OAKLAND COUNTY WATER RESOURCES COMMISSIONER
WASTEWATER TREATMENT UNIT

Walled Lake - Novi Wastewater Treatment Plant Laboratory

STANDARD OPERATING PROCEDURES MANUAL

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Section I

INTRODUCTION

The purpose of this manual is to describe the procedures currently in use at the Walled Lake-Novi Laboratory. The procedures described are intended for the analysis of water. These waters include, ground water, cooling water, boiler water, boiler feed water, domestic or industrial supplies, drinking water, treated or untreated municipal or industrial wastewater and saline water.

This manual also contains information about terminology and practices generally used in laboratory analysis.

Laboratory Analysis Information

Abbreviations

The following abbreviations may be used throughout the text of the procedure section:

C	degree(s) Celsius (Centigrade)
F	degree(s) Fahrenheit
AA	Atomic Absorption
ACS	American Chemical Society reagent grade purity
ASTM	American Society for Testing and Materials
BOD	Biochemical Oxygen Demand
CBOD	Biochemical Oxygen Demand, carbonaceous
CFU	Colony Forming Units
COD	Chemical Oxygen Demand
conc	concentrated, concentration
g	grams
h	hour
H2SO4	Sulfuric Acid
H3PO4	Phosphoric Acid
HCl	Hydrochloric Acid
HNO3	Nitric Acid
L	liter
M	Molarity, Molar
mg/L	milligrams per liter (ppm)
min	minute
ml	milliliter
MPN	Most Probable Number
N	Normality, Normal
NIST	National Institute of Standards and Technology
nm	nanometer
NPDES	National Pollutant Discharge Elimination System
O2	Oxygen
PPM	parts per million
ppb	parts per billion
QA	Quality Assurance
QC	Quality Control
s	second
TOC	Total Organic Carbon
ug/L	micrograms per liter (ppb)
um	micrometer
USEPA	United States Environmental Protection Agency
WEF	Water Environment Federation

Standard Definitions of Terms Relating to Water

Accuracy – combination of bias and precision of an analytical procedure, which reflects the closeness of a measured value to a true value.

Bias – consistent deviation of measured values from the true value, caused by systematic errors in a procedure.

Composite Sample – a combination of two or more grab samples collected at same sampling point, but at different times.

Detection Limits, minimum – the lowest value that can be determined within the stated precision of a method expressed quantitatively in the same dimension that is used for reporting results of the test.

Duplicate – two samples taken at the same time from one location.

Grab Sample – a sample collected at a particular time and place and represents the composition of the source at that time and place.

Meniscus – the curved upper surface of a liquid in a container that is concave if the liquid wets the container walls.

Precision – measure of the degree of agreement among replicate analyses of a sample usually expressed as the standard deviation.

Quality Assurance – a definitive plan for laboratory operation that specifies the measures used to produce data of known precision and bias.

Quality Control – set of measures within a sample analysis methodology to assure that the process is in control.

Replicate – two or more analyses for the same constituent in an extract of a single sample.

Spiked Sample – a sample where a known volume of standard is added to the sample and the recovery of the standard is obtained to test the efficiency of the analyst and the analytical procedure.

Laboratory Practice and Techniques

Boiling Aids

Boiling is included as a necessary step in some procedures. It may be convenient to use a boiling aid to reduce bumping. Bumping is caused by the sudden, almost explosive conversion of water to steam as it is heated. Bumping may cause sample loss or a hazardous condition and should be avoided. All boiling aids should be checked to verify they will not contaminate the sample. Boiling aids (except glass beads) should not be used again.

Filtration of Samples

Filtering is the process of separating particles from the sample by using a medium, generally filter paper, to retain particles but allow the solution to pass through. This is especially helpful when sample turbidity interferes with colorimetric analysis. Two general methods are gravity and vacuum. Gravity filtration uses the force of gravity to pull the sample through the filter paper. Vacuum filtration uses the pressure difference created by an aspirator or vacuum pump plus the force of gravity to move the sample through the filter. Vacuum filtration is faster than gravity filtration. Gravity filtration provides better retention of fine particles.

Reagent and Standard Stability

Most chemicals and prepared reagents do not deteriorate after manufacture, but storage conditions and packaging have a great influence on their stability. Absorption of moisture, carbon dioxide or other gases from the atmosphere, bacterial action, or light may affect the reagent shelf life. Reaction with other reagents may occur. Always check product label for special storage conditions. It is always good practice to date chemicals upon receipt and upon opening. Always follow procedure guidelines for use and disposal of standards and reagents. Most reagents used in our procedures are certified ACS grade.

Reagent Grade Water

Reagent grade water by definition is the quality of the water produced by a process for use in preparation of reagents, standards, or sample dilutions. There are three types of reagent grade water: Type I, Type II, and Type III. Type I has no detectable concentration of compounds or elements, while Type III is for washing and qualitative analysis. Type II falls in the middle. There are seven major water purification processes: distillation, deionization, reverse osmosis, carbon adsorption, filtration, ultrafiltration, and ultraviolet oxidation used to make the three types of water. Any method of preparation of reagent grade water is acceptable provided that the quality can be met. Generally various combinations of the processes are used to produce reagent grade water. Currently at Walled Lake-Novi lab Type I is confined to the metals detection area and Type II is for general use throughout the lab.

Reagent Blank

The term "reagent blank" refers to the portion of the test result contributed solely by the reagent and deionized water. In most cases the reagent blank is used to zero the instrument. The reagent blank is carried out through the procedure as if it were a sample. It is used to represent a positive error in the analysis. It is good practice to produce reagents with the lowest possible blank. In most cases the blank is less than 0.009 absorbance units.

Safety

Safety is the responsibility of each individual when performing analytical procedures. The analyst must develop and maintain good safety habits. Following are several rules that apply to laboratory analysis. They are not all inclusive.

Read Labels Carefully: Each reagent label should be read carefully. Never remove the label from a reagent container while it contains reagent. Do not put a different reagent into a labeled container without changing the label. When preparing a reagent or standard solution, be sure to label the container clearly.

Wear Protective Clothing: Protective clothing should be worn when handling chemicals that cause irritation or burns. Always wear eye protection while working in the lab to guard against spattering and splashes.

Pipettes: Never pipette by mouth. Mouth pipetting could result in accidentally ingesting a dangerous chemical. Always use mechanical pipetting devices for all pipetting.

Temperature Considerations

For best results, most tests described in this manual should be performed with sample temperatures between 20°C and 25°C. If certain tests require closer temperature control, that requirement will be indicated in notes following those procedures.

Use of Pipettes, Graduated Cylinders and Burettes

When small sample quantities are used, the accuracy of measurements is important. The proper way of reading sample levels in burette, cylinders or pipette is to find the meniscus formed when the liquid wets the walls. Rinse the burette, pipette or cylinder two or three times with the sample to be tested before filling.

Use a pipette bulb to draw the sample into the pipette. When filling a pipette, keep the tip of the pipette below the surface of the sample as the sample is drawn into the pipette. Serological pipettes are long tubes with a series of calibrated marks to indicate the volume of liquid delivered by the pipette. Fill serological pipettes to the zero mark and discharge the sample by allowing the sample to drain until the meniscus is level with the desired mark. If the serological pipette has calibrated marks extended to the tip of the pipette, the sample must be blown out of the tip for accurate sample measurements.

Volumetric (transfer) pipettes are long tubes with a bulb in the middle and a single ring above the bulb to indicate the volume of liquid to be delivered when it is filled to the mark. To discharge the sample from a volumetric pipette, hold the tip of the pipette at a slight angle against the container wall and drain. Do not attempt to discharge sample or reagents remaining in the tip of the pipette after draining. Volumetric pipettes are designed to always retain a small reproducible amount of sample in the tip of the pipette.

Burettes are long glass tubes with graduations and a stopcock at the bottom. To use, rinse the burette by closing the stopcock and filling with small amount of liquid to be measured. While holding onto the burette, horizontally twirl to wet all sides. Drain the rinse through the tip. Refill to above zero mark and drain excess through stopcock, being certain all bubbles are removed. Adjust meniscus to zero mark. To titrate, dispense reagent in a steady stream until reaction endpoint nears. As endpoint nears, add reagent in a dropwise manner.

Sampling and Storage

Correct sampling and storage are critical to the accuracy of each test. For greatest accuracy minimize contamination from the sampling device, remove residues of previous samples from sample container and preserve the sample properly.

Taking Water Samples

Samples for analysis should be collected carefully to make sure the most representative sample possible is obtained. Use only clean containers for collecting samples. Rinse the container several times first with the water to be sampled unless there is a reagent in the sample bottle. Generally analyze samples as soon as possible after collection. Sample preservatives and storage techniques are described in each procedure for sample held for later testing.

Sample Chain of Custody

All outside lab samples received in the lab follow chain of custody procedure. Outside lab samples are those samples not routinely analyzed at our facility. Our sample chain of custody forms contain the following information: person sampling; date and time sample collected; weather conditions; facility name and address; sampling location and type; observations and comments; bottle ID marking; sample split with another facility; name of facility representative; analysis required; relinquished by; received by; date and time received. A chain of custody form accompanies any sample leaving our facility.

Acid Washing Bottles

A procedure may suggest the sample bottles be acid-washed to minimize the effect of interferences. This is accomplished by using a detergent to clean the glassware or plasticware, rinsing with tap water, rinsing with a 1:1 Hydrochloric Acid Solution or 1:1 Nitric Acid Solution, rinsing with deionized water. Rinse a minimum of 5 times with deionized water. Chromium-free acid may be used to remove organic deposits from glass containers. Rinse thoroughly to remove the acid. Glassware for phosphate determinations should be washed with phosphate-free detergents and acid washed with 1:1 HCl.

Storage and Preservation

Preservation techniques can retard the chemical and biological changes continuing after sample is taken. As a general rule, it is best to analyze the samples as soon as possible after collection. Preservation methods are limited generally to pH control, chemical addition, refrigeration and freezing. The recommended preservation techniques are included with each procedure.

Laboratory Equipment and Instrumentation

The Walled Lake-Novi lab constantly updates its analytical instrumentation. All instrumentation is state-of-the-art and most is fully automated to increase precision, accuracy and efficiency. Our instrumentation is generally dedicated to a particular analysis.

Equipment Maintenance

A separate maintenance file is maintained on all equipment and analytical instruments. All repairs and routine preventative maintenance are recorded in these files. Service contracts are maintained on most of the equipment. These contracts include periodic preventative maintenance visits by manufacturer's service technicians.

Section II

INSTRUCTIONS AND METHODS

The processes included in this manual are primarily used for analysis of wastewater Treatment plant samples operated by Oakland County WATER RESOURCES COMMISSIONER's Office. Most samples are analyzed at the Walled Lake-Novi Wastewater Treatment Plant. Authorized contract labs manage special laboratory analysis when needed.

All methods in this manual are reviewed and updated to stay current with EPA regulations. Any analysis or method added to the lab's workload will be included in the SOP manual at the time they are initiated.

ALKALINITY
Titration Method
(Water/Wastewater Matrices)

1.0 APPLICATION

This method is based on 2320(B) of the 21st Edition of Standard Methods for Examination of Water and Wastewater. It is suitable for minimum concentrations of 4 mg/L.

2.0 SUMMARY

Alkalinity of water is its acid-neutralizing capacity. It is the sum of components in the water that tend to elevate the pH of the water above a value of about 4.5. It is measured by titration with standardized acid to a pH value of about 4.5. It is expressed commonly as mg/L of calcium carbonate. It is therefore, a measure of the buffering capacity of the water.

3.0 PRESERVATION

3.1 Collect sample in an unpreserved bottle.

3.2 Refrigerate at $\leq$ 6°C.

3.3 Maximum recommended holding time - 14 days.

3.4 Avoid sample agitation and prolonged exposure to air.

4.0 APPARATUS

4.1 Glass burette, 50 ml and burette stand.

4.2 Magnetic stirrer

4.3 Beakers - 300 ml

4.4 Graduated cylinders - 50 - 100 ml capacity

4.5 Volumetric flask - 500 ml

4.6 pH Meter

5.0 REAGENTS

5.1 pH buffers (4, 7, and 10) from supplier.

5.2 Standard sulfuric acid solution, 0.02 N: purchase from supplier.

5.3 Total alkalinity standard: Dry sodium carbonate (Na2CO3) in a 104°C oven for four hours. Cool in a desiccator for two hours. Weigh 5.295 g into a 500-ml volumetric flask. Dilute to volume. 1 ml = 10 mg total alkalinity as CaCO3 (10,000 mg/L).

6.0 INTERFERENCES

6.1 Use of pH meter for endpoint of 4.5 eliminates difficulties presented by highly colored and turbid samples.

7.0 QA/QC

7.1 Test at least one total alkalinity standard with each set of samples.

7.2 One in twenty samples should be replicates.

7.3 One in twenty samples should be spiked.

8.0 PROCEDURE

8.1 Calibrate pH meter as per SOP for pH meter on page 98.

8.2 Measure 100 ml of sample into 300-ml beaker.

8.3 Mix sample on stirrer.

8.4 Pour 0.02 N H_2SO_4 into titrating burette. Zero the burette.

8.5 Titrate sample with 0.02 N H_2SO_4 until pH of 4.5 is obtained.

8.6 Record titration volume on proper bench sheet.

8.7 Repeat procedure for all samples.

9.0 CALCULATIONS

9.1 Total Alkalinity, as $CaCO_3$ mg/L =

$$\frac{(A) \times (N) \times (50{,}000)}{\text{ml of sample titration}}$$

Where: A = ml of H_2SO_4 used
N = normality of H_2SO_4

QuikChem® Method 10-107-06-1-J

DETERMINATION OF AMMONIA BY FLOW INJECTION ANALYSIS

(LOW FLOW METHOD)

Written by Ninglan Liao

Applications Group

Revision Date:

15 March 2001

LACHAT INSTRUMENTS
5600 LINDBURGH DRIVE
LOVELAND, CO 80539 USA
QuikChem® Method 10-107-06-1-J

Ammonia (Phenolate) in Potable and Surface Waters

0.01 to 2.0 mg N/L as NH_3

– Principle –

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

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

– Interferences –

1. Calcium and magnesium ions may precipitate if present in sufficient concentration. Tartrate or EDTA is added to the sample in-line in order to prevent this.
2. Color, turbidity, and certain organic species may interfere. Turbidity is removed by manual filtration. Sample color may be corrected for by running the samples through the manifold without color formation.
3. Cyanate, which may be encountered in certain industrial effluents, will hydrolyze to some extent even at a pH of 9.5 at which distillation is carried out.
4. Residual chlorine must be removed by pretreatment of the sample with sodium thiosulfate or other reagents before distillation.
5. Method interference may be caused by contaminants in the reagent water, reagents, glassware, and other sample processing apparatus that bias analyte response.
6. Eliminate any marked variation in acidity or alkalinity among samples because intensity of measured color is pH – dependent. Likewise, ensure that pH of standard ammonia solutions approximates that of sample.

– Special Apparatus –

Please see Parts and Price list for Ordering Information

1. Heating Unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)
2. PVC PUMP TUBES MUST BE USED FOR THIS METHOD

QuikChem® Method 10-107-06-1-J

DETERMINATION OF AMMONIA (PHENOLATE) BY FLOW INJECTION ANALYSIS

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination ammonia (phenolate) in drinking, ground, and surface waters. It can also be used for industrial an domestic waters.

1.2 The method is based on reactions that are specific for the ammonium (NH_4^+) ion.

1.3 The applicable range is 0.01 to 2.0 mg N/L. The statistically determined detection limit as determined is 0.002 mg N/L in non acid preserved samples and 0.003 mg N/L in acid preserved samples. The method throughput is 55 injections per hour.

2.0 SUMMARY OF METHOD

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

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

3.0 DEFINITIONS

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

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

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

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

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

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

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

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

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

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

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

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

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

3.13 **USEPA** -- United States Environmental Protection Agency.

4.0 <u>INTERFERENCES</u>

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

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

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

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

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

4.6 Eliminate any marked variation in acidity or alkalinity among samples because intensity of measure color is pH dependent. Likewise, ensure that pH of standard ammonia solutions approximates that of sample.

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.

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

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

5.3.1 Phenol
5.3.2 Sodium nitroferricyanide
5.3.3 Sulfuric acid

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 pipettes or plastic containers as required. Samples may be stored in plastic or glass.

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

6.3.1 Sampler
6.3.2 Multichannel proportioning pump
6.3.3 Reaction unit or manifold
6.3.4 Colorimetric detector
6.3.5 Data system

6.4. Special Apparatus

6.4.1 Heating unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)
6.4.2 **PVC PUMP TUBES MUST BE USED FOR THIS METHOD**

7.0 REAGENTS AND STANDARDS

7.1. PREPARATION OF REAGENTS

Use ASTM Type I water for all solutions. (See Standard Specification for Reagent Water D1193-77 for more information).

Degassing with helium:

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

Reagent 1. Sodium Phenolate

CAUTION: Wear gloves. Phenol causes severe burns and is rapidly absorbed into the body through the skin.

By Volume: In a **1 L** volumetric flask, dissolve **88 mL of 88% liquefied phenol** or **83 g crystalline phenol** (C_6H_5OH) is approximately **600 mL DI water**. While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool, dilute to the mark, and invert to mix. Do not degas this reagent. Prepare fresh every 3 to 5 days. Discard when reagent turns brown.

By Weight: To a tared **1 L** container, add **888 g DI water**. Add **94.2 g 88% liquefied phenol** or **83 g crystalline phenol** (C_6H_5OH). While stirring, slowly add **32 g sodium hydroxide** (NaOH). Cool and invert to mix thoroughly. Do not degas this reagent. Prepare fresh every 3 to 5 days. Discard when reagent turns brown.

Reagent 2. Sodium Hypochlorite

By Volume: In a **500 mL** volumetric flask, dilute **250 mL 5.25% sodium hypochlorite** (NaOCl) to the mark with **DI water**. Invert to mix. Prepare fresh daily.

By Weight: To a tared **500 mL** container add **250 g 5.25% sodium hypochlorite** (NaOCl) and **250 g DI water**. Stir or shake to mix. Prepare fresh daily.

Reagent 3. Sodium Nitroprusside

By Volume: In a **1 L** volumetric flask, dissolve **3.5 g sodium nitroprusside** (Sodium Nitroferricyanide [$Na_2FE(CN)_5NO\ 2H_2O$]). Dilute to the mark with **DI water** and invert to mix. Prepare fresh every 1 to 2 weeks.

By Weight: To a tared **1 L** container add **3.5 g sodium nitroprusside** (Sodium Nitroferricyanide [$Na_2FE(CN)_5NO \cdot 2H_2O$]) and **1000 g DI water**. Invert to mix. Prepare fresh every 1 to 2 weeks.

Reagent 4. **1 M Sodium Hydroxide Solution**

By Volume: In a **1 L** volumetric flask, dissolve **40.0 g sodium hydroxide** (NaOH) in approximately **900 mL DI water**. Dilute to the mark and mix with a magnetic stirrer until dissolved.

Reagent 5. **Buffer for Non Acid Preserved Samples**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetic acid** (Na_2EDTA) and **225 mL 1 M sodium hydroxide** (Reagent 4) in approximately **700 mL DI water**. Dilute to the mark and mix with a magnetic stirrer until dissolved. Prepare fresh monthly.
If the samples are preserved at a pH less than two with sulfuric acid, the following reagent and standard recipe changes apply:

Reagent 6. **Buffer for Acid Preserved Samples**

By Volume: In a **1 L** volumetric flask, dissolve **50.0 g disodium ethylenediamine tetraacetic acid** (Na_2EDTA) and **254 mL 1 M sodium hydroxide** (Reagent 4) in approximately **700 mL DI water**. Dilute to the mark and mix with a magnetic stirrer until dissolved. Prepare fresh monthly.

Reagent 7. **Sulfuric Acid Diluent for Carrier and Standards**

By Volume: In a **1 L** volumetric flask, add approximately **800 mL DI water** followed by **2 mL concentrated sulfuric acid**. Dilute to the mark. Keep flask sealed when not in use with parafilm to avoid ambient ammonia contamination.

7.2. PREPARATION OF STANDARDS FOR NON ACID PRESERVED SAMPLES

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

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

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

Standard 1. Stock Standard 1000 mg N/L as NH_3

By Volume: In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 110°C in about 800 mL DI water. Dilute to the mark and invert to mix.

Standard 2. Intermediate Stock Standard 20 mg N/L as NH_3

By Volume: In a **1 L** volumetric flask add **20 mL of Stock Standard 1** (1000 mg N/L). Dilute to the mark with **DI water** and invert to mix. Prepare fresh weekly.

By Weight: To a tared **1 L** container, add about **20 g Stock Standard 1** (1000 mg N/L). Measure the exact weight of the solution added and divide this weight by **0.02**. This will give you the total weight of the diluted solution to be made. Make up the solution to this weight with **DI water**.

Working Standards (Prepare Weekly)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Concentration mg N/L as NH_3	2.0	0.8	0.2	0.05	0.02	0.01	0.0

By Volume

Volume (mL) of **stock standard 2** diluted to 250 mL with DI water	25	10	---	---	---	---	---
Volume (mL) of **standard A** diluted to 250 mL with DI water	---	---	25	6.25	2.5	1.25	---

By Weight

Weight (g) of **stock standard 2** diluted to final weight (~250 g) divided by **factor** below with DI water	25	10	---	---	---	---	---
Weight (g) **standard A** diluted to final weight (~250 g) divided by **factor** below with DI water	---	---	25	6.25	2.5	1.25	---
Division Factor Divide exact weight of the standard by this **factor** to give the final weight	0.1	0.04	0.1	0.025	0.01	0.005	---

7.3 PREPARATION OF STANDARDS FOR ACID PRESERVED SAMPLES

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

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

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

Standard 1. Stock Standard 1000 mg N/L as NH_3

By Volume: In a **1 L** volumetric flask dissolve **3.819 g ammonium chloride** (NH_4Cl) that has been dried for two hours at 11°C in about **800 mL DI water**. Dilute to the mark and invert to mix.

Standard 2. Intermediate Stock Standard 20 mg N/L as NH_3

By Volume: In a **1 L** volumetric flask add **20 mL of Stock Standard 1** (1000 mg N/L). Dilute to the mark with **Reagent 7** and invert to mix. Prepare fresh weekly.

By Weight: To a tared **1 L** container, add about **20 g Stock Standard 1** (1000 mg N/L). Measure the exact weight of the solution added and divide this weight by **0.02**. This will give you the total weight of the diluted solution to be made. Make up the solution to this weight with **Reagent 7**. Shake before using.

Working Standards (Prepare Weekly)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Concentration mg N/L as NH_3	2.0	0.8	0.2	0.05	0.02	0.01	0.0

By Volume

	A	B	C	D	E	F	G
Volume (mL) of **stock standard 3** diluted to 250 mL with **Reagent 7**	25	10	---	---	---	---	---
Volume (mL) of **standard A** diluted to 250 mL with **Reagent 7**	---	---	25	6.25	2.5	1.25	---

By Weight

	A	B	C	D	E	F	G
Weight (g) of **stock standard 3** diluted to final weight (~250 g) divided by **factor** below with **Reagent 7**	25	10	---	---	---	---	---
Weight (g) **standard A** diluted to final weight (~250 g) divided by **factor** below with **Reagent 7**	---	---	25	6.25	2.5	1.25	---
Division Factor Divide exact weight of the standard by this **factor** to give the final weight	0.1	0.04	0.1	0.025	0.01	0.005	---

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. The volume collected should be sufficient to ensure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

8.2 Samples must be preserved with sulfuric acid at pH < 2 and cooled to 4°C at the time of collection for NPDES monitoring. Do not add more than 2 mL sulfuric acid/L unless it is required to obtain pH < 2.

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

8.4 The Federal Register entry which defines standard EPA NPDES and NPDWR methods states that "Manual Distillation is NOT required if comparability data on representative effluent samples are on company file to show that this preliminary distillation step is not necessary; however, manual distillation will be required to resolve any controversies" (See Sources). This suggests that the user perform recovery studies on representative sample types, studies that show that the non-distilled samples give the same recoveries as the manually distilled samples. Follow EPA Method 350.1 for the complete distillation procedure.

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

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

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

$$MDL = t \times S$$

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

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

9.3. ASSESSING LABORATORY PERFORMANCE

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

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

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

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

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

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

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

9.4 ASSESSING ANALYTE RECOVERY AND DATA QUALITY

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

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

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

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

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

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

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

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

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

9.4.5 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.

10.0 CALIBRATION AND STANDARDIZATION

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

10.2 Calibrate the instrument as described in Section 11.

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

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

11.0 PROCEDURE

11.1 CALIBRATION PROCEDURE

11.1.1 Prepare reagent and standards as described in Section 7.

11.1.2 Set up manifold as shown in Section 17.

11.1.3 Input data system parameters as shown in Section 17.

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

11.1.5 Place samples and/or standards in the sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See Section 17).

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

11.2 SYSTEM NOTES

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

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

11.2.3 If baseline drifts, peaks are too wide, or other problems with precision arise, clean the manifold by the following procedure.

11.2.3.1 Place all reagents lines in DI Water and pump to clear reagents (2 to 5 minutes).
11.2.3.2 Place all reagent lines in 1 M hydrochloric acid (1 volume concentrated HCl added to 11 volumes of DI water) and pump for several minutes.
11.2.3.3 Place all reagent lines in DI water and pump until the HCl is thoroughly washed out.
11.2.3.4 Resume pumping reagents.

