INTERNATIONAL STANDARD

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Water quality — Determination of phosphorus — Ammonium molybdate spectrometric method

Qualité de l'eau — Dosage du phosphore — Méthode spectrométrique au molybdate d'ammonium

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 6878 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

This second edition cancels and replaces the first edition (ISO 6878:1998), which has been technically revised.

Introduction

This International Standard specifies the determination of different forms of phosphorus compounds present in ground, surface and waste waters in various concentrations in the dissolved and undissolved state.

The user should be aware that particular problems could require the specification of additional marginal conditions.

Water quality — Determination of phosphorus — Ammonium molybdate spectrometric method

WARNING — Persons using this International Standard should be familiar with normal laboratory practice. This International Standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions. It is absolutely essential that tests conducted according to this International Standard be carried out by suitably qualified staff. Molybdate and antimony waste solutions should be disposed of properly.

1 Scope

This International Standard specifies methods for the determination of

— orthophosphate (see Clause 4);

— orthophosphate after solvent extraction (see Clause 5);

— hydrolysable phosphate plus orthophosphate (see Clause 6);

— total phosphorus after decomposition (see Clauses 7 and 8).

The methods are applicable to all kinds of water including seawater and effluents. Phosphorus concentrations within the range of 0,005 mg/l to 0,8 mg/l may be determined in such samples without dilution.

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

2 Interferences

See Annex A for some known interferences. There may be others and it is recommended to verify whether any such interferences exist and take action to eliminate them.

3 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 complex to determine the concentration of orthophosphate present.

Polyphosphate and some organophosphorus compounds are determined if converted to molybdate reactive orthophosphate formed by sulfuric acid hydrolysis.

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

4 Determination of orthophosphate

4.1 Reagents

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

For low phosphate contents, double-distilled water from an all-glass apparatus is recommended.

4.1.1 Sulfuric acid solution, $c(H_2SO_4) \approx 9$ mol/l.

Add 500 ml ± 5 ml of water to a 2 l beaker. Cautiously add, with continuous stirring and cooling, 500 ml ± 5 ml of sulfuric acid, $\rho = 1{,}84$ g/ml. Mix well and allow the solution to cool to room temperature.

4.1.2 Sulfuric acid solution, $c(H_2SO_4) \approx 4{,}5$ mol/l.

Add 500 ml ± 5 ml of water to a 2 l beaker. Cautiously add, with continuous stirring and cooling, 500 ml ± 5 ml of sulfuric acid (4.1.1). Mix well and allow to cool to room temperature.

4.1.3 Sulfuric acid solution, $c(H_2SO_4) \approx 2$ mol/l.

Add 300 ml ± 3 ml of water to a 1 l beaker. Cautiously add 110 ml ± 2 ml of sulfuric acid solution (4.1.1), with continuous stirring and cooling. In a measuring flask, dilute to 500 ml ± 2 ml with water and mix well.

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

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

4.1.5 Ascorbic acid solution, $\rho = 100$ g/l.

Dissolve 10 g ± 0,5 g of ascorbic acid ($C_6H_8O_6$) in 100 ml ± 5 ml water.

NOTE 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.

4.1.6 Acid molybdate, Solution I.

Dissolve 13 g ± 0,5 g of ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in 100 ml ± 5 ml of water. Dissolve 0,35 g ± 0,05 g of antimony potassium tartrate hemihydrate [$K(SbO)C_4H_4O_6\cdot ½H_2O$] in 100 ml ± 5 ml of water.

Add the molybdate solution to 300 ml ± 5 ml of sulfuric acid (4.1.1) with continuous stirring. Add the tartrate solution and mix well.

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

4.1.7 Acid molybdate, Solution II.

Cautiously add 230 ml ± 0,5 ml of sulfuric acid (4.1.1) to 70 ml ± 5 ml of water, cool. Dissolve 13 g ± 0,5 g of ammonium heptamolybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in 100 ml ± 5 ml of water. Add to the acid solution and mix well. Dissolve 0,35 g ± 0,05 g of antimony potassium tartrate hemihydrate [$K(SbO)C_4H_4O_6\cdot ½H_2O$] in 100 ml ± 5 ml of water. Add to the molybdate-acid solution and mix well.

