BS EN 15911:2010

BSI Standards Publication

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

raising standards worldwide™

National foreword

This British Standard is the UK implementation of EN 15911:2010.

The UK participation in its preparation was entrusted to Technical Committee AW/-/3, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

ISBN 978 0 580 66428 1

ICS 67.180.10

Compliance with a British Standard cannot confer immunity from legal obligations.

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 November 2010.

Amendments issued since publication

Date	Text affected

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.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

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.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

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).

5.5 Volumetric flasks, of suitable capacity, e.g. 10 ml, 25 ml, 50 ml, 100 ml and 500 ml.

5.6 Centrifuge tubes, made of polypropylene, of suitable capacity, e.g. 50 ml.

5.7 Graduated test tubes, of suitable capacity, e.g. 5 ml.

5.8 Food blender, suitable for homogenisation of food samples.

5.9 Ultrasonic bath.

5.10 Centrifuge, capable of maintaining 4 000 min^{-1}.

5.11 SPE Vacuum system, or equivalent.

5.12 Equipment for solvent evaporation.

5.13 pH meter.

5.14 C_{18} SPE cartridges.

5.15 Analytical reverse phase column, fully end-capped, allowing sufficient separation of all nine sweeteners.

E.g. with:

— an RP C 18 stationary phase of 5 µm;

— a length of 250 mm;

— internal diameter of 3 mm.

5.16 HPLC system, equipped with a binary pump capable of maintaining a flow rate of 0,5 ml/min, preferably an automatic injection system, and an evaporative light scattering detector.

Other detection systems such as MS as substitute for ELSD or UV and DAD when substances do absorb in the UV region can also be used provided that the equivalent performance characteristics can be obtained.

5.17 Data acquisition and analysis software.

6 Procedure

6.1 General

Comminute the entire test sample to give a homogenous suspension (5.8). Liquid samples can be subjected directly to the extraction procedure.

6.2 Preparation of test sample

6.2.1 Step 1

Weigh approximately 5 g (M_1, recorded to two decimal places) of the homogenised test sample (6.1) into a volumetric flask of 50 ml (V_1). Make up to the mark with buffer solution (4.14), mix thoroughly by hand to obtain a homogeneous suspension and sonicate (5.9) for 15 min.

6.2.2 Step 2

Transfer the obtained suspension to a 50 ml centrifuge tube. Centrifuge at 4 000 min^{-1} for 10 min.

NOTE In case the test sample gives a clear solution (e.g. some beverages), this step can be ignored.

6.3 Solid phase extraction

6.3.1 Step 1

Condition the cartridges (5.14) by applying 3 ml of methanol (4.12) and let it pass through using a slight vacuum resulting in a flow rate of 1 ml/min to 2 ml/min. Make sure that a small portion of methanol remains above the sorbent bed (1 mm).

6.3.2 Step 2

Equilibrate the cartridges (5.14) by applying 2 ml of buffer solution (4.14) and let it pass through using a slight vacuum resulting in a flow rate of 1 ml/min to 2 ml/min. Make sure that a small portion of buffer solution remains above the sorbent bed (1 mm). Repeat the procedure two times.

6.3.3 Step 3

Load the cartridges (5.14) with 5 ml of sample extract (V_2 first loading), i.e. the supernatant from (6.2.2), and let it pass through using a slight vacuum resulting in a flow rate of 1 ml/min to 2 ml/min. Make sure that a small portion remains above the sorbent bed (1 mm). Repeat the procedure once more (V_2 in total 10 ml).

6.3.4 Step 4

Wash the cartridges (5.14) with 3 ml of buffer solution (4.14) and let it pass through using a slight vacuum resulting in a flow rate of 1 ml/min to 2 ml/min. Make sure that a small portion of buffer solution remains above the sorbent bed (1 mm).

6.3.5 Step 5

Elute the sweeteners from the cartridges (5.14) by applying 2 ml of methanol (4.12) and collecting the eluate in a graduated 5 ml test tube. Use a slight vacuum to obtain a flow rate of 1 ml/min. Make sure that a small portion of methanol remains above the sorbent bed (1 mm). Wait 10 min before applying a second portion of 2 ml of methanol and elute it subsequently to the same 5 ml test tube using the same vacuum conditions but this time letting the cartridges (5.14) run dry.

Avoid in all steps (6.2.1 to 6.3.5) that the sorbent bed runs dry with the only exception of the last step, i.e. second elution of analytes (6.3.5).

6.3.6 Step 6

Evaporate the solvent from the methanolic SPE extract to 3 ml under a stream of nitrogen at ambient temperature.

Temperatures above 40 °C have to be avoided, since aspartame can degrade.

6.3.7 Step 7

Fill the graduated test tube containing the SPE extract (6.3.6) up to the 5 ml mark with buffer solution (4.14) (V_3). Mix thoroughly and transfer the content into a suitable HPLC vial and analyse by HPLC.

6.4 HPLC conditions

Establish suitable HPLC conditions to meet the predefined performance criteria (6.5). The separation and quantification have proven to be satisfactory using the following experimental conditions and HPLC gradient conditions as outlined in Table 4:

- Column: see 5.15;
- Column temperature: ambient temperature;
- Injection volume: 10 µl;
- Mobile phase: see 4.15 and 4.16;
- Separation mode: gradient;
- Detector: evaporative light scattering detector (ELSD);
- ELSD drift tube temperature: 85 °C;
- ELSD nitrogen flow: 2,5 l/min;
- ELSD gain: 1;
- ELSD impactor: Off.

Table 4 — gradient analysis by HPLC, Flow rate 0,5 ml/min

Time min	**Mobile phase A** %	**Mobile phase B** %
0	0	100
4	0	100
11	53	47
23	100	0
24	100	0
26	0	100
36	0	100

NOTE The given detector parameters are applicable to the Alltech ELSD 2000ES system[1)]. Alternative ELSD systems and experimental conditions, used in an inter-laboratory study, are listed in Annex A. HPLC and ELSD operating conditions can be changed to obtain optimum separation.

