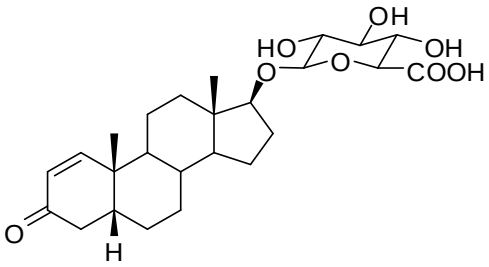
Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D935: 5β-Androst-1-en-17β-ol-3-one-17-β-D-glucuronide (free acid)



Report ID: D935.2024.01 (Ampouled 081105)

Chemical Formula: $C_{25}H_{36}O_8$

Molecular Weight: 464.6 g/mol

Property value

Batch No.	CAS No.	Mass per ampoule
08-S-11	**361432-77-1**	**948 ± 35 µg**

Synonyms: β-D-Glucopyranosiduronic acid, (5β,17β)-3-oxoandrost-1-en-17-yl.

Expiration of certification: The property values are valid until 24 April 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 expiry date/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 and is intended for a single use to prepare a standard solution containing D935. Material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This reference material should be used for qualitative analysis 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 948 ± 35 µg of anhydrous 5β-androst-1-en-17β-ol-3-one-17(3β)-D-glucuronide (free acid).

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 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 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.
1 May 2024

This report supersedes any issued prior to 01 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 Vanquish Flex
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	[Acetonitrile:20 mM ammonium acetate, pH 4.2] [30:70]
	Flow Rate:	1.0 mL/min
	Detector:	Vanquish PDA at 237 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 97.79%, s= 0.12% (5 ampoules in duplicate, April 2024)

HPLC:	Instrument:	Waters HPLC 1525 pump, 717 autosampler or alliance 2695 separations Module
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	A: 20 mM ammonium acetate, pH 4.2, B: Acetonitrile 30% B (0-10 mins), 30%-50% B (10-11 min), 50% B (11-15 min), 50%-70% B (15-16 min), 70% B (16-25 min), 70%-30% B (25-26 min), 30% B (26-35 min)
	Flow Rate:	1.0 mL/min Gradient
	Detector:	Waters 996 UV at 237 nm (2009-2010)
	Detector:	Waters 2998 UV at 237 nm (2011-2019)
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.4%, s= 0.01% (7 ampoules in duplicate, November 2008)
	Re-analysis:	Mean = 99.2%, s= 0.02% (5 ampoules in duplicate, November 2009)
	Re-analysis:	Mean = 99.0%, s= 0.02% (5 ampoules in duplicate, November 2010)
	Re-analysis:	Mean = 99.0%, s= 0.04% (5 ampoules in duplicate, November 2011)
	Re-analysis:	Mean = 98.7%, s = 0.06% (5 ampoules in duplicate, September 2014)
	Re-analysis:	Mean = 98.3%, s = 0.10% (5 ampoules in duplicate, September 2019)

The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

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

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

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

HPLC:	Instrument:	Waters HPLC 1525 pump, 717 autosampler
	Column:	Alltima C-18 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	[Acetonitrile:20 mM ammonium acetate, pH 4.2] [30:70]
	Flow Rate:	1.0 mL/min
	Detector:	Waters 996 UV at 237 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 98.9%, s= 0.07% (8 sub samples in duplicate, September 2008)
Karl Fischer analysis:		Moisture content 5.2% mass fraction (September 2008)
Thermogravimetric analysis:		Volatile content 3.7% and non volatile residue < 0.2% mass fraction (September 2008)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Micromass Quatro Micro
	Operation:	Negative ion mode, direct infusion at 5 µL/min
	Ionisation:	ESI spray voltage at 3.2 kV negative ion
	EM voltage:	500 V
	Cone voltage:	20 V
	Peak:	463 $(M-H)^-$ *m/z*
TLC:	Conditions:	Kieselgel 60F254. Chloroform/methanol (65/35) Single spot observed, Rf = 0.2-0.3. Visualisation with UV at 254 nm
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3529, 3452, 3286, 3156, 2924, 1654, 1439, 1344, 1265, 1179, 1091, 1028, 945,710 cm^{-1}
^{1}H NMR:	Instrument:	Bruker DMX600
	Field strength:	600 MHz
	Solvent:	MeOH-d_4 (3.31ppm)
	Spectral data:	δ 0.87 (3H, s), 1.04-1.32 (4H, m), 1.24 (3H, s), 1.37-1.67 (8H, m), 1.90-2.09 (5H, m), 2.85 (1H, dd, *J* = 15.5, 17.8 Hz), 3.20 (1H, dd, *J* = 7.9, 9.2 Hz), 3.35 (1H, t, *J* = 9.2 Hz), 3.51 (1H, t, *J* = 9.2 Hz), 3.68 (1H, t, *J* = 8.6 Hz), 3.73 (1H, d, *J* = 9.8 Hz), 4.36 (1H, d, *J* = 7.9 Hz), 5.88 (1H, d, *J* = 10.2 Hz), 7.00 (1H, d, *J* = 10.1 Hz) ppm
^{13}C NMR:	Instrument:	Bruker DMX600
	Field strength:	151 MHz
	Solvent:	MeOH-d_4 (49.0ppm)
	Spectral data:	δ 12.0, 21.2, 23.1, 24.3, 26.5, 27.4, 29.7, 36.4, 38.5, 39.86, 39.95, 42.5, 44.3, 47.6, 51.3, 73.2, 75.0, 76.6, 77.5, 90.4, 105.2, 127.4, 164.4, 172.6, 203.4 ppm
Melting point:		204-206 °C