

CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

NMIA MX020: Boldenone and Boldenone Metabolite 1 certified for Carbon Isotope Delta Value

Certified values

Steroid	CAS No.	$\delta^{13}\text{C}_{\text{VPDB}} / \text{‰}$	Coverage Factor (k)	V_{eff}
Boldenone	846-48-0	-30.38 ± 0.29	2.0	53
Boldenone Metabolite 1	10529-96-1	-29.71 ± 0.28	2.0	39

The measurands are the carbon isotope ratio delta values of the stated steroids relative to that embodied in the primary isotopic reference material VPDB. The uncertainties are expanded to provide a level of confidence of 95%.

Expiry: 30 August 2026

Batch No.: 2019.08

Description: 2 mL sealed ampoule containing a dry steroid mixture. The ampoule contains approximately 200 µg of each 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 1 mL of an appropriate solvent such as 2-propanol and allowed to equilibrate for 3 hours before use.

Storage: Store 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 USGS-40 (L-glutamic acid) and IAEA-CH-7 (polyethylene).²

Stability: The stability of the material under the recommended storage conditions has been verified using GC-C-IRMS and will continue to be monitored. The material was stable in an accelerated stability trial of two weeks at 40°C.

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

Production: The stock solution for the mixture was prepared by dissolving nominally 0.25 g of pure steroid in 250 mL of a mixture of 2-propanol and methanol prior to dispensing 0.2 mL aliquots into ampoules. The ampoules were vacuum dried for two days then flame sealed under argon and stored at 4°C.

Analytical method: EA-IRMS was employed to assign $\delta^{13}\text{C}_{\text{VPDB}}$ values to the pure steroid materials used for preparation of MX020. Calibration was performed using a two-point normalization approach³ with USGS-40 and IAEA-CH-7 providing metrological traceability to the VPDB scale. The uncertainty contribution from scale calibration and ¹⁷O correction by instrument software was estimated in each batch of analysis by using two CRMs (NBS 22 and IAEA-600) as quality control samples.

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 Welch-Satterthwaite equation. Based on the purity analysis of the starting materials the potential for bias was estimated and included in the expanded measurement uncertainty. The major contributions to the combined measurement uncertainty were potential biases, between-unit homogeneity, measurement reproducibility, potential instability due to transport conditions and uncertainty in the $\delta^{13}\text{C}_{\text{VPDB}}$ values of the calibration materials.



Raluca Iavetz
Manager Chemical Reference Values
30 August 2021
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Accreditation No. 198

References:

1. WADA. *Technical Document - TD2021IRMS: Detection of Synthetic Forms of Endogenous Anabolic Androgenic Steroids, Version 1.0*
2. IUPAC *Technical Report: Assessment of international reference materials for isotope-ratio analysis*, Pure Appl Chem, 2014, 86, 425-467
3. Debajyoti P, Skrzypek G and Istvan F, Normalisation of measured stable isotopic compositions to isotope reference scales – a review, *Rapid Comm in Mass Spectrometry*, 2007; 21:3006-3014.
4. JCGM, *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*. JCGM100:2008

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