Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

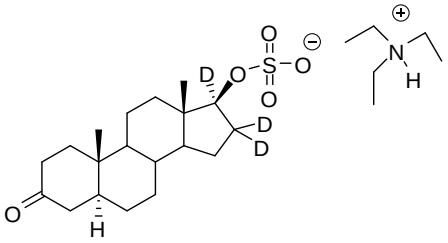
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D590: d_3-5α-Dihydrotestosterone sulfate (triethylammonium salt)

Report ID: D590.2025.01 (Ampouled 201124)

Chemical Formula: $C_{25}H_{42}D_3NO_5S$

Molecular Weight: 474.4 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
98-001909	N/A	1143 ± 166 μg

IUPAC name: Triethylammonium 3-Oxo-(16,16,17-$^2H_3$)-5α-androstan-17-yl sulfate

Expiration of certification: The property values are valid till 15 January 2030, 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D590. The material was prepared by synthesis and certified for identity and purity by NMI Australia.

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 1143 ± 166 μg of anhydrous d_3-5α-dihydrotestosterone sulfate triethylammonium salt. 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 ELS detection 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 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.
29 January 2025

This report supersedes any issued prior to 29 January 2025.

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:

HPLC:	Column:	Waters X-Bridge C-18 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = MilliQ water containing 0.1% formic acid; B = Methanol 0-4 min 30% B, 4-6 min 75% B; 6-16 min 75% B; 16-20 min 75-30% B; 20-30 min 30%B
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Retention time:	8.2 min
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.05% (7 ampoules in duplicate, December 2020)
HPLC:	Column:	Waters X-Bridge C-18 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	A = MilliQ water containing 0.1% formic acid; B = Methanol 0-4 min 30% B, 4-6 min 75% B; 6-16 min 75% B; 16-20 min 75-30% B; 20-30 min 30%B
	Flow Rate:	1.0 mL/min
	Detector:	ELSD
	Retention time:	10.6 min
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.6%, s = 0.06% (5 ampoules in duplicate, January 2025)

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 quantitative nuclear magnetic resonance (qNMR). The one-proton multiplet at 2.1 ppm was measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by elemental microanalysis and ^{1}H NMR.

Isotopic Purity: $d_3 \approx 94\%$ [$= d_3/(d_3 + d_2 + d_1 + d_0) \times 100$]

$d_0 < 0.5\%$ [$= d_0/(d_3 + d_2 + d_1 + d_0) \times 100$]

[from SIM analysis of the parent steroid D552]

The main component of this material is d_3-5α-dihydrotestosterone sulfate (triethylammonium salt). d_2-, d_1- and d_0-5α-Dihydrotestosterone 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_0-5α-dihydrotestosterone sulfate (triethylammonium salt) in the material.

QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Purity estimate:	Mean (2.1 ppm) = 93.9%, s = 0.8% (5 sub samples, December 2007)
	The purity estimate by qNMR is a measure of the mass fraction of *d*$_3$-5α-dihydrotestosterone sulfate triethylammonium salt in D590.	
HPLC:	Column:	Alltima C-18 5 μm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/Milli-Q water (35:65 v/v, both with 0.05% TFA)
	Flow Rate:	0.8 mL/min
	Detector:	ELSD
	Retention time:	6.5 min
	Relative peak area of main component:	
	Initial analysis:	Mean > 99% (3 sub samples in duplicate, December 2000)
	Re-analysis:	Mean = 99.7%, s = 0.06% (5 sub samples in duplicate, November 2007)
	Re-analysis:	Mean = 99.9%, s = 0.004% (7 sub samples in duplicate, March 2011)
Karl Fischer analysis:		Moisture content 4.4% mass fraction (2 sub samples, November 2007) Moisture content 3.0% mass fraction (2 sub samples, February 2011) Moisture content 4.3% mass fraction (2 sub samples, February 2016)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI probe at 4.5 kV
	Peak:	372 $(MSO_3)^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:20:2) Single spot observed, R_f = 0.3 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3500, 2741, 2680, 2492, 1719, 1224, 1025, 826, 608 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	Acetone-d_6 (2.05 ppm)
	Spectral data:	δ 0.65 (3H, s), 0.95 (3H, s), 1.14 (9H, t), 3.08 (6H, q) ppm As a result of successful deuteration, no absorptions or couplings observed due to hydrogens at the 16- or 17α-position
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	Acetone-d_6 (29.8 ppm)
	Spectral data:	δ 9.0, 11.4, 12.1, 20.9, 23.2, 28.7, 31.2, 35.7, 36.9, 38.0, 38.3, 42.5, 44.5, 46.2, 46.4, 50.4, 53.6, 210.8 ppm As a result of successful deuteration, signals due to C-16 and C-17 are not observed above baseline noise.
Melting point:		125-126 °C (December 2007)
Microanalysis:	Found:	C = 59.5%, H = 9.7%, N = 2.4% (1999)
	Found:	C = 58.5%, H = 9.3%, N = 2.3% (December 2007)
	Calculated:	C = 63.3%, H = 10.2%, N = 3.0% (Calculated for $C_{25}H_{45}NO_5S$)