SLOVENSKI STANDARD
SIST EN 14122:2003

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Živila - Določevanje vitamina B1 s HPLC

Foodstuffs - Determination of vitamin B1 by HPLC

Lebensmittel - Bestimmung von Vitamin B1 mit HPLC

Produits alimentaires - Détermination de la vitamine B1 par CLHP

Ta slovenski standard je istoveten z: **EN 14122:2003**

ICS:

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

SIST EN 14122:2003 **en**

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English version

Foodstuffs - Determination of vitamin B1 by HPLC

Produits alimentaires - Dosage de la vitamine B1 par CLHP

Lebensmittel - Bestimmung von Vitamin B1 mit HPLC

This European Standard was approved by CEN on 17 March 2003.

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

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Contents

Foreword

This document (EN 14122:2003) 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of an identical text or by endorsement, at the latest by November 2003, and conflicting national standards shall be withdrawn at the latest by November 2003.

Annexes A, B and C are informative.

WARNING — The use of this European Standard can involve hazardous materials, operations and equipment. This European Standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this European 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 a method for the determination of vitamin B_1 in foodstuffs by high performance liquid chromatography (HPLC). Vitamin B_1 is the mass fraction of total thiamin including its phosphorylated derivatives.

2 Normative references

This European Standard incorporates by dated or undated references, 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 3696:1995, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987).*

3 Principle

Thiamin is extracted from food after acid hydrolysis followed by dephosphorylation using an enzymatic treatment and quantified by HPLC with pre- or post-column derivatization to thiochrome [1] to [6].

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 3696:1995, or double distilled water.

4.2 Chemicals and solutions

4.2.1 Methanol, HPLC grade mass fraction $w(CH_3OH) \geq 99{,}8$ %

4.2.2 Acetic acid solution, substance concentration $c(CH_3COOH) = 0{,}02$ mol/l

4.2.3 Isobutanol, $w(C_4H_{10}O) \geq 98$ %

4.2.4 Sodium dihydrogen phosphate, $w(NaH_2PO_4) \geq 99{,}8$ %

4.2.5 Hydrochloric acid, $w(HCl) = 36$ %

4.2.6 Hydrochloric acid, $c(HCl) = 0{,}1$ mol/l

4.2.7 Sulfuric acid, $c(H_2SO_4) = 0{,}05$ mol/l

4.2.8 Sodium hydroxide, $w(NaOH) \geq 99$ %

4.2.9 Sodium hydroxide solution, mass concentration $\rho(NaOH) = 150$ g/l

4.2.10 Sodium hydroxide solution, $\rho(NaOH) = 200$ g/l

4.2.11 Potassium hexacyanoferrate III, $w\{K_3[Fe(CN)_6]\} \geq 99$ %

4.2.12 Potassium hexacyanoferrate III solution, $\rho\{K_3[Fe(CN)_6]\}$ = 10 g/l

4.2.13 Alkaline potassium hexacyanoferrate III solution (pre-column derivatization), $\rho\{K_3[Fe(CN)_6]\}$ = 0,4 g/l

Dilute 2,0 ml of the hexacyanoferrate solution (4.2.12) to 50 ml with sodium hydroxide solution (4.2.9). Prepare fresh each day of analysis.

4.2.14 Alkaline potassium hexacyanoferrate III solution (post-column derivatization), $\rho\{K_3[Fe(CN)_6]\}$ = 0,5 g/l

Dilute 2,5 ml of the hexacyanoferrate solution (4.2.12) to 50 ml with sodium hydroxide solution (4.2.10).

4.2.15 Taka diastase or suitable alternative [1)]

4.2.16 Sodium acetate solution, $c(CH_3COONa \cdot 3H_2O)$ = 2,5 mol/l

4.2.17 Sodium acetate solution, $c(CH_3COONa \cdot 3H_2O)$ = 0,5 mol/l

4.2.18 HPLC mobile phases

Examples of appropriate mixtures with volume fractions of e.g. 10 % to 50 % methanol (4.2.1) in water or using phosphate or acetate buffer are given in annex C. The possibility of using ion pairing agents is also given.

4.2.19 Phosphate buffer (pH 3,5), $c(KH_2PO_4)$ = 9,0 mmol/l

4.2.20 Tetraethylammoniumchloride, $w(C_8H_{20}NCl) \geq 98$ %

4.2.21 Sodium heptanesulfonate, $w(C_7H_{15}NaO_3S) \geq 98$ %

4.2.22 Acetate buffer, (pH 4,0), $c(CH_3COOH)$ = 50 mmol/l

4.3 Standard substances

4.3.1 General

Thiamin chloride hydrochloride can be obtained from various suppliers. The purity of the thiamin standard substances can vary and it is therefore necessary to determine the concentration of the calibration solution by UV-spectrometry (see 4.4.4).

4.3.2 Thiamin chloride hydrochloride, $w(C_{12}H_{17}ClN_4OS \cdot HCl) \geq 99$ %

4.3.3 Thiamin monophosphate chloride, $w(C_{12}H_{17}ClN_4O_4PS) \geq 98$ %

4.3.4 Thiamin pyrophosphate chloride (cocarboxylase), $w(C_{12}H_{19}ClN_4O_7P_2S) \geq 98$ %

4.4 Stock solutions

4.4.1 Thiamin chloride hydrochloride, $\rho(C_{12}H_{17}ClN_4OS \cdot HCl) \approx 0,1$ mg/ml

Dissolve an accurately weighed amount of the thiamin chloride hydrochloride standard substance (4.3.2) in a defined volume of an appropriate solvent, for example 10 mg of vitamin B_1 standard substance in 100 ml of hydrochloric acid (4.2.6). This solution can be stored for four weeks at + 4 °C.

