



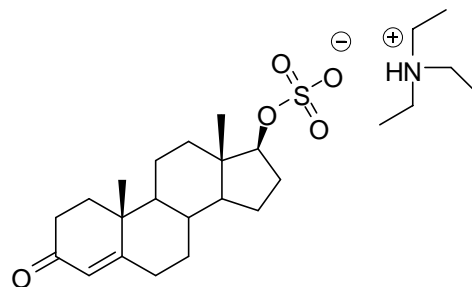
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D508: Testosterone sulfate (Et₃N salt)

Report ID: D508.2023.01 (Ampouled 230112)

Chemical Formula: C₂₅H₄₃NO₅S

Molecular Weight: 469.7 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-000340	N/A	971 ± 24 µg

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

IUPAC name: 17β-3-Oxoandrost-4-en-17-yl hydrogen sulfate-N,N-3-diethylethanamine (1:1)

Expiration of certification: The property values are valid till 20 March 2027, i.e. three years from the date of 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. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

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 D508. 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 971 ± 24 µg of anhydrous testosterone sulfate triethylammonium 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 °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%.

Stability: This material has demonstrated stability over a minimum period of five 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 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.
12 April 2024.

This report supersedes any issued prior to 12 April 2024.

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: Column: X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
 Mobile Phase: 20 mM Ammonium Acetate, pH 10/Acetonitrile (A:B)
 0-15 min 23% B, 15-21 min 63% B, 21-22 min 23% B
 Flow Rate: Gradient 1.0 mL/min
 Detector: PDA at 246 nm
 Relative mass fraction of the main component:
 Initial analysis: Mean = 98%, s = 0.014% (7 ampoules in duplicate, March 2023)
 Re-analysis: Mean = 98.04%, s = 0.005% (5 ampoules in duplicate, March 2024)

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 mass balance from a combination of traditional analytical techniques, including HPLC with UV detection and Karl Fischer analysis. 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.

Supporting evidence is provided by quantitative nuclear magnetic resonance (QNMR) spectroscopy, elemental microanalysis and thermogravimetric analysis. The purity estimate by QNMR was obtained using a one proton singlet at 5.7 ppm against a certified internal standard of 1,4-bis(trimethylsilyl)benzene.

HPLC: Column: Alltech Alltima C-18, 5 µm (4.6 mm x 150 mm)
 Mobile Phase: 20 mM Ammonium Acetate, pH 4.2/Acetonitrile (A:B)
 0-17 min 74 % A, 22-25 min 20 % A, 30-35 min 74 % A
 Flow Rate: Gradient 1.0 mL/min
 Detector: PDA at 246 nm
 Retention time: 12.5 min
 Relative mass fraction of the main component:
 Initial analysis: Mean = 98.5%, s = 0.03% (5 sub samples in duplicate, September 2007)
 Re-analysis: Mean = 98.3%, s = 0.01% (5 sub samples in duplicate, June 2011)

HPLC: Column: X-Bridge C-18, 5 µm (4.6 mm x 150 mm)
 Mobile Phase: 20 mM Ammonium Acetate, pH 10/Acetonitrile (A:B)
 0-15 min 23% B, 15-21 min 63% B, 21-22 min 23% B
 Flow Rate: Gradient 1.0 mL/min
 Detector: PDA at 246 nm
 Relative mass fraction of the main component:
 Initial analysis: Mean = 97.92%, s = 0.018% (7 sub samples in duplicate, March 2023)

Thermogravimetric analysis: Volatiles content < 0.1 % and non-volatile residue < 0.2 % mass fraction (October 2007)

Karl Fischer analysis: Moisture content 0.25 % mass fraction (October 2007)
 Moisture content 0.34 % mass fraction (July 2011)
 Moisture content 0.93 % mass fraction (Feb 2020)
 Moisture content 1.02 % mass fraction (March 2023)

QNMR: Instrument: Bruker Avance-600
 Field strength: 600 MHz
 Solvent: MeOH- d_4 (3.31 ppm)
 Internal standard: 1, 4-Bis(trimethylsilyl)benzene (100% mass fraction)
 Initial analysis: Mean (5.7 ppm) = 97.9%, u_c = 0.4% (5 sub samples, October 2011)

QNMR: Instrument: Bruker Ascend-500
 Field strength: 500 MHz
 Solvent: AcOH- d_4 (2.1 ppm)
 Internal standard: Dimethyl fumarate (99.9% mass fraction)
 Initial analysis: Mean (5.8 ppm) = 96.1%, u_c = 0.1% (4 sub samples, December 2019)

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:	367.3 (M-Et ₃ NH) ⁻ m/z
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Chloroform/methanol/water (70:30:2) Single spot observed, R _f = 0.45
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm ⁻¹ , KBr pellet
	Peaks:	3480, 1675, 1623, 1468, 1261, 1209, 1006, 846, 617 cm ⁻¹
¹ H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	MeOH- <i>d</i> ₄ (3.31 ppm)
	Key spectral data:	δ 0.85 (3H, s), 1.23 (3H, s), 1.31 (9H, t), 3.21 (6H, q), 4.22 (1H, t), 5.70 (1H, s) ppm
¹³ C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz
	Solvent:	MeOH- <i>d</i> ₄ (49.00 ppm)
	Spectral data:	δ 9.25, 12.1, 17.7, 21.6, 24.3, 29.2, 32.8, 33.8, 34.7, 36.7, 36.8, 37.8, 40.0, 43.8, 48.0, 51.3, 55.4, 87.9, 124.3, 175.1, 202.3 ppm
Microanalysis:	Found:	C = 63.8%; H = 9.5%; N = 3.1%; S = 6.8% (June 2000)
	Calculated:	C = 63.9%; H = 9.2%; N = 3.0%; S = 6.8% (C ₂₅ H ₄₃ NO ₅ S)