6.5 System suitability test – Resolution of separation system

The details of the chromatographic procedure depend, among other factors, on equipment, type, age, and supplier of the column, sample size and detector. Different columns can be used, and injection volumes can be varied, if the requirements of the system suitability tests are met.

1) This system 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.

The HPLC-ELSD system shall be capable of separating all nine sweeteners from each other with at least baseline separation. This requirement can be proven by using calibration solution 1 (4.18) as shown in Figure B.1.

Moreover, the system shall be capable of separating all nine sweeteners from other components of the matrix. Many matrix components, such as sodium benzoate, sorbic acid, citric acid, phosphoric acid, malic acid, ascorbic acid, glutamic acid, sucrose, glucose, fructose, lactose, caffeine, taurine, D-glucurono-γ-lactone and sorbitol, etc., are removed throughout the SPE clean-up. A commonly encountered critical pair is alitame (sweetener not authorised by the current EU legislation) and quinine, which is not removed by the SPE clean-up [7].

In case of failure, the chromatographic conditions (e.g. sample volume injected, mobile phase rate, gradient program, etc.) or the ELSD conditions (e.g. drift tube temperature, nitrogen/air flow) have to be optimized.

6.6 Construction of calibration graph

Analyse all standard solutions (4.18, Table 2) using HPLC conditions (6.4) identical to those used for the test samples, i.e. inject 10 µl of each solution into the HPLC system. Construct a calibration chart for each sweetener *i* from the results of the analysis of the standard solutions.

Plot the obtained peak area as log_{10}*(Peak area i)* (y-axis) against the log_{10}*(Concentration i)* (x-axis). Fit a straight line (y = a + bx) 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. If the results of the analyses of the standard solutions are linear, the calibration line can be used to calculate the concentration of sweetener i in the sample extract.

If detectors other than ELSD are used, other calibration functions may be used.

6.7 HPLC analysis of sample test solution

Analyse 10 µl of the sample test solution obtained from step in 6.3.7.

6.8 Interpretation of chromatographic data

Identify the individual sweeteners in the test samples by comparison of the retention time of sweeteners observed during the analysis of standard solutions analysed in the same batch as samples with the retention time of compounds eluted during the analysis of the test samples. The elution order of the individual sweeteners together with the retention times are given in an example chromatogram in Figure B.1.

Measure the peak area response (R_i) observed for sweetener *i* in each solution. In case the peak area of sweetener *i* in the chromatogram of the test sample solution exceeds the area of the respective sweetener peak in the chromatogram obtained for the standard solution with the highest concentration, the test sample solution is diluted with buffer solution (4.14) and the diluted extract re-analysed.

7 Calculation of results

Quantify sweetener *i* by integration of the peak area *i* (R_i) obtained from the analysis of the injected SPE extract. Use the resulting calibration function, i.e. y = a + bx (6.6) to calculate the mass concentration of sweetener *i* (ρ_{1i}) in the measured sample extract solution using Equations (1) and (2).

$$\log_{10} \rho_{1i} = \frac{(\log_{10} R_i) - a_i}{b_i} \quad (1)$$

$$\rho_{1i} = 10^{(\log_{10} \rho_{1i})} \quad (2)$$

where

R_i is the peak area response (6.8) for sweetener *i*;

a_i is the intercept of the calibration line (6.6) for sweetener *i*;

b_i is the slope of the calibration line (6.6) for sweetener *i*;

ρ_{1i} is the mass concentration of sweetener *i* in the SPE extract in micrograms per millilitre.

Calculate the concentration/mass fraction w/ρ_{2i} of sweetener *i* in the test sample according to Equation (3).

$$w/\rho_{2i} = \frac{\rho_{1i} \times V_1 \times V_3}{M_1 \times V_2} \quad (3)$$

where

ρ_{1i} is the mass concentration of sweetener *i* in the SPE extract in micrograms per millilitre (as determined in Equation (2));

M_1 is the mass of the sample taken for extraction in grams, i.e. 5 g (6.2.1);

V_1 is the total volume of the sample solution in millilitres, i.e. 50 ml (6.2.1);

V_2 is the volume of the sample solution loaded onto the SPE cartridge in millilitres, i.e. 10 ml (6.3.3);

V_3 is the final volume of the SPE extract in millilitres, i.e. 5 ml (6.3.7).

8 Precision

8.1 General

Details of the methods used by the individual laboratories in the inter-laboratory test are listed in Table A.1. Details of the inter-laboratory test of the precision of the method are summarized in Annex C. The values derived from the inter-laboratory test may not be applicable to analyte concentration ranges and matrices other than those detailed in Annex C.

8.2 Repeatability and reproducibility

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 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 values r, R are summarized below (see Table 5 and Table 6) and further data from the validation study are given in Annex C and [1].

Table 5 — Values for the nine sweeteners in beverages according to Tables C.1 to C.9

		Sample 2 mg/l	Sample 3 mg/l	Sample 4 mg/l	Sample 5 mg/l
acesulfame-K	$\bar{x}$	38,3	266,6	324,1	383,5
	r	7,4	16,9	29,7	25,7
	R	11,6	43,8	56,2	54,0
alitame	$\bar{x}$	31,1	69,1	96,4	114,5
	r	6,2	7,7	6,3	4,3
	R	8,3	21,1	7,2	11,0
aspartame	$\bar{x}$	38,1	485,1	584,8	702
	r	5,2	26,5	14,1	16,2
	R	17,1	93,3	86,6	65,9
cyclamate	$\bar{x}$	28,3	248,9	256,8	307,2
	r	3,5	18,4	10,2	16,5
	R	16,3	43,1	39.2	43,4
dulcin	$\bar{x}$	55,0	79,6	95,7	115,1
	r	3,8	8,2	2,8	4,3
	R	9,4	10,9	14,7	14,7
neotame	$\bar{x}$	37,6	77,9	97,2	115,3
	r	2,4	5,2	6,7	7,7
	R	6,8	12,9	13,5	14,4
neohesperidine dihydrochalcone	$\bar{x}$	31,4	42,8	51,0	59,3
	r	9,3	4,7	4,9	7,3
	R	25,1	18,7	12,4	14,5
saccharin	$\bar{x}$	36,2	60,1	74,1	87,6
	r	3,9	4,7	8,3	2,7
	R	11,3	7,7	13,6	14,5
sucralose	$\bar{x}$	36,8	245,1	282,9	346,8
	r	3,8	10,6	7,4	22,9
	R	14,7	28,2	45,3	37,4

