
**Water quality — Determination of
nitrate in water using small-scale
sealed tubes —**

**Part 2:
Chromotropic acid colour reaction**

*Qualité de l'eau — Détermination du nitrate dans l'eau par la
méthode à petite échelle en tubes fermés —*

Partie 2: Réaction colorimétrique à l'acide chromotropique

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

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Foreword

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

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

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

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

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

A list of all parts in the ISO 23696 series can be found on the ISO website.

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

Introduction

Nitrate plays an important role in nature and are naturally present in ecosystems. The main anthropic sources are agriculture (fertilizers and manure) and industrial sources such as food, chemical, paper companies, etc. and as a result of natural nitrification processes of reduced forms of nitrogen. In turn, nitrate can be converted to nitrogen gas by denitrifying bacteria. This process takes on strategic importance within biological water purification plants.

The presence of significant concentrations of nitrate in surface and bathing water, drinking water and wastewater can pose a risk to human health and/or environment.

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Water quality — Determination of nitrate in water using small-scale sealed tubes —

Part 2: Chromotropic acid colour reaction

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices.

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

1 Scope

This document specifies a method for the determination of nitrate as $\text{NO}_3\text{-N}$ in water of various origin such as natural water (including groundwater, surface water and bathing water), drinking water and wastewater, in a measuring range of concentration between 0,20 mg/l and 30 mg/l of $\text{NO}_3\text{-N}$ using the small-scale sealed tube method. Different measuring ranges of small-scale sealed tube methods can be required.

The measuring ranges can vary depending on the type of the small-scale sealed tube method of different manufacturers.

It is up to the user to choose the small-scale sealed tube test with the appropriate application range or to adapt samples with concentrations exceeding the measuring range of a test by preliminary dilution.

NOTE 1 The results of a small-scale sealed tube test are most precise in the middle of the application range of the test.

Manufacturers' small-scale sealed tube methods are based on chromotropic colour reaction, depending on the typical operating procedure of the small-scale sealed tube used, see [Clause 9](#).

NOTE 2 Laws, regulations or standards can require that the data is expressed as NO_3 after conversion with the stoichiometric conversion factor 4,426 81 in [Clause 11](#).

NOTE 3 In the habitual language, use of sewage treatment and on the displays of automated sealed-tube test devices, NO_3 without indication of the negative charge has become the common notation for the parameter nitrate and especially for the parameter nitrate-N. This notation is adopted in this document even though not being quite correct chemical nomenclature.

2 Normative references

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

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

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

ISO 5667-10, *Water quality — Sampling — Part 10: Guidance on sampling of waste water*

ISO 8466-1, *Water quality — Calibration and evaluation of analytical methods — Part 1: Linear calibration function*

ISO 8655-2, *Piston-operated volumetric apparatus — Part 2: Pipettes*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

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

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <https://www.electropedia.org/>

3.1
small-scale sealed tube
glass tube commercially available on the market, prefilled by the manufacturer with reagent(s), to develop a colour to be read by a photometer or a spectrophotometer

4 Principle

The nitrate content of the sample to be analysed reacts in acidic solution with chromotropic acid, which is read at maximum absorption by a photometer or a spectrophotometer.

The method serves to obtain the concentration of nitrate present in the sample.

5 Interferences

Typical interfering substances are metals, chloride and organic load [chemical oxygen demand (COD)]. Examples of maximum compatible concentrations are listed in [Table 1](#).

Every manufacturer of small-scale sealed tube shall provide information about interference levels above which the ion interferes. The concentration of interfering substances can depend on the ratio of sampled volume and pre-dosed reagents in the small-scale sealed tube.

Table 1 — Examples of interfering ions and organic load

| Substances | Maximum tolerable concentrations |
|------------------|----------------------------------|
| | mg/l |
| K ⁺ | 500 |
| Na ⁺ | 500 |
| Cl [−] | 500 |
| COD | 200 |
| Ag ⁺ | 100 |
| Ca ²⁺ | 50 |
| Fe ²⁺ | 10 |
| Cr(VI) | 5 |

High concentrations of chloride require precipitation in form of silver chloride with silver sulfate or, if applicable, by dilution as described below in this clause.

