SLOVENSKI STANDARD
oSIST prEN 16987:2016

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Živila - Določevanje akrilamida v kavi in proizvodih iz kave s HPLC-MS/MS in GC-MS

Foodstuffs - Determination of acrylamide in coffee and coffee products by HPLC-MS/MS and GC-MS

Lebensmittel - Bestimmung von Acrylamid in Kaffee und Kaffee-Erzeugnissen mit HPLC-MS/MS und GC-MS

Produits alimentaires - Détermination de la teneur en acrylamide dans le café et les produits du café par CLHP-SM/SM et CG-SM

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Foodstuffs - Determination of acrylamide in coffee and coffee products by HPLC-MS/MS and GC-MS

Lebensmittel - Bestimmung von Acrylamid in Kaffee und Kaffee-Erzeugnissen mit HPLC-MS/MS und GC-MS

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

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European foreword

This document (prEN 16987:2016) has been prepared by Technical Committee CEN/TC 275 “Food analysis - horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

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Introduction

When applying this European Standard, all existing safety regulations should be followed.

Annexes A to C are informative.

WARNING — The use of this document 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.

1 Scope

This standard specifies methods for the determination of acrylamide in coffee and coffee products by extraction with water, clean-up by solid-phase extraction and determination by HPLC-MS/MS and GC-MS. It was validated in a method validation study on roasted coffee, soluble coffee, coffee substitutes and coffee products with ranges from 53 µg/kg to 612,1 µg/kg.

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, *Water for analytical laboratory use — Specification and test methods (ISO 3696)*

3 Principle

The coffee sample is extracted with water or, in the case of soluble products, dissolved in water. A clean-up by solid phase extraction is employed to remove interfering matrix compounds. Two alternative methods can be used for the determination: high performance liquid chromatography with mass spectrometric detection (HPLC-MS/MS) or, after a bromination of the acrylamide, gas chromatography with mass spectrometric detection (GC-MS). In both cases isotopic labelled internal standards are used.

4 Reagents

WARNING — In view of health risks when working with acrylamide, appropriate preventive and protection measures shall be taken, such as using a fume cupboard, aspirating acrylamide-containing solutions only with a pipette, and avoiding skin and eye contact or inhalation of acrylamide-containing vapour

If available, reagents of “residue analysis grade” or “analytical reagent grade” shall be used. The level of impurities in the reagents that contribute to the blank should be negligibly small. The blank shall be checked regularly.

4.1 Water, of grade 1 according to EN ISO 3696, MS-grade is recommended.

4.2 Operating gases of high purity, suitable for GC and mass spectrometry according to the instructions of the manufacturer of the apparatus.

4.3 Solvents, such as methanol, ethyl acetate, acetonitrile, n-hexane, MS-grade is recommended.

4.4 Acrylamide, C_3H_5NO, purity > 98 %, reference compound.

4.4.1 Acrylamide stock solution, mass concentration ρ = 1 000 µg/ml.

Weigh (0,10 ± 0,001) g of acrylamide into a 100 ml one-mark volumetric flask and swirl it in 30 ml of water in order to dissolve the acrylamide. Fill up to the mark with water and mix well. The stock solution is stable for at least three months when stored protected from light at a maximum of 6 °C.

Alternatively a commercially available solution with a mass concentration of ρ = 1 000 µg/ml may be used. The information of the manufacturer regarding the stability of the solution shall be observed.

4.4.2 Acrylamide calibration solution, ρ = 10 µg/ml.

Using a pipette, transfer (1,0 ± 0,001) ml of the acrylamide stock solution (4.4.1) into a 100 ml one-mark volumetric flask and fill up to the mark with water. This solution shall be stored protected from light at a maximum of 6 °C and shall be freshly prepared every working day. Depending on the working range more dilution steps might be necessary.

4.5 D3-acrylamide (acrylamide-2,3,3-d3) internal standard, $C_3H_2D_3NO$, purity > 98 %, reference compound.

4.5.1 D3-acrylamide stock solution (internal standard),

Weigh (0,10 ± 0,001) g of D3-acrylamide into a 100 ml one-mark volumetric flask and swirl it in 30 ml of water in order to dissolve the D3-acrylamide. Fill up to the mark with water and mix well. The stock solution is stable for at least three months when stored protected from light at a maximum of 6 °C.

Alternatively a commercially available solution with a mass concentration of ρ = 1 000 µg/ml may be used. The information of the manufacturer regarding the stability of the solution shall be observed.

4.5.2 D3-acrylamide internal standard solution.

Using a pipette, transfer (1,0 ± 0,001) ml of the D3-acrylamide stock solution (4.5.1) into a 100 ml one-mark volumetric flask and fill up to the mark with water. This solution shall be stored protected from light at a maximum of 6 °C and shall be freshly prepared every working day. Depending on the working range more dilution steps might be necessary.