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

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

12.0 DATA ANALYSIS AND CALCULATIONS

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

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

12.3 Report results in mg N/L.

13.0 METHOD PERFORMANCE

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

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

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

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

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

15.0 WASTE MANAGEMENT

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

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

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 350.1.

16.2 Standard Methods, For the Examination of Water and Wastewater, 18th ed., p. 4-77, Methods 4500 NH_3 B and H (1992).

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

17.0 TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

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

Sample throughput: 55 samples/h, 65 s/sample
Pump Speed: 35
Cycle Period: 65

Analyte Data:

Concentration Units: mg N/L
Peak Base Width: 44 s
% Width Tolerance: 75
Threshold: 7000
Inject to Peak Start: 50 s
Chemistry: Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg N/L	2.0	0.8	0.2	0.05	0.02	0.01	0.0

Calibration Rep Handling: Weighted Avg
Calibration Fit Type: 1st Order Polynomial
Weighting Method: None
Force through zero: no

Sampler Timing:

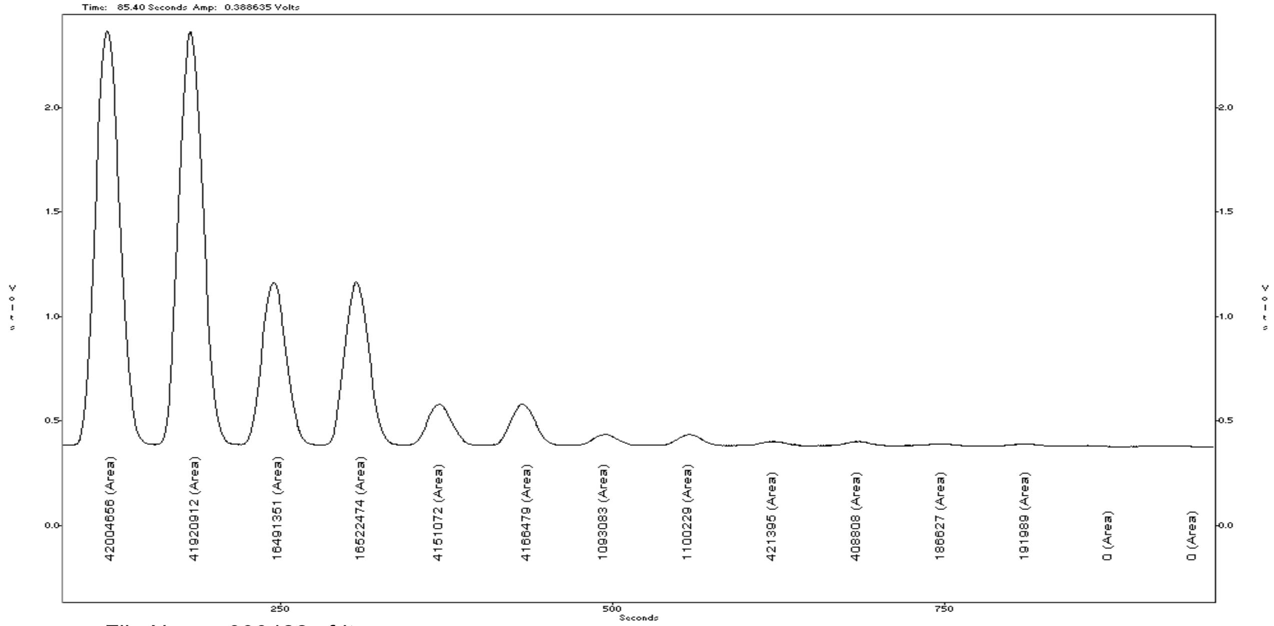
Min. Probe in Wash Period: 12 s
Probe in Sample Period: 32 s

Valve Timing:

Load Time: 0 s
Load Period: 28 s
Inject Period: 37 s

17.2 SUPPORT DATA FOR QUIKCHEM 8000 IN DI WATER

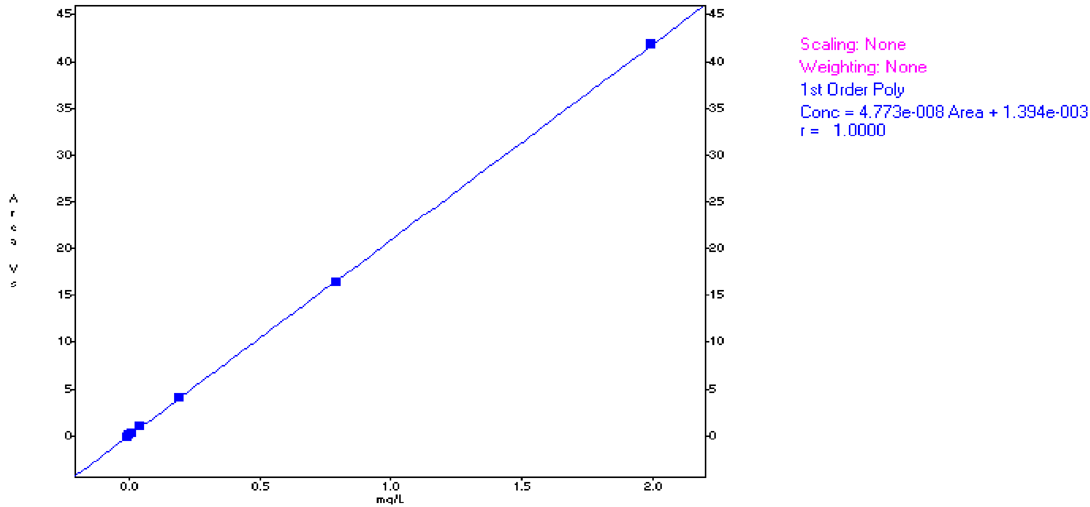
Calibration Data for Ammonia in DI Water

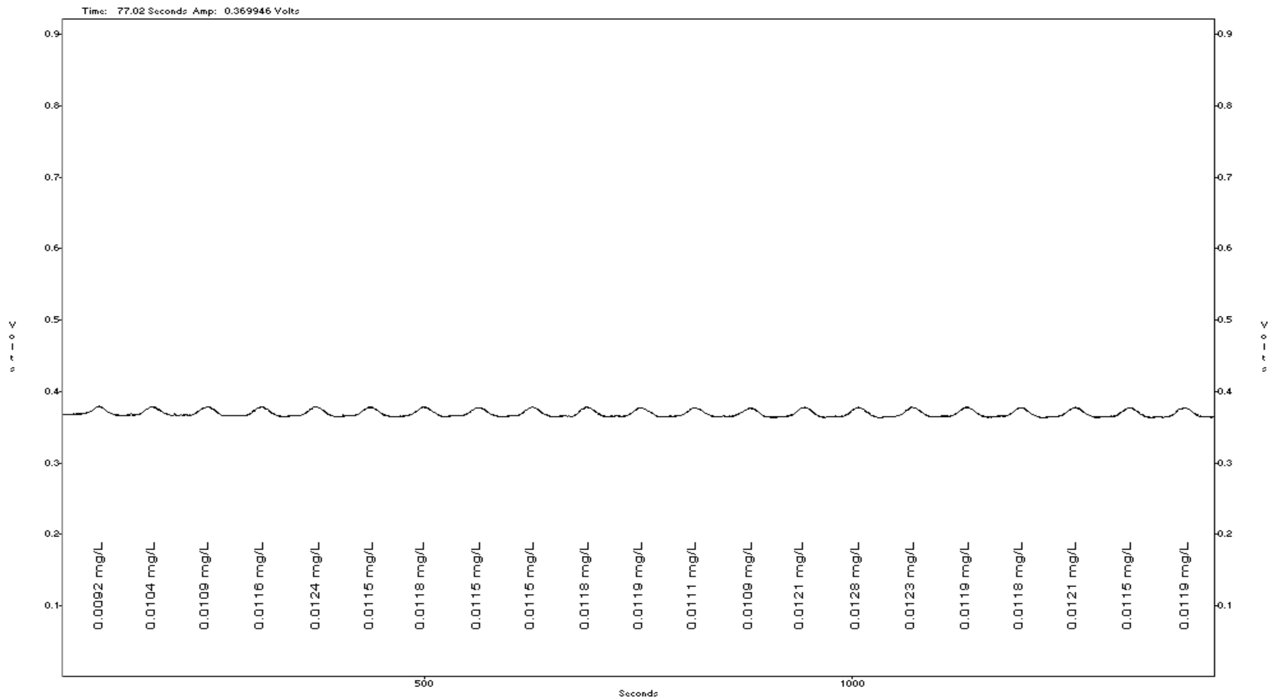


File Name: 990422c.fdt
Acq. Date: 22 April 1999

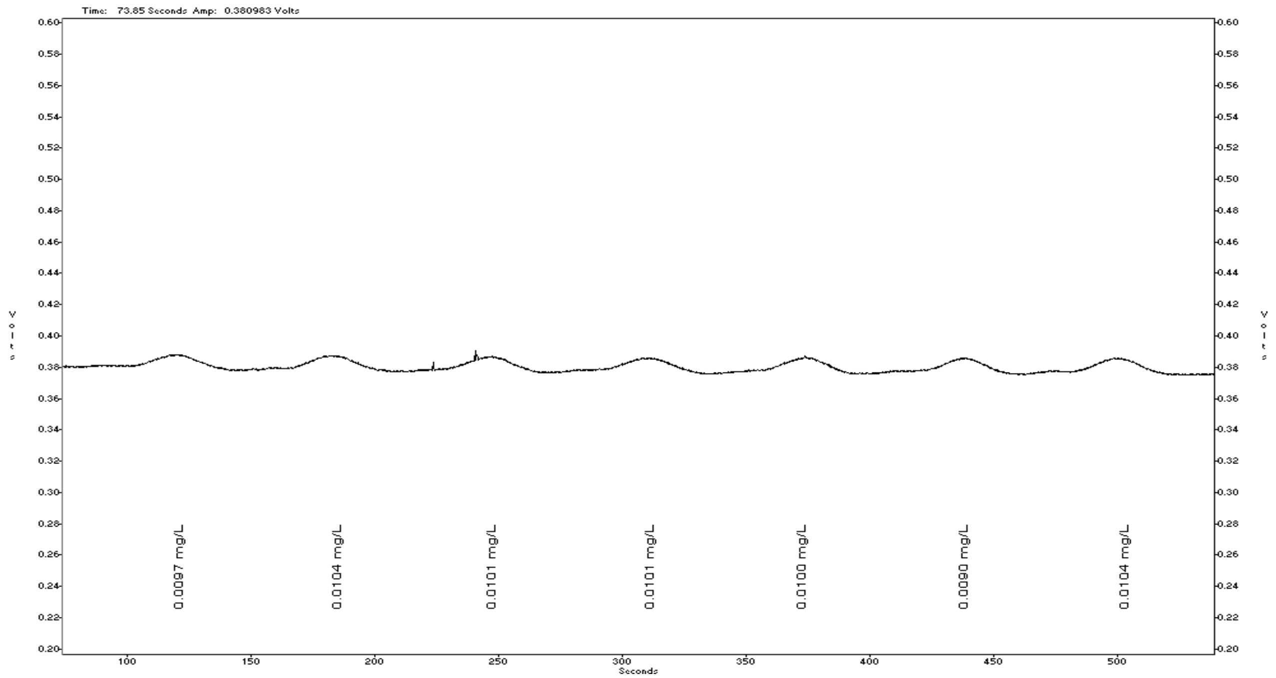
Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Replicate %RSD	% residual
1	41962784	2.00	2.00	0.1	-0.2
2	16506912	0.80	0.81	0.1	1.3
3	4158776	0.20	0.20	0.3	0.1
4	1096656	0.05	0.05	0.5	-7.5
5	415102	0.02	0.02	2.1	-6.0
6	189308	0.01	0.01	2.0	-4.3
7	0	0.00	---	0.0	---

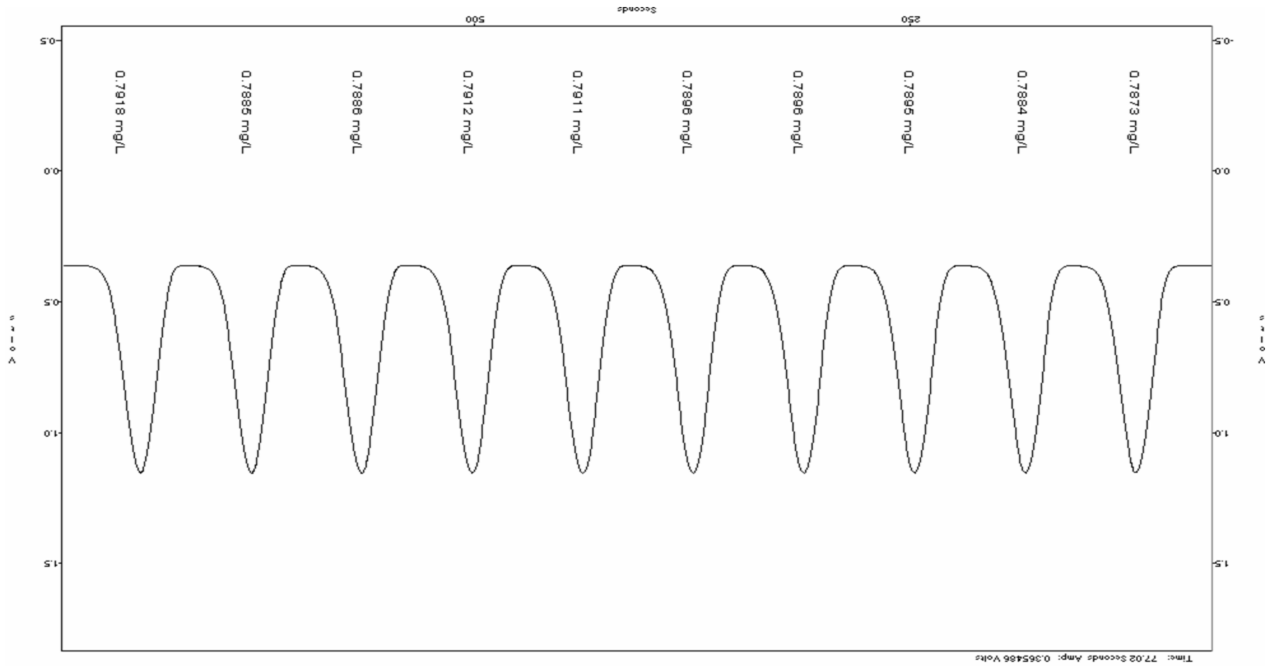




Method Detection Limit for ammonia using 0.01 mg N/L standard in DI water
MDL= 0.002 mg N/L
Standard Deviation (s) = 0.0008 mg N/L, Mean (x) = 0.012 mg N/L, Known value = 0.01 mg N/L
Acq. Date: 21 April 1999
File Name: 990421m1.fdt



Method Detection Limit for ammonia using 7 replicates of 0.01 mg N/L standard in DI water
MDL= 0.0015 mg N/L
Standard Deviation (s) = 0.0005 mg N/L, Mean (x) = 0.010 mg N/L, Known value = 0.01 mg N/L
Acq. Date: 21 April 1999
File Name: 990421m.fdt



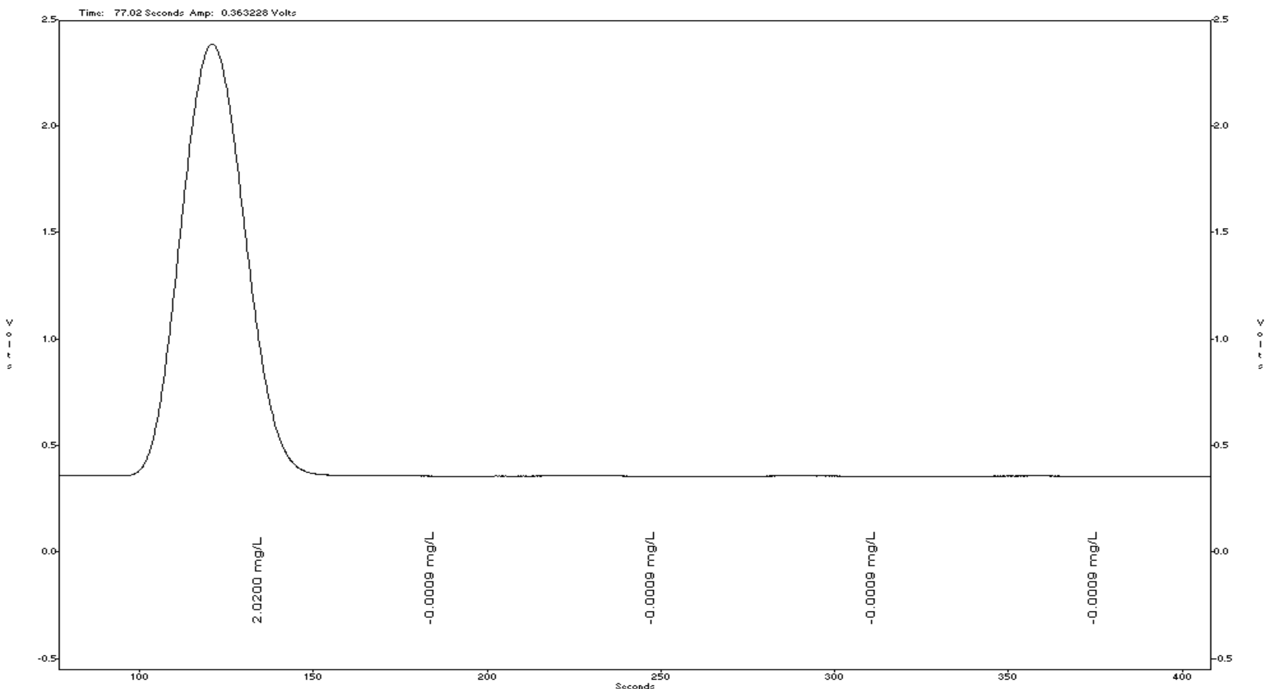
Precision data for ammonia using 0.8 mg N/L standard in DI water

% RSD = 0.18

Standard Deviation (s) = 0.0014 mg N/L, Mean (x) = 0.790 mg N/L, Known value = 0.8 mg N/L

Acq. Date: 21 April 1999

File Name: 990421p.fdt



Carryover Study: 2.0 mg N/L standard followed by 4 DI water blanks

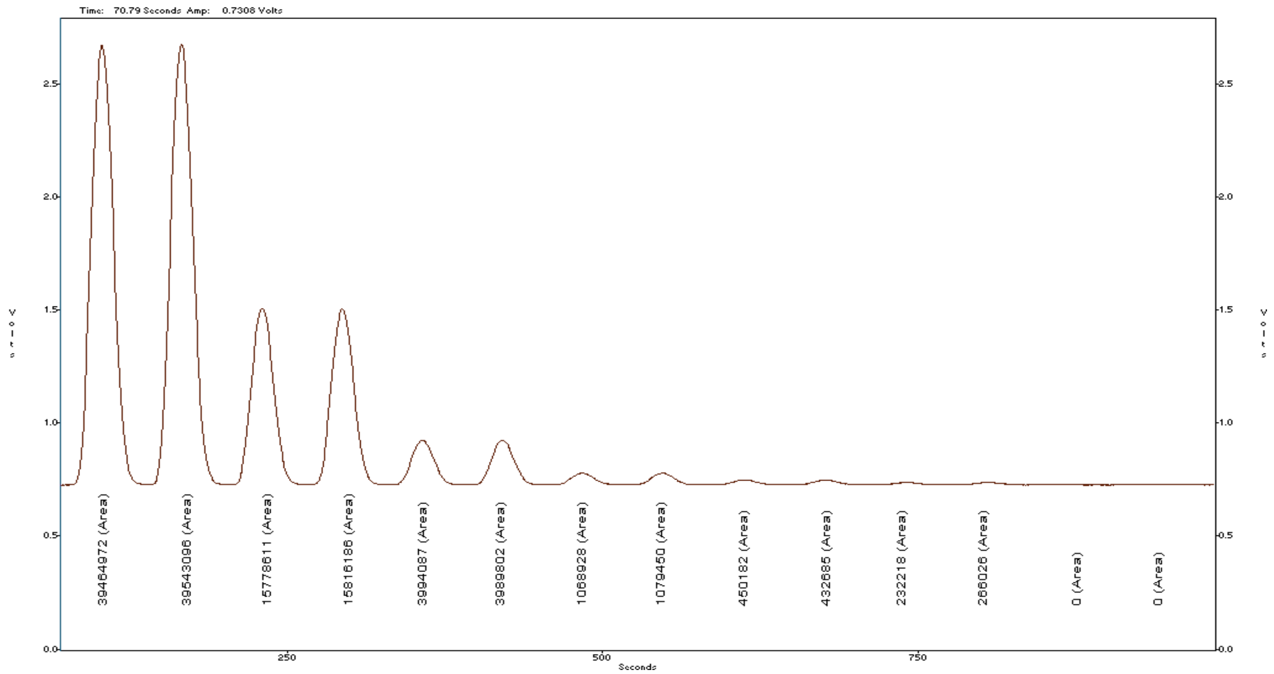
Carryover Passed

Acq. Date: 21 April 1999

File Name: 990421cr.fdt

17.3 SUPPORT DATA FOR QUIKCHEM 8000 IN 2 mL/L SULFURIC ACID

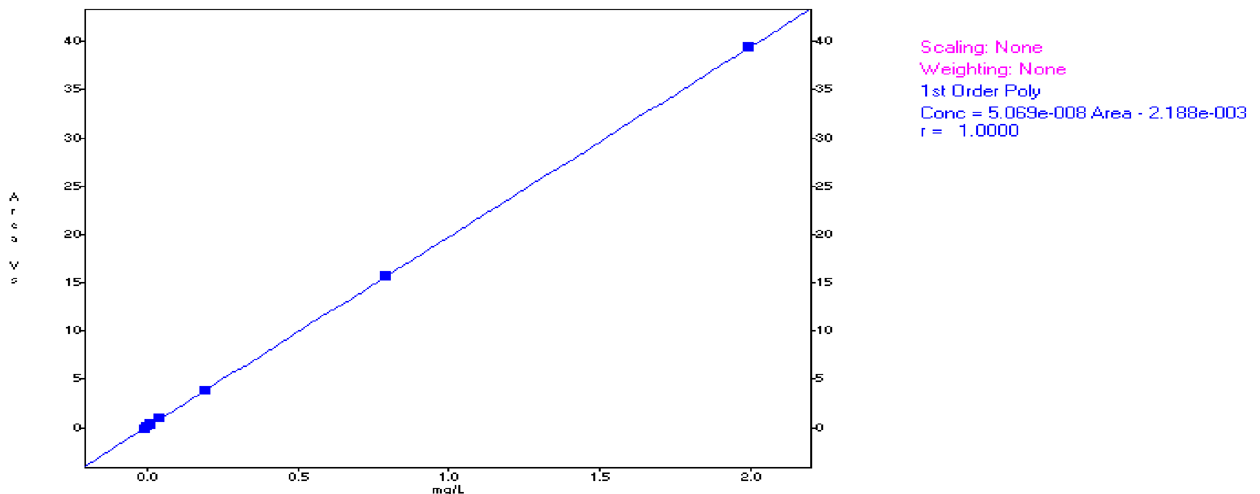
Calibration Data for Ammonia in 2 mL/L H_2SO_4

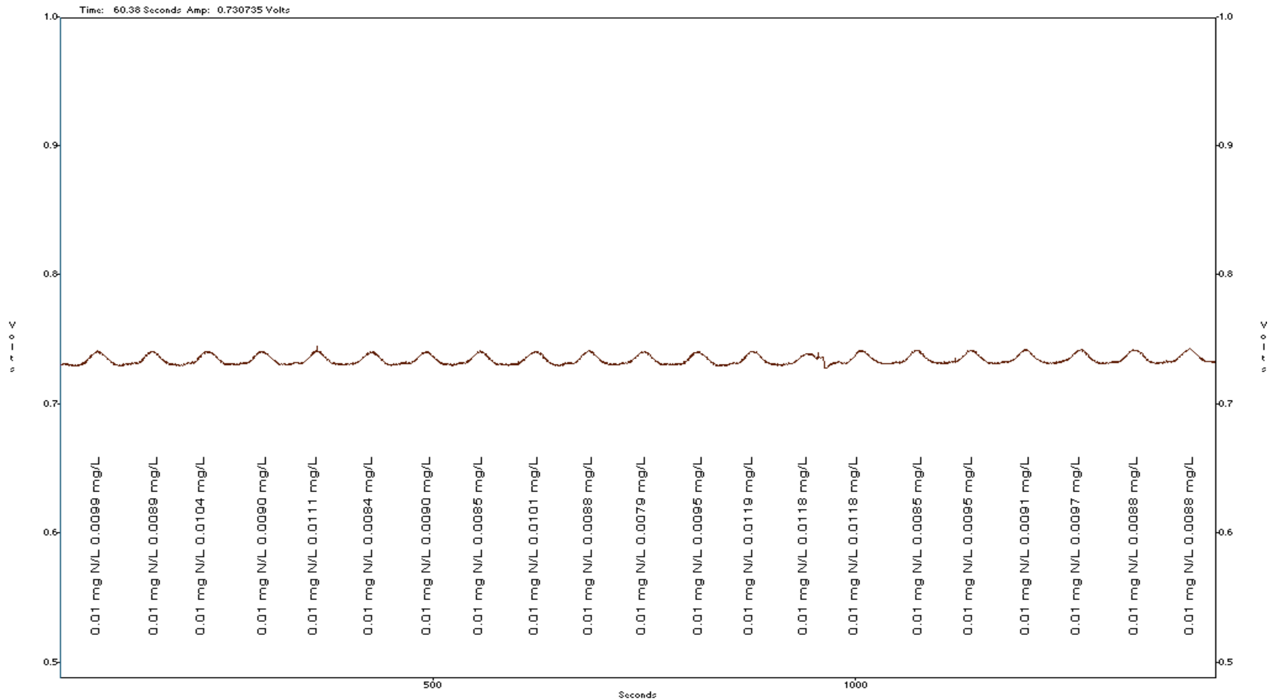


File Name: 062299c2.fdt
Acq. Date: 22 June 1999

Calibration Graph and Statistics

Level	Area	mg N/L	Determined	Replicate %RSD	% residual
1	39504032	2.00	2.00	0.1	-0.0
2	15797398	0.80	0.79	0.2	0.2
3	3991945	0.20	0.20	0.1	-0.1
4	1074189	0.05	0.05	0.7	-4.5
5	441434	0.02	0.02	2.8	-1.0
6	249122	0.01	0.01	9.6	-4.4
7	0	0.00	---	0.0	---