When calcium concentrations are greater than those indicated as examples in [Table 1](#), the reaction can produce turbidity.

In case the concentration of interfering ion in the sample exceeds the maximum compatible concentration, the user can dilute the sample and proceed with the appropriate measuring range of the small-scale sealed tube method.

In case of turbid samples, preliminary filtration is necessary using filters with 0,45 µm porosity. Before filtering, the filter shall be washed with water with the same pH as the sample. Generally, it can be done with the sample itself by discarding the first millilitres of sample, already filtered.

For coloured sample interference, the sample can be handled as follows:

- a) add sample to the small-scale sealed tube without adding any additional reagents and measure;
- b) measure the concentration value of the coloured solution with the addition of reagents;
- c) subtract the value obtained in a) from the value in b).

It is the laboratory's responsibility to identify interferences by, for example, spiking with standards containing known concentration of nitrogen to the sample containing the suspected ion, see [Clause 10](#).

6 Sampling and sample preparation

For methods of sampling and storing samples before analysis, proceed according to ISO 5667-1, ISO 5667-3 and ISO 5667-10.

Samples should be analysed as soon as possible. For longer storage periods, it is recommended to freeze below -18 °C as prescribed in ISO 5667-3. For nitrate in wastewater and surface water, on-site filtration is mandatory.

Due to the differences in small-scale sealed tube used, between all the manufacturers, it is recommended to follow manufacturers' manuals:

7 Reagents and standard solutions

7.1 Water, the concentration of nitrogen in the used water shall be below 25 % of the lower measurement range of the used sealed tube.

7.2 Potassium nitrate, KNO_3 , salt previously dried at $(105 \pm 5) ^\circ\text{C}$ for 2 h; commercially available potassium nitrate solutions of known concentrations can also be used as reference material.

7.3 Reagents provided by the manufacturers for the small-scale sealed tube method and used to determine $\text{NO}_3\text{-N}$ with the chromotropic acid colour reactions (see [Clause 9](#)).

7.3.1 Sulfuric acid, H_2SO_4 .

7.3.2 Sodium metabisulfite, $\text{Na}_2\text{S}_2\text{O}_5$.

7.3.3 Chromotropic acid, $\text{C}_{10}\text{H}_8\text{O}_8\text{S}_2$.

8 Apparatus

Usual laboratory apparatus and, in particular, the following.

8.1 Small-scale sealed tubes, for different measuring ranges of $\text{NO}_3\text{-N}$ concentrations: low, medium and high.

8.2 Photometer or spectrophotometer for measuring cuvettes.

NOTE Usually, an automated instrument has the calibration curves required for routine use stored in the memory and can also record new ones.

8.3 Calibrated pipettes and micropipettes of various volumes, according to ISO 8655-2.

8.4 Drying oven, able to maintain the temperature at $(105 \pm 5) ^\circ\text{C}$ to dry reference material.

9 Procedure

9.1 Reaction condition for colour reaction

It is important to keep in mind that the colour development that occurs in the small-scale sealed tube method is influenced by the temperature and pH of the sample. In order to obtain reproducible data, it is generally recommended that the temperature of the sample and reagents is between $15 ^\circ\text{C}$ and $25 ^\circ\text{C}$ when the reaction takes place. Sample pH should be kept between 3 and 10.

9.2 Chromotropic acid-based colour reaction

The reagents in the small-scale sealed tubes given as mass fraction including sample for the chromotropic acid colour reaction is given in [Table 2](#).

Nitrate ions react in acidic condition with chromotropic acid ([7.3.3](#)) into a small-scale sealed tube which is read in a filter photometer ([8.2](#)) at a wavelength of $(430 \pm 5) \text{ nm}$ or in a spectrophotometer ([8.2](#)) at a wavelength of $(410 \pm 5) \text{ nm}$.