This reagent is used when the sample is acidified with sulfuric acid (4.1.2) (see also Clauses 6, 7 and 8).

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

4.1.8 Turbidity-colour compensation solution.

On a volume/volume basis, mix two parts of sulfuric acid (4.1.2) and one part of ascorbic acid (4.1.5).

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

4.1.9 Sodium thiosulfate pentahydrate solution, ρ = 12,0 g/l.

Dissolve 1,20 g ± 0,05 g of sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$) in 100 ml ± 5 ml of water. Add 0,05 g ± 0,005 g of anhydrous sodium carbonate (Na_2CO_3) as preservative.

NOTE The reagent is stable for at least 4 weeks if stored in an amber glass bottle.

4.1.10 Orthophosphate stock standard solution, ρ_P = 50 mg/l.

Dry a few grams of potassium dihydrogen phosphate to constant mass at 105 °C. Dissolve 0,219 7 g ± 0,000 2 g of KH_2PO_4 in about 800 ml ± 10 ml of water in a 1 000 ml volumetric flask. Add 10 ml ± 0,5 ml of sulfuric acid (4.1.2) and make up to the mark with water.

Alternatively, use a commercially available stock solution.

The solution is stable for at least 3 months if stored in a well stoppered glass bottle. Refrigeration to about 4 °C is recommended.

4.1.11 Orthophosphate standard solution, ρ_P = 2 mg/l.

Pipette 20 ml ± 0,01 ml of orthophosphate stock standard solution (4.1.10) into a 500 ml volumetric flask. Make up to the mark with water and mix well.

Prepare and use this solution each day as required.

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

4.1.12 Hydrochloric acid, ρ(HCl) = 1,19 g/ml.

4.1.13 Hydrochloric acid, c(HCl) = 2,5 mol/l.

Cautiously add 200 ml ± 10 ml of hydrochloric acid (4.1.12) to 500 ml ± 10 ml of water. Mix and cool to room temperature. Make up to 1 000 ml with water.

4.2 Apparatus

4.2.1 Spectrometer, “prism”- or “grating-type” or filter type, capable of accepting optical cells of thickness 10 mm 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 can be accepted, absorbance may be measured at 700 nm.

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

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

4.2.3 Glassware.

Before use, wash all glassware, for example with hydrochloric acid (4.1.13), at approximately 40 °C to 50 °C and rinse thoroughly with water. Detergents containing phosphate shall not be used.

Preferably the glassware should be used only for the determination of phosphorus. After use, clean it as described above and keep covered until needed again.

Rinse glassware used for the colour development stage occasionally with sodium hydroxide solution (4.1.4), followed by thorough rinsing with water (4.1), to remove deposits of the coloured complex which has a tendency to stick as a thin film on the wall of glassware.

4.3 Sampling and samples

4.3.1 Sampling

Collect the laboratory samples in polyethene, polyvinylchloride or preferably glass bottles. In the case of low phosphate concentrations, use glass bottles.

The use of sampling bottles with cap lines should be avoided as these may contain phosphorus.

4.3.2 Preparation of the test sample

Filter the laboratory sample (4.3.1) within 4 h after sampling. If the sample has been kept cool in the meantime, bring to room temperature before filtration.

Wash a membrane filter of nominal pore size 0,45 µm to ensure it is free of phosphate by passing 200 ml of water, previously heated to approximately 30 °C to 40 °C. Discard these washings. Filter the sample and reject the first 10 ml of sample filtrate. Collect the remainder in a clean, dry glass bottle for the immediate determination of orthophosphate (4.4.4).

If the filtrate is not within the range of pH 3 to pH 10, adjust it with sodium hydroxide (4.1.4) or sulfuric acid solution (4.1.3).

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

The membrane filter should either be checked for phosphorus content or washed as described. Commercially available membrane filters that are sold free from phosphorus should be washed as described.

4.4 Procedure

4.4.1 Test portion

Take a volume of test portion not exceeding 40 ml. This maximum volume 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. Smaller test portions shall be used in order to accommodate higher phosphate concentrations as shown in Table 1. Similarly, low phosphate concentrations can be determined by measuring the absorbance in an optical cell of thickness 40 mm or 50 mm.

Table 1 — Sample volumes and concentrations

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

4.4.2 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.

