
Živila - Določevanje vsebnosti nitratov in/ali nitritov - 4. del: Metoda ionske izmenjalne kromatografije za določevanje vsebnosti 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

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 a base de viande

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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

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

Produits alimentaires - Détermination de la teneur en
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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

This European Prestandard (ENV) was approved by CEN on 6 June 1998 as a prospective standard for provisional application.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This European Prestandard has been prepared by Technical Committee CEN/TC 275 "Food analysis - Horizontal methods", the secretariat of which is held by DIN.

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

- Part 1: General considerations; [SIST ENV 12014-4:1999](http://standards.iteh.ai/SIST/ENV/12014-4-1999)
- Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products; <http://standards.iteh.ai/SIST/ENV/12014-4-1999>
- Part 3: Spectrometric determination of nitrate and nitrite content of meat products after enzymatic reduction of nitrate to nitrite <http://standards.iteh.ai/SIST/ENV/12014-4-1999>
- 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 announce this European Prestandard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.



1 Scope

This European Prestandard specifies an ion-exchange chromatographic method for the determination of the nitrate and nitrite contents of meat products having a nitrate content of 50 mg/kg to 300 mg/kg as nitrate ions 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 of greater than 40 mg/kg.

2 Normative references

This European Prestandard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this draft European Prestandard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

EN 12014-1

Foodstuffs - Determination of nitrate and/or nitrite content - Part 1: General considerations

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.

4 Reagents

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.

4.1 Acetonitrile

4.2 Glycerol

4.3 Lithium hydroxide, anhydrous, or lithium hydroxide monohydrate

4.4 Boric acid, having a mass fraction of 99 %

4.5 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.

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

Dilute 5 ml of hydrochloric acid (4.5) in a 100 ml volumetric flask to the mark with water and mix.

4.7 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.

4.8 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 (4.7) 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.

¹⁾ c is the substance concentration

4.9 Gluconic acid solution, having a mass concentration of 50 g/100 ml, optionally clarified on solid phase extraction columns (5.8).

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.

4.10 Lithium borate gluconate buffer solution

To 500 ml of water in a 1000 ml volumetric flask add 34,00 g of boric acid (4.4) and 19,6 ml of gluconic acid solution (4.9). Dissolve completely in the solution 11,00 g of anhydrous lithium hydroxide or 19,26 g of lithium hydroxide monohydrate (4.3). Add 125 ml of glycerol (4.2), 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.

4.11 Mobile phase

To 500 ml of water in a 1000 ml volumetric flask, add 17 ml of buffer solution (4.10) and 125 ml of acetonitrile (4.1). Dilute to the mark with water. Mix well.

Adjust the pH to $6,5 \pm 0,1$ by adding hydrochloric acid (4.5 then 4.6). Filter through a membrane filter of pore size of approximately $0,22 \mu\text{m}$ (5.5). 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.

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

5.1 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.

5.2 Magnetic stirrer (optional)

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

5.4 Membrane filter, for aqueous and acetonitrile solutions, with a pore size of approximately $0,45 \mu\text{m}$

5.5 Membrane filter, for aqueous and acetonitrile solutions, with a pore size of approximately $0,22 \mu\text{m}$

5.6 Filter holder for membrane filter, with suitable syringe

5.7 pH meter

5.8 Solid phase extraction column RP C_{18} , with suitable syringe (optional)

5.9 IC apparatus, comprising the following

5.9.1 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).

5.9.2 Analytical separating column, Anion exchanger, 4,6 mm × 150 mm, packing material; polymethacrylate resin with a quarternary ammonium functional group, particle size of $10 \mu\text{m}$, capacity $(30 \pm 3) \mu\text{eq/ml}$ 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 (5.1). 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 (5.1). 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 (4.1). Mix gently. Allow to cool to room temperature. Dilute to the mark with water.

Filter first through the fluted filter paper (5.3) and then through a membrane filter of pore size of approximately $0,45 \mu\text{m}$ (5.4). 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 $0,22 \mu\text{m}$ (5.5) (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 (4.8) 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 5.9.2 is used, it has been found satisfactory to adopt the following parameters.

Mobile phase:	as described in 4.11
UV:	205 nm
Injection volume:	$40 \mu\text{l}$ (minimum)
Flow rate:	1 ml/min

If other columns than the one described in 5.9.2 are used, adjust chromatographic conditions.

6.4.2 IC measurement

Inject the standard solutions (4.8) 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 (4.8) 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:

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

where:

$A_{\text{NO}_2^-}$ is the value for nitrite, read off the calibration graph, in milligrams per litre;

200 is the volume of the diluted test portion (see 6.2), in millilitres;;

m is the initial mass of the test portion, in grams;

F is the dilution factor.

Round the results without any decimals.