Table 2 — Reagents in the small-scale sealed tubes given as mass fraction, including a sample for the chromotropic acid colour reaction

| Reagent name | Mass fraction range percentage |
|--|--------------------------------|
| Sodium metabisulfite (7.3.2) | 0,01 to 5 |
| Chromotropic acid (7.3.3) | 0,01 to 1 |
| Sulfuric acid (7.3.1) | 75 to 85 |

10 Quality control

10.1 Verification of the calibration

In general, the photometer or spectrophotometer ([8.2](#)) for reading the small-scale sealed tube is calibrated by the manufacturer and the corresponding curves are saved. In addition, the instrument has a program for calculating the concentration of the analyte from the absorbance measurement of the sample being analysed through the parameters of the stored calibration curve.

The acceptance of the calibration curve should be evaluated using reference material as quality control solutions (diluted from [7.2](#)) of different concentrations within the calibration range and a blank. This shall be repeated whenever the manufacturer modifies the stored curve (information about calibration changes shall be communicated by manufacturers) and/or the analyte concentration calculation program or as described by ISO/IEC 17025.

The user establishes its own reference materials levels and acceptability criteria in accordance with the statistical variability provided by the photometer or spectrophotometer manufacturer, or according to current standards, or according to its specific requirements. If the data are acceptable, the photometer or spectrophotometer can be used. Otherwise, recalibration or appropriate maintenance and recalibration is necessary.

Each laboratory is responsible for system check including used reference material, frequency and chosen concentration levels of reference material.

It is also possible to create an own calibration curve starting from reference solution (7.2) and diluting to achieve the desired calibration points (at least five shall be prescribed in accordance with ISO 8466-1) and a blank in the measuring range. Proceed to read the small-scale sealed tubes with the photometer or spectrophotometer (8.2). It is recommended to store these own calibration curves on the photometer or spectrophotometer.

10.2 System and interferences checks

It is recommended to check the system using a reference material (7.2), because of potential variability in manufacturers practices of photometer or spectrophotometer, method followed, reagents used.

It is at least necessary to check the main potential interferences that can be present in the matrix (see Clause 5). It is recommended to run spiked known concentration of reference material or known concentration of interference ion on sample. To check nitrate recovery, it is recommended to run a number of samples representative of laboratory activity in terms of repetitions and levels.

It is up to the laboratory to define the criteria concerning quality control frequency.

The acceptance criteria selected by the laboratory shall be compatible with the declared uncertainty.

11 Calculation

The concentration of $\text{NO}_3\text{-N}$ in the sample being analysed is calculated using Formula (1):

$$C_{\text{NO}_3\text{-N}} = \rho \times d \quad (1)$$

where

$C_{\text{NO}_3\text{-N}}$ is the concentration of nitrate-N in the sample, expressed in mg/l;

ρ is the concentration read on the photometer or spectrophotometer, expressed in mg/l;

d is the dilution factor, which takes into account both the potential need to bring the concentration within the method's scope of application and the potential addition of acid or base for pH correction.

If the analysis was performed with a commercially available automated photometer or spectrophotometer, $\text{NO}_3\text{-N}$ is provided by the same instrument by setting the dilution factor.

If the data are expressed as nitrate (NO_3^-), the $\text{NO}_3\text{-N}$ concentration value has to be multiplied by the conversion factor 4,426 81.

Concentrations ρ even slightly higher than the upper limit of the application range indicated in the manufacturer's manual for the small-scale sealed tube method applied shall not be used for calculation of results or be reported as such.

12 Expression of results

The analysis results obtained when applying this document are subject to a measurement uncertainty that is to be considered in the interpretation of the results.

Results shall be reported to a maximum of 3 significant figures.

EXAMPLE

$$C_{\text{NO}_3\text{-N}} = 0,125 \text{ mg/l}$$

$$C_{\text{NO}_3\text{-N}} = 1,36 \text{ mg/l}$$

$C_{\text{NO}_3\text{-N}} = 12,3 \text{ mg/l}$

13 Test report

The test report shall contain at least the following information:

- a) the test method used, together with a reference to this document, i.e. ISO 23696-2:2023;
- b) identity of the sample;
- c) expression of the results according to [Clause 12](#);
- d) any deviation from this method;
- e) report of all circumstances that can have affected the results;
- f) any unusual features observed;
- g) the date of the test.

The method performance of this document is presented in [Annex A](#).

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