Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

NMIA MX013: Folic Acid in Wheat Flour

Certified value

	CAS No.	Mass fraction (mg/kg)
Folic acid	**59-30-3**	**2.28 ± 0.16**

The reported uncertainty is expanded to provide a level of confidence of 95%.

Expiry: 19 May 2027
When stored in accordance with the instructions given on this certificate.

Batch No.: 2011.01

Description: This reference material consists of wheat flour fortified with folic acid and is contained in a translucent screw capped polypropylene jar.

Intended use: The reference material is intended to be used to validate analytical methods for the measurement of folic acid in wheat flour. It may also be used to calibrate secondary reference materials of similar composition or as a matrix calibration standard.

Instructions for use: The recommended minimum sample size for analysis is 1 g of flour on an as received basis. Shake the jar to mix contents prior to sub-sampling. The exposure of the reference material to light should be minimized.

Storage: The material should be stored at -18°C out of direct light in the closed container as issued.

Metrological traceability: The certified mass fraction for folic acid in MX013 is traceable to the SI unit for mass (kg) through the Australian national standard for mass. All sample and standard preparation was performed gravimetrically using appropriately calibrated equipment. The purity of the standard reference material of folic acid was assigned using the primary method of quantitative nuclear magnetic resonance spectrometry (QNMR) calibrated with NMI reference material QNMR004 (trioxane). The primary ratio method of isotope dilution mass spectrometry was used for quantification of the mass fraction of folic acid in flour and the measurement system factor fully investigated to ensure the metrological traceability of the results [1].

Stability: The stability of the material, when stored under the storage conditions listed above, was demonstrated by an isochronous stability trial conducted at -16 to -18°C. Stability was also demonstrated in an accelerated stability study at 40°C. The long term stability of the certified value was re-assessed in April 2016. The uncertainty in the mass fraction was expanded to incorporate short term stability at 40 °C for 7 days and the long-term storage stability component extrapolated to cover the period of certification until the expiration date of this certificate [2].

Homogeneity: Assessment of homogeneity of CRM NMIA MX013 was conducted in accordance with ISO Guide 35 [2] and involved analysis of 25 bottles of the CRM selected at random from the batch. Duplicate sub-samples were analysed from 10 of these bottles and single sub-samples were analysed from the remaining 15 bottles. Homogeneity testing was carried out using the analytical procedures described in “Analytical method” below and the results used to calculate the within-bottle and between-bottle variances. The uncertainty in the certified value incorporates these variances.

Production: The material was prepared in January 2011 using unfortified wheat flour. Production involved the combination of two solid materials in a number of stages. A concentrated pre-mix of fortified flour was prepared and used to fortify the bulk material. The unfortified sieved flour was added incrementally to the pre-mix over a period of ten days in quantities ranging from 500 g to 3000 g with continual mixing. The fortified flour sample was then divided into 20 g portions using a Retsch PT 100 rotary sample divider. Five hundred units of MX013 were produced. The prepared samples were stored in sequentially numbered polypropylene screw-capped jars at -18°C to prevent deterioration from microbiological, enzymatic or chemical activity.

Analytical method: The folic acid mass fraction was measured by isotope dilution with liquid chromatography tandem mass spectrometry (LC-MSMS) in selected reaction mode for detection [3]. Isotopically labeled $^{13}C_5$-folic acid was used as the internal standard and added to the sample prior to extraction with aqueous ammonia solution (0.025% w/w). Samples were extracted in the dark overnight by end-over-end rotation to ensure equilibration of the isotopically-labeled internal standard with the folic acid and then allowed to settle prior to centrifugation. An aliquot of the supernatant was filtered through a 0.2 µm nylon filter then analysed by LC-MSMS with on-line Turbulent Flow clean up.

Measurement uncertainty: Measurement uncertainties were estimated according to international standards [2, 4] and National Measurement Institute standard operating procedures. All factors that could reasonably be expected to affect the measurement result were identified and the standard uncertainty of each estimated from experimental data. The standard uncertainties of the various components were combined as described in the ISO Guide to the Expression of Uncertainty in Measurement [4]. The combined standard uncertainties were expanded to a level of confidence of 95% using a coverage factor calculated from the effective degrees of freedom obtained from the Welch-Satterthwaite equation.

The individual components contributing to the measurement uncertainty estimates were the mass fractions assigned to calibration standards, gravimetric mass measurements, precision of the analytical method, batch homogeneity, long-term storage stability of the material at -18°C, stability of the material during transportation and potential sources of bias in the analytical procedure. The major contributing factors to the measurement uncertainties were mass fraction of the calibration solution, batch homogeneity and the long-term storage stability component extrapolated to cover the period of certification. The coverage factor (k) used and the effective degrees of freedom (v_{eff}) associated with the measurement uncertainty in the certified value is shown in Table 1.

Table 1: Coverage factor and the effective degree of freedom associated with the measurement uncertainty in the certified value for folic acid in CRM NMIA MX013

	k	v_{eff}
Folic acid	2.06	25

The certified value and its associated expanded uncertainty applies to any correctly stored and un-opened bottle of Batch 2011.01 of NMIA MX013 until the expiration date specified on this certificate. The corresponding expanded uncertainty represents the 95% confidence limits for the property value certified until the date of expiry. The validity of the certification of the material may be extended by the National Measurement Institute as additional information about the long-term storage stability of the material becomes available.

Raluca Iavetz
Manager, Chemical Reference Values
14 November 2023
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Accreditation no: 198

This report supersedes any issued prior to 14 November 2023

References:

1. D.G. Burke and L.G. Mackay, *Anal. Chem.* **80**, 2008, 5071-5078.
2. Reference Materials – Guidance for characterisation and assessment of homogeneity and stability. ISO Guide 35:2017
3. L.G. Mackay, C.P. Taylor, R.B. Myors, R. Hearn and B. King; *Accred. Qual. Assur.*, **8**, 2003, 191-194.
4. Joint Committee for Guides in Metrology; Evaluation of measurement data — Guide to the Expression of Uncertainty in Measurement; JCGM 100:2008.

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