FIAlab®

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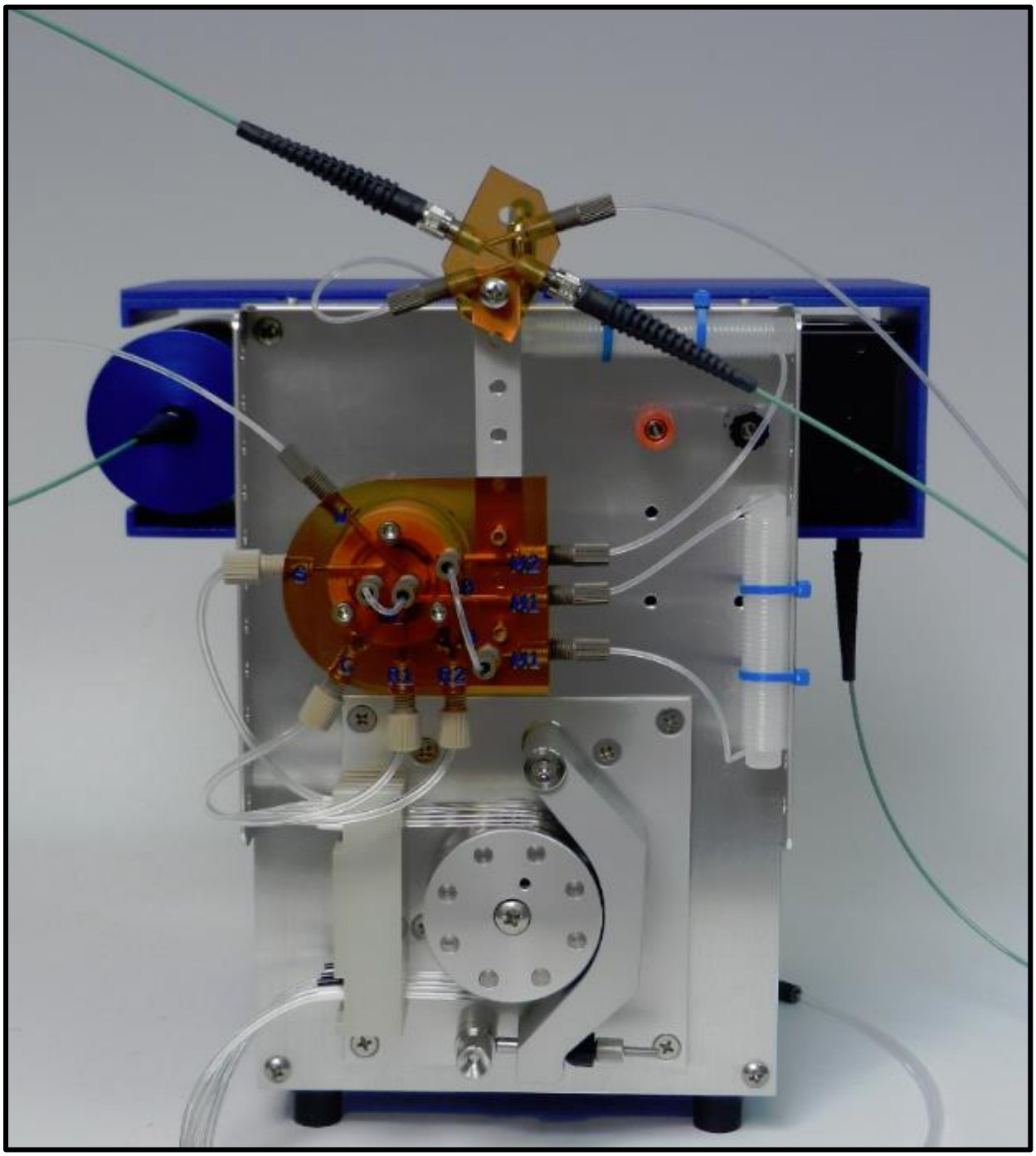

Method for Nitrite determination on Mid-Range Samples

1. PRINCIPLE

This method is designed for the determination of nitrite in soil, water and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIAlab analyzer. Nitrite ions react with sulfanilamide and couple to N- (1-Naphthyl) ethylenediamine dihydrochloride to form a magenta colored azo dye.
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2. SUMMARY

