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Foodstuffs – Determination of elements and their chemical species – Determination of aluminium by inductively coupled plasma optical emission spectrometry (ICP-OES)

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Produits alimentaires - Dosage des éléments et de leurs espèces chimiques - Dosage de l'aluminium par spectrométrie d'émission optique avec plasma à couplage inductif (ICP-OES)

Lebensmittel - Bestimmung von Elementen und ihren Verbindungen - Bestimmung von Aluminium mittels optischer Emissionsspektrometrie mit induktiv gekoppeltem Plasma (ICP-OES)

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European foreword

This document (EN 17265:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2020, and conflicting national standards shall be withdrawn at the latest by March 2020.

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1 Scope

This document specifies a method for the determination of aluminium in food by inductively coupled plasma optical emission spectrometry (ICP-OES) after pressure digestion. This method was validated for wheat noodle, cheese, liver, beetroot and cocoa powder at mass fractions in the range of 15 mg/kg to 200 mg/kg. At concentrations above 200 mg/kg digestion temperatures higher than 220 °C can be necessary to recover the aluminium as completely as possible.

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.

EN 13804, *Foodstuffs - Determination of elements and their chemical species - General considerations and specific requirements*

EN 13805, *Foodstuffs - Determination of trace elements - Pressure digestion*

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

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 <https://www.iso.org/obp>

4 Principle

Aluminium is determined quantitatively by ICP-OES after digestion of the sample with nitric acid (with addition of water in case of foods with low water content), according to the pressure digestion process described in EN 13805 but without the use of hydrofluoric acid. The digestion conditions are chosen in such a way that even for samples with aluminium compounds of low solubility (e.g. silicates, oxides) low findings are kept to a minimum.

5 Reagents

The mass concentration of aluminium shall be low enough in the reagents and water not to affect the results.

All reagents shall be of analytical grade, i.e. pro analysi, p.a. or similar unless otherwise specified.

Use water conforming to grade 2 of EN ISO 3696.

5.1 Nitric acid, mass fraction w = at least 65 %, density = 1,4 g/ml.

5.2 Aluminium stock solution, mass concentration ρ = 1 000 mg/l.

5.3 Scandium stock solution, $\rho = 1\ 000\ \text{mg/l}$ as internal standard.

Scandium is mentioned as an example of an internal standard. Yttrium and Ytterbium are suitable as well. Only a negligible quantity of the internal standard may be present in the sample. Wavelengths of Scandium, Yttrium and Ytterbium can be found in the manufacturer requirements of the ICP-OES.

5.4 Standard solutions

5.4.1 General

When preparing solutions, special care shall be taken to avoid contaminations. For aluminium there are various sources of contamination, e.g. volumetric flasks made of glass. Annex B of this document requires special attention.

5.4.2 Aluminium standard solution, $\rho = 10\ \text{mg/l}$.

Fill 10 ml of water into a 50-ml volumetric flask, add 2 ml of nitric acid (5.1) and mix. After cooling down to room temperature, pipette exactly 500 μl of the aluminium stock solution (5.2) to the flask and fill up with water to the mark. This standard solution is stable for at least 3 months.

5.4.3 Scandium standard solution (internal standard solution), $\rho = 10\ \text{mg/l}$.

Fill approximately 10 ml of water into a 50-ml volumetric flask, add 2 ml of nitric acid (5.1) and mix. After cooling down to room temperature, pipette exactly 500 μl of the scandium stock solution (5.3) to the flask and fill up with water to the mark. This standard solution is stable for at least 3 months.

5.5 Aluminium calibration solutions.

The concentrations of the calibration solutions described are examples and may be changed according to the sensitivity of the measuring instrument and the concentration range to be analysed. Carry out the calibration within the linear range of the detector system. At least 3 calibration solutions of different concentrations should be prepared. Make sure that the acid concentration of the calibration solutions corresponds to the test solution.

In order to avoid contaminations originating from the flask material, do not use volumetric flasks made from borosilicate glass. The information on contaminations provided in B.1 is to be regarded.

Mix all calibration and test solutions as well as the zero-point solution (5.6) with an equal proportion of internal standard solution (5.4.3).

