SLOVENSKI STANDARD
SIST EN 1379:1998

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Živila - Določevanje ciklamata in saharina v tekočih namiznih sladilih - Metoda tekočinske kromatografije visoke ločljivosti

Foodstuffs - Determination of cyclamate and saccharin in liquid table top sweetener preparations - Method by high performance liquid chromatography

Lebensmittel - Bestimmung von Cyclamat und Saccharin in Flüssigtafelsüßen - Hochleistungs-flüssigkeitschromatographisches Verfahren

Produits alimentaires - Dosage du cyclamate et de la saccharine dans les édulcorants de table liquides - Méthode par chromatographie liquide a haute performance

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English version

Foodstuffs - Determination of cyclamate and saccharin in liquid table top sweetener preparations - Method by high performance liquid chromatography

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CEN

European Committee for Standardization
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Europäisches Komitee für Normung

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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of an identical text or by endorsement, at the latest by March 1997, and conflicting national standards shall be withdrawn at the latest by March 1997.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 sodium cyclamate and saccharin in liquid table top sweetener preparations. It also allows the determination of sorbic acid in liquid table top sweetener preparations.

An inter-laboratory test has been carried out with liquid table top sweetener preparation [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 sodium cyclamate, saccharin and sorbic acid in an appropriate dilution of a liquid table top sweetener preparation in water by HPLC and subsequent photometric detection in the ultraviolet (UV) range. Identification on the basis of the retention times, and quantitative 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 Potassium dihydrogen orthophosphate solution, c (KH_2PO_4) = 0,0125 mol/l[1])

4.2 Methanol, suitable for HPLC analysis

4.3 Phosphoric acid

4.4 HPLC mobile phase

Mix 70 parts by volume of the potassium dihydrogen orthophosphate solution (4.1) and 30 parts by volume of methanol (4.2) and adjust the pH to 4,5 with phosphoric acid (4.3). 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.

4.5 Standard substances

4.5.1 Sodium cyclamate standard substance, with a known content of at least 98 % in dry matter (105 °C ± 2 °C, to constant mass). The loss in mass on drying shall not exceed 1 %.

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

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[1]) *c* is the substance concentration

4.5.2 Sodium saccharin standard substance, with a known content of at least 98 % in dry matter (105 °C ± 2 °C, to constant mass). The loss in mass on drying shall not exceed 15 %.

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

4.5.3 Sorbic acid standard substance, with a known content of at least 99 % after being stored for 4 h in a vacuum of about 33 hPa over concentrated sulfuric acid at room temperature.

4.6 Stock solutions

4.6.1 Sodium cyclamate stock solution, ρ ($C_6H_{11}NHSO_3Na$) ≈ 4 mg/ml[2])

Finely grind at least about 1,7 g of the sodium cyclamate standard substance (4.5.1), then, without delay, dissolve about 400 mg of the undried sodium cyclamate standard substance (equivalent to about 356 mg of cyclamate as free acid)[3]), weighed to the nearest 0,1 mg, in the mobile phase (4.4) in a 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4). Reserve the remaining finely ground sodium cyclamate standard substance for the determination of loss in mass on drying (see 6.1.1). The determination of loss in mass on drying is carried out immediately.

4.6.2 Saccharin stock solution, ρ ($C_7H_5NO_3S$) ≈ 2 mg/ml

Finely grind at least about 1,5 g of the undried sodium saccharin standard substance (4.5.2) then, without delay, dissolve about 263 mg of the undried sodium saccharin standard substance (equivalent to about 200 mg of saccharin as free imide)[4]), weighed to the nearest 0,1 mg, in the mobile phase (4.4) in a 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4). Reserve the remaining finely ground sodium saccharin standard substance for the determination of loss in mass on drying (see 6.1.2). The determination of loss in mass on drying is carried out immediately.

4.6.3 Calculation of the exact concentrations of sodium cyclamate and saccharin stock solutions

Calculate the exact concentration of anhydrous sodium cyclamate and anhydrous saccharin, r, in milligrams per litre of the stock solutions, using the following equation:

$$\rho = \frac{m_1 \times (100 - L_D) \times 10}{100} \quad \text{... (1)}$$

where:

m_1 is the mass of standard substances of sodium cyclamate or saccharin, in V_2 (see equation (2)), in milligrams;

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

4.6.4 Sorbic acid stock solution, ρ ($CH_3(CH)_4COOH$) ≈ 0,1 mg/ml

Dissolve about 100 mg of sorbic acid standard substance (4.5.3), weighed to the nearest 0,1 mg, in the mobile phase (4.4) in a 100 ml volumetric flask and dilute to the mark. Pipette 10 ml of this solution in an 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4).

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[2]) ρ is the mass concentration
[3]) conversion factor from sodium cyclamate to cyclohexane sulfamic acid = 0,8906
[4]) conversion factor from sodium saccharin to saccharin as free imide = 0,7593

4.7 Standard test solutions

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

4.7.1 Standard test solution I

Pipette 50 ml of the sodium cyclamate stock solution (4.6.1), 10 ml of the saccharin stock solution (4.6.2) and 10 ml of the sorbic acid stock solution (4.6.4) into a 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4). 1 l of this solution contains about 2000 mg of sodium cyclamate, 200 mg of saccharin and 10 mg of sorbic acid.

