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Standard Operating Procedure for:
QuickChem® 8500 Flow Injection Analysis System
Operation for
Orthophosphate (PO_4^{3-}) in waters
Department of Health and Environmental
Sciences

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1. Principle

The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs light at 880nm. The absorbance is proportional to the concentration of orthophosphate ion (PO_4^{3-}) in the samples.

2. Scope and application

2.1 Samples include drinking, ground, and surface waters, and domestic and industrial waters.

2.2 The applicable range is 0.01 to 10 mg P/L.

3. Health and safety

3.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential hazard and exposure should be as low as reasonably achievable. Laboratory personal who prepare or use them should wear protective clothing, safety goggles and clean disposable gloves.

3.2 The following chemicals have the potential to be highly toxic or hazardous, for detailed explanation consult the MSDS.

3.2.1 Sulfuric Acid

3.2.2 Dodecyl Sulfate

4. Reagents and standards

Use deionized water (DI) for all procedure.

4.1 Reagents

Reagent1. Stock Ammonium Molybdate Solution

To a tared 1L beaker add 40.0g ammonium molybdate tetrahydrate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ and 983g DI water. Stir for four hours, store in plastic refrigerator. (Two months)

Reagent2. Stock Antimony Potassium Tartrate solution

To a 1L dark, tared beaker add 3.0 g antimony potassium tartrate ($\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2\text{H}_2\text{O}$) and 995g DI water. Stir to dissolve, and store in a dark bottle and refrigerate. (Two months)

Reagent3. Molybdate Color Reagent

To a 1L volumetric flask add about **500 mL DI water**, then add **35.0 mL concentrated sulfuric acid**. Swirl to mix. When it can be comfortably handled, add 72.0 ml **Reagent2** and 213.0 mL **Reagent 1**. Dilute to the mark and invert 3 times. (Weekly)

Reagent4. Ascorbic Acid Reducing Solution. 0.33mol

To a tared 1L beaker, add 60.0g **granular acid** and 975g DI water. Stir to dissolve, and add 1.0g dodecyl sulfate ($\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$). (Weekly)

Reagent5. Sodium Hydroxide – EDTA Rinse

To a tared 1L beaker add 65g sodium hydroxide (**NaOH**), 6g tetrasodium ethylenediamine tetraacetic acid (**Na4EDTA**), and 1.0 kg DI water. Stir to dissolve.

4.2 Standards

Stock Standard 250.0 mg P/L

In a 500 mL beaker, add approximately 400 mL DI water and 1.099g primary grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for one hour at 105 °C. Stir to dissolve and transfer to a 1L volumetric flask. Dilute to the mark and invert to mix.

Working Standards (0-10 mg P/L)

Prepare the working standards through weighing corresponding volume (μl) of stock standard (shown in Table 1) and DI water to 40g in a 50 mL centrifuge tube.

Table 1 Working standards preparation

Working standards (Daily)	A	B	C	D	E	F
Concentration mg P/L	0	0.5	1	2	5	10
Volume (μl) of stock standard	0	80	160	320	800	1600

5. Operation procedure

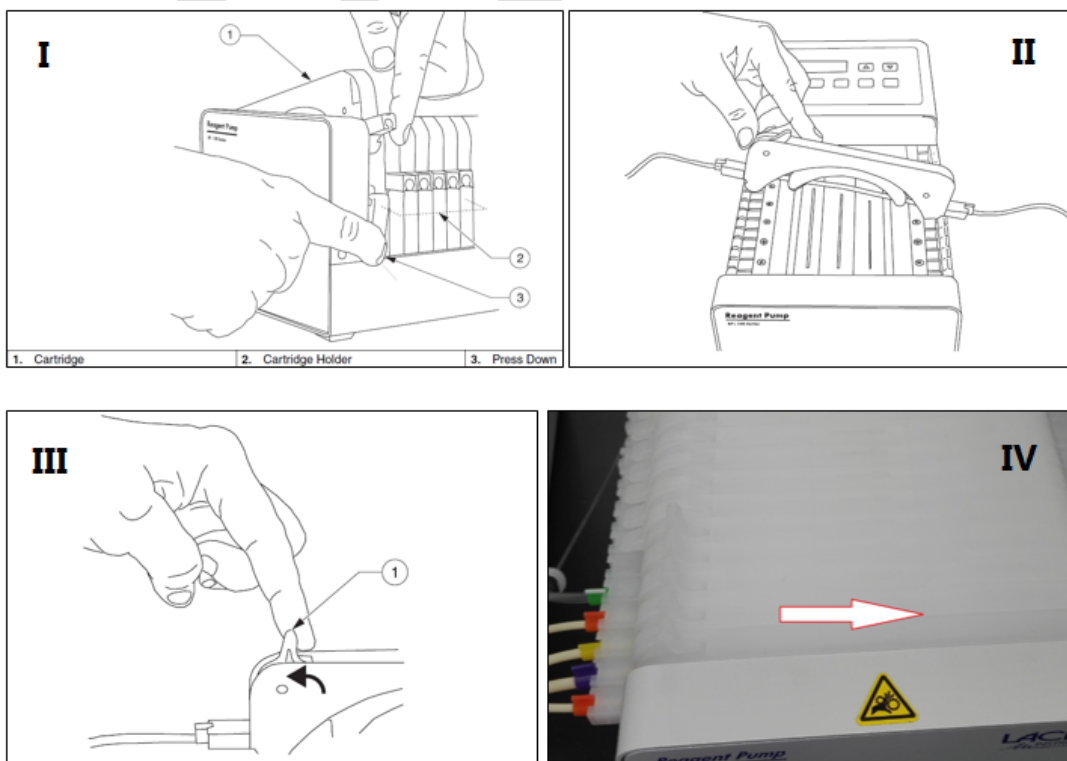
5.1 Prepare reagents and standards as in section 4. Check whether waste lines are put in waste container properly and whether two waste containers are empty.



5.2 Install tubes in pump

Check the five tubes for orthophosphate measurement channel (middle one for our machine). If there is broken tube, ask Xiao to change.

- I. Remove five pump cartridges by pressing the cartridge holder against the pump at both sides, then lifting the cartridges out.



- II. Install the pump cartridge as shown in the graph, you can hear “Ka” sound at both sides.
- III. Adjust tension lever to twelve O'clock direction.
- IV. Install all the tubes and make sure the arrows of cartridges point to the right direction.

5.3 Open the six switches as the following order



5.4 System check and washing

Insert the reagent lines into DI water container, press the start button on pump for leaching check. This check needs 5min, check lines from beginning to the end during this time. (If there is any leaching, taking lines out from DI water, and stop pump, and ask LFST to fix it.)

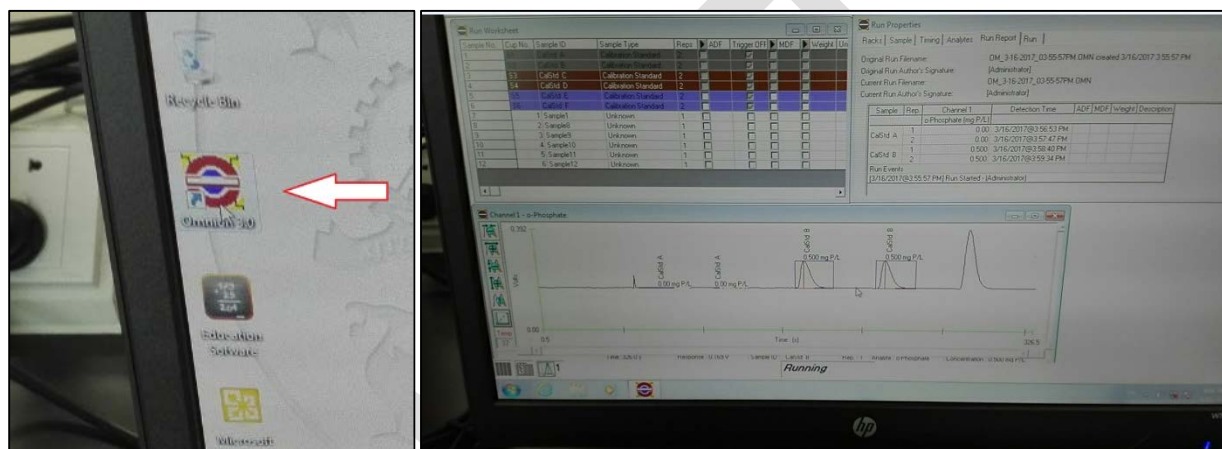


After leaching check, insert the lines into **Reagent 5** for 5 min. Use DI water to rinse before inserting it to DI water container. And then, pump DI water for 10min.

After the washing, insert “Molybdate Color Reagent” and “Ascorbic Acid” lines into Reagent 3 and 4, respectively.

5.5 Software setting

Administer → Omnion 3.0 → OPEN → Desktop → “Method” file → “Orthophosphate” → Save as → “...your run...”



Set up the Run Worksheet to match the sample sequence. To add a row right click in the grey area of the Run Worksheet box and insert or append one or many rows. ID samples automatically by clicking right on ID column. Choose “Channel” in “Analytes” of “Run Properties”, set heating equipment to 37°C.

5.6 Analysis

- I. Check that the reagent lines are in the correct reagents.
- II. Check that your standard solutions are in the correct standard vials and are set in the correct sequence in the Run Worksheet.
- III. Check that the waste lines are connected and direct waste to a container that is not close to being full. Check waste container often throughout procedure.
- IV. When the run worksheet is set up and all components of the the system are ready, click Preview to see the baseline. If the base line is constant and the value is around 0.18 V, that means you start test.

- V. Press Start to run the template and analyze the entire rack of samples.
- VI. Export data from “Run” button of “Run Properties”.

5.7 Shutdown

- i. Remove reagent lines from each reagent and rinse with DI water before placing them in the DI H₂O container. Pump DI H₂O through lines for 5min. Then, put reagent lines to Reagent 5 for 5min, and rinse with DI water before placing them in the DI H₂O container. Pump DI H₂O through lines for at least 10min.
- ii. Remove the lines from the DI H₂O and allow all liquid to be pumped out of the manifold (5min).
- iii. Release the cartridges from the pump and turn the pump off, and also release the tubes from the pump.
- iv. Turn the power strip off.
- v. Remove samples, standards, and reagents and store appropriately.
- vi. Empty two waste containers, and wash them with tap water for three times, and put waste lines into them properly.

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