Table 6 — Values for nine sweeteners in canned fruits according to Tables C.1 to C.9

		Sample 7 mg/kg	Sample 8 mg/kg	Sample 9 mg/kg	Sample 10 mg/kg
acesulfame-K	$\bar{x}$	38,4	259,2	323	391,3
	r	7,4	25,6	11,5	32
	R	15,9	35,5	44,8	49,1
alitame	$\bar{x}$	36	113,7	142,5	175,2
	r	9,7	6,9	8,8	18
	R	9,7	10,6	12,3	21,1
aspartame	$\bar{x}$	37,2	739,8	951,9	1 120,2
	r	10,1	46,3	12,5	37,8
	R	10,1	82,0	77,1	88,8
cyclamate	$\bar{x}$	27,5	749,7	924,7	1 100,6
	r	12,4	19,6	40,5	35,6
	R	13,7	86,5	124,2	104,3
dulcin	$\bar{x}$	49,8	111	141,7	172,6
	r	10,3	8,4	10,1	8,6
	R	12,0	13,4	13,1	15,2
neotame	$\bar{x}$	37,3	116,2	140,6	173,7
	r	3,6	10,1	6,2	13,5
	R	6,2	17,6	21,1	21,7
neohesperidine dihydrochalcone	$\bar{x}$	35,3	40,5	49,8	59,3
	r	6,1	2,8	5,6	6,5
	R	12,2	13,0	9,2	15,3
saccharin	$\bar{x}$	44,3	151,9	193,4	235,3
	r	6,8	11,3	12,0	18,8
	R	23,6	29,6	37,7	42,0
sucralose	$\bar{x}$	35,3	306,1	380,2	462,4
	r	6,3	20,6	23,8	27,1
	R	10,8	24,4	29,1	27,1

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 (i.e. EN 15911) or to the method used;

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

— the date of receipt;

— the date of test;

— the results and the units in which the results have been expressed;

— 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.

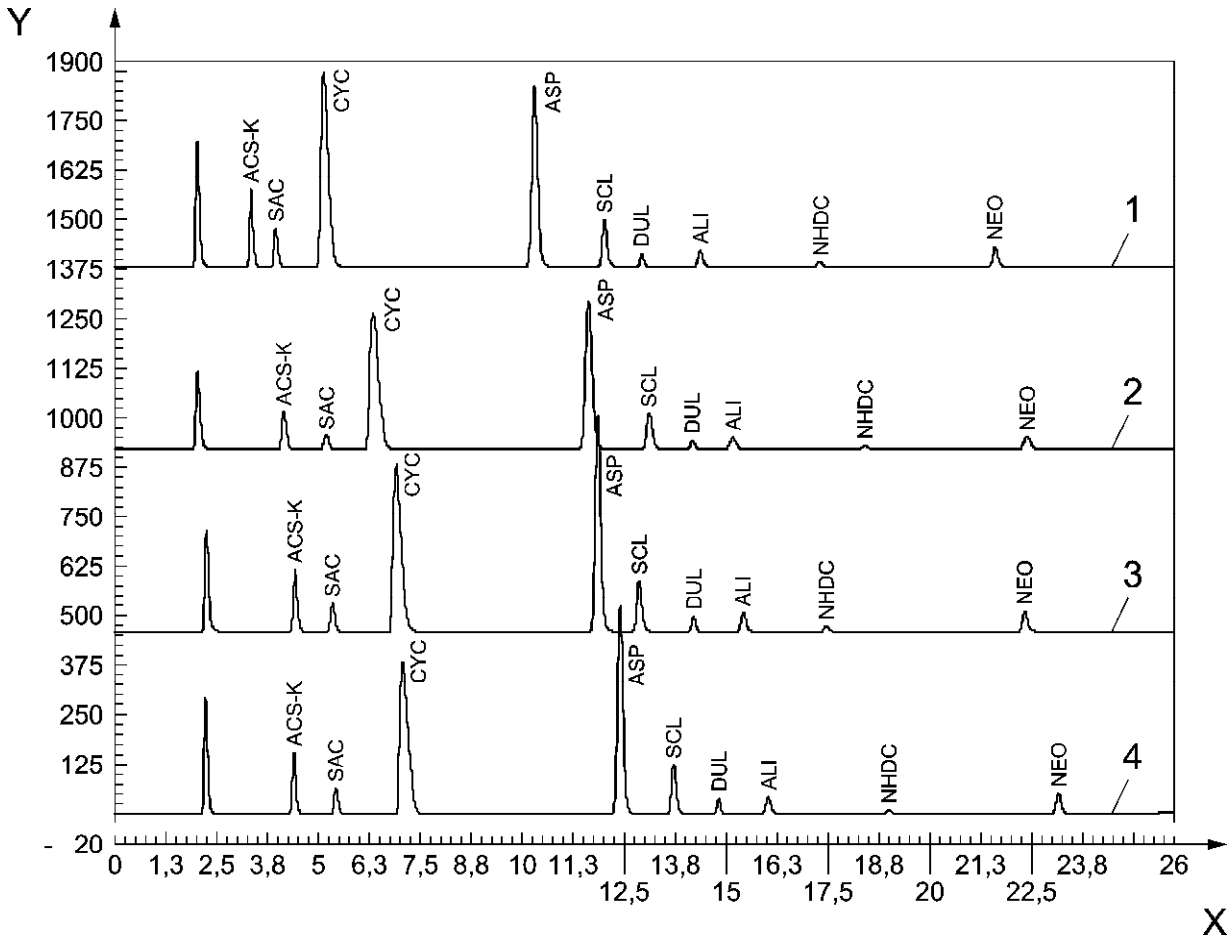
Annex A
(informative)

