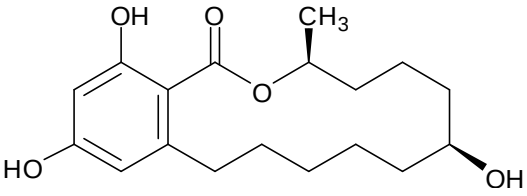
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA P1802: β-Zearalanol



Report ID: P1802.2019.01 (Bottled 190827)

Chemical Formula: $C_{18}H_{26}O_5$

Molecular Weight: 322.4 g/mol

Certified value

Batch No.	CAS No.	Purity (mass fraction)
03-AV-03	**42422-68-4**	**94.0 ± 1.8%**

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

IUPAC name: (3S,7S)-7,14,16-Trihydroxy-3-methyl-3,4,5,6,7,8,9,10,11,12-decahydro-1H-2-benzoxacyclotetradecin-1-one

Expiration of certification: The property values are valid till 20 December 2024, i.e. five 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 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 ºC 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%.

Stability: This material has demonstrated stability over a minimum period of 5 years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 seven 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.
3 January 2020

This report supersedes any issued prior to 3 January 2020

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 by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

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

Supporting evidence is provided by ^{1}H NMR and elemental microanalysis.

HPLC:	Instrument:	Alliance Waters 2695 or Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltech Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol: Acetonitrile: MilliQ water (35:20:45) or Methanol: MilliQ water (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot (200-300nm)
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 91.4%, s = 0.5% (7 sub samples in duplicate, May 2004)
	Re-analysis:	Mean = 92.5%, s = 0.7% (5 sub samples in duplicate, September 2007)
	Re-analysis:	Mean = 94.2%, s = 0.3% (5 sub samples in duplicate, December 2019)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (54:46 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot (200-300 nm)
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 92.7%, s = 0.6% (5 sub samples in duplicate, October 2010)
	Re-analysis:	Mean = 94.0%, s = 0.1% (5 sub samples in duplicate, September 2015)
Karl Fischer analysis:		Moisture content 0.33% mass fraction (August 2007) Moisture content ≤ 0.3% mass fraction (September 2010, August 2015 and September 2019)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (May 2004, September 2006 & 2010)

Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	Phenomenex ZB1701, 15 m x 0.25 mm I.D.x 0.30 μm
	Program:	175 °C (1 min), 5 °C/min to 230 °C, 30 °C/min to 275 °C (3.5 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention time of the *tris*-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.	
	Parent (12.6 min):	538 (M^+, 12), 523 (19), 433 (74), 335 (43), 307 (56) and 73 (100) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol (9:1) Single spot observed, R_f = 0.4
IR:	Instrument:	BioRad FTS 3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3522, 3215, 2943, 1641, 1613, 1587, 1464, 1311, 1255, 1199, 1096, 981, 817 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX500
	Field strength:	500 MHz
	Solvent:	Acetone-d_6 (2.09 ppm)
	Spectral data:	δ 1.33 (3H, d), 3.40 (1H, bs, OH), 6.23 (1H, d), 6.32 (1H, d), 8.94 (1H, bs, OH), 11.07 (1H, bs, OH) ppm
^{13}C NMR:	Instrument:	Bruker ARX500
	Field strength:	126 MHz
	Solvent:	Acetone-d_6 (205.9 and 30.6 ppm)
	Spectral data:	δ 20.5, 20.6, 21.9, 28.9, 31.9, 32.2, 35.6, 36.1, 36.5, 70.2, 73.7, 101.7, 106.9, 111.1, 148.6, 162.6, 164.7, 171.8 ppm
Melting point:		151-153 °C
Microanalysis:	Found:	C = 67.1%; H = 8.1% (2004)
	Calculated:	C = 67.1%; H = 8.1% (Calculated for $C_{18}H_{26}O_5$)