



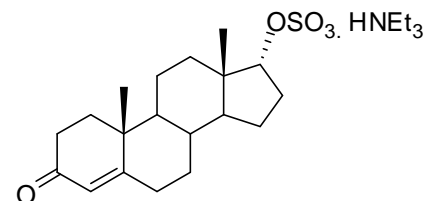
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D605: Epitestosterone sulfate triethylammonium salt

Report ID: D605.2019.02 (Ampouled 090429)

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

Molecular Weight: 469.7 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-000341	Not available	961 ± 28 µg

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

Synonyms: Epitestosterone sulfate, triethylammonium salt
17 α -Sulfoxyandrost-4-en-3-one triethylammonium salt
4-Androsten-17 α -ol-3-one sulfate, triethylammonium salt

Expiration of certification: The property values are valid till 28 June 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 under an atmosphere of argon. The certified reference material is intended for a single use to prepare a standard solution containing D605. 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 961 ± 28 µg of anhydrous epitestosterone 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 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.
17 February 2020

This report supersedes any issued prior to 17 February 2020

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 HPLC
	Column:	X-bridge C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	35 $^{\circ}\text{C}$
	Mobile Phase:	A= 20 mM $\text{NH}_4^+ \text{HCO}_2^-$ (aq) buffered to pH 10, B=Methanol 0-15 min 50% B, 15-22 min 50-90% B, 22-27 min 90% B, 27-28 min 90-50% B, 28-35 min 50% B
	Flow rate:	1.0 mL/min, Gradient
	Detector:	Waters Photodiode Array Detector operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.2%, s = 0.03% (6 Ampoules in duplicate, July 2014)
	Re-analysis:	Mean = 99.2%, s = 0.03% (5 ampoules in duplicate, June 2019)
HPLC:	Instrument:	Waters HPLC
	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/20 mM ammonium acetate pH 4.2
	Flow Rate:	1.0 mL/min Gradient: 27% acetonitrile for 13 min, increase acetonitrile to 50% in 5 min, elute for 5 min
	Detection:	Waters PDA 2998 operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, August 2011)
HPLC:	Instrument:	Shimadzu or Waters HPLC
	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/50 mM ammonium acetate/pH 4.2 [28:72]
	Flow Rate:	1.0 mL/min
	Detection:	Shimadzu SPD-M20APDA or Waters PDA 996 operating at 247nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.4%, s = 0.01% (7 ampoules in duplicate, June 2009)
	Re-analysis:	Mean = 99.4%, s = 0.005% (5 ampoules in duplicate, July 2010)

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

The certified purity value by qNMR was obtained using potassium hydrogen maleate as an internal standard. Impurity estimates by HPLC with UV detection at 247 nm, Karl Fischer and ^1H NMR analysis are also provided.

QNMR:	Instrument:	Bruker DMX-400
	Field strength:	400 MHz Solvent: D_2O
	Internal standard:	KH maleate (98.8% m/m)
	Purity estimate:	Mean = 96.5%, s = 0.4% (5 sub samples, June 2008)
HPLC:	Instrument:	Waters HPLC
	Column:	Alltima C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	Acetonitrile/50 mM ammonium acetate/pH 4.2 [28:72]
	Flow Rate:	1.0 mL/min
	Detection:	Waters PDA 996 operating at 247 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean > 99.5% (7 sub samples, July 1999)
	Re-analysis:	Mean = 99.5%, s = 0.01% (3 ampoules and 1 sub sample in duplicate, March 2007)
HPLC:	Instrument:	Shimadzu HPLC
	Column:	X-Bridge C-18 5 μm (4.6 mm x 150 mm)
	Mobile Phase:	0.1% TFA solution/MeOH
	Flow Rate:	1.0 mL/min
	Detection:	Shimadzu SPD-M20APDA operating at max plot
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.3%, s = 0.03% (5 sub samples in duplicate, March 2009)
Thermogravimetric analysis:	Volatile and non-volatile content not determined due to the nature of the material (March 2009)	
Karl Fischer analysis:	Moisture content 0.6% mass fraction (3 sub samples, March 2009)	

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:	367.0 (M-Et ₃ NH) ⁻ m/z
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Chloroform/methanol/water (70:20:2) Single spot observed, R _f = 0.20 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm ⁻¹ , KBr powder
	Peaks:	2723, 1671, 1617, 1380, 1271, 1194, 999, 963, 583 cm ⁻¹
¹ H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	d ₆ -DMSO (2.5 ppm)
	Key spectral data:	δ 0.68 (3H, s), 1.14 (3H, s), 1.18 (9H, t), 3.09 (6H, q), 4.05 (1H, d), 5.60 (1H, s) ppm Dichloromethane present at 0.1% mass fraction
¹³ C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz
	Solvent:	d ₆ -DMSO (39.52 ppm)
	Spectral data:	δ 9.0, 17.1, 17.4, 20.5, 24.5, 30.1, 31.6, 32.4, 34.0, 35.5, 35.6, 38.6, 39.7, 44.5, 46.2, 49.3, 53.6, 83.6, 123.5, 171.5, 198.4 ppm
Melting point:		183-185 °C
Microanalysis:	Found:	C = 62.9%; H = 9.2%; N = 3.0% (August 1999)
	Calculated:	C = 63.9%; H = 9.2%; N = 3.0% (Calculated for C ₂₅ H ₄₃ NO ₅ S)