DETERMINATION OF NITRATE AND NITRITE BY ZONE FLUIDICS

SPECTROPHOTOMETRIC DETECTION

WRITTEN BY GRAHAM MARSHALL

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1 SCOPE AND APPLICATION

This method is used for the determination of nitrate and nitrite in drinking water, surface water, domestic and industrial wastes, as well as estuarine, coastal, and seawater.

The method detection limit (DL) for NO_x is 0.1 µM with an applicable range of 0.2-10 µM. The method detection limit (DL) for NO_2 is 0.08 with an applicable range of 0.2 -10µM. The range may be extended to analyze higher concentrations by decreasing the sample volume or dilution.

2 SUMMARY OF METHOD

The method automates the familiar cadmium reduction method which involves contact of the nitrate in the sample with copper-coated cadmium particles under pH-buffered conditions. This causes nitrates to be converted to nitrites. The nitrites then react with a coloring reagent comprised of a mixture of sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride (NED) acidified with phosphoric acid to form a red color whose intensity is proportional to the nitrate plus nitrite and designated as NO_x. The nitrites present in the sample can be measured by omitting the cadmium reduction step. The nitrate is then determined by difference $[NO_3] = [NO_x] – [NO_2]$.

The absorbance of the resulting colored compound is measured at 540nm less any non-specific absorbance at 670nm. The spectrum of the measured product is given in Figure 1. Calibration curves are used to quantitate the NO_x and NO_2. The NO_2 measurement can be carried out on an aliquot of sample while another aliquot of sample is reacting in the cadmium reactor.

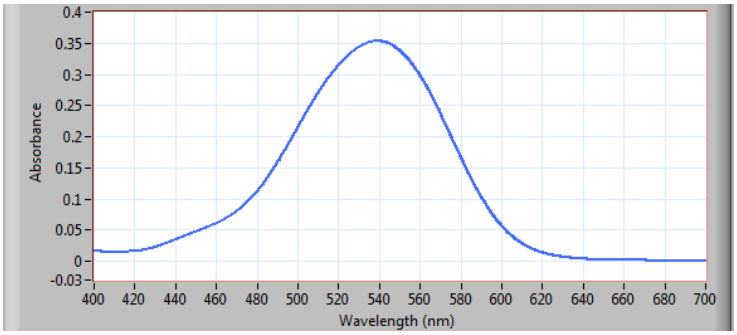


Figure 1: Spectrum of nitrite complex (10 µM)

3 INTERFERENCES

Method interferences which may bias the results can be caused by contaminants in the reagents, reagent water, glassware, and other sample processing apparatus. Care must be taken to ensure that contaminants are not inadvertently introduced.

Build of suspended matter in the cadmium reactor will restrict sample flow. Samples may be pre-filtered as nitrates and nitrites are soluble.

Interference from iron, copper, and other metals can be eliminated by adding ethylenediaminetetraacetic acid (EDTA) in the buffer solution.

Residual chlorine can produce a negative interference by limiting reduction efficiency. If residual chlorine is found in samples, de-chlorination with sodium thiosulfate is necessary prior to cadmium reduction.

Samples that contain large concentrations of oil and grease will coat the surface of the cadmium in the reduction reactor. Oil and grease can be eliminated with pre-extraction with an organic solvent.

4 REAGENTS AND STANDARDS

4.1 SAFETY INSTRUCTIONS AND GOOD LABORATORY PRACTICE

Consult reagent MSDS sheets to determine appropriate reagent handling practices and required personal protective equipment.

Always employ Good Laboratory Practice in the preparation and storage of reagent.

Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. The following chemical have the potential to be highly toxic or hazardous – consult MSDS and OSHA regulations regarding safe handling of these reagents. The preparation of a formal safety plan is also advisable.

