Foodstuffs - Determination of vitamin B6 by high performance chromatography

EESTI STANDARDIKESKUS
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NATIONAL FOREWORD

See Eesti standard EVS-EN 14164:2014 sisaldab Euroopa standardi EN 14164:2014 inglisekeelset teksti.	This Estonian standard EVS-EN 14164:2014 consists of the English text of the European standard EN 14164:2014.
Standard on jõustunud sellekohase teate avaldamisega EVS Teatajas.	This standard has been endorsed with a notification published in the official bulletin of the Estonian Centre for Standardisation.
Euroopa standardimisorganisatsioonid on teinud Euroopa standardi rahvuslikele liikmetele kättesaadavaks 04.06.2014.	Date of Availability of the European standard is 04.06.2014.
Standard on kättesaadav Eesti Standardikeskusest.	The standard is available from the Estonian Centre for Standardisation.

Tagasisidet standardi sisu kohta on võimalik edastada, kasutades EVS-i veebilehel asuvat tagasiside vormi või saates e-kirja meiliaadressile standardiosakond@evs.ee.

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Foodstuffs - Determination of vitamin B6 by high performance chromatography

Produits alimentaires - Détermination de la teneur en vitamine B6 par chromatographie liquide haute performance

Lebensmittel - Bestimmung von Vitamin B6 mit Hochleistungs-Flüssigchromatographie

This European Standard was approved by CEN on 17 April 2014.

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Foreword

This document (EN 14164:2014) 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of an identical text or by endorsement, at the latest by December 2014 and conflicting national standards shall be withdrawn at the latest by December 2014.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14164:2008.

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

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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.

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 vitamins of vitamin B_6 (pyridoxal, pyridoxamine and pyridoxine) are separated and individually quantified. A third European Standard, EN 14166 [2], determines the total vitamin B_6 by microbiological assay.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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)*

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

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

4.1 Acid phosphatase, (CAS 9001-77-8), from potatoes, enzyme activity is 33 nkat/mg[1)] with substrate p-nitrophenyl phosphate at pH = 4,8 and T = 37 °C, for example from Boehringer or Sigma[2)]. 33 nkat/mg corresponds to 2 U/mg.

4.1.1 Acid phosphatase solution

Prepare a solution of 20 mg/ml acid phosphatase in sodium acetate solution (4.14).

It is necessary to use acid phosphatase rather than Taka-diastase to obtain a complete hydrolysis of phosphorylated forms of vitamin B_6. Where 300 mg of Taka-diastase is needed to obtain good dephosphorylation, only 0,5 mg of acid phosphatase is needed, see [5].

1) 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.

2) This information is given for the convenience of users of this European standard and does not constitute an endorsement by CEN of the supplier. Equivalent products may be used if they can be shown to lead to the same results.