STANDARD OPERATING PROCEDURE (SOP)

"Simultaneous analysis of Nitrate+Nitrite and Phosphate using the Lachat Flow-injection System"

MED-SOP-CHA033 N+P LACHAT-TJ 4-2015

Revision 0

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PROCEDURAL SECTION

1.0 SCOPE AND APPLICATION

1.1 The principle, background, and details of this analysis are contained in the LACHAT QuikChem AE Instrument Manual-QuikChem Method, 10-107-04-1-0 Nitrate+Nitrite as NO_2+NO_3-N and QuikChem Method 10-115-01-1-B Orthophosphate as PO_4-P stored with the Lachat instrument.

1.2 This method can be used to analyze total nitrogen and total phosphorus after persulfate digestion or dissolve nitrate+nitrite and orthophosphate of water after filtration through a 0.45um membrane filter.

1.3 This SOP is a combination of MED SOPs CHA011 and CHA013 are for the analysis of Nitrate+Nitrite as NO_2+NO_3-N or Orthophosphate as PO_4-P individually.

1.4 Samples are stored frozen and analyzed within 6 months of collection.

2.0 SUMMARY OF METHOD

2.1 DEFINITIONS - DIW deionized water

2.2 HEALTH AND SAFETY WARNINGS - use of chemical(s) are required in the analysis procedures; therefore safety glasses, nitrile gloves and lab coat should be worn.

2.3 INTERFERENCES - Phosphorus: Arsenic and silica compounds react with reagents producing falsely elevated values at the 880nm wavelength. Calcium and magnesium will interfere by forming a white precipitate. High iron or humic concentrations can also form precipitates removing phosphorus which sorb to it. Bisulfite treatment may be used if iron interference is suspected.

Nitrate: Chlorine can interfere by oxidizing the Cd column and reducing its efficiency. Adding sodium thiosulfate ($Na_2S_2O_3$) can reduce this interference.

3.0 PERSONNEL QUALIFICATIONS

3.1 This SOP provides the basic steps necessary for use of the LACHAT Analyzer, however, proper training in the use of the instrument is necessary.

4.0 MATERIALS AND PROCEDURES

4.1 Materials

4.1.1 13x100mm borosilicate test tubes and 90 tube racks

4.1.2 Lachat Quikchem 10-107-04-1-0 nitrate+nitrite with 50cm sample loop

4.1.3 QuikChem 10-115-01-1-B orthophosphate manifold with 133cm sample loop.

4.1.4 ASX-520 XYZ autosampler by CETAC Technologies.

4.2 Reagents and standards

All reagents and standards are to be prepared with deionized water (DIW) of 10 megohm or better, as indicated by reading on deionized water system meter.

Nitrate+Nitrite:

4.2.1. Reagent 1: Ammonium chloride buffer, pH= 8.5:
Use the alternative recipe (by Volume): Add 210 mL concentrated HCl, 190 ml ammonium hydroxide (NH_4OH), and 2.0 gm disodium EDTA to 2 L volumetric half-filled with DIW. Dilute to mark with DIW after the flask cools.

4.2.2 Reagent 2: Color Reagent.
1L beaker + 876g DIW + 170g 85% H_3PO_4 + 40g Sulfanilamide + 2g N-1-naphthyl ethylenediamine dihydrochloride (NED). Make fresh weekly

4.2.3 Reagent 3: Carrier
1L DIW degassed with Helium. Make fresh daily.

4.2.4 Nitrate Stock Standard: 100.0 mg N/L as NO_3
Prepare 500.0 ml using 0.3610 gm potassium nitrate (KNO_3) dried for one hour at 105°C in DIW, but **DO NOT** add chloroform. Keep refrigerated.

4.2.5 Nitrite Stock Standard: 100.0 mg N/L as NO_2
Prepare 500.0 ml using 0.3035 gm potassium nitrite (KNO_2) dried for one hour at 105°C in DIW, but **DO NOT** add chloroform. Keep refrigerated.

Phosphate:

4.2.6 Reagent 1: Ammonium Molybdate Stock

By Weight: To a tared 1 L container, dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] and 983 g DIW. Mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

4.2.7 Reagent 2: Stock Antimony Potassium Tartrate Solution
By Weight: To a 1 L dark, tared container add 3.22 g antimony potassium tartrate (potassium antimonyl tartrate hemihydrate $K(SbO)C_2H_4O_6\cdot 1/2\ H_2O$) and 995 g DIW. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

4.2.8 Reagent 3: Molybdate Color Reagent

By Weight: To a tared 1 L container add 680 g DIW, then add 64.4 g concentrated sulfuric acid (H_2SO_4). Swirl to mix. When it can be comfortably handled add 72 g Antimony Potassium Tartrate Solution (Reagent 2) and 213 g Ammonium Molybdate Solution (Reagent 1). Invert a few times and degas with helium.

