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Foodstuffs - Determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc by ICP-OES

National foreword

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Foodstuffs - Determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc by ICP-OES

Produits alimentaires - Dosage du calcium, du cuivre, du fer, du magnésium, du manganèse, du phosphore, du potassium, du sodium, du soufre et du zinc par ICP-OES

Lebensmittel - Bestimmung von Calcium, Kupfer, Eisen, Magnesium, Mangan, Phosphor, Kalium, Natrium, Schwefel und Zink mit ICP OES

This European Standard was approved by CEN on 20 February 2017.

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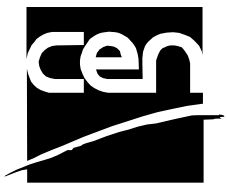

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Contents

Page

European foreword

This document (EN 16943:2017) 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of an identical text or by endorsement, at the latest by November 2017, and conflicting national standards shall be withdrawn at the latest by November 2017.

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1 Scope

This European Standard describes a method for the determination of calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc in different foodstuffs using optical emission spectrometry with inductively coupled plasma (ICP-OES) after pressure digestion.

This method has been validated in an interlaboratory study according to ISO 5725 [1] on infant formula soya based, cheese, chicken meat, wheat flour, apple juice, lobster and milk (see elements ranges Table 1 and validation data in Annex B).

HorRat values greater than 2 have been observed for certain analyte/matrix combinations during the validation study.

Table 1 — Validated element ranges

Element	**Range** mg/kg
Calcium	70 to 7178
Copper	0,60 to 16,40
Iron	0,88 to 77
Magnesium	45 to 1 174
Manganese	0,44 to 5,12
Phosphorus	72 to 9 708
Potassium	605 to 14 312
Sodium	11 to 2 220
Sulfur	26 to 8 542
Zinc	0,16 to 43,5

At European or International level, vertical standards for the determination of specific minerals can exist, e.g. for milk and milk products or for animal and vegetable fats and oils [2].

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*

3 Principle

After digestion of the sample with the pressure digestion process described in EN 13805, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc are determined quantitatively with the ICP-OES [2]. The digestion solution is nebulized, the aerosol is directed into an inductively coupled argon plasma, where the elements are atomized and excited for radiation. The emission radiation is resolved spectrally and its intensity determined at element-specific wavelengths with a detector system. Ionization interference can be minimized using an ionization buffer.

4 Reagents

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

4.1 Nitric acid, minimum w = 65 % [1], density about 1,4 g/ml

4.2 Hydrochloric acid, w = 30 %, density = 1,15 g/ml

4.3 Hydrogen peroxide, w = 30 %

Hydrogen peroxide (4.3) can contain phosphoric acid as a stabilizing agent. Hydrogen peroxide stabilized with phosphoric acid contaminates the samples with phosphorus and thus leads to incorrect results.

4.4 Caesium chloride solution as ionization buffer, e.g. w = 10 % (see 6.2.2)

4.5 Internal standard, e.g. scandium, ytterbium, yttrium

Depending on the matrix, e.g. for samples with high total salt content, the application of an internal standard can be advantageous in order to reduce physical interference. In doing so, it shall be ensured that no faults from line interference result due to the internal standard.

NOTE It has been shown that if an internal standard is used, it is applicable to all elements mentioned in the scope.

4.6 Stock solutions, ρ = 1 000 mg/l [2] per element

Calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur and zinc individual element stock solutions and internal standard used.

The individual element stock solutions may not contain any of the elements to be determined here as accompanying substances or impurities, which could affect the correctness of the results.

Alternatively, commercial mixed stock solutions can also be used.

4.7 Standard solutions, ρ = 100 mg/l per element

Copper, iron, manganese and zinc standard solution, produced by dilution of the respective stock solution (4.6).

Pour 10 ml of water into a 50-ml volumetric flask, add 2 ml of nitric acid (4.1) and mix. After cooling to room temperature, add exactly 5 ml respectively of the copper, iron, manganese and zinc stock solution (4.6) by pipette and fill up with water to the mark. These standard solutions are stable for at least six months.

4.8 Element reference solutions

4.8.1 General

The concentrations of the reference solutions indicated in 4.8.3 to 4.8.6 serve as examples and shall be modified corresponding to the instrument sensitivity, the direction of viewing (axial or radial) and the concentration range to be examined, if necessary. If possible, the calibration shall be performed in the

[1] w = mass fraction

[2] ρ = mass concentration

linear range. Minimum 3, in the nonlinear range 5, reference solutions of different concentrations should be prepared for the calibration. The concentration of the reagents (acids, if applicable ionization buffer and internal standard) shall correspond to the sample measuring solution.

For reasons of stability and purity, the phosphorus and sulfur reference solutions should be prepared separately from the multi-element reference solutions of the cations.

4.8.2 Preparation of the flask

Pour 10 ml to 20 ml of water into a 100-ml volumetric flask, add 10 ml of nitric acid (4.1) and 2 ml of hydrochloric acid (4.2) and mix. After cooling to room temperature, add stock (4.6) and standard solutions (4.7) by pipette. If using an ionization buffer, add 10 ml of 10 % caesium chloride solution (4.4). If using an internal standard (4.5), add the corresponding amount. Alternatively, the ionization buffer and/or the internal standard can be pumped via a Y-piece into the sample flow during the measurement.

