SLOVENSKI STANDARD
SIST EN 12014-2:1999

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Živila - Določevanje vsebnosti nitratov in/ali nitritov - 2. del: HPLC/IC metoda za določevanje vsebnosti nitratov v zelenjavi in zelenjavnih proizvodih

Foodstuffs - Determination of nitrate and/or nitrite content - Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products

Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 2: HPLC/IC Verfahren für die Bestimmung des Nitratgehaltes in Gemüsen und Gemüseerzeugnissen

Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites - Partie 2: Détermination par CLHP/CI de la teneur en nitrates des légumes et produits a base de légumes

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67.080.20	Zelenjava in zelenjavni proizvodi	Vegetables and derived products

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English version

Foodstuffs - Determination of nitrate and/or nitrite content - Part 2: HPLC/IC method for the determination of nitrate content of vegetables and vegetable products

Produits alimentaires - Détermination de la teneur en nitrates et/ou en nitrites - Partie 2: Détermination par CLHP/CI de la teneur en nitrates des légumes et produits à base de légumes

Lebensmittel - Bestimmung des Nitrat- und/oder Nitritgehaltes - Teil 2: HPLC/IC Verfahren für die Bestimmung des Nitratgehaltes in Gemüsen und Gemüseerzeugnissen

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CEN

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Foreword

This European Standard has been prepared by 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

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: 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,

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 October 1997 and conflicting national standards shall be withdrawn at the latest by October 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC)/ion-exchange high performance liquid chromatography (IC) method for the determination of nitrate contents of vegetables and vegetable products. This method is applicable to nitrate contents in the range of 50 mg/kg to 3000 mg/kg.

2 Normative references

This European Standard 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 European Standard 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

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

3 Principle

Extraction of nitrate from the food with hot water and removal of interfering substances by clarification with Carrez reagents or by purification with solid phase extraction columns. Determination by reversed phase HPLC with ultraviolet (UV) detection or IC with conductivity detection [1].

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 as defined in EN ISO 3696. When preparing solutions, the purities of the reagents available shall be taken into account.

4.1 Methanol, for HPLC

4.2 Acetonitrile, for HPLC

4.3 Sulfuric acid, $c(H_2SO_4)$ = 0,0125 mol/l [1]

Carefully pipette 20 ml of sulfuric acid of 96 % [$\rho(H_2SO_4)$ = 1,84 g/ml [2]] into a 1 000 ml volumetric flask containing 800 ml of water, mix, dilute to the mark with water and mix again. Transfer, e.g. by means of a graduated cylinder 33 ml of this dilution into a further 1 000 ml volumetric flask containing 500 ml of water. Dilute to the mark with water and mix.

4.4 Carrez solution No 1

Dissolve 150 g of potassium hexacyanoferrate(II) ($K_4[Fe(CN)_6] \cdot 3\ H_2O$) in water, mix well and dilute to 1 000 ml with water. Store in a brown bottle and replace it every week.

4.5 Carrez solution No 2

Dissolve 220 g of zinc acetate ($Zn(CH_3COO)_2 \cdot 2\ H_2O$) in water, add 30 ml of glacial acetic acid, mix and dilute to 1 000 ml with water.

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[1] c is the substance concentration

[2] ρ is the mass concentration

4.6 Nitrate stock solution, ρ (NO_3^-) = 1 mg/ml

Dissolve approximately 1,6307 g of potassium nitrate (KNO_3), weighed to the nearest 0,1 mg in water in a 1 000 ml volumetric flask, add 15 ml of sulfuric acid of 96 % [ρ (H_2SO_4) = 1,84 g/ml] for conservation purposes, mix, dilute to the mark with water and mix again. This solution is stable for at least 2 months if stored at 4 °C.

Also ready to use standard solutions may be used.

4.7 Nitrate standard solution I, ρ (NO_3^-) = 50 μg/ml

Pipette 5 ml of nitrate stock solution (4.6) into a 100 ml volumetric flask, dilute to the mark with water, mix, and filter through a membrane filter (5.4). Prepare this solution each day of the analysis. The solution may not be turbid.

4.8 Nitrate standard solution II, ρ (NO_3^-) = 5 μg/ml

Pipette 5 ml of nitrate stock solution (4.6) into a 1 000 ml volumetric flask, dilute to the mark with water, mix, and filter through a membrane filter (5.4). Prepare this solution each day of the analysis.

