



*Setting standards
in analytical science*

CCQM-P13 pilot study Metals in artificial food digest

C.S.J. Wolff Briche

LGC
Queens Road
Teddington
Middlesex TW11 0LY
United Kingdom

1. Abstract

The accuracy of elemental analyses in complex matrices is usually assessed by analysis of a suitable matrix reference material. The reference value is ascribed by consensus mean and by application of primary methods of analysis. However, the quality of this value will be affected by problems such as matrix-induced interferences, moisture corrections and heterogeneity.

Pilot study CCQM-P13 was undertaken to assess the capabilities of National Metrology Institutes to analyse Ca, Cu and Cd in an acidic solution that simulates the digest of a food sample. This study filled the gap between the analysis of a gravimetrically prepared calibration solution, and the analysis of an unknown in a complex matrix requiring extensive sample preparation. Having an independent reference value, with a small uncertainty, allowed a more rigorous estimation of the reliability of the institutes' analysis and uncertainty estimates, without including issues around sample digestion.

The reference values were: 1.6617 ± 0.0020 $\mu\text{mol/g}$ for Ca, 7.037 ± 0.012 nmol/g for Cu, and 45.57 ± 0.10 pmol/g for Cd (expanded uncertainties are quoted with coverage factor of 2). The other elements in the matrix were: Na (~ 25 $\mu\text{g/g}$), K (~ 90 $\mu\text{g/g}$), Cl (~ 120 $\mu\text{g/g}$), Fe (~ 100 ng/g), Mg (~ 5 $\mu\text{g/g}$), P (~ 5 $\mu\text{g/g}$), Sn (~ 80 ng/g), and Zn (~ 200 ng/g).

Twelve international laboratories, representing 8 countries, determined the amount content of the analytes. A range of techniques that include ID-ICP-MS (high resolution and collision cell), ICP-MS, ICP-OES, AAS, voltametry and potentiometry were used. The results for this pilot study averaged: 1.654 ± 0.058 $\mu\text{mol/g}$ for Ca ($n = 10$), 7.26 ± 0.53 nmol/g for Cu ($n = 12$), and 45.2 ± 5.1 pmol/g for Cd ($n = 11$) where the values associated with the averages are the standard deviations of n participants.

2. Introduction

There is a continuing need to measure a number of important elements in food. The intention of this study was to establish the capability to measure nutritional, essential and toxic elements in a synthetic food digest. Specifically the elements Ca (nutritional), Cu (essential) and Cd (toxic) were targeted. It is expected that the laboratories that can demonstrate the capability to measure these analytes in such a matrix, will be able to apply such expertise to a range of other types of sample.

By reviewing a cross section of food types, it was possible to propose a generic food matrix made up of the major components left following destructive digestion. The aim was to spike such a synthetic matrix solution with gravimetrically known amounts of selected target analytes. Preparing the final solution in this manner enables target reference values for selected trace elements to be established. A generic food matrix, made of the major components left after destructive digestion, was spiked with 3 elements (Ca, Cu, Cd) reflecting 3 different levels in food: nutritional, essential and toxic elements.

3. Rationale of this comparison

Trace inorganic CCQM studies have been done either on solutions with prepared gravimetric concentrations or on more complex matrix samples without known concentrations. In the latter case, it has sometimes been a problem to agree on how to calculate the reference value and its uncertainty estimate. This study filled the gap between analysis of a gravimetrically prepared calibration solution, and the analysis of an unknown in a complex matrix requiring sample digestion. Having an independent reference value, with a small uncertainty, allowed a more rigorous estimation of the reliability of the institutes' analysis and uncertainty estimates, without including issues around sample digestion.

This pilot study investigated the influence of a typical food matrix on high accuracy elemental analysis. The advantages of this approach are that it separates the complicated issues of homogeneity and sample digestion from the direct influence of the matrix on the end measurement. This enables a direct evaluation to be made of the influence of the matrix on high accuracy measurements, when compared to earlier CCQM trace metal studies, which have been conducted on simple acid solutions of the pure analyte (CCQM-K2 and K8).

4. Participation in CCQM-P13

Participants in CCQM-P13 are listed in Table 1.

Table 1. CCQM-P13 participants

institution / organisation	origin
CIENTEC Fundação de Ciência e Tecnologia	Brazil
Instituto Adolfo Lutz	Brazil
INCQS Instituto Nacional de Controle de Qualidade em Saude	Brazil
IRMM Institute for Reference Materials and Measurements	European Union
KRISS Korean Research Institute of Standards and Science	South Korea
LGC Laboratory of the Government Chemist	United Kingdom
BNM-LNE Bureau National de Métrologie - Laboratoire National d'Essais	France
NARL National Analytical Reference Laboratory	Australia
NIST National Institute for Standards and Technology	United States of America
NMi VSL Nederlands Meetinstituut	The Netherlands
VNIIFTRI National Scientific and Research Institute for Physical-technical and Radio-technical Measurements	Russian Federation
VNIIM Mendelevief Institute of Metrology	Russian Federation

5. Sample

The CCQM-P13 sample was made up into a final solution containing 7 % nitric acid. The nominal values for the elements to be analysed were 25 –100 $\mu\text{g g}^{-1}$ for Ca, 50 – 500 ng g^{-1} for Cu, and 1 – 10 ng g^{-1} for Cd. Two duplicate samples were sent to the participants in 60 mL Nalgene HDPE bottles.

The solution was made in 7 % nitric acid, to reflect a typical composition following microwave digestion of a food sample. The major matrix components aim to reflect the equivalent of a 1g sample of food digested and made up into 100g of solution. The matrix components were Cl (100-120 $\mu\text{g g}^{-1}$), K (80-100 $\mu\text{g g}^{-1}$), Mg (5-10 $\mu\text{g g}^{-1}$), Na (20-50 $\mu\text{g g}^{-1}$), P (20-60 $\mu\text{g g}^{-1}$), Fe ($\sim 0.1 \mu\text{g g}^{-1}$), Zn ($\sim 0.2 \mu\text{g g}^{-1}$), and Sn ($\sim 0.08 \mu\text{g g}^{-1}$).

To inhibit transpiration of the samples while in transit, the bottles were sealed in mylar foil and recommended to be kept in the mylar bags until they were used.

6. Instructions to the participants

The CCQM-P13 samples with the information documents were sent to all participants who had expressed their interest in participating on February 15, 2002.

The participants were free to choose one or more suitable methods of measurement for each element they analysed.

