Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

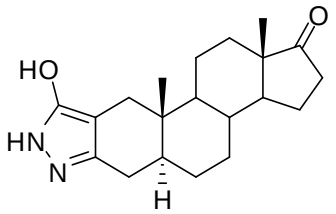
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA S016: 3'-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one

Report ID: S016.2019.01 (Ampouled 121025)

Chemical Formula: $C_{20}H_{28}N_2O_2$

Molecular Weight: 328.5 g/mol



Reference value

Batch No.	CAS No.	Mass per ampoule
12-S-04	1173998-80-5	781 μg

Synonyms: 3'-Hydroxy-2'H-5α-androst-2-eno[3,2-c]pyrazol-17-one.

Expiration of certification: The property values are valid till 3 December 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 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 S016. This 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. acetonitrile). This will transfer approximately 781 μg of 3'-hydroxy-5α-androstan[3,2-c]pyrazol-17-one.

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: This material has been shown to decompose over time (see HPLC summary). 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 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.
7 January 2020

This report supersedes any issued prior to 07 January 2020

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:

HPLC:	Instrument:	Waters Alliance 2695 Separations Module
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Acetonitrile/MilliQ water (30:70 v/v) or A = MilliQ water; B = Acetonitrile 0-24 min 30% B; 24-25 min 30-70% B; 25-35 min 70% B; 35-36 min 70-30% B; 36-45 min 30% B
	Flow rate:	1 mL/min
	Detector:	Waters 2998 PDA operating at 250 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.7%, s = 0.03% (7 ampoules in duplicate, October 2012)
	Re-analysis:	Mean = 99.4%, s = 0.2% (5 ampoules in duplicate, October 2013)
	Re-analysis:	Mean = 99.1%, s = 0.2% (5 ampoules in duplicate, October 2014)
	Re-analysis:	Mean = 97.4%, s = 1.4% (5 ampoules in duplicate, November 2015)
	Re-analysis:	Mean = 95.9%, s = 0.4% (5 ampoules in duplicate, December 2016)
	Re-analysis:	Mean = 94.5%, s = 0.3% (5 ampoules in duplicate, December 2019)

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.

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

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

Supporting evidence is provided by elemental microanalysis.

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:	Acetonitrile/MilliQ water (30:70 v/v)
	Flow rate:	1 mL/min
	Detector:	Waters 2998 PDA operating at 250 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.9%, s = 0.01% (7 sub samples in duplicate, July 2012)
Thermogravimetric analysis:		Volatile content 11.2% and non volatile residue < 0.2% mass fraction (July 2012)
Karl Fischer analysis:		Moisture content 17.6% mass fraction (July 2012)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2795 (HPLC)/Micromass Quatro		
	Column:	Prevail C-18, 50 mm × 2.1 mm I.D. × 3.5 μm		
	Column temp:	32 °C		
	Solvent system:	Solvent A: 10% Formic acid in Milli Q water Solvent B: Milli Q water Solvent C: Acetonitrile Gradient elution: 0 min 10% A 90% B; 1 min 10% A 90% B, 6.5 min 10% A 10% B 80% C; 8 min 10% A 90% B; 11 min 10% A 90% B		
	Flow rate:	0.2 mL/min		
	Sample prep:	50 μg/g in acetonitrile/MilliQ water		
	Injection volume:	10 μL		
	Ionisation mode:	Electrospray positive ion		
	Capillary voltage:	3.4 kV	Cone voltage:	54 V
	Source temp:	120 ºC	Desolvation gas temp:	300 ºC
	Cone gas flow rate:	52 L/hr	Desolvation gas flow:	550 L/hr
	The retention time of 3'-hydroxy-2'H-5α-androstan[3,2-c]pyrazol-17-one is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.			
	8.68 min:	329.06 ($M+H^+$) *m/z*		
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/methanol (95/5) Single spot observed, R_f = 0.15. Visualisation with UV at 254 nm		
IR:	Instrument:	Biorad FTS3000MX FT-IR		
	Range:	4000-400 cm^{-1}, KBr powder		
	Peaks:	3525, 3233, 3079, 2935, 1711, 1599, 1453, 1380 cm^{-1}		
^{1}H NMR:	Instrument:	Bruker Avance-400		
	Field strength:	400 MHz		
	Solvent:	DMSO-d_6 (2.50 ppm)		
	Spectral data:	δ 0.68 (3H, s), 0.80 (3H, s), 0.85 (1H, m), 0.95 (1H, m), 1.15-1.89 (13H, m), 1.96-2.09 (2H, m), 2.28-2.42 (3H, m), 10.17 (2H, bs) ppm		
^{13}C NMR:	Instrument:	Bruker Avance-400		
	Field strength:	101 MHz		
	Solvent:	DMSO-d_6 (39.5 ppm)		
	Spectral data:	δ 11.5, 13.4, 20.1 21.5, 25.8, 28.4, 30.2, 31.4, 33.4, 34.7, 35.4, 36.0, 41.5, 47.0, 50.6, 53.3, 97.8, 138.5, 158.8, 219.9 ppm		
Microanalysis:	Found:	C = 60.5%; H = 9.0%; N = 7.1% (July, 2012)		
	Calculated:	C = 73.1%; H = 8.6%; N = 8.5% (Calculated for $C_{20}H_{28}N_2O_2$)		
	Calculated:	C = 60.5%; H = 9.0%; N = 7.1% (Calculated for $C_{20}H_{28}N_2O_2$ + 17% water)		