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Water quality — Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA) —

Part 2: Method by continuous flow analysis (CFA)

Qualité de l'eau — Dosage des orthophosphates et du phosphore total par analyse en flux (FIA et CFA) —

Partie 2: Méthode par analyse en flux continu (CFA)

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ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

This document 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 15681-2:2003), which has been technically revised. The main changes compared to the previous edition are as follows:

a) the reagents have been adjusted to decrease the pH to enhance the colour reaction;

b) the figures in Annex A have been revised.

A list of all parts in the ISO 15681 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Methods of determining water quality using flow analysis automated wet chemical procedures are particularly suitable for the processing of many analytes in water in large sample series at a high analysis frequency.

Analysis can be performed by flow injection analysis (FIA)[6][8] or continuous flow analysis (CFA)[9]. Both methods share the feature of an automatic dosage of the sample into a flow system (manifold) where the analyte in the sample reacts with the reagent solutions on its way through the manifold. The sample preparation may be integrated in the manifold. The amount of reaction product is measured in a flow detector (e.g. flow photometer). This document describes the CFA method.

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

Water quality — Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA) —

Part 2: Method by continuous flow analysis (CFA)

WARNING — Persons using this document should be familiar with normal laboratory practice. This document 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.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably qualified staff.

1 Scope

This document specifies continuous flow analysis (CFA) methods for the determination of orthophosphate in the mass concentration range from 0,01 mg/l to 1,00 mg/l P, and total phosphorus in the mass concentration range from 0,10 mg/l to 10,0 mg/l P. The method includes the digestion of organic phosphorus compounds and the hydrolysis of inorganic polyphosphate compounds, performed either manually, as described in ISO 6878 and in References [4], [5] and [7], or with an integrated ultraviolet (UV) digestion and hydrolysis unit.

This document is applicable to various types of water, such as ground, drinking, surface, leachate and waste water. The range of application can be changed by varying the operating conditions.

This method is also applicable to the analysis of seawater, but with changes in sensitivity by adapting the carrier and calibration solutions to the salinity of the samples.

It is also applicable to analysis using 10 mm to 50 mm cuvettes depending on the desired range. For extreme sensitivity, 250 mm and 500 mm long way capillary flow cells (LCFCs) can be used. However, the method is not validated for these two uses. Changes in sensitivity and calibration solutions could be required.

Annex A provides examples of a CFA system. Annex B gives performance data from interlaboratory trials. Annex C gives information of determining orthophosphate-P and total-P by CFA and tin(II) chloride reduction.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 5667-1, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes and sampling techniques*

ISO 5667-3:2018, *Water quality — Sampling — Part 3: Preservation and handling of water samples*

ISO 6878:2004, *Water quality — Determination of phosphorus — Ammonium molybdate spectrometric method*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 1: Statistical evaluation of the linear calibration function*

ISO 8466-2, *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics — Part 2: Calibration strategy for non-linear second-order calibration functions*

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

4 Interferences

4.1 General interferences

Refer to ISO 6878:2004, Annex A for a list of general interferences. In addition, or contrary to the cited standard, the following applies:

a) arsenate causes serious interference: 100 µg/l As, present as arsenate, results in a response comparable to approximately 30 µg/l P;

b) if the silicate concentration in samples is not greater than 60 times the phosphorus concentration, interferences by silicate can be neglected;

c) fluoride interference is significant above 50 mg/l;

d) nitrite interference is significant above 5 mg/l; the interference can be eliminated by acidifying samples after collection;

e) for samples containing high concentrations of oxidizing agents, the amount of added reduction reagent can be insufficient; in this case, remove the oxidizing material prior to digestion;

f) the self-absorption of the sample can be compensated for by measuring, in addition to the sample signal (9.6), the signal of the sample without the admixture of the reagents; in this case, the difference of the two responses is used for the evaluation (Clause 10).

4.2 Interferences in the determination of total-P

Samples containing solids or suspended particles can show low values when analysed by the UV method, if the particles are not completely transported into the UV unit. The error can be minimized by stirring the sample immediately before or during sampling, in order to ensure that a representative sample is delivered into the analyser, and by reducing the particle size.

The interferences from silicate, nitrite, fluoride and iron described for the orthophosphate determination are generally not observed in the UV method, due to the pre-digestion and the higher analytical range.

The efficiency of the UV digestion can be affected for water samples with chemical oxygen demand (COD) values of more than 10 times the highest concentrations of the calibration solutions (6.22). In this case, the sample should be diluted.

5 Principle

5.1 Determination of orthophosphate

The sample is mixed with a surfactant solution, followed by an acidic solution containing molybdate and antimony ions. The resulting phospho-antimony-molybdate complex is reduced by ascorbic acid to molybdenum blue[4][7]. The pH of the reaction mixture shall be between pH 0,6 and pH 0,9[3].

