Australian Government
Department of Industry, Science and Resources

National Measurement Institute

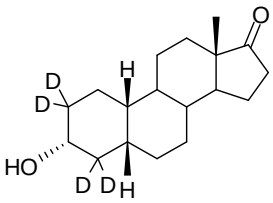
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D742: d_4-19-Noretiocholanolone

Report ID: D742.2021.02 (Ampouled 090319)

Chemical Formula: $C_{18}H_{24}D_4O_2$

Molecular Weight: 280.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
02-S-04	**361432-50-0**	**980 ± 76 μg**

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

IUPAC name: (3α, 5β)-3-Hydroxy(2,2,4,4-$^2H_4$)estran-17-one.

Expiration of certification: The property values are valid till 23 September 2031, i.e. ten 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 D742. 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 and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

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 980 μg of anhydrous 19-noretiocholanolone (d_4, 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 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.
14 November 2022.

This report supersedes any issued prior to 14 November 2022.

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:

GC-FID:	Instrument:	Agilent 6890/7890/8890
	Column:	HP-1MS, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	200 °C (10 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 = 99.6%, s = 0.01% (7 ampoules in duplicate, March 2009)
	Re-analysis	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, March 2010)
	Re-analysis:	Mean = 99.6%, s = 0.03% (5 ampoules in duplicate, February 2013)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, December 2015)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, October 2018)
	Re-analysis:	Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, September 2021)

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_4-19-noretiocholanolone. d_3-, d_2-, d_1- and d_0-19-noretiocholanolone are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_4, d_3, d_2 and d_1) and d_0-19-noretiocholanolone 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 ≈ 96% [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
	d_0 < 0.5% [= $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]	
GC-FID:	Instrument:	Varian CP-3800
	Column:	VF-1, 30 m x 0.32 mm I.D. x 0.25 µm
	Program:	180 °C (1 min), 10 °C /min to 230 °C, (6 min), 20 °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 = 99.7%, s = 0.03% (10 sub samples in duplicate, June 2002)
	Current re-analysis:	Mean = 99.5%, s = 0.01% (5 sub samples in duplicate, February 2009)
Thermogravimetric analysis:		Volatile content < 0.1% and non volatile residue < 0.2 % mass fraction (May 2002, April 2005 and February 2006)
Karl Fischer analysis:		Moisture content 0.33 % mass fraction (February, 2009)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	HP5890/5971A
Column:	BPX-5, 30m x 0.25mm I.D x 0.25 μm
Program:	200 °C (1 min), 10 °C /min to 300 °C (2 min)
Injector:	280 °C
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Split ratio:	20/1

Bis-trimethylsilyl derivative:

Column:	BPX-5, 30 m x 0.25 mm I.D x 0.25 μm
Program:	200 °C (1 min), 10 °C /min to 300 °C (2 min)
Injector:	250 °C
Transfer line temp:	280 °C
Carrier:	Helium 1.0 mL/min
Split ratio:	20/1

The retention times of the parent material and its *bis*-TMS derivative are reported with the major peaks in their mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.

Parent (8.2 min): 280 (M^+, 100), 236 (83), 218 (52), 206 (61), 191 (48), 91 (49), 79 (49) *m/z*
Bis-TMS (9.7 min): 424, (M^+, 35), 409 (32), 319 (15), 182 (10), 169 (23), 73 (100) *m/z*

The *bis*-silylated derivative of d_4-noretiocholanolone co-elutes with a comparison sample of silylated unlabelled noretiocholanolone under these conditions.

Deuteration yield determined by SIM analysis of the *bis*-TMS derivative (mean of 7 samples)
Relative areas of each ion are reported (deuteration state, % rel. to d_4-isomer at 424)

420 (d_0, 0), 421 (d_1, 0), 422 (d_2, 0.5), 423 (d_3, 3), 424 (d_4, 100) *m/z*

Results are uncorrected for possible small contributions due to $[M-H]^+$, $[M-2H]^+$ and ^{13}C isotope peaks of partially labelled steroids.

TLC:

Conditions:	Kieselgel 60F_{254} Chloroform/ethylacetate (4:1) Single spot observed, R_f = 0.41 (3 sub samples)

IR:

Instrument:	Biorad FTS3000MX
Range:	4000-400 cm^{-1}, KBr
Peaks:	3310, 2914, 2870, 2194, 2113, 1737, 1454, 1176, 1024, 1007, 919 cm^{-1} The IR spectrum obtained for D742 conforms with that obtained for a previous batch of d_4-19-noretiocholanolone (NMI D623)

^1H NMR:

Instrument:	Bruker DMX-500
Field strength:	500 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Key spectral data:	δ 0.86 (3H, s), 2.08 (1H, ddd, *J* = 9.0, 9.0, 18.2 Hz), 2.43 (1H, dd, *J* = 9.4, 19.4 Hz), 3.61 (1H, s) ppm

^2H NMR:

Instrument:	Bruker DMX-500
Field Strength	77 MHz
Solvent:	$CHCl_3$ (7.26 ppm)
Spectral data:	δ 1.18, 1.55, 1.64 ppm

^{13}C NMR:

Instrument:	Bruker DMX-300
Field strength:	126 MHz
Solvent:	$CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.8, 21.7, 24.9, 25.1, 25.7, 31.2, 31.6, 35.4, 35.9, 38.7, 39.8, 41.2, 47.9, 50.5, 71.3, 221.5 ppm The NMR spectra obtained for D742 conforms with that obtained for a previous batch of d_4-19-noretiocholanolone (NMI D623)

Melting Point: 164-165 °C

Microanalysis:

Found:	C = 77.2%; H/D = 11.3% (July 2002)
Calculated:	C = 77.1%; H/D = 11.5% (Calculated for $C_{18}H_{24}D_4O_2$)