The following recommendations were made to the participants concerning reporting the results and their uncertainty:

- a detailed description of the applied method of measurement.
- information about sample preparation (e.g. dilution, including type, origin and quality of the used diluent)
- a description of their equipment (e.g. type, technical specifications)
- information about the materials used for calibration (origin, purity, isotopic ratio if necessary) or any other material used during the analytical procedure
- information on how the uncertainty of the reference material used has been evaluated
- the complete calculation of the final result including all equations, and corrections e.g. of blanks and interferences. Because P13 is a study and not a key comparison, results of more than one method could be reported. Detailed information of all the different methods used was required. If the final result had been calculated as an average of more than one method, the individual results from the individual methods must also be reported.
- the identification and quantification of all uncertainty sources (list or table).
- the calculation of the uncertainty expressed as combined standard uncertainty, which must include the complete specification of the measurand.
- the calculation of the combined standard uncertainty u_c (complete formula) and information on the number of effective degrees of freedom

7. Methods and instrumentation used

The methods and instruments used for the elements Ca, Cu and Cd are given in Table 2, Table 3, and Table 4 respectively.

Table 2. Analytical methods and instrumental techniques used by CCQM-P13 participants for Ca measurements

<i>participant</i>	<i>method</i>	<i>instrumentation</i>
NMi VSL	Double IDMS	magnetic sector ICP-MS (R = 3000)
VNIIM	IDMS	ICP-MS
NIST	Standard addition	ICP-OES
LGC	Double IDMS	magnetic sector ICP-MS (R = 3000)
CIENTEC	Calibration curve	Flame AAS
Instituto Adolfo Lutz	Calibration curve	ICP-OES
INCQS	Calibration curve	ICP-OES
NARL	Double IDMS	Quadrupole ICP-MS with reaction cell
VNIIFTRI	Potentiometry	Ion-selective electrode
KRISS	Double IDMS	Quadrupole ICP-MS with reaction cell

Table 3. Analytical methods and instrumental techniques used by CCQM-P13 participants for Cu measurements

<i>participant</i>	<i>method</i>	<i>instrumentation</i>
NMi VSL	Double IDMS	magnetic sector ICP-MS (R = 3000)
IRMM	Direct IDMS	Quadrupole ICP-MS
VNIIM	IDMS	ICP-MS
NIST	Standard addition	Quadrupole ICP-MS
LGC	Double IDMS	magnetic sector ICP-MS (R = 3000)
CIEN TEC	Calibration curve	ET AAS/ Zeeman
Instituto Adolfo Lutz	Calibration curve	ICP-OES
INCQS	Calibration curve	ICP-OES
NARL	Double IDMS	Quadrupole ICP-MS with reaction cell
VNIIFTRI	Standard addition	Stripping voltametry
KRISS	Double IDMS	Quadrupole ICP-MS with reaction cell
BNM-LNE	Double IDMS	Quadrupole ICP-MS

Table 4. Analytical methods and instrumental techniques used by CCQM-P13 participants for Cd measurements

<i>participant</i>	<i>method</i>	<i>instrumentation</i>
NMi VSL	Double IDMS	magnetic sector ICP-MS (R = 300)
IRMM	Direct IDMS	Quadrupole ICP-MS
VNIIM	IDMS	ICP-MS
LGC	Double IDMS	magnetic sector ICP-MS (R = 300)
CIEN TEC	Calibration curve	ET AAS/ Zeeman
Instituto Adolfo Lutz	Calibration curve	ET AAS/ Zeeman
INCQS	Standard addition	ET AAS
NARL	Double IDMS	magnetic sector ICP-MS (R = 300)
VNIIFTRI	Standard addition	Stripping voltametry
KRISS	Double IDMS	magnetic sector ICP-MS (R = 300)
BNM-LNE	Double IDMS	magnetic sector ICP-MS (low resolution)

8. CCQM-P13 Participants' results

The participants' results are given in Table 5, Table 6, and Table 7 for Ca, Cu and Cd respectively. The mean values were calculated for each element from the results for the different participants. The standard deviations of all the results are also given as well as the relative standard deviations.

Table 5. CCQM-P13 participants' measurement results for Calcium

<i>participant</i>	<i>reported result</i> $\mu\text{mol}\cdot\text{g}^{-1}$	<i>expanded</i> <i>uncertainty (k=2)</i> $\mu\text{mol}\cdot\text{g}^{-1}$	<i>relative</i> <i>uncertainty (%)</i>	<i>reported result</i> $\mu\text{g}\cdot\text{g}^{-1}$	<i>expanded</i> <i>uncertainty (k=2)</i> $\mu\text{g}\cdot\text{g}^{-1}$	<i>relative</i> <i>uncertainty (%)</i>
NMi VSL	1.615	0.023	1.4	64.7	0.9	1.4
IRMM	-	-	-	-	-	-
VNIIM	1.522	0.015	0.99	61.0	0.6	0.98
NIST	1.684	0.013	0.77	67.49	0.52	0.77
LGC	1.678	0.018	1.1	67.27	0.72	1.1
CIENTEC	1.70	0.10	5.9	68.3	5	7.3
Instituto Adolfo Lutz	1.61	0.04	2.2	64.75	1.41	2.2
INCQS	1.71	0.02	0.94	68.5	0.6	0.88
NARL	1.666	0.05	3.0	66.76	1.99	3.0
VNIIFTRI	1.70	0.15	8.8	68	6.1	9.0
KRISS	1.657	0.030	1.8	66.4	1.2	1.8
BNM-LNE	-	-	-	-	-	-

<i>Mean</i>	1.654			66.3		
<i>Standard deviation</i>	0.058			2.3		
<i>Relative standard deviation (%)</i>	3.5			3.5		
<i>Median</i>	1.672			67.0		

Table 6. CCQM-P13 participants' measurement results for Copper

<i>participant</i>	<i>reported result nmol·g⁻¹</i>	<i>expanded uncertainty (k=2) nmol·g⁻¹</i>	<i>relative uncertainty (%)</i>	<i>reported result µg·g⁻¹</i>	<i>expanded uncertainty (k=2) µg g⁻¹</i>	<i>relative uncertainty (%)</i>
NMi VSL	7.04	0.11	1.6	0.448	0.007	1.6
IRMM	6.995	0.055	0.79	0.4445	0.0035	0.79
VNIIM	8.75	0.35	4.0	0.556	0.022	4.0
NIST	6.982	0.050	0.72	0.4437	0.0032	0.72
LGC	7.14	0.13	1.8	0.4537	0.0082	1.8
CIENTEC	7.54	0.10	1.3	0.479	0.009	1.9
Instituto Adolfo Lutz	7.49	0.25	3.3	0.48	0.016	3.3
INCQS	7.34	0.19	2.6	0.466	0.012	2.6
NARL	7.090	0.123	1.7	0.4506	0.0078	1.7
VNIIFTRI	6.60	0.72	10.9	0.420	0.046	10.9
KRISS	7.048	0.084	1.2	0.4479	0.0054	1.2
BNM-LNE	7.066	0.117	1.7	0.4490	0.0074	1.6
<i>Mean</i>	7.26			0.462		
<i>Standard deviation</i>	0.53			0.034		
<i>Relative standard deviation (%)</i>	7.3			7.3		
<i>Median</i>	7.12			0.452		

