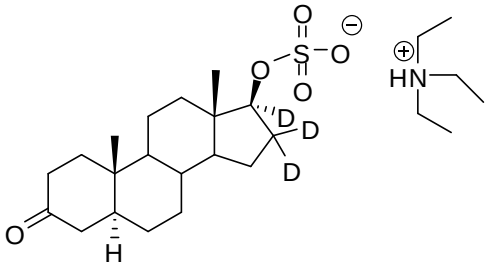
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D590.2016.01

Compound Name: **d_3-5α-Dihydrotestosterone sulfate (NEt_3 salt)**
Description: White crystals
Collection Number: D590
Chemical Formula: $C_{25}H_{42}D_3NO_5S$
CAS Registry Number: N/A
Structure:



Batch Number: 98-001909
Molecular Weight: 474.7
Release Date: August 2001

Synonyms: (16,16,17α-d_3)-5α-Dihydrotestosterone sulfate, triethylammonium salt
5α-d_3-Androstan-17β-ol-3-one sulfate, triethylammonium salt
d_3-17β-Sulfooxy-5α-androstan-3-one triethylammonium salt

Purity (mass fraction %): 72.7 ± 2.6% (as d_3-5α-dihydrotestosterone sulfate anion at 95% coverage interval)

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

Purity estimate obtained from Quantitative NMR using potassium hydrogen maleate as internal standard to quantify the amount of steroid sulfate anion in the sample. Supporting evidence is provided by elemental microanalysis and ^{1}H NMR.

Isotopic Purity:		$d_3 \approx 94\%$	$[= (d_3/d_0 + d_1 + d_2 + d_3) \times 100]$
		$d_0 < 0.5\%$	$[= (d_0/d_3) \times 100]$
		[from SIM analysis of the parent steroid D552]	
QNMR:	Instrument:	Bruker DMX-600	
	Field strength:	600 MHz	Solvent: d_6-DMSO
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)	
	Purity estimate:	Mean (2.1 ppm) = 73.7%, s = 0.59% (5 sub samples, December 2007)	
	The purity estimate by QNMR is a measure of the mass fraction of d_3-5α-dihydrotestosterone sulfate anion in D590.		
HPLC:	Column:	X-bridge C-18 5 μm (4.6mm × 150mm)	
	Mobile Phase:	A = Formic acid, pH = 2.3; B = methanol	
		0-5 min 35% B, 6-13 min 70% B, 20 min 35% B	
	Flow Rate:	1 mL/min	
	Detector:	ELSD	
	Relative peak area response of main component:		
	Initial analysis:	Mean = 98.9%, s = 0.2% (5 sub samples in duplicate, April 2016)	

HPLC:	Column:	Alltima C-18 5 μm (4.6mm × 150mm)
	Mobile Phase:	35% Acetonitrile/65% Milli-Q water (both with 0.05% TFA)
	Flow Rate:	0.8 mL/min
	Detector:	ELSD
	Relative peak area response 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:		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
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: d_6-Acetone (2.05 ppm)
	Spectral data:	δ 0.65 (3H, s), δ 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: d6-Acetone (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) Calc: C = 63.3%; H = 10.2%; N = 3.0% (for $C_{25}H_{45}NO_5S$)
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:20:2) Single spot observed, R_f = 0.3 (3 sub samples)

Expiration of certification
The property values are valid till 5th April 2021, 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 sample bottles 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% coverage 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 HPLC with ELS detection on seven randomly selected 1-2 mg sub samples 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 ºC in a closed container in a dry, dark area.

Intended Use
For ***in vitro*** laboratory 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: 25 May, 2016.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 25th May 2016.

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