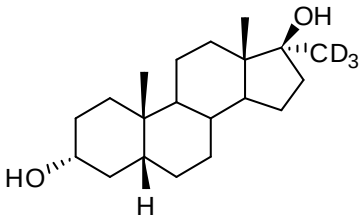
Australian Government

National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D928.2015.01 (Ampouled 090701)
This batch of ampoules was prepared from the bulk material on 1st July 2009.

Compound Name: **d_3-17α-Methyl-5β-androstan-3α-17β-diol**
Collection No: D928
Chemical Formula: $C_{20}H_{31}D_3O_2$
CAS No: NA
Structure:



Description: White crystals
Batch No: 08-S-08
Molecular Weight: 309.5
Batch production completed: July 2008

The main component of this material is d_3-17α-methyl-5β-androstan-3α-17β-diol. d_2-, d_1- and d_0-17α-Methyl-5β-androstan-3α-17β-diol are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_3, d_2 and d_1) and d_0-17α-methyl-5β-androstan-3α-17β-diol in the material.

The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D928. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform). This will transfer approximately 986 µg of anhydrous 17α-methyl-5β-androstan-3α-17β-diol (d_3, d_2, $d_1$and d_0).

The isotopic purity of this material is an estimate only. The deuterium analysis was carried out on d_3-17α-methyltestosterone, an intermediate that was used to synthesis D928. Deuterium analysis was not carried out on D928 due to the weak parent ion in the mass spectrum. This material should be considered for use as an internal standard only.

Isotopic Purity:		$d_3 \approx 99.3\%$ [= (d_3 / d_0 + d_1 + d_2 + d_3) × 100]
		$d_0 < 0.1\%$ [= (d_0 / d_0 + d_1 + d_2 + d_3) × 100]
GC-FID:	Instrument:	Varian CP-3800
	Column:	VF-1, 29.21 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 240 °C (5 min), 30 °C/min to 300 °C (3 min)
	Injector:	250 °C Detector Temp: 320 °C
	Carrier:	Helium Split ratio: 20/1
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.2%, s = 0.04% (7 ampoules in duplicate, July 2009)
	Re analysis:	Mean = 99.3%, s = 0.06% (5 ampoules in duplicate, May 2012)
	Re analysis:	Mean = 99.1%, s = 0.02% (5 ampoules in duplicate, April 2015)

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

Purity estimate obtained by subtraction from 100% of total impurities by GC-FID, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR.

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973	
Column:	ZB-5MS, 30 m x 0.25 mm I.D. x 0.25 μm	
Program:	180 °C (1 min), 10 °C/min to 300 °C (1 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 30/1

Bis-TMS derivative:

Instrument:	Agilent 6890 / 5973	
Column:	Ultra 1, 17 m x 0.2 mm I.D.x 0.11 μm	
Program:	187 °C (0.2 min), 3 °C/min to 238 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C (2 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 12/1

The retention times of the parent compound and *bis*-TMS derivative are reported along 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 (8.9 min): 309 (M^+, 2), 291 (30), 273 (27), 258 (22), 248 (13), 230 (100), 217 (42), 215 (55), 175 (14), 161 (19), 149 (20), 135 (37), 121 (26), 107 (29), 93 (29), 81 (27), 67 (22), 55 (18), 46 (21) m/z

Bis-TMS (11.0 min): 453 (M^+, 3), 435 (10), 318 (4), 273 (19), 258 (9), 228 (8), 146 (100), 132 (14), 73 (22) m/z

The parent compound co-elutes with a comparison sample of native 17α-methyl-5β-androstan-3α-17β-diol (NMI Collection # D561).

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1)
Single spot observed, R_f = 0.22. Visualisation with vanillin

IR: Instrument: Biorad FTS300MX FT-IR
Range: 4000-400cm^{-1}, KBr powder
Peaks: 3326, 2928, 2864, 2227, 1449, 1376, 1296, 1139, 1067, 1037 cm^{-1}

^{1}H NMR: Instrument: DMX-600
Field strength: 600 MHz Solvent: CD_3OD (3.30 ppm)
Spectral data: δ 0.82 (3H, s), 0.96 (3H, s), 0.99 (1H, m), 1.11, (1H, m), 1.20-1.35 (6H, m), 1.36-1.68 (10H, m), 1.75 (1H, quartet, *J* = 12.8 Hz), 1.79-1.85 (2H, m), 1.90 (1H, m), 3.53 (1H, m) ppm

^{13}C NMR: Instrument: DMX-600
Field strength: 151 MHz Solvent: CD_3OD (49 ppm)
Spectral data: δ 14.7, 21.6, 24.0, 24.3, 27.5, 28.3, 31.2, 33.1, 35.8, 36.6, 37.2, 38.1, 39.2, 42.0, 43.6, 46.9, 52.2, 72.4, 82.1 ppm

Melting point: 166 °C

Microanalysis: Found: C = 77.8%; H = 11.5% (May 2008)
Calc: C = 77.5%; H = 12.1% (Calculated for $C_{20}H_{31}D_3O_2$)

Thermogravimetric analysis: Volatile content not determined by TGA and non volatile residue 0.44 % mass fraction (July 2008 and July 2009).

Karl Fischer analysis: Moisture content 0.3% mass fraction (July 2009).

Expiration of certification

The property values are valid till 10th April 2020, 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.

The long-term stability of the compound in solution has not been examined.

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

Homogeneity assessment

The homogeneity of the material was assessed using purity assay by GC-FID on 7 randomly selected ampoules of the material. The material was judged to be 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.

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.

Intended Use

For ***in vitro*** laboratory analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI'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 certificate.

Authorised by: S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 22 April, 2015.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 22nd April 2015.