

# Orthophosphate analysis

## 1 - OBJECTIVE

The purpose of this procedure is to describe the analysis of orthophosphates ( $\text{PO}_4^{3-}$ ) by calorimetry in lake and river waters.

## 2 - APPLICATION

Analysis of orthophosphates in a range of 0 to 0,5 mgP/L

## 3 – SAFETY

Wear a labcoat, gloves, glasses and work under a hood when handling acids.

## 4 – PREPARATIONS

### 4.1- PRINCIPE

According to Murphy and Riley, ammonium molybdate and potassium antimony tartrate react with orthophosphates in an acidic environment to form an antimony phosphorus molybdate complex. This complex is reduced by ascorbic acid to a blue-colored compound whose maximum absorption is at 882 nm.

### 4.2- MATERIAL

- UV-vis spectrophotometer
- 2 cuvettes QS 10 cm

### 4.3- PRODUITS CHIMIQUES ET CONSOMMABLES SPECIFIQUES

- Concentrated sulphuric acid 98%  $\text{H}_2\text{SO}_4$ ;
- Ammonium molybdate  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ ;
- Ascorbic acid  $\text{C}_6\text{H}_8\text{O}_6$ ;
- Potassium hydrogenophosphate  $\text{KH}_2\text{PO}_4$ ;
- Potassium antimony tartrate (emetite)  $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6, 1/2 \text{H}_2\text{O}$ ;
- Sterile 50mL Falcon tubes

### 4.4- PRÉPARATION OF SOLUTIONS AND STANDARDS

#### **Sulphuric acid 5 N (2.5mol/L)**

Sulphuric acid 98%	140 mL
Ultrapure water	complete to 1000 mL

**Slowly pour the acid into the water**

#### **Note :**

*Adapt the volumes of solutions according to the quantity of samples to be analyzed!!*

### **Emetic solution**

Potassium antimony tartrate	2,743 g
Ultrapure water	complete to 1000 mL

*Storage: Store 2 months in an opaque glass bottle at 4 °C*

### **Ammonium molybdate solution**

Ammonium molybdate	20 g
Ultrapure water	complete to 500 mL

*Storage: Store 2 months in polyethylene bottle or opaque bottle at 4 °C*

### **Colorimetric reagent P ORTHO without reducer (ascorbic acid)**

Sulphuric acid 2.5 mol/L	500 mL
Ammonium molybdate solution	150 mL
Emetic solution	50 mL
Ultrapure water	complete to 1000 mL

*Storage: Store in a glass bottle at 4 °C*

### **Colorimetric reagent P ORTHO with reducer (ascorbic acid)**

**!! To be prepared at the time of employment!!**

In a test tube, take the necessary quantity of P ORTHO colorimetric reagent without reducing agent and add the reducing agent (ascorbic acid) in a proportion of 0.53 g per 100 ml.

Stir until the ascorbic acid is dissolved.

*Conservation: 24 hours.*

- **Standards**

#### **Phosphate solution 100 mgP/L**

Potassium hydrogenophosphate $\text{KH}_2\text{PO}_4$	0,4394 g
Ultrapure water	complete to 1000ml

*Storage: 1 year*

#### **Phosphate solution 1 mgP/L**

Stock solution 100 mgP/L	10 ml
Ultrapure water	complete to 1000 ml

*Storage: 1 month*

- **Calibration range**

Prepare in 100mL volumetric flasks.

Description	Phosphate solution 1 mgP/L	Complete with ultrapure water
STD 0.01 mgP/L de PO <sub>4</sub> <sup>3-</sup>	1mL	100mL
STD 0.05 mgP/L de PO <sub>4</sub> <sup>3-</sup>	5mL	
STD 0.10 mgP/L de PO <sub>4</sub> <sup>3-</sup>	10mL	
STD 0.20 mgP/L de PO <sub>4</sub> <sup>3-</sup>	20mL	
STD 0.30 mgP/L de PO <sub>4</sub> <sup>3-</sup>	30mL	
STD 0.40 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40mL	
STD 0.50 mgP/L de PO <sub>4</sub> <sup>3-</sup>	50mL	

Storage: 1 week

#### 4.5- INSTRUCTIONS

- The day before analysis: take out the samples and put them to defrost. The samples must have been filtered on a PES 0.22µm syringe filter. Prepare the solutions and standards.
- On the day of the analysis: prepare the two P ORTHO reagents (with and without reducer) and the samples for analysis.

Use 50mL Falcon tubes for the preparation of reaction mixtures.

Description	Samples Volume	P ORTHO reagent with reducer
Ultrapure water (blank)	40 mL	8 mL
STD 0.01 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.05 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.10 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.20 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.30 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.40 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
STD 0.50 mgP/L de PO <sub>4</sub> <sup>3-</sup>	40 mL	8 mL
Collected water	40mL	8 mL

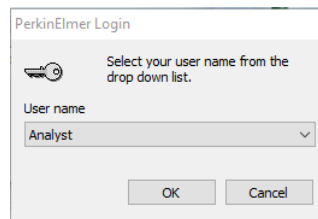
Mix the tubes on a vortex mixer (2 times per tube).

Leave to stand for 15 min (30 min max) and measure.

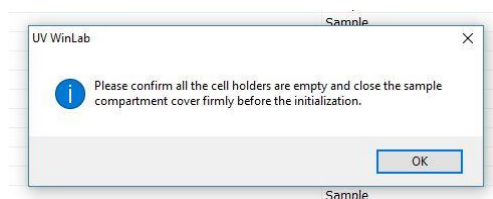
#### 4.6- MEASURE

- Switch on the Perkin UV-VIS spectrophotometer 30 min before analysis.
- Switch on the computer: ID: Administrator PW: administrator

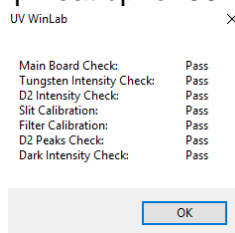
- Open the program : *PerkinElmer UV Winlab*
- The PerkinElmer Login window opens, click on « OK »



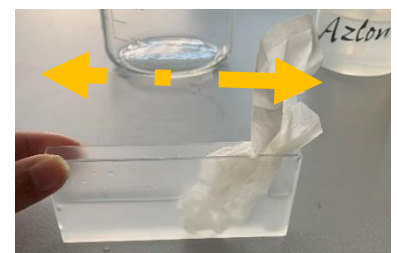
- Be sure that there are no cuvettes in the instrument, otherwise remove them.
- Double-click on the method « Phosphate 0.0005 - 0.05 mg/L »
- Initializing the instrument: Wait until the dialogue box opens. Start the initialization by clicking « OK ».



- When initialization is complete a report is displayed: all tests should indicate "PASS". Click "OK".  
If not, close the program, let the lamp heat up for 30min and start again.

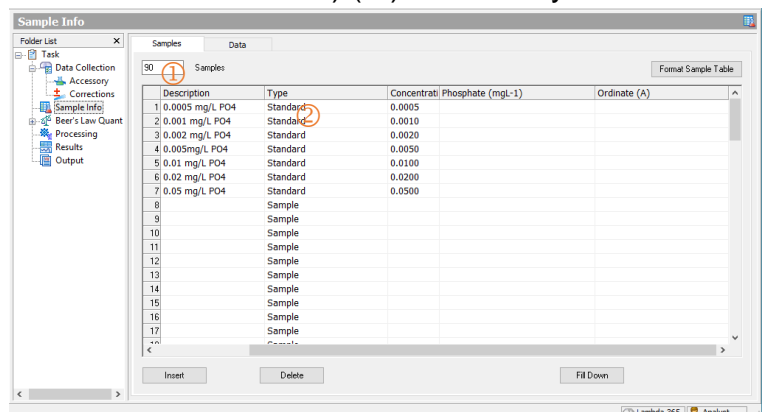


- Carefully clean the cuvettes :
  - a) Rinse the cuvette with ultrapure water.
  - b) Fill the cuvette with 70% ethanol.
  - c) Using a Kimtech paper, clean the inside of the cuvette and the edges (don't do this dry).
  - d) Rinse the cuvette again with water and let it dry.



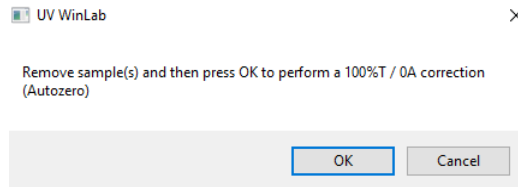
- Indicate the number of samples (include standards & blanks) (①) to be analyzed in the Sample Info.

Insert the name of each sample(②) in the table starting with the standards (!! The samples will be analyzed in the same order as the list!!)



- Fill the 2 cuvettes of 10 cm to 2/3 with the blank (ultrapure water + reagent)
- Place a cuvette in each holder.


- Click on « Autozero » 
- The dialogue box opens, click on « OK » to start the autozero.

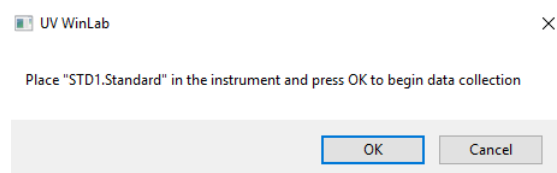


- Once the autozero has been carried out, only the front cuvette must be emptied. Rinse the cuvette with ultrapure water and fill with your first standard.

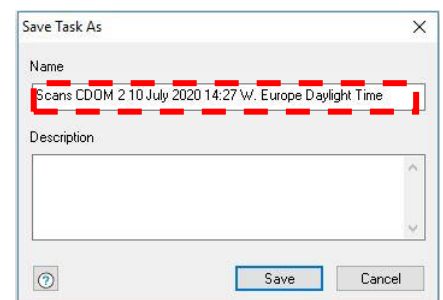


**Note :**  
Please do not empty your solutions, reagents, standards, and samples with the reagent in the sink but in a waste container!

- Insert your first standard in the machine.
- Start the analysis  then click "OK" when the dialogue box opens.



- Rinse the cuvette with ultrapure water between each sample measurement.
- Save all results in a folder: File > Save > New task. In the box, insert the name of the project and the date of analysis.



- The results will be saved under Documents > Data > CDOM\_scans
- Once the analyses are finished, clean both cuvettes as initially described. Switch off the instrument and the computer. Clean your work place.

