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

Part 1: Method by flow injection analysis (FIA)

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

Partie 1: Méthode par analyse avec injection en flux (FIA)

Reference number
ISO 15681-1:2003(E)

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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.

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 15681-1 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

ISO 15681 consists of the following parts, under the general title *Water quality — Determination of orthophosphate and total phosphorus contents by flow analysis (FIA and CFA)*:

— *Part 1: Method by flow injection analysis (FIA)*

— *Part 2: Method by continuous flow analysis (CFA)*

Introduction

Methods of determining water quality using flow analysis automated wet chemical procedures, and 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) [1], [2] or continuous flow analysis (CFA) [3]. 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 part of ISO 15681 describes the FIA 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 1: Method by flow injection analysis (FIA)

WARNING — Persons using this part of ISO 15681 should be familiar with normal laboratory practice. This part of ISO 15681 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. Molybdate and antimony waste solutions should be disposed of properly. It is absolutely essential that tests conducted according to this part of ISO 15681 be carried out by suitably qualified staff.

1 Scope

This part of ISO 15681 specifies flow injection analysis (FIA) methods for the determination of orthophosphate in the mass concentration range from 0,01 mg/l to 1,0 mg/l (P), and total phosphorus by manual digestion in accordance with ISO 6878 [5], [6] for the mass concentration range from 0,1 mg/l to 10 mg/l (P). The range of application can be changed by varying the operating conditions.

This part of ISO 15681 is applicable to various types of water (such as ground, drinking, surface, leachate and waste waters).

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

2 Normative references

The following reference documents are indispensable for the application 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 on sampling programmes*

ISO 5667-2, *Water quality — Sampling — Part 2: Guidance on sampling techniques*

ISO 5667-3, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of water samples*

ISO 6878:— [1)], *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*

1) To be published.

3 Interferences

3.1 General interferences

ISO 6878:—, Annex B gives a list of general interferences. In addition, or contrary to the cited standard, the following guidelines apply.

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 reducing reagent can be insufficient. In this case it is advisable to remove the oxidizing material prior to digestion.

f) The self-absorption of the sample can be compensated by measuring, in addition to the sample signal (8.6), 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 (see Clause 9).

3.2 Interferences in the determination of total-P

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

The efficiency of the digestion can be affected for water samples with a chemical oxygen demand (COD) value of more than 10 times the highest concentrations of the calibration solutions (5.16). In this case the sample should be diluted.

4 Principle

4.1 Determination of orthophosphate

The sample is injected into a carrier stream, which is merged with an acidic ammonium molybdate solution.

The resulting molybdophosphoric acid is reduced by tin(II) chloride to molybdenum blue [4], [5].

4.2 Total phosphorus with manual digestion

Phosphorus compounds in the sample are oxidized manually with a potassium peroxodisulfate solution, in accordance with ISO 6878. The resulting orthophosphate is determined by the molybdenum blue reaction as in 4.1 [5], [6].

5 Reagents

Use analytical grade chemicals unless otherwise specified. The phosphate blank value shall be checked (8.3).

Degas carefully all carrier and reagent solutions for the FIA determinations before use, e.g. by vacuum filtration or purging with helium (for at least 10 min).

5.1 Water, complying to grade 1 of ISO 3696.

5.2 Sulfuric acid, H_2SO_4.

5.2.1 Sulfuric acid (I), ρ = 1,84 g/ml; 98 % (mass fraction).

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

To approximately 800 ml of water (5.1) carefully add 136 ml of sulfuric acid (I) (5.2.1) while stirring. Cool and dilute to 1 000 ml with water (5.1).

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

5.4 Hydrazine sulfate, $N_2H_6SO_4$.

5.5 Tin(II) chloride dihydrate, $SnCl_2 \cdot 2\ H_2O$.

5.6 Potassium peroxodisulfate, $K_2S_2O_8$.

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

5.8 Potassium pyrophosphate, $K_4P_2O_7$.

5.9 Organophosphorus compounds to check the digestion.

5.9.1 Pyridoxal-5-phosphate monohydrate, $C_8H_{10}NO_6P \cdot H_2O$ *or alternatively*:

5.9.2 Disodium phenylphosphate, $C_6H_5Na_2PO_4$.

5.10 Molybdate solution (R1 in Figure A.1).

Dissolve 35 ml of sulfuric acid (I) (5.2.1) and 10 g of ammonium heptamolybdate tetrahydrate (5.3) in about 800 ml water (5.1), cool and dilute to 1 000 ml.

The solution is stable for 3 months if stored at room temperature.