Table 7. CCQM-P13 participants' measurement results for Cadmium

<i>participant</i>	<i>reported result nmol·g⁻¹</i>	<i>expanded uncertainty (k=2) nmol·g⁻¹</i>	<i>relative uncertainty (%)</i>	<i>reported result ng·g⁻¹</i>	<i>expanded uncertainty (k=2) ng g⁻¹</i>	<i>relative uncertainty (%)</i>
NMi VSL	0.0459	0.0004	0.87	5.16	0.05	1.0
IRMM	0.04553	0.00064	1.4	5.118	0.072	1.4
VNIIM	0.0459	0.0014	3.1	5.16	0.16	3.1
NIST	-	-	-	-	-	-
LGC	0.04535	0.00020	0.4	5.098	0.023	0.45
CIENTEC	0.0406	0.0060	14.8	4.57	0.7	15.3
Instituto Adolfo Lutz	0.039	0.003	7.7	4.39	0.34	7.7
INCQS	0.0587	0.0053	9.0	6.6	0.6	9.1
NARL	0.04450	0.00092	2.1	5.002	0.1	2.0
VNIIFTRI	0.0410	0.0048	11.7	4.60	0.54	11.7
KRISS	0.04555	0.00049	1.1	5.120	0.055	1.1
BNM-LNE	0.04540	0.00038	0.8	5.104	0.043	0.84
<i>Mean</i>	0.0452			5.08		
<i>Standard deviation</i>	0.0051			0.57		
<i>Relative standard deviation (%)</i>	11.3			11.3		
<i>Median</i>	0.0455			5.11		

Graphical representation for amount content of Ca, Cu and Cd are shown in Figure 1, Figure 2, and Figure 3 respectively. The line next to the scale represents 2 % of the gravimetric value.

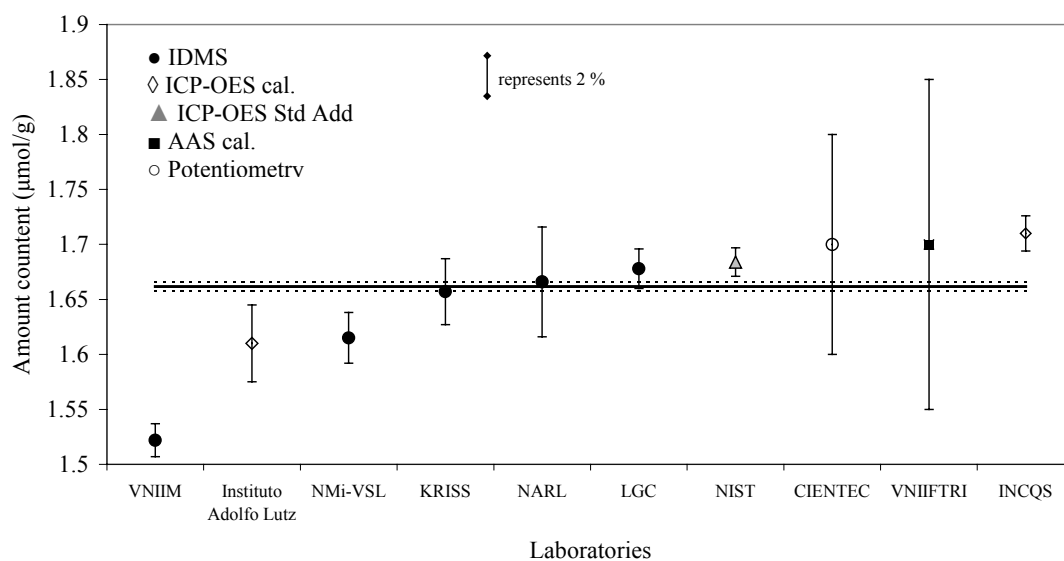


Figure 1: Results of the participants for the amount content of Ca in CCQM-P13 sample

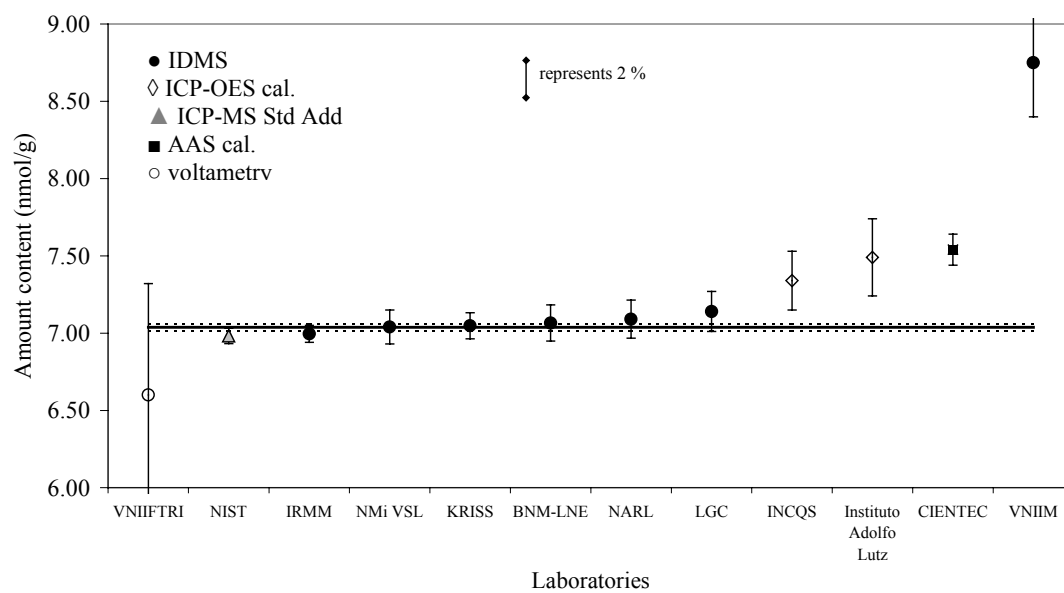


Figure 2: Results of the participants for the amount content of Cu in CCQM-P13 sample

