

## **Nitrate+ Nitrite Nitrogen in Soil, Plant Tissue, and Water**

### **1. Application (analytes and matrices)**

In this procedure nitrogen, in the form of nitrate and nitrite ions, in water, soil, sludges, sediments or plant tissue samples and analyzed by flow injection analyzer.

### **2. Method Sensitivity**

The approximate working ranges are 0.03 to 2.00 mg/N (as NO<sub>3</sub> + NO<sub>2</sub>)/L in water, and 0.25 -40 mg/Kg for solids. This range might be extended by diluting the sample prior to analysis.

### **3. Summary of Methods**

KCl is used to extract NO<sub>3</sub><sup>-</sup>-N from the soil and tissue samples. While water samples are analyzed directly without extraction. Nitrate in sample is quantitatively reduced to nitrite by passing the sample through a copper-coated cadmium column. The nitrite (reduced nitrate plus original nitrite) is determined by diazotizing with sulfanilamide dihydrochloride. The remaining water soluble dye has a magenta color which is read at 520 nm.

### **4. Safety and Waste Management**

- 4.1 Each chemical compound should be treated as a potential health hazard. The laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material handling data sheets should be made available to all personnel involved in the chemical analysis.
- 4.2 All laboratory wastes, excess reagents and samples must be disposed of in a manner that is consistent with applicable rules and regulations.
- 4.3 Waste disposal guidelines are described in the University of Wisconsin Chemical Safety and Disposal Guide.

### **5. Sample Collection, Preservation and Handling**

- 5.1 Samples are collected by clients in clean containers and submitted to the laboratory for analysis.
- 5.2 A chain-of-custody form is submitted with the samples. If samples were not preserved by client they will be preserved immediately be preserved at the lab. Soil and plant tissue samples are dried at 55C and 65C, respectively.
- 5.3 Drying is followed by grinding and extraction by 2N KCl solution.

- 5.4 Aqueous solutions are preserved by addition of 1 mL of concentrated sulfuric acid per liter of sample and kept in a fridge at 0-5C until analyzed.

## **6. Potential Interferences**

- 6.1 Residual chlorine can interfere by oxidizing the cadmium column.  
6.2 Low results will be obtained for samples containing high concentrations of iron, copper and other metals. EDTA is added to the buffer to reduce such interference.  
6.3 Color and turbidity may interfere with results. Turbidity is removed by manual filtration. Sample color may be corrected for by running the sample through the manifold without the color reagent and subtracting the result obtained from the result using the color reagent.

## **7. Equipment and Analytical Instruments**

- 7.1 Weigh boat (metal or glass)  
7.2 Erlenmeyer flasks (50-ml)  
7.3 Pipette bank (15-ml)  
7.4 Time-controlled, oscillating shaker.  
7.5 Filter paper, 9-cm (Whatman No. 2 or equivalent)  
7.6 Funnel tubes (15-ml)  
7.7 Glass test tubes (6.2-ml)  
7.8 Flow injection analyzer  
7.9 XYZ sampler

## **8. Consumable Supplies, Reagents and Standards**

- 8.1 All reagents and standards should be stored in the appropriate bottles and labeled with the following information: identity, supplier, lot number, date, preparer's initials and concentrations. Use de-ionized water for all solutions.  
8.2 2 N KCl-solution (1044.40 g of KCl to 7 liters of de-ionized water).  
8.3 15 N Sodium hydroxide solution (150 g of NaOH added slowly to 250 mL of DI water and swirled until dissolved).  
8.4 Ammonium chloride buffer, pH 8.5: In a 1 L flask, dissolve 85.0 g ammonium chloride and 1 g disodium ethylenediamine tetraacetic acid dehydrate in about 800 mL de-ionized water. Adjust pH to 8.5 with 15 N NaOH.  
8.5 Sulfanilamide color reagent: To a 1 L flask add about 600 mL de-ionized water/ Then add 100 mL of 85% phosphoric acid, 40 g sulfanilamide, and 1 g N-(1-naphthyl ) ethylenediamine dihydrochloride (NED). Stir to dissolve and dilute to mark and mix. Store in a dark bottle.

## **9. Method of Extraction and Analysis**

- 9.1 Weigh out 1.50 g of soil or .25 g of tissue into a weigh boat.  
9.2 Transfer sample to a 50-ML Erlenmeyer flask.

- 9.3 Add 15-ml of 2 N KCl solution using constant suction pipette.
- 9.4 Shake for 15 minutes on oscillating shaker.
- 9.5 Filter immediately.
- 9.6 Pipette 5-ml of filtrate into glass test tube.
- 9.7 Analyze by flow injection.
- 9.8 The sample preparation procedure is taken from section 7.1.1 of Lachat QuikChem Method 12-107-04-1-B.

## **10 Calibration, Standardization and Calculations**

- 10.1 The instrument is calibrated using a set of 6 calibration standards of the following concentrations 20, 10, 2, 1, .3, 0 mg/L of carrier solution. The standards are prepared from primary stock standards purchased from an outside vendor. Date of preparation, lot number, initials of the person who prepared the standards and expiration date of standards are marked on the standards containers. Working standards are preserved in concentrated sulfuric acid and kept in a fridge at 0-4°C.
- 10.2 Correlation coefficient of standard curve of calibrators must be at least 0.995 for the calibration test to pass. Sample concentration is calculated from a regression equation by plotting response versus standard concentration

## **11. Quality Control**

- 11.1 Method Blank (MB) – At least one MB must be analyzed with each batch of samples in order to assess contamination from the laboratory environment. If MB values exceed the method detection limit, laboratory or reagent contamination should be suspected, take correction action before continuing the analysis.
- 11.2 Laboratory Control Sample (LCS) – At least one LCS must be analyzed with each batch of sample. Calculate accuracy as percent recovery. If the recovery of the analyte falls outside the required control limits of 90-110%, the analyte is judged out of control, take corrective action before continuing analysis.
- 11.3 Replicates and Spikes\_ At least 10% of samples of a sample batch are replicates and spikes. If spikes and replicates do not fall within the specified limits of  $\pm 10\%$  of true value then re-digest and analyze sample batch including QC samples.
- 11.4 Continuing Calibration Verification (CCV) – For all determinations, a mid-range check standard must be analyzed immediately after daily instrument calibration, after every tenth sample, and at the end of the sample run. This process verifies that the instrument is within 10% of calibration. If the CCV standard indicates that the calibration is outside of present limits, take corrective action before continuing analysis.

## **12. Data Assessment and Reporting of Results**

- 12.1 Data are accepted when quality control samples fall within the set limits. Corrective actions of re-digestion and/or re-analyzing of samples and quality control samples will take place to handle out of control data.

- 12.2 Data are reported as mg/l of N for water and soil and as a % N for plant tissue on a dry weight basis.
- 12.3 Results are reported as mg/L or mg/Kg of nitrogen in the form of nitrate  $\text{NO}_3^-$ -N and/or  $\text{NO}_2^-$ - N in the different matrices.

### **13. References**

- 13.1 EPA method 353.2. Determination of Nitrate+Nitrite Nitrogen by automated colorimetry. Revision 2.0. August 1993.