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Coffee and coffee products — Determination of acrylamide — Methods using HPLC-MS/MS and GC-MS after derivatization

Café et de ses dérivés — Dosage de l'acrylamide — Méthodes utilisant CLHP-MS/MS et CG-MS après dérivation

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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The committee responsible for this document is ISO/TC 34, *Food products*, Subcommittee SC 15, *Coffee*.

Introduction

When applying this document, all existing safety regulations have to be followed.

Coffee and coffee products — Determination of acrylamide — Methods using HPLC-MS/MS and GC-MS after derivatization

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 take appropriate measures for ensuring the safety and health of the personnel prior to application of this document and to fulfil statutory requirements for this purpose.

1 Scope

This document 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 are referred to in the text in such a way that some or all of their content constitutes requirements 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.

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

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

4 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 standard solutions are used.

5 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.

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

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

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

5.4 Acrylamide, C_3H_5NO, purity >98 %, reference substance.

5.4.1 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 3 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.

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

Using a pipette, transfer (1,0 ± 0,001) ml of the acrylamide stock solution (5.4.1) 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.

5.5 D3-acrylamide (acrylamide-2,3,3-d3) internal standard solution, $C_3H_2D_3NO$, purity >98 %, reference substance.

5.5.1 D3-acrylamide stock solution (internal standard solution).

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 3 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.

5.5.2 D3-acrylamide internal standard solution.

Using a pipette, transfer (1,0 ± 0,001) ml of the D3-acrylamide stock solution (5.5.1) 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.

NOTE 1 For HPLC-MS/MS, the solutions according to 5.4.1 to 5.5.2 can be prepared using the HPLC eluent as a solvent. The stability of these solutions depends on the mobile phase used and has to be validated.

When using GC-MS, all standard solutions according to 5.4.2 and 5.5.2 shall be subjected to the derivatization step according to 8.5.1.

NOTE 2 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.

5.6 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/cm^3).

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

5.7 Potassium bromide, KBr.

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

5.9 Triethylamine, $(C_2H_5)_3N$.

5.10 Sodium sulfate (anhydrous, granular), Na_2SO_4.

5.11 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.

5.12 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.

5.13 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.

6 Apparatus

Usual laboratory apparatus and, in particular, apparatus according to 6.1 to 6.14 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.

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

6.2 Coffee mill, suitable for grinding roasted coffee beans.

6.3 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).

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

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

6.6 Centrifuge tubes, of 15 ml and 50 ml.

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

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

6.9 Glass or polypropylene cartridges, with sorbents for the solid phase extraction (SPE), and for the clean-up of extracts in 8.3.2 and 8.5.1 (examples are given in Table B.1).

6.10 High performance liquid chromatograph (for the test procedure according to 8.4), equipped with ESI and mass spectrometric detector (HPLC-MS/MS); gas supply as specified by the manufacturer.

6.11 HPLC column (for the test procedure according to 8.4), suitable for acrylamide chromatography (examples are given in Table C.1).

6.12 Gas chromatograph (for the test procedure according to 8.5) with mass spectrometric detector (GC-MS) and operating gas supply (5.2) as specified by the manufacturer.

6.13 GC column, (for the test procedure according to 8.5) capillary column, suitable for acrylamide chromatography (examples are given in Table C.2).

6.14 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.

7 Sampling

Sampling is not part of the method specified in this document. The sampling procedure shall be subject to agreement by the interested parties. A representative, thoroughly mixed sample shall be used, which has not been damaged or adulterated during transport or storage.

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.

8 Procedure

8.1 General

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

8.2 Preparation of the sample extract

If necessary, grind the sample in a coffee mill (6.2) 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 (6.1) and transfer it into a 50 ml centrifuge tube (6.6).

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 solution 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 (6.4) for 15 min at approximately 40 °C.

Allow a few minutes for precipitation and in the case of non-sedimenting samples centrifuge (6.5) for 15 min at 2 000 *g* to separate suspended solids. Before liquid chromatography (8.4) or derivatization and gas chromatographic separation (8.5), take 10 ml from the lower aqueous phase and use it for a further clean-up according to 8.3. 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.

8.3 Clean-up of the extracts

8.3.1 Carrez precipitation

Clean-up the sample extract prepared according to 8.2 by Carrez precipitation. Add 1 000 µl of Carrez solution I (5.11) and shake. Add 1 000 µl of Carrez solution II (5.12) and shake again. After a short exposure time, centrifuge for 4 min at 2 000 *g*. Decant the supernatant, wash the residue with 2 ml to 3 ml of water, centrifuge and decant again. Combine both aqueous solutions.

8.3.2 Solid phase extraction

Clean-up the sample extract after Carrez precipitation (8.3.1) 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 (8.3.1) 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.

8.4 HPLC-MS/MS measurement

8.4.1 High-performance liquid chromatography (HPLC)

Prior to the HPLC-MS/MS analysis, add organic solvent to the cleaned-up extract (8.3.2) in order to make up the desired eluent composition and filter through a membrane filter (6.14) 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 8.3.1 and 8.3.2 are essential for the chromatographic separation of the analyte peaks from the interfering peaks. An example of chromatogram is given in Figures C.1 and C.2.