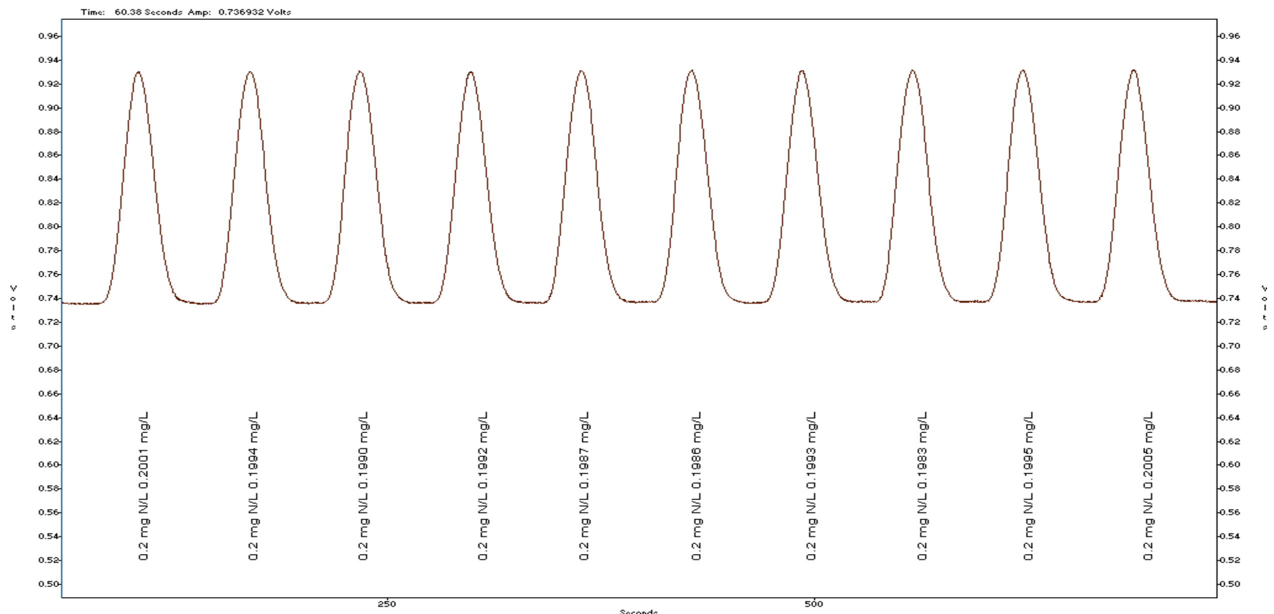




Method Detection Limit for ammonia using 0.01 mg N/L standard in 2mL/L H_2SO_4

MDL= 0.003 mg N/L

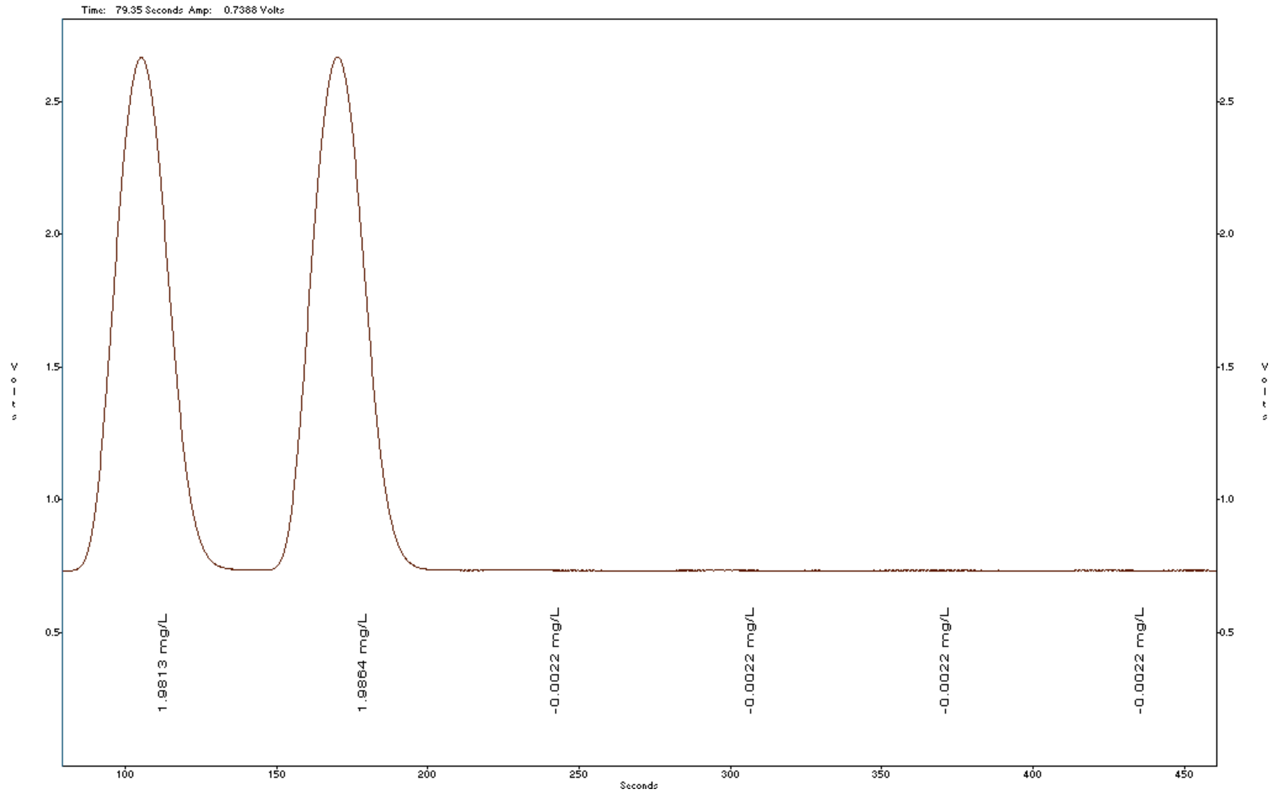
Standard Deviation (s) = 0.0012 mg N/L, Mean (x) = 0.01 mg N/L, Known value = 0.01 mg N/L
Acq. Date: 22 June 1999
File Name: 062299m1.fdt



Precision data for ammonia using 0.2 mg N/L standard in 2 mL/L H_2SO_4

% RSD = 0.33

Standard Deviation (s) = 0.0007 mg N/L, Mean (x) = 0.1993 mg N/L, Known value = 0.2 mg N/L
Acq. Date: 22 June 1999
File Name: 062299p1.fdt



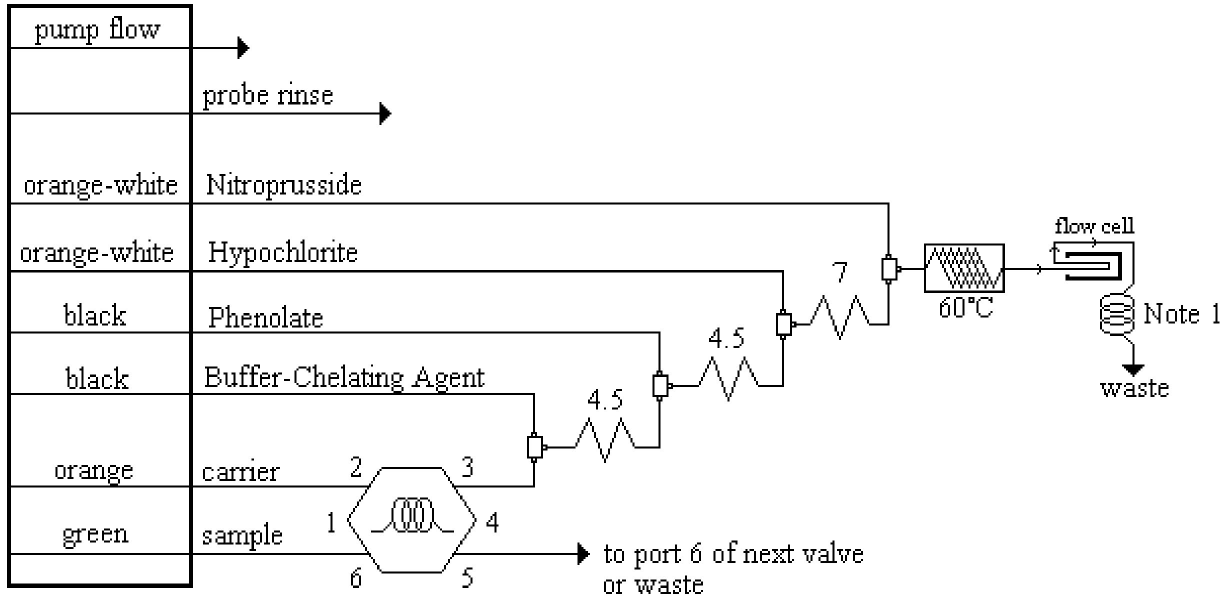
Carryover Study: 2.0 mg N/L standard followed by 4 blanks in 2 mL/L H_2SO_4

Carryover Passed

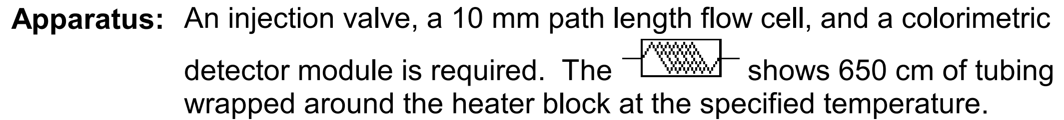
Acq. Date: 22 June 1999

File Name: 062299r1.fdt

17.4 AMMONIA MANIFOLD DIAGRAM



Carrier: **DI water**
Manifold Tubing: **0.5 mm (0.022 in) i.d.** This is **2.5 μL/cm**.
AE Sample Loop: 125 cm x 0.022 in. i.d.
QC8000 Sample Loop: 125 cm x 0.022 in. i.d.
Interference Filter: 630 nm

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

4.5: **70** cm of tubing on a 4.5 cm coil support
7: **135** cm of tubing on a 7 cm coil support
8: **168** cm of tubing on a 8 cm coil support
12: **255** cm of tubing on a 12 cm coil support
22: **550** cm of tubing on a 22 cm coil support

Note 1: 200 cm backpressure loop (0.022 in. i.d.)
Note 2: PVC PUMP TUBES MUST BE USED FOR THIS METHOD

BIOCHEMICAL OXYGEN DEMAND
Probe Method
(Water/Wastewater Matrices)

1.0 APPLICATION

This method is based on 5210(B) of the 21st Edition of Standard Methods for Examination of Water and Wastewater. It is used to determine the effect an effluent will have on the oxygen concentration of the receiving water.

2.0 SUMMARY

The method consists of filling with sample an airtight bottle of the specified size and incubating it at the specified temperature for 5 d. Dissolved oxygen is measured initially and after incubation, and the BOD is computed from the difference between initial and final DO. CBOD includes the addition of a nitrifying inhibitor (i.e. HACH 2533) or other TCM compound.

3.0 PRESERVATION

3.1 Collect samples in unpreserved bottle.

3.2 Refrigerate at $\leq$ 6 C until analysis.

3.3 Holding time - Analyze as soon as possible, but no longer than 24 hours after sample collection.

4.0 APPARATUS

4.1 Incubation bottles, 300-ml capacity. Clean bottles with a detergent or no-chromix rinse thoroughly, and drain before use. Obtain water seal and place a plastic cap over mouth of bottle to reduce evaporation during incubation.

4.2 Air incubator, thermostatically controlled at 20 $\pm$ 1°C.

4.3 Oxygen-sensitive membrane electrode with meter readable at $\pm$ 0.1 PPM O2.

4.4 Misc. Class A glassware: graduated cylinders, pipettes

5.0 REAGENTS

5.1 Make buffer water using commercially purchased BOD nitrification buffer pillows from HACH.

5.2 Sodium sulfite solution: Dissolve .1575 g NaSO3 in 100 ml distilled water. This solution is not stable; prepare daily.

5.3 Nitrification inhibitor, 2-chloro-6- (trichloromethyl) pyridine; Nitrification inhibitor 2579-24 (2.2 % TCMP), HACH Co., or equivalent.

5.4 Glucose-glutamic acid solution: Dry reagent grade glucose and reagent grade glutamic acid at 103°C for 1 h. Add 37.5 mg glucose and 37.5 mg glutamic acid to distilled water and dilute to 250 ml. Prepare fresh immediately before use unless solution is maintained in a sterile condition below 4ºC.

6.0 INTERFERENCES

6.1 Sample must be brought to 20°C before analysis.

6.2 Samples must be between pH 6 to 8.

6.3 Samples containing toxic substances (such as cyanide, chlorine, or heavy metals) interfere with the BOD analysis by killing the microorganisms. Using very small sample aliquot (0.1, 0.05 etc) may resolve this problem for some samples. Special study is required for these samples.

6.4 Inadequate shaking of sample may results in erratic results.

6.5 Samples containing residual chlorine must be dechlorinated using sodium sulfite solution.

7.0 QA/QC

7.1 Use a 2% dilution of the glucose-glutamic acid standard check solution for a standard. Glucose-glutamic acid check should have results in the range of 198 +/- 30.5 mg/L.

7.2 Always prepare a method blank using 300 mLs of dilution water. Two blanks are run each time a new container of nutrient water is prepared for a daily batch.

8.0 PROCEDURE

8.1 PREPARATION OF DILUTION WATER

8.1.1 Rinse carboy and fill with deionized water.

8.1.2 Add BOD nutrient buffer pillows to appropriate amount of DI water.

8.1.3 Make dilution water 1 day prior and place in incubator to stabilize temp.

8.1.4 Aerate water in container for at least 15 minutes. If DO is not between 7 and 9 mg/L after aeration, continue aerating until desired DO is obtained.

8.2 ANALYSIS

8.2.1 Calibrate D.O. meter according to manufacturer's instructions.

8.2.2 For routine samples refer to section 10.0 Helpful Hints for sample aliquot.

8.2.3 Vigorously shake sample bottle and measure out required volume. After putting the aliquot in the dilution bottle, fill the bottle to the neck with dilution water. Do not overflow bottle since some of the sample aliquot would be lost.

Note: If sample contains residual chlorine, add 0.5 mL of sodium sulfite solution prior to filling sample bottle with dilution water. If analyzing for carbonaceous BOD add 0.16 g on nitrification inhibitor to bottle. For samples that need seeding, add 2ml of primary effluent (clarifier). WLN clarifier effluent has negligible BOD present but introduces plenty of microorganisms to seed a sterile sample.

8.2.4 Using the DO meter, measure the initial DO of the blank, standards, and samples. Record the initial DO on the sample bench sheet along with sample description, sample name, and sample volume.

8.2.5 Add enough dilution water to form a water seal when stopper is placed in bottle. Make sure no air bubbles are in the bottle. Place a plastic cap over the stopper.

8.2.6 Incubate all bottles for 5 days in the dark at 20°C ± 1°C.

8.2.7 On the fifth day, ± 6 hours, take the final DO reading using the calibrated DO meter.

8.2.8 The depletion of the dilution water blank is used as a check on the quality of the dilution water. The depletion should not be more than 0.2 mg/L.

8.2.9 The samples must show a depletion of 2 mg/L and a residual of 1 mg/L in the final computation.

9.0 CALCULATIONS

9.1 Non-seeded BOD's

$$\text{BOD, mg/L} = \frac{(\text{IDO-FDO})}{\text{mL Sample}} \times 300$$

Where: IDO = Initial Dissolved Oxygen

FDO = Final Dissolved Oxygen

9.2 Seeded BOD's

$$\text{BOD, mg/L} = \frac{(\text{D1 - D2})}{\text{mL Sample}} \times 300$$

Where: D1 = DO depletion due to sample and seed

D2 = DO depletion due to seed

10.0 HELPFUL HINTS

10.1 Expected BOD ranges:

Expected BOD range	Sample volume, ml	Sample type
2-20 mg/L	75-300	Streams, storm water, Lakes, drinking water, Effluent
20-100 mg/L	15-75	Streams, storm water, Lakes, effluent
100-500 mg/L	3-15	Influent

10.2 If total suspended solids are ran on a unknown sample, the TSS value is a good starting point for BOD dilutions, because TSS is approximately equal to BOD.

10.3 Past experience and the above information will help in determining the proper sample aliquot to use.

QuikChem® Method 10-117-07-1-A

DETERMINATION OF CHLORIDE BY FLOW INJECTION ANALYSIS COLORIMETRY

(LOW FLOW METHOD)

Written by Dori Lewis

Applications Group

Revision Date:

7 September 2001

LACHAT INSTRUMENTS
5600 LINDBURGH DRIVE
LOVELAND, COLORADO 80539 USA
QuikChem® Method 10-117-07-1-A

Chloride in Waters

6.0 to 300.0 mg Cl/L

– Principle –

Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms the highly colored ferric thiocyanate, of which the absorbance is proportional to the chloride concentration. Ferric thiocyanate absorbs strongly at 480 nm. The calibration curve fits a second order polynomial.

– Interferences –

1. Substances which reduce iron (III) to iron (II) and mercury (III) to mercury (II) (e.g. sulfite, thiosulfate).
2. Halides which also form strong complexes with mercuric ion (e.g. Br^-, I^-) give a positive interference.

QuikChem® Method 10-117-07-1-A

DETERMINATION OF CHLORIDE BY FLOW INJECTION ANALYSIS COLORIMETRY

1.0 SCOPE AND APPLICATION

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

1.2 The applicable range is 6.0 to 300.0 mg Cl/L. The method detection limit is 0.15 mg Cl/L. The method throughput is 60 injections per hour.

2.0 SUMMARY OF METHOD

2.1 Thiocyanate ion is liberated from mercuric thiocyanate by the formation of soluble mercuric chloride. In the presence of ferric ion, free thiocyanate ion forms the highly colored ferric thiocyanate, of which the absorbance is proportional to the chloride concentration. Ferric thiocyanate absorbs strongly at 480 nm. The calibration curve fits a second order polynomial.

3.0 DEFINITIONS

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

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

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

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

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

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

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

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

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

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

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

4.0 INTERFERENCES

4.1 Substances which reduce iron (III) to iron (II) and mercury (III) to mercury (II) (e.g. sulfite, thiosulfate).

4.2 Halides which also form strong complexes with mercuric ion (e.g. Br^-, I^-) give a positive interference.
If any question of interferences arise, calibration curves should be prepared in water and in the suspected interfering matrix. If the two curves differ significantly, then there is interference, and the standards must be prepared in the interfering matrix instead of in water.

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.

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

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

5.3.1 Mercuric thiocyanate solution

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 pipettes or plastic containers as required. Samples may be stored in plastic or glass.

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

6.3.1 Sampler
6.3.2 Multichannel proportioning pump
6.3.3 Reaction unit or manifold
6.3.4 Colorimetric detector
6.3.5 Data system
6.3.6 10 mm pathlength, 80 µL, glass flow cell
6.3.7 480 nm interference filter

7.0 REAGENTS AND STANDARDS

7.1 PREPARATION OF REAGENTS

Use ASTM Type II water for all solutions. (See Standard Specification for Reagent Water D1193-77 for more information).

Reagent 1. Combined Color Reagent

This reagent may be purchased from GFS Chemicals, Powell, OH, USA (Chloride Color Solution Mercuric Thiocyanate 0.06% Solution), Cat. No. 50041, Item # 1991. Alternatively, it can be prepared as described below:

A. Stock Mercuric Thiocyanate Solution

In a **1 L** volumetric flask, dissolve **4.17 g mercuric thiocyanate** ($Hg(SCN)_2$) in about **500 mL methanol**. Dilute to the mark with **methanol** and invert to mix.

CAUTION: Mercuric thiocyanate is toxic. Wear gloves!

B. Stock Ferric Nitrate Reagent, 0.5 M

In a **1 L** volumetric flask, dissolve **202 g ferric nitrate** ($Fe(NO_3)_3 \cdot 9H_2O$) in approximately **800 mL water**. Add **25 mL conc. nitric acid** and dilute to the mark. Invert to mix.

C. Combined Color Reagent

In a **500 mL** volumetric flask, mix **75 mL stock Mercuric Thiocyanate Solution** with **75 mL Stock Ferric Nitrate Reagent** and dilute to the mark with water. Invert to mix. Vacuum filter through a 0.45 micrometer membrane filter.

7.2. PREPARATION OF STANDARDS

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

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

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

Standard 1. Stock Standard 1000 mg Cl/L

By Volume: In a 105° C oven, dry **3 g primary standard grade sodium chloride** (NaCl) overnight. In a **1 L** volumetric flask, dissolve **1.648 g primary grade sodium chloride** in about **500 mL water**. Dilute to the mark and invert to mix.

Standard 2. Working High Standard, 300 mg Cl/L

By Volume: To a **1 L** volumetric flask, add **300 mL** of **Standard 1**, above. Dilute to the mark with **DI water** and invert to mix.

Standard 3. Working Middle Standard, 100 mg/L

By Volume: To the **1 L** volumetric flask, add **100 mL** of **Standard 1**. Dilute to the mark with **DI water** and invert to mix.

Working Standards (Prepare Daily) Concentration mg Cl/L	**A** 300	**B** 225	**C** 150	**D** 60	**E** 30	**F** 20	**G** 10	**H** 6	**I** 0
By Volume									
Volume (mL) of **standard 2** diluted to 250 mL with **DI water**	250	187.5	125	50	---	---	---	---	---
Volume (mL) of **standard 3** diluted to 250 mL with **DI water**	---	---	---	---	75	50	25	15	---
By Weight									
Weight (g) of **standard 2** diluted to final weight (~250 g) divide by **factor** below with **DI water**.	250	187.5	125	50	---	---	---	---	---
Weight (g) of **standard 3** diluted to final weight (~250 g) divide by **factor** below with **DI water**.	---	---	---	---	75	50	25	15	---
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.0	0.75	0.5	0.2	0.3	0.2	0.1	0.06	---

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Collect samples in polyethylene bottles. Chemical preservation is not required. The maximum holding time is 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.

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

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

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

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

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

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

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

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

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

9.3 Matrix spikes - The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

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

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

9.3.2 Analyze one sample aliquot out of each set of ten samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4.1 Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

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

9.5 **Calibration Verification** - Verify calibration using the procedure described in Section 10

9.6 **On-going Precision and Recovery (OPR)** - With every analytical batch, a midrange standard must be prepared using the procedure described in Section 11.

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

9.7 **Quality Control Samples (QCS)** It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 7. The QCS is used to verify the concentrations of the calibration standards.

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

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare reagents and standards as described in Section 7.

10.2 Set up manifold as shown in Section 17.

10.3 Input data system parameters as shown in Section 17.

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

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

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

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

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

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

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

11.0 PROCEDURE

11.1 CALIBRATION PROCEDURE

11.1.1 Prepare reagent and standards as described in section 5.

11.1.2 Set up manifold as shown in section 11.

11.1.3 Input data system parameters as shown in section 11.

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

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

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

11.2 SYSTEM NOTES

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

11.2.2 Filtration of the color reagent has been found to decrease baseline noise.

11.2.3 QC 8000 users may improve accuracy by choosing a 1/X fit.

12.0 DATA ANALYSIS AND CALCULATIONS

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

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

12.3 Report results in mg Cl/L.

13.0 METHOD PERFORMANCE

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

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

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

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

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

15.0 WASTE MANAGEMENT

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

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

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 325.2.

