



Nitrate plus Nitrite Nitrogen and Nitrite Nitrogen in Soil and Plant Extracts by Segmented Flow Analysis (SFA)

(Cartridge Part #A002690)

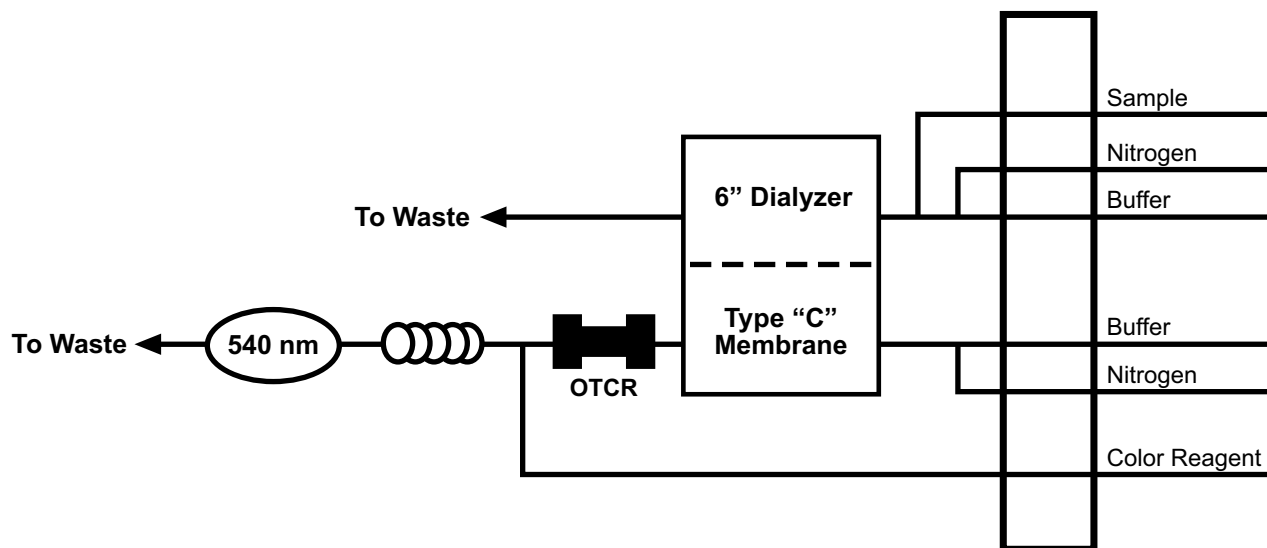
1.0 Scope and Application

- 1.1 This method is used to determine the concentration of nitrate (NO_3^-) nitrogen plus nitrite (NO_2^-) nitrogen or nitrite nitrogen singly in soil and/or plant extracts.
- 1.2 The Method Detection Limit (MDL) for this method is 0.004 mg/L nitrate plus nitrite nitrogen. The range of this method is 0.10–50 mg/L. The range may be extended to analyze higher concentrations by sample dilution or by the use of higher order curve fitting techniques.

2.0 Summary of Method

- 2.1 Nitrate is reduced quantitatively to nitrite by cadmium metal. Nydahl (Reference 15.6) provides a good discussion of nitrate reduction by cadmium metal. The nitrite formed, in addition to any nitrite originally present in the sample, is diazotized with sulfanilamide and subsequently coupled with *N*-1-naphthylethylenediamine dihydrochloride. The resulting highly colored azo dye is colorimetrically detected at 540 nm (Reference 15.10). A calibration curve allows for accurate quantitation of the detected nitrite.
- 2.2 Nitrite singly may be measured by performing the same analysis as in Section 2.1 but without the cadmium reduction. Without the cadmium, nitrate is not reduced to nitrite and is not detected since only nitrite forms the azo dye.
- 2.3 Both nitrate and nitrite may be measured simultaneously by using a two channel flow analyzer. One channel is used to measure nitrate plus nitrite (Section 2.1), while the second channel is used to measure nitrite only (Section 2.2). Using WinFLOW™ software, the results of the nitrite analysis may be subtracted from the results of the nitrate plus nitrite analysis, thus providing quantitative nitrate results.
- 2.4 For additional references, Patton (Reference 15.7) and Fox (Reference 15.1) provide discussions of the mechanisms and kinetics of the color forming reactions used in this method.

2.5 A general flow diagram of the SFA system used in development of this method is shown below:



3.0 Definitions

Definitions for terms used in this method are provided in Section 16.0, “Glossary of Definitions and Purposes.”

4.0 Contamination and Interferences

- 4.1 Turbid samples may interfere with the photometric detector’s ability to measure the true absorbance of the sample. Filter turbid samples prior to analysis.
- 4.2 Iron, copper, and other metals may interfere with the analysis by binding with the nitrate and/or nitrite in the sample, thus blocking the color formation reaction. Eliminate this interference by using ethylenediaminetetraacetic acid (EDTA) in the buffer solution.
- 4.3 Samples that are outside the functional pH range of the ammonium chloride buffer may affect the results obtained from this method. Adjust the pH of these samples to within a range of 5–9 using either concentrated hydrochloric acid (HCl) or ammonium hydroxide (NH₄OH).
- 4.4 Oil and grease will coat the cadmium surface, thus reducing its reduction efficiency. Extract samples containing large concentrations of oil and grease with an appropriate organic solvent (Reference 15.5).
- 4.5 Sulfide in the presence of cadmium will form cadmium sulfide (CdS), which will precipitate from solution. Samples containing sulfide cannot be determined by this method without first removing the sulfide by precipitation with cadmium salts (Reference 15.10).

