"Production of a Certified Reference Material of Boldenone and Formestane to support GC-C-IRMS"

Paul Armishaw, Fong Liu (MNI, Commonwealth of Australia)

Project Overview

The aim of this project is to produce certified reference materials (CRMs) to ensure the accuracy and traceability of measurements of the stable carbon isotope ratios of steroids used to confirm Adverse Analytical Findings (AAF) in sports doping analysis. These CRMs will be used for validation of GC-C-IRMS methods and confirmation of Adverse Analytical Findings in accordance with WADA Technical Document TD2016IRMS. The ability of WADA-accredited laboratories to comply with this document is reliant on the availability of reference materials of appropriate steroids certified with traceable values for 13C isotope ratios. The availability of such materials is currently limited.

The proposed substances are boldenone, boldenone M1 and formestane. Certified values for the $\delta^{13}C$ values of each steroid and their associated measurement uncertainties will be determined by a combination of reference measurements with metrological traceability to VPDB made by NMIA using Elemental Analysis (EA-IRMS) and Gas Chromatography (GC-C-IRMS) Carbon Isotope Ratio Mass Spectrometry.

Result and Conclusion:

Two new CRMs have been prepared providing three steroids certified for stable carbon isotope delta values (*δ13CVPDB*). These materials have been designed to assist anti-doping laboratories to validate their calibration method for stable carbon isotope measurements of boldenone, boldenone metabolite and formestane to ensure accuracy and traceability in compliance with WADA Technical Document TD2019IRMS. The CRMs are packaged as dried steroids sealed in ampoules. MX020 consists of a mixture of boldenone and boldenone metabolite 1 and MX021 contains a single analyte, formestane.

Elemental Analyser Isotope Ratio Mass Spectrometry (EA-IRMS) was employed as the primary reference method to assign *δ*-values for pure steroid starting materials due to the low uncertainty associated with this technique. Calibration was performed using a two-point normalization approach [2] which employed USGS-40 and IAEA-CH-7 as calibration standards, permitting traceability to the internationally recognized Vienna Pee Dee Belemnite (VPDB) carbon isotope reference standard. Potential bias in the assigned EA-IRMS values was investigated both through theoretical modelling using the steroid starting material's purity data and through data obtained using the gas chromatography coupled to combustion isotope ratio mass spectrometry (GC-C-IRMS). Calibration and normalization of the GC-C-IRMS results were performed using the MX018 certified steroid standards with traceability to the VPDB reference. Homogeneity assessments of MX020 and MX021 were carried out using fifteen randomly selected ampoules.

Stability of the CRMs were verified by analysis of randomly selected ampoules after storage at 4 °C for a period of up to 5 months and at 40 °C for period up to 30 days.