be employed.

6.0 EQUIPMENT AND SUPPLIES

6.1 The turbidimeter shall consist of a nephelometer, with light source for illuminating the sample, and one or more photo-electric detectors with a readout device to indicate the intensity of light scattered at right angles to the path of the incident light. The turbidimeter should be designed so that little stray light reaches the detector in the absence of turbidity and should be free from significant drift after a short warm-up period.

6.2 Differences in physical design of turbidimeters will cause differences in measured values for turbidity, even though the same suspension is used for calibration. To minimize such differences, the following design criteria should be observed:

6.2.1 Light source: Tungsten lamp operated at a color temperature between 2200-3000°K.

6.2.2 Distance traversed by incident light and scattered light within the sample tube: Total not to exceed 10 cm.

6.2.3 Detector: Centered at 90° to the incident light path and not to exceed ± 30° from 90°. The detector, and filter system if used, shall have a spectral peak response between 400 and 600 nm.

6.3 The sensitivity of the instrument should permit detection of a turbidity difference of 0.02 NTU or less in waters having turbidities less than 1 unit. The instrument should measure from 0 to 40 units turbidity. Several ranges may be necessary to obtain both adequate coverage and sufficient sensitivity for low turbidities.

6.4 The sample tubes to be used with the available instrument must be of clear, colorless glass or plastic. They should be kept scrupulously clean, both inside and out, and discarded when they become scratched or etched. A light coating of silicon oil may be used to mask minor imperfections in glass tubes. They must not be handled at all where the light strikes them, but should be provided with sufficient extra length, or with a protective case, so that they may be handled.

Tubes should be checked, indexed and read at the orientation that produces the lowest background blank value.

6.5 Balance -- Analytical, capable of accurately weighing to the nearest 0.0001 g.

6.6 Glassware -- Class A volumetric flasks and pipets as required.

7.0 REAGENTS AND STANDARDS

7.1 Reagent water, turbidity-free: Pass deionized distilled water through a 0.45μ pore size membrane filter, if such filtered water shows a lower turbidity than unfiltered distilled water.

7.2 Stock standard suspension (Formazin):

7.2.1 Dissolve 1.00 g hydrazine sulfate, $(NH_2)_2.H_2SO_4$, (CASRN 10034-93-2) in reagent water and dilute to 100 mL in a volumetric flask. **CAUTION--CARCINOGEN**

7.2.2 Dissolve 10.00 g hexamethylenetetramine (CASRN 100-97-0) in reagent water and dilute to 100 mL in a volumetric flask. In a 100 mL volumetric flask, mix 5.0 mL of each solution (7.2.1 + 7.2.2). Allow to stand 24 hours at 25 ± 3°C, then dilute to the mark with reagent water.

7.3 Primary calibration standards: Mix and dilute 10.00 mL of stock standard suspension (7.2) to 100 mL with reagent water. The turbidity of this suspension is defined as 40 NTU. For other values, mix and dilute portions of this suspension as required.

7.3.1 A new stock standard suspension (7.2) should be prepared each month. Primary calibration standards (7.3) should be prepared daily by dilution of the stock standard suspension.

7.4 Formazin in commercially prepared primary concentrated stock standard suspension (SSS) may be diluted and used as required. Dilute turbidity standards should be prepared daily.

7.5 AMCO-AEPA-1 Styrene Divinylbenzene polymer primary standards are available for specific instruments and require no preparation or dilution prior to use.

7.6 Secondary standards may be acceptable as a daily calibration check, but must be monitored on a routine basis for deterioration and replaced as required.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with turbidity free water. Volume collected should be sufficient to insure a

representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 No chemical preservation is required. Cool sample to 4°C.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, samples maintained at 4°C may be held for up to 48 h.

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

9.2 INITIAL DEMONSTRATION OF PERFORMANCE.

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS).

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analysis of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before continuing with on-going analyses.

9.3 ASSESSING LABORATORY PERFORMANCE

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data produced

are used to assess contamination from the laboratory environment.

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

9.3.3 Where additional reference materials such as Performance Evaluation samples 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 Turbidimeter calibration: The manufacturer's operating instructions should be followed. Measure standards on the turbidimeter covering the range of interest. If the instrument is already calibrated in standard turbidity units, this procedure will check the accuracy of the calibration scales. At least one standard should be run in each instrument range to be used. Some instruments permit adjustments of sensitivity so that scale values will correspond to turbidities. Solid standards, such as those made of lucite blocks, should never be used due to potential calibration changes caused by surface scratches. If a pre-calibrated scale is not supplied, calibration curves should be prepared for each range of the instrument.

11.0 PROCEDURE

11.1 Turbidities less than 40 units: If possible, allow samples to come to room temperature before analysis. Mix the sample to thoroughly disperse the solids. Wait until air bubbles disappear then pour the sample into the turbidimeter tube. Read the turbidity directly from the instrument scale or from the appropriate calibration curve.

11.2 Turbidities exceeding 40 units: Dilute the sample with one or more volumes of turbidity-free water until the turbidity falls below 40 units. The turbidity of the original sample is then computed from the turbidity of the diluted sample and the dilution factor. For example, if 5 volumes of turbidity-free water were added to 1 volume of sample, and the diluted sample showed a turbidity of 30 units, then the turbidity of the original sample was 180 units.

11.2.1 Some turbidimeters are equipped with several separate scales. The higher scales are to be used only as indicators of required dilution volumes to reduce readings to less than 40 NTU.

NOTE 1: Comparative work performed in the Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) indicates a progressive error on sample turbidities in excess of 40 units.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Multiply sample readings by appropriate dilution to obtain final reading.

12.2 Report results as follows:

NTU	Record to Nearest:
0.0 - 1.0	0.05
1 - 10	0.1
10 - 40	1
40 - 100	5
100 - 400	10
400 - 1000	50
> 1000	100

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), using surface water samples at levels of 26, 41, 75 and 180 NTU, the standard deviations were ± 0.60, ± 0.94, ± 1.2 and ± 4.7 units, respectively.

13.2 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in NTU.

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

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

15.0 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. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Annual Book of ASTM Standards, Volume 11.01 Water (1), Standard D1889-88A, p. 359, (1993).

2. Standard Methods for the Examination of Water and Wastewater, 18th Edition, pp. 2-9, Method 2130B, (1992).

17.0 TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
373	0.450	0.4864	0.0027	0.1071	-0.0078
374	0.600	0.6026	-0.0244	0.1048	-0.0211
289	0.65	0.6931	0.0183	0.1301	0.0005
482	0.910	0.9244	0.0013	0.2512	0.1024
484	0.910	0.9919	0.0688	0.1486	-0.0002
489	1.00	0.9405	-0.0686	0.1318	-0.0236
640	1.36	1.3456	-0.0074	0.1894	0.0075
487	3.40	3.2616	-0.0401	0.3219	-0.0103
288	4.8	4.5684	-0.0706	0.3776	-0.0577
714	5.60	5.6984	0.2952	0.4411	-0.0531
641	5.95	5.6026	-0.1350	0.4122	-0.1078

REGRESSIONS: $X = 0.955T + 0.54$, $S = 0.074T + 0.082$

METHOD 300.0

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY

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Revision 2.1
August 1993

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METHOD 300.0

DETERMINATION OF INORGANIC ANIONS BY ION CHROMATOGRAPHY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of the following inorganic anions:

PART A.

Bromide	Nitrite
Chloride	Ortho-Phosphate-P
Fluoride	Sulfate
Nitrate	

PART B.

Bromate	Chlorite
Chlorate	

1.2 The matrices applicable to each method are shown below:

A. Drinking water, surface water, mixed domestic and industrial wastewaters, groundwater, reagent waters, solids (after extraction 11.7), leachates (when no acetic acid is used).

B. Drinking water and reagent waters

1.3 The single laboratory Method Detection Limit (MDL defined in Sect. 3.2) for the above analytes is listed in Tables 1A and 1B. The MDL for a specific matrix may differ from those listed, depending upon the nature of the sample.

1.4 Method A is recommended for drinking and wastewaters. The multilaboratory ranges tested for each anion are as follows:

Analyte	mg/L
Bromide	0.63 - 21.0
Chloride	0.78 - 26.0
Fluoride	0.26 - 8.49
Nitrate-N	0.42 - 14.0
Nitrite-N	0.36 - 12.0
Ortho-Phosphate-P	0.69 - 23.1
Sulfate	2.85 - 95.0

1.5 This method is recommended for use only by or under the supervision of analysts experienced in the use of ion chromatography and in the interpretation of the resulting ion chromatograms.

1.6 When this method is used to analyze unfamiliar samples for any of the above anions, anion identification should be supported by the use of a fortified sample matrix covering the anions of interest. The fortification procedure is described in Sect. 11.6.

1.7 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must demonstrate the ability to generate acceptable results with this method, using the procedures described in Sect. 9.0.

2.0 SUMMARY OF METHOD

2.1 A small volume of sample, typically 2 to 3 mL, is introduced into an ion chromatograph. The anions of interest are separated and measured, using a system comprised of a guard column, analytical column, suppressor device, and conductivity detector.

2.2 The main differences between Parts A and B are the separator columns and guard columns. Sections 6.0 and 7.0 will elicit the differences.

2.3 An extraction procedure must be performed to use this method for solids (See 11.7).

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 DUPLICATES (FD) -- 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 field duplicates indicate the precision associated with sample collection, preservation and storage, as well as with laboratory procedures.

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

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

3.9 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.

3.10 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 PERFORMANCE EVALUATION SAMPLE (PE) -- A solution of method analytes distributed by the Quality Assurance Research Division (QARD), Environmental Monitoring Systems Laboratory (EMSL-Cincinnati), U. S. Environmental Protection Agency, Cincinnati, Ohio, to multiple laboratories for analysis. A volume of the solution is added to a known volume of reagent water and analyzed with procedures used for samples. Results of analyses are used by QARD to determine statistically the accuracy and precision that can be expected when a method is performed by a competent analyst. Analyte true values are unknown to the analyst.

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.

4.0 INTERFERENCES

4.1 Interferences can be caused by substances with retention times that are similar to and overlap those of the anion of interest. Large amounts of an anion can interfere with the peak resolution of an adjacent anion. Sample dilution and/or fortification can be used to solve most interference problems associated with retention times.

4.2 The water dip or negative peak that elutes near, and can interfere with, the fluoride peak can usually be eliminated by the addition of the equivalent of 1 mL of concentrated eluent (7.3 100X) to 100 mL of each standard and sample.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated baseline in ion chromatograms.

4.4 Samples that contain particles larger than 0.45 microns and reagent solutions that contain particles larger than 0.20 microns require filtration to prevent damage to instrument columns and flow systems.

4.5 Any anion that is not retained by the column or only slightly retained will elute in the area of fluoride and interfere. Known coelution is caused by carbonate and other small organic anions. At concentrations of fluoride above 1.5 mg/L, this interference may not be significant, however, it is the responsibility of the user to generate precision and accuracy information in each sample matrix.

4.6 The acetate anion elutes early during the chromatographic run. The retention times of the anions also seem to differ when large amounts of acetate are present. Therefore, this method is not recommended for leachates of solid samples when acetic acid is used for pH adjustment.

4.7 The quantitation of unretained peaks should be avoided, such as low molecular weight organic acids (formate, acetate, propionate etc.) which are conductive and coelute with or near fluoride and would bias the fluoride quantitation in some drinking and most waste waters.

4.8 Any residual chlorine dioxide present in the sample will result in the formation of additional chlorite prior to analysis. If any

concentration of chlorine dioxide is suspected in the sample purge the sample with an inert gas (argon or nitrogen) for about five minutes or until no chlorine dioxide remains.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Sulfuric acid (7.4)

6.0 Equipment and Supplies

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

6.2 Ion chromatograph -- Analytical system complete with ion chromatograph and all required accessories including syringes, analytical columns, compressed gasses and detectors.

6.2.1 Anion guard column: A protector of the separator column. If omitted from the system the retention times will be shorter. Usually packed with a substrate the same as that in the separator column.

6.2.2 Anion separator column: This column produces the separation shown in Figures 1 and 2.

6.2.2.1 Anion analytical column (Method A): The separation shown in Figure 1 was generated using a Dionex AS4A column (P/N 37041). An optional column may be used if comparable resolution of peaks is obtained, and the requirements of Sect. 9.2 can be met.

6.2.2.2 Anion analytical column (Method B). The separation shown in Figure 2 was generated using a Dionex AS9 column (P/N 42025). An optional column may be used if comparable resolution of peaks is

obtained and the requirements of Sect. 9.2 can be met.

6.2.3 Anion suppressor device: The data presented in this method were generated using a Dionex anion micro membrane suppressor (P/N 37106).

6.2.4 Detector -- Conductivity cell: approximately 1.25 μL internal volume, (Dionex, or equivalent) capable of providing data as required in Sect. 9.2.

6.3 The Dionex AI-450 Data Chromatography Software was used to generate all the data in the attached tables. Systems using a stripchart recorder and integrator or other computer based data system may achieve approximately the same MDL's but the user should demonstrate this by the procedure outlined in Sect. 9.2.

7.0 Reagents and Standards

7.1 Sample bottles: Glass or polyethylene of sufficient volume to allow replicate analyses of anions of interest.

7.2 Reagent water: Distilled or deionized water, free of the anions of interest. Water should contain particles no larger than 0.20 microns.

7.3 Eluent solution (Method A and Method B): Sodium bicarbonate (CASRN 144-55-8) 1.7 mM, sodium carbonate (CASRN 497-19-8) 1.8 mM. Dissolve 0.2856 g sodium bicarbonate ($NaHCO_3$) and 0.3816 g of sodium carbonate (Na_2CO_3) in reagent water (7.2) and dilute to 2 L.

7.4 Regeneration solution (micro membrane suppressor): Sulfuric acid (CASRN-7664-93-9) 0.025N. Dilute 2.8 mL conc. sulfuric acid (H_2SO_4) to 4 L with reagent water.

7.5 Stock standard solutions, 1000 mg/L (1 mg/mL): Stock standard solutions may be purchased as certified solutions or prepared from ACS reagent grade materials (dried at 105°C for 30 min) as listed below.

7.5.1 Bromide (Br^-) 1000 mg/L: Dissolve 1.2876 g sodium bromide (NaBr, CASRN 7647-15-6) in reagent water and dilute to 1 L.

7.5.2 Bromate (BrO_3^-) 1000 mg/L: Dissolve 1.1798g of sodium bromate ($NaBrO_3$, CASRN 7789-38-0) in reagent water and dilute to 1 L.

7.5.3 Chlorate (ClO_3^-) 1000 mg/L: Dissolve 1.2753g of sodium chlorate ($NaClO_3$, CASRN 7775-09-9) in reagent water and dilute to 1 L.

7.5.4 Chloride (Cl^-) 1000 mg/L: Dissolve 1.6485 g sodium chloride (NaCl, CASRN 7647-14-5) in reagent water and dilute to 1 L.

7.5.5 Chlorite (ClO_2^-) 1000 mg/L: Dissolve 1.3410g of sodium chlorite ($NaClO_2$, CASRN 7758-19-2) in reagent water and dilute to 1 L.

7.5.6 Fluoride (F^-) 1000 mg/L: Dissolve 2.2100g sodium fluoride (NaF, CASRN 7681-49-4) in reagent water and dilute to 1 L.

7.5.7 Nitrate (NO_3^--N) 1000 mg/L: Dissolve 6.0679 g sodium nitrate ($NaNO_3$, CASRN 7631-99-4) in reagent water and dilute to 1 L.

7.5.8 Nitrite (NO_2^--N) 1000 mg/L: Dissolve 4.9257 g sodium nitrite ($NaNO_2$, CASRN 7632-00-0) in reagent water and dilute to 1 L.

7.5.9 Phosphate ($PO_4^{=}$-P) 1000 mg/L: Dissolve 4.3937 g potassium phosphate (KH_2PO_4, CASRN 7778-77-0) in reagent water and dilute to 1 L.

7.5.10 Sulfate ($SO_4^{=}$) 1000 mg/L: Dissolve 1.8141 g potassium sulfate (K_2SO_4, CASRN 7778-80-5) in reagent water and dilute to 1 L.

NOTE: Stability of standards: Stock standards (7.5) are stable for at least 1 month when stored at 4°C. Except for the chlorite standard which is only stable for two weeks. Dilute working standards should be prepared weekly, except those that contain nitrite and phosphate should be prepared fresh daily.

7.6 Ethylenediamine preservation solution: Dilute 10 mL of ethylenediamine (99%) (CASRN 107-15-3) to 200 mL with reagent water. Use 1 mL of this dilution to each 1 L of sample taken.

8.0 Sample Collection, Preservation and Storage

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis, if required, and minimize waste disposal.

8.2 Sample preservation and holding times for the anions that can be determined by this method are as follows:

Analyte	Preservation	Holding Time
Bromate	None required	28 days

Bromide	None required	28 days
Chlorate	None required	28 days
Chloride	None required	28 days
Chlorite	Cool to 4°C	immediately
Fluoride	None required	28 days
Nitrate-N	Cool to 4°C	48 hours
Combined (Nitrate/Nitrite)	conc. H_2SO_4 to a pH < 2	28 days
Nitrite-N	Cool to 4°C	48 hours
O-Phosphate-P	Cool to 4°C	48 hours
Sulfate	Cool to 4°C	28 days

NOTE: If the determined value for the combined nitrate/nitrite exceeds 0.5 mg/L as N^-, a resample must be analyzed for the individual concentrations of nitrate and nitrite.

