Australian Government
Department of Industry, Science and Resources

National Measurement Institute

CIPM MRA

NATA
WORLD RECOGNISED ACCREDITATION

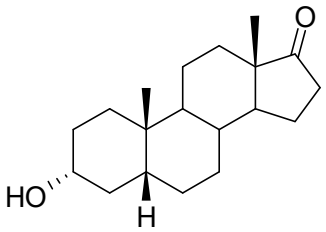
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D551c: Etiocholanolone

Report ID: D551c.2024.01 (Ampouled 190221)

Chemical Formula: $C_{19}H_{30}O_2$

Molecular Weight: 290.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
10-S-07	**53-42-9**	**990 ± 18 µg**

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

IUPAC: (3α,5β)-3-Hydroxyandrostan-17-one

Expiration of certification: The property values are valid till 10 September 2029, 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

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 D551c. This material was sourced from an external supplier, 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. *tert*-butyl methyl ether). This will transfer 990 ± 18 µg of anhydrous etiocholanolone. 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 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%. 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 minimum period of three 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 GC-FID 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.
11 September 2024

This report supersedes any issued prior to 11 September 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: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

GC-FID:	Instrument:	Agilent 6890 or 8890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	215 °C (20 min), 20 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.01% (7 ampoules in duplicate, February 2019)
	Re-analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, November 2021)
	Re-analysis:	Mean = 99.3%, s = 0.02% (5 ampoules in duplicate, September 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 GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by qualitative headspace GC-MS analysis of occluded solvents, quantitative nuclear magnetic resonance (qNMR) and elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890 or 7890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	215 °C (20 min), 20 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.05% (10 sub samples in duplicate, July 2010)
	Re-analysis:	Mean = 99.3%, s = 0.09% (5 sub samples in duplicate, August 2012)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, April 2015)
GC-FID:	Instrument:	Varian 3800
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	215 °C (20 min), 20 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Detector Temp:	320 °
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.06% (10 sub samples in duplicate, July 2010)
GC-FID:	Instrument:	Varian 3800
	Column:	VF-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	215 °C (20 min), 20 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.05% (10 sub samples in duplicate, July 2010)
Karl Fischer analysis:		Moisture content ≤ 0.1% mass fraction (August 2010, July 2012, April 2015 and July 2019)
Thermogravimetric analysis:		Volatiles content 0.6% and non-volatile residue < 0.2% mass fraction (August 2010)
QNMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26ppm)
	Internal standard:	Dimethyl terephthalate (100.0% mass fraction)
	Initial analysis:	Mean (2.4 ppm) = 98.6%, s = 0.8% (5 sub samples, August 2010)

Spectroscopic and other characterisation data

GC-MS:	Instrument:	HP6890/5973
	Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 20 °C/min to 240 °C (10 min), 20 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Split ratio:	30/1
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.0 mL/min
	Scan range:	50-550 *m/z*
	The retention time of the parent compound 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 (11.12 min):	290 (M^+, 84), 272 (100), 257 (100), 244 (78), 228 (32), 215 (63), 201 (56), 176 (17), 161 (56), 147 (56), 133 (41), 119 (51), 107 (77), 93 (81), 79 (96), 55 (69) *m/z*
	The material was shown to co-elute with a previously registered sample of etiocholanolone (D551b) and also afford an identical mass spectrum.	
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 μm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Ethyl acetate
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1) Single spot observed, R_f = 0.2-0.3. Visualisation with vanillin
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3469, 2937, 2866, 2850, 1729, 1718, 1456, 1364, 1045, 1009 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance 400
	Field strength:	400 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Spectral data:	δ 0.84 (3H, s), 0.94 (3H, s), 1.00 (1H, ddd, *J* = 3.4, 14.3, 14.3 Hz), 1.13-1.95 (19H, m), 2.07 (1H, dt, *J* = 9.1, 19.3 Hz), 2.43 (1H, ddd, *J* = 0.9, 8.3, 19.3 Hz), 3.63 (1H, heptet, *J* = 4.8 Hz) ppm
	Ethyl acetate estimated at 0.9% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance 400
	Field strength:	100.6 MHz
	Solvent:	$CDCl_3$ (77.0 ppm)
	Spectral data:	δ 13.8, 20.1, 21.8, 23.3, 25.3, 26.9, 30.5, 31.7, 34.7, 35.3, 35.4, 35.9, 36.3, 40.7, 42.0, 47.8, 51.5, 71.6, 221.3 ppm
Melting point:		150-151 °C
Microanalysis:	Found:	C = 78.7%; H = 10.4% (August, 2010)
	Calculated:	C = 78.6%; H = 10.4% (Calculated for $C_{19}H_{30}O_2$)