16.2 Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

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

17.0 TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

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

Sample throughput: 60 samples/h, 60 s/sample
Pump Speed: 35
Cycle Period: 60

Analyte Data:

Concentration Units: mg Cl/L
Peak Base Width: 40 s
% Width Tolerance: 100
Threshold: 6000
Inject to Peak Start: 13 s
Chemistry: Direct

Calibration Data:

Level	1	2	3	4	5	6	7	8	9
Concentration mg Cl/L	300	225	150	60	30	20	10	6	0

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

Sampler Timing:

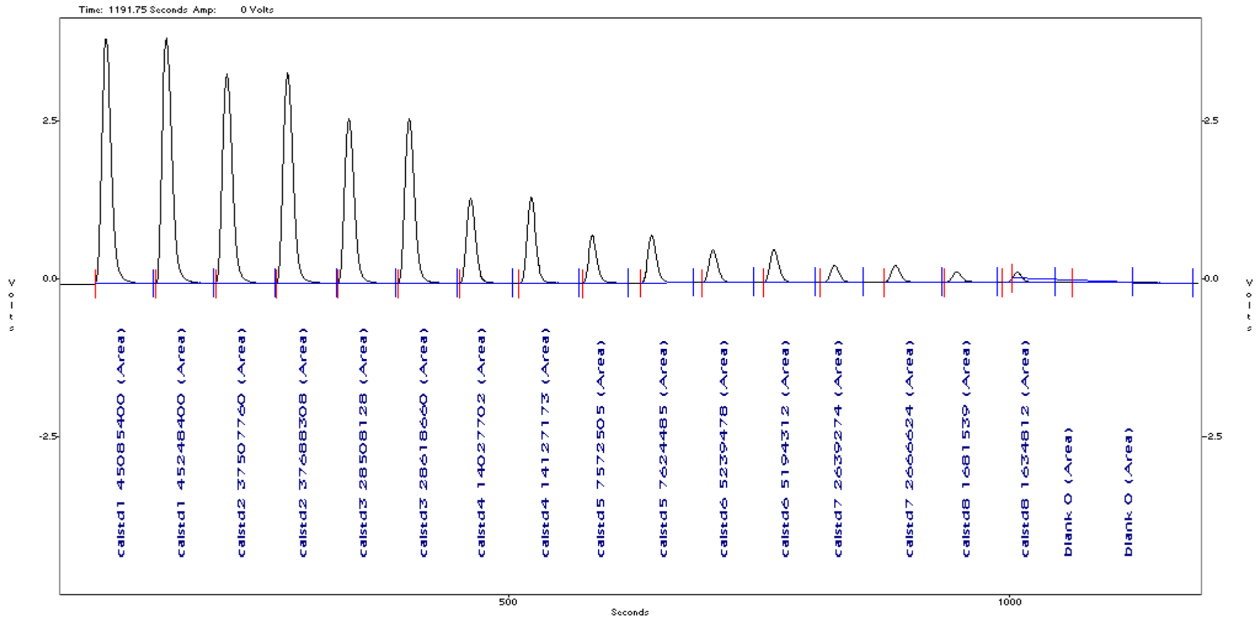
Min. Probe in Wash Period: 9 s
Probe in Sample Period: 15 s

Valve Timing:

Load Time: 0 s
Load Period: 10 s
Inject Period: 50 s

17.2 SUPPORT DATA FOR QUIKCHEM 8000

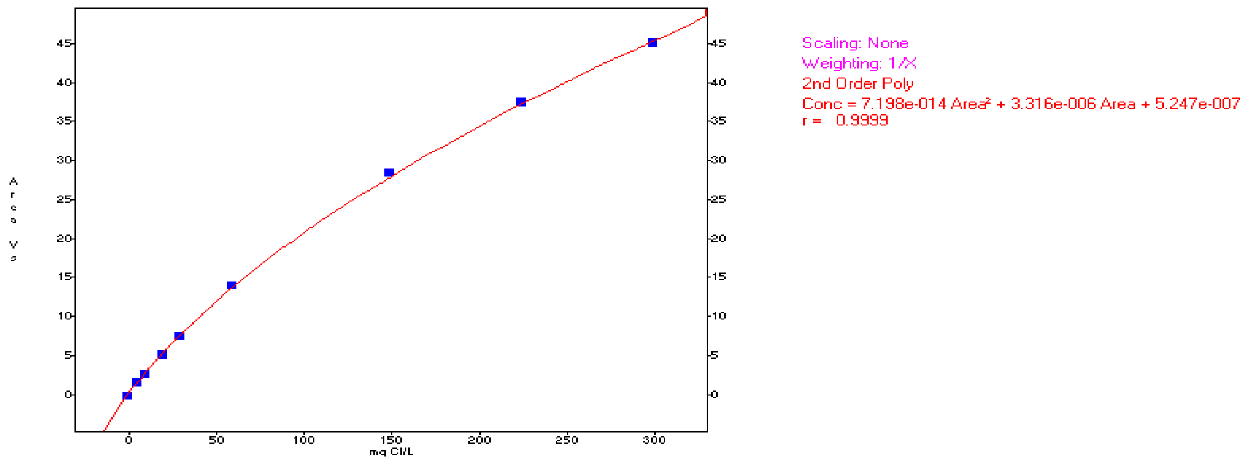
Calibration Data for Chloride

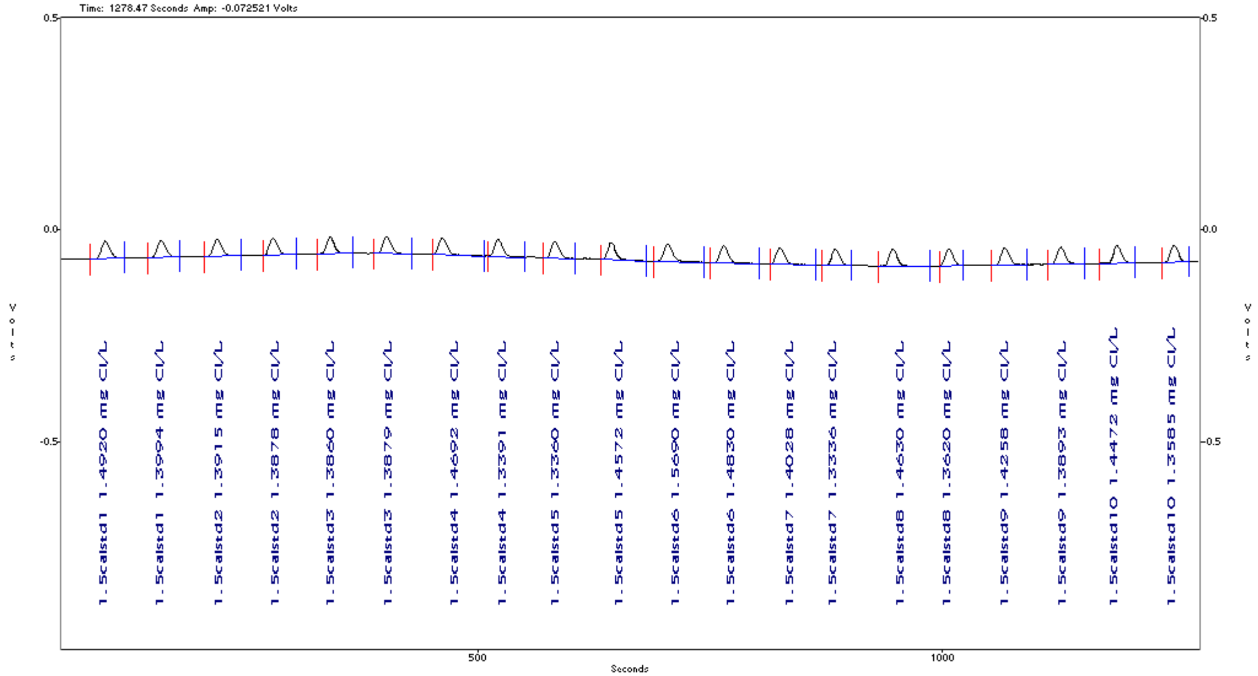


File Name: 1Clcal21.fdt
Acq. Date: 21 August 2001

Calibration Graph and Statistics

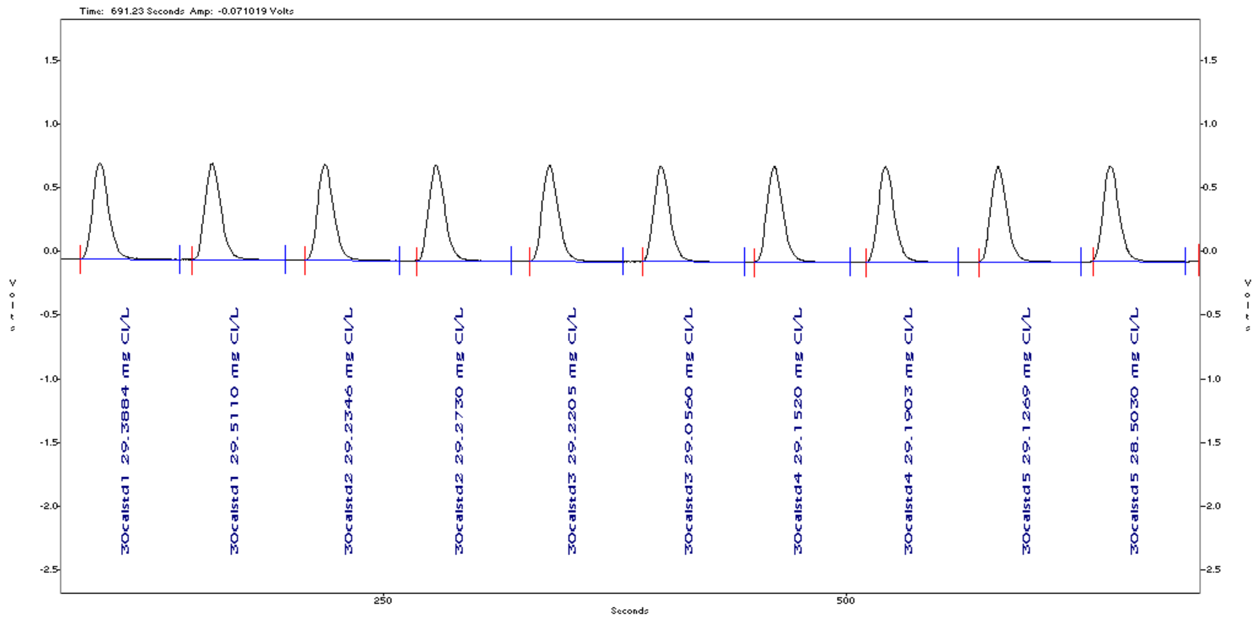
Level	Area	mg Cl/L	Determined	Replicate %RSD	% residual
1	45166900	300	296.7	0.3	1.1
2	37598032	225	226.4	0.3	-0.6
3	28563394	150	153.5	0.3	-2.3
4	14077438	60	60.96	0.5	-1.6
5	7598495	30	29.3	0.5	2.2
6	5216895	20	19.3	0.6	3.7
7	2652949	10	9.3	0.7	7.0
8	1658176	6	5.7	2.0	5.1
9	0	0	---	0.0	---





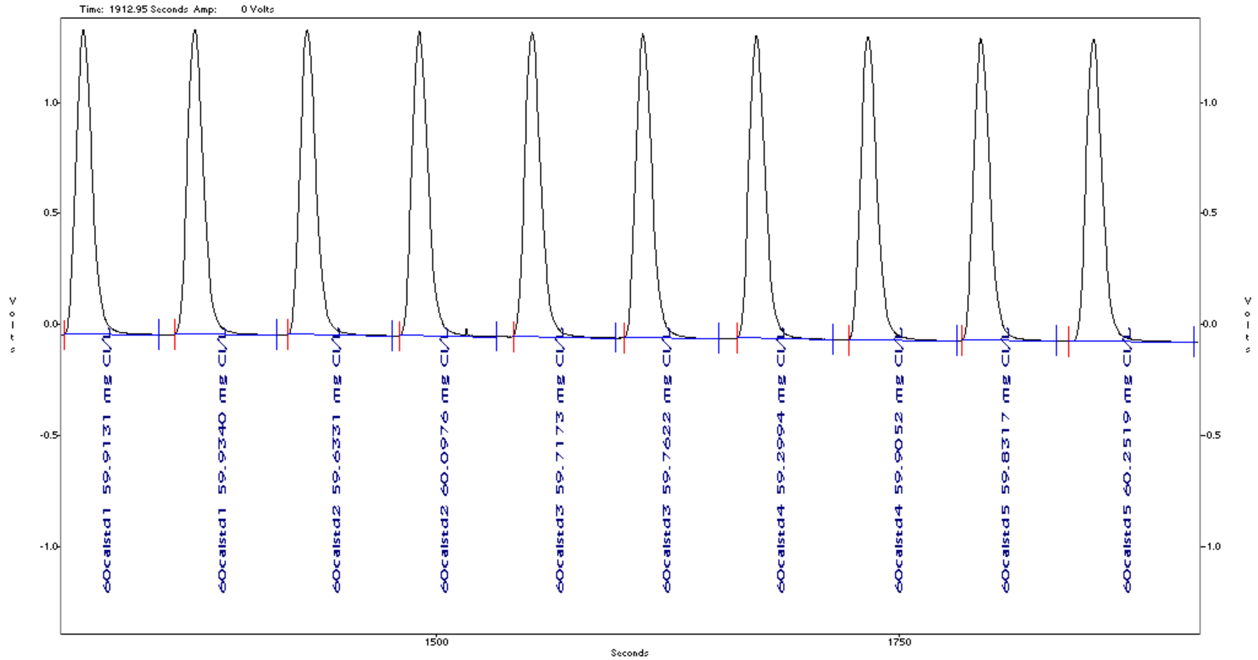
Method Detection Limit for chloride using 1.5 mg Cl/L standard

MDL= 0.15 mg Cl/L
Standard Deviation (s) = 0.05897 mg Cl/L, Mean (x) = 1.411 mg Cl/L, Known value = 1.5 mg Cl/L
File Name: 1mdl21.fdt
Acq. Date: 21 August 2001



Precision data for chloride using 30 mg Cl/L standard

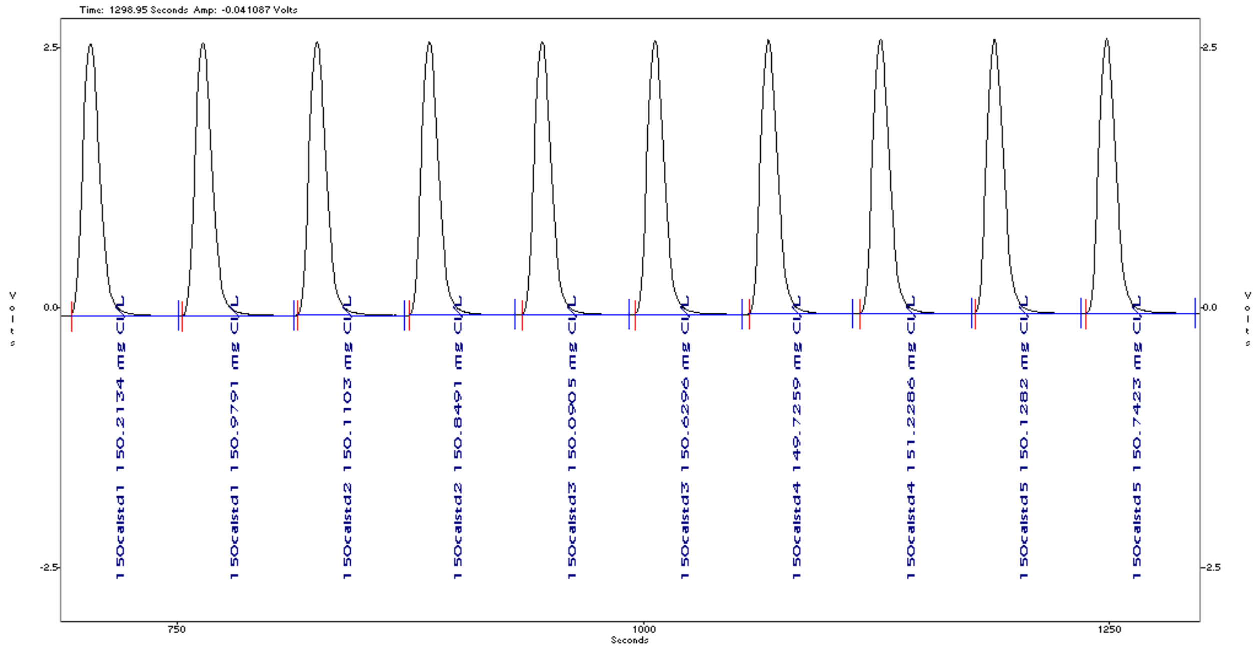
% RSD = 0.915
Standard Deviation (s) = 0.267 mg Cl/L, Mean (x) = 29.166 mg Cl/L, Known value = 30 mg Cl/L
File Name: Clprec21.fdt
Acq. Date: 21 August 2001



Precision data for chloride using 60 mg Cl/L standard

% RSD = 0.435

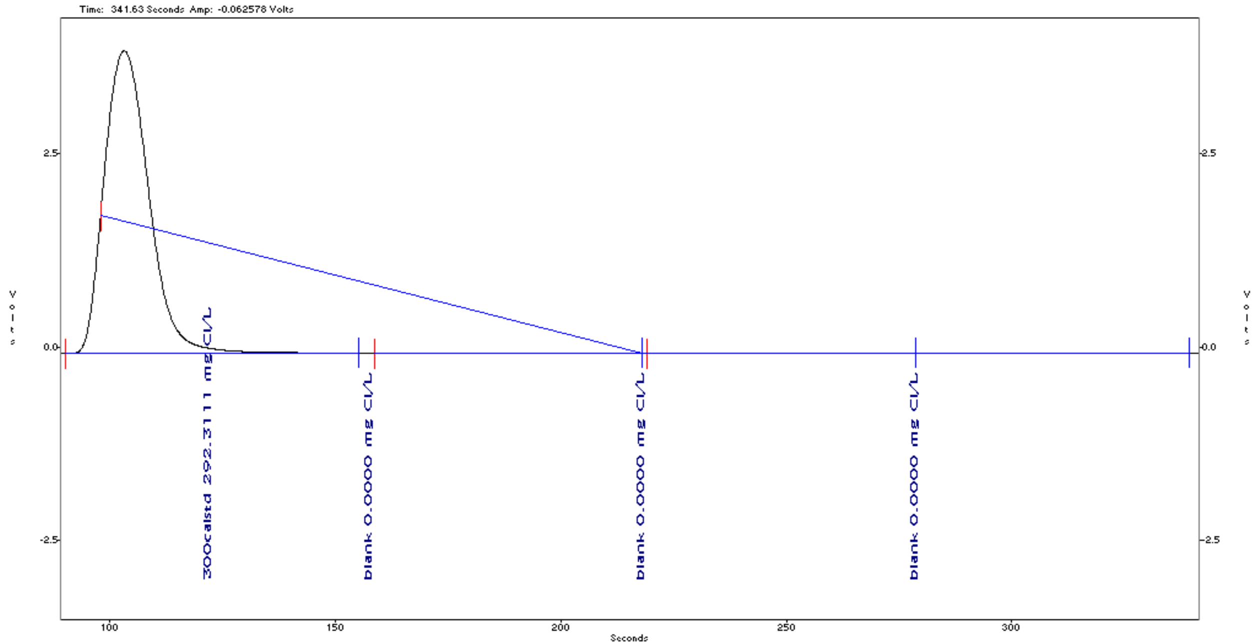
Standard Deviation (s) = 0.2604 mg Cl/L, Mean (x) = 59.835 mg Cl/L, Known value = 60 mg Cl/L
File Name: Clprec21.fdt
Acq. Date: 21 August 2001



Precision data for chloride using 150 mg Cl/L standard

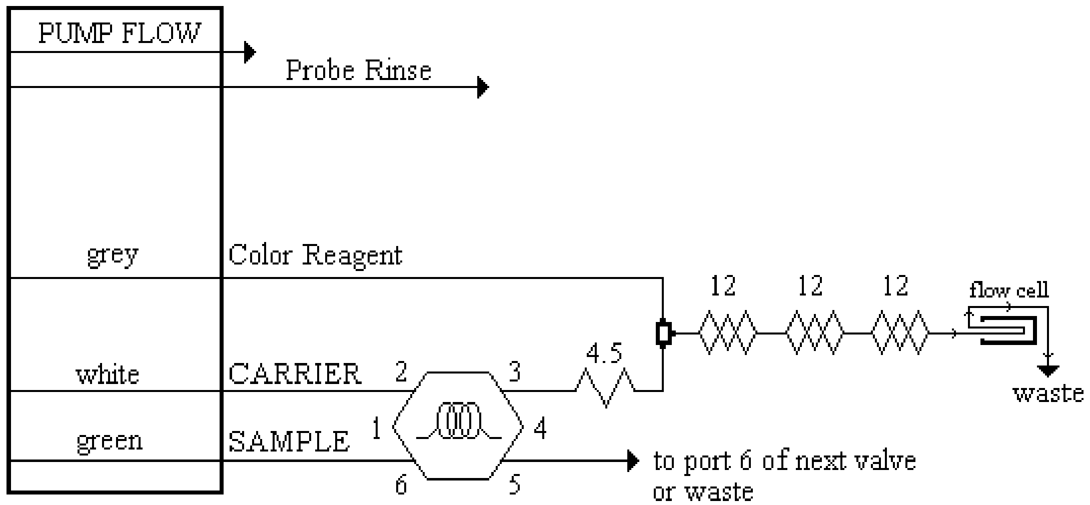
% RSD = 0.3200

Standard Deviation (s) = 0.48165 mg Cl/L, Mean (x) = 150.4697 mg Cl/L, Known value = 150 mg Cl/L
File Name: Clprec21.fdt
Acq. Date: 21 August 2001



Carryover Study: 300 mg Cl/L standard followed by 3 blanks
Carryover Passed
File Name: cryover21.fdt
Acq. Date: 21 August 2001

17.3 CHLORIDE MANIFOLD DIAGRAM



Carrier: **DI water**
Manifold Tubing: **0.5 mm (0.022 in) i.d.** This is **2.5 µL/cm**.
AE Sample Loop: 8.5 cm
QC8000 Sample Loop: 13 cm x 0.5 mm i.d. (0.022 in) i.d.
Interference Filter: 480 nm

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

4.5: **70** cm of tubing on a 7 cm coil support
12: **255** cm of tubing on a 12 cm alternating coil support

FECAL COLIFORM
Membrane Filter Technique
(Water/Wastewater Matrices)

1.0 APPLICATION

This method is based on 9222(D) of the 21st Edition of Standard Methods for Examination of Water and Wastewater and the DNR Laboratory Training Manual for Wastewater Treatment Plant Operators.

2.0 SUMMARY

A known volume of sample is filtered through a sterile 0.45 μm filter membrane. Microorganisms are trapped on the filter. The filter membrane is placed on an appropriate culture medium in a Petri dish and incubated in a water bath at 44.5°C for 24 (+/-2) hours. The colonies, which appear as various shades of blue, are then counted. Fecal coliforms are reported as # of colonies/100 mL of sample.

3.0 PRESERVATION

3.1 Start microbiological examination of a water sample promptly after collection to avoid unpredictable changes. If samples cannot be processed within 1 hr after collection, use an iced cooler for storage during transport to the laboratory.

3.2 Hold samples below 10°C during a maximum transport time of 6 hrs. Refrigerate these samples upon receipt in the laboratory and process within 2 h.

3.3 Collect samples in sterile 100-ml plastic or glass bottles. Bottles may contain sodium thiosulfate prior to sample delivery.

4.0 APPARATUS

4.1 **Sample bottles** - Plastic or glass, at least 100-ml capacity with large mouth opening.

4.2 **Pipettes** - Class A: Range from 0.1 ml to 10 ml with disposable tips.

4.3 **Graduated cylinders** - Class A: 25 ml to 100 ml

4.4 **Plastic Petri dishes** - Pre-sterilized containing pad.

4.5 **Membrane filters** - Pre-sterilized 0.45 micron filters; 47 mm diameter; gridded.

4.6 **Forceps** - Round tipped without corrugations on the inner side of the tips.

4.7 **Membrane filter holder and funnel** - Constructed of plastic, glass or stainless steel; connected to filtering flask.

4.8 **Water bath incubator** - Must provide a temperature of 44.5°C ± 0.2°C.

4.9 **Plastic bags** - Pre-sterilized

4.10 **Impulse sealer**

4.11 Drying oven set at 170°C or **autoclave**

5.0 REAGENTS

5.1 MF-C broth – Place 3.7 g of dehydrated broth into a beaker. Add 100 mL of DI water and 1 mL of 1% rosolic acid solution into beaker and mix. Heat solution to near boiling, promptly remove from heat, and cool to below 50 degrees C. The media should be stored in a refrigerator and must be discarded after 96 hours.

5.2 Rosolic Acid Solution, 1% - Weigh 0.5 g of dehydrated rosolic acid and place in vial. Add 50 mL of 0.2 N sodium hydroxide solution; swirl to mix. Do not sterilize this solution. Discard if color changes from dark red to muddy brown.

5.3 Sodium hydroxide solution, 0.2N – Dissolved 2 g of sodium hydroxide into 250 mL of DI water volumetrically.

5.4 Sterile buffered dilution water - Add 1.25 ml of stock phosphate buffer solution and 5mL of magnesium chloride solution to 1 liter of DI water. This solution should be dispensed into milk dilution bottles and autoclaved before use.

5.5 Stock phosphate buffer solution - Dissolve 34.0 g of potassium dihydrogen phosphate in 500 ml of DI water. By using a pH meter, adjust pH to 7.2 with 1N sodium hydroxide solution. Dilute to 1 liter.

5.6 Magnesium Chloride solution – Dissolve 81.1 g of magnesium chloride hexahydrate into DI water and dilute to 1 liter.

5.7 Sodium hydroxide solution, 1N - Dissolve 40 g of sodium hydroxide in 500 ml of DI water. Dilute to 1 liter.

5.8 10% Sodium thiosulfate solution - Dissolve 100 g of sodium thiosulfate in 1 liter of DI water.

6.0 INTERFERENCES

6.1 If growth is apparent on the Petri dish, but the colonies are not blue-(i.e. cream or gray-green) record as non-specific growth.

6.2 High solids will interfere with colony growth. Use a smaller aliquot.

6.3 If chlorine is present in sample, the microorganisms will be destroyed. To eliminate this interference, use sodium thiosulfate treated bottles when sampling.

6.4 This analysis is extremely temperature dependent. The water bath should be maintained at a constant temperature of 44.5°C ± 0.2°C.

