SLOVENSKI STANDARD
SIST EN 1378:1998

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Živila - Določevanje aspartama v namiznih sladilih - Metoda tekočinske kromatografije visoke ločljivosti

Foodstuffs - Determination of aspartame in table top sweetener preparations - Method by high performance liquid chromatography

Lebensmittel - Bestimmung von Aspartam in Tafelsüßen - Hochleistungs-flüssigkeitschromatographisches Verfahren

Produits alimentaires - Dosage de l'aspartame dans les édulcorants de table - Méthode par chromatographie liquide a haute performance

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67.180.10 Sladkor in sladkorni izdelki Sugar and sugar products

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English version

Foodstuffs - Determination of aspartame in table top sweetener preparations - Method by high performance liquid chromatography

Produits alimentaires - Dosage de l'aspartame dans les édulcorants de table - Méthode par chromatographie liquide à haute performance

Lebensmittel - Bestimmung von Aspartam in Tafelsüßen - Hochleistungs-flüssigkeitschromatographisches Verfahren

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Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat: rue de Stassart,36 B-1050 Brussels

Contents

Foreword

This European Standard has been prepared by the Technical Commitee CEN/TC 275 "Food analysis - Horizontal methods" the secretariat of 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of an identical text or by endorsement, at the latest by March 1997, and conflicting national standards shall be withdrawn at the latest by March 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

1 Scope

This European Standard specifies a high performance liquid chromatography (HPLC) method for the determination of aspartame in table top sweetener preparations.

An inter-laboratory test has been carried out on sweetener tablets [1].

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.

EN ISO 3696
Water for analytical laboratory use - Specification and test methods

3 Principle

Determination of aspartame in an appropriate solution of table top sweetener preparation in water by HPLC and subsequent photometric detection in the ultraviolet (UV) range. Identification of the aspartame on the basis of the retention time and determination by the external standard method using peak areas or peak heights.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and water of at least grade 1 as defined in EN ISO 3696.

4.1 Aspartame standard substance, with a known content of at least 98 % and not more than 102 % in dry matter (see 6.2). The loss in mass on drying shall not exceed 4,5 %. The material shall be chromatographically pure and be kept cool and dry.

NOTE: For further information on identification and purity, see [2].

4.2 Potassium dihydrogen orthophosphate solution, c (KH_2PO_4) = 0,0125 mol/l [1])

4.3 Methanol, suitable for HPLC analysis.

4.4 Orthophosphoric acid

4.5 Mobile phase for HPLC

Mix 70 parts by volume of the potassium dihydrogen orthophosphate solution (4.2) and 30 parts by volume of methanol (4.3) and adjust the pH to 4,5 with phosphoric acid (4.4). Remove particles by membrane filtration (5.2).

In order to avoid corrosion problems due to prolonged contact with phosphate containing eluents and as a precaution against blockages occurring due to precipitation of phosphate, water should be pumped through the HPLC equipment after carrying out this method.

4.6 Aspartame standard solution

Finely grind at least about 1,5 g of the aspartame standard substance (4.1), then, without delay, dissolve about 200 mg (m_1) of the undried, ground aspartame standard substance, weighed to the nearest 0,1 mg, in the mobile phase (4.5) in a 1000 ml (V_2) volumetric flask and dilute to the mark with the mobile phase.

Reserve the remaining finely ground aspartame standard substance for the determination of loss in mass on drying (L_D) (see 6.2). The determination of the loss in mass on drying is carried out immediately.

Prepare the standard solution on the day of the examination.

Additional solutions with concentrations within the linear range may be prepared for the calibration graph.

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[1]) c is the substance concentration

Calculate the mass concentration, ρ, of anhydrous aspartame in milligrams per litre of the standard solution, using the following equation:

$$\rho = \frac{m_1 \times (100 - L_D)}{100} \quad (1)$$

where:

m_1 is the mass of undried aspartame standard substance used for the stock solution, in milligrams;

L_D is the loss in mass on drying, in percent.

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following.

5.1 Filtration unit, e.g. glass vacuum filtration unit consisting of a glass sintered disk (diameter 50 mm), a 250 ml top section and a 1 l conical flask, all with ground glass joints.

5.2 Membrane filter, suitable for the mobile phase (4.5), pore size $\leq$ 5 μm.

5.3 High performance liquid chromatograph consisting of a pump, a sample applicator, a UV detector with variable wavelength setting and an evaluation system e.g. a chart recorder or integrator.

5.4 Analytical reversed phase separating column, e.g. C 18 reversed phase, particle size 10 μm, diameter 4 mm, length 250 mm.

Other particle sizes than specified in this standard may be used. Separation parameters have to be adapted to such materials to guarantee equivalent results.

NOTE: Minimal theoretical plates at the retention volume of the resolved analyte should preferably be not less than 1900 under the conditions of chromatography employed.

6 Procedure

6.1 Determination of the average tablet mass

Determine the mass of at least 20 sweetener tablets to the nearest 0,1 mg and calculate the average mass of one tablet.

NOTE: For improved accuracy the use of 100 tablets is recommended.

6.2 Determination of loss in mass on drying of standard substance

Weigh, to the nearest 0,1 mg, about 1,0 g of the remaining finely ground aspartame standard substance (4.1). Dry this portion to constant mass at 105 °C ± 2 °C and determine the loss in mass on drying (L_D) in percent by weighing.

