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Water quality — Determination of total cyanide and free cyanide using flow analysis (FIA and CFA) —

Part 2:
Method using continuous flow analysis (CFA)

Qualité de l'eau — Dosage des cyanures totaux et des cyanures libres par analyse en flux (FIA et CFA) —

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

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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 14403-2 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

This first edition of ISO 14403-2 cancels and replaces ISO 14403:2002, which has been technically revised.

ISO 14403 consists of the following parts, under the general title *Water quality — Determination of total cyanide and free cyanide using flow analysis (FIA and CFA)*:

- *Part 1: Method using flow injection analysis (FIA)*
- *Part 2: Method using continuous flow analysis (CFA)*

Introduction

Methods using flow analysis automate wet chemical procedures and are particularly suitable for the processing of many analytes in water in large series of samples at a high frequency of analysis.

Analysis can be performed by flow injection analysis (FIA) or continuous flow analysis (CFA). Both methods share the feature of an automatic introduction of the sample into a flow system (manifold) in which the analytes in the sample react with reagent solutions on their way through the manifold. Sample preparation may be integrated in the manifold. The reaction product is measured in a flow detector (e.g. flow photometer).

See the foreword for a list of parts of this International Standard.

It should be investigated whether and to what extent particular problems require the specification of additional marginal conditions.

Water quality — Determination of total cyanide and free cyanide using flow analysis (FIA and CFA) —

Part 2:
Method using continuous flow analysis (CFA)

WARNING — Persons using this part of ISO 14403 should be familiar with normal laboratory practice. This part of ISO 14403 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.

IMPORTANT — It is absolutely essential that tests conducted according to this part of ISO 14403 be carried out by suitably trained staff.

1 Scope

This part of ISO 14403 specifies methods for the determination of cyanide in various types of water (such as ground, drinking, surface, leachate, and waste water) with cyanide concentrations usually from 2 µg/l to 500 µg/l expressed as cyanide ions in the undiluted sample. The range of application can be changed by varying the operation conditions, e.g. by diluting the original sample or changing the pathlength of the flow cell.

In this method, a suitable mass concentration range from10 µg/l to 100 µg/l is described.

Seawater can be analysed with possible changes in sensitivity and adaptation of the reagent and calibration solutions to the salinity of the samples.

2 Normative references

The following referenced 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 and laboratory use — Specification and test methods*

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

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

For the purpose of this part of ISO 14403, the following definitions apply:

3.1
free cyanide
easily liberatable cyanide
sum of cyanide ions and the cyanide bound in weak metal cyanide complexes that liberate HCN at pH 3,8

3.2
total cyanide
free cyanide (3.1), and in addition stronger metal–cyanide complex compounds, with the exception of cyanide bound in gold, cobalt, platinum, ruthenium, and rhodium complexes, from which recovery can be partial

NOTE 1 Thiocyanate, organically bound cyanide and cyanate are not included in this definition of total cyanide.

NOTE 2 Distillation methods may recover some organic cyanide. Use the diffusion method when organic cyanide complexes are suspected to be present.

4 Interferences

4.1 Interferences by oxidizing agents

Oxidizing agents such as chlorine decompose most of the cyanides. If the presence of oxidizing agents cannot be excluded, treat the sample immediately after sampling. Test a drop of the sample with potassium iodide-starch test paper (KI starch paper); a blue colour indicates the need for treatment. Add sodium thiosulfate, a few crystals at a time, until a drop of sample produces no colour on the indicator paper. Then add an additional portion of 0,6 g of ascorbic acid (6.9) for each 1 000 ml of sample volume.

Do not add ascorbic acid unless samples will be analysed within 24 h.

4.2 Interferences by sulfide, sulfite, nitrite and carbonyl compounds

Interferences by sulfide start at 100 mg/l. It affects the colorimetric procedure, especially the gas diffusion method, and the amperometric procedure (see Annex C). If a drop of the sample on lead acetate test paper indicates the presence of sulfide, treat an additional 25 ml of the stabilized sample (pH >12) to that required for the cyanide determination with powdered lead carbonate.

Lead sulfide precipitates if the sample contains sulfide.

Repeat this operation until a drop of the treated sample solution does not darken the lead acetate test paper.

Filter the solution through a dry filter paper into a dry beaker, and from the filtrate measure the sample to be used for analysis. Avoid a large excess of lead and a long contact time in order to minimize loss by complexation or occlusion of cyanide on the precipitated material.

If the amperometric method (Annex C) is applied, it is necessary systematically to add lead carbonate to samples during the analysis (a few milligrams for a 10 ml sample), followed by filtration or decantation performed before the filtered sample aliquot is placed on the sample tray of the continuous flow analyser.

Aldehydes and ketones can, under certain conditions, absorb cyanide by nucleophilic addition. To avoid this interference ethylenediamine can be added to the sample.

Under the given distillation conditions, aldehydes can transform cyanide to nitrite. Aldehydes can be removed by adding silver nitrate to the sample. The addition of $AgNO_3$ can alter the ratio of the concentrations of free and total cyanide. The user should evaluate this procedure.

Interference by nitrite above concentrations of 5 mg/l can be avoided by addition of sulfamic acid (6.10) to the buffer (pH 3,8) for the distillation and gas diffusion method (6.21.1).

Sulfite interferes above concentrations of 1 mg/l.

4.3 Other interferences

Samples containing particulate matter can lead to losses if the particles clog the transport tubes and are not transported completely into the UV unit. This effect can be minimized by homogenizing (e.g. stirring) the sample immediately prior to analysis to ensure that a representative sample is taken and to reduce the particle size. Remaining particles with diameters >0,1 mm should be removed by filtration.

When using in-line distillation for separation of the hydrogen cyanide, salt concentrations higher than 10 g/l of salts can cause clogging of the distillation coil. Dilute these samples prior to measurement or use a gas diffusion method in order to overcome this problem.

Thiocyanate can slightly interfere and lead to positive bias (9.3.2). Significant interferences can arise from cyanide impurities in thiocyanate or from inappropriate distillation procedures (7.1).

5 Principle

5.1 Determination of total cyanide

Complex-bound cyanide is decomposed by UV light at pH 3,8. An UV-B lamp (312 nm to 400 nm) and a digestion coil of borosilicate glass, quartz glass or polytetrafluorethylene (PTFE) is used. The UV unit shall ensure that UV light with a wavelength of $<$290 nm is filtered off thus preventing the conversion of thiocyanate into cyanide.