8.3 The method of preservation and the holding time for samples analyzed by this method are determined by the anions of interest. In a given sample, the anion that requires the most preservation treatment and the shortest holding time will determine the preservation treatment. It is recommended that all samples be cooled to 4°C and held for no longer than 28 days for Method A and analyzed immediately in Method B.

NOTE: If the sample cannot be analyzed for chlorite within ≤ 10 minutes, the sample may be preserved by adding 1 mL of the ethylenediamine (EDA) preservation solution (7.6) to 1 L of sample. This will preserve the concentration of the chlorite for up to 14 days. This addition of EDA has no effect on bromate or chlorate, so they can also be determined in a sample preserved with EDA. Residual chlorine dioxide should be removed from the sample (per 4.8) prior to the addition of EDA.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of LCRs and analysis of QCS) and laboratory performance

(determination of MDLs) prior to performing analyses by this method.

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[6] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

9.4.1.1 If the concentration of fortification is less than 25% of the background concentration of the matrix the matrix recovery should not be calculated.

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

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

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

9.4.3 Until sufficient data becomes available (usually a minimum of 20 to 30 analysis), assess laboratory performance against recovery limits for method A of 80 to 120% and 75 to 125% for method B. When sufficient internal performance data becomes available develop control limits from percent mean recovery and the standard deviation of the mean recovery.

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 (Sect. 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.5 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.

9.4.6 In recognition of the rapid advances occurring in chromatography, the analyst is permitted certain options, such as the use of different columns and/or eluents, to improve the separations or lower the cost of measurements. Each time such modifications to the method are made, the analyst is required to repeat the procedure in Sect. 9.2.

9.4.7 It is recommended that the laboratory adopt additional quality assurance practices for use with this method. The specific practices that are most productive depend upon the needs of the laboratory and the nature of the samples. Field duplicates may be analyzed to monitor the precision of the sampling technique. When doubt exists over the identification of a peak in the chromatogram, confirmatory techniques such as sample dilution and fortification, must be used. Whenever possible, the laboratory should perform analysis of quality control check samples and participate in relevant performance evaluation sample studies.

9.4.8 At least quarterly, replicates of LFBs should be analyzed to determine the precision of the laboratory measurements. Add these results to the on-going control charts to document data quality.

9.4.9 When using Part B, the analyst should be aware of the purity of the reagents used to prepare standards. Allowances must be made when the solid materials are less than 99% pure.

10.0 Calibration and Standardization

10.1 Establish ion chromatographic operating parameters equivalent to those indicated in Tables 1A or 1B.

10.2 For each analyte of interest, prepare calibration standards at a minimum of three concentration levels and a blank by adding accurately measured volumes of one or more stock standards (7.5) to a volumetric flask and diluting to volume with reagent water. If a sample analyte concentration exceeds the calibration range the sample may be diluted to fall within the range. If this is not possible then three new calibration concentrations must be chosen, two of which must bracket the concentration of the sample analyte of interest. Each attenuation range of the instrument used to analyze a sample must be calibrated individually.

10.3 Using injections of 0.1 to 1.0 mL (determined by injection loop volume) of each calibration standard, tabulate peak height or area responses against the concentration. The results are used to prepare a calibration curve for each analyte. During this procedure, retention times must be recorded.

10.4 The calibration curve must be verified on each working day, or whenever the anion eluent is changed, and after every 20 samples. If the response or retention time for any analyte varies from the expected values by more than ± 10%, the test must be repeated, using fresh calibration standards. If the results are still more than ± 10%, a new calibration curve must be prepared for that analyte.

10.5 Nonlinear response can result when the separator column capacity is exceeded (overloading). The response of the detector to the sample when diluted 1:1, and when not diluted, should be compared. If the calculated responses are the same, samples of this total anionic concentration need not be diluted.

11.0 Procedure

11.1 Tables 1A and 1B summarize the recommended operating conditions for the ion chromatograph. Included in these tables are estimated retention times that can be achieved by this method. Other columns, chromatographic conditions, or detectors may be used if the requirements of Sect. 9.2 are met.

11.2 Check system calibration daily and, if required, recalibrate as described in Sect. 10.

11.3 Load and inject a fixed amount of well mixed sample. Flush injection loop thoroughly, using each new sample. Use the same size loop for standards and samples. Record the resulting peak size in area or peak height units. An automated constant volume injection system may also be used.

11.4 The width of the retention time window used to make identifications should be based upon measurements of actual retention time variations of standards over the course of a day. Three times the standard deviation of a retention time can be used to calculate a suggested window size for each analyte. However, the experience of the analyst should weigh heavily in the interpretation of chromatograms.

11.5 If the response for the peak exceeds the working range of the system, dilute the sample with an appropriate amount of reagent water and reanalyze.

11.6 If the resulting chromatogram fails to produce adequate resolution, or if identification of specific anions is questionable, fortify the sample with an appropriate amount of standard and reanalyze.

NOTE: Retention time is inversely proportional to concentration. Nitrate and sulfate exhibit the greatest amount of change, although all anions are affected to some degree. In some cases this peak migration may produce poor resolution or identification.

11.7 The following extraction should be used for solid materials. Add an amount of reagent water equal to ten times the weight of dry solid material taken as a sample. This slurry is mixed for ten minutes using a magnetic stirring device. Filter the resulting slurry before injecting using a 0.45 μ membrane type filter. This can be the type that attaches directly to the end of the syringe. Care should be taken to show that good recovery and identification of peaks is obtained with the user's matrix through the use of fortified samples.

11.8 It has been reported that lower detection limits for bromate ($\approx$7 μg/L) can be obtained using a borate based eluent[7]. The use of this eluent or other eluents that improve method performance may be considered as a minor modification of the method and as such still are acceptable.

11.9 Should more complete resolution be needed between peaks the eluent (7.3) can be diluted. This will spread out the run but will also cause the later eluting anions to be retained longer. The analyst must determine to what extent the eluent is diluted. This dilution should not be considered a deviation from the method.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve for each analyte by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg/L.

12.4 Report NO_2^- as N
NO_3^- as N
$HPO_4^=$ as P

13.0 METHODS PERFORMANCE

13.1 Tables 1A and 2A give the single laboratory (EMSL-Cincinnati) MDL for each anion included in the method under the conditions listed.

13.2 Tables 2A and 2B give the single laboratory (EMSL-Cincinnati) standard deviation for each anion included in the method in a variety of waters for the listed conditions.

13.3 Multiple laboratory accuracy and bias data (S_t) and estimated single operator values (S_o) for reagent, drinking and waste water using

method A are given for each anion in Tables 3 through 9. Data from 19 laboratories were used for this data.

13.4 Some of the bias statements, for example chloride and sulfate, may be misleading due to spiking small increments of the anion into large naturally occurring concentrations of the same anion.

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 Quantity of the chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

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2. Standard Methods for the Examination of Water and Wastewater, Method 4110B, "Anions by Ion Chromatography", 18th Edition of Standard Methods (1992).

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5. American Society for Testing and Materials. Test Method for Anions in Water by Chemically-Suppressed Ion Chromatography D4327-91. Annual Book of Standards, Vol 11.01 (1993).

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

7. Hautman, D.P. & Bolyard, M. Analysis of Oxyhalide Disinfection By-products and other Anions of Interest in Drinking Water by Ion Chromatography. Jour. of Chromatog., 602, (1992), 65-74.

17.0 TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1A. CHROMATOGRAPHIC CONDITIONS AND DETECTION LIMITS IN REAGENT WATER (PART A)

ANALYTE	PEAK # *	RETENTION TIME(MIN)	MDL mg/L
Fluoride	1	1.2	0.01
Chloride	2	1.7	0.02
Nitrite-N	3	2.0	0.004
Bromide	4	2.9	0.01
Nitrate-N	5	3.2	0.002
o-Phosphate-P	6	5.4	0.003
Sulfate	7	6.9	0.02

Standard Conditions:

Columns: as specified in 6.2.2.1
Detector: as specified in 6.2.4
Eluent: as specified in 7.3

Pump Rate: 2.0 mL/min.
Sample Loop: 50 μL

MDL calculated from data system using a y-axis selection of 1000 ns and with a stripchart recorder with an attenuator setting of 1 uMHO full scale.

* See Figure 1

TABLE 1B. CHROMATOGRAPHIC CONDITIONS AND DETECTION LIMITS IN REAGENT WATER (PART B)

ANALYTE	PEAK # *	RETENTION TIME(MIN)	MDL mg/L
Chlorite	1	2.8	0.01
Bromate	2	3.2	0.02
Chlorate	4	7.1	0.003

Standard Conditions:

Column: as specified in 6.2.2.2
Detector: as specified in 6.2.4
Eluent: as specified in 7.3

Pump Rate: 1.0 mL/min.
Sample Loop: 50 μL
Attentuation - 1
y-axis - 500 ns

* See Figure 2

TABLE 2A. SINGLE-OPERATOR ACCURACY AND BIAS OF STANDARD ANIONS (METHOD A)

ANALYTE	SAMPLE TYPE	KNOWN CONC. (mg/L)	NUMBER OF REPLICATES	MEAN RECOVERY %	STANDARD DEVIATION (mg/L)
Bromide	RW	5.0	7	99	0.08
	DW	5.0	7	105	0.10
	SW	5.0	7	95	0.13
	WW	5.0	7	105	0.34
	GW	5.0	7	92	0.34
	SD	2.0	7	82	0.06
Chloride	RW	20.0	7	96	0.35
	DW	20.0	7	108	1.19
	SW	10.0	7	86	0.33
	WW	20.0	7	101	5.2
	GW	20.0	7	114	1.3
	SD	20.0	7	90	0.32
Fluoride	RW	2.0	7	91	0.05
	DW	1.0	7	92	0.06
	SW	1.0	7	73	0.05
	WW	1.0	7	87	0.07
	GW	0.4	7	95	0.07
	SD	5.0	7	101	0.35
Nitrate- N	RW	10.0	7	103	0.21
	DW	10.0	7	104	0.27
	SW	10.0	7	93	0.17
	WW	10.0	7	101	0.82
	GW	10.0	7	97	0.47
	SD	10.0	7	82	0.28
Nitrite- N	RW	10.0	7	97	0.14
	DW	10.0	7	121	0.25
	SW	5.0	7	92	0.14
	WW	5.0	7	91	0.50
	GW	10.0	7	96	0.35
	SD	2.0	7	98	0.08
o-Phosphate- P	RW	10.0	7	99	0.17
	DW	10.0	7	99	0.26
	SW	10.0	7	98	0.22
	WW	10.0	7	106	0.85
	GW	10.0	7	95	0.33

TABLE 2A (CONT'D)

Sulfate	RW	20.0	7	99	0.40
	DW	50.0	7	105	3.35
	SW	40.0	7	95	1.7
	WW	40.0	7	102	6.4
	GW	40.0	7	112	3.2

RW = Reagent Water
DW = Drinking Water
SW = Surface Water
WW = Mixed Domestic and Industrial Wastewater
GW = Groundwater
SD = USEPA QC Solid (shale)

TABLE 2B. SINGLE-OPERATOR ACCURACY AND BIAS OF BY-PRODUCT (PART B)

ANALYTE	SAMPLE TYPE	SPIKE (mg/L)	NUMBER OF REPLICATES	MEAN RECOVERY %	STANDARD DEVIATION (mg/L)
Bromate	RW	5.0	7	103	0.07
		1.0	7	98	0.04
		0.1	7	155	0.005
		0.05	7	122	0.01
	DW	5.0	7	95	0.04
		1.0	7	85	0.02
		0.1	7	98	0.005
		0.05	7	98	0.005
Chlorate	RW	5.0	7	101	0.06
		1.0	7	97	0.01
		0.1	7	100	0.01
		0.05	7	119	0.05
	DW	5.0	7	101	0.04
		1.0	7	115	0.01
		0.1	7	121	0.005
		0.05	7	110	0.01
Chlorite	RW	5.0	7	100	0.04
		1.0	7	98	0.01
		0.1	7	86	0.01
		0.05	7	94	0.01
	DW	5.0	7	96	0.03
		1.0	7	100	0.02
		0.1	7	76	0.00
		0.05	7	96	0.01

RW = Reagent Water
DW = Drinking Water

TABLE 3. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR FLUORIDE

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.26	0.25	0.08	0.11	-3.8
	0.34	0.29	0.11		-14.7
	2.12	2.12	0.07	0.12	0.0
	2.55	2.48	0.14		-2.7
	6.79	6.76	0.20	0.19	-0.4
	8.49	8.46	0.30		-0.4
Drinking	0.26	0.24	0.08	0.05	-7.7
	0.34	0.34	0.11		0.0
	2.12	2.09	0.18	0.06	-1.4
	2.55	2.55	0.16		0.0
	6.79	6.84	0.54	0.25	+0.7
	8.49	8.37	0.75		-1.4
Waste	0.26	0.25	0.15	0.06	-3.8
	0.34	0.32	0.08		-5.9
	2.12	2.13	0.22	0.15	+0.5
	2.55	2.48	0.16		-2.7
	6.79	6.65	0.41	0.20	-2.1
	8.49	8.27	0.36		-2.6

TABLE 4. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR CHLORIDE

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.78	0.79	0.17	0.29	+1.3
	1.04	1.12	0.46		+7.7
	6.50	6.31	0.27	0.14	-2.9
	7.80	7.76	0.39		-0.5
	20.8	20.7	0.54	0.62	-0.5
	26.0	25.9	0.58		-0.4
Drinking	0.78	0.54	0.35	0.20	-30.8
	1.04	0.51	0.38		-51.0
	6.50	5.24	1.35	1.48	-19.4
	7.80	6.02	1.90		-22.8
	20.8	20.0	2.26	1.14	-3.8
	26.0	24.0	2.65		-7.7
Waste	0.78	0.43	0.32	0.39	-44.9
	1.04	0.65	0.48		-37.5
	6.50	4.59	1.82	0.83	-29.4
	7.80	5.45	2.02		-30.1
	20.8	18.3	2.41	1.57	-11.8
	26.0	23.0	2.50		-11.5

TABLE 5. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR NITRITE - NITROGEN

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.36	0.37	0.04	0.04	+2.8
	0.48	0.48	0.06		0.0
	3.00	3.18	0.12	0.06	+6.0
	3.60	3.83	0.12		+6.4
	9.60	9.84	0.36	0.26	+2.5
	12.0	12.1	0.27		+0.6
Drinking	0.36	0.30	0.13	0.03	-16.7
	0.48	0.40	0.14		-16.7
	3.00	3.02	0.23	0.12	+0.7
	3.60	3.62	0.22		+0.6
	9.60	9.59	0.44	0.28	-0.1
	12.0	11.6	0.59		-3.1
Waste	0.36	0.34	0.06	0.04	-5.6
	0.48	0.46	0.07		-4.2
	3.00	3.18	0.13	0.10	+6.0
	3.60	3.76	0.18		+4.4
	9.60	9.74	0.49	0.26	+1.5
	12.0	12.0	0.56		+0.3

TABLE 6. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR BROMIDE

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.63	0.69	0.11	0.05	+9.5
	0.84	0.85	0.12		+1.2
	5.24	5.21	0.22	0.21	-0.6
	6.29	6.17	0.35		-1.9
	16.8	17.1	0.70	0.36	+1.6
	21.0	21.3	0.93		+1.5
Drinking	0.63	0.63	0.13	0.04	0.0
	0.84	0.81	0.13		-3.6
	5.24	5.11	0.23	0.13	-2.5
	6.29	6.18	0.30		-1.7
	16.8	17.0	0.55	0.57	+0.9
	21.0	20.9	0.65		-0.4
Waste	0.63	0.63	0.15	0.09	0.0
	0.84	0.85	0.15		+1.2
	5.24	5.23	0.36	0.11	-0.2
	6.29	6.27	0.46		-0.3
	16.8	16.6	0.69	0.43	-1.0
	21.0	21.1	0.63		+0.3

TABLE 7. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR NITRATE - NITROGEN

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.42	0.42	0.04	0.02	0.0
	0.56	0.56	0.06		0.0
	3.51	3.34	0.15	0.08	-4.8
	4.21	4.05	0.28		-3.8
	11.2	11.1	0.47	0.34	-1.1
	14.0	14.4	0.61		+2.6
Drinking	0.42	0.46	0.08	0.03	+9.5
	0.56	0.58	0.09		+3.6
	3.51	3.45	0.27	0.10	-1.7
	4.21	4.21	0.38		0.0
	11.2	11.5	0.50	0.48	+2.3
	14.0	14.2	0.70		+1.6
Waste	0.42	0.36	0.07	0.06	-14.6
	0.56	0.40	0.16		-28.6
	3.51	3.19	0.31	0.07	-9.1
	4.21	3.84	0.28		-8.8
	11.2	10.9	0.35	0.51	-3.0
	14.0	14.1	0.74		+0.4

TABLE 8. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR ORTHO-PHOSPHATE

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	0.69	0.69	0.06	0.06	0.0
	0.92	0.98	0.15		+6.5
	5.77	5.72	0.36	0.18	-0.9
	6.92	6.78	0.42		-2.0
	18.4	18.8	1.04	0.63	+2.1
	23.1	23.2	0.35		+0.4
Drinking	0.69	0.70	0.17	0.17	+1.4
	0.92	0.96	0.20		+4.3
	5.77	5.43	0.52	0.40	-5.9
	6.92	6.29	0.72		-9.1
	18.4	18.0	0.68	0.59	-2.2
	23.1	22.6	1.07		-2.0
Waste	0.68	0.64	0.26	0.09	-7.2
	0.92	0.82	0.28		-10.9
	5.77	5.18	0.66	0.34	-10.2
	6.92	6.24	0.74		-9.8
	18.4	17.6	2.08	1.27	-4.1
	23.1	22.4	0.87		-3.0

