National Standards Authority of Ireland

IRISH STANDARD

I.S. ENV 14164:2002

ICS 67.050

National Standards
Authority of Ireland
Dublin 9
Ireland

Tel. (01) 807 3800
Tel (01) 807 3838

FOODSTUFFS - DETERMINATION OF VITAMIN B6 BY HPLC

This Irish Standard was published under the authority of the National Standards Authority of Ireland and comes into effect on. February 22. 2002

Price Code F

Údarás um Chaighdeáin Náisiúnta na hÉireann

EUROPEAN PRESTANDARD
PRÉNORME EUROPÉENNE
EUROPÄISCHE VORNORM

ENV 14164

February 2002

ICS 67.050

English version

Foodstuffs - Determination of vitamin B6 by HPLC

Produits alimentaires - Détermination de la vitamine B6 par CLHP

Lebensmittel - Bestimmung von Vitamin B6 mit HPLC

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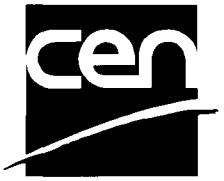

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPAISCHES KOMITEE FUR NORMUNG

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. ENV 14164:2002 E

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.

The annexes A, B, C and D are informative.

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, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Prestandard specifies a method for the determination of vitamin B_6 in foodstuffs by HPLC.

Vitamin B_6 is the mass fraction of the sum of pyridoxine, pyridoxal, pyridoxamine including their phosphorylated derivatives determined as pyridoxine. The β-glycosylated forms are not taken into account.

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 European Prestandard 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

Pyridoxal, pyridoxamine and pyridoxine are extracted from food by acid hydrolysis and dephosphorylated enzymatically using acid phosphatase.

By reaction with glyoxylic acid in presence of Fe++ as catalyst, pyridoxamine is transformed into pyridoxal, which is then reduced to pyridoxine by the action of sodium borohydride in alkaline medium. Pyridoxine is then quantified in the sample solution by HPLC with a fluorometric detection [1], [2].

It is also possible to quantify pyridoxine, pyridoxal and pyridoxamine separately and to sum the different contents obtained. In that case all risk of interference with the matrix will have to be verified and the analytical conditions adapted. The procedure is not described in this European Prestandard because it has not been validated. Adapted conditions can be found in the bibliography [1] to [4].

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 Acid phosphatase, from potatoes (it can be obtained from Boehringer)[1] Enzymatic activity: 2 U/mg.

4.2.2 Sodium acetate, trihydrate, *w* ($CH_3COONa \cdot 3\ H_2O$) ≥ 99,0 %

4.2.3 Glacial acetic acid, *w* (CH_3COOH) ≥ 99,8 %

4.2.4 Glyoxylic acid, *w* ($C_2H_2O_3 \cdot H_2O$) ≥ 97,0 %

4.2.5 Ferrous sulfate II, heptahydrate, *w* ($FeSO_4 \cdot 7H_2O$) ≥ 99,5 %

4.2.6 Sodium hydroxide, *w* (NaOH) ≥ 98,0 %

4.2.7 Sodium borohydride, *w* ($NaBH_4$) ≥ 97,0 %

4.2.8 Potassium dihydrogen phosphate, *w* (KH_2PO_4) ≥ 99,0 %

4.2.9 Orthophosphoric acid, *w* (H_3PO_4) ≥ 84,0 %

4.2.10 Sodium octanesulfonate, *w* ($C_8H_{17}NaO_3S$) ≥ 98,0 %, *or sodium heptanesulfonate,* *w* ($C_7H_{15}NaO_3S$) ≥ 98,0 %

4.2.11 Acetonitrile (HPLC grade), *w* (C_2H_3N) ≥ 99,8 %

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

Dissolve 170,1 g of sodium acetate, trihydrate (4.2.2) in 500 ml of water.

