Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

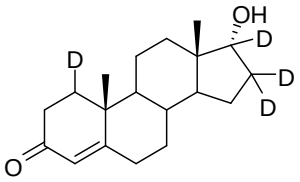
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S001: d_4-[1,16,16,17]-Epitestosterone

Report ID: S001.2023.01 (Ampouled 170706)

Chemical Formula: $C_{19}H_{24}D_4O_2$

Molecular Weight: 292.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
09-S-05	**Not available**	**978 ± 18 μg**

IUPAC name: (17β)-17-Hydroxy(1,16,16,17-$^2H_4$)androst-4-en-3-one.

Expiration of certification: The property values are valid till 27 April 2028, 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 CRM is intended for a single use to prepare a standard solution containing S001. 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. chloroform). This will transfer approximately 978 ± 18μg of anhydrous d_4-[1,16,16,17]-epitestosterone (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: 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 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.
28 April 2023.

This report supersedes any issued prior to 28 April 2023.

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:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °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 main component:	
	Initial analysis:	Mean = 98.1%, s = 0.02% (5 ampoules in duplicate, July 2017)
	Re-analysis:	Mean = 98.4%, s = 0.02% (5 ampoules in duplicate, June 2018)
	Re-analysis:	Mean = 98.4%, s = 0.01% (5 ampoules in duplicate, April 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 headspace GC-MS analysis of occluded solvents and elemental microanalysis.

The main component of this material is d_4-[1,16,16,17]-epitestosterone. d_3-, d_2-, d_1- and d_0- epitestosterone are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_4, d_3, d_2 and d_1) and d_0-epitestosterone 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:	$d_4 \approx 92.8\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	$d_0 < 0.2\%$ [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
GC-FID:	Instrument:	Agilent 6890 or Varian CP-3800
	Column:	HP-1, HP-5 or VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C/min to 300 °C (2 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of main component:	
	Initial analysis:	Mean = 98.4%, s = 0.06% (10 sub samples in duplicate, December 2009)
	Re-analysis:	Mean = 98.4%, s = 0.1% (5 sub samples in duplicate, November 2012)
Thermogravimetric analysis:		N/A
Karl Fischer analysis:		Moisture content < 0.3% mass fraction (December 2009 and November 2012)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	VF-1MS, 14.9 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
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

Bis-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	Ultra 1, 17 m × 0.22 mm I.D. × 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 (3 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 (8.1 min): 292 (M^+, 56), 274 (23), 250 (29), 232 (49), 217 (19), 206 (31), 188 (17), 168 (18), 149 (63), 125 (100), 110 (26), 91 (31), 79 (26), 55 (14), 41 (15) *m/z*

Bis-TMS (11.1 min): 436 (M^+, 100), 421 (8), 345 (3), 331 (6), 302 (2), 248 (2), 209 (6), 194 (4) 73 (87) *m/z*

The parent compound co-elutes with a comparison sample of native epitestosterone (NMI Collection # D547).

TLC:
Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1).
Single spot observed, R_f = 0.3. Visualisation with UV at 254 nm.

IR:

Instrument:	Biorad FTS300MX FT-IR.
Range:	4000-400cm^{-1}, KBr powder
Peaks:	3419, 2933, 2879, 2867, 2823, 2225, 2194, 2150, 2127, 1652, 1609, 1453, 1432, 1380, 1359, 1248, 1107, 880 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance 300
Field strength:	300 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.69 (3H, s), 0.89-1.27 (3H, m), 1.18 (3H, s), 1.34-1.67 (7H, m),1.76 (1H, dd, J = 6.9, 12.1 Hz), 1.87 (1H, m), 2.01 (1H, m), 2.22-2.45 (4H, m), 5.71 (1H, s) ppm

Ethyl acetate at 0.5% mass fraction was observed in the ^{1}H NMR (MeOH-d_4)
Chloroform at 0.4% mass fraction was observed in the ^{1}H NMR (MeOH-d_4)

^{13}C NMR:

Instrument:	Bruker Avance 300
Field strength:	75 MHz
Solvent:	$CDCl_3$ (77.0 ppm)
Spectral data:	δ 16.8, 17.4, 20.5, 24.3, 31.1, 31.5 (quintet, *J* = 18.8 Hz), 32.3, 32.9m, 33.8, 35.3 (t, *J* = 19.4 Hz), 35.8, 38.5, 45.0, 48.2, 53.5, 79.1 (t, *J* = 22.1), 123.8, 171.4, 199.6 ppm

Melting point: 213-216 °C

Microanalysis:

Found:	C = 77.4 %; H = 9.7 % (December 2009)
Calculated:	C = 78.0 %; H = 9.8 % (Calculated for $C_{19}H_{24}D_4O_2$)