

# STANDARD OPERATING PROCEDURE

For

## USEPA METHOD 350.1

### Determination of Ammonia-Nitrogen by the Colorimetric-Automated Phenate Method

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SOP #: EPA 350.1

REVISION #: 1.2

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# MassDEP

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## Massachusetts Department of Environmental Protection

### Division of Environmental Analysis

Senator William X. Wall Experiment Station

37 Shattuck Street, Lawrence, MA 01843

Prepared by:	<u>Maria E. Ruiz</u> Maria E. Ruiz, Chemist	Date:	<u>November 2000</u>
Revised by:	<u>Maria E. Ruiz</u> Maria E. Ruiz, Chemist	Date:	<u>June 4, 2003</u>
Approved by:	<u>James H. Sullivan</u> James Sullivan, Laboratory Supervisor	Date:	<u>April 14, 2003</u>
Approved by:	<u>Robert Serabian</u> Robert Serabian, Quality Assurance Officer	Date:	<u>April 14, 2003</u>
Approved by:	<u>Oscar C. Pancorbo</u> Oscar Pancorbo, Director	Date:	<u>May 2, 2003</u>



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## LIST OF REVISIONS

Rev. #	Date	Description of Revision	Page #
0	November 2000	None	
1.0	October 2001	TABLE 1 – Updated	17
		TABLE 2 – Updated	17
		TABLE 3 – Renumbered to TABLE 4	18
		New Table 3	18
1.1	November 2002	Section 1.1 – “ground” added	5
		Section 2.1 – “and measured colorimetrically” added to first sentence	5
		Section 3.1 renumbered to 3.2, Section 3.2 through 3.12 renumbered to 3.4 through 3.14, new Section 3.1 and 3.3	5
		Section 5.6 – added	6
		Section 6.4 – new printer, Hewlett-Packard Deskjet 692C	8
		Section 7.7 – “to liter” changed to “to 1 L with reagent water”	8
		Section 7.13 – added	9
		Section 8.1 and 8.2 renumbered to 8.2 and 8.3, new Section 8.1 added	9
		Section 8.3 – “at 4°C” added	9
		Section 9.1 – “LRB” changed to “Continuing Calibration Blank”	9
		Section 9.2 – “A Continuing Calibration Standard (1.0 mg/L)” changed to “The Instrument Performance Check Solution (IPC - midrange check standard)”	9
		Section 13.3 – “three to five times” changed to “two to three times”	17
		Tables 1 – 4 updated and renumbered to Tables 2 – 5	18-20
		New Table 1 added	18
1.2	June 2003	Sections 14.3 and 15.1 were updated	17
		Table 2, 3, 4, and 5 values were updated	19 - 20



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## 1.0 SCOPE AND APPLICATION

- 1.1 This method covers the determination of ammonia-nitrogen in drinking, ground, surface, and saline waters, and in domestic and industrial wastes.
- 1.2 The applicable range of this method is 0.02 to 2.0 mg/L  $\text{NH}_3$  as N. The range may be extended with sample dilution.
- 1.3 It uses the principle of air-segmented continuous-flow analysis (CFA) for fully automatic sample analysis of liquid samples using wet-chemistry methods. Samples are mixed with reagents in a continuously flowing stream. The individual sample segments are kept separate by means of air bubbles.
- 1.4 Operates in STEADY STATE mode characteristic of segmented flow systems, where the absorbance of the reaction stream is not changing with time.

## 2.0 SUMMARY OF METHOD

- 2.1 Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured colorimetrically. A solution of EDTA is added to the sample stream to eliminate the precipitation of the hydroxides of calcium and magnesium.

## 3.0 DEFINITIONS

- 3.1 Calibration Blank (CB) – A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes.
- 3.2 Calibration Standard (CAL) – A solution prepared from the dilution of stock standard solutions or from the primary dilution standard solution. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.
- 3.3 Instrument Performance Check Solution (IPC) – A solution of one or more method analytes used to evaluate the performance of the instrument system with respect to a defined set of criteria.
- 3.4 Laboratory Duplicates – Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures.
- 3.5 Laboratory Fortified Sample Matrix (LFM) – An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory.
- 3.6 Laboratory Reagent Blank (LRB) – An aliquot of reagent water that is treated exactly as a sample. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, reagents, or apparatus.
- 3.7 Method Detection Limit (MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte is greater than zero.
- 3.8 Standard Reference Material (SRM) – A solution of method analyte of known concentrations, that is obtained from a source external to the laboratory and different from the source of calibration standards.