4.4.3 Calibration

4.4.3.1 Preparation of calibration solutions

Transfer, by means of a volumetric pipette, appropriate volumes, for example, 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml, 5,0 ml, 6,0 ml, 7,0 ml, 8,0 ml, 9,0 ml and 10,0 ml of the orthophosphate standard solution (4.1.11) to 50 ml volumetric flasks. Dilute with water to about 40 ml. These solutions represent orthophosphate concentrations ρ_P = 0,04 mg/l to 0,4 mg/l.

Proceed accordingly for other ranges of phosphate concentrations shown in Table 1.

4.4.3.2 Colour development

Add to each flask, while swirling, 1 ml of ascorbic acid (4.1.5) followed by 2 ml of acid molybdate Solution I (4.1.6). Make up to the mark with water and mix well.

NOTE Absorbance measured at 700 nm causes a loss of about 30 % of the sensitivity at 880 nm.

4.4.3.3 Spectrometric measurements

Measure the absorbance of each solution using the spectrometer (4.2.1) at 880 nm after a period between 10 min and 30 min, or, if a loss of sensitivity can be accepted, at 700 nm. Use water in the reference cell.

4.4.3.4 Plotting the calibration graph

Plot a graph of absorbance (as the y-axis) against the phosphorus content (as the x-axis) in milligrams of phosphorus per litre of the calibration solutions. The relationship between absorbance and concentration is linear. Determine the slope of the graph.

Verify the graph from time to time for linearity, especially if new batches of chemicals are used.

4.4.4 Determination

4.4.4.1 Colour development

4.4.4.1.1 Standard procedure

Pipette the selected volume of test portion (4.4.1), V_S , into a 50 ml one-mark volumetric flask and, if necessary, dilute to approximately 40 ml $\pm$ 2 ml with water. Proceed as specified in 4.4.3.2.

If the test sample contains arsenate, this should be reduced to arsenite with thiosulfate in acidic medium. The reduction to arsenite is quantitative for arsenate concentrations up to at least 2 mg/l As, as described below.

Transfer, by means of a volumetric pipette, up to a maximum of 40 ml of the test sample to a 50 ml volumetric flask. Add 0,4 ml of sulfuric acid (4.1.2), 1 ml of ascorbic acid solution (4.1.5), and 1 ml of thiosulfate solution (4.1.9). Mix and allow the reduction to proceed for 10 min $\pm$ 1 min. Add 2 ml acid molybdate Solution II (4.1.7). Make up to the mark with water. Mix well. Proceed as described in 4.4.3.3.

4.4.4.1.2 Procedure in case of turbid samples

If the test sample is turbid and/or coloured, proceed as follows.

Add 3 ml of the turbidity-colour compensation reagent (4.1.8) to the selected volume of test portion. Dilute to 50 ml and measure the absorbance. Subtract the absorbance of this solution from the value measured as specified in 4.4.3.3.

4.4.4.2 Spectrometric measurements

See 4.4.3.3.

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

4.5 Expression of results

4.5.1 Calculation

Calculate the orthophosphate concentration, ρ_P, expressed in milligrams per litre, using the equation

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

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the slope of the calibration graph (4.4.3.4), expressed in litres per milligram (l/mg);

V_{max} is the volume of the volumetric flask (50 ml), expressed in millilitres (ml);

V_s is the actual volume of the test portion, expressed in millilitres (ml).

Report the mass concentrations of phosphorus as follows, but to no more than three significant figures:

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

— $\rho_P < 10$ mg/l to the nearest 0,01 mg/l;

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

4.5.2 Precision

The precision data in Table B.1 were obtained in an interlaboratory trial involving 16 laboratories.

NOTE For interferences, see Annex A.

4.6 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 International Standard (ISO 6878:2004);

c) a reference to the method used, and the number of the clause;

d) the results obtained;

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

5 Determination of orthophosphate after solvent extraction

5.1 Applicability

This method can be applied only if the phosphate concentration in the sample is less than 0,01 mg/l P. This method is especially suitable for marine water.

5.2 Reagents

Use the reagents specified in 4.1.5, 4.1.6 and 4.1.10 and in addition:

5.2.1 Hexan-1-ol ($C_6H_{13}OH$).

5.2.2 Ethanol (C_2H_5OH).

5.2.3 Orthophosphate, standard solution, $\rho_P = 0{,}5$ mg/l P.

Pipette 5,0 ml $\pm$ 0,01 ml of orthophosphate stock standard solution (4.1.10) into a 500 ml one-mark volumetric flask. Make up to the mark with water and mix well.