4.9 Sodium carbonate/sodium hydrogencarbonate solution

Weigh out approximately 29,676 g of sodium carbonate (Na_2CO_3) and about 18,482 g of sodium hydrogencarbonate ($NaHCO_3$) to the nearest milligram, into a 1 000 ml volumetric flask, dissolve in water, and dilute to the mark with water. 1 l of this solution contains 0,28 mol of sodium carbonate and 0,22 mol of sodium hydrogencarbonate. It will be stable for not more than 2 months if stored at 4 °C.

4.10 IC mobile phase

Pipette 50 ml of the sodium carbonate/sodium hydrogencarbonate solution (4.9) into a 5 l volumetric flask, dilute to the mark with water and mix. 1 l of this solution contains 0,0028 mol of sodium carbonate and 0,0022 mol of sodium hydrogencarbonate.

4.11 HPLC mobile phase

Weigh out 10 g of dipotassium hydrogenphosphate (K_2HPO_4) into a 1 000 ml volumetric flask, add 50 ml of acetonitrile (4.2), dilute to the mark with water and mix. Adjust the pH value to 3,0 using orthophosphoric acid with a mass fraction of 85 % and filter the solution through a membrane filter (5.4).

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following

5.1 Laboratory mixer (e.g. laboratory cutter)

5.2 Homogenizer.

5.3 Fluted filter paper.

5.4 Membrane filter, for aqueous solutions, with a pore size of 0,45 μm.

5.5 Filter holder for membrane filter, with suitable syringe.

5.6 Solid phase extraction column, with reversed phase RP C_{18} cartridge.

5.7 IC apparatus, comprising the following.

5.7.1 Ion-exchange chromatography unit, consisting of an eluent reservoir, a suitable pump, a sample applicator, a conductivity detector or UV detector and an evaluation unit (e.g. an integrator with plotter).

5.7.2 Analytical separating column (e.g. a low-capacity anion exchanger, such as Dionex Ion Pac AS 4A® and/or Dionex IonPac AS 4 ®[3]), with a membrane suppressor and a precolumn, which ensures a baseline resolved resolution (res = 1,3) of the nitrate peak from all other peaks.

Calculate the resolution (res) between peak A and peak B with equation 1:

$$res = 1{,}18 \frac{t_{R(B)} - t_{R(A)}}{w_{0,5(A)} + w_{0,5(B)}} \quad \ldots (1)$$

where

$t_{R(A)}$ is the retention time of substance A;

$t_{R(B)}$ is the retention time of substance B;

$w_{0,5(A)}$ is the peak width of half peak height of peak A;

$w_{0,5(B)}$ is the peak width of half peak height of peak B.

5.8 HPLC apparatus, comprising the following

5.8.1 High performance liquid chromatograph, consisting of an eluent reservoir, a pump, a sample applicator, a UV detector and an evaluation unit (e.g. an integrator with plotter).

5.8.2 Analytical (reversed phase) separating column of medium polarity with aminopropyl bonded phase and with a particle size of 5 µm to 10 µm, at least 250 mm long, internal diameter 4,6 mm and a precolumn with the same packing, such as LiChrosorb-NH_2®[3]).

6 Procedure

6.1 Sample preparation

6.1.1 Solid samples (e.g. leaf vegetables)

Take a representative sample and, if necessary, remove adhering animals, soil or dirty particles. Shred the sample, e.g. in a laboratory cutter (5.1) and thoroughly homogenize using a homogenizer (5.2).

NOTE: When washing samples, it should be taken into account that adhering water drops could increase the sample mass of samples with great surface area dramatically.

6.1.2 Liquid samples (e.g. vegetable juices)

Shake a representative sample vigorously until thoroughly mixed.

6.1.3 Paste-like samples (e.g. mashed vegetables)

Repeatedly stir a representative sample, e.g. by using a homogenizer until thoroughly mixed.

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[3]) Dionex IonPac® AS 4A, Dionex IonPac® AS 4 and LiChrosorb-NH_2® are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

6.2 Sample extraction

6.2.1 Solid samples

Weigh out a portion (not less than 10 g), containing approximately 15 mg of nitrate, of the sample prepared in accordance with 6.1.1 into a 500 ml conical flask, add approximately 400 ml of hot water and stand the flask in a boiling water bath for 15 min. Cool the flask to room temperature, transfer the solution into a 500 ml volumetric flask, dilute to the mark, shake thoroughly and filter the contents through a fluted filter paper (5.3). If necessary, clarify or purify the filtrate, i.e. the sample solution, as described in 6.3.

