



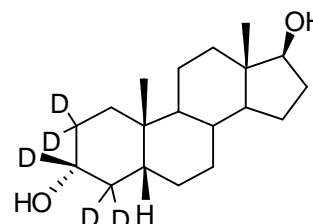
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

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

Report ID: D580.2020.01 (Ampouled 200305)

Chemical Formula: C₁₉H₂₇D₅O₂

Molecular Weight: 297.4 g/mol



Property value

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

IUPAC name: (2,2,3β,4,4-d₅)-(3α, 5β, 17β)-17-Methylandrostane-3,17-diol.

Expiration of certification: The property values are valid till 7 March 2023, i.e. three years from the date of 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 d₅-5β-androstane-3α,17β-diol. d₄-, d₃-, d₂-, d₁- and d₀-5β-androstane-3α,17β-diol are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d₅, d₄, d₃, d₂ and d₁) and d₀-5β-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 (d₅, d₄-, d₃-, d₂-, d₁- and d₀). 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 °C 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.
31 March 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:

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 = 0.02% (7 ampoules in duplicate, March 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 value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

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

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 d₅-5β-androstane-3α, 17β-diol. d₄-, d₃-, d₂-, d₁- and d₀-5β-Androstane-3α,17β-diol are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d₅, d₄, d₃, d₂ and d₁) and d₀-5β-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: d₄ ≈ 89% [= d₅/(d₅ + d₄ + d₃ + d₂ + d₁ + d₀) × 100]
 d₀ < 0.3% [= d₀/(d₅ + d₄ + d₃ + d₂ + d₁ + d₀) × 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 x 0.22 mm I.D. x 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 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/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 d₅-5β-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 d₅-[M⁺-CH₃] for *bis*-TMS derivative at 426 *m/z*)
421 (d₀, 0.2), 422 (d₁, 0.2), 423 (d₂, 0.2), 424 (d₃, 1.3), 425 (d₄, 9.8), 426 (d₅, 100)

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

TLC: Conditions: Kieselgel 60F₂₅₄. 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

¹H NMR: Instrument: Bruker DMX-500
Field strength: 500 MHz
Solvent: CDCl₃/d₆-DMSO
Spectral data: δ 0.65 (3H, s), 0.68 (3H, s), 3.29 (1H, t) ppm

²H NMR: Instrument: Bruker DMX-500
Field strength: 75 MHz
Solvent: CHCl₃/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 C₁₉H₂₇D₅O₂)