

# Method for Nitrate Determination by Cadmium Reduction, Version 2.3

## 1 PRINCIPLE

This method is designed for the determination of nitrate in soil, water, and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIALab analyzer. Nitrate is reduced to nitrite via a cadmium column placed on the instrument. The nitrite is then reacted with sulfanilamide and coupled with N- (1-Naphthyl) ethylenediamine dihydrochloride to form a magenta colored azo dye.

This method is designed to be run on the FIAlyzer-1000 or the FIAlyzer-FLEX.

## 2 SUMMARY

This method is designed for the determination of nitrate in samples of various matrices. The method is capable of detecting nitrate in the range of 0.003-50.0mg N-NO<sub>3</sub> / L. For more information, see the section on performance metrics.

## 3 SAFETY

The toxicity or carcinogenicity of all reagents used in this method must be taken into account and therefore each chemical listed below should be handled accordingly.

Each laboratory is responsible for maintaining compliance with OSHA regulations regarding the safe handling of the chemicals specified in this method. Material Safety Data Sheets (MSDS) should be made available to all personnel using the method.

All waste materials should be disposed of in a responsible manner, in accordance with federal, state, local, and any other applicable regulations.

The following chemicals have the potential to be highly toxic or highly hazardous; for detailed explanations consult the MSDS:

- **Phosphoric Acid**
- **Cadmium**
- **Sodium Hydroxide**
- **Copper (II) Sulfate**

## 4 EQUIPMENT AND SUPPLIES

### ▪ Equipment:

- Balance, analytical, with a 0.01 g resolution
- pH meter, accurate to 0.1 pH
- Pipettes for making standards

### ▪ Glassware:

- Class A volumetric flask, 1L, QTY 2
- Graduated cylinder, 100mL, QTY 1
- Glass storage container, 1L, clear, QTY 2
- Glass storage container, 1L, brown tinted, QTY 1

### ▪ Autosampler (for high sample loads):

- Cetac ASX-280/560 (recommended) or AIM-3200/3300

## 5 REAGENTS AND STANDARDS

Chemical part numbers refer to Sigma-Aldrich unless noted otherwise.

Chemical	Chem. Formula	CAS#	Supplier PN
Deionized Water	H <sub>2</sub> O	7732-18-5	
Ammonium Chloride	NH <sub>4</sub> Cl	12125-02-9	213330
EDTA, disodium form	C <sub>10</sub> H <sub>14</sub> N <sub>2</sub> Na <sub>2</sub> O <sub>8</sub> · 2H <sub>2</sub> O	6381-92-6	E4884
Sodium hydroxide	NaOH	1310-73-2	221465
Brij L23 Solution (30%)	C <sub>12</sub> E <sub>23</sub>	9002-92-0	B4184
Sulfanilamide	H <sub>2</sub> NC <sub>6</sub> H <sub>4</sub> SO <sub>2</sub> NH <sub>2</sub>	63-74-1	S-9251
85% Phosphoric acid	H <sub>3</sub> PO <sub>4</sub>	7664-38-2	695017
N-1-Naphthylethylene diamine dihydrochloride	C <sub>10</sub> H <sub>7</sub> NHCH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub> · 2HCl	1465-25-4	222488
Copper (II) sulfate pentahydrate	CuSO <sub>4</sub> · 5H <sub>2</sub> O	7758-99-8	C7631

