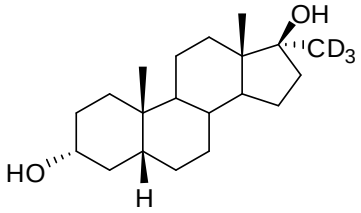
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D928: d3-17α-Methyl-5β-Androstane-3α, 17β-diol



Report ID: D928.2020.03 (Ampouled 090701)

Chemical Formula: $C_{20}H_{31}D_3O_2$

Molecular Weight: 309.5 g/mol

Property value

Batch No.	CAS No.	Mass per ampoule
08-S-08	**Not available**	**986 ± 20 μg**

IUPAC name: (3α,5β,17β)-17-[$^2H_3$]Methylandrostane-3,17-diol

Expiration of certification: The property values are valid till 19 May 2030, 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: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The deuterated internal standard is intended for a single use to prepare a standard solution containing D928. The material was prepared by synthesis, and certified for identity and purity by NMIA. 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 approximate combined masses of deuterated (d_4, d_3, d_2 and d_1) and d_0-17α-methyl-5β-androstan-3α-17β-diol in the material.

Intended use: The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. 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 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.

Stability: 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 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.
15 November 2022.

This report supersedes any issued prior to 27 July 2021.

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.

Characterisation Report:

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 of the 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)
	Re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, May 2020)

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 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.

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.

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 chemical purity of the analyte represents the combined mass fractions of deuterated (d_3, d_2 and d_1) and d_0-17α-methyl-5β-androstan-3α-17β-diol in the material.

The isotopic purity of this material is an estimate only. 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, 29.82 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 240 °C (5 min), 40 °C/min to 300 °C (3 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.3%, s= 0.1% (10 sub samples in duplicate, July 2008)
	Re-analysis:	Mean = 99.2%, s= 0.02% (5 sub samples in duplicate, July 2009)
Karl Fischer analysis:		Moisture content 0.29% mass fraction (July 2009)
Thermogravimetric analysis:		Volatile content not determined by TGA and non volatile residue 0.44 % mass fraction (July 2008 and July 2009)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP6890/5973
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 (3 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*

Bis-TMS derivative:

Instrument:	HP 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
Split ratio:	12/1
Transfer line temp:	300 °C
Carrier:	Helium
Scan range:	50-550 *m/z*

The retention times of the parent compound and *bis*-TMS derivative are 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 (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:	4000-400 cm^{-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:	MeOH-d_4 (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:	MeOH-d_4 (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)
Calculated:	C = 77.5 %; H = 12.1 % (Calculated for $C_{20}H_{31}D_3O_2$)