6.2.2 Liquid samples

If necessary, either clarify or purify the sample prepared in accordance with in 6.1.2 as it is (this will result in the sample test solution, see 6.3.1), or dilute the sample beforehand with water to give a solution containing approximately 25 mg/l of nitrate.

6.2.3 Paste-like samples

Weigh out a portion (not less than 10 g), containing approximately 15 mg of nitrate, of the sample prepared in accordance with 6.1.3 into a 500 ml conical flask, add approximately 400 ml of hot water and stand the flask in a boiling water bath for 15 min. Cool the flask to room temperature, transfer to a 500 ml volumetric flask, dilute to the mark, shake thoroughly and filter the contents through a fluted filter paper (5.3). If necessary, clarify or purify the filtrate, i.e. the sample solution, as described in 6.3.

For sample material with a low nitrate content, make appropriate adjustments, if necessary, to the initial sample masses and volumetric ratios specified in 6.2.1 to 6.2.3.

6.3 Sample test solution preparation

6.3.1 General

To protect the analytical column used in the determination and to avoid interference with matrix components, purify the sample solutions if necessary by one of the following methods.

6.3.2 Method 1

Pipette 40 ml of each sample solution from 6.2.1 to 6.2.3 into a 50 ml volumetric flask, add 2 ml of Carrez solution No 1 (4.4), mix, add 2 ml of Carrez solution No 2 (4.5) and dilute to the mark with water. Agitate the flask, filter the entire solution through a fluted filter paper (5.3) and then filter a portion of the filtrate through a membrane filter (5.4) (i.e. sample test solution). Proceed with the sample test solution as described in 6.5.2 or 6.5.4.

6.3.3 Method 2

Draw up 10 ml of each sample solution as in 6.2.1 to 6.2.3 using a millilitre syringe (5.5) and force first 2 ml and then 8 ml through a solid phase extraction column (5.6) which has been previously flushed with 2 ml of methanol (4.1) and 5 ml of water. Discard the first 2 ml fraction and filter the remainder through a membrane filter (i.e. sample test solution). Proceed with the sample test solution as described in 6.5.2 or 6.5.4.

6.4 Preparation of the calibration graph

To plot a calibration graph, prepare a series of standard solutions (at least three different solutions and a blank) having appropriate concentrations.

Inject equal volumes of the sample test and standard solutions as described in 6.5.2 or 6.5.4. Check the linearity of the calibration function.

6.5 Determination

6.5.1 IC operating conditions

If the column specified in 5.7.2 is used, it has been found satisfactory to adopt the following parameters.

Mobile phase	as described in 4.10
Regenerant (for suppressor):	0,0125 mol/l sulfuric acid (4.3)
Injection volume:	50 µl

6.5.2 IC measurement

Identify the nitrate peak by comparing the retention times for the standard solution (4.7) and the sample test solution (see 6.3.2 or 6.3.3). For the determination, inject the standard solution into the chromatograph and measure the height/area of the resulting peak. It is advisable to construct a calibration curve (see 6.4) on the basis of not less than four standard solutions having different nitrate contents, before injecting the sample test solution and measuring the height/area of the resulting peak. If the peak obtained for the sample falls outside the range of the calibration graph, dilute the sample test solution and repeat the analysis. Read off the nitrate content of the sample test solution from the calibration curve.

Ensure that the injected volumes of the sample and standard solutions are the same.

6.5.3 HPLC operating conditions

If the column specified in 5.8.2 is used, it has been found satisfactory to adopt the following parameters.

Mobile phase:	as described in 4.11
Injection volume:	20 µl
Detection (UV):	205 nm

6.5.4 HPLC measurement

Measure and determine the nitrate content as described in 6.5.2.

7 Calculation

Calculate the content, w, in milligrams per kilogram, or ρ, in milligrams per litre, of nitrate in the sample using equation 2 (external standard method):

$$w \text{ or } \rho = \frac{x \cdot F_1 \cdot F_2 \cdot 1\,000}{m} \qquad \text{... (2)}$$

where:

x is the concentration of nitrate in the sample test solution, in milligrams per litre read off from the calibration curve;

F_1 is the dilution factor for the purification method used (e.g. column purification: $F_1 = 1$, Carrez clarification: $F_1 = 1{,}25$);

F_2 is the dilution factor for any other dilution steps carried out;

m is the amount of sample, in grams or millilitres, per litre of sample solution (for undiluted liquids with low nitrate contents $m = 1\,000$).

Report the result in milligrams per kilogram or milligrams per litre without decimal places and according to current legislation.