4.2.13 Sodium acetate solution,*c* ($CH_3COONa \cdot 3H_2O$) = 0,05 mol/l (pH 4,5):

Dissolve 6,8 g of sodium acetate, trihydrate (4.2.2) in 1 l of water. Adjust the pH to 4,5 with glacial acetic acid (4.2.3).

4.2.14 Ferrous sulfate solution,*c* ($FeSO_4 \cdot 7H_2O$) = 0,0132 mol/l

Dissolve 36,6 mg of ferrous sulfate II, heptahydrate (4.2.5) in 10 ml of sodium acetate solution (4.2.13).

4.2.15 Sodium hydroxide solution,*c* (NaOH) = 0,2 mol/l

Dissolve 800 mg of sodium hydroxide (4.2.6) in 100 ml of water.

4.2.16 Sodium borohydride solution,*c* ($NaBH_4$) = 0,1 mol/l

Dissolve 378 mg of sodium borohydride (4.2.7) in 100 ml of sodium hydroxide solution (4.2.15).

4.2.17 Glyoxylic acid solution,*c* ($C_2H_2O_3 \cdot H_2O$) = 1 mol/l (pH 4.5):

Dissolve 4,7 g of glyoxylic acid monohydrate (4.2.4) in 30 ml of sodium acetate solution (4.2.12). Adjust the pH to 4,5 with the sodium acetate solution (4.2.12) and dilute to 50 ml with water in a volumetric flask.

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1) This information is given for the convenience of users of this standard method 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.

4.2.18 Hydrochloric acid, c (HCl) = 0,1 mol/l

4.2.19 HPLC mobile phase

In a beaker add 940 ml of water, 40 ml of acetonitrile (4.2.11), 160 mg of sodium octanesulfonate or sodium heptanesulfonate (4.2.10) and 6,8 g of potassium dihydrogen phosphate (4.2.8).

After shaking, adjust the pH to 2,5 with orthophosphoric acid (4.2.9). Transfer the solution in a 1 l volumetric flask. Adjust to the mark with water.

Filter through a 0,45 µm filter.

4.3 Vitamin B_6 standard substance

Pyridoxine hydrochloride, w ($C_8H_{11}NO_3 \cdot HCl$) ≥ 99 %

4.4 Stock solution

4.4.1 Vitamin B_6 stock solution, ρ ($C_8H_{11}NO_3 \cdot HCl$) ≈ 0,5 mg/ml

Dissolve an accurately weighed amount of the vitamin B_6 standard substance (4.3), e.g. approximately 50 mg in a defined volume, e.g. 100 ml, of water. The stock solution is stable for 4 weeks if stored at 4 °C in the dark.

4.4.2 Concentration test

Dilute 0,5 ml of vitamin B_6 stock solution (4.4.1) to 20 ml with 0,1 mol/l HCl (4.2.18) and measure the absorbance at 290 nm in a 1 cm cell using a UV-spectrometer (5.2) against 0,1 mol/l HCl solution as reference.

Calculate the mass concentration ρ, in microgram per millilitre of the stock solution according to equation (1):

$$\rho = \frac{A \cdot M_w}{8{,}6} \cdot F \qquad (1)$$

where

A is the absorbance value of the solution at 290 nm;

M_w is the molecular weight of vitamin B_6 standard substance, in gram per mol;

F is the dilution factor, i.e. 40.

8,6 is the molar extinction coefficient ε of pyridoxine hydrochloride in 0,1 mol/l hydrochloric acid at 290 nm [4], [5], in $mmol^{-1}\ cm^{-1}$;

Further information on molar extinction coefficients in other solutions than 0,1 mol/l HCl (pH ~ 1) can be seen in annex D.

4.5 Standard solutions

4.5.1 Vitamin B_6 intermediate standard solution, ρ ($C_8H_{11}NO_3 \cdot HCl$) ≈ 10 µg/ml

Pipette 1 ml of the vitamin B_6 stock solution (4.4.1) into a 50 ml volumetric flask and dilute to the mark with water.

Prepare this solution each day of analysis.