



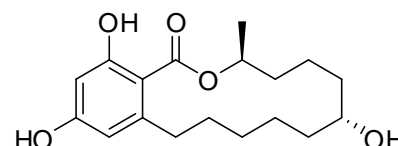
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA P1801b: $\alpha$ -Zearalanol

Report ID: P1801b.2024.01 (Ampouled 230412)

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

Molecular Weight: 322.4 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
22-AV-01	26538-44-3	983 $\pm$ 32 $\mu$ g

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

**IUPAC name:** (3S,7R)-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 until 1 May 2027, three 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

**Description:** The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing  $\alpha$ -zearalanol. This material was prepared by synthesis and certified for identity and purity by NMIA.

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

**Instructions for use:** Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer 983  $\pm$  32  $\mu$ g of anhydrous  $\alpha$ -zearalanol. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

**Recommended storage:** When not in use, this material should be stored at or below 4  $^{\circ}$ 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 all impurities are quantified as a mass fraction and subtracted from 100%.

**Stability:** In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from short term accelerated 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 ampoules 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 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.  
9 May 2024

This report supersedes any issued prior to 9 May 2024.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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

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### Characterisation Report:

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm x 150 mm)  
 Column oven: 40 °C  
 Mobile Phase: Methanol/MilliQ water (60:40 v/v)  
 Flow rate: 1.0 mL/min  
 Detector: Shimadzu SPD-M20A PDA operating at 262 nm  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 98.9%, s = 0.003% (8 ampoules in duplicate, May 2023)  
 Re analysis: Mean = 98.8%, s = 0.03% (5 ampoules in duplicate, May 2024)

### The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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-UV detection, thermogravimetric analysis, Karl Fischer analysis, and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by elemental microanalysis.

HPLC: Instrument: Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm x 150 mm)  
 Column oven: 40 °C  
 Mobile Phase: Methanol/Milli-Q water (60:40 v/v)  
 Flow rate: 1.0 mL/min  
 Detector: Shimadzu SPD-M20A PDA operating at 262 nm  
 Relative mass fraction of the main component:  
 Initial analysis: Mean = 99.0%, s = 0.01% (10 sub samples in duplicate, January 2023)

Karl Fischer analysis: Moisture content 0.3% mass fraction (February 2023)

Thermogravimetric analysis: Volatiles content 0.8 % and non-volatile residue < 0.2% mass fraction (January 2023)

### Spectroscopic and other characterisation data

LC-MS: Instrument: Waters Alliance/ Micromass Quattro TQ Detector  
 Column: Alltima C-18, 5  $\mu$ m (4.6 mm x 150 mm)  
 Column temp: 40 °C  
 Solvent system: Methanol/MilliQ water (60 :40 v/v)  
 Flow rate: 1 mL/min  
 Sample prep: 10  $\mu$ g/g in MeOH/MilliQ water (60 :40)  
 Injection volume: 10  $\mu$ L  
 Ionisation mode: Electrospray negative ion  
 Capillary voltage: 3.0 kV  
 Cone voltage: 20 V  
 Source temp: 120 °C  
 Desolvation gas temp: 400°C  
 Cone gas flow rate: 23 L/hr  
 Desolvation gas flow: 650 L/hr

The retention time of  $\alpha$ -zearalanol is reported with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.

11.9 min: 320.9 (M-H<sup>+</sup>) *m/z*

TLC: Conditions: Kieselgel 60F<sub>254</sub>. Hexane/ethyl acetate (4:1)  
 Single spot observed, R<sub>f</sub> = 0.1. Visualisation with UV at 254 nm.

IR: Instrument: BioRad FTS 3000MX FT-IR  
 Range: 4000-400 cm<sup>-1</sup>, KBr powder  
 Peaks: 3496, 3165, 2945, 1649, 1616, 1586, 1463, 1314, 1259, 1198, 1166, 1096, 1074, 989, 840, 762 cm<sup>-1</sup>

<sup>1</sup>H NMR: Instrument: Bruker Avance III-500  
 Field strength: 500 MHz  
 Solvent: MeOH-d<sub>4</sub> (3.31 ppm)  
 Spectral data:  $\delta$  1.34 (3H, d, *J* = 6.0 Hz), 1.21-1.87 (14H, m), 2.43 (1H, ddd, *J* = 5.0, 12.5, 17.5 Hz), 3.18 (1H, ddd, *J* = 4.0, 12.0, 16.0 Hz), 3.75 (1H, m), 5.17 (1H, m), 6.16 (1H, d, *J* = 2.5 Hz), 6.21 (1H, d, *J* = 2.0 Hz) ppm  
 Acetone was quantified at 0.5% mass fraction.

<sup>13</sup>C NMR: Instrument: Bruker Avance III-500  
 Field strength: 126 MHz  
 Solvent: Acetone-d<sub>6</sub> (29.0 ppm)  
 Spectral data:  $\delta$  20.6, 21.7, 23.8, 27.0, 31.3, 31.7, 34.9, 36.2, 36.9, 67.1, 73.5, 101.1, 104.2, 110.9, 148.7, 162.4, 165.8, 171.8 ppm

Melting point: 181-183 °C

Microanalysis: Found: C = 67.1%; H = 8.2% (February 2023)  
 Calculated: C = 67.1%; H = 8.1% (Calculated for C<sub>18</sub>H<sub>26</sub>O<sub>5</sub>)