TABLE 9. MULTIPLE LABORATORY (n=19)
DETERMINATION OF BIAS FOR SULFATE

WATER	AM'T ADDED mg/L	AM'T FOUND mg/L	S_t	S_o	BIAS %
Reagent	2.85	2.83	0.32	0.52	-0.7
	3.80	3.83	0.92		+0.8
	23.8	24.0	1.67	0.68	+0.8
	28.5	28.5	1.56		-0.1
	76.0	76.8	3.42	2.33	+1.1
	95.0	95.7	3.59		+0.7
Drinking	2.85	1.12	0.37	0.41	-60.7
	3.80	2.26	0.97		-40.3
	23.8	21.8	1.26	0.51	-8.4
	28.5	25.9	2.48		-9.1
	76.0	74.5	4.63	2.70	-2.0
	95.0	92.3	5.19		-2.8
Waste	2.85	1.89	0.37	0.24	-33.7
	3.80	2.10	1.25		-44.7
	23.8	20.3	3.19	0.58	-14.7
	28.5	24.5	3.24		-14.0
	76.0	71.4	5.65	3.39	-6.1
	95.0	90.3	6.80		-5.0

Method A

Peak	Ret. Time	Ion	mg/L
1	1.17	F^-	2
2	1.73	Cl^-	20
3	2.02	NO_2^-	2
4	2.95	Br^-	2
5	3.20	NO_3^-	10
6	5.38	HPO_4^{2-}	2
7	6.92	SO_4^{2-}	60

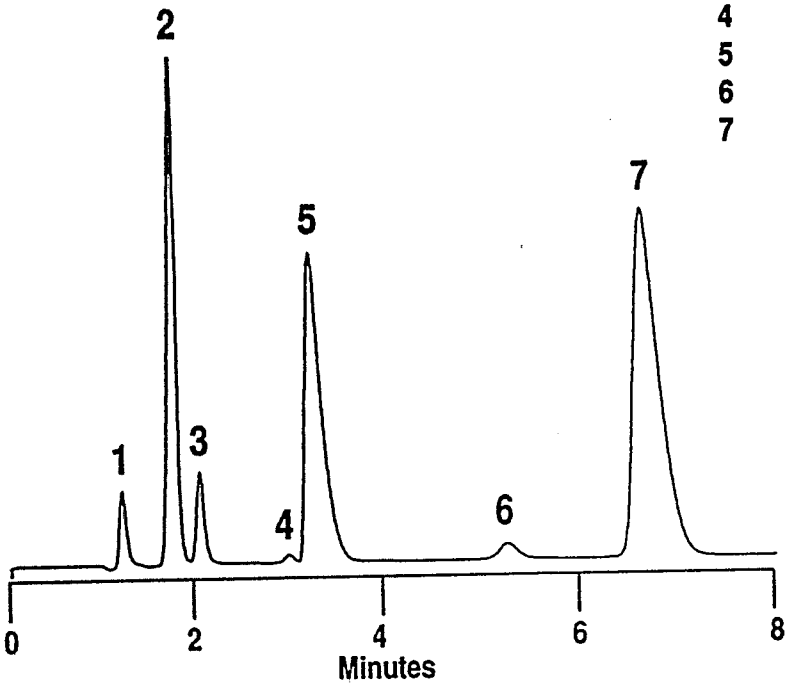

Figure 1. Chromatogram showing separation using the AS4A column

Method B

Peak	Ret. Time	Ion	mg/L
1	2.75	ClO_2^-	0.1
2	3.23	BrO_3^-	0.1
3	3.63	Cl^-	0.1
4	7.08	ClO_3^-	0.1

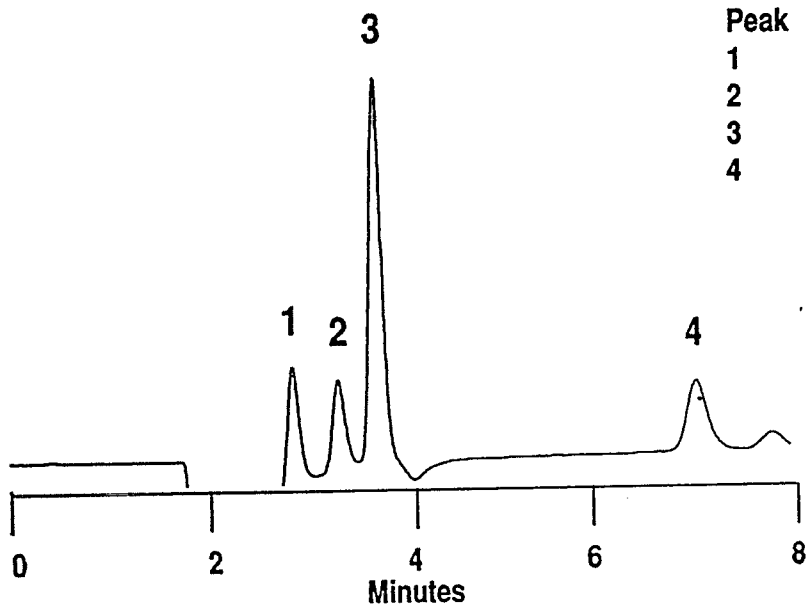

Figure 2. Chromatogram showing separation using the AS9 column

METHOD 335.4

DETERMINATION OF TOTAL CYANIDE BY SEMI-AUTOMATED COLORIMETRY

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METHOD 335.4

DETERMINATION OF TOTAL CYANIDE BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The applicable range is 5 to 500 µg/L.

2.0 SUMMARY OF METHOD

2.1 The cyanide as hydrocyanic acid (HCN) is released from cyanide complexes by means of a manual reflux-distillation operation and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is converted to cyanogen chloride by reactions with chloramine-T, that subsequently reacts with pyridine and barbituric acid to give a red-colored complex.

2.2 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.2 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 Several interferences are encountered with this method. Some of the known interferences are aldehydes, nitrate-nitrite, oxidizing agents, such as chlorine, thiocyanate, thiosulfate and sulfide. Multiple interferences may require the analysis of a series of laboratory fortified sample matrices (LFM) to verify the suitability of the chosen treatment. Some interferences are eliminated or reduced by the distillation.

4.2 Sulfides adversely affect the procedure by producing hydrogen sulfide during distillation. If a drop of the sample on lead acetate test paper indicates the presence of sulfide, treat 25 mL more of the stabilized sample (pH $\geq$ 12) than that required for the cyanide determination with powdered cadmium carbonate. Yellow cadmium sulfide precipitates if the sample contains sulfide. Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper. Filter the solution through a dry filter paper into a dry beaker, and from the filtrate, measure the sample to be used for analysis. Avoid a large excess of cadmium and a long contact time in order to minimize a loss by complexation or occlusion of cyanide on the precipitated material.

4.3 High results may be obtained for samples that contain nitrate and/or nitrite. During the distillation nitrate and nitrite will form nitrous acid that will react with some organic compounds to form oximes. These oximes will decompose under test conditions to generate HCN. The interference of nitrate and nitrite is eliminated by pretreatment with sulfamic acid.

4.4 Oxidizing agents, such as chlorine, decompose most of the cyanides. Test a drop of the sample with potassium iodide-starch paper (KI-starch paper) at time of collection; a blue color indicates the need for treatment. Add ascorbic acid, a few crystals at a time, until a drop of sample produces no color on the indicator paper; then add an additional 0.06 g of ascorbic acid for each liter of sample volume. Sodium arsenite has also been employed to remove oxidizing agents.

4.5 Other compatible procedures for the removal or suppression of interferences may be employed provided they do not adversely effect the overall performance of the method.

4.6 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

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

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

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

5.3.1 Hydrochloric acid (7.5)

5.3.2 Silver nitrate (7.9)

5.3.3 Potassium cyanide (7.10)

5.3.4 Sulfuric acid (7.14)

5.4 Because of the toxicity of evolved hydrogen cyanide (HCN), distillation should be performed in a well vented hood.

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Midi reflux distillation apparatus including boiling flask condenser, and absorber as shown in Figure 1.

6.4 Heating mantel or heating block as required.

6.5 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.5.1 Sampling device (sampler)

6.5.2 Multichannel pump

6.5.3 Reaction unit or manifold

6.5.4 Colorimetric detector

6.5.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Ascorbic acid: Crystal (CASRN-50-81-7)

7.3 Chloramine-T: Dissolve 2.0 g of chloramine-T (CASRN-127-65-1) in 500 mL of reagent water.

7.4 Magnesium Chloride Solution: Weigh 510 g of $MgCl_2.6H_2O$ (CASRN-7786-30-3) into a 1000 mL flask, dissolve and dilute to 1 L with reagent water.

7.5 Pyridine Barbituric Acid Reagent: Place 15 g of barbituric acid (CASRN-67-52-7) in a 1 L beaker. Wash the sides of the beaker with about 100 mL of reagent water. Add 75 mL of pyridine (CASRN-110-86-1) and mix. Add 15 mL of conc. HCl (CASRN-7647-01-0) and mix. Dilute to 900 mL with reagent water and mix until all the barbituric acid has dissolved. Transfer the solution to a 1-L flask and dilute to the mark.

7.6 Sodium dihydrogenphosphate buffer, 1 M: Dissolve 138 g of $NaH_2PO_4.H_2O$ (CASRN-10049-21-5) in 1 L of reagent water. Refrigerate this solution.

7.7 Sodium Hydroxide Solution, 1.25 N: Dissolve 50 g of NaOH (CASRN-1310-73-2) in reagent water, and dilute to 1 L with reagent water.

7.8 Sodium Hydroxide, 0.25 N: Dilute 200 mL of 1.25 N Sodium hydroxide solution (7.7) to 1 L with reagent water.

7.9 Standard Silver Nitrate Solution, 0.0192 N: Prepare by crushing approximately 5 g $AgNO_3$ (CASRN-7761-88-8) crystals and drying to constant weight at 40°C. Weigh out 3.2647 g of dried $AgNO_3$, dissolve in reagent water, and dilute to 1000 mL (1 mL = 1 mg CN).

7.10 Stock Cyanide Solution: Dissolve 2.51 g of KCN (CASRN-151-50-8) and 2 g KOH (CASRN-1310-58-3) in 900 mL of reagent water. Standardize with 0.0192 N $AgNO_3$ (7.9). Dilute to appropriate concentration so that 1 mL = 1 mg CN.

7.11 Standard Cyanide Solution, intermediate: Dilute 10.0 mL of stock (1 mL = 1 mg CN) (7.10) to 100.0 with reagent water (1 mL = 100.0 μg CN.

7.12 Working Standard Cyanide Solution: Prepare fresh daily by diluting 20.0 mL of intermediate cyanide solution (7.11) to 200.0 mL with reagent water and store in a glass stoppered bottle. 1 mL = 10.0 μg CN.

7.13 Sulfamic Acid: (CASRN-212-57-3).

7.14 Sulfuric Acid, 18N: Slowly add 500 mL of concentrated H_2SO_4 (CASRN-5329-14-6) to 500 mL of reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 If the sample contains chlorine or hydrogen sulfide, see Sect. 4.0 for treatment.

8.3 Samples must be preserved with sodium hydroxide pH ≥ 12 and cooled to 4°C at the time of collection.

8.4 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 14 days.

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

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding on with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[4] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data becomes 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 90-110%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

where, R = percent recovery.
C_s = fortified sample concentration.
C = sample background concentration.

s = concentration equivalent of analyte added to sample.

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by pipetting suitable volumes of working standard solution (7.12) into 100 mL volumetric flasks. To each standard (except those to be distilled) add 20 mL of 1.25 N sodium hydroxide and dilute to 100 mL with reagent water.

10.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least two standards (a high and low) and a blank be distilled and compared to similar values on the standard curve to insure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards the analyst should find the cause of the apparent error before proceeding. Before distillation, standards should contain 4 mL 0.25N NaOH (7.8) per 50 mL.

10.3 Set up the manifold as shown in Figure 2 in a hood or a well-ventilated area.

10.4 Allow the instrument to warm up as required. Pump all reagents, with 0.25N NaOH in the sample line, until a stable baseline is achieved.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

10.7 After the calibration has been established, it must be verified by the analysis of a suitable QCS. If measurements exceed ± 10% of the established QCS value, the analysis should be terminated and the

instrument recalibrated. The new calibration must be verified before continuing analysis. Periodic reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Pipet 50 mL of sample or an aliquot diluted to 50 mL into the MIDI distillation boiling flask. Add boiling chips as required. Pipet 50 mL of sodium hydroxide 0.25 N (7.8) into the absorbing tube. Connect the boiling flask, condenser, and absorber in the train as shown in Figure 1.

11.2 Start a slow stream of air entering the boiling flask by adjusting the vacuum source to maintain about 3 bubbles per minute.

11.3 If samples contain NO_3 and/or NO_2, add 0.2 g of sulfamic acid (7.13) after the air rate is set through the air inlet tube. Mix for 3 min prior to addition of H_2SO_4.

11.4 Slowly add 5 mL 18 N sulfuric acid (7.14) through the air inlet tube. Rinse the tube with distilled water and allow the airflow to mix the flask contents for 3 min. Pour 2 mL of magnesium chloride (7.4) into the air inlet and wash down with a stream of water.

11.5 Heat the solution to boiling. Reflux for one and one half hours. Turn off heat and continue the airflow for at least 15 min. After cooling the boiling flask, disconnect absorber and close off the vacuum source and remove absorber tube.

11.6 Fill and connect reagent containers and start system. Allow the instrument to warm up as required. Pump all reagents, with 0.25N NaOH in the sample line, until a stable baseline is achieved.

11.7 Place standards, distilled standards and unknown samples (ALL in 0.25N NaOH) in sampler tray. Calibrate instrument and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg/L.

13.0 METHOD PERFORMANCE

13.1 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg CN/L.

13.2 Single laboratory precision data can be estimated at 50 to 75% of the interlaboratory precision estimates.

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

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

15.0 WASTE MANAGEMENT

15.1 The U.S. Environmental Protection Agency requires that laboratory waste management practices conducted be consistent with all applicable rules and regulations. Excess Reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

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3. USEPA Contract Laboratory Program, Document Number ILMO 1.0, Method for Total Cyanide Analysis by MIDI Distillation #335.2 CLP-M.

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

17. TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
126	0.020	0.0182	0.0002	0.0055	0.0000
94	0.055	0.0501	-0.0014	0.0092	-0.0007
158	0.090	0.0843	-0.0008	0.0171	0.0027
118	0.110	0.1045	0.0003	0.0165	-0.0004
148	0.180	0.1683	-0.0030	0.0236	-0.0023
92	0.270	0.2538	-0.0038	0.0275	-0.0099
132	0.530	0.5019	-0.0049	0.0775	0.0069
119	0.540	0.5262	0.0098	0.0679	-0.0039
148	0.610	0.5803	-0.0032	0.0851	0.0043
94	0.700	0.6803	0.0105	0.1082	0.0159
92	0.800	0.7726	0.0069	0.0880	-0.0170
158	0.970	0.9508	0.0222	0.1464	0.0197

