

SLOVENSKI STANDARD
SIST EN 12014-4:2005**01-september-2005****Nadomešča:****SIST ENV 12014-4:1999**

Živila – Določevanje nitratov in/ali nitritov - 4. del: Metoda ionske izmenjalne kromatografije za določevanje nitratov in nitritov v mesnih proizvodih

Foodstuffs - Determination of nitrate and/or nitrite content - Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products

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Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 4: Ionenchromatographisches Verfahren (IC) für die Bestimmung des Nitrat- und Nitritgehaltes in Fleischerzeugnissen

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Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites - Partie 4 : Détermination par chromatographie ionique (CI) de la teneur en nitrates et en nitrites dans les produits carnés

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.120.10	Meso in mesni proizvodi	Meat and meat products

SIST EN 12014-4:2005**en**

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EUROPEAN STANDARD
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English version

**Foodstuffs - Determination of nitrate and/or nitrite content - Part
4: Ion-exchange chromatographic (IC) method for the
determination of nitrate and nitrite content of meat products**

Produits alimentaires - Détermination de la teneur en
nitrates et/ou en nitrites - Partie 4: Détermination par
chromatographie ionique (CI) de la teneur en nitrates et en
nitrites dans les produits à base de viande

Lebensmittel - Bestimmung des Nitrat- und/oder
Nitritgehaltes - Teil 4: Ionenchromatographisches
Verfahren (IC) für die Bestimmung des Nitrat- und
Nitritgehaltes in Fleischerzeugnissen

This European Standard was approved by CEN on 1 April 2005.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.



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Foreword

This European Standard (EN 12014-4:2005) has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2005, and conflicting national standards shall be withdrawn at the latest by November 2005.

This document supersedes ENV 12014-4:1998.

This series *Foodstuffs - Determination of nitrate and/or nitrite content* consist of the following parts:

Part 1: General considerations;

Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products;

Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite;

Part 4: Ion-exchange chromatographic (IC) method for the determination of nitrate and nitrite content of meat products;

Part 5: Enzymatic determination of nitrate content of vegetable-containing food for babies and infants;

Part 7: Continuous flow method for the determination of nitrate content of vegetables and vegetable products after Cadmium reduction.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

EN 12014-4:2005 (E)**1 Scope**

This European Standard specifies an ion-exchange chromatographic method for the determination of the nitrate and nitrite contents of meat products and has been validated for different meat products with a nitrate content of 50 mg/kg to 300 mg/kg as nitrate ion and a nitrite content of approximately 40 mg/kg as nitrite ion.

NOTE Validation data obtained from interlaboratory studies show that this method may also be applied to the determination of nitrate in vegetables and baby food, see [1], [2]. Furthermore, the method may be applied for the determination of nitrite in meat products having a nitrite content greater than 40 mg/kg.

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.

EN ISO 3696, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

3 Principle

Nitrate and nitrite are extracted from the test sample with hot water. The aqueous solution is treated with acetonitrile to remove any interfering substance. The nitrate and nitrite contents of the solution are then determined by ion-exchange chromatography (IC) and ultraviolet (UV) detection at a wavelength of 205 nm.

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4 Reagents

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4.1 General

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 3 according to EN ISO 3696. When preparing solutions, the purities of the reagents available shall be taken into account.

4.2 Acetonitrile**4.3 Glycerol****4.4 Lithium hydroxide, anhydrous, or lithium hydroxide monohydrate****4.5 Boric acid**, having a mass fraction of 99 %**4.6 Hydrochloric acid**, $c(\text{HCl}) = 1,8 \text{ mol/l}^{1)}$

Dilute 15 ml of hydrochloric acid of $\rho_{20}(\text{HCl}) = 1,18 \text{ g/ml}$ in a 100 ml volumetric flask to the mark with water and mix.

4.7 Hydrochloric acid, $c(\text{HCl}) \approx 0,1 \text{ mol/l}$

Dilute 5 ml of hydrochloric acid (4.6) in a 100 ml volumetric flask to the mark with water and mix.

1) c is the substance concentration

4.8 Stock solution of nitrate and nitrite

Dissolve 1,500 g of sodium nitrite and 3,258 g of potassium nitrate in a 1000 ml volumetric flask in water, dilute to the mark with water and mix well. The stock solution may be used for 2 weeks if stored in a refrigerator at 4 °C.

Sodium nitrite is a hygroscopic substance.

4.9 Standard solutions of nitrate and nitrite

Pipette 0 ml, 0,1 ml, 0,5 ml, 1,0 ml, 1,5 ml and 2,0 ml, respectively, of the stock solution (4.8) into six 200 ml volumetric flasks, dilute to the mark with water and mix. 1 l of these solutions contains 0 mg, 0,5 mg, 2,5 mg, 5,0 mg, 7,5 mg and 10 mg of nitrite ion and 0 mg, 1 mg, 5 mg, 10 mg, 15 mg and 20 mg of nitrate ion, respectively. It is recommended to prepare the standard solutions on the day of use.

4.10 Gluconic acid solution²⁾, optionally clarified on solid phase extraction columns (5.9)

Dilute 50 g of gluconic acid in a 100 ml volumetric flask to the mark with demineralized water and mix.

NOTE Gluconic acid sometimes has a dark brown colour. In this case it is recommended to clarify it until a slight yellow colour is obtained. Generally two cartridges activated with methanol are necessary.

