

Foodstuffs — Determination of vitamin B₆ by HPLC

ICS 67.050

National foreword

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This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion into a European Standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its three-year life. The commencement of the review period will be notified by an announcement in *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into a European Standard, to extend the life of the prestandard or to withdraw it. Comments should be sent in writing to the Secretary of BSI Technical Committee AW/-/3, Food analysis — Horizontal methods, at 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled “International Standards Correspondence Index”, or by using the “Find” facility of the BSI Standards Electronic Catalogue.

Summary of pages

This document comprises a front cover, an inside front cover, the ENV title page, pages 2 to 12, an inside back cover and a back cover.

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This Draft for Development, having been prepared under the direction of the Consumer Products and Services Sector Policy and Strategy Committee, was published under the authority of the Standards Policy and Strategy Committee on 26 February 2002

Amendments issued since publication

Amd. No.	Date	Comments

© BSI 26 February 2002

ISBN 0 580 39030 6

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

This European Prestandard (ENV) was approved by CEN on 15 November 2001 as a prospective standard for provisional application.

The period of validity of this ENV is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the ENV can be converted into a European Standard.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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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.

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 B₆ in foodstuffs by HPLC.

Vitamin B₆ 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, $w(\text{CH}_3\text{COONa} \cdot 3 \text{H}_2\text{O}) \geq 99,0 \%$

4.2.3 Glacial acetic acid, $w(\text{CH}_3\text{COOH}) \geq 99,8 \%$

4.2.4 Glyoxylic acid, $w(\text{C}_2\text{H}_2\text{O}_3 \cdot \text{H}_2\text{O}) \geq 97,0 \%$

4.2.5 Ferrous sulfate II, heptahydrate, $w(\text{FeSO}_4 \cdot 7\text{H}_2\text{O}) \geq 99,5 \%$

4.2.6 Sodium hydroxide, $w(\text{NaOH}) \geq 98,0 \%$

4.2.7 Sodium borohydride, $w(\text{NaBH}_4) \geq 97,0 \%$

4.2.8 Potassium dihydrogen phosphate, $w(\text{KH}_2\text{PO}_4) \geq 99,0 \%$

4.2.9 Orthophosphoric acid, $w(\text{H}_3\text{PO}_4) \geq 84,0 \%$

4.2.10 Sodium octanesulfonate, $w(\text{C}_8\text{H}_{17}\text{NaO}_3\text{S}) \geq 98,0 \%$, or sodium heptanesulfonate, $w(\text{C}_7\text{H}_{15}\text{NaO}_3\text{S}) \geq 98,0 \%$

4.2.11 Acetonitrile (HPLC grade), $w(\text{C}_2\text{H}_3\text{N}) \geq 99,8 \%$

4.2.12 Sodium acetate solution, $c(\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}) = 2,5 \text{ mol/l}$

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

4.2.13 Sodium acetate solution, $c(\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}) = 0,05 \text{ mol/l}$ (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, $c(\text{FeSO}_4 \cdot 7\text{H}_2\text{O}) = 0,0132 \text{ mol/l}$

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, $c(\text{NaOH}) = 0,2 \text{ mol/l}$

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

4.2.16 Sodium borohydride solution, $c(\text{NaBH}_4) = 0,1 \text{ mol/l}$

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, $c(\text{C}_2\text{H}_2\text{O}_3 \cdot \text{H}_2\text{O}) = 1 \text{ mol/l}$ (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.

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.