REGRESSIONS: $X = 0.959T - 0.001$, $S = 0.128T + 0.003$

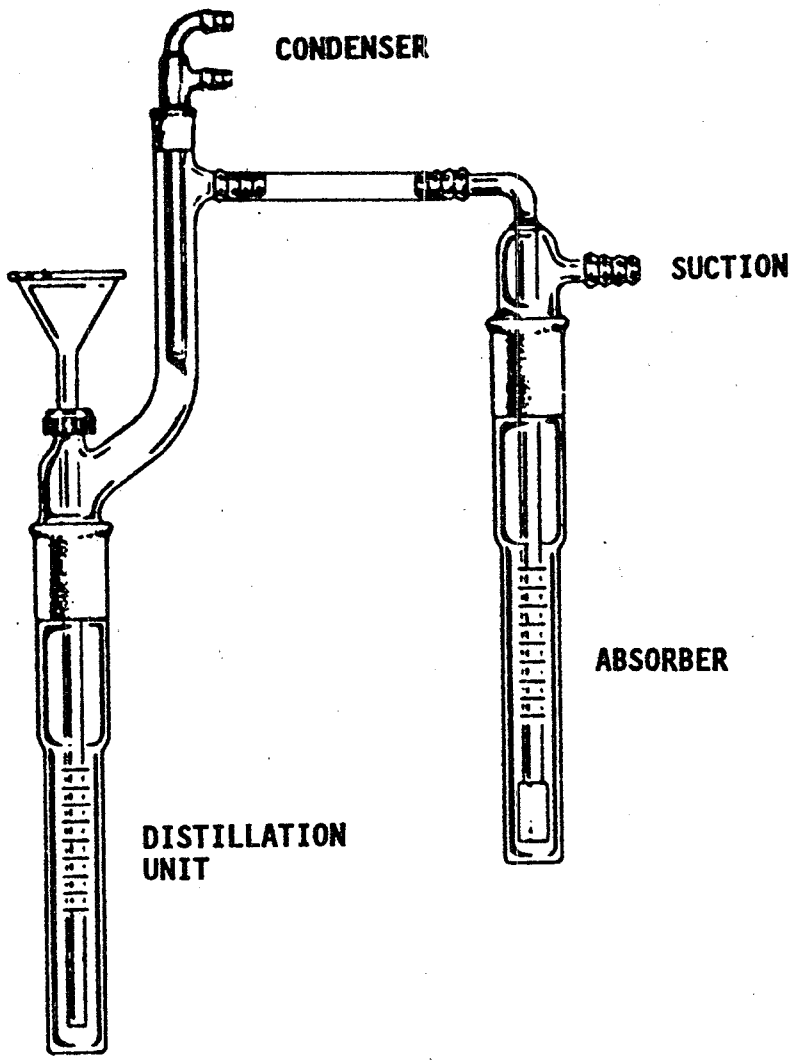

FIGURE 1. MIDI DISTILLATION APPARATUS

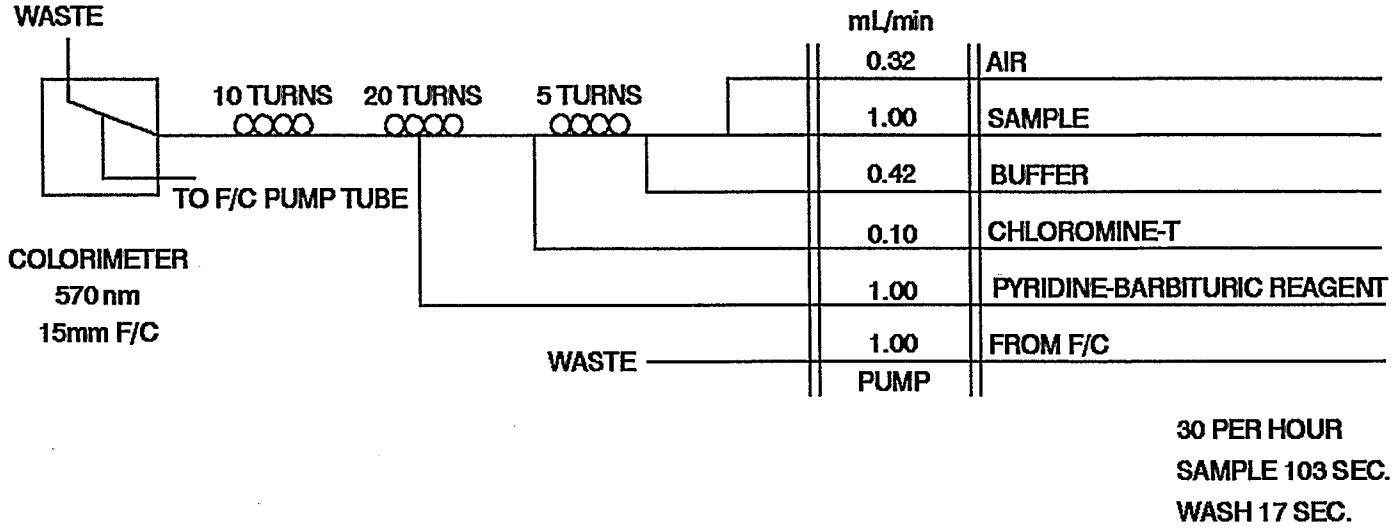

Figure 2
Cyanide Manifold

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 350.1

DETERMINATION OF AMMONIA NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The applicable range is 0.01 to 2.0 mg/L NH_3 as N. Higher concentrations can be determined by sample dilution. Approximately 60 samples per hour can be analyzed.

1.3 This method is described for macro glassware; however, micro distillation equipment may also be used.

2.0 SUMMARY OF METHOD

2.1 The sample is buffered at a pH of 9.5 with a borate buffer in order to decrease hydrolysis of cyanates and organic nitrogen compounds, and is distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at the pH of 9.5 at which distillation is carried out.

4.2 Residual chorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation.

4.3 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Sulfuric acid (7.6)

5.3.2 Phenol (7.7)

5.3.3 Sodium nitroprusside (7.10)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 An all-glass distilling apparatus with an 800-1000-mL flask.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water - Ammonia free: Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.

NOTE 1: All solutions must be made with ammonia-free water.

7.2 Boric acid solution (20 g/L): Dissolve 20 g H_3BO_3 (CASRN 10043-35-3) in reagent water and dilute to 1 L.

7.3 Borate buffer: Add 88 mL of 0.1 N NaOH (CASRN 1310-73-2) solution to 500 mL of 0.025 M sodium tetraborate solution (5.0 g anhydrous $Na_2B_4O_7$ [CASRN 1330-43-4] or 9.5 g $Na_2B_4O_7 \cdot 10H_2O$ [CASRN 1303-96-4] per L) and dilute to 1 L with reagent water.

7.4 Sodium hydroxide, 1 N: Dissolve 40 g NaOH in reagent water and dilute to 1 L.

7.5 Dechlorinating reagents: A number of dechlorinating reagents may be used to remove residual chlorine prior to distillation. These include:

7.5.1 Sodium thiosulfate: Dissolve 3.5 g $Na_2S_2O_3 \cdot 5H_2O$ (CASRN 10102-17-7) in reagent water and dilute to 1 L. One mL of this solution will remove 1 mg/L of residual chlorine in 500 mL of sample.

7.5.2 Sodium sulfite: Dissolve 0.9 g $Na2SO_3$ (CASRN 7757-83-7) in reagent water and dilute to 1 L. One ml removes 1 mg/L Cl per 500 mL of sample.

7.6 Sulfuric acid 5 N: Air scrubber solution. Carefully add 139 mL of conc. sulfuric acid (CASRN 7664-93-9) to approximately 500 mL of reagent water. Cool to room temperature and dilute to 1 L with reagent water.

7.7 Sodium phenolate: Using a 1-L Erlenmeyer flask, dissolve 83 g phenol (CASRN 108-95-2) in 500 mL of distilled water. In small increments, cautiously add with agitation, 32 g of NaOH. Periodically cool flask under water faucet. When cool, dilute to 1 L with reagent water.

7.8 Sodium hypochlorite solution: Dilute 250 mL of a bleach solution containing 5.25% NaOCl (CASRN 7681-52-9) (such as "Clorox") to 500 mL with reagent water. Available chlorine level should approximate 2% to 3%. Since "Clorox" is a proprietary product, its formulation is subject to change. The analyst must remain alert to detecting any variation in this product significant to its use in this procedure. Due to the instability of this product, storage over an extended period should be avoided.

7.9 Disodium ethylenediamine-tetraacetate (EDTA) (5%): Dissolve 50 g of EDTA (disodium salt) (CASRN 6381-92-6) and approximately six pellets of NaOH in 1 L of reagent water.

7.10 Sodium nitroprusside (0.05%): Dissolve 0.5 g of sodium nitroprusside (CASRN 14402-89-2) in 1 L of reagent water.

7.11 Stock solution: Dissolve 3.819 g of anhydrous ammonium chloride, NH_4Cl (CASRN 12125-02-9), dried at 105°C, in reagent water, and dilute to 1 L. 1.0 mL = 1.0 mg NH_3-N.

7.12 Standard Solution A: Dilute 10.0 mL of stock solution (7.11) to 1 L with reagent water. 1.0 mL = 0.01 mg NH_3-N.

7.13 Standard Solution B: Dilute 10.0 mL of standard solution A (7.12) to 100.0 mL with reagent water. 1.0 mL = 0.001 mg NH_3-N.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check Solution (IPC) -- For all determinations the laboratory must analyze the IPC (a mid-range check standard) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ± 10% of calibration. Subsequent analyses of the IPC solution must verify the calibration is

still within ± 10%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift, the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions (7.12, 7.13) to 100 mL with reagent water.

10.2 Process standards and blanks as described in Sect. 11, Procedure.

10.3 Set up manifold as shown in Figure 1.

10.4 Prepare flow system as described in Sect. 11, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

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

11.0 PROCEDURE

11.1 Preparation of equipment: Add 500 mL of reagent water to an 800-mL Kjeldahl flask. The addition of boiling chips that have been previously treated with dilute NaOH will prevent bumping. Steam out the distillation apparatus until the distillate shows no trace of ammonia.

11.2 Sample preparation: Remove the residual chorine in the sample by adding dechlorinating agent (7.5) equivalent to the chlorine residual. To 400 mL of sample add 1 N NaOH (7.4), until the pH is 9.5, check the pH during addition with a pH meter or by use of a short range pH paper.

11.3 Distillation: Transfer the sample, the pH of which has been adjusted to 9.5, to an 800-mL Kjeldahl flask and add 25 mL of the borate buffer (7.3). Distill 300 mL at the rate of 6-10 mL/min. into 50 mL of 2% boric acid (7.2) contained in a 500-mL Erlenmeyer flask.

NOTE 4: The condenser tip or an extension of the condenser tip must extend below the level of the boric acid solution.

11.4 Since the intensity of the color used to quantify the concentration is pH dependent, the acid concentration of the wash water and the standard ammonia solutions should approximate that of the samples.

11.5 Allow analysis system to warm up as required. Feed wash water through sample line.

11.6 Arrange ammonia standards in sampler in order of decreasing concentration of nitrogen. Complete loading of sampler tray with unknown samples.

11.7 Switch sample line from reagent water to sampler and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg NH_3-N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 1.41, 0.77, 0.59 and 0.43 mg NH_3-N/L, the standard deviation was ± 0.005.

13.2 In a single laboratory (EMSL-Cincinnati), using surface water samples at concentrations of 0.16 and 1.44 mg NH_3-N/L, recoveries were 107% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg NH_3-N/L.

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.

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

15.0 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. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
134	0.270	0.2670	-0.0011	0.0342	0.0015
157	0.692	0.6972	0.0059	0.0476	-0.0070
136	1.20	1.2008	0.0001	0.0698	-0.0112
195	1.60	1.6095	0.0076	0.1023	0.0006
142	3.00	3.0128	0.0069	0.1677	-0.0067
159	3.50	3.4991	-0.0083	0.2168	0.0165
156	3.60	3.5955	-0.0122	0.1821	-0.0234
200	4.20	4.2271	0.0177	0.2855	0.0488
196	8.76	8.7257	-0.0568	0.4606	-0.0127
156	11.0	11.0747	0.0457	0.5401	-0.0495
142	13.0	12.9883	-0.0465	0.6961	0.0027
199	18.0	17.9727	-0.0765	1.1635	0.2106

REGRESSIONS: $X = 1.003T - 0.003$, $S = 0.052T + 0.019$

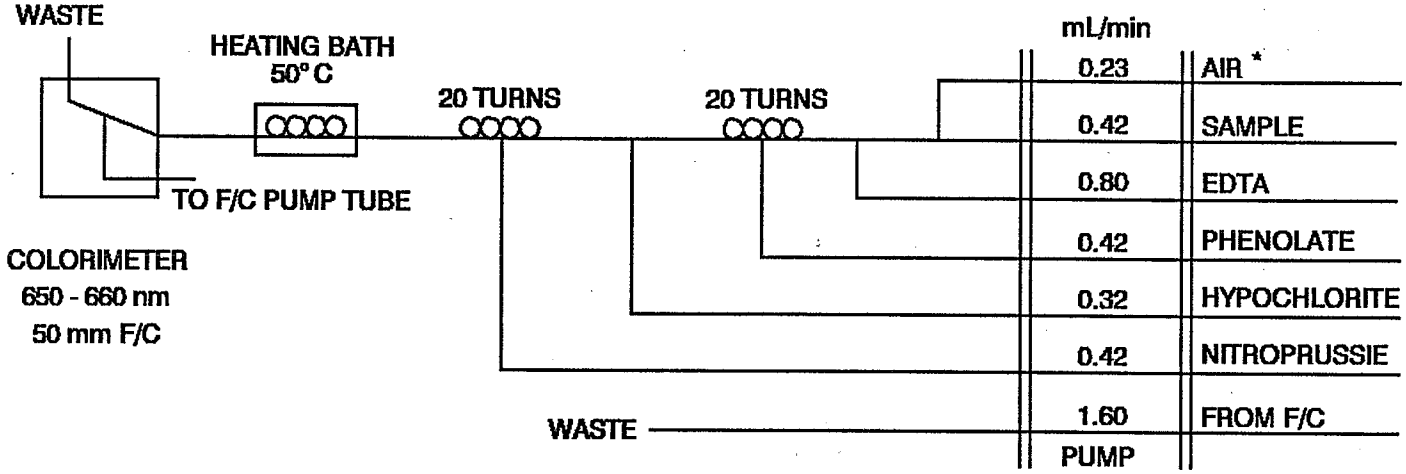


Figure 1
Ammonia Manifold

METHOD 351.2

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 351.2

DETERMINATION OF TOTAL KJELDAHL NITROGEN BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, ground, and surface waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 The applicable range is 0.1 to 20 mg/L TKN. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 The sample is heated in the presence of sulfuric acid, H_2SO_4 for two and one half hours. The residue is cooled, diluted to 25 mL and analyzed for ammonia. This digested sample may also be used for phosphorus determination.

2.2 Total Kjeldahl nitrogen is the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described.

2.3 Organic Kjeldahl nitrogen is the difference obtained by subtracting the free-ammonia value from the total Kjeldahl nitrogen value.

2.4 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.5 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 High nitrate concentrations (10X or more than the TKN level) result in low TKN values. If interference is suspected, samples should be diluted and reanalyzed.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Mercury (7.2, 7.3)

5.3.2 Sulfuric acid (7.2, 7.3, 7.4)

5.3.3 Sodium nitroprusside (7.9)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware - Class A volumetric flasks and pipets as required.

6.3 Block digestor with tubes.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Ammonia free distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Mercuric sulfate: Dissolve 8 g red mercuric oxide (HgO) (CASRN 21908-53-2) in 50 mL of 1:4 sulfuric acid (10 mL conc. H_2SO_4: [CASRN 7664-93-9] 40 mL reagent water) and dilute to 100 mL with reagent water.

7.3 Digestion solution: (Sulfuric acid-mercuric sulfate-potassium sulfate solution): Dissolve 133 g of K_2SO_4 (CASRN 7778-80-5) in 700 mL of reagent water and 200 mL of conc. H_2SO_4. Add 25 mL of mercuric sulfate solution (7.1) and dilute to 1 L.

NOTE 1: An alternate mercury-free digestion solution can be prepared by dissolving 134 g K_2SO_4 and 7.3 g $CuSO_4$ in 800 mL reagent water and then adding 134 mL conc. H_2SO_4 and diluting to 1 L. Use 10 mL solution per 25 mL of sample.

7.4 Sulfuric Acid solution (4%): Add 40 mL of conc. sulfuric acid to 800 mL of reagent water, cool and dilute to 1 L.

NOTE 2: If alternate mercury-free digestion solution is used, adjust the above solution to equal the acid concentration of the digested sample (11.6).

7.5 Stock Sodium Hydroxide (20%): Dissolve 200 g of sodium hydroxide (CASRN 1310-73-2) in 900 mL of reagent water and dilute to 1 L.

7.6 Stock Sodium Potassium Tartrate solution (20%): Dissolve 200 g sodium potassium tartrate (CASRN 6381-59-5) in about 800 mL of reagent water and dilute to 1 L.

7.7 Stock Buffer solution: Dissolve 134.0 g of sodium phosphate, dibasic (Na_2HPO_4) (CASRN 7558-79-4) in about 800 mL of reagent water. Add 20 g of sodium hydroxide and dilute to 1 L.

7.8 Working Buffer solution: Combine the reagents in the stated order, add 250 mL of stock sodium potassium tartrate solution (7.6) to 200 mL of stock buffer solution (7.7) and mix. Add xx mL sodium hydroxide solution (7.5) and dilute to 1 L. See concentration ranges, Table 2, for composition of working buffer.

7.9 Sodium Salicylate/Sodium Nitroprusside solution: Dissolve 150 g of sodium salicylate (CASRN 54-21-7) and 0.3 g of sodium nitroprusside (CASRN 13755-38-9 or 14402-89-2) in about 600 mL of reagent water and dilute to 1 L.

7.10 Sodium Hypochlorite solution: Dilute 6.0 mL sodium hypochlorite solution (CASRN 7681-52-9) (Clorox) to 100 mL with reagent water.

7.11 Ammonium chloride, stock solution: Dissolve 3.819 g NH_4Cl (CASRN 12125-02-9) in reagent water and bring to volume in a 1 L volumetric flask. 1 mL = 1.0 mg NH_3-N.

7.12 Teflon boiling chips.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis, or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[6] 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.

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

9.3 ASSESSING LABORATORY PERFORMANCE

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of

control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four

times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by diluting suitable volumes of standard solution (7.11) with reagent water.

10.2 Process standards and blanks as described in Sect. 11, Procedure.

10.3 Set up manifold as shown in Figure 1 and Table 2.

10.4 Prepare flow system as described in Sect. 11, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

10.6 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based regression curve fitting techniques. Acceptance or control limits should be established using the difference between

the measured value of the calibration solution and the "true value" concentration.

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

11.0 PROCEDURE

11.1 Pipet 25.0 mL of sample, standard or blank in the digestor tube.

11.2 Add 5 mL of digestion solution (7.3) and mix with a vortex mixer (See Note 1).

11.3 Add 4-8 Teflon boiling chips (7.12). **CAUTION:** An excess of Teflon chips may cause the sample to boil over.

11.4 Place tubes in block digestor preheated to 160°C and maintain temperature for 1 h.

11.5 Reset temperature to 380°C and continue to heat for one and one half hour.

(380°C MUST BE MAINTAINED FOR 30 MIN.)

11.6 Remove digestion tubes, cool and dilute to 25 mL with reagent water.

11.7 Excluding the salicylate line, place all reagent lines in their respective containers, connect the sample probe to the sampler and start the pump.

11.8 Flush the sampler wash receptacle with about 25 mL of 4% sulfuric acid (7.4) (See Note 2).

11.9 When reagents have been pumping for at least 5 min, place the salicylate line in its respective container and allow the system to equilibrate. If a precipitate forms after the addition of salicylate, the pH is too low. Immediately stop the proportioning pump and flush the coils with water using a syringe. Before restarting the system, check the concentration of the sulfuric acid solutions and/or the working buffer solution.