5.2 Total phosphorus with manual digestion

Phosphorus compounds in the sample are oxidized manually with a potassium peroxodisulfate solution, in accordance with ISO 6878 or with an equivalent procedure. The resulting orthophosphate is determined by the molybdenum blue reaction using the colour reaction described in 5.1. The samples can be neutralized manually in accordance with ISO 6878 or by taking into account the amount of acid used in this procedure when calculating the acid to be used in the molybdenum reagent.

5.3 Total phosphorus with integral UV digestion and hydrolysis

The sample is mixed with potassium peroxodisulfate and passed through a UV digester, followed by acid digestion to hydrolyse polyphosphates. The resulting orthophosphate is measured using the colour reaction described in 5.1. The pH of the reaction mixture shall be between pH 0,6 and pH 0,9[3]. The pH of the reaction mixture is critical to avoid interferences from silicate.

6 Reagents

Use analytical grade chemicals unless otherwise specified. Molybdate and antimony waste solutions should be disposed properly.

6.1 Water, conforming to grade 1 of ISO 3696.

The phosphate blank value shall be checked (see 9.3).

6.2 Sulfuric acid, H_2SO_4.

6.2.1 Sulfuric acid I, ρ = 1,84 g/ml; 95 % to 98 %.

6.2.2 Sulfuric acid II, $c(H_2SO_4)$ = 2,45 mol/l.

To approximately 800 ml of water (6.1), carefully add 136 ml of sulfuric acid I (6.2.1) while stirring. Cool and dilute to 1 000 ml with water (6.1).

6.2.3 Sulfuric acid III, $c(H_2SO_4)$ = 2,45 mol/l.

To 1 000 ml of sulfuric acid II (6.2.2), add 1 g of sodium dodecyl sulfate (6.7) and mix.

6.3 Sodium hydroxide, NaOH.

6.4 Ammonium heptamolybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$.

6.5 Antimony potassium tartrate trihydrate, $K_2(SbO)_2C_8H_4O_{10}{\cdot}3H_2O$.

6.6 Ascorbic acid, $C_6H_8O_6$.

6.7 Sodium dodecyl sulfate, $NaC_{12}H_{25}SO_4$.

6.8 Potassium peroxodisulfate, $K_2S_2O_8$.

6.9 Potassium dihydrogen phosphate, KH_2PO_4, dried at 105 °C ± 5 °C to constant mass.

6.10 Potassium pyrophosphate, $K_4P_2O_7$.

6.11 Organophosphorus compounds, to check the UV digestion.

6.11.1 Pyridoxal-5-phosphate monohydrate, $C_8H_{10}NO_6P{\cdot}H_2O$.

6.11.2 Disodium phenylphosphate, $C_6H_5Na_2PO_4$.

6.12 Surfactant solutions.

6.12.1 Surfactant solution I, see (A) or (B) in Figure A.1.

Dissolve 1 g of sodium dodecyl sulfate (6.7) in about 800 ml of water (6.1) and dilute to 1 000 ml with water (6.1).

The solution is stable for six months if stored at room temperature.

6.12.2 Surfactant solution II, see (A) or (B) in Figure A.1.

Dissolve 10 g of sodium dodecyl sulfate (6.7) in about 800 ml of water (6.1) and dilute to 1 000 ml with water (6.1).

The solution is stable for six months if stored at room temperature.

6.13 Molybdate solution.

Dissolve 40 g of ammonium heptamolybdate tetrahydrate (6.4) in about 800 ml of water (6.1) and dilute to 1 000 ml with water (6.1).

Do not use a metal spatula when weighing the ammonium heptamolybdate tetrahydrate (6.4). The solution is stable for three months if stored at room temperature. Avoid any contact between metal and the ammonium heptamolybdate.

6.14 Antimony potassium tartrate solution.

Dissolve 2,5 g of antimony potassium tartrate trihydrate (6.5) in about 800 ml of water (6.1) and dilute to 1 000 ml with water (6.1).

The solution is stable for three months if stored at room temperature.

6.15 Antimony tartrate molybdate reagents.

6.15.1 Antimony tartrate molybdate reagent I, for determination of orthophosphate and total P after manual digestion (R1 in Figure A.1).

Mix 500 ml of sulfuric acid II (6.2.2), 150 ml of molybdate solution (6.13) and 50 ml of antimony potassium tartrate solution (6.14).

The solution is stable for two weeks if stored at room temperature.

6.15.2 Antimony tartrate molybdate reagent II, for total phosphorus determination after integrated UV digestion (R5 in Figure A.2).

Add to 440 ml sulfuric acid solution II (6.2.2), 150 ml molybdate solution (6.13) and 90 ml of antimony potassium tartrate trihydrate solution (6.14) and dilute with water (6.1) to 1 000 ml.

The solution is stable for two weeks if stored in a polyethylene bottle at room temperature.

6.15.3 Antimony tartrate molybdate reagent III, for total phosphorus determination after integrated UV digestion (R4 in Figure A.3).

