Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

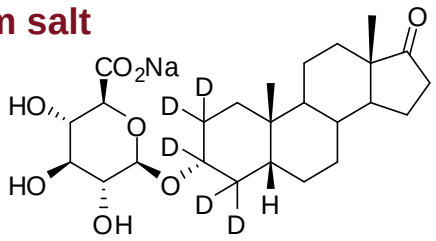
# DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

## NMIA S020b: d5-Etiocholanolone glucuronide sodium salt

Report ID: S020b.2024.01

Chemical Formula: $C_{25}H_{32}D_5NaO_8$

Molecular Weight: 493.6 g/mol




## Property value

| Batch No. | CAS No. | Purity by HPLC-ELSD-CAD |
|---|---|---|
| 24-S-06 | Not available | 99.8 ± 0.2% |

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)-17-oxo(2,2,3,4,4-$^2H_5$)androstan-3-yl β-D-glucopyranosiduronate.

**Expiration of certification:** The property values are valid till 23 August 2027, 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 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 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:** The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

**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 ºC in a closed container in a dry, dark area.

**Stability:** At the recommended storage conditions this material has demonstrated stability for a period of three 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 with charged aerosol detection 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.
27 September 2024

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

## Characterisation Report:

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The indicative purity value was obtained by mass balance from a combination of traditional analytical techniques, including HPLCevaporative light scattering/charged aerosol detection, 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.

Supporting evidence is provided by elemental microanalysis.

The main component of this material is $d_5$-etiocholanolone-β-glucuronide. $d_4$-,$d_3$-, $d_2$-, $d_1$- and $d_0$- etiocholanolone -β-glucuronide are also present. The stated chemical purity of the analyte represents the combined mass fractions of deuterated ($d_4$, $d_3$, $d_2$ and $d_1$) and $d_0$- etiocholanolone-β-glucuronide in the material.

The isotopic purity of this material is an estimate only. This material should be considered for use as an internal standard only.

| | | |
|---|---|---|
| Isotopic Purity: | $d_4$ ≈ 90% [ = $d_5/(d_5+d_4 + d_3 + d_2 + d_1 + d_0)$ x 100] | |
| | $d_0$ < 0.2% [ = $d_0/(d_5+d_4 + d_3 + d_2 + d_1 + d_0)$ x 100] | |
| HPLC: | Instrument: | Thermo Scientific Vanquish |
| | Column: | ACE Excel 5 Super C18, 150 mm × 4.6 mm I.D. × 5 μm |
| | Column oven: | 40°C |
| | Mobile Phase: | Methanol/0.5 percent formic acid (65:35 v/v) |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Vanquish detector |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.6%, s = 0.09% (10 sub samples in duplicate, August 2024) |
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler |
| | Column: | ACE Excel 5 Super C18, 150 mm × 4.6 mm I.D. × 5 μm |
| | Column oven: | 40 °C |
| | Mobile Phase: | Methanol/0.5 percent formic acid (65:35 v/v) |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Shimadzu ELSD-LT II |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 100.0%, s = 0.00% (10 sub samples in duplicate, August 2024) |
| Karl Fischer analysis: | | Moisture content 10.7% mass fraction (August 2024) |

## Spectroscopic and other characterisation data

| | | |
|---|---|---|
| ESI-MS: | Instrument: | Shimadzu LC-TQ-MS 8045 |
| | Operation: | Direct infusion at 10 μL/min |
| | Ionisation mode: | Electrospray negative ion |
| | Interface voltage: | 4.0 kV |
| | Peak: | 470 $(M\text{-}Na^{+})^{-}$ *m/z* |
| IR: | Instrument: | Bruker Alpha Platinum ATR |
| | Range: | 4000-400 $cm^{-1}$, neat |
| | Peaks: | 3615, 3520, 3454, 2934, 2923, 2866, 2853, 2200, 2130, 1730, 1612, 1408, 1376, 1295, 1167, 1087, 1070, 1042, 1027 $cm^{-1}$ |
| $^{1}$H NMR: | Instrument: | Bruker Avance III-500 |
| | Field strength: | 500 MHz |
| | Solvent: | MeOH-$d_4$ (3.31 ppm) |
| | Spectral data: | δ 0.87 (3H, s), 0.98 (3H, s), 0.99 (1H, d, *J* =14.5 Hz), 1.21-1.45 (6H, m), 1.51-1.69 (5H, m), 1.76 (1H, m), 1.82 (1H, d, *J* = 14.0 Hz),1.89-1.99 (2H, m), 2.06 (1H, ddd, *J* = 9.0, 9.0, 18.5 Hz), 2.43 (1H, dd, *J* = 8.5, 19.0 Hz), 3.18 (1H, t, *J* = 8.0 Hz), 3.39 (1H, t, *J* = 8.5 Hz), 3.43 (1H, t, *J* = 9.0 Hz), 3.54 (1H, d, *J* = 9.5 Hz), 4.40 (1H, d, *J* = 8.0 Hz), ppm |
| $^{13}$C NMR: | Instrument: | Bruker Avance III-500 |
| | Field strength: | 126 MHz |
| | Solvent: | MeOH-$d_4$ (49 ppm) |
| | Spectral data: | δ 14.2, 21.2, 22.8, 23.8, 26.5, 28.0, 33.0, 35.9, 36.1, 36.7, 36.8, 42.1, 43.4, 52.8, 73.7, 75.0, 76.1, 77.9, 101.8, 177.0, 224.2 ppm |
| Microanalysis: | Found: | C = 53.6%; H = 7.6% (August 2024) |
| | Calculated: | C = 60.8%; H = 7.7% (Calculated for $C_{25}H_{32}D_5O_8Na$) |
| | Calculated: | C = 54.8%; H = 8.0% (Calculated for $C_{25}H_{32}D_5O_8Na$ + 10% $H_2O$) |