



## REFERENCE MATERIAL PRODUCT INFORMATION SHEET

### Report ID: D602.2018.01 (Ampouled 100429)

This batch of ampoules was prepared from the bulk material on 29<sup>th</sup> April 2010.

Compound Name: **16 $\beta$ -Hydroxyfurazabol**

Collection Number D602

Chemical Formula: C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>3</sub>

CAS Registry Number: 36455-74-0

Metabolite of furazabol

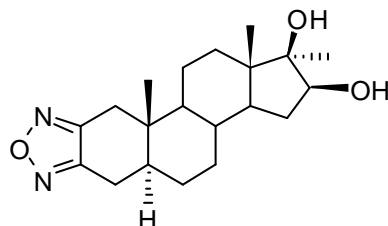
Structure:

Description: White crystals

Batch No: 99-S-12

Molecular Weight: 346.5

Release Date: May 1999



Synonym: 16 $\beta$ ,17 $\beta$ -Dihydroxy-17 $\alpha$ -methyl-5 $\alpha$ -androstano[2,3-c]furazan

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 D602. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (methanol). This will transfer  $923 \pm 4$   $\mu$ g of anhydrous 16 $\beta$ -hydroxyfurazabol. The uncertainty is stated at the 95% coverage interval.

This reference material has been shown to contain an isomeric impurity at 6-7% mass fraction, which could only be resolved from 16 $\beta$ -hydroxyfurazabol when using a 2.7  $\mu$ m 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 autosampler  
Column: Ascentis Express C-18, 2.7  $\mu$ m (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/water (35:65)  
Flow Rate: 1.0 mL/min  
Detector: Shimadzu SPD-M20A Photodiode Array Detector operating at 219 nm  
Relative peak area of 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)

HPLC: Instrument: Waters Model 1525 Binary pump, 717 plus autosampler  
Column: Alltima C-18, 5  $\mu$ m (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/water (55:45)  
Flow Rate: 1.0 mL/min  
Detector: Waters PDA 996 operating at 219 nm  
Relative peak area of main component:  
Initial analysis: Mean = 99.0%, s = 0.02% (7 ampoules in duplicate, May 2010)

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**The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.**

The purity estimate by traditional analytical techniques was obtained by subtraction from 100% of total impurities by HPLC with UV detection, Karl Fischer analysis and <sup>1</sup>H NMR. Supporting evidence is provided by thermogravimetric analysis and elemental microanalysis.

HPLC:	Instrument:	Waters Model 1525 Binary pump, 717 plus autosampler
	Column:	Alltima C-18, 5 µm (4.6 mm × 150 mm)
	Mobile Phase:	Acetonitrile/water (55:45)
	Flow Rate:	1.0 mL/min
	Detector:	Waters PDA 996 operating at 219 nm
	Retention time:	9.0 min
	Relative peak area of 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)
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Ethyl acetate/dichloromethane (1:1) Single spot observed, R <sub>f</sub> = 0.21 (3 sub samples)
Microanalysis:	Found: C = 69.4%; H = 8.8%; N = 8.1%	
	Calc: C = 69.3%; H = 8.7%; N = 8.1% (Calculated for C <sub>20</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub> )	
Thermogravimetric analysis:	Volatiles content < 0.1 and non-volatile residue < 0.2% mass fraction (November 1999 and October 2006)	
Karl Fischer analysis:	Moisture content 0.22% mass fraction (April 2010)	

### Spectroscopic and other characterisation data

LC-MS:	Instrument:	Waters 2695 (HPLC)/Micromass Quatro		
	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 °C	Desolvation gas temperature:	350 °C
	Cone gas flow rate:	26 L/hr	Desolvation gas flow rate:	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 <sup>-</sup> ) m/z				
GC-MS:	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	Transfer line temp:	300 °C
	Carrier:	Helium	Split ratio:	15/1
	The retention time of the <i>bis</i> -TMS derivative is reported with the major peaks in the mass spectrum. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the intensity of the base peak.			
	16.4 min: 490 (M <sup>+</sup> , 22), 474 (10), 328 (12), 231 (43), 218 (75), 73 (100) <i>m/z</i>			
IR:	Instrument:	FT-IR, Biorad WIN FTS40		
	Range:	4000-400 cm <sup>-1</sup> , KBr powder		
	Peaks:	3378, 1451, 1382, 1298, 1223, 1045, 875 cm <sup>-1</sup>		
<sup>1</sup> H NMR:	Instrument:	Bruker ARX-500		
	Field strength:	500 MHz	Solvent:	CDCl <sub>3</sub> (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 <sup>1</sup> H NMR.			
<sup>13</sup> C NMR:	Instrument:	Bruker ARX-500		
	Field strength:	126 MHz	Solvent:	CDCl <sub>3</sub> (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			

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### Expiration of certification

The property values are valid till 15<sup>th</sup> May 2023, 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.

The long-term stability of the compound in solution has not been examined.

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

### 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% coverage level from that observed on repeat analysis of the same sample.

### Recommended storage

When not in use this material should be stored at or below 4 °C in a closed container in a dry, dark area.

### Intended use

For *in vitro* laboratory analysis only.

### Caution

Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust.

### Legal notice

Neither NMI nor any person acting on NMI's behalf assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this certificate.

Authorised by:

S. R. Davies

Dr Stephen R. Davies,  
Team Leader,  
Chemical Reference Materials, NMI.  
Dated: 17 May, 2018.

Characterisation data and property values specified in this report supersede those in all reports issued prior to 17 May, 2018.