4.7.2 Standard test solution II

Pipette 25 ml of the sodium cyclamate stock solution (4.6.1), 5 ml of the saccharin stock solution (4.6.2) and 5 ml of the sorbic acid stock solution (4.6.4) into a 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4). 1 l of this solution contains about 1000 mg of sodium cyclamate, 100 mg of saccharin and 5 mg of sorbic acid.

4.7.3 Standard test solution III

Pipette 10 ml of the sodium cyclamate stock solution (4.6.1), 2 ml of the saccharin stock solution (4.6.2) and 2 ml of the sorbic acid stock solution (4.6.4) into a 100 ml volumetric flask and dilute to the mark with the mobile phase (4.4). 1 l of this solution contains about 400 mg of sodium cyclamate, 40 mg of saccharin and 2 mg of sorbic acid.

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 sinter 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 mobile phase (4.4), 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 4,6 mm, length 250 mm.

Other particle sizes than specified in this European 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 7700 for saccharin and not less than 4600 for sodium cyclamate under the conditions of chromatography employed.

6 Procedure

6.1 Determination of loss in mass on drying of standard substances

6.1.1 Determination of loss in mass on drying of sodium cyclamate standard substance

Weigh, to the nearest 0,1 mg, about 1,0 g of the reserved finely ground sodium cyclamate standard substance (4.5.1). Dry this portion to constant mass at 105 °C ± 2 °C and determine the loss in mass on drying (LD) in percent by weighing. The loss in mass on drying shall not exceed 1 %.

6.1.2 Determination of loss in mass on drying of sodium saccharin standard substance

Weigh, to the nearest 0,1 mg, about 1,0 g of the reserved finely ground sodium saccharin standard substance (4.5.2). Dry this portion to constant mass at 105 °C ± 2 °C and determine the loss in mass on drying (LD) in percent by weighing. The loss in mass on drying shall not exceed 15 %.

6.2 Preparation of the sample test solution

Dilute 10 ml of the liquid table top sweetener preparation to 100 ml (V_1) with the mobile phase (4.4) and filter through a membrane filter (5.2). Dilute 10 ml of this solution to 100 ml.

6.3 Identification by HPLC

Identify the sweeteners to be determined and the sorbic acid either by comparing the retention times in the sample with those of the standard substances 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.

Laboratories equipped with fixed-wavelength detectors shall carry out separate runs for determination of cyclamate and saccharin at the wavelengths concerned. This European Standard allows the determination of sorbic acid at the same conditions as chosen for saccharin (of wavelength 265 nm). Whenever the determination of sorbic acid with improved sensitivity is required, an additional wavelength switch to 260 nm is recommended.

NOTE 1: As sorbic acid is a late eluting compound, the risk of interference in the next run has to be taken into account.

NOTE 2: If the separating column (5.4) and the mobile phase (4.4) are used, it has been found satisfactory to adopt the following experimental conditions (see figure A.1).

Flow	1,7 ml/min
UV detection	200 nm (sodium cyclamate)
	265 nm (saccharin)
	260 nm (sorbic acid)
Volume injected	20 µl

6.4 Determination by HPLC

To carry out the determination by the external standard method, integrate the peak areas or determine the peak heights (see 7.3) and compare the results with the corresponding values for the standard substance with the nearest peak area/height, or use a calibration curve. In the case of a calibration curve additional solutions with concentrations within the linear range may be prepared for the calibration graph.

Inject equal volumes of the sample and standard test solutions. Check the linearity of the calibration function.

NOTE: The chromatogram in figure A.1 was prepared using a C 18 reversed phase, particle size 10 µm, diameter 4,6 mm, length 250 mm column.

7 Expression of results

7.1 Base the calculation on a calibration graph. An alternative evaluation using the regression graph may be used.

7.2 For routine and repeat tests, base the calculation either on a calibration graph or use the following simplified procedure.

Calculate the content, ρ, of sodium cyclamate and saccharin in grams per litre or of sorbic acid in milligrams per litre of the sample using the following equation:

$$\rho = \frac{A_1 \times V_1 \times m \times 10^3 \times F}{A_2 \times V_2 \times m_0} \quad \text{... (2)}$$

where:

A_1 is the peak area or peak height for sodium cyclamate, saccharin or sorbic acid obtained with the sample test solution, in units of area or length;

A_2 is the peak area or peak height for sodium cyclamate, saccharin or sorbic acid obtained with the standard test solution, in units of area or length;

V_1 is the total volume of sample test solution (6.2), in millilitres; (here: 100 ml)

V_2 is the total volume of standard test solution (4.7), in millilitres; (here: 100 ml);

m is the mass of standard substances of sodium cyclamate and saccharin, in grams, or of sorbic acid, in V_2, in milligrams, respectively, corrected for the loss in mass on drying;

F is the dilution factor (here: 10);

m_0 is the initial amount of sample, in millilitres.

Report the result for sodium cyclamate or cyclamic acid after rounding to one decimal place, for sodium saccharin or saccharin as free imide after rounding to two decimal places and for sorbic acid without any decimal places, according to current legislation.

7.3 When determining sodium cyclamate only peak areas can be used for evaluation since the peak is not symmetrical.

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 values are:

sodium cyclamate: r = 0,69 g/100 ml for liquid table top sweetener preparation.
saccharin: r = 0,03 g/100 ml for liquid table top sweetener preparation.
sorbic acid: r = 2 mg/100 ml for liquid table top sweetener preparation.