Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

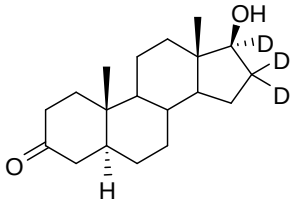
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D552: d_3-5α-Dihydrotestosterone

Report ID: D552.2021.03 (Ampouled 110404)

Chemical Formula: $C_{19}H_{27}D_3O_2$

Molecular Weight: 293.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-001008	**361432-57-7**	**889 ± 20μg**

IUPAC name: (5α,17β)-17-Hydroxy(16,16,17-$^2H_3$)androstan-3-one.

Expiration of certification: The property values are valid till 21 January 2031, i.e. ten 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 D552. The material was prepared by synthesisand 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 889 μg of anhydrous 5α-dihydrotestsoterone (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. 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.
1 November 2022.

This report supersedes any issued prior to 1 November 2022.

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), 10 °C/min to 240 °C (3 min), 10 °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 = 93.9%, s = 0.06% (7 ampoules in duplicate, June 2011)	
	Re-analysis:	Mean = 93.9%, s = 0.09% (5 ampoules in duplicate, March 2016)	
	Re-analysis:	Mean = 93.8%, s = 0.05% (5 ampoules in duplicate, January 2021)	

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, 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_3-5α-dihydrotestosterone. d_2-, d_1- and d_0-5α-Dihydrotestosterone 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_0-5α-dihydrotestosterone 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_3 ≈ 94% [= $d_3/(d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.5% [= $d_0/(d_3 + d_2 + d_1 + d_0)$ x 100]	
GC-FID:	Instrument:	HP5890
	Column:	ZB-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 15 °C/min to 240 °C, 10 °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 = 99.8%, s = 0.15% (7 sub samples, December 1998)
	Re-analysis:	Mean = 98.9%, s = 0.12% (3 sub samples in duplicate, June, 2005)
	Re-analysis:	Mean = 99.2%, s = 0.03% (5 sub samples in duplicate, July, 2006)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 240 °C (3 min), 10 °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 = 99.1%, s = 0.03% (7 sub samples in duplicate, April 2011)
HPLC:	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/MilliQ water (63:37)
	Flow rate:	1.0 mL/min
	Detector:	R.I detector
	Relative peak area of the main component:	
	Initial analysis:	Mean > 99.8% (3 sub samples, May 1999)
Thermogravimetric analysis:	Volatile content 4.2% and non volatile residue 0.24 % mass fraction (May 2005)	
Karl Fischer analysis:	Moisture content 5.4% mass fraction (July 2006) Moisture content 5.4% mass fraction (April 2011)	

Spectroscopic and other characterisation data

GC-MS:

Instrument:	Saturn 3400/2000 GC-MS Ion Trap	
Column:	J&W DB-17MS, 30 m × 0.25 mm I.D. × 0.17 μm	
Program:	220 °C (1 min), 10 °C /min to 280 °C (3 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 10/1

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 (8.5 min): 293 (M+, 14), 275 (13), 260 (22), 231 (100), 213 (31) *m/z*
Bis-TMS (6.1 min): 437 (M+, 58), 408 (100), 380 (21), 143 (47), 73 (74) *m/z*

The *bis*-TMS derivative of d_3-5α dihydrotestosterone co-elutes with a comparison sample of silylated unlabelled 5α-dihydrotestosterone under these conditions. Fragmentation pattern matches published data for the *bis*-TMS derivative of d_3-5α-dihydrotestosterone.

Deuteration yield (by SIM analysis of the *bis*-TMS derivative, mean of 3 sub samples)

Instrument:	Agilent 6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 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	Split ratio: 15/1
Bis-TMS:	(Deuteration state, % rel. to d_3-5α-dihydrotestosterone *bis*-TMS at 437 m/z) 434 (d_0, 0), 435 (d_1, 1), 436 (d_2, 5), 437 (d_3, 100)	

Results are uncorrected for potential small contributions due to $[M-H]^+$, $[M-2H]^+$ and ^{13}C isotope peaks of partially labeled steroids.

TLC:

Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20) Single spot observed, R_f = 0.4. Visualisation with UV at 254 nm

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3557, 3432, 1697, 1445, 1328, 1183, 1112 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.76 (3H, s), 1.02 (3H, s) ppm

^{2}H NMR:

Instrument:	Bruker DMX-500
Field strength:	76 MHz
Solvent:	$CHCl_3$ (7.26 ppm)
Spectral data:	δ 1.41 (1D), 2.04 (1D), 3.63 (1D)

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	125 MHz
Solvent:	$CDCl_3$ (77.16 ppm)
Spectral data:	δ 11.1, 11.5, 21.0, 23.2, 28.8, 29.7, 31.3, 35.5, 35.7, 36.6, 38.1, 38.6, 42.9, 44.7, 46.7, 50.8, 54.0, 81.2, 211.9 ppm

Melting point: 180-181 °C

Microanalysis:

Found:	C = 77.4%; H/D = 11.7% (January, 1999)
Calculated:	C = 77.8%; H/D = 11.3% (Calculated for $C_{19}H_{27}D_3O_2$)