CCQM-K60: Total selenium and selenomethionine in selenised wheat flour

Draft B Report

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Contents

1. Abstract

Key comparison CCQM-K60 was performed to assess the analytical capabilities of National Metrology Institutes (NMIs) to accurately quantitate the mass fraction of selenomethionine (SeMet) and total selenium (at low mg kg^{-1} levels) in selenised wheat flour. CCQM-K60 was organised as a follow-up key comparison to the previous pilot study CCQM-P86 on selenised yeast tablets. CCQM-K60 was an activity of the Inorganic Analysis Working Group (IAWG) of the Comité Consultatif pour la Quantité de Matière (CCQM) and was coordinated by LGC Limited (Teddington, UK) and the Institute for National Measurement Standards, National Research Council Canada (NRCC, Ottawa, Canada).

Nine results for total Se and four results for SeMet were reported by the participant NMIs. Methods used for sample preparation were microwave assisted acid digestion for total Se and, multiple-step enzymatic hydrolysis and hydrolysis with methanesulfonic acid for SeMet. For total Se, detection techniques included inductively coupled plasma mass spectrometry (ICP-MS) with external calibration, standard additions or isotope dilution analysis (IDMS), instrumental neutron activation analysis (INAA) and graphite furnace atomic absorption spectrometry (GFAAS) with external calibration. For determination of SeMet in the wheat flour sample, the four NMIs relied upon measurements using species-specific IDMS (using ^{76}Se-enriched SeMet) with HPLC-ICP-MS.

Eight of the nine participating NMIs reported results for total Se within 3.5% deviation from the KCRV. For SeMet, the four participating NMIs reported results within 3.2% deviation from the KCRV. This shows that, overall, an excellent agreement amongst participants was achieved.

Finally, the pilot study CCQM-P86.1 was carried out in parallel to this key comparison for the same measurands in the same wheat flour sample. Participation was meant for NMIs that did not take part in the key comparison, and invited expert laboratories.

CMC claims based on SeMet measurements in this study may be applied to other foods or supplement matrices (e.g., vegetables, meat, cereals) provided that the concentration range is similar and due diligence is taken to ensure an appropriate extraction process is achieved and species specific spikes are available for quantitation by ID. Indeed, having accepted such conditions, application to quantitation of other organometallic species and other elements in similar matrices should be possible with the same level of performance. CMC claims based on total Se measurements in this study may include other elements in similar matrices at a similar level of performance using the same measurement technique applied in CCQM-K60 provided that there are no additional factors (e.g. blank or dissolution issues).

2. Introduction

The previous pilot study CCQM-P86.1 [1] demonstrated the ability of NMIs and expert laboratories worldwide to deliver accurate results for total Se and SeMet in Se-yeast tablets (containing approximately 300 mg kg^{-1} Se) with 10 % expanded uncertainty. Therefore, the IAWG agreed that it should be succeeded by a key comparison with its associated pilot study.

The purpose of CCQM-K60&P86.1 was to test the ability of laboratories to accurately quantify total Se and seleno-amino acids of relevance to health products (e.g., selenomethionine), which are present at low mg/kg levels in complex food bio-fortified with selenium. The candidate wheat flour sample to be used in both CCQM-K60 and P86.1 is also of high complexity but contains much lower concentrations of Se than those encountered in the CCQM-P86 Se-yeast tablets (approx. 15-fold lower). This broadens the scope and degree of difficulty of earlier measurements in this field.

3. Rationale for this comparison

Due to the often-insufficient content of Se in the ordinary diet, it is advantageous to add selenium through production of food bio-fortified with this essential mineral. Wheat is one of the most important selenium sources for humans, with selenomethionine (SeMet) as the predominant selenium species [2]. The decline in the importation of Se-rich high protein wheat flour from North America is reported to have contributed to the substantial fall of selenium in the European mammalian diet. Therefore the production of Se-enriched wheat flour offers an effective bio-fortified food for increased human Se-intake. It is likely that, within Europe, new products of this type will be sold to the public. Those products are poorly characterised (e.g., they may contain higher Se levels than the maximum tolerable daily intake and most Se is present as inorganic Se, which is highly toxic) and it is evident from earlier studies that they are inconsistent in their makeup relative to label indications. The EC requested the Scientific Committee on Food (SFC) to review the upper level of daily intake of minerals, amongst them selenium, and to provide the basis for the establishment of safety factors [3]. The accurate assessment of Se, and especially the SeMet content, in food enriched with Se is, therefore, urgently needed but still presents a significant analytical challenge. Major problems are the relatively low food-Se concentration, high complexity of the matrix and the strong dependence of extraction efficiency of Se species on the sample extraction conditions [4].

4. Participation in CCQM-K60

A total of nine NMIs participated in CCQM-K60, as summarised in Table 1.

5. The CCQM K60 wheat flour sample and calibration standards

The matrix sample was a wheat flour material to be characterised for its total Se and selenomethionine content. Wheat grain, provided by Nottingham University, UK, was cleaned with water, milled at a temperature between 18 and 20 °C and 60% relative humidity, sieved twice to a final particle size of 140 μm, thoroughly homogenised, gamma-irradiated (25-40 kGy) and, finally, freeze dried to a moisture content of approx. 5% (w/w) before being bottled. The form of the sample is 15 g of dry wheat flour contained in a 30 mL amber glass bottle. One bottle was sent to each of the participants summarised in Table 1.

Between bottle homogeneity of the wheat flour for total Se and SeMet amount contents was tested at LGC. Ten bottles were analysed in duplicate. For total Se, double IDMS measurements (using

^{77}Se spike) by collision cell ICP-MS were performed after microwave acid digestion of the sample (0.3 g). The RSD (1s, n = 20) was 1.5%. For SeMet, HPLC-ICP-MS with species-specific IDMS (using ^{76}SeMet spike) after two-step enzymatic hydrolysis of the gamma-irradiated K60 sample (0.3 g) was used. The RSD (1s, n = 20) was 1.9%.

The CCQM-P86 selenised yeast tablets will soon be available as a certified reference material. Participants who did not take part in CCQM-P86 were sent one blister packet of tablets. This sample was intended to assist participants with their own method evaluation; it was not required to report results for it.

Table 1. CCQM-K60 participants

NMI	Country	Contact person
CSIR-NML (CSIR-National Metrology Laboratory)	South Africa	Alex Barzev
NRCC (Institute for National Measurement Standards)	Canada	Ralph Sturgeon
BAM (Bundesanstalt für Materialforschung und –prüfung)	Germany	Achim Berger
LGC	United Kingdom	Heidi Goenaga
LNE (Laboratoire National d'Essais)	France	Guillaume Labarraque
INTI (Instituto Nacional de Tecnología Industrial)	Argentina	Liliana Valiente
NMIA(National Measurement Institute, Australia)	Australia	Lindsey Mackay
NIM (National Institute of Metrology, P. R. China)	China	Wang Jun
NMIJ(National Measurement Institute, Japan)	Japan	Tomohiro Narukawa

Participants who indicated at registration that they intend to use isotope dilution for the selenomethionine analysis were provided with an isotopically enriched ^{76}Se standard of selenomethionine (1.5 mg). This material was not certified for isotope composition which, if required, had to be determined by each participant.

