Australian Government

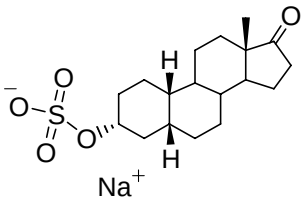
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D849.2011.03

Compound Name: **19-Noretiocholanolone sulfate (Na salt)**
Collection Number: D849
Chemical Formula: $C_{18}H_{27}NaO_5S$
CAS Number: N/A
Description: White solid
Batch Number: 03-S-24
Molecular Weight: 378.5 (355.5 free sulfate)
Release Date: 27th February 2006

Structure:



Synonyms: 3α-Hydroxy-5β-estran-17-one sulfate (Na salt)

Purity (mass fraction): 91.7 ± 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 ^{1}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 µm (4.6 mm × 150 mm)
	Column oven:	N/A
	Mobile Phase:	ACN / H_2O (33:67), buffered at pH 4.2 using 10mM NH_4OAc / 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_6-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 µL/min
	Ionisation:	ESI spray voltage at 3.2 kV negative ion
	EM voltage:	650 V
	Cone voltage:	45 V
	Peak:	355 (M-Na^+) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. 100% isopropanol Single spot observed, R_f = 0.45. Visualisation with vanillin dip
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3506, 2919, 2858, 1739, 1239, 1214, 1069, 957 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz Solvent: d_6-DMSO (2.50 ppm)
	Spectral data:	δ 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, *J* = 8.7, 8.7, 19.2 Hz), 2.36 (1H, dd, *J* = 8.5, 19.2 Hz), 3.96 (1H, m) ppm
^{13}C NMR:	Instrument:	Bruker DMX-500
	Field strength:	125 MHz Solvent: d_6-DMSO (2.50 ppm)
	Spectral data:	δ 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%; ($C_{18}H_{27}NaO_5S$ + 5.6% m/m H_2O)

Expiration of certification
The property values are valid till 9th 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 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: 21 November, 2013.

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