SVENSK STANDARD
SS-EN 15911:2010

Fastställd/Approved: 2010-11-04
Publicerad/Published: 2010-11-30
Utgåva/Edition: 1
Språk/Language: engelska/English
ICS: 67.180.10

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Foodstuffs – Simultaneous determination of nine sweeteners by high performance liquid chromatography and evaporative light scattering detection

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Europastandarden EN 15911:2010 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN 15911:2010.

The European Standard EN 15911:2010 has the status of a Swedish Standard. This document contains the official version of EN 15911:2010.

Upplysningar om sakinnehållet i standarden lämnas av SIS, Swedish Standards Institute, telefon 08-555 520 00. Standarder kan beställas hos SIS Förlag AB som även lämnar allmänna upplysningar om svensk och utländsk standard.

Information about the content of the standard is available from the Swedish Standards Institute (SIS), telephone +46 8 555 520 00. Standards may be ordered from SIS Förlag AB, who can also provide general information about Swedish and foreign standards.

Denna standard är framtagen av kommittén för Livsmedel och foder, SIS/TK 435.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 15911

October 2010

ICS 67.180.10

English Version

Foodstuffs - Simultaneous determination of nine sweeteners by high performance liquid chromatography and evaporative light scattering detection

Denrées alimentaires - Détermination simultanée de neuf édulcorants par chromatographie liquide haute performance et détection à diffusion de lumière

Lebensmittel - Gleichzeitige Bestimmung von neun Süßungsmitteln mit Hochleistungs-Flüssigchromatographie und Verdampfungs-Lichtstreu-Detektion

This European Standard was approved by CEN on 18 September 2010.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

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

Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 15911:2010: E

Contents

Page

Foreword

This document (EN 15911:2010) 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 April 2011, and conflicting national standards shall be withdrawn at the latest by April 2011.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

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1 Scope

This European Standard specifies a method for the simultaneous determination of nine sweeteners in beverages and canned or bottled fruits by high performance liquid chromatography (HPLC) with evaporative light scattering detection (HPLC-ELSD). This method has been validated in an interlaboratory study via the analysis of spiked samples on the following matrices:

— acesulfame-K (ACS-K) (from 38,3 mg/l to 383,5 mg/l) in beverages and (from 38,4 mg/kg to 391,3 mg/kg) in canned fruits;

— alitame (ALI) (from 31,1 mg/l to 114,5 mg/l) in beverages and (from 36 mg/kg to 175,2 mg/kg) in canned fruits;

— aspartame (ASP) (from 38,1 mg/l to 702 mg/l) in beverages and (from 37,2 mg/kg to 1 120,2 mg/kg) in canned fruits;

— cyclamic acid (CYC) (from 28,3 mg/l to 307,2 mg/l) in beverages and (from 27,5 mg/kg to 1 100,6 mg/kg) in canned fruits;

— dulcin (DUL) (from 55,0 mg/l to 115,1 mg/l) in beverages and (from 49,8 mg/kg to 172,6 mg/kg) in canned fruits;

— neotame (NEO) (from 37,6 mg/l to 115,3 mg/l) in beverages and (from 37,3 mg/kg to 173,7 mg/kg) in canned fruits;

— neohesperidine dihydrochalcone (NHDC) (from 31,4 mg/l to 59,3 mg/l) in beverages and (from 35,3 mg/kg to 59,3 mg/kg) in canned fruits;

— saccharin (SAC) (from 36,2 mg/l to 87,6 mg/l) in beverages and (from 44,3 mg/kg to 235,3 mg/kg) in canned fruits;

— sucralose (SCL) (from 36,8 mg/l to 346,8 mg/l) in beverages and (from 35,3 mg/kg to 462,4 mg/kg) in canned fruits.

For further information on the validation see Clause 8 and Annex C.

NOTE The method has been fully validated [1] through collaborative trial, according to the IUPAC Harmonised Protocol [2], on analyte-matrix combinations of all nine sweeteners in beverages and canned or bottled fruits.