These reference solutions are stable for a month.

The following data under 4.8.3 to 4.8.6 serve as examples and shall be adapted corresponding to the device sensitivity and the emission wavelength selected, and the direction of viewing (axial or radial), if necessary.

4.8.3 Reference solution 1

Pipette the following amounts of stock solutions (4.6) into the prepared 100-ml volumetric flask (4.8.2):

— 2,0 ml calcium →[3] ρ = 20 mg/l calcium;

— 1,0 ml magnesium → ρ = 10 mg/l magnesium;

— 2,0 ml potassium → ρ = 20 mg/l potassium;

— 1,0 ml sodium → ρ = 10 mg/l sodium;

Furthermore, pipette the following amounts of standard solutions (4.7) into the same flask and fill up to the mark with water:

— 0,50 ml copper → ρ = 0,5 mg/l copper;

— 0,50 ml iron → ρ = 0,5 mg/l iron;

— 0,50 ml manganese → ρ = 0,5 mg/l manganese;

— 0,50 ml zinc → ρ = 0,5 mg/l zinc.

4.8.4 Reference solution 2

Pipette the following amounts of stock solutions (4.6) into the prepared 100-ml volumetric flask (4.8.2):

— 10,0 ml calcium → ρ = 100 mg/l calcium;

— 5,0 ml magnesium → ρ = 50 mg/l magnesium;

— 10,0 ml potassium → ρ = 100 mg/l potassium;

— 5,0 ml sodium → ρ = 50 mg/l sodium.

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[3] the sign "→" means: "which leads to a mass concentration of"

Furthermore, pipette the following amounts of standard solutions (4.7) into the same flask and fill up to the mark with water:

— 2,0 ml copper → ρ = 2,0 mg/l copper;

— 2,0 ml iron → ρ = 2,0 mg/l iron;

— 2,0 ml manganese → ρ = 2,0 mg/l manganese;

— 2,0 ml zinc → ρ = 2,0 mg/l zinc.

4.8.5 Reference solution 3

Pipette the following amounts of stock solutions (4.6) into the prepared 100-ml volumetric flask (4.8.2):

— 20,0 ml calcium → ρ = 200 mg/l calcium;

— 10,0 ml magnesium → ρ = 100 mg/l magnesium;

— 20,0 ml potassium → ρ = 200 mg/l potassium;

— 10,0 ml sodium → ρ = 100 mg/l sodium.

Furthermore, pipette the following amounts of standard solutions (4.7) into the same flask and fill up to the mark with water:

— 5,0 ml copper → ρ = 5,0 mg/l copper;

— 5,0 ml iron → ρ = 5,0 mg/l iron;

— 5,0 ml manganese → ρ = 5,0 mg/l manganese;

— 5,0 ml zinc → ρ = 5,0 mg/l zinc.

4.8.6 Reference solutions phosphorus and sulfur

Pipette the following amounts of stock solutions (4.6) into the prepared 100-ml volumetric flask (4.8.2) and fill up to the mark with water:

— 1,0 ml respectively phosphorus and sulfur → ρ = 10,0 mg/l phosphorus and sulfur;

— 5,0 ml respectively phosphorus and sulfur → ρ = 50,0 mg/l phosphorus and sulfur;

— 10,0 ml respectively phosphorus and sulfur → ρ = 100,0 mg/l phosphorus and sulfur.

4.9 Zero value solution

The zero value solution contains water, 10 ml of nitric acid (4.1), 2 ml of hydrochloric acid (4.2), if applicable 10 ml caesium chloride solution (4.4) and a corresponding amount of internal standard (4.5) in 100 ml.

5 Apparatus

5.1 General

All equipment and labware that come into direct contact with the sample and the solutions used shall be carefully pretreated/cleaned corresponding to EN 13804 to minimize the blank value.

5.2 ICP-OES, optical emission spectrometer with inductively coupled argon plasma, sample supply and atomization system as well as device control and evaluation unit.

6 Procedure

6.1 Sample preparation

Mineralize the sample in a pressure digestion corresponding to EN 13805.

In the case of dry, powdery or free-flowing materials (water content below 20 %), add 2 ml of water in relation to a test portion of 400 mg, and mix intensively so that the sample is well suspended in the water. Add nitric acid (4.1) and mix. For the determination of iron in order to avoid adsorption losses, add 0,5 ml to 1 ml of hydrochloric acid (4.2) to the digestion vessel, in addition to the nitric acid (4.1). The amount of hydrochloric acid depends on the amount of nitric acid used. Do not add the hydrochloric acid before/until the termination/expiration of the spontaneous reaction caused by the nitric acid. Seal the digestion vessel immediately after addition of the hydrochloric acid. The pressure digestion should be started shortly afterwards. Use digestion conditions according to the device manufacturer, the reactivity of the sample, the maximum pressure stability of the digestion vessel and the attainable temperature.