5.11 Tin (II) chloride reagent (R2 in Figure A.1).

Dissolve 28 ml of sulfuric acid (I) (5.2.1), 200 mg of tin(II) chloride (5.5) and 2 g of hydrazine sulfate (5.4) in about 800 ml water (5.1), cool and dilute to 1 000 ml. Store at 4 °C ± 2 °C.

The solution is stable for 1 week if stored at 4 °C ± 2 °C.

NOTE Instead of hydrazine sulfate (5.4), *N,N*-diethylhydroxylamine, DEHA (Annex D) may be used. This change was not part of the validation in the interlaboratory trial cited in Annex B.

5.12 Carrier solutions

5.12.1 Carrier solution I, for orthophosphate determination (C1 in Figure A.1).

The carrier solution I is water (5.1).

5.12.2 Carrier solution II, for total phosphorus (P) determination after manual digestion (C1 in Figure A.1).

Add 5 ml of sulfuric acid (I) (5.2.1) to 1 000 ml of water (5.1) and mix.

5.13 Orthophosphate stock solution I, ρ = 50,0 mg/l P.

Dissolve 220 mg ± 1 mg of potassium dihydrogenphosphate (5.7) in water (5.1) and dilute to 1 000 ml. Store in a tightly closed glass bottle.

The solution is stable for 2 months if stored at 4 °C ± 2 °C.

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

Dilute 20 ml of orthophosphate stock solution I (5.13) to 100 ml with water (5.1).

Prepare freshly each day of use.

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

Dilute 2 ml of orthophosphate stock solution I (5.13) to 100 ml with water (5.1).

Prepare freshly each day of use.

5.16 Calibration solutions

Prepare at least 5 calibration solutions, evenly distributed over the working range, by diluting solutions 5.13 to 5.15 according to the range required.

Ranges:

For orthophosphate:	Range II:	0,01 mg/l to 0,10 mg/l P
	Range I:	0,10 mg/l to 1,00 mg/l P
For total phosphorus:	Range II:	0,10 mg/l to 1,00 mg/l P
	Range I:	1,00 mg/l to 10,0 mg/l P

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 (5.15) 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 (0,1 mg/l to 1,0 mg/l P)

Millilitres of orthophosphate stock solution II (5.14) 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 mg/l to 10 mg/l P)

Millilitres of orthophosphate stock solution I (5.13) 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.

5.17 Standards for verifying hydrolysis and digestion efficiency.

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

Dissolve 533 mg ± 3 mg of potassium pyrophosphate (5.8) in about 800 ml of water (5.1) and dilute to 1 000 ml. Store in a sealed glass container at 4 °C ± 2 °C.

The solution is stable for 6 months.

5.17.2 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 potassium pyrophosphate stock solution (5.17.1) and 100 µl of sulfuric acid (II) (5.2.2) to 100 ml with water (5.1).

The solution is stable for 1 month if stored at 4 °C ± 2 °C.

5.17.3 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 potassium pyrophosphate stock solution (5.17.1) and 100 µl of sulfuric acid (II) (5.2.2) to 100 ml with water (5.1).

The solution is stable for 1 month if stored at 4 °C ± 2 °C.

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

Dissolve 856 mg ± 4 mg of pyridoxal-5-phosphate monohydrate (5.9.1) in about 800 ml of water (5.1) and dilute to 1 000 ml.

The solution is stable for 6 months if stored in a tightly closed glass container at 4 °C ± 2 °C.

Alternatively:

Dissolve 704 mg ± 3 mg of disodium phenylphosphate (5.9.2) in about 800 ml of water (5.1), acidify with sulfuric acid II (5.2.2) to pH ≈ 2 and dilute to 1 000 ml with water (5.1).

The solution is stable for 3 months if stored in the dark at 4 °C ± 2 °C.

5.17.5 Organophosphorus solution I, to check the digestion 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 organophosphorus stock solution (5.17.4) and 100 µl of sulfuric acid (II) (5.2.2) to 100 ml with water (5.1).

The solution is stable for 1 month at 4 °C ± 2 °C.

5.17.6 Organophosphorus solution II, to check the 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 organophosphorus stock solution (5.17.4) and 100 µl of sulfuric acid (II) (5.2.2) to 100 ml with water (5.1).

The solution is stable for 1 month if stored at 4 °C ± 2 °C.

5.18 Rinsing solution.

Dissolve 65 g of sodium hydroxide, NaOH, and 6 g of tetrasodium ethylenediaminetetraacetic acid, (Na_4-EDTA, $C_{10}H_{12}O_8N_2Na_4$) in 1 000 ml of water (5.1).