For HPLC-MS/MS the solutions according to 4.4.1 to 4.5.2 can be prepared using the HPLC eluent as a solvent. The stability of these solutions depends on the solvent used and has to be validated.

When using GC-MS, all standards according to 4.4.2 and 4.5.2 shall be subjected to the derivatization step according to 6.5.1.

Instead of D3-acrylamide, it is also possible to use $^{13}C_3$ acrylamide for the preparation of the internal standard solution. However in the following clauses the procedure and calculation are described for D3-acrylamide only.

4.6 Saturated bromine water.

Saturate distilled water with bromine in a 100 ml one-mark volumetric flask (with a glass stopper) until a phase of bromine is formed at the bottom of the flask (around 3,5 % of bromine at 4 °C). Acidify the bromine water to a pH of about 1 using concentrated hydrobromic acid, (HBr, with a specific gravity of 1,48 g/cm³).

If stored at 4 °C and protected from light, the solution can be used for about 4 weeks.

4.7 Potassium bromide, KBr.

4.8 Sodium thiosulfate (pentahydrate), $Na_2S_2O_3 \cdot 5\ H_2O$.

4.9 Triethylamine, $(C_2H_5)_3N$.

4.10 Sodium sulfate (anhydrous, granular), Na_2SO_4.

4.11 Carrez solution I:

Dissolve 10,6 g of potassium hexacyanoferrate trihydrate (II) $K_4[Fe(CN)_6] \cdot 3\ H_2O$ in 100 ml of water. If stored at 4 °C and protected from light, the solution is stable for 6 months.

4.12 Carrez solution II:

Dissolve 21,9 g of zinc acetate dihydrate $Zn(CH_3COO)_2 \cdot 2\ H_2O$ in 100 ml of water. If stored at 4 °C and protected from light, the solution is stable for 6 months.

4.13 Borate buffer, pH 8,6.

Mix 68 ml of a 0,1 molar sodium borate solution (20,12 g $Na_2B_4O_7$ per litre of water) and 32 ml of 0,1 molar hydrochloric acid, *c*(HCl) = 0,1 mol/l, in a 100 ml one-mark volumetric flask.

5 Apparatus

5.1 General

Standard laboratory apparatus and, in particular, apparatus according to 5.2 to 5.15 are required.

Apparatus and parts of the apparatus which come into contact with the sample and extract shall be free of residues which can cause blank values. Preferably glassware or equipment made of stainless steel or PTFE (polytetrafluoroethylene) shall be used.

5.2 Analytical balance, capable of weighing to an accuracy of 0,1 mg.

5.3 Coffee mill, suitable for grinding roasted coffee beans.

5.4 Glassware, for collecting and storing the extracts, preferably made of amber glass, as sample vials for manual or automatic use, equipped with an inert seal (e.g. vials with PTFE coated septum).

5.5 Ultrasonic bath, capable of being maintained at 40 °C.

5.6 Laboratory centrifuge, suitable for 15 ml and 50 ml centrifugal tubes and with a minimum *g*-force of 2 000 *g*.

5.7 Centrifuge tubes, of 15 ml and 50 ml.

5.8 One-mark volumetric flask, of 20 ml and 100 ml.

5.9 Pipettes, glass or automatic, suitable for measuring volume ranges of standard and sample extract dilutions.

5.10 Glass- or polypropylene cartridges, with sorbents for the solid phase extraction (SPE), and for the clean-up of extracts in 6.3.2 and/or 6.5.1 (examples are given in Table B.1).

5.11 High performance liquid chromatograph (for the test procedure according to 6.4), equipped with ESI and mass spectrometric detector (HPLC-MS/MS); gas supply as specified by the manufacturer.

5.12 HPLC column (for the test procedure according to 7.4), suitable for acrylamide chromatography (examples are given in Table C.1).

5.13 Gas chromatograph (for the test procedure according to 6.5) with mass spectrometric detector (GC-MS) and operating gas supply (4.2) as specified by the manufacturer.

5.14 GC column, (for the test procedure according to 6.5) capillary column, suitable for acrylamide chromatography (examples are given in Table C.2).

5.15 Membrane filter units, syringe filter (e.g. cellulose acetate filters (0,45 µm pore size) suitable for filtration of sample eluate obtained by solid phase extraction before injection into the chromatographic system.

6 Procedure

6.1 General

To avoid losses of the analyte, it is necessary that the samples are protected from light during extraction and further preparation. Therefore, amber glassware should always be used. Otherwise, the content of the vessels and flasks shall be protected from incident light using aluminium foil.

In order to exclude changes in the acrylamide levels, the analysis shall be performed shortly after reception of the sample. The samples shall be stored under cool conditions below 6 °C at a maximum of 6 months, under the exclusion of light and they shall be exposed to room temperature only for analysis.

