Certificate of Analysis

NRC·CNRC Certified Reference Material

OTAL-1

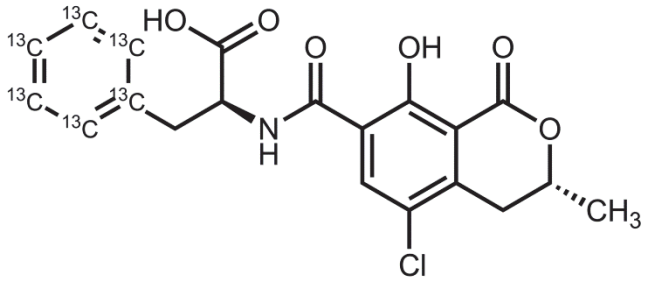
[$^{13}C_6$]-Ochratoxin A Calibration Solution Certified Reference Material

The following tables show those constituents for which certified values have been established for this [$^{13}C_6$]-ochratoxin A calibration solution certified reference material (CRM).

The certified values represent the mass fraction and mass concentration of [$^{13}C_6$]-ochratoxin A in a solution of acetonitrile with 0.1 % formic acid based on results generated at the National Research Council Canada (NRC) using quantitative proton nuclear magnetic resonance spectroscopy (^{1}H-qNMR) with external calibration [1]. The expanded uncertainty (U_{CRM}) in the certified value is equal to $U = ku_c$ where u_c is the combined standard uncertainty calculated according to the JCGM Guide [2] and k is the coverage factor. A coverage factor of two (2) was applied for [$^{13}C_6$]-ochratoxin A. It is intended that U_{CRM} accounts for every aspect that reasonably contributes to the uncertainty of the certified value.

Table 1: Certified quantity values for OTAL-1

Substance	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
[$^{13}C_6$]-ochratoxin A	$^{13}C_6C_{14}H_{18}ClNO_6$	4.89 ± 0.16	3.78 ± 0.13



[$^{13}C_6$]-ochratoxin A

CAS registry number for native ochratoxin A: 303-47-9
InChI Key: RWQKHEORZBHNRI-SSDYQDAKSA-N
Molecular formula: $^{13}C_6C_{14}H_{18}ClNO_6$
Molar mass: 409.752 ± 0.012 g/mol

Certified values

Certified values are considered to be those for which NRC has the highest confidence in accuracy and that all known and suspected sources of bias have been taken into account and are reflected in the stated expanded uncertainties. Certified values are the best estimate of the mean and uncertainty (Table 1).

Intended use

This reference material is primarily intended for use as an instrument calibration solution to assist in the quantitative analysis of ochratoxin A. The concentration of [$^{13}C_6$]-ochratoxin A in the OTAL-1 CRM is suitable for use as an internal standard during liquid chromatography – mass spectrometry (LC–MS).

Storage

It is recommended that the material be stored in a controlled cold temperature environment such as a freezer at approximately –20 °C.

Instructions for use

Prior to opening, each ampule should be allowed to warm to room temperature and the contents should be thoroughly mixed. The ampule should be opened at the pre-scored mark immediately prior to use. The CRM is sensitive to light, so caution should be taken to avoid exposure. Please note that the volume of the solution is not certified; only the concentration is certified. Therefore, the entire contents of the ampule should not be diluted to volume. Once opened, the contents of the ampule should be transferred to an amber glass vial (preferably silanized), tightly sealed and stored in the dark at –20 °C. It is recommended that the CRM solution should not be evaporated to dryness, solvents containing 0.1 % formic acid should be used for dilution, and all glassware should be silanized to minimize the risk of adhesion onto glass surfaces. A minimum sample size required to prepare accurate dilutions, such as 50 μL, is recommended.

The mass concentration value reported was calculated from the mass fraction value using a density of 0.773 ± 0.008 g/mL (k = 2) at 21 °C determined at NRC on the actual CRM solution. However, note that the density of acetonitrile changes by 0.14 % per degree Celsius (in the interval of 10 to 30 °C; decreasing density with increasing temperature).

