Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

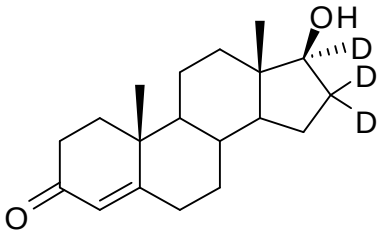
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D546: d_3-Testosterone

Report ID: D546.2019.01 (Ampouled 190926)

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

Molecular Weight: 291.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
98-002931	**77546-39-5**	**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 3 October 2022, i.e. three years from the date of 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 and is intended for a single use to prepare a standard solution containing D546. The main component of this material is d_3-testosterone. d_2-, d_1- and d_0-testosterone 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-testosterone in the material. 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: Each ampoule contains approximately 958 µg of anhydrous d_3-testosterone (d_3, d_2, d_1 and d_0). Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform).

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.
18 October 2019.

Characterisation Report:

Warning: **This material is sensitive to the quality of the silanised glass liner when injected at elevated temperature (~ 250 ºC) into a GC instrument.**

GC-FID:
Instrument: Varian CP3800
Column: VF-1MS, 30 m x 0.32 mm I.D. x 0.25 μm
Program: 200 °C (1 min), 15 °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 = 99.9%, s = 0.01% (7 ampoules in duplicate, October 2019)

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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID and Karl Fischer analysis. The purity value is calculated as per Equation 1.

Supporting evidence is provided by thermogravimetric analysis, ^{1}H NMR spectroscopy, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis.

Isotopic Purity: $d_3 \approx 91\%$ [$= d_3 / (d_0 + d_1 + d_2 + d_3) \times 100$]
$d_0 < 0.5\%$ [$= d_0 / (d_0 + d_1 + d_2 + d_3) \times 100$]

GC-FID:
Instrument: HP 5890
Column: J&W DB-5MS, 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: 325 °C
Carrier: Helium Split ratio: 20/1
Relative peak area of the main component:
Initial analysis: Mean = 99.9%, s = 0.1% (7 sub samples, November 1998)

GC-FID:
Instrument: HP 5890
Column: ZB-1, 30 m x 0.32 mm I.D. x 0.25 μm
Program: 200 °C (1 min), 15 °C/min to 300 °C (6 min)
Injector: 250 °C Detector Temp: 300 °C
Carrier: Helium Split ratio: 20/1
Relative peak area of the main component:
Initial analysis: Mean = 99.6%, s = 0.02% (5 sub samples, June 2005)

GC-FID:
Instrument: Varian 3800
Column: VF1-MS Capillary, 30 m x 0.32 mm I.D. x 0.25 μm
Program: 200 °C (1 min), 15 °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 = 99.8%, s = 0.004% (5 sub samples, March 2009)
Re-analysis: Mean = 99.9%, s = 0.006% (7 sub samples, October 2019)

GC-FID:
Instrument: Agilent 6890
Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
Program: 200 °C (1 min), 15 °C/min to 300 ° (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 = 0.02% (7 sub samples, November 2015)

HPLC:
Column: Alltima C-18, 5 μm (4.6 mm x 150 mm)
Mobile Phase: Acetonitrile/water (50:50)
Flow Rate: 0.5 mL/min
Detector: UV at 240 nm
Relative peak area of the main component:
Initial analysis: Mean = > 99.9% (3 sub samples, September 1998)

Thermogravimetric analysis: Volatile content 0.3% and non volatile residue < 0.2% mass fraction (August 2005)

Karl Fischer analysis: Moisture content 1.0 % mass fraction (March 2009)
Moisture content 5.0 % mass fraction (November 2015)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973	
Column:	HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11 μm	
Program:	190 °C (1 min), 12 °C/min to 300 °C (3 min)	
Injector:	280 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Splitless injection

Bis-TMS derivative:

Instrument:	Agilent 6890/5973	
Column:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μm	
Program:	170 °C (0.5 min), 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	Splitless injection

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 (5.5 min): 291 (M^+, 50), 276 (7), 273 (9), 249 (40), 231 (16), 206 (20), 124 (100) *m/z*
Bis-TMS (11.3 min): 435 (M^+, 100), 420 (12), 208 (6), 73 (52) *m/z*

The *bis*-TMS derivative of d_3-testosterone co-elutes with a comparison sample of silylated unlabelled testosterone under these conditions. The fragmentation pattern matches published data for the *bis*-TMS derivative of d_3-epitestosterone.

Deuteration yield (by SIM analysis of the *bis*-TMS derivative, mean of 3 sub samples)
SIM ions quantified (deuteration state, % relative intensity to d_3-testosterone *bis*-TMS at 435 *m/z*)
432 (d_0, 0), 433 (d_1, 1), 434 (d_2, 9), 435 (d_3, 100)

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20)
Single spot observed, R_f = 0.28 (3 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3529, 3385, 1665, 1612, 1235, 1187, 1045, 868 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500	
Field strength:	500 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.77 (3H, s), 1.17 (3H, s), 5.71 (1H, s, 4H) ppm	

^{2}H NMR:

Instrument:	Bruker DMX-500	
Field strength:	76 MHz	Solvent: $CHCl_3$
Spectral data:	δ 1.44 (1D, 16α-D), 2.06 (1D, 16β-D), 3.64 (1D, 17α-D) ppm	

^{13}C NMR:

Instrument:	Bruker DMX-500	
Field strength:	125 MHz	Solvent: $CDCl_3$ (77.2 ppm)
Spectral data:	δ 11.0, 17.4, 20.6, 23.1, (29.5), 31.5, 32.8, 33.9, 35.6, 35.7, 36.4, 38.7, 42.7, 50.5, 53.9, (81.0), 123.8, 171.3, 199.6 ppm	

Melting point: 153-155 °C

Microanalysis:

Found:	C = 78.1%; H/D = 10.9% (January, 1999)
Calculated:	C = 78.3%; H/D = 10.7% (Calculated for $C_{19}H_{25}D_3O_2$)