Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

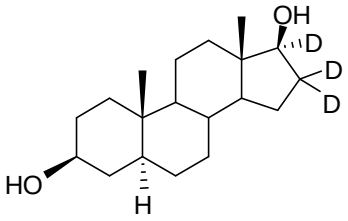
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D594: d_3-5α-Androstane-3β,17β -diol

Report ID: D594.2020.03 (Ampouled 081127)

Chemical Formula: $C_{19}H_{29}D_3O_2$

Molecular Weight: 295.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-09	**361432-67-9**	**931 ± 29 μg**

IUPAC name: (3β,5α,17β)-(16,16,17-$^2H_3$)androstane-3,17-diol.

Expiration of certification: The property values are valid till 14 August 2025, 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 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 D594. 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. methanol, chloroform). This will transfer approximately 931 μg of anhydrous 5α-androstane-3β,17β-diol (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: Varian CP-3800
Column: TG-17 or DB-17, 30 m x 0.32 mm I.D. x 0.25 µm
Program: 160 °C (1 min), 30 °C/min to 240 °C (10 min), 20 °C /min to 280 °C (10 min)
Injector: 250 °C
Detector: 320 °C
Carrier: Helium
Split rate: 20/1

Relative peak area of main component as the *bis*-TMS derivative:

Initial analysis: Mean = 99.5%, s = 0.02% (5 sub samples in duplicate, January 2013)
Re-analysis: Mean = 98.8%, s = 0.05% (5 ampoules in duplicate, November 2015)
Re-analysis: Mean = 98.7%, s = 0.15% (5 ampoules in duplicate, August 2020)

GC-FID:
Instrument: Agilent 6890N/Agilent 7890A
Column: HP-1, 30 m x 0.32 mm I.D. x 0.25 µm
HP-1MS, 30 m x 0.32 mm I.D. x 0.25 µm
Program: 180 °C (1 min), 10 °C/min to 200 °C (15 min), 20 °C /min to 300 °C (4 min)
Injector: 250 °C Detector: 320 °C
Carrier: Helium Split rate: 20/1

Relative peak area of main component:

Initial analysis: Mean = 99.5%, s = 0.54% (7 sub samples in duplicate, July 1999)
Re-analysis: Mean = 96.9%, s = 0.27% (7 ampoules in duplicate, December 2008)
Re-analysis: Mean = 97.7%, s = 0.11% (5 ampoules in duplicate, February 2012)

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 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.

$$\text{Purity} = (100\ \% - I_{ORG}) \times (100\ \% - I_{VOL} - I_{NVR}) \qquad \text{Equation 1}$$

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-androstane-3β,17β-diol. d_2-, d_1- and d_0- 5α-Androstane-3β,17β-diol 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-androstane-3β,17β-diol 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: $d_4 \approx 93\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 1\%$ [= $d_0/(d_3 + d_2 + d_1 + d_0) \times 100$]

GC-FID:
Instrument: Agilent 6890N
Column: HP-1, 30 m x 0.32 mm I.D. x 0.25 µm
Program: 180 °C (1 min), 10 °C to 200 °C (15min), 20 °C /min to 300 °C (4 min)
Injector: 250 °C Detector: 320 °C
Carrier: Helium Split rate: 20/1

Relative peak area of the main component:

Initial analysis: Mean = 99.54%, s = 0.54% (7 sub samples, in duplicate, July 1999)
Re-analysis: Mean = 97.17%, s = 0.23% (7 sub samples in duplicate, January 2008)

Thermogravimetric analysis: Volatiles content residue 5.2% mass fraction. (February 2000)

Karl Fischer analysis: Moisture content 5.2% mass fraction (January 2008)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP5890/5970	
Columns:	HP Ultra 2, 17 m x 0.22 mm I.D. x 0.11 µm	
Program:	180 °C (1 min), 12 °C/min to 310 °C (3 min)	
Injector:	260 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split injection

Bis-TMS derivative:

Instrument:	HP6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 µm	
Program:	170 °C (1 min), 10 °C/min to 300 °C (3 min)	
Injector:	260 °C	Transfer line temp: 300 °C
Carrier:	Helium 1.0 mL/min	Split ratio: 40/1

The retention times of the parent compound and its *bis*-TMS derivative are reported along with the major peaks in the mass spectra. The latter are reported as mass to charge ratios and (in brackets) as a percentage relative to the base peak.

Parent compound (6.1 min): 295 (M^+, 100), 280 (37), 233 (59), 215 (61), 165 (36), 107 (45) *m/z*
Bis-TMS derivative (5.8 min): 439 (M^+, 20), 424 (47), 349 (29), 244 (56), 131 (100), 75 (94) *m/z*
The *bis*-TMS derivative co-elutes with a comparison sample of silylated unlabelled 5α-androstane-3β,17β-diol under these conditions.

Deuteration yield (by SIM analysis of the bis-TMS derivative, mean of 3 sub 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	Transfer line temp: 300 °C
Carrier:	Helium	Split ratio: 15/1

Bis-TMS: (Deuteration state, % rel. to d_3-5α-Androstane-3β,17β-diol *bis*-TMS at 439 m/z)
436 (d_0, 1), 437 (d_1, 2), 438 (d_2, 5), 439 (d_3, 100)
Results uncorrected for contributions due to $[M-H]^+$, $[M-2H]^+$ or ^{13}C isotope peaks.

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm-1, KBr pellet
Peaks:	3400, 2213, 1448, 1379, 1186, 1075, 1032 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	CD_3OD
Key spectral data:	δ 0.72 (3H, s), 0.84 (3H, s), 3.50 (1H, m) ppm

^{2}H NMR:

Instrument:	Bruker DMX-500	
Field strength:	77 MHz	Solvent: CH_3OH
Spectral data:	δ 1.38 (1D), 1.90 (1D), 3.50 (1D) ppm	

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	126 MHz
Solvent:	CD_3OD
Spectral data:	δ 10.2, 11.3, 20.5, 22.6, 28.4, 30.6, 31.4, 35.2, 35.4, 36.5, 36.8, 37.4, 42.5, 44.8, 50.9, 54.6, 70.3 ppm

Microanalysis:

Found:	C = 71.8%, H = 10.5%;
Calculated:	C = 77.2%, H = 11.9% (January 2008)