NOTE The trueness of the determination of the other elements is not impaired by the addition of hydrochloric acid during the digestion.

Fill up the digestion solution obtained according to EN 13805 after the pressure digestion to a definite volume, e.g. 20 ml. It can be used directly or after dilution for the following element determinations. The same concentration of acids, if applicable internal standard and ionization buffer, shall be present in all measuring solutions as in the reference solutions.

6.2 Optical emission spectrometry with inductively coupled plasma

6.2.1 ICP-OES working conditions

Ensure that the device is set corresponding to the manufacturer's data and the plasma is ignited. After sufficient heating and stabilization of the device, optimize the settings [3], [4].

6.2.2 Determination with ICP-OES

Start the measurements after optimizing the device. The following wavelengths (Table 2), which have been tested for possible interference (see Table A.1), are recommended for determination of elements and for internal standards (Table 3). The wavelength data in the device-specific spectral libraries can deviate. Further wavelengths can be used, although these shall be tested for potential interference in individual cases.

Table 2 — Recommended wavelengths for element determination

Element	**Wavelengths** nm	**Possible interference**
Calcium	317,93 315,89 422,67	Ce, Fe, Mo, V Co, Mo, OH band Mo, V, Zr
Copper	324,75 327,40	Cr, Fe, Mo, Nb, Ti, U Co, Nb, Ti, U
Iron	259,94 238,20	Co, Mn, Nb Co
Magnesium	279,08 285,21	Fe, Nb, Rh Cr, Fe, Mo, W
Manganese	257,61 293,31	Cr, Fe, Mo, W Al, Cr, Fe, Ti
Phosphorus	213,62 214,91 177,43	Co, Cu, Fe, Mo, Zn Al, Cu, Fe, Mg Cu
Potassium	766,49 769,90 404,72	Ar, Ba, Mg, Ba Ba
Sodium	589,59 330,24	Ar, Ba, V Zn
Sulfur	181,97 180,67 182,56	B, Nb, Ni, Pb As, Ca, Co, Ni, Si B, Cu, Mg
Zinc	213,86 206,20	Al, As, Cu, Fe, Mg, Ni Cr, Nb

Table 3 — Recommended wavelengths for internal standards

Element	**Wavelength** nm
Scandium	361,38
Ytterbium	328,94
Yttrium	371,03

When determining sodium and potassium, ionization interferences are generally present. In general, an ionization buffer, e.g. caesium chloride (w = 1 % in the measurement solution), should be used for the axial recording of the plasma emission. In the case of radial recording of the emission intensity, the user of this standard shall establish whether the use of an ionization buffer can be omitted by optimizing the observation height and the gas settings. If element lines below 190 nm are applied, ensure sufficient inert gas purging between the plasma and inlet gap as well as the spectrometer so that oxygen absorption cannot cause any drifting of intensities.

If the ionization buffer is added via a Y-piece, adjust volume ratios so that the solution that enters the atomizer or plasma contains an excess of caesium chloride.

Measure zero value solution (4.9) and reference solutions (4.8) and prepare a calibration curve using emission intensities or peak areas and concentrations. In the case of complex matrices, the standard addition method may be necessary.

Aspirate sample solution and measure it. The determined peak areas or intensities are converted via calibration curve using linear regression into concentration units.

Ensure sufficient time for rinsing before the next measurement in case of samples with high element concentrations. Rinse performance can be monitored with zero value solution (4.9).

When making dilutions, ensure that the diluted measuring solutions have the same concentrations of reagents as the original measuring solutions.

6.3 Quality control of the analysis

For quality control, analyse control samples with reliably known contents of the elements to be determined in parallel to every measurement series. The control samples should pass through all analytical steps, beginning with the digestion. Likewise, prepare reagent blank solutions and measure for every digestion series, taking into account all process steps.

To check for matrix effect spiking experiments should be performed by adding a known concentration of the elements to the measurement solution.

7 Calculation

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

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

where

a is the mass fraction of the element in the sample solution in milligrams 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 grams or the sample volume in millilitres used for digestion.

Causes of increased element contents in the blank value solution shall be clarified and, if necessary, deducted from the calculation of the results.

8 Precision

8.1 General

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

8.2 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 time interval, will in no more than 5 % of the cases exceed the values of *r* given in Table 4.

8.3 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 *R* given in Table 4.

Table 4 — Precision data

Sample/elements	$\bar{x}$ mg/kg	*r* mg/kg	*R* mg/kg
Infant formula (soya based)			
Calcium	6 191	591	1 381
Copper	4,51	0,54	1,40
Iron	77,0	5,9	15,0
Magnesium	599	72	128
Manganese	2,19	0,29	0,84
Phosphorus	4 129	399	909
Potassium	6 733	769	1 035
Sodium	2 220	228	304
Sulfur	1 234	133	370
Zinc	43,5	3,2	8,4
Cheese			
Calcium	7 178	394	930
Copper	14,66	0,96	2,53
Iron	2,3	0,4	1,1
Magnesium	587	58	104
Manganese	0,2	-	-
Phosphorus	9 708	1 080	782
Potassium	8 397	879	1 293
Sodium	2 039	132	235