- Cadmium
- Phosphoric acid
- Hydrochloric acid
- Ammonium hydroxide
- Chloroform

4.2 DEGASSING PROCEDURES

In order to prevent outgassing of reagents resulting in micro-bubble formation that may interfere with mixing and detection, especially when operating temperatures are >20° C, it is recommended to degas the carrier stream using one of the following methods:

- Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonication aids in the degassing process.
- Purge distilled/deionized water with helium or nitrogen gas through a glass frit for 5 min.
- Boil distilled/deionized water in an Erlenmeyer flask for 15–20 minutes. Remove the flask from the heat source, cover it with an inverted beaker, and allow it to cool to room temperature.

DI water used to prepare standards and reagents should be allowed to de-gas for 24 hours.

4.3 CHEMICALS REQUIRED

The following chemical are used in this method. Alternate equivalent suppliers may be used.

Table 1: Required chemicals

CAS no.	Name	FW	Supplier	SKU
7664-38-2	Phosphoric Acid, concentrated 85%, H_3PO_4	98.0	Sigma	452289
63-74-1	Sulfanilamide, $C_6H_8N_2O_2S$	172.21	Sigma	S9251
1465-25-4	N-(1-naphthyl)ethylenediamine dihydrochloride (NED), $C_{10}H_7NHCH_2CH_2NH_2 \cdot 2HCl$	259.18	Sigma	N9125
7440-43-9	Cadmium granules, 0.3-1.6 mm	112.41	Sigma	7440-43-9
7758-99-8	Cupric Sulfate Pentahydrate, $CuSO_4 \bullet 5H_2O$	249.68	Fisher Sci	C493
7647-01-0	Hydrochloric acid, concentrated 37%, HCl	36.46	Integra	H627.10.40P
12125-02-9	Ammonium chloride, NH_4Cl	53.49	Sigma	326372
1336-21-6	Ammonium hydroxide, 5.0 M, NH_4OH	35.05	Sigma	318612
6381-92-6	Ethylenediaminetetraacetic acid disodium salt dehydrate, $C_{10}H_{14}N_2Na_2O_8 \bullet 2H_2O$	372.24	Integra	E865.10.20
7758-09-0	Potassium nitrite, KNO_2	85.10	Integra	P770.10.20
7757-79-1	Potassium nitrate, KNO_3	101.1	Sigma	204110
67-66-3	Chloroform, CCl_4	119.38	Integra	C455.10.30
	Capstone FS-31 Nonionic surfactant, 20-25%	-	Global FIA	FS-31

4.4 PREPARATION OF REAGENTS

Carrier: Use deionized water as carrier. Because of possible contamination, water used in the carrier and for reagent and standard preparation should be passed through a mixed-bed ion exchange column containing both strong acidic-cation and strongly basic-anion exchange resins. Add 25 µL of Capstone FS-31 to 250 mL DI water to improve flow conditions.

Hydrochloric acid, 6 N: Add 50 mL of conc. HCl to 25 mL DI water, cool, and dilute to 100 mL.

Copper sulfate solution, 2%: Dissolve 2 g of $CuSO_4 \bullet 5H_2O$ in 50 mL DI water and dilute to 100 mL.

Ammonium chloride-EDTA buffer: Dissolve 4 g of NH_4Cl and 0.04 g of Na_2EDTA in 90 mL DI water. Adjust the pH to 9.1 for preserved samples and 8.5 for non-preserved samples with NH_4OH (5 M). Dilute to 100 mL.

Cadmium reactor: Transfer cadmium granules to a reactor column while tapping the column gently to ensure a compact packing of granules in the reactor. Insert the column end piece thereby trapping the cadmium in the reactor (see Figure 2).

Figure 2: Cadmium reactor before activation

Clean and activate new or used the cadmium reactors using the **Activate Cd Reactor** sequence in FloZF as follows:

1. Pump 3 x 300 µL of dilute 1:1 HCl (6 N) through the column. Tap the column gently to dislodge any air bubbles. The granules will appear silver in color.
2. Rinse acid from the column with 3 x 300 µL of DI water pumped through the column.
3. Pump 3 x 300 µL of the $CuSO_4$ (2%) solution through the column. Wait 5 minutes to allow the coating process to proceed. A brown colloidal precipitate will form on the cadmium surface.
4. Wash the column with 3 x 300 µL of ammonium chloride-EDTA solution. Tap the column during this wash to dislodge any remaining bubbles. The color of the cadmium should be black.
5. Periodically measure the percentage of nitrate converted to nitrite. If this percentage drops below 80%. Repeat this procedure.
6. Avoid introducing air or any other oxidants into the column.
7. Store the column filled with ammonium chloride buffer. If the column dries out, clean and activate using this procedure.
8. In some instances the first few measurements after activation may have to be discarded.
9. The cadmium is consumed with use. Periodically top up the reactor with fresh granules. When new granules are added, it will be necessary to activate them using this procedure.
10. Oils and greases can be removed from the column by washing with acetone.

Color reagent, Sulfanilamide NED (S-NED): To approximately 15 mL DI water while stirring add 2 mL H_3PO_4, 0.4 g sulfanilamide, and 0.04 g of N-1-naphthylethylenediamine dihydrochloride. Stir until dissolved and dilute to 20 mL with DI water. Store in a brown bottle and keep in the dark when not in use. This solution is stable for several months if stored at 4°C. If a light pink color is observed, discard the solution and prepare a fresh batch.

4.5 PREPARATION OF STANDARDS

Diluent: Use DI water as diluent and blank. Because of possible contamination, water used for standard preparation should be passed through a mixed-bed ion exchange column containing both strong acidic-cation and strongly basic-anion exchange resins.

Stock Standard, KNO_3, 0.1 M: Dissolve 0.506 g of KNO_3 and dilute to 50 mL in a volumetric flask with DI water. Preserve with 200 µL chloroform. Solution is stable at room temperature for 6 months. Store the standard in a glass bottle.

Stock Standard, KNO_2, 0.1 M: Dissolve 0.425 g of KNO_2 and dilute to 50mL in a volumetric flask with DI water. Preserve with 200 µL chloroform. Solution is stable at 4°C for 6 months. Store the standard in a glass bottle.

Working Stock, KNO_3, 0.5 mM: Using a pipette, carry out serial dilutions of the stock standard using volumes specified in Table 2.

Table 2: Dilution volumes for working KNO_3 standards

Concentration	Volume	Stock conc	Final volume
10 mM	500 µL	0.1 M	5 mL
0.5 mM	500 µL	10 mM	10 mL

Working Stock, KNO_2, 0.5 mM: Using a pipette, carry out serial dilutions of the stock standard using volumes specified in Table 3.

Table 3: Dilution volumes for working KNO_3 standards

Concentration	Volume	Stock conc	Final volume
10 mM	500 µL	0.1 M	5 mL
0.5 mM	500 µL	10 mM	10 mL

Calibration standard, KNO_3 10 µM KNO_2 10 µM: Using a pipette, meter 500 µL of the 0.5 mM KNO_3 solution and 500 µL of the 0.5 mM KNO_2 working stock into a 25 mL volumetric flask and make up to volume with DI water.

5 APPARATUS AND EQUIPMENT

Global FIA FloPro Zone Fluidics Analyzer equipped for phosphate measurement (refer to Figure 3).

The FloPro is equipped with

- an Ocean optics detector configured to measure absorbance between 420 and 1000 nm
- Two lights sources – a white LED and a Tungsten/Xenon lamp
- Global FIA bubble tolerant-flow cell
- milliGAT pump
- Valco valves
- PID-controlled heater
- NUC mini-PC (on some systems)
- GPS (on field deployable systems)

FloPro makes use of Global FIA FloZF device control and data acquisition and manipulation software

