



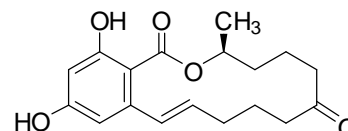
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA P1787b: Zearalenone

Report ID: P1787b.2021.01 (Bottled 200916)

Chemical Formula: C₁₈H₂₂O₅

Molecular Weight: 318.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
20-AV-03	17924-92-4	99.6 ± 0.8%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit ($k = 2$).

IUPAC name: (3S,11E)-14,16-Dihydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione.

Expiration of certification: The property values are valid till 25 March 2031, i.e. ten years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White powder prepared by sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

Recommended storage: When not in use this material should be stored at or below 4 °C in a closed container in a dry, dark area.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a period of ten years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from the demonstrated stability. The long-term stability of the compound in solution has not been examined.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by HPLC with UV detection on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

Safety: Treat as a hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
31 March 2021

This report supersedes any issued prior to 31 March 2021

NATA logo notice: Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with UV detection thermogravimetric analysis, Karl Fischer analysis, and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

The purity value obtained by quantitative nuclear magnetic resonance (qNMR) used a combination of the one-proton multiplet at 5.0 ppm, the one-proton multiplet at 5.7 ppm, and the one-proton doublet at 7.01 ppm measured against a certified internal standard of dimethyl terephthalate.

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz
	Solvent:	Acetic acid- <i>d</i> ₄ (2.04 ppm)
	Internal standard:	Dimethyl terephthalate (100.0% mass fraction)
	Initial analysis:	Mean (5.00 ppm) = 99.4%, s = 0.3% (8 sub samples, May 2020)
	Initial analysis:	Mean (5.71 ppm) = 99.4%, s = 0.3% (8 sub samples, May 2020)
	Initial analysis:	Mean (7.03 ppm) = 99.3%, s = 0.2% (5 sub samples, May 2020)
HPLC:	Instrument:	Waters Alliance 2695
	Column:	Phase Sep HICHROM C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/Milli-Q water (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 236 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, May 2020)
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, March 2021)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (May 2020 and March 2021)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (May 2020)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP 6890/5973
	Column:	DB-5MS 30 m x 0.25 mm I.D. x 0.25 μ m
	Program:	180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Split ratio:	20/1
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium
	Scan range:	50-550 <i>m/z</i>
	The retention time of the <i>bis</i> -TMS derivative is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	<i>Bis</i> -TMS (13.3 min):	462 (M^+ , 40), 447 (31), 444 (20), 429 (54), 333 (79), 305 (65), 260 (60), 232 (29), 151 (77), 125 (30), 73 (100) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	No solvents detected
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Hexane/ethyl acetate (1:1) Single spot observed, R_f = 0.5.
IR:	Instrument:	BioRad FTS 3000MX FT-IR
	Range:	4000-400 cm^{-1} , KBr powder
	Peaks:	3316, 2932, 1692, 1647, 1618, 1581, 1314, 1263, 1198, 970, 704 cm^{-1}
^1H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	CDCl_3 (7.26 ppm)
	Key spectral data:	δ 1.37 (3H, d, J = 6.2 Hz), 1.50 (1H, m), 1.59-1.70 (2H, m), 1.72-1.80 (2H, m), 2.13-2.23 (4H, m), 2.37 (1H, m), 2.62 (1H, m), 2.87 (1H, m), 4.99 (1H, m), 5.68 (1H, m), 6.15 (1H, s), 6.36 (1H, d, J = 2.5 Hz), 6.42 (1H, d, J = 2.5 Hz), 7.01 (1H, dd, J = 1.7, 15.3 Hz), 12.08 (1H, s) ppm Ethanol estimated at 0.02% mass fraction was observed in the ^1H NMR.
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	CDCl_3 (77.16 ppm)
	Spectral data:	δ 20.1 21.1, 22.4, 31.1, 34.9, 36.9, 43.1, 73.6, 102.6, 103.9, 108.6, 132.6, 133.4, 144.1, 160.8, 165.6, 171.5, 212.2 ppm
Melting point:		163-165 $^{\circ}$ C
Microanalysis:	Found:	C = 68.1%; H = 7.0% (May, 2020)
	Calculated:	C = 67.9%; H = 7.0% (Calculated for $\text{C}_{18}\text{H}_{22}\text{O}_5$)