This method is designed for the determination of nitrite in high range samples of various matrices. The method is capable of detecting nitrite in the range of 0.015-40mg 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**

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
- 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
- Fiber Optic Cables (2)

Autosampler (for high sample loads):

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

5. REAGENTS AND STANDARDS

5.1 List of chemicals

- Deionized water, (H_2O) [CAS - 7732-18-5].
- Sulfanilamide, ($H_2NC_6H_4SO_2NH_2$) [CAS - 63-74-1]. Sigma-Aldrich P/N S-9251 or corresponding.
- 85% Phosphoric acid, (H_3PO_4) [CAS - 7664-38-2]. Sigma-Aldrich P/N 79606 or corresponding.
- N-1-Naphthylethylene diamine dihydrochloride, ($C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$) [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: 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.

Reagent 2: Unnecessary. Block R2 port on LOV with plug tubing

Note: Use of high quality laboratory glass bottles is important.
Reagent 1 should be prepared every four weeks

5.3 Preparation of standards

- Dilute the 1000 mg/L N-(NO_2) stock solution with deionized water to the desired range of nitrite 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:

- Peristaltic pump tubing: Tygon, 1.02mm/0.04in ID (white/black), FIAlab P/N 270300
- Sample injection loop: 35µL - 3.0in/7.6cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIAlab P/N 270040
- 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: 60% pump speed (approx. 2.0 mL/min flow rate per pump channel)
- Bridge: 40µL – 3.5in/9cm of Teflon capillary tubing with 0.03 in/0.75 mm ID, FIAlab P/N 270040

Recommended spectrometer parameters:

Primary wavelength: 540nm
Second wavelength: 580nm
Third wavelength: 595nm
Fourth wavelength: 600nm