Add to 220 ml sulfuric acid solution II (6.2.2), 150 ml molybdate solution (6.13) and 90 ml of antimony potassium tartrate trihydrate solution (6.14) and dilute with water (6.1) to 1 000 ml.

The solution is stable for two weeks if stored in a polyethylene bottle at room temperature.

6.16 Ascorbic acid solution I, (R2 in Figure A.1).

Dissolve 1 g of ascorbic acid (6.6) in about 80 ml of water (6.1) and bring to a volume of 100 ml with water (6.1). Store in the dark. Prepare the solution daily before use.

6.17 Ascorbic acid solution II, (R6 in Figure A.2 and R5 in Figure A.3).

Dissolve 1,1 g of ascorbic acid (6.6) in about 80 ml of water (6.1), add 0,1 g of sodium dodecyl sulfate (6.7) and dilute with water (6.1) to 100 ml. Store in the dark. Prepare the solution daily before use.

6.18 Digestion reagent, for the determination of total phosphorus after integrated UV digestion, (R1 in Figures A.2 and A.3).

Dissolve 5 g of potassium peroxodisulfate (6.8) in about 900 ml of water (6.1). Adjust the pH to 1,1 to 1,2 with sulfuric acid II (6.2.2), cool and dilute with water (6.1) to 1 000 ml.

The solution is stable for two weeks if stored at room temperature.

6.19 Orthophosphate stock solution I, ρ = 50,0 mg/l orthophosphate-P.

Dissolve 220 mg ± 1 mg of potassium dihydrogenphosphate (6.9) in water (6.1) and dilute with water (6.1) to 1 000 ml. Store in a tightly closed glass bottle.

The solution is stable for two months if stored at 3 °C ± 2 °C.

6.20 Orthophosphate stock solution II, ρ = 10,0 mg/l P.

Dilute 20 ml of solution (6.19) to 100 ml with water (6.1). Prepare the solution daily before use.

6.21 Orthophosphate stock solution III, ρ = 1,00 mg/l P.

Dilute 2 ml of solution (6.19) to 100 ml with water (6.1). Prepare the solution daily before use.

6.22 Calibration solutions.

Prepare at least five calibration solutions by diluting solutions 6.19 to 6.21 according to the range required.

Ranges:

For orthophosphate-P:	range II:	0,01 mg/l to 0,10 mg/l P[a]
	range I:	0,10 mg/l to 1,00 mg/l P
For total-P:	range II:	0,10 mg/l to 1,00 mg/l P
	range I:	1,00 mg/l to 10,0 mg/l P

[a] This range is used for very clean water samples like surface- or drinking waters.

Tables 1 to 3 give examples for the preparation of 10 calibration solutions for the above-mentioned ranges.

Table 1 — Example for the preparation of 10 calibration solutions for the orthophosphate range II (0,01 mg/l to 0,10 mg/l P)

Millilitres of orthophosphate stock solution III (6.21) diluted to 100 ml	1	2	3	4	5	6	7	8	9	10
Concentration of calibration solutions, mg/l P	0,01	0,02	0,03	0,04	0,05	0,06	0,07	0,08	0,09	0,10

Table 2 — Example for the preparation of 10 calibration solutions for the orthophosphate range I and total phosphorus range II (0,10 mg/l to 1,00 mg/l P)

Millilitres of orthophosphate stock solution II (6.20) diluted to 100 ml	1	2	3	4	5	6	7	8	9	10
Concentration of calibration solutions, mg/l P	0,10	0,20	0,30	0,40	0,50	0,60	0,70	0,80	0,90	1,00

Table 3 — Example for the preparation of 10 calibration solutions for the total phosphorus range I (1,00 mg/l to 10,0 mg/l P)

Millilitres of orthophosphate stock solution I (6.19) diluted to 100 ml	2	4	6	8	10	12	14	16	18	20
Concentration of calibration solutions, mg/l P	1,00	2,00	3,00	4,00	5,00	6,00	7,00	8,00	9,00	10,0

Prepare the calibration solutions immediately before use.

6.23 Standards for verifying hydrolysis and digestion efficiency.

6.23.1 Potassium pyrophosphate stock solution, ρ = 100 mg/l P.

Dissolve 533 mg ± 3 mg of potassium pyrophosphate (6.10) in about 800 ml of water (6.1) and dilute with water (6.1) to 1 000 ml. Store in a sealed glass container at 3 °C ± 2 °C.

The solution is stable for six months.

6.23.2 Potassium pyrophosphate solution I, to check hydrolysis efficiency, ρ = 0,50 mg/l P, for the total-P working range II (0,10 mg/l to 1,00 mg/l P).

Dilute 0,5 ml of solution 6.23.1 and 100 µl of sulfuric acid (II) (6.2.2) to 100 ml with water (6.1).

The solution is stable for one month if stored at 3 °C ± 2 °C.

