CERTIFICATE OF ANALYSIS

FOR CERTIFIED REFERENCE MATERIAL

NMIA MX008

Trace Elements in Nutritional Supplement

Batch No. 2008.01

(i) Description of the material

Certified reference material MX008 is a nutritional supplement powder bottled in units of approximately 25 g. The material consists of a typical pharmaceutical excipient containing dietary nutrients and essential elements. A certified value is provided for the mass fraction of Cr. Information values are given in the appendix for the mass fractions of Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, P, Sr, Zn and for the mass fraction of moisture.

(ii) Certified property values

The certified values for mass fraction of Cr in MX008 are given in Table 1. The mass fraction of Cr is given on a dry mass basis and on an undried basis. Dry mass basis measurement gives a more accurate characterization of MX008 as the analyte mass fraction is not affected by moisture content variation over time. Instructions for use of dry mass basis and undried basis certified values are described in Section (v).

Table 1: Certified values for MX008.
Measurement uncertainty is reported as a 95% coverage interval.

	Mass fraction (dry mass basis)	Mass fraction (undried basis)
	mg/kg	mg/kg
Cr	14.3 ± 0.8	13.3 ± 1.0

Cr mass fraction was determined by isotope dilution ICP-MS analysis and is traceable to SI units. Details of the analytical methodology and measurement uncertainty estimation are provided in Sections (vii) and (x).

(iii) Expiration of certification

The MX008 certified values are valid until **15 March 2022** provided the material is stored and handled in accordance with the instructions given in this certificate. MX008 may be reassessed prior to the expiration date to investigate extension of the certification period.

(iv) Intended use

Certified reference material MX008 is intended to be used to verify and/or validate analytical methods for elemental analysis in nutritional supplements, pharmaceutical products or similar sample types.

(v) Instructions for use

Storage— MX008 should be stored tightly sealed in the original bottle at 20 °C ± 5 °C. Dry mass basis measurements are not affected by the humidity of storage conditions whereas undried basis measurements rely on avoiding extremes of humidity. Storage in a desiccator is therefore not recommended if undried basis results are to be used. MX008 should not be exposed to direct sunlight or intense sources of radiation.

Subsampling— The contents of the MX008 bottle should be mixed thoroughly shortly before subsampling. For trace element analysis, a minimum sample size of 0.5 g (dry mass basis) should be used for MX008 analysis. Trace element certified values may not be valid for sample sizes less than 0.5 g.

Contamination minimisation— MX008 should only be opened to the atmosphere in a clean environment such as a ULPA-filtered laminar flow hood. For trace element analysis, MX008 should be subsampled and handled in such a way as to avoid contamination of both the bulk material and the subsample.

Use of dry mass basis certified values— To remove the effect of moisture content variation on trace element mass fraction measurements, correction for moisture content should be applied. Gravimetric moisture content determination should be performed on a separate portion of MX008 to that used for trace element analysis to avoid contamination and/or loss of analytes through vaporization. A sample size not less than 0.5 g (dry mass basis) should be used for moisture content measurement. The drying protocol for MX008 is oven-drying at 100 °C for 24 hours. Longer oven drying should not be used as mass loss will continue but does not correspond to loss of moisture from the reference material. The moisture content measured at time of analysis should then be used to calculate the moisture content correction factor to convert undried basis measurements to dry mass basis measurements.

Use of undried basis certified values— Where correction for MX008 moisture content is not applied to analytical results, comparison can be made to the undried basis certified values. In this case, possible moisture content variation over time is accounted for in the measurement uncertainty estimate of the certified value. Consequently, the relative expanded uncertainty is larger for the undried basis certified values than for the dry mass basis certified values.

(vi) Production of MX008

MX008 is a composite of commercial dietary supplement products, pharmaceutical formulations and pharmaceutical placebo material. The major excipient component is calcium phosphate. The excipient contributes percent quantities of Ca, P, Mg, K and also contains silicates. Starting materials were crushed to a powder before combining. The bulk powder was blended thoroughly then passed through a coarse sieve (1000 µm) and a fine sieve (150 µm). The sieved powder was homogenised by tumbling in a drum-hoop mixer for several hours then bottled into 25 g units using a rotary sample divider. The entire production batch was gamma-irradiated (25 kGy minimum) to prevent microbial activity. Each unit of MX008 is labelled with a unique bottle number.