4.11 Lithium borate gluconate buffer solution

To 500 ml of water in a 1000 ml volumetric flask add 34,00 g of boric acid (4.5) and 19,6 ml of gluconic acid solution (4.10). Dissolve completely in the solution 11,00 g of anhydrous lithium hydroxide or 19,26 g of lithium hydroxide monohydrate (4.4). Add 125 ml of glycerol (4.3), dilute to the mark with water and mix well. This solution is stable for 6 months if stored in a refrigerator at approximately 4 °C.

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4.12 Mobile phase <https://standards.iteh.ai/catalog/standards/sist/98b45179-724f-4169-8f0b-49a8dccb9a65/sist-en-12014-4-2005>

To 500 ml of water in a 1000 ml volumetric flask, add 17 ml of buffer solution (4.11) and 125 ml of acetonitrile (4.2). Dilute to the mark with water. Mix well.

Adjust the pH to $6,5 \pm 0,1$ by adding hydrochloric acid (4.6 then 4.7). Filter through a membrane filter of pore size of approximately $0,22 \mu\text{m}$ (5.6). This solution is stable for not longer than one week and the pH value shall be checked every day before use. If the pH value falls outside the stated range, prepare a new solution.

²⁾ This solution is commercially available.

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5 Apparatus and equipment

5.1 General

Usual laboratory apparatus and, in particular, the following:

5.2 Homogenizing equipment, mechanical or electrical, capable of homogenizing the test sample, this includes a high-speed rotational cutter, or a mincer fitted with a plate with holes not exceeding 4,5 mm in diameter, and a homogenizer.

5.3 Magnetic stirrer (optional)

5.4 Fluted filter paper (nitrate/nitrite free), care is needed in the choice of filter paper as some can absorb nitrate.

5.5 Membrane filter, for aqueous and acetonitrile solutions, with a pore size of approximately 0,45 μm .

5.6 Membrane filter, for aqueous and acetonitrile solutions, with a pore size of approximately 0,22 μm .

5.7 Filter holder for membrane filter, with suitable syringe.

5.8 pH meter

5.9 Solid phase extraction column RP C₁₈, with suitable syringe (optional).

5.10 IC apparatus, comprising the following. [SIST EN 12014-4:2005](https://standards.iteh.ai/catalog/standards/sist/98b45179-724f-4169-8f0b-49a8dccb9a65/sist-en-12014-4-2005)
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5.10.1 Isocratic high performance liquid chromatography unit, with a UV detector, capable of measuring at a wavelength of 205 nm and an evaluation unit (e.g. an integrator with plotter).

5.10.2 Analytical separating column, Anion exchanger, 4,6 mm \times 150 mm, packing material; polymethacrylate resin with a quarternary ammonium functional group, particle size of 10 μm , capacity (30 \pm 3) μ eq/ml with a precolumn, e.g. 20 mm, having the same packing to protect the analytical column.

6 Procedure

6.1 Sample preparation

Homogenize the laboratory sample with the appropriate equipment (5.2). Take care that the temperature of the sample material does not rise above 25 °C. If a mincer is used, pass the sample at least twice through the equipment. Weigh, to the nearest 10 mg, 10 g of the homogenized sample into e.g. a 150 ml wide neck conical flask (test portion).

6.2 Extraction and clarification

The analysis should be performed in a single working day.

Add 50 ml of water at a temperature of 50 °C to 60 °C to the test portion in the wide neck flask. Mix thoroughly with the homogenizer (5.2). Rinse the homogenizer with water and add the washings to the flask. Quantitatively transfer this slurry into a 200 ml volumetric flask by rinsing the wide neck flask with water. Add 50 ml of acetonitrile (4.2). Mix gently. Allow to cool to room temperature. Dilute to the mark with water.

Filter first through the fluted filter paper (5.4) and then through a membrane filter of pore size of approximately 0,45 µm (5.5). If this solution is clear, it may be injected. If the solution is still not clear, filter through a membrane filter of pore size of approximately 0,22 µm (5.6) (Sample test solution). Prepare a blank replacing the test portion by 10 ml of water.

6.3 Preparation of the calibration graph

To plot a calibration graph, inject equal volumes of the standard solutions (4.9) first and then the blank solution (prepared according to 6.2) under the conditions as described in 6.4.1.

Check the linearity of the calibration graph.

6.4 Determination

6.4.1 IC operating conditions

If the column specified in 5.10.2 is used, it has been found satisfactory to adopt the following parameters.

Mobile phase: as described in 4.12

UV: 205 nm

Injection volume: 40 µl (minimum)

Flow rate: 1 ml/min

If other columns than the one described in 5.10.2 are used, adjust chromatographic conditions.

6.4.2 IC measurement

Inject the standard solutions (4.9) first and then the blank and the sample test solutions under the conditions as described in 6.4.1. One of the standard solutions should be injected every five sample test solutions when performing a series of analyses.

If the peak obtained for the sample falls outside the range of the calibration graph, dilute the sample test solution in the mobile phase and repeat the measurement step.

Identify the nitrate or nitrite peak by comparing the retention times for the standard solutions (4.9) and the sample test solutions (6.2).

Read off the content of nitrate or nitrite of the sample test solution from the calibration graph.

Check the blank value to ensure that there was no nitrate and/or nitrite contamination during the sample preparation.

6.5 Expression of results

Calculate the mass fraction of nitrite, $w_{(\text{NO}_2)}$, expressed in milligrams of ion per kilogram with Equation (1):

$$w_{(\text{NO}_2)} = \frac{200 \times A_{(\text{NO}_2)}}{m} \times F \quad (1)$$

where: