Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

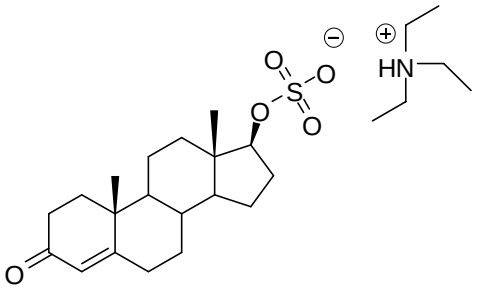
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D508: Testosterone sulfate (Et_3N salt)

Report ID: D508.2020.01 (Ampouled 110616)

Chemical Formula: $C_{25}H_{43}NO_5S$

Molecular Weight: 469.7 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-000340	N/A	982 ± 14 µg

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

Synonyms: Testosterone sulfate, triethylammonium salt,
17β Sulfooxyandrost-4-en-3-one triethylammonium salt,
Androst-4-en-17β-ol-3-one sulfate, triethylammonium salt

Expiration of certification: The property values are valid till 15 April 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 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 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 982 ± 14 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 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%.

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.
21 April 2020.

This report supersedes any issued prior to 21 April 2020

NATA logo notice: Accredited for compliance with ISO Guide 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:	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 26% B, 18-23 min 60% B, 24-30 min 26% B
	Flow Rate:	Gradient 1.0 mL/min
	Detector:	PDA at 246 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.3%, s = 0.004% (7 ampoules in duplicate, June 2011)
	Re-analysis:	Mean = 98.3%, s = 0.01% (5 ampoules in duplicate, May 2012)
	Re-analysis:	Mean = 98.3%, s = 0.03% (5 ampoules in duplicate, April 2015)
	Re-analysis:	Mean = 98.3%, s = 0.04% (5 ampoules in duplicate, April 2020)

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.

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

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)
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)
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)

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 $(M\text{-}Et_3NH)^-$ *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-*d*$_4$ (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-*d*$_4$ (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% ($C_{25}H_{43}NO_5S$)