6.3 Preparation of the sample test solution

Dissolve an amount of finely ground (m_0) table top sweetener preparation equivalent to about 400 mg aspartame (corresponding to 20 times the average mass of a tablet), and after transferring it to a 200 ml volumetric flask, dissolve in water and dilute to the mark. Dilute 20,0 ml of this solution with the mobile phase (4.5) to 200,0 ml (V_1) and filter through a membrane filter.

Prepare the sample test solution on the day of the analysis.

6.4 Identification by HPLC

Identify the sweetener to be determined either by comparing the retention time in the sample with that of the standard substance, or by comparing the absorption properties of the sample with those of the standard substance after either recording the absorption curve or taking measurements at different wavelengths in the relevant wavelength range for both sample and standard.

NOTE 1: If the separating column (5.4) and the mobile phase (4.5) are used, it has been found satisfactory to adopt the following experimental conditions (see figure A.1).

Flow	1,5 ml/min
UV detection	217 nm
Volume injected	20 µl

NOTE 2: When detecting at 210 nm, a higher sensitivity can be achieved. Possible interferences with decomposition products from aspartame such as diketopiperazine or phenylalanine can occur.

6.5 Determination by HPLC

To carry out the determination by the external standard method, integrate the peak areas or determine the peak heights and compare the results with the corresponding value for the standard substance or use a calibration graph.

Inject equal volumes of the sample and standard test solutions.

NOTE: The chromatogram shown in figure A.1 in was prepared using a C 18 reversed phase, particle size 10 µm, diameter 4,0 mm, length 250 mm column.

7 Expression of results

7.1 Graphical evaluation (optional)

Plot the absorption values of the aspartame standard solutions (4.6) on millimetre graph paper against the aspartame concentrations in milligrams per litre.

The calibration graph should be linear.

Read off the aspartame concentration, x, in milligrams per litre, corresponding to the absorption of the sample test solution from the calibration graph.

7.2 Calculation for graphical evaluation

7.2.1 If the calculation is based on the calibration graph, calculate the mass fraction, w_1, of anhydrous aspartame, in milligrams per kilogram table top sweetener preparation, using the following equation:

$$w_1 = \frac{x \times 10^6 \times 2}{m_0} \qquad \text{... (2)}$$

where:

x is the concentration of anhydrous aspartame in the sample test solution, read off from the calibration graph, in milligrams per litre;

10^6 is the conversion factor from milligrams to kilograms;

m_0 is the initial sample mass, in milligrams.

7.2.2 Calculate the mass fraction, w_2, of anhydrous aspartame, in milligrams per tablet, using the following equation:

$$w_2 = \frac{x \times m_2 \times 2}{m_0} \qquad \text{(3)}$$

where:

m_2 is the average tablet mass (6.1), in milligrams;

x, m_0 see equation (2).

An alternative calculative evaluation using the regression graph may be used.

7.3 Calculation for routine analysis

7.3.1 The calculation may either be carried out based on a calibration graph or, having demonstrated the linear range of response for standard solutions, for routine and repeat analyses calculate the mass fraction, w_1, of anhydrous aspartame, in milligrams per kilogram of the sample, using the following equation (external standard method):

$$w_1 = \frac{A_1 \times V_1 \times m \times 1000 \times F}{A_2 \times V_2 \times m_0} \quad \text{... (4)}$$

where:

A_1 is the peak area or peak height for the aspartame in the sample test solution, in units of area or length;

A_2 is the peak area or peak height for the aspartame in the standard test solution, in units of area or length;

V_1 is the total volume of the sample test solution (6.2), in millilitres; (here: 200 ml)

V_2 is the total volume of the standard test solution (4.6), in millilitres; (here: 1000 ml)

m is the mass of aspartame contained in V_2, corrected for the loss in mass on drying, in milligrams;

m_0 is the initial sample mass of 20 tablets, in grams;

1000 is the conversion factor from grams to kilograms;

F is the dilution factor (here: 10).

Report the result after rounding to one decimal place.

7.3.2 Calculate the the mass fraction, w_2, of anhydrous aspartame in milligrams per tablet using the following equation:

$$w_2 = \frac{w_1}{2000} \times m_0 \quad \text{(5)}$$

where:

w_1, m_0 see equation 4.

8 Precision

Details of the inter-laboratory test of the precision of the method according to ISO 5725 : 1986 [3] are summarized in annex B. The values derived from the inter-laboratory test may not be applicable to analyte concentration ranges and matrices other than given in annex B.

8.1 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 value is:

r = 0,66 g/100 g for commercially available aspartame tablets.

8.2 Reproducibility

The absolute difference between two single test results on identical test material reported by two laboratories will exceed the reproducibility limit R in not more than 5 % of the cases.

The value is:

R = 1,28 g/100 g for commercially available aspartame tablets.

9 Test report

The test report shall contain at least the following data:

- all information necessary for the identification of the sample;
- a reference to this European Standard or to the method used;
- the results and the units in which the results have been expressed;
- if the repeatability of the method has been verified;
- any particular points observed in the course of the test;
- any operations not specified in the method or regarded as optional which might have affected the results.