6.23.3 Potassium pyrophosphate solution II, to check hydrolysis efficiency, ρ = 5,00 mg/l P for the total-P working range I (1,00 mg/l to 10,0 mg/l P).

Dilute 5 ml of solution 6.23.1 and 100 µl of sulfuric acid (II) (6.2.2) to 100 ml with water (6.1).

The solution is stable for one month if stored at 3 °C ± 2 °C.

6.23.4 Organophosphorus stock solution, ρ = 100 mg/l P.

Dissolve 856 mg ± 4 mg of pyridoxal-5-phosphate monohydrate (6.11.1) in about 800 ml of water (6.1) and dilute with water (6.1) to 1 000 ml.

The solution is stable for six months in a closed glass container, if stored at 3 °C ± 2 °C.

Alternatively:

Dissolve 704 mg ± 3 mg of disodium phenylphosphate (6.11.2) in about 800 ml of water (6.1), acidify with sulfuric acid II (6.2.2) to approximately pH 2 and dilute to 1 000 ml with water (6.1).

The solution is stable for three months if stored in the dark at 3 °C ± 2 °C.

6.23.5 Organophosphorus solution I, to check UV digestion efficiency, ρ = 0,5 mg/l P, for the total-P working range II (0,10 mg/l to 1,00 mg/l P).

Dilute 0,5 ml of solution 6.23.4 and 100 µl of sulfuric acid II (6.2.2) to 100 ml with water (6.1).

The solution is stable for one month if stored at 3 °C ± 2 °C.

6.23.6 Organophosphorus solution II, to check UV digestion efficiency, ρ = 5,00 mg/l P, for the total-P working range I (1,00 mg/l to 10,0 mg/l P).

Dilute 5 ml of solution 6.23.4 and 100 µl of sulfuric acid II (6.2.2) to 100 ml with water (6.1).

The solution is stable for one month if stored at 3 °C ± 2 °C.

6.24 Cleaning solution.

Dissolve 65 g of sodium hydroxide, NaOH (6.3), and 6 g of tetrasodium ethylenedinitrilotetraacetic acid, Na_4-EDTA, $Na_4C_{10}H_{12}O_8N_2$, in 1 000 ml of water (6.1).

This solution is stable for one month if stored at 3 °C ± 2 °C.

6.25 Sodium hydroxide solution, ρ = 105 g/l.

Dissolve 105 g ± 1 g sodium hydroxide (6.3) in 800 ml of water (6.1). Cool and dilute to 1 000 ml with water (6.1).

7 Apparatus

7.1 Continuous-flow analysis (CFA)

The system generally consists of the following components (see Figures A.1, A.2 and A.3).

7.1.1 Sampler or other device, for reproducible sample introduction.

For the determination of total-P, consider using a device to mix the sample during sampling.

In order to ensure that a representative sample is delivered into the analyser, the sample should be stirred (ISO 5667-3:2018, 3.2). This may be achieved using a mechanical top-stirring device or bubbling air through the sample on the auto-sampler.

7.1.2 Reagent containers.

7.1.3 Peristaltic pump, with suitable pump tubes inert to the reagents used.

7.1.4 Manifold, with reproducible gas bubble introduction, sample and reagent introduction and components of chemically inert materials.

7.1.5 If necessary, **a dialyser** with a cellulose membrane to dilute the sample and eliminate interfering substances.

7.1.6 If necessary, **flow-through thermostats**, recommended temperature setting are 37 °C to 40 °C or 95 °C respectively (±1 °C).

7.1.7 Photometric flow-through detector, wavelength 880 nm ± 10 nm.

For the CFA method, the photometer may be equipped with 10 mm to 50 mm cuvettes depending on the desired range. For extreme sensitivity, also 250 mm and 500 mm LCFCs may be used.

7.1.8 Data display unit.

In general peak height signals are evaluated by use of PC with software for data acquisition and evaluation.

NOTE Figures A.1, A.2 and A.3 show CFA systems with tubing of 2 mm internal diameter. Other tubing diameters (e.g. 1 mm) can be used as long as the flowrates are in the same proportion and the recovery rates in 9.5 are achieved.

7.2 Additional apparatus

7.2.1 Graduated flasks, nominal capacity 100 ml, 200 ml and 1 000 ml.

7.2.2 Pipettes, nominal capacity 1 ml, 2 ml, 5 ml, 10 ml, 20 ml and 25 ml.

7.2.3 Beakers, nominal capacity 25 ml, 100 ml and 1 000 ml.

7.2.4 Disposable membrane filter assembly, with membrane filters of pore size 0,45 µm.

For samples with a large particulate load, the disposable filters may incorporate a glass fibre pre-filter.

7.2.5 pH meter.