The hydrogen cyanide present at pH 3,8 is separated by online distillation at 125 °C or by gas diffusion at 30 °C to 40 °C across a hydrophobic membrane. Using gas diffusion, hydrogen cyanide is absorbed in a sodium hydroxide solution.

The hydrogen cyanide is then determined photometrically by the reaction of cyanide with chloramine-T to cyanogen chloride. This reacts with pyridine-4-carboxylic acid and 1,3-dimethylbarbituric acid to give a red dye whose absorption is proportional to cyanide concentration.

5.2 Determination of free cyanide

During the procedure specified in 5.1, the UV-B lamp is switched off when determining the free cyanide content. During distillation at pH 3,8 for separation of the hydrogen cyanide present, a zinc sulfate solution is added to the sample flow in order to precipitate any iron cyanides present as the zinc-cyanoferrate complex.

When using the gas diffusion method for the liberation of cyanide from the nickel complex, 50 µl tetraethylenepentamine solution (6.12) per 30 ml sample is added prior to the analysis (see Reference [11]).

For detection see 5.1.

Alternatively, free and total cyanide can be determined after gas diffusion using an amperometric detector (see Annex C).

6 Reagents

WARNING — KCN, $K_2Zn(CN)_4$, their solutions, and wastes are toxic. Waste containing these substances shall be removed appropriately.

Use only reagents of recognized analytical grade.

Smaller portions of the following solutions can be applied provided the ratios of the prescribed volumes and mass concentrations are maintained.

6.1 **Water**, grade 1, as defined in ISO 3696.

6.2 **Hydrochloric acid**, $c(\text{HCl}) = 1$ mol/l.

6.3 **Sodium hydroxide solution I**, $c(\text{NaOH}) = 0{,}4$ mol/l.

6.4 **Sodium hydroxide solution II**, $c(\text{NaOH}) = 1{,}0$ mol/l.

6.5 **Sodium hydroxide solution III**, $c(\text{NaOH}) = 0{,}2$ mol/l.

6.6 Sodium hydroxide solution IV, rinsing solution, $c(\text{NaOH}) = 0{,}01$ mol/l.

6.7 Surfactant, polyoxyethylene laurylether, $HO\text{-}(CH_2CH_2\text{-}O)_n\text{-}C_{18}H_{37}$.

Add 30 g of polyoxyethylene laurylether in small quantities to 100 ml of water (6.1) and mix well.

Alternatively use a commercially available solution of the surfactant.

6.8 Citric acid monohydrate, $C_6H_8O_7 \cdot H_2O$.

6.9 Ascorbic acid, $C_6H_8O_6$.

6.10 Sulfamic acid, H_3SO_3N.

6.11 Tetraethylenepentamine, $C_8H_{23}N_5$.

6.12 Tetraethylenepentamine solution (for free cyanide only).

Dissolve 0,75 g of tetraethylenepentamine (6.11) in 250 ml of water.

This solution is stable for 1 month if stored at room temperature.

6.13 Zinc sulfate heptahydrate, $ZnSO_4 \cdot 7H_2O$.

6.14 Potassium hydrogenphthalate, $KHC_8H_4O_4$.

6.15 Chloramine-T trihydrate, $C_7H_7ClNNaO_2S \cdot 3H_2O$.

6.16 1,3-Dimethylbarbituric acid, $C_6H_8N_2O_3$.

6.17 Pyridine-4-carboxylic acid, $C_6H_5NO_2$.

6.18 Potassium thiocyanate, KSCN.

6.19 Potassium hexacyanoferrate(III), $K_3Fe(CN)_6$.

6.20 Cyanide standards.

6.20.1 Potassium cyanide, KCN.

6.20.2 Potassium cyanide solution, KCN, $\rho_{CN} = 1\,000$ mg/l, (see Annex B).

Dissolve 2 500 mg ± 1 mg of potassium cyanide, KCN (6.20.1), in sodium hydroxide solution IV (6.6) in a 1 000 ml volumetric flask, and make up to volume with sodium hydroxide solution IV (6.6).

A commercially available and certified 1 000 mg/l KCN stock solution may be used.

The solution is stable for 3 months at 1 °C to 8 °C.

Alternatively, a potassium tetracyanozincate solution (6.20.3) can be used:

6.20.3 Potassium tetracyanozincate solution, $K_2Zn(CN)_4$, $\rho_{CN} = 1\,000$ mg/l ± 2 mg/l, commercially available.

The solution is stable for 3 months at 1 °C to 8 °C.

6.20.4 Cyanide solution I, ρ_{CN} = 10 mg/l.

Pipette 1 ml of the potassium tetracyanozincate solution I (6.20.3) or 1 ml of the potassium cyanide solution (6.20.2) into a 100 ml volumetric flask, and make up to volume with sodium hydroxide solution IV (6.6).

This solution is stable for 1 week if stored at 1 °C to 8 °C.

6.20.5 Calibration solutions

Prepare at least five calibration solutions with cyanide concentrations, roughly regularly distributed over the working range, by appropriate dilution of the cyanide solution I (6.20.4). If, for example, six calibration standards should be prepared, proceed as follows.

Pipette 10 ml of the cyanide solution I (6.20.4) into a 100 ml volumetric flask, and make up to volume with sodium hydroxide solution IV (6.6). This solution contains 1mg/l cyanide.

Pipette, into 100 ml volumetric flasks, 1 ml, 3 ml, 5 ml, 6 ml, 8 ml, or 10 ml, respectively, of the previously mentioned 1 mg/l cyanide solution and make up to volume with sodium hydroxide solution IV (6.6).

These solutions contain 10 µg/l, 30 µg/l, 50 µg/l, 60 µg/l, 80 µg/l, and 100 µg/l of cyanide, respectively [except for corrections in the concentration found on titration of the potassium cyanide solution (6.20.2), (see Annex B)].

These solutions are stable for 2 d if stored in a refrigerator at 1 °C to 5 °C.