- 3.9 Laboratory Fortified Blank (LFB) – An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory.
- 3.10 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.
- 3.11 CAS – Chemical Abstract Service.
- 3.12 STORET – Storage and Retrieval of Water-Related Data.
- 3.13 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.
- 3.14 Linear Calibration Range (LCR) – The concentration range over which the instrument response is linear.

#### **4.0 INTERFERENCES**

- 4.1 Sample turbidity and color may interfere with this method. Turbidity must be removed by filtration prior to analysis. Sample color that absorbs in the photometric range used will also interfere.
- 4.2 Calcium and magnesium ions may be present in concentration sufficient to cause precipitation problems during analysis. EDTA solution is used to prevent the precipitation of calcium and magnesium ions from river water and industrial waste. For seawater, a sodium potassium tartrate solution is used.

#### **5.0 SAFETY**

- 5.1 Standard laboratory protective clothing and eye covering is required.
- 5.2 Acidification of samples should be done in a fume hood.
- 5.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.
- 5.4 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations.
- 5.5 All work is performed under a fume hood.
- 5.6 The following chemicals have the potential to be highly toxic or hazardous, consult MSDS.
- 5.6.1 Sulfuric acid
  - 5.6.2 Phenol
  - 5.6.3 Sodium nitroprusside

#### **6.0 EQUIPMENT AND SUPPLIES**

- 6.1 Analytical Balance – Capable of accurately weighing to the nearest 0.0001 g.



- 6.2 AutoAnalyzer 3 BRAN+LUEBBE – Continuous-flow analytical system consisting of the following main components:
- 6.2.1 Compact Sampler – BRAN + LUEBBE: A space-saving random access sampler with 100-cup capacity.
  - 6.2.2 AA3 2-Channel Digital Colorimeter – BRAN + LUEBBE: The detector module for the AA3 system as well as the control electronics for all AA3 modules. Colorimeter equipped with a 1.0-mm diameter and 10-mm path length flow cell (vol. 8  $\mu$ L) and 660-nm filter. The standard detector is a high-sensitivity photometer.
  - 6.2.3 AA3 Pump IV: A high-precision peristaltic pump that pumps samples, reagents, diluents, and air bubbles through a series of flexible pump tubes. By a steady process of first squeezing and then relaxing pressure on the tubes, the fluids are drawn into the system and pushed forward through the components on the Chem Tray until they arrive in the flowcell of the Digital Colorimeter. The air bubbles required to divide the sample and reagent streams into equally spaced segments are generated by a **valve module** that provides space for 4 air lines (silicone tubes).
  - 6.2.4 AA3 Chemistry Module: Contains all the components required for the reaction, e.g., mixing coils and heating bath at 37°C. The components for one method are mounted on a so-called Chem Trays and can be used to determine two parameters simultaneously.
- 6.3 PREMIO Computer, Inc., Model: PREMIO MTP 6C-PENTIUM II Mid-Tower.
- 6.3.1 Operating system: Windows 95.
  - 6.3.2 Control software: AACE – A BRAN + LUEBBE dedicated continuous-flow software package that controls the AA3 modules.
- 6.4 Printer: Hewlett Packard Deskjet 692C
- 6.5 All labware is washed with a detergent solution, rinsed with tap water, and prior to use, washed with H<sub>2</sub>SO<sub>4</sub> and rinsed with ASTM Type I reagent water.

## 7.0 REAGENTS

- 7.1 Alkaline Phenol: Add 83 g (approx. 78.4 mL) of liquified phenol to about 800 mL of reagent water. In small increments, cautiously add with agitation, 96.0 g (approx. 71.4 mL) of sodium hydroxide solution (50% w/w). Cool to room temperature under water faucet, dilute to one liter with reagent water, and mix thoroughly. Store in an amber glass container. This solution is corrosive. **STABILITY: TWO WEEKS.**
- 7.2 Sodium Hypochlorite Solution: Dilute 82 mL of sodium hypochlorite solution (5%) to 100 mL with ASTM Type I reagent water and mix thoroughly. Commercially available bleach is 5.25 % active. **STABILITY: ONE WEEK.**
- 7.3 Sodium Nitroprusside Solution: Dissolve 1.1 g of sodium nitroprusside in about 600 mL of ASTM Type I reagent water. Dilute to one liter with ASTM Type I reagent water and mix. **STABILITY: ONE MONTH.**