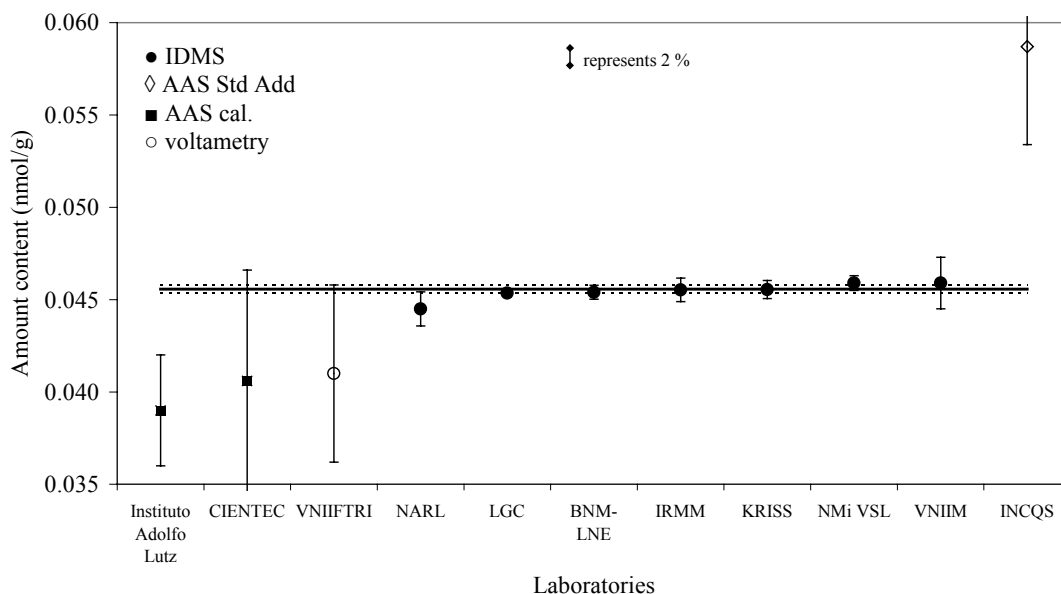


Figure 3: Results of the participants for the amount content of Cd in CCQM-P13 sample

9. Discussion

It is noticeable that the spread of results increases as the analyte concentration decreases, a trend that is often observed for analytical data.

In order to be able to compare the results, the reference values are given in Table 8 for the amount content and Table 9 for the mass fractions of Ca, Cu and Cd.

Table 8: Gravimetric reference values for Ca, Cu and Cd amount content in CCQM-P13 sample

	Value	Expanded uncertainty (k=2)	Unit	Relative deviation of results (%)
Ca	1.6617	0.0020	μmol/g	-0.45
Cu	7.037	0.012	nmol/g	3.1
Cd	0.04557	0.00010	nmol/g	-0.66

Table 9: Gravimetric reference for Ca, Cu and Cd mass fractions in CCQM-P13 sample

	Value	Expanded uncertainty (k=2)	Unit	Relative deviation of results (%)
Ca	66.596	0.078	μg/g	-0.42
Cu	447.17	0.76	ng/g	3.2
Cd	5.122	0.012	ng/g	-0.75

In order to draw some comparison with other CCQM studies, Table 10 shows the average mass fraction calculated using only the IDMS results provided by the participants.

Table 10: Average mass fraction for the IDMS results and relative deviation from the gravimetric reference values

	Value	Standard deviation	Unit	Relative deviation of results (%)
Ca	65.2	2.6	µg/g	-2.1
Cu	0.464	0.041	µg/g	3.8
Cd	5.109	0.053	ng/g	-0.26

For the Ca results the spread of IDMS results is only slightly larger (3.9%) than all the results (3.5 %). However, the deviation of the average of all IDMS results average to the gravimetric reference value (– 2.1 %) is much larger compared to – 0.42 % for all the results.

The IDMS results for Cu show a standard deviation of 8.7%, which is slightly larger than for all the results (7.3 %). And the deviation of the IDMS results and all results are about the same at 3.8 % and 3.2 % respectively.

The spread of results is much smaller for Cd (1 %) when only the IDMS results are taken into account (Figure 4). This also probably reflects the fact that Cd was the object of several previous intercomparisons where participants have used IDMS. The deviation of the mean of the IDMS results to the reference value (-0.26 %) is also smaller than the deviation between the mean of all results and the reference value (-0.75 %). For those using ICP-MS, Cd is usually analysed at low resolution. Ca and Cu require medium resolution to remove some interference. This could be more challenging.

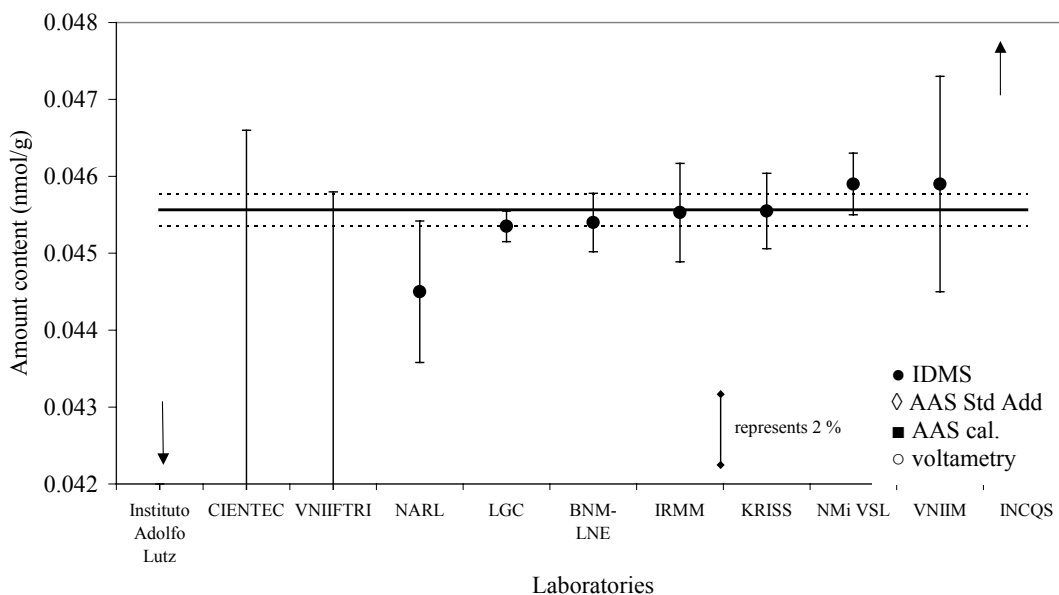


Figure 4: Results of the participants for the amount content of Cd in CCQM-P13 sample with a reduced scale to distinguish results close to the reference value

10. Acknowledgement

The work described here contains the contributions of many scientists: Vera Maria da Costa Dias from Fundação de Ciência e Tecnologia – CIENTEC, Odair Zenebon from Instituto Adolfo Lutz, Silvana do Couto Jacob from INCQS, Christophe Quénel, Emilia Vassileva, Ivan Petrov from IRMM, Euijin Hwang from KRISS, Peter Evans from LGC, Guillaume Labarraque from BNM-LNE, Lindsey Mackay, Richard Myors and Ruth Hearn from NARL, Michel van Son from NMi VSL, Jack Fassett, Laura Wood, Lee Wu, Lee Yu from NIST, Oleg V. Karpov, Igor Maksimov from VNIIFTRI, and Leonid A. Konopelko from VNIIM.