This solution is stable for 1 month if stored at 4 °C ± 2 °C .

6 Apparatus

6.1 Flow injection analysis (FIA)

The system generally consists of the following components (see Figure A.1).

6.1.1 Reagent containers.

6.1.2 Low-pulsation pump.

6.1.3 Suitable pump tubes, if necessary.

6.1.4 Injector with suitable injection volumes, e.g. 40 µl to 640 µl.

6.1.5 Manifold with transmission tubing 0,5 mm to 0,8 mm internal diameter, connectors and T-pieces of chemically inert materials**.**

6.1.6 If necessary, **dialyser** with a cellulose membrane to dilute the sample and to eliminate interfering substances (only for orthophosphate).

6.1.7 Photometric flow-through detector, wavelength 700 nm ± 20 nm**.**

6.1.8 Data display unit, such as a recorder, printer or plotter.

6.1.9 Autosampler, if required.

6.2 Additional apparatus

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

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

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

6.2.4 Membrane filter assembly with membrane filters, pore size 0,45 µm.

6.2.5 pH meter.

6.3 Additional apparatus for the determination of total phosphorus

6.3.1 Homogenizer.

6.3.2 Borosilicate flasks (see ISO 6878).

7 Sampling and sample preparation

Carry out sampling according to ISO 5667-1, ISO 5667-2 and ISO 5667-3. Prior to use, rinse with water (5.1) all containers which will come into contact with the sample.

For samples with low concentrations (e.g. $\leqslant$ 0,1 mg/l orthophosphate-P), use glass containers. For samples with higher concentrations (e.g. $>$ 0,1 mg/l phosphate-P or total-P), plastics bottles are also acceptable.

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 4 °C $\pm$ 2 °C. The filtration reduces biological reactions, and avoids interferences by sulfide and clogging of the analyser tubing (in case of solids of diameter larger than 100 µm). Maximum preservation time: 24 h.

Samples for the determination of total phosphorus should be preserved by adding sulfuric acid to a pH of $<$ 2 immediately after sampling. Maximum preservation time: 1 month.

Total phosphorus samples containing particles of diameter larger than 100 µm shall be homogenized (6.3.1).

8 Procedure

8.1 Analysis preparation

Set up the flow analyser for the desired procedure (orthophosphate or total phosphorus: see Figure A.1).

Pump the reagents for up to 10 min and set the baseline to zero.

The analyser is ready for use when the baseline is stable. Proceed according to steps 8.2 to 8.5.

8.2 Instrument performance check

In the analytical system, prepared according to 8.1, a calibration solution (5.16) 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 0,015 cm^{-1}. Otherwise the flow system is not suitable, and it shall be replaced by a system which fulfills this requirement.

If the photometric detector (6.1.7) does not allow any absorbance readings, the absorbance may be determined by comparison 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 5) should be prepared manually and measured in the external photometer.

A calibration solution (5.16) with a phosphate-P concentration of 0,01 mg/l shall exhibit a signal-to-noise ratio of at least 3:1.

8.3 Reagent blank check

Wait for a stable baseline.

Pump water (5.1) through all tubes. Record the change in absorbance.

If the absorbance per centimetre (see 8.2) is reduced by more than 0,01 cm^{-1}, the reagents or the water (5.1) may possibly be contaminated, and suitable measures shall be undertaken to eliminate the interference before starting the analysis.

Pump all the reagents, solutions again (8.1).

Alternatively, phosphate-free distilled water (5.1) may be injected.

8.4 Calibration

Select the desired working range and at least five appropriate calibration solutions (5.16), 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.

The analytical conditions for standards and samples are identical (8.6). The output signal is proportional to the phosphate-P concentration, or the total-P concentration, respectively. Use the following Equation (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 $\times$ litres per milligram;

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

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

8.5 Check of digestion efficiency for determination of total-P

Potassium pyrophosphate solution I or II (5.17.2, 5.17.3) and organophosphorus solution I or II (5.17.5, 5.17.6) at a concentration of 50 % of the selected working ranges I or II shall show a recovery rate of at least 90 %.

If these criteria are not met, a more vigorous treatment in accordance with ISO 6878 is required.

8.6 Measurement

Analyse samples, prepared according to Clause 7, in the same way as the calibration solutions (5.16).

If the sample concentration is higher than the selected working range, analyse the sample in a different working 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 (8.4). If necessary recalibrate the system.