6.21 Reagents for the determination of cyanide

6.21.1 Buffer, pH 3,8, for distillation and gas diffusion method (R1 in Figures A.1, A.2 and C.1).

Dissolve, in about 350 ml water (6.1), 10 g of citric acid (6.8). Add 50 ml of sodium hydroxide solution I (6.3) and, if necessary, adjust to pH 3,8 with hydrochloric acid (6.2) or sodium hydroxide solution IV (6.6). Add 12,5 ml of hydrochloric acid (6.2). Dilute to 500 ml with water.

NOTE Due to the addition of HCl, the pH of this buffer is about 3,4. After mixing with the sample a pH of 3,8 is achieved.

This solution is stable for 3 months if stored in a refrigerator at 1 °C to 5 °C.

6.21.2 Zinc sulfate solution, only for distillation method (R2 in Figure A.1).

Dissolve 10 g of zinc sulfate heptahydrate (6.13) in 750 ml of water (6.1), mix and dilute to 1 000 ml with water.

6.21.3 Recipient solution, only for gas diffusion (R3 in Figure A.2).

Sodium hydroxide solution III (6.5).

6.21.4 Buffer solutions for the final photometric determination (R4 in Figures A.1 and A.2)

6.21.4.1 For the distillation method (R4a in Figure A.1).

Dissolve 2,3 g of sodium hydroxide (NaOH) in 500 ml of water. Add 20,5 g of potassium hydrogenphthalate (6.14) and dilute to approximately 975 ml with water.

If necessary, adjust the pH of the solution to 5,2 with hydrochloric acid (6.2) or sodium hydroxide solution II (6.4).

Add 1 ml of surfactant (6.7) and make up to 1 000 ml with water.

For best results this solution is stable for 1 month if stored in an amber bottle in a refrigerator at 1 °C to 5 °C.

6.21.4.2 For the gas diffusion method (R4b in Figure A.2):

Dissolve 7,0 g of sodium hydroxide (NaOH) in 250 ml of water. Add 35,4 g of succinic acid (6.24) and dilute to 500 ml with water.

The solution has a pH of approximately 4.3. When mixed with sodium hydroxide solution I (6.21.3; R3 in Figure A.2), a pH of 5,2 shall be achieved.

For best results this solution is stable for 1 month if stored in an amber bottle in a refrigerator at 1 °C to 5 °C.

6.21.5 Chloramine-T-trihydrate solution (R5 in Figures A.1 and A.2).

Dissolve 2,0 g ± 0,05 g of chloramine-T (6.15) in 1 000 ml of water.

This solution is stable for 1 week if stored in a refrigerator at 1 °C to 5 °C; however, for best results prepare solution fresh daily.

6.21.6 Colour reagent (R6 in Figures A.1 and A.2).

Carefully dissolve in a 1 000 ml volumetric flask, 7,0 g of sodium hydroxide, NaOH, in about 500 ml of water (6.1). Add 16,8 g ± 0,1 g of 1,3-dimethylbarbituric acid (6.16), and 13,6 g ± 0,1 g of pyridine-4-carboxylic acid (6.17), and dilute to approximately 975 ml with water (6.1).

If necessary, adjust the solution to pH 5,2 with hydrochloric acid (6.2) or sodium hydroxide solution II (6.4).

Make up to 1 000 ml with water (6.1). Mix this solution intensively (e.g. by using a magnetic stirrer) for 1 h at 30 °C and then filter over a pleated filter (e.g. hardened ashless paper).

This solution is stable for 3 months if stored in a refrigerator at 2 °C to 5 °C.

NOTE Storage at 1 °C can cause precipitation of components of this solution.

6.22 Thiocyanate solution, calculated cyanide concentration: $\rho_{CN} = 100$ mg/l.

Dissolve in a 1 000 ml volumetric flask, 373 mg ± 1 mg of potassium thiocyanate (6.18) in sodium hydroxide solution IV (6.6), and make up to volume with sodium hydroxide solution IV (6.6).

This solution is stable for 2 months if stored in an amber bottle and refrigerated at 1 °C to 5 °C.

Verify each batch for absence of CN impurity by analysing for free cyanide.

6.23 Potassium hexacyanoferrate(III) solution, calculated cyanide concentration $\rho_{CN} = 10$ mg/l.

Dissolve in a 1 000 ml volumetric flask, 21,1 mg ± 0,1 mg of potassium hexacyanoferrate(III) (6.19) in sodium hydroxide solution IV (6.6), and make up to volume with sodium hydroxide solution IV (6.6).

This solution is stable for 2 months if stored in an amber bottle in a refrigerator at 1 °C to 5 °C.

Verify each batch for absence of CN impurity by analysing for free cyanide.

6.24 Succinic acid, $C_4H_6O_4$.

7 Apparatus

Usual laboratory apparatus and in particular the following.

7.1 Continuous flow analysis system for distillation method.

7.1.1 General. A suitable example of the system configuration contains the components specified in 7.1.2 to 7.1.10 (see Figure A.1). Alternative system configurations are also applicable if the requirements of Clause 9 are achieved.

7.1.2 Autosampler, or other device capable of introducing sample reproducibility.

7.1.3 Reagent reservoirs.

7.1.4 Low pulsation pump, having specific chemically inert pump tubes, for flow rates as shown in Figure A.1 as an example.

7.1.5 UV lamp, with:

— UV-B lamp (312 nm to 400 nm);

— a power of 8 W to 12 W;

— a digestion coil of borosilicate glass, quartz glass or polytetrafluorethylene (PTFE).

Suitable dimensions among others (using a UV lamp emitting at 351 nm) for a digestion coil made of quartz glass or PTFE are: capacity of approximately 4 ml (e.g. 30 or 60 turns with a diameter of 30 mm, a tube wall thickness of at maximum 1 mm and an internal diameter of 1 mm or 2 mm).

For an emission maximum of 312 nm ± 5 nm and a digestion coil made of borosilicate glass or quartz glass or PTFE, suitable dimensions among others are: a capacity of approximately 13 ml (e.g. 45 turns with a diameter of 30 mm).

The main criteria for a good choice of UV lamp and digestion system is the results of digestion recovery rates (9.3.2).

Make sure that no UV light with a wavelength below 290 nm reaches the sample flow in order to avoid the decomposition of thiocyanate to cyanide.

