CERTIFICATE OF ANALYSIS

ERM®- BC210a

Wheat Flour - Selenium and Selenomethionine		
	Certified value [1,2,3] (mg/kg)	Uncertainty [2,3,4] (mg/kg)
Total selenium	17.23	0.91
Selenomethionine	27.4	2.6
1) The certified values are mass fractions determined using isotope dilution mass spectrometry.		
2) The certified values are considered traceable to the SI through the use of pure reference materials (see page 2/3).		
3) Results are expressed on a dry weight basis.		
4) The quoted uncertainty is the half-width of the expanded uncertainty interval, calculated using a coverage factor (*k*) of 2.12, which gives a level of confidence of approximately 95 %.		

This certificate is valid for 12 months from the date of shipment provided the sample is stored unopened under the recommended conditions.

The minimum amount of sample to be used is 0.3 g.

NOTE

European Reference Material ERM®-BC210a was produced and certified under the responsibility of LGC according to the principles laid down in the Technical Guidelines of the European Reference Materials® co-operation agreement between BAM-LGC-IRMM.

Accepted as an ERM®, Teddington, June 2011.
Latest certificate revision January 2023.

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DESCRIPTION OF THE SAMPLE

Selenised wheat was obtained from a UK university. The grain was cleaned with water, milled at a temperature between 18 °C and 20 °C, and 60 % relative humidity, and sieved twice to a final particle size of 140 µm. The bulk material was thoroughly homogenised, freeze dried to a moisture content of approximately 5 % (m/m), and sub-sampled into 30 mL amber glass bottles fitted with tamper-evident screw caps. Approximately 400 units of 15 g were produced. Samples were irradiated at a dose of 25 - 40 kGy.

INTENDED USE

The primary use of this certified reference material is for the validation of methods for the determination of selenium and selenomethionine in food materials and dietary supplements. The material may also be applicable to other matrices where suitable reference materials are not available.

ANALYTICAL METHOD USED FOR CERTIFICATION

Selenomethionine by enzymatic hydrolysis:
An appropriate quantity of ^{76}selenomethionine solution was added to approx. 0.3 g of wheat flour, both being accurately weighed to give a $^{78}Se/^{76}Se$ ratio of approximately 0.3. This was followed by the addition of 60 mg of protease and 30 mg of lipase in 10 mL of a 30 mM Tris-HCl buffer solution (pH 7.5), previously degassed. Incubation at 37 ºC was then carried out in the dark for 20 hours. During enzymolysis, the sample slurries were constantly and gently homogenised, using a rotary shaker set at 60 rpm. Hydrolysed samples were centrifuged at 3000 rpm for 30 minutes and the supernatants filtered and stored at -20 °C. The residue was then subjected to proteolytic digestion with one more fresh enzymatic solution also containing 100 mg of driselase (used to release cell wall-bound components). Finally, the two supernatants were pooled, filtered and diluted 5-fold prior to their analysis for selenomethionine content.
Two selenomethionine standards with natural isotopic composition were purchased from Sigma-Aldrich (St. Louis, MO, USA) and LGC Mikromol GmbH (Luckenwalde, Germany). The LGC Mikromol material was used as the calibrant.
A ^{76}Se enriched selenomethionine spike material was purchased from IsoSciences (King of Prussia, PA, USA).
Quantification of selenomethionine in the digests was performed by ion pairing reversed phase HPLC-ICP isotope dilution MS. To do this, a 50 µL portion of the sample blend or calibration blend was analysed by RP-IP HPLC-ICP MS at a flow rate of 1 mL/minute using a 98:2 water:methanol mixture containing 0.1 % (v/v) trifluoroacetic acid (as ion pairing agent) as the mobile phase. Quantification was performed using a double match species-specific IDMS methodology. The selenium isotopes 76, 78 and 82 were measured in transient analysis mode using ICP MS. The isotope ratios 78/76 and 82/76 were used for quantification by IDMS analysis.

Total selenium by microwave digestion:
For each digestion approximately 0.4 g of wheat flour was accurately weighed into a teflon microwave digestion vessel followed by the addition of an appropriate quantity of ^{77}Se spike solution to give a $^{78}Se/^{77}Se$ isotope ratio of 1.0.
5 mL of an acid mixture containing HNO_3 (20 mL), H_2O_2 (15 mL), HCl (2 mL) and HF (2.4 mL) was added to each vessel which was then heated under the following conditions:

Step 1	10 minutes at 1000 W
Step 2	5 minutes at 0 W
Step 3	20 minutes at 1400 W
Step 4	10 minutes at 0 W

Within each microwave batch (maximum of 8 vessels) at least one blend of a matrix reference material, one blend of an independent standard solution and at least one blank were prepared.

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Two independent selenium standards with natural isotopic composition were used for the total selenium determinations. These were SRM3149 (10.11 ± 0.02) mg/g Se purchased from NIST (Gaithersburg, USA) and Se pellets with a certified purity of 99.999+ % purchased from Aldrich Company (Milwaukee, US). The NIST material was used as the calibrant. The ^{77}Se enriched total selenium spike material (68.7 % as ^{77}Se) was obtained from AEA Technologies (Didcot, UK). After digestion, the digest solutions were diluted to 50 g with deionised water before measurement. Selenium was measured using an Agilent 7500ce ICP-MS with collision cell.

CONFIRMATORY ANALYSIS

The samples were analysed by several other laboratories as part of the CCQM Key Comparison Study (K60). The results were in good agreement with the certified values.

The data are available on the BIPM website at
http://www.bipm.org/utils/common/pdf/final_reports/QM/K60/CCQM-K60.pdf

SAFETY INFORMATION

Refer to material safety data sheet.

ACCREDITATION

The certified values on this document are within LGC's scope of accreditation to ISO 17034.

INSTRUCTIONS FOR USE

The bottle should be brought to room temperature (20 ± 5) °C, and shaken gently before removing a sub-sample. To minimise any change in moisture content, the lid should be replaced as soon as possible after removing a sub-sample.

Moisture should be determined at the same time as selenium and/or selenomethionine, on a separate portion. Approximately 0.5 g of sample should be weighed into a moisture pan. Heat the pan for 3 hours at 100 °C. Remove the pan, place with the lid on in a desiccator, and reweigh when cooled (~20 minutes). Continue drying, in the oven, at 100 °C, and reweigh hourly until constant weight is achieved, i.e. results do not differ by more than 0.001 g.

STORAGE CONDITIONS

The material should be stored in the unopened bottle at (-20 ± 5) °C until it is required for use, and used immediately on opening. The opened bottle should not be stored for reuse.

CERTIFICATE REVISION

This certificate was revised in April 2018 to clarify the advice for users regarding opened units.

In January 2023, the UKAS symbol was updated, and a paragraph added to clarify the values within scope of LGC's ISO 17034 accreditation.

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Unit Number	Date of Shipment

LEGAL NOTICE

The values quoted in this certificate are the best estimate of the true values within the stated uncertainties and based on the techniques described herein. No warranty or representation, express or implied, is made that the use of the product or any information, material, apparatus, method or process which is the subject of or referred to in this certificate does not infringe any third party rights. Further, save to the extent: (a) prohibited by law; or (b) caused by a party's negligence; no party shall be liable for the use made of the product, any information, material, apparatus, method or process which is the subject of or referred to in this certificate. In no event shall the liability of any party exceed whichever is the lower of: (i) the value of the product; or (ii) £500,000; and any liability for loss of profit, loss of business or revenue, loss of anticipated savings, depletion of goodwill, any third-party claims or any indirect or consequential loss or damage in connection herewith is expressly excluded.

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