SLOVENSKI STANDARD
SIST-TS CEN/TS 17083:2017

01-september-2017

Živila - Določevanje akrilamida v živilih in kavi s plinsko kromatografijo/masno spektrometrijo (GC-MS)

Foodstuffs - Determination of acrylamide in food and coffee by gas chromatography-mass spectrometry (GC-MS)

Lebensmittel - Bestimmung von Acrylamid in Lebensmitteln und Kaffee mit Gaschromatographie-Massenspektrometrie (GC-MS)

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Produits alimentaires - Dosage par CG-SM des produits alimentaires et café avec GC-MS

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Ta slovenski standard je istoveten z: **CEN/TS 17083:2017**

ICS:

67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
71.040.50	Fizikalnokemijske analitske metode	Physicochemical methods of analysis

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2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

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ICS 67.050; 67.060; 67.140.20; 67.080.20

English Version

Foodstuffs - Determination of acrylamide in food and coffee by gas chromatography-mass spectrometry (GC-MS)

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This Technical Specification (CEN/TS) was approved by CEN on 14 May 2017 for provisional application.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (CEN/TS 17083:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association.

Annexes A, B, C and D are informative.

WARNING 1 — The use of this Technical Specification can involve hazardous materials, operations and equipment. This document does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

WARNING 2 — Some precaution is required when using polyacrylamide-based plastics because acrylamide may leach from these materials.

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1 Scope

This Technical Specification specifies a method for the determination of acrylamide in cereal-based products, potato-based products and coffee by gas-chromatography mass spectrometry (GC-MS).

The method has been single-laboratory validated via the analysis of spiked samples (French fries (uncooked), bread, water biscuit, infant cereal, biscuit, green coffee, roast coffee and instant coffee), ranging from 30 µg/kg to 1 500 µg/kg acrylamide.

The results from the single laboratory validation were obtained by a laboratory with significant experience in acrylamide analysis. In addition, this method has also been studied by inter laboratory trial via the analysis of samples containing incurred acrylamide, ranging from approximately 200 µg/kg to 2 000 µg/kg. Critical points of the method are identified in 7.5 and Clause 8.

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

The test portion is extracted with hot water and isotopically labelled acrylamide is added as internal standard. High-fat samples are defatted with hexane, cleared with Carrez solution and centrifuged. Sample extracts are brominated and extracted with ethyl acetate. Following removal of the ethyl acetate by evaporation, triethylamine is added to partially debrominate, after which a portion of the sample extract is injected onto a GC-MS system for quantification. The chromatographic separation is obtained on a mid-polarity capillary GC column. The acrylamide derivative is ionized at 70 eV and recorded in selected ion monitoring (SIM) mode, and quantified by comparison with the stable isotopically labelled analogue.

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4 Reagents

Use only reagents of recognized analytical grade and water complying with grade 1 of EN ISO 3696:1995 (electrical conductivity below 0,1 µS/cm at 25 °C), unless specified otherwise. Standard solutions are preferably prepared gravimetrically. An analytical balance (6.1) is used for the preparation of both native and stable isotope labelled acrylamide.

WARNING — Acrylamide has been classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (see [2]). Bromine is very toxic and corrosive and hydrobromic acid is corrosive and hazardous. The prepared brominating solution (see 4.17) shall be considered as very toxic and corrosive.

Protective equipment such as laboratory coat, disposable gloves and safety glasses shall be used. All handlings of acrylamide, bromine, hydrobromic acid, brominating solution and organic solvents shall be performed in a fume cupboard with adequate air flow.

IMPORTANT — Dispose chemical waste according to applicable environmental rules and regulations. Bromine is very toxic to aquatic organisms hence discharge into the environment shall be avoided. Waste shall be disposed of appropriately.

4.1 Acrylamide, purity ≥ 99 %.

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4.2 [1,2,3-$^{13}C_3$]-acrylamide, (*acrylamide-$^{13}C_3$*) isotopic ^{13}C purity 99 %, supplied as 1 000 µg/ml solution[1)]

It is permissible to use deuterated acrylamide (acrylamide-d_3) as an alternative internal standard. In the following sections of the procedure, MS detection and calculation are prescribed for acrylamide-$^{13}C_3$ only.

