DETERMINATION OF PHOSPHATE BY ZONE FLUIDICS

COLORIMETRIC DETECTION

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Revision: -

Date: May 5, 2014

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1 SCOPE AND APPLICATION

This method is used for the determination of phosphate in river and marine waters.

The method detection limit (DL) is 0.4 µM with an applicable range of 1-50 µM PO_4^{-3}. 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 molybdenum blue method in which orthophosphate is reacted with an acidified molybdenum/antimony reagent to form a complex which is then reduced by ascorbic acid to a colored antimony-phosphomolybdate heteropoly acid that is measured spectrophotometrically. The absorbance of the colored compound is measured at 810nm less any non-specific absorbance at 480nm. The spectrum of the measured product is given in Figure 1. Calibration curves are used to quantitate the PO_4^{-3}.

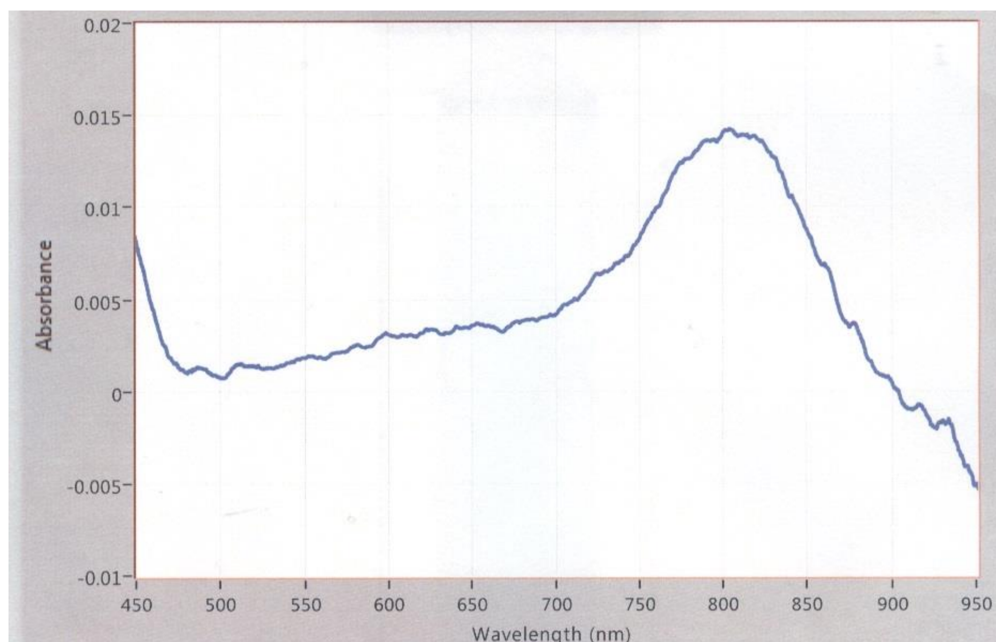


Figure 1: Spectrum of Reduced Antimony-phosphomolybdate Complex (2 uM)

3 INTERFERENCES

The following compounds are expected to interfere with this measurement:

1. Silica interference is generally insignificant if concentration is less than 30 mg/L.
2. Concentrations of ferric iron greater than 50 mg/L can cause a negative error due to reaction with the ascorbic acid reducing reagent. Higher concentrations may precipitate phosphate.
3. Solutions containing 5-20% salt can cause less than 1% error
4. Sample turbidity must be removed prior to measurement

For samples containing particles > 100 µm, filtration is recommended.

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.

The following chemical have the potential to be highly toxic or hazardous – consult MSDS.

- Sulfuric acid
- Antimony potassium tartrate

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:

1. Place distilled/deionized water under a strong vacuum for 15–20 minutes. Magnetic stirring or sonication aids in the degassing process.
2. Purge distilled/deionized water with helium or nitrogen gas through a glass frit for approx. five minutes.
3. 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.