Prepare and use this solution each day as required.

5.3 Sampling and samples

See 4.3.

5.4 Procedure

5.4.1 Test portion

Transfer, by means of a measuring cylinder, 350 ml $\pm$ 5 ml of the test sample (4.3) to a 500 ml separating funnel.

5.4.2 Blank test

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

5.4.3 Calibration

5.4.3.1 Preparation of calibration solutions

Add 300 ml $\pm$ 10 ml of water to five individual separating funnels. From a microburette add 1,4 ml, 2,8 ml, 4,2 ml, 5,6 ml and 7,0 ml of orthophosphate standard solution (5.2.3) to each 500 ml separating funnel. Dilute each solution to 350 ml $\pm$ 10 ml with water, stopper, swirl, and mix. These solutions represent orthophosphate concentrations, ρ_P, of 0,002 mg/l, 0,004 mg/l, 0,006 mg/l, 0,008 mg/l and 0,01 mg/l respectively.

5.4.3.2 Colour development

To each separating funnel, with swirling, add 7,0 ml ± 0,1 ml of ascorbic acid solution (4.1.5) and 14,0 ml ± 0,1 ml of acid molybdate Solution I (4.1.6).

After 15 min add 40,0 ml ± 0,1 ml of hexan-1-ol (5.2.1) to each separating funnel and stopper. Shake vigorously for 1 min. Allow the phases to separate and pipette 30 ml ± 0,01 ml of each of the upper hexan-1-ol extracts into a series of dry 50 ml one-mark volumetric flasks. Add 1,0 ml ± 0,2 ml of ethanol (5.2.2) to each flask and dilute each solution to the mark with 1-hexanol.

5.4.3.3 Spectrometric measurements

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

5.4.3.4 Plotting the calibration graph

Plot a graph of absorbance (as the y-axis) against the phosphorus content (as the x-axis), in milligrams per litre, of the calibration solutions. Determine the slope of the graph.

Verify the linearity of the calibration curve periodically, especially if new batches of chemicals are used.

5.4.4 Determination

5.4.4.1 Colour development

Treat the test portions (5.4.1) as specified in 5.4.3.2 for the calibration solutions.

5.4.4.2 Spectrometric measurements

See 5.4.3.3.

5.5 Expression of results

Calculate the orthophosphate concentration, ρ_P, expressed in milligrams per litre (mg/l), using the equation:

$$\rho_P = \frac{A - A_0}{f}$$

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the slope of the calibration graph (5.4.3.4), in litres per milligram (l/mg).

Report the value to the nearest 0,001 mg/l but give values below 0,000 5 mg/l as $\rho_P < 0{,}000\ 5$ mg/l.

NOTE For interferences, see Annex A.

5.6 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 International Standard (ISO 6878:2004);

c) a reference to the method used, and the number of the clause;

d) the results obtained;

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

6 Determination of hydrolysable phosphate and orthophosphate

6.1 Reagents

Use the reagents specified in 4.1.2, 4.1.4, 4.1.5, 4.1.7 and 4.1.11.

6.2 Apparatus

See 4.2.

6.3 Sampling and samples

6.3.1 Sampling

See 4.3.1.

6.3.2 Preparation of the test sample

Filter the sample (4.3.1) as described in 4.3.2 and analyse as soon as possible after sampling. If the sample has been kept cool (5 °C to 10 °C) in the meantime, bring to room temperature before filtration.

Add 1 ml of sulfuric acid (4.1.2) per 100 ml of filtered test sample to bring to about pH 1. Keep the filtrate cool and dark until analysis.

6.4 Procedure

6.4.1 Test portion

According to the expected phosphate concentration of the sample (see Table 1), transfer, by means of a volumetric pipette, up to a maximum of 40 ml of the test sample (6.3.2) to a conical flask. If necessary, dilute to 40 ml $\pm$ 2 ml with water. Acidify with sulfuric acid (4.1.2) to pH $<$ 1 and boil gently for approximately 30 min. Periodically, add sufficient water so that the volume remains between 25 ml and 35 ml. Cool, adjust to pH 3 to pH 10 with sodium hydroxide solution (4.1.4) and transfer to a 50 ml volumetric flask; dilute with water to about 40 ml.

