Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

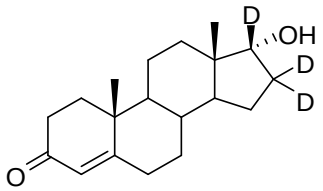
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D548: d_3-Epitestosterone

Report ID: D548.2020.04 (Ampouled 170323)

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

Molecular Weight: 291.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-000055	**171199-96-5**	**925 μg**

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

Expiration of certification: The property values are valid till 13 February 2030, 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 deuterated internal standard is intended for a single use to prepare a standard solution containing D548. 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 925 μg of d_3-epitestosterone (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 three years. 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.
20 July 2021.

This report supersedes any issued prior to 20 July 2021.

Characterisation Report:

GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m x 0.32 mm I.D. x 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 250 °C (5 min), 30 °C/min to 310 °C (10 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 = 0.02% (7 ampoules in duplicate, March 2017)
	Re-analysis:	Mean = 99.0%, s = 0.06% (5 ampoules in duplicate, February 2020)

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 estimate 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-epitestosterone. d_2-, d_1- and d_0-Epitestosterone 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-epitestosterone in the material.

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:	J&W DB-5MS, 30 m x 0.25 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:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.01% (10 sub samples, November 1998)
	Re-analysis:	Mean = 99.2%, s = 0.03% (5 sub samples in duplicate, February 2008)
GC-FID:	Instrument:	Agilent 6890
	Column:	HP-1, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	200 °C (1 min), 10 °C/min to 250 °C (5 min), 30°C/min to 310°C (10 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.2%, s = 0.02% (5 sub samples, March 2017)
HPLC:	Method:	Peak area percentage of total > 99.9% (3 samples)
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (50:50)
	Flow Rate:	0.8 mL/min
	Detector:	240 nm
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (February 2008 & March 2017)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (April 1999 & February 2008). The volatile content (e.g. organic solvents and/or water) could not be determined because of the inherent volatility of the material.

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP6890/5973	
Columns:	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	Splitless injection
Transfer line temp:	300 °C	Carrier: Helium, 1.0 mL/min

Bis-TMS derivative:

Instrument	HP6890/5973	
Columns:	HP Ultra 1, 17 m x 0.22 mm I.D. x 0.11 μm	
Program:	170 °C (1 min), 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)	
Injector:	280 °C	Splitless injection
Transfer line temp:	300 °C	Carrier: Helium, 1.0 mL/min

The retention times of the parent compound and *bis*-TMS derivative are reported 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 (5.2 min): 291 (M^+, 50), 276 (7), 273 (9), 249 (27), 231 (46), 149 (56), 124 (100) *m/z*.
Bis-TMS (10.7 min): 435 (M^+, 100), 420 (9), 330 (5), 208 (8), 73 (55) *m/z*.

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

GC-MS (ctd): Deuteration yield by SIM analysis of the *bis*-TMS derivative, mean of 3 samples:

Instrument	HP6890/5973	
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	Split ratio: 15/1
Transfer line temp:	300 °C	Carrier: Helium

SIM ions quantified (deuteration state, % rel. to d_3-epitestosterone *bis*-TMS at 435 m/z.)
432 (d_0, 0), 433 (d_1, 1), 434 (d_2, 6), 435 (d_3, 100)
Results are uncorrected for potential small contributions due to $[M\text{-}H]^+$, $[M\text{-}2H]^+$ and ^{13}C isotope peaks of partially labelled steroids.

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20)
Single spot observed, R_f = 0.22 (3 samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3420, 1656, 1610, 1381, 1231, 1188, 1108 cm^{-1}
	Weak, broad absorptions at 2200 and 2150 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$
Spectral data:	δ 0.69 (3H, s), 1.17 (3H, s), 5.71 (1H, s) ppm

As a result of successful deuteration, no signals observed due to hydrogen at 16α-, 16β- or 17β-position.

^{2}H NMR:

Instrument:	Bruker ACF-300
Field strength:	46 MHz
Solvent:	$CHCl_3$
Spectral data:	δ 1.47 (1D, 16β-D), 2.16 (1D, 16α-D), 3.76 (1D, 17β-D) ppm

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	125 MHz
Solvent:	$CDCl_3$
Spectral data:	δ 16.8, 17.3, 20.5, 24.2, 31.1, (31.5), 32.2, 32.8, 33.8, 35.7, 35.8, 38.6, 44.9, 48.1, 53.5, (79.0), 123.7, 171.2, 199.4 ppm
	As a result of successful deuteration, signals due to C-16 (31.5 ppm) and C-17 (79.0 ppm) are highly attenuated multiplets.

Melting point: 219-220 °C

Microanalysis:

Found:	C = 78.3%, H/D = 11.0%
Calculated:	C = 78.3%, H/D = 10.7% (Calculated for $C_{19}H_{25}D_3O_2$)