4.2.9 Reagent 4: Ascorbic Acid Reducing Solution.

By Weight: To a tared 1 L container add 60.0 g ascorbic acid and 975 g DIW. Stir or shake until dissolved. Add 2.0 g dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$). Dilute to the mark. Invert three times. Prepare fresh weekly.

4.2.10 Reagent 5: Carrier
1L DIW degassed with Helium. Make fresh daily.

4.2.11 Stock Standard: 100.0 mg P/L
Prepare 500.0 ml using 0.2197 gm potassium dihydrogen phosphate (KH_2P0_4) dried for one hour at 105°C in DIW. Keep refrigerated.

4.2.12 Working Standards: Working Calibration Standards: Prepare standards containing both nitrate and phosphate over the range of analysis from the stock standard.

Nitrate (ug/L)	5000	2500	1000	500	250	100	50	25	0
Phosphate (ug/L)	1000	500	200	100	50	25	10	2	0

4.2.14 Quality Control Standard: A second source standard should be prepared within the expected sample concentration range in DIW.

4.3 Procedure

4.3.1 Water samples are stored frozen until analysis within 6 months of

collection. Total nitrogen and phosphorus are digested by persulfate method according to MED-SOP-TNTP DIGESTION-CHA032. Nitrate+ Nitrite and Phosphate are filtered through a 0.45um membrane filter prior to analysis.

4.3.2 Prepare standards and reagent according to the above recipes in DIW.

4.3.3 Degas all reagents except the ascorbic acid and standards by bubbling helium gas thru a disposable glass pipette into the reagent for 5 minutes minimum. Adjust the flow just slow enough to prevent blowing the reagent out of its container.

4.3.4 Degas the ascorbic acid by connecting to a vacuum system for 5 minute minimum.

4.3.5 Lachat setup and analysis

4.3.5.1 Turn on instrument and software 15 minutes prior to starting analysis to allow for the heater block to warm up to 37°C for phosphate analysis.

4.3.5.2 Place reagent lines in appropriate reagents and start pump. Be sure to check that all reagents are flowing smoothly. Allow to run through system for minimum of 5 minutes ensure there is no air in the lines.

4.3.5.3 Turn the cadmium column on for nitrate+nitrite analysis and allow system to run an additional 5 minutes. Check column that there are no air bubbles. If there are air bubbles gently tap the column while reagents are pumping to vacate the air bubbles. (If nitrite is the analyte of interest skip this step)

4.3.5.3 Pour samples in tubes in racks and record on bench sheets. Note: that while the instrument is set up to handle 4 trays of 90. It is best to restrict batches to 2 racks of 90.

4.3.5.4 Open the Omnion software. Open NO3+PO4water_YYYY_method.omn (or TNTP_YYYY_method.omn), enter samples and cup numbers in the sample table.

4.3.5.5 Press the **Start** button when ready to run.

4.3.5.6 When run is complete, turn off the column, place reagent lines in DIW water and rinse through for 10 minutes, then pump air for another 10 minutes to dry the lines.

4.5 Data calculations

4.5.1 Lachat software yields results directly as mg/L NO_3+NO_2-N and PO_4. Occasionally it is appropriate to split a curve for greater sensitivity. If this is required the data can be exported to an excel spreadsheet where the analyst can recalculate the standard curve equations by regression and recalculate the sample concentrations using the peak areas based on the new curve equation.

5.0 QUALITY CONTROL AND QUALITY ASSURANCE

5.1 Laboratory replicates and spiked samples should be prepared for at least 5% of samples to be analyzed.

5.2 Duplicate field samples and field blanks should be collected for at least 5% of the total number collected.

5.3 Check standards and/or QC samples should be analyzed at least 5% of the total number of samples analyzed.

5.4 Method blanks and lab fortified blanks (LFB) should be ran a minimum of 2 per batch.

5.5 Nitrite check standard should be ran with each batch to ensure the column is working properly.

5.6 Check standards and/or QA samples should be analyzed at least 10% of the total number of samples analyzed.

5.7 Standard curve is automatically calculated by the software when the set of working Calibration Standards are ran as sample type "Calibration" in the sample table. A standard curve should be ran daily or at a minimum with each batch for fresh reagents. A set of Working Calibration Standards should be run at the end of each run as "unknown" samples to verify calibration. Should instrument drift occur this set of standards could be recalculate the standard curve equations by regression and recalculate the sample concentrations using the peak areas based on the new curve equation.

6.0 REFERENCES

6.1 LACHAT QuikChem AE Instrument Manual - QuikChem Method 10-115-01-1

B Orthophosphate August 1992

6.2 LACHAT QuikChem AE Instrument Manual- QuikChem Method 10-107-04-1-0 Nitrate + Nitrite. August 1992.

6.3 Lachat QuikChem 8000 Operating Manual

6.4 MED-SOP-CHA011 Nitrate Analysis

6.5 MED-SOP-CHA013 Orthophosphate Analysis