The recommended storage temperature for the wheat flour sample, CCQM-P86 tablets and

^{76}SeMet spike material was -20 °C.

Safety data sheets accompanied the isotopically enriched ^{76}Se standard of SeMet and tablets, as did instructions for their use and storage.

6. Instructions to the participants

The CCQM-K60 sample, P86 tablets and spike material were sent to all participants in May 2008 with an accompanying letter containing the protocol explaining the work to be conducted and a 'results report' form for submission of data (see Annexes 2 and 3). The protocol and 'results report' form included the following:

- scope of the study
- general instructions for handling and preservation of sample and calibration standards
- instructions for determination of wheat flour moisture content
- request for reporting results in mg kg^{-1}, on a dry weight basis and for at least three replicate analyses
- request for a full description of the extraction and measurement procedures
- request for uncertainty evaluation according to ISO principles (Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, 1993, ISBN 92-67-10188-9)
- request for a full uncertainty budget

No sample preparation/extraction and/or measurement techniques were prescribed by the coordinating laboratories. As a consequence, participants were free to develop and validate their own approaches, as summarised in Annex 4.

6.1. Instructions and results for determination of wheat flour moisture content

In view of the need for comparability of the results, CCQM K60 included a protocol for determination of wheat flour moisture content as follows: "The moisture content of the wheat should be determined for two independent wheat flour sub-samples from one bottle, independently of the sub-samples used for determination of total Se and/or SeMet. The bottle should be thoroughly shaken before a wheat flour sub-sample of 0.5 g is weighed and heated at 100 °C in an oven for 3 h. The sample should then be cooled to room temperature in a desiccator and weighed. The procedure should be repeated with 1 h heating cycles until constant weight is reached (difference between two consecutive values $\leq$ 0.0003 g). The overall drying time should be reported with the moisture content and should not normally exceed 8 h. Please note that it is important to minimise uptake of water during this procedure and, for laboratories in which the humidity is high, it is advisable to carry out the moisture determination at the time as that for Se and/or SeMet to ensure the same uptake of moisture by all the samples. It is recommended that the sample be weighed as quickly as possible in a pan with a lid for the remainder of the procedure. The lid should remain on the pan during all stages except heating in the oven, when it is placed under the pan".

The majority of participants followed the proposed procedure, except for BAM, which used their own procedure (see Table 2). Table 2 also summarises results submitted for moisture by all participants. Typically, a minor contribution to the overall uncertainty budget (0.1% or less) was derived from correction for moisture. Average moisture content based on measurements from 8 laboratories (except BAM, which did not used the recommended procedure and reported a moisture value 1.5-fold lower than the average value) was 5.6% with an RSD of 10%. It is interesting to note the small variation of moisture with laboratory location.

7. Methods and instrumentation used

For the determination of total Se, seven (out of nine) of the key comparison participants used microwave acid digestion with isotope dilution ICP-MS. BAM used INAA with external calibration (one-point) and INTI used graphite furnace AAS with external calibration (multi-point).

For the determination of SeMet, four (out of four) participants used HPLC-ICP-MS with species-specific IDA after multi-step enzymatic hydrolysis, except for NMIJ, which used hydrolysis with methanesulfonic acid.

An overview of the analytical methods and instrumental techniques used by each participant is given in Annex 4.

8. CCQM-K60 participant's results

8.1. Results for total Se

Results for total Se (dry-weight basis) are summarised in Table 3 and graphically displayed in Figure 1. All uncertainties reported are expanded uncertainties.

Table 2. Moisture content and methods

Lab/sample	Moisture content / g kg $^{-1}$	Method
NMIA/26	56.6; 56.8; 65.3; 60.8; 57.6; 57.6 (Mean: 59.1, n = 6)	Recommended method
LGC/25	54.8; 55.0; 55.1 (Mean: 55.0, n = 3)	Recommended method
NMIJ/7	55.95; 57.13; 57.82; 56.60; 56.02; 56.07; 55.48 (Mean: 56.44, n = 7)	Recommended method
NRC-CNRC/ 11	59.33; 59.34; 58.89 (Mean: 59.19 , n = 3)	Recommended method
LNE/5	60; 60; (Mean: 60, n = 2)	Recommended method
BAM/not reported	29.0; 43.0; 50.0; 23.7; 30.2; 41.5 (Mean: 36.2, n = 6)	0.1g heated at 50 °C for 9 days; check weight every other day until constant
INTI/10	44.8; 42.3; 41.7 (Mean: 42.9, n = 3)	Recommended method
NIM/9	53.7; 53.6; 58.5; 57.4 (Mean: 55.8, n = 4)	Recommended method
NMISA/1	59.17; 59.54 (Mean: 59.36, n = 2)	Recommended method

Table 3. Results for the determination of total Se in CCQM-K60 wheat flour

Participant	Mass fraction, mg kg^{-1}	Standard uncertainty, mg kg^{-1}	Expanded uncertainty, mg kg^{-1}	Relative expanded uncertainty, %	k =
NMISA	16.69	0.26	0.52	3.12	2
BAM	16.72	0.415	0.83	4.96	2
NRCC	16.99	0.14	0.28	1.65	2
LGC	17.23	0.135	0.27	1.57	2
NMIA	17.28	0.24	0.50	2.89	2.02
LNE	17.33	0.23	0.46	2.65	2
NMIJ	17.37	0.065	0.13	0.75	2
NIM	17.40	0.10	0.20	1.15	2
INTI	21.50	0.36	0.72	3.35	2