Special thanks are due to all colleagues at LGC who contributed to this study: Sheila Merson, Rita Harte, Ben Fairman, and Tim Catterick. To Michael Weber and Samuel Wunderli (EMPA) for fruitful discussions and information concerning buoyancy correction and uncertainty calculations. And to Mike Sargent (LGC) for his assistance as chairman of the Inorganic Analysis Working Group of CCQM.

This work was supported under contract with the Department of Trade and Industry as part of the National Measurement System Valid Analytical Measurement Program



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Annex A Calculation of the reference values

C.S.J. Wolff Briche

LGC
Queens Road
Teddington
Middlesex TW11 0LY
United Kingdom

1. Introduction

The preparation of the solution was made in 4 steps:

- Primary stock solutions of the analytes (Ca, Cu and Cd) were prepared by dissolving the metal or salt, to give a final solution in 7% HNO₃. This primary stock solution was diluted if necessary to produce the final stock solution.
- A solution A was prepared by mixing the 3 stock solutions of Ca, Cu and Cd in the required proportions.
- A “matrix” solution B was prepared by mixing the stock solutions of NaCl, KCl, Mg, P, Sn and Zn in the required proportions.
- Finally, the solutions A and B were mixed together to produce the final solution to be analysed.

It has been assumed that using the described procedures for dissolving and diluting the metals achieves quantitative dissolution of each metal. The uncertainty contributions of these steps are thus regarded as negligible.

The composition of the solutions is given as mass fraction w (metal mass m_M divided by total solution mass m_{Soln}) and as amount content c (number of moles of metal n_M divided by the total solution mass m_{Soln}).

2. Calculation of the gravimetric values

2.1. Equations used for different cases

For each primary solution prepared by dissolving a metal or salt, the mass fraction of the analyte was calculated according to Equation 1:

$$w = p \cdot \frac{m_2 - m_1}{m_4 - m_3} \cdot \frac{Bu(\text{Analyte})}{Bu(\text{Solution})} \quad \text{Equation 1}$$

With: p purity of the metal or salt

m_1 mass of the empty weighing boat

m_2 mass of the weighing boat + metal or salt

m_3 mass of the empty bottle

m_4 mass of the bottle + the solution

$Bu(\text{analyte})$ buoyancy correction factor for the mass of metal or salt

$Bu(\text{solution})$ buoyancy correction factor for the mass of solution

Further dilutions were made in 7 % HNO₃, whenever necessary to prepare the stock solution according to Equation 2

$$w_{Stock} = w \cdot \frac{m_2 - m_1}{m_3 - m_1} \quad \text{Equation 2}$$

With: w mass fraction of the analyte in the solution to dilute

w_{stock} mass fraction of the analyte in the stock solution

m_1 mass of the empty bottle

m_2 mass of the bottle + the solution to be diluted

m_3 mass of the bottle + the stock solution (after dilution in 7 % HNO₃)

For the preparation of solutions A, B and the final solution, Equation 3 was used for the calculation of the mass fraction of the different components as different solutions were weighed one after the other.

$$w_i = w_{stock} \cdot \frac{m_2 - m_1}{m_4 - m_3} \quad \text{Equation 3}$$

With: w_{stock} mass fraction of the analyte in the stock solution
 w_i mass fraction of the analyte i in the solution (A, B or the final one)
 m_1 mass of the bottle without stock solution i
 m_2 mass of the bottle + stock solution i
 m_3 mass of the empty bottle
 m_4 mass of the empty bottle + the solution (A, B or the final one)

As all dilutions were made in 7 % HNO₃, and solutions A, B and the final one were all in 7 % HNO₃, it was not necessary to consider the buoyancy correction factors as they would cancel out in the dilution equations.

The amount content c_i equals the mass fraction w_i divided by the molar mass of the element i M_i . Therefore the uncertainty budget of the amount content includes the uncertainty of the molar mass of the element. Further calculations were made for the mass fraction only.

$$c_i = \frac{w_i}{M_i} \quad \text{Equation 4}$$

3. Calculation of the uncertainty

All uncertainties were calculated using the “law of propagation of uncertainty”^{1,2,3}. When the result y of a measurand y is determined from N quantities x_i with input estimates x_i the combined standard uncertainty $u_c(y)$ of y is estimated by the equation

$$u_c(y)^2 = \sum_{i=1}^N \left(\frac{\partial f}{\partial x_i} \right)^2 \cdot u(x_i)^2 \quad \text{Equation 5}$$

where $y = f(x_1, x_2, \dots, x_N)$

The partial derivative is often called sensitivity coefficient because it describes how the measurement result varies with changes in the values of the input estimates. It has to be noted that this equation is an approximation that is valid only if all the quantities are independent (not correlated).

All combined standard uncertainties were calculated according to Equation 5.

4. Uncertainty of mass determination $u(m_i)$

The uncertainty of a weighing value is affected by several contributions, which can not all be taken into account. We considered the following contributions: repeatability, non-linearity, temperature coefficient and calibration weight effects. Further effects, with a smaller uncertainty than 10^{-7} , were neglected e.g. sample shape and height, eccentric loading effects etc.

An equation can therefore be written for the weighing value m'_i

$$m'_i = m'_i + \delta_{\text{Repeatability}} + \delta_{\text{Non-linearity}} + \delta_{\text{Temperature-coefficient}} + \delta_{\text{calibration-weights}} \quad \text{Equation 6}$$

where all the δ_i quantities are expected to be zero, but they all have associated uncertainties.

Balances used: For the preparation of all stock solutions and solutions A and B as well as the masses of solutions A and B added to the final solution, the balance used was a Sartorius, model R200D. For the weighing of the final solution, the balance used was an Oertling model MD31.

All weighings were done by difference, which means that some factors can be neglected in the calculation, The uncertainty associated with the temperature coefficient as well as the uncertainty associated with the calibration weights are negligible. Their contribution, as variances, was at least two orders of magnitude lower than the two other parameters, i.e. repeatability and linearity.

The standard uncertainty associated with the repeatability of the weighing was determined in two ways:

- If the weighing was repeated several times, then the standard deviation of the mean obtained from the observations was taken
- If only one weighing was made, then the values for the repeatability given on the balance certificate, divided by the square root of 3 (treated as a rectangular distribution) was used

The standard uncertainty associated with the non linearity factor of the weighing was calculated as the value for linearity given on the balance certificate, divided by the square root of 3 (treated as a rectangular distribution).