Reference wavelength: 650nm

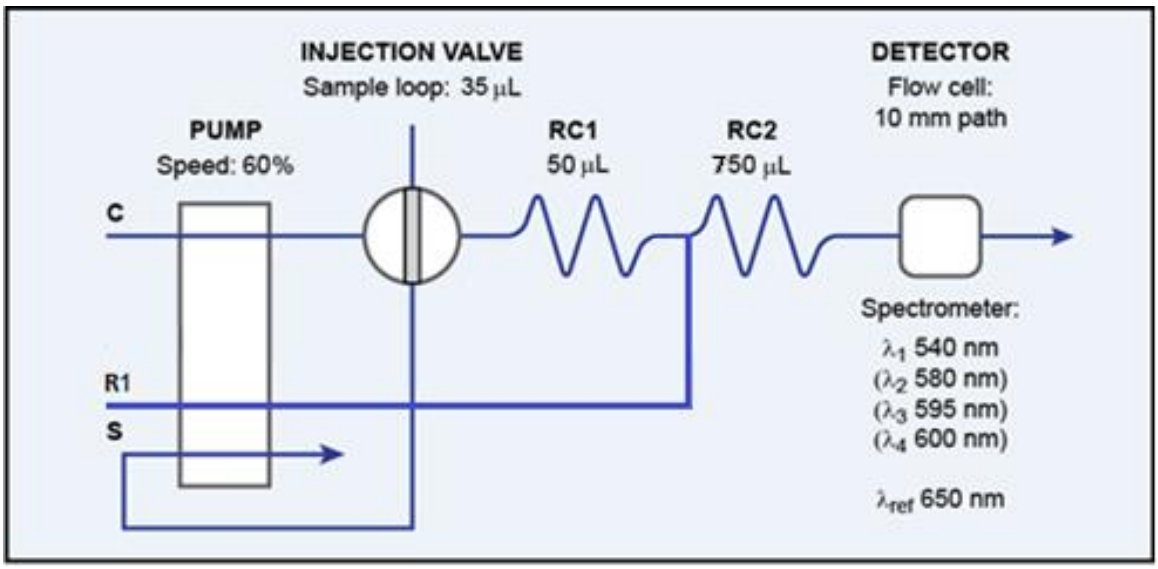

Fig. 1- Flow schematic for mid-range nitrite determination on a FIAlab-2500

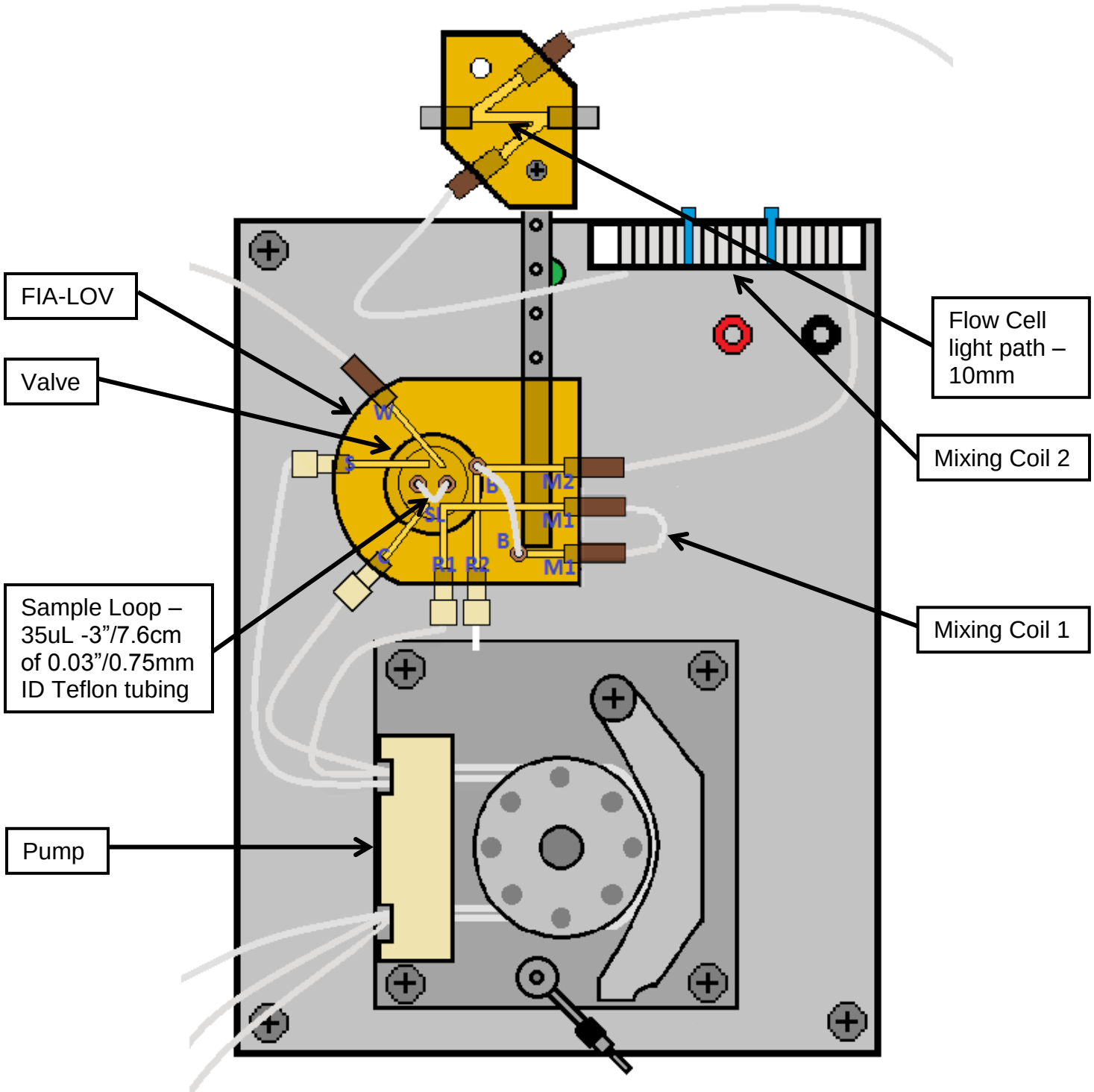

Fig. 2- Pictorial representation of mid-range nitrite setup on a FIAlab-2500

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(%) 60
 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

