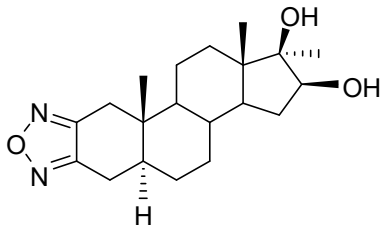
REFERENCE MATERIAL PRODUCT INFORMATION SHEET

NMIA D602: 16β-Hydroxyfurazabol

Report ID: D602.2023.02 (Ampouled 100429)

Chemical Formula: $C_{20}H_{30}N_2O_3$

Molecular Weight: 346.5 g/mol



Property value

Batch No.	CAS No.	Mass per ampoule
99-S-12	36455-74-0	929 ± 14 μg

IUPAC name: (1R,2S,3aS,3bR,5aS,10aS,10bS,12aS)-1,10a,12a-Trimethyl-2,3,3a,3b,4,5,5a,6,10,10a,10b,11,12,12a-tetradecahydro-1H-cyclopenta[7,8]phenanthro[2,3-c][1,2,5]oxadiazole-1,2-diol

Expiration of certification: The property values are valid till 25 January 2033, i.e. ten 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 reference material is intended for a single use to prepare a standard solution containing D602. The material was prepared by synthesis and certified for identity and purity by NMIA.

Intended use: This reference material is recommended 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 929 ± 14 μg of anhydrous 16β-hydroxyfurazabol. 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.

Stability: At the recommended storage conditions this material has demonstrated stability for a period of ten years. The measurement uncertainty includes components for long term stability at the recommended storage conditions.

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.
10 December 2025

This report supersedes any issued prior to 3 February 2023.

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:

This reference material has been shown to contain an isomeric impurity at 6-7% mass fraction, which could only be resolved from 16β-hydroxyfurazabol when using a 2.7 μm reverse phase HPLC column. Other steroidal impurities have not been identified and quantified as mass fractions, therefore it is recommended that this material be considered for use in qualitative analysis only.

HPLC:	Instrument:	Shimadzu Binary pump LC-20AB, SIL-20 A HT auto sampler or Thermo Scientific Ultimate 3000 RS Pump, RS Auto sampler
	Column:	Ascentis C-18, 2.7 μm (4.6 mm x 150 mm)
	Column oven:	Ambient or 40°C
	Mobile Phase:	Acetonitrile/MilliQ water (35:65 v/v)
		Flow rate: 1.0 mL/min
	Detector:	Shimadzu SPD-M20A PDA or RS PDA Detector operating at 219 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 92.4%, s = 0.05% (5 ampoules in duplicate, April 2013)
	Re-analysis:	Mean = 92.7%, s = 0.05% (5 ampoules in duplicate, May 2018)
	Re-analysis:	Mean = 93.0%, s = 0.09% (5 ampoules in duplicate, January 2023)
HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/MilliQ water (55:45 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 219 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.0%, s = 0.02% (7 ampoules in duplicate, June 2018)

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.

Supporting evidence is provided by elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus auto sampler
	Column:	Alltima C-18, 5 μm (4.6 mm × 150 mm)
	Column oven:	Ambient
	Mobile Phase:	Acetonitrile/MilliQ water (55:45 v/v)
	Flow rate:	1.0 mL/min
	Detector:	Waters 2998 PDA operating at 219 nm
	Relative peak area of the main component:	
	Initial analysis:	Mean = 99.3%, s = 0.03% (10 sub samples in duplicate, November 1999)
	Re-analysis:	Mean = 99.0%, s = 0.06% (7 sub samples in duplicate, October 2006)
	Re-analysis:	Mean = 99.0%, s = 0.08% (7 sub samples in duplicate, May 2010)
	Detector:	ELSD
	Relative peak area of main component:	
	Initial analysis:	Mean = 99.96%, s = 0.01% (7 sub samples in duplicate, May 2010)
Karl Fischer analysis:		Moisture content 0.22% mass fraction (April 2010)
Thermogravimetric analysis:		Volatiles content < 0.1 and non-volatile residue < 0.2% mass fraction (November 1999 and October 2006)

Spectroscopic and other characterisation data

GC-MS:	*Bis*-TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP Ultra 1, 17 m × 0.22 mm I.D. × 0.11 μm
	Program:	170 °C, 3 °C/min to 234 °C, 10 °C/min to 265 °C (3 min)
	Injector:	280 °C
	Split ratio:	15/1
	Transfer line temp:	300 °C
	Carrier:	Helium
	Scan range:	50-550 *m/z*
	The retention time of the *bis*-TMS derivative is reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (16.4 min):	490 (M^+, 22), 474 (10), 328 (12), 231 (43), 218 (75), 73 (100) *m/z*
LC-MS:	Instrument:	Waters 2695 (HPLC)/ Micromass Quattro TQ Detector
	Column:	Ascentis Express C-18, 2.7 μm (150 mm × 4.6 mm)
	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: 2% A; 35% B; 63% C
	Flow rate:	0.2 mL/min
	Sample prep:	Methanol
	Injection volume:	10 μL
	Ionisation mode:	Electrospray negative ion
	Capillary voltage:	3.0 kV
	Cone voltage:	20 V
	Source temp:	130 ºC
	Desolvation gas temp:	350 ºC
	Cone gas flow rate:	26 L/hr
	Desolvation gas flow:	691 L/hr
	The retention time of 16β-hydroxyfurazabol is reported along with the major peak in the mass spectrum. The latter is reported as a mass/charge ratio.	
	22.6 min:	391.5 ($M+HCOO^-$) *m/z*
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/dichloromethane (1:1) Single spot observed, R_f = 0.21 (3 sub samples)
IR:	Instrument:	FT-IR, Biorad WIN FTS40
	Range:	4000-400 cm^{-1}, KBr powder
	Peaks:	3378, 1451, 1382, 1298, 1223, 1045, 875 cm^{-1}
^{1}H NMR:	Instrument:	Bruker ARX-500
	Field strength:	500 MHz
	Solvent:	$CDCl_3$ (7.26 ppm)
	Key Spectral data:	δ 0.77 (3H, s), 0.88 (3H, s), 1.15 (3H, s), 3.68 (1H, m) ppm
	Dichloromethane, estimated at 0.1% mass fraction, has been quantified by ^{1}H NMR ^{13}C NMR: Instrument: BrukerARX-500	
	Field strength:	126 MHz
	Solvent:	$CDCl_3$ (77.16 ppm)
	Spectral data:	δ 11.8, 13.4, 20.5, 23.7, 23.8, 28.7, 31.2, 32.2, 33.6, 34.8, 35.8, 36.6, 37.3, 41.6, 44.7, 53.6, 77.6, 79.1, 150.8, 151.8 ppm
Melting point:		219-221 °C
Microanalysis:	Found:	C = 69.4%; H = 8.8%; N = 8.1%
	Calculated:	C = 69.3%; H = 8.7%; N = 8.1% (Calculated for $C_{20}H_{30}N_2O_3$)