



National Standards Authority of Ireland

IRISH STANDARD

ENV 12014-4:1998

ICS 67.120.10

**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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ICS 67.120.10

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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

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EUROPEAN COMMITTEE FOR STANDARDIZATION  
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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;
- 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 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, $c(\text{HCl}) = 0,1 \text{ mol/l}$ .

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.

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<sup>1)</sup> 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<sub>18</sub>**, 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

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