



SLOVENSKI STANDARD
oSIST prEN ISO 18862:2024
01-oktober-2024

Kava in proizvodi iz kave - Določevanje akrilamida - Metode z uporabo HPLC-MS/MS in GC-MS po derivatizaciji (ISO/DIS 18862:2024)

Coffee and coffee products - Determination of acrylamide - Methods using HPLC-MS/MS and GC-MS after derivatization (ISO/DIS 18862:2024)

Kaffee und Kaffee-Erzeugnisse- Bestimmung von Acrylamid- Verfahren mittels HPLC-MS/MS und mittels GC-MS nach Derivatisierung (ISO/DIS 18862:2024)

Café et dérivés du café - Dosage de l'acrylamide - Méthodes par CLHP-SM/SM et CG-SM après dérivation (ISO/DIS 18862:2024)

Ta slovenski standard je istoveten z: prEN ISO 18862

oSIST prEN ISO 18862:2024

<https://standards.slovenski-institut.si/standards/sist/99/00/000/0000/18862-2024/0000-0002/0002/18862-2024>

ICS:

67.140.20 Kava in kavni nadomestki Coffee and coffee substitutes

oSIST prEN ISO 18862:2024

en,fr,de



DRAFT International Standard

ISO/DIS 18862

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

*Café et dérivés du café — Dosage de l'acrylamide — Méthodes
par CLHP-SM/SM et CG-SM après dérivation*

ICS: 67.140.20

ISO/TC 34/SC 15

Secretariat: **ICONTEC**

Voting begins on:
2024-08-16

Voting terminates on:
2024-11-08

iteh Standards
<https://standards.iteh.ai/>
Document Preview

[oSIST prEN ISO 18862:2024](https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8cb5-5ed215eba547/osist-pren-iso-18862-2024)

<https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8cb5-5ed215eba547/osist-pren-iso-18862-2024>

This document is circulated as received from the committee secretariat.

ISO/CEN PARALLEL PROCESSING

Reference number
ISO/DIS 18862:2024(en)

THIS DOCUMENT IS A DRAFT CIRCULATED FOR COMMENTS AND APPROVAL. IT IS THEREFORE SUBJECT TO CHANGE AND MAY NOT BE REFERRED TO AS AN INTERNATIONAL STANDARD UNTIL PUBLISHED AS SUCH.

IN ADDITION TO THEIR EVALUATION AS BEING ACCEPTABLE FOR INDUSTRIAL, TECHNOLOGICAL, COMMERCIAL AND USER PURPOSES, DRAFT INTERNATIONAL STANDARDS MAY ON OCCASION HAVE TO BE CONSIDERED IN THE LIGHT OF THEIR POTENTIAL TO BECOME STANDARDS TO WHICH REFERENCE MAY BE MADE IN NATIONAL REGULATIONS.

RECIPIENTS OF THIS DRAFT ARE INVITED TO SUBMIT, WITH THEIR COMMENTS, NOTIFICATION OF ANY RELEVANT PATENT RIGHTS OF WHICH THEY ARE AWARE AND TO PROVIDE SUPPORTING DOCUMENTATION.

© ISO 2024

ISO/DIS 18862:2024(en)

iTeh Standards (<https://standards.iteh.ai>) Document Preview

[oSIST prEN ISO 18862:2024](https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024)

<https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024>



COPYRIGHT PROTECTED DOCUMENT

© ISO 2024

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

ISO/DIS 18862:2024(en)

Contents

	Page
Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	1
5 Reagents	1
6 Apparatus	3
7 Sampling	4
8 Procedure	4
8.1 General.....	4
8.2 Preparation of the sample extract.....	4
8.3 Clean-up of the extracts.....	5
8.3.1 Carrez precipitation.....	5
8.3.2 Solid phase extraction.....	5
8.4 HPLC-MS/MS measurement.....	5
8.4.1 High-performance liquid chromatography (HPLC).....	5
8.4.2 Identification and quantification by mass spectrometry (HPLC-MS/MS).....	5
8.5 Measurement with GC-MS.....	6
8.5.1 Derivatization and sample preparation for gas chromatography.....	6
8.5.2 Gas chromatography.....	6
8.5.3 Identification and quantification by mass spectrometry.....	6
9 Calibration	7
9.1 General advice.....	7
9.2 Determination of linearity and definition of the working range.....	7
9.3 Calibration with internal standard solution.....	7
9.4 Determination of the laboratory specific recovery.....	7
10 Evaluation	7
10.1 Criteria for identification.....	7
10.2 Calculation and final results.....	8
11 Precision data	8
11.1 General.....	8
11.2 Repeatability.....	8
11.3 Reproducibility.....	8
11.4 Recovery.....	9
12 Measurement uncertainty	9
13 Test report	9
Annex A (informative) Performance characteristics	10
Annex B (informative) Examples of absorber materials	11
Annex C (informative) Examples of columns and analysis conditions	12
Annex D (Informative) Examples for sample preparation and chromatographic conditions using LC-MS/MS	18
Bibliography	25

ISO/DIS 18862:2024(en)

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.

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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

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

(<https://standards.iteh.ai>)
Document Preview

[oSIST prEN ISO 18862:2024](https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024)

<https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024>

ISO/DIS 18862:2024(en)

Introduction

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

iTeh Standards
(<https://standards.iteh.ai>)
Document Preview

[oSIST prEN ISO 18862:2024](https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024)

<https://standards.iteh.ai/catalog/standards/sist/956a9366-0d05-43d8-8eb5-5ed215eba547/osist-pren-iso-18862-2024>

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 <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://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.

ISO/DIS 18862:2024(en)

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 $\rho = 1\ 000\ \mu\text{g/ml}$.

Weigh $(0,10 \pm 0,001)$ 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 $\rho = 1\ 000\ \mu\text{g/ml}$ may be used. The information of the manufacturer regarding the stability of the solution shall be observed.

5.4.2 Acrylamide calibration solution, $\rho = 10\ \mu\text{g/ml}$.

Using a pipette, transfer $(1,0 \pm 0,001)$ 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 $(0,10 \pm 0,001)$ 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 $\rho = 1\ 000\ \mu\text{g/ml}$ 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 $(1,0 \pm 0,001)$ 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.

ISO/DIS 18862:2024(en)

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/cm³).

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), Na₂S₂O₃ · 5 H₂O.

5.9 Triethylamine, (C₂H₅)₃N.

5.10 Sodium sulfate (anhydrous, granular), Na₂SO₄.

5.11 Carrez solution I.

Dissolve 10,6 g of potassium hexacyanoferrate trihydrate (II) K₄[Fe(CN)₆] · 3 H₂O 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(CH₃COO)₂ · 2 H₂O 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 Na₂B₄O₇ 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.