A NEW STEROID REFERENCE MATERIAL TO UNDERPIN STABLE CARBON ISOTOPE RATIO MEASUREMENT IN ANTI-DOPING ANALYSIS

Fong-Ha Liu[1], J.P. Merrick[1], J.E. Murby[1]

[1] National Measurement Institute, North Ryde, NSW 2113, Australia

Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) is the only technique approved by the World anti-doping Agency (WADA) to detect doping with illicit synthetic forms of endogenous steroids like testosterone. This technique is able to differentiate a synthetic form of endogenous steroid based on the $^{13}C/^{12}C$ ratio expressed as delta (‰) value. Isotope ratio measurements are not absolute but are reported relative to an international standard as:

$$\delta^{13}C_{sample} = \frac{R\left(\frac{^{13}C}{^{12}C}\right)_{sample} - R\left(\frac{^{13}C}{^{12}C}\right)_{standard}}{R\left(\frac{^{13}C}{^{12}C}\right)_{standard}}$$

The validity of these measurements therefore relies on the availability and selection of reference materials for calibration. The principle of 'identical treatment' where samples and standards are processed similarly is critical for carbon isotope ratio measurement of urinary steroids. Accuracy and comparability of data between WADA laboratories can only be realised through certified reference material traceable to an international standard.

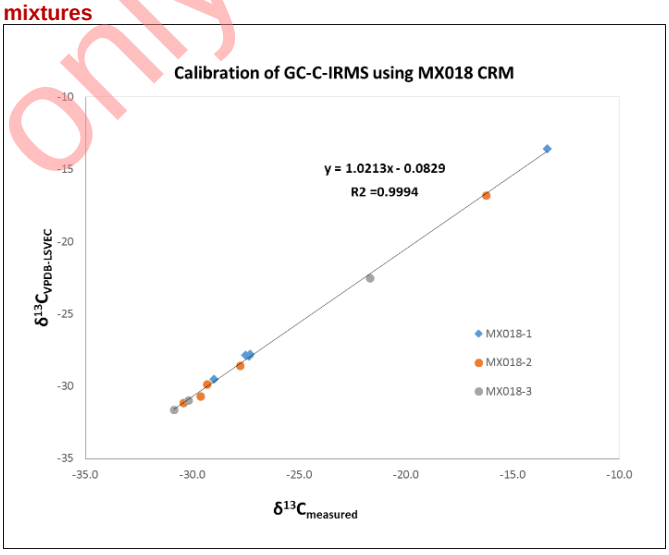
NMIA has produced a pure steroid CRM (MX018) certified by Elemental analyser-isotope ratio mass spectrometry (EA-IRMS) and GC-C-IRMS for $\delta^{13}C$ values with metrological traceability to the VPDB-LSVEC. This CRM facilitates a novel approach to carbon isotope calibration via GC-C-IRMS by eliminating the need to know accurately the $\delta^{13}C$ of reference CO_2 required by the conventional approach. This material assists the WADA laboratories in validating their accuracy and traceability of stable carbon isotope measurements in compliance with WADA protocol (TD2019IRMS).

The CRM is packaged as three ampoules containing thirteen steroids certified for $\delta^{13}C_{VPDB\text{-}LSVEC}$ from -13.58 to -31.63 ‰ (Table 1). A single injection of the MX018 mixtures following the identical treatment principle results in a typical calibration curve with linear correlation of R^2>0.999 (Figure 1). A measured $\delta^{13}C$ of an unknown sample within the calibration range can now be normalised to the international reference through $\delta^{13}C_{VPDB\text{-}LSVEC} = m.\delta^{13}C_{measured} + c$, where m refers to the slope and c the intercept of the linear regression equation.

Table 1. NMIA MX018 Steroid CRM

Ampoule		$\delta^{13}C_{VPDB\text{-}LSVEC}$ (‰)	k	V_{eff}
MX018-1	Etiocholanolone	–27.94 ± 0.24	2.0	41
	Androsterone	–27.79 ± 0.21	2.1	15
	11-oxoetiocholanolone	–13.58 ± 0.23	2.1	28
	Testosterone	–27.87 ± 0.24	2.1	24
	11β-hydroxyetiocholanolone	–29.51 ± 0.36	2.0	58
MX018-2	5β-androstane-3α,17β-diol	–29.86 ± 0.16	2.0	57
	5α-androstane-3α,17β-diol	–31.14 ± 0.24	2.0	52
	Pregnanediol	–16.79 ± 0.42	2.0	39
	Epitestosterone	–30.17 ± 0.36	2.0	50
	11β-hydroxyandrosterone	–28.59 ± 0.22	2.0	59
MX018-3	16-androstenol	–30.96 ± 0.37	2.0	47
	Dehydroepiandrosterone	–31.63 ± 0.54	2.0	40
	Testosterone	–22.52 ± 0.33	2.0	54

Figure 1: Calibration of GC-C-IRMS using MX018 steroid mixtures



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