Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
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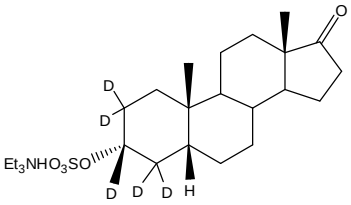
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA D606: d_5-Etiocholanolone sulfate (NEt_3 salt)

Report ID: D606.2019.02 (Ampouled 100716)

Chemical Formula: $C_{25}H_{40}D_5NO_5S$

Molecular Weight: 476.7 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-001744	**N/A**	**896 µg**

Synonyms: 5β-d_5-Androstan-3α-ol-17-one sulfate, triethylammonium salt

Expiration of certification: The property values are valid till 6 June 2024, 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 reference material is intended for a single use to prepare a standard solution containing D606. 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 896 µg of anhydrous etiocholanolone sulfate (NEt_3 salt) (d_5, d_4, 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: This material has demonstrated stability over a minimum period of three 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 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.
17 February 2020.

This report supersedes any issued prior to 17 February, 2020.

NATA logo notice: Accredited for compliance with ISO Guide 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.

Characterisation Report:

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler or Waters Model 1525 Binary Pump, 717 plus autosampler
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water, B = Acetonitrile 0-6 min 38%B, 6-7 min 38-60%B, 7-11 min 60%B, 11-12 min =60-38%B, 12-20 min 38%B The aqueous phase was buffered at pH 4.2 using 20 mM NH_4OAc and AcOH.
	Flow Rate:	0.8 mL/min
	Detector:	Shimadzu ELSD LT-II or Waters ELSD 2424
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.02% (August 2010)
	Re-analysis:	Mean = 99.98%, s = 0.004% (5 ampoules in duplicate, June 2013)
	Re-analysis:	Mean = 99.97%, s = 0.002% (5 ampoules in duplicate, June 2016)
	Re-analysis:	Mean = 99.98%, s = 0.008% (5 ampoules in duplicate, June 2019)

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 purity estimate by qNMR was obtained using a certified internal standard of potassium hydrogen maleate.

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

The main component of this material is d_5-etiocholanolone sulfate (NEt_3 salt). d_4-,d_3-,d_2-, d_1- and d_0-etiocholanolone sulfate (NEt_3 salt) are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_5, d_4, d_3, d_2 and d_1) and d_0-etiocholanolone sulfate (NEt_3 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:	$d_5 \approx 93\%$	$[= d_5 / (d_0 + d_1 + d_2 + d_3 + d_4 + d_5)) \times 100]$
	$d_0 < 0.5\%$	$[= d_0 / d_5) \times 100]$
	[from SIM analysis of the parent steroid NMI CRM D528]	
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: DMSO-d_6
	Internal standard:	Potassium hydrogen maleate (98.8% m/m)
	Purity estimate:	Mean = 89.2%, s = 1.3% (3 sub samples, August 2007)
HPLC:	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Flow Rate:	0.8 mL/min
	Detector:	ELSD
	Relative peak area of the main component:	
	Initial analysis:	Mean > 99% (December 2000)
	Re-analysis:	Mean = 99.9 %, s = 0.004% (5 sub samples in duplicate, July 2008)
Karl Fischer analysis:		Moisture content 2.4% mass fraction (two sub samples, September 2007 and two sub samples, August 2008)

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:	374.5 $(M\text{-}Et_3NH)^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Chloroform/methanol/water (70:20:2) Single spot observed, R_f = 0.30 (3 samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400cm^{-1}, KBr powder
	Peaks:	3500, 2740, 2679, 2492, 1738, 1234, 1058, 940, 618 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	d_6-DMSO
	Spectral data:	δ 0.75 (3H, s), δ 0.88 (3H, s), 1.16 (9H, t), 3.09 (6H, q) ppm As a result of successful deuteration, no absorptions or couplings observed due to hydrogen at 2-, 3β- or 4-position
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	d_6-DMSO
	Spectral data:	δ 9.0, 13.8, 20.0, 21.7, 23.4, 25.3, 26.9, 31.9, 34.6, 35.2, 35.3, 35.7, 40.5, 41.8, 46.2, 47.5, 51.0, 220.2 ppm As a result of successful deuteration, signals due to C-2, C-3 and C-4 are not observed above baseline noise
Microanalysis:	Found:	C = 60.5%, H/D = 10.6%, N = 3.1% (August 1999)
	Calculated:	C = 63.0%, H/D = 10.6%, N = 2.9% (Calculated for $C_{25}H_{40}D_5NO_5S$)