Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
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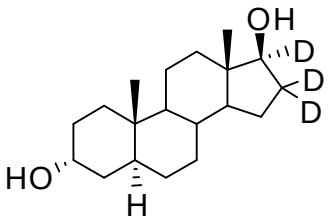
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D593: d_3-5α-Androstane-3α, 17β-diol

Report ID: D593.2020.01 (Ampouled 170316)

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-08	**361432-66-8**	**973 µg**

Synonyms: d_3-3α,17β-Dihydroxy-5α-androstane

Expiration of certification: The property values are valid till 03 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 D593. 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 973 µg of 5α-androstane-3α, 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: 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 February 2020.

This report supersedes any issued prior to 13 February, 2020.

NATA logo notice: Accredited for compliance with ISO Guide 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

Characterisation Report:

Note: Each ampoule contains approximately 905 μg of d_3-5α-androstane-3α, 17β-diol
[Calculated from the product of the chemical and isotopic purities]

GC-FID:

Instrument:	Varian CP-3800	
Column:	VF-1ms, 30 m × 0.32 mm I.D. × 0.25 μm	
Program:	180 °C (1 min), 10 °C/min to 240 °C (3 min), 20 °C/min to 300 °C (3 min)	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area response of main component:

Initial analysis:	Mean = 98.7%, s = 0.04% (7 ampoules in duplicate, March 2017)
Re-analysis:	Mean = 98.7%, s = 0.02% (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 certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID and Karl Fischer analysis. 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 ^{1}H NMR and elemental microanalysis.

The main component of this material is d_3-5α-androstane-3α, 17β-diol. d_2-, d_1-, and d_0-5α-Androstane-3α, 17β-diol 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_0-5α-androstane-3α, 17β-diol in the material.

Isotopic Purity: d_3 = 93% [= d_3 / (d_3 + d_2 + d_1 + d_0) ×100]
d_0 < 1% [= d_0 / (d_3 +d_2 +d_1 +d_0) × 100]

GC-FID:

Instrument:	HP5890	
Column:	J&W DB-5MS Capillary, 30 m × 0.32 mm I.D. × 0.25 μm or ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm	
Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (3 min)	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area response of main component:

Initial analysis:	Mean = 99.5%, s = 0.5% (7 sub samples, June 1999)
Re-analysis:	Mean = 98.8%, s = 0.07% (8 sub samples in duplicate, October 2005)

GC-FID:

Instrument:	Varian CP-3800	
Column:	VF-1ms, 30 m × 0.32 mm I.D. × 0.25 μm	
Program:	180 °C (1 min), 10 °C/min to 240 °C (3 min), 20 °C/min to 300 °C (3 min)	
Injector:	250 °C	Detector Temp: 320 °C
Carrier:	Helium	Split ratio: 20/1

Relative peak area response of main component:

Initial analysis:	Mean = 98.8%, s = 0.02% (5 sub samples in duplicate, August 2011)
Re-analysis:	Mean = 98.7%, s = 0.02% (6 sub samples in duplicate, March 2017)

Karl Fischer analysis: Moisture content 1.3% mass fraction (July 2011)
Moisture content 1.6% mass fraction (March 2017)

Thermogravimetric analysis: Volatiles content and non-volatile residue < 0.3% mass fraction (October 2005)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP5890/5970
Columns:	HP Ultra 2, 17 m × 0.22 mm I.D. × 0.11 μm
Program:	180 °C (1 min), 12 °C/min to 310 °C (3 min)
Injector:	260 °C Split ratio: 40/1
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min

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 Split ratio: 40/1
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min

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 (6.0 min):	295 (M^+, 100), 280 (64), 277 (58), 262 (40), 233 (72), 215 (85) *m*/z
Bis-TMS (5.3 min):	439 (M^+, 22), 424 (16), 349 (26), 334 (30), 259 (42), 244 (80), 73 (100) *m/z*

The *bis*-TMS derivative of D593 co-elutes with a comparison sample of silylated 5α-androstane-3α,17β-diol under these conditions.

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

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
Bis-TMS (12.5 min):	Deuteration state, % relative to d_3-5α-androstane-3α 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 of partially labeled steroids.

TLC:

Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20) Single spot observed, R_f = 0.2 (3 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3378, 1445, 1379, 1186, 1075, 1005 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	MeOH-*d*$_4$ (3.31 ppm)
Spectral data:	δ 0.72 (3H, s), 0.82 (3H, s), 3.94 (1H, m) ppm

^{2}H NMR:

Instrument:	Bruker DMX-500
Field strength:	77 MHz
Solvent:	MeOH-*d*$_4$ (3.31 ppm)
Spectral data:	δ 1.38 (1D), 1.90 (1D), 3.50 (1D)

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	126 MHz
Solvent:	MeOH-*d*$_4$ (49 ppm)
Spectral data:	δ 11.6, 11.7, 21.5, 24.0, 29.6, 29.7, 32.9, 33.5, 36.7, 36.9, 37.2, 38.0, 40.3, 44.0, 52.5, 56.1, 67.1 ppm

Melting point: 222-224 °C

Microanalysis:

Found:	C = 76.9%; H = 11.8% (June, 1999)
Calculated:	C = 77.2%; H = 11.9% (Calculated for $C_{19}H_{29}D_3O_2$)