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

The procedure involves extraction of the nine sweeteners with a buffer solution, sample clean-up using solid-phase extraction cartridges followed by HPLC-ELSD analysis.

4 Reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade for HPLC analysis and water of at least grade 1 as defined in EN ISO 3696:1995. When preparing solutions, the purity of the substances shall be taken into account.

4.1 Acesulfame-K, with a mass fraction w of at least 99,0 %.

4.2 Alitame, $w \geq 99{,}0$ %.

4.3 Aspartame, $w \geq 99{,}0$ %.

4.4 Dulcin, for HPLC.

4.5 Neotame, $w \geq 99{,}0$ %.

4.6 Neohesperidine dihydrochalcone, $w \geq 95{,}0$ %.

4.7 Saccharin, sodium salt dihydrate, $w \geq 98{,}0$ %.

4.8 Sodium cyclamate, $w \geq 99{,}0$ %.

4.9 Sucralose, $w \geq 99{,}0$ %.

4.10 Formic acid, HCOOH for HPLC.

4.11 Triethylamine, $(C_2H_5)_3N$, $w \geq 99{,}5$ %.

4.12 Methanol, for HPLC.

4.13 Acetone, for HPLC.

4.14 Buffer solution (pH = 4,5).

Dissolve 4 ml of formic acid (4.10) in 5 l of water. Adjust to pH 4,5 with approximately 12,5 ml triethylamine (4.11).

4.15 HPLC mobile phase A, methanol/buffer solution/acetone 69:24:7 (v/v/v).

Mix 690 ml of methanol (4.12) with 240 ml of buffer solution (4.14) and with 70 ml of acetone (4.13). Degas by sonication for 10 min.

4.16 HPLC Mobile phase B, methanol/buffer solution/acetone 11:82:7 (v/v/v).

Mix 110 ml of methanol (4.12) with 820 ml of buffer solution (4.14) and with 70 ml of acetone (4.13). Degas by sonication for 10 min.

4.17 Mixed stock solution, containing ACS-K, ALI, ASP, CYC-Na, DUL, NEO, NHDC, SAC-Na and SCL; mass concentration ρ(sweetener i) = 30 µg/ml to 250 µg/ml.

Prepare a mixed stock solution of all nine sweeteners by weighing the given masses of the individual sweetener standards (Table 1) first into a 100 ml beaker and dissolving them in 50 ml of methanol/water (1:1) until complete dissolution. Then transfer the obtained solution quantitatively into a 500 ml volumetric flask and make up to the mark with the buffer solution (4.14). Mix thoroughly by sonication until complete dissolution.

Table 1 — Masses of individual standards for preparation of mixed stock solution

Standard	Mass weighed into 500 ml volumetric flask [c] mg	Final mass concentration of sweetener *i* in mixed stock standard µg/ml
Acesulfame-K (ACS-K)	45	90
Alitame (ALI)	25	50
Aspartame (ASP)	125	250
Sodium cyclamate (CYC-Na)	140 [a]	–
Cyclamic acid (CYC) (free acid)	–	249,42
Dulcin (DUL)	25	50
Neotame (NEO)	25	50
Neohesperidine dihydrochalcone (NHDC)	15	30
Saccharin, sodium salt dihydrate (SAC-Na·2H_2O)	35 [b]	–
Saccharin (SAC) (free imide)	–	53,17
Sucralose (SCL)	50	100

[a] Equivalent to 124,71 mg free cyclamic acid; conversion factor to calculate mass of free cyclamic acid = 0,890 8; $m_{CYC} = 0{,}890\ 8 \times m_{CYC\text{-}Na}$.

[b] Equivalent to 26,58 mg free saccharin; conversion factor to calculate mass of free saccharin = 0,759 5; $m_{SAC} = 0{,}759\ 5 \times m_{SAC\text{-}Na\cdot 2H2O}$.

[c] First weigh into 100 ml beaker, dissolve in 50 ml of a methanol:water (1:1) mixture and then transfer quantitatively into 500 ml volumetric flask.