4.3 Bromine, purity ≥ 99 %.

4.4 Ethyl acetate, MS grade.

4.5 Helium purified compressed gas, (purity equivalent to 99,995 % or better).

4.6 n-Hexane, MS grade.

4.7 Hydrobromic acid, 48 % in water, assay ≥ 99,9 %.

4.8 Ice, crushed.

4.9 Magnesium sulfate, anhydrous powder.

4.10 Potassium bromide, purity ≥ 98,5 %.

4.11 Potassium hexacyanoferrate trihydrate (II), $K_4Fe(CN)_6.3H_2O$, purity ≥ 98,5 %.

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4.12 Sodium chloride.

4.13 Sodium thiosulfate pentahydrate, $Na_2S_2O_3 \cdot 5\ H_2O$, purity ≥ 99,5 %.

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4.14 Sodium thiosulfate solution, substance concentration c = 1 mol/l. Dissolve 24,82 g of sodium thiosulfate pentahydrate in 100 ml of water.

4.15 Triethylamine, $N(C_2H_5)_3$, purity ≥ 99 %.

4.16 Zinc acetate dihydrate, $Zn(CH_3COO)_2 \bullet 2\ H_2O$, purity ≥ 98,5 %.

4.17 Brominating solution.

Wear gloves and carry out the following steps in a fume cupboard. Prepare fresh reagent every three months.

For preparation of bromine water (saturated), place a 1 l glass bottle containing ca. 400 ml of water in a crushed ice bath and stir with a magnetic stirrer for ca. 10 min. Using a measuring cylinder, add 15 ml ± 1 ml of bromine (4.3), stopper the flask and continue stirring for at least 60 min. Cap securely and store at 4 °C to 6 °C.

Weigh 400 g ± 2 g of potassium bromide (4.10) into a 2 l screw-capped glass bottle and add 1 000 ml ± 5 ml of water. Shake or stir to dissolve then add 20 ml ± 1 ml of hydrobromic acid (4.7) using a measuring cylinder.

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1) This is an example of a suitable product available commercially, manufactured by Cambridge Isotope Laboratories Inc, Andover MA, USA. CLM-813-1.2 or similar. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement of these products by CEN. Equivalent products may be used if they provide similar results.

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Using measuring cylinders, add 320 ml ± 5 ml of saturated bromine water followed by 660 ml ± 5 ml of water to the glass bottle (6.3) and mix well. Stopper and store at 4 °C to 6 °C. Discard after 3 months. To dispose of excess saturated bromine water, add solid sodium thiosulfate pentahydrate (4.13) and swirl until the brown colour disappears.

4.18 Carrez solution I.

Dissolve 10,6 g of potassium hexacyanoferrate trihydrate (II) (4.11) in 100 ml of water. Discard after 6 months.

4.19 Carrez solution II.

Dissolve 21,9 g of zinc acetate dihydrate (4.16) in 100 ml of water. Discard after 6 months.

5 Standard solution preparation

5.1 General

Prepare all standard solutions preferably gravimetrically. The tare masses of all recipients and the masses after each preparation step are recorded and used for calculation of the standard concentrations. Volumetric standard preparation may also be applied provided that the volumetric glassware used complies with EN ISO 1042 (see [1]).

5.2 Acrylamide stock standard solution, mass concentration ρ approximately 1 000 µg/ml.

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Weigh 0,1 g ± 0,001 g of acrylamide into a 100 ml volumetric flask. Add 30 ml of water, swirl to dissolve and dilute to the mark with water and mix well. The stock solution is stable for 1 month when stored in a refrigerator (4 °C to 6 °C) and protected from light.

Alternatively, commercially-available certified standard solutions may be used if available.

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5.3 Acrylamide working standard solution, ρ approximately 1 µg/ml.

Pipette 100 µl of acrylamide stock standard solution (5.2) into a 100 ml volumetric flask, dilute to 100 ml with water and mix well. The solution is stable for 2 weeks when stored in a refrigerator (4 °C to 6 °C) and protected from light.

5.4 Acrylamide spiking solution, ρ approximately 100 µg/ml.

Pipette 10 ml of acrylamide stock solution (5.2) into a 100 ml volumetric flask, dilute to 100 ml with water and mix well. The solution is stable for 2 weeks when stored in a refrigerator (4 °C to 6 °C) and protected from light.