Alternatively, mineralize the acidified filtrate in a closed bottle for approximately 30 min in an autoclave at between 115 °C and 120 °C.

6.4.2 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 water acidified to the same extent as the test portion.

6.4.3 Calibration

6.4.3.1 Preparation of calibration solution

Transfer, by means of a volumetric pipette appropriate volumes, for example, 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml, 5,0 ml, 6,0 ml, 7,0 ml, 8,0 ml, 9,0 ml and 10,0 ml of the orthophosphate standard solution (4.1.11) to 50 ml conical flasks. Dilute with water to 40 ml $\pm$ 2 ml. These solutions represent orthophosphate concentrations ρ_P = 0,04 mg/l to 0,4 mg/l. Proceed accordingly for other ranges of phosphate concentration shown in Table 1. Acidify with sulfuric acid (4.1.2) to pH $<$ 1 and boil gently for approximately 30 min and continue as stated in 6.4.1.

6.4.3.2 Colour development

Add to each flask, while swirling, 1 ml of ascorbic acid (4.1.5) followed by 2 ml of acid molybdate Solution II (4.1.7). Make up to the mark with water.

6.4.3.3 Spectrometric measurements

See 4.4.3.3.

6.4.3.4 Plotting the calibration graph

See 4.4.3.4.

6.4.4 Determination

6.4.4.1 Colour development

Proceed according to 6.4.3.2, using the test portion (6.4.1).

6.4.4.2 Spectrometric measurements

See 4.4.3.3.

6.5 Expression of results

6.5.1 Calculation

Calculate the concentration of orthophosphate plus hydrolysable phosphate, ρ_P, expressed in milligrams per litre, using the equation

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

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the slope of the calibration graph (4.4.3.4), expressed in litres per milligram (l/mg);

V_{max} is the volume of the volumetric flask (50 ml), expressed in millilitres (ml);

V_s is the actual volume of the test portion, expressed in millilitres (ml).

Take into account any dilution steps and as well the dilution steps caused by the addition of sulfuric acid.

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

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

— $\rho_P < 10$ mg/l to the nearest 0,01 mg/l;

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

6.5.2 Precision

The precision data in Table B.2 were obtained in an interlaboratory trial involving 15 laboratories (see also Table B.1).

NOTE For interferences, see Annex A.

6.6 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 International Standard (ISO 6878:2004);

c) a reference to the method used, and the number of the clause;

d) the results obtained;

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

7 Determination of total phosphorus after peroxodisulfate oxidation

7.1 Reagents

Use the reagents specified in 4.1.2, 4.1.3, 4.1.4, 4.1.5, 4.1.7, 4.1.8, 4.1.9 and 4.1.11, and in addition:

7.1.1 Potassium peroxodisulfate solution.

Add 5 g $\pm$ 0,1 g of potassium peroxodisulfate ($K_2S_2O_8$) to 100 ml $\pm$ 5 ml of water, stir to dissolve.

NOTE The solution is stable for at least 2 weeks, if the supersaturated solution is stored at room temperature in an amber borosilicate bottle, protected from direct sunlight.

7.2 Apparatus

See 4.2, and in addition:

7.2.1 Borosilicate flasks, 100 ml, with glass stoppers, tightly fastened by metal clips (for the determination of total phosphorus using the peroxodisulfate method in an autoclave); polypropene bottles or conical flasks (screw capped) are also suitable.

Before use, clean the bottle or flasks by adding about 50 ml of water and 2 ml of sulfuric acid (8.1.1). Place in an autoclave for 30 min at operating temperature of between 115 °C and 120 °C, cool, and rinse with water, repeat the procedure several times and store covered.

7.3 Sampling and samples

7.3.1 Sampling

See 4.3.1.

7.3.2 Preparation of the test sample

Add 1 ml of sulfuric acid (4.1.2) per 100 ml of the unfiltered test sample. The acidity should be about pH 1; if not, adjust with sodium hydroxide solution (4.1.4) or sulfuric acid (4.1.3).

Store in a cool dark place until analysis.

If total dissolved phosphorus is to be determined, filter the sample as specified in 6.3.2.