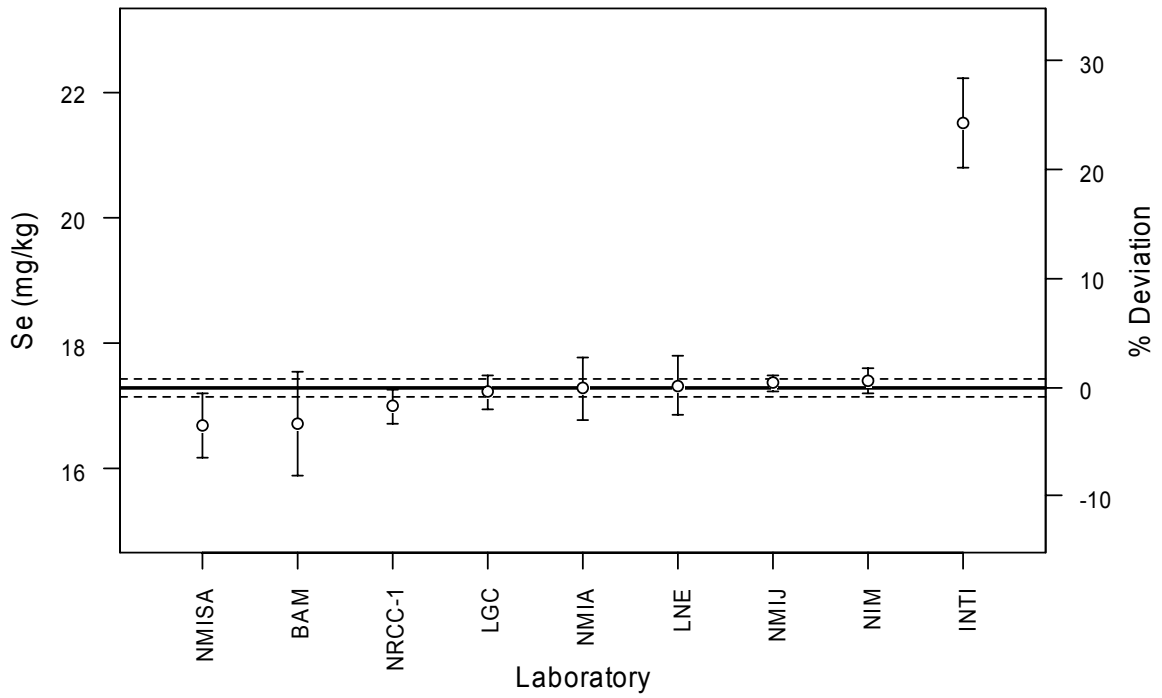

Figure 1. Results for total Se in K60 wheat flour. Error bars depict expanded uncertainties. The solid horizontal line is the suggested KCRV of 17.3 mg kg^{-1}; the dashed lines show the expanded uncertainty interval calculated with a coverage factor *k*=2.4

8.2. Results for SeMet

Results for SeMet (dry-weight basis) are summarised in Table 4 and graphically displayed in Figure 2. All uncertainties reported are expanded uncertainties for a coverage factor (k) of 2.

Table 4. Results for the determination of SeMet in CCQM-K60 wheat flour

Participant	Mass fraction, mg kg^{-1}	Standard uncertainty, mg kg^{-1}	Expanded uncertainty, mg kg^{-1}	Relative expanded uncertainty, %	k =
LGC	27.41	0.545	1.09	3.98	2
LNE	27.49	0.395	0.79	2.87	2
NMIJ	29.06	0.61	1.22	4.20	2
NIM	29.24	0.86	1.72	5.88	2

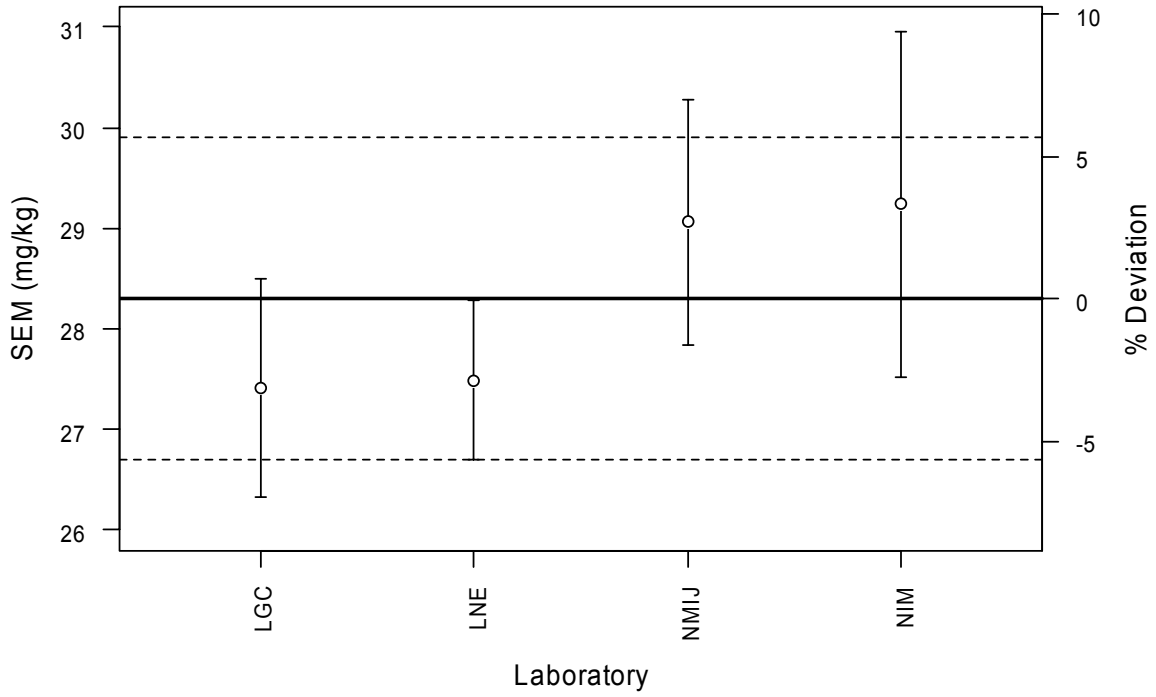

Figure 2. Results for SeMet in K60 wheat flour. Error bars depict expanded uncertainties The solid horizontal line is the suggested KCRV of 28.3 mg kg-1; the dashed lines show the expanded uncertainty interval calculated with a coverage factor k =3.2.

9. KCRV and associated uncertainty

9.1. Total Se

There is one clear outlier and some evidence of inconsistency among the remaining participants. A robust or outlier-rejected estimate is therefore indicated. The reported uncertainties are substantially different, making outweighed robust estimates difficult to justify. The modest inconsistency among the participants after outlier removal suggests that the uncertainty-weighted mean is inappropriate unless adjustments are made for the modest over dispersion.

A variety of robust estimates, with accompanying uncertainties, together with outlier-rejected statistics, are listed in Table 5. The mean and standard deviation of the mean are included for comparison. The weighted mean of all data was not calculated, as it is clearly inappropriate in the presence of marked inconsistency attributable to the extreme value at 21.5 mg kg^{-1}.

As expected, the mean, at 17.61 mg kg^{-1}, is higher than all other estimates, and the associated standard uncertainty inflated by the high value at 21.5 mg kg^{-1}. Robust and outlier-rejected estimates are broadly within the range 17.1 to 17.3 mg kg^{-1}, with standard uncertainties generally near 0.1 mg kg^{-1}. The simple outlier-rejected mean and Huber (H15) estimates are comparatively lower than weighted estimates because they are more influenced by the three lowest values with larger uncertainties than the weighted estimates, which tend to be close to the values with smaller

uncertainties; the uncertainty for the H15 estimate is also slightly influenced by the outlying high value and is therefore likely to be over-conservative. The median is similar to the weighted robust estimates as it is not greatly influenced by the two or three lowest values.

