

Orthophosphate analysis

1 - OBJECTIVE

The purpose of this procedure is to describe the analysis of orthophosphates (PO_4^{3-}) by colorimetry 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-BACKGROUND

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- EQUIPMENT

- UV-vis spectrophotometer
- 2 cuvettes QS 10 cm
- Sterile 50mL Falcon tubes

4.3- CHEMICALS

sulfuric acid 98%	H_2SO_4	Si. Aldrich : 1.12080.1000
ascorbic acid	$\text{C}_6\text{H}_8\text{O}_6$	Si. Aldrich : A7506
ammonium molybdate	$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	Si. Aldrich : A7302
potassium hydrogenophosphate	KH_2PO_4	Si. Aldrich : 795488
potassium antimony tartrate (emetic)	$\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot 1/2 \text{H}_2\text{O}$	Si. Aldrich : 244791

4.4- SOLUTIONS AND STANDARDS

Sulfuric acid 5 N (2.5mol/L)

Sulfuric acid 98% (H_2SO_4)	140mL
Milli-Q water	complete to 1000mL

Slowly pour the acid into the water

Emetic solution

Potassium antimony tartrate	2.743g
Milli-Q water	complete to 1000mL

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

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

Ammonium molybdate solution

Ammonium molybdate		20g
Milli-Q water	complete to	500mL

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

Colorimetric reagent P ORTHO without reducer (ascorbic acid)

Sulfuric acid 2.5 mol/L		500mL
Ammonium molybdate solution		150mL
Emetic solution		50mL
Milli-Q water	complete to	1000mL

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.

Phosphate standard 100mgP/L

Potassium hydrogenophosphate (KH ₂ PO ₄)		0.4394g
Milli-Q water	complete to	1000mL

Conservation: 1 year

Phosphate standard 1.0mgP/L

Stock solution phosphate standard 100mgP/L		10mL
Milli-Q water	complete to	1000mL

Conservation: 1 month

4.5- CALIBRATION RANGE

Prepare in 100mL volumetric flasks.

Description	Phosphate sTD 1.0 mgP/L	Complete with MilliQ water
STD 0.01 mgP/L de PO ₄ ³⁻	1mL	
STD 0.05 mgP/L de PO ₄ ³⁻	5mL	
STD 0.10 mgP/L de PO ₄ ³⁻	10mL	
STD 0.20 mgP/L de PO ₄ ³⁻	20mL	100mL
STD 0.30 mgP/L de PO ₄ ³⁻	30mL	
STD 0.40 mgP/L de PO ₄ ³⁻	40mL	
STD 0.50 mgP/L de PO ₄ ³⁻	50mL	

Storage: 1 week

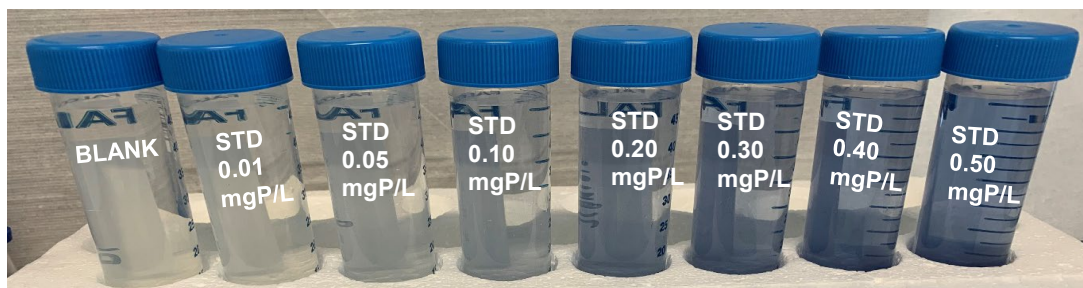
4.6- INSTRUCTIONS

- 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.

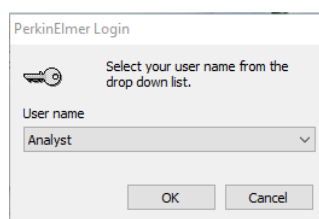
Description	Samples Volume	P ORTHO reagent with reducer
Ultrapure water (blank)	40 mL	8 mL
STD 0.01 mgP/L de PO ₄ ³⁻	40 mL	8 mL
STD 0.05 mgP/L de PO ₄ ³⁻	40 mL	8 mL
STD 0.10 mgP/L de PO ₄ ³⁻	40 mL	8 mL
STD 0.20 mgP/L de PO ₄ ³⁻	40 mL	8 mL
STD 0.30 mgP/L de PO ₄ ³⁻	40 mL	8 mL
STD 0.40 mgP/L de PO ₄ ³⁻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

Mix the tubes on a vortex mixer (2 times per tube).
Leave to stand for 15min (30min max) and measure.



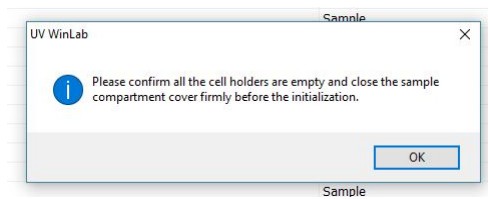
4.7- MESURE

- **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 »

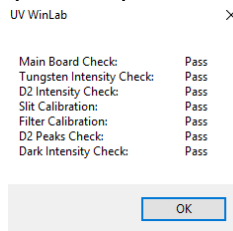


- Be sure that there are no cuvettes in the instrument, otherwise remove them.

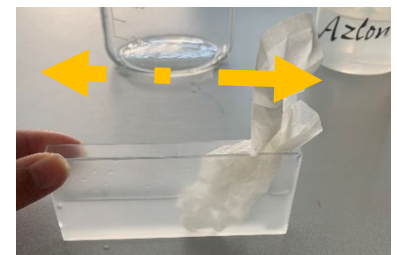
- Double-click on the method « Phosphate 0.0005 - 0.05 mg/L »
- Initializing the instrument: Wait until the dialogue box opens. Start the initialization by clicking « OK ».



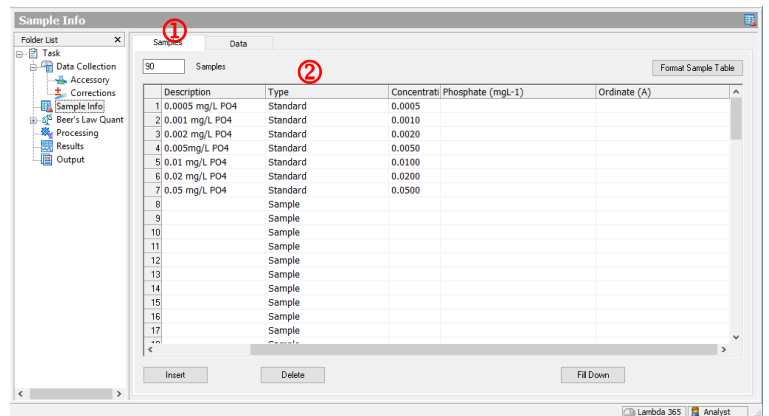
- When initialization is complete a report is displayed: all tests should indicate "PASS". Click "OK".
If not, close the program, let the lamp heat up for 30min and start again.



- Carefully clean the cuvette :
 - a) Rinse the cuvette with MilliQ 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. 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!!)

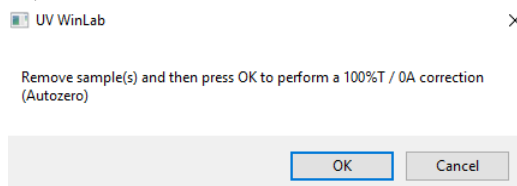


- Fill the cuvette of 10 cm to 2/3 with the blank (MilliQ water + reagent)



- Click on « Autozero »

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




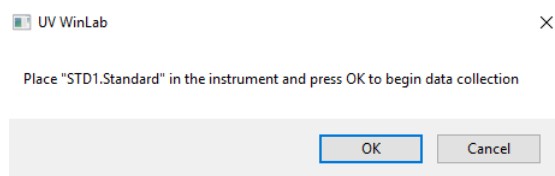
- Once the Autozero has been carried out, empty the cuvette. Rinse the cuvette with MilliQ water and fill with your first standard.



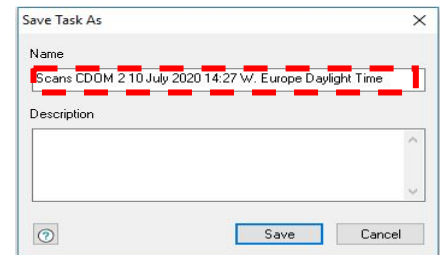
Note:

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 MilliQ 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 the cuvette as initially described. Switch off the instrument and the computer. **Clean your work place.**