7.4 Procedure

7.4.1 Test portion

The oxidation using peroxodisulfate will not be effective in the presence of large quantities of organic matter; in this case, oxidation using a mixture of nitric acid and sulfuric acid is necessary (see Clause 8).

Pipette up to a maximum of 40 ml of the test sample (7.3.2) into a 100 ml conical flask. If necessary, dilute with water to 40 ml $\pm$ 2 ml. Add 4 ml of potassium peroxodisulfate solution (7.1.1) and boil gently for approximately 30 min. Periodically, add sufficient water so that the volume remains between 25 ml and 35 ml. Cool, adjust to between pH 3 to pH 10 with sodium hydroxide solution (4.1.4) or sulfuric acid (4.1.3) and transfer to a 50 ml volumetric flask; dilute with water to about 40 ml.

Alternatively, mineralize for 30 min in an autoclave at between 115 °C and 120 °C.

NOTE 1 Thirty minutes are usually sufficient to mineralize phosphorus compounds; some polyphosphonic acids need up to 90 min for hydrolysis.

NOTE 2 Any arsenate present will cause interferences. Any arsenic originally present will be oxidized to arsenate under the conditions described in this subclause and will therefore also cause interference.

If arsenic is known or suspected to be present in the sample, the interference needs to be eliminated. Treat the solution with sodium thiosulfate solution (4.1.9) immediately after the mineralization step. In the case of seawater mineralized in an autoclave, remove free chlorine by boiling for about 2 min before the arsenate is reduced by thiosulfate.

7.4.2 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 water instead of the test portion.

7.4.3 Calibration

7.4.3.1 Preparation of calibration solutions

Transfer, by means of a volumetric pipette appropriate volumes, for example, 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml, 5,0 ml, 6,0 ml, 7,0 ml, 8,0 ml, 9,0 ml and 10,0 ml of the orthophosphate standard solution (4.1.11) to 100 ml conical flasks, dilute to about 40 ml with water. These solutions represent orthophosphate concentrations ρ_P = 0,04 mg/l to 0,4 mg/l. Proceed as specified in 7.4.1 from “Add 4 ml potassium peroxodisulfate solution (7.1.1) and boil gently for approximately 30 min”.

7.4.3.2 Colour development

Add to each 50 ml flask, while swirling, 1 ml of ascorbic acid (4.1.5) and after 30 s, 2 ml of acid molybdate Solution II (4.1.7). Make up to the mark with water and mix well.

7.4.3.3 Spectrometric measurements

See 4.4.3.3.

7.4.3.4 Plotting the calibration graph

See 4.4.3.4.

7.4.4 Determination

7.4.4.1 Colour development

Prepare the test portion from 7.4.1 and proceed as specified in 7.4.3.2.

If the test sample is turbid and/or coloured, the following procedure is recommended:

Add 3 ml of the turbidity-colour compensation reagent (4.1.8) to the selected volume of a test portion mineralized with peroxodisulfate. Dilute with water to 50 ml and measure the absorbance. Subtract the absorbance of the solution from the value measured according to 4.4.3.3.

7.4.4.2 Spectrometric measurements

See 4.4.3.3.

7.5 Expression of results

7.5.1 Calculation

Calculate the concentration of total phosphorus, ρ_P, expressed in milligrams per litre, using the equation

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

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the slope of the calibration graph (4.4.3.4), expressed in litres per milligram (l/mg);

V_{max} is the volume, of the volumetric flask (50 ml), expressed in millilitres (ml);

V_s is the actual volume of the test portion, expressed in millilitres (ml).

Take into account any dilution steps and as well the dilution caused by the addition of sulfuric acid.

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

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

— $\rho_P < 10$ mg/l to the nearest 0,01 mg/l;

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

7.5.2 Precision

The precision data in Table B.3 were obtained in an interlaboratory trial involving 16 laboratories.

NOTE For interferences see Annex A.

