FINAL
DRAFT

INTERNATIONAL
STANDARD

**ISO/FDIS
23637**

ISO/TC **34**/SC **4**

Secretariat: **SAC**

Voting begins on:
2021-09-29

Voting terminates on:
2021-11-24

Cereals — Determination of cadmium content by graphite furnace atomic absorption spectrometry with diluted nitric acid extraction

Reference number
ISO/FDIS 23637:2021(E)

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

Contents

Page

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 of the voluntary nature of standards, 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 www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 4, *Cereals and pulses*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Cereals — Determination of cadmium content by graphite furnace atomic absorption spectrometry with diluted nitric acid extraction

1 Scope

This document specifies a method for the determination of cadmium (Cd) in cereals.

It is applicable to rice, brown rice, wheat and maize by graphite furnace atomic absorption spectrometry (GFAAS) after extraction with diluted nitric acid (HNO_3). The limit of quantification is 0,002 mg/kg; it is approximate and dependent on the sample matrix as well as on the instrument conditions.

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:

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

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

4 Principle

Cadmium (Cd) is extracted from the cereals using diluted nitric acid and then determined by graphite furnace atomic absorption spectrometry (GFAAS).

5 Reagents and solution

During the analysis, unless otherwise stated, use only reagents of recognized analytical purity and only water of grade 1 in accordance with ISO 3696.

5.1 Nitric acid (HNO_3), not less than 65 % (mass fraction) of approximately $\rho(HNO_3)$ = 1,4 g/ml.

5.2 Nitric acid solution (0,5 %, volume fraction), mix 0,5 volume parts of HNO_3 (5.1) and 100 ml volume parts of water.

5.3 Nitric acid solution (50 %, volume fraction), mix HNO_3 (5.1) and water in equal volume.

5.4 Palladium nitrate hydrate ($Pd(NO_3)_2$, 99,9 % purity).

5.5 Palladium nitrate ($Pd(NO_3)_2$) solution, c($Pd(NO_3)_2$) = 100 mg/l, dissolve 0,1 g $Pd(NO_3)_2$ (5.4) and dilute to 1 000 ml with HNO_3 (5.2). Other matrix modifiers may also be used if their applicability is proven.

5.6 Cadmium standard solution.

5.6.1 Cadmium stock standard solution, with a cadmium mass concentration of 1 000 mg/l or 500 mg/l.

5.6.2 Cadmium standard solution, stepwise dilute cadmium stock solution (5.6.1) into concentration of 2 µg /l with HNO_3 (5.2).

5.6.3 Cadmium calibration solutions, pipette suitable volumes of cadmium standard solution (5.6.2), e.g. 2 ml, 4 ml, 6 ml, 8 ml and 10 ml, into, for example, a 10 ml volumetric flask (6.7) and dilute to the mark with diluted nitric acid solution (5.2). The concentration of cadmium in the calibration solutions should cover the range of 0,4 µg /l to 2,0 µg/l.

6 Apparatus and equipment

All glassware shall be cleaned several times with water after being soaked overnight with HNO_3 (5.3) and rinsed three times with ultrapure water before use.

6.1 Grinding mill, grinder suitable to obtain the particle sizes of 0,25 mm and 0,40 mm.

6.2 Atomic absorption spectrometer, equipped with graphite furnace, autosampler and background correction capability, such as Zeeman background correction.

6.3 Cadmium hollow cathode lamp, with wavelength 228,8 nm and stable lamp energy.

6.4 Centrifuge, with positions for 10 ml centrifuge tubes and 3 000 r/min of speed.

6.5 Analytical balance, accurate to 1 mg.

6.6 Sieve, with aperture sizes of 0,25 mm and 0,40 mm.

6.7 Plastic centrifuge tube, 10 ml.

7 Procedure

7.1 General

The measurement should be performed as soon as possible after extraction.

7.2 Sampling

A representative sample should be sent to the laboratory. It should not have been damaged or changed during transport and storage.

Sampling is not part of the method specified in this document. A recommended sampling method is given in ISO 24333.

7.3 Preparation of the test samples

Grind the laboratory sample using a grinding mill (6.1) until it passes through the sieve (6.6) and mix thoroughly.

The particle size of the maize and its milling products should be less than 0,25 mm. The particle size of other cereal varieties should be less than 0,40 mm.

7.4 Extraction

7.4.1 General

Weigh 0,200 g ± 0,001 g (*m*) of the sample flour into a 10 ml plastic centrifuge tube, and add 5,0 ml of diluted nitric acid (5.2). Close the centrifuge tube tightly and mix the content thoroughly to make sure that there are no remaining lumps. Afterwards, the extract is centrifuged at 3 000 r/min for 5 min, or let it stand for 5 min to get a supernatant for further determination.

7.4.2 Reagent blank

Prepare a blank solution in the same manner as the sample by transferring 5,0 ml of diluted nitric acid (5.2) into a 10 ml plastic centrifuge tube and following the same procedure as for the sample.

In cases of high cadmium concentration, take into consideration the further dilution with diluted nitric acid (5.2)

7.5 Sample analysis

7.5.1 General

Inspect the graphite furnace, the sample uptake system and the autosampler injector for any problems that can affect instrument performance. If necessary, clean the system and replace the graphite tube and/or platform.

