Australian Government
Department of Industry, Innovation and Science

National Measurement Institute

CIPM MRA

NATA
ACCREDITED FOR TECHNICAL COMPETENCE

CERTIFIED REFERENCE MATERIAL
CERTIFICATE OF ANALYSIS

NMIA MX018: Steroid Mixtures certified for Carbon Isotope Delta Value

Certified values

Ampoule		CAS No.	$\delta^{13}C_{\text{VPDB-LSVEC}}$ / ‰	k	V_{eff}
MX018-1	**Etiocholanolone**	**53-42-9**	**–27.94 ± 0.24**	**2.0**	**41**
Batch No. 2017.12	**Androsterone**	**53-41-8**	**–27.79 ± 0.21**	**2.1**	**15**
	11-oxoetiocholanolone	**739-27-5**	**–13.58 ± 0.23**	**2.1**	**28**
	Testosterone	**58-22-0**	**–27.87 ± 0.24**	**2.1**	**24**
	11β-hydroxyetiocholanolone	**739-26-4**	**–29.51 ± 0.36**	**2.0**	**58**
MX018-2	**5β-androstane-3α,17β-diol**	**1851-23-6**	**–29.86 ± 0.16**	**2.0**	**57**
Batch No. 2017.12	**5α-androstane-3α,17β-diol**	**1852-53-5**	**–31.14 ± 0.24**	**2.0**	**52**
	Pregnanediol	**80-92-2**	**–16.79 ± 0.42**	**2.0**	**39**
	Epitestosterone	**481-30-1**	**–30.17 ± 0.36**	**2.0**	**50**
	11β-hydroxyandrosterone	**57-61-4**	**–28.59 ± 0.22**	**2.0**	**59**
MX018-3	**16-androstenol**	**1153-51-1**	**–30.96 ± 0.37**	**2.0**	**47**
Batch No. 2018.01	**Dehydroepiandrosterone**	**53-43-0**	**–31.63 ± 0.54**	**2.0**	**40**
	Testosterone	**58-22-0**	**–22.52 ± 0.33**	**2.0**	**54**

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

Expiry: 30 September 2020

Description: Three ampoules containing dry steroid mixtures in a 2 mL flame sealed ampoule. The ampoules contain approximately 400 µg of each steroid with the exception of 16-androstenol supplied close to 280 µg.

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[2]. In-vitro use only.

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

Instructions for use: Each ampoule must be reconstituted in 2 mL of appropriate solvent such as 2-propanol and allowed to equilibrate for 3 hours before use.

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

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

Metrological traceability: The certified carbon isotope delta values are traceable to the stable carbon isotope ratios of the primary isotopic reference materials NBS19 and LSVEC via the secondary isotopic reference materials IAEA-CH 6 (sucrose) and IAEA-CH-7 (polyethylene)[1].

Production: The stock solution for each ampoule mixture was prepared by dissolving 0.3 to 0.5 g of pure steroid in 250 mL of 2-propanol 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 $\delta^{13}C_{\text{VPDB-LSVEC}}$ values to the pure steroid materials used for preparation of MX018. Calibration was performed using a two-point normalization approach[3] with IAEA-CH-6 and IAEA-CH-7 providing metrological traceability to the VPDB-LSVEC scale. The uncertainty contribution from scale calibration and ^{17}O correction by instrument software was estimated in each batch of analysis by using four CRMs (NBS 22, IAEA-600, USGS 40 and USGS-24) as quality control samples. A second reference method using GC-C-IRMS was used for confirmation of δ-values of each steroid after preparation and ampouling the mixtures. This also demonstrated absence of any effects from potential impurities in the steroid starting materials. The target steroids were analysed using two GC capillary columns (DB17ms and VF5ms) to reveal any potential bias between the two reference techniques. Calibration and normalization of the GC-C-IRMS results were performed using two calibration mixtures prepared from eight steroid standards with $\delta^{13}C_{\text{VPDB-LSVEC}}$ values assigned in-house by EA-IRMS. Ten ampoules were analysed in duplicate in random order by each capillary column bracketed by two calibration mixtures. Linear correlation of the measured and true δ-values of steroids in calibration solutions was >0.999. The slope and intercept from linear regression allowed the measured δ-value of an unknown sample to be normalised to the reference scale. The absolute difference in δ-value between the EA-IRMS and GC-C-IRMS reference methods 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]. The major contributions to the combined measurement standard uncertainty were observed differences between results of the two reference techniques, between-unit homogeneity, measurement reproducibility, potential instability due to transport conditions and uncertainty in the $\delta^{13}C_{\text{VPDB-LSVEC}}$ values of the calibration materials. The combined standard uncertainties were expanded with coverage factors calculated from degrees of freedom obtained from Welch-Satterthwaite equation.

P Armishaw

Paul Armishaw
Manager Chemical Reference Values
7 December 2018
Report ID: MX018.2018.01

References:

1. IUPAC *Technical Report: Assessment of international reference materials for isotope-ratio analysis,* Pure Appl Chem, 2014, 86, 425-467
2. WADA. *Technical Document - TD2016IRMS: Detection of Synthetic Forms of Endogenous Anabolic Androgenic Steroids, Version 1.0,* 2016
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

CIPM MRA Notice: This certificate is consistent with the capabilities that are included in Appendix C of the CIPM MRA drawn up by the CIPM. Under the CIPM MRA, all participating institutes recognize the validity of each other's calibration and measurement certificates for the quantities, ranges and measurement uncertainties specified in Appendix C. The "CIPM MRA Logo" and this statement attest only to the measurement(s) applied for determining the certified values on the certificate (for details see http://www.bipm.org).

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.

END