Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

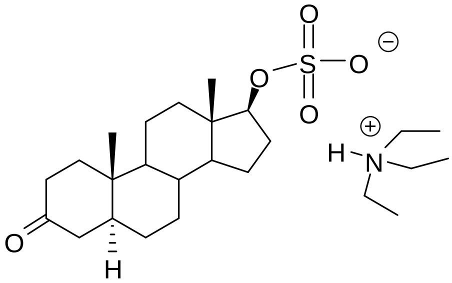
REFERENCE MATERIAL
PRODUCT INFORMATION SHEET

NMIA D609: 5α–Dihydrotestosterone sulfate anion (nominally NEt3 salt)

Report ID: D609.2025.01 (Ampouled 101109)

Chemical Formula: $C_{25}H_{45}NO_5S$

Molecular Weight: 471.7 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
97-000703	**1633766-47-8**	**776 ± 48 μg**

IUPAC name: Triethylammonium (5α,17β)-3-Oxoandrostan-17-yl sulfate

Expiration of certification: The property values are valid till 14 May 2035, ten 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 under an atmosphere of argon. The reference material is intended for a single use to prepare a standard solution containing D609. This material was prepared by synthesis and certified for identity and purity by NMI Australia.

Intended use: This reference material should be used for qualitative analysis only.

Instructions for use: Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. acetonitrile/water). This will transfer 776 ± 48 μg of anhydrous 5α-dihydrotestosterone sulfate anion. 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.

Stability: 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.
1 July 2025

This report supersedes any issued prior to 01 July 2025.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Characterisation Report:

HPLC:
Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltech Alltima C-18 5 µm (4.6 mm × 150 mm)
Column oven: 55 °C
Mobile Phase: A = MilliQ water; B = Acetonitrile
0-6 min 35% B; 6-13 min 35-70% B; 13-20 min 70%B; 20-25 min 70-35%B.
Both A and B were buffered with 0.05% trifluoroacetic acid
Flow rate: 0.8 mL/min (Gradient)
Detector: Waters ELSD 2424

Relative peak area of the main component:
Initial analysis: Mean = 99.6%, s = 0.01% (7 ampoules in duplicate, December 2010)
Re-analysis: Mean = 99.7%, s = 0.01% (5 ampoules in duplicate, October 2012)
Re-analysis: Mean = 99.7%, s = 0.01% (5 ampoules in duplicate, September 2015)
Re-analysis: Mean = 99.6%, s = 0.01% (5 ampoules in duplicate, September 2020)

Instrument: Shimadzu Binary pump LC-20AB, Shimadzu SIL-20 A HT autosampler
Column: Waters X-Bridge C-18 5 µm (4.6mm × 150 mm)
Column oven: 40 °C
Mobile Phase: A = MilliQ water; B = Acetonitrile
0-5 min 35% B; 5-10 min 35-80% B; 10-12 min 80%B; 12-13 min 80-35% B; 13-20 min 35% B.
Both A and B were buffered with 0.05% trifluoroacetic acid
Flow rate: 0.8 mL/min (Gradient)
Detector: Shimadzu LT-II ELSD

Relative peak area of the main component:
Initial analysis: Mean = 99.6%, s = 0.07% (5 ampoules in duplicate, May 2025)

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

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. 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 elemental microanalysis.

HPLC:
Instrument: Waters Model 1525 Binary pump, 717 plus autosampler
Column: Alltech Alltima C-18 5 µm (4.6 mm × 150 mm)
Column oven: 55 °C
Mobile Phase: A = MilliQ water; B = Acetonitrile
0-6 min 35% B; 6-13 min 35-70% B; 13-20 min 70%B; 20-25 min 70-35%B.
Both A and B were buffered with 0.05% trifluoroacetic acid
Flow rate: 0.8 mL/min (Gradient)
Detector: Waters ELSD 2424

Relative peak area of the main component:
Initial analysis: Mean = 98.1%, s = 0.2% (3 sub samples in duplicate, December 2000)
Re- analysis: Mean = 99.1%, s = 0.07% (5 sub samples in duplicate, November 2007)
Re- analysis: Mean = 99.2%, s = 0.09% (5 sub samples in duplicate, November 2008)
Re- analysis: Mean = 99.6%, s = 0.01% (7 sub samples in duplicate, November 2010)

Karl Fischer analysis: Moisture content 1.2% mass fraction. (2 sub samples, November 2007)
Moisture content 0.9 – 1.0% mass fraction (2 sub samples, December 2010)

Thermogravimetric analysis: Volatile content 0.6% mass fraction (November 2007)
Non volatile residue was not determined

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro LC Micro
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI spray voltage at 4.5 kV negative ion
	EM voltage:	650 V
	Cone voltage:	20 V
	Peak:	369 $(MSO_3)^-$ *m/z*
TLC:	Conditions:	Kieselgel $60F_{254}$. Chloroform/methanol/water (70:20:2) Single spot observed, $R_f = 0.3$
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3500, 2740, 2679, 2492, 1719, 1234, 1063, 1004, 856, 804 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	Acetone-d_6 (2.05 ppm)
	Spectral data:	δ 0.65 (3H, s), 0.95 (3H, s), 1.16 (9H, t, J = 7.3 Hz), 3.09 (6H, q, J = 7.3 Hz) 3.95 (1H, m) ppm ^{1}H NMR (6/12/2007) run under QNMR conditions (no internal standard) indicates that only 92% of the 5α-dihydrotestosterone sulfate anion is associated with the triethylammonium ion. The remaining 8% is protonated.
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	126 MHz
	Solvent:	Acetone-d_6 (29.8 ppm)
	Spectral data:	δ 9.0, 11.4, 12.1, 20.9, 23.4, 28.5, 28.7, 31.2, 35.2, 35.7, 37.0, 38.0, 38.3, 42.6, 44.5, 46.2, 46.4, 50.4, 53.6, 210.8 ppm
Microanalysis:	Found:	C = 63.0%; H = 9.5%; N = 2.8% (August 1999)
	Calculated:	C = 63.7%; H = 9.6%; N = 3.0% (Calculated for $C_{25}H_{45}NO_5S$)