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Foodstuffs — Determination of vitamin B2 by high performance liquid chromatography

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

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Foodstuffs - Determination of vitamin B2 by high performance liquid chromatography

Produits alimentaires - Détermination de la teneur en vitamine B2 par chromatographie liquide haute performance

Lebensmittel - Bestimmung von Vitamin B2 mit Hochleistungs-Flüssigchromatographie

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

This document (EN 14152:2014) 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of an identical text or by endorsement, at the latest by December 2014 and conflicting national standards shall be withdrawn at the latest by December 2014.

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This document supersedes EN 14152:2003.

Annexes A, B and C are informative.

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WARNING — The use of this standard can involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this 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_2 in food by high performance liquid chromatography (HPLC) and fluorescence detection. This method has been validated in two interlaboratory studies. The first study was for the analysis of samples of milk powder and pig's liver ranging from 1,45 mg/100 g to 10,68 mg/100 g. The second study was for the analysis of samples of tube feeding solution, baby food, powdered milk, meal with fruits, yeast, cereal and chocolate powder ranging from 0,21 mg/100 g to 87,1 mg/100 g. Vitamin B_2 is the mass fraction of total riboflavin including its phosphorylated derivatives.

For further information on the validation, see Clause 8 and Annex B.

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

Riboflavin is extracted from food after acid hydrolysis followed by dephosphorylation using an enzymatic treatment, and separated by HPLC, and detected by fluorometric detection. An external standard is used for quantification. For further information see [1] to [11].

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 Methanol, mass fraction $w(CH_3OH) \geq 99,8$ %, HPLC grade.

4.2 Sodium acetate trihydrate, $w(CH_3COONa \cdot 3H_2O) = 99$ %.

4.3 Sodium acetate solution, substance concentration $c(CH_3COONa \cdot 3H_2O) = 0,1$ mol/l.

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

4.5 Glacial acetic acid, $w(CH_3COOH) = 99,8$ %.

4.6 Acetic acid solution, $c(CH_3COOH) = 0,02$ mol/l.

4.7 Hydrochloric acid, $w(HCl) = 36$ %.

4.8 Hydrochloric acid, $c(HCl) = 0,1$ mol/l.

4.9 Hydrochloric acid, $c(HCl) = 0,01$ mol/l.

4.10 Sulfuric acid, $c(H_2SO_4) = 0,05$ mol/l.

4.11 Sodium hydroxide, $w(NaOH) \geq 99$ %.