

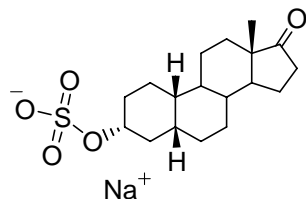


**REFERENCE MATERIAL ANALYSIS REPORT**

**Report ID: D849.2011.03**

Compound Name: <b>19-Noretiocholanolone sulfate (Na salt)</b>	Description: White solid
Collection Number: D849	Batch Number: 03-S-24
Chemical Formula: C <sub>18</sub> H <sub>27</sub> NaO <sub>5</sub> S	Molecular Weight: 378.5 (355.5 free sulfate)
CAS Number: N/A	Release Date: 27 <sup>th</sup> February 2006

Structure:



Synonyms: 3 $\alpha$ -Hydroxy-5 $\beta$ -estran-17-one sulfate (Na salt)

Purity (mass fraction): 91.7  $\pm$  5.3% (95% coverage interval)

Purity estimate obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (QNMR). The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis and <sup>1</sup>H NMR. The purity estimate by QNMR was obtained using a certified internal standard of potassium hydrogen maleate. Supporting evidence is provided by headspace GC-MS analysis of occluded solvent and elemental microanalysis.

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler  
Column: Alltima C18, 5  $\mu$ m (4.6 mm  $\times$  150 mm)  
Column oven: N/A  
Mobile Phase: ACN / H<sub>2</sub>O (33:67), buffered at pH 4.2 using 10mM NH<sub>4</sub>OAc / AcOH  
Flow rate: 1.0 mL/min  
Detector: Waters ELSD 2420  
Retention time: 6.6 min  
Relative peak area response of main component:  
Initial analysis: Mean = 100.0%, s = 0.0% (7 sub samples in duplicate, September 2005)  
Re-analysis: Mean = 100.0%, s = 0.0% (5 sub samples in duplicate, January 2008)  
Re-analysis: Mean = 100.0%, s = 0.0% (1 samples in triplicate, February 2011)

Thermogravimetric analysis: Volatile content 5.2% mass fraction (October 2005)  
Volatile content 6.1% mass fraction (October 2006)  
Non volatile residue was not determined

Karl Fischer analysis: Moisture content 7.1% mass fraction (December 2006)  
Moisture content 7.5% mass fraction (January 2008)  
Moisture content 6.9% mass fraction (January 2011)

QNMR: Instrument: Bruker DMX-500  
Field strength: 500 MHz Solvent: d<sub>6</sub>-DMSO (2.5 ppm)  
Internal standard: Potassium hydrogen maleate (98.8% m/m)  
Initial analysis: Mean = 96.0%, s = 0.3% (3 sub samples, November 2005)  
Re-analysis: Mean = 92.1%, s = 0.6% (3 sub samples, December 2006)

### Spectroscopic and other characterisation data

ESI -MS:	Instrument:	Micromass Quatro Micro
	Operation:	Negative ion mode, direct infusion at 5 $\mu$ L/min
	Ionisation:	ESI spray voltage at 3.2 kV negative ion
	EM voltage:	650 V
	Cone voltage:	45 V
	Peak:	355 (M-Na <sup>+</sup> ) m/z
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . 100% isopropanol Single spot observed, R <sub>f</sub> = 0.45. Visualisation with vanillin dip
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm <sup>-1</sup> , KBr powder
	Peaks:	3506, 2919, 2858, 1739, 1239, 1214, 1069, 957 cm <sup>-1</sup>
<sup>1</sup> H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz Solvent: d <sub>6</sub> -DMSO (2.50 ppm)
	Spectral data:	$\delta$ 0.78 (3H, s), 0.95-1.27 (7H, m), 1.29-1.76 (12H, m), 1.79-1.86 (2H, m), 2.00 (1H, ddd, <i>J</i> = 8.7, 8.7, 19.2 Hz), 2.36 (1H, dd, <i>J</i> = 8.5, 19.2 Hz), 3.96 (1H, m) ppm
<sup>13</sup> C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz Solvent: d <sub>6</sub> -DMSO (2.50 ppm)
	Spectral data:	$\delta$ 13.5, 21.3, 24.4, 24.7, 25.7, 26.8, 30.9, 31.4, 33.4, 35.3, 35.4, 38.1, 40.7, 47.3, 49.7, 75.5, 220.0 ppm
Melting point:		190 °C
Microanalysis:		Found: C = 53.8%; H = 7.6% (August, 2005) Calc: C = 53.9%; H = 7.4%; (C <sub>18</sub> H <sub>27</sub> NaO <sub>5</sub> S + 5.6% m/m H <sub>2</sub> O)

### Expiration of certification

The property values are valid till 9<sup>th</sup> February 2016, 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.

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

This material has demonstrated stability over a minimum period of five 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 with ELS detection on seven 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 °C 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: 21 November, 2013.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 21<sup>st</sup> November 2013.



This document is issued in accordance with NATA's accreditation requirements.

Accredited for compliance with ISO Guide 34.

This document shall not be reproduced except in full.

Accreditation Number : 198

Corporate Site Number : 14214