Table A.1 — Suitable method conditions

SPE characteristics							
- brand name	Chromabond®	Chromabond®	Bakerbond spe®	Chromabond®	Chromabond®	Chromabond®	Chromabond®
- stationary phase	C18ec	C18ec	C18	C18ec	C18ec	C18ec	C18ec
- capacity [ml/mg]	6/1 000	6/1 000	3/500	6/1 000	6/1 000	6/1 000	6/1 000
HPLC column characteristics							
- brand name	Purospher® Star	Purospher® Star	Purospher® Star	Nucleodur®	Purospher® Star	Purospher® Star	Purospher® Star
- stationary phase	RP-C18 endcapped	RP-C18 endcapped	RP-C18 endcapped	C-18ec Pyramid	RP-C18 endcapped	RP-C18 endcapped	RP-C18 endcapped
- length [mm]	250	250	250	250	250	250	250
- i.d. [mm]	3	3	3	3	3	3	3
- particle size [µm]	5	5	5	5	5	5	5
HPLC mobile phase							
- mobile phase A composition [v/v/v]	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7	Methanol:Buffer solution:Acetone; 69:24:7
- mobile phase B composition [v/v/v]	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7	Methanol:Buffer solution:Acetone; 11:82:7
- flow rate [ml/min]	0,5	0,5	0,5	0,5	0,6	0,55	0,5
HPLC separation mode							
- gradient program [min - mobile phase A %]	0 min - 100 % A; 4 min - 100 % A; 11 min - 47 % A; 23 min - 2 % A; 24 min -2 % A; 26 min -100 % A	0 min - 5 % A; 10 min - 60 % A; 30 min - 95 % A; 31 min - 95 % A; 32 min - 5 % A; 45 min - 5 % A	0 min - 0 % A; 15 min - 100 % A; 18 min - 100 % A; 20 min - 0 % A; 35 min - 0 % A	0 min - 0 % A; 4 min - 0 % A; 11 min - 53 % A; 23 min - 100 % A; 24 min - 100 % A; 26 min - 0 % A; 36 min - 0 % A	0 min - 0 % A; 4 min - 0 % A; 11 min - 53 % A; 21 min - 100 % A; 23 min - 100 % A; 25 min - 0 % A; 31 min - 0 % A	0 min - 0 % A; 4 min - 0 % A; 11 min - 53 % A; 23 min - 100 % A; 24 min - 100 % A; 26 min - 0 % A; 36 min - 0 % A	0 min - 0 % A; 4 min - 0 % A; 11 min - 53 % A; 23 min - 100 % A; 24 min - 100 % A; 26 min - 0 % A; 36 min - 0 % A
HPLC injection mode							
- manual/automatic	automatic	automatic	automatic	automatic	automatic	automatic	automatic
ELSD conditions							
- manufacturer	Sedex 85, Sedere	Varex MKIII, Alltech	ELSD-LT II, Shimadzu	Sedex, Sedere	Sedex 75, Sedere	ELSD 2000ES, Alltech	ELSD 2000ES, Alltech
- drift tube temperature [°C]	40	90	50	43	45	85	85
- nitrogen/air [pressure/flow]	nitrogen 3,2 bar	nitrogen 2,5 l/min	air 3 bar	nitrogen 3,5 bar	air 2,5 bar	nitrogen 2,5 l/min	nitrogen 2,5 l/min
- gain	7	1	9	10	2	1	1

Annex B
(informative)