7.3 Additional apparatus for the determination of total phosphorus after integral digestion

7.3.1 Homogenizer, dispersing instrument [e.g. Ultra-Turrax, Polytron[1)] if needed (see Clause 8)].

7.3.2 Apparatus integrated in the CFA system (7.1).

7.3.2.1 UV digestion unit, e.g. with ozone-producing lamp and reaction coil of quartz glass (see Figures A.2 and A.3).

NOTE In-line digesters with a power of 25 W are commercially available (see, for example, Figures A.2 and A.3).

7.3.2.2 Thermostat, for temperature control of the hydrolysis at 95 °C ± 1 °C.

1) Ultra-Turrax and Polytron are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

8 Sampling and sample preparation

Carry out sampling and sample preservation in accordance with ISO 5667-1 and ISO 5667-3. Prior to use, rinse all containers which will come into contact with the sample with water (6.1).

Field sampling equipment decontamination procedures shall be sensitive to potential for cross contamination by phosphate surfactants.

For samples with low concentrations (e.g. ≤ 0,1 mg/l orthophosphate-P), use glass containers. For samples with higher concentrations (e.g. > 0,1 mg/l orthophosphate-P or total-P), plastic bottles (e.g. made from high density polyethylene) are also acceptable.

NOTE Glass containers are preferred at the low concentration. These are easier to clean and phosphate free.

If filtering is required (in the case of particles of diameter > 0,1 mm), samples for the determination of orthophosphate should be filtered through a membrane filter (0,45 µm) immediately after sampling and stored at 3 °C ± 2 °C. The filtration reduces biological reactions, and reduces interferences by sulfide and clogging of the analyser tubing. The maximum preservation time is 24 h.

Samples for the determination of total-P shall be preserved either by freezing (–18 °C) or by adding sulfuric acid to a value of pH ≤ 2 immediately after sampling. The maximum preservation time is one month. Total phosphorus samples containing particles shall be homogenized (7.3.1).

Samples with particles should be homogenized for at least 15 s at about 12 500 rpm (7.3.1).

9 Procedure

9.1 Preparation for analysis

Set up the flow analyser for the desired procedure orthophosphate-P or total-P, see Figures A.1, A.2 and A.3.

Pump the reagents for up to 10 min (for total-P: up to 30 min) and adjust the baseline to zero.

The analyser is ready as soon as the baseline is stable. Proceed according to steps 9.2 to 9.5.

9.2 Instrument performance check

In the analytical system, prepared according to 9.1, a calibration solution (6.22) with a phosphate-P concentration of 0,05 mg/l shall exhibit an absorbance per centimetre in working range II (0,01 mg/l to 0,10 mg/l) of at least 0,010 cm^{-1}. Otherwise, the flow system is not suitable and it shall be replaced by a system which fulfils this requirement.

If the photometric detector (7.1.7) does not allow any absorbance readings, the absorbance may then be determined by comparing with an external absorbance measuring photometer. In this case, a sufficient quantity of the reaction mixture (containing the sample and the appropriate reagent solutions, see Clause 6) should be prepared manually and measured in the external photometer.

A calibration solution (6.22) with a phosphate-P concentration of 0,01 mg/l shall exhibit a signal-to-noise ratio of at least 3:1.

9.3 Reagent blank check

Wait for a stable baseline.

Pump water (6.1) through all tubes. Record the change in absorbance.

If the absorbance per centimetre (9.2) is decreased by more than 0,01 cm^{-1}, the reagents or the water (6.1) are possibly contaminated, and suitable measures to eliminate the interference shall be undertaken before starting the analysis.

Pump all the reagent solutions again (9.1).

9.4 Calibration

Select the desired range and the appropriate calibration solutions (6.22), at least five, equally distributed over the working range. Carry out a separate calibration for each working range.

Before starting the analysis, set the baseline as recommended by the instrument manufacturer, or as appropriate.

Obtain the measured values corresponding to the calibration solutions applied.

Calibrate by sequentially applying the calibration solutions and reagent blanks.

Determine the calibration curve in accordance with ISO 8466-1 or ISO 8466-2.

The analysis conditions for standards and samples are identical (9.6). The output signal is proportional to the phosphate-P concentration, or the total-P concentration, respectively. For linear calibration, use the following Formula (1):

$$y = b \cdot \rho + a \tag{1}$$

where

y is the measured value, in system-related units;

b is the calibration-curve slope, in system-related units × litres per milligram, l/mg;

ρ is the mass concentration of orthophosphate-P or total P, in milligrams per litre, mg/l;

a is the calibration-curve intercept, in system-related units.

9.5 Check of UV digestion and hydrolysis for total P determination (Figures A.2 and A.3)

Establish a stable baseline.

Potassium pyrophosphate solution I or II (6.23.2 and 6.23.3) and organophosphorus solution I or II (6.23.5 and 6.23.6) at a concentration of 50 % of the selected working ranges I or II shall show a recovery rate of at least 90 %.

The recovery rate depends on the manufacturer's equipment. If these criteria are not met, replace it by a system which fulfils these requirements.

