



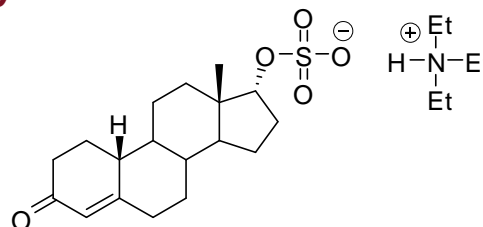
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D783b: Epinandrolone sulfate triethylammonium salt

Report ID: D783b.2020.01 (Bottled 190430)

Chemical Formula: C<sub>24</sub>H<sub>41</sub>NO<sub>5</sub>S

Molecular Weight: 455.7 g/mol



### Certified value

Batch No.	CAS No.	Purity (mass fraction)
17-S-03	N/A	88.7 ± 2.2%

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

**IUPAC name:** Estr-4-en-17 $\alpha$ -ol-3-one sulfate, triethylammonium salt.

**Expiration of certification:** The property values are valid till 10 December 2023, 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 material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

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

**Stability:** In the absence of long term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years. 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-UV 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.  
23 December 2020

This report supersedes any issued prior to 23 December 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:** Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA'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 document.

## 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 <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}})$$

Equation 1

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

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	X-Bridge C-18, 5 $\mu\text{m}$ (4.6 mm $\times$ 250 mm)
	Column oven:	40 $^{\circ}\text{C}$
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-20 min 25% B; 20-25 min 25-80% B; 25-30 min 80% B; 30-31 min 80-25% B; 31-40 min 25% B The aqueous phase was buffered at pH 6.0 using 20mM $\text{NH}_4\text{OAc}$ and $\text{AcOH}$ .
	Flow rate:	1 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 244 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 99.2%, s = 0.02% (10 sub samples in duplicate, November 2018)
	Re-analysis:	Mean = 98.9%, s = 0.01% (5 sub samples in duplicate, December 2019)
	Re-analysis:	Mean = 98.3%, s = 0.11% (5 sub samples in duplicate, December 2020)

Thermogravimetric analysis: Non volatile residue < 0.2% mass fraction (November 2018). The volatile content (e.g. organic solvents and/or water) could not be determined by thermogravimetric analysis.

Karl Fischer analysis: Moisture content ca 1.9 % mass fraction (November 2018)  
Moisture content 4.4% mass fraction (September 2019)  
Moisture content 5.0% mass fraction (November 2020)

## Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters Acquity/Waters TQ Detector	
	Column:	X-Bridge C-18, 250 mm × 4.6 mm I.D. × 5.0 μm	
	Column temp:	45 °C	
	Solvent system:	A = MilliQ water; B = Acetonitrile 0-20 min 25% B; 20-25 min 25-80% B; 25- 30min 80% B; 30-31 min 80-25% B; 31-40 min 25% B. The aqueous phase was buffered at pH 8.6 using 10mM ammonium formate and ammonia	
	Flow rate:	0.2 mL/min	
	Sample prep:	2000 μg/g in mobile phase	
	Injection volume:	10 μL	
	Ionisation mode:	Electrospray negative ion	
	Capillary voltage:	3 kV	Cone voltage: 25 V
	Source temp:	120 °C	Desolvation gas temperature: 400 °C
	Cone gas flow rate:	23 L/hr	Desolvation gas flow rate: 500 L/hr
		The retention time of the epi-nandrolone sulfate anion is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	5.93 min:	353.2 [M - Et <sub>3</sub> NH] <sup>-</sup>	
IR:	Instrument:	Bruker Alpha Platinum ATR	
	Range:	4000-400 cm <sup>-1</sup> , neat	
	Peaks:	2939, 2861, 2710, 1667, 1475, 1456, 1248, 1196, 1012, 995, 957, 938, 880, 825, 747, 580 cm <sup>-1</sup>	
<sup>1</sup> H NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	500 MHz	
	Solvent:	MeOH- <i>d</i> <sub>4</sub> (3.31 ppm)	
	Spectral data:	δ 0.83 (3H, s), 0.89 (1H, m), 1.14 (1H, m), 1.23-1.62 (6H, m), 1.32 (9H, t, <i>J</i> = 7.0 Hz), 1.76-1.82 (2H, m), 1.89-2.01 (3H, m), 2.15-2.22 (2H, m), 2.28-2.40 (4H, m), 2.51 (1H, m), 3.21 (6H, q, <i>J</i> = 7.0 Hz), 4.36 (1H, d, <i>J</i> = 5.7 Hz), 5.81 (1H, s) ppm Sulfur trioxide triethylamine complex estimated at 4.5 % mass fraction was observed in the <sup>1</sup> H NMR. Dichloromethane, diethyl ether, pyridine were each detected at 0.1 % mass fraction.	
<sup>13</sup> C NMR:	Instrument:	Bruker Avance III-500	
	Field strength:	126 MHz	
	Solvent:	MeCN- <i>d</i> <sub>3</sub> (1.32 ppm)	
	Spectral data:	δ 9.1, 17.3, 25.0, 26.7, 27.4, 30.9, 32.4, 32.6, 36.1, 37.1, 41.4, 43.2, 45.8, 47.4, 49.6, 50.3, 86.1, 124.6, 168.3, 200.1 ppm	
Melting point:		196-198 °C	
Microanalysis:	Found:	C = 61.2 %; H = 9.7 %; N = 2.8 %; S = 8.2 % (December 2018)	
	Calculated:	C = 63.3 %; H = 9.1 %; N = 3.1 %; S = 7.0 % (Calculated for C <sub>24</sub> H <sub>41</sub> NO <sub>5</sub> S)	