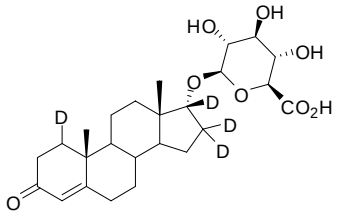
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S023: d_4-Epitestosterone-17-O-β-glucuronic acid

Report ID: S023.2019.02

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

Molecular Weight: 468.6 g/mol



Property value

Batch No.	CAS No.	Purity estimate
13-S-07	**Not Available**	**87.6%**

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 13 August 2024, i.e. five 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.

Description: White powder prepared by sourced from an external supplier, 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 ten 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 UV detection on seven 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.
24 April 2020

This report supersedes any issued prior to 24 April 2020

Characterisation Report:

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 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: $d_4 \approx 96\%$ [= $d_4/(d_4 + d_3 + d_2 + d_1 + d_0)$ 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) 0.5% Formic was present in the aqueous phase
	Flow rate:	1.0 mL/min
	Detector:	Shimazu 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) 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 $C_{25}H_{32}D_4O_8$)