

# INTERNATIONAL STANDARD

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**Water quality — Determination of nitrite  
nitrogen and nitrate nitrogen and the sum  
of both by flow analysis (CFA and FIA) and  
spectrometric detection**  
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*Qualité de l'eau — Détermination de l'azote nitreux et de l'azote nitrique  
et de la somme des deux par analyse en flux (CFA et FIA) et détection  
spectrométrique*

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## Foreword

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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.

International Standard ISO 13395 was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical, biochemical methods*.

Annexes A, B, C and D of this International Standard are for information only.

## Introduction

Methods using flow analysis enable wet chemistry procedures to be automatized and are particularly suitable for the processing of many analytes in water in large series of samples at a high analysis frequency (up to 100 samples per hour).

A differentiation is made between flow injection analysis (FIA) [1][2] and continuous flow analysis (CFA) [3]. Both methods share the feature of an automatic dosage of the sample into a flow system (manifold) where the analytes in the sample will react with the reagent solutions on their way through the manifold. The sample preparation may be integrated in the manifold. The reaction product is measured in a flow detector (e.g. flow photometer).

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# Water quality — Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis (CFA and FIA) and spectrometric detection

## 1 Scope

This International Standard specifies a method for the determination of nitrite(N) (see note 2), nitrate(N) or the sum of both [nitrite/nitrate(N)], in various types of waters (such as ground, drinking, surface, and waste waters) in mass concentrations ranging from 0,01 mg/l to 1 mg/l for nitrite(N) and from 0,2 mg/l to 20 mg/l for nitrite/nitrate(N), both in the undiluted sample. The range of application can be changed by varying the operating conditions.

### NOTES

1 Seawater may be analysed with changes in respect to sensitivity and adaptation of the carrier solution and calibration solutions to the salinity of the samples.

2 The following concise terms are used in the text of this International Standard:

<b>nitrite(N):</b>	(mass concentration of) nitrite, expressed as nitrogen
<b>nitrate(N):</b>	(mass concentration of) nitrate, expressed as nitrogen
<b>nitrite/nitrate(N):</b>	(mass concentration of) the sum of nitrite(N) and nitrate(N)

## 2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this International Standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 3696:1987, *Water for analytical laboratory use — Specification and test methods*.

ISO 5667-1:1980, *Water quality — Sampling — Part 1: Guidance on the design of sampling programmes*.

ISO 5667-2:1991, *Water quality — Sampling — Part 2: Guidance on sampling techniques*.

ISO 5667-3:1994, *Water quality — Sampling — Part 3: Guidance on the preservation and handling of samples*.

ISO 6777:1984, *Water quality — Determination of nitrite — Molecular absorption spectrometric method*.

## 3 Principle

### 3.1 Sum of nitrite(N) and nitrate(N), nitrite/nitrate(N)

With flow injection analysis (FIA), the sample is fed into a continuously flowing buffer solution (carrier stream) by means of an injection valve, or, with continuous flow analysis (CFA) being applied, it is continuously mixed with this buffer solution. Nitrate in the sample is reduced with metallic cadmium to nitrite[4]. Then, a phosphoric acid reagent solution that is also flowing continuously is admixed. Nitrite that is initially present and nitrite resulting from the reduction of nitrate will diazotize sulfanilamide in acid solution to the diazonium salt which is then coupled with *N*-(1-naphthyl)ethylenediamine to form a red dye [5] [6][7].

Waste containing cadmium in liquid or solid form shall be removed appropriately.

### 3.2 Nitrite(N)

Nitrite(N) is determined, omitting the cadmium reduction, directly by the above-mentioned diazotization and coupling reaction [8]. The chemical reactions of the nitrite are identical with those in the manual procedure (see ISO 6777).

### 3.3 Nitrate(N)

The mass concentration of nitrate(N) is given by the difference: nitrite/nitrate(N) – nitrite(N).

