Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

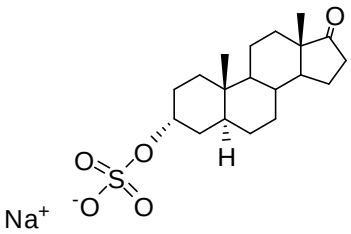
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D610b: Androsterone sulfate (Na salt)

Report ID: D610b.2017.02 (Ampouled 100315)

Chemical Formula: $C_{19}H_{29}O_5SNa$

Molecular Weight: 392.5 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
06-S-05	**2681-45-0**	**826 ± 17 μg**

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

IUPAC name: Sodium (3α,5α)-17-oxoandrostan-3-yl sulfate

Expiration of certification: The property values are valid till 4 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 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 and is intended for a single use to prepare a standard solution containing D610b. 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. methanol). This will transfer 826 ± 17 μg of anhydrous androsterone sulfate (Na salt).

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: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. 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.
12 February 2020

This report supersedes any issued prior to 12 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.

Characterisation Report:

HPLC:	Column:	X-Bridge C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	0.1% TFA in water/Methanol (40:60)
	Flow Rate:	1.0 mL/min
	Detector:	Waters ELSD 2420
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.8%, s = 0.005% (7 ampoules in duplicate, March 2010)
	Re-analysis:	Mean = 99.8%, s = 0.01% (5 ampoules in duplicate, June 2011)
	Re-analysis:	Mean = 99.6%, s = 0.02% (5 ampoules in duplicate, April 2014)
	Re-analysis:	Mean = 99.8%, s = 0.01% (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 certified purity value was obtained by quantitative nuclear magnetic resonance (qNMR). The one-proton multiplet at 2.0 ppm was measured against a certified internal standard of dimethyl sulfone.

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

HPLC:	Column:	X-Bridge C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	0.1% TFA in water/ Methanol (40:60)
	Flow Rate:	1.0 mL/min
	Detector:	Waters ELSD 2420
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (7 sub samples in duplicate, December 2006)
	Re-analysis:	Mean = 99.8%, s = 0.01% (7 sub samples in duplicate, May 2010)
Karl Fischer analysis:		Moisture content 9.6% mass fraction (August 2006 & March 2010)
Thermogravimetric analysis:		Volatile content 7.9% mass fraction (August 2006)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Internal standard:	Dimethyl sulfone (100.0% mass fraction)
	Initial analysis:	Mean (2.0 ppm) = 82.2%, s = 1.4% (3 sub samples, November 2006)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 2 μL/min
	Ionisation:	ESI spray voltage at 3.2 kV positive ion
	EM voltage:	650 V
	Cone voltage:	40 V
	Peak:	369.1 (M-Na^+) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Isopropanol Single spot observed, R_f = 0.35. Visualisation with vanillin
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3597, 3498, 2973, 2928, 2853, 1742, 1638, 1441, 1375, 1218, 1066, 923, 689 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.70 (1H, m), 0.74 (3H, s), 0.76 (3H, s), 0.94 (1H, m), 1.08-1.65 (15H, m), 1.69-1.86 (3H, m), 1.98 (1H, ddd, *J* = 8.9, 8.9, 18.2 Hz), 2.35 (1H, dd, *J* = 8.5, 19.0 Hz), 4.27 (1H, s) ppm
	^{13}C NMR:	Instrument: Bruker DMX-600
	Field strength:	150 MHz
	Solvent:	DMSO-d_6 (39.5 ppm)
	Spectral data:	δ 10.8, 13.0, 19.2, 20.9, 25.9, 27.4, 30.1, 30.9, 31.9, 32.9, 34.1, 34.8, 34.9, 38.8, 46.6, 50.3, 53.6, 70.6, 219.5 ppm
Melting point:		140-141 °C
Microanalysis:	Found:	C = 49.3%; H = 7.3%; S = 6.4% (August 2006)
	Calculated:	C = 58.1%; H = 7.5%; S = 8.2% (for $C_{19}H_{29}O_5SNa$)