

# FERTILIZER METHODS

Chapter

NITROGEN ANALYSIS

Subject

Urea Nitrogen – Flow Injection by Urease

**Adapted from QuickChem Method 14-206-00-4-A**

**By: Lachat Instruments 6645 West Mill Road, Milwaukee, WI 53218**

*Determination of Ammonia in Fertilizers by Flow Injection Analysis*

**4 to 600 mg N/L as Urea + NH<sub>4</sub> per 1 g sample  
(0.1 to 15.0 % N)**

## **PRINCIPLE:**

This is a two channel, low flow method, which measures ammonia on one channel (FM-471), and the sum of ammonia and urea on a second channel. The sample is injected into a urease solution.

The result on the ammonia channel (FM-471) consists of only the ammonia present in the sample. Urease cleaves urea (CH<sub>4</sub>N<sub>2</sub>O) to NH<sub>3</sub> + CO<sub>2</sub> on the second channel. The result consists of the ammonia initially present in the sample, plus the ammonia produced by the action of urease on urea.

When ammonia is heated with salicylate and hypochlorite in an alkaline phosphate buffer, a colored product results. Absorbance of the colored product at 660 nm is directly proportional to the ammonia concentration. The color is intensified by the addition of sodium nitroprusside. The method detection limit is 1 mg N/L as NH<sub>4</sub>.

At the end of the analysis, the ammonia channel (FM-471) result is subtracted from the urea + ammonia result to give the percent urea N present in the sample. An ammonia standard is used to calibrate both channels. Urea stock solutions are analyzed to determine the percent conversion of urea to ammonia. Percent conversions of urea to ammonia are generally in the range of 93-98%.

## **SCOPE &**

### **APPLICATION:**

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

The applicable range is 4 to 600 mg urea N/L. The method detection limit is 1 mg NH<sub>4</sub> N/L. The method throughput is 40 injections per hour.

**Note: This method is intended for use with methods FM-451 & FM-471, but may be used alone.**

## **DEFINITIONS**

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

### INTERFERENCES

Ammonia interferes with the method 100%, therefore the method must be set up as a two channel subtraction system.

Non-volatile amines such as cysteine, ethanolamine and ethylenediamine may cause a decrease in ammonia sensitivity.

### SAFETY

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.

### 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 sample and reagents in the required order and ratios.

Sampler

Multichannel proportioning pump

Reaction unit or manifold

Colorimetric detector

Data system

Special Apparatus

Heating unit

1 mm path length flow cell (Lachat Part No. 24950) **NOTE: PVC PUMP TUBES MUST BE USED WITH THIS METHOD.**

### REAGENTS AND STANDARDS

#### PREPARATION OF REAGENTS

Use deionized water (10 megohm) for all solutions.

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

#### Reagent 1. Buffer Solution

**By Volume:** In a 1 L volumetric flask, dissolve **20 g sodium hydroxide (NaOH)**, **42 g sodium phosphate heptahydrate (Na<sub>2</sub>HPO<sub>4</sub> · 7H<sub>2</sub>O)**, and **50 g potassium sodium tartrate tetrahydrate (KNaC<sub>4</sub>H<sub>4</sub>O<sub>6</sub> · 4H<sub>2</sub>O)**, in about **600 mL D.I. water**. Dilute to the mark with **D.I. water** and stir to dissolve.

**Reagent 2. Nitroprusside/Salicylate Color Reagent**

**By Volume:** In a 1 L volumetric flask, dissolve **150 g sodium salicylate**, **1.0 g sodium nitroprusside** and **0.4 g Brij-35** (C<sub>12</sub>H<sub>25</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>23</sub>OH) in about **700 mL D.I. water**. Dilute to the mark with **D.I. water** and stir until dissolved. (Brij-35 is a registered trademark of ICI Americas, Inc.).

**Reagent 3. Urease Solution**

**By Volume:** In a **250 mL** volumetric flask, dissolve **0.06 g urease** (Sigma # U-4002) in about **200 mL D.I. water**. Dilute to the mark with **D.I. water** and invert to mix. Urease solution will best maintain its activity level if kept in an ice-water bath while running. Prepare daily. This amount will last approximately 3 hours. Volume can be scaled up depending upon sample load, but fresh reagent should be prepared each day.