## 4 Reagents

If not stated otherwise, only reagents of recognized analytical grade and water according to grade 1 of ISO 3696 shall be used. The blank value of the reagents shall be checked regularly (see 9.3).

**4.1 Phosphoric acid** ( $\text{H}_3\text{PO}_4$ ),  $\rho = 1,71$  g/ml.

**4.2 Sulfanilamide** (4-aminobenzenesulfonamide,  $\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$ ).

**4.3 *N*-(1-naphthyl)ethylenediaminedihydrochloride** [*N*-(1-naphthyl)-1,2-diaminoethanedihydrochloride],  $\text{C}_{12}\text{H}_{16}\text{N}_2\text{Cl}_2$ .

**4.4 Sodium nitrite** ( $\text{NaNO}_2$ ), dried to constant mass at, for example, 150 °C.

**4.5 Potassium nitrate** ( $\text{KNO}_3$ ), dried to constant mass at, for example, 150 °C.

**4.6 Imidazole** ( $\text{C}_3\text{H}_4\text{N}_2$ ), for synthesis, or alternatively **ammonium chloride** ( $\text{NH}_4\text{Cl}$ ), dried to constant mass at 105 °C (see note 3).

**4.7 Hydrochloric acid I** (HCl) concentrated,  $w = 37$  %.

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

**4.9 Copper sulfate solution I**,  $\rho(\text{CuSO}_4 \cdot 5\text{H}_2\text{O}) = 2,5$  g/l; this solution is stable.

**4.10 Copper sulfate solution II**,  $\rho(\text{CuSO}_4 \cdot 5\text{H}_2\text{O}) = 20$  g/l; this solution is stable.

### 4.11 Polyethyleneglycol dodecyl ether

$[\text{HO} - (\text{CH}_2\text{CH}_2 - \text{O})_n - \text{C}_{12}\text{H}_{21}]$ , surfactant,  $\theta = 33$  °C to 41 °C, solution,  $w = 30$  %.

The solution is stable for approximately 4 weeks.

**4.12 Cadmium granulate (Cd)**, grain size, for example 0,3 mm to 1,5 mm (a minimum reduction capacity of 90 % shall be reached; see 5.1 and 5.2).

**4.13 Imidazole stock solution**,  $c = 0,25$  mol/l.

Dissolve, in a beaker of nominal capacity 1 litre, 17,0 g of imidazole (4.6) in approximately 900 ml of water.

While stirring with a magnetic stirrer, add hydrochloric acid I (4.7) and adjust, with the help of a pH electrode, the pH to 7.5.

Transfer to a volumetric flask, of nominal capacity 1 000 ml, and dilute to volume with water.

The solution is stable for 4 weeks if kept in a brown glass bottle at room temperature.

### 4.14 Buffer solution.

Mix 100 ml of the imidazole stock solution (4.13) with 100  $\mu\text{l}$  of copper sulfate solution I (4.9).

Prepare the solution freshly before use.

NOTE 3 Alternatively an ammonium buffer solution can be used, for example:

85 g of ammonium chloride ( $\text{NH}_4\text{Cl}$ ) should be dissolved in water and diluted to a volume of 1 000 ml, and the pH should be adjusted to approximately 7,5.

**4.15 Carrier or dilution solutions**, C and B in figures A.1, B.1 and C.1.

Table 1 shows some well-known options for preparing these solutions.

Prepare the solutions, containing the surfactant (4.11), freshly before use.

Prior to use, solutions C and B for FIA shall be degassed, for example by membrane filtration (vacuum).

**Table 1 — Options for preparing the solutions C and B in figures A.1, B.1 and C.1**

Method	Parameter	Content of solution C (see figures A.1, B.1 and C.1)	Content of solution B (see figures A.1, B.1 and C.1)
FIA	Nitrite	Water	Water <sup>1)</sup> Buffer (4.14) <sup>1)</sup> No solution B <sup>1)</sup>
	Nitrite/nitrate	Buffer (4.14)	Buffer (4.14)
CFA	Nitrite	"C" is not necessary	Water <sup>1),2)</sup> Buffer (4.14) <sup>1),2)</sup> No solution B <sup>1),2)</sup>
	Nitrite/nitrate	"C" is not necessary	Buffer (4.14) <sup>2)</sup>

1) Three different alternatives.

