SLOVENSKI STANDARD
SIST EN 14130:2003

01-november-2003

Živila - Določevanje vitamina C s HPLC

Foodstuffs - Determination of vitamin C by HPLC

Lebensmittel - Bestimmung von Vitamin C mit HPLC

Produits alimentaires - Dosage de la vitamine C par CLHP

Ta slovenski standard je istoveten z: **EN 14130:2003**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN 14130:2003 **en**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14130

June 2003

ICS 67.050

English version

Foodstuffs - Determination of vitamin C by HPLC

Produits alimentaires - Dosage de la vitamine C par CLHP

Lebensmittel - Bestimmung von Vitamin C mit HPLC

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. EN 14130:2003 E

Contents

Foreword

This document (EN 14130:2003) 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of an identical text or by endorsement, at the latest by December 2003, and conflicting national standards shall be withdrawn at the latest by December 2003.

Annexes A and B are informative.

WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

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

1 Scope

This European Standard specifies an HPLC-method for the determination of vitamin C in foodstuffs. Vitamin C is the sum of L(+) ascorbic acid and dehydro L(+) ascorbic acid.

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 (including amendments).

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

3 Principle

Vitamin C is extracted from the sample to be analysed using metaphosphoric acid solution. A reducing solution is used to transform dehydro L(+) ascorbic acid to L(+) ascorbic acid. Total L(+) ascorbic acid content is determined by HPLC with a UV detection at 265 nm [1], [2].

4 Reagents

4.1 General

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and water of at least grade 1 according to EN ISO 3696, or double distilled water.

4.2 Chemicals and solutions

4.2.1 Metaphosphoric acid, ($(HPO_3)_n$)

4.2.2 Trisodium phosphate, mass fraction $w(Na_3PO_4 \cdot 12H_2O) \geq 98,0$ %

4.2.3 Potassium dihydrogen phosphate, $w(KH_2PO_4) \geq 99,0$ %

4.2.4 L-Cysteine or another suitable reducing agent, $w(C_3H_7NO_2S) \geq 99,0$ %

4.2.5 N-cetyl-N,N,N--trimethylammonium bromide, $w(C_{19}H_{42}BrN) \geq 99,0$ %

4.2.6 Methanol (HPLC grade), $w(CH_3OH) \geq 99,0$ %

4.2.7 Metaphosphoric acid solution, mass concentration $\rho((HPO_3)_n) = 200$ g/l

Dissolve 200 g of metaphosphoric acid (4.2.1) in a 1 l volumetric flask with water to the mark. This solution is stable for one month if stored at + 4 °C.

4.2.8 Metaphosphoric acid solution, $\rho((HPO_3)_n) = 20$ g/l

Pipet 50 ml of the metaphosphoric solution (4.2.7) into a 500 ml volumetric flask. Dilute to the mark with water. Prepare fresh each day of the analysis.

4.2.9 Trisodium phosphate solution, $\rho(Na_3PO_4 \cdot 12H_2O) = 200$ g/l

Dissolve 200 g of trisodium phosphate (4.2.2) in a 1 l volumetric flask with water. Dilute to the mark with water.

4.2.10 L-Cysteine solution, $\rho(C_3H_7NO_2S) = 40$ g/l

Dissolve 20 g of cysteine (4.2.4) in a 500 ml volumetric flask with water. Dilute to the mark with water. Prepare fresh each day of analysis.

4.2.11 HPLC mobile phase:

Dissolve 13,6 g of potassium dihydrogen phosphate (4.2.3) in 900 ml of water in a beaker. Filter through a 0,45 µm filter (solution A). Dissolve 1,82 g of N-cetyl-N,N,N-trimethylammonium bromide (4.2.5) in 100 ml of methanol (4.2.6) in a beaker. Mix and filter through a 0,45 µm filter (solution B). Mix the 900 ml of solution A with the 100 ml of solution B. Degas the solution before use, if necessary.

