Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

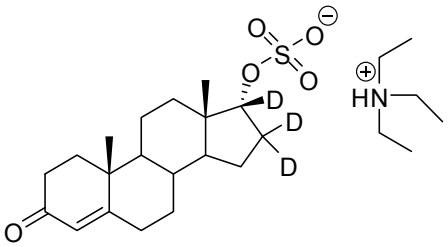
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D591: d_3-Epitestosterone sulfate (trethylammonium salt)

Report ID: D591.2017.02 (Ampouled 090521)

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

Molecular Weight: 472.7 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-000059	Not available	859 μg

IUPAC name: Triethylammonium (17α)-3-Oxo(16,16,17-$^2H_3$)-androst-4-en-17-yl sulfate

Expiration of certification: The property values are valid till 19 April 2022, 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 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 RM is intended for a single use to prepare a standard solution containing D591. The material was prepared by synthesis, and certified for identity and purity by NMIA.

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 859 μg of anhydrous epitestosterone 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: In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten 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 UV 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 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.
11 February 2020.

This report supersedes any issued prior to 11 February 2020

Characterisation Report:

HPLC:	Instrument:	Waters 1525 binary pump, 717 autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Column temp:	40 °C
	Mobile Phase:	Acetonitrile/50 mM ammonium acetate (28:72), pH 4.2
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 2998 at 247 nm
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.4%, s = 0.02% (7 ampoules in duplicate, June 2009)
	Re-analysis:	Mean = 99.4%, s = 0.01% (5 ampoules in duplicate, May 2012)
	Re-analysis:	Mean = 99.4%, s = 0.04% (5 ampoules in duplicate, April 2017)

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 by qNMR was obtained using the one-proton multiplets at 1.77 ppm measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by HPLC with UV detection, Karl Fischer analysis, ^{1}H NMR spectroscopy and elemental microanalysis.

The main component of this material is d_3-epitestosterone sulfate (triethylammonium salt). d_2-, d_1- and d_0-Epitestosterone sulfate (triethylammonium salt) are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated (d_4, d_3, d_2 and d_1) and d_0- epitestosterone 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:	$d_3 \approx 91\%$	$[= d_3/(d_3 + d_2 + d_1 + d_0) \times 100]$
	$d_0 < 0.5\%$	$[= d_0/(d_3 + d_2 + d_1 + d_0) \times 100]$
QNMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis	Mean (1.77 ppm) = 90.2%, s = 0.6% (3 sub samples, March 2007)
Karl Fischer analysis:		Moisture content ca. 0.7% mass fraction (February 2007 and June 2009)

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:	370.4 (M-Et_3NH) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:20:2) Single spot observed, R_f = 0.23
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr pellet
	Peaks:	3530, 3350, 2740, 2680, 2493, 2200, 1675, 1622, 1261, 1211, 1057, 1026, 1013, 991, 771, 606 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.85 (3H, s), 1.23 (3H, s), 1.31 (9H, t, *J* = 7.3 Hz), 3.21 (6H, m), 5.60 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz
	Solvent:	DMSO-d6 (39.52 ppm)
	Spectral data:	δ 9.0, 17.0, 17.4, 20.5, 24.3, 31.6, 32.4, 34.0, 35.5, 35.6, 38.6, 39.4, 44.4, 46.2, 49.3, 53.6, 123.5, 171.5, 198.4 ppm. As a result of successful deuteration, signals due to C-16 and C-17 are not observed above baseline noise
Melting point:		148-154 °C
Microanalysis:	Found:	C = 62.2%; H/D = 9.8%; N = 3.0% (August 1999)
	Found:	C = 62.2%; H/D = 9.8%; N = 3.0% (March 2007)
	Calculated:	C = 63.5%; H/D = 9.8%; N = 3.0% (Calculated for $C_{25}H_40D_3NO_5S$)