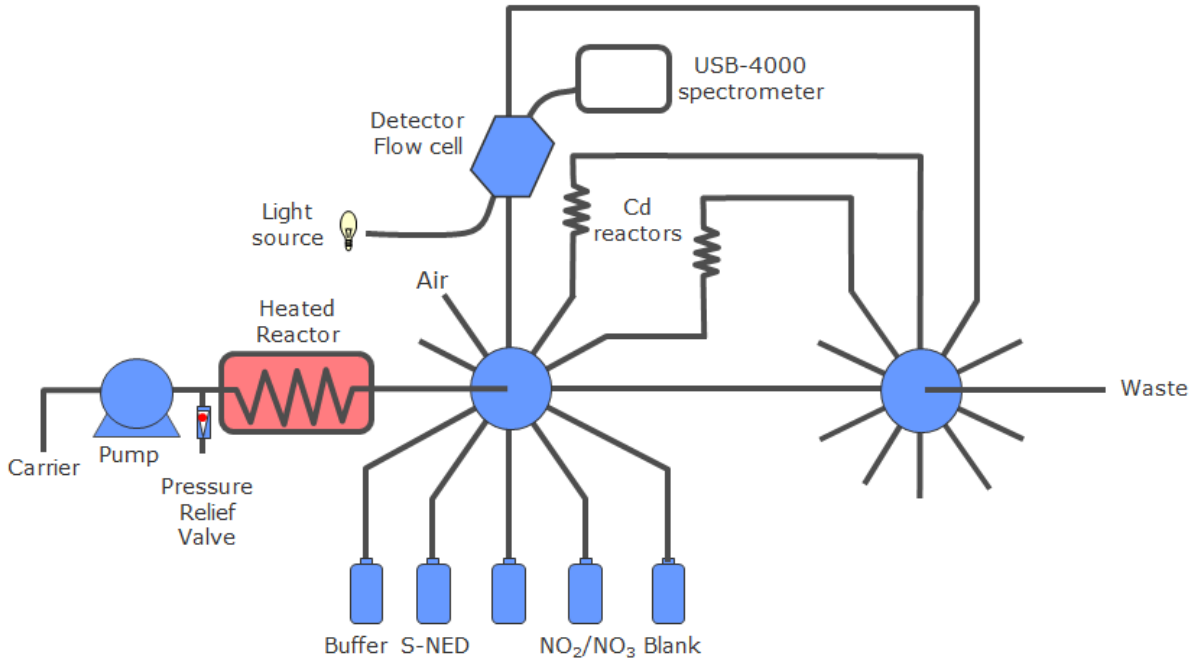


Figure 3: Zone Fluidics manifold for the determination of nitrate and nitrite

The following port assignments (Table 4) apply.

Table 4: Port assignments

18 Port valve		10 Port valve	
Port	**Function**	**Port**	**Function**
1	Link to 10 port valve	1	Link to 18 port valve
2	Detector	2	Detector
3	NH_4Cl buffer	3	N/C

18 Port valve		10 Port valve	
4	S-NED	4	N/C
5	N/C	5	N/C
6	NO_2/NO_3 standard	6	N/C
7	Blank / Diluent	7	N/C
8	Sample	8	N/C
9	Cadmium reactor 1	9	Cadmium reactor 1
10	Cadmium reactor 2	10	Cadmium reactor 2
11	N/C		
12	N/C		
13	N/C		
14	N/C		
15	N/C		
16	N/C		
17	N/C		
18	Air		
Common	Heated reactor	Common	Waste

6 METHOD

6.1 GENERAL DESCRIPTION

In this method, the sample is buffered and delivered to the cadmium reactor. While reduction is taking place, another aliquot of the sample is reacted with sulfanilamide and NED and the nitrite is determined by spectrophotometric detection. The reduced sample is then withdrawn from the cadmium reactor and reacted with sulfanilamide and NED and the nitrite plus nitrate is determined by spectrophotometric detection. Options are provided in an automated sequence to calibrate both methods, measure an individual standard / check standard or measure multiple samples (Sequence: NO2-NOx).

Two times the volume of analyte needed for the method is buffered and sent to the cadmium reactor. This is to compensate for dispersion that occurs in the reactor. The unreacted sample in the line between the valve and cadmium reactor is discarded prior to reaction with the coloring reagents. The sensitivity of the nitrate plus nitrite methodology is about half that of the nitrite alone. This is due to dilution with the buffer and dispersion in the reactor.

