

# Method for Phosphate Determination by Molybdenum Blue, Version 2.0

## 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-FLEX.

## 2 SUMMARY

This method is designed for the determination of ortho-phosphate in ultra low-range samples of water, soil, or other forms of samples. The method is capable of detecting ortho-phosphate in the range of 0.00015-1mg P/L. For more information, see the section on performance metrics.

## 3 COMPLIANCE

This method follows EPA 365.3.

## 4 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**

## 5 EQUIPMENT AND SUPPLIES

- **Equipment:**
  - Balance, analytical, with a 0.01 g resolution
  - Pipettes for making standards
- **Glassware:**
  - Class A volumetric flask, 1L, QTY 2
  - Graduated cylinder, 100mL, QTY 1
  - Glass storage container, 1L, clear, QTY 1
  - Glass storage container, 1L, brown tinted, QTY 1

- **FIALab Phosphate Analysis Package. Includes:**

| Component   | FIALab PN | QTY |
|---|-----------|-----|
| FIAlyzer-FLEX flow injection analyzer.  |           | 1   |
| FLAME-T-VIS-NIR Spectrometer. 350-1000 nm.  | 64019     | 1   |
| HL-2000-LL light source. Tungsten-halogen, 360-2000 nm, 10,000 hrs. Includes power supply.      | 17041     | 1   |
| SMA-Z-50 Cell - Plexiglas - 50mm optical path FIALab SMA-Z-Cell with Silica Windows, Plexiglas. | 29083     | 1   |

- **Autosampler (for high sample loads):**
  - Cetac ASX-280/560 (recommended) or AIM-3200/3300

## 6 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  |             |
| Brij L23 (30%)                          |   | 12054-85-2 | B4184       |
| 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 | 09880       |
| 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      |
| 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:** 600mM Ascorbic Acid (1 L)
  - Dissolve 60.0g ascorbic acid in 600mL of DI water.
  - Add 1.5g sodium dodecyl sulfate and mix until dissolved. Mix slowly to prevent foaming.
  - Add deionized water to a total volume of 1L.
  - Transfer solution to a dark glass bottle.
  - Reagent should be clear with no particles in it.
- **Reagent 2:** 6mM Ammonium Molybdate (1 L)
  - Add 8.52g of ammonium molybdate tetrahydrate and 0.232g potassium antimonyl tartrate trihydrate to 800mL of DI water and mix until completely dissolved.
  - Mix 40mL of sulfuric acid into solution, stir, let cool to room temperature.
  - Add deionized water to a total volume of 1L.
  - Mix well and transfer solution to a tightly capped glass bottle.
  - Reagent should be clear with no particles in it.
- **Probe Wash Solution:** Water with 0.1% Brij L23 (1L)
  - Add 3.3mL Brij L23 to 800mL of deionized water, dilute to 1L.
  - Mix well and store in a glass 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 day.
- Reagent 2 should be prepared every 4 weeks.

**Preparation of Standards**

- Dilute 1000 mg/L P-(PO<sub>4</sub>) stock solution with carrier to the desired range of nitrate standards.

## 7 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.

## 8 INSTRUMENT SETUP – MID-RANGE

### Flow injection analysis apparatus parameters:

| Component               | Specifications  | FIALab PN |
|-------------------------|---|-----------|
| Analyzer                | 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     |
| SMA-Z flow cell         | Light path – 50mm   | 79083     |
| Peristaltic pump tubing | Tygon, 1.02 mm/0.04 in ID (white/white)   | 270301    |
| Peristaltic pump tubing | Tygon, 0.76 mm/0.03 in ID (black/black)   | 270351    |
| Peristaltic pump tubing | Tygon, 0.51 mm/0.02 in ID (orange/yellow)   | 270353    |
| Peristaltic pump tubing | Tygon, 0.38 mm/0.02 in ID (orange/green)  | 270350    |
| Sample injection loop   | 417 $\mu$ L - 36.0 in/91.44 cm of Teflon capillary tubing with 0.03 in/0.75 mm ID | 270160    |
| Heated Reaction Coil    | 1.8 mL - 155.0 in/400 cm of Teflon capillary tubing with 0.03in/0.75mm ID.        | 77030     |

