Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

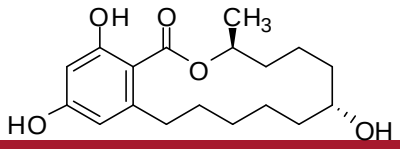
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA P1801: α-Zearalanol

Report ID: P1801.2021.01 (Bottled 170221)

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-02	26538-44-3	98.3 ± 1.0%

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

IUPAC name: (3*S*,7*R*)-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 7 June 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 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 ten 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.
22 June 2021

This report supersedes any issued prior to 22 June 2021

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 qualitative elemental microanalysis.

Note: Identified impurities:		Zearalanone (ca. 2% mass fraction)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltech C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (65:35 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.2%, s = 0.08% (7 sub samples in duplicate, May 2004)
	Re-analysis:	Mean = 98.6%, s = 0.03% (5 sub samples in duplicate, August 2008)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.4%, s = 0.05% (5 sub samples in duplicate, September 2011)
	Re-analysis:	Mean = 98.5%, s = 0.02% (5 sub samples in duplicate, August 2016)
HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	ACE Excel 5 Super C-18, 5μm (4.6 mm × 250 mm)
	Column oven:	Ambient
	Mobile Phase:	Methanol/MilliQ water (60:40 v/v)
	Flow rate:	1.0 mL/min
	Detector:	SPD-M20A PDA operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.7%, s = 0.04% (5 sub samples in duplicate, June 2021)
Karl Fischer analysis:		Moisture content is 0.3% mass fraction (August 2008) Moisture content is 0.2% mass fraction (September 2011) Moisture content is 0.6% mass fraction (August 2016) Moisture content is 0.4% mass fraction (June 2021)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (May 2004, August 2006 & September 2008)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	Agilent 6890/5973
	Column:	Phenomenex ZB1701, 15m x 0.25mm I.D.x 0.30 µm
	Program:	175 °C, 5 °C /min to 230 °C, 30 °C /min to 275 (3.5 min)
	Injector:	250 °C
	Split ratio:	3:2
	Transfer line temp:	280 °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.	
	Bis-TMS (12.6 min):	538 (M^+, 11), 523 (15), 433 (77), 335 (44), 307 (61), 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:	3496, 3165, 2945, 1649, 1616, 1586, 1463, 1314, 1259, 1198, 1166, 1096, 1074, 989, 840, 762 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX500
	Field strength:	500 MHz
	Solvent:	Acetone-d_6 (2.05 ppm)
	Key Spectral data:	δ 1.34 (3H, d), 3.46 (1H, bs, OH), 6.22 (1H, d), 6.29 (1H, dd), 9.08 (1H, bs, OH), 11.97 (1H, bs, OH) ppm
^{13}C NMR:	Instrument:	Bruker ARX500
	Field strength:	126 MHz
	Solvent:	Acetone-d_6 (30 ppm)
	Spectral data:	δ 21.4, 22.5, 24.6, 27.8, 32.0, 32.5, 35.7, 36.9, 37.7, 68.0, 74.3, 101.9, 105.0, 111.7, 149.4, 163.2, 166.7, 172.6 ppm
Melting point:		181-182 °C
Microanalysis:	Found:	C = 67.1%, H = 8.2%
	Calculated:	C = 67.1%, H = 8.1% (Calculated for $C_{18}H_{26}O_5$)