Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

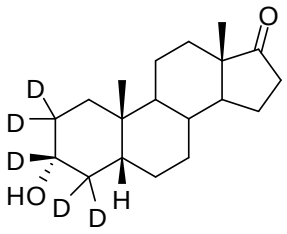
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D528d: d5-Etiocholanolone

Report ID: D528d.2023.01 (Ampouled 211021)

Chemical Formula: $C_{19}H_{25}D_5O_2$

Molecular Weight: 295.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
20-S-01	**1620102-33-1**	**991 ± 20 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(2,2,3,4,4-$^2H_5$)androstan-17-one.

Expiration of certification: The property values are valid till 26 March 2027, three 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The deuterated internal standard is intended for a single use to prepare a standard solution containing D528d. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d_5-etiocholanolone. d_4-, d_3-, d_2-, d_1- and d_0-Etiocholanolone are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-etiocholanolone in the material.

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. methanol). This will transfer approximately 991 μg of anhydrous etiocholanolone (d_5, 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: 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.
27 March 2024.

This report supersedes any issued prior to 27 March 2024.

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 7890 or 8890
	Column:	HP-1MS, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 10 °C/min to 220 °C (8 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 = 99.3%, s = 0.00% (7 ampoules in duplicate, October 2021)
	Re-analysis:	Mean = 99.3%, s = 0.00% (5 ampoules in duplicate, June 2022)
	Re-analysis:	Mean = 99.4%, s = 0.01% (5 ampoules in duplicate, April 2023)
	Re-analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, March 2024)

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_5- etiocholanolone. d_4-, d_3-, d_2-, d_1- and d_0- etiocholanolone are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0- etiocholanolone 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: $d_5 \approx 92\%$ [= $d_5/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]

$d_0 < 0.2\%$ [= $d_0/(d_5 + d_4 + d_3 + d_2 + d_1 + d_0)$ x 100]

GC-FID:	Instrument:	Agilent 7890
	Column:	VF-1ms, 30 m × 0.32 mm I.D. × 0.25 μm
	Program:	180 °C (1 min), 5 °C/min to 280 °C (10 min)
	Injector:	200 °C
	Detector Temp:	280 °C
	Carrier:	Helium
	Split ratio:	20/1
	Relative peak area of the main component as the *mono*-TMS-derivative:	
	Initial analysis:	Mean = 99.3%, s = 0.05% (7 sub samples in duplicate, February 2020)
Karl Fischer analysis:		Moisture content < 0.2% mass fraction (February 2020 and March 2021)
Thermogravimetric analysis:		Volatiles content 0.4% and non-volatile residue < 0.2% mass fraction (March 2020)

Spectroscopic and other characterisation data

GC-MS:

Parent compound:

Instrument:	Agilent 6890/5973
Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)
Injector:	250 °C Split ratio: 20/1
Transfer line temp:	280 °C
Carrier:	Helium, 1.0 mL/min
Scan range:	50-550 *m/z*

Bis-TMS derivative:

Instrument:	Agilent 6890/5973
Column:	TG-1MS, 30 m x 0.25 mm I.D. x 0.25 μm
Program:	180 °C (1 min), 10 °C/min to 300 °C (3 min)
Injector:	250 °C Split ratio: 20/1
Transfer line temp:	280 °C
Carrier:	Helium
Scan range:	50-550 *m/z*

The retention times of the parent compound 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 base peak.

Parent (8.9 min): 295 (M^+, 100), 277 (44), 251 (43), 247 (70), 233 (44), 206 (45), 176 (41), 163 (29), 150 (27), 134 (22), 121 (30), 112 (37), 108 (35), 97 (47), 79 (53), 67 (54), 55 (37) *m/z*

Bis-TMS (9.1 min): 439 (M^+, 59), 424 (60), 334 (33), 244 (11), 182 (18), 169 (30), 73 (100) *m/z*

The silylated compound co-elutes with a derivatised comparison sample of etiocholanolone.

TLC:

Conditions: Kieselgel 60F_{254}. Chloroform/ethyl acetate (4:1)
Single spot observed, R_f = 0.22. Visualisation with vanillin.

IR:

Instrument:	Biorad FTS3000MX FT-IR
Range:	4000-400 cm^{-1}, KBr powder
Peaks:	3470, 2930, 2902, 2849, 2189, 2106, 1729, 1456, 1379, 1094, 1052, 1011, 947 cm^{-1}

^{1}H NMR:

Instrument:	Bruker Avance III-400
Field strength:	400 MHz
Solvent:	$CDCl_3$ (7.26 ppm)
Spectral data:	δ 0.84 (3H, s), 0.94 (3H, s), 0.97 (1H, d, *J* = 14 Hz), 1.17 (1H, m), 1.22-1.36 (4H, m), 1.38-1.62 (7H, m), 1.74-1.96 (4H, m), 2.07 (1H, dt, *J* = 19.2, 9.0 Hz), 2.42 (1H, dd, *J* = 19.2, 8.3 Hz) ppm

Ethyl acetate estimated at 0.5% mass fraction was observed in the ^{1}H NMR

^{13}C NMR:

Instrument:	Bruker Avance III-500
Field strength:	126 MHz
Solvent:	$CDCl_3$ (77.2 ppm)
Spectral data:	δ 13.9, 20.2, 21.9, 23.4, 25.5, 27.0, 29.6 (m), 31.9, 34.8, 35.3, 35.4 (m), 35.5, 36.1, 40.9, 42.0, 48.0, 51.6, 71.0 (m), 221.5 ppm

Melting point: 152 - 153 °C

Microanalysis:

Found:	C = 77.6%; H = 10.4% (March 2020)
Calculated:	C = 77.2%; H = 10.4% (Calculated for $C_{19}H_{25}D_5O_2$)