5.5 $^{13}C_3$-Acrylamide internal standard solution, ρ approximately 20 µg/ml.

Pipette 200 µl of $^{13}C_3$-acrylamide stock standard solution (4.2) into a 10 ml volumetric flask dilute to 10 ml with water. The solution is stable for 2 weeks when stored in a refrigerator (4 °C to 6 °C) and protected from light.

5.6 Calibration standards

Prepare calibration standards of approximately 0 µg/l, 1 µg/l, 3 µg/l, 5 µg/l, 10 µg/l, 20 µg/l and 100 µg/l according to the following scheme. Pipette 15 ml of water into each of seven 40 ml screw-cap vials and add acrylamide working standard solution (5.3) and $^{13}C_3$-acrylamide internal standard solution (5.5) to each vial as detailed in Table 1. Carry out the bromination step (7.4) on each calibration standard.

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It should be noted that a little bias caused by the different volumes of the calibration standard solutions when calculated as given in 8.8 and 9.1 is assumed to be negligible. Alternatively, to avoid this bias, the calibration standards may be prepared as follows.

The volume of water may be adjusted to give the same concentration of internal standard in each vial (i.e. by using a 15 ml volumetric flask which will give a nominal internal standard concentration of 20 µg/l). The concentration (i.e. linear working) range of the calibration standards is for guidance only. Other standard concentrations may be prepared if required but shall take into account expected acrylamide levels.

Table 1 — Calibration standards (nominal values)

Water (ml)	**Working standard solution (5.3)** (µl)	**Internal standard solution (5.5)** (µl)	**Concentration acrylamide (A)** (µg/l)	**Concentration internal standard (B)** (µg/l)	**Ratio of A:B**
15	0	15	0	19,98	0
15	15	15	1,00	19,96	0,05
15	45	15	2,99	19,92	0,15
15	75	15	4,97	19,88	0,25
15	150	15	9,89	19,78	0,50
15	300	15	19,59	19,59	1,00
15	1 500	15	90,83	18,17	5,00

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6 Apparatus

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WARNING — All glassware shall be meticulously cleaned (except disposable glassware).

Usual laboratory glassware and equipment and, in particular, the following:

6.1 Analytical balance, capable of weighing to an accuracy of ± 0,0001 g.

6.2 Bottle, glass or plastic (polypropylene), ca. 175 ml to 250 ml capacity.

6.3 Bottle, glass 1 l and 2 l capacity.

6.4 Centrifuge, capable of ≥ 3 000 *g*, suitable for 50 ml centrifuge tubes.

6.5 Centrifuge tubes, 50 ml capacity.

6.6 Gas chromatography mass spectrometry (GC-MS) apparatus, comprising the following:

6.6.1 Injection system, split-splitless injector, suitable for temperatures up to 200 °C.

A programmed temperature vaporizing (PTV) injector may be used as an alternative to a split/splitless injector.

6.6.2 GC oven, suitable for temperatures up to 300 °C and capable of temperature programming.

6.6.3 Sample carousel, suitable for use with vials and caps (6.13).

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6.6.4 GC capillary column, DB 35[2] (35 %-Phenyl)-dimethylpolysiloxane), length of 30 m, internal diameter of 0,25 mm, d_f = 0,25 µm, or any column with comparable separation characteristics.

The use of a suitable guard column is highly recommended e.g. deactivated silica 5 m.

6.6.5 An interface to a mass spectrometer, with a temperature control device, suitable for temperatures up to 250 °C.

6.6.6 A mass spectrometer with the following characteristics:

— electron ionization source;

— ionization energy of 70 eV;

— mass resolution of at least 1;

— temperature control devices for the ion source (280 °C), the GC-MS interface (280 °C). Optionally a temperature control device for the quadrupole (150 °C);

— tuning stability of at least 48 h (allowing for the analysis of a sequence of samples and standards);

— response linearity range of at least two orders of magnitude.

6.7 Heater, dry-block or similar with nitrogen stream, thermostatically controlled at 40 °C ± 1 °C.

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6.8 Pipettes, calibrated positive displacement 15 µl to 1 500 µl capacity.

6.9 Pipettes, glass or calibrated air displacement 4 ml to 10 ml capacity.

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6.10 Shaker, orbital type.