2) If, in the case of CFA, water or buffer (4.14) is used as solution B and 1 ml of surfactant (4.11) per 1 litre of solution is added. If solution B is omitted, the surfactant (4.11) has to be added to the reagent solution R<sub>1</sub> (4.17, see figures A.1, B.1 and C.1).

#### 4.16 Buffered copper sulfate solution.

Mix 20 ml of the copper sulfate solution II (4.10) and 20 ml of the imidazole stock solution (4.13) in a beaker of nominal capacity 50 ml.

Prepare the solution freshly before use.

#### 4.17 Reagent solution, R<sub>1</sub> (in figures A.1, B.1 and C.1).

Dissolve, in a volumetric flask of nominal capacity 500 ml, 5 g of sulfanilamide (4.2), 0,5 g of *N*-(1-naphthyl)ethylenediaminedihydrochloride (4.3) in water, add 50 ml of phosphoric acid (4.1), and dilute to volume.

Stored in a brown glass bottle, the solution is stable for at least one week.

NOTE 4 For the preparation of this reagent solution, hydrochloric acid (4.7, 4.8) may be used instead of phosphoric acid, provided equivalent performance characteristics are obtained. The solutions of sulfanilamide (4.2) and *N*-(1-naphthyl)ethylenediaminedihydrochloride (4.3) can

also be made separately and dosed into the equipment by different lines.

Prior to use, solution R<sub>1</sub> for FIA shall be degassed, for example by membrane filtration (vacuum).

#### 4.18 Nitrite(N) stock solution, ρ<sub>N</sub> = 100 mg/l.

Dissolve 492,6 mg of sodium nitrite (4.4) in water, in a volumetric flask of nominal capacity 1 000 ml, and dilute to volume.

This solution is stable for at least 2 weeks if kept in a stoppered glass bottle at 4 °C.

#### 4.19 Nitrite(N) solution I, ρ<sub>N</sub> = 20 mg/l.

Pipette 20 ml of the stock solution (4.18) into a volumetric flask of nominal capacity 100 ml, and dilute to volume with water.

Prepare the solution freshly before use.

#### 4.20 Nitrite(N) solution II, ρ<sub>N</sub> = 1 mg/l.

Pipette 1 ml of the stock solution (4.18) into a volumetric flask of nominal capacity 100 ml, and dilute to volume with water.

Prepare the solution freshly before use.

#### 4.21 Nitrate(N) solution I, ρ<sub>N</sub> = 200 mg/l.

Dissolve 144,4 mg of potassium nitrate (4.5) in water, in a volumetric flask of nominal capacity 100 ml, and dilute to volume.

The solution is stable for at least 1 month.

#### 4.22 Nitrate(N) solution II, ρ<sub>N</sub> = 20 mg/l.

Pipette 10 ml of the nitrate solution I (4.21) into a volumetric flask of nominal capacity 100 ml, and dilute to volume with water.

Prepare the solution freshly before use.

#### 4.23 Calibration solutions

The calibration solutions are prepared by diluting the respective solutions described in 4.19 to 4.22. These solutions may be used in any combination for the preparation of calibration solutions for the determination of nitrite(N), nitrite/nitrate(N) or nitrate(N). At least five calibration standards per working range are recommended.

