

Certificate of Analysis

NRC-CMRC

Certified Reference Material

OTAN-1

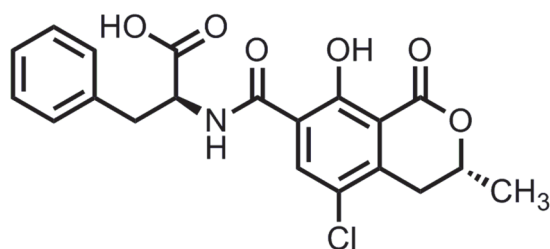
Ochratoxin A Calibration Solution Certified Reference Material

The following tables show those constituents for which certified and information values have been established for this ochratoxin A calibration solution certified reference material (CRM).

The certified values represent the mass fraction and mass concentration of 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 (^1H -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 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 OTAN-1

Substance	Molecular formula	Mass fraction $\mu\text{g/g}$	Mass concentration $\mu\text{g/mL}$
ochratoxin A	$\text{C}_{20}\text{H}_{18}\text{ClNO}_6$	11.03 ± 0.38	8.53 ± 0.31



ochratoxin A

CAS registry number: 303-47-9

InChI Key: RWQKHEORZBHNRI-BMIGLBTASA-N

Molecular formula: $\text{C}_{20}\text{H}_{18}\text{ClNO}_6$

Molar mass: 403.814 ± 0.012 g/mol



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Table 2: Information values for OTAN-1

Substance	CAS number	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
ochratoxin B	4825-86-9	C ₂₀ H ₁₉ NO ₆	0.062	0.048

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).

Information values

Information values are those for which insufficient data are available to provide a comprehensive estimate of uncertainty (Table 2).

Intended use

This reference material is primarily intended for use in method development and in the calibration of instrumentation for the quantitative analysis of ochratoxin A.

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 values reported were calculated from the mass fraction values 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

A sample of solid ochratoxin A, acquired from a commercial supplier, was dissolved in CD₃CN + 0.1 % DCOOH for analysis by ¹H-qNMR. Subsequent gravimetric dilution of the qNMR solution 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 40 % relative humidity.

Stability

The short-term stability of OTAN-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 –80 °C. No significant degradation was observed during this period at any temperature. The long-term stability was assessed by comparing samples of ochratoxin A in acetonitrile with 0.1 % formic acid stored at –20 °C for one year to a fresh gravimetrically prepared solution. Results were evaluated using the DerSimonian-Laird (DSL) random effects model [3] 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 [3]. 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 ($u_{\text{stability}}$). Expressed as standard uncertainties, these components are listed in Table 3.

Table 3: Uncertainty components for OTAN-1

Substance	$U_{k=2}$ µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{\text{stability}}$ µg/g
ochratoxin A	0.38	0.19	0.15	0.00	0.11

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of established purity (benzoic acid, NIST SRM 350b) and international measurement inter-comparisons. As such, OTAN-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. ^1H 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] R. DerSimonian, N. Laird (1986), Meta-analysis in clinical trials. *Controlled Clinical Trials*, 7: 177-188.

Authorship

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OTAN-1*Date of issue: March 2018**Date of expiry: March 2020***Approved by:**

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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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