4.3 CHEMICALS REQUIRED

The following chemical are used in this method. All reagents were of analytical reagent (AR) grade or exceed the specifications set by the American Chemical Society. Alternate equivalent suppliers may be used.

Table 1: Required chemicals

CAS no.	Name	FW	Supplier	SKU
7664-93-9	Sulfuric acid, 95-98%, H_2SO_4	98.08	Sigma	320501
28300-74-5	Antimony potassium tartrate, $C_8H_4\ K_2O_{12}\ Sb_2 \cdot xH_2O$	333.94	Sigma	244791
12027-67-7	Ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1236.86	Integra	A650
50-81-7	Ascorbic acid, $C_6H_8O_6$	176.12	Integra	A933
7778-77-0	Potassium phosphate monobasic	136.09	Integra	P792-10-30
	Capstone FS-3100 Nonionic fluoro surfactant	-	Global FIA	FS-31

4.4 PREPARATION OF REAGENTS

Carrier: Use distilled water as carrier. Add Capstone FS-31 to the carrier to improve flow conditions – concentration of surfactant in carrier - 0.01% (25 µL per 250 mL carrier)

Ascorbic acid solution: Dilute 0.18g of ascorbic acid in 10mL of DI water. Prepare fresh daily.

Ammonium molybdate stock solution: Dissolve 0.4g of ammonium molybdate in 10mL of DI water. Store in plastic at 4°C.

Potassium antimony tartrate stock solution: Dissolve 28 mg of potassium antimony tartrate in 10 mL of DI water. Store in plastic at 4°C.

5 N H_2SO_4: Dilute 7 mL of concentrated H_2SO_4 with DI water to 50 mL in a volumetric flask and mix. Add 25 mL of water first, then add the acid slowly, and finally dilute to the mark with water. The liquid will get very hot if mixed too rapidly. Let the 5N acid cool to room temperature before using it to prepare the Mixed Reagent.

Mixed Reagent: Pipette 5 mL of 5N H_2SO_4 into a 10 mL vial. Add 0.50 mL of the potassium antimony stock solution and mix. Then add 1.50 mL of the ammonium molybdate stock solution and mix. Prepare fresh daily.

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 , 0.0100 M: Dissolve 0.136 g of KH_2PO_4 in DI water in a 100 mL in a volumetric flask, dilute to volume, and mix. Solution is stable at room temperature for 3 months. Store the standard in a glass bottle.

Working Stock, 100 µM: Using a pipette, deliver 1.00 mL of the stock standard to a 100 mL volumetric flask, dilute to volume with DI water, and mix. The standard is stable at room temperature for 3 months.

Working Standard, 3.00 µM: Weigh into one of the FloPro vials 300 mg of the working stock standard. Add DI water to a total weight of 10.00 g. This will be the top standard for calibration

5 APPARATUS AND EQUIPMENT

Global FIA FloPro Zone Fluidics Analyzer equipped for phosphate measurement (refer to Figure 2).

