National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

MYCO-1

Certified Reference Material of mycotoxin contaminated rye flour

MYCO-1 is a certified reference material (CRM) consisting of rye flour that has been naturally contaminated with mycotoxins. This CRM is primarily intended for use in method development, validation, and quality control for the analysis of 4-deoxynivalenol (DON), ochratoxin A (OTA), and zearalenone (ZEA) in flour or related matrices. Certified values for the mass fraction of 4-deoxynivalenol, ochratoxin A, and zearalenone in MYCO-1 have been established, as listed in Table 1. An information value for the water content in MYCO-1 is provided in Table 2. A unit of MYCO-1 contains 100 g of contaminated flour.

The certified values represent the mass fraction of different mycotoxins in MYCO-1 as supplied and are based on results from data generated at the National Research Council Canada (NRC). Using the NRC certified reference materials DONN-1, OTAN-1, and ZERA-1 as primary calibrators, isotope dilution liquid chromatography – mass spectrometry (ID−LC−MS) was employed for value assignment [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, CI). 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 MYCO-1

Substance	Molecular formula	Mass fraction µg/kg
4-deoxynivalenol (a, b)	$C_{15}H_{20}O_6$	1280 ± 70
ochratoxin A (a, b)	$C_{20}H_{18}ClNO_6$	4.05 ± 0.84
zearalenone (a, b)	$C_{18}H_{22}O_5$	43 ± 12

Table 2: Information value for MYCO-1

Substance	Molecular formula	Mass fraction g/kg
water (c)	H_2O	130

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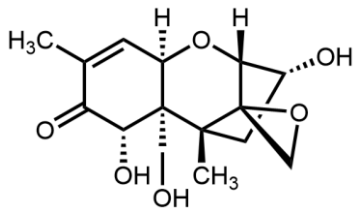
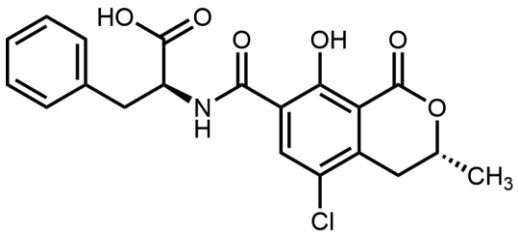


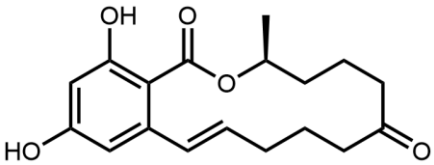
4-deoxynivalenol

CAS registry number: 51481-10-8
InChI Key: LINOMUASTDIRTM-QGRHZQQGSA-N
Molecular formula: $C_{15}H_{20}O_6$
Molar mass: 296.319 ± 0.016 g/mol



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



zearalenone

CAS registry number: 17924-92-4
InChI Key: MBMQEIFVQACCCH-QBODLPLBSA-N
Molecular formula: $C_{18}H_{22}O_5$
Molar mass: 318.367 ± 0.020 g/mol

Coding

The coding refers to the instrumental method used for value assignment:

- **a** Isotope dilution liquid chromatography – tandem mass spectrometry (ID−LC−MS/MS)
- **b** Isotope dilution liquid chromatography – high resolution mass spectrometry (ID−LC−HRMS)
- **c** Karl Fischer titration (KF)

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 100 g units, this certified reference material is primarily intended for use in method development, validation, and quality control for the analysis of mycotoxins in flour.

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 bottle should be allowed to warm to room temperature and the contents thoroughly mixed. After use, bottles should be tightly sealed and immediately returned to the freezer. Repeated sampling is permitted, although care must be taken not to introduce contamination. A minimum sample mass of 5 g is recommended.

Preparation of material

The material was prepared at the NRC using commercially sourced rye flour from local vendors blended with highly contaminated flour generated at the NRC. Mycotoxin producing fungal cultures were identified and incubated on sterile wheat kernels via solid state fermentation. The kernels were then autoclaved, freeze-dried, ground, blended, and sieved to generate highly contaminated sources of flour. Small amounts of contaminated flour were blended with the commercial rye flour in a multi-step process. The material was bottled into 100 g units in a controlled environment at 40 % relative humidity. MYCO-1 contains several other mycotoxins such as T-2 toxin, neosolaniol, 4-deoxynivalenol-3-glucoside, and the ergot alkaloids.

Stability

The freeze-thaw (F/T), transportation, and long-term stability of the mycotoxins in MYCO-1 were assessed at the NRC via ID–LC–MS/MS. The F/T stability was assessed over ten (ochratoxin A) or twenty (4-deoxynivalenol and zearalenone) F/T cycles from –20 to +20 °C. The results were evaluated as a function of the number of F/T cycles using ordinary least squares fitting and indicated no significant instability trend for all three mycotoxins. Therefore, the uncertainty due to F/T stability was considered negligible and assigned to zero.

Transportation stability was carried out using an isochronous approach. Bottles of MYCO-1 were stored at temperatures ranging from –20 to +60 °C for up to six months and compared to reference samples stored at –80 °C. No significant degradation was observed for ochratoxin A or 4-dexoyxnivalenol in MYCO-1, therefore the uncertainty due to transportation stability were considered negligible and assigned to zero for these two mycotoxins. Degradation was observed at elevated temperatures for zearalenone in MYCO-1. Therefore, the stability uncertainty includes an estimate of transportation stability at +40 °C for one-week to represent any significant shipping delays.

The long-term stability of ochratoxin A in MYCO-1 was assessed at –20 °C for up to sixty-seven months and for 4-deoxynivalenol and zearalenone at +4 and –20 °C for up to twelve months. No significant differences in the measured mass fraction were observed. Therefore, the uncertainty due to the long-term stability of the mycotoxins in MYCO-1 were considered negligible and assigned to zero.

Homogeneity

The material was tested for homogeneity at the NRC via ID−LC−HRMS. Results from sub-samples (5 g) from randomly selected bottles were evaluated using two-way Bayesian analysis of variance (ANOVA) [3] to determine both within-unit and between-unit heterogeneity components. These uncertainties were combined to assign an associated uncertainty component.

Uncertainty

Included in the combined uncertainty estimate (u_c) are uncertainties in the batch characterization and differences in the results obtained from ID−LC−MS/MS and ID−LC−HRMS (u_{char}), uncertainties related to within-unit and 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 values for MYCO-1

Substance	$U_{k=2}$ µg/kg	u_c µg/kg	u_{char} µg/kg	u_{hom} µg/kg	$u_{stability}$ µg/kg
4-deoxynivalenol	70	35	33	11	0
ochratoxin A	0.84	0.42	0.09	0.41	0.00
zearalenone	12	6	0.8	4.8	2.8

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of NRC DONN-1 (4-deoxynivalenol), OTAN-1 (ochratoxin A), and ZERA-1 (zearalenone) employed as the primary calibrators for ID−LC−MS. As such, MYCO-1 serves as 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. For updates, please refer to doi.org/10.4224/crm.2018.myco-1.

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] 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 MYCO-1 can be found at doi.org/10.4224/crm.2018.myco-1.

Authorship

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Revised: August 2022 (extended expiry date and editorial updates)
Revised: August 2023 (certified values updated (Table 1 and 3) including addition of 4-deoxynivalenol and zearalenone, information values updated (Table 2), and editorial updates)

Approved by:

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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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