Preparation of material

The [$^{13}C_6$]-ochratoxin A material was synthesized as a mixture of diastereomers at NRC from racemic alpha-ochratoxin and [$^{13}C_6$]-*L*-phenylalanine using a procedure adapted from that of Lindenmeier et al. [3]. The correct diastereomer of [$^{13}C_6$]-ochratoxin A was obtained as a lyophilized solid after reversed-phase preparative HPLC. Two separate portions of solid [$^{13}C_6$]-ochratoxin A were dissolved in CD_3CN + 0.1 % DCOOH for analysis by ^{1}H-qNMR. Subsequent gravimetric dilution of the combined qNMR solutions in acetonitrile with 0.1 % formic acid produced the calibration solution, which was dispensed in 1 mL aliquots in clean amber glass ampules. The ampules were immediately flame-sealed in a controlled environment at 25 % relative humidity.

Stability

The short-term stability of OTAL-1 was assessed using liquid chromatography with UV detection (LC–UV) at 1, 2, and 4-week time points using an isochronous approach at +37, +20, +4, and –20 °C temperatures, with reference to samples held at –40 °C. No significant degradation was observed during

this period at any temperature. Due to the high cost of [$^{13}C_6$]-ochratoxin A, the long-term stability was inferred [4] from native ochratoxin A in identical solvent. Samples of native ochratoxin A in acetonitrile with 0.1 % formic acid stored at –20 °C for one year were compared to a fresh gravimetrically prepared solution. Results were evaluated using the DerSimonian-Laird (DSL) random effects model [5] and included in the calculation of the certified value.

Homogeneity

The material is expected to have a high degree of homogeneity as it is a pure solution. The homogeneity was tested at NRC using LC–UV. Results from a representative number of ampules across the fill series (2 %) were evaluated using the DSL random effects model [5]. No between-bottle variability was observed, therefore, the material is deemed to be homogeneous.

Uncertainty

Included in the combined uncertainty estimate (u_c) are uncertainties in the batch characterization (u_{char}), uncertainties related to possible between-bottle variation (u_{hom}), and uncertainties related to stability ($u_{stability}$). Expressed as standard uncertainties, these components are listed in Table 2.

Table 2: Uncertainty components for OTAL-1

Substance	$U_{k=2}$ µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{stability}$ µg/g
[$^{13}C_6$]-ochratoxin A	0.16	0.08	0.06	0.00	0.05

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of established purity (potassium hydrogen phthalate, NIST SRM 84L) and international measurement inter-comparisons. As such, OTAL-1 serves as a suitable reference material for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

Quality System (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented NRC Measurement Science and Standards (MSS) Quality System, which conforms to the requirements of ISO/IEC 17025 and ISO Guide 34. The MSS Quality System supporting NRC calibration and measurement capabilities, as listed in the Bureau international des poids et mesures (BIPM) key comparison database (http://kcdb.bipm.org/), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM), and found to be in compliance with the expectations of the Comité international des poids et mesures (CIPM) Mutual Recognition Arrangement. The SIM certificate of approval is available upon request.

Updates

Users should ensure that the certificate they have is current. Our website at www.nrc.gc.ca/crm will contain any new information.

References

[1] Burton I.W., Quilliam M.A., Walter K.A. ^{1}H NMR with external standards: use in preparation of calibration solutions for algal toxins and other natural products. Anal Chem (2005), 77: 3123-3131.

[2] JCGM, Evaluation of measurement data: Guide to the expression of uncertainty in measurement, JCGM 100:2008.

[3] Lindenmeier, M., Schieberle, P., Rychlik, M. Quantification of Ochratoxin A in foods by a stable isotope dilution assay using high-performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A (2004), 1023: 57-99.

[4] ISO (2017), Reference materials – Guidance for characterization and assessment of homogeneity and stability. ISO Guide 35:2017.

[5] R. DerSimonian, N. Laird (1986), Meta-analysis in clinical trials. Controlled Clinical Trials, 7: 177-188.

Authorship

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This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our authorized vendors.

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