Calculate the mass fraction of nitrate, $w_{\text{NO}_3^-}$, expressed in milligrams of ion per kilogram with equation 2:

$$w_{(\text{NO}_3^-)} = \frac{200 \cdot A_{(\text{NO}_3^-)}}{m} \cdot F \quad (2)$$

where:

$A_{\text{NO}_3^-}$ is the value for nitrate, read off the calibration graph, in milligrams per litre;
200, m , F , see equation (1).

Round the result without any decimals.

7 Precision

7.1 General

Details of the interlaboratory test of the method are summarized in annex B. The values derived from the interlaboratory test may not be applicable to analyte concentration ranges and matrices other than those given in annex B.

7.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases.

The values are:

Nitrate	corned beef (NO_3^-)	$\bar{x} = 60,8 \text{ mg/kg}$	$r = 6,0 \text{ mg/kg}$
	corned beef (NO_3^-)	$\bar{x} = 289,6 \text{ mg/kg}$	$r = 25,0 \text{ mg/kg}$
Nitrite	corned beef (NO_2^-)	$\bar{x} = 38,9 \text{ mg/kg}$	$r = 4,4 \text{ mg/kg}$

7.3 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The values are:

Nitrate	corned beef (NO_3^-)	$\bar{x} = 60,8 \text{ mg/kg}$	$R = 27,7 \text{ mg/kg}$
	corned beef (NO_3^-)	$\bar{x} = 289,6 \text{ mg/kg}$	$R = 26,6 \text{ mg/kg}$
Nitrite	corned beef (NO_2^-)	$\bar{x} = 38,9 \text{ mg/kg}$	$R = 10,3 \text{ mg/kg}$

8 Test report

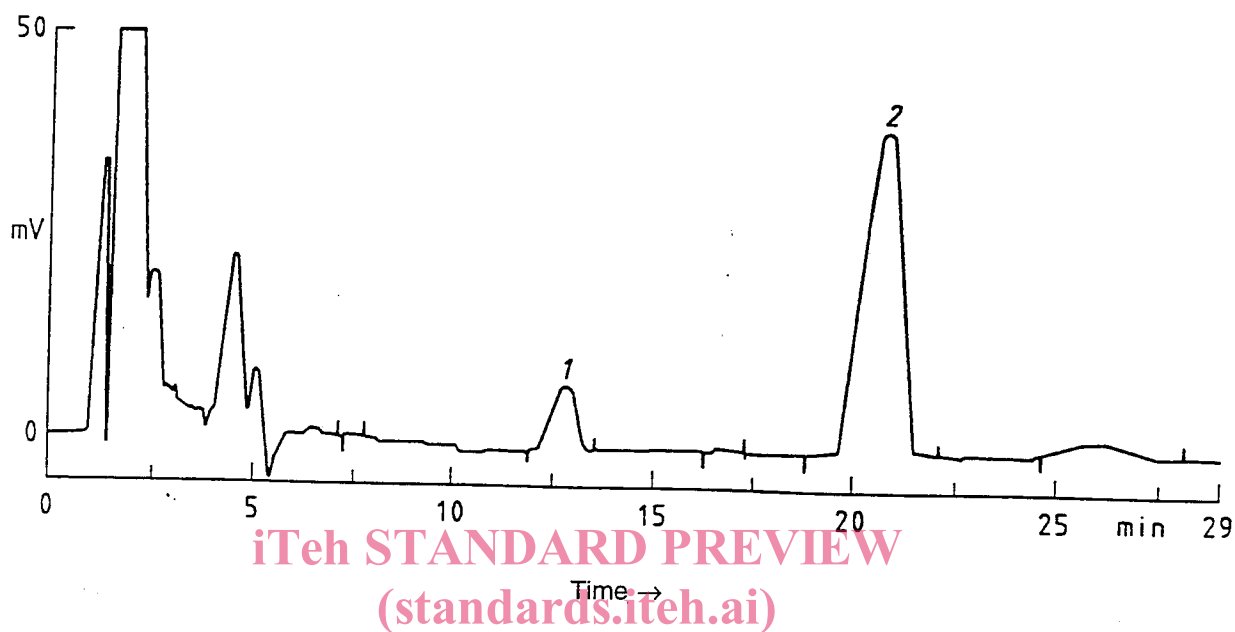
The test report shall contain at least :

- all information necessary for the identification of the sample;
- a reference to this European Standard or to the method used;
- the results and the units in which the results have been expressed;
- date and type of sampling procedure (if known);
- date of receipt;
- date of test;
- if the repeatability has been verified;
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results

as given in EN 12014-1.

Annex A (informative)

Figure



SIST ENV 12014-4:1999

1 = Nitrite, mass fraction of 38,9 mg/kg at retention time of 12,63 min.

2 = Nitrate, mass fraction of 289,6 mg/kg at retention time of 20,73 min.

Figure A.1 Example for an IC chromatogram of corned beef
(Sample from the interlaboratory trial)