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Analysis of coffee and coffee products — Determination of acrylamide — Methods using HPLC-MS/MS and GC-MS after derivatisation

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

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ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

Contents

Page

Foreword v
Introduction vi
1 Scope 1
2 Normative references 1
3 Principle 1
4 Reagents 1
5 Apparatus 3
6 Sampling 4
7 Procedure 4
7.1 General 4
7.2 Preparation of the extract 4
7.3 Clean-up of the extracts 4
7.3.1 Carrez precipitation 4
7.3.2 Solid phase extraction 4
7.4 HPLC-MS/MS measurement 5
7.4.1 High performance liquid chromatography (HPLC) 5
7.4.2 Identification and quantification by mass spectrometry (HPLC-MS/MS) 5
7.5 Measurement with GC-MS 6
7.5.1 Derivatisation and sample preparation for gas chromatography 6
7.5.2 Gas chromatography 6
7.5.3 Identification and quantification by mass spectrometry 6
8 Calibration 7
8.1 General advice 7
8.2 Determination of linearity and definition of the working range 7
8.3 Calibration with internal standard 7
8.4 Determination of the laboratory specific recovery 7
9 Evaluation 7
9.1 Criteria for identification 7
9.2 Calculation and final results 7
10 Procedure characteristics 8
10.1 General 8
10.2 Repeatability, r 8
10.3 Reproducibility, R 8
10.4 Recovery 9
11 Measurement uncertainty 9
12 Test report 9
Annex A (informative) Performance characteristics 10
Annex B (informative) Examples of absorber materials 11
Annex C (informative) Examples for recommended columns and analysis conditions 12
C.1 Generally suitable chromatographic columns 12
C.2 Example method and approximate retention time for HPLC-MS/MS 13
C.3 Example of suitable conditions and approximate retention time for GC-MS analysis 13
C.4 Example chromatograms for HPLC-MS/MS 1
C.5 Example of chromatogram for GC-MS 1

Bibliography **3**

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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

ISO 18862 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 15, *Coffee*.

Introduction

This Standard was set up by the German Institute for Standardization (DIN), Agricultural products, subcommittee Food Analysis – vertical methods, subcommittee coffee

This standard can only be applied by experts. It is necessary to stick to existing safety regulations. Annexes A to C are informative.

Warning — Users of this standard must be familiar with practical work in laboratories. This standard does not point out all risks which might occur during the use of this method. It is the responsibility of the user to make sure that all safety precautions necessary are taken and that all national safety regulations are obeyed.

Analysis of coffee and coffee products — Determination of acrylamide — Methods using HPLC-MS/MS and GC-MS after derivatisation

1 Scope

This standard specifies methods for the determination of the acrylamide content in coffee and coffee products by means of extraction, clean-up by solid-phase extraction and determination by HPLC-MS/MS and GC-MS. It is applicable to roasted coffee, soluble coffee, coffee substitutes and coffee products.

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.

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

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, by 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 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 analytical instruments.

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 standard 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 (ρ = 1 000 µg/ml) 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 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 working solution for calibration, ρ **=** 10 µg/ml

Using a pipette, transfer (1,0 ± 0,001) ml of the acrylamide stock standard 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 standard solution (internal standard), the D3-acrylamide stock standard solution (internal standard) shall be prepared analogously to the acrylamide stock standard solution in 4.4.1.

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

4.5.2 D3-acrylamide working solution (internal standard), the working solution of the internal standard is prepared analogously to 4.4.2 from commercially available or self-prepared stock solutions (4.5.1).

NOTE 1 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 liquid phase 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 7.5.1.

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.

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/cm^3).

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

4.7 Potassium bromide, KBr, p.a.

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

4.9 Triethylamine, $(C_2H_5)_3N$, p.a.

4.10 Sodium sulfate (anhydrous, granular), Na_2SO_4, p.a.

4.11 Carrez solution I

Dissolve 10,6 g of potassium hexacyanoferrate trihydrate (II) $K_4[Fe(CN)_6] \cdot 3\,H_2O$ p.a. 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 $Zn(CH_3COO)_2 \cdot 2\,H_2O$ p.a. 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, nominal capacities of 15 ml and 50 ml.

5.8 One-mark volumetric flask, nominal capacities 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 7.3.2 and/or 7.5.1 (examples are given in Table B.1).

5.11 High performance liquid chromatograph (for the test procedure according to 7.4), 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 7.5) 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 7.5) 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 Sampling

Sampling is not part of the method specified in this standard. 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 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.

7 Procedure

7.1 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.

7.2 Preparation of the sample extract

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

Weigh 2 g of the homogenised 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 (7.4) or derivatization and gas chromatographic separation (7.5), take 10 ml from the lower aqueous phase and use it for a further clean-up according to 7.3. 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.

7.3 Clean-up of the extracts

7.3.1 Carrez precipitation

Clean-up the sample prepared according to 7.2 by Carrez precipitation. Add 1 000 µl Carrez solution I (4.11) and shake. Add 1 000 µl 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 and decant again. Combine both aqueous solutions.

7.3.2 Solid phase extraction

Clean-up the sample extract from the Carrez precipitation (7.3.1) by solid phase extraction (SPE) using two sequential cartridges with adsorber material (examples are given in Table B.1).The first cartridge contains