### Software parameters:

| Parameter                       | Value |
|---------------------------------|-------|
| Pump Speed                      | 90%   |
| Primary Spectrometer Wavelength | 880nm |
| Reference Wavelength            | 490nm |

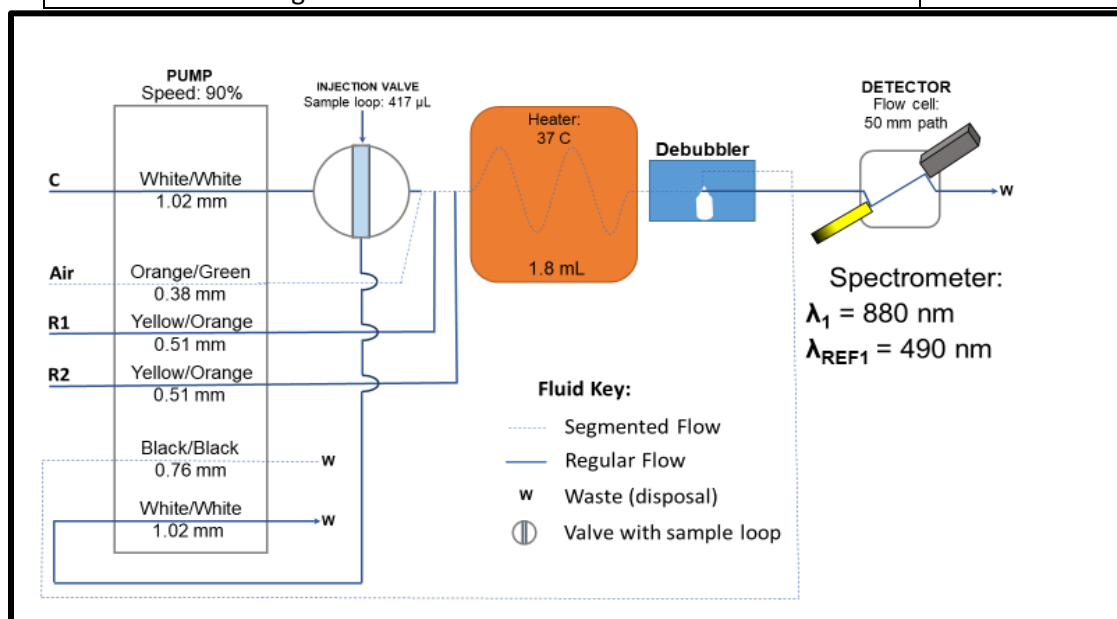


Fig. 1- Flow schematic for ortho-phosphate determination.