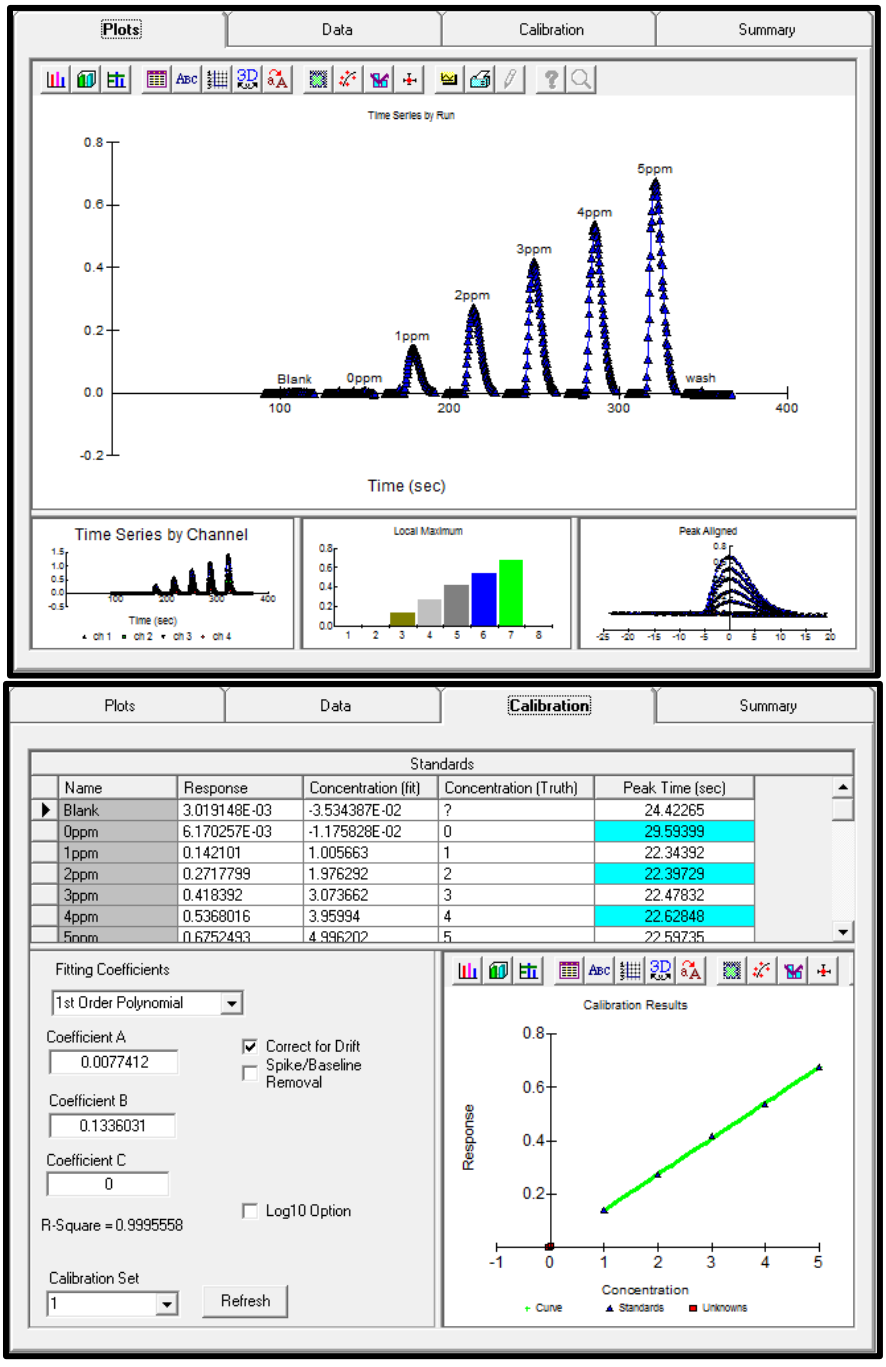

Fig. 3 – Example plot and calibration data for mid-range nitrite at 580nm

Lower limit of detection:	0.015mg/L – $1.1*10^{-6}$molN/L
Upper limit of detection:	40mg/L – $2.9*10^{-3}$molN/L
Sample throughput:	100 samples/hr
Startup:	5 minutes
Shutdown:	5 minutes