7.1.6 In-line distillation device, adjustable to a temperature of 125 °C ± 1 °C with a distillation coil of glass or polymer material, length of coil e.g. 80 cm, internal diameter e.g. 1,5 mm.

7.1.7 Manifold, capable of highly reproducible dosing of gas bubbles, sample and reagents, and having appropriate transport systems and connection assemblies of chemically inert glass, polymer or metal.

7.1.8 Heating bath, for colorimetric reaction, adjustable to a temperature of 37 °C ± 1 °C with a coil volume to allow a sample retention period of approximately 4 min.

7.1.9 Photometric detector, with a flow cell, and having a wavelength range 600 nm ± 10 nm.

Use an appropriate optical pathlength to achieve a minimum absorbance (absolute value) of 0,01 per 1 cm pathlength for a 10 µg/l cyanide solution.

7.1.10 Recording unit (e.g. strip chart recorder, integrator, printer and plotter or a computer data system). In general, peak height signals are measured.

7.2 Continuous flow analysis system for gas diffusion method.

7.2.1 General. A suitable example of the system contains the components specified in 7.2.2 to 7.2.11 (see Figure A.2). Alternative systems are also applicable if the requirements of Clause 9 are achieved.

7.2.2 Autosampler, or other device capable of introducing sample reproducibly.

7.2.3 Reagent reservoirs.

7.2.4 Low pulsation pump, having specific chemically inert pump tubes. Figure A:2 shows an example of flow rates.

7.2.5 UV lamp

See 7.1.5.

7.2.6 Gas diffusion cell, having a hydrophobic semipermeable membrane made from polypropylene or PTFE, for example, and having a typical thickness 90 µm to 200 µm, pore size 0,1 µm to 1 µm.

7.2.7 Heating bath for gas diffusion temperature stabilization, adjustable to a temperature between 30 °C to 40 °C (with a tolerance of ± 1 °C) with a coil capacity of typically 2 ml and internal diameter of, for example,. 1 mm.

7.2.8 Manifold, capable of highly reproducible dosing of gas bubbles, sample and reagents, and having appropriate transport systems and connection assemblies made of chemically inert glass, polymer or metal.

7.2.9 Heating bath for colorimetric reaction adjustable to a temperature of 37 °C ± 1 °C with a spiral dimension to allow a sample retention period of approximately 4 min.

7.2.10 Photometric detector, with flow cell, and having a wavelength range of 600 nm ± 10 nm. Use an appropriate optical pathlength to achieve a minimum absorbance (absolute value) of 0,01 per 1 cm pathlength for a 10 µg/l cyanide solution.

7.2.11 Recording unit (e.g. strip chart recorder, integrator, printerand plotter or a computer data system). In general, peak height signals are measured.

7.3 Additional apparatus.

7.3.1 Lead acetate test paper, commercially available.

7.3.2 Membrane filter assembly, with membrane filters having a pore size of 0,45 µm.

7.3.3 pH measuring device.

8 Sampling and sample preparation

Immediately after sampling, adjust the pH of the water samples to 12 with sodium hydroxide solutions I to IV (6.3 to 6.6) such that the quantity of added alkali yields a negligible dilution of the sample.

If necessary, remove particles of diameter >0,1 mm by filtration or decantation at the laboratory.

Test for interferences and treat if necessary (see Clause 4).

Analyse the sample in accordance with Clause 9 as soon as possible after sampling, but as specified in ISO 5667-3 at the latest within 7 d. Store the sample in the dark.

9 Procedure

9.1 Flow system set-up

Set up and adjust the continuous flow analysis system according to Table 1 or Table 2 depending on the determination to be performed.

Table 1 — Adjustment of continuous flow analysis system for distillation method

Procedure	Component or parameter	Setting for determination to be performed	
		Total cyanide	Free cyanide
UV decomposition	UV lamp (7.1.5)	on	off
Distillation	Solution after UV decomposition	Water (6.1)	Zinc sulfate solution (6.21.2)
	pH	3,8	3,8
	Temperature	125 °C	125 °C
Final photometric determination	Colour reagent (6.21.6)		
	pH	5,2	5,2
	Temperature	37 °C	37 °C
	Wavelength	590 nm to 610 nm	590 nm to 610 nm

Table 2 — Adjustment of flow analysis system for gas diffusion methods

Procedure	Component or parameter	Setting for determination to be performed	
		Total cyanide	Free cyanide
UV decomposition	UV lamp (7.2.5)	on	off
Gas diffusion	Buffer for distillation and gas diffusion method (6.21.1)		
	pH	3,8	3,8
	Temperature	30 °C to 40 °C	30 °C to 40 °C
Final photometric determination	Colour reagent (6.21.6)		
	pH	5,2	5,2
	Temperature	37 °C	37 °C
	Wavelength	590 nm to 610 nm	590 nm to 610 nm

9.2 Reagent blank measurement

Set the analysis system in operation by first pumping water through the system.

Wait for stabilization of the baseline and zero the baseline.

Run the reagents buffer (pH 3,8) for distillation and gas diffusion (6.21.1), zinc sulfate solution (6.21.2), recipient solution (6.21.3), buffer solution for the final photometric determination (6.21.4.1 or 6.21.4.2), chloramine-T solution (6.21.5), or colour reagent (6.21.6), respectively, through the system and measure the increase in absorbance against water.

If the absorbance per centimetre changes by more than 0,006 cm^{-1} of cell pathlength, it is possible that either the water or the reagent solutions is contaminated. Take appropriate measures to eliminate the interference.

NOTE If the photometric detector does not give absorbance readings, the absorbance can be determined with an external absorbance-measuring spectrometer.

9.3 Checking the suitability of the flow system

9.3.1 Minimum absorbance

Run a cyanide calibration solution (6.20.5) with a concentration of 50 µg/l.

This calibration solution (6.20.5) shall produce an absorbance of at least 0,027 cm^{-1}.

See note to 9.2.

9.3.2 Recovery rates

Prepare standard solutions of hexacyanoferrate(III), and thiocyanate respectively, by placing 1 ml of potassium hexacyanoferrate(III) solution (6.23), and 1 ml of thiocyanate solution (6.22) respectively, in 100 ml measuring flasks. Make each of the solutions up to volume with sodium hydroxide solution IV (6.6).

