International Standard 6878/1

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Water quality — Determination of phosphorus — Part 1: Ammonium molybdate spectrometric method

Qualité de l'eau — Dosage du phosphore — Partie 1: Dosage spectrométrique à l'aide du molybdate d'ammonium

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Foreword

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Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

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Water quality — Determination of phosphorus — Part 1 : Ammonium molybdate spectrometric method

0 Introduction

This part of ISO 6878 deals with the determination of phosphorus compounds present in ground, surface, and waste waters in various concentrations in the dissolved and undissolved state.

A spectrometric method after mineralization with sulfuric acid and perchloric acid, for heavily polluted waste water, will form the subject of ISO 6878/2.

1 Scope and field of application

This part of ISO 6878 specifies methods for the determination of

- orthophosphate (see **section one**);
- orthophosphate after extraction (see **section two**);
- hydrolysable phosphate plus orthophosphate (see **section three**);
- total soluble phosphorus and total phosphorus after decomposition (see **section four**).

The methods are applicable to all kinds of water including sea-water and effluents. Phosphorus contents within the range of 0,005 to 0,8 mg of P per litre may be determined in such samples without dilution.

An extraction procedure allows smaller phosphorus concentrations to be determined with a detection limit of about 0,000 5 mg/l.

See the annex for some known interferences. There may be others and it is necessary to verify whether any such exist and take action to remove them.

2 Principle

Reaction of orthophosphate ions with an acid solution containing molybdate and antimony ions to form an antimony phosphomolybdate complex.

Reduction of the complex with ascorbic acid to form a strongly coloured molybdenum blue complex. Measurement of the absorbance of this to determine the concentration of orthophosphate present.

Polyphosphates and some organophosphorus compounds are determined if converted to the molybdate reactive orthophosphate form by sulfuric acid hydrolysis.

Many organophosphorus compounds are converted to orthophosphate by mineralization with persulfate. Nitric acid-sulfuric acid mineralization is used if a more vigorous treatment is required.

Section one: Determination of orthophosphate

3 Reagents

During the analysis, use only reagents of recognized analytical grade and only distilled water having a phosphate content that is negligible compared with the smallest concentration to be determined in the samples.

For low phosphate contents, double distilled water from an all-glass apparatus is necessary. Deionized water shall be checked according to the procedures given in the bibliography.

3.1 Sulfuric acid, solution, $c(H_2SO_4)$ = 9 mol/l.

Add 500 ± 5 ml of water to a 2 l beaker. Cautiously add, with continuous stirring, 500 ± 5 ml of sulfuric acid (ϱ = 1,84 g/ml).

3.2 Sulfuric acid, solution, $c(H_2SO_4)$ = 4,5 mol/l.

Add 500 ± 5 ml of water to a 2 l beaker. Cautiously add, with continuous stirring, 500 ± 5 ml sulfuric acid (3.1) and mix well.

3.3 Sulfuric acid, solution, $c(H_2SO_4)$ = 2 mol/l.

Add 300 ± 3 ml of water to a 1 litre beaker. Cautiously add 110 ± 2 ml of sulfuric acid solution (3.1), with continuous stirring and cooling. Dilute to 500 ± 2 ml with water and mix well.

3.4 Sodium hydroxide, solution, $c(NaOH)$ = 2 mol/l.

Dissolve 80 g of sodium hydroxide pellets in water, cool and dilute to 1 litre with water.

3.5 Ascorbic acid, 100 g/l solution.

Dissolve 10 g of ascorbic acid ($C_6H_8O_6$) in 100 ml water.

The solution is stable for 2 weeks if stored in an amber glass bottle in a refrigerator and can be used as long as it remains colourless.

3.6 Acid molybdate, solution I.

Dissolve 13 g ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in 100 ml water. Dissolve 0,35 g antimony potassium tartrate hemihydrate [$K(SbO)C_4H_4O_6\cdot 1/2H_2O$] in 100 ml water.

Add the molybdate solution to 300 ml of 9 mol/l sulfuric acid (3.1) with continuous stirring. Add the tartrate solution and mix well.

The reagent is stable for at least 2 months if stored in an amber glass bottle.

