



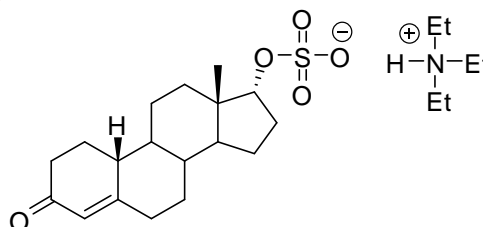
CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA D783b: Epinandrolone sulfate triethylammonium salt

Report ID: D783b.2021.02 (Bottled 190430)

Chemical Formula: C₂₄H₄₁NO₅S

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 α -ol-3-one sulfate, triethylammonium salt.

Expiration of certification: The property values are valid till 28 October 2024, 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.
14 November 2022

This report supersedes any issued prior to 14 November 2022.

NATA Accreditation No. 198 / Corporate Site No. 14214.

Legal notice: Terms and Conditions associated with the provision of this reference material can be found on the NMIA website.

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 ¹H 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_{ORG} = Organic impurities of related structure, I_{VOL} = volatile impurities, I_{NVR} = non-volatile residue.

| | | |
|-----------------------------|--|---|
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler Waters 2695 Separation module |
| | Column: | X-Bridge C-18, 5 μm (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 NH_4OAc and AcOH . |
| | Flow rate: | 1 mL/min |
| | Detector: | Shimadzu SPD-M20A PDA operating at 244 nm Waters 2998 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) |
| | Re-analysis: | Mean = 97.4%, s = 0.13% (6 sub samples in duplicate, October 2021) |
| 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) Moisture content 4.2% mass fraction (September 2021) | |

Spectroscopic and other characterisation data

| | | |
|----------------------|---|--|
| LC-MS: | Instrument: Column: Column temp: Solvent system: Flow rate: Sample prep: Injection volume: Ionisation mode: Capillary voltage: Source temp: Cone gas flow rate: | Waters Acquity/Waters TQ Detector X-Bridge C-18, 250 mm × 4.6 mm I.D. × 5.0 μm 45 °C 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 0.2 mL/min 2000 μg/g in mobile phase 10 μL Electrospray negative ion 3 kV 120 °C 23 L/hr |
| | | Cone voltage: 25 V Desolvation gas temperature: 400 °C 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 ₃ NH] ⁻ |
| IR: | Instrument: Range: Peaks: | Bruker Alpha Platinum ATR 4000-400 cm ⁻¹ , neat 2939, 2861, 2710, 1667, 1475, 1456, 1248, 1196, 1012, 995, 957, 938, 880, 825, 747, 580 cm ⁻¹ |
| ¹ H NMR: | Instrument: Field strength: Solvent: Spectral data: | Bruker Avance III-500 500 MHz MeOH- <i>d</i> ₄ (3.31 ppm) 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 ¹ H NMR. Dichloromethane, diethyl ether, pyridine were each detected at 0.1 % mass fraction. |
| ¹³ C NMR: | Instrument: Field strength: Solvent: Spectral data: | Bruker Avance III-500 126 MHz MeCN- <i>d</i> ₃ (1.32 ppm) 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: Calculated: | C = 61.2 %; H = 9.7 %; N = 2.8 %; S = 8.2 % (December 2018) C = 63.3 %; H = 9.1 %; N = 3.1 %; S = 7.0 % (Calculated for C ₂₄ H ₄₁ NO ₅ S) |