



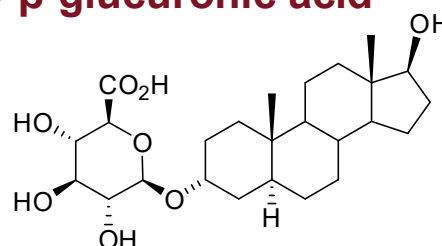
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA S004b: 5 $\alpha$ -Androstane-3 $\alpha$ ,17 $\beta$ -diol-3-O- $\beta$ -glucuronic acid

Report ID: S004b.2025.01 (Ampouled 250814)

Chemical Formula: C<sub>25</sub>H<sub>40</sub>O<sub>8</sub>

Molecular Weight: 468.4 g/mol



### Certified value

| Batch No. | CAS No.    | Mass per ampoule |
|-----------|------------|------------------|
| 24-S-01   | 65535-18-4 | 820 ± 21 μg      |

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

**IUPAC name:** (3 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-17-Hydroxyandrostane-3-yl  $\beta$ -D-glucopyranosiduronic acid.

**Expiration of certification:** The property values are valid till 29 August 2028, three years from the date of 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 ampoules 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 solid prepared by synthesis and certified for identity and purity by NMI Australia. The analyte is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon, intended for a single use to prepare a standard solution containing S004b.

**Intended use:** This certified reference material is suitable for use as a primary calibrator.

**Instructions for use:** Open the ampoule and carefully rinse the interior at least three times, each time with a minimum of 0.5 mL of a suitable organic solvent (e.g. methanol). This process will ensure the transfer of the stated mass per ampoule of anhydrous 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol-3-O- $\beta$ -glucuronic acid. The mass of analyte contained in each ampoule has been determined based on the assigned purity of the bulk material and the concentration of that bulk material in the stock solution used during ampoule preparation.

**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 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:** 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 ten years. The measurement uncertainty at the 95% confidence interval also includes a stability component determined from accelerated stability trials conducted at 40 °C and 75% humidity for 14 days.

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 charged aerosol detection on seven randomly selected ampoules 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 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.  
1 April 2026

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.

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**Characterisation Report:**

|       |   |  |
|-------|---|--|
| HPLC: | Instrument:                                   | Thermo Scientific Vanquish Flex  |
|       | Column:                                       | Grace Altima HP, C 18-HL, 5 $\mu$ m (4.6 mm x 150 mm)  |
|       | Column oven:                                  | 40 $^{\circ}$ C  |
|       | Mobile Phase:                                 | A = MilliQ water with 0.2% HCO <sub>2</sub> H; B = Acetonitrile<br>0-12 min 30% B; 12-20 min 30-38% B; 20-26 min 38%B; 26-29 min 38-30%B, 29-33 mins 30%B. |
|       | Flow rate:                                    | 1.0 mL/min   |
|       | Detector:                                     | Charged Aerosol Detector   |
|       | Relative mass fraction of the main component: |  |
|       | Initial analysis:                             | Mean = 93.7%, s = 0.05% (7 ampoules in duplicate, August 2025)   |

**The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.**

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 evaporative light scattering and charged aerosol detection, thermogravimetric analysis, Karl Fischer analysis, and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100\% - I_{\text{ORG}}) \times (100\% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

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

Supporting evidence is provided by quantitative NMR (qNMR), and elemental microanalysis. The purity value obtained by qNMR was determined using combination of the protons at region 3.3-4.6 ppm against a certified internal standard of maleic acid.

|       |               |  |
|-------|---------------|--|
| HPLC: | Instrument:   | Thermo Scientific Vanquish Flex  |
|       | Column:       | Grace Altima HP, C 18-HL, 5 $\mu$ m (4.6 mm x 150 mm)  |
|       | Column oven:  | 40 $^{\circ}$ C  |
|       | Mobile Phase: | A = MilliQ water with 0.2% HCO <sub>2</sub> H; B = Acetonitrile<br>0-12 min 30% B; 12-20 min 30-38% B; 20-26 min 38%B; 26-29 min 38-30%B, 29-33 mins 30%B. |
|       | Flow rate:    | 1.0 mL/min   |
|       | Detector:     | Charged Aerosol Detector   |

Relative mass fraction of the main component:

Initial analysis: Mean = 93.4%, s = 0.2% (10 sub samples in duplicate, July 2025)

|       |               |   |
|-------|---------------|---|
| HPLC: | Instrument:   | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler |
|       | Column:       | ACE Super 18, 5 $\mu$ m (4.6 mm x 150 mm)             |
|       | Column oven:  | 40 $^{\circ}$ C                                       |
|       | Mobile Phase: | Methanol/0.1 percent formic acid (65:35 v/v)          |
|       | Flow rate:    | 1.0 mL/min  |
|       | Detector:     | Shimadzu ELSD-LT II                                   |

Relative mass fraction of the main component:

Initial analysis: Mean = 99.8%, s = 0.03% (10 sub samples in duplicate, October 2024)

Karl Fischer analysis: Moisture content 13.2% (s = 0.3%, n = 6) mass fraction (July, October 2024 and April 2026)

Thermogravimetric analysis: Non-volatile residue 2.3% mass fraction (October 2024)

|       |                    |  |
|-------|--------------------|--|
| QNMR: | Instrument:        | Bruker Avance-III-500  |
|       | Field strength:    | 400 MHz  |
|       | Solvent:           | AcOH-d <sub>4</sub> (2.03 ppm)                                     |
|       | Internal standard: | Maleic acid (99.9% mass fraction)                                  |
|       | Initial analysis:  | Mean (3.3-4.6 ppm) = 82.3%, s = 0.1% (5 sub samples, October 2024) |
|       | Initial analysis:  | Mean 4.5 ppm = 82.7%, s = 0.1% (5 sub samples, October 2024)       |

**Spectroscopic and other characterisation data**

|                      |                    |   |
|----------------------|--------------------|---|
| ESI-MS:              | Instrument:        | Shimadzu LC-TQ-MS 8045  |
|                      | Operation:         | Direct infusion at 10 $\mu$ L/min   |
|                      | Ionisation mode:   | Electrospray negative ion   |
|                      | Interface voltage: | 3 kV  |
|                      | Peak:              | 467.4 (M-H <sup>+</sup> ) <i>m/z</i>  |
| IR:                  | Instrument:        | Bruker Alpha Platinum ATR   |
|                      | Range:             | 4000-400 $\text{cm}^{-1}$ , neat  |
|                      | Peaks:             | 3423, 2930, 1717, 1446, 1362, 1261, 1162, 1066, 1035 $\text{cm}^{-1}$   |
| <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:     | $\delta$ 0.72 (3H, s), 0.78 (1H, dt, <i>J</i> = 0.4, 12.5 Hz), 0.83 (3H, s), 0.87-1.05 (3H, m), 1.13-1.68 (15H, m), 1.80-1.85 (2H, m), 1.96 (1H, m), 3.24 (1H, dd, <i>J</i> = 8.0, 9.0 Hz), 3.38 (1H, t, <i>J</i> = 9.0 Hz), 3.53 (1H, t, <i>J</i> = 8.5 Hz), 3.55 (1H, t, <i>J</i> = 8.0 Hz), 3.76 (1H, d, <i>J</i> = 9.5 Hz), 3.94 (1H, m), 4.37 (1H, d, <i>J</i> = 7.5 Hz) ppm |
| <sup>13</sup> C NMR: | Instrument:        | Bruker Avance III-500   |
|                      | Field strength:    | 126 MHz   |
|                      | Solvent:           | MeOH- <i>d</i> <sub>4</sub> (49 ppm)  |
|                      | Spectral data:     | $\delta$ 11.7, 11.9, 21.5, 24.3, 26.5, 29.5, 30.6, 32.8, 33.7, 35.3, 36.9, 37.0, 38.1, 40.5, 44.1, 52.5, 55.8, 73.2, 74.8, 75.7, 76.6, 77.6, 82.6, 103.0, 172.6 ppm   |
| Microanalysis:       | Found:             | C = 54.6%; H = 8.8% (October 2024)  |
|                      | Calculated:        | C = 64.1%; H = 8.6% (Calculated for C <sub>25</sub> H <sub>40</sub> O <sub>8</sub> )  |
|                      | Calculated:        | C = 54.3%; H = 8.7% (Calculated for C <sub>25</sub> H <sub>40</sub> O <sub>8</sub> + 4 H <sub>2</sub> O + 1/4 NaCl)   |