8.7 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 rinsing solution (5.18), and then for about 5 min with water (5.1).

9 Calculation of results

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

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

where the symbols are as defined in 8.4.

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

10 Expression of results

Report results to not more than 2 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

11 Test report

The test report shall refer to this part of ISO 15681 and contain at least the following information:

a) identity of the sample;

b) procedure applied (FIA);

c) sample preparation, if any;

d) description of the analyser type or flow analysis conditions used;

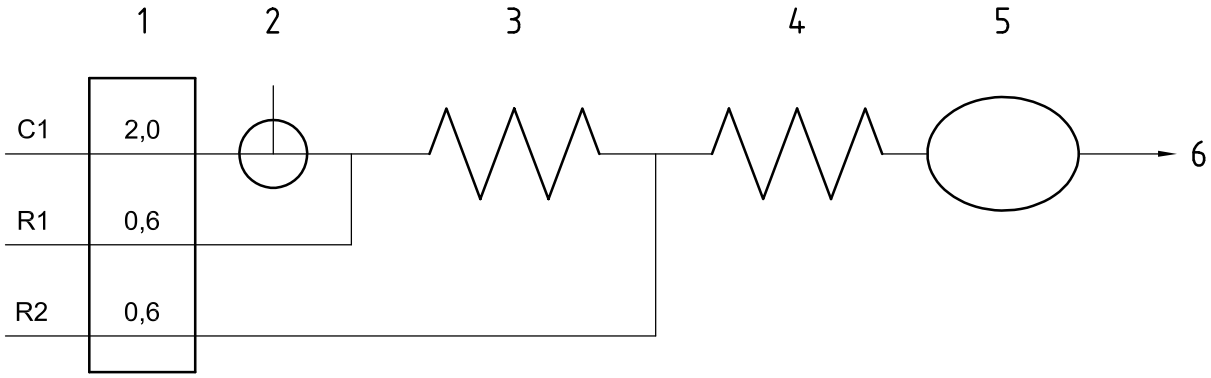
e) results, in accordance with Clause 10;

f) any deviation from this part of ISO 15681 or any circumstances which might affect the result.

Annex A
(informative)

Example of an FIA system

Figure A.1 gives an example of an FIA system (see 6.1).



Key

1 pump, flowrates in ml/min
2 injector for introducing the samples
injection volume for orthophosphate-P:
working range II (0,01 mg/l to 0,10 mg/l): 300 µl to 400 µl
working range I (0,10 mg/l to 1,00 mg/l): 40 µl
injection volume for total-P after manual digestion:
working range II (0,10 mg/l to 1,00 mg/l): 40 µl to 400 µl
working range I (1,00 mg/l to 10,0 mg/l): 10 µl to 40 µl
3 reaction coil, l = 30 cm, ∅ = 0,5 mm
4 reaction coil, l = 60 cm, ∅ = 0,5 mm
5 detector, wavelength: 700 nm ± 20 nm
6 waste
C1 carrier (5.12.1 or 5.12.2): flowrate 2,0 ml/min
R1 molybdate solution (5.10): flowrate 0,6 ml/min
R2 tin(II) chloride reagent (5.11): flowrate 0,6 ml/min

Figure A.1 — Example of an FIA system (6.1) for the determination of orthophosphate-P and total-P after manual digestion for all working ranges

Annex B
(informative)

Precision and accuracy

The statistical data in Tables B.1 to B.4 were obtained from an interlaboratory trial, carried out in May 2000 by DIN. The key to the tables is shown after Table 4.

Table B.1 — Statistical data for the determination of orthophosphate-P by FIA in accordance with ISO 5725-2

Sample	Matrix	l	n	o %	$\bar{x}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
P-1	Drinking water	12	39	17,0	73,8	5,43	7,36	0,733	0,99
P-2	Surface water	12	43	10,6	477	13,1	2,75	6,30	1,32
P-3	Waste water	12	42	10,6	510	49,0	9,61	3,98	0,78
P-4	Surface water	12	47	0	322	67,8	21,0	4,84	1,50

Table B.2 — Statistical data for the determination of orthophosphate-P by FIA in accordance with ISO 5725-2 (including the method described in Annex C)

Sample	Matrix	l	n	o %	$\bar{x}$ µg/l	s_R µg/l	CV_R %	s_r µg/l	CV_r %
P-1	Drinking water	15	51	12,1	72,0	6,90	9,59	0,69	0,958
P-2	Surface water	15	47	17,5	478	19,1	4,00	3,35	0,700
P-3	Waste water	14	43	20,4	513	57,7	11,3	2,28	0,444
P-4	Surface water	15	59	0	333	82,1	24,7	4,45	1,34

