



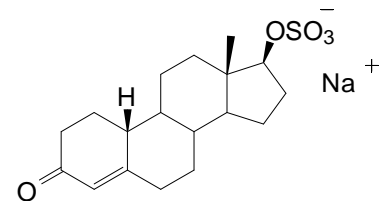
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D809b: Nandrolone sulfate, sodium salt

Report ID: D809b.2019.01 (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 18 April 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 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 °C 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.
27 May 2019

This report supersedes any issued prior to 27 May 2019

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: | Instrument: | Waters Model 1525 Binary pump, 717 plus autosampler |
| | Column: | X-Bridge C-18, 5 µm (4.6 mm × 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | A = MilliQ water (with 0.1% trifluoroacetic 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 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 = 0.004% (5 ampoules in duplicate, March 2013) |
| | Re analysis: | Mean = 98.3%, s = 0.01% (5 ampoules in duplicate, February 2016) |
| | Re analysis: | Mean = 98.3%, s = 0.06% (5 ampoules in duplicate, April 2019) |

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 was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with UV detection thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

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

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. Supporting evidence is provided by ¹H 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 = MilliQ 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) |
| QNMNR: | Instrument: | Bruker DMX-600 |
| | Field strength: | 600 MHz |
| | Internal standard: | Dimethyl sulfone (100.0% mass fraction) |
| | Initial analysis: | Mean (4.2 ppm) = 89.9%, s = 1.4% (5 sub samples, February 2008) |
| QNMNR: | Instrument: | Bruker DMX-400 |
| | Field strength: | 400 MHz |
| | 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

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|----------------------|-----------------|---|
| ESI-MS: | Instrument: | Micromass Quatro LC Micro |
| | Operation: | Negative ion mode, direct infusion at 5 μ L/min |
| | Ionisation: | ESI spray voltage at 3.0 kV positive ion |
| | EM voltage: | 650 V |
| | Cone voltage: | 30 V |
| | Peak: | 353.0 (M-Na ⁺) <i>m/z</i> |
| TLC: | Conditions: | Kieselgel 60F ₂₅₄ , ethyl acetate/methanol (4:1) Single spot observed, R _f = 0.42. 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- <i>d</i> ₄ (3.1 ppm) |
| | Spectral data: | δ δ 0.87 (1H, ddd, <i>J</i> = 4.2, 11.0, 21.6 Hz), 0.87 (3H, s), 1.02-1.11 (2H, m), 1.22 (1H, ddd, <i>J</i> = 3.5, 12.9, 12.9 Hz), 1.28-1.40 (2H, m), 1.44 (1H, ddd, <i>J</i> = 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, <i>J</i> = 2.6, 12.7 Hz), 2.14-2.23 (2H, m), 2.27-2.38 (4H, m), 2.49 (1H, ddd, <i>J</i> = 2.5, 3.9, 14.7 Hz), 4.24 (1H, t, <i>J</i> = 8.6 Hz), 5.80 (1H, s) ppm |
| ¹³ C NMR: | Instrument: | Bruker Gyro-500 |
| | Field strength: | 125 MHz |
| | Solvent: | MeOH- <i>d</i> 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 ₅ S) |
| | Calculated: | C = 54.8%; H = 6.9%; S = 8.1% (Calculated for C ₁₈ H ₂₅ NaO ₅ S.H ₂ O) |