These prepared solutions should contain:

Solution A	hexacyanoferrate(III):	100 µg/l CN, chemically bound
Solution B	thiocyanate:	1 000 µg/l CN, chemically bound

Measure the cyanide (CN) content of solutions A and B with the flow system adjusted to the free and total cyanide method.

Consider the system to be suitable for the measurement of total cyanide if the determined cyanide content of solution A is ≥90 % of the theoretical cyanide content. Lower yields (<90 %) indicate inadequate effectiveness of the UV decomposition.

For the determination of free cyanide, the previously mentioned recovery rate shall be ≤5 %.

For both determinations the determined cyanide content of solution B shall be ≤1 % of the theoretical cyanide content.

9.4 Calibration

Select the working mode of the flow system and calibrate by sequentially applying the calibration solutions (6.20.5) and the blank solution.

Prior to the calibration, zero the instrument, following the manufacturer’s instructions as long as they are in accordance with the specifications of this part of ISO 14403.

Determine the measured values from the calibration solutions.

The test conditions for the calibration and the measurement of samples (9.5) are the same. The magnitude of the measuring signal is proportional to the mass concentration of cyanide. Establish the regression line for the measuring series obtained.

Calibrate the flow system as specified in ISO 8466-1. In general, Equation (1) is appropriate (ISO 8466-1). If the linearity test described in ISO 8466-1 shows that the calibration curve is not linear, calculate the calibration curve as specified in ISO 8466-2.

The measured value for the calibration solutions, y, in terms of instrument related units (e.g. peak heights in centimetres or counts), is given by:

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

where

b is the slope of the calibration function, expressed in instrument-related units or micrograms per litre;

ρ is the mass concentration of the standard solutions, expressed in micrograms per litre;

a is the ordinate intercept, expressed in instrument-related units.

9.5 Sample measurement

Analyse the samples, pretreated according to Clause 8, in the same way as the calibration solutions with the continuous flow system.

If the mass concentrations of the samples exceed the validity of the calibrated working range, dilute the samples.

Check the validity of the calibration function after each sample series, but at least after the measurement of 10 to 20 samples, using one calibration solution each for the lower and upper part of the working range.

Recalibrate if necessary.

After completion of the measurement the system should be rinsed with water (6.1).

10 Calculation

Determine the mass concentrations of the samples using the measured values, obtained as specified in 9.4 for the calibration solutions.

Calculate ρ using Equation (2):

$$\rho = \frac{y - a}{b} \tag{2}$$

For an explanation of the symbols see 9.4.

11 Expression of results

Report the results to two significant figures at most.

EXAMPLE 1	ρ(total CN)	$2{,}9 \times 10^2$ µg/l
EXAMPLE 2	ρ(free CN)	45 µg/l

12 Test report

This test report shall contain at least the following information:

a) the test method used, together with a reference to this part of ISO 14403 (ISO 14403-2:2012);

b) all information necessary for identification of the sample;

c) the type of sample pretreatment;

d) the cyanide concentration (free or total cyanide) in micrograms per litre, expressed in accordance with Clause 11;

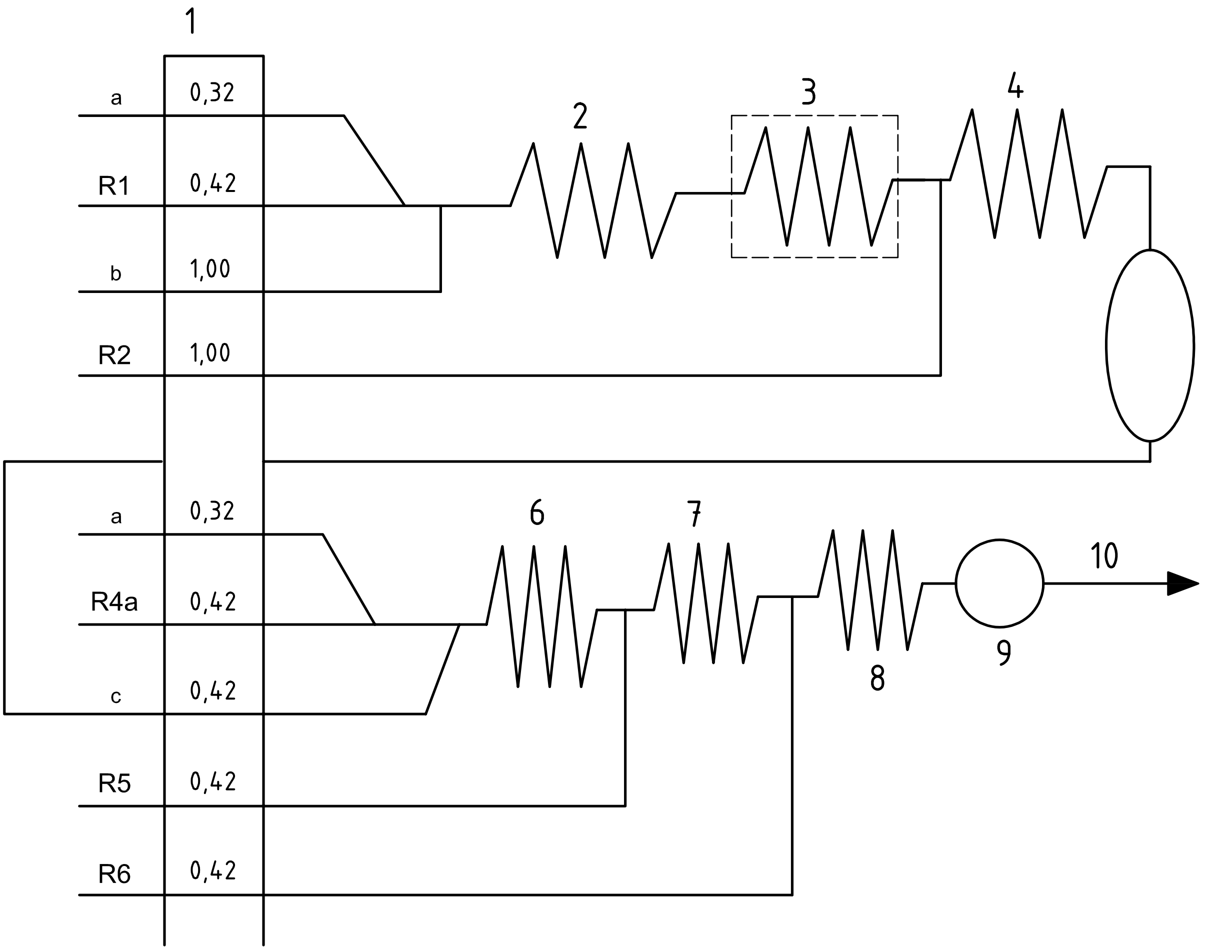
e) any special observations noted during the determination;

f) any deviations from this part of ISO 14403, which could have affected the result.

