Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

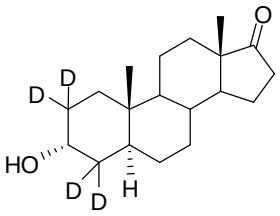
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D549: d_4-Androsterone

Report ID: D549.2020.04 (Ampouled 141014)

Chemical Formula: $C_{19}H_{26}D_4O_2$

Molecular Weight: 294.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-002003	**89685-10-9**	**988 ± 48 μg**

IUPAC name: (3α,5α)-3-Hydroxy(2,2,4,4-$^2H_4$)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 d_4-androsterone. d_3-, d_2-, d_1- and d_0-androsterone are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_4, d_3, d_2 and d_1) and d_0- 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 (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.
25 October 2023.

This report supersedes any issued prior to 25 October 2023.

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 ^{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_4-androsterone. d_2-, d_1- and d_0-Androsterone 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-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: $d_4 \approx 81\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 0.1\%$ [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

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 Detector Temp: 325 °C
Carrier: Helium 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 Detector Temp: 320 °C
Carrier: Helium 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 *bis*-TMS derivative:

Instrument:	Saturn 3400/2000 GC-MS Ion Trap
Column:	J&W DB-17MS, 30 m × 0.25 mm I.D. × 0.17 □m
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 *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 intensity of the base peak.

Parent (7.9 min):	294 (M+, 42), 279 (37), 261 (79), 243 (100), 217 (82), 93 (86) *m/z*
Bis-TMS (5.2 min):	438 (M+, 16), 423 (100), 333 (46), 243 (29), 169 (15), 73 (38) *m/z*

The *bis*-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 *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 (10.5 min):	(Deuteration state, % rel. to d_4-androsterone *bis*-TMS at 438 m/z) 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 $C_{19}H_{26}D_4O_2$)