SLOVENSKI STANDARD
SIST-TS CEN/TS 14537:2003

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Živila - Določevanje neohesperidin-dihidrohalkona

Foodstuffs - Determination of neohesperidin-dihydrochalcon

Lebensmittel - Bestimmung von Neohesperidin-Dihydrochalcon mit Hochleistungsflüssigkeitschromatographie (HPLC)

Denrées alimentaires - Dosage de la néohespéridine-dihydrochalcone par chromatographie liquide de haute performance (CLHP)

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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 neohesperidin-dihydrochalcon

Lebensmittel - Bestimmung von Neohesperidin-Dihydrochalcon mit Hochleistungsflüssigkeitschromatographie (HPLC)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents

Foreword

This document (CEN/TS 14537:2003) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

Annexes A, B and C are informative.

1 Scope

This Technical Specification specifies an HPLC-method for the determination of neohesperidin-dihydrochalcon (NHDC) in foodstuffs.

It has been validated in a collaborative test with cherry yoghurt containing 42,7 mg/kg and on a multi vitamin drink containing 35,6 mg/l NHDC [1].

The method has been successfully applied to a range of other foods including marzipan, bakery products, cream, custard powder, chocolate, ice cream.

2 Normative references

This Technical Specification 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 Technical Specification 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

The sample is diluted with methanol or extracted with a methanol/water – mixture and possibly filtered. NHDC is separated by HPLC on a reversed phase, detected spectrometrically and determined by the external standard method [1].

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 use distilled water.

4.2 tetra-n-butyl ammonium hydrogen sulfate-solution (mobile Phase A) for HPLC recommended

Substance concentration c = 0,01 mol/l

4.3 Methanol (mobile Phase B)

4.4 HPLC mobile phase

For the mobile phase, tetra-*n*-butyl ammonium hydrogen sulfate-solution (4.2) and methanol (4.3) are applied in appropriate ratios (gradient, e.g. as given in 6.3.1). Filter through a membrane filter (5.2) before use.

4.5 Standard substance

The NHDC standard substance shall fulfil the requirements according to EU directive 95/31/EU [2].

Commercially available NHDC can contain 4 mol of water. If using this commercially available substance, take its water content into account. The molecular weight of dried NHDC is 612,6 g/mol.

4.6 Standard test solutions

Dissolve an appropriate amount of NHDC with a mixture of methanol/water (50 + 50, volume fraction) and dissolve this solution again with a mixture of methanol/water (50 + 50, volume fraction) to obtain standard solutions which will give peaks comparable to those obtained with the sample solution.

This solution may be stored for 3 months in a refrigerator set at + 4 °C.

5 Apparatus

Use usual laboratory apparatus and, in particular, the following:

5.1 Filter device, e.g. filtration unit for vacuum, glass, consisting of glass sinter plate D2 (diameter 50 mm), 250 ml funnel and 1 l conical flask, each with ground joint.

5.2 Membrane filter for filtering the mobile phase, with pore size of 0,45 µm (or less).

NOTE Filtering of the mobile phase as well as of the sample solution through a membrane filter prior to use or injection is supposed to increase longevity of the columns.

5.3 Ultrasonic bath

5.4 Stomacher, optional, e. g. for extraction and preparation of solid samples.

5.5 HPLC system, consisting of

— a pump;

— a sample injecting device;

— a UV detector with variable wavelength setting;

— an evaluating system.

5.6 HPLC column

Analytical reversed phase column, acid resistant, diameter 4 mm, length 250 mm, filled with particle size 5 µm, e.g. LiChrospher® 60 RP-select B[1)], or another acid resistant RP phase. To protect the analytical column, it is recommended to use a pre-column of similar characteristics.

Column dimensions other than those specified in this Technical Specification may be used (e.g. of diameter 3,0 mm to 4,6 mm, length 100 mm to 250 mm). Separation parameters (flow, injection volume) have to be adapted to guarantee equivalent results.

6 Procedure

6.1 Preparation of the test sample

Homogenise the test sample. Shake liquid samples, homogenise viscous and semi-solid samples by stirring and grind solid samples with an appropriate mill and mix again.

