Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

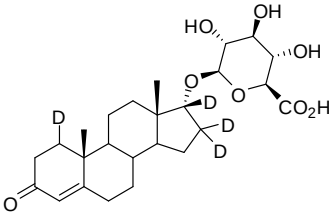
# DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

## NMIA S023: d4-Epitestosterone-17-O-β-glucuronic acid

Report ID: S023.2024.01 (Ampouled 210624)

Chemical Formula: $C_{25}H_{32}D_4O_8$

Molecular Weight: 468.6.6 g/mol




## Property value

| Batch No. | CAS No. | Mass per ampoule |
|---|---|---|
| 13-S-07 | Not available | 887 ± 23 µg |

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

**IUPAC name:** (17α)-3-Oxo-(1,16,16,17-$^2H_4$)-androst-4-en-17-yl β-D-glucopyranosiduronic acid.

**Expiration of certification:** The property values are valid till 8 August 2027, 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 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:** The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The deuterated internal standard is intended for a single use to prepare a standard solution containing S023. The material was sourced from an external supplier, and certified for identity and purity by NMIA. The main component of this material is $d_4$-epitestosterone-17-O-β-glucuronic acid. $d_3$-, $d_2$-, $d_1$- and $d_0$- epitestosterone-17-O-β-glucuronic acid are also present. The stated mass of the analyte per ampoule represents the approximate combined masses of deuterated ( $d_4$, $d_3$, $d_2$ and $d_1$) and $d_0$- epitestosterone-17-O-β-glucuronic acid in the material.

**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:** Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer approximately 887 µg of anhydrous epitestosterone-17-O-β-glucuronic acid ($d_4$, $d_3$, $d_2$, $d_1$ and $d_0$). The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

**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:** 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 UV 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 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 September 2024.

This report supersedes any issued prior to 23 September 2024.

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:

| | | |
|---|---|---|
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler |
| | Column: | X-Bridge C-18, 5 μm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | Methanol/MilliQ water (55:45 v/v)<br>0.2% Formic was present in the aqueous phase |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Shimadzu SPD-M20A PDA operating at 246 nm |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.2%, s = 0.03% (7 ampoules in duplicate, July 2021) |
| | Re-analysis: | Mean = 99.3%, s = 0.01% (5 ampoules in duplicate, July 2022) |
| | Re-analysis: | Mean = 99.0%, s = 0.06% (5 ampoules in duplicate, August 2024) |

**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 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 $^{1}$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 qualitative elemental microanalysis.

The main component of this material is $d_4$- epitestosterone-17-O-β-glucuronic acid. $d_3$-, $d_2$-, $d_1$- and $d_0$- epitestosterone-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 $d_0$- epitestosterone-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: | $d_4$ ≈ 96% [ = $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100] | |
| | $d_0$ < 0.2% [ = $d_0/(d_4 + d_3 + d_2 + d_1 + d_0)$ x 100] | |
| HPLC: | Instrument: | Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler |
| | Column: | X-Bridge C-18, 5 μm (4.6 mm x 150 mm) |
| | Column oven: | 40 °C |
| | Mobile Phase: | Methanol/MilliQ water (55:45 v/v)<br>0.5% Formic was present in the aqueous phase |
| | Flow rate: | 1.0 mL/min |
| | Detector: | Shimadzu SPD-M20A PDA operating at 246 nm |
| | Relative peak area of the main component: | |
| | Initial analysis: | Mean = 99.3%, s = 0.05% (7 sub samples in duplicate, August 2013) |
| | Re-analysis: | Mean = 99.4%, s = 0.03% (5 sub samples in duplicate, August 2016) |
| | Re-analysis: | Mean = 99.4%, s = 0.01% (5 sub samples in duplicate, August 2019) |
| Karl Fischer analysis: | | Moisture content 5.6% mass fraction (September 2016)<br>Moisture content 9.0% mass fraction (August 2019) |
| Thermogravimetric analysis: | | Volatiles content 5.6% and non-volatile residue < 0.2% mass fraction (September 2016) |

## Spectroscopic and other characterisation data

| | | |
|---|---|---|
| ESI-MS: | Instrument: | Waters Acquity, UPLC, QBA 119 |
| | Operation: | Negative ion mode, direct infusion at 10 μL/min |
| | Ionisation: | ESI spray voltage at 3.0 kV positive ion |
| | Cone voltage: | 20 V |
| | Peak: | 467.5 ($M-H^+$) *m/z* |
| GC-MS: | The free steroid was liberated upon treatment with β-glucuronidase enzyme (E. Coli K12) and derivatised with MSTFA. | |
| | Instrument: | Shimadzu GC-2010/GCMS-QP210 plus |
| | Column: | HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm |
| | Program: | 180 °C, 3 °C /min to 240 °C, 10 °C/min to 265 °C, 30 °C/min to 310 °C |
| | Injector: | 260 ºC |
| | Transfer line temp: | 300 ºC |
| | Carrier: | Helium, 1.0 mL/min |
| | Split ratio: | 14/1 |
| | The retention times of the *bis*-TMS derivative is reported along with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak. | |
| | *Bis*-TMS (10.8 min): | 436 (M+, 98), 421 (11), 331 (13), 210 (20), 73 (100) *m/z* |
| | The silylated compound co-elutes with a derivatised comparison sample of epitestosterone. | |
| IR: | Instrument: | Biorad FTS3000MX FT-IR |
| | Range: | 4000-400 $cm^{-1}$, KBr powder |
| | Peaks: | 2936, 2877, 2157, 1733, 1623, 1434, 1370, 1339, 1253, 1191, 1164, 1061, 1018, 935, 698, 654, 598 $cm^{-1}$ |
| $^1$H NMR: | Instrument: | Bruker Avance-400 |
| | Field strength: | 400 MHz |
| | Solvent: | $CD_3OD$ (3.31 ppm) |
| | Spectral data: | δ 0.77 (3H, s), 0.99 (1H, m), 1.10 (1H, m), 1.24 (3H, s), 1.26 (1H, t, *J* = 12.0 Hz), 1.44-1.71 (5H, m), 1.76-1.84 (2H, m), 1.93 (1H, m), 2.08 (1H, m), 2.26-2.33 (2H, m), 2.44-2.54 (2H, m), 3.18 (1H, dd, *J* = 7.8, 9.2 Hz), 3.36 (1H, t, *J* = 9.1 Hz), 3.52 (1H, t, *J* = 9.1 Hz), 3.72 (1H, d, *J* = 9.8 Hz), 4.28 (1H, d, *J* = 7.8 Hz), 5.71 (1H, s) ppm |
| | | Methanol estimated at 2.8% mass fraction was observed in the $^1$H NMR (2016) |
| $^{13}$C NMR: | Instrument: | Bruker Avance-400 |
| | Field strength: | 101 MHz |
| | Solvent: | $CD_3OD$ (49.0 ppm) |
| | Spectral data: | δ 17.4, 17.8, 21.7, 25.5, 32.8, 33.7, 34.1, 34.7, 37.2, 40.0, 45.8, 50.3, 55.3, 73.2, 74.7, 76.7, 77.6, 102.5, 124.1, 172.7, 175.5, 202.5 ppm |
| Melting point: | | 218 °C decomposition |
| Microanalysis: | Found: | C = 62.2%; H = 7.9% (August 2013) |
| | Calculated: | C = 64.1%; H = 7.8% (Calculated for $C_{25}H_{32}D_4O_8$) |