11.10 To prevent precipitation of sodium salicylate in the waste tray, which can clog the tray outlet, keep the nitrogen flowcell pump tube and the nitrogen Colorimeter "To Waste" tube separate from all other lines or keep tap water flowing in the waste tray.

11.11 After a stable baseline has been obtained, start the sampler and perform analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg N/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations of 1.2, 2.6, and 1.7 mg N/L, the precision was ± 0.07, ± 0.03, and ± 0.15, respectively.

13.2 In a single laboratory (EMSL-Cincinnati) using sewage samples at concentrations 4.7 and 8.74 mg N/L, the recoveries were 99% and 99%, respectively.

13.3 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg N/L.

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

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess Reagents and samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. McDaniel, W.H., Hemphill, R.N. and Donaldson, W.T., "Automatic Determination of total Kjeldahl Nitrogen in Estuarine Water," Technicon Symposia, pp. 362-367, Vol. 1, 1967.

2. Gales, M.E. and Booth, R.L., "Evaluation of Organic Nitrogen Methods," EPA Office of Research and Monitoring, June, 1972.

3. Gales, M.E. and Booth, R.L., "Simultaneous and Automated Determination of Total Phosphorus and Total Kjeldahl Nitrogen," Methods Development and Quality Assurance Research Laboratory, May 1974.

4. Technicon "Total Kjeldahl Nitrogen and Total Phosphorus BD-40 Digestion Procedure for Water," August 1974.

5. Gales, M.E., and Booth, R.L., "Evaluation of the Technicon Block Digestor System for the Measurement of Total Kjeldahl Nitrogen and Total Phosphorus," EPA-600/4-78-015, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, 1978.

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
115	0.380	0.3891	-0.0091	0.0750	-0.0135
134	0.451	0.4807	0.0125	0.1181	0.0238
127	1.00	1.0095	-0.0000	0.1170	-0.0227
164	3.10	3.0992	0.0191	0.2821	-0.0310
138	3.50	3.4765	0.0020	0.3973	0.0512
115	5.71	5.6083	-0.0452	0.4869	-0.0417
175	7.00	6.9246	-0.0008	0.6623	0.0272
121	8.00	7.9991	0.0877	0.6283	-0.0894
120	15.0	15.0213	0.2080	1.2495	-0.0462
127	21.0	20.4355	-0.2937	1.7267	-0.0644
164	25.0	24.7157	0.0426	2.0147	-0.1067
175	26.9	26.1464	-0.4000	2.9743	0.6960

REGRESSIONS: $X = 0.986T + 0.024$, $S = 0.083T + 0.057$

TABLE 2. CONCENTRATION RANGES

Range mg/LN	Pump mL/min Sample	Resample	mL NaOH Buffer (7.7)
0-1.5	0.80	0.32	250
0-5.0	0.16	0.32	120
0-10.0	0.16	0.16	80

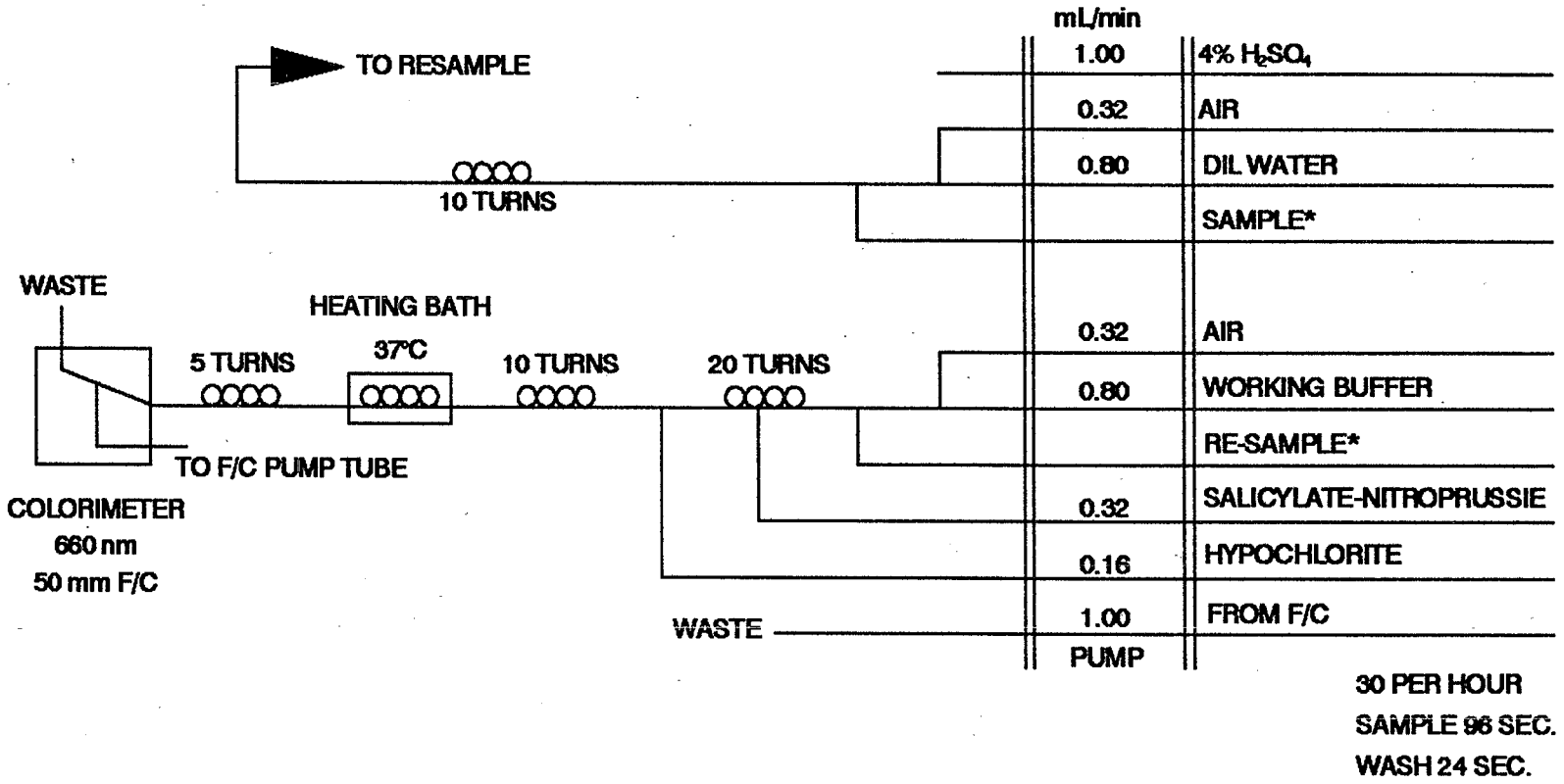


Figure 1
Ammonia Manifold

METHOD 353.2

DETERMINATION OF NITRATE-NITRITE NITROGEN BY AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

METHOD 353.2

DETERMINATION OF NITRATE-NITRITE NITROGEN BY AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of nitrite singly, or nitrite and nitrate combined in drinking, ground, surface, domestic and industrial wastes.

1.2 The applicable range is 0.05 to 10.0 mg/L nitrate-nitrite nitrogen. The range may be extended with sample dilution.

2.0 SUMMARY OF METHOD

2.1 A filtered sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite. The nitrite (that was originally present plus reduced nitrate) is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a highly colored azo dye which is measured colorimetrically. Separate, rather than combined nitrate-nitrite, values are readily obtained by carrying out the procedure first with, and then without, the Cu-Cd reduction step.

2.2 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.3 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 Build up of suspended matter in the reduction column will restrict sample flow. Since nitrate and nitrite are found in a soluble state, samples may be pre-filtered.

4.2 Low results might be obtained for samples that contain high concentrations of iron, copper or other metals. EDTA is added to the samples to eliminate this interference.

4.3 Residual chlorine can produce a negative interference by limiting reduction efficiency. Before analysis, samples should be checked and if required, dechlorinated with sodium thiosulfate.

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

4.5 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Cadmium (7.1)

5.3.2 Phosphoric acid (7.5)

5.3.3 Hydrochloric acid (7.6)

5.3.4 Sulfuric acid (7.8)

5.3.5 Chloroform (7.10, 7.11)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1 Sampling device (sampler)

6.3.2 Multichannel pump

6.3.3 Reaction unit or manifold

6.3.4 Colorimetric detector

6.3.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Granulated cadmium: 40-60 mesh (CASRN 7440-43-9). Other mesh sizes may be used.

7.2 Copper-cadmium: The cadmium granules (new or used) are cleaned with dilute HCl (7.6) and copperized with 2% solution of copper sulfate (7.7) in the following manner:

7.2.1 Wash the cadmium with HCl (7.6) and rinse with distilled water. The color of the cadmium so treated should be silver.

7.2.2 Swirl 10 g cadmium in 100 mL portions of 2% solution of copper sulfate (7.7) for 5 min or until blue color partially fades, decant and repeat with fresh copper sulfate until a brown colloidal precipitate forms.

7.2.3 Wash the copper-cadmium with reagent water (at least 10 times) to remove all the precipitated copper. The color of the cadmium so treated should be black.

7.3 Preparation of reduction column. The reduction column is a U-shaped, 35 cm length, 2 mm I.D. glass tube (Note 1). Fill the reduction column with distilled water to prevent entrapment of air bubbles during the filling operations. Transfer the copper-cadmium granules (7.2) to the reduction column and place a glass wool plug in each end. To prevent entrapment of air bubbles in the reduction column, be sure that all pump tubes are filled with reagents before putting the column into the analytical system.

NOTE 1: Other reduction tube configurations, including a 0.081 I.D. pump tube, can be used in place of the 2-mm glass tube, if checked as in 10.1.

7.4 Reagent water: Because of possible contamination, this should be prepared by passage through an ion exchange column comprised of a mixture of both strongly acidic-cation and strongly basic-anion

exchange resins. The regeneration of the ion exchange column should be carried out according to the manufacturer's instructions.

7.5 Color reagent: To approximately 800 mL of reagent water, add, while stirring, 100 mL conc. phosphoric acid (CASRN 7664-38-2), 40 g sulfanilamide (CASRN 63-74-1) and 2 g N-1-naphthylethylenediamine dihydrochloride (CASRN 1465-25-4). Stir until dissolved and dilute to 1 L. Store in brown bottle and keep in the dark when not in use. This solution is stable for several months.

7.6 Dilute hydrochloric acid, 6N: Add 50 mL of conc. HCl (CASRN 7647-01-0) to reagent water, cool and dilute to 100 ml.

7.7 Copper sulfate solution, 2%: Dissolve 20 g of $CuSO_4 \cdot 5H_2O$ (CASRN 7758-99-8) in 500 mL of reagent water and dilute to 1 L.

7.8 Wash solution: Use reagent water for unpreserved samples. For samples preserved with H_2SO_4, use 2 mL H_2SO_4 (CASRN 7764-93-9), per liter of wash water.

7.9 Ammonium chloride-EDTA solution: Dissolve 85 g of reagent grade ammonium chloride (CASRN 12125-02-9) and 0.1 g of disodium ethylenediamine tetracetate (CASRN 6381-92-6) in 900 mL of reagent water. Adjust the pH to 9.1 for preserved or 8.5 for non-preserved samples with conc. ammonium hydroxide (CASRN 1336-21-6) and dilute to 1 L. Add 0.5 mL Brij-35 (CASRN 9002-92-0).

7.10 Stock nitrate solution: Dissolve 7.218 g KNO_3 (CASRN 7757-79-1) and dilute to 1 L in a volumetric flask with reagent water. Preserve with 2 mL of chloroform (CASRN 67-66-3) per liter. Solution is stable for 6 months. 1 mL = 1.0 mg NO_3-N.

7.11 Stock nitrite solution: Dissolve 6.072 g KNO_2 in 500 mL of reagent water and dilute to 1 L in a volumetric flask. Preserve with 2 mL of chloroform and keep under refrigeration. 1.0 mL = 1.0 mg NO_2-N.

7.12 Standard nitrate solution: Dilute 1.0 mL of stock nitrate solution (7.10) to 100 mL. 1.0 mL = 0.01 mg NO_3-N. Preserve with .2 mL of chloroform. Solution is stable for 6 months.

7.13 Standard nitrite solution: Dilute 10.0 mL of stock nitrite (7.11) solution to 1000 mL. 1.0 mL = 0.01 mg NO_2-N. Solution is unstable; prepare as required.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

8.4 Samples to be analyzed for nitrate or nitrite only should be cooled to 4°C and analyzed within 48 hours.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[6] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by diluting suitable volumes of standard nitrate solution (7.12). At least one nitrite standard should be compared to a nitrate standard at the same concentration to verify the efficiency of the reduction column.

10.2 Set up manifold as shown in Figure 1. Care should be taken not to introduce air into the reduction column.

10.3 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

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

NOTE 3: Condition column by running 1 mg/L standard for 10 min if a new reduction column is being used. Subsequently wash the column with reagents for 20 min.

11.0 PROCEDURE

11.1 If the pH of the sample is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. NH_4OH.

11.2 Set up the manifold as shown in Figure 1. Care should be taken not to introduce air into reduction column.

11.3 Allow system to equilibrate as required. Obtain a stable baseline with all reagents, feeding reagent water through the sample line.

11.4 Place appropriate nitrate and/or nitrite standards in sampler in order of decreasing concentration and complete loading of sampler tray.

11.5 Switch sample line to sampler and start analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg/L as nitrogen.

13.0 METHOD PERFORMANCE

13.1 Three laboratories participating in an EPA Method Study analyzed four natural water samples containing exact increments of inorganic nitrate, with the following results:

Increment as Nitrate Nitrogen mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Accuracy as Bias, mg N/liter
0.29	0.012	+ 5.75	+ 0.017
0.35	0.092	+ 18.10	+ 0.063
2.31	0.318	+ 4.47	+ 0.103
2.48	0.176	- 2.69	- 0.067

13.2 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg NO_3-N/L.

13.3 Single laboratory precision data can be estimated at 50% to 75% of the interlaboratory precision estimates.

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

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples, and method process wastes should be characterized and disposed of in an acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Fiore, J., and O'Brien, J.E., "Automation in Sanitary Chemistry - Parts 1 & 2: Determination of Nitrates and Nitrites," Wastes Engineering 33, 128 &238 (1962).

2. Armstrong, F.A., Stearns, C.R., and Strickland, J.D., "The Measurement of Upwelling and Subsequent Biological Processes by Means of the Technicon AutoAnalyzer and Associated Equipment," Deep Sea Research 14, pp. 381-389 (1967).

3. Annual Book of ASTM Standards, Part 31, "Water," Standard D1254, p. 366 (1976).

4. Standard Methods for the Examination of Water and Wastewater, 17th Edition, pp. 4-91, Method 4500-NO3 F (1992).

5. Chemical Analyses for Water Quality Manual, Department of the Interior, FWPCA, R.A. Taft Engineering Center Training Program, Cincinnati, Ohio 45226 (January, 1966).

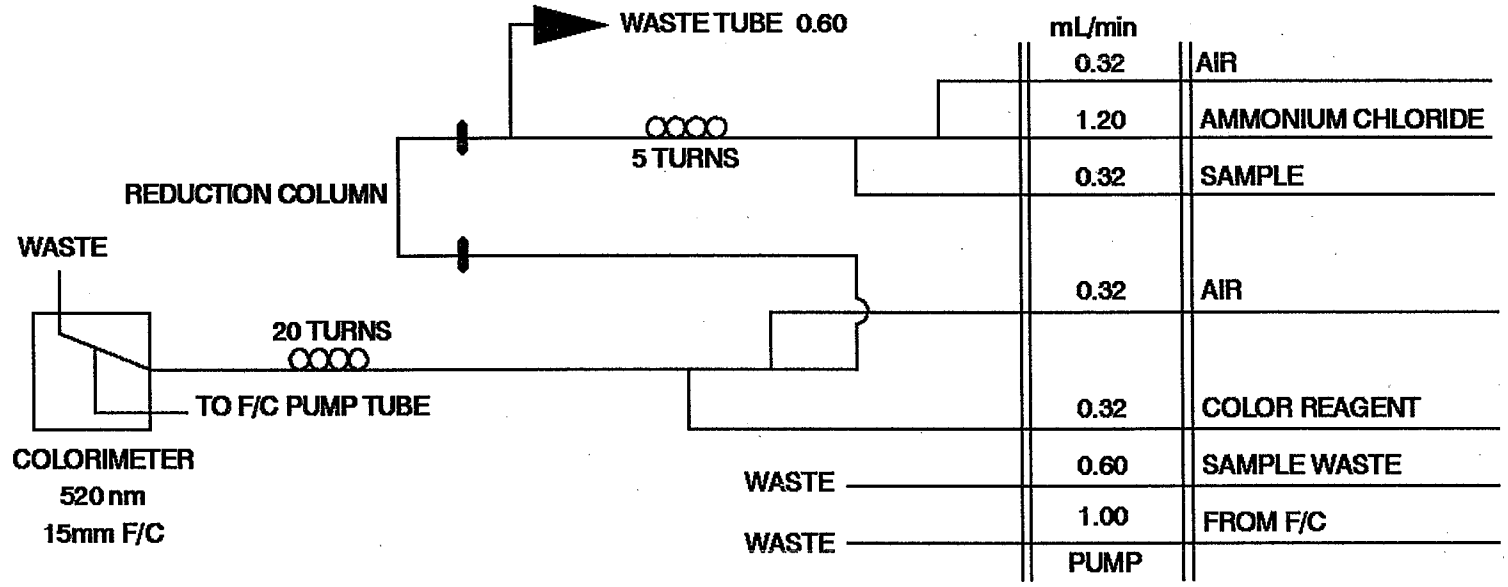
6. Code of Federal Regulations 40, Ch. 1, Pt. 136, Appendix B.

