Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

NATA ACCREDITED FOR TECHNICAL COMPETENCE

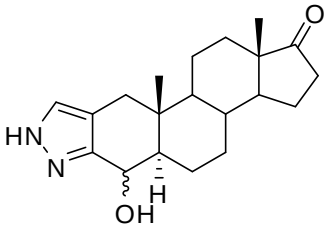
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA S019: 4α/β-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one

Report ID: S019.2019.01 (Ampouled 130325)

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

Molecular Weight: 328.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
12-S-08	**N/A**	**973 ± 23 μg**

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

Synonyms: 4α/β-Hydroxy-2′H-5α-androst-2-eno[3,2-c]pyrazol-17-one

Expiration of certification: The property values are valid till 5 April 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 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 S019. 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 973 ± 23 μg of anhydrous 4α/β-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 demonstrated stability over a minimum period of five years. The measurement uncertainty at the 95% confidence interval includes a stability component which has been estimated from annual stability trials. 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.
15 April 2019

This report supersedes any issued prior to 15 April 2019

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:

Note: This material has been certified as a mixture of 4α-hydroxy- and 4β-hydroxy-5α-androstan[3,2-c]pyrazol-17-one. The mass fraction of each individual isomer can be calculated from the certified purity value and the ratio of the two isomers by HPLC.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	45 °C
	Mobile Phase:	Methanol/MilliQ water (70:30 v/v)
	Flow rate:	0.4 mL/min
	Detector:	Shimadzu SPD-M20A PDA operating at 224 nm
	Relative peak area of 4α-hydroxy diastereomer:	
	Initial analysis:	Mean = 37.7%, s = 0.3% (7 ampoules in duplicate, April 2013)
	Re-analysis:	Mean = 37.5%, s = 0.1% (5 ampoules in duplicate, April 2014)
	Re-analysis:	Mean = 37.4%, s = 0.1% (5 ampoules in duplicate, April 2015)
	Re-analysis:	Mean = 37.4%, s = 0.1% (5 ampoules in duplicate, April 2016)
	Re-analysis:	Mean = 37.2%, s = 0.06% (5 ampoules in duplicate, April 2019)
	Relative peak area of 4β-hydroxy diastereomer:	
	Initial analysis:	Mean = 62.1%, s = 0.3% (7 ampoules in duplicate, April 2013)
	Re-analysis:	Mean = 62.4%, s = 0.02% (5 ampoules in duplicate, April 2014)
	Re-analysis:	Mean = 62.4%, s = 0.01% (5 ampoules in duplicate, April 2015)
	Re-analysis:	Mean = 62.3%, s = 0.01% (5 ampoules in duplicate, April 2016)
	Re-analysis:	Mean = 62.6%, s = 0.08% (5 ampoules in duplicate, April 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 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.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT autosampler
	Column:	Alltima C-18, 5 μm (4.6 mm x 150 mm)
	Column oven:	45 °C
	Mobile Phase:	MilliQ water/Methanol (30:70)
	Flow rate:	0.4 mL/min
	Detector:	Shimadzu SPD-M20A at 224 nm
	Relative peak area of the 4α-hydroxy diastereomer:	
	Initial analysis:	Mean = 37.7%, s = 0.3% (10 sub samples in duplicate, February 2013)
	Relative peak area of the 4β-hydroxy diastereomer:	
	Initial analysis:	Mean = 62.1%, s = 0.3% (10 sub samples in duplicate, February 2013))
Thermogravimetric analysis:		Volatile content 1.2% and non-volatile residue < 0.2% mass fraction (December 2012)
Karl Fischer analysis:		Moisture content 0.5% mass fraction (December 2012)

Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695/ Micromass Quattro TQ Detector
	Column:	Alltima C-18, 5 µm (4.6 mm x 150 mm)
	Column temp:	30 °C
	Solvent system:	200 mM NH_4^+ HCO_2^- (aq) buffered to pH 3 [10% v/v], Methanol [60% v/v], MilliQ water [30% v/v]
	Flow rate:	0.2 mL/min
	Sample prep:	1000 µg/g in MeOH
	Injection volume:	5 µL
	Ionisation mode:	Electrospray positive ion
	Capillary voltage:	3.5 kV
	Cone voltage:	25 V
	Source temp:	130 ºC Desolvation gas temperature: 350 ºC
	Cone gas flow rate:	27 L/hr Desolvation gas flow rate: 759 L/hr
	The retention time of 4α/β-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.	
	8.69 min:	329.2 ($M+H^+$, 20), 311.2 (100), 293 (8) m/z
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate Single spot observed, R_f = 0.45. Visualisation with vanillin
IR:	Instrument:	Biorad FTS3000MX FT-IR
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3429, 3345, 3128, 3095, 2927, 2865, 1720, 1736, 1447, 1400, 1379, 1330, 1050, 1014, 963 cm^{-1}
^{1}H NMR:	Instrument:	Bruker Avance-400
	Field strength:	400 MHz
	Solvent:	MeOH-d_4 (3.31 ppm)
	Key Spectral data for 4α-hydroxy-5α-androstan[3,2-c]pyrazol-17-one: δ 0.82 (3H, s), 0.90 (3H, s), 2.64 (1H, d, *J* = 14.4 Hz), 4.28 (1H, d, *J* = 9.2 Hz), 7.30 (1H, s) ppm	
	Key Spectral data for 4β-hydroxy-5α-androstan[3,2-c]pyrazol-17-one: δ 0.90 (3H, s), 0.98 (3H, s), 2.73 (1H, d, *J* = 14.8 Hz), 4.65 (1H, d, *J* = 4.4 Hz), 7.30 (1H, s) ppm	
	Tetrahydrofuran estimated at 2.1% mass fraction was observed in the ^{1}H NMR	
^{13}C NMR:	Instrument:	Bruker Avance-400
	Field strength:	101 MHz
	Solvent:	d_6-DMSO+D_2O (39.5 ppm)
	Spectral data:	δ 12.6, 13.6, 14.4, 19.8, 19.9, 21.7, 24.8, 25.4, 30.2, 30.6, 31.5, 34.5, 34.7, 35.0, 35.2, 35.6, 36.4, 47.2, 48.2, 50.7, 50.9, 51.0, 53.6, 54.2, 67.3, 113.8, 220.49, 220.53 ppm (This data is reported as a mixture of 4α/β-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one)
Melting point:		>230 °C
Microanalysis:	Found:	C = 72.9%; H = 8.8%; N = 8.4% (January, 2013)
	Calc:	C = 73.1%; H = 8.6%; N = 8.5% (Calculated for $C_{20}H_{28}N_2O_2$)