Annex A
(informative)

Examples of flow systems

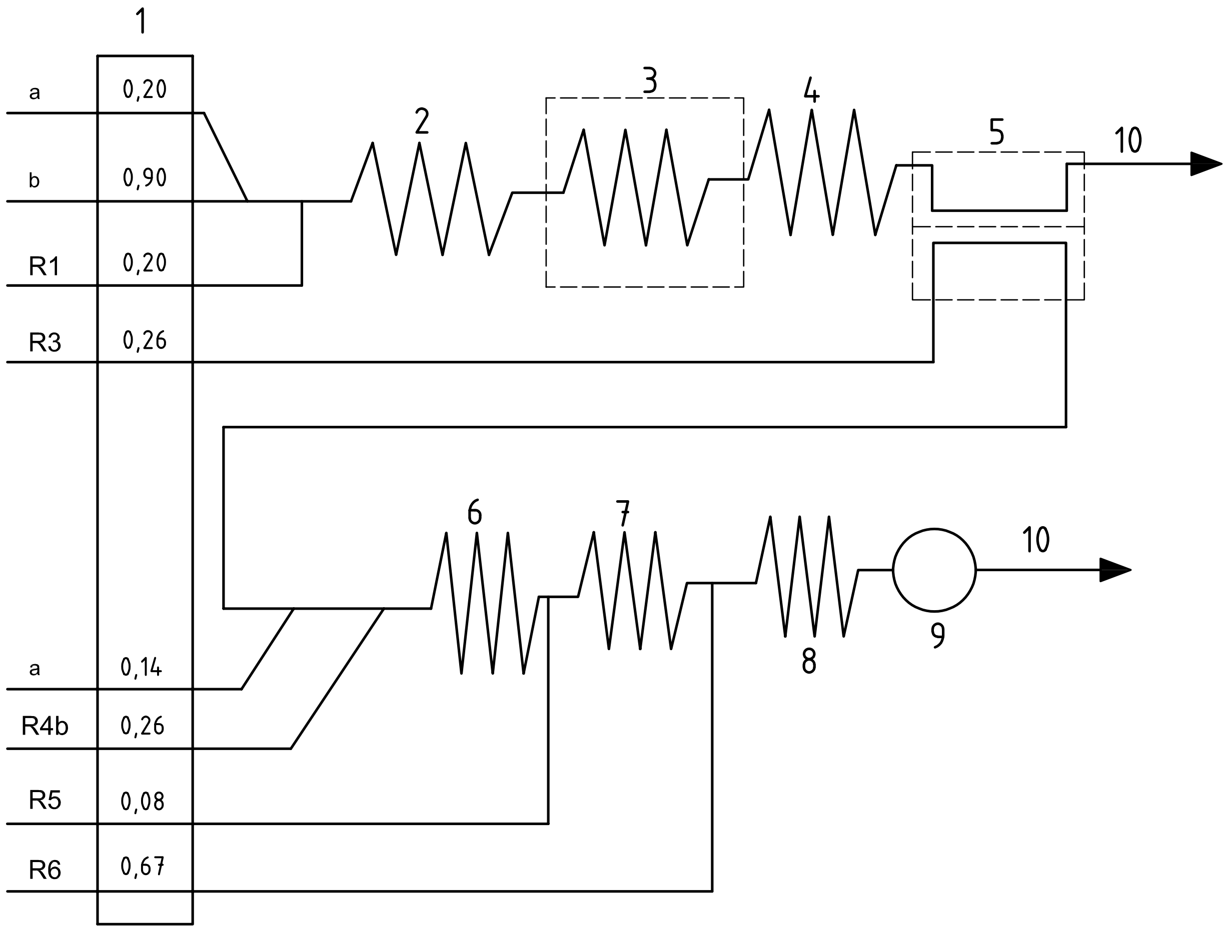
Photometric CFA configurations other than shown in Figures A.1 and A.2 may be used provided they meet the criteria of the method and achieve the quality control criteria in Clause 9.



Key

R1	buffer, pH 3,8, for distillation *and* gas diffusion method (6.21.1)
R2	$ZnSO_4$ solution (6.21.2) for free cyanide; water (6.1) for total cyanide
R4a	buffer solution for final photometric determination for distillation method (6.21.4.1)
R5	chloramine-T solution (6.21.5)
R6	colour reagent (6.21.6)
1	pump (flow rates in millilitres per minute)
2	reaction coil: length 50 cm, internal diameter 1,5 mm
3	UV decomposition unit, 351 nm
4	reaction coil: length 50 cm, internal diameter 1,5 mm
5	distillation unit, 125 °C
6	reaction coil: length 50 cm, internal diameter 1,5 m
7	reaction coil: length 50 cm, internal diameter 1,5 mm
8	heating bath: 37 °C, length 100 cm, internal diameter 2 mm
9	detector (e.g. 1 cm optical pathlength), wavelength 590 nm to 610 nm
10	waste
a	Air, segmentation gas.
b	Sample.
c	Resample.

Figure A.1 — Example of a CFA system for the spectrometric determination of free and total cyanide (10 µg/l to 100 µg/l) with a distillation procedure (according to 7.1)



Key

R1	buffer, pH 3,8, for distillation *and* gas diffusion method (6.21.1)
R3	recipient solution, *only* for gas diffusion (6.21.3)
R4b	buffer solution for the final photometric determination for gas diffusion method (6.21.4.2)
R5	chloramine-T solution (6.21.5)
R6	colour reagent (6.21.6)
1	pump (flow rates in millilitres per minute)
2	reaction coil: length 50 cm, internal diameter 1 mm
3	UV decomposition unit: 351 nm, 420 cm, internal diameter 2 mm
4	heating bath: 30 °C, length 50 cm, internal diameter 1 mm
5	gas diffusion unit
6	reaction coil: length 50 cm, internal diameter 1 mm
7	reaction coil: length 50 cm, internal diameter 1 mm
8	heating bath: 37 °C, length 100 cm, internal diameter 1 mm
9	detector (e.g. 1 cm optical pathlength), wavelength 590 nm to 610 nm
10	waste
[a]	Air, segmentation gas.
[b]	Sample.