Examples of chromatograms



Key

1 Zorbax Extend-C_{18}[2)]

2 Nucleodur® C_{18} Pyramid[2)]

3 Nucleodur® C_8 Gravity[2)]

4 Purospher® Star RP-18, 5 µm[3)]

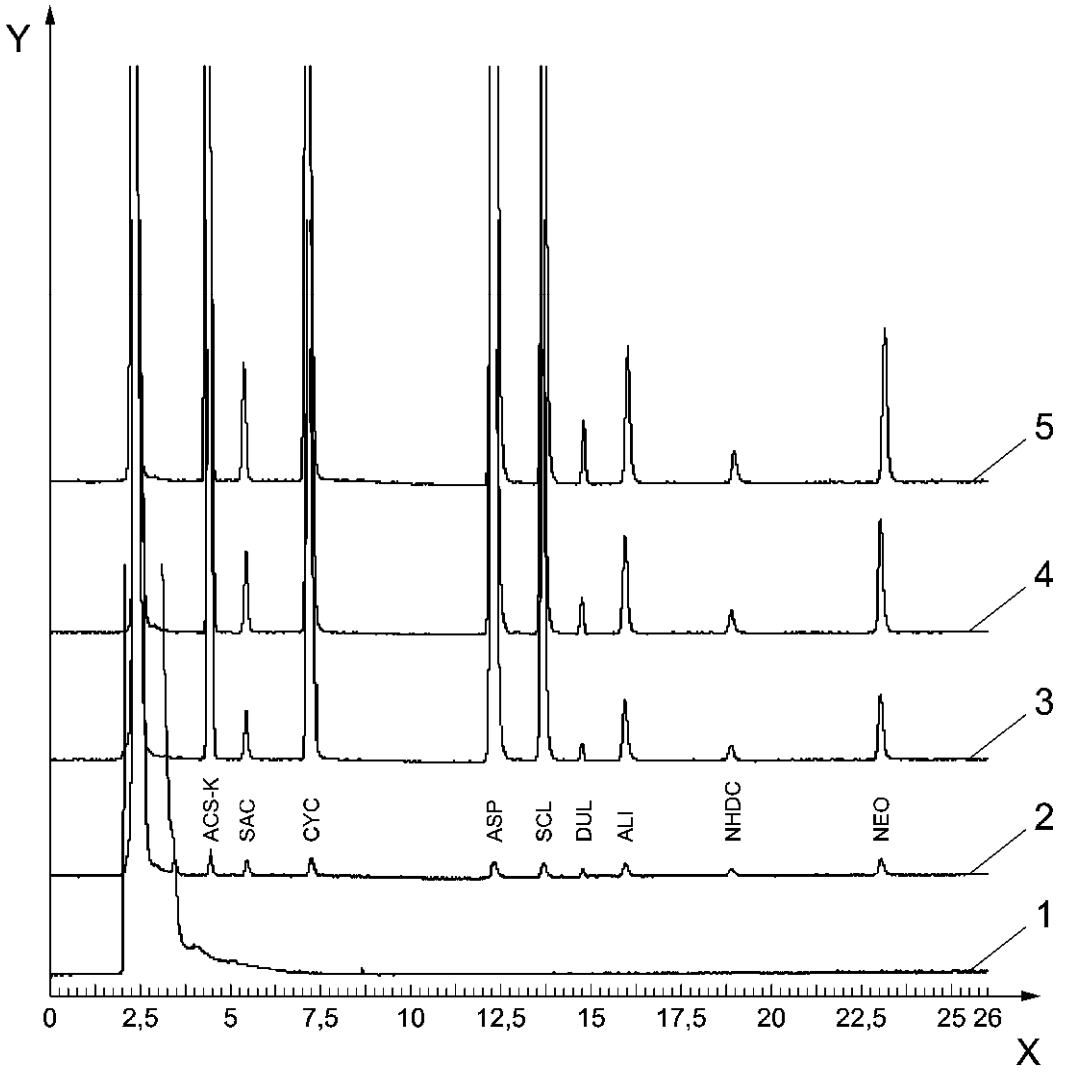
X time (min)

Y ELSD signal

Figure B.1 — Chromatograms of sweeteners standard solution obtained by using different HPLC columns

2) These are examples of suitable products available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of these products.

3) Purospher® Star RP-18, 5 µm is an example of 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.



Key

1 energy drink - blank
2 energy drink fortified at concentration level close to the limit of quantification
3 non-carbonated soft drink fortified at a concentration level of approximately 80 % of maximum usable dosage
4 carbonated soft drink fortified at a concentration level of approximately 100 % of maximum usable dosage
5 carbonated soft drink fortified at a concentration level of approximately 120 % of maximum usable dosage
X time (min)
Y ELSD signal
Separating column Purospher® Star RP-18, 5 µm[4)]
Diameter 3 mm;
Length 250 mm;
Experimental conditions as described in this European Standard.

Figure B.2 — Chromatograms of various beverages fortified with sweeteners obtained by using a fully end-capped reversed phase HPLC column

4) Purospher® Star RP-18, 5 µm is an example of 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.

Annex C
(informative)

Precision data

Seven laboratories took part in the interlaboratory tests. A pre-trial was organised to allow all participants to identify any potential problems before the main trial took place. During the pre-trial, participants were given two test samples with given composition of all nine sweeteners, i.e. one beverage with a low concentration and one with a high concentration of all nine sweeteners. The two test samples were used for optimisation purposes and demonstration of correctly functioning chromatographic systems.

During the interlaboratory tests, participants were sent 20 test materials (ten blind-duplicates) of various beverages, i.e.:

— Sample 1 – Energy drink – blank;

— Sample 2 – Energy drink fortified at concentration level close to the limit of quantification (LOQs);

— Sample 3 – Non-carbonated soft drink fortified at a concentration level of approximately 80 % of maximum usable dosage (MUDs);

— Sample 4 – Carbonated soft drink fortified at a concentration level of approximately 100 % of MUDs;

— Sample 5 – Carbonated soft drink fortified at a concentration level of approximately 120 % of MUDs;

and canned fruits, i.e.:

— Sample 6 – Canned cocktail fruits – blank;

— Sample 7 – Canned cocktail fruits fortified at concentration level close to the LOQs;

— Sample 8 – Canned pears fortified at a concentration level of approximately 75 % of MUDs;

— Sample 9 – Canned pears fortified at a concentration level of approximately 100 % of MUDs;

— Sample 10 – Canned pears fortified at a concentration level of approximately 115 % of MUDs,

differing in fortified concentration amounts of all nine sweeteners for analysis.

Method details as applied by the individual laboratories are given in Table A.1.

The following data were obtained in interlaboratory tests according to the IUPAC Harmonised Protocol [2] conducted by the Institute for Reference Materials and Measurements of the European Commission's Directorate General Joint Research Centre.

Table C.1 — Acesulfame-K

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	38,3	266,6	324,1	383,5
True value [mg/l]	42,1	282,5	354,2	421,7
Repeatability standard deviation s_r [mg/l]	2,6	6,0	10,6	9,2
Repeatability relative standard deviation RSD_r [%]	6,9	2,3	3,3	2,4
Repeatability limit r [mg/l]	7,4	16,9	29,7	25,7
Reproducibility standard deviation s_R [mg/l]	4,2	15,6	20,1	19,3
Reproducibility relative standard deviation RSD_R [%]	10,9	5,9	6,2	5,0
Reproducibility limit R [mg/l]	11,6	43,8	56,2	54,0
Horrat value	1,2	0,9	0,9	0,8
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	1	0
Number of laboratories retained after eliminating outliers	7	7	6	7
Number of accepted results	14	14	12	14
Mean value $\bar{x}$ [mg/kg]	38,4	259,2	323,0	391,3
True value [mg/kg]	36,5	265,6	338,8	410,0
Repeatability standard deviation s_r [mg/kg]	2,7	9,1	4,1	11,4
Repeatability relative standard deviation RSD_r	6,9	3,5	1,3	2,9
Repeatability limit r [mg/kg]	7,4	25,6	11,5	32,0
Reproducibility standard deviation s_R [mg/kg]	5,7	12,7	16,0	17,5
Reproducibility relative standard deviation RSD_R [%]	14,8	4,9	4,9	4,5
Reproducibility limit R [mg/kg]	15,9	35,5	44,8	49,1
Horrat value	1,6	0,7	0,7	0,7