The choice of KCRV estimator is largely motivated by the most appropriate assumptions for the data. If it is reasonable to suppose that the majority of reported uncertainties are sound, with occasional underestimation, robust or outlier-rejected estimates with weighting should be preferred. For simplicity, the outlier-rejected weighted mean (17.3 mg kg^{-1} with standard uncertainty 0.07 mg kg^{-1}, with 7 degrees of freedom) is recommended. The KCRV is 17.3 mg kg^{-1} with standard uncertainty 0.07 mg kg^{-1}. With 7 degrees of freedom, a coverage factor of 2.4 is appropriate, giving an expanded uncertainty of 0.2 (to 1 significant figure). The corresponding value and expanded uncertainty interval are shown in Figure 1.

9.2. SeMet

With only four data points, broadly consistent with one another, neither outlier rejection nor robust estimation is necessary. Given the approximately similar uncertainties, weighted estimation is also of little value. For these four observations, therefore, the simple arithmetic mean is suggested as the KCRV, with uncertainty based on the standard deviation of the mean. The suggested KCRV for selenomethionine is accordingly 28.3 mg kg^{-1} with standard uncertainty 0.5 mg kg^{-1}. With three degrees of freedom, a coverage factor k=3.2 is appropriate, giving an expanded uncertainty of 1.6 mg kg^{-1}. The suggested KCRV and expanded uncertainty interval are shown in Figure 2.

Table 5. Classical and robust estimates and standard uncertainties for total Se

Estimator	**Value (mg kg^{-1})**	**Uncertainty (mg kg^{-1})**	**Notes**
Classical and Outlier-rejected estimates			
Mean	17.61	0.49	1
Outlier-rejected mean	17.13	0.10	2
Outlier-rejected weighted mean	17.29	0.07	2,3
Robust estimates			
Median	17.28	0.07	4
A15 mean	17.22	0.07	4,5
H15 mean	17.19	0.16	4,5
Huber M-estimate (weighted)	17.31	0.09	6

MM-estimate	17.29	0.07	6,7

Note 1: Standard uncertainty calculated as the standard deviation of the mean.

Note 2: Highest value (21.6 mg kg^{-2}) removed as outlier.

Note 3: The weighted mean is calculated using the reciprocal variance ($1/u^2$) as weights. The uncertainty for the weighted mean is based on the reciprocal sum of the weights, $\sqrt{1/\sum 1/u^2}$, adjusted for over-dispersion by multiplication by $\sqrt{\chi^2_{\text{obs}}/(n-1)}$.

Note 4: Uncertainty estimated from scaled median absolute deviation adjusted for asymptotic efficiency by multiplying by $\sqrt{\pi/2}$.

Note 5: Calculated using RobStat.xla with tuning parameter set to 1.5

Note 6: Calculated using iterative reweighting with prior weights set to $2/u^2$ and using a Huber tuning parameter of 1.345 (corresponding to 95% asymptotic efficiency).

Note 7: The MM-estimate is as implemented in the R MASS package. The estimator uses a Tukey bisquare weighting function with tuning parameter set for 95% asymptotic efficiency, with initial scale values set using a specific S-estimator. A full description is provided by Yohai [5]

Note 8: Statistical assessment used R version 2.7.1 [6], supplemented by the AMC Software Add-in RobStat.xls. Weighted M- and MM-estimates used the R MASS package [7].

10. Degrees of equivalence

10.1. Total Se

Given the values x_i (submitted by the participating NMIs) with standard uncertainties u_i (see Table 3) and a KCRV x_K calculated as the variance-weighted mean with standard uncertainty u_K (see section 9), the degree of equivalence d_i is $(x_i\text{-}x_K)$ with standard uncertainty $u(d_i) = (u_i^2\text{-}u_K^2)^{0.5}$ for results included in the estimate, and $u(d_i) = (u_i^2\text{-}u_K^2)^{0.5}$ otherwise. The negative sign arises as a consequence of correlation between the individual values and the KCRV. Since the uncertainty u_K has been adjusted for over-dispersion by multiplication by $B = \sqrt{\chi^2_{\text{obs}}/(n-1)}$ (see note 3 above), so that $u_K = B\sqrt{1/\sum 1/u_i^2}$, the degree-of-equivalence uncertainties are necessarily adjusted to $u(d_i) = (B^2u_i^2\text{-}u_K^2)^{0.5}$ (See note below)

Degrees of equivalence and their associated standard uncertainties are shown for Se in Table 6.

Table 6. Degrees of equivalence for total Se

Participant	d_i	$u(d_i)$	$U(d_i)$ (k=2)
NMISA	-0.61	0.37	0.74
BAM	-0.58	0.60	1.20
NRCC	-0.31	0.19	0.38
LGC	-0.07	0.18	0.37
NMIA	-0.02	0.34	0.68
LNE	0.03	0.33	0.65
NMIJ	0.07	0.06	0.13
NIM	0.1	0.13	0.25
INTI	4.2	0.53	1.05

Note: The degree-of-equivalence uncertainty calculation follows from $u_{\mathrm{K}} = B\sqrt{1/\sum 1/u_i^2}$, $= \sqrt{1/\sum 1/\left(B^2 u_i^2\right)}$; that is, the adjustment is equivalent to taking the estimated laboratory standard uncertainties as Bu. The degree-of-equivalence uncertainties are therefore larger than would be expected from participant uncertainties alone and cannot be taken as a guide to the validity of participants' uncertainty estimates.

A plot of the degrees of equivalence and expanded uncertainties with coverage factor k=2 is shown in Figure 3.