- 7.4 Disodium EDTA: Dissolve 25 g (approx. 18.6 mL) of 50% (w/w) sodium hydroxide and 41.0 g of disodium EDTA in about 800 mL of ASTM Type I reagent water. Dilute to one liter. Add 3 mL (60 drops) of Brij-35 and mix well.
- 7.5 Complexing Reagent - Potassium Sodium Tartrate: Used for the analysis of seawater samples. Dissolve 33.0 g of potassium sodium tartrate and 24.0 g of sodium citrate in 950 mL of ASTM Type I reagent water. Adjust the pH of this solution to 5.0 with concentrated sulfuric acid. Dilute to 1 liter with ASTM Type I reagent water. Add 0.5 mL (10 drops) of Brij-35.
- 7.6 Stock Standard  $\text{NH}_3\text{-N}$ : Dissolve 3.8190 g of  $\text{NH}_4\text{Cl}$ , oven dried at 100-105° C for 24 hours, in ASTM Type I reagent water and dilute to 1 liter. Add 1 mL of chloroform ( $\text{CHCl}_3$ ) as a preservative. 1.0 mL = 1.0 mg  $\text{NH}_3\text{-N}$
- 7.7 Standard  $\text{NH}_3\text{-N}$  Solution (100 mg/L): Dilute 100 mL of stock standard  $\text{NH}_3\text{-N}$  (See Section 7.6) to 1 liter with ASTM Type I reagent water. 1.0 mL = 0.1 mg  $\text{NH}_3\text{-N}$

**NOTE:** This standard is prepared combined with  $\text{NO}_3\text{-N}$

- 7.8 Preparation of Calibration Standards: Using the standard  $\text{NO}_3\text{-N}/\text{NH}_3\text{-N}$  solution (See Section 7.7), prepare the following calibration standards in 100-mL volumetric flasks: 10, 2.0, 1.5, 1.0, 0.5, 0.1, and 0.05 mg/L (prepare 100 mL of each).

10.0 mg/L: 10 mL of 100 mg/L, QS to 100 mL with ASTM Type I reagent water.

2.00 mg/L: 2 mL of 100 mg/L, QS to 100 mL with ASTM Type I reagent water.

1.50 mg/L: 15 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water.

1.00 mg/L: 10 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water.

0.50 mg/L: 5 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water.

0.10 mg/L: 10 mL of 1 mg/L, QS to 100 mL with ASTM Type I reagent water.

0.05 mg/L: 5 mL of 1 mg/L, QS to 100 mL with ASTM Type I reagent water.

Calibration blank

0.02 mg/L: 0.2 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water (used as MDL standard).

0.40 mg/L: 4 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water (used as LFB).

0.06 mg/L: 0.6 mL of 10 mg/L, QS to 100 mL with ASTM Type I reagent water (used as RDL standard)

- 7.9 Reagent Water: ASTM Type I reagent-grade water. NOTE: For seawater analysis, use artificial seawater as diluent for the standards and the sampler wash solution.

#### Artificial Seawater

31 g Sodium chloride

5 g Magnesium chloride pentahydrate





7 g Magnesium sulfate heptahydrate

1.6 g Calcium chloride dihydrate

0.2 g Sodium hydrogen carbonate

ASTM Type I reagent water to 1000 mL

Dissolve 31 g of sodium chloride, 5 g of magnesium chloride, 7 g of magnesium sulfate, 1.6 g of calcium chloride, and 0.2 g of sodium hydrogen carbonate in about 900 mL of reagent water. Dilute to one liter with reagent water and mix thoroughly.

7.10 System Wash Solution: Use ASTM Type I reagent water containing 2 mL/L Brij-35, 30% solution.

7.11 Special Wash Solution: Use a 1-N HCl solution (about 83 mL/L conc. hydrochloric acid). **NOTE:** Sampler wash solution does not contain wetting agent.

7.12 Brij-35

7.13 Sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) for preservation of samples.

## 8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Samples should be collected in plastic or glass bottles.

8.2 If analysis can be performed within 24 hours, samples should be preserved by refrigeration at 4° C. When samples must be stored for more than 24 hours, they should be preserved with 2 mL of sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) per liter and refrigerated.

