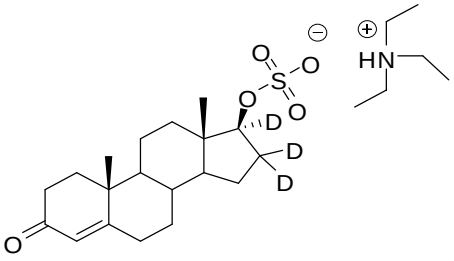
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D506b: d3-Testosterone sulfate (triethylammonium salt)

Report ID: D506b.2020.01 (Ampouled 200701)

Chemical Formula: $C_{25}H_{40}D_3NO_5S$

Molecular Weight: 472.7 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
20-S-02	**Not available**	**974 µg**

IUPAC name: (16,16,17α-d_3)- 17β-3-Oxoandrost-4-en-17-yl sulfate, triethylammonium salt (1:1)

Expiration of certification: The property values are valid till 2 July 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 D506b. The material was prepared by synthesis, and certified for identity and purity by NMIA. The main component of this material is d_3-testosterone sulfate (triethylammonium salt). d_2-, d_1- and d_0-Testosterone sulfate (triethylammonium salt) are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated (d_3, d_2 and d_1) and d_0- testosterone sulfate (triethylammonium salt) 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. acetonitrile). This will transfer approximately 974 µg of anhydrous testosterone sulfate triethylammonium salt (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 HPLC with UV 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.
17 July 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:

HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 20 mM ammonium acetate was present only in the aqueous phase 0-14 min 25% B; 14-17 min 25-75% B; 17-22 min 75% B; 22-25 min 75-25% B, 25-30 min 25% B.
	Flow rate:	1.0 mL/min
	Detector:	Thermo Scientific UltiMate 3000 RS PDA operating at 246 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.1%, s = 0.06% (7 sub samples in duplicate, July 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 purity estimate was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity estimate is calculated as per Equation 1.

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

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

Supporting evidence is provided by elemental microanalysis.

The main component of this material is d_3-testosterone sulfate (triethylammonium salt). d_2-, d_1- and d_0-Testosterone sulfate (triethylammonium salt) are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_3, d_2 and d_1) and d_0- testosterone sulfate (triethylammonium salt) 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:	$d_3 \approx 80\%$ [$= d_3/(d_3 + d_2 + d_1 + d_0) \times 100$]
	$d_0 < 1\%$ [$= d_0/(d_3 + d_2 + d_1 + d_0) \times 100$]

HPLC:	Instrument:	Thermo Scientific UltiMate 3000
	Column:	X-Bridge C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 20 mM ammonium acetate was present only in the aqueous phase 0-14 min 25% B; 14-17 min 25-75% B; 17-22 min 75% B; 22-25 min 75-25% B, 25-30 min 25% B.
	Flow rate:	1.0 mL/min
	Detector:	Thermo Scientific UltiMate 3000 RS PDA operating at 246 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.02% (7 sub samples in duplicate, June 2020)
Karl Fischer analysis:		Moisture content 0.8% mass fraction (June 2020)
Thermogravimetric analysis:		Volatiles content 0.4% and non-volatile residue 1.1% mass fraction (June 2020)

Spectroscopic and other characterisation data

GC-MS:	The free steroid was liberated upon treatment with acid and derivatised with MSTFA.	
	Instrument:	Agilent 6890/5973
	Column:	DB-5MS, 30 m x 0.25 mm I.D. x 0.25 μm
	Program:	180 °C (1 min), 30°C/min to 250°C (10 min), 30°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 time of the *bis*-TMS derivative of d_3-testosterone is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Bis-TMS (12.1 min):	435 (M^+, 100), 420 (12), 209 (9), 208 (8), 75 (13), 73 (55) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:28:2) Single spot observed, R_f = 0.5. Visualisation with UV at 254 nm
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3530, 1675, 1622, 1261, 1211, 1057, 1026, 1013, 991, 771, 606 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Spectral data:	δ 0.86 (3H, s), 0.95-1.08 (3H, m), 1.21 (1H, m), 1.24 (3H, s), 1.32 (9H, t, *J* = 7.3 Hz), 1.36 (1H, m), 1.48 (1H, m), 1.60-1.75 (4H, m), 1.90 (1H, m), 2.00 (1H, dt, *J* = 13.0, 3.4 Hz), 2.09 (1H, ddd, *J* = 3.1, 5.0, 13.5 Hz), 2.26-2.33 (2H, m), 2.44-2.52 (2H, m), 3.22 (6H, q, *J* = 7.2 Hz), 5.71 (1H, s) ppm
		Diethyl ether estimated at 0.2% mass fraction was observed in the ^{1}H NMR
^{13}C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 9.2, 12.0, 17.7, 21.6, 24.1, 32.8, 33.9, 34.7, 36.7, 36.8, 37.7, 40.0, 43.7, 48.0, 51.3, 55.4, 124.2, 175.2, 202.3 ppm
		As a result of successful deuteration, signals due to C-16 and C-17 are not observed above baseline noise
Melting point:		154-158 °C
Microanalysis:	Found:	C = 63.2%; H = 9.3%; N = 2.9%; S% = 6.5% (June, 2020)
	Calculated:	C = 63.5%; H = 9.2%; N = 3.0%; S% = 6.8% (Calculated for $C_{25}H_{40}D_3NO_5S$)