National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

OTAN-1

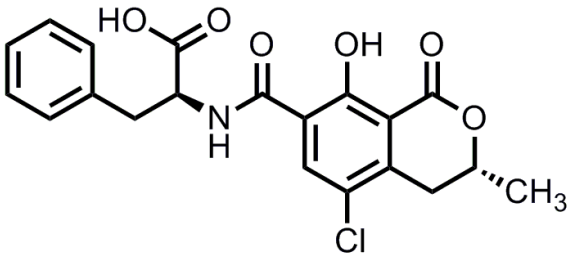
Ochratoxin A Calibration Solution Certified Reference Material

OTAN-1 is a calibration solution certified reference material (CRM) for the mycotoxin ochratoxin A, in acetonitrile. This material, distributed in 1 mL units, is intended for instrument calibration, method development, and validation for the quantitation of ochratoxin A. Certified values for the mass fraction and mass concentration of ochratoxin A in OTAN-1 have been established for this CRM, as listed in Table 1. The information values for the mass fraction and mass concentration of a minor impurity, ochratoxin B, in OTAN-1 are provided in Table 2.

The certified values of ochratoxin A in a solution of acetonitrile with 0.1 % formic acid are based on results from data 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) for all values 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 of two (k = 2, 95 % confidence interval). It is intended that the U for certified values accounts for every aspect that reasonably contributes to their uncertainties.

Table 1: Certified values and expanded uncertainties (k = 2, 95 % CI) for OTAN-1

Substance	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
ochratoxin A	$C_{20}H_{18}ClNO_6$	11.03 ± 0.32	8.53 ± 0.26



ochratoxin A

CAS registry number: 303-47-9
InChI Key: RWQKHEORZBHNRI-BMIGLBTASA-N
Molecular formula: $C_{20}H_{18}ClNO_6$
Molar mass: 403.814 ± 0.024 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 the 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 true value 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

Distributed in 1 mL units, this certified 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 or below.

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. 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 or below. A potential deviation from the certified value can occur due to solvent evaporation. 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. 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.

The mass concentration values reported were calculated from the mass fraction values using a density of 0.773 ± 0.008 g/mL (k = 2, 95 % confidence interval) at 21 °C determined at the 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 CD_3CN + 0.1 % DCOOH for analysis by ^{1}H-qNMR. Subsequent gravimetric dilution of the qNMR solution 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 40 % relative humidity.

Stability

The transportation stability of OTAN-1 was assessed using liquid chromatography with UV detection (LC–UV) at one-, two-, and four-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 of OTAN-1 stored at –20 and –40 °C for four and a half years was assessed using LC–UV and compared to the initial assigned concentration. No significant differences in the measured mass fraction were observed over this period. Therefore, the results for both the transportation and long-term stability showed no instability trends. However, an accelerated isochronous stability study was performed using LC–UV with OTAN-1 at +50 and +70 °C at two-, four-, six-, and eight-week time intervals. Samples were compared to those stored at the reference condition of –40 °C and the results were fitted to determine the degradation rates at both temperatures. Using the Arrhenius model, an uncertainty for OTAN-1 due to the transportation stability was estimated at +40 °C for two months and an uncertainty due to the long-term stability was estimated at +4 °C for four years. These uncertainties were combined to assign an associated uncertainty component. This estimate does not reflect an observed instability trend of the material but rather reflects a conservative estimate of possible instability.

Homogeneity

The material is expected to have a high degree of homogeneity as it is a pure solution. The homogeneity was tested at the NRC using LC–UV. Results from a representative number of ampules across the fill series were evaluated using the DerSimonian Laird (DSL) random effects model [3]. The over dispersion variance (dark uncertainty) was taken as the measure of the inhomogeneity and it's 95 % confidence interval showed absence of between-unit heterogeneity, 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-unit variation (u_{hom}), and uncertainties related to stability ($u_{stability}$). Expressed as standard uncertainties, these components are listed in Table 3.

Table 3: Uncertainty components of the certified value for OTAN-1

Substance	$U_{k=2}$ µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{stability}$ µg/g
ochratoxin A	0.32	0.16	0.15	0.00	0.04

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of benzoic acid (NIST SRM 350b) employed as an external standard for ^{1}H-qNMR. As such, OTAN-1 serves as a suitable reference material for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

Quality Management System (ISO 17034, ISO/IEC 17025)

This material was produced in compliance with the NRC Metrology Quality Management System, which conforms to the requirements of ISO 17034 and ISO/IEC 17025. The Metrology Quality Management System supporting the 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 approval is available upon request.

Updates

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

References

[1] Bates J, Bahadoor A, Cui Y, Meija J, Windust A, Melanson JE. Certification of Ochratoxin A Reference Materials: Calibration Solutions OTAN-1 and OTAL-1 and a Mycotoxin-Contaminated Rye Flour MYCO-1. J AOAC Int (2019), 102 (6): 1756-1766. https://doi.org/10.1093/jaoac/102.6.1756

[2] Evaluation of measurement data: Guide to the expression of uncertainty in measurement. JCGM 100:2008. https://www.bipm.org/en/publications/guides/gum.html

[3] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials (1986), 7: 177-188. https://doi.org/10.1016/0197-2456(86)90046-2

Cited by

A list of scientific publications citing OTAN-1 can be found at doi.org/10.4224/crm.2018.otan-1.

Authorship

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OTAN-1

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This Certificate is only valid if the corresponding material was obtained directly from NRC or an Authorized Reseller.

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