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

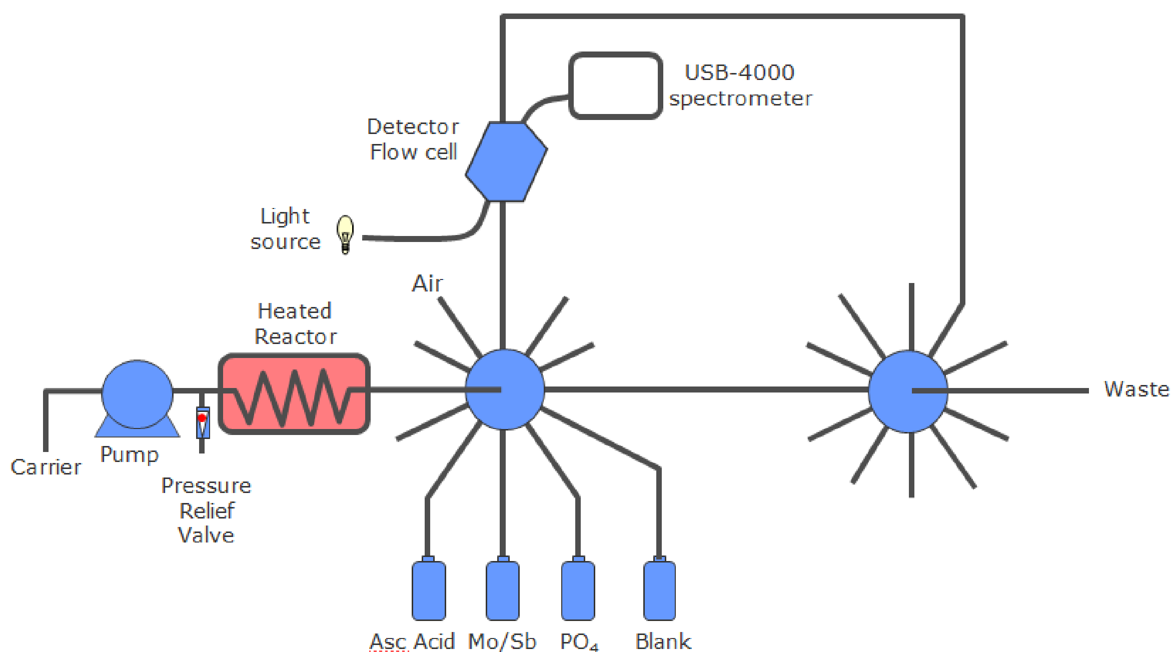


Figure 2: Zone Fluidics manifold for the determination of phosphate

The following port assignments (Table 2) apply.

Table 2: 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	Ascorbic Acid	3	N/C
4	Mo/Sb/H_2SO_4 Reagent	4	N/C
5	N/C	5	N/C
6	PO_4 Standard	6	N/C
7	Blank	7	N/C
8	Sample	8	N/C
9	N/C	9	N/C
10	N/C	10	N/C
11	N/C		
12	N/C		
13	N/C		
14	N/C		
15	N/C		

	18 Port valve		10 Port valve
Port	Function	Port	Function
16	N/C		
17	N/C		
18	Air		
Common	Heated reactor		Waste

6 METHOD

6.1 GENERAL DESCRIPTION

In this method, a bubble bracketed zone stack consisting of a zone of sample sandwiched between a zone of Mo/Sb/H_2SO_4 reagent and a zone of ascorbic acid reducing agent is aspirated into the heated reactor. The heated reactor is maintained at 50^0C. The zone stack is held in the heated reactor for 4 minutes while the two step reaction described in the Summary section takes place. The zone stack is then dispensed to the Flow Cell and centered in the light path. The flow is stopped for 60 seconds while the signal equilibrates to give a plateau profile. The signal is averaged over a period of 18 seconds to statistically enhance the S/N. The stack is then dispensed to waste.

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).

A sequence to insert a 5 µL bubble in the tips of the reagent and standard lines is provided to enhance thorough flush out of lines during priming of the lines.

6.3 USEFUL NOTES

If the instrument has been sitting idle overnight, prior to running STARTUP, insert a bubble in the tips of the reagent and standard lines using the sequence provided. Although the SHUTDOWN sequence leaves an air bubble at the tip of the lines, it often dissipates during a long idle period.

If time allows, it is generally good practice to run a blank first after STARTUP to confirm that the instrument is equilibrated and performing satisfactorily.