4.2.12 Starch solution (optional), ρ(soluble starch) = 1 g/100 ml

4.2.13 Iodine solution (optional), $c(I_2) = 0,05$ mol/l

4.2.14 Diluted sulfuric acid $c(H_2SO_4) = 0,1$ mol/l

4.3 Standard substances

4.3.1 Ascorbic acid, L(+) Ascorbic acid, $w(C_6H_8O_6) \geq 99,7$ %

(R)-5-[(S)-1,2-Dihydroxyethyl]-3,4-dihydroxy-5-H-furan-2-on

4.3.2 Erythorbic (Isoascorbic) acid, D (-) Ascorbic acid, $w(C_6H_8O_6) \geq 99,0$ %

(R)-5-[(R)-1,2-Dihydroxyethyl]-3,4-dihydroxy-5-H-furan-2-on

4.4 Stock solutions

4.4.1 Ascorbic acid stock solution, $\rho(C_6H_8O_6) \approx 1$ mg/ml

Dissolve an accurately weighed amount of ascorbic acid (4.3.1), e.g. approximately 100 mg to the nearest 0,1 mg in a defined volume, e.g. 100 ml, of the metaphosphoric acid solution (4.2.8). Prepare fresh each day of analysis.

The stability of the stock solution can be enhanced by addition of L-Cysteine.

4.4.2 Erythorbic acid stock solution, $\rho(C_6H_8O_6) \approx 1$ mg/ml

Dissolve an accurately weighed amount of the erythorbic acid (4.3.2), e.g. approximately 100 mg to the nearest 0,1 mg in a defined volume, e.g. 100 ml, of the metaphosphoric acid solution (4.2.8). Prepare fresh each day of analysis.

4.4.3 Purity test (optional)

Weigh accurately approximately 150 mg of the ascorbic acid standard substance in a conical flask and dissolve by adding 10 ml of diluted sulfuric acid (4.2.14) and 80 ml of carbon dioxide free water. After addition of starch or starch solution (4.2.12), titrate with iodine solution (4.2.13) until permanent coloration. 1 ml of iodine solution corresponds to 8,81 mg ascorbic acid.

Calculate the purity of the standard substance, w_{st}, in percent according to equation (1).

$$w_{st} = \frac{V_1 \times 8{,}81 \times 100}{m} \qquad (1)$$

where

V_1 is the volume of the iodine solution used, in millilitre;

m is the sample mass, in milligramm;

8,81 is the conversion factor;

100 is the conversion factor to get the result in percent.

4.5 Calibration solutions

4.5.1 Ascorbic acid calibration solutions, $\rho(C_6H_8O_6)$ = 5 µg/ml to 50 µg/ml

Pipette 0,5 ml to 5 ml of the ascorbic acid stock solution (4.4.1) into 100 ml volumetric flask and dilute with metaphosphoric acid (4.2.8) to the mark. Prepare fresh each day of analysis.

4.5.2 Erythorbic acid calibration solution, (optional), $\rho(C_6H_8O_6) \approx 10$ µg/ml

Pipet 1 ml of the erythorbic acid stock solution (4.4.2) into a 100 ml volumetric flask and dilute to the mark with metaphosphoric acid solution (4.2.8). The standard solution should have a concentration of 5 µg/ml to 20 µg/ml of erythorbic acid. Prepare fresh each day of analysis.

5 Apparatus

5.1 General

Usual laboratory apparatus, glassware, and, the following:

5.2 UV Spectrometer

Capable of measurement of absorbance at defined wavelengths.

5.3 High performance liquid chromatographic system

Consisting of a pump, sample injecting device, UV detector set at 265 nm and an evaluation system such as an integrator.

5.4 HPLC-Column

5.4.1 General

Other particle sizes or column dimensions than specified in this European standard may be used. Separation parameters have to be adapted to such materials to guarantee equivalent results. The performance criterion for suitable analytical columns is the baseline resolution of the L-ascorbic acid and the erythorbic acid [3].

5.4.2 Analytical column

Lichrospher® 100 RP 18 [1)] endcapped, particle size of 5 µm, diameter 4,0 mm, length 250 mm.

5.5 Filter device

A membrane filter with e.g. a pore size of 0,2 µm or of 0,45 µm. Filtering of the mobile phase as well as of the sample test solution through a membrane filter prior to use or injection will increase longevity of the columns.

6 Procedure

6.1 General

Standard solutions and sample solutions should be processed as soon as possible, kept under 25 °C during analysis and shall not be analysed after 8 h.