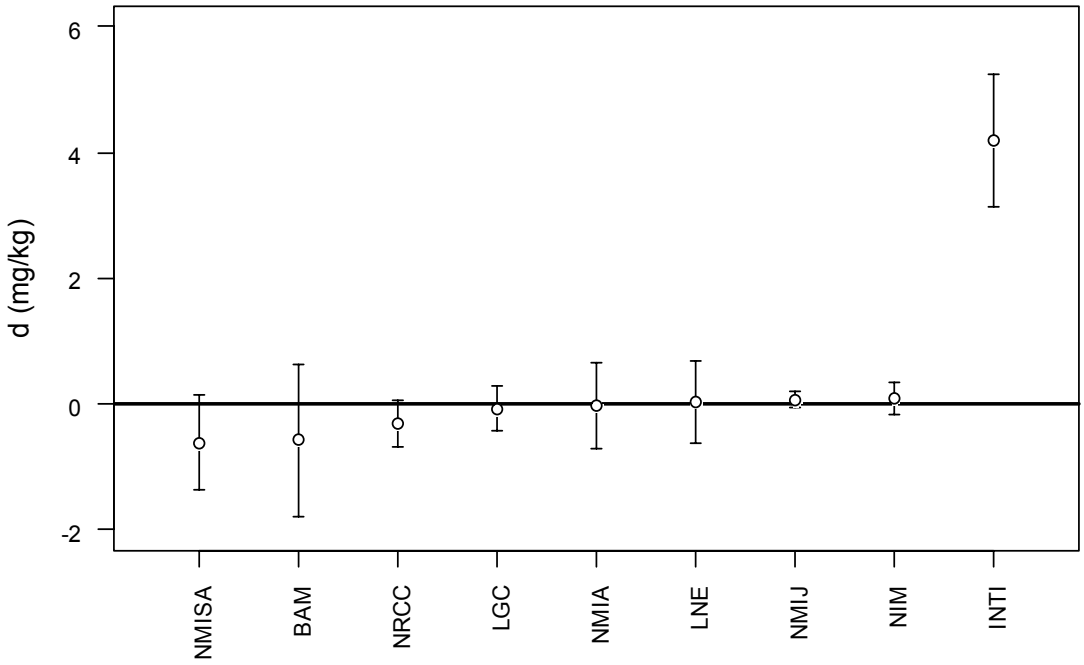

Figure 3. Degrees of equivalence for total Se

10.2. SeMet

For selenomethionine, the KCRV is calculated as the ordinary arithmetic mean, with uncertainty based on the dispersion of the four observations. To the level of approximation implied by this approach, degree-of-equivalence uncertainties are identical for all participants included in the estimated KCRV, with

$$u(d_i) = \sqrt{\left(1 - \frac{1}{m}\right) s(x)^2}$$

The degrees of equivalence and expanded uncertainty intervals (k=2) are listed in 7 and plotted in 4.

Note that as a consequence of the KCRV uncertainty using observed dispersion instead of participant uncertainties, degree-of-equivalence uncertainties can not provide a reliable assessment of the validity of participants' reported uncertainties.

11. Discussion

11.1. Total Se

Nine NMIs reported results for total Se in the CCQM-K60 wheat flour. Eight of the nine participating NMIs reported results for total Se within 3.5% deviation from the KCRV. For the

Table 7. Degrees of equivalence for SeMet

Participant	d_i	$u(d_i)$	$U(d_i)$ (k=2)
LGC	-0.89	0.86	1.7
LNE	-0.81	0.86	1.7
NMIJ	0.76	0.86	1.7
NIM	0.94	0.86	1.7

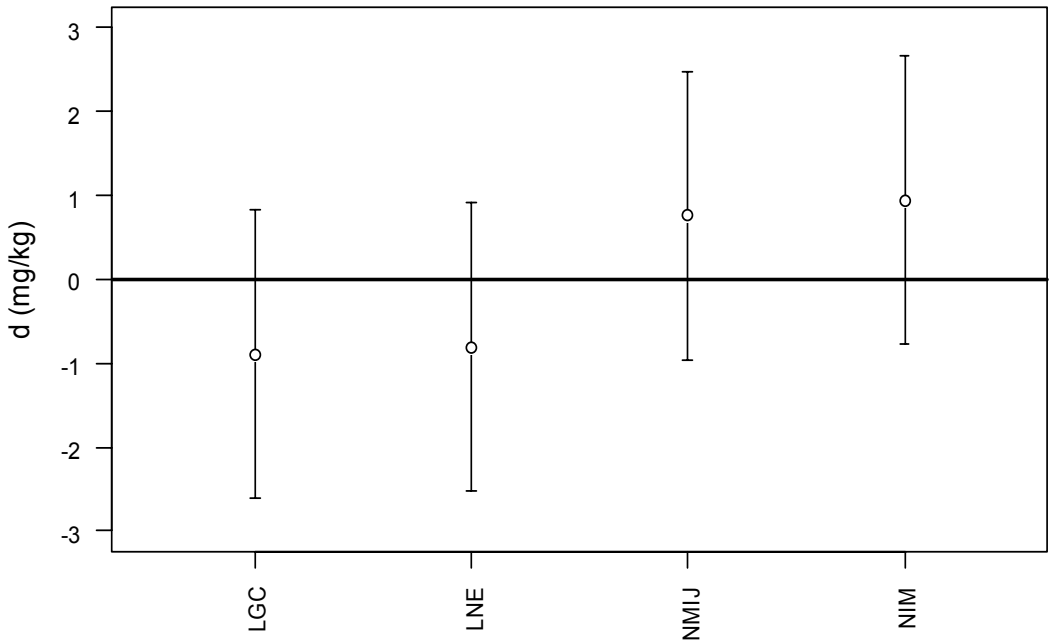

Figure 4. Degrees of equivalence for SeMet

seven participants that used IDMS and for BAM, which used INAA, the results and their expanded uncertainty overlapped with the window defined by the KCRV and its associated expanded uncertainty (± 1.1% relative to the KCRV) as shown in Figure 1. The relative expanded uncertainty was lower than 5% for all participants.

The result for INTI, which used GFAAS with external calibration, was determined to be an outlier. INTI, which reported an acceptable result for total Se in the CCQM-P86 tablets using FAAS with external calibration, provided a possible explanation for their unsatisfactory result for

total Se in the K60 wheat flour, suggesting that the lack of matching the nitric acid concentration of the calibration standards with that of the sample digests is responsible for a matrix-induced error in the determination of total Se using GFAAS with external calibration.

11.2. SeMet

Two out of the four K60 participant NMIs (LGC and NIM), had previously participated in the pilot study CCQM-P86 (Se and SeMet in selenised yeast tablets). Table 4 and Figure 2 show that the agreement of measurement results between NMIs is very good. The reported results fall within a range of ± 3.2% relative to the KCRV. Data presented in Table 4 show that the relative expanded uncertainties range from 2.87 to 5.88%, which appears very good considering that there is a 15-fold decrease in the total concentration of Se in the CCQM-K60 sample compared to the CCQM-P86 tablets [1] and the challenges posed by the accurate quantitation of SeMet in the complex wheat flour sample. A major contribution to such uncertainty was the concentration of the natural SeMet calibration standard. It is important to note that all NMIs followed methodology recommendations driven by the pilot CCQM-P86 study [1]; either hydrolysis with methanesulfonic acid or multi-step enzymatic extraction with quantitation by IC using HPLC-ICP-MS were used for determination of SeMet in the K60 sample. Since there are only a few KC results for SeMet, all the data for K60 and P86.1 are shown in Figure 5. Clearly, for seven out of ten P86.1 participants, results for the determination of SeMet in wheat flour were within the window defined by the KCRV and its associated expanded uncertainty. This supports the assertion that the KC reference value is an accurate representation of the true value. Moreover, this reflects the improvement in analytical capabilities of expert laboratories worldwide to accurately quantify this Se species at low mg/kg levels in complex food/supplement samples in comparison with previous studies.