7 PROCEDURES

7.1 INSTALLATION PROCEDURE

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

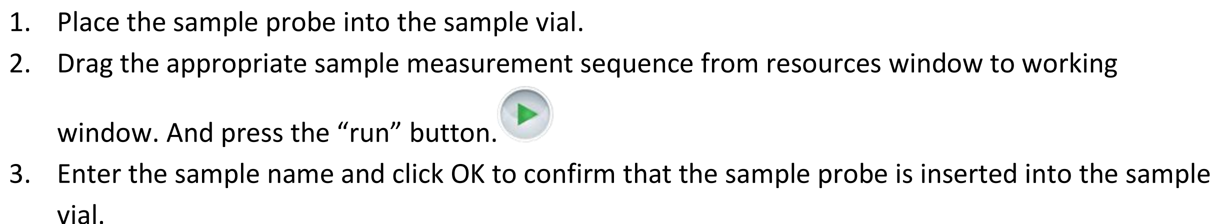
7.2 START UP PROCEDURE

1. Turn on the FloPro.
2. Prepare reagents and standards.
3. Fill reagent and standards vials and load them onto the FloPro according to the port assignment table.
4. Ensure that the carrier reservoir is full and the waste reservoir is empty.
5. The fluid manifold (Figure 2) and valve port assignment table (Table 2) 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.
6. Open FloZF software and load the applicable method project. The software will automatically check to ensure that all components are connected and communicating correctly. The method sequences will load into the Resources window. Troubleshoot the system by following instructions in the Troubleshooting section of the Operating Manual if the software indicates that any system component is not connected or communicating.
7. All of the sequences are located in the develop/resources-sequence window. Drag the desired sequence from resources window to working window.
8. Before any standard or sample test, run the appropriate STARTUP sequence, to prime all lines. At the end of the run, the control window for the spectrometer will popup, make sure that the detector response calculation is correct according to the Apparatus and Equipment section above. The spectral emission maximum of the reference spectrum should be greater than 20,000 at the measurement wavelength.

7.3 SYSTEM CALIBRATION PROCEDURE

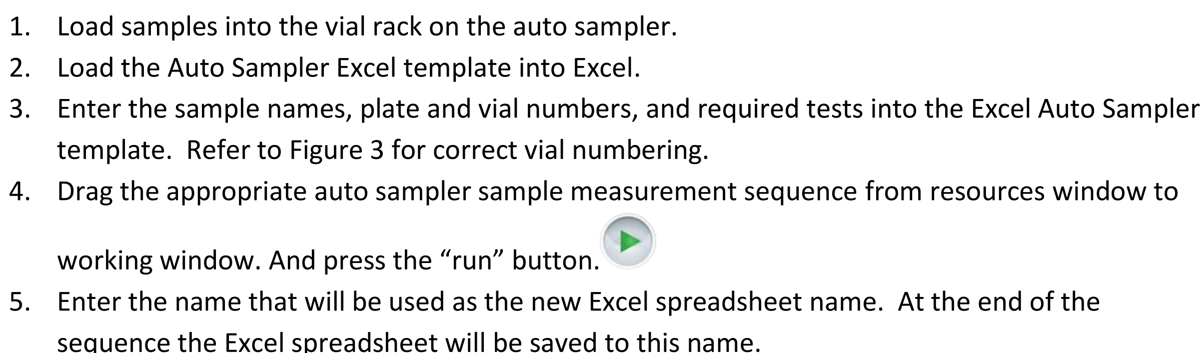
1. Set up calibration table in the “Measure” – “calibration” tab – “make new” or activate an existing calibration table.
2. Run the appropriate calibration sequence.
3. Check that the calibration curve meets the required range and that the correlation coefficient (r^2) is ≥ 0.990.

7.4 SAMPLE DETERMINATION PROCEDURE USING SAMPLE PROBE



1. Place the sample probe into the sample vial.
2. Drag the appropriate sample measurement sequence from resources window to working window. And press the “run” button.
3. Enter the sample name and click OK to confirm that the sample probe is inserted into the sample vial.

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 3 for correct vial numbering.
4. Drag the appropriate auto sampler sample measurement sequence from resources window to working window. And press the “run” button.
5. Enter the name that will be used as the new Excel spreadsheet name. At the end of the sequence the Excel spreadsheet will be saved to this name.