3.7 Acid molybdate, solution II.

Add 230 ml 9 mol/l sulfuric acid (3.1) to 70 ml water, cool, then add molybdate and tartrate solutions as in 3.6.

This reagent is used when samples are acidified with 1 ml of 4,5 mol/l sulfuric acid (3.2) per 100 ml (see sections three and four).

The reagent is stable for at least 2 months.

3.8 Turbidity-colour compensation solution.

Mix two parts by volume of 9 mol/l sulfuric acid (3.1) and one part by volume of ascorbic acid (3.5).

The reagent is stable for several weeks if stored in an amber glass bottle in a refrigerator.

3.9 Sodium thiosulfate pentahydrate, 12,0 g/l solution.

Dissolve 1,20 g sodium thiosulfate pentahydrate ($Na_2S_2O_3\cdot 5H_2O$) in 100 ml water. Add about 50 mg anhydrous sodium carbonate (Na_2CO_3) as preservative.

This reagent is stable for several weeks if stored in an amber glass bottle.

3.10 Orthophosphate, stock standard solution corresponding to 50 mg of P per litre.

Dry a few grams of potassium dihydrogenphosphate to constant mass at 105 °C. Dissolve 0,219 7 g KH_2PO_4 in about 800 ml water in a 1 000 ml volumetric flask. Add 10 ml of 4,5 mol/l sulfuric acid (3.2) and make up to the mark with water.

The solution is stable for at least 1 week if stored in a well-stoppered glass bottle. Refrigeration is recommended.

3.11 Orthophosphate, standard solution corresponding to 2 mg of P per litre.

Pipette 20 ml of orthophosphate stock standard solution (3.10) into a 500 ml volumetric flask. Make up to the mark with water.

Prepare this solution each day it is required.

1 ml of this standard solution contains 2 µg of P.

4 Apparatus

Ordinary laboratory apparatus, and

4.1 Spectrometer, prism or grating type, or filter type, capable of accepting optical cells of thickness 10 to 50 mm.

The spectrometer chosen shall be suitable for measuring absorbance in the visible and near infra-red regions of the spectrum. The most sensitive wavelength is 880 nm, but if a loss of sensitivity is acceptable, absorbance can be measured at 700 nm.

NOTE — The detection limit of the method is lowered if a spectrometer capable of accepting 100 mm optical cells is available.

4.2 Filter assembly, to hold a membrane filter of pore size 0,45 µm.

NOTE ON THE PREPARATION OF GLASSWARE

Before use all glassware should be washed with hot 2 mol/l hydrochloric acid and rinsed thoroughly with water. Do not use detergents containing phosphate.

Preferably the glassware should be used only for the determination of phosphorus. After use it should be cleaned as above and kept covered until needed again.

Glassware used for the colour development stage should be rinsed occasionally with sodium hydroxide solution (3.4) to remove deposits of the coloured complex which has a tendency to stick as a thin film on the walls of glassware.

5 Sampling and samples

5.1 Sampling

Collect laboratory samples in polyethylene, polyvinylchloride or preferably glass bottles. In the case of small phosphate concentrations the use of glass bottles is essential.

5.2 Preparation of the test sample

Filter the laboratory sample (5.1) within 4 h after sampling. If the sample has been kept cool in the meantime, bring to room temperature before filtration.

Filter the sample through a membrane filter of pore size 0,45 µm (see notes 1 and 2) that has been washed free of phosphates by passing through it approximately 200 ml water warmed to 30 to 40 °C. Discard these washings. Reject the first 10 ml of sample filtrate and collect the remainder in a clean dry glass bottle for the immediate determination of orthophosphate as specified in clause 6.

If the filtrate is not within the range of pH 3 to 10, adjust it with sodium hydroxide solution (3.4) or 2 mol/l sulfuric acid (3.3).

NOTES

1 The filtration time should not exceed 10 min. If necessary, choose a larger diameter filter.

2 The membrane filter must be checked for phosphorus content. Membrane filters free from phosphorus are commercially available.

