



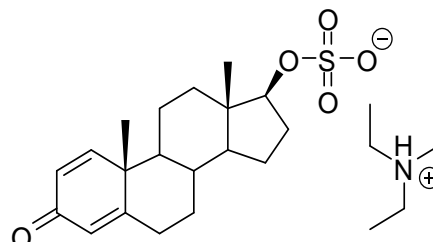
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D931: Boldenone sulfate, triethylamine salt

Report ID: D931.2020.01

Chemical Formula: $C_{25}H_{41}NO_5S$

Molecular Weight: 467.7 g/mol



Certified value

Batch No.	CAS No.	Purity (mass fraction)
08-S-09	Not available	94.5 ± 1.6%

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

Synonyms: 1,4-Androstadiene-3-one-17 β -ol sulfate, triethylamine salt.

Expiration of certification: The property values are valid till 7 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

Description: Off-white powder prepared by synthesis, and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap or screw top cap.

Intended use: This certified reference material is suitable for use as a primary calibrator.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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%. 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 three 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 ten randomly selected 1-2 mg sub samples 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.
15 May 2020

This report supersedes any issued prior to 15 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:

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, thermogravimetric analysis, Karl Fischer analysis and ¹H NMR spectroscopy. 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 LC-MS analysis, Q NMR analysis and elemental microanalysis.

HPLC:	Instrument:	Waters HPLC
	Column:	X-Bridge C-18, 5.0 μm (4.6 mm x 150 mm)
	Column oven:	40 $^{\circ}\text{C}$
	Mobile Phase:	A= 20 mM $\text{NH}_4^+ \text{HCO}_2^-$ (aq) buffered to pH 10, B=Methanol 0-15 min 22% B, 15-25 min 22-90% B, 25-30 min 90% B, 30-31 min 90-22% B, 31-46 min 22% B
	Flow rate:	1.0 mL/min, Gradient
	Detector:	Waters Photodiode Array Detector operating at 248 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.5%, s = 0.04% (6 sub samples in duplicate, June 2014)
	Re-analysis:	Mean = 98.7%, s = 0.02% (5 sub samples in duplicate, June 2017)
	Re-analysis:	Mean = 98.4%, s = 0.07% (5 sub samples in duplicate, May 2020)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz
	Solvent:	DMSO- d_6 (2.50 ppm)
	Internal standard:	Dimethyl terephthalate
	Purity estimate:	Mean = 94.4%, s = 0.49% (5 sub samples in duplicate, April 2011)
Thermogravimetric analysis:	Non volatile residue 0.4% mass fraction (May 2008) Non volatile residue 0.2% mass fraction (October 2010)	
Karl Fischer analysis:	Moisture content 2.6% mass fraction (September 2008) Moisture content 3.5% mass fraction (October 2009) Moisture content 3.8% mass fraction (October 2010) Moisture content 4.1% mass fraction (March 2014) Moisture content 4.5% mass fraction (June 2017) Moisture content 3.9% mass fraction (May 2020)	

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro	
	Column:	X-Bridge C-18, 150 mm × 4.6 mm I.D. × 5 μm	
	Column temp:	40 °C	
	Solvent system:	20 mM NH ₄ ⁺ HCO ₂ ⁻ (aq) buffered to pH 10, methanol	
	Flow rate:	0.2 mL/min	
	Sample prep:	2000 μg/g in MeOH/20 mM NH ₄ ⁺ HCO ₂ ⁻ (aq) buffered to pH 10 (22:78)	
	Injection volume:	10 μL	
	Ionisation mode:	Electrospray negative ion	
	Capillary voltage:	3.0 kV	Cone voltage: 20 V
	Source temp:	130 °C	Desolvation gas temperature: 350 °C
	Cone gas flow rate:	27 L/hr	Desolvation gas flow rate: 748 L/hr
ESI-MS:	Instrument	Micromass Quatro Micro	
	Operation:	Negative ion mode, direct infusion at 5 μL/min	
	Ionisation:	ESI spray voltage at 3.2 kV negative ion	
	EM voltage:	500 V	
	Cone voltage:	20 V	
	Peak:	365 (M-TEA ⁺) ⁻ <i>m/z</i>	
TLC:	Conditions:	Kieselgel 60F ₂₅₄ . Dichloromethane / Methanol (9/1) Single, broad spot observed, R _f = 0.23. Visualisation with UV at 254 nm	
IR:	Instrument:	Biorad FTS300MX FT-IR	
	Range:	4000-400 cm ⁻¹ , KBr powder	
	Peaks:	3362, 2939, 2676, 2492, 1659, 1474, 1446, 1257, 1220, 1000, 804 cm ⁻¹	
¹ H NMR:	Instrument:	Bruker Gauss-400	
	Field strength:	400 MHz	
	Solvent:	MeOH-d ₄ (3.30 ppm)	
	Spectral data:	δ 0.91 (3H, s), 0.98-1.09 (3H, m), 1.20 (1H, m), 1.28 (3H, s), 1.31 (9H, t, <i>J</i> = 7.3 Hz), 1.40 (1H, m), 1.59-1.84 (5H, m), 2.01 (2H, m), 2.16 (1H, m), 2.39 (1H, m), 2.58 (1H, m), 3.21 (6H, q, <i>J</i> = 7.3 Hz), 4.21 (1H, dd, <i>J</i> = 8.1, 8.9 Hz), 6.05 (1H, m), 6.20 (1H, dd, <i>J</i> = 1.9, 10.1 Hz), 7.29 (1H, d, <i>J</i> = 10.1 Hz) ppm	
¹³ C NMR:	Instrument:	Bruker Gauss-400	
	Field strength:	100 MHz	
	Solvent:	MeOH-d ₄ (49.0 ppm)	
	Spectral data:	δ 9.2, 12.1, 19.1, 23.5, 24.4, 29.1, 33.8, 34.5, 36.6, 37.7, 44.1, 45.4, 47.9, 50.9, 54.2, 87.7, 124.0, 127.5, 159.6, 173.5, 188.6 ppm	
Melting point:		108-112 °C	
Microanalysis:	Found:	C = 62.3 %; H = 8.7 %; N = 3.0% (September 2008)	
	Calculated:	C = 64.2 %; H = 8.8 %; N = 3.0% (Calc. for C ₂₅ H ₄₁ NO ₅ S)	
	Calculated:	C = 62.3 %; H = 8.9 %; N = 2.9% (Calc. for C ₂₅ H ₄₁ NO ₅ S containing 0.8 molecule of moisture)	