9.6 Measurement

Analyse samples, prepared according to Clause 8, in the same way as the calibration solutions (6.22).

If the sample concentration is higher than the selected working range, analyse in a different range or dilute prior to analysis.

After each set of sample measurements, at least after every 20 measurements, check the system calibration using calibration solutions in the lower and the upper third of the working range (9.4). If necessary recalibrate the system.

9.7 Closing down the system

To remove any precipitates, close down the flow system as follows.

At the end of a run, rinse the system for about 5 min with the cleaning solution (6.24), and then for about 25 min with water (6.1).

10 Calculation of results

Calculate the mass concentration of the samples using Formula (2):

$$\rho = (y - a)/b \quad (2)$$

where the symbols are as defined in 9.4.

Calculate sample concentrations according to the calibration range they fall into. Do not extrapolate a calibration curve.

11 Expression of results

Report results to not more than two significant figures.

EXAMPLES

Orthophosphate-P:	$2{,}7 \times 10^{-2}$ mg/l
Orthophosphate-P:	0,42 mg/l
Total-P:	0,69 mg/l
Total-P:	2,9 mg/l

12 Test report

The test report shall contain at least the following information:

a) the test method used, together with a reference to this document, i.e. ISO 15681-2:2018;

b) identity of the sample;

c) expression of the results according to Clause 11;

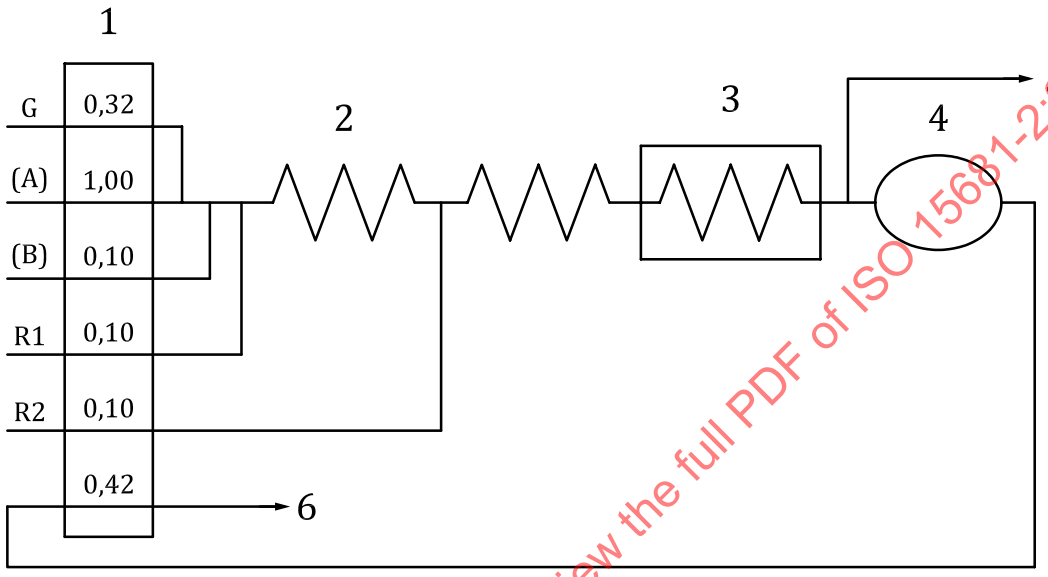
d) any deviation from this method;

e) report of all circumstances that can have affected the results.

Annex A
(informative)

Examples of a CFA system

Figures A.1, A.2 and A.3 give examples of a CFA system (7.1).



Key

1 pump, flowrates in ml/min
2 reaction coil, *l* = 30 cm, ID = 2 mm
3 thermostat coil 37 °C to 40 °C (precision: ± 1 °C), *l* = 120 cm, ID = 2 mm
4 detector, wavelength = 880 nm
5 waste
6 debubbled waste
G air: flowrate 0,32 ml/min
R1 antimony tartrate molybdate reagent I (6.15.1): flowrate 0,10 ml/min
R2 ascorbic acid solution I (6.16): flowrate 0,10 ml/min

For orthophosphate-P:

Working range II (0,01 mg/l to 0,10 mg/l P):

(A) sample: flowrate 1,00 ml/min

(B) surfactant solution II (6.12.2): flowrate 0,10 ml/min

Working range I (0,10 mg/l to 1,00 mg/l P):

(A) surfactant solution I (6.12.1): flowrate 1,00 ml/min

(B) sample: flowrate 0,10 ml/min

For total-P (obtained by manual digestion):

Working range II (0,10 mg/l to 1,00 mg/l P):