### ■ Preparation of Reagents

- **Carrier:** Matrix match carrier to samples. Salt solution to seawater samples, extraction solution for soil samples, etc.
- **Reagent 1:** Ammonium Chloride Buffer (1 L)
  - Dissolve 86g of ammonium chloride in 800 mL of deionized water.
  - Add 1.0g of disodium EDTA.
  - Adjust the pH of this solution to 8.5 +/- 0.05 using sodium hydroxide pellets as needed (approximately 3.5 g).
  - Add 0.5mL of 30% Brij L23. Mix until dissolved.
  - Fill container to 1 L mark using deionized water.
  - Mix well and store in a glass bottle.
  - The reagent should be clear with no particles in it.
- **Reagent 2:** Sulfanilamide Solution (1 L)
  - Mix 100ml of 85% phosphoric acid into 800 mL of deionized water.
  - Dissolve 40g of sulfanilamide and 1.0 g N-1-Naphthylethylene diamine dihydrochloride to this solution.
  - Add deionized water to a total volume of 1 L.
  - Mix well and store in a dark glass bottle.
  - The reagent should be clear or very faintly pink with no particles in it.
- **Probe Wash Solution:** Water with 0.1% Brij L23 (1L)
  - Add 3.3mL Brij L23 to 800mL of deionized water, dilute to 1L.
  - Mix well and store in a polyethylene bottle.
  - The wash solution should be clear with no particles in it.

- **Tips on Prolonging Cd Column Life [Optional]**
  - **CuSO<sub>4</sub> to Reagent 1:**
    - Prepare 2% CuSO<sub>4</sub> solution by addition of 10 g CuSO<sub>4</sub> 5H<sub>2</sub>O to 500 mL DI water.
    - Add 2 mL of 2% w/v CuSO<sub>4</sub> solution per 1 L of R1 solution.
  - **Degassing Carrier and Reagent 1:**
    - Degas Carrier and Reagent 1 *daily*.
    - If helium is available, sparge the solutions for one minute (approx. 20 psi sparging pressure).
    - If helium is not available, apply vacuum until solution bubbles for one minute.
    - Note that plastic bottles are gas permeable. Use glass containers.
- **Notes on reagent shelf life:**
  - Use of high quality laboratory glass bottles is important.
  - Reagent 1 should be prepared every twelve weeks.
  - Reagent 2 should be prepared every four weeks.
  - CuSO<sub>4</sub> solution, if used, should be prepared every twelve weeks.
- **Preparation of Standards**
  - Dilute 1000 mg/L N-(NO<sub>3</sub>) stock solution with carrier to the desired range of nitrate standards.

## 6 SAMPLE COLLECTION AND PRETREATMENT

This protocol only covers the analysis process. Sample collection and pretreatment depends on the type of sample and will have to be determined separately.

## 7 INSTRUMENT SETUP – MID-RANGE

### Flow injection analysis apparatus parameters:

Component	Specifications	FIALab PN
Analyzer	FIAlyzer-1000 or FIAlyzer-FLEX flow injection analyzer	*Inquire
Spectrometer	FLAME-T-VIS-NIR Spectrometer. 350-1000 nm.	64019
Light Source	HL-2000-LL. Tungsten-halogen, 360-2000 nm.	17041
Peristaltic Pump Tubing	1.02mm ID (white/white)	*Inquire
Sample Injection Loop	35 $\mu$ L – 3.0in/7.6cm of Teflon capillary tubing with 0.03in/0.71mm ID	*Inquire
Reaction coil 1	Cadmium column	71010
Reaction coil 2	750 $\mu$ L – 65in/165cm of Teflon capillary tubing with 0.03in/0.76mm ID	79209
SMA-Z Flow Cell	10mm light path	79028

### Software parameters:

Parameter	Value
Pump Speed	60%
Primary Spectrometer Wavelength	540nm
Secondary Wavelength (optional – for extended upper range)	600nm
Reference Wavelength	650nm

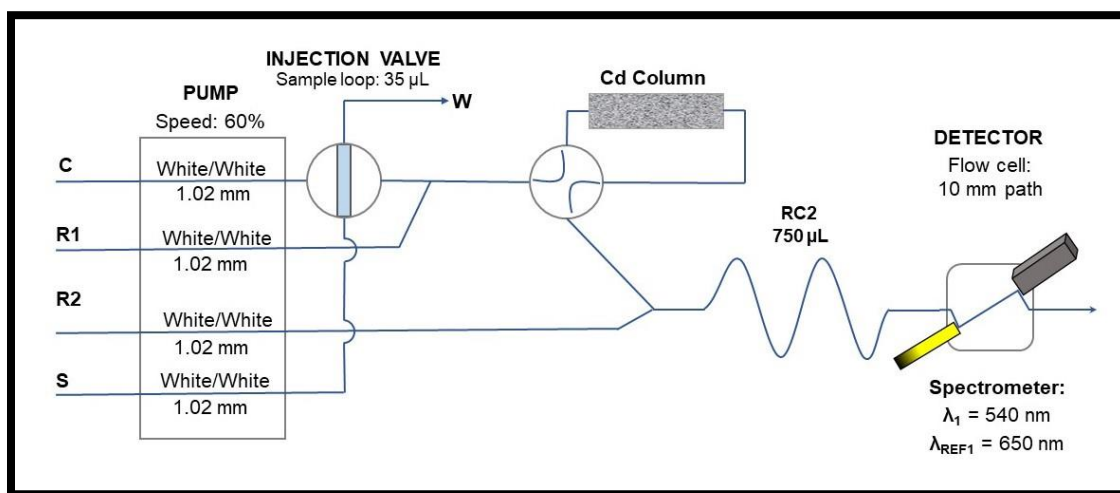


Fig. 1- Flow schematic for nitrate determination.

## 8 FIASOFT METHOD

- The program script using an autosampler is shown below. For manual sample introduction see the FIASoft manual.

### 'Nitrate Method Script

FIALab Injection Valve Sample Load  
Message Turn On Cadmium Column  
FIALab Peristaltic Pump CounterClockwise(%) 60  
Autosampler Wash  
Next Sample  
Delay (sec) 60

### Loop Start

Autosampler Wash  
Analyte New Sample  
Next Sample  
FIALab Injection Valve Sample Inject  
Delay (sec) 3  
FIALab Injection Valve Sample Load

Spectrometer Reference Scan  
Spectrometer Absorbance Scanning  
Delay (sec) 30  
Spectrometer Stop Scanning

### Loop End

Autosampler Rinse  
Delay (sec) 60  
Autosampler Wash  
Delay (sec) 30  
Autosave Data C:\Users\FIALab\Desktop\Autosave Data\DateTime  
FIALab Peristaltic Pump Off