12. Conclusions

The performance of the majority of the K60 participants was very good, illustrating their ability to obtain accurate results for such analytes in a complex food matrix (containing approximately 17 mg kg^{-1} Se, approximately 15-fold lower than that of the P86 tablets) with 5% and 6% expanded uncertainty for total Se and SeMet, respectively.

CCQM-K60 is a good example of a key comparison in parallel with a pilot study involving not only NMIs, but also expert laboratories worldwide, thus enabling them to assess their capabilities, discover problems and learn how to modify analytical procedures accordingly. The majority of NMIs have proven with their participation in CCQM-K60&P86.1 not only their measurement capability for trace total Se and Se species but also their capability with respect to sample preparation. Therefore, this key comparison will support CMCs of those NMIs in Table 1.

With regard to **'how far does the light shine'**, CMC claims based on SeMet measurements in this study may be applied to other foods or supplement matrices (e.g., vegetables, meat, cereals) provided that the concentration range is similar and due diligence is taken to ensure an appropriate extraction process is achieved and species specific spikes are available for quantitation by ID. Indeed, having accepted such conditions, application to quantitation of other organometallic

species and other elements in similar matrices should be possible with the same level of performance. CMC claims based on total Se measurements in this study may include other elements in similar matrices at a similar level of performance using the same measurement technique applied in CCQM-K60 provided that there are no additional factors (e.g. blank or dissolution issues).

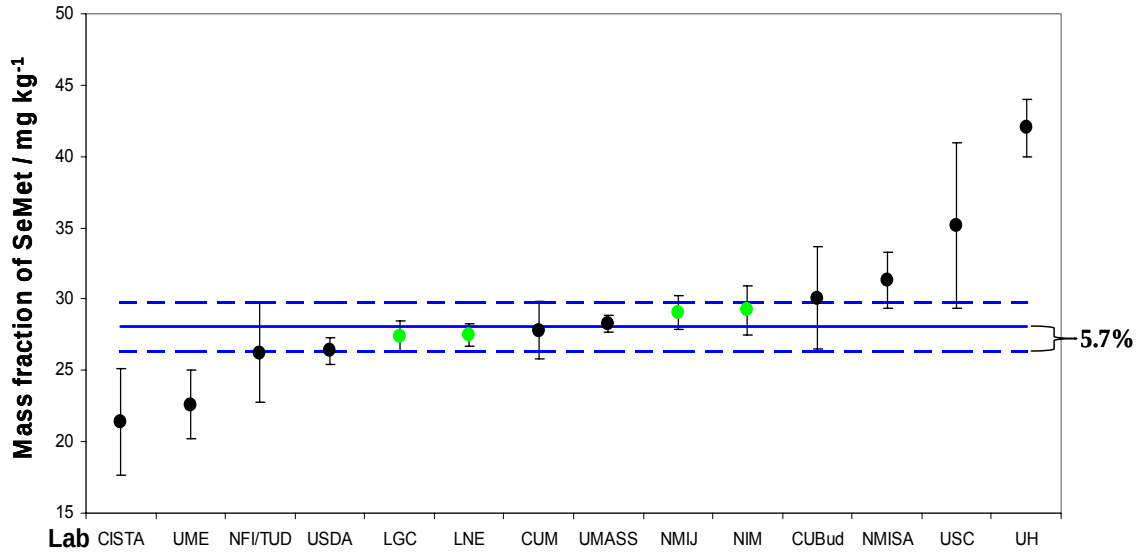

Figure 5. Selenomethionine results for the key comparison CCQM-K60 (light green) and parallel pilot CCQM-P86.1 (black) showing the performance of 14 individual laboratories against the key comparison reference value (solid blue line) and the expanded uncertainty interval for k=3.2 (defined by blue dashed lines)

13. Acknowledgements

The work described contains contributions of many scientists as described in Table 1.

LGC particularly acknowledges the efforts of the Reference Materials team, especially, G. Holcombe and D. Curtis for help with sending the samples and standards to the participants and S. Ellison for the statistical analysis of the K60 results. Special thanks to M. Broadley from Nottingham University, UK for providing the CCQM-K60 wheat grain and to E. Stokes from LGC for organising wheat flour preparation. The coordinating laboratories acknowledge, in particular, the efforts of R. Harte for sending the relevant documents to the participants and for collecting the data and R. Hearn, S. Merson and L. Evans for contributing to the homogeneity testing of the K60 sample. Also, special thanks to M. Sargent for very helpful discussions and for his assistance as chairman of the Inorganic Analysis WG of CCQM.

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Annex 1. Invitation to participate in the key comparison CCQM-K60

Key Comparison CCQM-K60 and Pilot Study CCQM-P86.1
Total Se and Se speciation analysis of Se-rich wheat flour

From:

The Co-ordinating Laboratories (LGC and NRC-CNRC)

To:

Members of the CCQM Inorganic Analysis Working Group (IAWG)

Other expert institutes

29 January 2008

(Reply is requested even if the institute does not wish to participate)

Dear Colleague,

This letter is an invitation to participate in the key comparison CCQM-K60 and the parallel pilot study CCQM-P86.1, total Se and Se speciation analysis of Se-rich wheat flour. These are follow-up studies to the previous pilot CCQM-P86 (on selenised yeast tablets), in which the ability of NMIs and expert laboratories worldwide to deliver accurate results for total Se and SeMet in such materials (containing approximately 300 mg kg^{-1} Se) with 10 % expanded uncertainty was demonstrated. Therefore, the IAWG agreed that it should be succeeded by a key comparison with its associated pilot study. The candidate wheat flour sample to be used in both CCQM-K60 and P86.1 is also of high complexity but contains much lower concentrations of Se than those encountered in the CCQM-P86 Se-yeast tablets (approx. 15-fold lower). This broadens the scope and degree of difficulty of earlier measurements in this field.

Organisations which are a national metrological institute (NMI), or an appropriate designated laboratory in accordance with the CIPM MRA, are invited to participate in the key comparison or the pilot study. Other expert institutes, from countries that are

members of the Metre Convention, may also participate in the pilot study provided that their contribution has added scientific value or where they may qualify later as a designated institute in the field under study. The process of nomination of expert laboratories for participation in a CCQM pilot study should preferably be nationally co-ordinated. Expert laboratories which respond to this invitation are requested to inform their national metrological institute of their participation in the pilot study and to advise the co-ordinating laboratory of the appropriate contact at their NMI. In accordance with the requirements of the CCQM President, the IAWG Chairman will be asked to formally notify each relevant NMI of the participation by an expert institute from their country.

The results of the key comparison will be presented in the form of a report to the CCQM, available to participants and to members of the IAWG. The report will identify the results with the names of the participating institutes. Preliminary (A) and final (B) drafts of the report will be circulated to participants for comment and correction. The approved report will be submitted to the BIPM's Key Comparison Database (KCDB) and the results will be publicly available. A similar report will be prepared for the pilot study, for participants and members of the IAWG. A scientific paper describing the study may be published separately in an appropriate journal provided participants agree to this.

