



REFERENCE MATERIAL ANALYSIS REPORT

Report ID: D622.2014.01 (Ampouled 090507)

This batch of ampoules was prepared from the bulk material on 7<sup>th</sup> May 2009.

Compound Name: 2-Hydroxymethyl-17 $\alpha$ -methylandrosta-1,4-diene-11 $\alpha$ ,17 $\beta$ -diol-3-one

Description: White crystalline solid

Batch Number: 99-S-29

Collection Number: D622

Formula Weight: 346.5

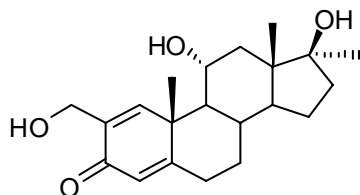
Chemical Formula: C<sub>21</sub>H<sub>30</sub>O<sub>4</sub>

Release Date: 10<sup>th</sup> December 1999

CAS Registry Number: 59400-02-1

Metabolite of formebolone

Structure:



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 D622. Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. methanol). This will transfer  $907 \pm 27 \mu\text{g}$  of anhydrous 2-hydroxymethyl-17 $\alpha$ -methylandrosta-1,4-diene-11 $\alpha$ ,17 $\beta$ -diol-3-one. The uncertainty is stated at the 95% coverage interval.

HPLC: Column: Alltima C-18, 5 $\mu\text{m}$  (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/water  
Flow Rate: 1.0 mL/min Gradient: 26% acetonitrile for 12 min, increase acetonitrile to 50% in 1 min, elute for 6 min  
Detector: Waters PDA 2998 operating at 254 nm  
Relative peak area response of main component:  
Initial analysis: Mean = 95.9%, s = 0.1% (5 ampoules in duplicate, August 2011)  
Re-analysis: Mean = 95.9%, s = 0.01% (5 ampoules in duplicate, July 2014)

HPLC: Column: Alltima C-18, 5 $\mu\text{m}$  (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/water (26/74)  
Flow Rate: 1.0 mL/min  
Detector: Waters PDA 996 operating at 254 nm  
Relative peak area response of main component:  
Initial analysis: Mean = 96.1%, s = 0.05% (7 ampoules in duplicate, June 2009)  
Re-analysis: Mean = 96.1%, s = 0.02% (5 ampoules in duplicate, July 2010)

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

Purity estimate obtained from HPLC with UV detection and Karl Fischer moisture analysis. Supporting evidence is provided by thermogravimetric analysis, microanalysis and <sup>1</sup>H NMR.

HPLC: Column: Alltima C-18, 5 $\mu\text{m}$  (4.6 mm  $\times$  150 mm)  
Mobile Phase: Acetonitrile/water (26:74)  
Flow Rate: 1.0 mL/min  
Detector: Waters PDA 996 operating at 254 nm  
Relative peak area response of main component:  
Initial analysis: Mean = 96.0%, s = 0.2% (10 sub samples in duplicate, February 2000)  
Re-analysis: Mean = 96.0%, s = 0.04% (10 sub samples, June 2009)

Thermogravimetric analysis: Volatiles content 4.8% (February 2000 & November 2005)  
Non-volatile residue 0.3% mass fraction (June 2009)

Karl Fischer analysis: Moisture content 5.2% mass fraction (May 2009)

Accredited for compliance with ISO Guide 34.

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### Expiration of certification

The property values are valid till 28<sup>th</sup> July 2019, 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 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: 4<sup>th</sup> August 2014

Characterisation data and property values specified in this report supersede those in all reports issued prior to 4<sup>th</sup> August 2014