BS EN 16155:2012

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Foodstuffs — Determination of sucralose — High performance liquid chromatographic method

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The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

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ISBN 978 0 580 72441 1

ICS 67.050

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2012.

Amendments issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16155

April 2012

ICS 67.050

English Version

Foodstuffs - Determination of sucralose - High performance liquid chromatographic method

Produits alimentaires - Dosage du sucralose - Méthode par chromatographie liquide à haute performance

Lebensmittel - Bestimmung von Sucralose - Hochleistungsflüssigchromatographisches Verfahren

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Contents

Page

Foreword

This document (EN 16155:2012) 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of an identical text or by endorsement, at the latest by October 2012, and conflicting national standards shall be withdrawn at the latest by October 2012.

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1 Scope

This European Standard specifies a method for the determination of sucralose in foodstuffs by high performance liquid chromatography (HPLC) by means of elution from a reversed-phased (RP) column using aqueous methanol, followed by RI detection [1]. This method has been validated in an inter-laboratory study via the analysis of sucralose (from 83 mg/kg to 737 mg/kg) in spiked samples of ketchup, mayonnaise, biscuits, yoghurt, instant beverage powder and sweets.

For further information on the validation results, see Annex A.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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

Depending on their consistency, the samples are either dissolved in water or diluted with water and, if appropriate, they are filtered or clarified with modified Carrez solutions. Afterwards, they are extracted on a solid phase extraction column and eluted with a mixture of methanol/water. The sucralose content is determined by high-performance liquid chromatography (HPLC) by means of elution from a reversed-phased (RP) column using aqueous methanol, followed by RI (refractive index) detection. Alternatively, an ELSD (Evaporative Light Scattering Detector) may be used. Quantification is performed by applying the external standard method. The content of sucralose in foodstuffs means identifying the content of 1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-D-galactopyranoside, as determined in accordance with the method described in this document.

4 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995, unless otherwise specified. Solvents shall be of the same quality used for HPLC analysis, unless otherwise specified. Commercially available solutions with equivalent properties to the reagents listed may be used.

4.1 Sucralose, ($C_{12}H_{19}Cl_3O_8$, MW: 397,63).

4.2 Potassium hexacyanoferrate(II), $K_4[Fe(CN)_6] \cdot 3H_2O$.

4.3 Zinc nitrate, $Zn(NO_3)_2 \cdot 6H_2O$.

4.4 Methanol for HPLC.

4.5 Stock solution ($\rho_{Suc} \approx 1\,000$ mg/l).

Weigh approximately 100 mg of sucralose to the nearest 0,1 mg in a 100 ml volumetric flask; dissolve in a small amount of water and dilute to the calibration mark with water. Prepare the solution fresh every day. The water content and the purity of the standard substance shall be taken into consideration.

4.6 Standard solutions (ρ_{Suc} = 20 mg/l to 100 mg/l).

The concentrations of the standard solution given in the following are examples only and may be changed depending on the devices' sensitivity and the concentration range to be covered. Care shall be taken not to exceed the linear range of the detector system.

Prepare from the stock solution (4.5) at least five standard solutions by diluting in such a way that sucralose concentrations of e.g., 20 mg/l, 40 mg/l, 60 mg/l, 80 mg/l and 100 mg/l are obtained. Prepare these solutions fresh every day of the analysis.

4.7 Modified Carrez solutions.

4.7.1 Solution A (potassium hexacyanoferrate(II)).

Dissolve 53,45 g of potassium hexacyanoferrate(II) (4.2) in water and make up to 500 ml.

4.7.2 Solution B (Zinc nitrate).

Dissolve 148,75 g of zinc nitrate (4.3) in water and make up to 500 ml.

4.8 Elution solution for HPLC.

Mix one volume fraction of methanol (4.4) with 3 volume fractions of water.

5 Apparatus and equipment

Usual laboratory apparatus, in particular:

5.1 Membrane filter, for sample filtration, pore size: 0,45 µm maximum.

5.2 Pleated filter.

5.3 C18 SPE cartridges, 500 mg.

5.4 High-performance liquid chromatograph, comprising:

— a pump;

— a sample injector;

— a temperature-controlled RI detector or, alternatively, an ELSD;

— a column oven;

— an evaluation system.

5.5 Analytical C-18 reversed-phase separating column, 250 mm × 4 mm, e.g., Lichrospher®[1)] 100 RP-18, 5 µm, or a similar column.

A guard column with a similar packing material should be used in order to protect the analytical separating column.

1) Lichrospher® 100 RP-18 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 this product.

6 Procedure

6.1 Sample preparation

Homogenize the test material in a suitable manner. Liquid samples, for example, may be stirred, solid products such as hard candies may be crushed, and products such as chewing gum deep-frozen and then crushed. Semi-solid products such as yoghurt or ketchup may be homogenized by stirring, and then clarified with a modified Carrez solution (4.7), if necessary.

