Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

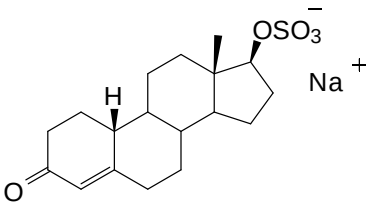
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D809b: Nandrolone sulfate, sodium salt

Report ID: D809b.2023.01 (Ampouled 120116)

Chemical Formula: $C_{18}H_{25}NaO_5S$

Molecular Weight: 376.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
08-S-01	60672-82-4	924 ± 26 µg

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

Synonyms: 4-Estren-17-β-ol-3-one sulfate, sodium salt, 19-nortestosterone sulfate, sodium salt

Expiration of certification: The property values are valid till 27 June 2028, 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 D809b. This material was sourced from an external supplier 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 924 ± 26 µg of nandrolone sulfate, sodium 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. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. 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 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 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.
30 June 2023

This report supersedes any issued prior to 30 June 2023.

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:	Waters Model 1525 Binary pump, 717 plus autosampler or Shimadzu Binary pump LC-20AB, SIL-20 A HT
	Column:	X-Bridge C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water (with 0.1% trifluoracetic acid), B = Methanol 0-8 min 40%-60% B; 8-18 min 60% B; 18-23 min 60%-40% B; 23-30 min 40% B.
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA or Shimadzu SPD-M20A PDA operating at Max plot
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.4%, s = 0.004% (7 ampoules in duplicate, February 2012)
	Re analysis:	Mean = 98.3%, s = < 0.02% (5 ampoules in duplicate, March 2013, February 2016 and April 2019)
	Re analysis:	Mean = 97.9%, s = 0.01% (5 ampoules in duplicate, June 2023)

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

The identity was confirmed by NMR, infrared and mass spectrometry. The purity value was obtained from a combination of mass balance and quantitative nuclear magnetic resonance (qNMR). The purity value by qNMR was obtained using a combination of the one-proton triplet at 4.2 ppm and the one proton multiplet at 5.8 ppm measured against a certified internal standard of potassium hydrogen maleate. The mass balance techniques included HPLC with UV detection thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The mass balance determined purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

Supporting evidence is provided by ^{1}H NMR and elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler (2008, 2011) Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler (2009, 2010)
	Column:	X-Bridge C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = Milli-Q water (with 0.1%Trifluoacetic acid), B = Methanol Gradient, 0-8 min 40%-60% B; 8-18 min 60% B; 18-23 min 60%-40% B; 23-30 min 40% B
	Flow rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at Max plot (2008, 2011) Shimadzu SPD-M20A (2009, 2010)
	Relative peak area response of main component:	
	Initial analysis:	Mean = 98.4%, s = 0.48% (10 sub samples in duplicate, January 2008)
	Re-analysis:	Mean = 98.4%, s = 0.04% (5 sub samples in duplicate, March 2009)
	Re-analysis:	Mean = 98.4%, s = 0.09% (5 sub samples in duplicate, March 2010)
	Re-analysis:	Mean = 98.4%, s = 0.08% (5 sub samples in duplicate, March 2011)
	Detector:	ELSD
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (10 sub samples in duplicate, January 2008)
	Re-analysis:	Mean = 99.5%, s = 0.05% (5 sub samples in duplicate, March 2009)
	Re-analysis:	Mean = 98.4%, s = 0.09% (5 sub samples in duplicate, March 2010)
Karl Fischer analysis:		Moisture content 4.6% mass fraction (2007, 2009, 2010 and 2011)
Thermogravimetric analysis:		Volatiles content 4.8% mass fraction (2007) Volatiles content 4.5% mass fraction (2009) Non volatile residue was not determined
Inorganic-sodium analysis:		Sodium content 6% mass fraction analysed by inductive coupled plasma optical emission spectroscopy (ICP-OES) (February 2008)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: Methanol-d_4 (3.31 ppm)
	Internal standard:	Dimethyl sulfone (100.0% mass fraction)
	Initial analysis:	Mean (4.2 ppm) = 89.9%, s = 1.4% (5 sub samples, February 2008)
QNMR:	Instrument:	Bruker DMX-400
	Field strength:	400 MHz Solvent: D_2O (4.79 ppm)
	Internal standard:	Potassium hydrogen maleate (98.8% mass fraction)
	Initial analysis:	Mean (5.8 ppm) = 89.8%, s = 0.5% (5 sub samples, April 2010)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion at 5 μL/min
	Ionisation:	ESI spray voltage at 3.0 kV positive ion
	EM voltage:	650 V
	Cone voltage:	30 V
	Peak:	353.0 ($M-Na^+$) *m/z*
TLC:	Conditions:	Kieselgel $60F_{254}$. Ethyl acetate/methanol (4:1) Single spot observed, R_f = 0.4. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3548, 3459, 2936, 2848, 1651, 1429, 1221, 988, 659 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Hertz-500
	Field strength:	500 MHz
	Solvent:	MeOH-d_4 (3.1 ppm)
	Spectral data:	δ 0.87 (1H, ddd, *J* = 4.2, 11.0, 21.6 Hz), 0.87 (3H, s), 1.02-1.11 (2H, m), 1.22 (1H, ddd, *J* = 3.5, 12.9, 12.9 Hz), 1.28-1.40 (2H, m), 1.44 (1H, ddd, *J* = 2.6, 10.9, 10.9 Hz), 1.52 (1H, m), 1.65 (1H, m), 1.75 (1H, m), 1.85-1.88 (2H, m), 1.98 (1H, dt, *J* =2.6, 12.7 Hz), 2.14-2.23 (2H, m), 2.27-2.38 (4H, m), 2.49 (1H, ddd, *J* = 2.5, 3.9, 14.7 Hz), 4.24 (1H, t, *J* = 8.6 Hz), 5.80 (1H, s) ppm
^{13}C NMR:	Instrument:	Bruker Gyro-500
	Field strength:	125 MHz
	Solvent:	MeOH-d_4 (49 ppm)
	Spectral data:	δ 10.6, 22.6, 25.6, 26.2, 27.6, 30.4, 35.0, 35.8, 36.2, 39.9, 42.2, 42.5, 49.0, 49.5, 86.4, 123.3, 169.3, 201.4 ppm
Melting point:		166-170 °C
Microanalysis:	Found:	C = 53.0%; H = 6.9%; S = 6.3% (December 2007)
	Calculated:	C = 57.4%; H = 6.7%; S = 8.5% (Calculated for $C_{18}H_{25}NaO_5S$)
	Calculated:	C = 54.8%; H = 6.9%; S = 8.1% (Calculated for $C_{18}H_{25}NaO_5S.H_2O$)