The standard uncertainty of a weighing was calculated as:

$$u^2(m'_i) = u^2(\delta_{\text{Repeatability}}) + u^2(\delta_{\text{Non-linearity}}) \quad \text{Equation 7}$$

5. Calculation of the buoyancy correction factors

The buoyancy correction factor Bu is calculated according to Equation 8.

$$Bu = \frac{1 - \frac{\rho_{air}}{\rho_{ref}}}{1 - \frac{\rho_{air}}{\rho_{sample}}} \quad \text{Equation 8}$$

ρ_{sample} Mean mass density of the sample

ρ_{air} Mass density of the air ($\rho_{air} = \rho_{air}(t, p, h_r, composition)$)

ρ_{ref} Mass density of the certified reference weights used

Bu Buoyancy correction factor

As no record of pressure, temperature and humidity could be made with accurate instruments, it was decided to use values in the middle of the ranges encountered and

to associate them with large uncertainties: pressure $P = 1000$ mbar $u(P) = 50$ mbar, relative humidity $h_r = 50$ % $u(h_r) = 20$ %, and temperature $t = 20.0$ °C $u(t) = 2.0$ °C.

The air density was calculated according to Equation 9^{4,5}.

$$\rho_{air} = \frac{A \cdot p + B \cdot h_r \cdot \exp\{C \cdot t\}}{(273.15 + t)} \quad \text{Equation 9}$$

With: $A = 0.34848$, $B = -0.009024$ and $C = 0.0612$

With these values, the density of air is equal to:

$$\rho_{air} = 1.184 \text{ kg/m}^3 \text{ with } u(\rho_{air}) = 0.011 \text{ kg/m}^3$$

Concerning the density of the other variables: the calibration weights were considered to have a density of $\rho_{ref} = 8000$ kg/m³ with $u(\rho_{ref}) = 10$ kg/m³. For the sample (whether it is the analyte solid or the solution) the uncertainty associated with its density was conservatively estimate as $u(\rho_{sample}) = 30$ kg/m³. The values themselves for the different analytes (Cu, Cd, CaCO₃, and 7 % HNO₃) were taken from the 60th edition of the CRC Handbook of Chemistry and Physics. The density of the materials used to prepare the primary stock solutions are given in Table 11.

Table 11: Density of the materials used to prepare the solution

Analyte	Density (ρ) kg/m ³	Uncertainty $u(\rho)$ kg/m ³
Calcium carbonate	2710	30
Copper	8920	30
Cadmium	8642	30
7 % HNO ₃	1037	30

6. Metals purity

For Cu and Cd, a piece of metal rod from Johnson Matthey was used. The total certified impurities were 9 ppm for Cu and 6 ppm for Cd. As a conservative approach, the associated uncertainty for the metals impurity of both metals was considered to be 10 ppm. As no analysis was performed to quantify the amount of included gases present in either of the pure metals (Cu or Cd), an additional standard uncertainty of 0.05 % was also added, to reflect on the possible presence of included gases that were not determined.

For the Ca solution, the salt CaCO₃ from Alfa Aesar Puratronic was used. The certificate stated a purity of 99.999% with Ca content certified as 40.0 %. It also stated a total of about 10 ppm impurities. As stoichiometry could be a problem with the possible presence of CaO, Ca(OH)₂ the calcium content was considered to be 40.00 ± 0.04 %. As a consequence, the relative uncertainty associated with the purity (including stoichiometry) was considered to be 0.1 % and treated as a rectangular distribution.

The uncertainty of the metal purity $u(p)$ was also an estimation and it is based on a solely type B uncertainty. The purity of the materials used to prepare the primary stock solutions, with their estimated uncertainties, are given in Table 12.

Table 12: purity of the materials used to prepare the solution

Analyte	Purity (p)	Uncertainty $u(p)$
Calcium carbonate	0.99999	0.00058
Copper	0.99999	0.00050
Cadmium	0.99999	0.00050

7. Preparation of the final stock solutions of Ca, Cu and Cd

7.1. Mass fraction of Ca in the solution

The primary stock solution (which is the final stock solution as no dilution was necessary) of Ca was prepared directly from the dissolution of CaCO_3 (after it was dried in oven at 110 C for 5 days, and cooled in a desiccator), and made up in 7 % HNO_3 . No dilution was necessary to achieve the desired mass fraction. The data for the preparation of the Ca stock solution is given in Table 13. The mass fraction was calculated according to Equation 1. As CaCO_3 was weighed and not Ca, a correction had to be made and Equation 1 was multiplied by the molar mass of Ca ($40.0780 \text{ g mol}^{-1} \pm 0.0023 \text{ g mol}^{-1}$, cf Table 28) and divided by the molar mass of CaCO_3 ($100.0869 \text{ g mol}^{-1} \pm 0.0024 \text{ g mol}^{-1}$, calculated using individual atomic weights⁷).

Table 13: Data for the preparation of the final stock solution of Ca (weighed as CaCO_3)

variable	x_i	$u(x_i)$	unit
$Bu(\text{CaCO}_3)$	1.0002889	0.0000055	-
$Bu(\text{HNO}_3)$	1.000994	0.000034	-
m_1	20.91980	0.00011	g
m_2	25.258500	0.000020	g
m_3	136.25440	0.00013	g
p	0.99999	0.00058	-

Mass fraction of the final stock solution:

$$w_{stock}(\text{Ca}) = 15.0528 \text{ mg g}^{-1} \text{ with } u_c(w_{stock}(\text{Ca})) = 0.0087 \text{ mg g}^{-1}.$$

7.2. Mass fraction of Cu in the solution

The final stock solution of Cu was prepared by diluting the primary stock solution obtained from the dissolution of Cu and made up in 7 % HNO_3 . Cu was abraded with SiC paper, then washed in 50% HCl rinsed and dried. The data for the preparation of the Cu primary stock solution was calculated according to Equation 1 from the data given in Table 14.

Table 14: Data for the preparation of the primary stock solution of Cu

variable	x_i	$u(x_i)$	unit
$Bu(Cu)$	0.99998474	0.00000050	-
$Bu(HNO_3)$	1.000994	0.000034	-
m_1	8.57626	0.00012	g
m_2	10.21284	0.00013	g
m_3	21.08900	0.00013	g
m_4	124.01128	0.00030	g
p	0.99999	0.00050	-

Mass fraction of the primary stock solution:

$$w_{stock1} (Cu) = 15.8849 \text{ mg g}^{-1} \text{ with } u_c(w_{stock1} (Cu)) = 0.0081 \text{ mg g}^{-1}.$$

The Cu primary stock solution was diluted according to Equation 2, using the data given in Table 15. To get the final Cu stock solution.

