FERTILIZER METHODS	Chapter: NITROGEN ANALYSIS
	Subject: Nitrate Nitrogen – Flow Injection

Adapted from QuickChem Method 14-107-04-1-B
By: Lachat Instruments 6645 West Mill Road, Milwaukee, WI 53218
Determination of Ammonia in Fertilizers by Flow Injection Analysis Colorimetry
5 to 300 mg N/L as NO_3
0.13 to 7.5 % N/1 gram sample

PRINCIPLE

The sample is injected into a D.I. water carrier stream. The carrier stream is merged with an ammonium chloride buffer. The nitrate in the sample is further diluted by dialyzing across a membrane. The nitrate in the acceptor stream is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate and 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. The method determines nitrate plus nitrite.

SCOPE AND APPLICATION

This method covers the determination of nitrate in fertilizer samples prepared using FM-430 or FM-701.

This method is based on reactions that are specific for the nitrate/nitrite ion.
The applicable range is 5 to 300 mg N/L as NO_3. 0.13 to 7.5% N assuming a 1 g sample diluted to 250 mL. The method detection limit is 0.38 mg N/L. **Note: This method is intended for use with methods FM-462 & FM-471 at 40 injections per hour, but may be used alone at up to 60 injections per hour.**

DEFINITIONS

See: BUREAU OF FEED, SEED & FERTILIZER LABORATORIES, STANDARD OPERATING PROCEDURES, and LACHAT DEFINITIONS

INTERFERENCES

Low results may be obtained for samples that contain high concentrations of iron, copper or other metals.

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

SAFETY

Each laboratory is responsible for maintaining a current 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.

EQUIPMENT AND SUPPLIES

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

Glassware – Class A volumetric flasks and pipettes or plastic containers as required. Samples may be stored in plastic or glass containers.

Flow Injection Analysis equipment designed to deliver and react samples and reagents in the required order and ratios.

Autosampler

Multichannel Proportioning Pump

Reaction Unit or Manifold

Colorimetric Detector

Data System

6 Inch Pathlength Dialysis Block (Lachat Part # 50311)

Type C Membrane (Lachat Part # 50304)

10mm flow cell (Lachat Part No. 31933)

Pump tubes **NOTE: PVC PUMP TUBES WILL LAST LONGER AND MAY BE USED FOR THIS METHOD**

REAGENTS AND STANDARDS

PREPARATION OF REAGENTS

Use deionized (D.I.) water (10 megohm) for all solutions.

Reagent 1. **10 % w/v Sodium Hydroxide for pH adjustment**
In a **100 mL** volumetric flask add **10 g sodium hydroxide (NaOH)** to approximately **50 mL of D.I. water.**
Caution : The solution gets very hot! Swirl until dissolved. Cool solution to room temperature. Dilute to the mark with **D.I. water**. Mix by inversion.

Reagent 2. **20 % v/v Hydrochloric Acid for pH adjustment**
In a **100 mL** volumetric flask add approximately **60 mL of D.I. water**. In a fume hood, cautiously add **20 mL of concentrated hydrochloric acid (HCl).** Gently swirl flask, cool to room temperature and add **D.I. water** to mark. Mix by inversion.

Reagent 3. **Ammonium Chloride Buffer – Acceptor.**
(By Volume)
In a **2 L** graduated beaker, dissolve **42.5 g ammonium chloride (NH_4Cl**) in approximately **1500 mL D.I. water.** Adjust the **pH** to **5.5 – 6.0** with either **20 % hydrochloric acid** (Reagent 2) or **10% sodium hydroxide** (Reagent 1). Only a few drops may be necessary for the pH adjustment. Transfer to a volumetric flask and dilute to **2000 mL** using **D.I. water.** Mix with a magnetic stirrer. Stable for one month.

Reagent 4. **Ammonium Chloride Buffer – Donor.**
(By Volume)
In a **2 L** graduated beaker, dissolve **127.5 g ammonium chloride (NH_4Cl**) in approximately **1500 mL D.I. water**. Adjust the pH to **5.5 – 6.0** with either **20 % hydrochloric acid** (Reagent 2) or **10% sodium hydroxide** (Reagent 1). Only a few drops may be necessary for the pH adjustment. Transfer to a volumetric flask and dilute to **2000 mL** using **D.I. water.** Mix with a magnetic stirrer. Stable for one month.

