

Foodstuffs — Determination of d- biotin by HPLC

ICS 67.050

National foreword

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Contents

Page

Foreword.....	3
1 Scope	4
2 Normative references	4
3 Principle	4
4 Reagents	4
5 Apparatus	7
6 Procedure	8
7 Calculation	9
8 Precision	9
9 Test report	10
Annex A (informative) Typical chromatogram	11
Annex B (informative) Precision data	13
Bibliography	14

Foreword

This document (EN 15607:2009) 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of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by November 2009.

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1 Scope

This European Standard specifies a method for the determination of the mass fraction of d-biotin by high performance liquid chromatography (HPLC). The method has been validated in an inter-laboratory test on fortified and non-fortified samples such as cereal breakfast powder, infant milk powder, lyophilized green peas with ham, lyophilized chicken soup and on nutritive orange juice, at levels from 16 µg/100 g to 200 µg/100 g. For further information on the validation data, see Annex B.

NOTE 1 d-biocytyl can also be estimated by this method. But none of the samples used for the validation step contained d-biocytyl. Nonetheless the recovery rate is more than 90 % for d-biotin and d-biocytyl, see [2] and [3].

NOTE 2 The method underestimates the real biotin content when used for samples containing egg.

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 3696:1995, *Water for analytical laboratory use – Specification and test methods (ISO 3696:1987)*

3 Principle

D-biotin is extracted from food after an enzymatic treatment and quantified by HPLC with post-column binding reaction, see [2] and [3].

The complexation of d-biotin with avidin appears to be very specific. On that account, this protein, covalently bound to a fluorescent marker, fluorescein 5-isothiocyanate, can be used as a reagent for a post-column binding of d-biotin, see [4] and [5].

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 3696:1995, or double distilled water.

4.2 Chemicals and solutions

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

4.2.2 Sulfuric acid solution, substance concentration $c(\text{H}_2\text{SO}_4) = 1 \text{ mol/l}$

4.2.3 Sulfuric acid solution, $c(\text{H}_2\text{SO}_4) = 1,5 \text{ mol/l}$

4.2.4 Citric acid monohydrate, $w(\text{C}_6\text{H}_8\text{O}_7 \cdot \text{H}_2\text{O}) \geq 99,7 \%$

4.2.5 Sodium monohydrogen phosphate dihydrate, $w(\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}) \geq 99,8 \%$