17.0 TABLES, DIAGRAMS, FLOWCHARTS AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
163	0.250	0.2479	0.0007	0.0200	-0.0001
183	0.451	0.4441	-0.0039	0.0289	-0.0002
213	0.650	0.6479	0.0012	0.0398	0.0017
170	0.950	0.9537	0.0074	0.0484	-0.0031
163	1.90	1.8987	0.0037	0.0918	-0.0024
172	2.20	2.1971	0.0025	0.1164	0.0087
183	2.41	2.3732	-0.0312	0.1273	0.0102
214	3.20	3.2042	0.0109	0.1456	-0.0070
172	6.50	6.4978	0.0089	0.3156	0.0148
213	8.00	7.9814	-0.0055	0.3673	-0.0008
170	8.50	8.5135	0.0273	0.3635	-0.0271
214	10.0	9.9736	-0.0106	0.4353	-0.0227

REGRESSIONS: $X = 0.999T + 0.002$, $S = 0.045T + 0.009$



40 PER HOUR
SAMPLE 72 SEC.
WASH 18 SEC.

Figure 1
Nitrate-Nitrite Manifold

METHOD 365.1

DETERMINATION OF PHOSPHORUS BY SEMI-AUTOMATED COLORIMETRY

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METHOD 365.1

DETERMINATION OF PHOSPHORUS BY AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The methods are based on reactions that are specific for the orthophosphate ion. Thus, depending on the prescribed pretreatment of the sample, the various forms of phosphorus that may be determined are defined in Section 3 and given in Figure 1.

1.2.1 Except for in-depth and detailed studies, the most commonly measured forms are total and dissolved phosphorus, total and dissolved orthophosphate. Hydrolyzable phosphorus is normally found only in sewage-type samples. Insoluble forms of phosphorus are determined by calculation.

1.3 The applicable range is 0.01 to 1.0 mg P/L. Approximately 20-30 samples per hour can be analyzed.

2.0 SUMMARY OF METHOD

2.1 Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

2.2 Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by manual sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by manual persulfate digestion.[2] The developed color is measured automatically.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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

3.12 TOTAL PHOSPHORUS (P) -- All of the phosphorus present in the sample regardless of forms, as measured by the persulfate digestion procedure.

3.12.1 TOTAL ORTHOPHOSPHATE (P-ortho) -- Inorganic phosphorus [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure.

3.12.2 TOTAL HYDROLYZABLE PHOSPHORUS (P-hydro) -- Phosphorus in the sample as measured by the sulfuric acid hydrolysis procedure, and minus predetermined orthophosphates. This hydrolyzable phosphorus includes polyphosphates [$(P_2O_7)^{-4}$, $(P_3O_{10})^{-5}$, etc.] plus some organic phosphorus.

3.12.3 TOTAL ORGANIC PHOSPHORUS (P-org) -- Phosphorus (inorganic plus oxidizable organic) in the sample as measured by the persulfate digestion procedure, and minus hydrolyzable phosphorus and orthophosphate.

3.13 DISSOLVED PHOSPHORUS (P-D) -- All of the phosphorus present in the filtrate of a sample filtered through a phosphorus-free filter of 0.45 micron pore size and measured by the persulfate digestion procedure.

3.13.1 DISSOLVED ORTHOPHOSPHATE (P-D ortho) -- As measured by he direct colorimetric analysis procedure.

3.13.2 DISSOLVED HYDROLYZABLE PHOSPHORUS (P-D, hydro) -- As measured by the sulfuric acid hydrolysis procedure and minus predetermined dissolved orthophosphates.

3.13.3 DISSOLVED ORGANIC PHOSPHORUS (P-D, org) -- As measured by the persulfate digestion procedure, and minus dissolved hydrolyzable phosphorus and orthophosphate.

3.14 The following forms, when sufficient amounts of phosphorus are present in the sample to warrant such consideration, may be calculated:

3.14.1 INSOLUBLE PHOSPHORUS (P-I) = (P)-(P-D).

3.14.1.1 INSOLUBLE ORTHOPHOSPHATE (P-I, ortho) =(P, ortho) - (P-D, ortho).

3.14.1.2 INSOLUBLE HYDROLYZABLE PHOSPHORUS (P-I, hydro) = (P, hydro) - (P-D, hydro).

3.14.1.3 INSOLUBLE ORGANIC PHOSPHORUS (P-I, org) = (P, org) -(P-D, org).

3.15 All phosphorus forms shall be reported as P, mg/L, to the third place.

4.0 INTERFERENCES

4.1 No interference is caused by copper, iron, or silicate at concentrations many times greater than their reported concentration in seawater. However, high iron concentrations can cause precipitation of, and subsequent loss, of phosphorus.

4.2 The salt error for samples ranging from 5% to 20% salt content was found to be less than 1%.

4.3 Arsenate is determined similarly to phosphorus and should be considered when present in concentrations higher than phosphorus. However, at concentrations found in sea water, it does not interfere.

4.4 Sample turbidity must be removed by filtration prior to analysis for orthophosphate. Samples for total or total hydrolyzable phosphorus should be filtered only after digestion. Sample color that absorbs in the photometric range used for analysis will also interfere.

4.5 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Sulfuric acid (7.2, 7.7)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Hot plate or autoclave.

6.4 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.4.1 Sampling device (sampler)

6.4.2 Multichannel pump

6.4.3 Reaction unit or manifold

6.4.4 Colorimetric detector

6.4.5 Data recording device

6.5 Acid-washed glassware: All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove the last traces of phosphorus that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphorus and after use it should be rinsed with distilled water and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is only required occasionally. Commercial detergent should never be used.

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Sulfuric acid solution, 5N: Slowly add 70 mL of conc. H_2SO_4 (CASRN 7664-93-9) to approximately 400 mL of distilled water. Cool to room temperature and dilute to 500 mL with distilled water.

7.3 Antimony potassium tartrate solution: Weight 0.3 g $K(SbO)C_4H_4O_6.1/2H_2O$ (CASRN 28300-74-5), dissolved in 50 mL distilled water in 100-mL volumetric flask, dilute to volume. Store at 4°C in a dark, glass-stoppered bottle.

7.4 Ammonium molybdate solution: Dissolve 4 g $(NH_4)_6Mo_7O_{24}.4H_2O$ (CASRN 12027-67-7) in 100 mL reagent water. Store in a plastic bottle at 4°C.

7.5 Ascorbic acid, 0.1M: Dissolve 1.8 g of ascorbic acid (CASRN 50-81-7) in 100 mL of reagent water. The solution is stable for about a week if prepared with water containing no more than trace amounts of heavy metals and stored at 4°C.

7.6 Combined reagent: Mix the above reagents in the following proportions for 100 mL of the mixed reagent: 50 mL of 5N H_2SO_4 (7.2), 5 mL of antimony potassium tartrate solution (7.3), 15 mL of ammonium molybdate solution (7.4), and 30 mL of ascorbic acid solution (7.5). Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until the turbidity disappears before processing. This volume is sufficient for 4 h operation. Since the stability of this solution is limited, it must be freshly prepared for each run.

NOTE 1: A stable solution can be prepared by not including the ascorbic acid in the combined reagent. If this is done, the mixed reagent (molybdate, tartrate, and acid) is pumped through the distilled water line and the ascorbic acid solution (30 mL of 7.5 diluted to 100 mL with reagent water) through the original mixed reagent line.

7.7 Sulfuric acid solution, 11 N: Slowly add 155 mL conc. H_2SO_4 to 600 mL reagent water. When cool, dilute to 500 ml.

7.8 Ammonium persulfate (CASRN 7727-54-0).

7.9 Acid wash water: Add 40 mL of sulfuric acid solution (7.7) to 1 L of reagent water and dilute to 2 L. (Not to be used when only orthophosphate is being determined).

7.10 Phenolphthalein indicator solution (5 g/L): Dissolve 0.5 g of phenolphthalein (CASRN 77-09-8) in a solution of 50 mL of isopropyl alcohol (CASRN 67-63-0) and 50 mL of reagent water.

7.11 Stock phosphorus solution: Dissolve 0.4393 g of predried (105°C for 1 h) Potassium phosphate monobasic KH_2PO_4 (CASRN 7778-77-0) in reagent water and dilute to 1000 mL. 1.0 mL = 0.1 mg P.

7.12 Standard phosphorus solution: Dilute 10.0 mL of stock solution (7.11) to 100 mL with reagent water. 1.0 mL = 0.01 mg P.

7.13 Standard phosphorus solution: Dilute 10.0 mL of standard solution (7.12) to 100 mL with reagent water. 1.0 mL = 0.001 mg P.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held for up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the

determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[5] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by pipetting and diluting suitable volumes of working standard solutions (7.12 or 7.13) into 100 mL volumetric flasks. Suggested ranges include 0.00 to 0.10 and 0.20 to 1.00 mg/L.

10.2 Process standards and blanks as described in Sect. 11, Procedure.

10.3 Set up manifold as shown in Figure 2.

10.4 Prepare flow system as described in Sect. 11, Procedure.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

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

reanalysis of the QCS is recommended as a continuing calibration check.

11.0 PROCEDURE

11.1 Phosphorus

11.1.1 Add 1 mL of sulfuric acid solution (7.7) to a 50 mL sample and/or standard in a 125-mL Erlenmeyer flask.

11.1.2 Add 0.4 g of ammonium persulfate (7.8).

11.1.3 Boil gently on a pre-heated hot plate for approximately 30-40 min or until a final volume of about 10 mL is reached. Do not allow sample to go to dryness. Alternately, heat for 30 min in an autoclave at 121°C (15-20 psi).

11.1.4 Cool and dilute the sample to 50 mL. If sample is not clear at this point, filter.

11.1.5 Determine phosphorus as outlined (11.3.2) with acid wash water (7.9) in wash tubes.

11.2 Hydrolyzable Phosphorus

11.2.1. Add 1 mL of sulfuric acid solution (7.7) to a 50 mL sample and/or standard in a 125 mL Erlenmeyer flask.

11.2.2 Boil gently on a pre-heated hot plate for 30-40 min until a final volume of about 10 mL is reached. Do not allow sample to go to dryness. Alternatively, heat for 30 min in an autoclave at 121°C (15-20 psi).

11.2.3 Determine phosphorus as outlined (11.3.2) with acid wash water (7.9) in wash tubes.

11.3 Orthophosphate

11.3.1 Add 1 drop of phenolphthalein indicator solution (7.10) to approximately 50 mL of sample. If a red color develops, add sulfuric acid solution (7.7) drop-wise to just discharge the color. Acid samples must be neutralized with 1 N sodium hydroxide (40 g NaOH/L).

11.3.2 Set up manifold as shown in Figure 1.

11.3.3 Allow system to equilibrate as required. Obtain a stable baseline with all reagents, feeding reagent water through the sample line.

11.3.4 Place standards in sampler in order of decreasing concentration. and complete filling of sampler tray.

11.3.5 Switch sample line from reagent water to Sampler and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

12.2 Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed. Any sample whose computed value is less than 5% of its immediate predecessor must be rerun.

12.3 Report results in mg P/L.

13.0 METHOD PERFORMANCE

13.1 Six laboratories (using Technicon AAI equipment) participating in an EPA Method Study, analyzed four natural water samples containing exact increments of orthophosphate, with the following results:

Increment as Orthophosphate mg P/liter	Precision as Standard Deviation mg P/liter	Accuracy as Bias %	Accuracy as Bias mg P/liter
0.04	0.019	+16.7	+0.007
0.04	0.014	-8.3	-0.003
0.29	0.087	-15.5	-0.05
0.30	0.066	-12.8	-0.04

13.2 In a single laboratory (EMSL), using surface water samples at concentrations of 0.04, 0.19, 0.35, and 0.84 mg P/L, standard deviations were ±0.005, ±0.000, ±0.003, and ±0.000, respectively.

13.3 In a single laboratory (EMSL), using surface water samples at concentrations of 0.07 and 0.76 mg P/L, recoveries were 99% and 100%, respectively.

13.4 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg PO_4-P/L.

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

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples and method process wastes should be characterized and disposed of in an acceptable manner. 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 waster discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Murphy, J. and Riley, J., "A Modified Single Solution for the Determination of Phosphate in Natural Waters." Anal. Chim. Acta., 27, 31 (1962).

2. Gales, M., Jr., Julian, E., and Kroner, R., "Method for Quantitative Determination of Total Phosphorus in Water." Jour. AWWA, 58, No. 10, 1363 (1966).

3. Lobring, L.B. and Booth, R.L., "Evaluation of the AutoAnalyzer II; A Progress Report," Technicon International Symposium, June, 1972, New York, N.Y.

4. Standard Methods for the Examination of Water and Wastewater, 18th Edition, p. 4-116, Method 4500-P F (1992).

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
54	0.150	0.1530	-0.0017	0.0128	-0.0010
69	0.351	0.3670	0.0140	0.0368	0.0084
88	0.625	0.6090	-0.0141	0.0413	-0.0069
87	1.80	1.7374	-0.0444	0.1259	-0.0072
57	2.50	2.4867	0.0146	0.1637	-0.0200
69	2.75	2.8344	0.1158	0.2019	0.0002
53	3.50	3.5619	0.1038	0.2854	0.0295
87	3.60	3.4957	-0.0610	0.2137	-0.0495
64	4.00	3.8523	-0.0989	0.3158	0.0237
57	7.01	6.9576	0.0383	0.5728	0.0632
88	8.20	8.0995	0.0068	0.5428	-0.0528
63	9.00	8.6717	-0.2099	0.6770	0.0236

REGRESSIONS: $X = 0.986T + 0.007$, $S = 0.072T + 0.003$

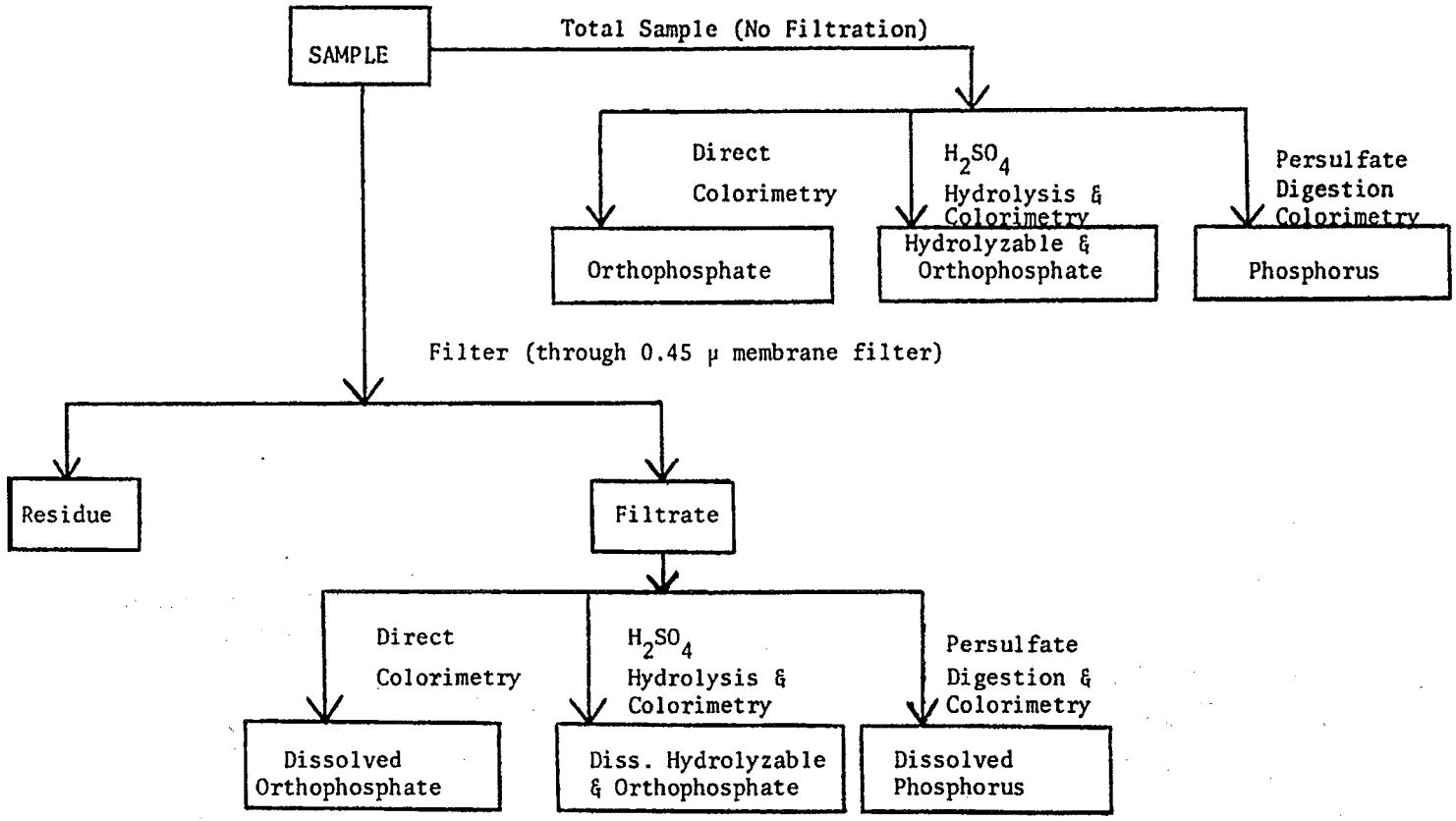

FIGURE 1. ANALYTICAL SCHEME FOR DIFFERENTIATION OF PHOSPHORUS FORMS

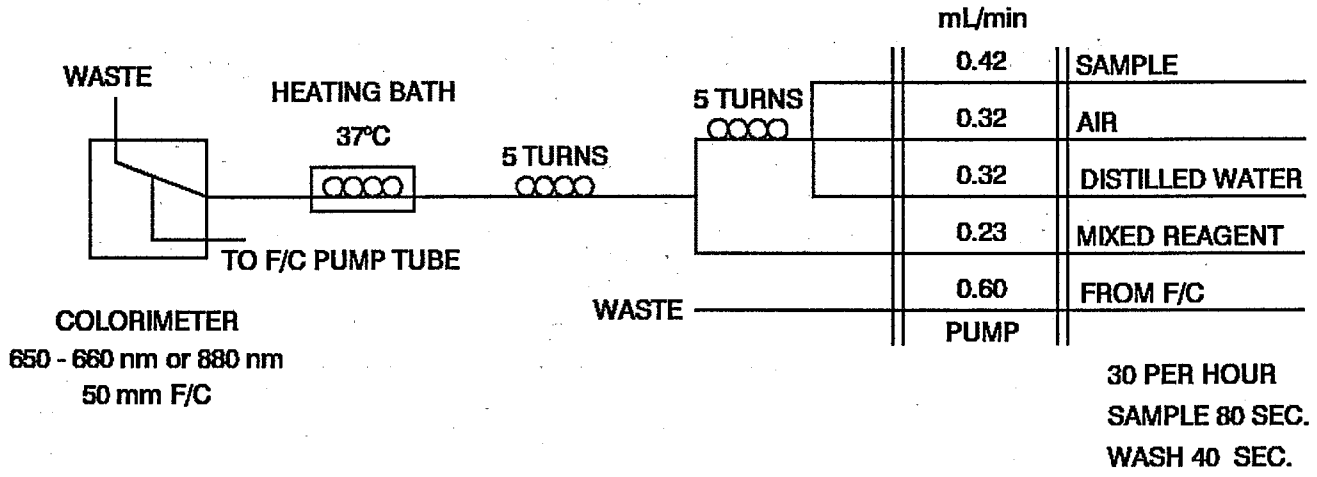

Figure 2
Phosphorus Manifold

METHOD 375.2

DETERMINATION OF SULFATE BY AUTOMATED COLORIMETRY

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Revision 2.0
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METHOD 375.2

DETERMINATION OF SULFATE IN WATER BY AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This automated method is applicable to drinking, ground and surface water, domestic and industrial wastes.