6.2 Preparation of the test sample

Homogenise the test sample. Grind coarse material with an appropriate mill and mix again. Immediately analyse the sample after homogenisation. Preparation of the test sample for raw vegetables and fruits shall be conducted with the extraction (see 6.3.1).

6.3 Preparation of the sample test solution

6.3.1 Extraction

Weigh an appropriate amount of the sample to the nearest 1 mg, e.g. 3 g if the vitamin C content is around 50 mg/100 g, in a 100 ml volumetric flask. Add 80 ml of the metaphosphoric acid solution (4.2.8) and shake. Dilute to the mark with metaphosphoric acid, shake and filter. This is the sample extract solution.

For raw vegetables and fruits, cut an appropriate amount with a knife, mix, and weigh an appropriate amount of the sample to the nearest mg, e.g. 2 g to 10 g of the sample directly in a 100 ml beaker containing metaphosphoric acid (4.2.8). Homogenize, transfer quantitatively into a 100 ml volumetric flask. Shake and filter. This is the sample extract solution.

[1)] Lichrospher® 100 RP 18 endcapped is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product. Equivalent products may be used if they can be shown to lead to the same results

NOTE The stability of ascorbic acid solution can be enhanced e.g. by adding 125 ml of the cysteine solution (4.2.10) or dithiothreitol (DTTA) or diethylene triamine phosphoric acid (DTPA) to the metaphosphoric acid solution. However, these stabilisers can influence the chromatography and have not been used for the interlaboratory test.

6.3.2 Reduction step

Immediately introduce 20 ml of the sample extract solution (6.3.1) into a 50 ml beaker. Add 10 ml of the L-Cysteine solution (4.2.10). Magnetically stir and adjust the pH to a value between 7,0 and 7,2 by adding the trisodium phosphate solution (4.2.9) and stir for exactly 5 min. Then decrease the pH to a value between 2,5 and 2,8 by adding metaphosphoric acid solution (4.2.7). Quantitatively transfer to a 50 ml volumetric flask by rinsing the electrode, the magnetic bar and the beaker with water. Adjust to the mark with water. Filter through a membrane filter (5.5). This filtrate is used for chromatography.

If the sample contains thickeners or solidifying agents, it is useful to precipitate them in order to avoid all risks of column obstruction. In that case, add 1 ml of methanol (4.2.6) to 4 ml of the reduced sample solution. Filter through a membrane filter (5.5). This filtrate is used for chromatography.

6.4 Identification

Identify the L-ascorbic acid by comparison of the retention time of the individual peaks in the chromatograms obtained with the sample test solution and with the standard test solution. Peak identification can also be performed by adding the standard substance to the sample test solution.

The separation and the quantification have proven to be satisfactory if following experimental conditions are followed (see also Figure A.1). The following conditions were used in the interlaboratory study:

Stationary phase:	Lichrospher® 100 RP 18 endcapped, 5 µm, 250 mm x 4,0 mm
Mobile phase:	solution A + solution B (4.2.11)
Flow rate:	0,7 ml/min
Injection volume:	30 µl
Detection:	UV 265 nm

This procedure can also be used to quantify erythorbic acid, which has not to be included as vitamin C.

NOTE Other suitable chromatographic conditions that can be used are: mobile phase with 10 % of acetonitril and 90 % of water containing 13,6 g/l of potassium dihydrogen phosphate and 2 g/l of cetrimid, a C8 type column with particle size of 10 µm, 250 mm × 4,6 mm and a flow rate of (1,0 ± 0,1) ml/min.

6.5 Determination

Depending on the system, inject appropriate volumes (not more than 50 µl) of the standard solution and of the sample test solution into the HPLC system. To carry out a determination by external calibration, integrate the peak areas or peak heights compare the results with the corresponding values for the standard substance, or use a calibration graph. Check the linearity of the calibration graph.

7 Calculation

Base the calculation on a calibration graph, or use the corresponding programs of the integrator, or use the following simplified procedure.

Calculate the mass fraction, *w*, of ascorbic acid in mg/100 g of the sample using equation (2):

$$w = \frac{A_s \times \rho \times V \times F \times 100}{A_{st} \times m \times 1000} \qquad (2)$$