

Method for Nitrate determination on High-Range Samples

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.

2. SUMMARY

This method is designed for the determination of nitrate in high range samples of various matrices. The method is capable of detecting nitrate in the range of 0.15-80mg N / L.

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 and other local 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**

4. EQUIPMENT AND SUPPLIES

Balance:

- Analytical, with a 0.01 g resolution

Glassware:

- Class A volumetric flasks of at least 50 ml
- Pipettes and appropriate beakers
- Tinted glass storage containers

Flow injection analysis apparatus:

- FIALab-2500 flow injection analyzer
- Cadmium column
- Tungsten-halogen light source, Ocean Optics HL-2000-LL or corresponding
- SMA-Z absorbance flow cell with 10 mm light path
- Spectrophotometer, Ocean Optics USB4000-VIS/NIR, USB4000-UV/VIS, or corresponding

Autosampler (for high sample loads):

- Cetac ASX-260/520 or AIM-3200/3300

5. REAGENTS AND STANDARDS

5.1 List of chemicals

- Deionized water, (H₂O) [CAS - 7732-18-5].
- Ammonium chloride, (NH₄Cl) [CAS - 12125-02-9]. Sigma-Aldrich P/N 213330 or corresponding.
- Ethylenediaminetetraacetic acid, ((HO₂CCH₂)₂NCH₂CH₂N(CH₂CO₂H)₂) [CAS - 60-00-4]. Sigma-Aldrich P/N E9884 or corresponding.
- Sodium hydroxide, (NaOH) [CAS - 1310-73-2]. Sigma-Aldrich P/N 221465 or corresponding.
- Sulfanilamide, (H₂NC₆H₄SO₂NH₂) [CAS - 63-74-1]. Sigma-Aldrich P/N S-9251 or corresponding.
- 85% Phosphoric acid, (H₃PO₄) [CAS - 7664-38-2]. Sigma-Aldrich P/N 79606 or corresponding.
- N-1-Naphthylethylene diamine dihydrochloride, (C₁₀H₇NHCH₂CH₂NH₂ · 2HCl) [CAS - 1465-25-4]. Sigma-Aldrich P/N 222488 or corresponding.

5.2 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 (1L)

- Dissolve 86g of ammonium chloride in 800ml of deionized water.
- Add 1.0g of ethylenediaminetetraacetic acid (**only if metals may be present**).
- Adjust the pH of this solution to ~8.5 using sodium hydroxide pellets as needed.
- Fill container to 1L mark using deionized water.
- Store in a glass bottle.

Reagent 2: Sulfanilamide Solution (1L)

- Mix 100ml of 85% phosphoric acid into 800ml of deionized water.
- Dissolve 40g of sulfanilamide and 1.0g N-1-Naphthylethylene diamine dihydrochloride to this solution.
- Add deionized water to a total volume of 1L.
- Mix well and store in a dark glass bottle.

Note: 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

5.3 Preparation of standards

- Dilute the 1000 mg/L N-(NO₃) stock solution with deionized water 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 – HIGH-RANGE

Flow injection analysis apparatus parameters:

- Peristaltic pump tubing: Tygon, 1.02mm/0.04in ID (white/black), FIALab P/N 270300
- Sample injection loop: 15µL - 3.0in/7.6cm of Teflon capillary tubing with 0.02 in/0.51 mm ID, FIALab P/N 270160
- Reaction coil 1: 50µL - 4.0in/10cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040
- Reaction coil 2: 750µL - 65.0in/165cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIALab P/N 270040
- SMA-Z flow cell: light path -10mm
- Flow rate: 50% pump speed (approx. 2.0 mL/min flow rate per pump channel)
- Bridge (“B” ports) connects to cadmium column

Recommended spectrometer parameters:

Primary wavelength: 540nm

Second wavelength: 580nm

Third wavelength: 595nm

Fourth wavelength: 600nm

Reference wavelength: 650nm

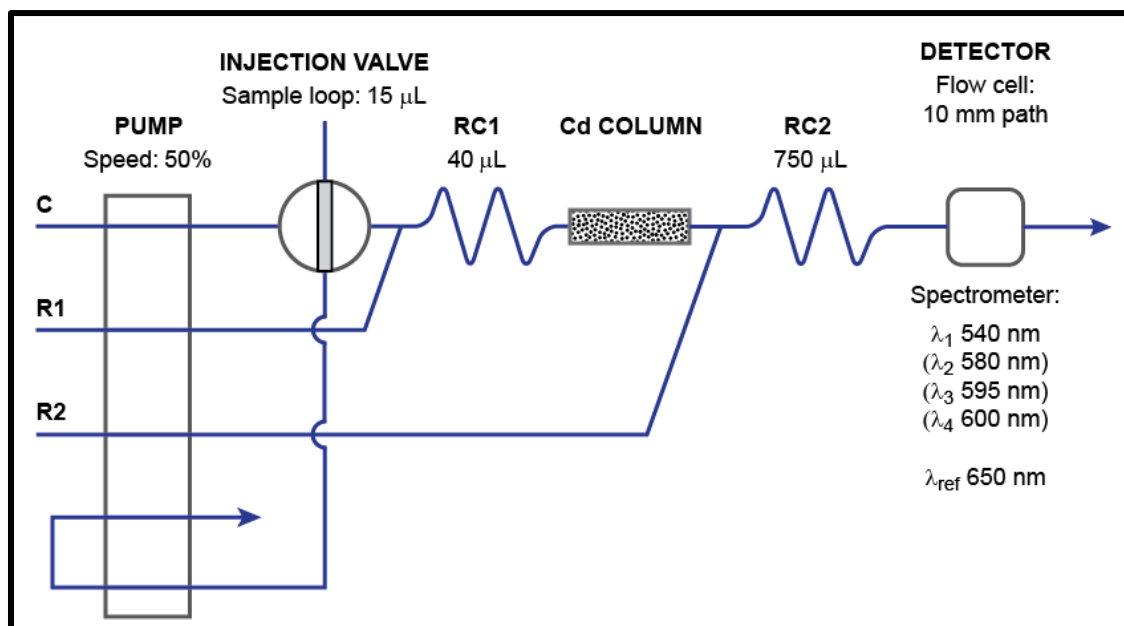


Fig. 1- Flow schematic for high-range nitrate determination on a FIALab-2500

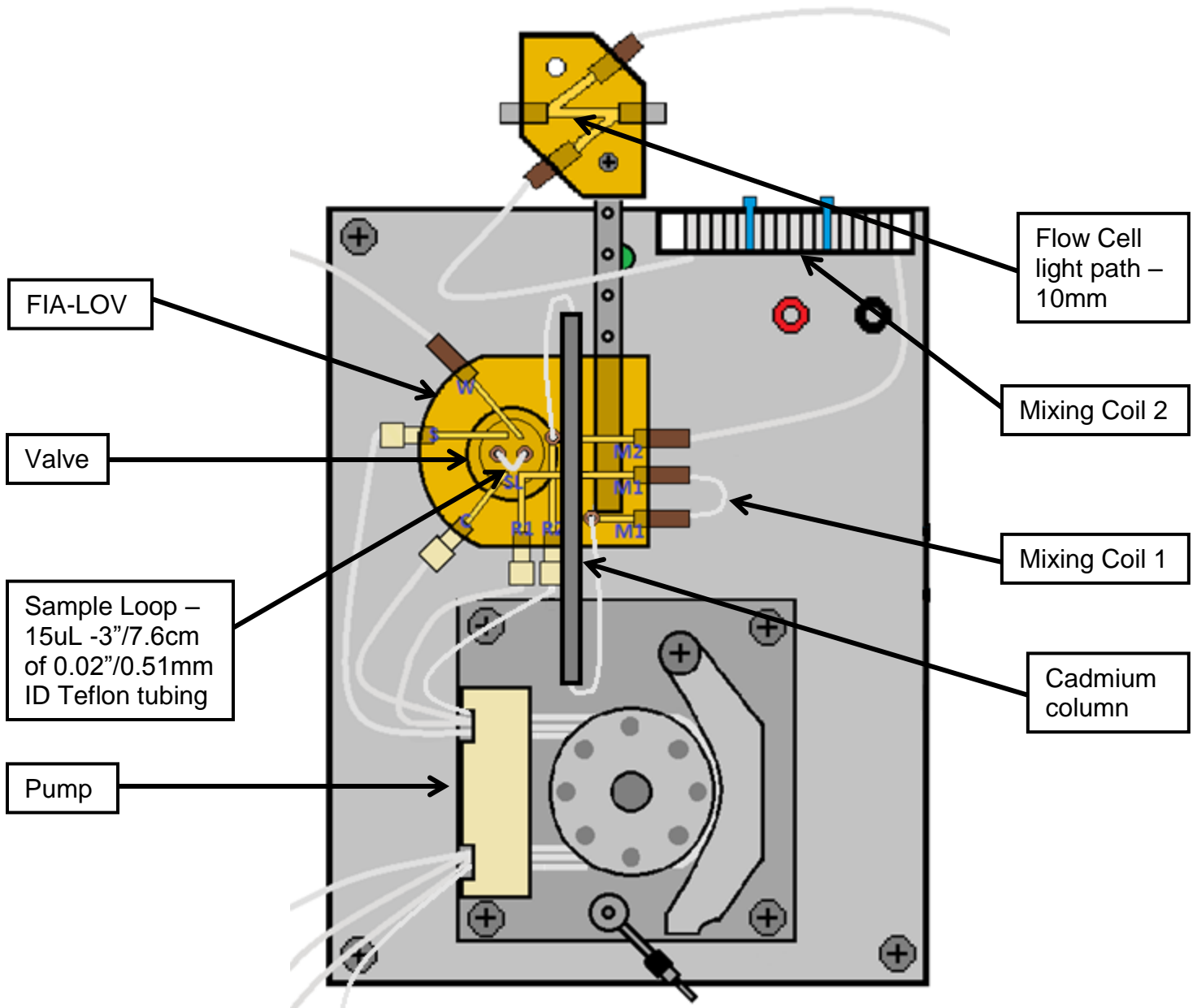


Fig. 2- Pictorial representation of high-range nitrate setup on a FIAlab-2500

The program script using an autosampler is shown below. For manual sample introduction see the FIALab-2500 software manual.

'Nitrate/Nitrite Assay

```
'FIA Template For FIALab-2500 System
Global Logon      ' logon to all components
Sample Description ' load sample description file
Injection Valve Sample Load
Optimize_FIALab2500
```

```
'Set Wavelengths
Hardware Settings Wavelength 1 (nm) 540
Hardware Settings Wavelength 2 (nm) 580
Hardware Settings Wavelength 3 (nm) 595
Hardware Settings Wavelength 4 (nm) 600
Reference Wavelength1 650
```

```
'Set delay time, start pump to prime lines
Valve Delay 5000      ' sample inject (msec)
Peristaltic Pump Clockwise(%) 50
Injection Valve Sample Load
Delay (sec) 40
Hardware Settings Optimize Integration
```

```
'Put autosampler in first sample (usually a blank)
Next Sample
Delay (sec) 35
```

LoopStart (#) 5000

```
'Inject sample, load next sample
'Autosampler wash
Analyte New Sample
Next Sample
Injection Valve Sample Inject
Delay (sec) 3
```

```
'Perform reference scan and start absorbance scans
Spectrometer Reference Scan
Spectrometer Absorbance Scanning
Delay (sec) 30
Spectrometer Stop Scanning
```

```
'Refresh plots and update concentrations
Refresh Plot
```

```
'Method clean up (at end of run)
If sampleid < 0 Then
    autosampler Wash
    ' Save Data Date-Time .dat
End If
```

