National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

DONN-1

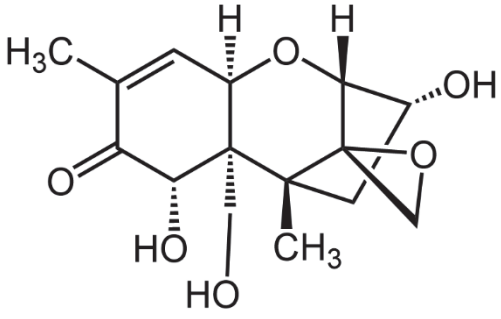
4-Deoxynivalenol Calibration Solution Certified Reference Material

DONN-1 is a calibration solution certified reference material (CRM) for the regulated mycotoxin 4-deoxynivalenol, in acetonitrile. The calibration solution is intended for method development and instrument calibration to produce accurate and traceable measurements for 4-deoxynivalenol. Certified values for the mass fraction and mass concentration of 4-deoxynivalenol in DONN-1 have been established, as listed in Table 1.

The certified values of 4-deoxynivalenol in a solution of acetonitrile are based on results from data generated at the National Research Council of Canada (NRC). Quantitative proton nuclear magnetic resonance spectroscopy with internal calibration was employed for value assignment of 4-deoxynivalenol [1, 2]. 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 [3] 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 for DONN-1 (k = 2, 95 % CI)

Substance	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
4-deoxynivalenol	$C_{15}H_{20}O_6$	56.6 ± 1.6	43.8 ± 1.5



4-deoxynivalenol

CAS registry number: 51481-10-8
InChI Key: LINOMUASTDIRTM-QGRHZQQGSA-N
Molecular formula: $C_{15}H_{20}O_6$
Molar mass: 296.32 ± 0.02 g/mol

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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).

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 4-deoxynivalenol.

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. Once opened, the contents of the ampule should be transferred to an amber glass vial, 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 material 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.

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. 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 calibration solution was prepared by dissolving a sample of solid 4-deoxynivalenol in acetonitrile. The purified 4-deoxynivalenol material was produced at the NRC from starting materials supplied by Agriculture and Agri-Food Canada. The calibration solution was dispensed in 1 mL aliquots in clean amber glass ampules. The ampules were immediately flame-sealed in a controlled environment at 20 % relative humidity.

Stability

The transportation stability of DONN-1 was assessed using liquid chromatography with UV detection (LC–UV) at one-, two-, four-, and eight-week time points using an isochronous approach at +60, +40, +20, +4, and –20 °C with reference to samples held at –40 °C. No degradation was observed over the span of four weeks at temperatures up to +40 °C. Therefore, the uncertainty associated with transportation stability was set to zero.

The long-term stability of DONN-1 was estimated from a pseudo first-order kinetic model based on the Arrhenius equation. The data from an isochronous study at +60, +40, +20, +4 °C at one-, two-, four-, and eight-week time points, was analysed by the Arrhenius equation and a prediction for potential degradation of DONN-1 after two years at –20 °C was obtained. From these calculations the best estimate for the

uncertainty component due to long-term stability was determined to be 0.6 µg/g. It is important to note that this is a conservative estimate and does not reflect any instability trend for DONN-1.

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 analysis of variance (ANOVA) random effects model [4]. The between-unit variability was determined to be negligible, 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 2.

Table 2: Uncertainty components of the certified value for DONN-1

Substance	$U_{k=2}$ µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{stability}$ µg/g
4-deoxynivalenol	1.6	0.8	0.5	0.0	0.6

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of benzoic acid (NIST PS1) which was used to assign purity to dimethyl terephthalate, employed as an internal standard for quantitative proton NMR (^{1}H-qNMR) and quantitative proton NMR with ^{13}C-decoupling (^{1}H{^{13}C}-qNMR). As such, DONN-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] Bahadoor A, Watt S, Rajotte, I. Bates, J. Tautomerization and Isomerization in qNMR: A Case Study with 4-deoxynivalenol (DON). J Agric Food Chem. (2022), 70 (8): 2733-2740. https://doi.org/10.1021/acs.jafc.1c08053

[2] Bahadoor A, Brinkmann A, Melanson JE. ^{13}C-Satellite Decoupling Strategies for Improving Accuracy in Quantitative Nuclear Magnetic Resonance. Anal Chem. (2021), 93: 851-858. https://doi.org/10.1021/acs.analchem.0c03428

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

[4] van der Veen AMH. Bayesian analysis of homogeneity studies in the production of reference materials. Accred Qual Assur. (2017), 22 (6): 307-19. https://doi.org/10.1007/s00769-017-1292-6

Cited by

A list of scientific publications citing DONN-1 can be found at doi.org/10.4224/crm.2022.donn-1.

Authorship

Adilah Bahadoor, Isabelle Rajotte, Jennifer Bates, Sarah Watt, Juris Meija, and Jeremy Melanson.

National Research Council Canada, 1200 Montreal Rd, Ottawa, Ontario, K1A 0R6, Canada.

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Approved by: _____

Jeremy E. Melanson, Ph.D.
Team Leader, Organic Chemical Metrology
NRC Metrology

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

National Research Council Canada
Metrology
1200 Montreal Road
Building M36, Room 1029
Ottawa, Ontario K1A 0R6

Telephone: 613-993-2359
Fax: 613-993-8915
Email: CRM-MRCOttawa@nrc-cnrc.gc.ca

Canada