Arikaree Environmental Laboratory
Revision 1
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NH_4(N) and Soluble Reactive Phosphorus by FIA

Title: Lachat Quickchem Method 10-107-06-1-A
Lachat Quickchem Method 10-115-01-1-Q

ANALYTES: NH_4(N), PO_4(P)

1) Applicable Matrices

a) This method is applicable to ground, surface, KCl extract samples, and waste waters**.**

b) This method is applicable for ammonium in the range from 0.005mg/L N to 2.000mg/L N

c) This method is applicable for soluble reactive phosphorus in the range from 0.0004mg/L P to 0.2000mg/L P.

2) Scope and Application

a) Ammonia reacts the alkaline phenol, then with hypochlorite to form indophenol blue. Sodium nitroprusside enhances sensitivity. The absorbance is measured at 630nm and is directly proportional to the ammonia concentration in the sample.

b) Orthophosphate reacts with ammonium molybdate and antimony potassium tartrate in acidic conditions to form a complex. The complex is reduced using ascorbic acid to form a blue color which absorbs light at 880nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

3) Interferences

Ammonia

1.) Calcium and magnesium may precipitate if in high concentrations. EDTA is added to mitigate this problem

2.) Color, turbidity and certain organic species may interfere. Filtration removes turbidity.

4) Equipment and Supplies

a) Analytical balance capable with 0.0001g sensitivity.

b) Top loading balance with 0.01g sensitivity.

c) Pipets capable of precisely dispensing1.0-10.0mL and 0.1-1.0mL

d) Pipet tips appropriate for each pipet.

e) Class A volumetric flasks and pipets for reagent and standard preparation.

f) Lachat 8500 flow-injected autoanalyzer with Ammonia(N) manifold, and SRP manifold,

10.00mm flow cells, and interference filter at 630 nm for ammonia and 880nm for SRP.

g) Water purifier for preparation of ASTM Type I water (Ultrapure).

h) Various Class A volumetric pipets for dilution of calibration standards.

5) Reagents and Standards

Ammonia

1,000ppm NH_4(N) Stock Standard

1.) In a 1,000mL class A volumetric flask, dissolve 3.8190g NH_4Cl primary standard that has been dried @ 110°C for 2 hours, and allowed to cool in a desiccator. Do not add stir bar to flask, instead swirl solution to completely dissolve NH_4Cl. Add 1mL conc. sulfuric acid for preservation. Dilute to 1L mark, and invert to mix. Store in 1L Nalgene. Refrigerate.

1,000ppm PO_4(P) Stock Standard

1.) In a 1,000mL class A volumetric flask, dissolve 4.3936g of anhydrous potassium phosphate monobasic primary standard which has been dried at 105°C for 1 hour. Do not use a stir bar to mix solution, instead swirl flask contents manually by swirling the contents. After the crystals have dissolved, preserve the standard by pipetting 1mL of conc. sulfuric acid into the flask. Carefully dilute to 1L mark on the flask, replace stopper, and invert to mix. Store in 1L Nalgene and refrigerate.

NH_4/Reactive Phosphorus Intermediate Standard

1.) In a 1,000mL class A volumetric flask, using class A volumetric pipets, dilute 20.0mL of 1,000ppm NH_4(N) Stock Standard and 2.0mL of 1,000ppm PO_4(P) Stock standard. Preserve standard by pipetting 1mL of conc. sulfuric acid into flask. Carefully dilute to the 1L mark, replace stopper, and invert to mix. Store in 1L Nalgene and refrigerate.

NH_4/Reactive Phosphorus Matrix Spike Solution

1.) Prepare a 10mg/L NH_4^+(N)/1.0mg/L PO_4^{3-}(P) matrix spike solution as follows:

Pipet 50.0mL of NH_4^+/Reactive Phosphorus Intermediate Standard into a class A 100mL volumetric flask. Preserve matrix spike solution by pipetting 0.1mL of conc. sulfuric acid into flask. Dilute to the mark, replace the stopper and invert to mix. Store in a 100mL Nalgene bottle, and refrigerate.

Stock Ammonium Molybdate Solution

1.) In a 1,000mL volumetric flask with a stir bar, dissolve 40.0g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ in ~500mL of ultrapure water. Remove the stir bar, dilute to the mark and invert to mix. Store in 1L Nalgene container, record date prepared and refrigerate. Stable for 6 months.

(Reagents and Standards continued)

Stock Antimony Potassium Tartrate Solution

1.) In a 1,000mL volumetric flask with a stir bar, dissolve 3.0g antimony potassium tartrate $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ in ~500mL of ultrapure water. Remove the stir bar, dilute to the mark and invert to mix. Store in a brown 1L Nalgene container and refrigerate. Stable for 6 months.

Molybdate Color Reagent

1.) In a 500mL volumetric flask, using graduated cylinders, dissolve 17.5mL conc. sulfuric acid, 36mL antimony potassium tartrate stock solution, and 106.5mL ammonium molybdate solution. Dilute to the 500mL mark, replace stopper, and invert to mix. Degas with helium for 5 minutes.