The holding coil is maintained at 40 °C to ensure uniform reaction conditions under varying ambient and sample temperatures.

6.2 SUPPORTING SEQUENCES

A startup sequence switches on the light sources, sets the heater set point, flushes reactors, and primes all lines (Sequence: STARTUP).

A shutdown sequence switches off the light sources, sets the heater set point to ambient temperature, returns reagents and standards to their vials, and flushes reactors (Sequence: SHUTDOWN). Buffer solution is loaded into the cadmium columns. If the instrument will be out of service for more than 1 week, it is a good idea to empty all lines including the cadmium reactors. When starting up again it will be necessary to reactivate the cadmium reactors.

Care must be taken not to introduce air into the cadmium reactor as it deactivated the reduction surface. If air is inadvertently introduced then the cadmium reactor must be flushed with fresh buffer (Sequence: Flush reactors and flow cell). It is also a good idea to confirm reactor performance by measuring a check standard and if necessary reactivate the column (Sequence: Activate cadmium reactor 1 and Activate cadmium reactor 2).

Two cadmium reactors are provided in case one begins to fail. To swap from one reactor to another it is necessary to specify in the NO2-NOx sequence which port is connected to the desired cadmium reactor.

7 PROCEDURES

7.1 INSTALLATION PROCEDURE

Follow instructions in the Instrument Operating Manual for installation of a new system.

7.2 START UP PROCEDURE

1. Prepare reagents and standards.
2. Fill reagent and standards vials and load them onto the FloPro according to the port assignment table.
3. Fill the carrier reservoir and ensure that the waste reservoir is empty.
4. The fluid manifold (Figure 3) and valve port assignment table (Table 4) provide details for ensuring correct tubing connections and appropriate loading or reagent and standards. Follow the diagram to ensure that all connections are made according to the diagram and table and reagents and standards are loaded into their correct positions.
5. Power up the FloPro and computer.
6. Open the FloZF software and load the Nitrate method project. The software will automatically check to ensure that all devices are connected and communicating correctly. If the software

indicates that any device are not connected or communicating, follow the instructions in the Troubleshooting section of the Operating Manual.

7. The available sequences are located on the Develop tab in the Resources sequence panel. Drag the desired sequence from Resources sequences panel to the Working sequence window.
8. Verify that the concentration of the calibration solution is the same as the concentration of the top standard in the calibration tables.
9. Run the STARTUP sequence, to prime all lines and prepare devices for measurement.
10. The spectral transmission of the reference spectrum should be greater than 20,000 at the measurement wavelength and less than 60,000 across the entire spectrum. If not then ensure that bubbles have been flushed from the flow cell. If the response still falls outside of the above specifications then adjust the integration time in the USB-4000 setup.

7.3 SYSTEM CALIBRATION PROCEDURE

1. The concentrations of the standards in the calibration table are specified in the active calibration table on the Measure – Calibrate tab. The concentration of the top standard corresponds to the concentration of the stock s
2. To execute a calibration select and run the NO2-NOx sequence and select the Calibrate button.
3. Confirm that the calibration curve covers the required range and that the correlation coefficient (r^2) is ≥ 0.990.

7.4 SAMPLE DETERMINATION PROCEDURE USING SAMPLE PROBE

1. Place the sample probe into the sample vial.
2. Select the appropriate sample measurement sequence in the Resources sequences panel. And run the sequence by pressing the  button.
3. Enter the sample name and click OK to confirm that the sample probe is inserted into the sample vial.
4. The sequence will prime the sample line and execute the measurement. Results will be displayed in the Results table on the Measure tab.

7.5 SAMPLE DETERMINATION PROCEDURE USING AUTO SAMPLER

1. Load samples into the vial rack on the auto sampler.
2. Load the Auto Sampler Excel template into Excel.
3. Enter the sample names, plate and vial numbers, and required tests into the Excel Auto Sampler template. Refer to Figure 4 for correct vial numbering.

4. Select the auto sampler sample measurement sequence in the Resource sequences panel and press the button.
5. Enter the name of the Excel spreadsheet where the data will be stored. At the end of the sequence the Excel spreadsheet will be saved to this Excel file.