7.6 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 International Standard (ISO 6878:2004);

c) a reference to the method used, and the number of the clause;

d) the results obtained;

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

8 Determination of total phosphorus after nitric acid-sulfuric acid digestion

8.1 Reagents

Use the reagents specified in 4.1.2, 4.1.5, 4.1.7, 4.1.9 and in addition:

8.1.1 Sulfuric acid, $\rho(H_2SO_4) = 1{,}84$ g/ml.

8.1.2 Nitric acid, $\rho(HNO_3) = 1{,}40$ g/ml.

8.1.3 Sodium hydroxide, $c(NaOH) = 8$ mol/l solution.

Dissolve 64 g $\pm$ 1 g of sodium hydroxide pellets in 150 ml $\pm$ 10 ml of water, cool, and dilute with water to 200 ml $\pm$ 10 ml. Store in a polyethylene bottle.

8.2 Apparatus

See 4.2 and in addition:

8.2.1 Kjeldahl flask, 200 ml.

8.3 Sampling and samples

8.3.1 Sampling

See 4.3.1.

8.3.2 Preparation of the test sample

Add 1 ml of sulfuric acid (4.1.2) per 100 ml of the unfiltered test sample. The acidity should be about pH 1; if not, adjust the pH with sodium hydroxide solution (4.1.4) or sulfuric acid (4.1.3). Store in a cool dark place until analysis.

If total dissolved phosphorus is to be determined, the sample is filtered according to 6.3.2.

8.4 Procedure

8.4.1 Test portion

WARNING — It is necessary to carry out this procedure in a well-ventilated fume cupboard.

Pipette up to a maximum of 40 ml of the test sample (8.3.2) into a Kjeldahl flask (8.2.1). Cautiously add 2 ml of sulfuric acid (8.1.1) and swirl to mix. Add anti-bumping granules and heat gently to the appearance of white fumes. After cooling, cautiously add 0,5 ml of nitric acid (8.1.2) dropwise while swirling, and heat until brown fumes cease to be evolved. After cooling continue to treat as necessary with nitric acid dropwise while swirling, until a clear and colourless solution is obtained. Cool and cautiously add 10 ml of water with continuous swirling and heat to the appearance of white fumes. After cooling, cautiously add 20 ml of water with continuous swirling. While cooling, cautiously add sodium hydroxide solution (8.1.3) with continuous swirling to adjust the solution to between pH 3 to pH 10. After cooling, transfer the solution to a 50 ml volumetric flask. Rinse the Kjeldahl flask with a small amount of water and add the washings to the flask.

For arsenic interference, see 4.4.4 and A.2.

8.4.2 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 water instead of the test portion.

8.4.3 Calibration

8.4.3.1 Preparation of calibration solutions

Transfer, by means of a volumetric pipette, appropriate volumes, for example 1,0 ml, 2,0 ml, 3,0 ml, 4,0 ml, 5,0 ml, 6,0 ml, 7,0 ml, 8,0 ml, 9,0 ml and 10,0 ml of the orthophosphate standard solution (4.1.11) to 200 ml Kjeldahl flasks.

These solutions represent orthophosphate concentrations $\rho_P = 0{,}04$ mg/l to 0,4 mg/l. Proceed as specified in 8.4.1 from “Cautiously add 2 ml of sulfuric acid (8.1.1) and swirl to mix.”

8.4.3.2 Colour development

Add to each 50 ml flask, while swirling, 1 ml of ascorbic acid (4.1.5) and after 30 s, 2 ml of acid molybdate Solution II (4.1.7). Make up to the mark with water and mix well.

8.4.3.3 Spectrometric measurements

See 4.4.3.3.

8.4.3.4 Plotting the calibration graph

See 4.4.3.4.

8.4.4 Determination

8.4.4.1 Colour development

Proceed according to 8.4.3.2 using the test portion from 8.4.1.

8.4.4.2 Spectrometric measurements

See 4.4.3.3.

8.5 Expression of results

8.5.1 Calculation

Calculate the concentration of total phosphorus, ρ_P, expressed in milligrams per litre, using the equation

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

where

A is the absorbance of the test portion;

A_0 is the absorbance of the blank test;

f is the slope of the calibration graph (4.4.3.4), expressed in litres per milligram (l/mg);

V_{max} is the volume, of the volumetric flask (50 ml), expressed in millilitres (ml);

V_s is the actual volume of the test portion, expressed in millilitres (ml).

Take into account any dilution steps and as well the dilution caused by the addition of sulfuric acid.

