Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

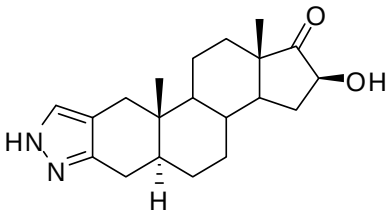
CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

NMIA S017: 16β-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one

Report ID: S017.2018.03 (Ampouled 121115)

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

Molecular Weight: 328.4 g/mol



Certified value

Batch No.	CAS No.	Mass per ampoule
12-S-05	Not available	921 ± 21 μg

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

IUPAC name: 16β-Hydroxy-2'H-5α-androst-2-eno[3,2-c]pyrazol-17-one .

Expiration of certification: The property values are valid till 18 September 2021, 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 under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing S017. This material was sourced from an external supplier, and certified for identity and purity by NMIA.

Intended use: This certified reference material is suitable for use as a primary calibrator.

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 921 ± 21 μg of anhydrous 16β-hydroxy-5α-androstan[3,2-c]pyrazol-17-one. 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.

Metrological traceability: The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%.

Stability: This material has demonstrated stability over a minimum period of three years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials.

This compound is very susceptible to rearranging to 17β-hydroxy-5α-androstan[3,2-c]pyrazol-16-one when subjected to heating or exposure to strong alkali.

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.
17 February 2020

This report supersedes any issued prior to 17 February 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 Model 1525 Binary pump, 717 plus autosampler
	Column:	Grace Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	Methanol/MilliQ water (60:40)
	Flow rate:	1.0 mL/min
	Detector:	Waters PDA 2998 operating at 224 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.7%, s = 0.17% (7 ampoules in duplicate, February 2013)
	Re-analysis:	Mean = 97.8%, s = 0.09% (5 ampoules in duplicate, November 2013)
	Re-analysis:	Mean = 97.9%, s = 0.04% (5 ampoules in duplicate, October 2014)
	Re-analysis:	Mean = 97.9%, s = 0.05% (5 ampoules in duplicate, November 2015)
	Re-analysis:	Mean = 97.4%, s = 0.18% (5 ampoules in duplicate, September 2018)

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 certified 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 qualitative 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:	30 °C
	Mobile Phase:	Acetonitrile/MilliQ water (30:70)
	Flow rate:	1 mL/min
	Detector:	Waters PDA 2998 operating at 224 nm
	Relative mass fraction of the main component:	
	Initial analysis:	Mean = 98.8%, s = 0.2% (7 sub samples in duplicate, July 2012)
Thermogravimetric analysis:		Non volatile residue < 0.2% mass fraction (July 2012). The volatile content (e.g. organic solvents and/or water) could not be determined because of the stability of the material at elevated temperature.
Karl Fischer analysis:		Moisture content 1.0% mass fraction (July 2012)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro	
	Column:	Alltima C-18, 150 mm × 4.6 mm I.D. × 5 μm	
	Column temp:	40 °C	
	Solvent system:	Solvent A: 2% formic acid in Milli Q water Solvent B: Acetonitrile Solvent C: Milli Q water Solvent D: Methanol	
	Isocratic:	10% A; 30% C; 60% D	
	Flow rate:	0.2 mL/min	
	Sample prep:	Methanol	
	Injection volume:	10 μL	
	Ionisation mode:	Electrospray positive ion	
	Capillary voltage:	3.5 kV	
	Cone voltage:	25 V	
	Source temp:	130 °C	Desolvation gas temperature: 350 °C
	Cone gas flow rate:	26 L/hr	Desolvation gas flow rate: 756 L/hr
	The retention time of 16β-hydroxy-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.		
	7.9 min:	329.0 ($M+H^+$) *m/z*	
IR:	Instrument:	Biorad FTS3000MX FT-IR	
	Range:	4000-400 cm^{-1}, KBr powder	
	Peaks:	3264, 2921, 2850, 1732, 1453, 1376, 1312, 1169, 1049, 954, 910, 750 cm^{-1}	
^{1}H NMR:	Instrument:	Bruker Avance-400	
	Field strength:	400 MHz	
	Solvent:	d_6-DMSO (2.50 ppm)	
	Spectral data:	δ 0.69 (3H, s), 0.83 (3H, s), 0.83-1.01 (2H, m), 1.14-1.78 (11H, m), 2.03 (1H, d, *J* = 15.2 Hz), 2.11-2.23 (2H, m), ~2.49 (1H, m), 2.52 (1H, m), 3.79 (1H, m), 5.61 (1H, d, *J* = 6 Hz), 7.23 (1H, s), 12.21 (1H, m) ppm	
		Chloroform estimated at 5.3% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance-400	
	Field strength:	101 MHz	
	Solvent:	d_6-DMSO (39.5 ppm)	
	Spectral data:	δ 11.3, 14.1, 19.8, 28.6, 30.4, 31.2, 31.6, 33.9, 34.4, 36.2, 42.1, 44.5, 46.0, 53.5, 73.8, 79.2, 113.1, 219.5 ppm	
Microanalysis:	Found:	C = 68.2%; H = 8.0%; N = 8.1% (July, 2012)	
	Calculated:	C = 73.1%; H = 8.6%; N = 8.5% (Calculated for $C_{20}H_{28}N_2O_2$)	