7.0 QA/QC

7.1 Monitor refrigerator and water bath temperature daily. Record and initial in logbook.

7.2 Method blank - Run dilution water (section 5.2) once each sample batch.

7.3 Replicate samples - Frequency of 1 in 20 samples.

7.4 Analysis of known positive sample (i.e. influent sewer sample) - Frequency of once per month.

7.5 Positive confirmation test - Frequency of once per month.

7.6 Random analysis of sample containers - Frequency of 1 container per month.

8.0 PROCEDURE

8.1 WASHING AND STERILIZATION

8.1.1 Cleanse all glassware thoroughly with a suitable detergent and hot water, rinse with hot water to remove all traces of residual washing compound, and finally rinse with laboratory-pure water. If mechanical glassware washers are used, use stainless steel or other nontoxic material for the rinse water system.

8.1.2 Sterilize all glassware for not less than 60 min in a dry oven at a temperature of 170°C. Alternatively, use an autoclave at 121°C for 15 min to sterilize glassware. The use of a disinfectant, such as Zepynamic A, with thorough rinsing with DI water is also acceptable.

8.2 ANALYSIS

Before testing starts have all necessary materials at hand. Arrange in such a pattern as to minimize lost time in useless motions.

NOTE: If samples are suspected of containing chlorine, dechlorinate sample bottles with 0.1 ml of 10% sodium thiosulfate solution before sample collection..

8.2.1 Prepare Petri dishes by labeling them with the sample name, date, amount filtered, and time. After labeling, pipette 2 mL of MF-C broth onto each pad. For sample dilutions, refer to bench sheets.

8.2.2 Connect vacuum tubing to filtering flask. Using forceps, place a membrane filter (grid side up) on base of filter holder assembly. Handle filters on the edges only.

8.2.3 For dilutions less than 20 mL, pour at least 20 mL of the sterile dilution water into the filter funnel. Vigorously shake the sample (about 25 times) and using the appropriate pipette, add the sample to the filter funnel. For dilutions greater than 20 ml, use a graduated cylinder. Before adding sample, be sure the control valves on the filter assembly are closed.

8.2.4 Turn on vacuum, open control valves, and filter the sample.

8.2.5 After the entire sample has passed through the membrane filter, rinse down the sides of the funnel walls with dilution water. Repeat the rinse two more times.

8.2.6 Close the control valve. Carefully remove the membrane filter and place the filter on the absorbent pad in the appropriate Petri dish. Be sure that no air bubbles have been trapped between the membrane filter and the pad.

Repeat for all samples.

8.2.7 Place the Petri dishes into a watertight plastic bag. Seal bag using impulse sealer.

8.2.8 Submerge in water bath in an inverted position. Incubate for 24 $\pm$ 2 hrs. Anchor bags below water surface during incubation.

8.2.9 After 24-hr. Incubation period, remove bag (s) from water bath.

8.2.10 Typical fecal coliform colonies are blue in color.

8.2.11 Count colonies and record value as per Section 9.0 Calculations.

9.0 CALCULATIONS

9.1 Coliform density is reported in terms of fecal coliforms per 100 ml.

9.2 Total fecal coliform/100 ml = $\frac{\text{coliform colonies counted X 100}}{\text{mL sample filtered}}$

9.3 Report Fecal coliform numbers using “Counting and Reporting Bacterial Colonies Membrane Filtration Methods” procedure provided by the MDEQ in the 2010 Laboratory Training Manual pp. 233-1 to 233-4.

10.0 CLEAN-UP

10.1 Dispose of used Petri dishes in waste container.

10.2 Wash filtering equipment and sterilize as per section 8.1 Washing and Sterilization.

QuikChem® Method 10-107-04-1-J

DETERMINATION OF NITRATE/NITRITE IN SURFACE AND WASTEWATERS BY FLOW INJECTION ANALYSIS

(LOW FLOW METHOD)

Written by David Diamond

Applications Group

Revision Date:

27 March 2001

LACHAT INSTRUMENTS
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QuikChem® Method 10-107-04-1-J

Nitrate/Nitrite in Waters

0.10 to 10.0 mg N/L as NO_3^- or NO_2^-

– Principle –

Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone also can be determined by removing the cadmium column.

– Interferences –

1. Residual chlorine can interfere by oxidizing the cadmium column.

2. Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce thisinterference.

3. 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. Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45 μM pore diameter membrane filter prior to analysis.

QuikChem® Method 10-107-04-1-J

DETERMINATION OF NITRATE/NITRITE IN SURFACE AND WASTEWATERS BY FLOW INJECTION ANALYSIS

1.0 SCOPE AND APPLICATION

1.1 This purpose of this method is to determine nitrate/nitrite in surface waters, and industrial wastewaters.

1.2 The applicable range is 0.10 to 10.0 mg N/L. The statistically determined detection limit as determined in water is 0.012 mg N/L. The method throughput is 90 injections per hour.

1.3 Each laboratory that uses this method must demonstration the ability to generate acceptable results using the procedure in Section 9.

2.0 SUMMARY OF METHOD

2.1 Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus original nitrite) is then determined by diazotizing with sulfanilamide followed by coupling with N-(1-naphthyl)ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm. Nitrite alone also can be determined by removing the cadmium column.

3.0 DEFINITIONS

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

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

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

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

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

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

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

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

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

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

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

4.0 INTERFERENCES

4.1 Residual chlorine can interfere by oxidizing the cadmium column.

4.2 Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this interference.

4.3 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.

4.4 Sample turbidity may interfere. Turbidity can be removed by filtration through a 0.45 μM pore diameter membrane filter prior to analysis.

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.

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

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

5.3.1 Cadmium
5.3.2 Ammonium Hydroxide
5.3.3 Sodium Hydroxide
5.3.4 Phosphoric Acid
5.3.5 Sulfanilamide

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 pipettes or plastic containers as required. Samples may be stored in plastic or glass.

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

6.3.1 Sampler
6.3.2 Multichannel proportioning pump
6.3.3 Reaction unit or manifold
6.3.4 Colorimetric detector
6.3.5 Data system

7.0 REAGENTS AND STANDARDS

7.1 PREPARATION OF REAGENTS

Use ASTM Type I water for all solutions. (See Standard Specification for Reagent Water D1193-77 for more information).

Degassing with helium:

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

Reagent 1. 15 N Sodium Hydroxide

By Volume: Add **150 g NaOH** very slowly to **250 mL or g of water**.

CAUTION: The solution will get very hot! Swirl until dissolved. Cool and store in a plastic bottle.

Reagent 2. Ammonium Chloride buffer, pH 8.5

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

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

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

By Volume: CAUTION: Fumes!!! In a hood, to a **1 L** volumetric flask add **500 mL water, 105 mL concentrated hydrochloric acid** (HCl), **95 mL ammonium hydroxide** (NH_4OH), and **1.0 g disodium EDTA.** Dissolve and dilute to the mark. Invert to mix. Adjust the pH to 8.5 with **HCl** or **15 N NaOH solution.**

By Weight: CAUTION: Fumes!!! In a hood, to a tared **1 L** container add **800 g water**, **126 g concentrated hydrochloric acid** (HCl), **85 g ammonium hydroxide** (NH_4OH) and **1.0 g disodium EDTA**. Stir until dissolved. Adjust the pH to 8.5 with **HCl** or **15 N NaOH**.

Reagent 3. Sulfanilamide color reagent

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

By Weight: To a tared, dark **1 L** container add **876 g water**, **170 g 85% phosphoric acid** (H_3PO_4), **40.0 g sulfanilamide**, and **1.0 g N-(1-naphthyl)ethylenediamine dihydrochloride** (NED). Shake until wetted and stir with stir bar for 30 minutes until dissolved. This solution is stable for one month.

7.2 PREPARATION OF STANDARDS

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

By Volume: Four 1 and six 250 mL volumetric flasks.

By Weight: Four 1 L and six 250 mL containers.

NOTE: Following are standards preparations for a 1 channel system determining NO_2^- + NO_3^- or NO_2^- and a 2 channel system where one channel is used for NO_2^- + NO_3^- and the other channel is used for determining NO_2^-. For the 1 channel system, either NO_2^- or NO_3^- standards may be used. We recommend the use of NO_3^- standards when running a 1 channel method for NO_2^- + NO_3^-. For the 2 channel system, we recommend the use of both NO_2^- + NO_3^- standard sets.

Standard 1. Stock Nitrate Standard 500 mg N/L as NO_3^-

By Volume: In a **1 L** volumetric flask dissolve **3.610 g potassium nitrate** (KNO_3) in about **600 mL water**. Dilute to the mark and invert to mix. This solution is stable for six months.

Standard 2. Stock Nitrite Standard, 500 mg N/L as NO_2^-

By Volume: In a **1 L** volumetric flask dissolve **2.464 g sodium nitrite** ($NaNO_2$) or **3.038 g potassium nitrite** (KNO_2) in approximately **800 mL water.** Dilute to the mark and invert to mix. Refrigerate. This solution is stable for 3-5 days.

Standard 3. Stock Nitrate Standard 50 mg N/L as NO_3^-

In a **1 L volumetric flask**, add **100 mL of Stock Nitrate Standard 1,** 500.0 mg N/L. Dilute to the mark and invert to mix. This solution is stable for one week.

Standard 4. Stock Nitrite Standard 50 mg N/L as NO_2^-

In a **1 L volumetric flask**, add **100 mL of Stock Nitrite Standard 2**, 500.0 mg N/L. Dilute to the mark and invert to mix. This solution is stable for 3-5 days.

Nitrate Standards

Working Standards (Prepare Daily) Concentration mg N/L as NO_3^-	**A** 10.0	**B** 5.00	**C** 1.00	**D** 0.50	**E** 0.10	**F** 0.00
By Volume						
Volume (mL) **of stock nitrate standard 2** diluted to 250 mL with DI water	50	25	5.00	2.50	0.50	0.00
By Weight						
Weight (g) **of stock nitrate standard 2** diluted to final weight (~250 g) divided by **factor** below with DI water	50	25	5.0	2.5	0.5	0.0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	0.2	0.1	0.02	0.01	0.002	-----

Nitrite Standards

Working Standards Concentration mg N/L as NO_2^-	**A** 10.0	**B** 5.00	**C** 1.00	**D** 0.50	**E** 0.10	**F** 0.00
By Volume						
Volume (mL) of **stock nitrite standard 4** diluted to 250 mL with DI water	50	25	5.00	2.50	0.50	0.00
By Weight						
Weight (g) of **stock nitrite standard 4** diluted to final weight (~250 g) divided by **factor** below with DI water	50	25	5.0	2.5	0.5	0.0
Division Factor Divide exact weight of the standard by this **factor** to give final weight	0.2	0.1	0.02	0.01	0.002	-----

8.0 SAMPLE COLLECTION, PRESERVATION AND STORAGE

8.1 Nitrite will be oxidized by air O_2 to nitrate in a few days. If analysis can be made within 24 hours, the sample should be preserved by refrigeration at 4°C. When samples must be stored for more than 24 hours, they should be preserved with sulfuric acid (2 mL conc. H_2SO_4 per liter) and refrigerated. **CAUTION:** Samples must NOT be preserved with mercuric chloride or thiosulfate because this will degrade the cadmium column.

8.2 If build-up of suspended matter in the reduction column restricts sample flow, the samples may be prefiltered.

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.

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

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

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

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

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

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

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

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

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

9.3 Matrix spikes - The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

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

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

9.3.2 Analyze one sample aliquot out of each set of ten samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4.1 Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

9.4.2 If analyte is detected in the blank at a concentration greater than MDL (Section 3.10.), analysis of the samples is halted until the source of contamination is eliminated and a blank shows no evidence of contamination. All samples must be associated with an uncontaminated method blank before the results may be reported for regulatory compliance purposes.

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

9.6 On-going Precision and Recovery (OPR) - With every analytical batch, a midrange standard must be prepared using the procedure described in Section 11.

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

9.7 Quality Control Samples (QCS) - It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in Section 7. The QCS is used to verify the concentrations of the calibration standards.

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

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare reagents and standards as described in Section 7.

10.2 Set up manifold as shown in Section 17.

10.3 Input data system parameters as shown in Section 17.

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

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

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

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

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

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

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

11.0 PROCEDURE

11.1 Prepare reagents and standards as described in Section 7.

11.2 Set up manifold as shown in Section 17.

11.3 Input data system parameters as in Section 17.

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

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

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

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

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

12.0 DATA ANALYSIS AND CALCULATIONS

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

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

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

13.0 METHOD PERFORMANCE

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

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

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

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

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

15.0 WASTE MANAGEMENT

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

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

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, **Methods for Chemical Analysis of Water and Wastes** Method 353.2

16.2 **Methods for Determination of Inorganic Substances in Water and Fluvial Sediments.** Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey.

17.0 TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

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

Sample throughput: 60 samples/h, 60 s/sample
Pump Speed: 35
Cycle Period: 60

Analyte Data:

Concentration Units: mg N/L
Peak Base Width: 30 s
% Width Tolerance: 100
Threshold: 9360
Inject to Peak Start: 23 s without column
30 s with column
Chemistry: Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg N/L	10.0	5.00	2.00	1.00	0.25	0.10	0.00

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

Sampler Timing:

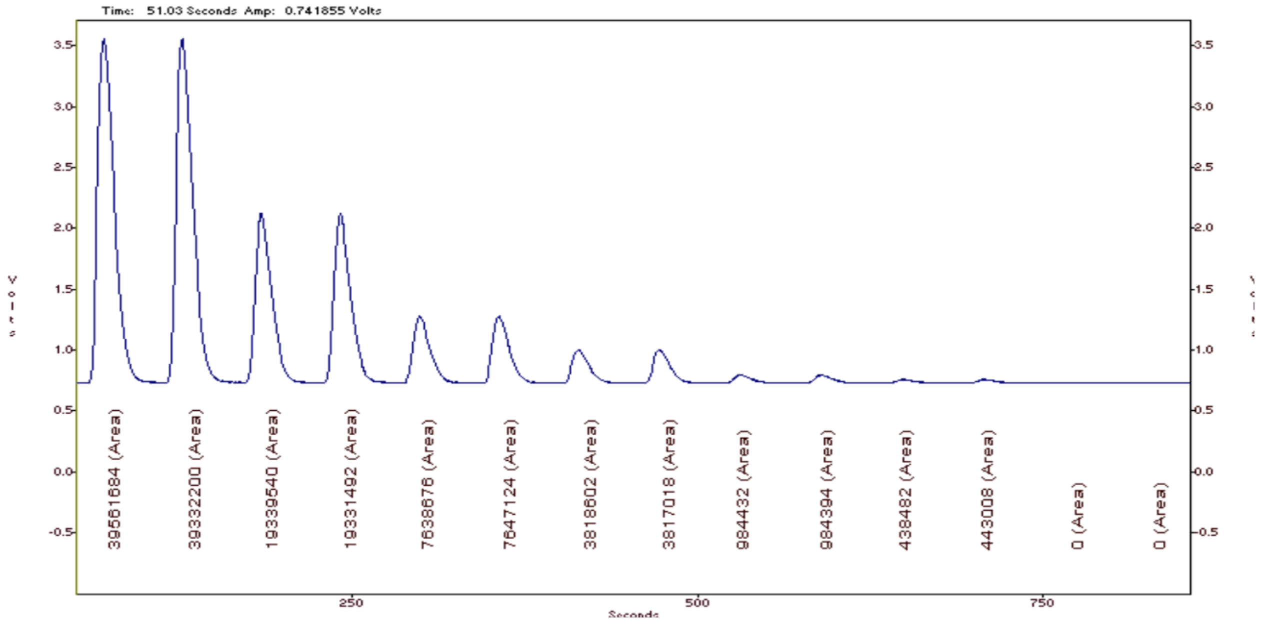
Min. Probe in Wash Period: 9 s
Probe in Sample Period: 20 s

Valve Timing:

Load Time: 0 s
Load Period: 6 s
Inject Period: 54 s

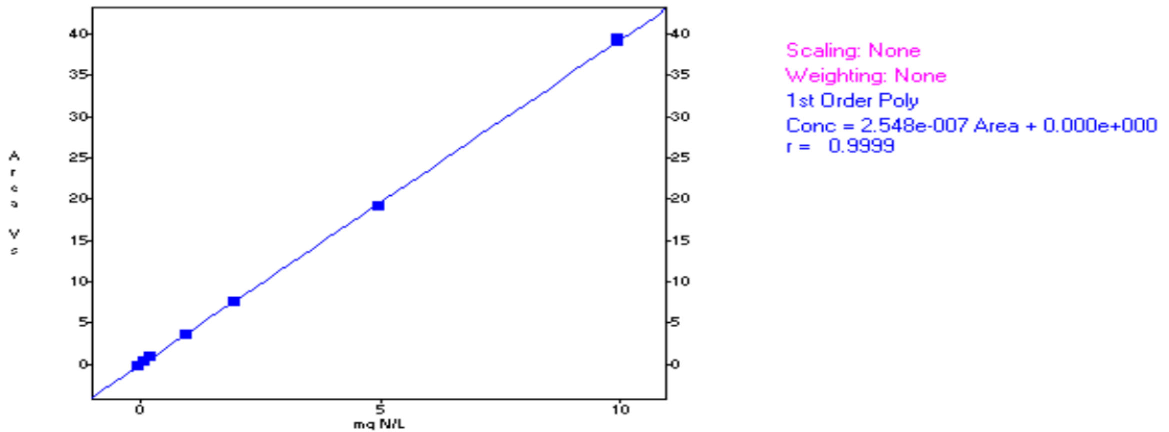
17.2 SUPPORT DATA FOR QUIKCHEM 8000

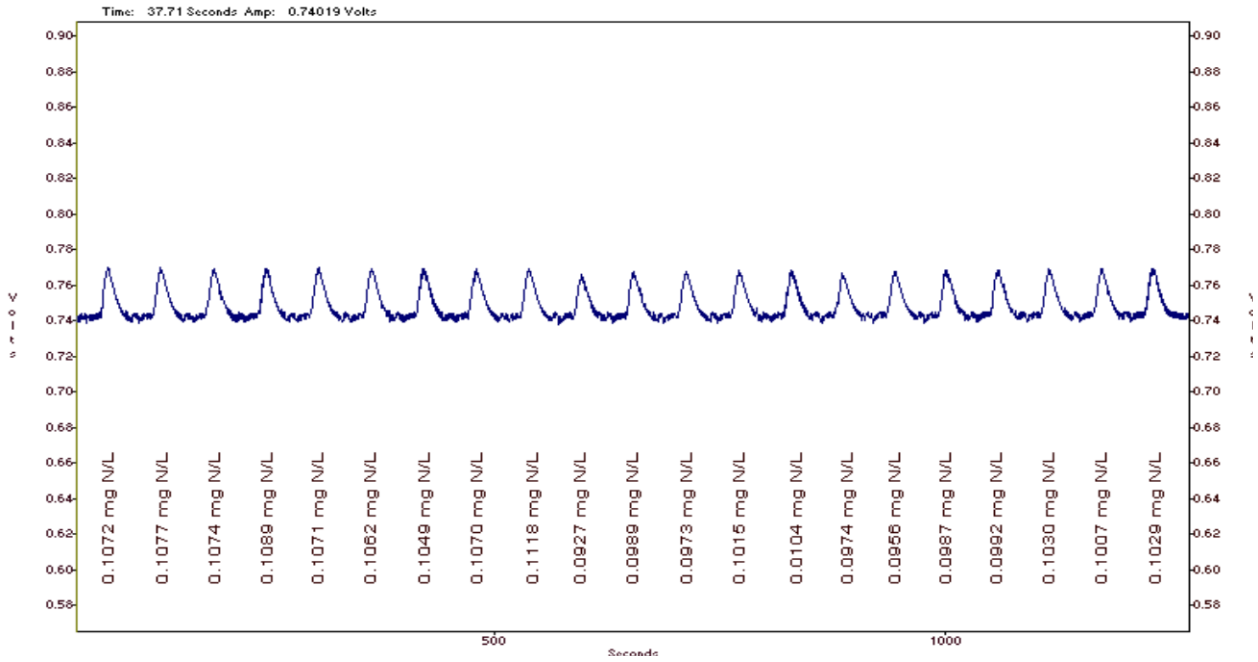
Calibration Data for Nitrate/Nitrite



Nitrate Calibration Graph and Statistics

Level	Area (V-s)	mg N/L	Determined	Replicate %RSD	% residual
1	39446944	10.0	10.05	0.4	-0.5
2	19335516	5.00	4.93	0.0	1.5
3	7642900	2.00	1.95	0.1	2.6
4	3817810	1.00	0.97	0.0	2.7
5	984413	0.25	0.25	0.0	-0.3
6	440745	0.10	0.11	0.7	-12.3
	0	0.00	---	0.0	---

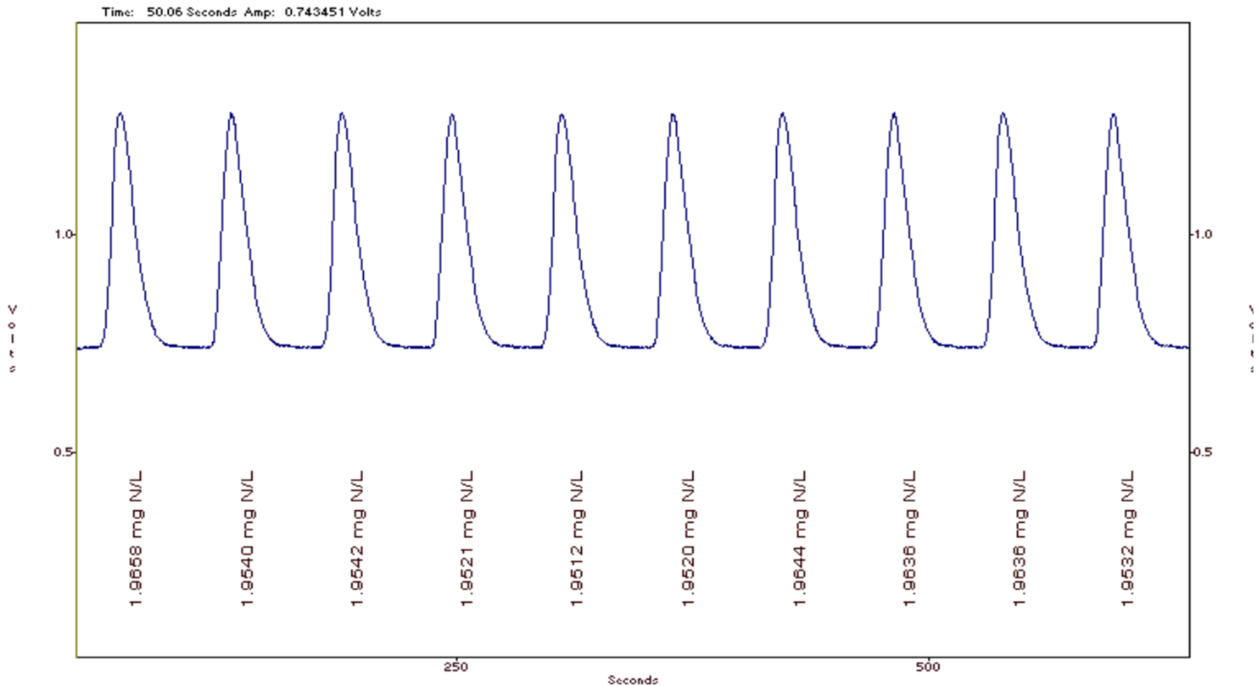




Method Detection Limit using 0.10 mg N/L standard

MDL = 0.012 mg N/L

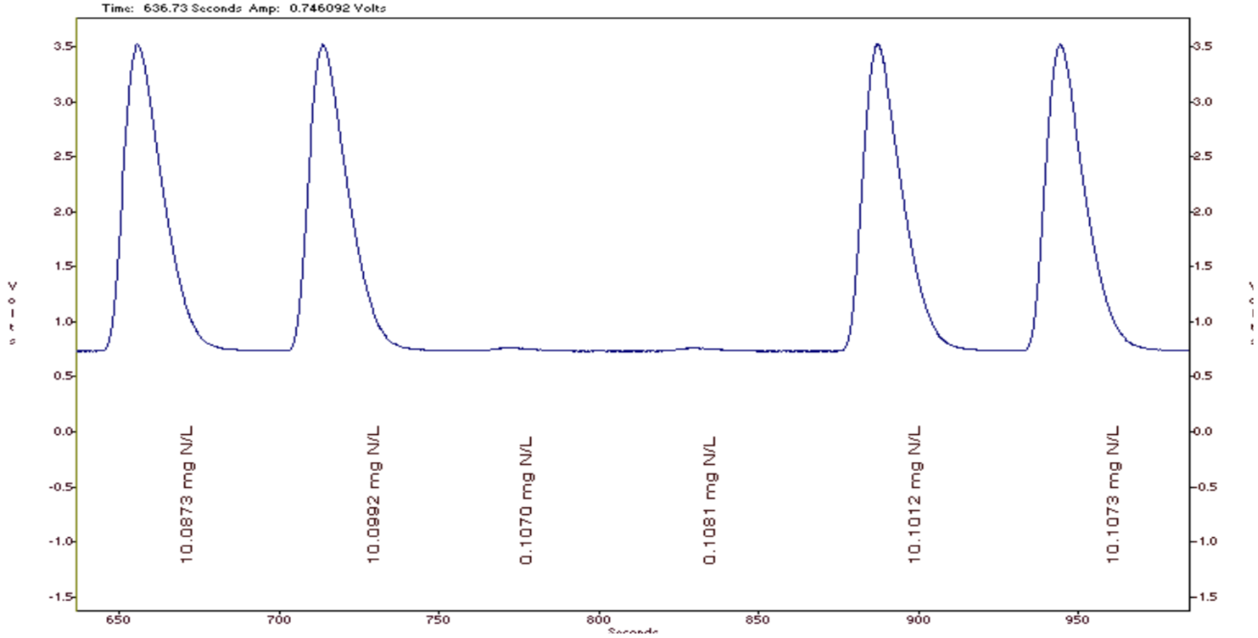
Standard Deviation (s) = 0.0049 mg N/L, Mean (X) = 0.102 mg N/L, Known Value = 0.10 mg N/L
ACQ Date: 3 December 1998



