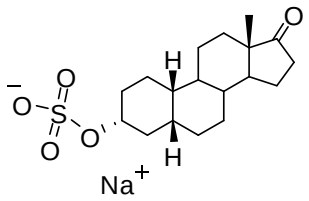
Australian Government
Department of Industry, Science, Energy and Resources

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D849: 19-Noretiocholanolone sulfate (Na salt)



Report ID: D849.2011.04

Chemical Formula: $C_{18}H_{27}NaO_5S$

Molecular Weight: 378.5 g/mol (355.5 free sulfate)

Certified value

Batch No.	CAS No.	Purity (mass fraction)
03-S-24	**NA**	**91.7 ± 5.3%**

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

IUPAC name: Sodium (3α,5β)-3-Hydroxyestran-17-one sulfate.

Expiration of certification: The property values are valid till 9 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.

Description: 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 25 ºC 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 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 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.
5 May 2020

This report supersedes any issued prior to 21 April 2020.

Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained from a combination of traditional analytical techniques and quantitative nuclear magnetic resonance (qNMR). The techniques used in the mass balance approach include HPLC with ELS detection, thermogravimetric analysis, Karl Fischer analysis and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) Equation 1

I_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue

The certified purity value by qNMR was obtained using the one-proton multiplet at 4.0 ppm measured against a certified internal standard of potassium hydrogen maleate.

Supporting evidence is provided by 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 mass fraction of the 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 sample in triplicate, February 2011)
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)
Thermogravimetric analysis:		Volatile content 5.2% mass fraction (October 2005) Volatile content 6.1% mass fraction (October 2006) Non volatile residue was not determined
QNMR:	Instrument:	Bruker DMX-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 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 LC 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 ($M\text{-}Na^+$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. 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:	DMSO-d_6 (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:	DMSO-d_6 (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)
	Calculated:	C = 53.9%; H = 7.4%; ($C_{18}H_{27}NaO_5S$ + 5.6% m/m H_2O)