8.3 Maximum holding time is 28 days for preserved samples (pH < 2 at 4°C).

## 9.0 QUALITY CONTROL

9.1 A Continuing Calibration Blank is run after calibration, after each batch of samples, and at the end of the run.

9.2 The Instrument Performance Check Solution (IPC - a midrange check standard) is run after calibration, every 10 samples, and at the end of the run.

9.3 Two QCS are analyzed after calibration and at the end of the run. Reading must be within ± 10%.

9.4 Duplicates and LFM's are run every 10 or fewer samples.

9.5 A LFB is analyzed with each batch of samples analyzed.

9.6 An MDL standard is analyzed with each run.

9.7 A RDL standard is analyzed with each run.

## 10.0 CALIBRATION AND STANDARDIZATION

10.1 Remove reagents, 100 mg/L NO<sub>3</sub>-N and NH<sub>3</sub>-N combined stock standard, QCS, and samples from refrigerator. Prepare reagents as needed. Prepare working standards.



- 10.2 Rinse diluent-wash solution flask with reagent water and refill with reagent water and BRIJ –35\* (30% solution) from BRAN + LUEBBE, Part # T21-0110-04 (15 drops of BRIJ per 500 mL of reagent water). Place tubing into diluent-wash solution flask again.
- 10.3 Make sure waste line tubes are placed in container.
- 10.4 Latch plate to compress manifold tubing by engaging the hooks with the holding pins. Raise the cover plate away from the platen until it is fully open and then press the cover plate down until the latches lock. Then lower the cover plate onto the top of the platen. Set the power switch at the side of the pump to position **on**. Set the **red** switch on top of the pump to position **run** and the **black** switch to position **normal**. The rollers will start rotating, and the status LED on top of the pump will light up in green.
- 10.5 Switch on the power to the computer, monitor, printer, and analyzer modules (sampler and colorimeter with cover on); pump reagent water + wetting agent through the reagent lines.

**NOTE:** Sampler wash solution does not contain wetting agent.

- 10.6 When Windows 95 has started, click the **START** button in the lower left-hand corner of the screen and click **AACE**. After starting **AACE**, the Main Menu screen will be displayed. It contains the Menu bar, Toolbar, System Window, Desktop, and Taskbar.
- 10.7 Click on **CONFIGURE** menu to enter the configuration of your analyzing system and to choose software options. The entered configuration will influence the appearance of the menus when working with ACE.

**NOTE:** This is done in the first application to be prepared with AACE. You don't need to enter the system configuration daily, unless you change any setting.

- 10.8 **Programming the Analysis:** Select **SET UP-Analysis** in the Main Menu or click the SET UP Analysis tool button. For each application to be run on your Analyzer, create a separate Analysis in Set UP- Analysis, it will then be used as the template for the runs. The list on the **left** shows the **analyses (.ani)** that are contained in the AACE/Data directory. Each Analysis is stored in a separate subdirectory (folder). The list on the **right** shows the **Analysis and run files (.run)** belonging to the Analysis currently selected. Each run consists of a number of files with different extensions. The **\*.run** stores Main Page, Channel, and Tray Protocol information. Enter the following information to create an Analysis subdirectory or folder. Only the samples ID will be different after you create the Analysis for  $\text{NH}_3\text{N}\&\text{NO}_3\text{NO}_2\text{-N}$ .

- 10.8.1 **Analysis Main Page**, including general data, for example the sampling rate or sample and wash time.

Name of Analysis:  $\text{NH}_3\text{-N}\&\text{NO}_3\text{NO}_2\text{-N}$

Samples per Hour: 80

Sample/Wash: 6.0

Sample Time: 39

Wash time: 6.0

Method 1 EPA 350.1



Method 2 EPA 353.1

**Note:** A high sample to wash ratio gives more accurate results. The reason is that the steady-state plateau is increased, allowing a better average to be taken.

- 10.8.2 **Tray protocol** reflects the arrangements of the cups on the sample tray. On the left-hand side, the Tray Protocol shows a table with the following columns: peak, icon, type, cup, and sample ID. The Primer must be the first cup on the tray. It starts the peak window and must reach a certain peak height. The highest standard (2.0 mg/L) is used as Primer. The Primer acceptance criteria are set in the Peak Detection tab in **Configure-Software**. The concentration of the standards must be entered for each channel. In a run, their **peak height** is measured and sample concentrations are calculated by comparison of their peak height to that of the standards. The calibrants should come immediately after the Primer, in **descending** order of concentration (i.e., the highest standard first). On the right-hand side of the Tray Protocol are buttons to enter the cup types and their position on the sample tray.