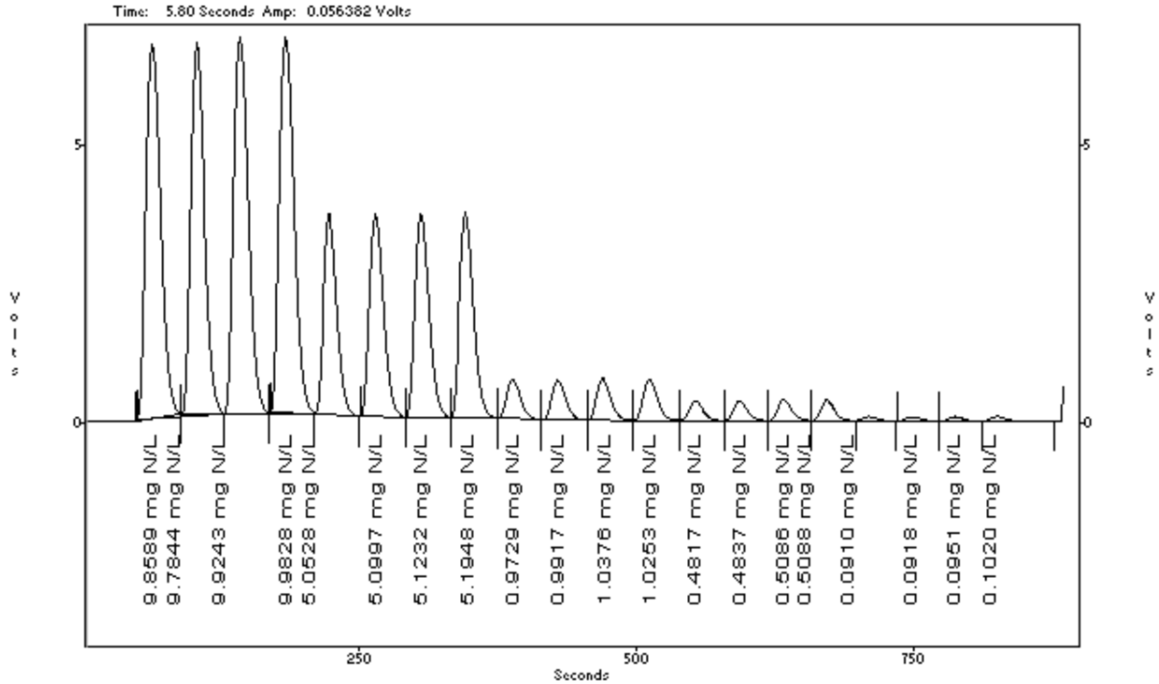
Precision Data using a 2.0 mg N/L standard

%RSD = 0.31

Standard Deviation (s) = 0.006 mg N/L, Mean (X) = 1.95 mg N/L, Known Value = 2.00 mg N/L
ACQ Date: 3 December 1998



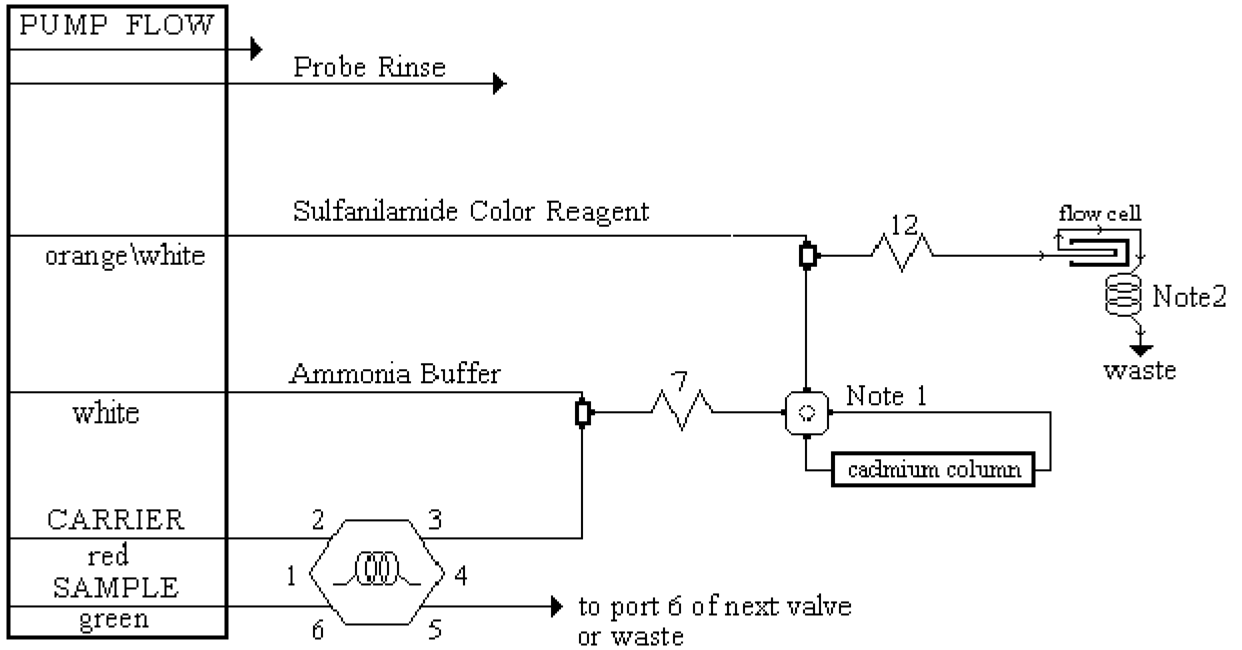
Carryover Study using two 10.0 mg N/L standards followed by two blanks followed by two 10.0 mg N/L standards
Carryover passed
ACQ Date: 3 December 1998



Column Efficiency

Concentration (mg N/L)	10.0	5.00	1.00	0.50	0.10
Efficiency (%)	98.6	98.5	95.2	94.9	92.9

17.3 NITRATE/NITRITE MANIFOLD DIAGRAM

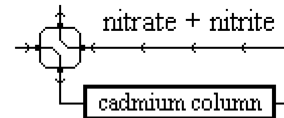


Carrier: **DI water**
Manifold Tubing: **0.5 mm (0.022 in) i.d.** This is **2.5 µL/cm**.
AE Sample Loop: Microloop
QC8000 Sample Loop: Microloop
Interference Filter: 520 nm

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

7: **135** cm of tubing on a 7 cm coil support
12: **255** cm of tubing on a 12 cm coil support

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



Note 2: This is a backpressure loop of 100 cm x 0.022" i.d. tubing.

pH
Thermo Orion 4 Star System
(Water/Wastewater Matrices)

1.0 APPLICATION

The pH method is based on 4500-H (B) of the 21st Edition of Standard Methods for Examination of Water and Wastewater. The operation of the Thermo Orion 4 Star system is taken from its operational manual.

2.0 SUMMARY

pH is the determination of the activity of the Hydrogen ions by potentiometer measurement using an electrode. The pH meter and electrode are standardized against three reference buffer solutions that closely bracket the anticipated sample pH.

3.0 PRESERVATION

3.1 Collect sample in unpreserved container.

3.2 pH must be measured at room temperature or temperature compensation probe must be used.

3.3 Holding time - analyze immediately

4.0 APPARATUS

4.1 Thermo Orion 4 Star pH meter: meter, refillable Ag/AgCl pH/ATC probe, and stirrer.

4.2 Beakers - 150 ml

5.0 REAGENTS

5.1 pH buffer = 4.00 ± 0.01, NIST Traceable

5.2 pH buffer = 7.00 ± 0.01, NIST Traceable

5.3 pH buffer = 10.00 ± 0.01, NIST Traceable

5.2 Ag/AgCl Reference Electrode Filling Solution

5.5 Electrode storage solution

6.0 INTERFERENCES

6.1 Coatings of oily material or particulate matter can impair electrode response. These coatings may be removed by gentle wiping or detergent washing, followed by distilled water rinsing.

7.0 QA/QC

7.1 **Check standards** - 4.00, 7.00 and 10.00 - Frequency every 10 samples.

7.2 **Replicate samples** - Frequency of 1 in 20 samples.

8.0 PROCEDURE

8.1 Place fresh buffer in correctly labeled beakers.

8.2 Turn on the power to the meter.

8.3 Rinse, blot dry, and place pH probe in the pH4 buffer and activate the stirrer.

8.4 Press the Calibrate button to begin.

8.5 When the arrow next to the pH value begins to flash, the reading has become stable. Press the Calibrate button again. Rinse, blot dry, and place pH probe in the pH7 buffer.

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8.6 Again, when the arrow next to the pH value begins to flash, the reading has become stable. Press the Calibrate button once more. Rinse, blot dry, and place pH probe in the ph10 buffer.

8.7 Wait for the reading to become stable and press the Measure button. The bench unit will display and print the slope. The slope should be between 92.0 – 102.0, if not, repeat calibration with fresh buffers. Refer to pH electrode manual if problems persist. Record slope in log book and initial.

8.8 Place electrode back into the pH 7.0 buffer and read. The reading should be 7.00 ± 0.05 S.U.

8.9 Repeat until all samples are analyzed rinsing and blotting dry the electrode between samples. Record results on bench sheets.

9.0 CALCULATIONS

9.1 None

10.0 CLEAN-UP

10.1 Keep electrode submerged in pH 7 buffer or electrode storage solution when not in use.

TOTAL COLIFORM, FECAL COLIFORM, AND E. COLI
Colilert/Colilert-18 MPN Method
(Wastewater Only)

1.0 APPLICATION

Colilert/Colilert-18 is used for the simultaneous detection, specific identification, and confirmation of total coliform, fecal coliform, and E. coli in water. It is based on Method 9223 (B) of the 21st Edition, Standard Methods for the Examination of Water and Wastewater and IDEXX's Colilert-18 procedure. Total coliform, fecal coliform, and E. coli are detected at 1 CFU/100 ml of an undiluted sample in 24-28 hours for Colilert and 18-22 hours for Colilert-18.

2.0 SUMMARY

It is based on IDEXX'S patented Defined Substrate Technology (DST)7. DST utilizes indicator nutrients, which cause target microbes contained in the sample and incubated to produce a color change or another signal, fluorescence, indicating and confirming the presence of the target microbe.

Colilert/Colilert-18 are a specially designed reagent that is specific to total coliform. It provides specific indicator nutrients: ONPG (O-Nitrophenyl-β-d-galactopyranoside and MUG (4-Methylumbelliferyl-β-d-glucuronide) for the target microbes, total coliform and E. Coli. As these nutrients are metabolized, yellow color (from ONPG) and fluorescence (from MUG) are released confirming the presence of total coliform and E. coli, respectively. Non-coliform bacteria are suppressed and cannot metabolize the indicator nutrients. Consequently, they do not interfere with the specific identification of the target microbes during the test incubation period.

3.0 PRESERVATION

3.1 Colilert/Colilert-18 are stored at 4°C to 30°C, away from light.

3.2 Start examination of water sample promptly after collection. If sample can't be processed within 1 hr after collection refrigerate below 10°C. Maximum transport time of 6 hrs and process time of 2 hours is allowed following guidelines from Standard Methods 9060 (B).

4.0 APPARATUS

4.1 Various sterile pipette tips and pipettes.

4.2 Air incubator capable of maintaining 35 ± 0.5°C for total coliform and E. coli analysis. Water bath incubator capable of maintaining 44.5 ± 0.2°C for fecal coliform analysis.

4.3 Long wavelength (365 nm) ultraviolet lamp.

4.4 Rack for holding trays.

4.5 Heated sealer for Quanti-Trays.

5.0 REAGENTS

5.1 Colilert/Colilert-18 reagent ampules.

5.2 **Quanti-Cult cultures** - use for QA/QC analysis. Each culture set contains Pseudomonas aeruginosa, Klebsiella Pneumoniae, and Escherichia coli, which are pre-quantitated.

5.3 Color and fluorescence comparator

INTERFERENCES

6.1 Do not autoclave Colilert/Colilert-18 prior to use. This process destroys the reagent, which is heat liable.

6.2 Incubation beyond allotted time may cause non-coliform heterotrophic bacteria to overcome the suppressant systems causing a false positive.

6.3 Do not transfer colonies or cultures pre-grown in any enrichment media to Colilert/Colilert-18.

6.4 Do not pre-filter a sample and then place that filter in Colilert/Colilert-18. The filtration step can concentrate coliform and non-coliform heterotrophs, particulates, and certain chemicals, which can overlay and suppress coliform affecting the sensitivity of the test. Also, coliform bacteria can become trapped in the filter, restrict their access to the indicator nutrients.

6.5 Do not dilute the sample in buffered water before addition to Colilert/Colilert-18. Colilert/Colilert-18 is already buffered and additional buffer compounds may affect the growth of the target microbes.

6.6 Upon mixing Colilert/Colilert-18 with the sample, a transient blue color appears the sample contains an excessive amount of free chlorine. The sample should be considered invalid and testing discontinued.

7.0 QA/QC

7.1 Quality control should be conducted on each lot of Colilert/Colilert-18, Tryptic Soy Broth (TSB), and sample bottles received to ensure integrity and proper product performance.

7.1.1 Each lot of Colilert/Colilert-18 is tested using Quanti-Cult cultures. Each culture set contains Pseudomonas aeruginosa, Klebsiella Pneumoniae, and Escherichia coli. Follow Quanti-Cult procedure provided with each new culture set and fill out Form DC-597-C. Expected results are below:

Quanti-Cult Organism	Expected Result
E. coli	yellow, fluorescent
Klebsiella Pneumoniae	yellow, no fluorescent
Pseudomonas aeruginosa	clear, no fluorescent

7.1.2 Each lot of microbiological sample containers need to be tested. Follow form DC-597-E. Each new lot needs the lot number recorded, manufacturer’s certification filed, volume check, fluorescence check, and sterility check with TSB.

7.1.3 Each lot of TSB is tested using the TSB Media Acceptability Log (Form DC-597-D). Each new lot need the lot number recorded, manufacturer’s certification filed, pH check with supporting records, and a positive and negative sterility check performed.

7.2 Monitor incubator and refrigerator temperatures daily. Record and initial in log book. Thermometers are verified/calibrated twice a year referencing a NIST traceable digital thermometer.

7.3 A blank DI water sample and duplicate will be run weekly for WWTP fecal analysis and results recorded on Form DC-525. During basin spill events a blank will be run a minimum of daily or every 20 samples, whichever occurs most often, for each parameter tested. These results will be recorded on Form DC-464.

7.4 A Laboratory Control Sample (LCS) composed of influent sewer sample or clarifier sample will be performed weekly for WWTP fecal analysis and results recorded on Form DC-525. During basin spill events an LCS will be run a minimum of daily or every 20 samples, whichever occurs most often, for each parameter tested. These results will be recorded on Form DC-464.

7.5 The color comparator should be kept in the dark. It has an expiration date and a new one should be purchased prior to this date.

7.5 The analytical balance needs to be kept clean. A weight check should be performed on a monthly basis using the 100, 50, 5, 0.5, and 0.05 gram weights with a tolerance within +/- 0.5 mg. Balance will be properly inspected yearly by a certified outside vendor.

7.6 Two sets of outside QC/QA samples for each parameter will be run every year with a DMR-QA set being one of them.

8.0 PROCEDURE

8.1 Set-up three Quanti-Tray/2000's per sample and label with the sample ID and dilutions that will be performed.

8.2 Samples received by the laboratory should be kept below 10°C and processed within 8 hours from sampling time. If samples are from the Basins for Total coliform, fecal, and E. coli, Colilert-18 must be used and samples must be incubated for 18-22 hours. If samples are from IDEP/SWAT or SSO and time is not an issue, Colilert will be used and samples will be incubated for 24-28 hours. After hours SSO's can be inoculated with Colilert-18 and incubated for 18-22 hours if it helps with the read-back time.

8.3 Thoroughly mix all samples immediately before making necessary dilutions. Aseptically transfer appropriate amount of sample to sterile sample container and fill with DI water to the 100 mL mark. Inoculate sample with either Colilert or Colilert-18 depending upon analysis and shake vessel till dissolved. Transfer to appropriate Quanti-Tray/2000.

8.4 Place the Quanti-Tray/2000 in the sealer template and insert in the heat sealer.

8.5 Place the sealed tray in a 35+/-0.5°C for Total coliform and/or E. coli (or 44.5+/-0.2°C for fecal coliforms) incubator for 18-22 hours when Colilert-18 is used or 24-28 hours when regular Colilert is used.

8.6 Read results according to the Result Interpretation table below. Count the number of positive wells, record numbers on appropriate Form DC-464, and refer to the MPN

table provided with the trays to obtain a Most Probable number. Look for fluorescence with a 6-watt, 365-nm UV light within 5 inches of the sample in a dark environment.

Results Interpretation

Appearance	Results
Less yellow than the comparator at 35°C	Negative for total coliforms and E. coli
Less yellow than the comparator at 44.5°C	Negative for fecal coliforms
Yellow = or > comparator at 35°C	Positive for total coliforms
Yellow = or > comparator at 44.5°C	Positive for fecal coliforms
Yellow and fluorescence = or > Comparator at 35°C	Positive for E. coli

9.0 CALCULATIONS

9.1 When the MPN is obtained using the chart provided by the manufacturer, record the value on the appropriate Form DC-464. The MPN value obtained along with the dilutions can then be put into the Excel chart labeled “Formulas” under the E. coli tab. This chart will generate the MPN based on the dilutions used along with the bacteria average and bacteria Geo Mean for reporting purposes.

TOTAL SUSPENDED SOLIDS
Non-filterable residue, 103°C - 105°C
TOTAL VOLATILE SUSPENDED SOLIDS
Non-filterable residue, ignition at 550°C
(Water/Wastewater Matrices)

1.0 APPLICATION

Total suspended solids (TSS) is based on 2540(D) of the 21st Edition of Standard Methods for Examination of Water and Wastewater. Volatile suspended solids (VSS) method follows 2540(E).

2.0 SUMMARY

The sample is filtered through a 0.45 micron glass fiber filter. The nonfilterable residue (suspended matter) is determined by weighing the filter before and after filtering. The difference in weights is measured and reported as mg suspended solids per liter of sample. The total volatile suspended solids is the difference between the solids before and after ashing.

3.0 PRESERVATION

3.1 Collect samples in an unpreserved bottle.

3.2 Refrigerate sample at $\leq$ 6°C.

3.3 Holding time - 7 days

4.0 APPARATUS

4.1 Analytical balance, capable of weighing to 0.1 mg.

4.2 Desiccator, provided with a desiccant containing a color indicator of moisture concentration.

4.3 Gooch crucible with Gooch crucible adapter.

4.4 Glass fiber filter disks without organic binder, 934 AH type or equivalent.

4.5 Drying oven, for operation at 103°C to 105°C.

4.6 Filtration apparatus with reservoir.

4.7 Muffle furnace, for operation at 550°C ± 50°C.

4.8 Wide-bore pipettes

4.9 Graduated cylinders

4.10 Crucible tongs, 9 and 20 inch

4.11 Tweezers

4.12 Heat reflective/resistant gloves

5.0 REAGENTS

5.1 Deionized wash water

6.0 INTERFERENCES

6.1 Samples with high oil and grease will produce inaccurate results because of the difficulty in completely drying the residue.

6.2 Errors from ashing may result from loss of ammonium carbonate and volatile organic matter while drying.

7.0 QA/QC

7.1 **Replicate/Duplicate samples** - Frequency 1 in 20 samples.

8.0 PROCEDURE

8.1 PREPARATION OF GLASS FIBER DISKS

8.1.1 Insert one disk with wrinkled side up in Gooch crucible.

8.1.2 Place crucible onto filtration apparatus and apply vacuum.

8.1.3 Using a water bottle, wash filter with 10-20 ml portions of reagent grade water. Continue suction to remove all traces of water.

8.1.4 Remove crucible from filtration apparatus and place in 550°C muffle furnace for 15 minutes. Remove and cool in desiccator. Store in desiccator until needed.

8.2 SAMPLE ANALYSIS

8.2.1 Remove crucible(s) from desiccator and weigh on balance. Record crucible weight on bench sheet in grams. Place crucible(s) on filtering assembly.

8.2.2 Mix sample by shaking thoroughly. Influent samples should filter 25 mL to 50 mL. Effluent samples should filter 100 mL to 2000 mL, depending on integrity of the sample. Mixed liquor samples should filter 10 mL.

8.2.3 Continue suction of crucible until all traces of water are gone.

8.2.4 Remove crucible(s) from filtering assembly and place in drying oven for at least 1 hour.

8.2.4 Remove crucible(s) from oven and place in desiccator for at least 1 hour.

8.2.5 Weigh crucible(s) on balance and record dried residue + crucible on bench sheet in grams.

8.2.6 Place crucible(s) in muffle furnace for at least 15 minutes. For more than one sample or heavier solids, longer ignition time may be necessary.

8.2.7 Remove from furnace and allow to air cool until most of the heat has dissipated. Transfer to desiccator and cool for a minimum of 1 hour. Record crucible + ash on bench sheet.

8.2.8 Remove used filter disk and replace with new. Re-pad crucible(s) in same manner described above in preparation of filter disk.

9.0 CALCULATIONS

9.1 Total suspended solids, mg/L =

$$\frac{(A - B)\ g}{\text{sample volume, mL}} \times \frac{1{,}000\ mg}{g} \times \frac{1{,}000\ mL}{L}$$

where: A = weight of crucible + dried residue, g
B = weight of crucible, g

9.2 Volatile suspended solids, mg/L =

$$\frac{(A - C)\ g}{\text{sample volume, mL}} \times \frac{1{,}000\ mg}{g} \times \frac{1{,}000\ mL}{L}$$

where: A = weight of crucible + dried residue, g
C = weight of crucible + ash residue, g

10.0 CLEAN-UP

10.1 Dispose of used filters in waste container.

10.2 Re-pad crucible following step 8.1.

TOTAL COLIFORM AND E. COLI
COLILERT7 PRESENCE-ABSENCE (P-A) METHOD
(Drinking Water Only)

1.0 APPLICATION

Colilert is used for the simultaneous detection, specific identification, and confirmation of total coliform and E. coli in water. It is based on Method 9223 (B) of the 21st Edition, Standard Methods for the Examination of Water and Wastewater. Total coliform and E. Coli are detected at 1 CFU/100 ml of sample in 24-28 hours.

2.0 SUMMARY

It is based on IDEXX'S patented Defined Substrate Technology (DST)7. DST utilizes indicator nutrients, which cause target microbes contained in the sample and incubated to produce a color change or another signal, fluorescence, indicating and confirming the presence of the target microbe.

Colilert is a specially designed reagent that is specific to total coliform. It provides specific indicator nutrients: ONPG (O-Nitrophenyl-β-d-galactopyranoside and MUG (4-Methylumbelliferyl-β-d-glucuronide) for the target microbes, total coliform and E. Coli. As these nutrients are metabolized, yellow color (from ONPG) and fluorescence (from MUG) are released confirming the presence of total coliform and E. coli, respectively. Non-coliform bacteria are suppressed and cannot metabolize the indicator nutrients. Consequently, they do not interfere with the specific identification of the target microbes during the test incubation period.

3.0 PRESERVATION

3.1 Colilert is stored at 4°C - 30°C, away from light.

3.2 Start examination of water sample promptly after collection. If sample can't be processed within 1 hr after collection refrigerate below 10°C. Maximum process time is 30 hrs.

4.0 APPARATUS

4.1 Sterile, transparent, non-fluorescent bottle (120 ml). Follow QA/QC procedure for acceptance of new lots of bottles.

4.2 Incubator capable of maintaining 35°C ± 0.5°C.

4.3 Long wavelength (365 nm) ultraviolet lamp.

4.2 Autoclave.

4.3 Analytical balance, capable of weighing to 0.1 mg and 100 g

5.0 REAGENTS

5.1 Colilert/Colilert-18 reagent, snap packs

5.2 **Quanti-Cult cultures** - use for QA/QC analysis. Follow QA/QC test procedure.

5.3 Color and fluorescence comparator

5.4 **Chem strips** - used for confirmation of autoclave operation

5.5 **Tryptic Soy Broth** - non-selective broth for sterility checks

5.6 **Rinse/dilution water** - sterile water used to check sterility of Colilert/Colilert-18 reagent. Use 100 ml of water and follow test procedure.

6.0 INTERFERENCES

6.1 Do not autoclave Colilert/Colilert-18 prior to use. This process destroys the reagent, which is heat liable.

6.2 Incubate samples for 24-28 hrs at 35°C ± 0.5°C. Incubation beyond 28 hrs. may cause non-coliform heterotrophic bacteria to overcome the suppressant systems causing a false positive. Positives for both total coliforms and E. coli observed before 24 hours and negatives observed after 28 hours are also valid.

6.3 Do not transfer colonies or cultures pre-grown in any enrichment media to Colilert/Colilert-18.

6.4 Do not pre-filter a sample and then place that filter in Colilert/Colilert-18. The filtration step can concentrate coliform and non-coliform heterotrophs, particulates, and certain chemicals, which can overlay and suppress coliform affecting the sensitivity of the test. Also, coliform bacteria can become trapped in the filter, restrict their access to the indicator nutrients.

