

# Method for Phosphate Determination by Molybdenum Blue, Version 2.1

## 1 PRINCIPLE

This method is designed for the determination of ortho-phosphate in water, soil, and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIALab analyzer. Ortho-phosphates react with molybdate anions to form a yellow colored complex; this complex is reduced to a molybdenum blue species by ascorbic acid.

This method is designed to be run on the FIAlyzer-1000. It can be run on the FIAlyzer-FLEX as well, although timing will be affected.

## 2 SUMMARY

This method is designed for the determination of orthophosphate in water, soil, and other forms of samples. The method is capable of detecting phosphate in the range of 0.07 – 35 mg P / 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:

- **Potassium Antimonyl Tartrate Trihydrate**
- **Sulfuric Acid**
- **Sodium Dodecyl Sulfate**

## 4 EQUIPMENT AND SUPPLIES

- **Equipment:**
  - Balance, analytical, with a 0.01 g resolution
  - Pipettes for making standards
- **Glassware:**
  - Class A volumetric flask, 1 L, QTY 2
  - Graduated cylinder, 100 mL, QTY 1
  - Glass storage container, 1 L, clear, QTY 1
  - Glass storage container, 1 L, 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 molybdate tetrahydrate	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> ·4H <sub>2</sub> O	12054-85-2	09878
Potassium antimonyl tartrate trihydrate	C <sub>8</sub> H <sub>4</sub> K <sub>2</sub> O <sub>12</sub> Sb <sub>2</sub> ·3H <sub>2</sub> O	28300-74-5	383376
Sulfuric acid (36N)	H <sub>2</sub> SO <sub>4</sub>	7664-93-9	258105
L-Ascorbic acid	C <sub>6</sub> H <sub>8</sub> O <sub>6</sub>	50-81-7	255564
Sodium dodecyl sulfate	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>11</sub> OSO <sub>3</sub> Na	151-21-3	436143

## ▪ Preparation of Reagents

- **Carrier:** Matrix match carrier to samples. Salt solution to seawater samples, extraction solution for soil samples, etc.
- **Reagent 1:** 6mM Ammonium Molybdate (1 L)
  - Add 10.0g of ammonium molybdate tetrahydrate and 0.2g potassium antimonyl tartrate trihydrate into 800mL of DI water and mix until dissolved.
  - Add 40mL of sulfuric acid and mix. Let cool to room temperature.
  - Add DI water to a total volume of 1 L.
  - Transfer solution to a capped glass bottle.
  - The reagent should be clear with no particles in it.
- **Reagent 2:** 300mM Ascorbic Acid (1 L)
  - Dissolve 30.0 g ascorbic acid in 600 mL of DI water.
  - Add 1.0 g sodium dodecyl sulfate and mix until dissolved. Mix slowly to prevent foaming.
  - Add DI water to a total volume of 1 L.
  - Transfer solution to a capped dark glass bottle.
  - The reagent should be clear with no particles in it.
- **Probe Wash Solution:** Water with 0.1% Brij L23 (1 L)
  - Add 3.3 mL Brij L23 to 800 mL of deionized water, dilute to 1 L.
  - Mix well and store in a polyethylene bottle.
  - The wash solution should be clear with no particles in it.

## ▪ Notes on reagent shelf life:

- Use of high quality laboratory glass bottles is important.
- Reagent 1 should be prepared every 4 weeks.
- Reagent 2 degrades quickly and should be prepared daily.

