Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
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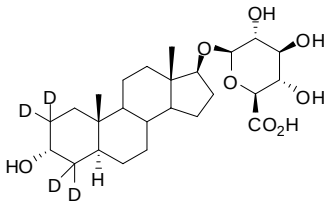
# DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

## NMIA S009: $d_4$-5α-Androstan-3α, 17β-diol-17-*O*- β-glucuronic acid

Report ID: S009.2019.01

Chemical Formula: $C_{25}H_{36}D_4O_8$

Molecular Weight: 472.6 g/mol




## Property value

| Batch No. | CAS No. | Purity estimate |
|---|---|---|
| **11-S-06** | **NA** | **89.8% ± 1.4%** |

**IUPAC name:** NA

**Expiration of certification:** The property values are valid till 12 June 2022, 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:** White solid 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.

**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:** 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 with ELS 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.
17 June 2019

This report supersedes any issued prior to 17 June 2019

**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:** 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 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.

Supporting evidence is provided by elemental microanalysis.

The main component of this material is $d_4$-5α-androstan-3α, 17β-diol-17-*O*-β-glucuronic acid. $d_3$-, $d_2$-, $d_1$- and $d_0$- 5α-androstan-3α, 17β-diol-17-*O*-β-glucuronic acid 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 $d_0$- 5α-androstan-3α, 17β-diol-17-*O*-β-glucuronic acid 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: $d_4 \approx 83\%$ [ = $d_4/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

$d_0 < 0.2\%$ [ = $d_0/(d_4 + d_3 + d_2 + d_1 + d_0) \times 100$]

[from SIM analysis of the *bis*-TMS derivatised free steroid]

| | | |
|---|---|---|
| HPLC: | Instrument: | Waters Model 1525 Binary pump, 717 plus autosampler<br>Or Shimadzu Binary pump LC-20A HT autosampler |
| | Column: | X-Bridge C-18, 5 μm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | Methanol/0.5% Formic acid in MilliQ water (60:40) |
| | Flow rate: | 1 mL/min |
| | Detector: | Waters 2424 ELS detector or Shimadzu LT-II ELSD |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 100%, s = 0.1% (10 sub samples in duplicate, September 2012) |
| | Re-analysis: | Mean = 99.99%, s = 0.02% (7 sub samples in duplicate, July 2014) |
| | Re-analysis: | Mean = 99.6%, s = 0.1% (5 sub samples in duplicate, July 2015) |
| | Re-analysis: | Mean = 100%, s = 0.01% (5 sub samples in duplicate, June 2019) |
| Thermogravimetric analysis: | | Volatile content 3.7% and non volatile residue < 0.2% mass fraction (September 2012) |
| Karl Fischer analysis: | | Moisture content 2.4% mass fraction (September 2012)<br>Moisture content 11.5% mass fraction (July 2014)<br>Moisture content 11.2% mass fraction (July 2015)<br>Moisture content 11.8% mass fraction (June 2016)<br>Moisture content 10.2% mass fraction (May 2019) |

## Spectroscopic and other characterisation data

| | | | |
|---|---|---|---|
| LC-MS: | Instrument: | Waters 2695 (HPLC)/Micromass Quatro | |
| | Column: | Ascentis C-18, 150 mm × 4.6 mm I.D. × 2.7 µm | |
| | Column temp: | 40 °C | |
| | Solvent system: | A = MilliQ water [2% Formic acid v/v]; B = ACN; C = MilliQ water<br>Gradient: A (10%); B (20%); C (70%) to A (10%); B (65%); C (25%) | |
| | Flow rate: | 0.5 mL/min | |
| | Sample prep: | 50 µg/g in MeOH | |
| | Injection volume: | 30 µL | |
| | Ionisation mode: | Electrospray negative ion | |
| | Capillary voltage: | 3.0 kV | |
| | Cone voltage: | 20 V | |
| | Source temp: | 130 ºC | Desolvation gas temperature: 350 ºC |
| | Cone gas flow rate: | 27 L/hr | Desolvation gas flow rate: 762 L/hr |
| | The retention time of $d_4$-5α-androstan-3α, 17β-diol-17-*O*-β-glucuronic acid is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio. | | |
| | 12.2 min: | 471.3 ($M\text{-}H^+$) *m/z* | |
| TLC: | Conditions: | Kieselgel 60F254. Chloroform/methanol (2/1)<br>Single spot observed, Rf = 0.71. Visualisation with vanillin | |
| IR: | Instrument: | Biorad FTS3000MX FT-IR | |
| | Range: | 4000-400 $cm^{-1}$, KBr powder | |
| | Peaks: | 3528, 3424, 2917, 2194, 2008, 1692, 1441, 1380, 1350, 1288, 1229, 1159, 1076, 1019 $cm^{-1}$ | |
| $^1$H NMR: | Instrument: | Bruker Avance III-400 | |
| | Field strength: | 400 MHz | |
| | Solvent: | $CD_3OD$ (3.31 ppm) | |
| | Spectral data: | δ 0.76 (1H, m), 0.82 (3H, s), 0.83 (3H, s), 0.88-1.03 (2H, m), 1.12-1.47 (8H, m), 1.53-1.71 (5H, m), 1.93-2.04 (2H, m), 3.21 (1H, dd, *J* = 7.9, 8.4 Hz), 3.35 (1H, t, *J* = 9.1 Hz), 3.51 (1H, t, J = 9.5 Hz), 3.67 (1H, t, *J* = 8.6 Hz), 3.74 (1H, d, *J* = 9.7), 3.94 (1H, s), 4.37 (1H, d, *J* = 7.8 Hz) ppm | |
| $^{13}$C NMR: | Instrument: | Bruker Avance III-400 | |
| | Field strength: | 100 MHz | |
| | Solvent: | $CD_3OD$ (49.0 ppm) | |
| | Spectral data: | δ 11.7, 12.1, 21.5, 24.3, 29.6, 29.8, 32.8, 33.3, 36.8, 37.2, 38.8, 40.2, 44.4, 52.3, 56.0, 70.0, 73.2, 75.1, 76.6, 77.6, 90.5, 105.1, 172.5 ppm | |
| Melting point: | | > 230 °C | |
| Microanalysis: | Found: | C = 62.8%; H = 8.7%; (September 2012) | |
| | Calculated: | C = 64.1%; H = 8.6%; (Calculated for $C_{25}H_{34}D_4O_8$) | |
| | Calculated: | C = 62.0%; H = 8.7%; (Calculated for $C_{25}H_{34}D_4O_8$ + 2.4% water) | |