(A) sample: flowrate 1,00 ml/min

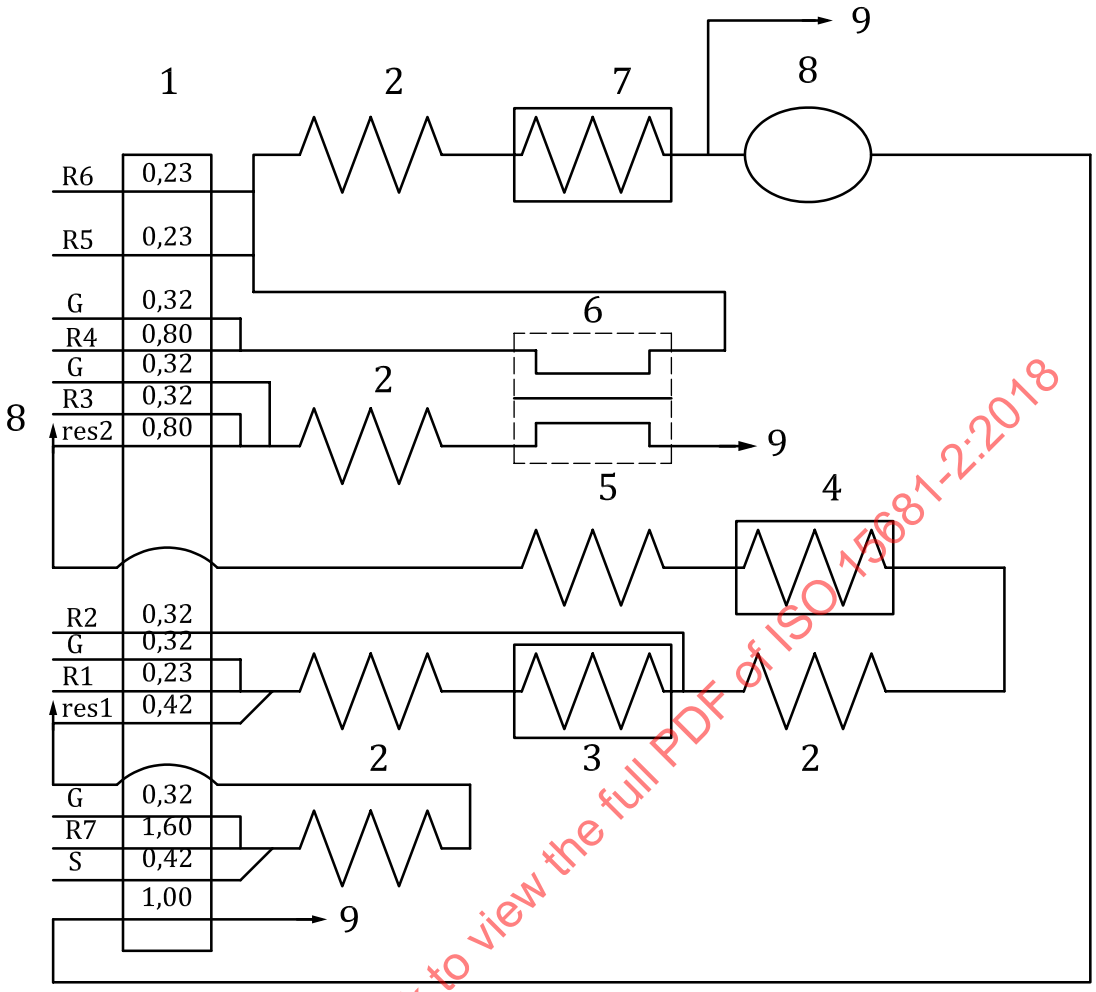
(B) surfactant solution II (6.12.2): flowrate 0,10 ml/min

Working range I (1,00 mg/l to 10,0 mg/l P):