**Reagent 4. Hypochlorite Solution**

**By Volume:** In a **500 mL** volumetric flask, dilute **30 mL 5.25 % sodium hypochlorite** (NaOCl) in **400 mL D.I. water**. Dilute to the mark with **D.I. water** and invert to mix. Prepare fresh daily.

**By Weight:** To a tared **500 mL** container, add **30 g 5.25 % sodium hypochlorite** (NaOCl) and **470 g D.I. water**. Invert to mix. Prepare fresh daily.

**PREPARATION OF STANDARDS**

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

**By Volume:** One 1 L and five 500 mL volumetric flasks.

**By Weight:** One 1 L and five 500 mL containers.

**Standard 1. Stock Standard 1000 mg N/L as ammonia**

**By Volume:** In a 1 L volumetric flask, dissolve **4.750 g ammonium sulfate** (reagent grade) in approximately **900 mL D.I. water**. Dilute to the mark with **D.I. water** and invert to mix.

Working Standards (Prepare Daily)	A	B	C	D	E
Concentration mg N/L as ammonia	600	300	150	60	0.0

**By Volume**

Volume (mL) of stock standard 1 diluted to 500 mL with DI water	300	150	75	30	---
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**By Weight**

Weight (g) of stock standard 1 diluted to final weight (~500g) divided by factor below with DI water	300	150	75	30	---
Division Factor  Divide exact weight of the standard by this factor to give the final weight	0.6	0.3	0.15	0.06	---

**Urea to ammonia conversion check standard (2000 ug N/L)**

**Dissolve 1.073 g of Electrophoresis grade urea in D.I. water and bring to volume in 250 mL volumetric flask.**

**Dilute 15 mL of “Urea to ammonia conversion check standard” in D.I. water and bring to volume in 100 mL volumetric flask. (300 ug N/L)**

**SAMPLE PRESERVATION AND STORAGE**

For sample analysis, fertilizers (liquid or solid) containing up to 15% NH<sub>3</sub>-N plus CH<sub>4</sub>N<sub>2</sub>O-N by weight are diluted. One gram of sample is diluted to 250 mL in D.I. water. 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 possibility 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.

**CALIBRATION PROCEDURE**

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

Set up manifold as shown in UREA/AMMONIA MANIFOLD DIAGRAM.

Input data system parameters as shown in **DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000 FOR UREA**.

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 sampler. Input the information required by the data system, such as concentration, replicates and QuikChem scheme (See **DATA ANALYSIS AND CALCULATIONS**).

Both channels are calibrated with ammonia standards. The first “samples” run following the calibration are urea check standards. Then, during the analysis, ammonia and urea check standards are run every 10-20 samples, depending upon requirements. Calibration must be re-run whenever new reagents are prepared.

**SYSTEM NOTES**

For information on system maintenance and troubleshooting refer to the Troubleshooting Guide in the System Operation Manual.

Let reagents run for 10-15 minutes prior to starting analysis. The heater must be at 60°C before beginning. If diluted sample concentrations are greater than 600 ppm N total (urea and ammonia), the samples need to be further diluted to bring them within the method range. This method can be run with a second order calibration, however the accuracy will not be of the same quality as with a third-order calibration.

**DATA ANALYSIS AND CALCULATIONS**

Calibration is performed 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. Report only those values that fall between the lowest and highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.  
Report results in % N as Urea in fertilizer.

**METHOD PERFORMANCE**

See: Quikchem method 14-206-00-4-A from Lachat Instruments.

**FLOWCHARTS, AND VALIDATION DATA:**

See: Quikchem method 14-206-00-4-A from Lachat Instruments.

