Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

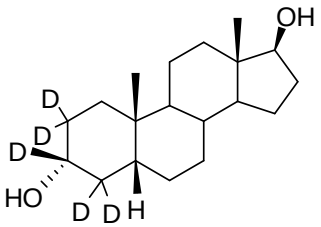
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D580: d5-5β-Androstane-3α, 17β-diol

Report ID: D580.2022.02 (Ampouled 200305)

Chemical Formula: $C_{19}H_{27}D_5O_2$

Molecular Weight: 297.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-001007	**361432-68-0**	**987 ± 19 μg**

IUPAC name: (2,2,3β,4,4-d_5)-(3α, 5β, 17β)-17-Methylandrostane-3,17-diol.

Expiration of certification: The property values are valid till 18 July 2032, 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 D580. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d_5-5β-androstane-3α,17β-diol. d_4-, d_3-, d_2-, d_1- and d_0-5β-androstane-3α,17β-diol are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-5β-androstane-3α, 17β-diol in the material.

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. 2-propanol). This will transfer approximately 987 μg of anhydrous 5β-androstane-3α, 17β-diol (d_5, d_4-, 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.
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 6890N or 7890
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	170 °C (1 min), 10 °C/min to 220 °C (5 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 of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.02% (7 ampoules in duplicate, March 2020)
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, July 2022)

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.

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_5-5β-androstane-3α, 17β-diol. d_4-, d_3-, d_2-, d_1- and d_0-5β-Androstane-3α,17β-diol are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-5β-androstane-3α, 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: $d_5 \approx 89\%$ [= $d_5/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]

$d_0 < 0.3\%$ [= $d_0/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]

GC-FID:	Instrument:	Agilent 5890
	Column:	ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 20 °C/min to 250 °C (5 min), 20 °C/min to 310 °C
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component:	
	Initial analysis:	Mean > 99.9% (7 sub samples, May 1999)
	Re-analysis:	Mean = 99.1%, s = 0.2% (*bis*-TMS derivative, 7 sub samples, April 1998)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	170 °C (1 min), 10 °C/min to 220 °C (5 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 of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.01% (5 sub samples in duplicate, March 2009)
	Re-analysis:	Mean = 99.7%, s = 0.07% (7 sub samples in duplicate, January 2013)
Karl Fischer analysis:		Moisture content 0.1% mass fraction (March 2009 & February 2013)

Spectroscopic and other characterisation data

GC-MS: Parent compound:

Instrument:	HP5890/5970
Column:	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 (2 min)
Injector:	260 °C
Split injection:	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 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 (5.8 min): 297 (M^+, 13), 279 (82), 261 (64), 246 (43), 235 (33), 220 (100) *m/z*
Bis-TMS (6.8 min): 441 (M^+, 6), 426 (11), 351 (30), 261 (100), 246 (88), 129 (80), 73 (93) *m/z*

The *bis*-silylated derivative of D580 co-elutes with a derivatised comparison sample of unlabelled 5β-androstane-3α,17β-diol.

GC-MS: Deuteration yield (by SIM analysis of the *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), 3 °C/min to 234 °C, 10°C/min to 265°C/min, 30°C/min to 310 °C
Injector:	280°C
Transfer line temp:	300 °C
Carrier:	Helium
Split ratio:	40/1

The retention time of the *bis*-TMS derivative of d_5-5β-androstane-3α,17β-diol is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Bis-TMS (7.52 min): (Deuteration state, % rel. to d_5-[M^+-CH_3] for *bis*-TMS derivative at 426 m/z)
421 (d_0, 0.2), 422 (d_1, 0.2), 423 (d_2, 0.2), 424 (d_3, 1.3), 425 (d_4, 9.8), 426 (d_5, 100)

Results uncorrected for contributions due to $[M-H]^+$, $[M-2H]^+$ and ^{13}C isotopes of partially labelled steroids.

TLC: Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (80:20)
Single spot observed, R_f = 0.13 (3 samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3339, 2196, 2106, 1445, 1314, 1277, 1105, 1058, 1032, 944 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$/d_6-DMSO
Spectral data:	δ 0.65 (3H, s), 0.68 (3H, s), 3.29 (1H, t) ppm

^{2}H NMR:

Instrument:	Bruker DMX-500
Field strength:	75 MHz
Solvent:	$CHCl_3$/DMSO
Spectral data:	δ 1.00 (1D), 1.19 (1D), 1.34 (1D), 1.44 (1D), 3.27 (1D) ppm

Melting point: 230-234 °C

Microanalysis:
Found: C = 76.5%; H/D = 12.5% (May 1999)
Calculated: C = 76.7%; H/D = 12.5% (Calculated for $C_{19}H_{27}D_5O_2$)