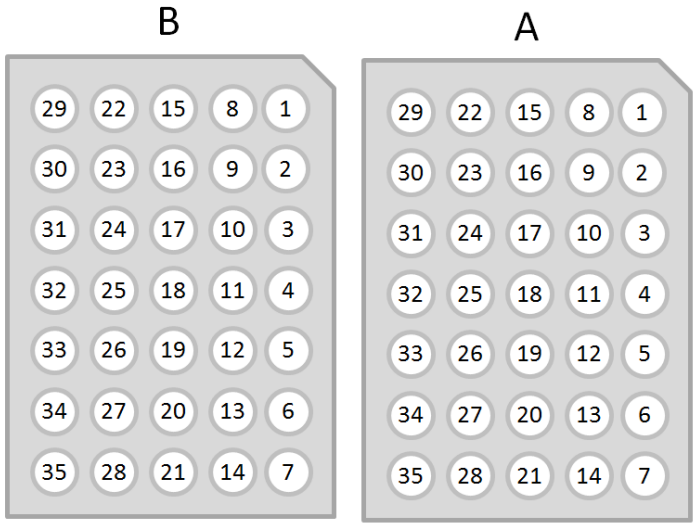


Figure 4: Vial numbers in autosampler

7.6 SAMPLE DETERINATION PROCEDURE USING AUTOMATED SAMPLING

1. Ensure that the sampling system is operational and delivering sample to the analyzer.
2. Select the appropriate sample measurement sequence in the Resources sequences panel. And run the sequence by pressing the button.
3. After calibrating the instrument (see section 7.3), select the Sample button.
4. Enter the Run name. This name will be included in the sample name in the results table.
5. Enter the number of samples to measure. You can enter more than you need and stop the run at an appropriate time.
6. The sequence will prime the sample line with fresh sample and execute the measurement. Results will be displayed in the Results table on the Measure tab.

7.7 SYSTEM SHUT DOWN PROCEDURE

1. Run SHUTDOWN sequence. Reagents and standards will be returned to the vial. Vials can be removed and stored till next use.

2. If the system needs to be transported or will not be used for several days, remove the carrier reservoir and empty all tubing lines.

7.8 SYSTEM DECONTAMINATION PROCEDURE

This method allows for the determination of trace levels of analyte. In some instances, the analyte is a common component of reagents used in other methods at high concentration levels, e.g. as part of a pH buffer or chromogenic reagent. In such instances, it is necessary to decontaminate the fluidics manifold prior to measurement. When swapping from another method that has left the system contaminated with the analyte, the following washout procedure should be used to decontaminate the system. This procedure is not suitable for decontamination prior to determination of total nitrogen or ammonia:

1. Prepare a 2% solution of Micro-90 wash solution by diluting 4 mL of Micro-90 (Cole-Parmer P/N: S-18100-01) in 200 mL of DI water.
2. Remove the reagent reservoirs and safely dispose of any remaining reagent.
3. Thoroughly wash reagent vials using the Micro-90 wash solution, followed by a rinse with DI water. Allow the vials to air dry.
4. Replace the carrier reservoir with a reservoir containing the prepared Micro-90 wash solution.
5. Load all reagents vials that have tubes plumbed to them with the prepared Micro-90 wash solution.
6. Remove the Cadmium column and connect the cadmium reactor tubes with a union.
7. Run the STARTUP sequence 5 times.
8. Remove the vials and run STARTUP once.
9. Replace the carrier reservoir with a reservoir containing DI water.
10. Load all reagents vials that have tubes plumbed to them with DI water.
11. Run the STARTUP sequence 10 times.

8 SYSTEM MAINTENANCE AND TROUBLESHOOTING PROCEDURES

Keep the system clean and dust free.

Clean up any reagent spills immediately.

It may be necessary to flush out all lines from time to time with a cleaning solution as described in section 7.7.

Ensure that the holding coil is free of bubbles before starting a measurement sequence. If bubbles are present, run the Flush reactors and flow cell sequence.