Table B.3 — Statistical data for the determination of total-P by FIA in accordance with ISO 5725-2

Sample	Matrix	l	n	o %	x_{corr} µg/l	$\bar{x}$ µg/l	RR %	s_R µg/l	CV_R %	s_r µg/l	CV_r %
P-1	Drinking water	10	34	10,5	275	265	96	12,9	4,87	4,66	1,76
P-2	Surface water	10	34	10,5	500	499	100	14,5	2,90	4,96	0,99
P-3	Waste water	10	33	10,8	$4{,}36 \times 10^3$	$4{,}29 \times 10^3$	98	85,7	2,00	42,9	1,00
P-4	Surface water	10	37	2,6	$3{,}12 \times 10^3$	$3{,}20 \times 10^3$	103	110	3,44	27,5	0,88

Table B.4 — Statistical data for the determination of total-P by FIA in accordance with ISO 5725-2
(including the method described in Annex C)

Sample	Matrix	l	n	o	x_{corr}	$\bar{x}$	RR	s_R	CV_R	s_r	CV_r
				%	µg/l	µg/l	%	µg/l	%	µg/l	%
P-1	Drinking water	14	54	0	275	245	89	50,6	20,7	4,22	1,72
P-2	Surface water	14	46	14,8	500	499	100	20,7	4,14	5,61	1,12
P-3	Waste water	13	41	16,3	$4{,}36 \times 10^3$	$4{,}13 \times 10^3$	95	311	7,53	45,9	1,11
P-4	Surface water	14	57	1,7	$3{,}12 \times 10^3$	$3{,}17 \times 10^3$	102	180	5,68	32,7	1,03

Key to Tables B.1 to B.4

l is the number of laboratory data sets received (including outliers);

n is the number of outlier-free individual analytical values;

o is the relative portion of outliers;

x_{corr} is the correct value of the concentration by convention;

$\bar{x}$ is the total mean of the concentrations, obtained from outlier-free values;

RR is the recovery rate;

s_R is the reproducibility standard deviation;

CV_R is the reproducibility coefficient of variation;

s_r is the repeatability standard deviation;

CV_r is the repeatability coefficient of variation.

The results of the mean values, the recovery rates, and the precision data are equivalent with the corresponding results obtained by the interlaboratory trial on the CFA method (see ISO 15681-2:2003, Annex B).

Annex C
(informative)

Determination of orthophosphate-P and total-P by FIA using ascorbic acid reduction

C.1 General

Due to the limited reaction time in FIA and a faster reduction of the phosphorus molybdate complex formed with tin(II) chloride, the method described in the body of this part of ISO 15681 is generally used.

Alternatively, a method, described here, using ascorbic acid as reduction reagent may be used. This alternative, which was not validated by the interlaboratory trial (Annex B), is recommended in some cases, e.g. because of the lower toxicity of reagents.

In contrast to the normative part of this part of ISO 15681, the method described below also allows the determination of total-P with integral UV digestion and hydrolysis (see C.3.3 and Figure C.2).

The method described in Clauses 3 to 11 is then altered as given below.

C.2 Interferences in the determination of total-P

— Samples containing solids or suspended particles may exhibit 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 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 a COD value of more than 10 times the highest concentrations of the calibration solutions (5.16).

C.3 Principle

C.3.1 Determination of orthophosphate-P

The sample is injected into a carrier stream, which is merged with an acidic solution of molybdate and antimony ions and an ascorbic acid solution. The phospho-antimony-molybdate complex formed is reduced by ascorbic acid to molybdenum blue [4], [5].

C.3.2 Total-P with manual digestion

Phosphorus compounds in the sample are oxidized manually with a potassium peroxodisulfate solution, according to the procedure in ISO 6878. The resulting orthophosphate is determined by the molybdenum blue reaction as in C.3.1 [5], [6].

C.3.3 Total-P with integral UV digestion and hydrolysis

The sample is passed through a heated digestion coil for the acid hydrolysis of polyphosphates, and through a UV digestor for the conversion of organic phosphorus into orthophosphate using a peroxodisulfate reagent. The resulting orthophosphate is determined by the molybdenum blue reaction as in C.3.1.

C.4 Reagents

The appropriate reagents are listed in Clause 5.

Degas carefully all reagent solutions for the FIA determinations before use, e.g. by vacuum filtration or by purging with helium for 1 min.

Additionally the following reagents are used.

C.4.1 Sodium chloride, NaCl.

