CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA MX012B: 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 (AOZ)	80-65-9	30.2 ± 1.8	4.53 ± 0.27	2.09
Semicarbazide (SEM)	563-41-7	70.3 ± 3.1	10.5 ± 0.5	2.23

[#]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) and fortified semicarbazide (marker for nitrofurazone) 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 and semicarbazide 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 values for NMIA MX012B are 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 purity of the standard reference material of semicarbazide was assigned using QNMR calibrated with NMI reference material QNMR004 (trioxane). The primary ratio method of isotope dilution mass spectrometry was used for quantification of the mass fractions of AOZ and SEM in NMIA MX012B. 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 MX012B 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 MX012B was conducted in accordance with ISO Guide 35 [2] by analysing 15 bottles of freeze-dried prawn material selected at random from the batch. Duplicate sub-samples were analysed from 10 of the bottles with single sub-samples analysed from the remaining 5 bottles. 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 MX012B is intended for in-vitro diagnostic analysis only.

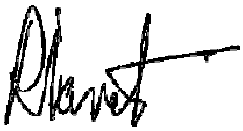
Production: The material was prepared in April 2008 using frozen fresh prawn with incurred levels of AOZ in excess of 100 ng/g. This material was homogenised with blank prawn to obtain a material with a mass fraction of AOZ of approximately 20 ng/g, which was then freeze-dried, blended and sieved to produce NMIA MX012A, certified for the mass fraction of AOZ only. The NMIA MX012A material was mixed with further freeze-dried blank prawn and the diluted material homogenised with a solution of semicarbazide (SEM). The fortified material was then freeze-dried, blended and sieved to prepare 550 units of NMIA MX012B, certified for the mass fractions of AOZ and SEM.

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 and semicarbazide were 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 mixed solution of $^{2}H_4$-3-amino-2-oxazolidinone and [1,2-$^{15}N_2$,^{13}C]-semicarbazide. The prawn samples were hydrolysed overnight at 60 °C with acid and 2-nitrobenzaldehyde to free protein-bound residues and derivatise analytes to their 2-nitrobenzylidene derivatives. Derivatised analytes were 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 uncertainty for AOZ were reproducibility effects (between batch) and homogeneity (due to one anomalous result of 25 results), as well as the stability components extrapolated to cover possible transportation conditions and the period of certification. For SEM, the major contributing factor was the stability component extrapolated to cover the period of certification. The coverage factors (k) used and the effective degrees of freedom (ν_{eff}) associated with the measurement uncertainties in the certified values are: for 3-amino-2-oxazolidinone, k = 2.09, ν_{eff} = 19.9; for semicarbazide k = 2.23, ν_{eff} = 10.8.



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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