NOTE In case of cyclamic acid and saccharin, their sodium salts are used, since they are either not available in free form or poorly soluble.

The final concentrations of the individual sweeteners in micrograms per millilitre in the mixed stock solution have to be calculated by using the actually weighed masses.

4.18 Standard solutions.

From the mixed stock solution (4.17) prepare a series of standard solutions containing the sweeteners at levels fitting appropriate limits, e.g. the highest concentration of the calibration shall be at least equivalent to 125 % of the given limits, such as those in Commission Directives [3], [4], [5] (see Table D.1), whilst taking the dilution steps within the procedure into account (see Table 2). For sweeteners not authorised by the current EU legislation (ALI, DUL and NEO) fictitious maximum usable dosages (MUD) are assumed at approximately 200 mg/l or 200 mg/kg.

The user of the standard has to check whether the limits in Table D.1 are still valid. If not, the mass concentration of the standard substance in the calibration solution shall be adjusted to meet the current MUDs.

NOTE The present procedure is simplified by preparing one calibration series for both food matrices. The described calibration series is fitted to canned fruits as the MUDs for canned fruits are in some cases higher than the MUDs for beverages. In case only the latter matrix is analysed the calibration series can be fitted to the MUDs of beverages.

Pipette the following volumes (see Table 2) from the mixed stock solution (4.17) into appropriate volumetric flasks (10 ml to 50 ml) and make up to the mark with buffer solution (4.14) and shake thoroughly.

Table 2 — Preparation of series of standard solutions

Calibration solution	Volume of volumetric flask ml	Volume taken from mixed stock solution (4.17) ml	Volume taken from buffer solution (4.14) ml
1[a]	10	10	0
2	10	8	2
3	10	6	4
4	10	4	6
5	10	2	8
6	25	3	22
7	50	3	47
8	50	1,5	48,5

[a] Undiluted mixed stock solution (4.17).

Table 3 details the concentration of sweetener *i* in each calibration standard following preparation described in Table 2.

If not all of the sweeteners covered by this standard are the subject of analysis in routine use of the method, when applied to a particular set of samples consideration may be given to reduce the levels of the calibration solutions used for those samples.

Table 3 — Concentration of sweetener *i* in the individual standard solutions

Sweetener	Calibration solution mg/ml							
	1	2	3	4	5	6	7	8
ACS-K	90,0	72,0	54,0	36,0	18,0	10,8	5,4	2,7 [a]
ALI	50,0	40,0	30,0	20,0	10,0	6,0	3,0 [a]	1,5 [a]
ASP	250,0	200,0	150,0	100,0	50,0	30,0	15,0	7,5
CYC	249,4	199,5	149,7	99,8	49,9	29,9	15,0	7,5
DUL	50,0	40,0	30,0	20,0	10,0	6,0 [a]	3,0 [a]	1,5 [a]
NEO	50,0	40,0	30,0	20,0	10,0	6,0	3,0 [a]	1,5 [a]
NHDC	30,0	24,0	18,0	12,0	6,0	3,6 [a]	1,8 [a]	0,9 [a]
SAC	53,2	42,5	31,9	21,3	10,6	6,4	3,2 [a]	1,6 [a]
SCL	100,0	80,0	60,0	40,0	20,0	12,0	6,0	3,0 [a]

[a] The concentration level might be below the limit of quantification (LOQ). If yes, the result obtained by HPLC analysis is not included in the construction of the calibration graph, e.g. in case of ACS-K a seven point calibration is performed, ignoring the result obtained for calibration solution 8.

5 Apparatus and equipment

Usual laboratory apparatus and, in particular, the following:

5.1 Common laboratory glassware, such as graduated cylinders, volumetric pipettes, glass beakers.

5.2 Analytical balance, capable of weighing to 0,01 mg.

5.3 Laboratory balance, capable of weighing to 0,01 g.

5.4 Positive displacement pipette, or equivalent, capable of delivering 1 ml to 10 ml (variable volume).