Table C.2 — Alitame

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	31,1	69,1	96,4	114,5
True value [mg/l]	36,5	80,5	102,6	122,2
Repeatability standard deviation s_r [mg/l]	2,2	2,8	2,3	1,5
Repeatability relative standard deviation RSD_r [%]	7,1	4,0	2,3	1,3
Repeatability limit r [mg/l]	6,2	7,7	6,3	4,3
Reproducibility standard deviation s_R [mg/l]	3,0	7,5	2,6	3,9
Reproducibility relative standard deviation RSD_R [%]	9,5	10,9	2,7	3,4
Reproducibility limit R [mg/l]	8,3	21,1	7,2	11,0
Horrat value	1,0	1,3	0,3	0,4
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/kg]	36,0	113,7	142,5	175,2
True value [mg/kg]	34,6	116,1	145,1	175,5
Repeatability standard deviation s_r [mg/kg]	3,5	2,5	3,1	6,4
Repeatability relative standard deviation RSD_r	9,7	2,2	2,2	3,7
Repeatability limit r [mg/kg]	9,7	6,9	8,8	18,0
Reproducibility standard deviation s_R [mg/kg]	3,5	3,8	4,4	7,5
Reproducibility relative standard deviation RSD_R [%]	9,7	3,3	3,1	4,3
Reproducibility limit R [mg/kg]	9,7	10,6	12,3	21,1
Horrat value	1,0	0,4	0,4	0,6

Table C.3 — Aspartame

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	1	0	0	1
Number of laboratories retained after eliminating outliers	6	7	7	6
Number of accepted results	12	14	14	12
Mean value $\bar{x}$ [mg/l]	38,1	485,1	584,8	702,0
True value [mg/l]	42,0	485,0	605,0	720,3
Repeatability standard deviation s_r [mg/l]	1,9	9,5	5,0	5,8
Repeatability relative standard deviation RSD_r [%]	4,9	1,9	0,9	0,8
Repeatability limit r [mg/l]	5,2	26,5	14,1	16,2
Reproducibility standard deviation s_R [mg/l]	6,1	33,3	30,9	23,5
Reproducibility relative standard deviation RSD_R [%]	16,0	6,9	5,3	3,4
Reproducibility limit R [mg/l]	17,1	93,3	86,6	65,9
Horrat value	1,7	1,1	0,9	0,6
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	1	0	2	1
Number of laboratories retained after eliminating outliers	6	7	5	6
Number of accepted results	12	14	10	12
Mean value $\bar{x}$ [mg/kg]	37,2	739,8	951,9	1 120,2
True value [mg/kg]	37,3	752,1	967,8	1 171,1
Repeatability standard deviation s_r [mg/kg]	3,6	16,5	4,5	13,5
Repeatability relative standard deviation RSD_r	9,7	2,2	0,5	1,2
Repeatability limit r [mg/kg]	10,1	46,3	12,5	37,8
Reproducibility standard deviation s_R [mg/kg]	3,6	29,3	27,5	31,7
Reproducibility relative standard deviation RSD_R [%]	9,7	4,0	2,9	2,8
Reproducibility limit R [mg/kg]	10,1	82,0	77,1	88,8
Horrat value	1,0	0,7	0,5	0,5

Table C.4 — Cyclamate

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	28,3	248,9	256,8	307,2
True value [mg/l]	36,9	239,0	252,7	300,8
Repeatability standard deviation s_r [mg/l]	1,2	6,6	3,6	5,9
Repeatability relative standard deviation RSD_r [%]	4,4	2,6	1,4	1,9
Repeatability limit r [mg/l]	3,5	18,4	10,2	16,5
Reproducibility standard deviation s_R [mg/l]	5,8	15,4	14,0	15,5
Reproducibility relative standard deviation RSD_R [%]	20,6	6,2	5,5	5,0
Reproducibility limit R [mg/l]	16,3	43,1	39,2	43,4
Horrat value	2,1	0,9	0,8	0,7
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	1	0	1
Number of laboratories retained after eliminating outliers	7	6	7	6
Number of accepted results	14	12	14	12
Mean value $\bar{x}$ [mg/kg]	27,5	749,7	924,7	1 100,6
True value [mg/kg]	32,2	752,6	968,8	1 172,3
Repeatability standard deviation s_r [mg/kg]	4,4	7,0	14,5	12,7
Repeatability relative standard deviation RSD_r	16,1	0,9	1,6	1,2
Repeatability limit r [mg/kg]	12,4	19,6	40,5	35,6
Reproducibility standard deviation s_R [mg/kg]	4,9	30,9	44,4	37,2
Reproducibility relative standard deviation RSD_R [%]	17,9	4,1	4,8	3,4
Reproducibility limit R [mg/kg]	13,7	86,5	124,2	104,3
Horrat value	1,8	0,7	0,8	0,6