Table 15: Data for the preparation of the final stock solution of Cu (from the primary stock solution of Cu)

variable	x_i	$u(x_i)$	unit
m_1	0.000000	0.000020	g
m_2	2.064200	0.000020	g
m_3	151.23200	0.00013	g
$w_{stock1} (Cu)$	15.8849	0.0081	mg g ⁻¹

Mass fraction of the final stock solution:

$$w_{stock} (Cu) = 216.82 \text{ } \mu\text{g g}^{-1} \text{ with } u_c(w_{stock} (Cu)) = 0.11 \text{ } \mu\text{g g}^{-1}.$$

7.3. Mass fraction of Cd in the solution

The final stock solution of Cd was prepared diluting twice the primary stock solution obtained from the dissolution of Cd and made up in 7 % HNO₃. Cd was abraded with SiC paper, then washed in 30 % HNO₃, rinsed and dried. The data for the preparation of the Cd primary stock solution was calculated according to Equation 1 from the data given in Table 16

Table 16: Data for the preparation of the primary stock solution of Cd

variable	x_i	$u(x_i)$	unit
$Bu(Cd)$	0.99998901	0.00000052	-
$Bu(HNO_3)$	1.0009945	0.0000343	-
m_1	5.131650	0.000051	g
m_2	5.576100	0.000020	g
m_3	21.026760	0.000061	g
m_4	112.73720	0.00021	g
p	0.99999	0.00050	-

Mass fraction of the primary stock solution:

$$w_{stock1} (Cd) = 4.8413 \text{ mg g}^{-1} \text{ with } u_c(w_{stock1} (Cd)) = 0.0025 \text{ mg g}^{-1}.$$

Two successive dilutions were made in order to reach the low mass fraction required according to Equation 2. The data in Table 17 represents the preparation of an intermediate stock solution. The data from Table 18 represents the preparation of the final stock solution of Cd.

Table 17: Data for the preparation of the intermediate stock solution of Cd (from the primary stock solution)

variable	x_i	$u(x_i)$	unit
m_1	0.000000	0.000020	g
m_2	2.033100	0.000020	g
m_3	50.10310	0.00013	g
w_{stock1} (Cd)	4.8413	0.0025	mg g ⁻¹

Mass fraction of the intermediate stock solution:

$$w_{stock2}(\text{Cd}) = 196.45 \mu\text{g g}^{-1} \text{ with } u_c(w_{stock2}(\text{Cd})) = 0.10 \mu\text{g g}^{-1}.$$

Table 18: Data for the preparation of the final stock solution of Cd (weighing of Cd intermediate stock solution)

variable	x_i	$u(x_i)$	unit
m_1	0.000000	0.000020	g
m_2	2.036800	0.000020	g
m_3	150.57770	0.00013	g
w_{stock2} (Cd)	196.45	0.10	$\mu\text{g g}^{-1}$

Mass fraction of the final stock solution:

$$w_{stock}(\text{Cd}) = 2.6573 \mu\text{g g}^{-1} \text{ with } u_c(w_{stock}(\text{Cd})) = 0.0014 \mu\text{g g}^{-1}.$$

8. Preparation of solutions A and B

8.1. Preparation of solution A, containing Ca, Cu, and Cd

Solution A was prepared by adding an appropriate amount of the final stock solution of Ca, Cu and Cd followed by the further addition of 7 % HNO₃.

The mass fraction of Ca in solution A was calculated according to Equation 2, using the data in Table 19.

Table 19: Data for the preparation of Ca in solution A (weighing of Ca stock solution)

variable	x_i	$u(x_i)$	unit
m_1	20.275000	0.000050	g
m_2	35.827700	0.000050	g
m_3	109.40560	0.00020	g
w_{stock} (Ca)	15.0528	0.0087	mg g ⁻¹

Mass fraction of the solution A:

$$w_A(\text{Ca}) = 2626.6 \mu\text{g g}^{-1} \text{ with } u_c(w_{stock}(\text{Ca})) = 1.5 \mu\text{g g}^{-1}.$$

The mass fraction of Cu in solution A was calculated according to Equation 3, using the data in Table 20.

Table 20: Data for the preparation of Cu in solution A (weighing of Cu stock solution)

variable	x_i	$u(x_i)$	unit
m_1	42.60390	0.00020	g
m_2	49.85420	0.00020	g
m_3	20.275000	0.000050	g
m_4	109.40560	0.00020	g
$w_{stock}(\text{Cu})$	216.82	0.011	$\mu\text{g g}^{-1}$

Mass fraction of the solution A:

$$w_A(\text{Cu}) = 17.6369 \mu\text{g g}^{-1} \text{ with } u_c(w_{stock}(\text{Cu})) = 0.0091 \mu\text{g g}^{-1}.$$

The mass fraction of Cd in the solution A was calculated according to Equation 3, using the data in Table 21.

Table 21: Data for the preparation of Cd in solution A (weighing of Cd stock solution)

variable	x_i	$u(x_i)$	unit
m_1	35.827700	0.000050	g
m_2	42.60390	0.00020	g
m_3	20.275000	0.000050	g
m_4	109.40560	0.00020	g
$w_{stock}(\text{Cd})$	2.6573	0.0014	$\mu\text{g g}^{-1}$

Mass fraction of the solution A:

$$w_A(\text{Cd}) = 202.02 \text{ ng g}^{-1} \text{ with } u_c(w_{stock}(\text{Cd})) = 0.10 \text{ ng g}^{-1}.$$

8.2. Preparation of the matrix solution B

The matrix solution was prepared by mixing together stock solutions of NaCl, KCl, Fe, Mg, P, Sn and Zn, and additional 7 % HNO₃ to get the desired mass fractions. The mass fractions of the different stock solutions used for the matrix are given in Table 22.

Table 22: The mass fractions of the different stock solutions used for the matrix solution and their respective masses.

variable	$w_{stock}(x_i)$	$u(w_{stock})$	Unit	m_i (g) mixed	$u(m_i)$ (g)
w_{stock} (Na)	6.22401	0.00036	mg g ⁻¹	13.47270	0.00021
w_{stock} (Cl from NaCl)	9.59818	0.00054	mg g ⁻¹	13.47270	0.00021
w_{stock} (K)	15.91985	0.00124	mg g ⁻¹	19.558700	0.000071
w_{stock} (Cl from KCl)	14.43558	0.00113	mg g ⁻¹	19.558700	0.000071
w_{stock} (Fe)	400.15	0.60	µg g ⁻¹	0.87320	0.00028
w_{stock} (Mg)	2.7978	0.0010	mg g ⁻¹	7.49230	0.00028
w_{stock} (P)	0.950	0.015	mg g ⁻¹	18.92200	0.00028
w_{stock} (Sn)	196.2	1.1	µg g ⁻¹	1.41670	0.00028
w_{stock} (Zn)	758.7	2.7	µg g ⁻¹	0.89880	0.00028
<i>Solution mass</i>	-	-	-	78.38990	0.00021

The mass fractions of the different elements mixed to produce the matrix solution B are given in Table 23.

