Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

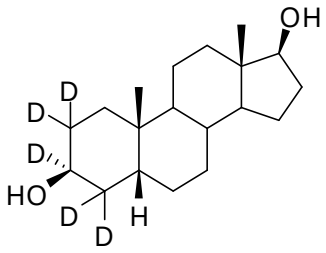
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA S014: d_5-5β-Androstane-3β,17β-diol

Report ID: S014.2023.01 (Ampouled 160630)

Chemical Formula: $C_{19}H_{27}D_5O_2$

Molecular Weight: 297.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
12-S-01	**Not available**	**974 µg ± 45 µg**

IUPAC name: (3β,17β)-3,17-Dihydroxy(2,2,3,4,4-$^2H_5$)androstane.

Expiration of certification: The property values are valid till 15 March 2028, 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 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 RM is intended for a single use to prepare a standard solution containing S014. The material was prepared by synthesis and certified for identity and purity by NMIA.

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. methanol). This will transfer approximately 974 µg of anhydrous 5β-androstane-3β,17β-diol (d_5, d_4, 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: This material has demonstrated stability over a minimum period of five years. 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 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.
21 April 2023.

This report supersedes any issued prior to 21 April 2023.
NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

GC-FID:	Instrument:	Agilent 6890N, Agilent 8890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 220 °C (10 min), 30 °C/min to 300 °C (5 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.002% (7 ampoules in duplicate, July 2016)
	Re-analysis:	Mean = 99.6%, s= 0.02% (5 ampoules in duplicate, May 2017)
	Re-analysis:	Mean = 99.6%, s= 0.01% (5 ampoules in duplicate, June 2020)
	Re-analysis:	Mean = 99.6%, s= 0.01% (5 ampoules in duplicate, March 2023)

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_5-5β-androstane-3β,17β-diol. d_4-, d_3-, d_2-, d_1- and d_0-5β-Androstane-3β,17β-diol are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-5β-androstane-3β,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:	$d_5 \approx 92.7\%$ [= $d_5/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	$d_0 < 0.1\%$ [= $d_0/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
GC-FID:	Instrument:	Varian CP-3800
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 220 °C (10 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 = 99.6%, s = 0.01% (10 sub samples in duplicate, September 2012)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 220 °C (10 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 = 99.6%, s = 0.003% (5 sub samples in duplicate, July 2016)
Thermogravimetric analysis:		Volatile content 6.1% and non volatile residue < 0.2% mass fraction (September 2012)
Karl Fischer analysis:		Moisture content 3.3% mass fraction (May 2012) Moisture content 2.5% mass fraction (September 2012) Moisture content 3.0% mass fraction (July 2016)

Spectroscopic and other characterisation data

GC-MS: Parent compound/*Bis*-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	TG1-MS, 30 m x 0.25 mm I.D. x 0.25 µm
Program:	180 °C (1 min), 10 °C/min to 300 °C (2 min)
Injector:	250 °C
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

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 (10.1 min):	297 (M^+, 10), 282 (24), 279 (100), 261 (30), 246 (27), 238 (36), 220 (77), 154 (41), 149 (25), 133 (22), 112 (38), 93 (44), 81 (43), 67 (33) *m/z*
Bis-TMS (10.8 min):	441 (M^+, 8), 426 (16), 381 (15), 351 (45), 336 (23), 261 (75), 246 (55), 220 (32), 162 (25), 148 (22), 129 (100), 73 (89) *m/z*

TLC: Conditions: Kieselgel 60F254. Chloroform/ethyl acetate (2/1)
Single spot observed, Rf = 0.47. Visualisation with vanillin

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3308, 2977, 2948, 2932, 2899, 2854, 2200, 2190, 2131, 1442, 1374, 1104, 1074, 1055, 956, 833 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance-600
Field strength:	600 MHz
Solvent:	MeOH-d_4 (3.31 ppm)
Spectral data:	δ 0.72 (3H, s), 0.98 (3H, s), 1.00-1.11 (3H, m), 1.15-1.50 (10H, m), 1.59 (1H, m), 1.74 (1H, m), 1.83 (1H, dt, *J* = 3.3, 12.5 Hz), 1.89-2.00 (2H, m), 3.57 (1H, t, *J* = 8.7 Hz) ppm

Ethanol estimated at 0.1% mass fraction was observed in the ^{1}H NMR.

^{13}C NMR:

Instrument:	Bruker Avance-600
Field strength:	150 MHz
Solvent:	MeOH-d_4 (49.0 ppm)
Spectral data:	δ 11.7, 21.8, 24.3, 24.5, 27.1, 27.7, 30.7, 30.9, 33.5, 36.2, 37.1, 37.7, 38.2, 41.4, 44.2, 52.5, 67.2, 82.6 ppm

Melting point: 180-184 °C

Microanalysis:

Found:	C = 75.3%; H/D = 11.1% (September, 2012)
Calculated:	C = 76.7%; H/D = 11.0% (Calculated for $C_{19}H_{27}D_5O_2$)
Calculated:	C = 74.8%; H/D = 11.0% (Calculated for $C_{19}H_{27}D_5O_2$ + 2.5% water)