



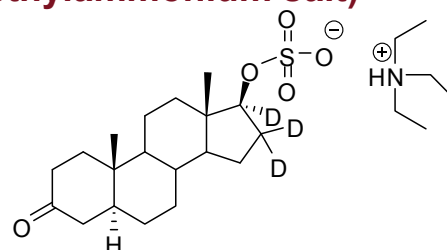
## DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

### NMIA D590: d<sub>3</sub>-5 $\alpha$ -Dihydrotestosterone sulfate (triethylammonium salt)

Report ID: D590.2016.02

Chemical Formula: C<sub>25</sub>H<sub>42</sub>D<sub>3</sub>NO<sub>5</sub>S

Molecular Weight: 474.7 g/mol



### Property value

Batch No.	CAS No.	Purity estimate
98-001909	Not available	72.7 %

**IUPAC name:** Triethylammonium 3-Oxo-(16,16,17-<sup>2</sup>H<sub>3</sub>)-5 $\alpha$ -androstan-17-yl sulfate

**Expiration of certification:** The property values are valid till 5 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.

**Description:** White powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

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

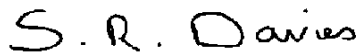
**Instructions for use:** Equilibrate the bottled material to room temperature before opening.

**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 five 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 HPLC with ELS detection on five 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.

**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.



Dr Stephen R. Davies,  
Team Leader,  
Chemical Reference Materials, NMI.  
11 February 2020

This report supersedes any issued prior to 11 February 2020

**NATA logo notice:** Accredited for compliance with ISO 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.

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**Characterisation Report:**

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 headspace GC-MS analysis of occluded solvents and elemental microanalysis.

The main component of this material is d<sub>3</sub>-5α-dihydrotestosterone sulfate (triethylammonium salt). d<sub>2</sub>-, d<sub>1</sub>- and d<sub>0</sub>-5α-Dihydrotestosterone sulfate (triethylammonium salt) are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d<sub>3</sub>, d<sub>2</sub> and d<sub>1</sub>) and d<sub>0</sub>-5α-Dihydrotestosterone 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<sub>3</sub> ≈ 94% [ = d<sub>3</sub>/(d<sub>3</sub> +d<sub>2</sub> +d<sub>1</sub> +d<sub>0</sub>) x 100]

d<sub>0</sub> < 0.5% [ = d<sub>0</sub>/(d<sub>3</sub> +d<sub>2</sub> +d<sub>1</sub> +d<sub>0</sub>) x 100]

QNMR: Instrument: Bruker DMX-600  
Field strength: 600 MHz  
Solvent: d<sub>6</sub>-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<sub>3</sub>-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 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 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 <sub>3</sub> ) <sup>-</sup> <i>m/z</i>
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm <sup>-1</sup> , KBr pellet
	Peaks:	3500, 2741, 2680, 2492, 1719, 1224, 1025, 826, 608 cm <sup>-1</sup>
<sup>1</sup> H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	d <sub>6</sub> -Acetone (2.05 ppm)
	Spectral data:	$\delta$ 0.65 (3H, s), 0.95 (3H, s), 1.14 (9H, t, <i>J</i> = 7.3 Hz), 3.08 (6H, q, <i>J</i> = 7.3 Hz) ppm
	As a result of successful deuteration, no absorptions or couplings observed due to hydrogens at the 16- or 17 $\alpha$ -position	
<sup>13</sup> C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	d <sub>6</sub> -Acetone (29.8 ppm)
	Spectral data:	$\delta$ 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% (for C <sub>25</sub> H <sub>45</sub> NO <sub>5</sub> S)
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Chloroform/methanol/water (70:20:2) Single spot observed, R <sub>f</sub> = 0.3 (3 sub samples)