C.4.2 Ascorbic acid, $C_6H_8O_6$.

C.4.3 Antimony potassium tartrate hemihydrate, $K(SbO)C_4H_4O_6 \cdot 0{,}5\ H_2O$.

C.4.4 Antimony tartrate molybdate reagents.

C.4.4.1 Molybdate solution.

Dissolve 40 g of hexa-ammonium heptamolybdate tetrahydrate (5.3) in about 800 ml of water (5.1) and dilute to 1 000 ml with water (5.1).

The solution is stable for 3 months if stored at room temperature.

C.4.4.2 Antimony potassium tartrate solution.

Dissolve 3,0 g of antimony potassium tartrate hemihydrate (C.4.3) in about 800 ml of water (5.1) and dilute to 1 000 ml with water (5.1).

The solution is stable for 3 months if stored at room temperature.

C.4.4.3 Antimony tartrate molybdate reagent I, for the determination of orthophosphate-P and total-P after manual digestion (R3 in Figure C.1).

Carefully add 35 ml of sulfuric acid (I) (5.2.1) to about 500 ml of water (5.1). After cooling, add 213 ml of molybdate solution (C.4.4.1) and 72 ml of antimony potassium tartrate solution (C.4.4.2) and make up to 1 000 ml with water (5.1).

The solution is stable for 2 weeks if stored at room temperature.

C.4.4.4 Antimony tartrate molybdate reagent II, for total-P determination after integrated UV digestion (R5 in Figure C.2).

Add 213 ml of molybdate solution (C.4.4.1) to about 700 ml water (5.1) and 72 ml of antimony potassium tartrate solution (C.4.4.2). Add 22,8 g of sodium hydroxide, NaOH, to the solution and make up to 1 000 ml with water (5.1).

The solution is stable for 1 week if stored at room temperature.

C.4.5 Sodium dodecyl sulfate, $NaC_{12}H_{25}SO_4$.

C.4.6 Ascorbic acid solution (R4 in Figures C.1 and C.2).

Dissolve 6,0 g of ascorbic acid (C.4.2) in about 80 ml water (5.1), add 0,1 g of sodium dodecyl sulfate (C.4.5) and make up to 100 ml with water (5.1). Prepare the solution daily before use.

C.4.7 Carrier solutions for the FIA systems

C.4.7.1 Carrier solution I, for orthophosphate-P determination (C1 in Figure C.1).

The carrier solution is water (5.1) (without surfactant).

C.4.7.2 Carrier solution II, for total-P determination after manual digestion (C1 in Figure C.1).

Add 5 ml of sulfuric acid I (5.2.1) to 1 000 ml water (5.1) and mix.

C.4.7.3 Carrier solution III, for total-P determination after integrated UV digestion; (C2 in Figure C.2).

To approximately 800 ml water (5.1) carefully add 37,8 ml of sulfuric acid I (5.2.1) while stirring. Cool, and add 5 g of sodium chloride (C.4.1) and 1,0 g of sodium dodecyl sulfate (C.4.5). Dilute to 1 000 ml with water (5.1).

C.4.8 Digestion reagents for the determination of total-P after integrated UV digestion.

C.4.8.1 Digestion solution I (D1 in Figure C.2)**.**

To approximately 800 ml water (5.1) carefully add 106,5 ml of sulfuric acid I (5.2.1) while stirring.

CAUTION — This solution gets very hot. Cool, and dilute to 1 000 ml with water (5.1).

C.4.8.2 Digestion solution II (D2 in Figure C.2)**.**

Dissolve 26 g of potassium peroxodisulfate (5.6) in about 800 ml water (5.1) and dilute to 1 000 ml with water (5.1).

C.5 Apparatus

C.5.1 Flow injection analysis (FIA)

The apparatus described in 6.1 is required.

C.5.2 Additional apparatus

The apparatus described in 6.2 is required.

C.5.3 Additional apparatus for determination of total phosphorus

The apparatus described in 6.3 is required.

C.5.4 Additional apparatus for determination of total phosphorus after integral digestion

C.5.4.1 Apparatus integrated in the FIA system (6.1).

C.5.4.1.1 UV digestion unit.

C.5.4.1.2 Thermostat for temperature control of hydrolysis at 95 °C ± 1 °C .

C.6 Sampling and sample preparation

Sampling and sample preparation are carried out in accordance with Clause 7.

C.7 Procedure

The procedure described in Clause 8 is followed.