Sample/elements	$\bar{x}$ mg/kg	r mg/kg	R mg/kg
Sulfur	5 764	504	1 176
Zinc	38,4	3,5	7,1
Chicken meat			
Calcium	186	18	33
Copper[a]	1,50	0,70	1,35
Iron	24,0	4,4	7,7
Magnesium	1 147	116	242
Manganese	1,35	0,17	0,46
Phosphorus	9 034	1 022	1 911
Potassium	14 312	473	2 459
Sodium	1 303	124	145
Sulfur	8 542	465	1 464
Zinc	24,8	3,0	4,6
Wheat flour			
Calcium	305	23	34
Copper	2,51	0,33	0,90
Iron	15,3	1,9	3,9
Magnesium	397	27	74
Manganese	5,12	0,40	0,92
Phosphorus	1 466	119	258
Potassium	1 325	163	203
Sodium[a]	11	3	8
Sulfur	1 694	149	320
Zinc	11,2	1,2	1,5
Apple juice			
Calcium	70	6	9
Copper	0,04	-	-
Iron	0,88	0,11	0,19
Magnesium	45	3	10
Manganese	0,44	0,05	0,09
Phosphorus	72	7	15
Potassium	1 090	99	196

Sample/elements	$\bar{x}$ mg/kg	r mg/kg	R mg/kg
Sodium	19	4	9
Sulfur	26	6	13
Zinc	0,16	0,04	0,09
Lobster			
Calcium	183	25	32
Copper	16,40	2,63	3,13
Iron	12,1	2,2	2,9
Magnesium	85	9	20
Manganese	1,20	0,16	0,27
Phosphorus	973	86	194
Potassium	871	88	153
Sodium	1 175	9	217
Sulfur	876	77	185
Zinc	13,9	1,8	3,1
Milk			
Calcium [a]	516	45	209
Copper	0,60	0,12	0,21
Iron	7,7	0,7	1,4
Magnesium	53	4	11
Manganese	0,04	-	-
Phosphorus [a]	319	30	130
Potassium	605	85	148
Sodium	186	14	34
Sulfur	177	13	44
Zinc	5,1	0,4	0,9

[a] This element/matrix combination lead to a Horrat value of greater than 2 in the validation study, see Annex B.

9 Test report

The test report should comply with EN ISO/IEC 17025 [5] and shall contain at least the following data:

a) all information necessary for the complete identification of the sample;

b) the test method used and the elements to be determined 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 European Standard 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)

Spectral interferences

The line interferences were checked with three device systems of different design and resolution capacity (Perkin Elmer Optima 3000, Spectro Ciros, Varian Vista). The interferences listed in the following Table A.1 have been checked with the concentrations indicated.

Line interference is generally affected by the resolution of the spectrometer used and the background correction and shall therefore always be checked on the spectrometer used for the measurement. Table A.1 can thus only serve as a starting point for the level of an interference.

Table A.1 — Line interferences

Element (Analyte)	**Wavelength** nm	**Interfering element**	**Concentration of interfering element** mg/l	**Apparent analyte concentration** mg/l
Calcium	317,93	Ce	10	> 0,01
		Fe	50	< 0,01
		Mo	10	< 0,01
		V	10	> 0,01
	315,89	Co	10	> 0,01
		Mo	10	> 0,01
	422,67	Mo	10	< 0,01
		V	10	> 0,01
		Zr	10	< 0,01
Copper	324,75	Cr	10	< 0,01
		Fe	50	< 0,01
		Mo	10	< 0,01
		Nb	10	> 0,1
		Ti	10	< 0,01
		U	10	< 0,01
	327,40	Co	10	< 0,01
		Nb	10	> 0,01
		Ti	10	< 0,01
		U	10	> 0,01
Iron	259,94	Co	10	< 0,01
		Mn	10	< 0,01
		Nb	10	< 0,01
	238,20	Co	10	< 0,01

Element (Analyte)	**Wavelength** nm	**Interfering element**	**Concentration of interfering element** mg/l	**Apparent analyte concentration** mg/l
Magnesium	279,08	Fe	50	> 0,01
		Nb	10	> 0,1
		Rh	10	> 0,1
	285,21	Cr	10	< 0,01
		Fe	50	< 0,01
		Mo	10	< 0,01
		W	10	< 0,01
Manganese	257,61	Cr	10	< 0,01
		Fe	50	< 0,01
		Mo	10	< 0,01
		W	10	< 0,01
	293,31	Al	50	< 0,01
		Cr	10	< 0,01
		Fe	50	< 0,01
		Ti	10	< 0,01
Phosphorus	213,62	Co	10	< 0,01
		Cu	10	> 0,2
		Fe	50	< 0,01
		Mo	10	> 0,01
		Zn	50	> 0,01
	214,91	Al	50	< 0,01
		Cu	10	> 0,05
		Fe	50	> 0,05
		Mg	200	< 0,01
	177,43	Cu	10	> 0,01
Potassium	766,49	Ba	10	< 0,01
		Mg	200	< 0,01
	769,90	Ba	10	< 0,01
	404,72	Ba	10	< 0,01
Sodium	589,59	Ba	10	> 1
		V	10	< 0,01
	330,24	Zn	10	> 5