P, 7C,3N,CARRYOVER SET,2QCS, 10S,...2N... 2QCS,2N,E

- 10.8.3 **Channel** - contains data related to each channel, such as method name, calibrator concentrations, and corrections.

Units: mg/L

Calibration Fit: Linear

Peak Window:

Start: 50 End: 105

Autobase Reference (%): 5

Calibrant concentrations: 2.000, 1.000, 0.5000, 0.1000, 0.1000, 0.0500,0.0000

Base Correction: NO

Drift Correction: NO

Carry Over Corr: Yes

- 10.9 When setting up an Analysis or run, you can generate a report. This can be useful before preparing the cups on the sample tray. At the bottom of the window, click **OK** to store the settings.
- 10.10 To select NH<sub>3</sub>-N&NO<sub>3</sub>NO<sub>2</sub>-N Analysis, double-click the Data folder and then double-click it. The Analysis and run files belonging to this Analysis will appear in the list on the right. Then continue in one of the following ways:
- 10.10.1 Click **NEW RUN** to create a new run based on the Analyses template.
- 10.10.2 Select a run file in the list and click **COPY RUN** to create a new run based on the selected run. Conveniently, only ID samples will be changed.



## 11.0 PROCEDURE

### 11.1 CHARTING allows you to:

- 11.1.1 View and print the charts to check if the baseline is stable before starting a run.
- 11.1.2 Check the reagent absorbance (once a week or whenever you use new reagents or pump tubes, or when you start an Analysis for the first time). See Section 11.14.
- 11.1.3 Adjust baseline and sensitivity (once a week or whenever you use new reagents or pump tubes, or when you start an Analysis for the first time). See Section 11.15.
- 11.1.4 Adjust the lamp intensity for AA3 Colorimeters.
- 11.1.5 Manually control the analyzer modules.
- 11.1.6 The cover of the Digital Colorimeter must be closed, because stray light could distort the dark current measurement which is carried out after the download.

### 11.2 To open Charting windows for the channels:

- 11.2.1 Click **CHARTING** in the System window on the AACE desktop, or
- 11.2.2 Click **RUN** in the Main Menu, then point to Chart. The lower left corner of the system window displays the Analysis that was last used for Charting or a run. When Charting is started, the Charting button in the System window will change to **Print**. This button allows you to print the charts up to the current moment or save the corresponding Charting values to a file. Make sure you print or save the charts before you stop Charting. The Charting values are only temporarily stored and will be deleted when the charts are closed. Click the **right** mouse button in a Charting window to access the following options:
  - 11.2.3 **Set Base:** Select this option to automatically adjust the baseline reading to the value defined in the Run Chart tab in **Configure- Software**. The default is **5%**.
  - 11.2.4 **Set Gain:** Select this option to automatically adjust the Gain value while the highest standard is in the flow cell. The Gain value (95%) is adjusted to the Primer Height value entered in the Run Chart tab in **Configure-Software**.
  - 11.2.5 **Auto Lamp:** Click this option to automatically adjust the intensity of the lamp in the AA3 colorimeter. When you adjust the lamp intensity, you should check if the value on the scale considerably increases and/or the Reference value (see below) falls under 80%. This can be an indication that the lamp needs to be replaced. The values are shown as % of the 2<sup>21</sup>A/D converter resolution of the photometer. To manually set Base and Gain values and lamp intensity, double-click the Channel icon for the desired colorimeter channel while Charting is running.

### 11.3 During Charting, each Charting window contains the following elements:

- 11.3.1 A scroll bar at the top to move forwards and backwards through the chart.
- 11.3.2 Horizontal lines representing the percent full scale and vertical lines representing the minutes.



