Australian Government
Department of Industry, Science and Resources

National Measurement Institute

NATA
WORLD RECOGNISED ACCREDITATION

CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

NMIA MX024: Etiocholanolone certified for Carbon Isotope Delta Value

Certified value

Steroid	CAS No.	$\delta^{13}C_{VPDB-LSVEC}$ / ‰	Coverage Factor (k)	V_{eff}
Etiocholanolone	**53-42-9**	**-29.69 ± 0.27**	**2.1**	**19**

The measurand is the carbon isotope delta value of the stated steroid reported as relative to the primary reference Vienna Pee Dee Belemnite (VPDB), normalised by consensus $\delta^{13}C_{VPDB}$ values of +1.95 ‰ (NBS 19) and -46.6 ‰ (LSVEC).[a] The uncertainty has been expanded to provide a level of confidence of 95%.

Expiry: 1 August 2026

Batch No: 2023.01

Description: 2 mL sealed ampoule containing approximately 1004 µg of dried steroid.

Intended use: Validation and calibration of Gas Chromatography Combustion Isotope Ratio Mass Spectrometry (GC-C-IRMS) for steroid carbon isotope ratio measurements in anti-doping analysis.[1] In-vitro use only.

Instructions for use: Each ampoule must be reconstituted in a minimum of 2 mL of an appropriate solvent such as 2-propanol and allowed to equilibrate for 3 hours before use. The user must take appropriate care during reconstitution, including avoiding plasticisers contamination from vials and pipette tips. The user is responsible for appropriate handling and storage of the reconstituted material. It is recommended for the reconstituted material to be stored at 4°C when not in use.

Storage: Store sealed ampoule in a refrigerator at 4°C out of direct light.

Metrological traceability: The certified carbon isotope delta values are traceable to the stable carbon isotope ratios of the primary isotopic reference material NBS19 via the secondary isotopic reference materials USGS61 ($\delta^{13}C_{VPDB-LSVEC}$ -35.05 ± 0.04‰), NBS 22 ($\delta^{13}C_{VPDB}$ -30.03 ± 0.05‰), USGS40 ($\delta^{13}C_{VPDB}$ -26.39 ± 0.04‰), USGS62 ($\delta^{13}C_{VPDB-LSVEC}$ -14.79 ± 0.04‰) and IAEA-CH-6 ($\delta^{13}C_{VPDB-LSVEC}$ -10.45 ± 0.04‰).[2]

Stability: The stability of the material in the sealed ampoule under the recommended storage conditions has been verified using GC-C-IRMS and will continue to be monitored. The material in the sealed ampoule was also shown to be stable in an accelerated stability trial of four weeks at 40°C. However, the stability of the reconstituted material will have to be verified by the user under the storage condition and the reconstitution solvent employed.

Homogeneity: Ten ampoules were selected using a stratified sampling plan and each ampoule was analysed three times by GC-C-IRMS. No significant inhomogeneity was detected.

Production: The stock solution was prepared by dissolving 0.5 g etiocholanolone in 250 mL of 2-propanol/methanol (5:1) prior to dispensing 0.5 mL aliquots into ampoules. Each batch of ampoules were vacuum dried for two days then flame sealed under argon and stored at 4°C.

Analytical method: EA-IRMS was employed for assignment of $\delta^{13}C_{\text{VPDB-LSVEC}}$ value to the bulk steroid material. All carbon isotope delta measurements were normalised[3] using five reference materials with metrological traceability to the VPDB-LSVEC scale. The GC-C-IRMS was employed for homogeneity, stability, and confirmatory testing after CRM production.

Measurement uncertainty: Standard uncertainties were estimated and combined as described in the JCGM Guide to the Expression of Uncertainty in Measurement.[4] The combined standard uncertainties were expanded with coverage factors calculated from degrees of freedom obtained from the Welch-Satterthwaite equation. Based on the purity analysis of the starting materials, a potential for bias was estimated and included in the expanded measurement uncertainty using the SUMU_{max} approach.[5] The major contributors to the combined measurement uncertainty were potential bias from any impurity in bulk steroid material, method reproducibility, potential instability due to transport conditions and a very small contribution from the uncertainty associated with the calibrators. The uncertainty contribution from the calibration materials used for normalisation was estimated using Monte Carlo simulation. For each simulation iteration, 'true' values for each calibrator were randomly drawn from their normal distributions, and ordinary least squares regression used to generate a calibration function with the 'measured' values as the independent variable, and the 'true' values as the dependent variable. The output from 300,000 iterations of simulated values were normally distributed and the standard deviations were taken as the uncertainty related to the calibrator materials.

Raluca Iavetz
Manager Chemical Reference Values
7 August 2025
Report ID: MX024.2025.01

Accreditation No. 198

The property values specified in this report supersede any issued prior to 7 August 2025.

References:

1. WADA. *Technical Document - TD2022IRMS: Detection of Synthetic Forms of Prohibited Substances by GC/C/IRMS, Version 1.0, 1 January 2022.*
2. IUPAC *Technical Report: Assessment of international reference materials for isotope-ratio analysis,* Pure Appl Chem, 2014, 86, 425-467
3. Meier-Augenstein W, Schimmelmann A. *A guide for proper utilisation of stable isotope reference materials. Isotopes in Environmental and Health*, 2019; 55 (2): 113-128.
4. JCGM, *Evaluation of measurement data — Guide to the expression of uncertainty in measurement.* JCGM100:2008
5. Magnusson B and Ellison SLR. *Treatment of uncorrected measurement bias in uncertainty estimation for chemical measurements. Anal Bioanal Chem* 2008; 390:201-213.

[a] **IUPAC CIAWW Notice:** In 2017, IUPAC advised that LSVEC is no longer suitable for normalization of the VPDB scale but all carbon isotope delta measurements are to be normalized to VPDB using at least two appropriate international reference materials *(online: https://www.ciaaw.org/carbon-references.htm)*

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