National Research Council Canada | Conseil national de recherches Canada

Certificate of Analysis

Certified Reference Material

ZERA-1

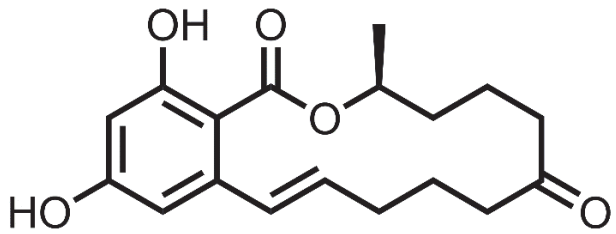
Zearalenone Calibration Solution Certified Reference Material

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

The certified values of zearalenone in a solution of acetonitrile are based on results from data generated at the National Research Council of Canada (NRC) using quantitative nuclear magnetic resonance spectroscopy (^{1}H-qNMR) with internal calibration. The zearalenone mass fraction in ZERA-1 was also confirmed in an international comparison exercise coordinated within the International Bureau of Weights and Measures (BIPM) [1]. The expanded uncertainty (U_{CRM}) in the certified value is equal to $U_{CRM} = ku_c$, where u_c is the combined standard uncertainty calculated according to the JCGM Guide [2] and k is the coverage factor. The U_{CRM} for ZERA-1 was obtained by applying a coverage factor of two (k = 2, 95 % confidence interval). It is intended that U_{CRM} accounts for every aspect that reasonably contributes to the uncertainty of the certified value.

Table 1: Certified values and expanded uncertainties (k = 2, 95 % confidence interval) for ZERA-1

Substance	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
zearalenone	$C_{18}H_{22}O_5$	66.5 ± 0.7	51.9 ± 0.8



Zearalenone

CAS registry number: 17924-92-4
InChI Key: MBMQEIFVQACCCH-QBODLPLBSA-N
Molecular formula: $C_{18}H_{22}O_5$
Molar mass: 318.367 ± 0.010 g/mol

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Table 2: Information values for ZERA-1

Substance	CAS number	Molecular formula	Mass fraction µg/g	Mass concentration µg/mL
zearalanone (ZAN)	5975-78-0	$C_{18}H_{24}O_5$	0.035	0.027

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

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

Storage

It is recommended that the material be stored in a controlled cold temperature environment such as a refrigerator at approximately +4 °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. Zearalenone is a photosensitive compound, therefore precautionary measures to avoid exposure of ZERA-1 to light should be taken. 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, tightly sealed, and stored in the dark at +4 °C or below. 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 0.780 ± 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

The calibration solution was prepared by dissolving a sample of solid zearalenone, provided by Agriculture and Agri-Food Canada, in acetonitrile. The purity of the solid zearalenone was certified by ^{1}H-qNMR with an internal standard. 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 40 % relative humidity.

Stability

The transportation stability of ZERA-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 –40 °C. No significant degradation was observed during this period at any temperature. The long-term stability of samples of zearalenone in acetonitrile stored at –40 °C for two and seven months was assessed using LC–UV and compared to the expected concentration. Linear regression was fitted to the results and the regression slopes were not significantly different from zero. Therefore, the results for both the transportation and long-term stability showed no instability trends. An accelerated isochronous stability study was performed using LC–UV with ZERA-1 at +50 °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 by linear regression. The regression slopes were not significantly different from zero, confirming no instability trends for ZERA-1 at +50 °C. In the absence of any observed instability trend under the conditions tested, the long-term uncertainty component for ZERA-1 was set to zero.

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 (1 %) were evaluated using the analysis of variance (ANOVA) random effects model [3, 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 3.

Table 3: Uncertainty components for ZERA-1

Substance	$U_{k=2}$ µg/g	u_c µg/g	u_{char} µg/g	u_{hom} µg/g	$u_{stability}$ µg/g
zearalenone	0.70	0.35	0.35	0.00	0.00

Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of NIST PS1 benzoic acid, which was used to assign purity to dimethyl terephthalate, employed as an internal standard for ^{1}H-qNMR. As such, ZERA-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.gc.ca/crm will contain any new information.

References

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Cited by

A list of scientific publications citing ZERA-1 can be found at doi.org/10.4224/crm.2020.zera-1.

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ZERA-1

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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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