The following working ranges are provided:

<b>For nitrite(N):</b>	Working range II: 0,01 mg/l to 0,1 mg/l	Proceed, for example, as recommended in tables 2 and 3 (if 10 standards are used).
	Working range I: 0,1 mg/l to 1,0 mg/l	All calibration solutions shall be prepared immediately before measurement.
<b>For nitrite/nitrate(N):</b>	Working range II: 0,2 mg/l to 2 mg/l	
	Working range I: 2 mg/l to 20 mg/l	

**Table 2 — Preparation of the calibration solutions for nitrite(N)**

Nitrite(N) concentration mg/l	Volume of nitrite(N) solution II (4.20) diluted with water to 100 ml ml	Nitrite(N) concentration mg/l	Volume of nitrite(N) solution I (4.19) diluted with water to 200 ml ml
0,01	1	0,1	1
0,02	2	0,2	2
0,03	3	0,3	3
0,04	4	0,4	4
0,05	5	0,5	5
0,06	6	0,6	6
0,07	7	0,7	7
0,08	8	0,8	8
0,09	9	0,9	9
0,10	10	1,0	10

**Table 3 — Preparation of the calibration solutions for nitrite/nitrate(N)**

Nitrate(N) concentration mg/l	Volume of nitrate(N) solution II (4.22) diluted with water to 100 ml ml	Nitrate(N) concentration mg/l	Volume of nitrate(N) solution I (4.21) diluted with water to 100 ml ml
0,2	1	2	1
0,4	2	4	2
0,6	3	6	3
0,8	4	8	4
1,0	5	10	5
1,2	6	12	6
1,4	7	14	7
1,6	8	16	8
1,8	9	18	9
2,0	10	20	10



## 5 Apparatus

Usual laboratory apparatus and

**5.1 Flow injection system (FIA)**, normally comprising the following components (see figure A.1).

- Reagent reservoirs.
- Low pulsation pump.
- Suitable pump tubing, if required.
- Sample injector with an injection volume of 10  $\mu$ l to 300  $\mu$ l.
- If nitrite/nitrate(N) or nitrate(N) is to be determined: cadmium reductor with a minimum reduction efficiency of 90 %, e.g. packed cadmium column with granulate (4.12), of internal diameter 4,0 mm, for example, and minimum length 5 cm.
- Transport tubes and reaction coils of internal diameter 0,5 mm to 0,8 mm, with tube connections and T-connections of chemically inert plastics.
- Dialysis cell, if required, with, for example, a cellulose membrane, suitable for the predilution of the sample or the elimination of interfering compounds.

NOTE 5 The dialysis cell should be placed after the injector (see figure A.1). The carrier solution C may serve as a donor and also as a recipient solution. The flow rates of these two streams should be equal.

- Photometric detector with flow cell, wavelength range 520 nm to 560 nm.
- Recording unit (e.g. strip chart recorder, integrator or printer/plotter). In general, peak height signals are evaluated.
- Autosampler, if required.

**5.2 Continuous flow analysis (CFA)**, normally comprising the following components (see figure B.1):

- Autosampler or any other device allowing a reproducible application of the sample.
- Reagent reservoirs.
- Low pulsation pump with suitable, chemically inert pump tubes.

- If nitrite/nitrate(N) or nitrate(N) is to be determined: cadmium reductor with a minimum reduction efficiency of 90 %, e.g. cadmium tube<sup>[10]</sup> of internal diameter 1,1 mm or cadmium column (see 5.1).
- Manifold with highly reproducible gas-bubble feeding (nitrogen is recommended), sample and reagent feeding, with appropriate transport systems and connection assemblies of chemically inert plastics or metal. The application of the cadmium reductor requires oxygen-free gas. Before entering the cadmium column the flow stream has to be debubbled, if air is used for segmenting the flow stream.
- Dialysis cell, if required, with, for example, a cellulose membrane, suitable for the predilution of the sample or the elimination of interfering compounds.
- Photometric detector with flow cell, wavelength range 520 nm to 560 nm.
- Recording unit (e.g. strip chart recorder, integrator or printer/plotter). In general, peak height signals are evaluated.

NOTE 6 Figure B.1 describes two flow systems with an internal diameter of approximately 1 mm. Figure C.1 shows a flow system (for the determination of nitrite/nitrate(N) with an internal diameter of 2,2 mm.

**5.3 Volumetric flasks**, of nominal capacity 100 ml, 500 ml and 1 000 ml.

**5.4 Pipettes**, of nominal capacity 100  $\mu$ l, 1 ml to 10 ml, 20 ml, 50 ml and 100 ml.

**5.5 Beakers**, of nominal capacity 25 ml, 50 ml and 1 litre.

**5.6 Syringe**, of nominal capacity 25 ml.

**5.7 Membrane filter assembly**, with membrane filters of pore size 0,45  $\mu$ m.

**5.8 pH electrode**.

## 6 Checking the flow system

A calibration solution (4.23) with a nitrite(N) concentration of 0,05 mg/l, or a nitrate(N) concentration of 1 mg/l, measured in the system (FIA or CFA, respectively) which is adjusted to the lower working range, should give an absorbance of at least 0,04 per 10 mm path length.

NOTE 7 If the photometric detector (see 5.1 or 5.2) does not give any absorbance readings, the absorbance may then be determined by comparing with an external absorbance-measuring photometer.

## 7 Sampling and sample preparation

Before use, all containers coming into contact with the sample shall be cleaned thoroughly with water and shall be rinsed several times with the samples. (See ISO 5667-3.)

If only nitrite is to be determined, collect the sample in a glass or polyethylene bottle and analyse it at once. Proceed in the same way, if nitrate is to be determined and if the nitrite(N) concentration is at least in the same working range as the nitrate(N) concentration.

If only nitrite/nitrate(N) is to be determined, or nitrate(N) in samples with nitrite(N) concentrations below the respective nitrite/nitrate(N) working range, sample containers made of polyalkylene and polytetrafluoroethylene (PTFE) are also suitable. Acidify these samples with hydrochloric acid (4.7 and 4.8) to approximately pH 2, store at 2 °C to 5 °C and analyse within 24 h.

As an exception, the samples may be stored in the freezer at approximately – 20 °C for 8 d, provided the applicability of this preservation has previously been checked.

Provided that significant loss of nitrate/nitrite is excluded, filtration of the sample is necessary if it contains particulate matter of a particle size > 0,1 mm (risk of clogging the transport tubes).

## 8 Interferences

### 8.1 Interferences of a general nature

Nitrite cannot be stabilized and shall immediately be analysed (see clause 7).

Particulate matter present in the sample may lead to clogging of the transport tubes and will interfere with the photometric measurement. Larger particles (> 0,1 mm) can be removed by membrane filtration, smaller particles can be removed suitably by dialysis.

In order to remove an interfering organic matrix (compounds with a higher molar mass), the sample may be dialysed, if need be in an online process. As an alternative, the sample may be filtered through activated carbon, provided changes of the nitrite or

nitrate mass concentration in the sample can be ruled out.

The self-absorption of the sample can be compensated by measuring, in addition to the sample signal (9.5), 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 (according to clause 10).

Prior to the measurement, dilute samples with a total salt concentration of > 30 g/l.

### 8.2 Interference with the reduction of nitrate to nitrite

Interferences may occur if the sample, after the admixture of the buffer solution, does not reach a pH of 6,5 to 7,5. This may happen with strongly acid, basic or buffered samples. In this event, the sample shall, prior to the measurement, be treated appropriately with bases or acids so as to reach the above mentioned pH in the solution streaming through the cadmium reductor (see 5.1 and 5.2).

### 8.3 Interferences with the formation of the azo dye

Surfactant concentrations > 10 mg/l may interfere with the determination; this interference can be prevented by a dialysis of the sample, if need be in the online process. (See [9] in annex D.)

## 9 Procedure

### 9.1 Preparation, activation and checking of the cadmium reductor

#### 9.1.1 Cadmium column with granulate

Place a sufficient quantity of the cadmium granulate (4.12) to fill the column (see 5.1) in a beaker (5.5) of nominal capacity 25 ml. Stir with hydrochloric acid II (4.8) until the surface of the granulates shows a metallic shine.

Remove the acid by washing with water.