Element (Analyte)	**Wavelength** nm	**Interfering element**	**Concentration of interfering element** mg/l	**Apparent analyte concentration** mg/l
Sulfur	181,97	B	10	< 0,1
		Nb	10	< 0,1
		Ni	10	< 0,1
		Pb	10	< 0,1
	180,67	As	10	< 0,1
		Ca	200	> 2
		Co	10	< 0,1
		Ni	10	< 0,1
		Si	50	< 0,1
	182,56	B	10	> 1
		Cu	10	< 0,1
		Mg	200	< 0,1
Zinc	213,86	Al	50	< 0,01
		As	10	< 0,01
		Cu	10	> 0,01
		Fe	50	> 0,01
		Mg	200	< 0,01
		Ni	10	> 0,05
	206,20	Cr	10	> 0,2
		Nb	10	< 0,01

Annex B
(informative)

Precision Data

B.1 Details on the inter-laboratory study

The data given in Table B.1 to Table B.10 were obtained in an inter-laboratory study [2] organized in 2011 by the Working Group "Elementanalytik" of the Federal Office for Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit — BVL) according to the German Food and Feed Act, Paragraph 64 (Lebensmittel- und Futtermittelgesetzbuch-LFGB, § 64) in accordance with ISO 5725-2, −4 and −6 with 13 participating laboratories. Each quantitative value of a laboratory results from a double determination or, in the case of cheese (double blind), from a fourfold determination. The materials used in the interlaboratory study have been fresh materials (milk, apple juice and lobster) and dry materials (wheat flour, infant formula, cheese and chicken meat). Four materials were reference materials (NCS ZC73009 wheat, NCS ZC73016 chicken, NRC LUTS-1 non defatted lobster hepatopancreas (approximately 8 % fat) and dried low fat fresh cheese (approximately 4,5 % fat, 23 % carbohydrates and 67 % protein) as a former proficiency test material). The infant formula soya based material contained 58 % carbohydrates, 24 % fat and 13 % protein. The apple juice was a commercial available product mainly containing 10 % carbohydrates.

The milk with a typical fat content of 4 %, 2,5 % protein, a dry weight of 12 % and a carbohydrate content of 8,5 % was enriched with minerals and proteins. For calcium and phosphorous the reproducibility was relatively high. The laboratories did not use the same procedure for homogenization which led to insufficiently homogenous partition of the casein fraction in the sample during sample preparation.

In Table B.11 the data concerning trueness determined by the use of reference material is given.

Table B.1 — Validation data for calcium

Statistical parameter	Calcium						
	Infant formula soya based	**Cheese**	**Chicken meat**	**Wheat flour**	**Apple juice**	**Lobster**	**Milk**
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	13	13	13	13
Number of laboratories after elimination of outliers	13	12	12	12	12	13	13
Number of outlier laboratories	0	1	1	1	1	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	6 191 ±261	7 178 ±176	186 ±6	305 ±6	70 ±2	183 ±5	516 ±41
Reproducibility standard deviation s_R, mg/kg	493	332	12	12	3	12	75
Relative reproducibility standard deviation $s_{R,rel}$	7,97 %	4,62 %	6,24 %	3,97 %	4,67 %	6,31 %	14,50 %
Reproducibility limit R, mg/kg	1 381	930	33	34	9	32	209
Relative reproducibility limit R_{rel}	22,31 %	12,95 %	17,48 %	11,10 %	13,06 %	17,67 %	40,60 %
Repeatability standard deviation s_r, mg/kg	211	141	6	8	2	9	16
Relative repeatability standard deviation $s_{r,rel}$	3,41 %	1,96 %	3,37 %	2,70 %	3,03 %	4,90 %	3,09 %
Repeatability limit r, mg/kg	591	394	18	23	6	25	45
Relative repeatability limit r_{rel}	9,55 %	5,48 %	9,45 %	7,56 %	8,49 %	13,71 %	8,64 %
Relative Horwitz standard deviation	4,30 %	4,20 %	7,28 %	6,76 %	8,45 %	7,30 %	6,25 %
HorRat	1,85	1,10	0,86	0,59	0,55	0,86	2,32

Table B.2 — Validation data for copper

Statistical parameter	Copper						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice[a]	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	11	13	5	13	13
Number of laboratories after elimination of outliers	13	12	11	12	4	13	13
Number of outlier laboratories	0	1	0	1	1	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	4,51 ±0,27	14,66 ±0,48	1,50 ±0,27	2,51 ±0,18	0,04	16,40 ±0,50	0,60 ±0,04
Reproducibility standard deviation s_R, mg/kg	0,50	0,90	0,48	0,32		1,12	0,07
Relative reproducibility standard deviation $s_{R,rel}$	11,06 %	6,15 %	32,25 %	12,76 %		6,82 %	12,20 %
Reproducibility limit R, mg/kg	1,40	2,53	1,35	0,90		3,13	0,21
Relative reproducibility limit R_{rel}	30,98 %	17,23 %	90,31 %	35,74 %		19,11 %	34,15 %
Repeatability standard deviation s_r, mg/kg	0,19	0,34	0,25	0,12		0,94	0,04
Relative repeatability standard deviation $s_{r,rel}$	4,30 %	2,34 %	16,75 %	4,67 %		5,72 %	7,04 %
Repeatability limit r, mg/kg	0,54	0,96	0,70	0,33		2,63	0,12
Relative repeatability limit r_{rel}	12,03 %	6,54 %	46,89 %	13,08 %		16,01 %	19,70 %
Relative Horwitz standard deviation	12,75 %	10,68 %	15,06 %	13,93 %		10,50 %	17,27 %
HorRat	0,87	0,58	2,14	0,92		0,65	0,71

