Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

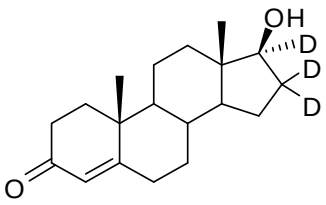
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D546: d3-Testosterone

Report ID: D546.2025.01 (Ampouled 230216)

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	947 ± 19 μg

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

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

Expiration of certification: The property values are valid till 08 January 2028, three 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 deuterated internal standard is intended for a single use to prepare a standard solution containing D546. The material was prepared by synthesis, and certified for identity and purity by NMI Australia. 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 approximate 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 and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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 947 ± 19 μg of anhydrous d_3-testosterone (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: At the recommended storage conditions this material has demonstrated stability for a period of five years. The measurement uncertainty includes components for long term stability at the recommended storage conditions.

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.
13 January 2025.

This report supersedes any issued prior to 13 January 2025.

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:

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:	Agilent 8890
Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
Program:	200 °C (1 min), 10 °C/min to 300 °C (3 min)
Injector:	200 °C
Detector Temp:	320 °C
Carrier:	Helium
Split ratio:	20/1

Relative peak area of the main component:

Initial analysis:	Mean = 99.8%, s = 0.06% (7 ampoules in duplicate, March 2023
Re-analysis:	Mean = 99.94%, s = 0.002% (5 ampoules in duplicate, March 2024)
Re-analysis:	Mean = 99.87%, s = 0.018% (5 ampoules in duplicate, January 2025)

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 indicative 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 elemental microanalysis.

The main component of this material is d_3-testosterone. d_2-, d_1- and d_0-Testosterone 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- testosterone 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: $d_4 \approx 91\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0)$ x 100]

$d_0 < 0.5\%$ [= $d_0/(d_3 + d_2 + d_1 + d_0)$ x 100]

GC-FID:

Instrument:	Agilent 8890	
Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
Program:	200 °C (1 min), 10 °C/min to 300 °C (3 min)	
Injector:	200 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area of the main component:

Initial analysis:	Mean = 99.8%, s = 0.02% (7 sub samples, March 2023)

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)
Moisture content 5.2 % mass fraction (November 2023)

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 = 0.3 (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 $C_{19}H_{25}D_3O_2$)