

Foodstuffs — Determination of vitamin B2 by HPLC

The European Standard EN 14152:2003 has the status of a
British Standard

ICS 67.050

National foreword

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Summary of pages

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English version

Foodstuffs - Determination of vitamin B2 by HPLC

Produits alimentaires - Dosage de la vitamine B2 par CLHP

Lebensmittel - Bestimmung von Vitamin B2 mit HPLC

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Foreword

This document (EN 14152:2003) 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of an identical text or by endorsement, at the latest by January 2004, and conflicting national standards shall be withdrawn at the latest by January 2004.

Annexes A, B and C are informative.

Warning – The use of this standard may 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.

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1 Scope

This European Standard specifies a method for the determination of vitamin B₂ in foodstuffs by high performance liquid chromatography (HPLC). The determination of vitamin B₂ content is carried out by measurement of riboflavin.

2 Normative references

This European Standard 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 Standard 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

Riboflavin in an appropriate sample solution is determined after acid hydrolysis followed by dephosphorylation using an enzymatic treatment by high performance liquid chromatographic (HPLC) separation with a fluorometric detection [1] to [8].

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 Methanol, mass fraction, $w(\text{CH}_3\text{OH}) \geq 99,8 \%$ HPLC grade

4.2.2 Sodium acetate trihydrate, $w(\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}) = 99 \%$

4.2.3 Sodium acetate solution, substance concentration $c(\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}) = 0,1 \text{ mol/l}$

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

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

4.2.6 Acetic acid solution, $c(\text{CH}_3\text{COOH}) = 0,02 \text{ mol/l}$

4.2.7 Hydrochloric acid, $w(\text{HCl}) = 36 \%$

4.2.8 Hydrochloric acid, $c(\text{HCl}) = 0,1 \text{ mol/l}$

4.2.9 Hydrochloric acid, $c(\text{HCl}) = 0,01 \text{ mol/l}$

4.2.10 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$

4.2.11 Sodium hydroxide, $w(\text{NaOH}) = 99 \%$

4.2.12 Sodium hydroxide solution, $c(\text{NaOH}) = 0,5 \text{ mol/l}$

4.2.13 Phosphorous pentoxide, $w(\text{P}_2\text{O}_5) = 98 \%$

4.2.14 Dephosphorylation enzyme, with the ability to hydrolyse bound riboflavin from food

NOTE Taka-Diastase¹⁾ has been used for the determination of the precision data.

4.2.15 HPLC Mobile phase

Examples of appropriate mixtures of e. g. 10 % to 50 % methanol (4.2.1) in water or using phosphate or acetate buffer are given in annex A1 and C; possibility of using ion-pairing agents is also given.

¹⁾ Taka-Diastase Nr. T00040 is the trade name of a product supplied by Pfaltz & Bauer, Waterbury, CT 06708, USA. This information is given for the convenience of users of this European standard 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.