7.5.2 Operating conditions

Cadmium is determined at 228,8 nm. An exemplary furnace programme is given in Table 1.

Prior to the use of this method, the instrument operating conditions shall be optimized. The analyst should follow the instructions provided by the manufacturer while using the reference conditions as a guide.

Table 1 — Furnace programme of atomic absorption spectrometry

Programme		Temperature	Ramp	Hold	Gas		Run
No	Step	°C	°C/s	s	Ar	Assist gas	s
1	Dry 1	70	3	10	Max.	Stop	
2	Dry 2	90	1	10	Max.	Stop	
3	Dry 3	110	2	2	Max.	Stop	
4	Pyrolysis	550	250	35	Max.	Max.	
5	Auto Zero	550	0	4	Stop	Stop	3,0
6	Atomize	1 100	1 500	3	Stop	Stop	
7	Cleanout	2 300	500	4	Max.	Max.	

7.5.3 Calibration graph

Prepare a calibration graph at the beginning of the analysis.

Inject at least four calibration solutions of different suitable concentrations (see 5.6.3). Plot the absorbance (peak height or peak area) of the cadmium calibration solutions (5.6.3) against the cadmium concentrations. Ensure that the linearity check is carried out[2].

7.5.4 Determination

The spectrum absorptions are obtained by detecting supernatant samples injected into the graphite furnace. The concentration of cadmium is determined from the regression equation by interpolating the spectrum absorption.

7.5.5 Application of matrix-modifier

If there is an obvious background absorption peak, 100 mg/l of $Pd(NO_3)_2$ solution(5.5) can be used as matrix-modified. Adding 5 µl of the $Pd(NO_3)_2$ solution (5.5) while injecting will eliminate the matrix interference during calibration curve plotting and the measuring of samples.

7.6 Analytical quality control

For the purpose of quality control, analyse the control sample (CRM/RM) in every analytical run including all the steps in the method.

8 Calculation

Calculate the mass fraction X of cadmium in mg/kg using Formula (1) (external standard method):

$$X = \frac{(C_1 - C_2) \times V \times N}{m \times 1\,000} \quad (1)$$

where

C_1 is the concentration of cadmium in final solution used for GFAAS determination, in µg/l;

C_2 is the concentration of cadmium in the blank solution, in µg /l;

V is the volume of the nitric acid solution used for the extraction, in ml;

N is the dilution factor;, in cases of high cadmium concentrations, take into consideration the further dilutions;

m is the mass of the test portion used for extraction, in g;

Express the result to two significant figures.

Indicate whether or not a correction for recovery has been applied.

9 Precision

9.1 General

Details of the interlaboratory test of the precision of the method in accordance with ISO 5725:1986[1)] are summarized in Annex A. It is possible that the values derived from the interlaboratory tests are not applicable to analyte concentration ranges and matrices other than those given in Annex A.

1) Cancelled and replaced by the ISO 5725 series.

9.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will not be greater than the repeatability limit, *r*, given in Formula (2) in more than 5 % of cases:

$$r = 2{,}77\ s_r = 2{,}77\ (0{,}020\ 1x + 0{,}002\ 7) \tag{2}$$

where

s_r is the repeatability standard deviation;

x is the concentration of cadmium (mg/kg).

9.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will not be greater than the reproducibility limit, *R*, given in Formula (3) in more than 5 % of cases:

$$R = 2{,}77\ s_R = 2{,}77\ (0{,}111\ 1x - 0{,}000\ 03) \tag{3}$$

where

s_R is the reproducibility standard deviation;

x is the concentration of cadmium (mg/kg).

9.4 Critical difference, *D*

9.4.1 General

The critical difference is the difference between two averaged values obtained from two test results under repeatability conditions.

9.4.2 Comparison of two groups of measurements in one laboratory

The critical difference (D_r) between two averaged values obtained in one laboratory from two test results under repeatability conditions is shown by Formula (4):

$$D_r = 2{,}77 s_r \sqrt{\frac{1}{2n_1} + \frac{1}{2n_2}} = 2{,}77 s_r \sqrt{\frac{1}{2}} = 1{,}98 s_r \tag{4}$$

where

s_r is the standard deviation of repeatability;

n_1 and n_2 are the number of test results corresponding to each of the averaged values ($n_1 = n_2 = 2$).

9.4.3 Comparison of two groups of measurements in two laboratories

The critical difference (D_R) between two averaged values obtained in one laboratory from two test results under repeatability conditions is shown by Formula (5):

$$D_R = 2{,}77\sqrt{s_R^2 - s_r^2\left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2{,}77\sqrt{s_R^2 - 0{,}5s_r^2} \quad (5)$$

where

s_r is the standard deviation of repeatability;

s_R is the standard deviation of reproducibility;

n_1 and n_2 are the number of test results corresponding to each of the averaged values ($n_1 = n_2 = 2$).

10 Test report

The test report shall contain at least the following:

— all the information necessary for the identification of the sample;

— the results and the units in which the results have been expressed;

— a reference to this document, i.e. ISO 23637;

— the date and type of sampling (if known);

— the date of receipt of the laboratory sample;

— the date of the test;

— any particular points observed in the course of the test;

— any operations not specified in the method or regarded as optional which might have affected the results.