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

— $\rho_P > 0{,}1$ mg/l to the nearest 0,001 mg/l;

— $\rho_P < 10$ mg/l to the nearest 0,01 mg/l;

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

8.5.2 Precision

The precision data in Table B.3 were obtained in an interlaboratory trial involving 16 laboratories.

NOTE For interferences, see Annex A.

8.6 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 International Standard (ISO 6878:2004);

c) a reference to the method used, and the number of the clause;

d) the results obtained;

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

Annex A
(informative)

Interferences

A.1 Silicate

Silicate concentrations up to 5 mg/l Si do not interfere. However, higher concentrations cause an increase in absorbance.

After a reaction time of 30 min the values in Table A.1 were obtained.

Table A.1 — Influence of silicate ions on the analytical result

Silicate concentration, as Si mg/l	Equivalent phosphate concentration, as P mg/l
10	0,005
25	0,015
50	0,025

A.2 Arsenate

Arsenate produces a colour similar to that produced by orthophosphate. This interference can be eliminated by reducing arsenate to arsenite (see 4.4.4.1.1) with sodium thiosulfate (4.1.9).

A.3 Sulfidic sulfur

Sulfidic sulfur concentrations up to 2 mg/l S are tolerable. Higher concentrations can be reduced to an acceptable level by passing nitrogen gas through an acidified sample (acidification as in 6.4.1).

A.4 Fluoride

Fluoride concentrations up to 70 mg/l of fluoride are tolerable. Concentrations higher than 200 mg/l totally inhibit colour development.

A.5 Transition metals

A.5.1 Iron affects the colour intensity, but at a concentration of 10 mg/l Fe the effect is less than 5 %. An increase in colour caused by vanadate is linear and is about 5 % at a concentration of 10 mg/l of vanadium.

A.5.2 Chromium(III) and chromium(VI) in concentrations up to 10 mg/l do not interfere, but at a concentration of about 50 mg/l Cr absorbance increases by about 5 %.

A.5.3 Copper in concentrations up to 10 mg/l does not interfere.

A.6 Seawater

Variations in salinity have a negligible influence on colour intensity.

A.7 Nitrite

If the nitrite concentration exceeds 3,29 mg/l, colour bleaching may occur. A slight excess of sulfamic acid is effective in breaking down nitrite; 100 mg of the acid will deal with a nitrite concentration of 32,9 mg/l.

Annex B
(informative)

Precision data

The precision data in Table B.1 were obtained in an interlaboratory trial organized by Finland involving 16 laboratories using the method given in Clause 4.

Table B.1 — Precision data for Clause 4

Description of samples	Number of samples n	Mean	Standard deviation		
			Repeatability	Reproducibility	
			Absolute	Absolute	Relative
		mg/l	mg/l	mg/l	%
Orthophosphate in presence of polyphosphate	70	0,057 6	0,002 2	0,010 8	18,8
Orthophosphate	69	0,312 7	0,004 81	0,032 4	10,4
Orthophosphate in presence of arsenate and polyphosphate	78	0,192	0,004 01	0,034 8	18.1
Orthophosphate in presence of arsenate	78	0,101 3	0,005 77	0,022 1	21,8

The precision data in Table B.2 were obtained in an interlaboratory trial involving 15 laboratories using the method given in Clause 6.

Table B.2 — Precision data for Clause 6

Description of samples	Number of samples n	Mean	Standard deviation		
			Repeatability	Reproducibility	
			Absolute	Absolute	Relative
		mg/l	mg/l	mg/l	%
Polyphosphate	79	0,179 2	0,006 59	0,044 6	24,8
Polyphosphate in presence of organically bound phosphorus	65	0,174 9	0,007 09	0,025 9	14,8

The precision data presented in Table B.3 were obtained in an interlaboratory trial involving 16 laboratories. Both peroxodisulfate oxidation and “nitric acid/sulfuric acid” digestion procedures were used and no significant differences were observed in the samples analysed.

Table B.3 — Precision data for Clauses 7 and 8

Description of samples	Number of samples	Mean	Standard deviation		
			Repeatability	Reproducibility	
			Absolute	Absolute	Relative
	n	mg/l	mg/l	mg/l	%
Organically bound phosphorus and indigosulfonate	70	0,068 7	0,003 83	0,008 32	12,0
Organically bound phosphorus plus phloroglucine	58	0,438 1	0,012 8	0,036 9	8,4

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