Table C.5 — Dulcin

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	55,0	79,6	95,7	115,1
True value [mg/l]	60,7	81,3	101,8	121,1
Repeatability standard deviation s_r [mg/l]	1,4	2,9	1,0	1,5
Repeatability relative standard deviation RSD_r [%]	2,5	3,7	1,0	1,3
Repeatability limit r [mg/l]	3,8	8,2	2,8	4,3
Reproducibility standard deviation s_R [mg/l]	3,3	3,9	5,2	5,2
Reproducibility relative standard deviation RSD_R [%]	6,1	4,9	5,5	4,6
Reproducibility limit R [mg/l]	9,4	10,9	14,7	14,7
Horrat value	0,7	0,6	0,7	0,6
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	1	0	0	0
Number of laboratories retained after eliminating outliers	6	7	7	7
Number of accepted results	12	14	14	14
Mean value $\bar{x}$ [mg/kg]	49,8	111,0	141,7	172,6
True value [mg/kg]	50,2	114,3	145,7	176,3
Repeatability standard deviation s_r [mg/kg]	3,7	3,0	3,6	3,1
Repeatability relative standard deviation RSD_r	7,4	2,7	2,5	1,8
Repeatability limit r [mg/kg]	10,3	8,4	10,1	8,6
Reproducibility standard deviation s_R [mg/kg]	4,3	4,8	4,7	5,4
Reproducibility relative standard deviation RSD_R [%]	8,6	4,3	3,3	3,1
Reproducibility limit R [mg/kg]	12,0	13,4	13,1	15,2
Horrat value	1,0	0,5	0,4	0,4

Table C.6 — Neotame

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	37,6	77,9	97,2	115,3
True value [mg/l]	37,5	80,5	102,2	121,7
Repeatability standard deviation s_r [mg/l]	0,9	1,9	2,4	2,8
Repeatability relative standard deviation RSD_r [%]	2,3	2,4	2,4	2,4
Repeatability limit r [mg/l]	2,4	5,2	6,7	7,7
Reproducibility standard deviation s_R [mg/l]	2,4	4,6	4,8	5,2
Reproducibility relative standard deviation RSD_R [%]	6,4	5,9	5,0	4,5
Reproducibility limit R [mg/l]	6,8	12,9	13,5	14,4
Horrat value	0,7	0,7	0,6	0,6
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/kg]	37,3	116,2	140,6	173,7
True value [mg/kg]	36,2	118,3	145,4	175,9
Repeatability standard deviation s_r [mg/kg]	1,3	3,6	2,2	4,8
Repeatability relative standard deviation RSD_r	3,5	3,1	1,6	2,8
Repeatability limit r [mg/kg]	3,6	10,1	6,2	13,5
Reproducibility standard deviation s_R [mg/kg]	2,2	6,3	7,5	7,7
Reproducibility relative standard deviation RSD_R [%]	5,9	5,4	5,3	4,5
Reproducibility limit R [mg/kg]	6,2	17,6	21,1	21,7
Horrat value	0,6	0,7	0,7	0,6

Table C.7 — Neohesperidine dihydrochalcone

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	31,4	42,8	51,0	59,3
True value [mg/l]	36,7	40,2	50,7	60,4
Repeatability standard deviation s_r [mg/l]	3,3	1,7	1,8	2,6
Repeatability relative standard deviation RSD_r [%]	10,6	3,9	3,5	4,4
Repeatability limit r [mg/l]	9,3	4,7	4,9	7,3
Reproducibility standard deviation s_R [mg/l]	9,0	6,7	4,4	5,2
Reproducibility relative standard deviation RSD_R [%]	28,5	15,6	8,7	8,8
Reproducibility limit R [mg/l]	25,1	18,7	12,4	14,5
Horrat value	3,0	1,7	1,0	1,0
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	1	0	0
Number of laboratories retained after eliminating outliers	7	6	7	7
Number of accepted results	14	12	14	14
Mean value $\bar{x}$ [mg/kg]	35,3	40,5	49,8	59,3
True value [mg/kg]	33,4	37,5	48,9	59,1
Repeatability standard deviation s_r [mg/kg]	105,6	108,0	102,0	100,4
Repeatability relative standard deviation RSD_r	6,1	2,5	4,0	3,9
Repeatability limit r [mg/kg]	6,1	2,8	5,6	6,5
Reproducibility standard deviation s_R [mg/kg]	4,4	4,6	3,3	5,5
Reproducibility relative standard deviation RSD_R [%]	12,4	11,5	6,6	9,2
Reproducibility limit R [mg/kg]	12,2	13,0	9,2	15,3
Horrat value	1,3	1,3	0,7	1,1

Table C.8 — Saccharin

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	1	0	1
Number of laboratories retained after eliminating outliers	7	6	7	6
Number of accepted results	14	12	14	12
Mean value $\bar{x}$ [mg/l]	36,2	60,1	74,1	87,6
True value [mg/l]	40,3	65,2	80,9	96,3
Repeatability standard deviation s_r [mg/l]	1,4	1,7	3,0	1,0
Repeatability relative standard deviation RSD_r [%]	3,8	2,8	4,0	1,1
Repeatability limit r [mg/l]	3,9	4,7	8,3	2,7
Reproducibility standard deviation s_R [mg/l]	4,0	2,8	4,9	5,2
Reproducibility relative standard deviation RSD_R [%]	11,1	4,6	6,6	5,9
Reproducibility limit R [mg/l]	11,3	7,7	13,6	14,5
Horrat value	1,2	0,5	0,8	0,7
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/kg]	44,3	151,9	193,4	235,3
True value [mg/kg]	38,0	150,0	194,0	234,8
Repeatability standard deviation s_r [mg/kg]	2,4	4,0	4,3	6,7
Repeatability relative standard deviation RSD_r	5,5	2,7	2,2	2,9
Repeatability limit r [mg/kg]	6,8	11,3	12,0	18,8
Reproducibility standard deviation s_R [mg/kg]	8,4	10,6	13,5	15,0
Reproducibility relative standard deviation RSD_R [%]	19,0	7,0	7,0	6,4
Reproducibility limit R [mg/kg]	23,6	29,6	37,7	42,0
Horrat value	2,1	0,9	1,0	0,9