The date of receipt of the sample as well as the date of roasting or the best-before date shall be documented along with the date of analysis.

6.2 Preparation of the sample extract

If necessary, grind the sample in a coffee mill (5.3) and homogenize thoroughly.

Weigh 2 g of the homogenized sample of roasted coffee, soluble coffee or coffee substitute or 5 g of liquid coffee beverage to the nearest 1 mg using an analytical balance (5.2) and transfer it into a 50 ml centrifuge tube.

Add 2 ml of n-hexane to the test sample and shake briefly. Then spike the test sample with D3-acrylamide as the internal standard in a concentration corresponding to the expected acrylamide level of the sample.

EXAMPLE Weigh 2 g of coffee and add 100 µl internal standard solution (ρ = 10 µg/ml), which is equivalent to an acrylamide mass fraction of 500 µg/kg in the coffee sample.

Add 20 ml of distilled water, shake briefly but vigorously, and sonicate (5.5) for 15 min at approximately 40 °C.

Allow a few minutes for precipitation and in the case of non-sedimenting samples centrifuge (5.6) for 15 min at 2000 *g* to separate suspended solids. Before liquid chromatography (6.4) or derivatization and gas chromatographic separation (6.5), take 10 ml from the lower aqueous phase and use it for a further clean-up according to 6.3. Take the lower aqueous phase through the upper hexane phase using a pipette without removing the hexane phase. If necessary, the hexane phase may also be removed cautiously using a Pasteur pipette.

6.3 Clean-up of the extracts

6.3.1 Carrez precipitation

Clean-up the sample extract prepared according to 6.2 by Carrez precipitation. Add 1 000 µl of Carrez solution I (4.11) and shake. Add 1 000 µl of Carrez solution II (4.12) and shake again. After a short exposure time centrifuge for 4 min at 2000 *g*. Decant the supernatant, wash the residue with 2 ml to 3 ml of water, centrifuge again and decant. Combine both aqueous solutions.

6.3.2 Solid phase extraction

Clean-up the sample extract after Carrez precipitation (6.3.1) by solid phase extraction (SPE) using two sequential cartridges with adsorber material (examples are given in Table B.1). The first cartridge

contains 500 mg of C18 material, the second cartridge 500 mg of ion exchanger. The cartridges can be used in a serial alignment. If appropriate, a combined cartridge can be used.

Condition both SPE columns according to the manufacturer's instructions successively with methanol and distilled water. Place the complete sample extract (6.3.1) on top of the upper (first) SPE column, allow to soak and add 2 ml to 3 ml of water. Collect the eluate until the cartridge is dry. Place the eluate on top of the second or lower conditioned ion exchange column, add 2 ml to 3 ml of water and collect the eluate. A complete elution can be achieved by using a light vacuum or pressure. Collect the eluate including washing water in a 20 ml one-mark volumetric flask and fill up to 20 ml with water.

6.4 HPLC-MS/MS measurement

6.4.1 High performance liquid chromatography (HPLC)

Prior to the HPLC-MS/MS analysis, add organic solvent to the cleaned-up extract (6.3.2) in order to make up the desired eluent composition and filter through a membrane filter (5.15) before injecting a suitable volume (e.g.10 µl to 100 µl depending on the column used) onto the HPLC column.

Optimize the device parameters of the HPLC system in accordance with the manufacturer's instructions. The chromatographic conditions shall be adjusted to suit the selected column (examples are given in Table C.1).

The clean-up stages according to 6.3.1 and 6.3.2 are essential for the chromatographic separation of the analyte peaks from the interfering peaks. An example chromatogram is given in Figure C.1 and C.2.

6.4.2 Identification and quantification by mass spectrometry (HPLC-MS/MS)

Detect acrylamide using MS/MS in the positive ionization mode (electrospray ionization, ESI).

For identification use the mass transition m/z = 72 → 55. Acrylamide is identified as present if a signal at the mass track of the daughter ion (m/z 55) appears in the MS/MS chromatogram and the deviation of the retention time from that of an authentic reference compound, analysed under the same HPLC conditions, is less than 5 %.

Possible transitions for acrylamide and D3-acrylamide are given in Table 1.

Quantify the analyte by comparing the abundance of the parent-daughter ions of acrylamide with the isotope-labelled internal standard using the mass transitions 72 → 55 (acrylamide) and 75 → 58 (D3-acrylamide).

A third mass transition 72 → 54 may be used for further confirmation of the results. The evaluation of this transition was not part of the inter-laboratory study (Annex A).

Table 1 — Mass spectrometric transitions used for the identification and quantification of acrylamide

Reference compound	Selected transitions for the identification and quantification of acrylamide using MS/MS modus m/z	
Acrylamide	72 → 55	Identification and quantification
	72 → 44	Identification (informative, qualifier)
D3-acrylamide	75 → 58	Identification and quantification
	75 → 44	Identification (informative, qualifier)