A short description of the study is given below. A detailed study protocol will be sent to registered participants later.

Background

Due to the often-insufficient content of Se in the ordinary diet, it is advantageous to add selenium through production of food bio-fortified with this essential mineral. Wheat is one of the most important selenium sources for humans, with selenomethionine (SeMet) as the predominant selenium species. The decline in the importation of Se-rich high protein wheat flour from North America and Canada is reported to have contributed to the substantial fall of selenium in the European mammalian diet. Therefore the production of Se-enriched wheat flour offers an effective biofortified food for increased human Se-intake. It is likely that within Europe new products of this type will be sold to the public but accurate assessment of Se, and especially the SeMet content, in food enriched with Se presents a significant analytical challenge. Major problems are the relatively low food-Se concentration and the lack of knowledge on the efficiency of the extraction procedures used for quantification of selenomethionine in complex food-type matrices. Participants will be required to determine the analytes in the matrix material using their own calibration solutions. The proposed study will fully test the ability of laboratories to accurately quantify total Se and seleno-amino acids of relevance to health, which are present at low parts per million levels in complex food bio-fortified with selenium.

Sample materials

The matrix sample will be a wheat flour material to be characterised for its total Se and selenomethionine content. The form of the sample will be 15 g of dry wheat flour contained in a 30 ml amber glass bottle. The homogeneity of the candidate material will be fully investigated before it is distributed. One bottle will be sent but, if requested, the co-ordinating laboratory may be able to provide additional bottles.

Other materials

The existing NRC certified reference material of selenised yeast (SELM-1) will be provided to new participants, who did not contribute to the pilot CCQM-P86, to assist in method evaluation. Participants choosing to use isotope dilution for the selenomethionine analysis will be provided with an isotopically enriched ^{76}Se standard of selenomethionine.

Measurands

Total Se (~20 mg kg^{-1}) and selenomethionine (~ 10 mg kg^{-1}) in a wheat flour sample.

Method of analysis

Participants are encouraged to use any method of their choice. Results of analysis for the matrix sample should be corrected for dry weight and a moisture content determination procedure will be provided. It is recommended that preparation and dilution of solutions be carried out by weighing.

Time schedule

Deadline for registration of participation:	31 March 2008
Shipment of samples:	April 2008
Deadline for delivery of results:	31 August 2008
Draft report:	October 2008
Final report:	**December 2008**

Registration

If you wish to participate in the key comparison K-60 or pilot study CCQM-P86.1 **please use the registration sheet provided** and specify which analyte(s) you wish to analyse and which method(s) will be used. Institutes not wishing to participate are also requested to sign and return the registration form.

Please return the form by fax no later than 31 March 2008 to:

Dr Heidi Goenaga-Infante
LGC Limited
Queens Road
Teddington,
Middlesex
TW11 0LY
United Kingdom
☎ +44-20-8943 7555
Fax: +44-20-8943 2767
E-Mail: hgi@lgc.co.uk

I look forward to your participation in this study.

Yours sincerely,

Heidi Goenaga-Infante

Annex 2. Protocol distributed to participants

Key Comparison CCQM-K60 and Pilot Study CCQM-P86.1
Analysis of total Se and selenomethionine in selenised wheat flour

Protocol

Introduction

Due to the often-insufficient content of Se in the ordinary diet, it is advantageous to add selenium through production of food bio-fortified with this essential mineral. Wheat is one of the most important selenium sources for humans, with selenomethionine (SeMet) as the predominant selenium species. The decline in the importation of Se-rich high protein wheat flour from North America and Canada is reported to have contributed to the substantial fall of selenium in the European mammalian diet. Therefore the production of Se-enriched wheat flour offers an effective bio-fortified food for increased human Se-intake. It is likely that within Europe new products of this type will be sold to the public but accurate assessment of Se, and especially the SeMet content, in food enriched with Se presents a significant analytical challenge. Major problems are the relatively low food-Se concentration and the lack of knowledge on the efficiency of the extraction procedures used for quantification of selenomethionine in complex food-type matrices.

The study will fully test the ability of laboratories to accurately quantify total Se and seleno-amino acids of relevance to health, which are present at low parts per million levels in complex food bio-fortified with selenium. The wheat flour sample to be used in both CCQM-K60 and P86.1 is of high complexity and contains much lower concentrations of Se than those encountered in the CCQM-P86 Se-yeast tablets (approx. 15-fold lower). This broadens the scope and degree of difficulty of earlier measurements in this field. Participants will be required to determine the analytes in the matrix material using their own calibration solutions.

Sample

The matrix sample is a wheat flour material to be characterised for its total Se and selenomethionine content. The form of the sample is 15 g of dry wheat flour contained in a 30 mL amber glass bottle. The homogeneity of the candidate material was fully investigated before it was distributed. One bottle will be sent to each participant but, if requested, the co-ordinating laboratory may be able to provide additional bottles. The recommended storage temperature is 4 °C.

Other materials

1. The CCQM-P86 selenised yeast tablets are available as a certified reference material. Participants who did not take place in CCQM-P86 will be sent one blister of tablets. This sample is intended to assist participants in their own method evaluation and there is no need to report results for it. However, information about the experience of the participants in analysing this material would be useful in evaluating any inconsistencies in the results for the CCQM-P86-K60 wheat flour. To assist in providing this information, there is space at the end of the results form to include any data obtained for Se and/or SeMet in the Se-yeast tablets. Please include any additional comments you may have about the method(s) used or your experience with the analysis of this material. The recommended storage temperature is 4 °C.

2. Participants who indicated at registration that they intend to use isotope dilution for the selenomethionine analysis will also be provided with an isotopically enriched ^{76}Se standard of selenomethionine (1.5 mg). This material is not certified for isotope composition which, if required, must be determined by each participant. The recommended storage temperature is -20 °C.

Analysis

At least three replicate analyses should be carried out on the wheat sample. Participants are free to use any suitable method but **please include a full description of your method of analysis** when reporting the results. Participants may, if they wish, obtain results by more than one method for each analyte. If these are reported separately, only results from one method may be submitted for CCQM-K60; any results from additional methods should be submitted as part of CCQM-P86.1. A full uncertainty budget should also be included with your results, as indicated below. Results should be reported on a dry weight basis. The recommended protocol for moisture determination is given below and for this part of the study participants are requested to adhere to the protocol to ensure consistent data between laboratories.

Determination of wheat flour moisture content

The moisture content of the wheat should be determined for two independent wheat flour sub-samples from one bottle, independently of the sub-samples used for total Se and/or SeMet determination. The bottle should be thoroughly shaken before a wheat flour sub-sample of 0.5 g is weighed and heated at 100 °C in an oven for 3 h. The sample should then be cooled down to room temperature in a desiccator and weighed. The procedure should be repeated with 1 h heating cycles until constant weight is reached (difference between two consecutive values ≤ 0.0003 g). The overall drying time should be reported with the moisture content and should not normally exceed 8 h.