(A) surfactant solution I (6.12.1): flowrate 1,00 ml/min

(B) sample, diluted 1:10 (offline or online): flowrate 0,10 ml/min

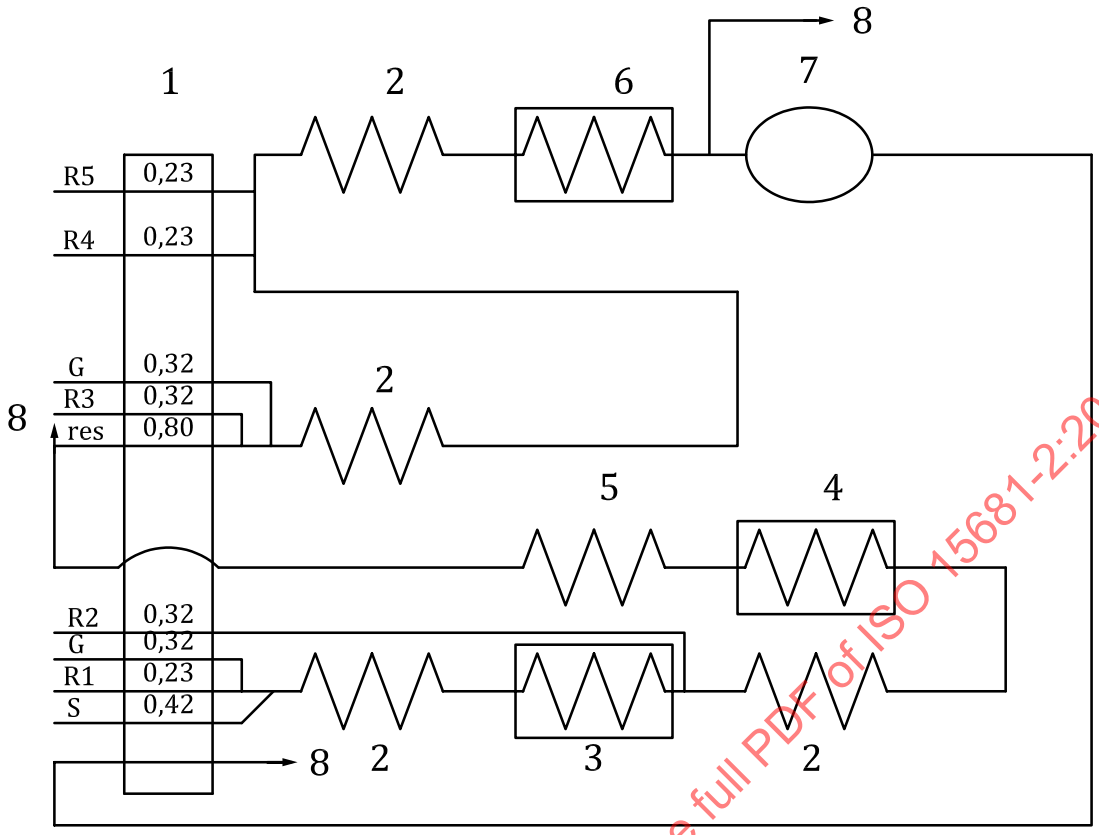
Figure A.1 — Example of a CFA system (7.1) for determination of orthophosphate-P and total-P (obtained by manual digestion) in all concentration ranges



Key

G	air: flowrate 0,32 ml/min	1	pump (flowrates in ml/min)
S	sample: flowrate 0,42 ml/min	2	reaction coil, *l* = 35 cm, ID = 1,5 mm
res1	resample: flowrate 0,42 ml/min		
res2	resample: flowrate 0,80 ml/min	3	UV digester; e.g. with ozone-producing lamp and reaction coil of quartz glass, power of the UV lamp = 25 W, reaction path: *l* = 500 cm, ID 2 mm
R1	digestion reagent (6.18): flowrate 0,23 ml/min	4	thermostat coil, *l* = 500 cm, temp = 95 °C, ID 2 mm
R2	sulfuric acid III (6.2.3): flowrate 0,32 ml/min	5	reaction coil, *l* = 134 cm, ID = 1,5 mm
R3	sodium hydroxide solution (6.25): flowrate 0,32 ml/min	6	dialyzer; path length 700 mm
R4	surfactant solution I (6.12.1): flowrate 0,80 ml/min	7	thermostat coil, *l* = 140 cm, temp = 40 °C, ID 2 mm
R5	antimony tartrate molybdate reagent II (6.15.2): flowrate 0,23 ml/min	8	detector, wavelength = 880 nm
R6	ascorbic acid solution II (6.17): flowrate 0,23 ml/min	9	waste
R7	water (6.1): flowrate 1,60 ml/min		

Figure A.2 — Example of a CFA system (7.1) for determination of total-P with integrated UV digestion in working range I (1,00 mg/l to 10,0 mg/l P)



Key

G	air: flowrate 0,32 ml/min	1	pump (flowrates in ml/min)
S	sample: flowrate 0,42 ml/min	2	reaction coil, *l* = 35 cm, ID = 1,5 mm
res	resample: flowrate 0,80 ml/min	3	UV digester; e.g. with ozone-producing lamp and reaction coil of quartz glass, power of the UV lamp = 25 W, reaction path: *l* = 500 cm, ID = 2 mm
R1	digestion reagent (6.18): flowrate 0,23 ml/min	4	thermostat coil, *l* = 500 cm, temp = 95 °C, ID = 2 mm
R2	sulfuric acid III (6.2.3): flowrate 0,32 ml/min	5	reaction coil, *l* = 134 cm, ID = 1,5 mm
R3	sodium hydroxide solution (6.25): flowrate 0,32 ml/min	6	thermostat coil, *l* = 140 cm, temp = 40 °C, ID = 2 mm
R4	antimony tartrate molybdate reagent III (6.15.3): flowrate 0,23 ml/min	7	detector, wavelength = 880 nm
R5	ascorbic acid solution II (6.17): flowrate 0,23 ml/min	8	waste

Figure A.3 — Example of a CFA system (7.1) for determination of total-P with integrated UV digestion in working range II (0,10 mg/l to 1,00 mg/l P)