## ▪ Preparation of Standards

- Dilute 1000 mg/L P-(PO<sub>4</sub>) stock solution with carrier to the desired range of phosphate 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	1.02 mm ID (white/white)	*Inquire
Sample injection loop	35 $\mu$ L - 3.0in/7.6cm of Teflon capillary tubing with 0.03in/0.75mm ID	270040
Reaction coil 1	750 $\mu$ L - 65.0in/165cm of Teflon capillary tubing with 0.03in/0.75mm ID	79209
Reaction coil 2, heated	800 $\mu$ L - 69.0in/175cm of Teflon capillary tubing with 0.03 in/0.75 mm ID - heated	77030
Waste/back pressure coil	600 $\mu$ L – 120.0in/300cm of Teflon capillary tubing with 0.02 in/0.51mm ID	270160
SMA-Z flow cell	10 mm light path	29028

### Software parameters:

Parameter	Value
Pump Speed	50%
Heater Temperature	45°C
Primary Spectrometer Wavelength	880 nm
Secondary Wavelength (optional – for extended upper range)	600 nm
Reference Wavelength	490 nm

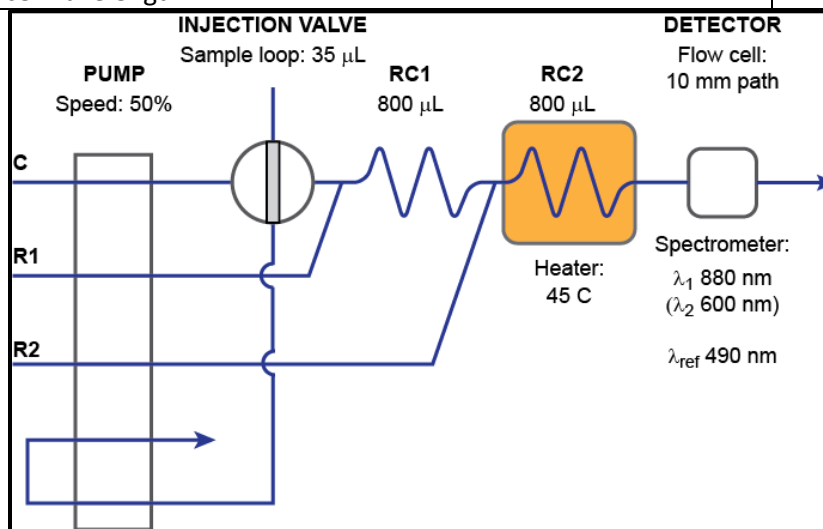


Fig. 1- Flow schematic for phosphate determination.

## 8 FIASOFT METHOD

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

FIALab Injection Valve Sample Load  
FIALab Peristaltic Pump CounterClockwise(%) 50  
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  
Autosave Data C:\Users\FIALab\Desktop\Autosave Data\DateTime  
FIALab Peristaltic Pump Off

## 9 PERFORMANCE METRICS

- Lower limit of detection: 0.02mg P-PO<sub>4</sub> / L – 6.5×10<sup>-7</sup>mol P-PO<sub>4</sub> / L
- Lower limit of quantification: 0.07mg P-PO<sub>4</sub> / L – 2.3×10<sup>-6</sup>mol P-PO<sub>4</sub> / L
- Upper limit of quantification: 50 mg P-PO<sub>4</sub>/ L – 1.6×10<sup>-3</sup>mol P-PO<sub>4</sub>/ L
- Sample throughput: 100 samples / hour
- Startup + Calibration: 5 minutes
- Shutdown: 5 minutes

## 10 ALTERNATE CONFIGURATIONS

- **Low-Range Setup**

Component	Specifications	FIALab PN
Sample Loop	140µL – 12.0in/30.0cm of Teflon capillary tubing with 0.03in/0.71mm ID	270040
SMA-Z flow cell	100mm light path	29038

- Lower limit of detection: 0.007 mg P-PO<sub>4</sub> / L – 2.3×10<sup>-7</sup>mol P-PO<sub>4</sub> / L
- Lower limit of quantification: 0.02 mg P-PO<sub>4</sub> / L – 6.5×10<sup>-7</sup>mol P-PO<sub>4</sub> / L
- Upper limit of quantification: 1 mg P-PO<sub>4</sub>/ L – 3.2×10<sup>-3</sup>mol P-PO<sub>4</sub>/ L
- Sample throughput: 90 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes

## 11 REFERENCES

Nagul E.A., McKelvie I.D., Worsfold P., Kolev S.D., "The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box", *Analytica Chimica Acta* 890 (2015) 60-82.