[a] Only some of the participating laboratories have determined quantitative values for the copper content in the apple juice. As less than 7 values were available for determination of the precision data after eliminating the outliers, precision data will not be indicated at this point. The copper content of this sample was in the range of the quantification limit at 0,04 mg/kg.

Table B.3 — Validation data for iron

Statistical parameter	Iron						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	12	13	13	12	13	13
Number of laboratories after elimination of outliers	13	11	13	12	12	13	13
Number of outlier laboratories	0	1	0	1	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	77,0 ±2,9	2,3 ±0,2	24,0 ±1,4	15,3 ±0,8	0,88 ±0,04	12,1 ±0,5	7,7 ±0,3
Reproducibility standard deviation s_R, mg/kg	5,4	0,4	2,8	1,4	0,07	1,0	0,5
Relative reproducibility standard deviation $s_{R,rel}$	6,98 %	16,83 %	11,49 %	9,19 %	7,81 %	8,59 %	6,59 %
Reproducibility limit R, mg/kg	15,0	1,1	7,7	3,9	0,19	2,9	1,4
Relative reproducibility limit R_{rel}	19,55 %	47,13 %	32,17 %	25,73 %	21,86 %	24,05 %	18,44 %
Repeatability standard deviation s_r, mg/kg	2,1	0,1	1,6	0,7	0,04	0,8	0,3
Relative repeatability standard deviation $s_{r,rel}$	2,75 %	6,13 %	6,61 %	4,48 %	4,38 %	6,45 %	3,47 %
Repeatability limit r, mg/kg	5,9	0,4	4,4	1,9	0,11	2,2	0,7
Relative repeatability limit r_{rel}	7,69 %	17,15 %	18,50 %	12,54 %	12,27 %	18,06 %	9,72 %
Relative Horwitz standard deviation	8,32 %	14,10 %	9,92 %	10,62 %	16,31 %	11,00 %	11,78 %
HorRat	0,84	1,19	1,16	0,87	0,48	0,78	0,56

Table B.4 — Validation data for magnesium

Statistical parameter	Magnesium						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	13	13	13	13
Number of laboratories after elimination of outliers	13	13	13	13	13	13	13
Number of outlier laboratories	0	0	0	0	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	599 ±23	587 ±18	1 174 ±45	397 ±14	45 ±2	85 ±4	53 ±2
Reproducibility standard deviation s_R, mg/kg	46	37	86	26	4	7	4
Relative reproducibility standard deviation $s_{R,rel}$	7,64 %	6,35 %	7,35 %	6,67 %	7,97 %	8,63 %	7,68 %
Reproducibility limit R, mg/kg	128	104	242	74	10	20	11
Relative reproducibility limit R_{rel}	21,38 %	17,79 %	20,59 %	18,67 %	22,31 %	24,18 %	21,50 %
Repeatability standard deviation s_r, mg/kg	26	21	41	10	1	3	1
Relative repeatability standard deviation $s_{r,rel}$	4,30 %	3,55 %	3,52 %	2,44 %	2,29 %	3,73 %	2,55 %
Repeatability limit r, mg/kg	72	58	116	27	3	9	4
Relative repeatability limit r_{rel}	12,05 %	9,95 %	9,87 %	6,84 %	6,42 %	10,44 %	7,13 %
Relative Horwitz standard deviation	6,11 %	6,13 %	5,52 %	6,50 %	9,01 %	8,20 %	8,81 %
HorRat	1,25	1,04	1,33	1,03	0,88	1,05	0,87

Table B.5 — Validation data for manganese

Statistical parameter	Manganese						
	Infant formula soya based	Cheese[a]	Chicken meat	Wheat flour	Apple juice	Lobster	Milk[b]
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	7	11	13	13	13	6
Number of laboratories after elimination of outliers	13	6	11	13	13	13	6
Number of outlier laboratories	0	1	0	0	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	2,19 ±0,16	0,2	1,35 ±0,10	5,12 ±0,17	0,44 ±0,02	1,20 ±0,05	0,04
Reproducibility standard deviation s_R, mg/kg	0,30		0,16	0,33	0,03	0,10	
Relative reproducibility standard deviation $s_{R,rel}$	13,71 %		12,15 %	6,39 %	6,85 %	7,95 %	
Reproducibility limit R, mg/kg	0,84		0,46	0,92	0,09	0,27	
Relative reproducibility limit R_{rel}	38,38 %		34,03 %	17,90 %	19,17 %	22,26 %	
Repeatability standard deviation s_r, mg/kg	0,10		0,06	0,14	0,02	0,06	
Relative repeatability standard deviation $s_{r,rel}$	4,67 %		4,46 %	2,81 %	3,71 %	4,74 %	
Repeatability limit r, mg/kg	0,29		0,17	0,40	0,05	0,16	
Relative repeatability limit r_{rel}	13,09 %		12,48 %	7,88 %	10,38 %	13,28 %	
Relative Horwitz standard deviation	14,21 %		15,29 %	12,51 %	18,07 %	15,57 %	
HorRat	0,96		0,79	0,51	0,38	0,51	