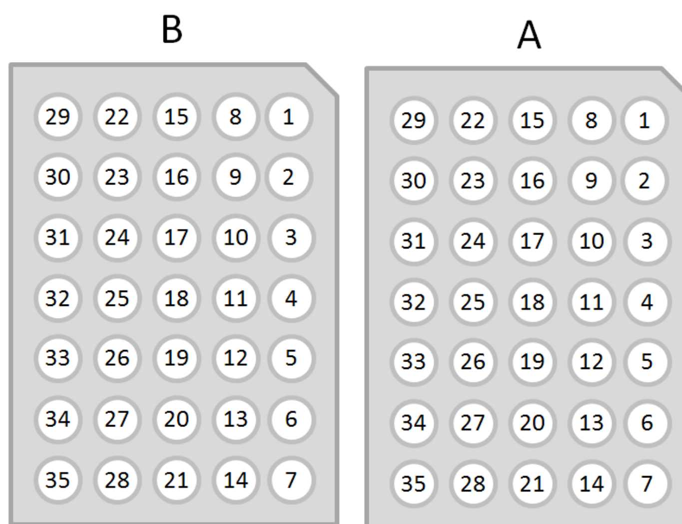
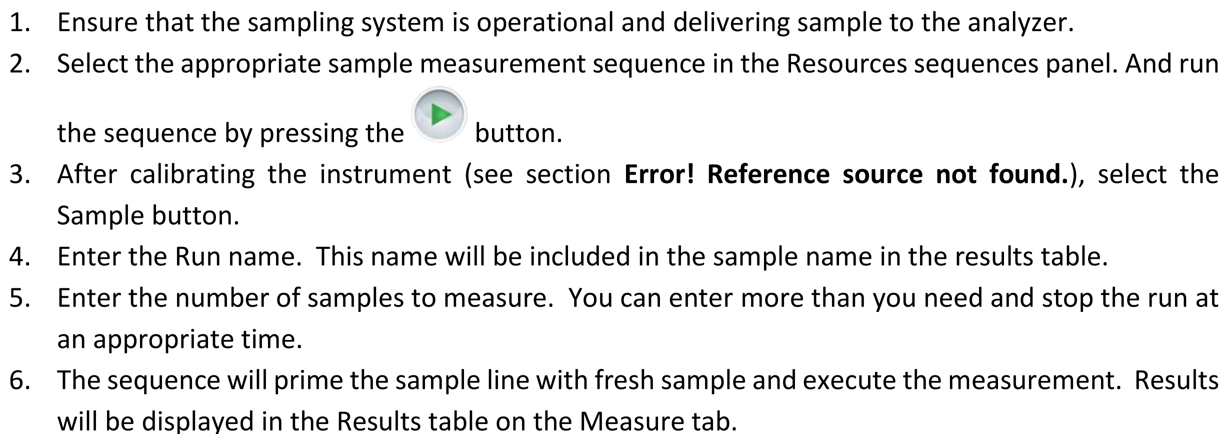


Figure 3: Vial numbers in autosampler

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 **Error! Reference source not found.**), 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. Remove reagents and carrier, replace with DI water.
2. Run SHUTDOWN sequence.
3. If the system needs to be transported or will not be used for several days, remove DI water from rack and carrier and then run SHUTDOWN sequence again to remove all of liquid from the tubing.

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. Run the method *Wash All* sequence 5 times.
7. Remove the reagent cartridge and run *Wash All* once
8. Replace the carrier reservoir with a reservoir containing DI water.
9. Load all reagents vials that have tubes plumbed to them with DI water.
10. Run the method *Wash All* 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 **Error! Reference source not found.**.

The system may need to be flushed periodically.

Ensure that the holding coil is free of bubbles before commencing. If not, then run the flush sequence until there are no bubbles present.

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

9 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

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

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

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

Perform sample analyses of unpreserved samples within 24 hours or as soon as possible to eliminate analyte loss.

