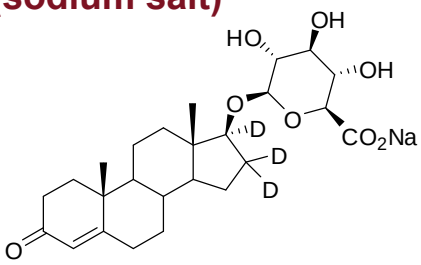
DEUTERATED INTERNAL STANDARD PRODUCT INFORMATION SHEET

NMIA S036: d3-Testosterone-17-O-β-glucuronic acid (sodium salt)

Report ID: S036.2024.01 (Ampouled 200923)

Chemical Formula: $C_{25}H_{32}D_3O_8Na$

Molecular Weight: 489.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
15-S-06	Not available	986 ± 24 μg

IUPAC name: Testosterone-1,16,16,17-d_3 glucuronide sodium salt

Expiration of certification: The property values are valid till 6 May 2029, 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 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.

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 S036. The material was prepared by synthesis and certified for identity and purity by NMIA. The main component of this material is d_3-testosterone-17-*O*-β-glucuronic acid sodium salt. d_2, d_1 and d_0-testosterone-17-*O*-β-glucuronic acid sodium salt are also present. The stated mass of the analyte per ampoule represents the combined masses of deuterated (d_3, d_2 and d_1) and d_0-testosterone-17-*O*-β-glucuronic acid sodium salt 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 986 ± 24 μg of anhydrous testosterone-17-*O*-β-glucuronic acid sodium salt (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.
13 May 2024.

This report supersedes any issued prior to 9 May 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:	Thermo Scientific UltiMate 3000
	Column:	Alltima or ACE Excel Super C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water contained 0.1% formic acid; B = Methanol 0-12 min 55% B; 12-14 min 55-80% B; 14-20 min 80% B; 20-21 min 80-55% B, 21-30 min 55% B
	Flow rate:	1.0 mL/min
	Detector:	UltiMate 3000 RS Diode Array detector operating at 246 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 97.0%, s = 0.05% (7 ampoules in duplicate, October 2020) Re-analysis: Mean = 96.5%, s = 0.02% (7 ampoules in duplicate, May 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 and ^{1}H NMR. The purity value is calculated as per Equation 1.

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

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

Supporting evidence is provided by elemental microanalysis.

Isotopic Purity:		$d_3 \approx 96\%$ [= $d_3 / (d_0 + d_1 + d_2 + d_3) \times 100$] $d_0 < 0.4\%$ [= $d_0 / (d_0 + d_1 + d_2 + d_3) \times 100$] [from SIM analysis of the *bis*-TMS derivative of the free steroid]
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/Milli-Q water (55:45) The aqueous phase contained 0.1% formic acid
	Flow rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating at 246 nm
	Relative mass fraction of the main component:	
	Re-analysis:	Mean = 97.0%, s = 0.017% (5 sub samples in duplicate, August 2020
Thermogravimetric analysis:		Volatile content 7.2% (July 2015)
Karl Fischer analysis:		Moisture content 9.6% mass fraction (July 2015) Moisture content 9.4% mass fraction (June 2020)

Spectroscopic and other characterisation data

GC-MS: The free steroid was liberated upon treatment with methanolic HCl, and derivatised with MSTFA.

Instrument:	Agilent 6890/5973	
Column:	HP-1MS, 30 m x 0.25 mm I.D. x 0.25 μm	
Program:	180 °C (1 min), 30 °C/min to 250 °C (10 min), 30 °C/min to 300 °C (2 min)	
Injector:	250 °C	Transfer line temp: 280 °C
Carrier:	Helium, 1.0 mL/min	Split ratio: 20/1

The retention time of the *bis*-TMS derivative of d_3-testosterone 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 (11.6 min): 435 (M+, 100), 420 (14), 209 (10), 73 (52) *m/z*

ESI-MS:

Instrument:	Micromass Quatro LC Micro
Operation:	Negative ion mode, direct infusion at 10 μL/min
Ionisation:	ESI spray voltage at 3.5 kV positive ion
EM voltage:	650 V
Cone voltage:	50 V
Peak:	466.4 $(M\text{-}Na^+)^-$ *m/z*

TLC:

Conditions:	Kieselgel 60F254. Chloroform/methanol/water (70:30:2) Single spot observed, Rf = 0.1-0.2. Visualisation with UV at 254 nm

IR:

Instrument:	Bruker Alpha FT-IR
Range:	4000-400 cm-1, neat
Peaks:	3454 (br), 1667, 1613, 1417 cm^{-1}

(This IR data is for the corresponding d_3-testosterone-17-O-β-glucuronic acid.)

^{1}H NMR:

Instrument:	Bruker Avance III 500
Field strength:	500 MHz
Solvent:	MeOH-d_4 (3.31 ppm)
Spectral data:	δ 0.90 (3H, s), 0.94-1.06 (3H, m), 1.20-1.32 (2H, m), 1.24 (3H, s), 1.51 (1H, dddd, *J* = 4.0, 13.3, 13.3, 13.3 Hz), 1.56-1.66 (3H, m), 1.70 (1H, ddd, *J* = 4.2, 13.9, 13.9 Hz), 1.89 (1H, m), 2.02-2.10 (2H, m), 2.25-2.33 (2H, m), 2.44-2.51 (2H, m), 3.20 (1H, t, *J* = 8.5 Hz), 3.37 (1H, t, *J* = 8.7 Hz), 3.42 (1H, t, *J* = 9.5 Hz), 3.50 (1H, d, *J* = 9.6 Hz), 4.35 (1H, d, *J* = 7.7 Hz), 5.71 (1H, s) ppm

^{13}C NMR:

Instrument:	Bruker Avance III 500
Field strength:	126 MHz
Solvent:	MeOH-d_4 (49.0 ppm)
Spectral data:	δ 12.0, 17.7, 21.8, 23.9, 32.8, 33.9, 34.7, 36.7, 38.5, 40.0, 44.1, 51.7, 55.4, 73.8, 75.3, 76.3, 77.9, 104.4, 124.1, 175.3, 176.9, 202.4 ppm

Melting point: > 250 °C (decomposition)

Microanalysis:

Found:	C = 55.1%; H = 7.5% (August 2015)
Calculated:	C = 55.4%; H = 7.7% (Calculated for $C_{25}H_{32}D_3O_8Na$ plus 9.6% water)