

## Potato Petioles Nitrate-Nitrogen

### 1. Application

In this procedure, nitrogen in the form of nitrate ( $\text{NO}_3\text{-N}$ ) is extracted from potato leaf petioles with 2% acetic acid (at room temperature) and analyzed by flow injection.

### 2. Summary of Methods

Inorganic nitrate in plant tissue are readily water soluble and is extracted by water from samples that have been oven dried and finely ground. Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. The nitrite (reduced nitrate plus 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. Results are reported on a dry weight basis.

### 3. Safety

All chemicals should be considered 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. Interferences

- 4.1 Efficiency of nitrate reduction by the cadmium reduction tube can be adversely affected by interferences in plant extracts. A dilution ratio of no less than 1:10 should be used when nitrate is measured in extracts of oven-dried samples (0.1 g to 25 ml 2% acetic acid).
- 4.2 Build-up of suspended matter in the reduction column will restrict sample flow. Since  $\text{NO}_3\text{-N}$  is soluble, the sample may be pre-filtered.
- 4.3 Low results would be obtained for samples that contain high concentrations of iron, copper or other metals. In this method, EDTA is added to the buffer to reduce this effect.
- 4.4 Samples that contain large concentrations of oil and grease will coat the surface of the cadmium in the reduction column. This interference can be eliminated by pre-extracting the sample with an organic solvent (e.g. methanol).

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

The youngest fully-expanded leaves of potato plants, usually the 4<sup>th</sup> or 5<sup>th</sup> leaf from the apex, are to be sampled. Leaflets should be stripped from the petioles by hand. Petioles are to be dried at 50-55°C and ground in a Wiley Mill and sieved through a 12 mesh screen. When stripping could not be done immediately after sampling, leaflets should be kept cool during transit to the laboratory where they can be stored at 4°C.

## 6. Apparatus and Materials

- 6.1 Weigh paper
- 6.2 Erlenmeyer flasks (125ml)
- 6.3 Pipette bank (15ml)
- 6.4 Time-controlled, oscillating shaker
- 6.5 Filter paper, 9cm (Whatman No. 2 or equivalent)
- 6.6 Funnel tubes (15ml)
- 6.7 Glass test tubes (6.2ml)
- 6.8 Flow injection analyzer (Lachat QuikChem 8000)

## 7. Reagents

- 7.1 2% acetic acid solution (40 ml acetic acid, bring to 2L with distilled water)
- 7.2 15 M sodium hydroxide
- 7.3 Ammonium chloride buffer, pH = 8.5
- 7.4 Sulfanilamide color reagent
- 7.5 Potassium nitrate standards

## 8. Methods

- 8.1 Weigh out 0.10 g of dried leaf petiole sample into weigh paper.
- 8.2 Transfer sample to a 125ml Erlenmeyer flask.
- 8.3 Add 25 ml of 2% acetic acid.
- 8.4 Shake for 15 minutes on oscillating shaker.
- 8.5 Filter immediately into funnel tubes.
- 8.6 Pour filtrate into glass test tubes.
- 8.7 Analysis using flow injection analyzer.
- 8.8 Dilute sample with DI water as necessary.

## 9. Calculations

- 9.1 Sample concentration (mg/L) is calculated from a regression equation by plotting response versus standard concentration. Final nitrate content of sample is calculated as follows:
- 9.2 Nitrate content (mg/L) = 
$$\frac{\text{measure value (mg/L)} \times \text{sample volume (ml)}}{\text{weight of sample (g)}}$$
- 9.3 Original nitrite in the sample is assumed to be negligible and not included in the calculations.

## 10. Quality Control

- 10.1 Laboratory Reagent Blank (LRB) – At least one LRB is analyzed with each batch of samples to assess contamination from the laboratory environment. Contamination from the laboratory or reagents is suspected if LRB values exceed the detection limit of the method. Corrective action must be taken before proceeding.
- 10.2 Potato Petioles Standard – One or more standards of known extractable nitrate content are analyzed with each batch of samples to check instrument calibration and procedural accuracy.

## 11. Reporting

Results are reported as ppm (mg/L) nitrogen in the form of nitrate  $\text{NO}_3^-$ -N.

## 12. References

- 12.1 AOAC 1980. Methods of Analysis. Nitrogen. W. Horwitz (ed.). Association of Official Analytical Chemists. Washington DC.
- 12.2 Lachat Instruments 2000. Determination of Nitrate/Nitrite in Surface and Wastewaters by Flow Injection Analysis. QuickChem Method 10-107-04-1-A.
- 12.3 MacKown and Weik 2004. Comparison of Laboratory and Quick-test Methods for Forage Nitrate. Crop Sci. 44:218-226.