The calibration solutions are prepared from the standard solution (5.4.2) according to the following scheme in Table 1:

Table 1 — Example of aluminium calibration solutions

Calibration solution no	Volume of standard solution (5.4.2) μl	Volume of internal standard solution (5.4.3) μl	Mass concentration of aluminium in the calibration solution mg/l
1	100	2 000	0,05
2	200	2 000	0,10
3	500	2 000	0,25
4	1 000	2 000	0,50
5	2 000	2 000	1,00

EXAMPLE Fill 4 ml to 5 ml of water into a 20-ml volumetric flask, add 1,25 ml of nitric acid (5.1) and mix. After cooling down to room temperature, pipette standard solution and internal standard solution according to Table 1 above to the flask, fill up to the mark with water, and mix.

Alternatively the internal standard solution can be pumped into the sample flow via a Y-piece during measurement. In this case the internal standard solution is not pipetted into the calibration solutions.

NOTE The acid concentration of the calibration solution in the example is adapted to a digestion with 2,5 ml of nitric acid (5.1), a filling volume of 20 ml, and a dilution factor of 2 (in case of a dilution with water).

The calibration solutions shall be freshly prepared each working day.

5.6 Zero-point solution

20 ml of zero-point solution contain water, 1,25 ml of nitric acid (5.1) and 2 000 µl internal standard solution (5.4.3) according to the example given in 5.5.

6 Apparatus

All equipment and labware that come into direct contact with the sample and the solutions used shall be carefully pretreated/cleaned according to EN 13804 to minimize the blank value (see Annex B for details). In addition to standard laboratory equipment, use the following:

6.1 Inductively coupled plasma optical emission spectrometer (ICP-OES).

Optical emission spectrometer with inductively coupled argon plasma, sample supply and atomization system as well as device control and data acquisition. The instrument settings shall allow the measurement of the emission intensities on both sides of the emission line and enable a spectral background correction on both sides [1] [2].

6.2 Digestion vessels, e.g. of polyfluoropolymers or quartz with volumes from 70 ml to 100 ml.

6.3 Test tube shaker, optional.

6.4 Analytical balance, capable to weigh to the nearest milligram.

7 Procedure

7.1 Digestion

7.1.1 General

To ensure complete dissolution of aluminium the following shall be respected:

- ensure that the sample is sufficiently homogeneous;
- in case of incomplete digestion or high aluminium contents, it may be beneficial to use a test portion as small as possible (at least 200 mg for dry samples) in order to digest aluminium compounds as completely as possible;
- for samples containing silicates, digestion temperatures above 220 °C may be necessary in order to dissolve the aluminium as completely as possible;
- in case of samples with low water content, first add water and mix intensively, before adding concentrated nitric acid;
- do not use hydrogen peroxide for digestion.

Further instructions regarding test portions and digestion are given in B.2 and B.3.

For pressure digestion according to EN 13805, different vessels may be used depending on the instrument type and manufacturer. The maximum test portion and the minimum liquid volume depend on the pressure stability of the respective vessels. The specifications according to 7.1.2 refer to digestion vessels with volumes from 70 ml to 100 ml and a minimum liquid volume of 5 ml.

All indications in 7.1.2 shall be adjusted to the digestion instrument used. For safety reasons the manufacturer's specifications shall be strictly followed.

7.1.2 Digestion procedure

Before digestion, different amounts of water, depending on the different types of food [3], are added in order to obtain comparable acid concentrations in the final digestion solution. The amount of water to be added depends on the test portion and thus on the content of carbon and water in the food type.

Add just as much water to the test portion that is necessary to suspend the food completely. Then complete the test portion with water to reach 3 g. Water is also added to fat-containing foods, even if a suspension is hardly possible.

Example for digestion of powdery food: Add 2,5 ml of water to the dry food, e.g. 0,5 g of flour. Mix the contents of the digestion vessel intensively, e.g. on a test-tube shaker. Repeat shaking every 10 min to 15 min until the suspension is homogeneous. No agglutinated particles shall remain, and the sample material shall be welled. Fat-containing samples require more time and repeated shaking. After at least 30 min, add 2,5 ml of nitric acid (5.1) to the sample. Mix thoroughly again to obtain a homogeneous suspension. After the pre-reaction has subsided, close the digestion vessel, and start the digestion.

Example for digestion of a sample with high water content, e.g. lettuce: Directly add 2,5 ml of nitric acid (5.1) to 3 g of lettuce and mix thoroughly. It is not necessary to add water. After the pre-reaction has subsided, close the digestion vessel, and start the digestion.

A digestion temperature of at least 200 °C to be kept for at least 20 min is required for a complete digestion in the microwave.

The digestion conditions depend on the manufacturer's specifications, the reactivity of the sample, the maximum pressure stability of the digestion vessel, and the attainable temperature. To monitor the laboratory-specific digestion conditions, it is recommended to carry out measurements with reference materials containing certified contents [4].