Some samples may require pre-treatment using SPE cartridges.

6.2 Preparation of the sample test solutions

6.2.1 Soluble samples (e. g. hard candies and similar products)

Dissolve about 5 g to the nearest 1 mg of the homogenized sample in water in a 50 ml volumetric flask and make up with water to the stated volume. Filter through a membrane filter if the solution is turbid.

6.2.2 Incompletely soluble samples (e. g. pastries, chewing gum, yoghurt, ketchup, mayonnaise)

Mix about 5 g to the nearest 1 mg of the homogenized sample with approximately 25 ml of water in a 50 ml volumetric flask and stir for 30 min at about 40 °C to 60 °C (magnetic stirrer) or treat in the ultrasonic bath. Mix protein-containing samples with 1 ml each of the modified Carrez solutions (4.7.1 and 4.7.2) and shake after each addition. Afterwards, bring the sample test solution to room temperature and make up to the mark with water. Filter through a pleated filter and then through the membrane filter if the solution is turbid.

6.2.3 Solid phase extraction

Condition the solid phase extraction columns (5.3) with 5 ml each of methanol and water. Inject 5 ml of the sample solution given in 6.2.1 or 6.2.2 respectively onto the conditioned columns. Afterwards, wash the cartridges three times with 5 ml water, taking care not to let the columns run dry during these steps. Subsequently, elute the sucralose from the column with 5 ml of the HPLC elution solution (4.8). Collect in a 10 ml volumetric flask until the columns are dry, and make up to volume. Filter the eluate through a membrane filter (5.1) and fill into HPLC glass vials.

6.3 High-performance liquid chromatography (HPLC)

When using a column in accordance with 5.5, compliance with the following parameters has proven useful:

— injection volume: up to 100 µl

— eluent: (4.8)

— column oven temperature: 35 °C

— RI-detector temperature: 30 °C

— flow: approximately 1,2 ml/min.

6.4 Identification

Inject an aliquot of the sample test solution into the HPLC-System, preferably under the condition as mentioned in 6.3. Identify sucralose in the sample by comparing the retention time in the sample with that of the standard sucralose solution. An additional identification consists of adding sucralose to the sample and in determining if the peaks overlap.

6.5 Quantitative determination

Quantitative determination is performed by integrating the peak area of sucralose and relating it to concentration via a calibration function.

The obtained peak areas are plotted against the concentrations. A straight line (y = a + bx) is fitted to the results, where b is the value of the slope of the linear function and a is the value where the calibration function intercepts the y-axis.

The appropriate character of the calibration function shall be checked.

7 Calculation

Quantify the mass concentration ρ_{suc} in milligram per litre or the mass fraction w_{suc} in milligram per kilogram of sucralose (*suc*) by integration of the peak area (R) obtained from the analysis of the injected sample solution. The concentration of sucralose in the sample is then calculated using the calibration function according to Formula (1):

$$\rho_{suc} \text{ or } w_{suc} = \frac{(R - a) \cdot V}{b \cdot m} \quad (1)$$

where

R is the peak area response;

a is the intercept of the calibration line (6.5);

b is the slope of the calibration line (6.5);

V is the total volume of the sample solution (e.g. 50 ml);

m is the mass of the sample (e.g. 5 g).

The result is expressed rounded with no decimal point.

8 Precision

8.1 General

The statistical data for the determination of sucralose were obtained in 2007/2008 in two inter-laboratory trials on the following products: ketchup, mayonnaise with high and low sucralose concentrations, biscuits, yoghurt, 2 types of instant beverage powders and 2 compressed tablets (sweets) with different flavours and concentrations. The values derived from the inter-laboratory test may not be applicable to analyte concentration ranges and matrices other than those detailed in Annex A.

8.2 Repeatability

The absolute difference between two single test results determined 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 cases. The repeatability depends on the concentration level of the analyte in the sample.

Ketchup	$\bar{x}$ = 128,83 mg/kg	r = 8,38 mg/kg
Mayonnaise 1	$\bar{x}$ = 463,03 mg/kg	r = 38,58 mg/kg
Mayonnaise 2	$\bar{x}$ = 175,84 mg/kg	r = 27,87 mg/kg
Biscuits	$\bar{x}$ = 737,30 mg/kg	r = 31,12 mg/kg
Yoghurt	$\bar{x}$ = 87,53 mg/kg	r = 7,88 mg/kg
Instant beverage powder 1	$\bar{x}$ = 249,52 mg/kg	r = 24,61 mg/kg
Instant beverage powder 2	$\bar{x}$ = 225,21 mg/kg	r = 16,88 mg/kg
Sweets 1	$\bar{x}$ = 471,14 mg/kg	r = 25,30 mg/kg
Sweets 2	$\bar{x}$ = 139,35 mg/kg	r = 10,73 mg/kg

8.3 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.

