Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

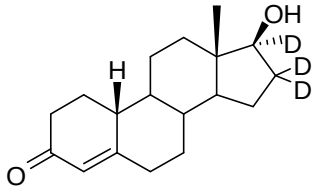
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D583: d_3-Nandrolone

Report ID: D583.2023.01 (Bottled 160913)

Chemical Formula: $C_{18}H_{23}D_3O_2$

Molecular Weight: 277.4 g/mol



Property value

Batch No.	CAS No.	Purity estimate
98-002941	**361432-70-4**	**99.4 ± 2.1%**

Synonyms: (16,16,17-d_3)-Nandrolone
d_3-19-Nortestosterone
d_3-17β-Hydroxy-4-estren-3-one

Expiration of certification: The property values are valid till 07 June 2028, 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 expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: White crystals prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap.

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: Equilibrate the bottled material to room temperature before opening.

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 five years. The stability of this material is reduced when it is taken into solution. Reference solutions containing this material should be stored out of direct light at below 4 °C and monitored regularly for possible decomposition. This material is also sensitive to the quality of the silanised glass liner when injected at elevated temperature (~250 ºC) into a GC instrument.

Homogeneity assessment: The homogeneity of the material was assessed using purity assay by GC-FID on seven randomly selected 1-2 mg sub samples 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.
5 July 2023

This report supersedes any issued prior to 5 July 2023.

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:

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 elemental microanalysis.

The main component of this material is d_3-nandrolone. d_{2-}, d_1- and d_0-Nandrolone are also present. The stated chemical purity represents the combined mass fraction of deuterated (d_3, d_2 and d_1) and d_0-nandrolone 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: $d_3 \approx 92\%$ [= $d_3/(d_3 + d_2 + d_1 + d_0) \times 100$]

$d_0 < 0.5\%$ [= $(d_0/d_3) \times 100$]

Note: Each mg of material contains <u>approximately</u> 913 µg of d_3-nandrolone.

GC-FID:	Instrument:	HP5890
	Column:	J&W DB-5MS or ZB-1 Capillary, 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 the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.08% (7 sub samples in duplicate, June 1999)
	Re-analysis:	Mean = 99.0%, s = 0.03% (3 sub samples in duplicate, July 2003)
	Re-analysis:	Mean = 99.0%, s = 0.04% (5 sub samples in duplicate, June 2006)
GC-FID:	Instrument:	Agilent 6890N
	Column:	HP-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 240 °C (5 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 the main component:	
	Initial analysis:	Mean = 99.2%, s = 0.02% (5 sub samples in duplicate, June 2009)
	Re-analysis:	Mean = 98.9%, s = 0.16% (5 sub samples in duplicate, April 2014)
	Re-analysis:	Mean = 98.9%, s = 0.02% (5 sub samples in duplicate, March 2019)
	Re-analysis:	Mean = 99.4%, s = 0.02% (5 sub samples in duplicate, June 2023)
Karl Fischer analysis:		Moisture content < 0.1% mass fraction (July 2009, March 2019 and June 2023) Moisture content 0.12% mass fraction (May 2014)
Thermogravimetric analysis:		Volatiles content < 0.1% and non-volatile residue < 0.2% mass fraction (June 2006)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP6890/5973
Column:	HP Ultra 2, 17 m x 0.20 mm I.D. x 0.10 µm
Program:	140 °C (1 min), 8 °C/min to 250 °C, 30 °C/min to 300 °C (3 min)
Injector:	280°C
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min

Splitless injection

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, 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

The retention times of the parent material 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 intensity of the base peak.

Parent (12.1 min): 277 (M+, 100), 259 (21), 231 (18), 215 (20), 110 (47), 91 (47) *m/z*
Bis-TMS (10.9 min): 421 (M+, 100), 331 (2), 288 (3), 247 (1), 233 (1), 194 (2) *m/z*

The reported data are for the major Δ-3,5-dienylsilylether derivative.
The *bis*-TMS derivative of D583 co-elutes with a derivatised sample of nandrolone.

Deuteration yield (by SIM analysis of the *bis*-TMS derivative, mean of three samples)
Bis-TMS (10.9 min): (Deuteration state, % rel. to d_3-nandrolone *bis*-TMS at 421 *m/z*)
418 (d_0, 0), 419 (d_1, 0), 420 (d_2, 8), 421 (d_3, 100)
Results uncorrected for 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, Rf = 0.22 (3 sub samples)

IR:

Instrument:	FT-IR, Biorad WIN FTS40
Range:	4000-400 cm^{-1}, KBr pellet
Peaks:	3423, 1667, 1619, 1453, 1335, 1178, 962, 886 cm^{-1}

^{1}H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$
Spectral data:	δ 0.78 (3H, s), 5.80 (1H, s) ppm

^{2}H NMR:

Instrument:	Bruker DMX-500
Field strength:	77 MHz
Solvent:	CH_2Cl_2 ($CDCl_3$ ref)
Spectral data:	δ 1.33 (1D, br s), 1.94 (1D, br s), 3.54 (1D, br s) ppm

^{13}C NMR:

Instrument:	Bruker DMX-500
Field strength:	126 MHz
Solvent:	$CDCl_3$
Spectral data:	δ 11.0, 23.0, 26.1, 26.6, (29.6), 30.7, 35.5, 36.4, 36.5, 40.5, 42.6, 42.9, 49.6, 49.7, (81.0), 124.5, 166.6, 199.9 ppm

Melting point: 117-118 °C

Microanalysis:

Found:	C = 78.1%, H/D = 10.6% (August 1999)
Calculated:	C = 77.9%, H/D = 10.5% (Calculated for $C_{18}H_{23}D_3O_2$)