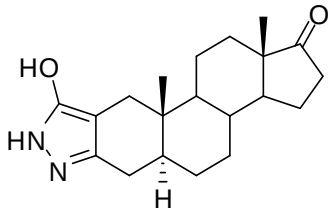
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA S016b: 3'-Hydroxy-5α-androstan[3,2-c]pyrazol-17-one

Report ID: S016b.2024.01

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

Molecular Weight: 328.5 g/mol



Property value

Batch No.	CAS No.	Purity by GC-FID or Purity estimate
24-S-15	**1173998-80-5**	**81.3 ± 1.6%**

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

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

Expiration of certification: The property values are valid till 6 September 2027, three years from the date of 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 sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials. The material will be re-tested on an annual basis to ensure that the property values are still valid. In the event a product fails the stability trial, notification will be sent to all impacted customers.

Description: White powder sourced from an external supplier and certified for identity and purity by NMIA. Packaged in amber glass bottles with a septum and crimped aluminium cap.

Intended use: This reference material is recommended for qualitative analysis only and is not intended for use as a calibrator. The material does not have certified reference material status as metrological traceability of the stated purity value to the SI unit for mass (kg) has not been established.

Instructions for use: Equilibrate the bottled material to room temperature before opening.

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: In the absence of long-term stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last ten years. 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 on ten randomly selected 1-2 mg sub samples 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.
25 September 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:

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-UV, thermogravimetric analysis, Karl Fischer analysis, and ^{1}H NMR spectroscopy. The purity value is calculated as per Equation 1.

Purity = (100 % - I_{ORG}) x (100 % - I_{VOL} – I_{NVR}) 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:	Thermo Scientific Vanquish Flex
	Column:	ACE Super C18, 5um (4.6 mm x 150 mm)
	Column oven:	40 °C
	Mobile Phase:	A = MilliQ water; B = Acetonitrile 0-10 min 33% B; 10-30 min 33-70% B; 30-35 min 70% B; 35-36 min 70-33% B; 36-45 min 33% B.
	Flow rate:	1.0 mL/min
	Detector:	Vanquish PDA operating at 250 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.24%, s = 0.03% (10 sub samples in duplicate, September 2024)
Karl Fischer analysis:		Moisture content 17.4% mass fraction (November 2023)
Thermogravimetric analysis:		Volatiles content 16.4% and non-volatile residue 0.6% mass fraction (October 2023)

Spectroscopic and other characterisation data

ESI-MS:	Instrument:	Shimadzu LC-TQ-MS 8045
	Operation:	Direct infusion at 10 µL/min
	Ionisation mode:	Electrospray positive ion
	Interface voltage:	4.0 kV
	Peak:	329 *m/z*
IR:	Instrument:	Bruker Alpha Platinum ATR
	Range:	4000-400 cm^{-1}, neat
	Peaks:	3525, 3233, 3079, 2935, 2855, 2338, 1711, 1599, 1453, 1380, 1204, 1012, 829, 765, 605, 545.
^{1}H NMR:	Instrument:	Bruker Avance III-500
	Field strength:	500 MHz
	Solvent:	DMSO-d_6 (2.50 ppm)
	Spectral data:	δ 0.69 (3H, *s*), 0.80 (3H, *s*), 0.86 (1H, ddd, *J* = 2,4,12 Hz), 0.96 (1H, dddd, *J* = 4,4, 13, 25 Hz), 1.15-1.57 (8H, *m*), 1.61-1.69 (2H, *m*), 1.75-1.89 (3H, *m*), 1.97-2.085 (2H, *m*), 2.30 (1H, *d*, *J* = 15 Hz), 2.36-2.41 (2H, *m*) 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
Melting point:		208-214 °C
Microanalysis:	Found:	C = 60.3%; H = 8.4%; N = 7.0%; (February 2024)
	Calculated:	C = 60.5%; H = 9.0%; N = 7.1%; (Calculated for $C_{20}H_{28}N_2O_2$ .3.8 H_2O)
	Calculated:	C = 73.1%; H = 8.6%; N = 8.5%; (Calculated for $C_{20}H_{28}N_2O_2$)