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1) LiChrospher® 60 RP-select B is an example of a suitable product available commercially. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of this product.

6.2 Preparation of the sample test solution

6.2.1 Liquid samples

Dilute 50 ml of sample in a 100 ml volumetric flask with methanol to the mark, mix and place the flask for 10 min in an ultrasonic bath. Filter this suspension through a folded filter and than though a membrane filter (5.2).

6.2.2 Viscous and semi-solid samples

Weigh 10 g of the homogenised sample into a 100 ml volumetric flask and add 50 ml of methanol. Shake and place for 10 min into an ultrasonic bath. Let the flask stand until the content reaches room temperature and dilute with water to the mark. Place this suspension again in an ultrasonic bath for 10 min, filter this suspension through a folded filter and then though a membrane filter (5.2).

NOTE In the collaborative trial with cherry yoghurt, it is unlikely that the volume of the insoluble material exceeded 1 % of the final suspension volume. In such circumstances, any correction by calculation for the reduced volume of extract can be considered unnecessary.

6.2.3 Solid samples

Weigh 10 g of the homogenised sample into a stomacher bag, add 50 ml of methanol, add 50 ml of water less than the water which is already included in the sample. Shake, place in the stomacher and stomach for 10 min. Filter the suspension through a folded filter and than through a membrane filter (5.2).

6.3 HPLC determination

6.3.1 HPLC conditions

The separation and the quantification have proven to be satisfactory if following experimental conditions are followed:

Stationary Phase: according to 5.6.

Mobile phase: apply a gradient as in Table 1:

Table 1 — Gradient of mobile phases

Time, min	Eluent A, %	Eluent B, %
0	85	15
4	85	15
10	70	30
25	40	60
30	5	95
40	5	95

Flow rate: 1,2 ml /min

Injection volume: 20 µl

Detection: UV, at 285 nm

6.3.2 Identification

Inject the same appropriate volumes, e.g. 20 µl of the standard test solution (4.6) as well as of the sample test solution (6.2) into the HPLC-system (5.5).

Identify NHDC by comparison of the retention time of the peak in the chromatograms obtained with the sample test solution and with the standard solution. Peak identification can also be performed by adding small amounts of the appropriate standard solutions to the sample test solution and by checking the peak purity by UV-VIS-spectrum, e. g. using a diode array spectrometer.

6.3.3 Determination

To carry out the determination by external calibration, integrate the peak areas or determine the peak heights of the sample and compare the results with the corresponding values for the standard substance or use a calibration graph. Check the linearity of the calibration graph.

7 Calculation

Calculate the mass concentration, ρ, of NHDC in milligram per litre or the mass fraction, w, in milligram per kilogram of the sample using equation (1):

$$w \text{ or } \rho = \frac{A_1 \cdot V_1 \cdot m_1}{A_2 \cdot V_2 \cdot m_0} \cdot 1000 \qquad (1)$$

where

A_1 is the peak area for NHDC obtained with the sample test solution (6.2), in units of area;

A_2 is the peak area for NHDC obtained with the standard test solution (4.6), in units of area;

V_1 is the total volume of sample test solution (6.2), in millilitre;

V_2 is the total volume of standard test solution (4.6), in millilitre;

m_1 is the mass of (dried) NHDC contained in V_2, in milligram;

m_0 is the sample mass, in millilitre or gram.

Report the result without decimal places.

8 Precision

8.1 General

The precision data for the determination of NHDC were established in 1999 by a collaborative study on cherry yoghurt and on a multi vitamin drink as shown in annex A. The data derived from this collaborative study may not be applicable to analyte concentration ranges and sample matrices other than those given in annex A.

8.2 Repeatability

The absolute difference between two single test results found on identical test material by one operator using the same apparatus within the shortest feasible time interval will exceed the repeatability limit r in not more than 5 % of the cases. The repeatability is dependent on the concentration level of the analyte in the sample.

The values for NHDC are:

cherry yoghurt	$\bar{x}$ = 42,7 mg/kg	r = 2,6 mg/kg
multi vitamin drink	$\bar{x}$ = 35,6 mg/l	r = 1,8 mg/l