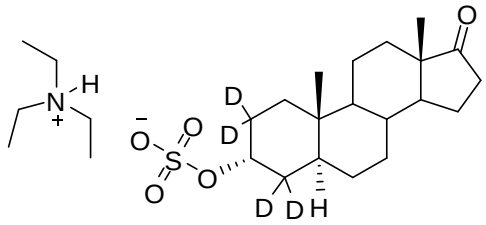
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D587.2016.01 (Ampouled 101014)
This batch of ampoules was prepared from the bulk material on 14th October 2010.

Compound Name: **d_4-Androsterone sulfate (NEt_3 salt)**
Collection Number: D587
Chemical Formula: $C_{25}H_{41}D_4NO_5S$
CAS Registry Number:
Structure:



Description: White crystals
Batch Number: 98-000501
Molecular Weight: 475.7
Release Date: January 2001

Synonyms: (2,2,4,4-d_4)-Androsterone sulfate, triethylammonium salt
5α-d_4-Androstan-3α-ol-17-one sulfate, triethylammonium salt
d_4-3α-Sulfoxy-5α-androstan-3-one triethylammonium salt

The main component of this material is d_4-androsterone sulfate (NEt_3 salt). d_3-, d_2-, d_1- and d_0-androsterone sulfate (NEt_3 salt) are also present. The stated mass of the material per ampoule represents the combined masses of deuterated (d_4, d_3, d_2 and d_1) and d_0-androsterone sulfate (NEt_3 salt) in the material.

The material is supplied as a dried aliquot in a sealed ampoule and is intended for a single use to prepare a standard solution containing D587. Each ampoule contains approximately 959 µg of material. The organic purity of the androsterone sulfate (NEt_3 salt) (d_4, d_3, d_2, d_1 and d_0) was estimated as 99.9% by HPLC-ELSD. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol).

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

Isotopic Purity: $d_4 \approx 94\%$ [$= d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]
$d_0 < 0.5\%$ [$= d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

HPLC:		
	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	35 °C
	Mobile Phase:	Methanol/MilliQ water (55:45) Trifluoro acetic acid (0.05% v/v) was present in both aqueous and organic phases
	Flow Rate:	1.0 mL/min
	Detector:	Waters ELSD
	Relative peak area response of main component:	
	Re-analysis:	Mean = 99.9%, s = 0.01% (5 ampoules in duplicate, September 2016)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The one-proton singlet at 4.7 ppm was measured against a certified internal standard of dimethylterephthalate. Supporting evidence is provided by HPLC with ELS detection, Karl Fischer moisture analysis, thermogravimetric analysis and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-III-500
	Field strength:	500 MHz Solvent: $CDCl_3$ (7.26 ppm)
	Internal standard:	Dimethylterephthalate (100.0% mass fraction)
	Initial analysis:	Mean (4.7 ppm) = 94.2%, s = 0.7% (5 sub samples, March 2017)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
	Column oven:	35 °C
	Mobile Phase:	Methanol/MilliQ water (55:45) Trifluoro acetic acid (0.05% v/v) was present in both aqueous and organic phases
	Flow rate:	1 mL/min
	Detector:	Waters ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, October 2010)
ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI probe at 4.5 kV
	Peak:	373 $(MSO_3)^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:20:2) Single spot observed, R_f = 0.30 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3500, 2712, 2361, 1738, 1269, 1198, 1014, 888, 659 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz Solvent: d_6-DMSO (2.5 ppm)
	Key spectral data:	δ 0.74 (3H, s), δ 0.76 (3H, s), 1.16 (9H, t), 3.09 (6H, q), 4.25 (1H, s) ppm As a result of successful deuteration, no absorptions or couplings observed due to hydrogen at 2- or 4-position.
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz Solvent: d_6-DMSO (39.5 ppm)
	Spectral data:	δ 9.0, 11.6, 13.8, 20.0, 21.7, 28.2, 30.9, 31.8, 32.6, 34.9, 35.7, 39.4, 40.2, 46.2, 47.5, 51.1, 54.4, 71.1, 220.2 ppm As a result of successful deuteration, signals due to C-2 and C-4 are not observed above baseline noise.
Microanalysis:		Found: C = 62.8%; H/D = 10.3%; N = 3.0% (June 1999) Calc: C = 63.1%; H/D = 10.4%; N = 2.9% (Calculated for $C_{25}H_{41}D_4NO_5S$)
Thermogravimetric analysis:		Non-volatile residue 0.4% mass fraction (September 2016)
Karl Fischer analysis:		Moisture content 0.3-0.4% mass fraction (July 2006, September 2007 and September 2010), 2.6% mass fraction (March 2017)

Expiration of certification
The property values are valid till 24th August 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 ampoules 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 three 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 ampoules of the material. The material was judged to be 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: 2nd May 2017.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 2nd May 2017.