Reagent 5. **Sulfanilamide Color Reagent (By Volume)**
To a **2 L** volumetric flask add approximately **1200 mL D.I. water**. Then add **80.00 g sulfanilamide** and **2.0 g N-(1-naphthyl)ethylenediamine dihydrochloride (NED).** Shake to wet and stir with a magnetic stirrer. Add **200 mL 85 % phosphoric acid** (H_3PO_4). Stir until dissolved. Dilute to mark using **D.I. water** and invert to mix. Store in a dark bottle. This solution is stable for one month.

PREPARATION OF REAGENTS FOR CADMIUM REDUCTION COLUMN

Reagent A. **1 M Hydrochloric Acid (HCl)**

By Volume :
In **a 100 mL** container, add **8 mL concentrated hydrochloric acid** to **92 mL D.I. water**. Stir or shake to mix.

By Weight :
To a **100 mL** container, add **92 g D.I. water** and then add **9.6 g concentrated hydrochloric acid**. Stir or shake to mix.

Reagent B. **2% Copper Sulfate Solution**.

By Volume :
In a **1 L** volumetric flask, dissolve **20 g copper sulfate** ($CuSO_4 \bullet 5H_2O$) in about **800 mL D.I. water**. Dilute to the mark with **D.I. water.** Invert to mix thoroughly.

By Weight :
To a **1 L** container, add **20 g copper sulfate** **(** $CuSO_4 \bullet 5H_2O$) to **991 g D.I. water**. Stir or shake to dissolve.

Reagent C. **Column Efficiency Check Standard.**

The column efficiency check standard is prepared using sodium nitrite ($NaNO_2$).

Preparation Of Stock Check Standard
(1000 mg N/L as NO_2)

Dissolve **1.232 g of sodium nitrite** ($NaNO_2$) **in D.I. water**. Bring the volume up to **250 mL.**

Preparation Of Working Check Standard
(150 mg N/L as NO_2)

Dilute **15 mL** of NO_2 **Stock Standard** to **100 mL**. using **D.I. water.**

PREPARATION OF CADMIUM REDUCTION COLUMN

Cadmium Preparation

Place 10 – 20 g of coarse cadmium granules (0.3 – 1.5 mm diameter, Lachat Part # 50231) in a 250 mL beaker. Wash with two 50 mL portions of D.I. water, then two 50 mL portions of 1 M hydrochloric acid (Reagent A). Rinse several times with D.I. water.
CAUTION : Collect and store all waste cadmium. **Cadmium is toxic and carcinogenic.** Wear gloves and follow the precautions described on the Material Safety Data Sheet.

Copperization

Add a 100 mL portion of 2 % copper sulfate solution (Reagent B) to the cadmium prepared above. Swirl for about 5 minutes, then decant the liquid and repeat with a fresh 100 mL portion of the 2 % copper sulfate solution. Continue this process until the blue aqueous copper persists. Decant and wash with at least five portions of ammonium chloride buffer (Reagent 4) to remove colloidal copper. The cadmium should be black or dark gray. The copperized cadmium granules may be stored in ammonium chloride buffer (Reagent 4).

Packing the Column

The empty cadmium column is available as Lachat Part # 50230. Wear gloves and do all cadmium transfers over a special tray or beaker dedicated to this purpose. Clamp the empty column upright so that your hands are free. Unscrew one of the colored fittings from an end of the column and pull out and save the foam plug. The column and threads are glass so be careful not to break or chip them. Fasten this fitting up higher than the open end of the column and completely fill the column, attached fittings and tubing with ammonium chloride buffer (Reagent 4).

Scoop up prepared copperized cadmium granules with a spatula and pour them into the top of the filled column so that they sink down to the bottom of the column. Continue pouring the cadmium in and tapping the column with a screwdriver handle to dislodge any air bubbles and to prevent gaps in the cadmium filling. When the cadmium granules reach to about 5 mm from the open end of the column, push in the foam plug and screw on the top fitting. Rinse the outside of the column with D.I. water.

If air remains in the column or is introduced accidentally, connect the column into the manifold, turn the pump on maximum and tap firmly with a screwdriver handle, working up the column until all air is removed.

CADMIUM COLUMN INSERTION PROCEDURE

a. Before inserting the column, pump all reagents into manifold.

b. Turn the pump off and immediately connect to the outlet tubing of buffer mixing coil.

c. Connect the open tubing on the column to the tee fitting where the color reagent is.

d. Return the pump to normal speed.

e. The direction of the reagent flow through the column is not relevant.