10 METHOD PERFORMANCE

The following analytical figures of merit were obtained:

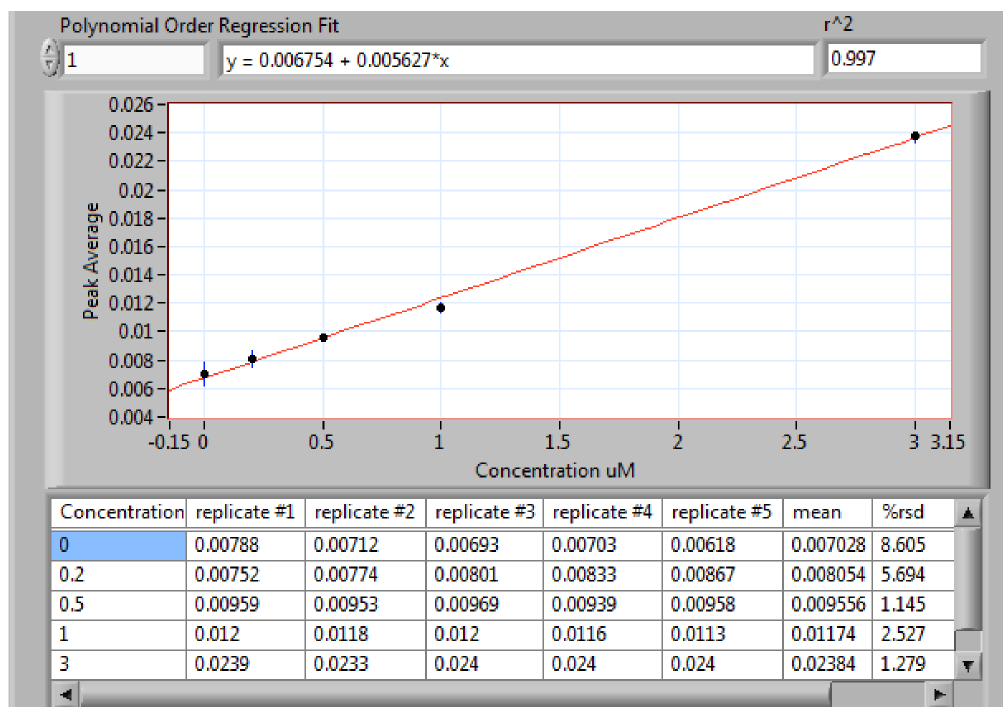
Table 3: Analytical figures of merit

Parameter	Units	Value
Calibration Range	µM	1-50
Precision	1.3 % RSD at 3 µM	
Method Detection Limit*	µM	0.4
Measurement time	420 sec	

Precision is measured as % RSD of 5 replicates shown in the calibration in Figure 4.

* The method detection limit is calculated as follows:

The standard deviation and average response 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	replicate #5	mean	%rsd
0	0.00788	0.00712	0.00693	0.00703	0.00618	0.007028	8.605
0.2	0.00752	0.00774	0.00801	0.00833	0.00867	0.008054	5.694
0.5	0.00959	0.00953	0.00969	0.00939	0.00958	0.009556	1.145
1	0.012	0.0118	0.012	0.0116	0.0113	0.01174	2.527
3	0.0239	0.0233	0.024	0.024	0.024	0.02384	1.279

Figure 4: Phosphate calibration curve

11 REFERENCES

O’deill, James. Determination of Phosphorus by Semi-Automated Colorimetry. USEPA Method 365.1 (1993)

Going, J.E. and Eisenrich, S.J. Spectrophotometric studies of reduced molybdoantimonylphosphoric acid, Anal. Chim. Acta 70, (1974), 95-106

12 REVISION HISTORY

Revision history for FloPro Method: Phosphate

Rev	Description	Date	By
-	Initial release	4-28-2014	Don Olson