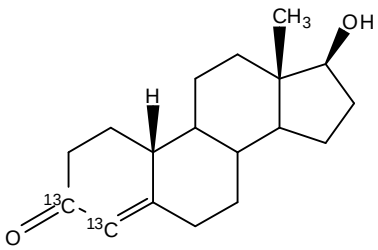
Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

ISOTOPICALLY LABELLED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D679: 3,4-$^{13}C_2$-Nandrolone



Report ID: D679.2019.01

Chemical Formula: $^{12}C_{16}{}^{13}C_2H_{26}O_2$

Molecular Weight: 276.4 g/mol

Certified value

Batch No.	CAS No.	Purity estimate
01-S-01	82952-73-6	99.0 ± 1.0%

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: (17β)-17-Hydroxy(3,4-$^{13}C_2$)estr-4-en-3-one

Expiration of certification: The property values are valid till 3 April 2024, 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: Off-white crystals sourced from an external supplier, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top 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 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 five 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.
30 April 2019

This report supersedes any issued prior to 30 April 2019.

Characterisation Report:

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 isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

Isotopic Purity: $^{13}C_2$ = 98% [= $^{13}C_2$ / ($^{13}C_0$ + $^{13}C_1$ + $^{13}C_2$) × 100]

Warning: This material is sensitive to the quality of the silanised glass liner when injected into a GC instrument.

GC-FID:	Instrument:	HP5890
	Column:	ZB-1 Capillary, 30 m × 0.32 mm I.D. × 0.25 µm
	Program:	180 °C (1 min), 10 °C/min to 220 °C, 20 °C/min to 300 °C (6 min)
	Injector:	250 °C
	Detector Temp:	320 °C
	Carrier:	Helium
	Split ratio:	15/1
	Relative peak area of main component:	
	Re-analysis:	Mean = 99.1%, s = 0.05% (3 sub samples in duplicate, May 2004)
	Re-analysis:	Mean = 99.1%, s = 0.025% (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 main component:	
	Initial analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, June 2009)
	Re-analysis:	Mean = 99.0%, s = 0.03% (5 sub samples in duplicate, April 2014)
	Re-analysis:	Mean = 99.2%, s = 0.04% (5 sub samples in duplicate, April 2019)
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (May 2009 and 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 (Splitless injection)
Transfer line temp:	300 °C
Carrier:	Helium, 1.0 mL/min
Scan range:	50-550 *m/z*

The retention time of the parent compound isreported 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 (12.1 min): 276 (M^+, 100), 275 (3), 274 (4), 258 (21), 233 (18), 217 (20), 112 (47) *m/z*

$^{13}C_2$ isotopic purity (from HRMS-SIM analysis of the parent)

Major HRMS peaks observed (Peak ID, % rel. To $^{13}C_2$-nandrolone at 276.19999 m/z.)
274.1858($^{13}C_2$-H_2, 4), 275.1899($^{13}C_2$-H, 3), 276.1998($^{13}C_2$, 100).

HRMS results indicate peaks at 275 and 274 in the low res. spectrum are consistent with $[^{13}C_2\text{-H}]^+$ and $[^{13}C_2\text{-}H_2]^+$ rather than $^{13}C_1$ and $^{13}C_0$ species.

HPLC:

Method:	Peak area percentage of total: 98.9% (Feb 2001)
Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
Mobile Phase:	Acetonitrile/MilliQ water (50:50 v/v)
Flow rate:	1.0 mL/min
Detector:	U.V. at 214 nm

TLC:

Conditions:	Kieselgel 60F_{254}. Hexane/ethyl acetate (2:1) Single spot observed, R_f = 0.25

IR:

Instrument:	FT-IR, Perkin-Elmer
Range:	4000-400 cm^{-1}, Nujol mull
Peaks:	3414, 1625, 1608, 1452, 1326, 1197, 1053, 880 cm^{-1}

Close agreement observed with a reference IR spectrum for unlabelled nandrolone

^{1}H NMR:

Instrument:	Bruker Advance-300
Field strength:	300 MHz
Solvent:	$CDCl_3$
Spectral data:	0.77 (3H, s), 5.78 (1H, d, $J_{1H\text{-}13C}$ = 160 Hz) ppm

^{13}C NMR:

Instrument:	Bruker Advance-300
Field strength:	75 MHz
Solvent:	$CDCl_3$
Spectral data:	δ 11.0, 23.1, 26.1, 26.5, 30.3, 30.7, 35.4, 36.4, 36.5 (m), 40.4, 42.5, 43.0, 49.5, 49.7, 81.6, 124.9 (d, *J* = 52 Hz), 167.1 (d, *J* = 64 Hz), 200.3 (d, *J* = 53 Hz) ppm

Microanalysis:

Found:	C = 78.7%, H= 9.2% (March 2001)
Calculated:	C = 78.9%, H = 9.5% (Calculated for $^{12}C_{16}{}^{13}C_2H_{26}O_2$)