## 9 PERFORMANCE METRICS

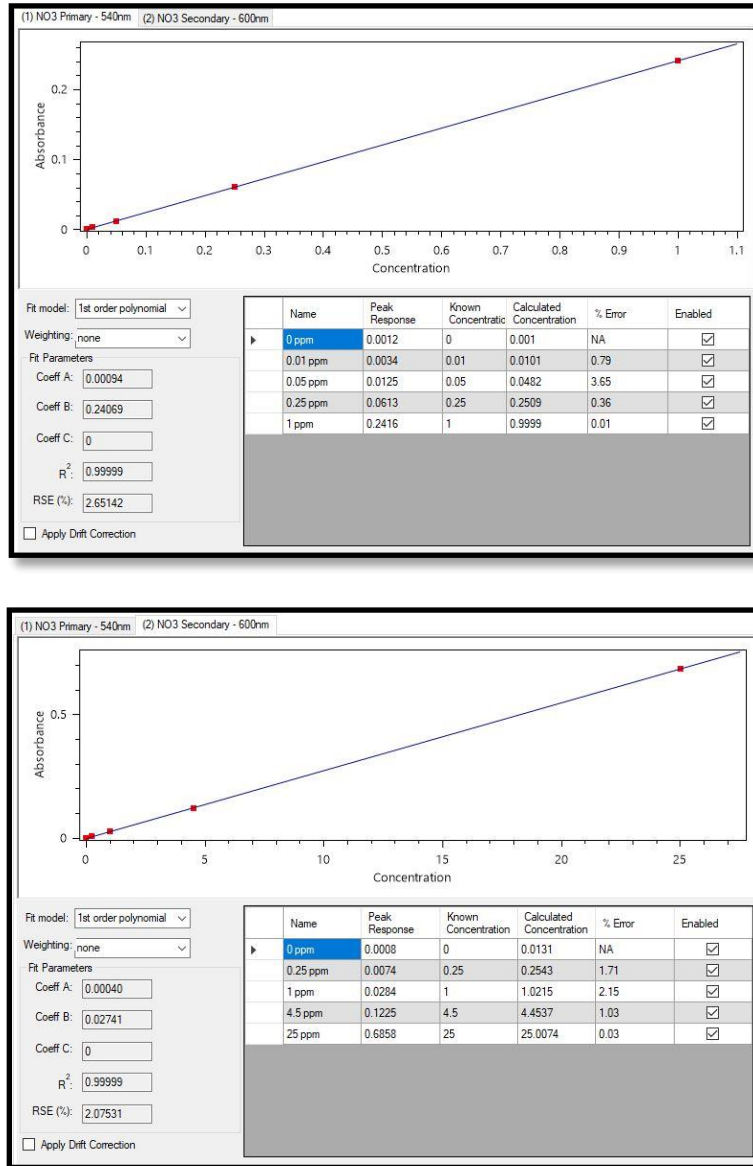


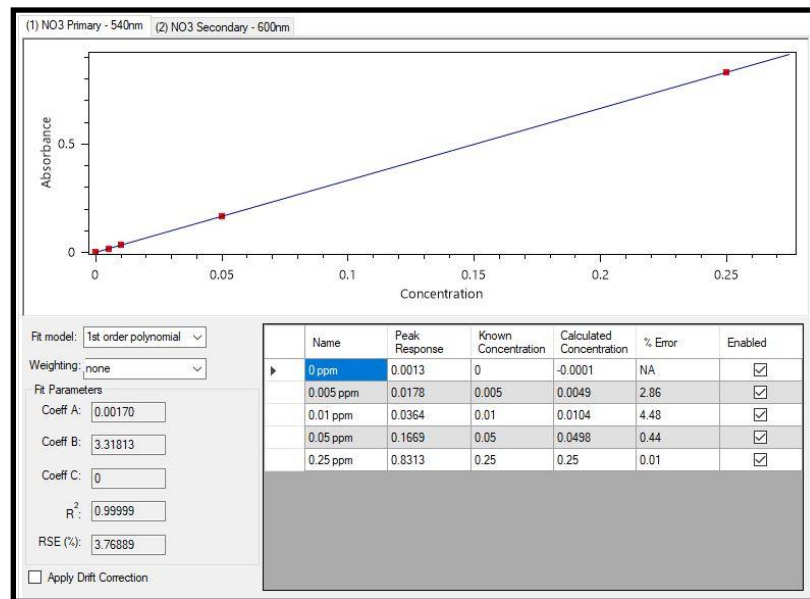
Fig. 2 – Example plot and calibration data for nitrate.

- Lower limit of detection: 0.003mg N-NO<sub>3</sub> / L – 4.84\*10<sup>-8</sup> mol N / L
- Lower limit of quantification: 0.008mg N-NO<sub>3</sub> / L – 1.29\*10<sup>-7</sup> mol N / L
- Upper limit of detection: 25.0mg N-NO<sub>3</sub> / L – 4.03\*10<sup>-4</sup> mol N / L
- Sample throughput: 100 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes

## 10 ALTERNATE CONFIGURATIONS

### ▪ Low-Range Setup

Component	Specifications	FIALab PN
Sample Injection Loop	140µL – 12.0in/30cm of Teflon capillary tubing with 0.03in/0.71mm ID	270160
SMA-Z Flow Cell	100mm light path	79038



- Lower limit of detection: 0.001mg N-NO<sub>3</sub> / L – 1.61\*10<sup>-8</sup> mol N / L
- Lower limit of quantification: 0.003mg N-NO<sub>3</sub> / L – 4.84\*10<sup>-8</sup> mol N / L
- Upper limit of detection: 1.0mg N-NO<sub>3</sub> / L – 1.61\*10<sup>-8</sup> mol N / L
- Sample throughput: 100 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes