UNIVERSITY OF KWAZULU-NATAL	*Standard Operating Procedure* PRG pollution research group	Effective Date: 20 June 2013	Version: *002*
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SOP_Chem_011 Chemical Analysis_Spectroquant Total and Orthophosphates			Page #: *1 of 4*

Standard Operation Procedure – Total and Ortho Phosphates, Spectroquant Method Test (Cat. No. 1.14543)

1. Scope and Application

- Test measures the total phosphorous, in a concentration range of 0.05 – 5.0 mg/l PO_4-P.

2. Summary

- In sulfuric solution orthophosphate ions react with molybdate ions to form molybdophosphoric acid.
- Ascorbic acid reduces this to phosphomolybdenum blue (PMB) that is determined photometrically.

3. Apparatus and Glassware

- Heating Block for Total P measurement.
- Spectrophotometer.
- Glassware: Use acid washed glassware for determining low concentrations of orthophosphates.
- Phosphate contamination is common because of its absorption on glass surfaces.
- Avoid using commercial detergents containing phosphate.
- Clean all glassware with hot dilute HCL and rinse well with distilled water.
- Preferably, reserve the glassware only for phosphate determination and after use, wash and keep filled with water until needed.
- If this is done, acid treatment is required only occasionally.

4. Interferences

Concentrations of foreign substances in mg/l or %					
Ag^{+}	1000	Fe^{3+}	1000	EDTA	1000
AsO_4^{3-}	0.2	Hg^{2+}	10	Surfactants	100
Ca^{2+}	1000	Mg^{2+}	1000	COD (K-hydrogen	150

Cd^{2+}	1000	Mn^{2+}	1000	phthalate)	
CN^-	1000	NH_4^+	1000	Na–acetate	1 %
Cr^{3+}	1000	Ni^{2+}	500	NaCl	5 %
$Cr_2O_7^{2+}$	5	NO_2^-	1000	$NaNO_3$	10 %
Cu^{2+}	250	Pb^{2+}	25	Na_2SO_4	10 %
F^-	50	S^{2-}	2.5		
SiO_3^{2-}	1000	Zn^{2+}	1000		
SO_3^{2-}	1000				

- Sample for phosphate analysis must be pretreated by filtration (0.45µm) to remove most of turbidity (interferes with photometric measurement).
- In case of total P, sample must not be filtrated.
- The filtration step would remove already precipitated struvite during urine storage and thus false the analysis.
- In any case urine should be diluted at least 1:100 to avoid matrix effects.

5. Collection, Preservation and Storage

- Collect faecal samples in 1L plastic buckets.
- Preferably, analyse samples immediately after sampling.
- Store samples at 4 °C or freeze dry samples.
- Preserve wastewater samples by acidifying with concentrated sulphuric acid to pH 2 and faecal samples by freeze drying or freezing.
- Reclose the reagent bottles immediately after use.

6. Safety Precautions

- Handle concentrated acid with care.
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are mixed.After the analysis, clean bottles and beakers with clear water keep it for drying.
- Dispose the used gloves after completion of analysis.
- Clean the hands using antiseptic soap.
- Disinfect hands after washing with soap.
- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.

7. Sample Preparation –Faecal Sludge

1. Weigh out 2.0000g of well-mixed faecal sludge sample.

2. Blend the weighed sample with 500ml of distilled water in a 1L blender for 30 seconds on the highest speed.
3. Add 250ml distilled water and blend on highest speed until the sample is homogenised (this could range from 30 to 60 seconds).
4. Transfer the blended mixture into a 1L volumetric flask.
5. Add 200ml of blender washings into the flask and top up to 1L with distilled water.
6. Transfer the 1L solution to a plastic bottle and store at 4 °C.

8. Reagents

- Sulphuric acid: 10-15 % concentration (phosphate test).
- Sodium nitrate: 50-100 % concentration (total P test).
- Potassium persulfate: 25-100 % concentration (total P test).

9. Calibration

- To check the photometric measurement system (test reagents, measurement device, and handling) and the mode of working, Spectroquant® CombiCheck 10 can be used.
- Besides a standard solution with 0.80 mg/l PO_4-P, the CombiCheck 10 also contains an addition solution for determining sample-dependent interferences (matrix effects).
- Prepare a series of at least three standards, covering the desired range, and a blank by diluting suitable volumes of standard solutions. Prepare a calibration curve by plotting instrument response against standard concentration. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and re-analyzed. Report results in mg/L.

10. Procedure

Ortho-Phosphate measurement:

- Pipette 5 ml pre-treated sample into a test tube.
- Reagent PO_4-1, add 5 drops and mix.
- Reagent PO_4-2, add 1 level blue micro-spoon, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then fill the sample into the cell, and measure in the photometer.

Total P measurement:

- Digestion for the determination of total phosphorus (Wear eye protection):
- Pipette 5 ml pre-treated sample into a reaction cell.
- Add 1 dose Reagent P-1K, close the cell tightly, and mix.

- Heat the cell at 120°C in the preheated thermoreactor for 30 min.
- Allow the closed cell to cool to room temperature in a test-tube rack.
- Do not cool with cold water.
- Shake the tightly closed cell vigorously after cooling.
- Add 5 drops reagent P-2K, close the cell tightly, and mix.
- Add 1 dose reagent P-3K, close the cell tightly, and shake vigorously until the reagent is completely dissolved.
- Leave to stand for 5 min (reaction time), then measure the sample in the photometer.

Notes on the measurement:

- For photometric measurement the cells must be clean. Wipe, if necessary, with a clean, dry paper towel.
- Measurement of turbid solutions yields false-high readings.
- The pH of the measurement solution must be within the range 0.80 - 0.95.
- The colour of the measurement solution remains stable for at least 60 min after the end of the reaction time stated above.

11. Waste Disposal

- Dilute 10 ml into 1000ml.
- Slowly add $NaCO_3$ until pH 6-8 is reached.
- Flush down the sink with excess water.

12. Data Quality

Measurement	0.05 – 5.0 mg/l PO_4-P
Standard Deviation (mg/l PO_4-P)	± 0.024
Confidence Interval (mg/l PO_4-P)	± 0.04
Sensitivity (mg/l PO_4-P)	0.02
Accuracy (mg/l PO_4-P)	± 0.06

13. References

http://www.merckmillipore.com/ZA/en/products/analytics-sample-prep/test-kits-and-photometric-methods/instrumental-test-systems-for-quantitative-analyses/photometric-measurements-spectroquant-system/spectroquant-tests/

APPROVAL OF STANDARD OPERATING PROCEDURE

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