Foodstuffs - Determination of vitamin B6 by HPLC

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NATIONAL FOREWORD

Käesolev Eesti standard EVS-EN 14164:2008 sisaldab Euroopa standardi EN 14164:2008 ingliskeelset teksti.	This Estonian standard EVS-EN 14164:2008 consists of the English text of the European standard EN 14164:2008.
Standard on kinnitatud Eesti Standardikeskuse 21.07.2008 käskkirjaga ja jõustub sellekohase teate avaldamisel EVS Teatajas.	This standard is ratified with the order of Estonian Centre for Standardisation dated 21.07.2008 and is endorsed with the notification published in the official bulletin of the Estonian national standardisation organisation.
Euroopa standardimisorganisatsioonide poolt rahvuslikele liikmetele Euroopa standardi teksti kättesaadavaks tegemise kuupäev on 04.06.2008.	Date of Availability of the European standard text 04.06.2008.
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English Version

Foodstuffs - Determination of vitamin B_6 by HPLC

Produits alimentaires - Dosage de la vitamine B_6 par CLHP

Lebensmittel - Bestimmung von Vitamin B_6 mit HPLC

This European Standard was approved by CEN on 5 December 2007.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 14164:2008) 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of an identical text or by endorsement, at the latest by December 2008, and conflicting national standards shall be withdrawn at the latest by December 2008.

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1 Scope

This European Standard specifies a method for the determination of vitamin B_6 in foodstuffs by high performance liquid chromatography (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. These can be determined with the method given in EN 14663 [1] by which the different vitamers of vitamin B_6 (pyridoxal, pyridoxamine and pyridoxine) are separated and individually quantified. A third European Standard (EN 14166 [1]) [2] determines the total vitamin B_6 by microbiological assay.

2 Normative references

The following referenced document is indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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 the presence of Fe^{2+} as a 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 [3], [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, (CAS 9001-77-8), from potatoes, enzyme activity is 33 nkat/mg [2)] with substrate p-nitrophenyl phosphate at pH = 4,8 and T = 37 °C, for example from Boehringer or Sigma [3)]. 33 nkat/mg corresponds to 2 U/mg.

4.2.1.1 Acid phosphatase solution

Prepare a solution of 20 mg/ml acid phosphatase in sodium acetate solution (4.2.14).

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[1] Under elaboration.

2) Katal (symbol "kat") is a derived SI unit of enzyme activity. One katal is that catalytic activity which will raise the rate of reaction by one mol/s in a specified assay system.

3) 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.