Table 23: Mass fraction of the different elements in the matrix stock solution

variable	$w(x_i)$	$u(w)$	Unit
w_{stock} (Na)	1.069708	0.000064	mg g ⁻¹
w_{stock} (K)	3.97209	0.00031	mg g ⁻¹
w_{stock} (Cl)	5.25137	0.00030	mg g ⁻¹
w_{stock} (Fe)	4.4574	0.0069	µg g ⁻¹
w_{stock} (Mg)	0.267406	0.000096	mg g ⁻¹
w_{stock} (P)	0.2293	0.0036	mg g ⁻¹
w_{stock} (Sn)	3.545	0.019	µg g ⁻¹
w_{stock} (Zn)	8.699	0.031	µg g ⁻¹

8.3. Blank analysis

The possible presence of Ca, Cd and Cu as contaminants in the matrix constituents was checked. An equivalent blank matrix solution was prepared by diluting the stock solutions of NaCl, KCl, P, Fe, Mg, Sn and Zn in 7 % HNO₃. This solution was then analysed for Ca, Cu and Cd. This analysis was performed on an Agilent 7500. The level of counts for that solution was compared to the level of counts obtained for the 7 % HNO₃ solution, and 5 ng/g solutions of Ca, Cu and Cd were used to quantify the level of the elements found.

No Cd or Ca were detected in the blank matrix solution. Cu was detected at about 0.3 ng/g. It was not possible to get a very precise value for this blank measurement which was close to the detection limit. As a consequence, the gravimetric value for the copper solution was not altered for the amount of copper in the blank solution but it was added to the uncertainty of the gravimetric value.

9. Preparation of the final solution

9.1. Gravimetric mixing

Mass of solution A (Ca, Cu and Cd) added was: 88.74340 g with an associated uncertainty of 0.00021 g. The mass of matrix solution B added was: 78.01750 g with an associated uncertainty of 0.00021 g. The total mass of the solution prepared was 3500.13 g with an associated uncertainty of 0.14 g.

Table 24: Data for the preparation of the final solution

variable	x_i	$u(x_i)$	unit
$m_1(A)$	20.666100	0.000050	g
$m_2(A)$	109.40950	0.00020	g
$m_1(B)$	20.797600	0.000050	g
$m_2(B)$	98.81510	0.00020	g
m_3	1128.07	0.25	g
m_4	4628.20	0.25	g

The final mass fractions for the matrix elements are given in Table 25 and in Table 26 for the target analytes (i.e. Ca, Cu and Cd).

Table 25: Mass fraction of the matrix elements in the solution

variable	$w(x_i)$	$u(w)$	Unit
$w(\text{Na})$	23.8436	0.0017	$\mu\text{g g}^{-1}$
$w(\text{K})$	88.5373	0.0078	$\mu\text{g g}^{-1}$
$w(\text{Cl})$	117.0524	0.0082	$\mu\text{g g}^{-1}$
$w(\text{Fe})$	99.35	0.15	ng g^{-1}
$w_k(\text{Mg})$	5.9605	0.0022	$\mu\text{g g}^{-1}$
$w(\text{P})$	5.111	0.081	$\mu\text{g g}^{-1}$
$w(\text{Sn})$	79.03	0.43	ng g^{-1}
$w(\text{Zn})$	193.91	0.70	ng g^{-1}

Table 26: Mass fraction of the target analytes

variable	$w(x_i)$	$u(w)$	Unit
$w(\text{Ca})$	66.596	0.039	$\mu\text{g g}^{-1}$
$w(\text{Cu})$	447.171	0.057	ng g^{-1}
$w(\text{Cd})$	5.1222	0.0027	ng g^{-1}

9.2. Homogeneity of the samples

The bulk solution was divided into 50 bottles, For the homogeneity study, ten bottles were randomly selected and analysed in duplicate by ICP-OES for Ca and Cu, and by ICP-MS for Cd. Two aliquots per bottle were spiked with a relevant internal standard: Sc for Ca, Mn for Cu and Rh for Cd. The experimental conditions for the analyses are given in Table 27.

Table 27: Instrumental conditions

Conditions for the ICP-OES	
Integration time	0.1 s
Read time	10 s
Replicate	10
Wavelength Ca	315.88 nm
Wavelength Sc	361.38 nm
Wavelength Cu	324.752 nm
Wavelength Mn	259.372 nm
Conditions for the ICP-MS	
Isotope used	¹¹⁰ Cd and ¹⁰³ Rh
Number of repeats	7

The homogeneity was assessed by analysis of variance (one-way ANOVA) on the set of results of each analyte. For all 3 elements, the calculated F value was smaller than the critical F value, As a consequence, no significant difference was found between the 10 bottles analysed. According to Ellison et al⁶, “...for CRMs which are true solutions, i.e. u_{hom} can reasonably be assumed to be insignificant compared with the final uncertainty value and can therefore be ignored”. As the CCQM-P13 solution was prepared from solutions prepared with pure elements, P13 sample falls into the described category by Ellison et al.⁶ As a consequence, no contribution was added to the gravimetric value uncertainty for any heterogeneity for the Ca and Cu values. For the Cd value, which is around 5 ng g⁻¹, other factors could cause heterogeneity, such as adsorption on the walls of the different bottles. As a consequence, for this value, a relative uncertainty of 0.1 % (corresponding to $u(hom) = 0.0051$ ng g⁻¹) was added in the calculation of the final combined standard uncertainty, increasing it from 0.027 ng g⁻¹ to 0.0058 ng g⁻¹.

9.3. Uncertainty of the molar mass of the elements

The data were taken from IUPAC Technical report “Atomic weights of the elements 1999”⁷. The atomic weight of the elements Ca, Cu and Cd are given in Table 28.

Table 28: Atomic weight of the elements

Analyte	Molar mass M_i (g/mol)	Uncertainty $u(M_i)$ (g/mol)
Calcium	40.078	0.004
Copper	63.546	0.003
Cadmium	112.411	0.008

The uncertainties were treated as having a rectangular distribution to calculate the standard uncertainties (i.e. dividing then by $\sqrt{3}$).

9.4. Reference values

The reference values for Ca, Cu and Cd are given in Table 8, Table 9, and Table 31 respectively, with the amount content values derived from data in Table 28.

Table 29: Reference values for Ca in CCQM-P13 sample

	Value	Expanded uncertainty (k=2)	Unit	Relative uncertainty (%)
Mass fraction	66.596	0.078	µg/g	0.12
Amount content	1.6617	0.0020	µmol/g	0.12

Table 30: Reference values for Cu in CCQM-P13 sample

	Value	Expanded uncertainty (k=2)	Unit	Relative uncertainty (%)
Mass fraction	447.17	0.76	ng/g	0.17
Amount content	7.037	0.012	nmol/g	0.17

Table 31: Reference values for Cd in CCQM-P13 sample

	Value	Expanded uncertainty (k=2)	Unit	Relative uncertainty (%)
Mass fraction	5.122	0.012	ng/g	0.23
Amount content	0.04557	0.00010	nmol/g	0.23

10. References

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