If precipitations or turbidities are visible in the digestion solutions, low findings can occur. In these cases, use smaller test portions and higher digestion temperatures up to 300 °C in order to minimize or avoid possible low findings.

NOTE Turbidities or small amounts of precipitates adhering to the wall or bottom of the vessel are only visible in vessels with high transparency (e.g. aluminium-free quartz vessels). Plastic vessels are often not completely transparent, making precipitates difficult to discern.

Fill up the digestion solution obtained by pressure digestion to a defined volume, e.g. 20 ml. Add the internal standard (5.4.3) (e.g. 1 000 µl) to an aliquot of the digestion solution, e.g. 5 ml, and fill up with water to 10 ml. Separate insoluble residues before measurement. If the digestion solution has a different final volume than 20 ml and a different dilution factor than 2 after addition of the internal standard, adapt the acid concentration of the calibration solutions respectively. All test solutions shall have approximately the same acid concentration as the calibration solutions. The digestion solution shall be measured within 2 weeks.

Matrix effects can occur in digestion solutions. It is recommended to dilute the digestion solution by a factor of 2 to reduce matrix effects and avoid contamination of instruments.

In order to determine low aluminium contents reliably, choose a small filling-up volume after digestion. During sample preparation special care should be taken to avoid contaminations. See also explanation notes in Annex B.

7.2 Inductively coupled plasma optical emission spectrometry

7.2.1 ICP-OES operating conditions

Set the instrument according to the manufacturer's specifications and ignite the plasma. After sufficient warming-up and stabilization of the instrument, optimize the settings.

7.2.2 Determination by ICP-OES

Once the instrument is optimized, start the measurement. For the determination of aluminium a wavelength of 396,15 nm is recommended. It is necessary to measure the emission intensity of the spectral background on both sides of the emission line and to subtract it from the intensity measured at a wavelength of 396,15 nm (spectral background correction on both sides).

Alternatively use the emission lines from Table C.1, which show different sensitivities, but also more interferences than the wavelength recommended.

Measure the zero-point solution (5.6) and the calibration solutions 1 to 5 (5.5, Table 1) and generate a calibration curve from the emission intensities and concentrations. In case of complex matrices, it can be of advantage to perform a standard addition.

Aspirate and measure the sample test solution. Determine the emission intensity and correct it by the background intensity, then convert it into concentration units with the help of the calibration curve.

Check the stability of the emission intensities of the calibration samples by measuring a control sample at sufficiently frequent intervals (e.g. after 10 samples). If necessary, re-calibrate the system.

In case of samples with high element concentrations, sufficient rinsing is necessary before analysing the next test solution. The rinsing behaviour can be monitored with the help of the zero-point solution (5.6).

When preparing dilutions, make sure that the diluted test solutions have the same concentration of acids as the original test solutions.

7.2.3 Quality control

For quality control purposes, analyse control samples with reliably known contents of aluminium (see B.4) in parallel to every series of measurement. Include all steps of the procedure, beginning with the digestion. Prepare and measure blank solutions for every digestion series, also including all steps of the procedure.

8 Evaluation

8.1 Calculation

Calculate the mass fraction w in milligrams per kilogram according to Formula (1) or the mass concentration, ρ , in milligrams per litre of sample according to Formula (2):

$$w = \frac{a \cdot V \cdot F}{m} \quad (1)$$

where

a is the mass fraction of aluminium in the sample solution in micrograms per litre;

V is the volume of the sample solution after digestion in millilitres;

F is the dilution factor of the sample measuring solution;

m is the mass of the test portion in milligrams used for digestion.

$$\rho = \frac{a \cdot V \cdot F}{v} \quad (2)$$

where

a is the mass fraction of aluminium in the sample solution in micrograms per litre;

V is the volume of the sample solution after digestion in millilitres;

F is the dilution factor of the sample measuring solution;

v is the sample volume in microlitres used for digestion.

It is not recommended to subtract the blank value. The aluminium content of the blank solution should be low enough not to considerably change the test result when subtracted. If this is not the case, determine the origin of the aluminium content in the blank solution, and if necessary, repeat the digestion series.

8.2 Precision

Details of the interlaboratory test of the precision of the methods are summarized in Annex A. The values derived from this test may not be applicable to analyte concentration ranges and matrices other than given in Annex A.

