Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

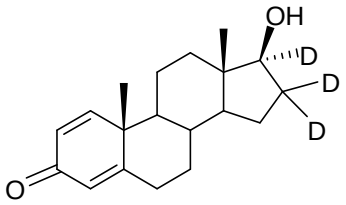
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D581b: d_3-Boldenone

Report ID: D581b.2020.01 (Ampouled 150401)

Chemical Formula: $C_{19}H_{23}D_3O_2$

Molecular Weight: 289.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
04-S-10	1224710-35-3	932 μg

IUPAC name: (17β)-17-Hydroxy(16,16,17-$^2H_3$)androsta-1,4-dien-3-one.

Expiration of certification: The property values are valid till 16 December 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 RM is intended for a single use to prepare a standard solution containing D581b. 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 932 μg of anhydrous boldenone (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: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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.
19 January 2021.

This report supersedes any issued prior to 19 January 2021.

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), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (2 min)	
	Injector:	250 °C	Detector Temp: 320 °C
	Carrier:	Helium	Split ratio: 20/1
	Relative peak area of main component		
	Initial analysis:	Mean = 97.8%, s = 0.08% (7 ampoules in duplicate, April 2015)	
	Re-analysis:	Mean = 98.8%, s = 0.03% (5 ampoules in duplicate, March 2018)	
	Re-analysis:	Mean = 98.8%, s = 0.02% (5 ampoules in duplicate, December 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 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 elemental microanalysis.

The main component of this material is d_3-boldenone. d_2-, d_1- and d_0-Boldenone 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-boldenone 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 ≈ 92.9% [= $d_3/(d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 2.4% [= $d_0/(d_3 + d_2 + d_1 + d_0)$ x 100]	
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 40 °C/min to 250 °C (10 min), 40 °C/min to 300 °C (2 min)
	Injector:	250 °C Detector Temp: 320 °C
	Carrier:	Helium Split ratio: 20/1
	Relative peak area of main component	
	Initial analysis:	Mean = 99.0%, s = 0.02% (10 sub samples in duplicate, August 2004)
	Re-analysis:	Mean = 98.9%, s = 0.01% (5 sub samples in duplicate, September 2008)
	Re-analysis:	Mean = 98.1%, s = 0.04% (7 sub samples in duplicate, April 2015)
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2% mass fraction (August 2004 & August 2005)
Karl Fischer analysis:		Moisture content 0.9% mass fraction (September 2008) Moisture content 5.6% mass fraction (March 2015)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973	
Column:	Zebron ZB-5, 30 m x 0.25 mm I.D. x 0.30 μm	
Program:	220 °C (1 min), 10 °C/min to 300 °C (5 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

Bis-TMS derivative:

Instrument:	HP 6890/5973	
Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm	
Program:	187 °C (0.2 min), 3 °C/min to 238 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C (2 min)	
Injector:	250 °C	Transfer line temp: 300 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 12/1

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 base peak.

Parent (9.60 min):	289 (M^+, 3), 122 (100), 108 (8), 107 (8), 91 (14), 79 (6), 77 (6), 41 (4) *m/z*
Bis-TMS (11.4 min):	433 (M^+, 54), 418 (19), 328 (19), 229 (14), 206 (100), 191 (22), 73 (65) *m/z*

Deuteration yield (by SIM analysis of the *bis*-TMS derivative, mean of 7 sub samples)

Column:	BPX-5, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	220 °C (2 min), 10 °C/min to 300 °C (5 min)
7.12 min:	Percentage deuteration state, 430 (d_0, 2.4%), 431 (d_1, 1.3%), 432 (d_2, 3.4%), 433 (d_3, 92.9%)

TLC:

Conditions:	Kieselgel 60F_{254}. Chloroform/ethyl acetate (3/1) Single spot observed, R_f = 0.2. Visualisation with UV at 254 nm

IR:

Instrument:	Biorad FTS300MX FT-IR
Range:	4000-400cm^{-1}, KBr pellet
Peaks:	3479, 2939, 2873, 2851, 2216, 2158, 2119, 1661, 1619, 1599, 1453, 1378, 1299, 1240, 1188, 1166, 1040, 958, 885, 821 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-600
Field strength:	600 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.81 (3H, s), 0.9-1.11 (4H, m), 1.23 (3H, s), 1.30 (1H, t, *J* = 12.5 Hz), 1.58 (1H, dd, *J* = 7.3, 12.5 Hz), 1.62-1.71 (2H, m), 1.75 (1H, m), 1.86 (1H, ddd, *J* = 3.6, 3.6, 12.4 Hz), 1.94 (1H, m), 2.35 (1H, m), 2.46 (1H, m), 6.05 (1H, s), 6.21 (1H, dd, *J* = 1.9, 10.1 Hz), 7.05 (1H, d, *J* = 10.1 Hz) ppm

^{2}H NMR:

Instrument:	Bruker DMX-600
Field strength:	76.8 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 1.43 (1D, s), 2.02 (1D, s), 3.60 (1D, s) ppm

^{13}C NMR:

Instrument:	Bruker DMX-600
Field strength:	151 MHz
Solvent:	$CDCl_3$ (77.16 ppm)
Spectral data:	δ 11.1, 18.7, 22.4, 23.3, 32.7, 33.1, 35.5, 36.2, 43.0, 43.6, 50.0, 52.5, 123.8, 127.4, 155.9, 169.2, 186.4 ppm

Melting point: 167-169 °C

Microanalysis:

Found:	C = 78.8%; H = 9.9% (September, 2004)
Calculated:	C = 78.9%; H = 10.1% (Calculated for $C_{19}H_{23}D_3O_2$)