(vii) Analytical method

The certified Cr mass fraction of MX008 was measured by double isotope dilution ICP-MS [1]. ^{53}Cr-enriched chromium was used as isotope dilution internal standard added prior to sample digestion. IDMS measurements were made using a sample size of 0.5 g. The sample was digested with nitric acid and microwave heating in sealed high-pressure vessels. A small amount of undissolved silicate residue from the excipient was left after digestion. More aggressive digestion using hydrofluoric acid demonstrated that this undissolved residue did not contribute to the total Cr content.

Cr isotope ratio measurements were made using ICP-SF-MS. The ^{52}Cr/^{53}Cr isotope ratio was measured for isotope dilution quantification. Instrumental analysis was optimised to minimize systematic error from spectral interferences, linearity, mass bias and detector deadtime.

Analysis was calibrated using a Cr standard solution traceable to SI units (NIST SRM 3112a lot no. 030730). The ^{53}Cr-enriched material was from Oak Ridge National Laboratory (batch no. 103580, ^{53}Cr = 95.74%).

(viii) Homogeneity assessment

Homogeneity assessment for MX008 certified values was conducted according to ISO Guide 35 [2]. Ten sample bottles of MX008 were selected using a stratified random sampling plan to assess the homogeneity of the reference material batch. Measurements were made on duplicate subsamples taken from these 10 bottles using the method described in Section (vii). Analysis of variance (ANOVA) was used to estimate the measurement uncertainty due to method precision and reference material inhomogeneity.

(ix) Stability assessment

The main source of instability for MX008 is variation of moisture content in transport and/or storage which will directly affect elemental mass fraction values. Applying a moisture content correction and using dry mass basis certified values is recommended to negate the effect of moisture content variation. Undried-basis certified values include a measurement uncertainty contribution for variation in the moisture content of MX008 under recommended storage conditions.

The long-term stability of certified values has been demonstrated through periodic analysis of MX008 for twelve months. Statistical assessment following ISO Guide 35 [2] showed that the linear regression model for stability assessment data had a gradient not statistically different from zero (α = 0.05). The gradient of the linear regression stability model for Cr was 0.04 mg/kg/year.

The long term stability of certified values of MX008 was reassessed in January 2014 and March 2017. Measurements indicated that it was not valid to extend the expiry date of the certified value for mass fraction of Se. Based on this long term stability testing the certificate has been re-issued for Cr only.

(x) Measurement uncertainty

Measurement uncertainty was estimated according to international standards [2,3] and NMIA standard procedures. All factors that could reasonably be expected to affect the measurement result were identified and the standard uncertainty of each was estimated from experimental data. The standard uncertainty estimates were combined and expanded to a 95% coverage interval using the coverage factors given in Table 2.

For the MX008 certified values, the measurement uncertainty contributors examined were the calibration standard, gravimetric preparation, isotopic composition, moisture content, isotopic equilibration, isotope ratio measurement, method precision, method bias, between-bottle homogeneity and long-term stability.

Table 2: Coverage Factors for MX008 Certified Values.

	Coverage Factor	
	Mass fraction (dry mass basis)	Mass fraction (undried basis)
Cr	2.10	2.18

(xi) Metrological traceability

Certified values are traceable to the SI units for mass (kilogram) and amount of substance (mole). Gravimetric preparation is traceable to the SI kilogram through balance calibrations. Isotope ratio measurements are traceable to the mole through double isotope dilution (a primary ratio method). Isotope dilution mass fraction quantification is traceable to SI units through NIST single element calibration solutions. For dry mass basis certified values, moisture content correction is traceable to the method described in Section (v).

(xii) Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

References

1. L.G. Mackay, C.P. Taylor, R.B. Myors, R. Hearn and B. King; "High accuracy analysis by isotope dilution mass spectrometry using an iterative exact matching technique"; *Accred. Qual. Assur.* (2003) 8:191-194.
2. *Reference materials — General and statistical principles for certification*; ISO Guide 35, 3rd edition (2006).
3. *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*; JCGM 100, 1st edition (2008).