This method was validated in an interlaboratory study (in 2014) with 11 participating laboratories. Altogether the aluminium content was determined in eight samples of different contents of aluminium - partly as natural and partly as added content, given in Table 2.

8.3 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 time interval, will in no more than 5 % of the cases exceed the values of repeatability r given in Table 2.

8.4 Reproducibility

The absolute difference between two single test results obtained using the same method on identical test material in different laboratories by different operators using the equivalent equipment will in no more than 5 % of the cases exceed the values of reproducibility R given in Table 2.

Table 2 — Validation data

Sample	Mean value \bar{x} mg/kg	Repeatability r mg/kg	Reproducibility R mg/kg
Infant formula	1,38 ± 0,48	0,37	1,7
Wheat noodle 1	18,2 ± 1,5	2,6	7,3
Wheat noodle 2	18,7 ± 1,4	1,7	6,6
Cheese	31,0 ± 1,3	2,5	6,3
Liver	38,5 ± 1,6	2,9	7,6
Beetroot	170 ± 11	11	49
Cocoa powder	210 ± 11	11	51
Herbs	586 ± 86	45	383

9 Test report

The test report should fulfil the requirements in EN ISO/IEC 17025 and shall specify at least the following:

- a) all information necessary for the complete identification of the sample;
- b) the test method used with reference to this European Standard;
- c) the results obtained and the units in which they are specified;
- d) the date of the sampling procedure (if known);
- e) the date when the analyses was finished;
- f) all operating details not specified in this document or regarded as optional, together with details of any incidents occurred when performing the method which might have influenced the test results.

Annex A (informative)

Results of interlaboratory study

This method was elaborated by working group “Elementanalytik” of the Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit — BVL) according to the German Food and Feed Act, Paragraph 64. It was tested in an interlaboratory study with a total number of 11 participants from four European countries. Each quantitative value of a laboratory results from a double determination or, in the case of wheat noodle (double blind), from a fourfold determination. All materials used in the interlaboratory study have been dry materials. Four materials were reference materials characterized by former proficiency tests (dried low fat fresh cheese, lyophilized pig liver, beetroot powder and cereal infant formula (FAPAS T07123)). The herbs are a certified reference material (Mixed Polish Herbs INCT-MPH-1). The wheat noodle and the cocoa powder (slightly defatted) are commercial available products. The plant materials beetroot, herbs and cocoa powder had an aluminium content of natural origin. In some cases, the digestion solutions of these materials showed fine precipitates. The animal materials cheese and liver had artificial aluminium contents. The origin of the aluminium in the infant formula and wheat noodles is unknown.

The statistical analysis of data was done in accordance with ISO 5725-3 [5].

The results are given in Table A.1, see also Figure B.1.

Table A.1 — Validation data

Statistical parameter	Infant formula	Wheat noodle 1 ^a	Wheat noodle 2 ^a	Cheese	Liver	Beetroot	Cocoa powder	Herbs
Number of participating laboratories	11	11	11	11	11	11	11	11
Number of laboratories with quantitative values	7	11	11	11	11	11	11	11
Number of outlier laboratories	1	0	0	0	1	1	0	1
Number of laboratories after elimination of outliers	6	11	11	11	10	10	11	10
Reference value ± expanded uncertainty U ($k = 2$), mg/kg	0,972 (U not available)	-	-	30,25 ± 1,09	39,21 ± 1,43	184,4 ± 9,0	-	670 ± 111
Mean value \bar{x}	1,38 ± 0,48	18,2 ± 1,5	18,7 ± 1,4	31,0 ± 1,3	38,5 ± 1,6	170 ± 11	210 ± 11	586 ± 86
95 % confidence interval, mg/kg								
Repeatability limit r , mg/kg	0,37	2,6	1,7	2,5	2,9	11	11	45
Repeatability standard deviation s_r , mg/kg	0,13	0,9	0,6	0,9	1,0	4	4	16
Relative repeatability standard deviation (RSD) $s_{r,rel}$	9,7 %	5,1 %	3,2 %	2,9 %	2,7 %	2,2 %	1,9 %	2,7 %
Reproducibility limit R , mg/kg	1,7	7,3	6,6	6,3	7,6	49	51	383
Reproducibility standard deviation s_R , mg/kg	0,60	2,6	2,4	2,3	2,7	18	18	137
Relative reproducibility standard deviation (RSD) $s_{R,rel}$	43 %	14 %	13 %	7,3 %	7,0 %	10 %	8,7 %	23 %
HorRat (Horwitz ratio)	2,8 ^b	1,4	1,2	0,8	0,8	1,4	1,2	3,8 ^b

^a Identical material.

