Foodstuffs - Determination of vitamin B6 (including its glycosylated forms) by HPLC

Foodstuffs - Determination of vitamin B6 (including its glycosylated forms) by HPLC

EESTI STANDARDIKESKUS

EESTI STANDARDI EESSÕNA **NATIONAL FOREWORD**

Käesolev Eesti standard EVS-EN 14663:2006 sisaldab Euroopa standardi EN 14663:2005 ingliskeelset teksti. Käesolev dokument on jõustatud 27.02.2006 ja selle kohta on avaldatud teade Eesti standardiorganisatsiooni ametlikus väljaandes. Standard on kättesaadav Eesti standardiorganisatsioonist.	This Estonian standard EVS-EN 14663:2006 consists of the English text of the European standard EN 14663:2005. This document is endorsed on 27.02.2006 with the notification being published in the official publication of the Estonian national standardisation organisation. The standard is available from Estonian standardisation organisation.

Käsitlusala: This document specifies a method for the determination of vitamin B6 in foodstuffs by high performance liquid chromatography (HPLC).	**Scope:** This document specifies a method for the determination of vitamin B6 in foodstuffs by high performance liquid chromatography (HPLC).

ICS 67.050

Võtmesõnad:

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14663

December 2005

ICS 67.050

English Version

Foodstuffs - Determination of vitamin B6 (including its glycosylated forms) by HPLC

Produits alimentaires - Dosage de la vitamine B6 (y compris ses formes glycosylées) par CLHP

Lebensmittel - Bestimmung von Vitamin B6 (einschließlich glucosidisch gebundener Verbindungen) mit HPLC

This European Standard was approved by CEN on 26 October 2005.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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

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

Ref. No. EN 14663:2005: E

Contents

Page

Figure

Tables

Foreword

This document (EN 14663:2005) 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 June 2006, and conflicting national standards shall be withdrawn at the latest by June 2006.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

1 Scope

This document 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 as well as the β-glycosylated forms, calculated as pyridoxine.

This method has been successfully validated with semolina with milk (infant food), potato puree, vegetables with ham (convenient products) and a multi vitamin drink at levels from 0,034 mg/100 g to 1,21 mg/100 g.

2 Normative references

The following referenced documents are 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 and deglycosilated enzymatically using acid phosphatase and β-glucosidase.

The different derivatives of vitamin B_6 (pyridoxal, pyridoxamine and pyridoxine) are separated by HPLC and quantified by fluorometric detection [1], [2].

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 Di-potassium hydrogen phosphate, mass fraction $w(K_2HPO_4 \cdot 3\ H_2O) \geq 99{,}9\ \%$

4.3 Sodium acetate, without crystal water, $w(CH_3COONa) \geq 99{,}0\ \%$

4.4 Trichloroacetic acid (TCA), $w(Cl_3CCOOH) \geq 99{,}0\ \%$

4.5 Sodium acetate solution, substance concentration $c(CH_3COONa) = 2{,}5$ mol/l

Dissolve 205 g of sodium acetate (4.3) in 1 l of water.

4.6 Post-column reagent (optional), K_2HPO_4 solution $c(K_2HPO_4) = 0{,}15$ mol/l

Dissolve 34,2 g of di-potassium hydrogen phosphate (4.2) in water, dilute to 1 000 ml, mix and degas.