SLOVENSKI STANDARD
SIST EN 15607:2009

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Živila - Določevanje d-biotina s HPLC

Foodstuffs - Determination of d-biotin by HPLC

Lebensmittel - Bestimmung von d-Biotin mit HPLC

Produits alimentaires - Dosage de la d-biotine par CLHP

Ta slovenski standard je istoveten z: **EN 15607:2009**

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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

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Foodstuffs - Determination of d-biotin by HPLC

Produits alimentaires - Dosage de la d-biotine par CLHP

Lebensmittel - Bestimmung von d-Biotin mit HPLC

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

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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of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by November 2009.

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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 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-fortifed 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-biocytin can also be estimated by this method. But none of the samples used for the validation step contained d-biocytin. Nonetheless the recovery rate is more than 90 % for d-biotin and d-biocytin, 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 complexion 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 $w(CH_3OH) \geq 99{,}8$ %

4.2.2 Sulfuric acid solution, substance concentration $c(H_2SO_4) = 1$ mol/l

4.2.3 Sulfuric acid solution, $c(H_2SO_4) = 1{,}5$ mol/l

4.2.4 Citric acid monohydrate, $w(C_6H_8O_7 \cdot H_2O) \geq 99{,}7$ %

4.2.5 Sodium monohydrogen phosphate dihydrate, $w(Na_2HPO_4 \cdot 2H_2O) \geq 99{,}8$ %

4.2.6 Gluthatione, $w(C_{10}H_{17}N_3O_6S) \geq 98$ %

4.2.7 EDTA sodium salt dihydrate, $w(C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O) \geq 99$ %

4.2.8 Potassium monohydrogen phosphate, $w(K_2HPO_4) \geq 96$ %

4.2.9 Potassium dihydrogen phosphate, $w(KH_2PO_4) \geq 99{,}5$ %

4.2.10 Citrate buffer solution

Dissolve 0,462 g of citric acid monohydrate (4.2.4) and 1,05 g of sodium monohydrogen phosphate dihydrate (4.2.5) in 450 ml of distilled water. Adjust the solution to pH = 5,7 with sulfuric acid solution (4.2.3), and then dilute to 500 ml with distilled water. This solution is stable for 1 day.

4.2.11 Gluthatione solution, mass concentration $\rho(C_{10}H_{17}N_3O_6S) = 10$ g/l

Dissolve 30 mg of gluthatione (4.2.6) in 3 ml of distilled water. This solution is stable for 1 day.

4.2.12 EDTA solution, $\rho(C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O) = 10$ g/l

Dissolve 0,1 g of EDTA (4.2.7) in 10 ml of distilled water. This solution is stable for 1 day.

4.2.13 Potassium monohydrogen phosphate solution, $c(K_2HPO_4) = 0{,}1$ mol/l

Dissolve 17,4 g of potassium monohydrogen phosphate (4.2.8) in 1000 ml of water. This solution is stable for 2 days.

4.2.14 Potassium dihydrogen phosphate solution, $c(KH_2PO_4) = 0{,}1$ mol/l

Dissolve 13,6 g of potassium dihydrogen phosphate (4.2.9) in 1000 ml of water. This solution is stable for 2 days.

4.2.15 Phosphate buffer solution pH = 6,0

Mix 4.2.13 and 4.2.14 in such a proportion that the final solution has a pH of 6,0 (e.g. 30 parts per volume of 4.2.13 and 70 parts per volume of 4.2.14). This solution is stable for 1 week at room temperature.

4.2.16 Phosphate buffer solution pH = 7,0

Mix 4.2.13 and 4.2.14 in such a proportion that the final solution has a pH of 7,0 (e.g. 40 parts per volume of 4.2.13 and 60 parts per volume of 4.2.14). This solution is stable for 1 week at room temperature.

4.2.17 Papain powder, (CAS 9001-73-4), enzyme activity is 15 nkat/mg[1] with substrate N-benzoyl-L-arginine ethyl ester (BAEE) at pH = 6,2 and t = 25 °C. 15 nkat/mg corresponds to 1 U/mg.

4.2.18 Papain solution, ρ(papain) = 20 g/l

4.2.18.1 General

Dissolve 1 g of papain powder (4.2.17) in 50 ml of citrate buffer solution (4.2.10). This solution is stable for 5 days at 4 °C.

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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.

4.2.18.2 Activity check of papain

The activity of papain can be checked by making a second extract (see 6.2) with a double amount of enzyme. Verify that the level of d-biotin calculated is the same and not higher.