Figure A.2 — Example of a CFA system for the spectrometric determination of free and total cyanide (10 µg/l to 100 µg/l) with gas-diffusion separation (according to 7.2)

Annex B
(normative)

Determination of the real cyanide concentration in the potassium cyanide solution

B.1 General

If KCN is used to prepare the cyanide calibration solutions (6.20.5), proceed as follows.

B.2 Additional reagents

B.2.1 *p*-Dimethylaminobenzylidene rhodanine.

B.2.2 Indicator solution.

Dissolve 0,02 g of *p*-dimethylaminobenzylidene rhodanine (B.2.1) in 100 ml of acetone (C_3H_6O).

This solution is stable for 1 week if stored in a refrigerator at 1 °C to 5 °C.

B.2.3 Silver nitrate solution, $c(\mathrm{AgNO_3})$ = 1 mmol/l.

B.3 Determination of cyanide concentration in potassium cyanide solution

Pipette 10 ml of potassium cyanide solution (6.20.2) into a beaker. Add 0,25 ml of indicator solution (B.2.2).

Titrate with the silver nitrate solution (B.2.3) until the colour changes from yellow to yellow-red (consumption V_1).

Calculate the cyanide concentration in the potassium cyanide solution using Equation (B.1):

$$\rho_{\mathrm{CN}} = \frac{V_1 c\left(\mathrm{AgNO_3}\right) M_{\mathrm{2CN}}}{V} \qquad \text{(B.1)}$$

where

ρ_{CN}	is the cyanide contentration in the potassium cyanide solution (6.20.2) in milligrams per litre;
V_1	is the volume of silver nitrate solution (B.2.3) consumed, in millilitres;
$c(\mathrm{AgNO_3})$	is the concentration of silver nitrate solution in millimoles per litre;
M_{2CN}	is the molar mass of 2CN (= 52 g/mol);
V	is the volume of the potassium cyanide solution (6.20.2) in millilitres.

Annex C
(informative)

Example for the determination of total cyanide and free cyanide by continuous flow analysis (CFA) with gas diffusion and amperometric detection

C.1 Amperometric detection

Amperometric detection may be used as an alternative to the photometric detection method. According to this detection, the use of toxic and hazardous substances is avoided and the sensitivity of the method for the determination of free and total cyanide is improved to the lower microgram per litre level (e.g. a typical value for lower limit for total cyanide is 3 µg/l).

Figure C.1 is a flow diagram for the determination of free and total cyanide with gas diffusion and amperometric detection. An appropriate combination of the distillation method with an amperometric detection is also applicable.

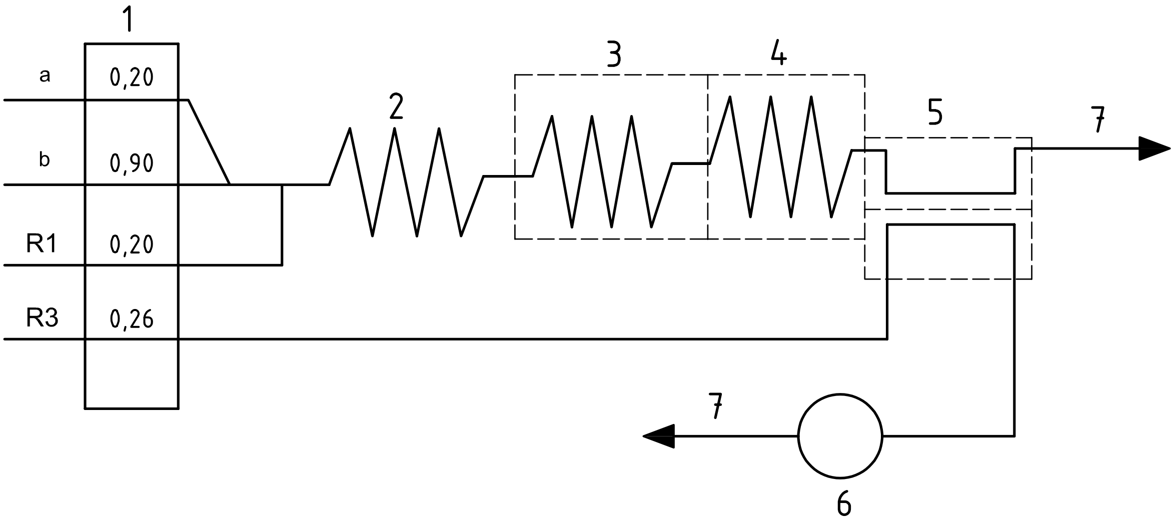
The amperometric detector may consist of:

- working electrode: silver;
- reference electrode: Ag|AgCl;
- auxiliary electrode: platinum;
- potential applied: 0,0 V.

Other amperometric CFA configurations may be used provided they meet the criteria of the method and achieve the quality control criteria in Clause 9.

C.2 Additional bibliography for the application of amperometric detection

See [7] to [10] and [12].



Key

R1	buffer, pH 3,8, for distillation *and* gas diffusion method (6.21.1)
R3	recipient solution for gas diffusion (6.21.3)
1	pump (flow rates in millilitres per minute)
2	reaction coil: length 50 cm, internal diameter 1 mm
3	UV decomposition unit: 351 nm, length 420 cm, internal diameter 2 mm
4	heating bath: 30 °C, length 50 cm, internal diameter 1 mm
5	gas diffusion unit
6	amperometric detector (specification see C.1)
7	waste
a	Air, segmentation gas.
b	Sample.

Figure C.1 — Example of a CFA system for the amperometric determination of free and total cyanide (10 µg/l to 100 µg/l) gas-diffusion separation (according to C.1)

Annex D
(informative)

Performance data

An interlaboratory trial for flow analysis systems as described in Clause 7 was carried out in Spring 2009. The results are shown in Tables D.1 and D.2.

