



DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

Report ID: D742.2018.01 (Ampouled 090319)

This batch of ampoules was prepared from the bulk material on 19th March 2009.

Compound Name: **d₄-19-Noretiocholanolone**

Collection Number: D742

Chemical Formula: C₁₈H₂₄D₄O₂

CAS Registry Number: 361432-50-0

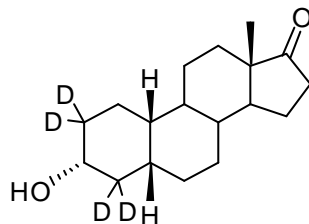
Structure:

Description: White powder

Batch Number: 02-S-04

Molecular Weight: 280.4

Release Date: 10th June 2003



Synonyms: d₄-5β-Estran-3α-ol-17-one
d₄-3α-Hydroxy-5β-estran-17-one

The main component of this material is d₄-19-noretiocholanolone. d₃-, d₂-, d₁- and d₀-19-noretiocholanolone are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d₄, d₃, d₂ and d₁) and d₀-19-noretiocholanolone in the material.

The material is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D742. Each ampoule contains approximately 980 µg of anhydrous 19-noretiocholanolone (d₄, d₃, d₂, d₁ and d₀). Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (chloroform).

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

Isotopic Purity: d₄ ≈ 96% [= d₄ / (d₀ + d₁ + d₂ + d₃ + d₄) × 100]
d₀ < 0.5% [= (d₀ / d₄) × 100]

Note: Each ampoule contains approximately 941 µg of d₄-19-noretiocholanolone.
[Calculated from the product of the chemical and isotopic purities]

GC-FID: Instrument: Agilent 6890/7890
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 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)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The purity value was obtained from a combination of traditional analytical techniques, by subtraction from 100% of total impurities by GC-FID, Thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. Supporting evidence is provided by elemental microanalysis.

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 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 characterization data

GC-MS:	Parent compound:	
	Instrument:	HP5890/5971A
	Column:	BPX-5, 30m x 0.25mm I.D x 0.25 μ m
	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
	<i>Bis</i> -trimethylsilyl derivative:	
	Column:	BPX-5, 30 m x 0.25 mm I.D x 0.25 μ m
	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 <i>bis</i> -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) <i>m/z</i>	
	<i>Bis</i> -TMS (9.7 min): 424, (M ⁺ , 35), 409 (32), 319 (15), 182 (10), 169 (23), 73 (100) <i>m/z</i>	
	The <i>bis</i> -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 <i>bis</i> -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) <i>m/z</i>	
	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)
¹ H 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), 2.43 (1H, dd), 3.61 (1H, s) ppm
² H 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) Calc: C = 77.1%; H/D = 11.5% (Calculated for C ₁₈ H ₂₄ D ₄ O ₂)	

Expiration of certification

The property values are valid till 31st October 2023, 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 ampoules that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

The long-term stability of the compound in solution has not been examined.

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.

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.

Recommended storage

When not in use this material should be stored at or below 4 °C in a closed container in a dry, dark area.

Intended use

This reference material should be used for qualitative analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 26 November, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 26th November 2018.