COLUMN EFFICIENCY PROCEDURE

a. Visually inspect the column. Check for air bubbles in the column or lines, gaps in the column or any change in the cadmium surface characteristics. (Cadmium granules should be dark.)

b. If air bubbles are present in the column, connect the column into the manifold, turn the pump on maximum and tap firmly with a screwdriver handle, being careful not to break the column, working up the column until all air is removed. If air can not be removed, the column should be repacked. Cadmium columns should be stored filled with buffer. If air enters the column, efficiency will decrease.

c. Check the flow efficiency by disconnecting the cadmium column from the manifold and reconnecting to a green pump tube. Pump buffer through the packed column and collect in a graduated cylinder. The flow rate with the column connected should be greater than 4.0 mL/min.

d. There are two procedures for determining column efficiency, as follows :

Slope Ratio Method

1. Calibrate with the mid range NO_3 – N standards.

2 Calibrate with a matching range of NO_2 – N standards.

3. The column efficiency is determined by the equation :

$$\frac{S\ NO_3 - N}{S\ NO_2 - N} \times 100 = E,$$

where
$S\ NO_3 - N$ = Slope of NO_3 calibration
$S\ NO_2 - N$ = Slope of NO_2 calibration
E = % Efficiency

4. If the efficiency is less than 90 %, the column should be repacked.

Concentration Ratio Method

1. Calibrate with the mid range NO_2 – N or NO_3 – N standards.

2. Run a known concentration of NO_2 – N standard.

3. Run a matching concentration of NO_3 – N standard.

4. The column efficiency is determined by the equation :

$$\frac{C\ NO_3 - N}{C\ NO_2 - N} \times 100 = E,$$

where
$C\ NO_3 - N$ = Concentration of NO_3 standard.
$C\ NO_2 - N$ = Concentration of NO_2 standard
E = % Efficiency

5. If the efficiency is less than 90 %, the column should be repacked.

PREPARATION OF STANDARDS

Calibration Standards

The calibration standard is prepared using potassium nitrate.

Preparation of Stock Calibration Standard (1000 mg N/L as NO_3)

In a single **500 mL** volumetric flask add **350 mL D.I. water**. Weigh and add **3.611 g potassium nitrate (KNO_3).** Dilute to mark and mix with a magnetic stirrer. Refrigerate stock standard when not in use. Prepare fresh stock standard weekly or when the flask is about half empty, which ever is earlier.

Preparation of Working Calibration Standards :

Standard 1 : 300mg N/L as NO_3

Pipette **30 mL of Stock Standard** into a **100 mL volumetric flask.**
Dilute to the mark with **D.I. water**. Invert to mix.

Standard 2 : 150 mg N/L as NO_3

Pipette **15 mL of Stock Standard** into a **100 mL volumetric flask.**
Dilute to the mark with **D.I. water**. Invert to mix.

Standard 3 : 75 mg N/L as NO_3

Pipette **15 mL of Stock Standard** into a **200 mL volumetric flask.**
Dilute to the mark with **D.I. water**. Invert to mix.

Standard 4 : 30 mg N/L as NO_3

Pipette **3 mL of Stock Standard** into a **100 mL volumetric flask.**
Dilute to the mark with **D.I. water**. Invert to mix.

Standard 5 : 0 mg N/L as NO_3 -Blank

100 mL volumetric flask of 10 meg ohm D.I. water.

SAMPLE PRESERVATION AND STORAGE

If the sample contains particulate matter, it must be filtered through at least a Whatman # 4 or equivalent filter to avoid plugging the valve or manifold. Once the sample has been filtered, it should be allowed to settle for about 5 minutes before analysis.

To further decrease the chance that particulate matter will be aspirated with the sample, set the probe so that there is about ½ inch of clearance from the bottom of the sample tube.

The diluted samples should be analyzed as soon as possible. If they can not be analyzed immediately, they should be refrigerated.

CALIBRATION PROCEDURE

Prepare a series of at least 3 standards, covering the desired range and a blank by diluting suitable volumes of standard solution.

Calibrate the instrument as described in **DATA ANALYSIS AND CALCULATIONS**.

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.

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

CALIBRATION PROCEDURE

(DATA SYSTEM PARAMETER)

Prepare reagents and standards as described in **REAGENTS AND STANDARDS**.

Set up manifold as shown in **NITRATE MANIFOLD DIAGRAM**.

Input data system parameters as in **DATA SYSTEM PARAMETERS**.

Pump D.I. 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.

Place samples and / or standards in the autosampler. Input the information required by the data system, such as concentration, replicates and QC scheme.

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

SYSTEM NOTES

All the reagents and the carrier must be degassed for one minute with helium.

Upon completion of the final analysis of the day, place transmission lines into D.I. water.