^b The HorRat values are higher than the usually accepted value of 2, however these values are given for information.

Annex B (normative)

Explanation notes

B.1 Information on contaminations

Due to the frequent use of the material aluminium and the partly high concentrations present in food, special attention has to be paid to the danger of contaminations and blank values in all steps of the procedure.

Dust from the air is a frequent source of contamination with aluminium. Working under dust-free or low-dust conditions minimizes the risk of contamination with aluminium. When containers and solutions have to be left open, they should be covered with plastic film or exposed to filtrated air only.

If volumetric flasks are used, they shall not release any aluminium into the solutions they contain. In general it is recommended to use volumetric flasks made of plastic or aluminium-free silica glass. Depending on quality and batch, also pipette tips can contribute to the contamination of solutions [6].

Plastic tubes used for sample introduction with peristaltic pumps could have been treated with substances containing aluminium in order to prevent the tubes from gluing together.

In aged polyfluoropolymer or silica digestion vessels, poorly soluble aluminium-containing deposits can form, can lead to elevated aluminium blank values or higher findings in the samples. It is indispensable that digestion vessels are cleaned carefully before use. It is recommended to strip out the vessels with acid (see EN 13805), or, in case of fluoroplastics use hydrofluoric acid. Due to the low solubility of aluminium-containing residues, the cleaning can only help to a limited extent.

Visible deposits in silica vessels can be removed by treatment with approximately 3 % hydrofluoric acid (e.g. 70 ml of water + 20 ml of nitric acid (5.1) + 7 ml of 40 % hydrofluoric acid) in an ultrasonic bath for 1 min to 2 min.

In general, it is recommended not to carry out digestions of materials containing high contents of aluminium (e.g. feeding stuffs, cosmetics) in digestion vessels used for food analysis.

B.2 Information on test portions

It is recommended to choose the smallest possible test portion depending on the homogeneity of the prepared sample. The smallest test portion for powdery samples is usually around 0,2 g. Larger test portions can lead to low findings in samples in which the aluminium is difficult to dissolve. Test portions of more than 0,5 g should be avoided for powdery materials. If the aluminium content is not influenced by the size of the different test portions, there are no critical problems regarding the solubility of the aluminium compounds present in these samples.

Foods of animal origin usually have low to very low aluminium contents, which are, in general, easily soluble. In contrast, foods of plant origin can partly contain very high aluminium contents, and poorly soluble aluminium compounds (presumably aluminium oxides and silicates) can occur.

B.3 Information on digestion

Digestion temperatures of 200 °C or higher are recommended to keep the remaining carbon contents in the digestion solutions as low as possible.

In case of poorly soluble aluminium contents, more aluminium can be transferred into a soluble form by digesting at higher temperatures. Digestion temperatures of up to 300 °C may be necessary to obtain results which are comparable to digestions performed with hydrofluoric acid. Figure B.1 presents results for the determination of aluminium in the tested herbs by ICP-OES together with the digestion temperatures in degrees centigrade reported by the laboratories.