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APPENDIX

Additional Information

Additional property values for MX008 are given in Table 3 and Table 4. These values do not satisfy NMIA criteria for certification.

Information values for trace and major elements in MX008

Information values for 14 additional trace and major elements in MX008 are given in Table 3. Information values were obtained from interlaboratory testing coordinated by NMIA in which MX008 was the test sample. Information values are given on a dry mass basis and also on an undried basis. The number of results used to generate the information value for each element is given in Table 3.

Table 3: Information values for MX008 from interlaboratory testing. Measurement uncertainty is reported as a 95% coverage interval.

	Mass fraction (dry mass basis)	Mass fraction (undried basis)	
	mg/kg	mg/kg	*n*
Al	36 ± 5	33 ± 5	19
B	85 ± 7	79 ± 8	21
Ba	4.5 ± 0.3	4.2 ± 0.4	20
Ca	42000 ± 3000	39000 ± 3000	21
Cu	94 ± 6	87 ± 7	22
Fe	1040 ± 60	960 ± 70	21
K	8200 ± 600	7600 ± 600	13
Mg	13000 ± 500	12000 ± 700	20
Mn	556 ± 21	516 ± 31	21
Mo	6.2 ± 0.5	5.7 ± 0.5	21
Na	3800 ± 400	3600 ± 400	14
P	30000 ± 1200	27800 ± 1700	20
Sr	20.6 ± 0.8	19.2 ± 1.2	19
Zn	3300 ± 120	3060 ± 180	21

Interlaboratory testing— Data for information values was collected from two NMIA interlaboratory studies using MX008 as the test sample. Proficiency testing scheme PTC01 [4]

was conducted in February 2008 and measured all the elements listed in Table 3. Proficiency testing scheme AQA 09-07 [5] was conducted in September 2009 and measured all the elements listed in Table 3 except Na and K. All interlaboratory results collected were for the reference material as received (no moisture content correction was performed by participants). The dry mass basis information values in Table 3 were calculated using the MX008 moisture content measured at time of certification analysis (see Section (v) and Table 4). The two sets of interlaboratory data were combined for determination of information values for MX008 and data was evaluated using robust statistics [6]. The robust average of participants' results has been used as the information value.

Homogeneity assessment— Homogeneity assessment for all elements in Table 3 was performed by NMIA. The method used a sample size of 1 g, acid extraction (1:1 nitric acid/hydrochloric acid with steam bath heating and shaking) and quantification by ICP-OES and ICP-MS. Ten sample bottles of MX008 were selected in a stratified random sampling plan to assess homogeneity of the entire production batch. Measurements were made on duplicate subsamples taken from these 10 bottles. Cochran's test for duplicate data was used to identify widely discrepant pairs which, if found, were removed from the homogeneity assessment data set. Analysis of variance (ANOVA) was used to estimate the standard uncertainty due to method precision and sample homogeneity.

Stability assessment— The long-term stability of MX008 information values has not been thoroughly assessed. For elements measured in both interlaboratory studies (all except K and Na), comparison of results gives no indication of instability in MX008.

Measurement uncertainty— The measurement uncertainty estimates for information values combines estimates for measurement uncertainty due to characterization by interlaboratory comparison, moisture content and homogeneity. The uncertainty of the robust average (derived from the robust standard deviation of interlaboratory data) [6] has been used to estimate the measurement uncertainty due to characterization.

Metrological traceability— Information values are traceable to SI units through the consensus of interlaboratory results from a variety of analytical methods. For dry mass basis information values, moisture content correction is traceable to the method described in Section (v).

Information value for MX008 moisture content

Table 4 contains an information value for the moisture content of MX008 at time of certification (August 2008) measured according to the procedure described in Section (v). Moisture content determination must be performed at the time of analysis for dry mass basis measurements.

Table 4: Information value for moisture content of reference material MX008. Measurement uncertainty is reported as a 95% coverage interval.

	Mass fraction
	mg/g
MX008 moisture content	**72 ± 9**

Additional References

4. *Proficiency Study PTC01*; NMIA (2008)
5. *Proficiency Study AQA 09-07*; NMIA (2010)
6. *Statistical methods for use in proficiency testing by interlaboratory comparisons*; ISO 13528, 1st edition (2005)