**TABLE AND DIAGRAMS:****DATA SYSTEM PARAMETERS FOR QUIKCHEM 8000 FOR UREA**

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

Sample throughput: 40 samples/h, 90 s/sample  
Pump Speed: 35  
Cycle Period: 90

**Analyte Data:**

Concentration Units: mg NH<sub>3</sub>-N/L as urea  
Peak Base Width: 47 s  
% Width Tolerance: 100  
Threshold: 7900  
Inject to Peak Start: 85 s  
Chemistry: Direct

**Calibration Data:**

Level	1	2	3	4	5
Concentration mg N/L	600	300	150	60	0.0

Calibration Rep Handling: Average  
Calibration Fit Type: 3rd Order Polynomial  
Weighting Method: None  
Force through zero: no

**Sampler Timing:**

Min. Probe in Wash Period: 20 s

Probe in Sample Period: 35 s

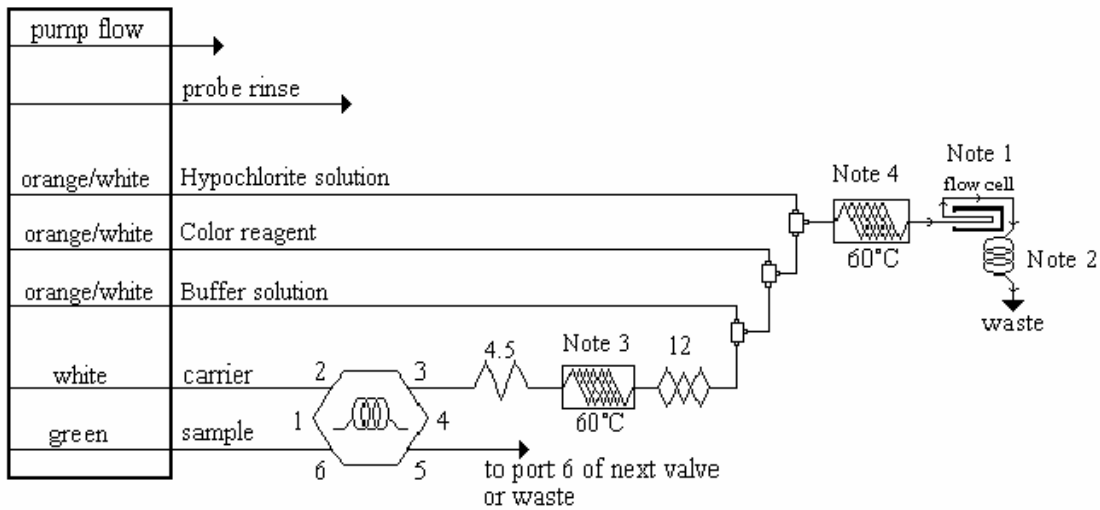
**Valve Timing:**

Load Time: 0 s

Load Period: 25 s

Inject Period: 65 s

**UREA/AMMONIA MANIFOLD DIAGRAM**




**Carrier:** Urease Solution (Reagent 3)

**Manifold Tubing:** 0.5 mm (0.022 in) i.d. This is 2.5 µL/cm.

**AE Sample Loop:** 7.5 cm x 0.5 mm i.d.

**QC8000 Sample Loop:** 13 cm x 0.5 mm i.d.

**Interference Filter:** 660 nm

**Apparatus:** An injection valve, a 1 mm path length flow cell, and a colorimetric detector module is required. The  shows xxx cm (see notes below) of tubing wrapped around the heater block at the specified temperature.

**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:** The flow cell is 1 mm path length


**Note 2:** 50 cm backpressure loop x 0.5 mm i.d.

**Note 3:** 175 cm of tubing on the heater block

**Note 4:** 650 cm of tubing on the heater block

**Note 5:** The same heating module is used for both heaters (see notes 3 and 4)

**APPROVAL:**

Approved by:  Date: 1/27/03  
Signature

**Bureau Chief**

**Title**

**METHOD REVISION HISTORY:**

Version	Date	Description	Author
Original	6/11/98	Replaces N-450.00	W.M. Bell
Original	2/20/99	Replaces FM-460	W.M. Bell
Original	1/27/03	Replaces FM-461	W.M. Bell

**REFERENCES**

U.S. Environmental Protection Agency, Methods for the Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Revised March 1983,  
Quikchem method 14-206-00-4-A from Lachat Instruments.