Table C.9 — Sucralose

	Beverages			
Sample No.	**2**	**3**	**4**	**5**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/l]	36,8	245,1	282,9	346,8
True value [mg/l]	38,9	251,8	302,6	360,3
Repeatability standard deviation s_r [mg/l]	1,4	3,8	2,7	8,2
Repeatability relative standard deviation RSD_r [%]	3,7	1,5	0,9	2,4
Repeatability limit r [mg/l]	3,8	10,6	7,4	22,9
Reproducibility standard deviation s_R [mg/l]	5,2	10,1	16,2	13,3
Reproducibility relative standard deviation RSD_R [%]	14,2	4,1	5,7	3,8
Reproducibility limit R [mg/l]	14,7	28,2	45,3	37,4
Horrat value	1,5	0,6	0,8	0,6
	Canned fruits			
Sample No.	**7**	**8**	**9**	**10**
Year of inter-laboratory test	2007	2007	2007	2007
Number of laboratories	7	7	7	7
Number of samples	2	2	2	2
Number of outliers (laboratories)	0	0	0	0
Number of laboratories retained after eliminating outliers	7	7	7	7
Number of accepted results	14	14	14	14
Mean value $\bar{x}$ [mg/kg]	35,3	306,1	380,2	462,4
True value [mg/kg]	34,6	313,1	388,2	469,7
Repeatability standard deviation s_r [mg/kg]	2,2	7,4	8,5	9,7
Repeatability relative standard deviation RSD_r [%]	6,3	2,4	2,2	2,1
Repeatability limit r [mg/kg]	6,3	20,6	23,8	27,1
Reproducibility standard deviation s_R [mg/kg]	3,8	8,7	10,4	9,7
Reproducibility relative standard deviation RSD_R [%]	10,9	2,8	2,7	2,1
Reproducibility limit R [mg/kg]	10,8	24,4	29,1	27,1
Horrat value	1,2	0,4	0,4	0,3

Annex D
(informative)

Present EU limits for the nine sweeteners

Table D.1 — Present EU limits for the nine sweeteners in beverages and canned fruits

Sweetener	MUD [a] for beverages mg/l	MUD [a] for canned fruits mg/kg
ACS-K	350	350
ALI [b]	-	-
ASP	600	1 000
CYC	250	1 000
DUL [b]	-	-
NEO	20	-
NHDC	30	50
SAC	80	200
SCL	300	400

[a] MUD = maximum usable dosage according to present EU limits [3] to [6].

[b] Sweeteners not authorised by the current EU legislation.

Bibliography

[1] Buchgraber, M. and Wasik, A.: Validation of an analytical method for the simultaneous determination of nine intense sweeteners by HPLC-ELSD – Report on the collaborative trial. EUR 22726 EN, 2007

[2] IUPAC Harmonised Protocol for the Design, Conduct and Interpretation of method-Performance Studies. Pure & Appl Chem, 1995, 67, 331-343

[3] Directive 94/35/EC of European Parliament and of the Council of 30 June 1994 on sweeteners for use in foodstuffs. Official Journal of European Communities 1994, L237/13

[4] Directive 96/83/EC of the European Parliament and of the Council of 19 December 1996 amending Directive 94/35/EC on sweeteners for use in foodstuffs. Official Journal of European Union 1997, L048, 16-19

[5] Directive 2003/115/EC of the European Parliament and of the Council of 22 December 2003 amending Directive 94/35/EC on sweeteners for use in foodstuffs. Official Journal of European Union 2004, L024, 65-71

[6] Commission Directive 2009/163/EU of 22 December 2009 amending Directive 94/35/EC of the European Parliament and of the Council on sweeteners for use in foodstuffs with regard to neotame. Official Journal of European Union 2009, L344, 37-40

[7] Wasik, A., McCourt, J. and Buchgraber, M.: Simultaneous determination of nine sweeteners in foodstuffs by high performance liquid chromatography and evaporative light scattering detection – Single-laboratory validation. J Chromat A, 2007, 1157, 187-196

This page deliberately left blank

British Standards Institution (BSI)

BSI is the independent national body responsible for preparing British Standards and other standards-related publications, information and services.
It presents the UK view on standards in Europe and at the international level.

It is incorporated by Royal Charter.

Revisions

British Standards are updated by amendment or revision. Users of British Standards should make sure that they possess the latest amendments or editions.

It is the constant aim of BSI to improve the quality of our products and services. We would be grateful if anyone finding an inaccuracy or ambiguity while using this British Standard would inform the Secretary of the technical committee responsible, the identity of which can be found on the inside front cover.

Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001

BSI offers Members an individual updating service called PLUS which ensures that subscribers automatically receive the latest editions of standards.

Tel: +44 (0)20 8996 7669 Fax: +44 (0)20 8996 7001
Email: plus@bsigroup.com

Buying standards

You may buy PDF and hard copy versions of standards directly using a credit card from the BSI Shop on the website **www.bsigroup.com/shop.** In addition all orders for BSI, international and foreign standards publications can be addressed to BSI Customer Services.

Tel: +44 (0)20 8996 9001 Fax: +44 (0)20 8996 7001
Email: orders@bsigroup.com

In response to orders for international standards, it is BSI policy to supply the BSI implementation of those that have been published as British Standards, unless otherwise requested.

Information on standards

BSI provides a wide range of information on national, European and international standards through its Knowledge Centre.

Tel: +44 (0)20 8996 7004 Fax: +44 (0)20 8996 7005
Email: knowledgecentre@bsigroup.com

Various BSI electronic information services are also available which give details on all its products and services.

Tel: +44 (0)20 8996 7111 Fax: +44 (0)20 8996 7048
Email: info@bsigroup.com

BSI Subscribing Members are kept up to date with standards developments and receive substantial discounts on the purchase price of standards. For details of these and other benefits contact Membership Administration.

Tel: +44 (0)20 8996 7002 Fax: +44 (0)20 8996 7001
Email: membership@bsigroup.com

Information regarding online access to British Standards via British Standards Online can be found at **www.bsigroup.com/BSOL**

Further information about BSI is available on the BSI website at **www.bsigroup.com/standards**

Copyright

BSI Group Headquarters

389 Chiswick High Road London W4 4AL UK

Tel +44 (0)20 8996 9001
Fax +44 (0)20 8996 7001
www.bsigroup.com/standards

raising standards worldwide™