Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
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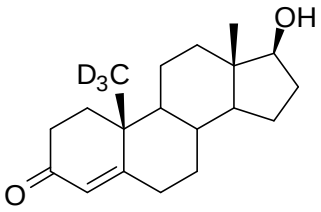
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D644: 19-d_3-Testosterone

Report ID: D644.2016.02 (Ampouled 071105)

Chemical Formula: $C_{19}H_{25}D_3O_2$

Molecular Weight: 291.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
00-S-10	69660-28-2	958 µg

IUPAC name: (17β)-17-Hydroxy(16,16,17-$^2H_3$)androst-4-en-3-one

Expiration of certification: The property values are valid till 16th August 2021, 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 CRM is intended for a single use to prepare a standard solution containing D644. The material was sourced from external supplier, 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 928 µg of anhydrous testosterone (d3, d2, d1 and d0). 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 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 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.
10 February 2020.

This report supersedes any issued prior to 10 February 2020.

Characterisation Report:

GC-FID:	Instrument:	Varian 3800	
	Column:	VF-1, 30 m x 0.32 mm I.D. x 0.25 µm	
	Program:	180 °C (1 min), 20 °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 = 99.2%, s = 0.02% (7 ampoules in duplicate, November 2007)	
	Re-analysis:	Mean = 99.3 %, s = 0.02% (5 ampoules in duplicate, November 2008)	
	Re-analysis:	Mean = 99.1 %, s = 0.02% (5 ampoules in duplicate, October 2011)	
	Re-analysis:	Mean = 99.1 %, s = 0.02% (5 ampoules in duplicate, August 2016)	

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 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.

Isotopic Purity:		$d_3 \approx 72\%$ [$= d_3 / (d_0 + d_1 + d_2 + d_3) \times 100$]
		$d_0 < 0.5\%$ [$= d_0 / (d_0 + d_1 + d_2 + d_3) \times 100$]
Note:		Each ampoule contains approximately 690 µgs of 19-d_3-testosterone
GC-FID:	Instrument:	Agilent 6890
	Column:	ZB-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °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 = 99.1%, s = 0.04% (5 subsamples in duplicate, June 2005)
Thermogravimetric analysis:		Volatiles content 3.2% and non-volatile residue 0.34% mass fraction (November 2007)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP5890 / 5971	
Column:	BPX-5, 30m x 0.25mm I.D. x 0.25 µm	
Program:	180 °C (1 min), 15 °C/min to 300 °C (3 min)	
Injector:	260°C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 30/1

Bis-TMS derivative:

Instrument:	Agilent 6890 / 5973	
Column:	HP Ultra 1, 17m x 0.25mm ID x 0.22 µm	
Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)	
Injector:	280°C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 15/1

The retention time of the parent compound and its *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 (11.6 min):	291 (M^+, 26), 273 (13), 249 (36), 203 (27), 147 (32), 127 (100) *m/z*
Bis-TMS (11.9 min):	435 (M^+, 100), 420 (8), 304 (3), 211 (7), 73 (48) *m/z*

TLC:

Conditions:	Kieselgel 60F254. Chloroform/ethyl acetate (80:20) Single spot observed, Rf =0.28 (3 samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3530, 3388, 2225, 1667, 1614, 1353, 1230, 1058, 870 cm^{-1}

^{1}H NMR:

Instrument:	Bruker AMX-400	
Field strength:	400 MHz	Solvent: CDCl3
Key spectral data:	δ 0.77 (3H, s), 3.63 (1H, t), 5.71 (1H, s) ppm	

^{13}C NMR:

Instrument:	Bruker DMX-500	
Field strength:	126 MHz	Solvent: CDCl3
Spectral data:	δ 11.0, 20.7, 23.3, 30.5, 31.5, 32.8, 34.0, 35.7 (x 2), 36.4, 38.5, 42.8, 50.5, 53.9, 81.6, 123.9, 171.2, 199.5 ppm	