C.8 Calculation of results

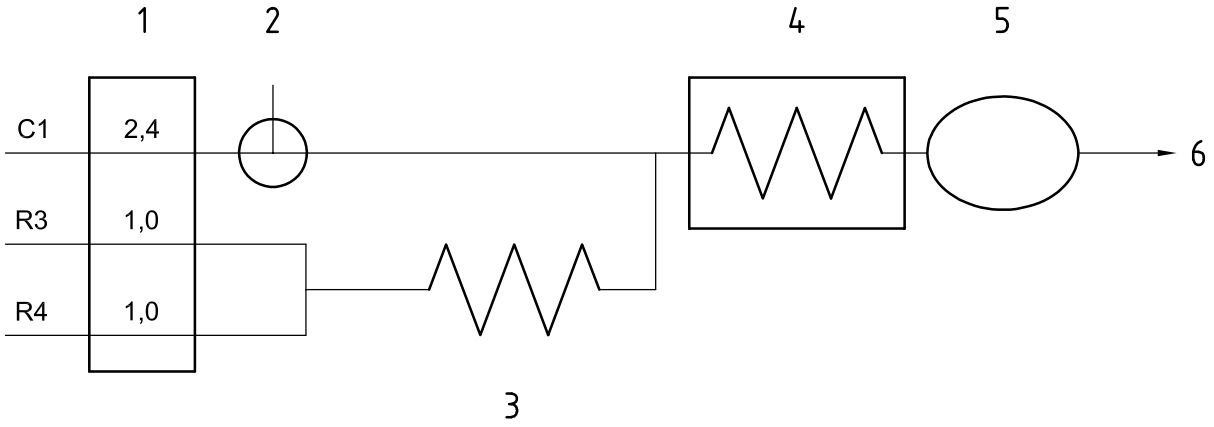
The procedure described in Clause 9 is followed.

C.9 Expression of results

The procedure described in Clause 10 is followed.

C.10 Examples of FIA systems for the determination of orthophosphate-P and total P using ascorbic acid as reduction reagent

Figures C.1 and C.2 shown below are examples only. The methods described in this annex have not been validated in the interlaboratory trial cited in Annex B. Flow schemes, flowrates and tubing volumes are examples only. They may be changed proportionally.



Key

1 pump, flowrates in ml/min

2 injector for introducing the samples; injection volume:
- 640 µl for range II orthophosphate-P (0,01 mg/l to 0,10 mg/l P)
- 100 µl for range I orthophosphate-P and range II total-P (0,10 mg/l to 1,00 mg/l P)
- 12 µl for range II total-P (1,00 mg/l to 10,0 mg/l P)

