



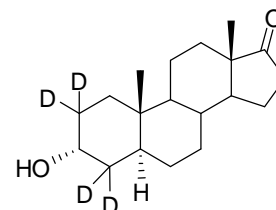
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D549: d₄-Androsterone

Report ID: D549.2020.01 (Ampouled 141014)

Chemical Formula: C₁₉H₂₆D₄O₂

Molecular Weight: 294.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-002003	361432-60-2	988 µg

IUPAC name: (3 α ,5 α)-3-Hydroxy(2,2,4,4-²H₄)androstan-17-one.

Expiration of certification: The property values are valid till 22 June 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 D549. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d₄-androsterone. d₃-, d₂-, d₁- and d₀-androsterone are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d₄, d₃, d₂ and d₁) and d₀-androsterone 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. dichloromethane). This will transfer approximately 988 µg of anhydrous androsterone (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.
16 July 2020.

This report supersedes any issued prior to 16 July, 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, 30 m × 0.32 mm I.D. × 0.25 µm
 Program: 180 °C (1 min), 10 °C/min to 200 °C (22 min), 30 °C/min to 300 °C (3 min)
 Injector: 250 °C
 Detector Temp: 320 °C
 Carrier: Helium
 Split ratio: 20/1
 Relative peak area of main component:
 Initial analysis: Mean = 99.6%, s = 0.03% (7 ampoules in duplicate, October 2014)
 Re-analysis: Mean = 99.5%, s = 0.01% (5 ampoules in duplicate, July 2017)
 Re-analysis: Mean = 99.5%, s = 0.05% (5 ampoules in duplicate, June 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 d₄-androsterone. d₂-, d₁- and d₀-Androsterone are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d₃, d₂ and d₁) and d₀-androsterone 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 ≈ 81% [= d₄/(d₄ + d₃ + d₂ + d₁ + d₀) × 100]
 d₀ < 0.1% [= d₀/(d₄ + d₃ + d₂ + d₁ + d₀) × 100]

GC-FID: Instrument: HP5890
 Column: ZB-1, 30 m × 0.32 mm I.D. × 0.25 µm
 Program: 180 °C (1 min), 10 °C/min to 240 °C, 20 °C/min to 280 °C (3 min)
 Injector: 250 °C
 Carrier: Helium
 Detector Temp: 325 °C
 Split ratio: 20/1
 Relative peak area of main component:
 Initial analysis: Mean = 99.5%, s = 0.06% (7 sub samples, January 1999)
 Re-analysis: Mean = 99.1%, s = 0.02% (8 sub samples in duplicate, July 2006)

GC-FID: Instrument: Agilent 6890N
 Column: HP-1, 30 m × 0.32 mm I.D. × 0.25 µm
 Program: 180 °C (1 min), 10 °C/min to 200 °C (22 min), 30 °C/min to 300 °C (3 min)
 Injector: 250 °C
 Carrier: Helium
 Detector Temp: 320 °C
 Split ratio: 20/1
 Relative peak area of main component:
 Initial analysis: Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, September 2009)
 Re-analysis: Mean = 99.6%, s = 0.03% (7 sub samples in duplicate, October 2014)

HPLC: Method: Peak area percentage of total > 99% (3 sub samples)
 Column: Alltima C-18, 5 µm (4.6 mm × 150 mm)
 Mobile Phase: Acetonitrile/water (63:37)
 Flow Rate: 1.0 mL/min
 Detector: U.V. at 205 nm and refractive index

Karl Fischer analysis: Moisture content 0.2% mass fraction (September 2009)
 Moisture content < 0.2% mass fraction (October 2014)

Thermogravimetric analysis: Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction
 (April 1999 and June 2006)

Spectroscopic and other characterisation data

GC-MS:	Parent compound and <i>bis</i> -TMS derivative: Instrument: Saturn 3400/2000 GC-MS Ion Trap Column: J&W DB-17MS, 30 m × 0.25 mm I.D. × 0.17 μm Program: 220 °C (1 min), 10 °C /min to 280 °C (3 min) Injector: 250 °C Transfer line temp: 280 °C Carrier: Helium (1.0 mL/min) Split ratio: 10/1
	The retention times of the parent compound and <i>bis</i> -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 intensity of the base peak. Parent (7.9 min): 294 (M+, 42), 279 (37), 261 (79), 243 (100), 217 (82), 93 (86) <i>m/z</i> <i>Bis</i> -TMS (5.2 min): 438 (M+, 16), 423 (100), 333 (46), 243 (29), 169 (15), 73 (38) <i>m/z</i>
	The <i>bis</i> -TMS derivative of d ₄ -androsterone co-elutes with a comparison sample of silylated unlabelled androsterone under these conditions. Deuteration yield (by SIM analysis of the <i>bis</i> -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 <i>Bis</i> -TMS (10.5 min): (Deuteration state, % rel. to d ₄ -androsterone <i>bis</i> -TMS at 438 <i>m/z</i>) 434 (d ₀ , 1), 435 (d ₁ , 1), 436 (d ₂ , 3), 437 (d ₃ , 19), 438 (d ₄ , 100) Results are uncorrected for potential small contributions due to [M-H] ⁺ , [M-2H] ⁺ and ¹³ C isotope peaks of partially labelled steroids
TLC:	Conditions: Kieselgel 60F ₂₅₄ . Chloroform/ethyl acetate (80:20) Single spot observed, R _f = 0.39 (3 sub samples)
IR:	Instrument: FT-IR, Biorad WIN FTS40 Range: 4000-400 cm ⁻¹ , KBr pellet Peaks: 3529, 1722, 1449, 1385, 1094, 1013 cm ⁻¹
¹ H NMR:	Instrument: Bruker DPX-300 Field strength: 300 MHz Solvent: CDCl ₃ (7.26 ppm) Key spectral data: δ 0.80 (3H, s), 0.86 (3H, s), 4.06 (1H, s) ppm
² H NMR:	Instrument: Bruker DMX-500 Field strength: 76 MHz Solvent: CHCl ₃ (7.26 ppm) Spectral data: δ 1.38 (1D), 1.49 (1D), 1.60 (1D), 1.66 (1D) ppm
¹³ C NMR:	Instrument: Bruker DPX-300 Field strength: 75 MHz Solvent: CDCl ₃ (76.9 ppm) Spectral data: δ 11.1, 13.7, 19.9, 21.7, 28.1, 30.7, 31.5, 31.9, 34.9, 35.8, 36.1, 38.9, 47.7, 51.4, 54.3, 66.1, 221.4 ppm
Melting point:	183-186 °C
Microanalysis:	Found: C = 77.5%, H/D = 11.6% (May, 1999) Calculated: C = 77.5%, H/D = 11.6% (Calculated for C ₁₉ H ₂₆ D ₄ O ₂)