Loop End

8. PERFORMANCE METRICS

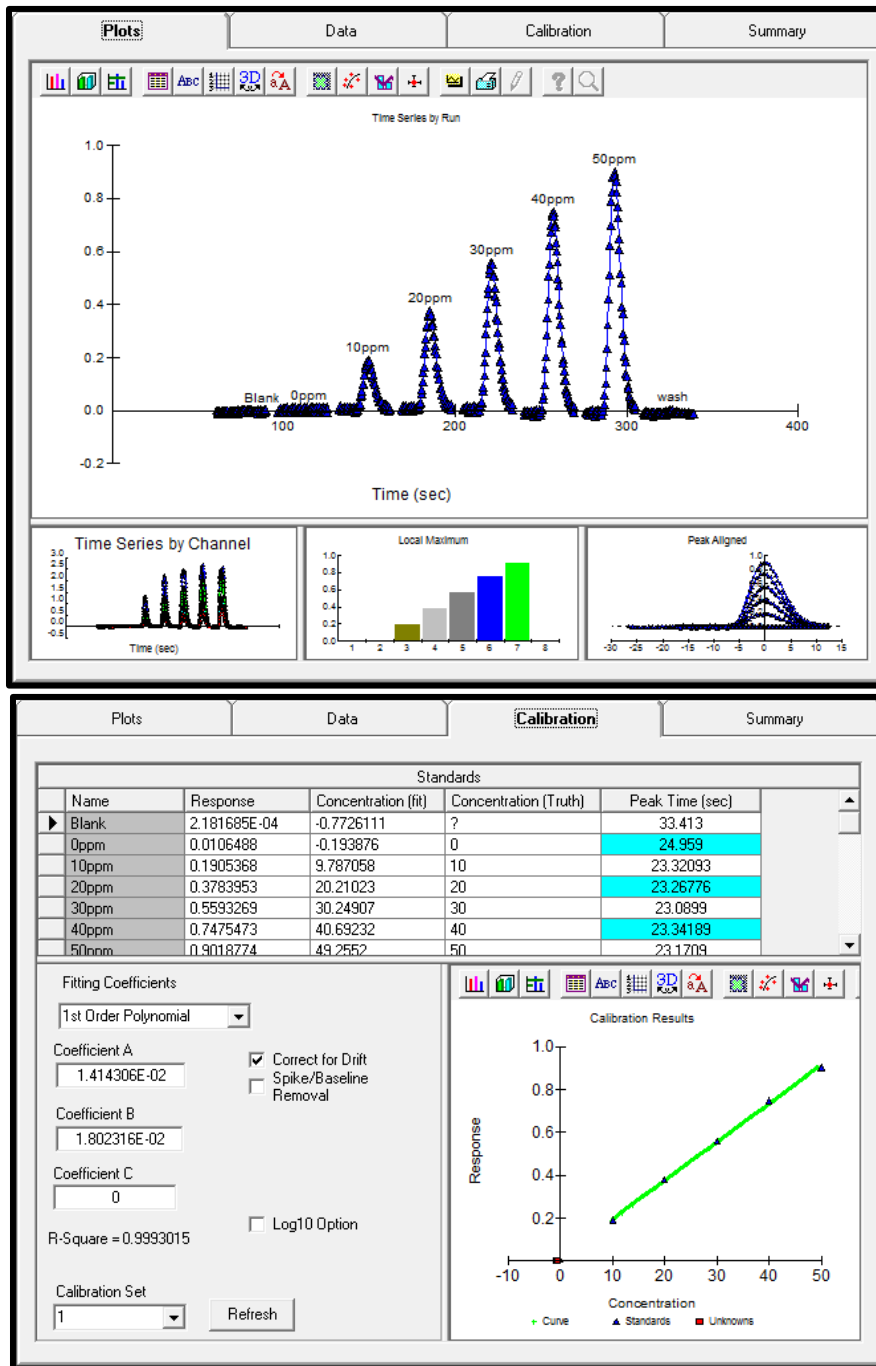


Fig. 3 – Example plot and calibration data for high-range nitrate

Lower limit of detection: 0.15mg/L – $1.1 \cdot 10^{-5}$ molN/L
 Upper limit of detection: 80 mg/L – $5.7 \cdot 10^{-3}$ molN/L
 Sample throughput: 100 samples/hr
 Startup: 5 minutes
 Shutdown: 5 minutes

FIAlab[®]



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