NOTE For the interlaboratory study, the papain powder from VWR International GmbH, Hilpertstraße 20a, 64295 Darmstadt, ref. nr. 26.146.180 has been used [2].

4.2.19 Avidin fluoresceine isothiocyanate (Avidin-FITC), labelled, 80 % protein, 2 mol to 4 mol FITC per mol of avidin

4.2.20 Stock solution reagent for post-column binding reaction, ρ(avidin-FITC) = 50 mg/ml

Dissolve 2,5 mg of avidin-FITC (4.2.19) in 50 ml of phosphate buffer solution pH = 7,0 (4.2.16). This solution is stable for 2 weeks at 4 °C.

4.2.21 Reagent for post-column binding reaction, ρ(avidin-FITC) = 2 mg/ml

Add 600 ml of phosphate buffer solution pH = 7,0 (4.2.16) to 25 ml of the stock solution (4.2.20). Filter this solution through a 0,45 µm filter (5.5). This solution is stable for 8 hours, screened from light.

4.2.22 HPLC mobile phase

Mix 80 parts per volume of phosphate buffer solution pH = 6,0 (4.2.15) with 20 parts per volume of methanol (4.2.1). Filter this solution through a 0,45 µm filter (5.5).

4.2.23 Taka-diastase from *Aspergillus Oryzae*, enzyme activity is 1 500 nkat/mg (1 500 nkat/mg corresponds to 100 U/mg), suitable for samples with a high starch content.

4.3 Standard substances

4.3.1 General

D-biotin and d-biocytin can be obtained from various suppliers. The baseline separation of d-biotin and d-biocytin shall be verified. So it is necessary to prepare a standard solution.

The biotin content of the standard can be confirmed according to the European Pharmacopoeia procedure [6].

4.3.2 d-biotin, $w(C_{10}H_{16}N_2O_3S) \geq 99$ %

4.3.3 d-biocytin, $w(C_{16}H_{28}N_4O_4S) \geq 98$ %

4.4 Stock solutions

4.4.1 d-biotin, $\rho(C_{10}H_{16}N_2O_3S) = 100$ µg/ml

Dissolve an amount of the d-biotin standard substance (4.3.2), approximately 10 mg to the nearest 0,1 mg in 100 ml of distilled water. It may take 4 h to 5 h to dissolve. This solution is stable for 2 months at –18 °C.

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2 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.

4.4.2 d-biocytin, $\rho(C_{16}H_{28}N_4O_4S)$ = 100 µg/ml

Dissolve an amount of the d-biocytin standard substance (4.3.3), approximately 10 mg to the nearest 0,1 mg in 100 ml of distilled water. This solution is stable for 2 months at –18 °C.

4.5 Standard solutions

4.5.1 d-biotin solution, $\rho(C_{10}H_{16}N_2O_3S)$ = 0,05 µg/ml to 0,30 µg/ml

Prepare for example a solution with 1 ml of the stock solution (4.4.1) in 10 ml of distilled water. Then prepare six calibration solutions (0,5 ml, 1,0 ml, 1,5 ml, 2,0 ml, 2,5 ml and 3 ml) in 100 ml of distilled water. These solutions are stable for 1 day.

4.5.2 d-biocytin solution, $\rho(C_{16}H_{28}N_4O_4S)$ = 0,30 µg/ml

Prepare for example a solution with 1 ml of the stock solution (4.4.2) in 10 ml of distilled water. Then prepare a standard solution with 3 ml in 100 ml of distilled water. Solution is stable for 1 day.

5 Apparatus

5.1 General

Usual laboratory apparatus and glassware, and the following.

5.2 Oven

Capable of maintaining a temperature of 37 °C ± 2 °C.

5.3 HPLC system

Consisting of a pump, sample injecting device, fluorescence detector with excitation wavelength set at 490 nm and emission wavelength set at 520 nm, and an evaluation system such as an integrator.

5.4 Analytical reverse-phase separating column, e.g. LiChrospher® 100 RP-18 endcapped [3]

The column shall ensure a baseline resolution of the analytes concerned with the following characteristics:

a) a length of 250 mm;

b) an inner diameter of 4,0 mm;

c) a particle size of 5 µm.

Other particle sizes or column dimensions than specified in this European Standard may be used. Separation parameters shall be adapted to such other materials to guarantee equivalent results.

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3 LiChrospher® 100 RP-18 endcapped is an example of a suitable product available commercially. 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.