Please note that it is important to minimise uptake of water during the moisture determination procedure and, for laboratories in which the humidity is high, it is advisable to carry out moisture determination at the time of Se and/or SeMet analysis to ensure the same uptake of moisture by all the samples. It is recommended to weigh the sample as quickly as possible in a pan with a lid for the remainder of the procedure. The lid should

remain on the pan during all stages except heating in the oven, when it is placed under the pan.

Uncertainty Evaluation

Each laboratory should make an assessment of the experimental uncertainty according to ISO principles (Guide to the Expression of Uncertainty in Measurement, ISO, Geneva, 1993, ISBN 92-67-10188-9). Each variable contributing to the uncertainty of the results should be identified and quantified in order to be included in the combined standard uncertainty of the result. A full uncertainty budget must be reported, as part of the results.

Contributions to the overall uncertainty will arise from the repeatability of the sample preparation, the repeatability of instrumental determination, determination of masses and volumes, concentration of primary and internal standards, and any other parameter specific to each method of analysis chosen by the participant.

Results should be submitted using the results report form provided and sent to **Rita Harte (E-mail: Rita.Harte@lgc.co.uk) at LGC**, by post, e-mail or fax, **no later than 31 August 2008.**

Study Co-ordinator

Dr. Heidi Goenaga-Infante

LGC Limited

Queens Road

Teddington

Middlesex

TW11 0LY

United Kingdom

☎ +44-20-8943 7644

Fax: +44-20-8943 2767

E-mail: hgi@lgc.co.uk

Annex 3. Results report form

Key Comparison CCQM-K60
Analysis of total Se and selenomethionine in selenised wheat flour

Results Report

Please return this results report by 31 August, 2008.

NAME :
INSTITUTE :
DEPARTMENT :
ADDRESS :

COUNTRY :
TEL :
FAX :
E-MAIL :

Report your results and uncertainties in mass fraction on a dry weight basis using the units in the table below. Details concerning the analysis of replicates, details of the method, calculation of results, and associated uncertainties should be given in the following pages of your report.

Analyte	Matrix	Unit	Mass fraction	Expanded uncertainty	k
Total Se	Wheat flour	mg kg^{-1}			
SeMet	Wheat flour	mg kg^{-1}			

Method used for each analyte:
Sample preparation:
Determination:

DATE:

SIGNATURE:

Additional information

Results for the analysis of replicate samples

Date of Analysis:

Determination	Result Se (mg kg^{-1}) in wheat flour	Result SeMet (mg kg^{-1}) in wheat flour	Sample no.
1			
2			
3			

Report at least 3 replicates. If more than 5 determinations are carried out, please insert more lines

Results for moisture determination in wheat flour

Date of Analysis:

Determination	Drying time (h)	Result (g kg^{-1})	Sample no.
1			
2			
3			
4			

If more than 4 determinations are carried out, please insert more lines

Method(s) used for Total Se and SeMet analysis

Further information and details can be added in pages below, or in a separate report if preferred. If you use a separate report, please provide a complete description of the method(s) used for the determination, including the following information as appropriate:

1. Details of sample handling, including sample digestion/extraction and weight taken.
2. Measurement technique.
3. Calibration procedure.
4. Details of the source of your calibration standard(s) together with the purity and associated uncertainty.
5. Any other relevant information.

An uncertainty calculation should be prepared as described in the study protocol.

Description

Brief outline, or a reference to a published procedure, any special precautions to minimise loss or transformation of analyte, interferences etc.

Sample digestion or extraction:

- Weight taken:

- Extraction conditions including extractant/acids used, digestion/extraction time:

- Procedure for extract/digest handling and preservation:

- Time between extraction and measurement:

Measurement procedure:

Principle (e.g. ICP-MS):

Conditions used:

Instrument used:

Calibration standard(s) used :

Source, purity and uncertainty of standard(s):

Typical standard solution concentration :

Typical uncertainty of concentration :

Type of calibration : IDMS [ ]; External calibration [ ]; Standard addition [ ]
- one-point [ ]
- two-point [ ]
- multi-point [ ]

Method used for moisture determination

1. **If the suggested protocol was not followed exactly,** please specify below the options you have used in the procedure.

a) Type of oven and temperature ……..

b) Drying cycle and time interval ……..

c) Other (please give details)……

……

Please add below any aspects of the specified procedure that could not be followed, or any modifications made.

2. **If another method was used**, please provide full details of the method below or in a separate report.

Additional information on Se-yeast tablets (QC sample)

Participants who used this material in their method development are requested to provide the following information where it is available.

1. Values for the analysis of Se and/or SeMet (expressed on a dry weight basis)

Determination	Se (mg kg^{-1})	SeMet (mg kg^{-1})
1		
2		
3		

2. Value for moisture determination: ……g kg^{-1}

3. Differences (if any) in the method(s) used from the information given on page 3

4. Any problems experienced in analysing the material

5. Other comments about the material and its use for method development

Annex 4. Overview of analytical methods and instrumental techniques used by the participants

Total Se

Lab	Digestion method	Instrumentation	Calibration
NMIA	Acid digest, microwave	ICPMS (crc)	IDA (^{74}Se spike)
LGC	Acid digest, microwave	ICPMS (crc)	IDA (^{77}Se spike)
NMIJ	Acid digest, microwave	ICPMS (crc)	IDA (^{82}Se spike)
NRC-NRCC	Acid digest, microwave	ICPMS GFAAS	IDA (^{82}Se spike) SA (multi-point)
LNE	Acid digest, microwave	ICPMS (crc)	IDA (^{78}Se spike)
BAM	None	INAA	EC (one point)
INTI	Acid digest, microwave	GFAAS	EC (multi-point)
NMISA	Acid digest, microwave	Hydride generation-ICPMS (magnetic sector)	IDA (^{82}Se spike)
NIM	Acid digest, microwave	ICPMS (crc)	IDA (^{78}Se spike)

Selenomethionine

Lab	Extraction / derivatization	Instrumentation	Calibration
LNE	Protease+lipase, 16 h at 37 °C +mixing (repeated three times)	HPLC-ICPMS (cc)	IDA (^{76}SeMet spike); natural SeMet (99.9% Acros)
LGC	Protease+lipase+driselase, 20 h at 37 °C + mixing (repeated two times)	HPLC-ICPMS (cc)	IDA (^{76}SeMet spike); natural SeMet (> 99.0% Sigma)
NIM	Protease+lipase+driselase, 20 h at 37 °C+mixing (repeated two times)	HPLC-ICPMS (cc)	IDA (^{76}SeMet spike
NMIJ	Methanesulfonic acid, 16 h, reflux at 130 °C, N_2 gas purge	HPLC-ICPMS (cc)	IDA (^{76}SeMet spike