Ascorbic Acid Reducing Solution

1.) In a 1,000mL volumetric flask, dissolve 60.0g ascorbic acid. Dilute to the mark and invert to mix. Transfer solution into bottle. Degas solution for 5 minutes with helium. Add 0.5g sodium lauryl sulfate (dodecyl sulfate) to solution. Store in 1L Nalgene. Discard after 1 week. Discard if yellow color develops

Ammonium Buffer Solution

1.) In a 1,000mL volumetric flask, dissolve 50.0g ethylenediamine tetraacetic acid, disodium salt (Na_2EDTA), and 9.0g sodium hydroxide (NaOH). Using a magnetic stir bar in the flask, stir until all crystals are dissolved. Rinse and remove stir bar from flask, and dilute to the 1L mark. Replace stopper and invert to mix. Store in 1L Nalgene. Degas with helium for 5 minutes.

Sodium Nitroprusside

1.) In a 1,000mL volumetric flask, dissolve 3.50g sodium nitroprusside (AKA sodium nitroferricyanide) using a magnetic stir bar in the flask. Once crystals have completely dissolved, rinse and remove stir bar. Dilute to the 1L mark with ultrapure water. Replace stopper and invert to mix. Store in brown Nalgene. Degas with helium for 5 minutes.

Sodium Hypochlorite Solution

1.) In a 500mL volumetric flask, dilute 250mL of 5.25% sodium hypochlorite (bleach). Dilute to 500mL mark. Replace stopper and invert to mix. Degas with helium for 5 minutes. Prepare fresh before each analytical run.

Sodium Phenolate

1.) Wearing proper personal protective equipment (lab coat and gloves), in a 500mL volumetric flask, dissolve 41.5g crystalline Phenol, and 16.0g sodium hydroxide (NaOH). Stir with a stir bar in the flask until completely dissolved. Dilute to 500mL mark, replace stopper and invert to mix. Store in 500mL Nalgene. Degas with helium

for 5 minutes. **WARNING: Phenol burns on contact with skin! Take special care when handling!**

Carrier Solution

1.) Fill a clean 1L Nalgene to the bottle shoulder with ultrapure water. Pipet 2mL of 1+1 sulfuric acid into the bottle. Cap and invert to mix. Degas with helium for 5 minutes. This also doubles as blank solution.

6) Sample Collection, Preservation, Shipment, and Storage

a) Collect and filter samples in clean plastic bottles. Each sample shall be preserved with sulfuric acid to a pH<2, or the samples may be stored frozen for preservation and preserved when thawed.

b) Acid preserved samples may be stored up to 28 days. Frozen samples may be stored indefinitely.

7) Quality Control

a) Calibrate before each analytical run. Analyze Instrument Performance Check (IPC)/ from a second source standard after calibration. Calibration r-value is required to be >0.995, if not re-calibrate.

b) Run a blank (ICVB) at the beginning of each run and then once every 20 samples. ICVB must be <LOD. If not, re-analyze, if still out of control qualify data. Subsequent blanks (CCVB) must be <LOD, or less than 10% of the measured concentration in the samples from the adjacent set.

c) Analyze a Continuing Calibration Verification Standard (CCVS) with every 20 samples.

d) A duplicate and matrix spike must be analyzed every 20 samples (5%). Prepare matrix spike samples by pipetting 0.1mL of matrix spike solution into a sample test tube, along with 4.9mL of sample. Cover tube with parafilm and invert to mix.

8) Calibration

a) Prepare calibration standards fresh before each analysis. Prepare standards in autosampler standard tubes as follows using calibrated adjustable pipets.

Standard	mL Std 1	mL Blank	[N]/[P]
Cal Std 1	20	0	2.00/0.200
Cal Std 2	10	10	1.00/0.100
Cal Std 3	5	15	0.50/.050
Cal Std 4	2	18	0.20/0.020
Cal Std 5	1	19	0.10/0.010
Cal Std 6	0.5	19.5	0.05/0.005
Cal Std 7	0.2	19.8	0.02/0.002
Cal Std 8	0	20	0.00/0.000

9) Procedure for Lachat 8500 operation

a) Turn on the Lachat autoanalyzer by moving the switch on the power strip behind the autosampler to the on position. The instrument will go through its normal startup routine (autosampler probe moves, switching valves on the instrument turn, etc.).

b) Place reagent tubing into water and start the peristaltic pump. Clamp each pump tube clamp onto the rollers. Push the tensioner cam lever back one click. Keep reagent lines for NH_4 and SRP in separate water flasks to avoid contamination.

c) Hit the space bar on the computer keyboard to "wake-up" the computer. If the computer is on, restart before continuing. After reboot, double click on the Omnion icon.

d) Click the open button on the top toolbar. Find the correct template for NH4_SRP analysis and double-click to open.