- 11.3.3 A blue line representing the chart reading over time. The current value is displayed in the upper-right corner.
- 11.3.4 An indicator bar (bar graph) on the right. The following colors are used:
- Yellow** if the current reading is below the baseline level of **5%**
- Green** if the reading is between baseline and primer level of **95%**.
- Red** if the reading is above the primer level.
- 11.4 Check the water baseline; it should be stable and flat. Check bubble pattern in all lines, especially flow cell waste line. If the bubbles are the wrong shape, in most cases there is insufficient wetting agent, or tubing is wrong sort or contaminated.
- 11.5 Check or adjust baseline, reagent absorbance and sensitivity when necessary.
- 11.6 Place the reagent lines from the wash receptacle into the reagent containers. Allow the reagent stream and bubble pattern to stabilize.
- 11.7 To close the Charting windows and exit the **Chart** option, you have the following possibilities:
- 11.7.1 Click **Stop** in the System window on the AACE desktop, or
- 11.7.2 Click **Run** in the Main Menu, then point to Stop and click the system you want, or
- 11.7.3 Click the Close button in each Charting window to stop each channel individually.
- 11.8 **Start A Run:** With the system pumping reagents and the sampler tray loaded according to the tray protocol, observe the bubble pattern and make any other final checks that may be necessary before starting the run. Click **Run** in the Main Menu, then point to **Start** and click the system you want, or in the System window on the desktop, click **Run**. The appearing dialog box shows the Analysis folder currently selected on the left and the runs belonging to this Analysis on the right. Usually the software measures the baseline for 3 minutes or less for a stable baseline and calculates the deviation. The Status line displays message, for example "Looking for stable baseline" or "Autobase in progress". If the baseline is stable, the actual run will start and the sample probe will move to the cups as specified in the tray protocol. If the baseline stays noisy, the run will not start. After starting a run, a run chart will appear for each selected channel.
- 11.9 Each Run Chart window contains the following elements:
- 11.9.1 **Window title** displaying the channel number, the system number, and the method name.
- 11.9.2 **Scroll bar** at the top to move forwards and backwards through the chart.
- 11.9.3 **Force Baseline tool button.**
- 11.9.4 **Calibration Curve tool button:** This tool button appears when the calibration has been completed. If you click it, another window will open, showing the calibration curve for this channel.
- 11.9.5 **Peak symbol:** The peak symbol appears when the Primer has been found and the cups are being analyzed. The peak turns red during each time the peak window is



active – i. e., when the peak-picking program looks for the actual peak. This time interval can be adjusted for each channel.

- 11.9.6 **Result field** displaying the number, the type, and the uncorrected concentration of the cup that was last analyzed.
- 11.9.7 **Window pane with the chart.**
- 11.10 The chart itself shows:
  - 11.10.1 A grid of horizontal lines representing the percent full scale and vertical lines representing the minutes.
  - 11.10.2 A blue line representing the chart reading over time.
  - 11.10.3 Green markers in the chart showing the approximate place where the program takes a peak.
  - 11.10.4 An indicator bar on the right if **View Bargraph** was activated during Charting.
  - 11.10.5 When the chart completely fills the window, it will automatically move to the left.
- 11.11 A run will stop automatically when all the samples have been analyzed and the final baseline as soon as it is steady is taken. The window with the curve will disappear, and a message saying that the run was successfully completed will appear instead. Automatically, a report corrected results for standards and samples, charts, and calibration curves are printed out as selected in the **Configure Software- Post Run**.
- 11.12 The Retrieve Menu: After the completion of a run, you can view and print charts, calibration curves, reports, and raw data of a run using the Retrieve option in the Main Menu if necessary.
- 11.13 System Shutdown: The following describes the steps that are required if the pump will be out of operation for more than an hour – e.g., overnight:
  - 11.13.1 Place the reagent lines and the sampler probe into wash solution.
  - 11.13.2 Set the pump to fast speed and pump wash solution through the complete system for a few minutes until the tubing is clean.
  - 11.13.3 Set the **red** switch to position **stop**.
  - 11.13.4 Remove the pump platen to relieve pressure on the pump tubes.
  - 11.13.5 Set the power switch at the side of the pump to position **off**.
  - 11.13.6 Switch off all AA3 modules.
  - 11.13.7 To exit AACE, select File-Exit in the Main Menu.
  - 11.13.8 To shut down the computer, click the **START** button in the lower left-hand corner of the screen, and then click SHUT DOWN.
- 11.14 Reagent Absorbance Check – **Repeat this procedure for each channel.**