6 Procedure

6.1 Test portion

The maximum volume of test portion to be used is 40,0 ml. This is suitable for the determination of orthophosphate concentrations of up to ϱ_P = 0,8 mg/l when using an optical cell of thickness 10 mm to measure the absorbance of the coloured complex formed by reaction with acid molybdate reagent. Smaller test portions may be used as appropriate in order to accommodate higher phosphate concentrations as shown in table 1. Phosphate concentrations at the lower end of the calibration ranges are best determined by measuring absorbance in an optical cell of thickness 40 or 50 mm.

Table 1

Orthophosphate concentration mg/l	Volume of test portion ml	Thickness of optical cell mm
0,0 to 0,8	40,0	10
0,0 to 1,6	20,0	10
0,0 to 3,2	10,0	10
0,0 to 6,4	5,0	10
0,0 to 0,2	40,0	40 or 50

6.2 Blank test

Carry out a blank test in parallel with the determination, by the same procedure, using the same quantities of all the reagents as in the determination, but using the appropriate volume of water instead of the test portion.

6.3 Calibration

6.3.1 Preparation of the set of calibration solutions

Transfer, by means of a pipette, 1,0; 2,0; 3,0; 4,0; 5,0; 6,0; 7,0; 8,0; 9,0; and 10,0 ml of the orthophosphate standard solution (3.11) to a series of 50 ml volumetric flasks. Dilute with water to about 40 ml. Proceed accordingly for other ranges of phosphate concentration.

6.3.2 Colour development

Add to each flask, while swirling, 1 ml of ascorbic acid (3.5) followed by 2 ml of acid molybdate solution I (3.6). Make up to the mark with water and mix well.

6.3.3 Spectrometric measurements

Measure the absorbance of each solution after between 10 and 30 min at 880 nm, or if a loss of sensitivity can be accepted, at 700 nm. Use water in the reference cell.

6.3.4 Plotting the calibration graph

Plot a graph of absorbance against the phosphorus content, in milligrams per litre, of the calibration solutions. The relationship between absorbance and concentration is linear. Determine the reciprocal of the slope of the graph.

Check the graph from time to time, especially if new packages of chemicals are used. Run a calibration solution with each series of samples.

6.4 Determination

6.4.1 Colour development

Pipette the selected volume of test portion into a 50 ml one-mark volumetric flask and if necessary dilute to 40 ± 2 ml with water. Proceed as specified in 6.3.2.

NOTES

1 If the test sample contains arsenate, this must be reduced to arsenite with thiosulfate. The reduction is quantitative for arsenate concentrations up to at least 2 mg of As per litre.

Transfer, by means of a pipette, up to a maximum of 40 ml of the test sample to a 50 ml volumetric flask. Add 1 ml of ascorbic acid solution (3.5) and 1 ml of the thiosulfate solution (3.9). Mix and allow the reduction to proceed for 10 ± 1 min, then add 2 ml of acid molybdate solution II (3.7). Make up to the mark with water.

2 If the test sample is turbid and/or coloured, compensate for this by adding 3 ml of turbidity-colour compensation reagent (3.8). The absorbance of this solution is subtracted from the value measured according to 6.4.2.

3 Absorbance measured at 700 nm represents a loss of about 30 % of the sensitivity at 880 nm.

6.4.2 Spectrometric measurements

See 6.3.3.

NOTE — If the test portion has been treated with thiosulfate due to interference by arsenate, take the measurements within 10 min; otherwise the colour will fade.

7 Expression of results

7.1 Calculation

The orthophosphate concentration, ϱ_P, expressed in milligrams per litre, is given by the equation

$$\varrho_P = \frac{(A - A_0) f V_{max}}{V_s}$$

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the reciprocal of the slope of the calibration graph;

V_{max} is the maximum volume, 40 ml, of the test portion;

V_s is the actual volume, in millilitres, of the test portion.

Report the mass concentrations of phosphorus as follows, but to not more than three significant figures:

$\varrho_P < 0{,}1$ mg/l to the nearest 0,001 mg/l;

$0{,}1 \leqslant \varrho_P < 10$ mg/l to the nearest 0,01 mg/l;

$\varrho_P \geqslant 10$ mg/l to the nearest 0,1 mg/l.