- 4.6 Chlorine may reduce the reduction efficiency of the cadmium reactor. Samples that may contain residual chlorine should be tested for reduction efficiency through the analysis of Matrix Spike/ Matrix Spike Duplicate (MS/MSD) samples (Section 9.3). When necessary, dechlorinate samples with sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$).
- 4.7 Method interferences may be caused by contaminants in the reagents, reagent water, glassware, etc., which may bias the results. Care should be taken to keep all such items free of contaminants.
- 4.8 Norwitz and Keliher (References 15.4 and 15.5) have compiled a comprehensive study of interferences in the spectrophotometric analysis of nitrite.

5.0 Safety

- 5.1 The toxicity or carcinogenicity of each compound or reagent used in this method has not been fully established. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be reduced to the lowest possible level.
- 5.2 For reference purposes, a file of Material Safety Data Sheets (MSDS) for each chemical used in this method should be available to all personnel involved in this chemical analysis. The preparation of a formal safety plan is also advisable.
- 5.3 The following chemicals used in this method may be highly toxic or hazardous and should be handled with extreme caution at all times. Consult the appropriate MSDS before handling.
 - 5.3.1 Ammonium Chloride, NH_4Cl (FW 53.50)
 - 5.3.2 Ammonium Hydroxide, NH_4OH (FW 35.05)
 - 5.3.3 Cadmium, Cd (FW 112.40)
 - 5.3.4 Chloroform, CHCl_3 (FW 119.38)
 - 5.3.5 Cupric Sulfate Pentahydrate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (FW 249.61)
 - 5.3.6 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $\text{C}_{10}\text{H}_{16}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$ (FW 372.24)
 - 5.3.7 Hydrochloric Acid, concentrated, HCl (FW 36.46)
 - 5.3.8 *N*-(1-naphthyl)ethylenediamine Dihydrochloride, $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$ (FW 259.18)
 - 5.3.9 Phosphoric Acid, concentrated, H_3PO_4 (FW 98.00)
 - 5.3.10 Potassium Nitrate, KNO_3 (FW 101.11)
 - 5.3.11 Potassium Nitrite, KNO_2 (FW 85.11)
 - 5.3.12 Sulfanilamide, $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ (FW 172.21)

- 5.4 Unknown samples may be potentially hazardous and should be handled with extreme caution at all times.
- 5.5 Proper personal protective equipment (PPE) should be used when handling or working in the presence of chemicals.
- 5.6 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.

6.0 Apparatus, Equipment, and Supplies

- 6.1 Segmented Flow Analysis (SFA) System (OI Analytical Flow Solution® IV) consisting of the following:
 - 6.1.1 Model 502 Multichannel Peristaltic Pump
 - 6.1.2 Random Access (RA) Autosampler
 - 6.1.3 Expanded Range (ER) Photometric Detector with 5-mm path length flowcell and 540-nm optical filter
 - 6.1.4 Data Acquisition System (PC or Notebook PC) with WinFLOW software
 - 6.1.5 Nitrate+Nitrite and Nitrite in Soil and Plant Extracts Cartridge (Part #A002690)
- 6.2 Sampling equipment—Sample bottle, amber glass, with polytetrafluoroethylene (PTFE)-lined cap. Clean by washing with detergent and water, rinsing with two aliquots of reagent water, and drying by baking at 110°–150°C for a minimum of one hour.
- 6.3 Standard laboratory equipment including volumetric flasks, pipettes, syringes, etc. should all be cleaned, rinsed, and dried per bottle cleaning procedure in Section 6.2.

7.0 Reagents and Calibrants

- 7.1 Raw Materials
 - 7.1.1 Ammonium Chloride, NH_4Cl (FW 53.50)
 - 7.1.2 Ammonium Hydroxide, NH_4OH (FW 35.05)
 - 7.1.3 Brij®-35, 30% w/v (Part #A21-0110-33)
 - 7.1.4 Chloroform, CHCl_3 (FW 119.38)
 - 7.1.5 Cupric Sulfate Pentahydrate, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (FW 249.61)

- 7.1.6 Deionized Water (ASTM Type I or II)
- 7.1.7 Ethylenediaminetetraacetic Acid, Disodium Salt Dihydrate (EDTA), $C_{10}H_{16}N_2Na_2O_8 \cdot 2H_2O$ (FW 372.24)
- 7.1.8 Hydrochloric Acid, concentrated, HCl (FW 36.46)
- 7.1.9 *N*-(1-naphthyl)ethylenediamine Dihydrochloride, $C_{12}H_{14}N_2 \cdot 2HCl$ (FW 259.18)
- 7.1.10 Phosphoric Acid, concentrated, H_3PO_4 (FW 98.00)
- 7.1.11 Potassium Nitrate, KNO_3 (FW 101.11)
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