6.11 Syringe filters, polytetrafluoroethylene (PTFE) 0,45 µm, 13 mm diameter.

6.12 Vials, glass screw cap 4 ml and 40 ml capacity.

6.13 Vials, glass for use with GC injection apparatus (6.6.3).

7 Procedure

7.1 General

Residues of acrylamide have occasionally been detected in laboratory wares e.g. filters. Acrylamide may also be formed as an artefact in certain analytical procedures e.g. during extraction or in the GC injector port. Reagent blank samples shall therefore be included as controls with each batch (series) of samples to ensure that there is no significant background interference.

It has been proven that acrylamide is extracted sufficiently from various types of food by shaking with water provided that the sample particle size is sufficiently small. To ensure that the particle size is < 1 mm before extraction, the use of mechanical homogenization is strongly advised.

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7.2 Sample treatment

If necessary, the sample is milled, ground or homogenized by suitable means. Allow frozen samples to defrost fully by storing in a refrigerator (4 °C to 6 °C) overnight and then allow them to reach room temperature before carrying out the following steps. Heat until boiling a sufficient amount of water to carry out step 7.3 for all samples.

7.3 Test sample preparation

Weigh 5 g of homogeneous food sample, or 1 g of coffee (roasted coffee, soluble coffee or substitute coffee) to the nearest 0,01 g into a bottle (6.2) and add 100 µl of internal standard solution (5.5) using a calibrated pipette. Add 100 ml ± 1 ml of freshly boiled hot water (see 7.2), secure the cap, shake to ensure adequate sample dispersion and place on a shaker for 1 h. Remove the samples from the shaker and ensure that they cooled to room temperature. Transfer 40 ml of the extract to a 50 ml centrifuge tube. Add 1 000 µl each of Carrez I (4.18) and Carrez II (4.19) solution to the 40 ml extract, shake after each addition. Centrifuge for 5 min at 3 000 *g*.

Transfer 25 ml of the supernatant to a clean 50 ml centrifuge tube, add 15 ml of *n*-hexane and shake vigorously for 30 s. Centrifuge for 5 min at 3 000 *g*. Proceed to bromination step.

7.4 Bromination

For all foodstuff extracts other than coffee, transfer 15 ml of the aqueous extract (7.3) to a 50 ml centrifuge tube. Add 15 ml of brominating solution (4.17), cap securely and shake. For coffee extracts, transfer 7,5 ml of the aqueous extract (7.3) to a 50 ml centrifuge tube. Add 30 ml of brominating solution (4.17), cap securely and shake.

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NOTE 1 The aqueous phase can be taken using a pipette without removing the n-hexane phase. If necessary, the *n*-hexane phase can be removed using a Pasteur pipette.

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Allow the bromination to take place in a refrigerator (4 °C to 6 °C) for at least 1 h. Using a pipette, add ca. 200 µl of the 1 mol/l of sodium thiosulfate solution (4.14) until the yellow colour disappears after shaking. For coffee extracts add approximately 400 µl of sodium thiosulfate solution. Avoid adding a high excess of sodium thiosulfate solution.

NOTE 2 Sufficient sodium thiosulfate is added to remove excess bromine. The addition of excess sodium thiosulfate may cause the formation of undesirable products such as hydrogen sulfide (H_2S).

7.5 Partition cleanup

Add 10 ml of ethyl acetate, 4 g to 5 g of magnesium sulfate and 1 g to 1,5 g of sodium chloride. Cap securely and shake on an orbital shaker for 15 min. Centrifuge for 5 min at 3 000 *g*. Using a pipette, transfer ca. 4 ml of the ethyl acetate layer to a 4 ml vial.

NOTE The volume of the ethyl acetate layer will be reduced due to dissolution in the aqueous phase.

Evaporate the ethyl acetate to a volume of ca. 0,5 ml under a gentle stream of nitrogen at ca. 40 °C ± 1 °C.

IMPORTANT — Do not allow the extract to go to dryness.

Add 50 µl of triethylamine (4.15), cap securely and shake to dissolve the residue. If a precipitate forms, filter the extract through a 0,45 µm PTFE syringe filter. Transfer the extract to a suitable container (6.13) for GC-MS analysis.