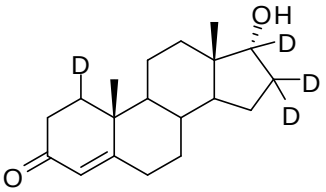
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

Report ID: S001.2018.01 (Ampouled 170706)
This batch of ampoules was prepared from the bulk material on 6th July 2017.

Compound Name: **d_4-[1,16,16,17]-Epitestosterone**
Collection Number: S001
Chemical Formula: $C_{19}H_{24}D_4O_2$
CAS Number: NA
Structure:



Description: Off white solid
Batch Number: 09-S-05
Molecular Weight: 292.45
Release date: 8th February 2010

Synonyms: d_4-[1,16,16,17]-17α-Testosterone
d_4-[1,16,16,17]-17α-Hydroxyandrost-4-en-3-one

The main component of this material is d_4-epitestosterone. d_3-, d_2-, d_1- and d_0-Epitestosterone are also present. The stated mass of the analyte per ampoule represents the combined mass of deuterated (d_4, d_3, d_2 and d_1) and d_0-epitestosterone in the material.

The material is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing S001. Each ampoule contains approximately 978 μg of anhydrous epitestosterone (d_4, 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 (chloroform).

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

The isotopic purity of S001 was assessed by mass spectroscopic analysis on the *bis*-TMS-derivative. Mass spectroscopic analysis of native epitestosterone highlighted a significant M-2 peak (loss of 2H), which appeared as an M-3 peak in the deuterated sample. This difference was attributed to the loss of H and D in the latter.

Isotopic Purity: $d_4 \approx 92.8\%$ [$= (d_4 / d_0 + d_1 + d_2 + d_3 + d_4) \times 100$]
$d_3 \approx 6.6\%$ [$= (d_3 / d_0 + d_1 + d_2 + d_3 + d_4) \times 100$]
$d_0 < 0.2\%$ [$= (d_0 / d_0 + d_1 + d_2 + d_3 + d_4) \times 100$

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

Note: Each ampoule contains approximately 907 μg of d_4-epitestosterone [calculated from the product of the chemical and isotopic purities]

GC-FID:

Instrument:	Agilent 6890	
Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C/min to 300 ºC (3 min)	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area of main component:

Initial analysis:	Mean = 98.1%, s = 0.02% (5 ampoules in duplicate, July 2017)
Re-analysis:	Mean = 98.4%, s = 0.02% (5 ampoules in duplicate, June 2018)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The purity value was obtained from a combination of traditional analytical techniques, by subtraction from 100% of total impurities by GC-FID, Karl Fischer analysis, and ^{1}H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

GC-FID:	Instrument:	Agilent 6890	
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component:		
	Initial analysis:	Mean = 98.4%, s = 0.06% (10 sub samples in duplicate, December 2009)	
	Re-analysis:	Mean = 98.4%, s = 0.1% (5 sub samples in duplicate, November 2012)	
GC-FID:	Instrument:	Varian CP-3800	
	Column:	VF-1MS, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C to 300 (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component:		
	Initial analysis:	Mean = 98.4%, s = 0.05 (10 sub samples in duplicate, December 2009)	
GC-FID:	Instrument:	Varian CP-3800	
	Column:	HP-5, 30 m × 0.32 mm I.D. × 0.25 μm	
	Program:	180 °C (1 min), 30 °C/min to 240 °C (10 min), 30 °C to 300 (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component:		
	Initial analysis:	Mean = 98.3%, s = 0.06 (10 sub samples in duplicate, December 2009)	
Thermogravimetric analysis:		N/A	
Karl Fischer analysis:		Moisture content < 0.3% mass fraction (December 2009 and November 2012)	

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	Agilent 6890/5973	
Column:	VF-1MS, 14.9 m x 0.25 mm I.D. x 0.25 µm	
Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

Bis-TMS derivative:

Instrument:	Agilent 6890/5973	
Column:	Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm	
Program:	187 °C (0.2 min), 3 °C/min to 238 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C (3 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 12/1

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 (8.1 min): 292 (M^+, 56), 274 (23), 250 (29), 232 (49), 217 (19), 206 (31), 188 (17), 168 (18), 149 (63), 125 (100), 110 (26), 91 (31), 79 (26), 55 (14), 41 (15) *m/z*

Bis-TMS (11.1 min): 436 (M^+, 100), 421 (8), 345 (3), 331 (6), 302 (2), 248 (2), 209 (6), 194 (4) 73 (87*) m/z*

The parent compound co-elutes with a comparison sample of native epitestosterone (NMI Collection # D547).

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4/1).
Single spot observed, R_f = 0.31. Visualisation with UV at 254 nm

IR:

Instrument:	Biorad FTS300MX FT-IR.
Range:	4000-400cm^{-1}, KBr powder
Peaks:	3419, 2933, 2879, 2867, 2823, 2225, 2194, 2150, 2127, 1652, 1609, 1453, 1432, 1380, 1359, 1248, 1107, 880 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance 300	
Field strength:	300 MHz	Solvent: $CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.69 (3H, s), 0.89-1.27 (3H, m), 1.18 (3H, s), 1.34-1.67 (7H, m),1.76 (1H, dd, *J* = 6.9, 12.1 Hz), 1.87 (1H, m), 2.01 (1H, m), 2.22-2.45 (4H, m), 5.71 (1H, s) ppm	

Ethyl acetate at 0.5% mass fraction was observed in the ^{1}H NMR (MeOH-d_4)
Chloroform at 0.4% mass fraction was observed in the ^{1}H NMR (MeOH-d_4)

^{13}C NMR:

Instrument:	Bruker Avance 300	
Field strength:	75 MHz	Solvent: $CDCl_3$ (77.0 ppm)
Spectral data:	δ 16.8, 17.4, 20.5, 24.3, 31.1, 31.5 (quintet, *J* = 18.8 Hz), 32.3, 32.9m, 33.8, 35.3 (t, *J* = 19.4 Hz), 35.8, 38.5, 45.0, 48.2, 53.5, 79.1 (t, *J* = 22.1), 123.8, 171.4, 199.6 ppm	

Melting point: 213-216 ºC

Microanalysis: Found: C = 77.4 %; H = 9.7 % (December 2009)
Calc: C = 78.0 %; H = 9.8 % (Calculated for $C_{19}H_{24}D_4O_2$)

The Synthesis and Certification of this Reference Material is supported by the Australian Government through the *Anti-Doping Research Program (ADRP)* of the Department of Health and Ageing.

Expiration of certification
The property values are valid till 20th June 2023, 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 expiry date/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.

The long-term stability of the compound in solution has not been examined.

This material has been given a shelf life of three years from the date of re-certification. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

This material has demonstrated stability over a minimum period of 3 years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

Homogeneity assessment
The homogeneity of the material was assessed using purity assay by GC-FID on five 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.

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.

Intended use
For use as an internal standard only.

Caution
Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice
Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 26 June, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 26th June 2018.