- 11.14.1 With the system pumping reagent water+wetting agent and Charting windows displayed on the screen, wait until the water baseline is stable and the bubble pattern is regular.
- 11.14.2 Double-click the Channel icon in the System window to open the Base&Gain dialog box and set the Gain value to **10**. This sets full scale absorbance to 1.
- 11.14.3 Click the right mouse button in the chart and select Auto lamp from the local menu to adjust the lamp intensity. The Reference channel should reach 80%. If not, the lamp needs to be replaced.
- 11.14.4 Click the right mouse button in the chart and select **Auto Base** from the local menu. This will adjust the baseline to the Chart % entered in **Configure-Software-Run Chart** to **5%**.
- 11.14.5 Write down the baseline reading in % as indicated in the chart.
- 11.14.6 Introduce reagents into the system and wait until they arrive in the flowcell.
- 11.14.7 When the reagent baseline is stable, write down the new reading.
- 11.14.8 Calculate the reagent absorbance as follows:

$$\text{reagent absorbance relative to water} = \frac{\text{reagent baseline (\%)} - \text{water baseline (\%)}}{100\%}$$

- 11.14.9 Check that the calculated reagent absorbance relative to water is  $\pm 15\%$  of the value specified in the method description.

Reagent absorbance for  $\text{NH}_3\text{-N}$  = 0.03 – 0.04 AU

Reagent absorbance for  $\text{NO}_3\text{-N}$  = 0.02 AU

- 11.14.10 If the calculated value exceeds this range, substitute each reagent and the wash solution to check for contamination.
  - 11.14.11 Click **OK** to close and save the new settings or **Cancel** to close it and restore the old settings.
- 11.15 Sensitivity Check – **Repeat this procedure for each channel:**

- 11.15.1 Make sure the system is pumping reagents, the baseline is stable and Charting windows are displayed on the screen.
- 11.15.2 Double-click the Channel icon in the System window and set the Gain value to **10**. This sets full scale absorbance to 1.
- 11.15.3 Click the right mouse button in the chart and select **Auto Base** from the local menu. This will adjust the baseline to the Chart % entered in **Configure-Software-Run Chart** to **5%**. Write down the baseline reading in % as indicated in the chart.





- 11.15.4 Aspirate a full-scale standard (should be a mixed standard if is used for more than a channel) into the sample probe.
- 11.15.5 Double-click the **Sampler** icon in the System window; enter the cup position containing the top standard (2.0 mg/L) under CUP Position and click Sample. Wait until the standard reaches the flowcell, and then check for noise and drift.
- 11.15.6 Note signal value in % and calculate the Sensitivity (also called Absorbance)

$$\text{Sensitivity} = \frac{(\% \text{ Std.} - \% \text{ Baseline})}{100\%}$$

- 11.15.7 Check that the calculated sensitivity is  $\pm 15\%$  of the value specified in the method description.

Sensitivity at 2.0 mg/L  $\text{NH}_3\text{-N}$  = 0.10 - 0.20 AU

Sensitivity at 2.0 mg/L  $\text{NO}_3\text{-N}$  = 0.40 AU

If the sensitivity is not correct, check if reagents are OK and if the heating bath temperature is correct. For  $\text{NH}_3$ , if chlorine reagent is OK. For  $\text{NO}_3$ , if reduction stage is OK.

## 12.0 DATA ANALYSIS AND CALCULATIONS

- 12.1 Direct measurement of ammonia-nitrogen is made using linear regression analysis. Sample data are reported in mg/L.
- 12.2 If dilution was needed, multiply READING by DILUTION FACTOR.

## 13.0 METHOD PERFORMANCE

- 13.1 Participate in annual proficiency test (PT) studies.
- 13.2 Precision and Accuracy Quality Control Charts are reviewed monthly.
- 13.3 MDL is determined annually or when a new operator begins work. Using reagent water fortified at a concentration of two to three times the estimated instrument detection limit, take seven replicates and process through the entire analytical method. Calculate the MDL as follows:

$$\text{MDL} = (\text{SD}) \times (t)$$

t = students' t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [ t = 3.14 for seven replicates ].

SD = standard deviation of the seven analyses.

## 14.0 POLLUTION PREVENTION

- 14.1 The quantity of chemicals purchased should be based on expected usage during its shelf life.





Massachusetts Department of Environmental Protection  
Division of Environmental Analysis  
Senator William X. Wall Experiment Station  
37 Shattuck St., Lawrence, MA 01843

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14.2 Actual reagent preparation volumes should reflect anticipated usage and reagent stability.