For information on general system maintenance and troubleshooting, refer to the Troubleshooting Guide in the Instrument Operation Manual.

9 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample can be directly pumped to the analyzer or collected in a vial for subsequent measurement. If samples are collected or preserved, then the following procedures must be followed.

Collect samples in glass bottles thoroughly cleaned and rinsed with DI water or other preferred cleaning method.

Ensure the volume of sample collected is sufficient to obtain a representative sample, analyze replicates, and minimize waste disposal.

Samples can be preserved by acidification to pH<2 (typically with H_2SO_4) and cooled and stored at 4°C and may be held for up to 28 days.

Perform sample analyses of samples that have not been preserved within 48 hours or as soon as possible to eliminate analyte loss.

If the sample pH is below 5 or above 9, adjust to between 5 and 9 with either conc. HCl or conc. NH_4OH.

Do not preserve samples with mercuric chloride. Mercuric chloride will deactivate the cadmium reactor.

10 METHOD PERFORMANCE

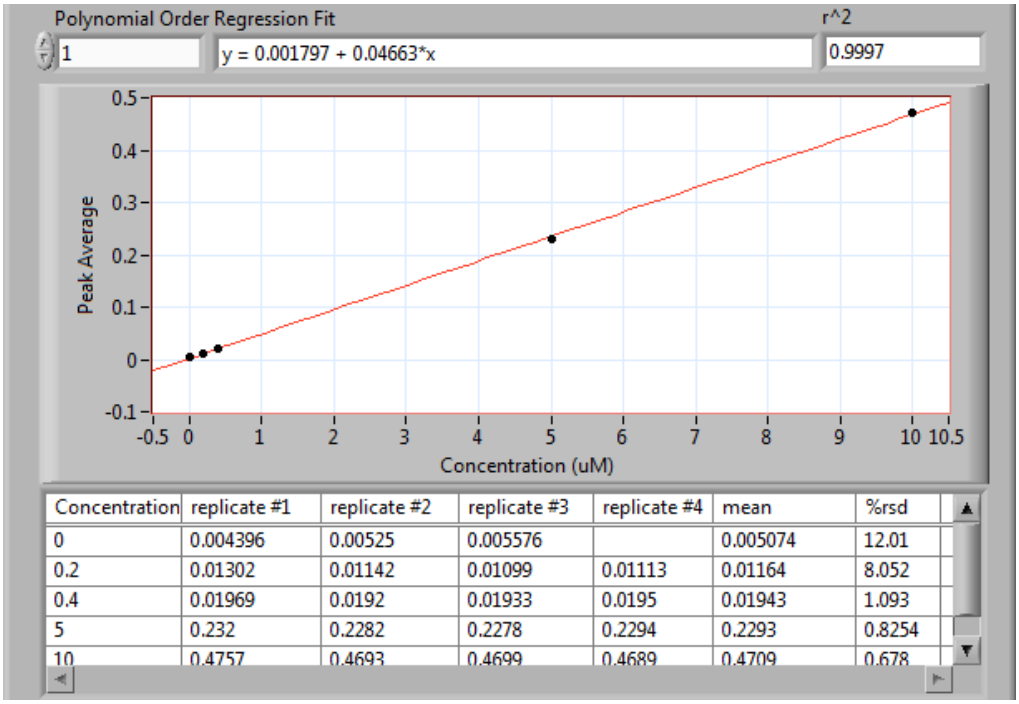
The following analytical figures of merit were obtained:

Table 5: Analytical figures of merit

Parameter	Units	NO_2	NO_2/NO_3
Calibration Range	µM	0.2-10	0.2-10
Regression fit		Resp=0.001797+0.04663*Conc	Resp=0.01007+0.02244*Conc
r^2		0.9997	0.9997
Reduction recovery			85-100%
%RSD at 10 µM		0.7%	1.1%
Detection limit*	µM	0.07	0.1
Measurement time	sec	372	

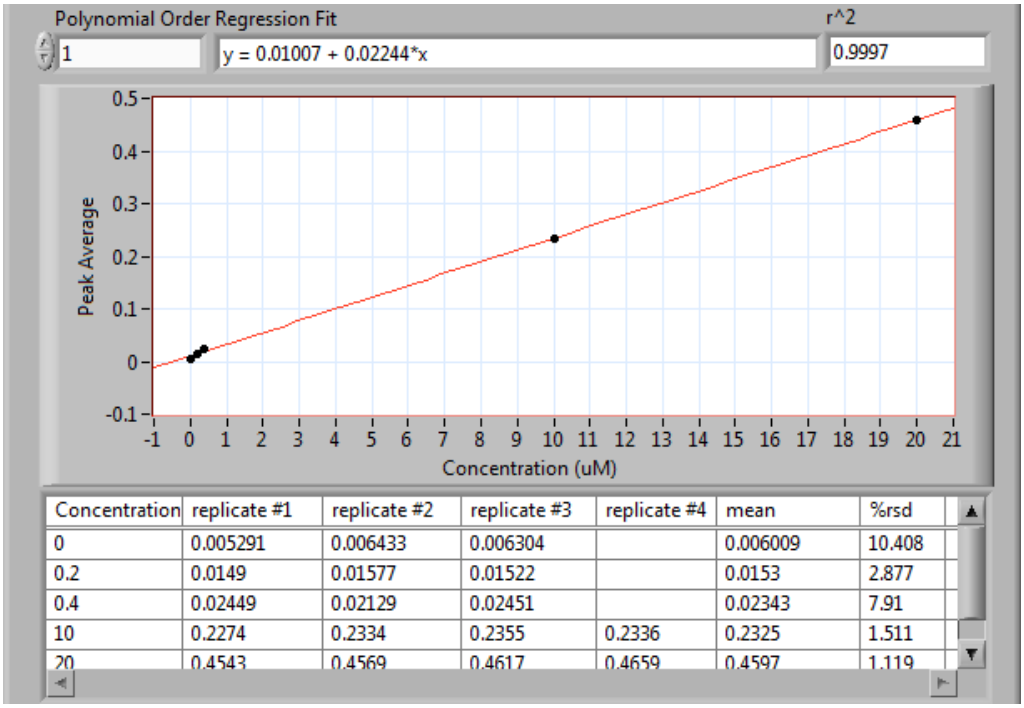
* The method detection limit is calculated as follows:

The standard deviation of the blank is calculated for 10 measurements. The concentration is calculated from the regression fit equation for a response equal to 3 times the standard deviation plus the average.



Concentration	replicate #1	replicate #2	replicate #3	replicate #4	mean	%rsd
0	0.004396	0.00525	0.005576		0.005074	12.01
0.2	0.01302	0.01142	0.01099	0.01113	0.01164	8.052
0.4	0.01969	0.0192	0.01933	0.0195	0.01943	1.093
5	0.232	0.2282	0.2278	0.2294	0.2293	0.8254
10	0.4757	0.4693	0.4699	0.4689	0.4709	0.678

Figure 5: NO_2 calibration curve



Concentration	replicate #1	replicate #2	replicate #3	replicate #4	mean	%rsd
0	0.005291	0.006433	0.006304		0.006009	10.408
0.2	0.0149	0.01577	0.01522		0.0153	2.877
0.4	0.02449	0.02129	0.02451		0.02343	7.91
10	0.2274	0.2334	0.2355	0.2336	0.2325	1.511
20	0.4543	0.4569	0.4617	0.4659	0.4597	1.119

Figure 6: NO_2/NO_3 calibration curve

11 REFERENCES

1. O'Dell, James, Determination of nitrate-nitrite nitrogen by automated colorimetry, USEPA Method 353.2 rev 2.0 (Aug 1993)

12 REVISION HISTORY

Revision history for FloPro Method: Nitrate/Nitrite

File name: met_NOx_-.docx

Last printed: December 10, 2015

Rev	Description	Date	By
-	Initial release	4-16-2014	Graham Marshall