



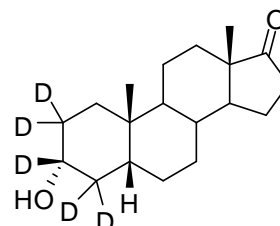
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D528c: d₅-Etiocholanolone

Report ID: D528c.2020.01 (Ampouled 200102)

Chemical Formula: C₁₉H₂₅D₅O₂

Molecular Weight: 295.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
13-S-10	1620102-33-1	996 µg

IUPAC name: (3 α ,5 β)-3-Hydroxy(2,2,3,4,4-²H₅)androstan-17-one.

Expiration of certification: The property values are valid till 23 January 2023, i.e. three years from the date of 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 deuterated internal standard is intended for a single use to prepare a standard solution containing D528c. The material was prepared by synthesis, and certified for identity and purity by NMIA. The compound is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D528c. The main component of this material is d₅-etiocholanolone. d₄-, d₃-, d₂-, d₁- and d₀-Etiocholanolone are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d₅, d₄, d₃, d₂ and d₁) and d₀-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.

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 906 µg of anhydrous d₅-etiocholanolone.

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.

Stability: This material has demonstrated stability over a minimum period of three years. 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.
11 February 2020.

NATA logo notice: Accredited for compliance with ISO Guide 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

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
 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, January 2020)

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 ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

Supporting evidence is provided by elemental microanalysis.

Isotopic Purity: $d_5 \approx 92\%$ [= $d_5 / (d_5 + d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
 $d_0 < 0.2\%$ [= $d_0 / (d_5 + d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
 [from SIM analysis of the TMS derivative]

GC-FID: Instrument: Agilent 7890
 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 = 99.1%, s = 0.03% (10 sub samples in duplicate, November 2013)

Re-analysis: Mean = 99.2%, s = 0.01% (5 sub samples in duplicate, March 2016)

Re-analysis: Mean = 99.2%, s = 0.03% (5 sub samples in duplicate, September 2019)

Karl Fischer analysis: Moisture content < 0.2% mass fraction (December 2013 and March 2016)

Thermogravimetric analysis: Non volatile residue < 0.2% mass fraction (December 2013). The volatile content (e.g. organic solvents and/or water) could not be determined because of the inherent volatility of the material and/or degradation at elevated temperatures.

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 μ m
Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
Injector: 250 $^{\circ}$ C, Split ratio: 20/1
Transfer line temp: 280 $^{\circ}$ 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 μ m
Program: 180 $^{\circ}$ C (1 min), 10 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
Injector: 250 $^{\circ}$ C Split ratio: 20/1
Transfer line temp: 280 $^{\circ}$ 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 (9.8 min): 295 (M^+ , 100), 277 (52), 251 (49), 247 (75), 233 (51), 206 (51), 176 (45), 163 (34), 150 (34), 134 (26), 121 (33), 112 (39), 108 (39), 97 (59), 79 (58), 67 (59), 55 (41) m/z

Bis-TMS (10.5 min): 439 (M^+ , 74), 424 (76), 334 (43), 244 (11), 182 (22), 169 (35), 73 (100) m/z

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

TLC: Conditions: Kieselgel 60F₂₅₄. Chloroform/ethyl acetate (4:1)
Single spot observed, R_f = 0.2. 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}

¹H NMR: Instrument: Bruker Avance III-400
Field strength: 400 MHz
Solvent: CDCl₃ (7.26 ppm)
Spectral data: δ 0.84 (3H, s), 0.94 (3H, s), 1.12-1.63 (12H, m), 1.76-1.96 (4H, m), 2.07 (1H, dt, J = 19.2, 9.0 Hz), 2.46 (1H, dd, J = 19.0, 8.8 Hz) ppm
Ethyl acetate estimated at 0.3% mass fraction was observed in the ¹H NMR

¹³C NMR: Instrument: Bruker Avance III-400
Field strength: 101 MHz
Solvent: CDCl₃ (77.2 ppm)
Spectral data: δ 14.0, 20.3, 22.0, 23.4, 25.5, 27.0, 29.7 (m), 31.9, 34.9, 35.3, 35.4 (m), 35.6, 36.1, 40.9, 42.0, 48.0, 51.7, 71.2 (m), 221.5 ppm

Melting point: 153 - 154 $^{\circ}$ C

Microanalysis: Found: C = 76.9%; H = 10.3% (December, 2013)
Calculated: C = 77.2%; H = 10.4% (Calculated for C₁₉H₂₅D₅O₂)