14.3 All chemical wastes are collected until removed for disposal by a licensed hazardous waste contractor.

## **15.0 WASTE MANAGEMENT**

15.1 Refer to the WES Environmental Management System (EMS) policy and SOPs regarding waste management.

## **16.0 REFERENCES**

16.1 U.S. EPA. 1978. Methods for Chemical Analysis of Water and Wastewater, Rev 1978.

16.2 Bran+Luebbe. AutoAnalyzer 3 Operation Manual & Methods.



## 17.0 TABLES AND VALIDATION DATA

**TABLE 1. Quality Control Elements and Acceptance Limits for EPA Method 350.1 – Determination of Ammonia-N by Colorimetric-Automated Phenate Method**

QC Elements	Frequency	Acceptance Criteria	Corrective Action
Initial Calibration	Every sample batch	Correlation > 0.99	Recalibrate
Calibration Blank	At the beginning, after calibration, at a 10% frequency, and at the END of the batch	< RDL	Check laboratory process.
IPC	After calibration, at a 10% frequency, and at the END of the batch	± 10% Recovery	Reanalyze. If the second analysis is outside the limits, sample analysis must be discontinued. Recalibrate
Laboratory Reagent Blank	At least one with each batch	< RDL	Check laboratory process
Quality Control Sample (QCS)	After calibration and at the END of the run	± 10% Recovery	Performance of the determinative step of the method is unacceptable
Laboratory Duplicates	For a minimum of 10% samples or at least one with every analysis batch.		
Laboratory Fortified Sample Matrix (LFM)	For a minimum of 10% samples or at least one with every analysis batch. At the same conc. used to prepare the LFB (should not be less than four times the MDL).	± 10% Recovery	Either matrix or solution related if the performance for that analyte is shown to be in control. If necessary, increase the amount used for spike and reanalyze.
Laboratory Fortified Blank (LFB)	One for batch, at the same conc. used to prepare the LFM.	± 10% Recovery	Should be identified and resolved before continuing analyses.
Method Detection Limit (MDL)	Annually or a new operator.		



**TABLE 2. Accuracy of Ammonia-Nitrogen Analysis in Water Samples by EPA Method 350.1**

Date of Study	Based on Sample Numbers	Accuracy (% Recovery)					
		Mean	SD <sup>a</sup>	Warning Limits ( $\pm 2$ SD)		Control Limits ( $\pm 3$ SD)	
				Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)
04-08-2003	L2002356-25 to L2003022-3	101	6.53	114	88.0	121	81.5
<sup>a</sup> SD = Standard deviation							

**TABLE 3. Precision of Ammonia-Nitrogen Analysis in Water Samples by EPA Method 350.1**

Date	Based on Sample Numbers	Precision (RPD) <sup>b</sup>					
		Mean	SD <sup>a</sup>	Warning Limits		Control Limits	
				Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)
04-08-2003	L2001145-3 to L2003022-3	7.86	8.04	23.93	0	32.0	0
<sup>a</sup> SD = standard deviation. <sup>b</sup> RPD = Relative percent difference							



**TABLE 4. Accuracy of Ammonia-Nitrogen Analysis in Reagent Water (LFB) by EPA Method 350.1**

Date of Study	Based on Dates	Accuracy (% Recovery)					
		Mean	SD <sup>a</sup>	Warning Limits ( $\pm 2$ SD)		Control Limits ( $\pm 3$ SD)	
				Upper (UWL)	Lower (LWL)	Upper (UCL)	Lower (LCL)
04-08-2003	7-17-2002 to 3-5-2003	103	6.27	116	90.5	122	84.3
<sup>a</sup> SD = Standard deviation							

**TABLE 5. Method Detection Limit (MDL) for Ammonia-N in Reagent Water by EPA Method 350.1**

Date of Study	No. of Samples Spiked (n)	Spiked Concentration (mg/L)	Accuracy (Mean % Recovery <sup>a</sup> )	Precision (SD <sup>b</sup> in mg/L)	MDL <sup>c</sup> (mg/L)
04-14-2003	7	0.09	107	0.005	0.02
<sup>a</sup> Recovery of spiked concentration <sup>b</sup> SD = standard deviation of mean concentration measured <sup>c</sup> Method Detection Level (MDL) is 0.02 mg/L. DEA-WES Reporting Detection Level (RDL) is 0.06 mg/L.					