Decant the water and stir the granulate twice for approximately 2 min with copper sulfate solution II (4.10). The surface of the granulate will turn black.

Decant and carefully wash with water.

Fill the column with the granulate, avoiding air bubbles and large cavities, and stopper the ends of the column (e.g. with glass wool).

Assemble the column in the flow system, and activate the reductor by applying nitrate(N) solution I (4.21) or II (4.22) three times.

Repeatedly measure the calibration solution (4.23) which has, for the respective working range, the highest permissible nitrite/nitrate(N) concentration (2 mg/l or 20 mg/l, respectively), until stable results are obtained.

NOTE 8 The cadmium column can be stored, free from air bubbles, in the imidazole stock solution (4.13). Prior to re-use, the column should be stabilized and activated as described above.

### 9.1.2 Cadmium tube

Using the syringe (5.6), aspirate approximately 5 ml of the buffered copper sulfate solution (4.16) into the cadmium tube (see 5.2) and allow to react for 5 min. Repeat the procedure, avoiding air bubbles.

Using the syringe, aspirate approximately 20 ml of imidazole stock solution (4.13) through the tube and allow to react, avoiding air bubbles.

Assemble the column in the flow system, stabilize and activate as described in 9.1.1.

NOTE 9 The cadmium tube can be stored, free from air bubbles, in the imidazole stock solution (4.13). Prior to measurement, stabilize or treat, if required, (see 9.1.3) with buffered copper sulfate solution (4.16).

### 9.1.3 Checking the reduction capacity [applies to the nitrate(N) or the nitrite/nitrate(N) determination only]

Sequentially analyse a nitrate(N) and a nitrite(N) solution with a nitrogen mass concentration of 2 mg/l each for the nitrite/nitrate(N) working range II, or of 20 mg/l each for the nitrite/nitrate(N) working range I, and compare the measured values obtained.

Transport reagent solutions (4.17) through the flow system with a built-in cadmium reductor and allow the baseline to stabilize.

For example, to check the nitrite/nitrate(N) working range I, use the nitrite(N) solution I (4.19) and the nitrate(N) solution II (4.22).

If the measured value for nitrate(N) is less than 90 % of the measured nitrite(N) value, appropriate measures according to 9.1.1 and 9.1.2 shall be taken to obtain a reduction capacity of at least 90 %.

Check the reduction capacity again, prior to the analysis of each series of samples.

## 9.2 Preparation for measurement

Assemble the flow system according to the method of determination desired (FIA or CFA, determination of nitrite/nitrate(N) or nitrite(N); see figures A.1, B.1 and C.1).

Prior to measurement of nitrite, continuously run the reagent solutions for approximately 10 min through the flow system. For the determination of nitrite/nitrate(N), run the reagent solutions through the system for approximately 10 min without the Cd reductor and after that for approximately 10 min with the Cd reductor. Record and zero the base absorbance.

The system is ready when the baseline no longer shows any drift. A satisfactory signal-noise relation should be obtained. Then perform the reaction steps in the sequence of 9.3 to 9.5.

## 9.3 Monitoring the blank of the reagents

Allow the baseline to stabilize.

Instead of the buffer solution B (4.15) and the reagent solution R<sub>1</sub> (4.17) transport water for 2 min and record changes in the measuring signal.

If the absorbance changes by more than 0,015 per 10 mm path length, either the water being used or the reagent solutions may be contaminated. Take appropriate measures to eliminate the interference.

Then transport the reagent solutions again.

## 9.4 Calibration

Select the respective working range for nitrite(N) or nitrite/nitrate(N) and prepare the calibration solutions (4.23) for the selected working range. Each working range requires its own calibration.

Calibrate by sequentially adding the calibration solutions and the blank solution.

Prior to the calibration, zero the instrument, if necessary following the manufacturer's instructions.

Determine the measured values from the calibration solutions used while following the manufacturer's instructions, as long as they do not contradict the specifications of this International Standard.

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 nitrite(N) or