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1) e.g Taka-Diastase Nr T00040, Pfalz & Bauer, Waterbury, CT 06708, USA. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

4.4.2 Thiamin monophosphate, $\rho(C_{12}H_{17}ClN_4O_4PS) \approx 0{,}1$ mg/ml

Dissolve an accurately weighed amount of the monophosphate standard substance (4.3.3) in a defined volume of an appropriate solvent, for example 10 mg of monophosphate standard substance in 100 ml of hydrochloric acid (4.2.6). This solution can be stored for four weeks at - 20 °C.

4.4.3 Thiamin pyrophosphate, $\rho(C_{12}H_{19}ClN_4O_7P_2S) \approx 0{,}1$ mg/ml

Dissolve an accurately weighed amount of the pyrophosphate standard substance (4.3.4) in a defined volume of an appropriate solvent, for example 10 mg of the pyrophosphate standard substance in 100 ml of hydrochloric acid (4.2.6).

4.4.4 Concentration tests

Dilute 10 ml of the thiamin chloride hydrochloride stock solution (4.4.1) with hydrochloric acid solution (4.2.6) in a 100 ml volumetric flask to the mark. Measure the absorbance of this solution at the maximum of about 247 nm in a 1 cm cell against hydrochloric acid solution (4.2.6) in the reference cell using an UV spectrometer (5.2). Calculate the mass concentration, ρ, in microgram per millilitre of the stock solution using equation (1):

$$\rho = \frac{\varepsilon_{247} \times 10^4 \times 10}{421} \qquad (1)$$

where

ε_{247} is the absorption value of the solution at the maximum wavelength of about 247 nm;

421 is the absorption coefficient $A^{1\%}_{1cm}$ of thiamin chloride hydrochloride in 0,1 mol/l hydrochloride acid (see [7])

10 is the dilution factor.

4.5 Standard solutions

4.5.1 Thiamin chloride hydrochloride, $\rho(C_{12}H_{17}ClN_4OS \cdot HCl) \approx 1$ µg/ml to 10 µg/ml

Pipette 1 ml to 10 ml of the thiamin chloride hydrochloride stock solution (4.4.1) into a 100 ml volumetric flask and dilute to the mark with the appropriate solvent, e.g. hydrochloric acid (4.2.6). This solution can be stored at 4 °C in the dark for 1 month.

4.5.2 Thiamin monophosphate, $\rho(C_{12}H_{17}ClN_4O_4PS) \approx 1$ µg/ml to 10 µg/ml

Pipette 1 ml to 10 ml of the monophosphate stock solution (4.4.2) into a 100 ml volumetric flask and dilute to the mark with the appropriate solvent, e.g. hydrochloric acid (4.2.6). This solution can be stored at 4 °C in the dark for 1 month.

4.5.3 Thiamin pyrophosphate, $\rho(C_{12}H_{19}ClN_4O_7P_2S) \approx 1$ µg/ml to 10 µg/ml

Pipette 1 ml to 10 ml of the pyrophosphate stock solution (4.4.3) into a 100 ml volumetric flask and dilute to the mark with the appropriate solvent, e.g. hydrochloric acid (4.2.6). This solution can be stored at 4 °C in the dark for 1 month.

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 Autoclave or heating device

Autoclave for extraction purpose, e.g. pressure cooker type, with pressure or temperature reading device, electrical heating device or water bath.

5.4 High performance liquid chromatographic system

Consisting of a pump, sample injecting device, fluorescence detector with excitation and emission wavelengths set e.g. at 366 nm and 420 nm, respectively (see annex C), and an evaluation system such as an integrator.

5.5 HPLC columns

5.5.1 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 analytes concerned [2)].

5.5.2 Pre-column oxidation

Analytical columns, e.g. Lichrospher® 60 RP Select B [2)], particle size of 5 µm, diameter 4,0 mm to 4,6 mm, length 100 mm to 250 mm.

5.5.3 Post-column oxidation

Analytical columns, e.g. Supelco® LC-18-DB [2)], particle size of 5 µm, diameter 4,0 mm to 4,6 mm, length 100 mm to 250 mm.

5.6 Filter device

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

5.7 Post-column reactor pump and derivatization tube

A suitable reagent delivery system, a T-type connecting tube and a derivatization tube (e.g. 10 m x 0,33 mm).

6 Procedure

6.1 Preparing of the sample solution

Homogenise the test sample. Grind coarse material with an appropriate mill and mix again. Measures such as pre-cooling have to be taken to avoid exposing to high temperature for long periods of time.

6.2 Preparation of the sample test solution

6.2.1 Extraction

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2) Suitable silica column packing materials available commercially are Lichrosorb® Si 60, Spherisorb® Si, Hypersil® Si and Lichrospher® 100 DIOL. Suitable RP column packing materials are Spherisorb® ODS, µ-Bondapak® radial C18, Supelco® LC-18-DB and Hypersil® ODS. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.