1.2 The applicable range is 3 to 300 mg SO_4/L. The sensitivity of the method can be increased by a minor modification to analyze samples in the range of 0.5 to 30 mg SO_4/L. Approximately 30 samples per hour can be analyzed.

2.0 SUMMARY OF METHOD

2.1 The sample is first passed through a sodium form cation-exchange column to remove multivalent metal ions. The sample containing sulfate is then reacted with an alcohol solution of barium chloride and methylthymol blue (MTB) at a pH of 2.5-3.0 to form barium sulfate. The combined solution is raised to a pH of 12.5-13.0 so that excess barium reacts with MTB. The uncomplexed MTB color is gray; if it is all chelated with barium, the color is blue. Initially, the barium and MTB are equimolar and equivalent to 300 mg SO_4/L; thus the amount of uncomplexed MTB is equal to the sulfate present.

2.2 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.3 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 The ion exchange column eliminates interferences from multivalent cations. A mid-scale sulfate standard containing Ca^{++} should be analyzed periodically to insure that the column is functioning properly.

4.2 Samples with pH below 2 should be neutralized because high acid concentrations elute cations from the ion exchange resin.

4.3 Turbid samples should be filtered or centrifuged.

4.4 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Barium chloride (7.2)

5.3.2 Hydrochloric acid (7.3)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.3.1 Sampling device (sampler)

6.3.2 Multichannel pump

6.3.3 Reaction unit or manifold

6.3.4 Colorimetric detector

6.3.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Barium chloride: Dissolve 0.7630 g of barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) (CASRN 10326-27-9) in 250 mL of reagent water and dilute to 500 mL.

7.3 Methylthymol blue: Dissolve 0.1182 g of methylthymol blue (3'3-bis-N,N-biscarboxymethyl)-amino methylthymolsulfone-phthalein pentasodium salt) (CASRN 1945-77-3) in 25 mL of barium chloride solution (7.2). Add 4 mL of 1.0 N hydrochloric acid (CASRN 7647-01-0) which changes the color to bright orange. Add 71 mL of reagent water and dilute to 500 mL with ethanol. The pH of this solution is 2.6. This reagent should be prepared the day before and stored in a brown plastic bottle in the refrigerator.

7.4 Buffer, pH 10.5 ± 0.5: Dissolve 6.75 g of ammonium chloride (CASRN 12125-02-9) in 500 mL of reagent water. Add 57 mL of concentrated ammonium hydroxide (CASRN 1336-21-6) and dilute to 1 L with distilled water.

7.5 Buffered EDTA: Dissolve 20 g of tetrasodium EDTA (CASRN 64-02-8) in pH 10.5 buffer (7.4), and dilute to 500 mL with buffer.

7.6 Sodium hydroxide solution (50%): Dissolve 250 g NaOH (CASRN 1310-73-2) in 300 mL of reagent water, cool, and dilute to 500 mL.

7.7 Sodium hydroxide, 0.18N: Dilute 7.2 mL of sodium hydroxide solution (7.6) to 500 mL.

7.8 Ion exchange resin: Bio-Rex 70, 20-50 mesh, sodium form, Bio-Rad Laboratories, Richmond, California. Free from fines by stirring with several portions of reagent water and decant the supernate before settling is complete.

7.9 Dilution Water: Add 0.75 mL of sulfate stock solution (7.10) and 3 drops of Brij-35 (CASRN 9002-92-0) to 2 L of reagent water.

7.10 Sulfate stock solution, 1 mL = 1 mg SO_4: Dissolve 1.479 g of dried (105°C) Na_2SO_4 (CASRN 7757-82-6) in reagent water and dilute to 1 L.

7.11 Dilute sulfate solution, 1 mL = 0.1 mg SO_4: Dilute 50 mL of sulfate stock solution (7.10) to 500 ml with reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleansed and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 No chemical preservation required. Cool sample to 4°C.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, samples maintained at 4°C may be held for up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the

determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[3] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Set up the manifold for high (0-300 mg SO_4/L) or low (0-30 mg SO_4/L) level samples as described in Figure 1.

10.1.1 High level working standards, 10-300 mg/L: As a minimum, prepare high level working standards by diluting the following volumes of stock standard (7.10) to 100 mL.

mL Stock	mg/L SO_4
1	10
5	50
10	100
15	150
25	250
30	300

10.1.2 Low level working standards, 1-30 mg/L: Prepare at least this number of low level working standards by diluting the following volumes of dilute sulfate solution (7.11) to 100 mL.

mL Stock	mg/L SO_4
1	1.0
5	5.0
10	10.0
15	15.0
25	25.0
30	30.0

10.2 The ion exchange column is prepared by pulling a slurry of the resin into a piece of glass tubing 7.5 inches long, 2.0 mm ID and 3.6 mm OD. This is conveniently done by using a pipet and a loose fitting glass wool plug in the tubing. Care should be taken to avoid allowing air bubbles to enter the column. If air bubbles become trapped, the column should be prepared over again. The column can exchange the equivalent of 35 mg of calcium. For the high level manifold this corresponds to about 900 samples with 200 mg/L Ca. The column should be prepared as often as necessary to assure that no more than 50% of its capacity is used up.

10.3 Allow the instrument to warm up as required. Pump all reagents until a stable baseline is achieved.

10.4 Analyze all working standards in duplicate at the beginning of a run to develop a standard curve. Control standards are analyzed every hour to assure that the system remains properly calibrated. Since the chemistry is non-linear, data recording devices should be adjusted accordingly.

10.5 Prepare standard curve by plotting instrument response against concentration values. A calibration curve may be fitted to the calibration solutions concentration/response data using computer or calculator based on regression curve fitting techniques. Acceptance or control limits should be established using the difference the measured value of the calibration solution and the "true value" concentration.

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

11.0 PROCEDURE

11.1 Set up instrument as specified under calibration and standardization (10.0).

11.2 Fill and connect reagent containers and start system. Allow the system to equilibrate as required. Pump all reagents until a stable baseline is achieved.

11.3 Place standards and samples in sampler tray. Calibrate instrument, and begin analysis.

11.4 At the end of each day, the system should be washed with the buffered EDTA solution (7.5). This is done by placing the methylthymol blue line and the sodium hydroxide line in reagent water for a few minutes and then in the buffered EDTA solution for 10 min. Wash the system with reagent water for 15 min before shutting down.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg/L.

13.0 METHOD PERFORMANCE

13.1 In a single laboratory the estimated standard deviation, calculated from duplicate analyses of 26 surface and wastewaters at a mean concentration of 100 mg/L was ± 1.6 mg/L.

13.2 The mean recovery from 24 surface and wastewaters was 102%.

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

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

15.0 WASTE MANAGEMENT

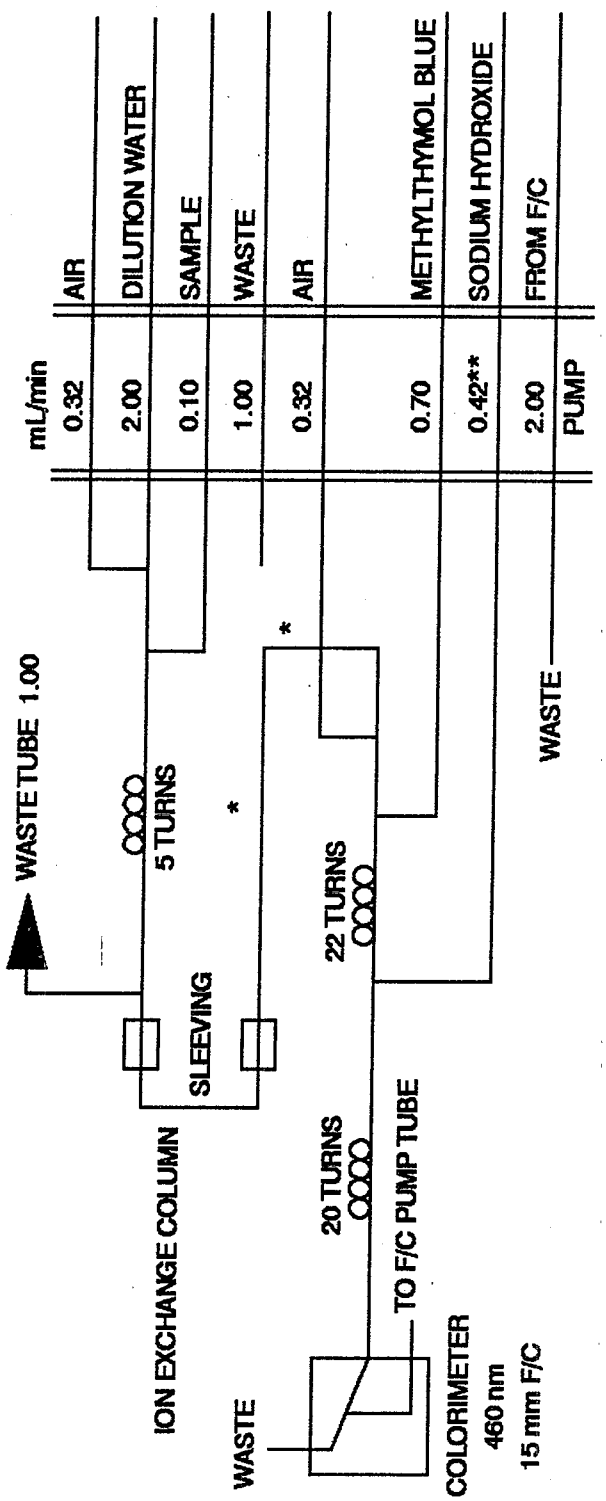
15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess Reagents and samples and method process wastes should be characterized and disposed of in an acceptable manner. The Agency urges laboratories 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 waster discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Lazrus, A.L., Hill, K.C. and Lodge, J.P., "Automation in Analytical Chemistry," Technicon Symposia, 1965.

2. Coloros, E., Panesar, M.R. and Parry, F.P., "Linearizing the Calibration Curve in Determination of Sulfate by the Methylthymol Blue Method," Anal. Chem. 48, 1693 (1976).

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



To analyze samples in the range 0-30 mg/L, change water and sample tubes to 1.0.

Figure 1
Sulfate Manifold

METHOD 410.4

THE DETERMINATION OF CHEMICAL OXYGEN DEMAND BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 2.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
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METHOD 410.4

THE DETERMINATION OF CHEMICAL OXYGEN DEMAND BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of chemical oxygen demand (COD) in ground and surface waters, domestic and industrial wastes.

1.2 The applicable range is 3-900 mg/L.

2.0 SUMMARY OF METHOD

2.1 Sample, blanks, and standards in sealed tubes are heated in an oven or block digestor in the presence of dichromate at 150°C. After two hours, the tubes are removed from the oven or digester, cooled, and measured spectrophotometrically at 600 nm. The colorimetric determination may also be performed manually.

2.2 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.3 Limited performance-based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 Chlorides are quantitatively oxidized by dichromate and represent a positive interference. Mercuric sulfate is added to the digestion tubes to complex the chlorides.

4.2 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

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

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

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

5.3.1 Mercuric sulfate (7.2)

5.3.2 Potassium dichromate (7.2)

5.3.3 Sulfuric acid (7.2, 7.3, 7.4)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Block digestor or drying oven capable of maintaining 150°C.

6.4 Muffle furnace capable of 500°C.

6.5 Culture tube with Teflon-lined screw cap, 16 x 100 mm or 25 x 150 mm.

6.6 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.6.1 Sampling device (sampler)

6.6.2 Multichannel pump

6.6.3 Reaction unit or manifold

6.6.4 Colorimetric detector

6.6.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Digestion solution: Add 5.1 g potassium dichromate $K_2Cr_2O_7$ (CASRN 7778-50-9), 84 mL conc. sulfuric acid H_2SO_4 (CASRN 8014-95-7) and 16.7 g mercuric sulfate $HgSO_4$ (CASRN 7783-35-9) to 250 mL of reagent water, cool and dilute to 500 mL. CAUTION: CAN BE VERY HOT!

7.3 Catalyst solution: Add 22 g silver sulfate Ag_2SO_4 (CASRN 10294-26-5) to a 4.09 kg bottle of conc. H_2SO_4. Stir until dissolved.

7.4 Sampler wash solution: Add 250 mL of conc. H_2SO_4 to 250 mL of reagent water. CAUTION: PREPARE CAREFULLY, HIGH HEAT GENERATION!

7.5 Stock potassium hydrogen phthalate standard: Dissolve 0.425 g KHP (CASRN 877-24-7) in 400 mL of reagent water and dilute to 500 mL. 1 mL = 1 mg COD.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleansed and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with H_2SO_4 to a pH < 2 and cooled to 4°C at the time of collection.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples maintained at 4°C may be held for up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of QCS) and laboratory

performance (determination of MDLs) prior to performing analyses by this method.

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

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[2] 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.

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

9.3.1 Laboratory Reagent Blank (LRB) -- The laboratory must analyze at least one LRB with each batch of samples. Data

produced are used to assess contamination from the laboratory environment. Values that exceed the MDL indicate laboratory or reagent contamination should be suspected and corrective actions must be taken before continuing the analysis.

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, by diluting appropriate volumes of the stock standard (7.5)and a blank.

10.2 Process standards and blanks as described under Procedure (11.0).

10.3 Set up manifold as shown in Figure 1.

10.4 Allow the instrument to warm up as required. Pump all reagents until a stable baseline is achieved.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

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

11.0 PROCEDURE

11.1 Wash all culture tubes and screw caps with 20% H_2SO_4 before their first use to prevent contamination. Trace contamination may be removed from the tubes by igniting them in a muffle furnace at 500°C for 1 h.

11.2 Pipet 2.5 mL of sample, standard or blank, into 16 x 100 mm tubes or 10 mL into 25 x 100 mm tubes.

11.3 Add 1.5 mL of digestion solution (7.2) to the 16 x 100 mm tubes or 6.0 mL to the 25 x 150 mm tubes and mix.

11.4 Add 3.5 mL of catalyst solution (7.3) carefully down the side of the 16 x 100 mm tubes or 14.0 mL to the 25 x 150 mm tubes.

11.5 Cap tubes tightly and shake to mix layer. **CAUTION:** Tubes are hot.

11.6 Place tubes into a block digester or oven at 150°C and heat for 2 h.

11.7 Remove, mix, and cool tubes. Allow any precipitate to settle.

11.8 Fill and connect reagent containers and start system. Allow the instrument to warm up as required. Pump all reagents until a stable baseline is achieved.

11.9 Place standards, blanks, and samples in sampler tray. Calibrate instrument, and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in mg/L.

13.0 METHOD PERFORMANCE

13.1 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg COD/L.

13.2 Single laboratory precision data can be estimated at 50 to 75% of the interlaboratory precision estimates.

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

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess reagents, samples, and method process wastes should be characterized and disposed of in an

acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Jirka, A.M., and M.J. Carter, "Micro-Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand." Anal. Chem. 47:1397, (1975).