[a] Only some of the participating laboratories have determined quantitative values for the manganese content in the cheese. As less than 7 values were available for determination of the precision data after eliminating the outliers, precision data will not be indicated at this point. The manganese content of this sample was in the range of the quantification limit at 0,2 mg/kg.

[b] Only some of the participating laboratories have determined quantitative values for the manganese content in milk. As less than 7 values were available for determination of the precision data, precision data will not be indicated at this point. The manganese content of this sample was in the range of the quantification limit at 0,04 mg/kg.

Table B.6 — Validation data for phosphorus

Statistical parameter	Phosphorus						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	13	13	13	13
Number of laboratories after elimination of outliers	13	13	13	13	13	13	13
Number of outlier laboratories	0	0	0	0	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	4 129 ±171	9 708 ±300	9 034 ±351	1 466 ±48	72 ±3	973 ±37	319 ±25
Reproducibility standard deviation s_R, mg/kg	325	636	683	92	5	69	46
Relative reproducibility standard deviation $s_{R,rel}$	7,87 %	6,55 %	7,56 %	6,28 %	7,31 %	7,13 %	14,58 %
Reproducibility limit R, mg/kg	909	1 782	1 911	258	15	194	130
Relative reproducibility limit R_{rel}	22,03 %	18,35 %	21,16 %	17,59 %	20,48 %	19,98 %	40,84 %
Repeatability standard deviation s_r, mg/kg	143	386	365	42	2	31	11
Relative repeatability standard deviation $s_{r,rel}$	3,45 %	3,97 %	4,04 %	2,89 %	3,34 %	3,16 %	3,38 %
Repeatability limit r, mg/kg	399	1 080	1 022	119	7	86	30
Relative repeatability limit r_{rel}	9,66 %	11,12 %	11,32 %	8,09 %	9,35 %	8,84 %	9,46 %
Relative Horwitz standard deviation	4,57 %	4,02 %	4,06 %	5,34 %	8,40 %	5,68 %	6,72 %
HorRat	1,72	1,63	1,86	1,18	0,87	1,26	2,17

Table B.7 — Validation data for potassium

Statistical parameter	Potassium						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	13	13	13	13
Number of laboratories after elimination of outliers	13	13	13	11	13	13	13
Number of outlier laboratories	0	0	0	2	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	6 733 ±174	8 397 ±207	14 312 ±441	1 325 ±36	1 090 ±36	871 ±28	605 ±27
Reproducibility standard deviation s_R, mg/kg	370	462	878	72	70	55	53
Relative reproducibility standard deviation $s_{R,rel}$	5,49 %	5,50 %	6,14 %	5,46 %	6,43 %	6,27 %	8,72 %
Reproducibility limit R, mg/kg	1 035	1 293	2 459	203	196	153	148
Relative reproducibility limit R_{rel}	15,37 %	15,39 %	17,18 %	15,28 %	18,01 %	17,55 %	24,41 %
Repeatability standard deviation s_r, mg/kg	275	314	526	58	35	32	30
Relative repeatability standard deviation $s_{r,rel}$	4,08 %	3,74 %	3,68 %	4,39 %	3,26 %	3,63 %	5,01 %
Repeatability limit r, mg/kg	769	879	1 473	163	99	88	85
Relative repeatability limit r_{rel}	11,43 %	10,47 %	10,29 %	12,28 %	9,12 %	10,15 %	14,03 %
Relative Horwitz standard deviation	4,25 %	4,11 %	3,79 %	5,42 %	5,58 %	5,78 %	6,10 %
HorRat	1,29	1,34	1,62	1,01	1,15	1,09	1,43

Table B.8 — Validation data for sodium

Statistical parameter	Sodium						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	11	12	13	13
Number of laboratories after elimination of outliers	13	12	13	10	12	13	13
Number of outlier laboratories	0	1	0	1	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	2 220 ±51	2 039 ±42	1 303 ±23	11 ±2	19 ±2	1 175 ±40	186 ±7
Reproducibility standard deviation s_R, mg/kg	108	84	52	3	3	78	12
Relative reproducibility standard deviation $s_{R,rel}$	4,89 %	4,11 %	3,97 %	26,32 %	16,94 %	6,60 %	6,60 %
Reproducibility limit R, mg/kg	304	235	145	8	9	217	34
Relative reproducibility limit R_{rel}	13,68 %	11,50 %	11,10 %	73,71 %	47,44 %	18,47 %	18,48 %
Repeatability standard deviation s_r, mg/kg	82	47	44	1	2	39	5
Relative repeatability standard deviation $s_{r,rel}$	3,67 %	2,30 %	3,40 %	8,15 %	8,17 %	3,31 %	2,71 %
Repeatability limit r, mg/kg	228	132	124	3	4	109	14
Relative repeatability limit r_{rel}	10,28 %	6,45 %	9,52 %	22,82 %	22,87 %	9,27 %	7,58 %
Relative Horwitz standard deviation	5,02 %	5,08 %	5,44 %	11,10 %	10,31 %	5,52 %	7,29 %
HorRat	0,97	0,81	0,73	2,37	1,64	1,19	0,91