e) Make sure the concentrations for the IPC/QCS sample are correct by clicking on the sample in the worksheet. If the values are incorrect, change them by highlighting all of the samples in the DQM set (most likely DQM1) and clicking "clear DQM set" from the menu. Change the concentration in the "Run Properties" window in the upper right of the screen. Highlight the DQM samples again and right click. Select define DQM set. Set the frequency to once.

f) Start inputting sample names/numbers into the worksheet. To add more spaces for samples, highlight a row and right click. Select "Insert many" and input how many rows to add. Remember to hit "enter' after each sample is input. Use the benchsheet to enter the sample identifiers. To enter multiple samples quickly, select multiple rows and right click. Go to "Columns" and select "Auto SampleID." Enter the part of the sample identifier that will not change in the "Fixed Part of Sample ID" then the number or letter to start. Click "Accept." It is helpful if the number of rows highlighted matches the number of samples to be input automatically.

g) Once finished, highlight the entire worksheet and right click. Go to "Columns" and select "Auto-number cups." Make sure the number of cups in the worksheet matches what is on the benchsheet. If the numbers do not match, check the duplicates and matrix spikes to see if they match. Once you find a matching duplicate and spike set, you will know where the discrepancy is located.

h) Remove the reagent lines from the water and begin placing them in their corresponding reagent bottles. Do this in this order, allowing reagents to pump for a minute or two before placing other tubes into reagents:

NH4

Carrier=>Buffer=>Nitroprusside=>Phenol=>Hypochlorite

PO4

Carrier=>Ascorbic=>Molybdate

i) Click on the “Configuration” menu at the top of the Omnion window. Go to “Autosamplers” and click the button “Intialize Autosampler.” This will put the sampler probe into the wash well to begin drawing water through the sample line.

j) After reagents have been pumping for ~5 min. click the preview button at the top of the Omnion window. This will allow you see the baseline before starting the run. The baseline is flat and the signal is where it is expected, click “Stop” next to the “Preview” button and then click “Start.”

k) During the beginning of the run, watch to make sure there is good Gaussian peak shape and the peak expection window is integrating the entire peak correctly.

l) After the calibration standards are integrated, check the calibration curve by clicking on the bottom button on the left side of the channel window. Make sure calibration coefficient is ≥0.995 and %RSD for each standard is under 10%. If either of these limits are exceeded begin by excluding peaks that may skew the curve. If this does not correct the problem, recalibrate.

m) Next, be sure the initial calibration check standards are within 10% of the expected value. It not, reanalyze the standards. If this does not correct the issue, recalibrate.

n) After everything in the beginning is in control, the rest of the run can be monitored for duplicate RPD’s/differences and matrix spike recoveries. If these are out of control, try reanalyzing the samples. Also, a continuing calibration verification blank (CCVB) and continuing calibration verification standard will be analyzed every 20 samples. Make sure these are within 10% of known value.

o) If everything in the run goes to plan, the instrument will stop after all of the samples in the worksheet have been analyzed. The reagent lines can be removed in reverse order of how they were put in, and placed into ultrapure water for a few minutes, then place the PO4 lines into an EDTA cleaning solution for a minute to clean any phosphate deposits left in the lines. Place tubes back into ultrapure water for ~10 min. After the 10min., remove tubes from water and hang them on the hook above the pump and allow air to be pumped through the manifold. Pump air until all water has been evacuated from the manifold lines.

p) Release the pump tubes on the pump and stop the pump. Turn off the Lachat by flipping the switch on the power strip behind the autosampler.

q) Samples in the test tubes can be disposed by dumping the sample into the waste drum. The tubes will be cleaned and used again.

10) Corrective Action for Out-of-Control Data

a) Any CCV blank that exceeds the LOD, the analyst must inspect the concentration of the previous sample. If the blank is greater than 10% of the previous sample, reanalyze the blank immediately following the CCVS. No samples can be run until the blank meets requirements.

b) CCV and LCS standards must fall within 10% of true value (90%-110% Recovery). If not re-mix and re-analyze, if still out of range re-calibrate. Fill out corrective action report.

11) Contingencies for Handling Out-of-Control Data

a) Samples that fail the CCVB or ICVS will have to be qualified back to the last sample that the quality control met the above conditions.

12) Waste Management

a) All waste must be collected (except for waste from probe wash well) and disposed of in the correct hazardous waste drum. Keep waste from SRP channel separate as it contains molybdate.

13) References

a) Standard Methods for the Examination of Water and Wastewater, 22nd ed. Clesceri, L.S.; Greenberg, A.

b) Lachat QuikChem Method 10-107-06-1-A. Ammonia (Phenolate) Potable and Surface Waters. K. Switwala, revision date 9 September, 1994.

c) Lachat Quickchem Method 10-115-01-1-M. Determination of Orthophosphate in Waters by Flow Injection Analysis Colorimetry. L. Egan, revision date 29 November, 2007.