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA

NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
241	18.2	18.9398	-0.4220	5.2026	-0.0964
144	26.3	26.1454	-1.0445	5.6142	-0.0888
140	28.5	32.7275	3.4115	6.2230	0.4103
112	43.5	42.8360	-0.9763	6.4351	-0.1257
261	46.6	45.3034	-1.5049	6.7677	0.0523
181	50.0	49.4740	-0.6201	7.0494	0.1644
262	65.4	63.2876	-1.6894	7.6041	-0.0489
182	76.2	75.7960	0.3816	8.4490	0.2573
141	91.7	94.0772	3.6833	7.9289	-1.0358
250	121	117.7424	-0.9678	9.6197	-0.8063
144	201	196.9391	0.9151	14.6995	0.2837
113	229	221.8109	-1.2730	17.3403	1.5280

REGRESSIONS: $X = 0.966T - 1.773$, $S = 0.050T + 4.391$

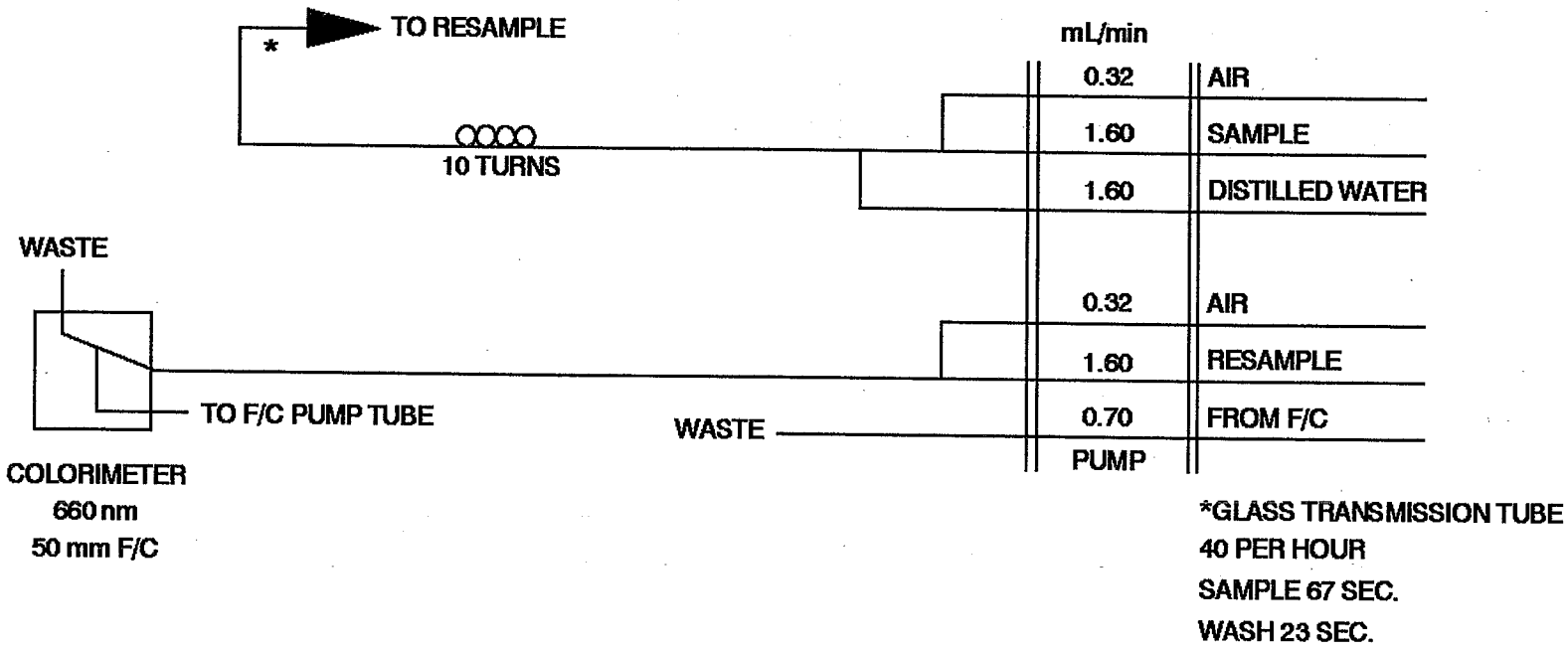

Figure 1
C O D Manifold

METHOD 420.4

DETERMINATION OF TOTAL RECOVERABLE PHENOLICS BY SEMI-AUTOMATED COLORIMETRY

Edited by James W. O'Dell
Inorganic Chemistry Branch
Chemistry Research Division

Revision 1.0
August 1993

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
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METHOD 420.4

DETERMINATION OF TOTAL RECOVERABLE PHENOLICS BY SEMI-AUTOMATED COLORIMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The applicable range is from 2 to 500 µg/L. The working ranges are 2 to 200 µg/L and 10 to 500 µg/L.

2.0 SUMMARY OF METHOD

2.1 This semi-automated method is based on the distillation of phenol and subsequent reaction of the distillate with alkaline ferricyanide and 4-aminoantipyrine to form a red complex which is measured at 505 or 520 nm.

2.2 Color response of phenolic materials with 4-aminoantipyrine is not the same for all compounds. Because phenolic type wastes usually contain a variety of phenols, it is not possible to duplicate a mixture of phenols to be used as a standard. For this reason, phenol has been selected as a standard and any color produced by the reaction of other phenolic compounds is reported as phenol. This value will represent the minimum concentration of phenolic compounds present in the sample.

2.3 Reduced volume versions of this method that use the same reagents and molar ratios are acceptable provided they meet the quality control and performance requirements stated in the method.

2.4 Limited performance based method modifications may be acceptable provided they are fully documented and meet or exceed requirements expressed in Sect. 9.0, Quality Control.

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 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.

3.4 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.

3.5 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.

3.6 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.

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

3.8 MATERIAL SAFETY DATA SHEET (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

3.9 METHOD DETECTION LIMIT (MDL) -- The minimum concentration of an analyte that can be identified, measured and reported with 99% confidence that the analyte concentration is greater than zero.

3.10 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.

3.11 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 Interferences from sulfur compounds are eliminated by acidifying the sample to a pH of 4.0 and aerating briefly by stirring.

4.2 Oxidizing agents such as chlorine, detected by the liberation of iodine upon acidification in the presence of potassium iodide, are removed immediately after sampling by the addition of an excess of ferrous ammonium sulfate (7.11). If chlorine is not removed, the phenolic compounds may be partially oxidized and the results may be low.

4.3 Background contamination from plastic tubing and sample containers is eliminated by filling the wash receptacle by siphon (using Kel-F tubing) and using glass tubes for the samples and standards.

4.4 Method interferences may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Cautions are included for known extremely hazardous materials or procedures.

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

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

5.3.1 Potassium ferricyanide (7.2)

5.3.2 Phenol (7.5)

5.3.3 Sulfuric acid (7.10)

6.0 EQUIPMENT AND SUPPLIES

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

6.2 Glassware -- Class A volumetric flasks and pipets as required.

6.3 Distillation apparatus, all glass consisting of a 1-L pyrex distilling apparatus with Graham condenser. Reduced volume apparatus also may be used.

6.4 pH meter with electrodes.

6.5 Automated continuous flow analysis equipment designed to deliver and react sample and reagents in the required order and ratios.

6.5.1 Sampling device (sampler)

6.5.2 Multichannel pump

6.5.3 Reaction unit or manifold

6.5.4 Colorimetric detector

6.5.5 Data recording device

7.0 REAGENTS AND STANDARDS

7.1 Reagent water: Distilled or deionized water, free of the analyte of interest. ASTM type II or equivalent.

7.2 Buffered potassium ferricyanide: Dissolve 1.0 g potassium ferricyanide (CSRN 13746-66-2), 1.55 g boric acid (CASRN 10043-35-3), and 1.875 g potassium chloride (CASRN 7447-40-7) in 400 mL of reagent water. Adjust to pH of 10.3 with 1 N sodium hydroxide (CASRN 1310-73-2) (7.3) and dilute to 500 mL. Add 0.25 mL of Brij-35 (CASRN 9002-92-0). Prepare fresh weekly.

7.3 Sodium hydroxide (1N): Dissolve 20 g NaOH in 250 mL of reagent water, cool and dilute to 500 mL.

7.4 4-Aminoantipyrine: Dissolve 0.13 g of 4-aminoantipyrine (CASRN 83-07-8) in 150 mL of reagent water and dilute to 200 mL. Prepare fresh each day.

7.5 Stock phenol: Dissolve 0.50 g phenol (CASRN 108-95-2) in 500 mL of reagent water and dilute to 500 mL. Add 0.25 mL conc. H_2SO_4 (CASRN 7664-93-9) as preservative. 1.0 mL = 1.0 mg phenol.

7.6 Standard phenol solution A: Dilute 1.0 mL of stock phenol solution (7.5) to 100 mL with reagent water. 1.0 mL = 0.01 mg phenol.

7.7 Standard phenol solution B: Dilute 10.0 mL of standard phenol solution A (7.6) to 100 mL with reagent water. 1.0 mL = 0.001 mg phenol.

7.8 Standard solution C: Dilute 10.0 mL of standard phenol solution B (7.7) to 100 mL with reagent water. 1.0 mL = 0.0001 mg phenol.

7.9 Sodium hydroxide, 1+9: Dilute 10 mL of 1N NaOH (7.3) to 100 mL with reagent water.

7.10 Sulfuric acid, 1+9 : Slowly add 10 mL conc. H_2SO_4 (CASRN 7764-93-9) to 70 mL of reagent water. Cool and dilute to 100 mL with reagent water.

7.11 Ferrous ammonium sulfate: Dissolve 0.55 g ferrous ammonium sulfate in 250 mL reagent water containing 0.5 mL H_2SO_4 and dilute to 500 mL with freshly boiled and cooled reagent water.

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Samples should be collected in glass bottles only. All bottles must be thoroughly cleansed and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved at time of collection with H_2SO_4 to a pH of < 2 and cooled to 4°C.

8.3 Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at 4°C and may be held up to 28 days.

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 that are generated.

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

9.2.2 Linear Calibration Range (LCR) -- The LCR must be determined initially and verified every 6 months or whenever a significant change in instrument response is observed or expected. The initial demonstration of linearity must use sufficient standards to insure that the resulting curve is linear. The verification of linearity must use a minimum of a blank and three standards. If any verification data

exceeds the initial values by ± 10%, linearity must be reestablished. If any portion of the range is shown to be nonlinear, sufficient standards must be used to clearly define the nonlinear portion.

9.2.3 Quality Control Sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS. If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of MDLs or continuing with on-going analyses.

9.2.4 Method Detection Limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[4] 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.

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

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

9.3.2 Laboratory Fortified Blank (LFB) -- The laboratory must analyze at least one LFB with each batch of samples. Calculate accuracy as percent recovery (Sect. 9.4.2). If the recovery of any analyte falls outside the required

control limits of 90-110%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 90-110%. When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the percent mean recovery (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 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 on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

9.4.1 Laboratory Fortified Sample Matrix (LFM) -- The laboratory must add a known amount of 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. The analyte concentration must be high enough to be detected above the original sample and should not be less than four

times the MDL. The added analyte concentration should be the same as that used in the laboratory fortified blank.

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

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

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

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

9.4.4 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 Prepare a series of at least 3 standards, covering the desired range, and a blank by pipetting suitable volumes of working standard solutions (7.6, 7.7, 7.8) into 100-mL volumetric flasks. Suggested ranges include 1 to 5, 10 to 100, and 200 to 500 µg/L.

10.2 It is not imperative that all standards be distilled in the same manner as the samples. It is recommended that at least one standard and a blank be distilled and compared to similar values on the standard curve to insure that the distillation technique is reliable. If distilled standards do not agree within ± 10% of the undistilled standards, the analyst should find the cause of the apparent error before proceeding. Before distillation, standards should be adjusted to a pH of 4 with H_2SO_4.

10.3 Set up the manifold as shown in Figure 1 in a hood or a well-ventilated area.

10.4 Allow the instrument to warm up as required. Pump all reagents until a stable baseline is achieved.

10.5 Place appropriate standards in the sampler in order of decreasing concentration and perform analysis.

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

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

11.0 PROCEDURE

11.1 Distillation

11.1.1 Measure 500 mL sample into a beaker. Adjust the pH to approximately 4 with 1+9 NAOH (7.9) or 1+9 H_2SO_4 (7.10), and transfer to the distillation apparatus.

11.1.2 Distill 450 mL of sample, stop the distillation, and when boiling ceases add 50 mL of warm reagent water to the flask and resume distillation until 500 mL have been collected.

11.1.3 If the distillate is turbid, filter through a prewashed membrane filter.

11.2 Set up the manifold as shown in Figure 1.

11.3 Fill the wash receptacle by siphon with reagent water. Use Kel-F tubing with a fast flow (1 L/h).

11.4 Allow the instrument to warm up as required. Run a baseline with all reagents, feeding reagent water through the sample line. Use polyethylene tubing for sample line. When new tubing is used, about 2 hours may be required to obtain a stable baseline. This two hour time period may be necessary to remove the residual phenol from the tubing.

11.5 Place appropriate phenol standards in sampler in order of decreasing concentration. Complete loading of sampler tray with unknown samples, using glass tubes.

11.6 Switch sample line from reagent water to sampler and begin analysis.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.

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

12.3 Report results in μg/L.

13.0 METHOD PERFORMANCE

13.1 The interlaboratory precision and accuracy data in Table 1 were developed using a reagent water matrix. Values are in mg Phenol/L.

13.2 Single laboratory precision data can be estimated at 50 to 75% of the interlaboratory precision estimates.

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

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

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. Excess Reagents and samples and method process wastes should be characterized and disposed of in an

acceptable manner. 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 waste discharge permit 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 Sect. 14.3.

16.0 REFERENCES

1. Technicon AutoAnalyzer II Methodology, Industrial Method No. 127-71W, AAII.

2. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p. 574, Method 510 (1975).

3. Gales, M.E. and Booth, R.L., "Automated 4 AAP Phenolic Method," AWWA 68, 540 (1976).

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1. INTERLABORATORY PRECISION AND ACCURACY DATA					
NUMBER OF VALUES REPORTED	TRUE VALUE (T)	MEAN (X)	RESIDUAL FOR X	STANDARD DEVIATION (S)	RESIDUAL FOR S
99	0.020	0.0149	0.0000	0.0074	0.0000
87	0.250	0.1443	-0.0052	0.0268	-0.0038
76	0.400	0.2352	-0.0021	0.0422	-0.0036
110	0.545	0.3364	0.0142	0.0681	0.0076
89	0.604	0.3610	0.0043	0.0625	-0.0039
107	0.660	0.3959	0.0064	0.0894	0.0173
86	0.800	0.4627	-0.0087	0.0806	-0.0057
62	0.817	0.4692	-0.0122	0.0776	-0.0104
76	0.970	0.5680	-0.0029	0.1017	-0.0017
89	2.96	1.7734	0.0377	0.3065	0.0018
61	4.18	2.3916	-0.0582	0.4044	-0.0237
110	4.54	2.7150	0.0545	0.5382	0.0737

REGRESSIONS: $X = 0.585T + 0.003$, $S = 0.101T + 0.005$

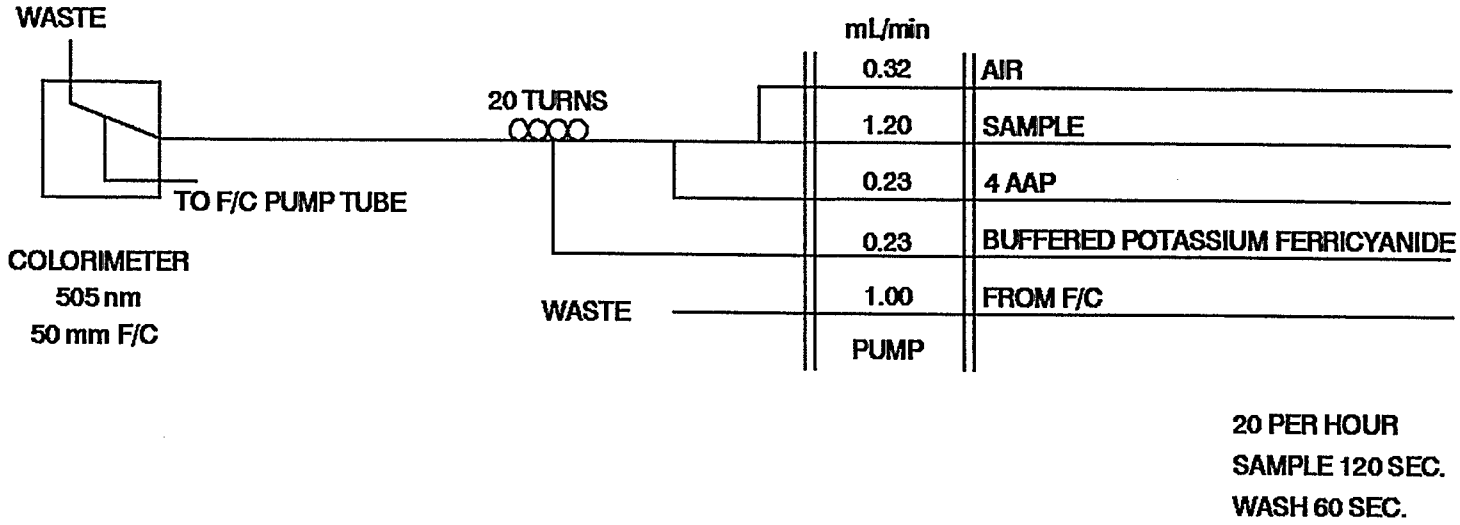

Figure 1
Phenol Manifold