Table D.1 — Statistical data for the determination of free cyanide by CFA (in accordance with ISO 5725-2[13])

Sample	Matrix[a]	l	n	o %	X µg/l	$\overline{\overline{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
1	Drinking water	21	92	1,1	30	27,9	92,9	2,21	7,9	0,45	1,6
2	Drinking water	21	93	0,0	25	25,3	101,1	2,12	8,4	0,53	2,1
3	Surface water	21	93	0,0	45	43,6	96,8	2,73	6,3	0,55	1,3
4	Surface water	20	89	4,3	30	30,8	102,6	2,32	7,5	0,45	1,5
5	Waste water	18	82	11,8	60	55,0	91,6	3,43	6,2	0,82	1,5
6	Waste water	20	90	3,2	25	24,0	96,1	2,80	11,7	0,61	2,5

l	number of laboratories after outlier rejection
n	number of individual test results after outlier rejection
o	percentage of outliers
X	assigned value
$\overline{\overline{x}}$	overall mean of results (without outliers)
η	recovery rate
s_R	reproducibility standard deviation
$C_{V,R}$	coefficient of variation of reproducibility
s_r	repeatability standard deviation
$C_{V,r}$	coefficient of variation of repeatability

[a] Origin of samples:

samples 1, 2, spiked, City of Berlin

samples 3, 4, spiked, Landwehrkanal, City of Berlin

samples 5, 6, spiked, Waster Water Plant, Berlin-Ruhleben

Table D.2 — Statistical data for the determination of total cyanide by CFA (in accordance with ISO 5725-2[13])

Sample	Matrix[a]	l	n	o %	X µg/l	$\bar{\bar{x}}$ µg/l	η %	s_R µg/l	$C_{V,R}$ %	s_r µg/l	$C_{V,r}$ %
1	Drinking water	21	93	9,7	30	28,8	96,1	1,64	5,7	0,57	2,0
2	Drinking water	20	91	13,3	50	47,5	94,9	2,32	4,9	0,78	1,6
3	Drinking water	20	91	9,9	28	25,8	92,3	2,00	7,7	0,50	1,9
4	Surface water	18	86	14,9	45	45,2	100,4	1,72	3,8	0,62	1,4
5	Surface water	21	96	7,7	55	52,9	96,2	2,65	5,0	1,05	2,0
6	Surface water	19	88	16,2	53	48,9	92,3	3,75	7,7	0,69	1,4
7	Waste water	18	84	20,0	60	57,7	96,1	3,00	5,2	0,68	1,2
8	Waste water	19	88	16,2	63	55,9	88,7	4,09	7,3	0,67	1,2
9	Waste water	20	93	11,4	45	39,9	88,7	4,14	10,4	0,75	1,9

Explanation of symbols see Table D.1

[a] Origin of the samples:

samples 1, 2, 3, spiked, City of Berlin

samples 4, 5, 6, spiked, Landwehrkanal, City of Berlin

samples 7, 8, 9, spiked, Waste Water Plant, Berlin-Ruhleben

NOTE Investigations have shown that the addition of potassium hexacyanoferrate(III) to the particular waste water matrix used in the interlaboratory trial on *total cyanide* was not fully recovered. The recovery actually achieved depends on the time delay between preparation of the spiked solution and the moment of analysis. The samples for the total cyanide test were spiked with hexacyanoferrate(III). It is assumed that a part of this reagent was reduced by components of the matrix and that therefore a negative bias could have arisen.

This finding explains the lower recovery of total cyanide in waste water samples 8 and 9 as observed in the interlaboratory trial by several participants.

These observations are expected to be of minor importance for practical purposes, because usually waste water does not contain hexacyanoferrate(III) in measurable concentrations. It can be also assumed that the possible reduction of hexacyanoferrate(III) by components of the waste water has finished when the sample is taken.

Bibliography

[1] NEN 6655,$^{1)}$ *Water en bodem — Fotometrische bepaling van het totale gehalte aan cyanide en het gehalte aan vrij cyanide met behulp van een doorstroomanalysesysteem* [Water and soil — Photometric determination of the content of total and free cyanide by continuous flow analysis]

[2] Kelada, N.P. Automated direct measurements of total cyanide species and thiocyanates, and their distribution in waste water and sludge. *J. Water Pollut. Contr. Fed.* 1989, **61**, pp. 350–356

[3] Meeussen, J.C.L., Temminghoff, E.J.M., Keizer, M.G., Novozamsky, I. Spectrophotometric determination of total cyanide, iron-cyanide complexes and thiocyanate in water by a continuous flow system. *Analyst* 1989, **114**, pp. 959–963

[4] TAUW Infra Consult BV. *Laboratory study on cyanide determination*, Project No 3162052, Deventer, 1992-05

[5] Berman, R., Christmann, D., Renn, C. Automated determination of weak acid dissociable and total cyanide without thiocyanate interference. *Am. Environ. Lab.* 1993, **5**, pp. 32–34

[6] Sekerka, I., Lechner, J.F. *Determination of cyanide by continuous flow isothermal distillation with amperometric detection.* National Water Research Institute of Canada, NWRI Report, pp. 91–108

[7] Pihlar, B., Kosta, L., Hristovski, B. Amperometric determination of cyanide by use of a flow-through electrode. *Talanta* 1979, **26**, pp. 805–810

[8] Nagy, A., Nagy, G. Amperometric air gap cell for the measurement of free cyanide. *Anal. Chim. Acta* 1993, **283**, pp. 795–802

[9] Nikolic, S., Milosavljevic, E., Hendrix, J., Nelson, J. Flow injection amperometric determination of cyanide on a modified silver electrode. *Analyst* 1992, **117**, pp. 47–50

[10] Eaton, A.D., Clesceri, L.S., Greenberg, A.E. Method 4500-CN^- Cyanide. In: *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association, 1999

[11] Sebrowski, J., Ode, R. Method comparison and evaluation for the analysis of weak acid-dissociable cyanide. *Environ. Sci. Technol.*1997, **31**, pp. 52–57

[12] ASTM D7511, *Standard test method for total cyanide by segmented flow injection analysis, in-line ultraviolet digestion and amperometric detection*

[13] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

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1) Also suitable for the determination of free and total cyanide in soil and sludge.

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