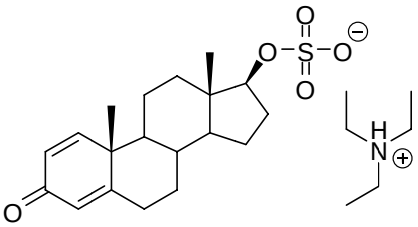
REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D931.2014.01

Compound Name: **Boldenone sulfate, triethylamine salt**
Collection Number: D931
Chemical Formula: $C_{25}H_{41}NO_5S$
CAS Number: N/A
Description: Off white solid
Batch Number: 08-S-09
Molecular Weight: 467.7
Release Date: 10th October 2008

Structure:



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

Purity (mass fraction): 94.5 ± 1.6 % (95 % coverage interval)

The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR. 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 °C
	Mobile Phase:	A= 20 mM NH_4^+ 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, 31-36 min 22% B
	Flow rate:	1.0 mL/min, Gradient
	Detector:	Waters Photodiode Array Detector operating at 248 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 98.5%, s = 0.04% (6 sub samples in duplicate, June 2014)
QNMR:	Instrument:	Bruker DMX-600
	Field strength:	600 MHz Solvent: DMSO-*d*6
	Internal standard:	Dimethyl terephthalate
	Purity estimate:	Mean = 94.4%, s = 0.49% (5 sub samples in duplicate, April 2011)
HPLC:	Column:	X-bridge C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Methanol with 0.05% TFA/(water with 0.05%TFA)
	Flow Rate:	1.0 mL/min
	Detector:	PDA, 248 nm
	Relative peak area response of main component:	
	Initial analysis:	Mean = 98.3%, s = 0.09% (10 sub samples in duplicate, September 2008)
	Re-analysis:	Mean = 98.9%, s = 0.16% (5 sub samples in duplicate, September 2009)
	Re-analysis:	Mean = 98.8%, s = 0.04% (5 sub samples in duplicate, October 2010)
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)

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 ºC	Desolvation gas temperature: 350 ºC
	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 $(M\text{-}TEA^+)^-$ m/z	
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:	4000-400cm^{-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: CD_3OD (3.30)
	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, *J* = 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, *J* = 7.3 Hz), 4.21 (1H, dd, *J* = 8.1, 8.9 Hz), 6.05 (1H, m), 6.20 (1H, dd, *J* = 1.9, 10.1 Hz), 7.29 (1H, d, *J* = 10.1 Hz) ppm	
^{13}C NMR:	Instrument:	Bruker Gauss-400	
	Field strength:	100 MHz	Solvent: CD_3OD (49.0)
	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 ºC	
Microanalysis:		Found: C = 62.3 %; H = 8.7 %; N = 3.0% (September 2008) Calc: C = 64.2 %; H = 8.8 %; N = 3.0% (Calc. for $C_{25}H_{41}NO_5S$) Calc: C = 62.3 %; H = 8.9 %; N = 2.9% (Calc. for $C_{25}H_{41}NO_5S$ containing 0.8 molecule of moisture)	

Expiration of certification

The property values are valid till 27th June 2017, i.e. three 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.

The long-term stability of the compound in solution has not been examined.

This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% coverage interval includes a stability component which has been estimated from annual stability trials.

Homogeneity assessment

The homogeneity of the material was assessed using purity assay by HPLC on ten randomly selected 1-2 mg sub samples of the material. The material was judged to be 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.

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.

Intended Use

For ***in vitro*** laboratory analysis only.

Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,
Team Leader,
Chemical Reference Materials, NMI.
Dated: 3 July, 2014.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 3rd July 2014.