3 reaction coil, l = 120 cm, ∅ = 0,7 mm

4 thermostat set at 60 °C (precision: ± 1 °C), l = 150 cm, ∅ = 0,7 mm

5 detector, wavelength = 880 nm

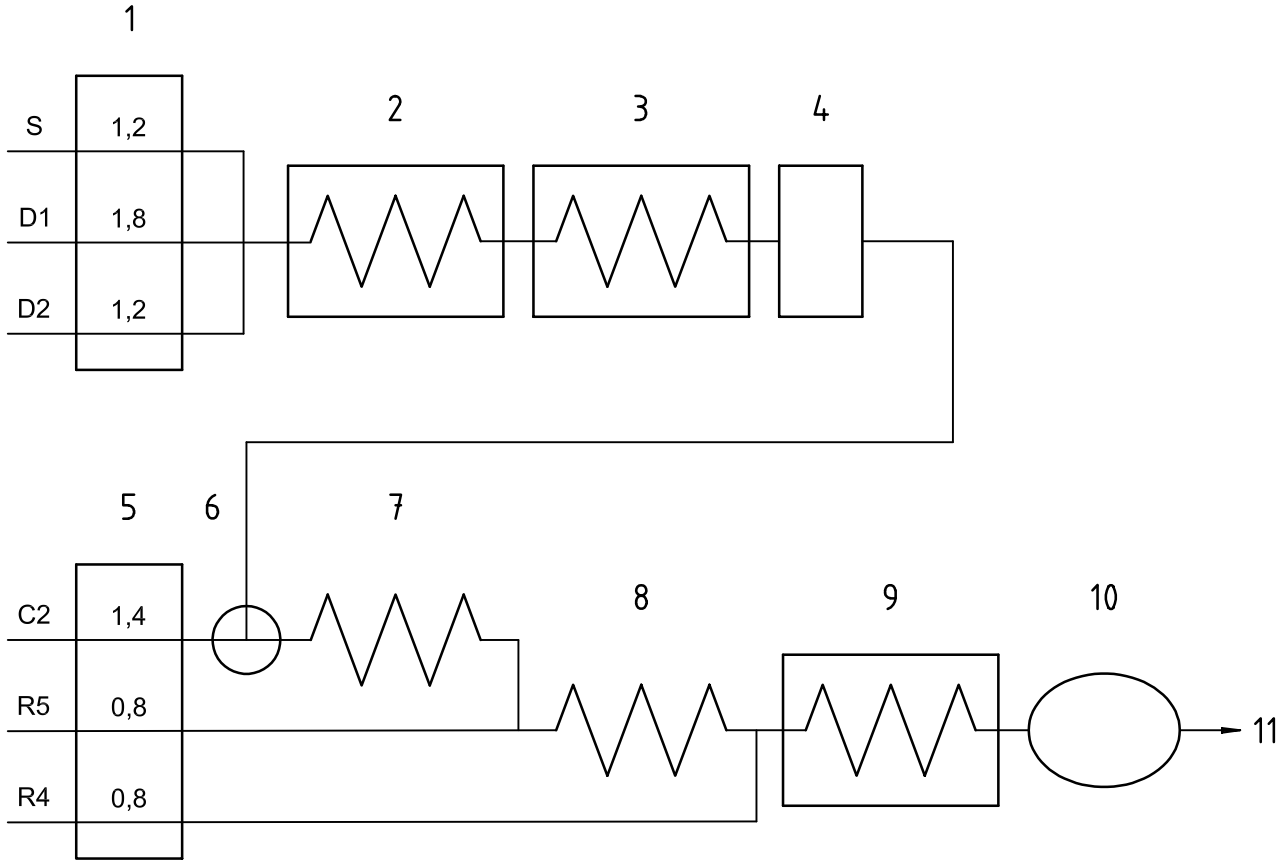
6 waste

C1 carrier solution I (C.4.7.1) for orthophosphate or carrier solution II (C.4.7.2) for total P: flowrate 2 ml/min

R3 antimony tartrate molybdate reagent I (C.4.4.3): flowrate 1,0 ml/min

R4 ascorbic acid solution (C.4.6): flowrate 1,0 ml/min

Figure C.1 — Example of an FIA system (C.5.1) for the determination of orthophosphate-P and total-P after manual digestion



Key

1 and 5	pump(s), flowrates in ml/min
2	thermostat set at 95 °C (precision: ± 1 °C), volume 6,2 ml
3	UV digestion unit, consisting of e.g. PTFE tubing (PTFE = polytetrafluoroethene) irradiated by a mercury-discharge ultraviolet lamp emitting radiation at 254 nm, volume 2,9 ml
4	debubbler or degassing device
6	sample injector; injection volume selected to match the appropriate ranges
7	mixing coil, l = 60 cm, ∅ = 0,7 mm
8	reaction coil, l = 120 cm, ∅ = 0,7 mm
9	thermostat set at 60 °C (precision: ± 1 °C), l = 150 cm, ∅ = 0,7 mm
10	detector, wavelength = 880 nm
11	waste
C2	carrier solution III (C.4.7.3) for total-P using in-line UV digestion, flowrate 1,4 ml/min
R5	antimony tartrate molybdate reagent II (C.4.4.4), flowrate 0,8 ml/min
R4	ascorbic acid solution (C.4.6), flowrate 0,8 ml/min
D1	digestion solution I (C.4.8.1), flowrate 1,8 ml/min
D2	digestion solution II (C.4.8.2), flowrate 1,2 ml/min
S	sample aspiration line, flowrate 1,2 ml/min

Figure C.2 — Example of an FIA system (C.5.1) for the determination of total-P using integrated UV digestion for all working ranges

Annex D
(informative)

Replacement of hydrazine sulfate by DEHA (*N,N*-diethylhydroxylamine)

The toxic hydrazine sulfate can be replaced by *N,N*-diethylhydroxylamine (DEHA), with the following changes in the described standard procedure:

a) Read **5.4** with the following alteration:

"*N,N*-Diethylhydroxylamine (DEHA), $C_4H_{11}NO$, $>$ 97 %".

b) Read **5.11** with the following alteration of paragraph 1:

"Dissolve 28 ml of sulfuric acid (I) (5.2.1), 200 mg of tin (II) chloride (5.5) and 1 ml of *N,N*-diethylhydroxylamine (DEHA) (5.4) in about 800 ml of water, cool and dilute to 1 000 ml. Store at 4 °C $\pm$ 2 °C."

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