Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

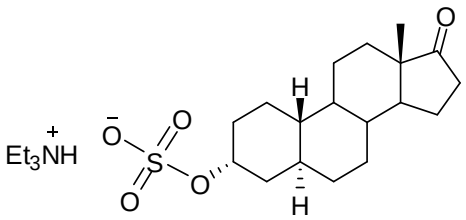
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D841b: 19-Norandrosterone sulfate (triethylamine salt)

Report ID: D841b.2020.03 (Ampouled 110705)

Chemical Formula: $C_{24}H_{43}NO_2S$

Molecular Weight: 457.7 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
10-S-05	**Not available**	**929 ± 25 μg**

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit (k = 2).

IUPAC name: Triethylammonium (3α,5α)-17-oxo-estran-3-yl sulfate

Expiration of certification: The property values are valid till 1 October 2025, 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.

Description: The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D841b. This material was prepared by synthesis, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile). This will transfer 929 ± 25 μg of anhydrous 19-norandrosterone sulfate (triethylamine salt). 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.

Metrological traceability: Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

Stability: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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 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.
15 November 2022

This report supersedes any issued prior to 15 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

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
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water 0-2 min 20% ACN, 14-20 min 65% ACN, 21-30 min 20% ACN 0.05% TFA was present in both aqueous and organic phases.
	Flow rate:	0.5 mL/min
	Detector:	Shimadzu LT II evaporative light scattering detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.04% (7 ampoules in duplicate, July 2011)
	Re-analysis:	Mean = 99.7%, s = 0.06% (5 ampoules in duplicate, November 2012)
HPLC:	Instrument:	Shimadzu binary pump LC-20AB
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water 0-2 min 20% ACN, 9-14 min 65% ACN, 20-30 min 20% ACN 0.05% TFA was present in both aqueous and organic phases.
	Flow rate:	0.5 mL/min
	Detector:	Shimadzu LT II evaporative light scattering detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.6%, s = 0.03% (5 ampoules in duplicate, November 2015)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, October 2020)

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

The certified purity value by qNMR was obtained using a combination of the one-proton doublet of doublets at 2.4 ppm and the one-proton multiplet at 4.6 ppm measured against a certified internal standard of maleic acid.

Supporting evidence is provided by HPLC with UV/ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy, headspace GC-MS analysis of occluded solvents and elemental microanalysis.

QNMR:	Instrument:	Bruker Avance-600
	Field strength:	600 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Internal standard:	Maleic acid (98.8% m/m)
	Initial analysis:	Mean (2.4 ppm) = 93.0%, s = 0.1% (5 sub samples, November 2010)
	Initial analysis:	Mean (4.6 ppm) = 91.9%, s = 0.3% (5 sub samples, November 2010)
HPLC:	Instrument:	Shimadzu binary pump LC-20AB
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water 0-2 min 20% ACN, 14-20 min 65% ACN, 21-30 min 20% ACN 0.05% TFA was present in both aqueous and organic phases.
	Flow rate:	0.5 mL/min
	Detector:	Shimadzu SPD-M20A photodiode array detector at 290 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 95.9%, s = 0.23% (10 sub samples in duplicate, January 2011)
	Detector:	Shimadzu LT II evaporative light scattering detector
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.5%, s = 0.06% (10 sub samples in duplicate, January 2011)
Thermogravimetric analysis:		Non volatile residue 0.4% mass fraction (November 2010). The volatile content (e.g. organic solvents and/or water) could not be determined accurately because of the volatility or degradation of the material at elevated temperatures.
Karl Fischer analysis:		Moisture content 0.7% mass fraction (November 2010)

Spectroscopic and other characterisation data

LC/ESI -MS:	Instrument:	Waters Acquity UPLC with TQD detector
	Operation:	Negative ion mode, direct infusion at 10 µL/min
	Ionisation:	ESI spray voltage at 3.0 kV negative ion
	EM voltage:	698 V
	Cone voltage:	37 V
	Peak:	354.7 ($M\text{-}H^+$) *m/z*
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 µm
	Program:	50 °C (5 min), 7 °C/min to 120 °C, 15 °C/min to 220 °C (8.3 min)
	Injector:	150 °C
	Transfer line temp:	280 °C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Triethylamine
TLC:	Conditions:	Kieselgel $60F_{254}$. Dichloromethane/methanol (4/1) Single spot observed, R_f = 0.30. Visualisation with UV vanillin stain
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3611, 3444, 2942, 2678, 2492, 1733, 1473, 1446, 1265, 1195, 1045, 959, 929, 814, 685 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.61-0.76 (2H, m), 0.79 (3H, s), 0.92-1.10 (5H, m), 1.12-1.35 (5H, m), 1.17 (9H, t, *J* = 7.3 Hz), 1.40-1.53 (2H, m), 1.56-1.66 (2H, m), 1.68-1.75 (1H, m), 1.75-1.87 (3H, m), 1.90-2.04 (2H, m), 2.36 (1H, dd, *J* = 8.1, 11.0 Hz), 3.09 (6H, q, *J* = 7.3 Hz), 4.31 (1H, m), 8.86 (1H, bs) ppm Excess triethylamine sulfur trioxide was detected at 2.0% mass fraction
^{13}C NMR:	Instrument:	Bruker Avance III-400
	Field strength:	100 MHz
	Solvent:	DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 8.7, 13.5, 21.2, 24.0, 24.5, 29.5, 30.5, 31.4, 33.0, 35.3, 36.1, 38.2, 40.2, 45.8, 45.9, 47.2, 48.0, 49.9, 70.7, 219.9 ppm
Melting point:		170-174 °C
Microanalysis:	Found:	C = 62.1%; H = 9.7%; N = 3.1%; S = 7.0% (December, 2010)
	Calculated:	C = 63.0%; H = 9.5%; N = 3.1%; S = 7.0% (Calculated for $C_{24}H_{43}NO_5S$)