## 9 FIASOFT METHOD

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

### 'Phosphate Method Script

FIALab Injection Valve Sample Load

Message Turn On Pump

Delay (sec) 40

Next Sample

Delay (sec) 35

Loop Start

Autosampler Wash

Analyte New Sample

Next Sample

FIALab Injection Valve Sample Inject

Delay (sec) 24

FIALab Injection Valve Sample Load

Spectrometer Reference Scan

Spectrometer Absorbance Scanning

Delay (sec) 100

Spectrometer Stop Scanning

Loop End

Autosampler Rinse

Delay (sec) 60

Autosampler Wash

Delay (sec) 30

Autosave Data C:\Users\FIALab\Desktop\Autosave Data\DateTime

## 10 PERFORMANCE METRICS

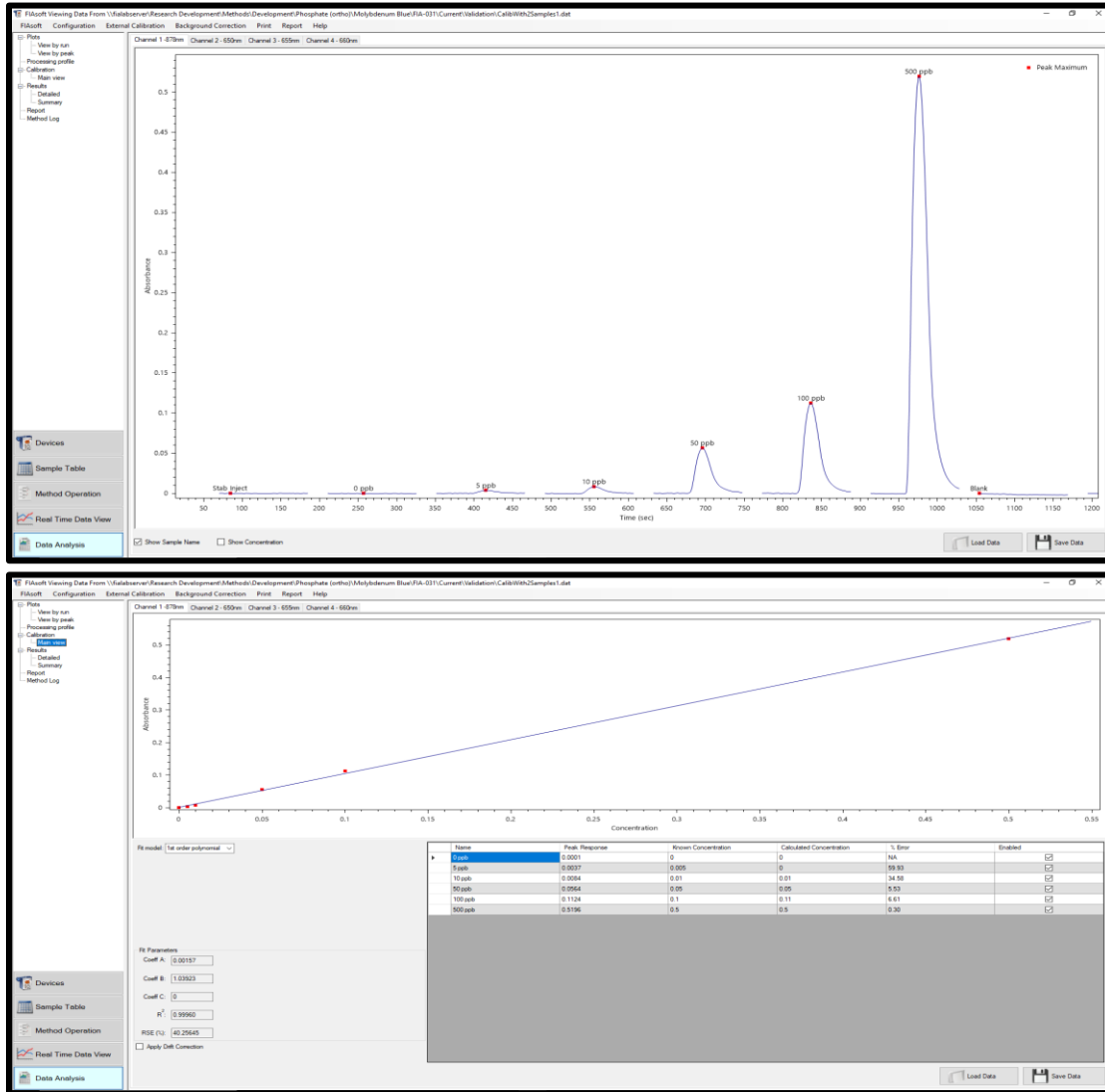


Fig. 2 – Example plot and calibration data for ortho-phosphate.

- Lower limit of detection: 0.00015 mg P/L –  $1.60 \cdot 10^{-9}$  mol P/L
- Lower limit of quantification: 0.00043 mg P/L –  $4.3 \cdot 10^{-9}$  mol P/L
- Upper limit of detection:  $4.3 \cdot 10^{-9}$  –  $1.0 \cdot 10^{-3}$  mol P/L
- Sample throughput: 30 samples / hour
- Startup + Calibration: 5 minutes
- Shutdown: 5 minutes