Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA MX012A: Nitrofuran Marker Residues in Freeze-Dried Prawn

Batch No.: 2008.01

Certified values

Analyte	CAS No.	Mass fraction as supplied (ng/g)	Mass fraction reconstituted[#] (ng/g)	Coverage Factor (k)
3-Amino-2-oxazolidinone	**80-65-9**	**137.5 ± 8.5**	**20.6 ± 1.3**	**2.13**

[#]0.150 g reconstituted to exactly 1.00 g with water as described in the instructions for use
Uncertainties are expanded to provide a level of confidence of 95%

Expiry: The certified values are valid for sealed bottles of the material **for one year from the date of receipt** provided that the bottles are handled and stored in accordance with the instructions given on this certificate.

Description: This reference material consists of naturally-incurred 3-amino-2-oxazolidinone (marker residue for nitrofuran antibiotic furazolidone) in freeze-dried prawn contained in a sealed glass bottle with a crimped rubber septum. The material must be reconstituted with water prior to use.

Intended use: The reference material is intended to be used in validation of analytical methods for the measurement of 3-amino-2-oxazolidinone in prawn in residue analysis. It may also be used to calibrate secondary reference materials of similar composition or as a matrix calibration standard.

Instructions for use: The material should be reconstituted with reagent water at least 30 minutes prior to use. The recommended minimum sample size is 150 mg of the freeze-dried sample. This should be reconstituted to a final mass of 1 g if a sample equivalent to the original fresh prawn tissue is required.

Storage: Store at -20 °C out of direct light in the closed container as issued. Exposure of the material to elevated temperatures should be avoided.

Metrological traceability: The certified property value for NMIA MX012A 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 3-amino-2-oxazolidinone was assigned using the primary method of quantitative nuclear magnetic resonance spectrometry (QNMR) calibrated with NMI reference material QNMR002 (dimethylsulfoxide). The primary ratio method of isotope dilution mass spectrometry was used for quantification of the mass fraction of AOZ in NMIA MX012A. The measurement system factor [1] for the analytical procedure was fully investigated to ensure the metrological traceability of the results.

Stability: The stability of the material under the recommended storage conditions has been verified via a protocol compliant with ISO Guide 35 [2] and will continue to be monitored. Long term stability was assessed at -20 °C. Accelerated stability studies conducted at 40 °C for up to two weeks demonstrated that some degradation of 3-amino-2-oxazolidinone in NMIA MX012A occurred at this temperature. **Exposure of the material to elevated temperatures, such as may occur during transportation, should be avoided.**

Homogeneity: Evaluation of the homogeneity of CRM NMIA MX012A was conducted in accordance with ISO Guide 35 [2] by analysing duplicate sub-samples from 10 bottles of freeze-dried prawn material selected at random from the batch. Homogeneity testing was carried out using the analytical procedures described below and the results obtained were used to calculate within-bottle and between-bottle variances. The uncertainty in the certified value incorporates these variances.

Safety: CRM NMIA MX012A is intended for in-vitro diagnostic analysis only.

Production: The material was prepared in April 2008 using frozen fresh prawn with incurred levels of AOZ in excess of 100 ng/g. This material was homogenised with blank prawn to obtain a material with a mass fraction of AOZ of approximately 20 ng/g, which was then freeze-dried, blended and sieved to produce 300 units of NMIA MX012A.

The residual water content of the freeze-dried material was measured by Karl Fischer titration as approximately 1.7%. The average mass of freeze-dried material in each bottle is approximately 1.2 g, equivalent to 8 g of fresh prawn.

Analytical method: The certified mass fraction of 3-amino-2-oxazolidinone was measured by isotope dilution mass spectrometry [3] involving the addition of an isotopically-labelled analogue prior to any sample preparation. Water was added to 150 mg of freeze-dried material, followed by a solution of $^{2}H_{4}$-3-amino-2-oxazolidinone. The prawn samples were hydrolysed overnight at 60 °C with acid and 2-nitrobenzaldehyde to free protein-bound residues and derivatise the analyte to its 2-nitrobenzylidene derivative. Derivatised analyte was extracted into ethyl acetate and the extracts evaporated and reconstituted in acidified aqueous 10% acetonitrile for solid-phase extraction clean-up. The eluate obtained was evaporated and reconstituted in acetonitrile/aqueous acetic acid (1:9) for analysis by LC-MSMS.

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 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 -20 ºC, stability of the material during transportation and potential sources of bias in the analytical procedure. The major contributing factors to the measurement uncertainties were reproducibility (between-batch) effects and the stability components extrapolated to cover possible transportation conditions and the period of certification. The coverage factor (k) used to expand the uncertainty to give a level of confidence of 95% was 2.13 and the effective degrees of freedom (v_{eff}) associated with the measurement uncertainty in the certified value is 16.

Raluca Iavetz
Manager Chemical Reference Values
16 October 2020

Accreditation No. 198

The property values specified in this report supersede any issued prior to 14 October 2020.

References:

1. D.G. Burke and L.G. Mackay, *Anal. Chem.* **80**, 2008, 5071-5078
2. ISO Guide 35:2017, Reference Materials – Guidance for characterization and assessment of homogeneity and stability
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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