Ketchup	$\bar{x}$ = 128,83 mg/kg	R = 18,16 mg/kg
Mayonnaise 1	$\bar{x}$ = 463,03 mg/kg	R = 69,92 mg/kg
Mayonnaise 2	$\bar{x}$ = 175,84 mg/kg	R = 53,55 mg/kg
Biscuits	$\bar{x}$ = 737,30 mg/kg	R = 130,89 mg/kg
Yoghurt	$\bar{x}$ = 87,53 mg/kg	R = 22,18 mg/kg
Instant beverage powder 1	$\bar{x}$ = 249,52 mg/kg	R = 64,44 mg/kg
Instant beverage powder 2	$\bar{x}$ = 225,21 mg/kg	R = 48,89 mg/kg
Sweets 1	$\bar{x}$ = 471,14 mg/kg	R = 43,64 mg/kg
Sweets 2	$\bar{x}$ = 139,35 mg/kg	R = 25,40 mg/kg

9 Test report

The test report shall contain at least the following data:

a) all information necessary for the identification of the sample;

b) a reference to this European Standard or to the method used;

c) the date and time of sampling procedure (if known);

d) the date of receipt;

e) the date of test;

f) the results and the units in which the results have been expressed;

g) any particular points observed in the course of the test;

h) any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Statistical data of the inter-laboratory trial

This method has been prepared by working group "Sweeteners" of the German Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, BVL) for the purposes of implementing § 64 LFGB. It was tested in two inter-laboratory trials with respectively 10 and 9 participants in total, see [2] and Table A.1.

Table A.1 — Statistical data of the inter-laboratory trial

Probe	Ketchup	Mayon-naise 1	Mayon-naise 2	Biscuit	Yoghurt	Instant bever-age powder (Peach-Icetea)	Instant bever-age powder (Apple)[a]	Sweets (Orange)[a]	Sweets (Pepper-mint)[a]
Year of inter-laboratory test	2007/2008	2007/2008	2007/2008	2007/2008	2007/2008	2007/2008	2007/2008	2007/2008	2007/2008
Number of laboratories	10	7	7	10	10	10	9	9	9
Number of replicates	5	5	5	5	5	5	6	6	6
Number of laboratories retained after eliminating outliers	9	7	7	9	9	9	8	8	8
Number of outliers (laboratories)	1	0	0	1	1	1	1	1	1
Mean value $\bar{x}$ [mg/kg]	128,83	463,03	175,84	737,30	87,53	249,52	225,21	471,14	139,35
Repeatability standard deviation sr [mg/kg]	2,99	13,78	9,95	11,11	2,81	8,79	6,03	9,04	3,83
Repeatability relative standard deviation RSD_r [%]	2,32 %	2,98 %	5,66 %	1,51 %	3,21 %	3,52 %	2,68 %	1,92 %	2,75 %
Repeatability limit r [mg/kg]	8,38	38,58	27,87	31,12	7,88	24,61	16,88	25,30	10,73
Reproducibility standard deviation s_R [mg/kg]	6,48	24,97	19,12	46,75	7,92	23,05	17,46	15,59	9,07
Reproducibility relative standard deviation RSD_R [%]	5,03 %	5,39 %	10,88 %	6,34 %	9,05 %	9,24 %	7,75 %	3,31 %	6,51 %
Reproducibility limit R [mg/kg]	18,16	69,92	53,55	130,89	22,18	64,55	48,89	43,64	25,40
Relative standard deviation according to Horwitz(H)	7,70	6,35	7,35	5,92	8,16	6,97	7,08	6,33	7,61
Horrat value (RSD_R/H)	0,65	0,85	1,48	1,07	1,11	1,33	1,09	0,52	0,86

[a] Inter-laboratory test 2.

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[2] Untersuchung von Lebensmitteln: Bestimmung des Gehaltes an Sucralose in Lebensmitteln L 00.00-126, Januar 2010 (Food Analysis: Determination of sucralose content in foodstuffs L 00.00-126, 2010-01) in: Amtliche Sammlung von Untersuchungsverfahren nach § 64 LFGB: Verfahren zur Probenahme und Untersuchung von Lebensmitteln, Tabakerzeugnissen, kosmetischen Mitteln und Bedarfsgegenständen/Bundesgesundheitsamt (In: Collection of official methods under article 64; Methods of sampling and analysis of foods, tobacco products, cosmetics and commodity goods/Federal Health Office) Loseblattausgabe, Stand Jan. 2010 Bd. 1 (Loose leaf edition, as of 2010-01 Vol. I.) Berlin, Köln: Beuth Verlag GmbH

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