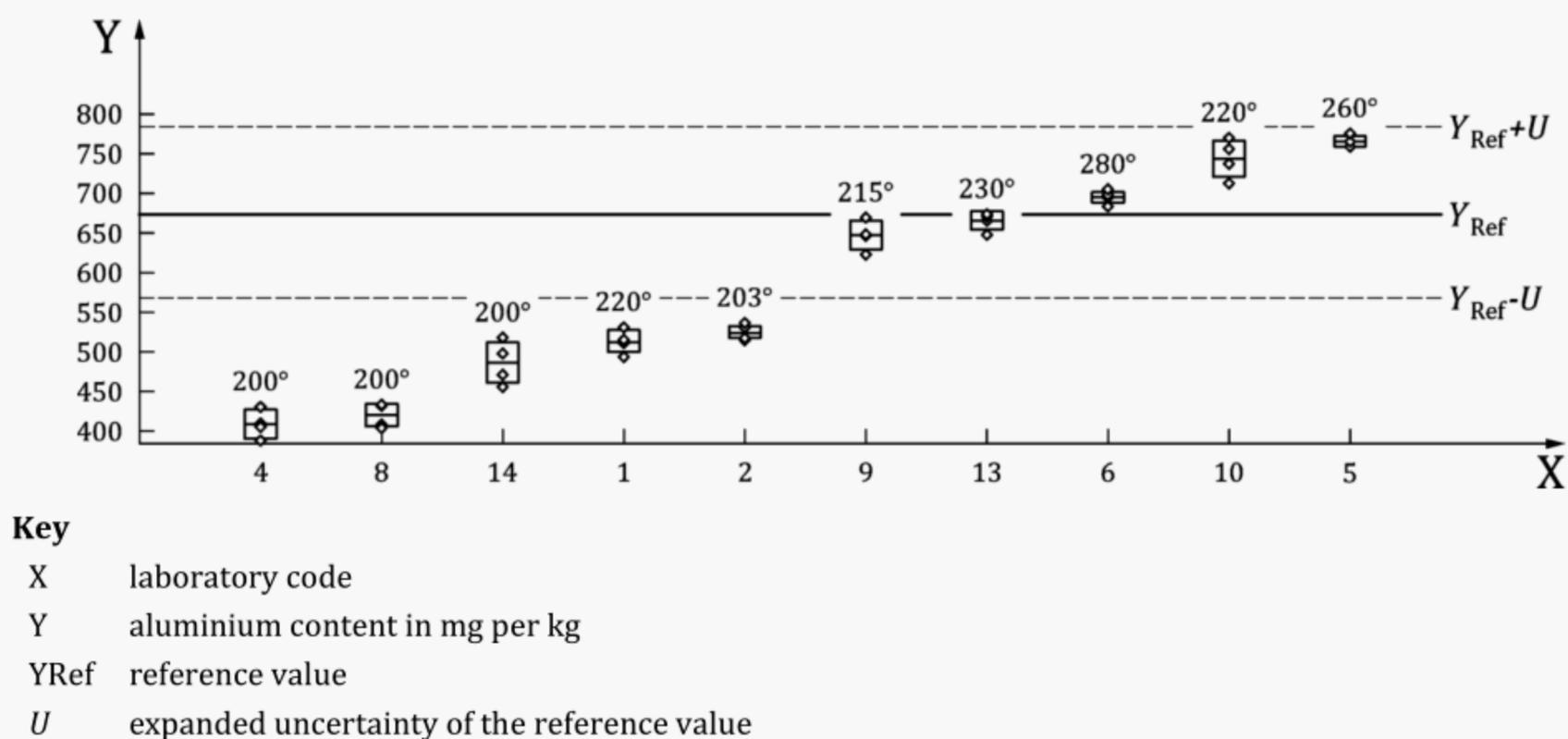


Figure B.1 — Results for the determination of aluminium in the tested herbs by ICP-OES

Only nitric acid is used to mineralise the sample. The use of both hydrofluoric acid and hydrogen peroxide is dispensable. In most cases, nitric acid is sufficient to determine the complete aluminium content. If there are poorly soluble aluminium compounds present in the samples, lower findings can occur.

B.4 Reference materials

The digestion of silicate containing samples has to be checked by digesting and measuring reference material with a certified value.

It is recommended to use a certified reference material, which is comparable to matrix and concentration range of the sample and shows a low uncertainty interval.

Examples of suitable reference materials are listed in Table B.1.

Table B.1 — Suitable reference materials

Name	Matrix	Certified aluminium content in dry mass mg/kg
NIST SRM 1570a	Spinach leaves	310 ± 11
NIST SRM 1547	Peach leaves	249 ± 8
NIST SRM 1566b	Oyster tissue	197 ± 6
NIST SRM 1573a	Tomato leaves	598 ± 12

Annex C (normative)

Spectral interferences

Verification of line interferences was performed during the pre-trials using an echelle spectrometer and axially viewed plasma (Varian Vista¹⁾) as well as a Paschen-Runge spectrometer and radially viewed plasma (Spectro CIROS CCD¹⁾), with the resolutions (half widths) listed in the table below.

Line interferences are generally influenced by the resolution and the background correction of the spectrometer used. Therefore, they always have to be checked before analysis. Table C.1 shows the extents of the most common spectral interferences.

Table C.1 — Potential spectral interferences

Wavelength nm	Interfering element	Concentration of the interfering element mg/l	Apparent concentration mg/l	Measured half width of the emission line nm
396,15	Cu	50	< 0,01	0,018
	Fe	10	< 0,01	
	Mo	10	> 0,1	
237,31	Co	50	< 0,05	0,010 to 0,018
	Cr	50	< 0,01	
	Mn	50	< 0,2	

The emission line at 167,08 nm is not recommended due to the interference caused by iron.

1) Varian Vista and Spectro CIROS CCD are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

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