Table B.9 — Validation data for sulfur

Statistical parameter	Sulfur						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	11	11	11	11	10	11	11
Number of laboratories after elimination of outliers	11	11	10	11	10	11	11
Number of outlier laboratories	0	0	1	0	0	0	0
Mean value $\bar{x}$ ± confidence interval, mg/kg	1 234 ±77	5 764 ±235	8 542 ±322	1 694 ±65	26 ±3	876 ±38	177 ±9
Reproducibility standard deviation s_R, mg/kg	132	420	523	114	5	66	16
Relative reproducibility standard deviation $s_{R,rel}$	10,71 %	7,29 %	6,12 %	6,75 %	17,85 %	7,54 %	8,94 %
Reproducibility limit R, mg/kg	370	1 176	1 464	320	13	185	44
Relative reproducibility limit R_{rel}	29,98 %	20,40 %	17,14 %	18,89 %	49,98 %	21,13 %	25,02 %
Repeatability standard deviation s_r, mg/kg	48	180	166	53	2	27	5
Relative repeatability standard deviation $s_{r,rel}$	3,86 %	3,12 %	1,94 %	3,15 %	7,76 %	3,13 %	2,59 %
Repeatability limit r, mg/kg	133	504	465	149	6	77	13
Relative repeatability limit r_{rel}	10,81 %	8,74 %	5,44 %	8,81 %	21,73 %	8,75 %	7,26 %
Relative Horwitz standard deviation	5,48 %	4,35 %	4,10 %	5,23 %	9,80 %	5,77 %	7,34 %
HorRat	1,95	1,68	1,49	1,29	1,82	1,31	1,22

Table B.10 — Validation data for zinc

Statistical parameter	Zinc						
	Infant formula soya based	Cheese	Chicken meat	Wheat flour	Apple juice[a]	Lobster	Milk
Number of participating laboratories	13	13	13	13	13	13	13
Number of laboratories with quantitative values	13	13	13	13	8	13	13
Number of laboratories after elimination of outliers	13	12	13	13	8	13	12
Number of outlier laboratories	0	1	0	0	0	0	1
Mean value $\bar{x}$ ± confidence interval, mg/kg	43,5 ±1,6	38,4 ±1,3	24,8 ±0,8	11,2 ±0,3	0,16 ±0,02	13,9 ±0,6	5,1 ±0,2
Reproducibility standard deviation s_R, mg/kg	3,0	2,5	1,7	0,5	0,03	1,1	0,3
Relative reproducibility standard deviation $s_{R,rel}$	6,89 %	6,61 %	6,66 %	4,88 %	19,80 %	7,90 %	6,52 %
Reproducibility limit R, mg/kg	8,4	7,1	4,6	1,5	0,09	3,1	0,9
Relative reproducibility limit R_{rel}	19,29 %	18,50 %	18,65 %	13,66 %	55,45 %	22,12 %	18,25 %
Repeatability standard deviation s_r, mg/kg	1,1	1,2	1,1	0,4	0,02	0,6	0,2
Relative repeatability standard deviation $s_{r,rel}$	2,60 %	3,25 %	4,25 %	3,87 %	9,83 %	4,63 %	2,96 %
Repeatability limit r, mg/kg	3,2	3,5	3,0	1,2	0,04	1,8	0,4
Relative repeatability limit r_{rel}	7,27 %	9,10 %	11,89 %	10,84 %	27,53 %	12,96 %	8,28 %
Relative Horwitz standard deviation	9,07 %	9,24 %	9,86 %	11,12 %	21,11 %	10,77 %	12,52 %
HorRat	0,76	0,72	0,68	0,44	0,94	0,73	0,52

[a] The zinc content of the apple juice is in the range of the quantification limit.

Table B.11 — Data concerning trueness determined by the use of reference material

Reference material/ element	Mean mg/kg	Uncertainty[a] of mean mg/kg	Reference value mg/kg	Uncertainty[a] of reference value mg/kg
Dried fresh cheese				
copper	14,66	0,48	14,49	0,31
zinc	38,4	1,3	37,56	1,13
NRCC LUTS-1				
calcium	183	5	203	33
copper	16,40	0,50	15,9	1,2
iron	12,1	0,5	11,6	0,9
magnesium	85	4	89,5	4,1
manganese	1,20	0,05	1,20	0,13
potassium	871	28	948	72
zinc	13,9	0,6	12,4	0,8

[a] 95% confidence interval.

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