



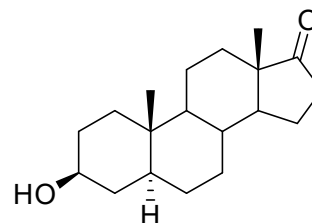
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA S057: Epiandrosterone

Report ID: S057.2024.01

Chemical Formula: C₁₉H₃₀O₂

Molecular Weight: 290.4 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
24-S-10	481-29-8	99.8 ± 0.5%

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

IUPAC name: (3β,5α)-3-Hydroxyandrostane-17-one.

Expiration of certification: The property values are valid until 10 May 2027, three years from the date of 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. 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: Off-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.

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 all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: At the recommended storage conditions this material has demonstrated stability for a period of three years. The measurement uncertainty includes components for long term stability at the recommended storage conditions, and accelerated stability trials conducted at 40 °C and 75% humidity for a 14 day period.

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 GC-FID 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.
2 August 2024

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

CIPM MRA notice: This certificate is consistent with the capabilities that are included in Appendix C of the MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognise the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in the KCDB (for details see <http://www.bipm.org/kcdb/>). The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate.

Legal notice: Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA's behalf, assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this document.

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 GC-FID, 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.

GC-FID:	Instrument:	Varian CP3800
	Column:	DB-17, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	200 °C (1 min), 5 °C/min to 260 °C, 30 °C/min to 280 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.8%, s = 0.02% (10 sub samples in duplicate, May 2024)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (May 2024)
Thermogravimetric analysis:		Non-volatile residue <0.1% mass fraction (June 2024)

Spectroscopic and other characterisation data

GC-MS: Instrument: Agilent 8890/5973
 Column: HP-5MS, 30 m x 0.25 mm I.D. x 0.25 μ m
 Program: 200 °C (0.5 min), 10 °C/min to 240 °C (10 min), 20 °C/min to 300 °C (3 min)
 Injector: 250 °C
 Split ratio: 20/1
 Transfer line temp: 280 °C
 Carrier: Helium, 1.0 mL/min
 Scan range: 50-550 *m/z*

The retention time of epiandrosterone 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 (11.9 min): 290 (M^+ , 100), 275 (14), 272 (25), 257 (30), 254 (15), 246 (46), 239 (26), 228 (14), 216 (22), 175 (14), 164 (20), 161 (19), 148 (29), 145 (34), 135 (26), 131 (31), 129 (19), 124 (38), 121 (35), 119 (40), 115 (21), 109 (34), 107 (84), 105 (57), 97 (34), 95 (39), 93 (65), 91 (79), 81 (52), 79 (89), 77 (46), 67 (61), 65 (19), 55 (39), 53 (16) *m/z*

IR: Instrument: Bruker Alpha Platinum ATR
 Range: 4000-400 cm^{-1} , neat
 Peaks: 3467, 2974, 2927, 2889, 2863, 2836, 1727, 1470, 1445, 1374, 1304, 1131, 1080, 1046, 1009, 837, 613, 586, 493 cm^{-1}

^1H NMR: Instrument: Bruker Avance III-500
 Field strength: 500 MHz
 Solvent: CDCl_3 (7.26 ppm)
 Spectral data: δ 0.69 (1H, ddd, $J = 4.0, 10.8, 12.3$ Hz), 0.83 (3H, s), 0.85 (3H, s), 0.92-1.02 (2H, m), 1.13 (1H, m), 1.20-1.37 (6H, m), 1.42 (1H, m), 1.47-1.60 (2H, m), 1.65 (1H, dm, $J = 13.2$ Hz), 1.72 (1H, dt, $J = 3.6, 13.2$ Hz), 1.77-1.84 (3H, m), 1.92 (1H, m), 2.06 (1H, dt, $J = 9.2, 19.2$ Hz), 2.43 (1H, ddd, $J = 0.7, 9.0, 19.2$ Hz), 3.59 (1H, m) ppm

^{13}C NMR: Instrument: Bruker Avance III-500
 Field strength: 126 MHz
 Solvent: CDCl_3 (77.0 ppm)
 Spectral data: δ 12.3, 13.8, 20.5, 21.8, 28.4, 30.9, 31.5, 31.6, 35.1, 35.7, 35.9, 37.0, 38.1, 44.9, 47.8, 51.5, 54.5, 71.2, 221.4 ppm

Melting point: 175-179 °C

Microanalysis: Found: C = 78.4%; H = 10.3% (July 2024)
 Calculated: C = 78.6%; H = 10.4% (Calculated for $\text{C}_{19}\text{H}_{30}\text{O}_2$)