6.5 Do not dilute the sample in buffered water for addition to Colilert/Colilert-18. Colilert/Colilert-18 is already buffered and additional buffer compounds may affect the growth of the target microbes.

6.6 Upon mixing Colilert/Colilert-18 with the sample, if a transient blue color appears the sample contains an excessive amount of free chlorine. The sample should be considered invalid and testing discontinued.

7.0 QA/QC

7.1 Quality control should be conducted on each lot of Colilert/Colilert-18, Tryptic Soy Broth(TSB), and sample bottles received to ensure integrity and proper product performance.

7.1.1 Each lot of Colilert/Colilert-18 is tested using Quanti-Cult cultures. Each culture set contains Pseudomonas aeruginosa, Klebsiella Pneumoniae, and Escherichia coli. Follow Quanti-Cult procedure provided with each new culture set and fill out Form DC-597-C. Expected results are below:

Quanti-Cult Organism	Expected Result
E. coli	yellow, fluorescent
Klebsiella Pneumoniae	yellow, no fluorescent
Pseudomonas aeruginosa	clear, no fluorescent

7.1.2 Each lot of microbiological sample containers need to be tested. Follow form DC-597-E. Each new lot needs the lot number recorded, manufacturer's certification filed, volume check, fluorescence check, and sterility check with TSB.

7.1.3 Each lot of TSB is tested using the TSB Media Acceptability Log (Form DC-597-D). Each new lot need the lot number recorded, manufacturer's

certification filed, pH check with supporting records, and a positive and negative sterility check performed.

7.3 Monitor incubator and refrigerator temperatures daily. Record and initial in log book. Record each incubator shelf temperature twice daily; am and p.m. Separated by a minimum of 4 hours. Thermometers are verified/calibrated twice a year referencing a NIST traceable digital thermometer.

7.3 A blank DI water sample will be run every 23 samples and results recorded on Form DC-463.

7.4 A Laboratory Control Sample (LCS) composed of influent sewer sample or clarifier sample will be performed every 23 samples and results recorded on Form DC-463.

7.5 The color comparator should be kept in the dark. It has an expiration date and a new one should be purchased prior to this date.

7.6 The analytical balance needs to be kept clean. A weight check should be performed on a monthly basis using the 100, 50, 5, 0.5, and 0.05 gram weights with a tolerance within +/- 0.5 mg. Balance will be properly inspected yearly by a certified outside vendor.

7.7 Twice a year, a Benchmark Performance Audit needs to be performed by laboratory personnel. These are outside unknown QA/QC samples for testing.

8.1 PROCEDURE

8.1 For sample groups of 8 bottles or less proceed to step 8.2 following the standard Colilert 24-hour test procedure. However, when there are more than eight samples, one of two procedures must occur. 1)If time is not an issue, let the samples warm to room temperature and proceed with the standard Colilert 24-hour test procedure following step 8.2. 2)If time is an issue, follow the Colilert-18 test procedure as outlined in step 8.3.

8.2 Colilert-the standard 24-hour test procedure

- **8.2.1** Carefully separate one snap pack of Colilert from the strip taking care not to accidentally open an adjacent pack
- **8.2.2** Tap the snap pack to ensure that all the Colilert powder is in the bottom part of the pack
- **8.2.3** Open the pack by snapping back the top at the score line. Caution: Do not touch the opening of pack
- **8.2.4** Add the Colilert to the water sample bottle
- **8.2.5** Aseptically cap and seal the bottle
- **8.2.6** Shake until dissolved
- **8.2.7** Incubate for 24-28 hours at 35°C ± 0.5°C.
- **8.2.8** Read the results at 24-28 hours. Interpret test results following step 8.4.

8.3 Colilert-18

- **8.3.1** Carefully separate one snap pack of Colilert-18 from the strip taking care not to accidentally open an adjacent pack
- **8.3.2** Tap the snap pack to ensure that all the Colilet-18 powder is in the bottom part of the pack
- **8.3.3** Open the pack by snapping back the top at the score line. Caution: Do not touch the opening of pack
- **8.3.4** Add the Colilert-18 to the water sample bottle and shake till dissolved.
- **8.3.5** Place the water bottle(s) in a 44.5°C water bath for 7-10 minutes. Take care not to submerse the bottle beyond the bottom of neck which could possible

contaminate sample.

8.3.6 Remove bottle from and bath, dry off with clean paper towel, and place in a 35°C ± 0.5°C incubator for the remainder of the 18-22 hours.

8.3.7 Read the result at 18-22 hours. Interpret test results following step 8.4.

8.4 TEST RESULTS AND INTERPRETATION(Colilert/Colilert-18)

8.4.1 If no yellow color is observed, the test is negative for total coliform and E. coli.

8.4.2 If any bottle has a yellow color greater or equal to the comparator, the presence of total coliform is confirmed.

8.4.3 If the sample is yellow, but lighter than the comparator, it may be incubated an additional 4 hours (up to 28 hours for Colilert or up to 22 hours for Colilert-18). If the sample is coliform positive, the color will intensify. If is does not intensify, the sample is negative.

8.4.4 If yellow is observed check for fluorescence by placing the UV lamp 3-5 inches in front of the tube and making sure it is facing away from your eyes and toward the tube. Observe for fluorescence in a dark environment. If fluorescence is greater or equal to fluorescence of the comparator, the presence of E. coli is specifically confirmed.

8.4.5 The comparator is the **lowest** level of yellow and fluorescence, which can be considered positive. A typical positive test is much more intense than the comparator.

9.0 CALCULATIONS

9.1 None

10.0 CLEAN-UP

10.1 Sterilize all positive bottles after analysis in autoclave following autoclave's procedure. Confirm sterilization with spore strips.

10.3 After sterilization, dispose properly.

Oil and Grease
Liquid/Gravimetric Method
(Water/Wastewater Matrices)

1.0 APPLICATION

This method is based on 5520 B of the 18th Edition of Standard Methods for Examination of Water and Wastewater. Grease and oils include hydrocarbons, fatty acids, soaps, fats, waxes, oils, and any other material that is extracted from an acidified sample and not volatilized during the test procedure.

2.0 SUMMARY

Many oils and greases can be extracted from water, soil, and sludge sample by extracting the material with either Freon, or hexane depending on the level of efficiency desired. Freon has a greater affinity to extract a wider range of grease and oils than hexane. However, the use of Freon has a detrimental effect on the atmosphere. Thus, hexane extraction is the current preferred method. Extracts performed with Freon will result in better recoveries, in general, than hexane. The analyst must indicate which extraction solvent was utilized when presenting data.

3.0 PRESERVATION

3.1 Collect sample in a one liter wide mouth glass bottle.

3.2 Add hydrochloric acid to pH <2.

3.3 Maximum recommended holding time - 28 days.

4.0 APPARATUS for liquid/liquid extraction

4.1 Two liter glass separatory funnel with Teflon stopcock

4.2 Distilling flask, 125 ml capacity

4.3 Filter paper, Whatman No. 40, 12.5 cm diameter

4.4 Filter paper, Whatman PS, 12.5 cm diameter

4.5 Water bath capable of maintaining 85°C

4.6 Centrifuge capable of spinning four 100 ml glass tubes at 2400 rpm.

4.7 Vacuum source

4.8 Distilling adapter with drip tip.

4.9 Dessicator large enough to accommodate the distilling flask.

4.10 Ice bath large enough to accommodate the solvent recovery container.

5.0 APPARATUS for soils and sludges

5.1 In addition to the above materials a Soxhlet extraction or similar apparatus will be needed.

5.2 Extraction thimble

5.3 Mortar and pestle

5.4 Glass wool

5.5 Manganese sulfate monohydrate

6.0 REAGENTS

6.1 Hydrochloric acid 1 + 1

6.2 Sodium sulfate, anhydrous crystal

6.3 Trichlorotrifluoroethane or hexane spectrophotometric grade

7.0 INTERFERENCES

7.1 Heavy residuals of petroleum may contain significant portions that are not solvent extractable. Organic solvents tend to dissolve other organic substances in addition to oil and grease. Strict adherence to drying time must be followed due to the possible absorption of moisture. Alternative techniques may be needed if intractable emulsions form that cannot be broken by centrifuging. Determine such modifications on a case by case basis.

8.0 QA/QC

8.1 Check recovery with a known mixture of Wesson oil and No. 2 fuel oil.

8.2 One in twenty samples should be replicates.

8.3 One in twenty samples should be spiked.

9.0 PROCEDURE – Liquid/Liquid Extraction

9.1 Acidify the sample with hydrochloric acid if it has not been previously done.

9.2 Shake the sample well immediately prior to pouring 1000 ml in to a pre-marked two liter separatory funnel.

9.3 Add 30 ml of the extracting solvent to the 1000 ml sample contained in the separatory funnel. Cap and shake for 2 minutes.

9.4 Carefully open the cap away from personnel and add another 30 ml of solvent.

9.5 Recap and shake vigorously again for 2 minutes.

9.6 Release the pressure on the separatory funnel safely and allow the layers to stratify.

9.7 If an emulsion formed, add 1 gram of sodium sulfate to the filter paper and slowly drain the emulsified solvent on to the crystals. Repeat as necessary.

9.8 Repeat the extraction twice more with the solvent, taking care to note that oil/Freon extracts will be in the lower layer while oil/hexane extracts will be in the upper layer.

9.9 Collect the cleaned extracts in the distilling flask. If the emulsion failed to break, centrifuge the emulsion for 5 minutes to achieve stratification.

9.10 Distill the solvent from the preweighed distilling flask to near dryness while collecting the condensing solvent in an appropriate container.

9.11 Allow the distilling flask to achieve a constant weight.

10.0 CALCULATIONS

10.1 mg/L grease or oil = (wt of flask + residue) – (wt of flask) x 1000/ml of sample

11.0 PROCEDURE for soils and sludges

11.1 Weigh out 20 grams of dry material. *

11.2 Acidify the material to pH <2

11.3 Place the material in the solvent prewashed thimble.

11.4 Top off the thimble with solvent prewashed glass wool.

11.5 Insert the thimble in the Soxhlet extractor; connect the appropriate glassware to ensure no leakage during cycling.

11.6 Heat the solvent at a rate to produce 20 extraction cycles per hour for 4 hours.

11.7 Distill the solvent from the extraction flask on a warm steam bath for about 15 minutes.

11.8 Draw air through the flask by means of vacuum for the final minute.

11.9 Cool in a dessicator for exactly 30 minutes and weigh.

***Note:** If sludge is wet, add 25 grams manganese sulfate monohydrate and allow to stand 15 to 30 minutes before grinding with mortar and pestle.

12.0 CALCULATION

Dry solids

Mg/g grease and oil= (wt of flask + residue)- (wt of flask) x 1000/grams of dry material

Wet solids

Grease and oil as % dry solids =(weight gain in flask, grams 100)/(weight of wet solids, g x %dry solids)

Total Residual Chlorine
Amperometric Method

1.0 APPLICATION

This method is based on 4500-Cl D of the 21st Edition of Standard Methods for Examination of Water and Wastewater. It is suitable for concentrations between 0.024 mg/L and 2.0 mg/L. Higher concentrations of chlorine may be determined by careful appropriate dilutions.

2.0 SUMMARY

Total residual chlorine can be determined with a high degree of accuracy by an adaptation of the polarographic principle when titrated in the presence of the proper amount of potassium iodide in the pH range of 3.5 to 4.5. A special amperometric cell is used to detect the endpoint of the residual chlorine phenylarsine oxide (PAO) titiration. One mole of PAO reacts with two equivalents of halogen. The greater the residual, the greater the microammeter reading. The meter acts as a null point indicator that deflects less and less as the endpoint is approached.

3.0 PRESERVATION

3.1 None, determine immediately - - at most 30-minute delay

4.0 APPARATUS

4.1 Amperometric titrator

4.2 Magnetic stirrer with repeatable settings

4.3 Beakers - 250 ml

4.4 Class A volumetric flasks for dilutions

4.5 Eppendorf precision pipets and disposable tips

5.0 REAGENTS

5.1 Phenylarsine oxide 0.00564 N solution.

5.2 Potassium bi-iodate 0.002256 N solution.

5.3 Phosphate buffer solution, pH 7. This is available commercially. It consists of 25.4 g anhydrous postassium dihydrogen phosphate and 34.1 g sodium hydrogen phosphate dissolved in 1000 ml of chorine demand free water distilled water.

5.4 Acetate buffer solution. This is available commercially. It consists of 146 g anhydrous sodium acetate dissolved in 400 ml of water to which 480 g of concentrated acetic acid has been added and the total volume adjusted to 1 liter.

6.0 INTERFERENCES

6.1 Nitrogen trichloride and chlorine dioxide titrate partly as free chlorine, free halogens will also titrate as free chlorine. Copper and silver will plate out on the electrodes causing slow or no response.

7.0 QA/QC

7.1 Test at least one known concentration with samples to verify electrode responses.

7.2 One in twenty samples should be replicates.

7.3 One in twenty samples should be spiked.

8.0 PROCEDURE

8.1 Measure 200 ml of sample into a 250-ml beaker.

8.2 Add 1 ml of acetate buffer to the sample.

8.3 Mix sample on stirrer.

8.4 Add 1 ml of phosphate buffer to the sample

8.5 Titrate sample with 0.00564 N PAO until the indicating needle does not deflect.

8.6 Record titration volume on proper bench sheet.

8.7 Repeat titration for subsequent samples

9.0 CALCULATIONS

9.1 Total Residual Chlorine mg/L = mls of titrant used to achieve a null deflection in a 100 ml sample.

QuikChem® Method 10-115-01-1-E

DETERMINATION OF TOTAL PHOSPHORUS BY FLOW INJECTION ANALYSIS (ACID PERSULFATE DIGESTION METHOD)

Written by David Diamond

Applications Group

Revision Date:

8 November 2001

LACHAT INSTRUMENTS
5600 LINDBURGH DRIVE
LOVELAND, CO 80539 USA
QuikChem® Method 10-115-01-1-E

Total Phosphorous in Persulfate Digests

0.2 to 10.0 mg P/L

– Principle –

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

– Interferences –

1. Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

2. Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

– Special Apparatus –

Please see Parts and Price list for Ordering Information

1. Heating Unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)

2. Autoclave or Hot Plate

3. Labware for Digestion

QuikChem® Method 10-115-01-1-E

DETERMINATION OF TOTAL PHOSPHORUS BY FLOW INJECTION ANALYSIS COLORIMETRY

1.0 SCOPE AND APPLICATION

1.1 This method covers the determination of total phosphorus in drinking, ground, and surface waters, and domestic and industrial wastes. This method determines total phosphorus or if the sample is filtered through a 0.45 micron pore size filter, the result is termed total dissolved phosphorus. The difference between the result of a sample determined directly and filtered is termed total insoluble phosphorus.

1.2 The method is based on reactions that are specific for the orthophosphate (PO_4^{3-}) ion.

1.3 The applicable range is 0.2 to 10.0 mg/L. The method detection limit is 0.1 mg P/L. The method throughput is 103 injections per hour.

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

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

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

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

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

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

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

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

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

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

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

4.0 INTERFERENCES

4.1 Silica forms a pale blue complex, which also absorbs at 880 nm. This interference is generally insignificant as a silicate concentration of approximately 30 mg SiO_2/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

4.2 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free preparations for lab glassware.

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.

5.2 Each laboratory is responsible for maintaining a current awareness file of the Occupational Health and Safety Act (OSHA) regulations regarding the safe handling

of the chemicals specified in this method. A reference file of Material Safety Data sheets (MSDS) should be made available to all personnel involved in the chemical analysis. The preparation of a formal safety plan is also advisable.

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

5.3.1 Sulfuric Acid

6.0 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 pipettes or plastic containers as required. Samples may be stored in plastic or glass.

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

6.3.1 Sampler
6.3.2 Multichannel proportioning pump
6.3.3 Reaction unit or manifold
6.3.4 Colorimetric detector
6.3.5 Data system

6.4 Special Apparatus

6.4.1 Heating unit Lachat Part No. A85X00 (X=1 for 110V, X=2 for 220V)
6.4.2 Autoclave or Hotplate
6.4.3 Labware for Digestion

7.0 REAGENTS AND STANDARDS

7.1 PREPARATION OF REAGENTS

Use ASTM Type I water for all solutions. (See Standard Specification for Reagent Water D1193-77 for more information).

Degassing with helium:

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

Reagent 1. Stock Ammonium Molybdate Solution

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

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

Reagent 2. Stock Antimony Potassium Tartrate Solution

By Volume: In a **1 L** volumetric flask, dissolve **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$) or dissolve **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) in approximately **800 mL DI water**. Dilute to the mark **DI water** and invert to mix. Store in a dark bottle and refrigerate. May be stored up to two months when kept refrigerated.

By Weight: To a **1 L** dark, tared container add **3.0 g antimony potassium tartrate** (potassium antimonyl tartrate hemihydrate $K(SbO)C_4H_4O_6\cdot 1/2H_2O$) or dissolve **3.22 g antimony potassium tartrate** (potassium antimonyl tartrate trihydrate $C_8H_4O_{12}K_2Sb_2\cdot 3H_2O$) and **995 g DI water**. Stir or shake until dissolved. Store in a dark bottle and refrigerate. May be stored up to two months when kept refrigerated.

Reagent 3. Molybdate Color Reagent

By Volume: To a **1 L** volumetric flask, add about **500 mL DI water**, and then add **21.0 mL concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2), **213 mL Ammonium Molybdate Solution** (Reagent 1). Dilute to the mark **DI water** and invert to mix.

By Weight: To a tared **1 L** container **694 g DI water**, and then add **38.4 g concentrated sulfuric acid** (CAUTION: The solution will get very hot!). Swirl to mix. When it can be comfortably handled, add **72.0 mL Stock Antimony Potassium Tartrate Solution** (Reagent 2), **213 mL Ammonium Molybdate Solution** (Reagent 1). Shake to mix.

Reagent 4. Ascorbic Acid Reducing Solution, 0.33 M

By Volume: In a **1 L** volumetric flask dissolve **60.0 g granular ascorbic acid** in about **700 mL DI water**. Dilute to the mark **DI water** and invert to mix. Add **1.0 g dodecyl sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Prepare fresh weekly. Discard if the solution becomes yellow.

By Weight: To a tared **1 L** container, add **60.0 g granular ascorbic acid and 975 g DI water**. Stir or shake until dissolved. Add **1.0 g dodecyl sulfate** ($CH_3(CH_2)_{11}OSO_3Na$). Prepare fresh weekly. Discard if the solution becomes yellow.

Reagent 5. Carrier: Sulfuric Acid, 0.13 M

By Volume: In a **1 L** volumetric flask add **500 mL DI water and 7.2 mL concentrated sulfuric acid** (H_2SO_4). Dilute to the mark **DI water** and invert to mix. Degas daily. Prepare fresh weekly.

By Weight: To a tared **1 L** container add **993 g DI water**, and then add **13.3 g concentrated sulfuric acid** (H_2SO_4). Shake carefully to mix. Degas daily. Prepare fresh weekly.

7.2. PREPARATION OF STANDARDS

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

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

By Weight: Two, 1 L and seven, 250 mL containers.

NOTE: Standards are prepared in DI water and digested by the procedure in section 11.

Standard 1. Stock Standard 1000 mg P/L

In a **1 L** volumetric flask dissolve **4.396 g primary standard grade anhydrous potassium phosphate monobasic** (KH_2PO_4) that has been dried for one hour at 105°C in about **800 mL DI water**. Dilute to the mark with **DI water** and invert to mix.

Standard 2. Working Stock Standard Solution 10.0 mg P/L

By Volume: In a **1 L** volumetric flask, about **550 mL DI water**, and **10.0 mL Stock Standard** (Standard 1). Dilute to the mark with **DI water**. Invert to mix.

By Weight: To a tared **1 L** container, add about **10 g Stock Standard** (Standard 1). Divide the actual weight of the solution added by 0.01 and make up to this resulting total weight with **DI water**. Shake to mix.

Working Standards (Prepare Weekly)	**A**	**B**	**C**	**D**	**E**	**F**	**G**
Concentration mg P/L	10.0	4.00	2.00	1.00	0.40	0.20	0.00
By Volume							
Volume (mL) of **stock standard 2** diluted to 250 mL with DI water	250	100	50	25	10	5.0	---
By Weight							
Weight (g) of **stock standard 2** diluted to final weight (~250 g) divided by **factor** below with DI water	250	100	50	25	10	5.0	---
Division Factor Divide exact weight of the standard by this **factor** to give final weight	1.0	0.4	0.2	0.1	0.04	0.02	---

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 acid rinsed. The 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 by addition of concentrated H_2SO_4 to pH < 2. This is accomplished by adding no more than 2 mL concentrated H_2SO_4 per liter and verifying that the pH is less than 2. If the pH is still greater than 2, more sulfuric acid is added until the pH is < 2. Samples are stored at 4^oC. Acid preserved samples have a holding time of 28 days. Sample digests should be run immediately after digestion.

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.

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

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

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

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

9.2 INITIAL DEMONSTRATION OF PERFORMANCE

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

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

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

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

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

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

9.3 **Matrix spikes** - The laboratory must spike, in duplicate, a minimum of 10 percent of all samples (one sample in each batch of ten samples) from a given sampling site or if for compliance monitoring, from a given discharge. The two sample aliquots shall be spiked with the stock standard (section 7.2).

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

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

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

9.3.2 Analyze one sample aliquot out of each set of ten samples from each site or discharge according to the procedure beginning in Section 11 to determine the background concentration of (B) of the analyte.

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

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

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

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

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

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

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

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

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

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

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

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

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

9.4.1 Analyze a laboratory reagent water blank initially (with the test in Section 9.2) and with each analytical batch. The blank must be subjected to the same procedural steps as a sample.

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

9.5 **Calibration Verification** - Verify calibration using the procedure described in Section 10

9.6 **On-going Precision and Recovery (OPR)** - With every analytical batch, a midrange standard must be prepared using the procedure described in Section 11.

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

9.7 **Quality Control Samples (QCS)** - It is suggested that the laboratory obtain and/or prepare a quality control sample using a source different from the source routinely used in section 7. The QCS is used to verify the concentrations of the calibration standards.

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

10.0 CALIBRATION AND STANDARDIZATION

10.1 Prepare reagents and standards as described in Section 7.

10.2 Set up manifold as shown in Section 17.

10.3 Input data system parameters as shown in Section 17.

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

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

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

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

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

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

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

11.0 PROCEDURE

11.1 SAMPLE DIGESTION PROCEDURE

Note: Both standards and samples should be carried through this procedure. If samples have been preserved with sulfuric acid, standards should be preserved in the same manner.

11.1.1 The standards must go through the digestion process because the pH will change during the digestion and this could in turn affect the peak height of the standards. Alternately standards can be prepared in 0.13M sulfuric acid. In this case, digest several standards to check digestion completeness. (See 11.1.4)

11.1.2 Persulfate Digestion

11.1.2.1 Add 1.0 mL 5.6 M H_2SO_4 (310 mL conc. H_2SO_4/L) to 50.00 mL sample in a 125 mL Erlenmeyer flask.
11.1.2.2 Add 0.40 g ammonium persulfate [ammonium peroxydisulfate, $(NH_4)_2S_2O_8$] and boil gently until volume is about 10 mL. Alternatively, autoclave for 30 minutes at 121°C, 15-20 psi.
11.1.2.3 For samples containing arsenic or high levels of iron, add 5 ml of sodium bisulfite mix (dissolve 5.2 g of $NaHSO_3$ in 100 ml of 1.0 N H2SO4) and place in a 95˚C water bath for 30 minutes (20 minutes after the temperature of the sample reaches 95˚C). Cool and dilute to 50 ml.
11.1.2.4 Cool, transfer, and dilute to 50.0 mL in a volumetric flask.
11.1.2.5 The sample or standard is now prepared for determination by this method.

11.1.3 Sulfuric Acid Digestion

11.1.3.1 Add 1.0 mL of 5.6 M H_2SO_4 (310 mL conc. H_2SO_4/L) to 50.00 mL sample in a 125 mL Erlenmeyer flask.
11.1.3.2 Boil gently 30-40 minutes or until about 10 mL volume is reached. Alternatively, autoclave 30 minutes at 121°C, 15-20 psi.
11.1.3.3 For samples containing arsenic or high levels of iron, add 5 ml of sodium bisulfite mix (dissolve 5.2 g of NaHSO3 in 100 ml of 1.0 N H2SO4) and place in a 95˚C water bath for 30 minutes (20 minutes after the temperature of the sample reaches 95˚C). Cool and dilute to 50 ml.
11.1.3.4 Cool, transfer, and dilute to 50.00 mL in a volumetric flask.
11.1.3.5 The sample is now ready for determination by this method.

11.1.4 Tripoly phosphate, phenyl phosphate and trimethyl phosphate can be used to prepare standards as a check on digestion completeness. Recoveries of >95% are routinely achieved.

11.2. CALIBRATION PROCEDURE

11.2.1 Prepare reagent and standards as described in section 7.

11.2.2 Set up manifold as shown in section 17.

11.2.3 Input data system parameters as in section 17.

11.2.4 Pump DI water through all reagent lines and check for leaks and smooth flow. Switch to reagents and allow the system to equilibrate.

11.2.5 Place samples and/or standards in the autosampler. Input the information required by the data system, such as concentration, replicates and QC scheme. (See section 17)

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

11.3 SYSTEM NOTES

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

11.3.2 Glassware contamination is a problem in low level phosphorus determinations. Glassware should be washed with 1:1 HCl and rinsed with deionized water. Commercial detergents should rarely be needed but, if they are used, use special phosphate-free detergents for lab glassware.