7.2 Precision

The precision data in table 2 were obtained in an interlaboratory trial involving 16 laboratories.

NOTE — For interferences, see the annex.

8 Test report

The test report shall contain the following information:

a) all information necessary for complete identification of the sample;

b) a reference to this part of ISO 6878;

c) a reference to the method used;

d) the results obtained;

e) the conditions of test;

f) details of any operations not included in this section or regarded as optional, together with any incidents likely to have had an influence upon the results.

Table 2

Description of sample	No. of samples, n	Mean (µg/l)	Standard deviation		
			repeatability	reproducibility	
			absolute (µg/l)	absolute (µg/l)	relative (%)
Orthophosphate in presence of polyphosphate	70	57,6	2,20	10,8	18,8
Orthophosphate	69	312,7	4,81	32,4	10,4
Orthophosphate in presence of arsenate and polyphosphate	78	192,0	4,01	34,8	17,6
Orthophosphate in presence of arsenate	78	101,3	5,77	22,1	21,8

Section two: Determination of orthophosphate after extraction

This method is only applied if the phosphate concentration in the sample is less than 10 µg/l.

9 Reagents

Use the reagents specified in 3.5 and 3.6, and in addition:

9.1 1-Hexanol ($C_6H_{13}OH$).

9.2 Ethanol (C_2H_5OH).

9.3 Orthophosphate, standard solution, corresponding to 0,5 mg of P per litre.

Pipette 5,0 ml of orthophosphate stock standard solution (3.10.1) into a 500 ml one-mark volumetric flask. Make up to the mark with water and mix well.

Prepare this solution each day as required.

10 Sampling and samples

See clause 5.

11 Procedure

11.1 Test portion

Transfer, by means of a measuring cylinder, 350 ml of the test sample (5.2) to a 500 ml separating funnel.

11.2 Blank test

Carry out a blank test in parallel with the determination, by the same procedure, using the same quantities of all the reagents as in the determination, but using 350 ml of water instead of the test portion.

11.3 Calibration

11.3.1 Preparation of the set of calibration solutions

From a microburette add 1,4; 2,8; 4,2; 5,6; and 7,0 ml of orthophosphate standard solution (9.3) to a series of five 500 ml separating funnels. Dilute each solution to 350 ± 10 ml with water and swirl to mix. These solutions represent orthophosphate concentrations, ϱ_P, of 2; 4; 6; 8; and 10 µg/l respectively.

11.3.2 Colour development

To each flask, with swirling, add 7,0 ± 0,1 ml of ascorbic acid solution (3.5) and 14,0 ± 0,1 ml of acid molybdate solution I (3.6).

After 15 min add 40,0 ± 0,1 ml of 1-hexanol (9.1) to each flask and stopper and shake the flasks vigorously for 1 min. Allow the phases to separate and pipette 30,0 ml of each of the upper layer 1-hexanol extracts into a series of dry 50 ml one-mark volumetric flasks. Add 1,0 ± 0,2 ml of ethanol (9.2) to each flask and dilute each solution to the mark with 1-hexanol (9.1). Discard the lower aqueous phase from the separating funnels.

11.3.3 Spectrometric measurements

Measure the absorbance of each solution at 680 nm in optical cells of thickness 40 or 50 mm against 1-hexanol in the reference cell.

11.3.4 Plotting the calibration graph

Plot a graph of absorbance against the phosphorus content, in micrograms per litre, of the calibration solutions. Determine the reciprocal of the slope of the graph.

Check the graph from time to time, especially if new packages of chemicals are used.

11.4 Determination

11.4.1 Colour development

Treat the test portion (11.1) as specified in 11.3.2 for the calibration solutions.

11.4.2 Spectrometric measurements

See 11.3.3.

12 Expression of results

The orthophosphate concentration, ϱ_P, expressed in milligrams per litre, is given by the equation

$$\varrho_P = (A - A_0)f$$

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the reciprocal of the slope of the calibration graph.

Report the value to the nearest 0,000 1 mg/l; give values below 0,000 5 mg/l as "ϱ_P < 0,000 5 mg/l".

NOTE — For interferences, see the annex.