Australian Government

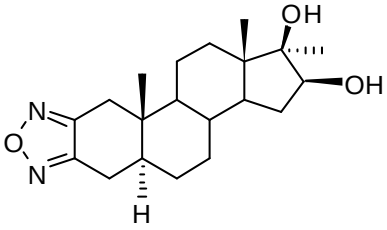
National Measurement Institute

REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D602.2013.01 (Ampouled 100429)
This batch of ampoules was prepared from the bulk material on 29th April 2010.

Compound Name: **16β-Hydroxyfurazabol**
Collection Number D602
Chemical Formula: $C_{20}H_{30}N_2O_3$
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: 16β,17β-Dihydroxy-17α-methyl-5α-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 ± 4 µg of anhydrous 16β-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 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 autosampler
	Column:	Ascentis Express C-18, 2.7 µm (4.6 mm × 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 response of main component:	
	Initial analysis:	Mean = 92.4%, s = 0.05% (5 ampoules in duplicate, April 2013)
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:	8.5 min
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.0%, s = 0.02% (7 ampoules in duplicate, May 2010)

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 ^{1}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 response 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
	Retention time:	9.1 min
	Relative peak area response of main component:	
	Initial analysis:	Mean = 99.96%, s = 0.01% (7 sub samples in duplicate, May 2010)
TLC:	Conditions:	Kieselgel 60F_{254}. Ethyl acetate/dichloromethane (1:1) Single spot observed, R_f = 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 $C_{20}H_{30}N_2O_3$)
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 ºC	Desolvation gas temperature: 350 ºC
	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 ($M+HCOO^-$) 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 *bis*-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^+, 22), 474 (10), 328 (12), 231 (43), 218 (75), 73 (100) *m/z*		
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:	Bruker ARX-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	

Expiration of certification
The property values are valid till 16th April 2018, 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 been given a shelf life of three years from the date of re-certification. 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.

This material has demonstrated stability over a minimum period of 3 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 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 ºC 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: 25 April, 2013

Characterisation data and property values specified in this report supersede those in all reports issued prior to 25th April 2013.