



**CERTIFICATE OF ANALYSIS**  
**FOR CERTIFIED REFERENCE MATERIAL NMIA MX003**  
**19-Norandrosterone in 1,2-dimethoxyethane solvent**

**(i) Description of the material**

This reference material is 1 mL of a solution of 19-norandrosterone (CAS Registry Number 1225-01-0) in 1, 2-dimethoxyethane solvent (CAS Registry number 110-71-4) contained in a sealed glass ampoule.

**(ii) Certified property values**

The certified mass fraction and concentration for 19-norandrosterone in the supplied sample of solution are given in Table 1. This value was obtained by high-accuracy GC-HRMS analysis. Details of the analytical methodology and uncertainty calculations are provided below.

**Table 1 Certified property values for 19-norandrosterone in 1,2-dimethoxyethane (glyme) in CRM NMIA MX003, the reported uncertainties are expanded uncertainties (U) with a level of confidence of 95%.**

<b>19-norandrosterone<sup>†</sup></b>	<b>221.4 ± 6.9 ng/g</b>	<b>192.0 ± 6.0 ng/mL*</b>
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<sup>†</sup> The measurand is defined as the free steroid form of 19-norandrosterone (CAS Registry Number 1225-01-0) in the solution in ng/g or ng/mL.

\* This mass concentration applies to 20°C. The conversion from mass fraction units of ng/g to mass concentration units of ng/mL at 20°C was carried out by measuring the density of the material. The density was measured as 866.98 ± 0.05 kg/m<sup>3</sup> at 20°C (± 0.02°C).

**(iii) Expiration of certification**

The certified values are valid until 31 March 2020 provided the material is handled and stored in accordance with the instructions given on this certificate.

**(iv) Intended use**

The reference material is intended to be used as a calibration standard for analytical methods for the measurement of 19-norandrosterone, a metabolite of 19-nortestosterone (nandrolone), in doping, clinical or forensic analysis.

**(v) Instructions for use**

Break the top off the ampoule at the etch mark and use immediately. The solvent is volatile and significant evaporation may occur that will change the concentration value and this should be avoided.

The smallest subsample used for homogeneity assessment was 0.35 g; the use of a smaller quantity may expand the stated uncertainties for the certified values.

**(vi) Production of the reference material**

A stock solution was prepared on 1 September 2004 by dissolving 10 mg of 19-norandrosterone (NMIA D555) in 10 g of 1,2-dimethoxyethane (glyme). A portion (0.29 g) of this solution was

diluted to 1305 g with glyme then dispensed into ampoules and flame sealed. The certified value agreed with the calculated gravimetric mass fraction of the solution.

### **(vii) Analytical method**

The certified mass fraction was measured by isotope dilution mass spectrometry involving the addition of an isotopically-labelled analogue of 19-norandrosterone prior to any sample preparation. The mass fraction determined analytically by GC-HRMS was converted to a mass concentration from the measured density of the material.

### **(viii) Uncertainty calculations**

Measurement uncertainty was estimated according to NMIA standard procedures. Standard uncertainties for all components were estimated and combined as described in the ISO Guide to the Expression of Uncertainty in Measurement [2]. The combined standard uncertainties were expanded to a 95% level of confidence using a coverage factor calculated from degrees of freedom obtained from the Welch-Satterthwaite equation.

The factors examined were calibration standards, mass measurements, isotope ratios, method precision, heterogeneity between ampoules, storage stability and method biases. The major contributor to the uncertainty was the calibration standard.

The certified expanded uncertainty was calculated to apply to any ampoule within the batch of NMIA MX003 over the entire certification period.

### **(ix) Homogeneity assessment**

The homogeneity of the material was assessed from 33 ampoules selected in a stratified sampling plan to test for homogeneity across the entire batch. Both within- and between-ampoule variance was estimated. Homogeneity testing was carried out on 0.35 g aliquots of the material. Both within- and between-bottle variance was estimated. The uncertainties in the certified values incorporate these results.

### **(x) Storage and stability assessment**

The material should be stored at -20°C out of direct light as issued. The stability of the material was demonstrated by an accelerated stability trial. No instability was observed between -20°C and 40°C. Stability at -20°C has been verified by on-going testing of the stored material. The uncertainties for the reference values were expanded to incorporate a factor relating to stability in storage [3].

### **(xi) Metrological traceability of property values**

The certified values are traceable to the SI unit for mass (kg) through the Australian national standards for mass. The primary ratio method of isotope dilution mass spectrometry (IDMS) was used and the quantities used in the calculation of the measurement values are traceable to the SI. The pure steroid standards used in the calibration of the IDMS method were traceable through certification or in-house analysis. Balances used in the preparation of samples were all appropriately calibrated.

The certified mass concentrations are traceable to the SI units for mass (kg) and length (m) through the relevant Australian national standards. The mass fraction values obtained by IDMS have been converted to mass concentration using a traceable density measurement carried out at NMIA's mass laboratory.

### **(xii) Legal Notice**

Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA'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.

**References:**

1. L.G. Mackay, C.P. Taylor, R.B. Myers, R. Hearn and B. King; *Accred. Qual. Assur.*, **8**, 2003, 191-194.
2. *Guide to the Expression of Uncertainty of Measurement*, ISO, Geneva (1995).
3. *Reference Materials – General and statistical principles for certification*. ISO Guide 35, 3<sup>rd</sup> Edition (2006), Annex B5.

Authorised by:



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The certified property values specified in this report supersede those in all reports issued prior to 22 October 2015.