Australian Government
Department of Industry, Innovation and Science
National Measurement Institute
CIPM MRA
NATA ACCREDITED FOR TECHNICAL COMPETENCE

REFERENCE MATERIAL CERTIFICATE

NMIA MX016: Carbon Isotope Delta Value of 19-norandrosterone

Certified value

	CAS No.	$\delta^{13}C_{VPDB}$ / ‰
19-norandrosterone	**1225-01-0**	**–29.7 ± 0.8**

The measurand is the carbon isotope delta value relative to Vienna peedee belemnite (VPDB) of free steroid after hydrolysis of the glucuronide conjugate. Uncertainty is an expanded uncertainty using a coverage factor of 2.1 and represents the 95% confidence limit.

Expiry: 31 October 2023. **Batch No.:** 2013.11
For sealed ampoules stored as described on this certificate.

Description: 1 mL of a solution of 19-norandrosterone glucuronide in water containing 20% methanol, sealed under argon in a glass ampoule. The approximate concentration of the solution is 200 ng/mL, expressed as the free steroid 19-norandrosterone.

Storage: Store in a freezer (-18 °C) out of direct light.

Metrological traceability: The certified carbon isotope delta values are traceable to the carbon isotope ratio embodied in the Vienna Pee Dee Belemnite [1] isotopic reference material via NIST RM 8559 (methane and ethane) used to assign delta values to the steroid mixture CU/USADA 34-1 [2].

Intended use: Quality control or calibration of the measurement of carbon isotope delta values of 19-norandrosterone in human urine in doping, clinical or forensic analysis. In-vitro use only.

Instructions for use: Equilibrate the ampoule to room temperature. Thoroughly mix the contents using a vortex mixer. Cautiously break off the top at the etch mark and use immediately.
Use an aliquot of 125 µL - 1000 µL to fortify 10 mL of urine or deionized water to produce a sample fortified with 19-norandrosterone in the range of 2.5 ng/mL to 20 ng/mL.

Stability: The stability of the material has been verified and will continue to be monitored.
The material was stable in accelerated stability trial of two weeks at 40 °C.

Production: 1 mg of 19-norandrosterone glucuronide (NMIA D596) was dissolved in 5 g of methanol. 1.91 g of this solution was diluted to 1 L with 20% methanol/water (v/v) and dispensed into ampoules. The headspace was purged with argon and the ampoule sealed.

Analytical method: The CRM (1 mL) was hydrolysed in 1 mL of phosphate buffer (pH 7) using β-glucuronidase for 2 hours at 37 °C. The resulting free steroid was extracted into hexane after the addition of carbonate buffer (pH 10) and NaCl. The extract was evaporated to dryness and reconstituted in a mixed steroid internal standard solution. During carbon isotope ratio analysis, carbon dioxide calibrated with a reference free steroid mixture, CU/USADA 34-1, was introduced into the mass spectrometer through the reference inlet as an internal standard (IS). A steroid internal standard added to the sample was also added to the calibration solution. The CU/USADA 34-1 standard was used to bracket sample analysis throughout the batch to allow an average batch $\delta^{13}C_{IS}$ value to be assigned to the reference gas and steroid internal standard. The correction for ^{17}O contribution was estimated from the $^{18}O/^{16}O$ abundance determined from ratio measurement of m/z 46/44 in the CO_2 reference gas or steroid internal standard and the reported relationship between the ^{17}O and ^{18}O isotopic abundances [3]. The carbon isotope delta value for 19-norandrosterone in extracts of the solution CRM was calculated using the instrument software (Isodat NT3.0) using the delta values ($\delta^{13}C_{IS/VPDB}$ and $\delta^{18}O_{IS/VPDB}$) determined during calibration input for the reference gas. The software employs the SSH algorithm for ^{17}O correction. Any difference between the results obtained using the reference gas and steroid internal standard was captured in the measurement uncertainty of the property value.

Measurement uncertainty: Standard uncertainties were estimated and combined as described in the JCGM Guide to the Expression of Uncertainty in Measurement [4]. Contributions to the combined standard measurement uncertainty were isotopic fractionation during the extraction procedure, uncertainty in the assignment of delta value to the reference gas, choice of internal standards, method precision, in-homogeneity, stability during transport and uncertainty in the calibration material. The combined standard uncertainty was multiplied by a coverage factor (*k*) of 2.1, corresponding to 15 effective degrees of freedom, to give an expanded uncertainty with an approximate 95% . The major contributors to the combined uncertainty were the choice of internal standard and the assignment of the carbon isotope ratio of the internal standard.

Homogeneity: 12 ampoules were selected using a stratified sampling plan. Homogeneity testing was carried out on 1 mL aliquots of the material. No significant inhomogeneity was detected.

P Armishaw

Paul Armishaw
Manager Chemical Reference Values
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This report supersedes any issued prior to 13 September 2018

References:

1. Coplen, T.B. Reporting of stable hydrogen, carbon, and oxygen isotopic abundances. *Pure Appl. Chem.,* (1994) **66,** 273-276
2. Zhang, Y.; Tobias, H. J.; Brenna, J. T., Steroid isotopic standards for gas chromatography-combustion isotope ratio mass spectrometry (GCC-IRMS). *Steroids (*2009) **74**, 369-378.
3. Santrock S.; Studley S.A; Hayes, J.M., Isotopic analyses based on the mass spectrum of carbon dioxide. *Analytical Chemistry* (1985) **57,** 1444-1448
4. *Evaluation of measurement data — Guide to the expression of uncertainty in measurement;* JCGM 100, 1st edition (2008).

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