NOTE : **T**urn the cadmium column switching valve, so the cadmium column is not connected to the rest of the manifold. Ammonium chloride buffer should be in the cadmium column when the column is not in use. Pump D.I. water for an additional 10 minutes. Release the tension on the pump tubing and keep the transmission lines in the D.I. water. The slow flow of water through the dialysis block will keep the type C membrane wet. The membrane must be kept wet in order to obtain good results the next morning.

DATA ANALYSIS AND CALCULATIONS

Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve.

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

Report results in % N as NO_3 in fertilizer.

METHOD PERFORMANCE

See: Quikchem method 14-107-04-1-B from Lachat Instruments.

FLOWCHARTS AND VALIDATION DATA

See: Quikchem method 14-107-04-1-B from Lachat Instruments.

TABLES AND DIAGRAMS

DATA SYSTEM PARAMETERS

ANALYTE DATA :

Analyte Name :	Nitrate
Concentration Units :	mg N/L
Chemistry :	Direct
Inject to peak start (s) :	20.0
Peak base width (s) :	39.706
% Width tolerance :	100.000
Threshold :	15000.00
Autodilution trigger :	Off
QuikChem Method :	14-107-04-1-B

CALIBRATION DATA :

Levels :	1:300, 2:150, 3:75, 4:30, 5:0
Calibration Rep Handling :	Weighted Average
Average Weight Factor :	100.00
Calibration Fit Type :	1st Order Poly
Force Through Zero :	No
Weighting Method :	None
Concentration Scaling :	None

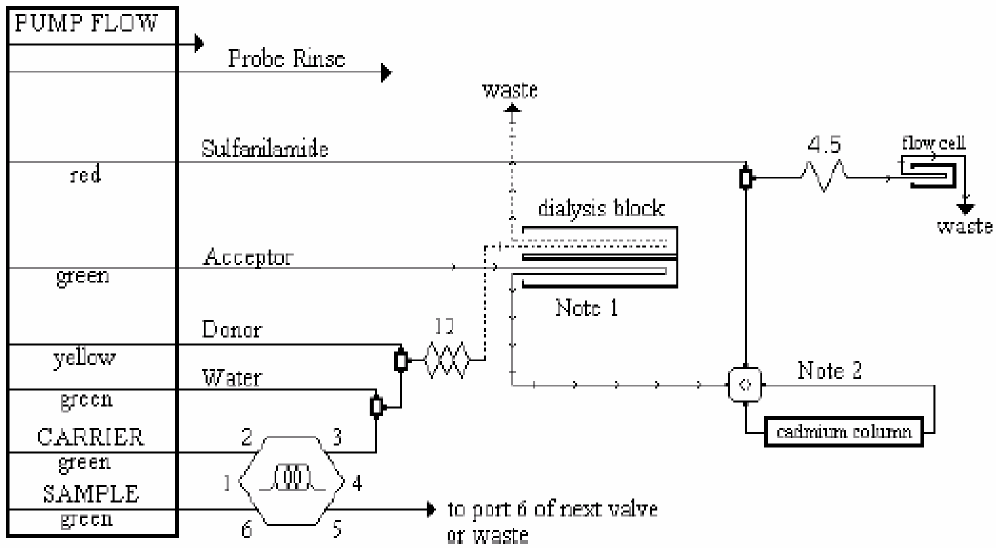
SAMPLER TIMING :

Method Cycle Period :	60.00
Min. Probe in Wash Period :	14.00
Probe in Sample Period :	28.00

VALVE TIMING :

Method Cycle Period :	60.00
Sample Reaches 1st Valve :	23.00
Valve :	On
Load Time :	0.00
Load Period :	20.00
Inject Period :	40.00

NITRATE MANIFOLD DIAGRAM

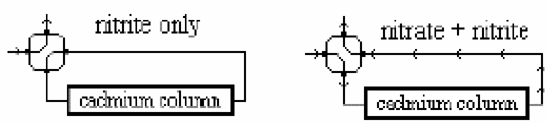


Carrier: **DI water**
Manifold Tubing: **0.8 mm (0.032 in) i.d.** This is **5.2 μL/cm**.
AE Sample Loop: 8 cm
QC8000 Sample Loop: 13.5 cm
Interference Filter: 520 cm

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

Note 1: 6 inch pathlength dialysis block and a Type C membrane.
Note 2: This is a 2 state switching valve used to place the cadmium column in-line with the manifold.



APPROVAL

Approved by: ______________________ **Date: 1/24/03**
Signature

Bureau Chief
Title

METHOD REVISION HISTORY

Version	Date	Description	Author
Original	01/24/03	Original	Bill Bell

REFERENCE

Adapted from QuickChem Method 14-107-04-1-B
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