11.3.3 Allow 15 minutes for the heating unit to warm up to 37°C.

11.3.4 If necessary, at end of a run place the color reagent and ascorbic acid transmission lines into the NaOH - EDTA solution (65 g NaOH + 6 g tetrasodium EDTA dissolved in 1L DI H_2O). Pump this solution for five minutes to remove any precipitated reaction products. Place these lines in water and pump for an additional five minutes. Then pump all lines dry.

11.3.5 If sample concentrations are greater than the high standard, the digested sample should be diluted with carrier (Reagent 4). When the automated diluter is used, carrier (Reagent 4) should be used. Do not dilute digested samples or standards with DI water.

12.0 DATA ANALYSIS AND CALCULATIONS

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

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

12.3 Report results in mg P/L.

13.0 METHOD PERFORMANCE

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

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

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

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

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

15.0 WASTE MANAGEMENT

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

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

16.0 REFERENCES

16.1 U.S. Environmental Protection Agency, Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983, Method 365.1, 365.3

16.2 Methods for Determination of Inorganic Substances in Water and Fluvial Sediments. Book 5. Chapter A1. U.S. Department of the Interior, U.S. Geological Survey, Method I-2601-78.

16.3 Lachat Instruments Inc., QuikChem Method 10-115-01-1-E written by W.R. Prokopy on 28 May 1992.

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

17.0 TABLE, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000

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

Sample throughput: 103 samples/h, 35 s/sample
Pump Speed: 35
Cycle Period: 35

Analyte Data:

Concentration Units: mg P/L
Peak Base Width: 28 s
% Width Tolerance: 100
Threshold: 2500
Inject to Peak Start: 10 s
Chemistry: Direct

Calibration Data:

Level	1	2	3	4	5	6	7
Concentration mg P/L	10.0	4.00	2.00	1.00	0.40	0.20	0.00

Calibration Rep Handling: Average
Calibration Fit Type: 2nd Order Polynomial
Weighting Method: None
Force through zero: No

Sampler Timing:

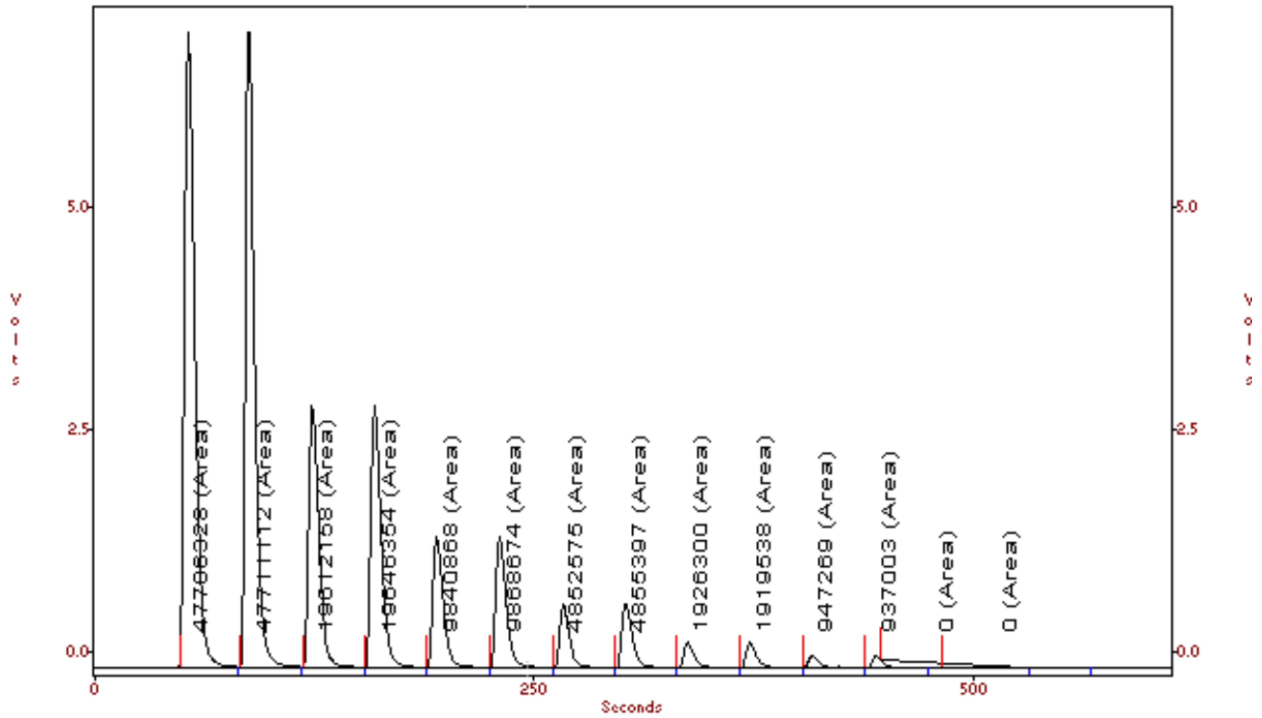
Min. Probe in Wash Period: 9 s
Probe in Sample Period: 20 s

Valve Timing:

Load Time: 0 s
Load Period: 15 s
Inject Period: 20 s

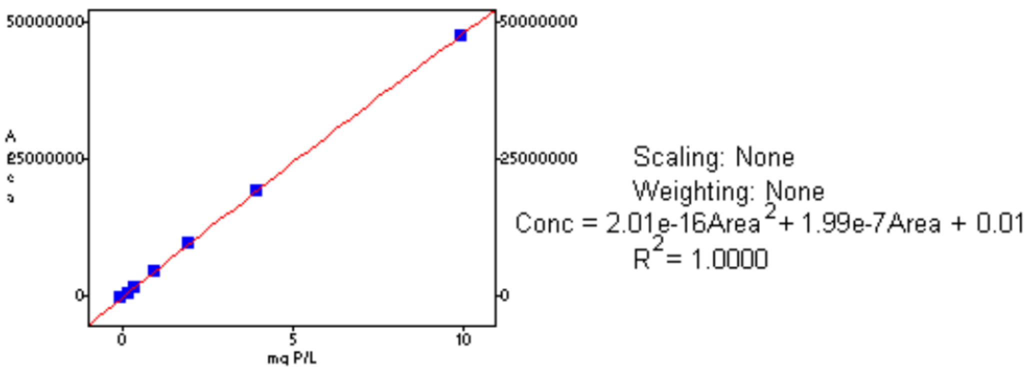
17.2 SUPPORT DATA FOR QUIKCHEM 8000

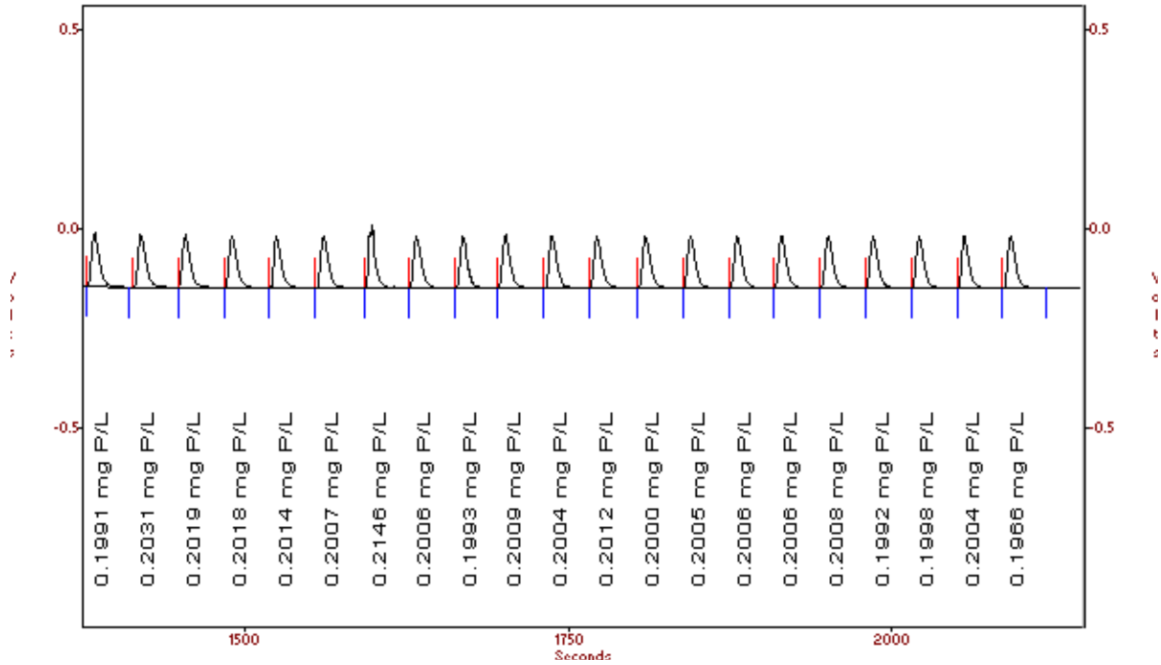
Calibration Data for Total Phosphorus



Calibration Graph and Statistics

Level	Area	mg P/L	Determined	Replicate %RSD	% residual
1	47748720	10.0	10.0	0.1	0.0
2	19629256	4.00	4.01	0.1	-0.2
3	9854771	2.00	2.00	0.2	0.1
4	4853986	1.00	1.00	0.0	1.4
5	1922919	0.40	0.40	0.2	0.8
6	942136	0.20	0.21	0.8	-0.3
7	0	0.00	0.01	0.0	0.0

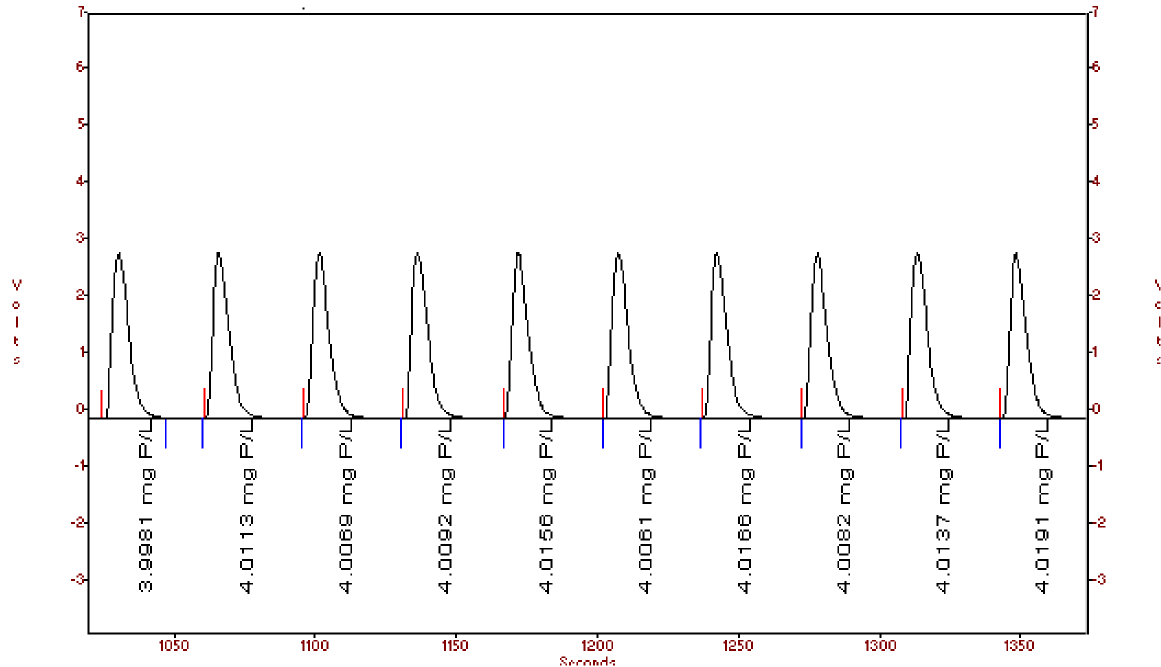




Method Detection Limit for phosphorus using 0.2 mg P/L standard

MDL= 0.097 mg P/L

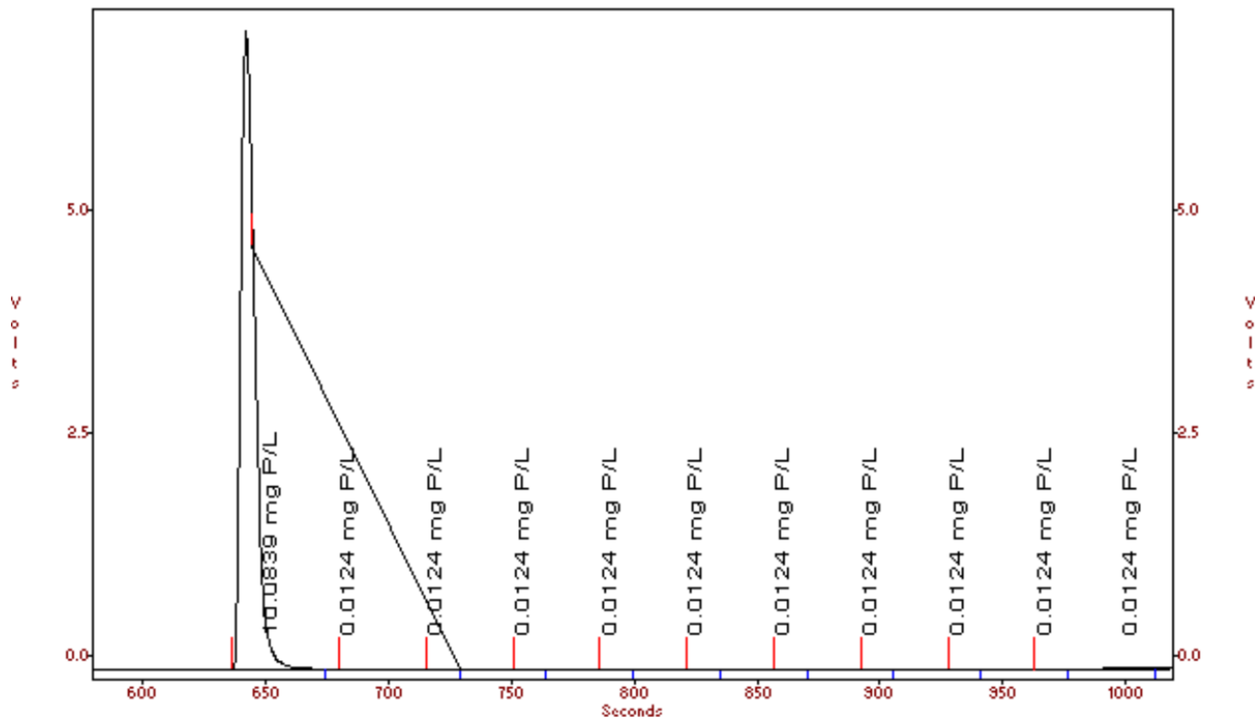
Standard Deviation (s) = 0.038 mg P/L, Mean (x) = 0.193 mg P/L, Known value = 0.2 mg P/L



Precision data for phosphorus using 4.0 mg P/L standard

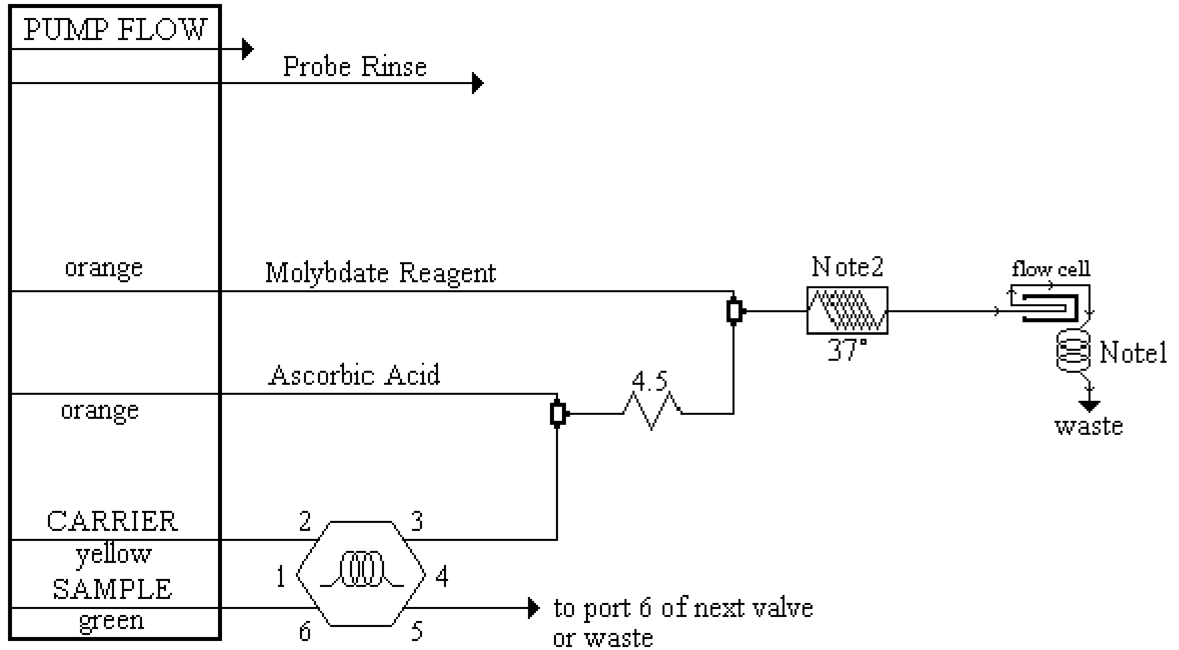
% RSD = 0.24

Standard Deviation (s) = 0.017 mg P/L, Mean (x) = 4.016 mg P/L, Known value = 4.0 mg P/L

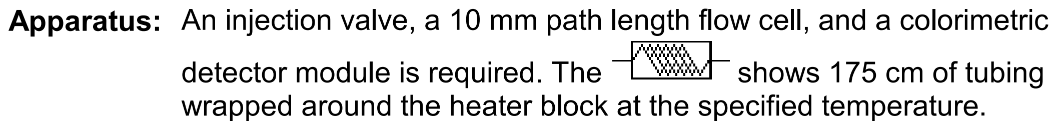


Carryover Study: 10.0 mg P/L standard followed by 10 blanks
Carryover Passed

17.3 TOTAL PHOSPHORUS MANIFOLD DIAGRAM



Carrier: **0.13 m sulfuric acid**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 μL/cm**.
AE Sample Loop: 15 cm
QC8000 Sample Loop: 20.5 cm
Interference Filter: 880 nm

Apparatus: An injection valve, a 10 mm path length flow cell, and a colorimetric detector module is required. The  shows 175 cm of tubing wrapped around the heater block at the specified temperature.

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

Note 1: 200 cm back pressure loop, 0.52 mm (0.22 in.) i.d.
Note 2: 175 cm of tubing on the heater.

Section III

APPENDIX

A. **References**

B. **Equipment and Instrumentation List**

C. **Table of Methodologies**

D. **Revisions**

A. REFERENCES

1. “Standard Methods for the Examination of Water and Wastewater.” 21st Edition. 2005. American Public Health Association, 800 I Street, NW, Washington, DC 20001-3710.

2. “Standard Methods for the Examination of Water and Wastewater.” 20th Edition. 1998. American Public Health Association, 101 18th Street, N.W., Washington, DC 20036.

3. “Standard Methods for the Examination of Water and Wastewater.” 18th Edition. 1992. American Public Health Association, 101 18th Street, N.W., Washington, DC 20036.

4. “State of Michigan DNR Laboratory Training Manual for Wastewater Treatment Plant Operators.” 1989

5. “Annual Book of ASTM Standards.” Water and Environmental Technology Section 11, Volume 11.01 & 11.02, 1985. 1916 Race Street, Philadelphia, PA 19103.

6. “Methods for Chemical Analysis of Water and Wastes” U.S. Environmental Protection Agency, Office of Research and Development, Water, Cincinnati, OH 45268, March 1983 (EPA-600-4-79-020).

7. “Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals.” 10th Edition. 1983.Merck & CO., Inc., Rahway, NJ.

8. Lange’s Handbook of Chemistry, 11th Edition

9. “HACH Water Analysis Handbook.” 1979, HACH Chemical Company, P.O. Box 389, Loveland, Colorado 80537.

10. “Manual for the Certification of Laboratories Analyzing Drinking Water”. 5th Edition, U.S. Environmental Protection Agency, Office of Ground Water & Drinking Water, Cincinnati, OH 45268, January, 2005 (EPA-815-13-97-001).

11. “State of Michigan DNR Laboratory Training Manual for Wastewater Treatment Plant Operators.” 2010.

12. “Colilert-18 Fecal Coliform Protocol Addendum.” 2018. IDEXX, One IDEXX Drive, Westbrook, Maine 04092.

B. EQUIPMENT AND INSTRUMENTATION LIST

Description	Manufacturer	Model No.	Cat. No.
Air compressor	Quincy	QR-25	
Analytical balance	Sartorius	R160P	
Automated Ion Analyzer	Lachat Instruments	8500	
Auto Temp Comp Probe	Orion		917002
Autochemistry system	Orion	960	
Autoclave-Steam sterilizer	Tuttnauer	2340E	
Automatic glassware washer	Miele	G7733	
Automatic sampler	American Sigma	704	
Automatic sampler	American Sigma	1600	
Barometer	Digital	469 NOVA	
Blender	Waring	34BL97	
Centrifuge	Dynac		0101
Circulating water bath	Precision Scientific	260	
Conductivity meter	YSI	35	
Conductivity probe	YSI	3402	
Desiccator	Labconco	55300	
Dissolved Oxygen meter	YSI	5100	
Dissolved Oxygen probe	YSI	5010	
Distillation unit	Buchii	321	
Drying oven	Baxter	DX41	
Drying oven	Fisher	106G	
Filter funnel manifold	Gelman		15402
Hot plate	Corning	PC 300	
Hot plate	Thermolyne	Cimerac 3	
Hot plate/ stir plate	Thermolyne	SP18425	
Impulse sealer	American Int'l Electric	AIE-200	
Incubator	Thelco	31706	
Incubator	Precision Scientific	815	
Membrane dispenser	Millipore		71AC000-6
Microscope	Reichert	310	
Microwave	CEM	MDS 81D	
Muffle furnace	Thermolyne Scientific	30400	
pH selective electrode	Orion	81-72 BN	
Printer for Orion 960	Seikosha	SP 2000	
Regent grade water system	Millipore	ZD40 115 84	
Refrigerator	Baxter	SLR512A	
Refrigerator	Kenmore	18	
Refrigerator	Lab-Line	FV19F5WXFB	
Scale	Sartorius	BP610	
Spectrophotometer	Hitachi	U-3110	
Stirrer	Corning	PC-353	
Stirrer	Lab Assistants	MR 200	
Stirrer	Phipps & Bird, Inc		5167
Stirrer	Troemner	500	
Timer	Fisher Scientific	14-649-15	
TOC Analyzer	Dohrman	DC-80	
UPS	TRIPP LITE	BC PRO 3-67S	
UV light	Blak-Ray	ULV-56	
Vacuum pump	Quincy	FR17	
Vortex mixer	Buchler Instruments	REAX 2000	
Water bath	Precision Scientific	Coli	66566
Water bath	Precision Scientific	184	
Water bath	Precision Scientific	186	

C. Methods for Analysis of Water and Wastewater Matrices

Parameter	EPA Method 3/93	Std. Meth 21st Ed.
Alkalinity	310.1	2320-B
Ammonia	350.3	4500-NH3-D
BOD	405.1	5210-B
Chloride	325.3	4500-Cl-C
Fecal Coliform		9222
Nitrate	352.1	4500-NO3-E
Nitrite	354.1	4500-NO2-B
pH	150.1	4500-H-B
Phosphorus, total	365.3	4500-P-E
Sulfate	375.4	4500-SO4-E
Suspended Solids	160.2	2540-D
Temperature	170.1	2550-B
TOC	415.2	5310-B
TRC	330.1	4500-Cl-D
Oil and Grease	1664	5520

Bray's Method—issued by MDNR (aka MDEQ)

Parameter	Lachat Instruments Method
Ammonia	QuikChem Method 10-107-06-1-J
Chloride	QuikChem Method 10-117-07-1-A
Nitrate/Nitrite	QuikChem Method 10-107-04-1-J
Phosphorus, total	QuikChem Method 10-115-01-1-E

D. Revisions

Signature	Date	Reason
s/Ronald Wintland	11/25/02	Updated Manual
WLN Supervisor II		
s/Ronald Wintland	12/04/02	Updated Manual
WLN Supervisor II		
	05/23/03	Updated Manual - 19th to 20th Edition
WLN Supervisor II		
WLN Supervisor I	11/23/04	Updated Manual – Pg. 58, 7.3
WLN Supervisor II	04/13/05	Added “Metals by Flame Atomic
		Absorption Spectrometry” section (pg 34-37). Changed front cover
Operations Engineer, Engineering Tech	09/12/06	Added Lachat Methods for P, NH_3, NO_2+NO_3 and CL
WLN Chemist	11/8/11	Updated manual- 21st Edition of Standard Methods accepted by EPA, updates to pH and Fecal Methods. Updated table of contents.
WLN Chemist	1/31/2019	Removal of discontinued procedures. Update to Total Coliform and E. Coli P/A procedure. Updated table of contents
WLN Chemist	2/15/19	Update of Total Coliform, Fecal, and E. Coli MPN procedure. Updated table of contents.