



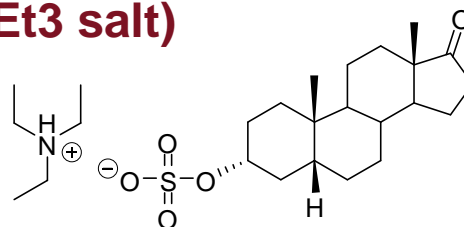
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D608: Etiocholanolone sulfate (NEt₃ salt)

Report ID: D608.2020.01 (Ampouled 130508)

Chemical Formula: C₂₄H₄₅NO₅S

Molecular Weight: 471.6 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
97-001741	20998-02-1	898 ± 22 µg

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

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

Expiration of certification: The property values are valid till 18 May 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 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 CRM is intended for a single use to prepare a standard solution containing D608. 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 898 ± 22 µg of etiocholanolone sulfate (NEt₃ 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. 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 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 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.
22 May 2020

This report supersedes any issued prior to 22 May 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 alliance 2650
Column: Alltima or X-Bridge C-18 5 µm (4.6 mm × 150 mm)
Column oven: 55 °C
Mobile Phase: Methanol/ MilliQ water with 0.05% TFA [65:35]
Flow rate: 1 mL/min
Detector: Waters ELSD 2424
Relative peak area of the main component:
Initial analysis: Mean = 99.95%, s = 0.02% (7 ampoules in duplicate, May 2013)
Re-analysis: Mean = 99.97%, s = 0.004% (5 ampoules in duplicate, April 2014)
Re-analysis: Mean = 99.9%, s = 0.02% (5 ampoules in duplicate, April 2015)
Re-analysis: Mean = 100.0%, s = 0.01% (5 ampoules in duplicate, May 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 quantitative nuclear magnetic resonance (qNMR). The one-proton doublet of doublets at 2.0 ppm was measured against a certified internal standard of potassium hydrogen maleate/ maleic acid.

Supporting evidence is provided by HPLC with ELS detection, Karl Fischer analysis, ¹H NMR and elemental microanalysisHPLC:

Instrument: Waters alliance 2650
Column: Alltima or X-Bridge C-18 5 µm (4.6 mm × 150 mm)
Column oven: ambient
Mobile Phase: Methanol/ MilliQ water with 0.05% TFA [65:35]
Flow rate: 0.8 mL/min
Detector: Waters ELSD 2424
Relative peak area of the main component:
Initial analysis: > 99% (December 2000)
Re-analysis: Mean = 99.9%, s = 0.01% (5 sub samples in duplicate, November 2007)
Re-analysis: Mean = 99.8%, s = 0.01% (5 sub samples in duplicate, October 2012)

Karl Fischer analysis: Moisture content mass fraction 2.3% (January 2007)
Moisture content mass fraction 2.4% (October 2012)

QNMR: Instrument: Bruker DMX-600
Field strength: 600 MHz
Solvent: DMSO-*d*₆ (2.50 ppm)
Internal standard: potassium hydrogen maleate (98.8% m/m) / maleic acid (98.7% m/m)
Initial analysis: Mean = 90.2%, s = 0.9% (4 sub samples, August 2007)
Re-analysis: Mean = 89.5%, s = N/A (1 sub sample, November 2012)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Finnigan MAT TSQ 700
	Operation:	Negative ion mode, direct infusion
	Ionisation:	ESI spray voltage at 4.5 kV positive ion
	Peak:	369.5 (MSO ₃) ⁻ <i>m/z</i>
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Kieselgel 60F ₂₅₄ . Chloroform/methanol/water (70:20:2) Single spot observed, R _f = 0.3
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm ⁻¹ , KBr pellet
	Peaks:	3500, 2680, 1739, 1248, 1195, 1054, 976, 950, 827, 624 cm ⁻¹
¹ H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	DMSO- <i>d</i> ₆ (2.50 ppm)
	Key spectral data:	δ 0.75 (3H, s), 0.89 (3H, s), 1.16 (9H, t, <i>J</i> = 7.3 Hz), 3.09 (6H, q, <i>J</i> = 7.3 Hz), 3.96 (1H, m) ppm
¹³ C NMR:	Instrument:	Bruker Avance III-500
	Field strength:	126 MHz
	Solvent:	DMSO- <i>d</i> ₆ (39.9 ppm)
	Spectral data:	δ 9.0, 13.8, 20.0, 21.7, 23.4, 25.3, 26.9, 28.0, 31.9, 33.7, 35.2, 35.5, 35.7, 40.2, 40.8, 42.0, 46.2, 47.5, 51.0, 75.9, 220.2 ppm
Microanalysis:	Found:	C = 61.7%; H = 9.5%; N = 3.1% (August 1999)
	Found:	C = 61.6%; H = 9.8%; N = 3.1% (January 2007)
	Calculated:	C = 63.7%; H = 9.6%; N = 3.0% (for C ₂₅ H ₄₅ NO ₅ S)