PROJECT REVIEW

"Uniform calibration standards and methodology for GC-C-IRMS analysis of endogenous anabolic steroids"

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The detection of the abuse of anabolic steroids which occur naturally in the body is a special problem facing all WADA Accredited laboratories. The measurement of the testosterone to epitestosterone ratio (T/E) has been and is used as an effective means of detecting doping with endogeous anabolic steroids. A significant change in T/E ratio over time is strongly indicative of doping. This can be confirmed by the use of gas chromatography carbon isotope ratio mass spectrometry (GC-C-IRMS) as the isotopic abundance of the carbon present in synthetic endogenous steroids is different to that of the same steroid produced in the body. With the lowering of the T/E investigatory threshold from 6 to 1 to 4 to 1 there are now many more samples to be investigated. In order to avoid many ongoing investigations GC-C-IRMS is being used by more WADA laboratories to confirm that an elevated T/E ratio is natural. Different laboratories use different instruments and protocols which at present do not have a common set of standards on which to base their measurements. This means that whilst measurements of carbon isotope ratios made in one laboratory will be perfectly consistent the values obtained for an identical sample in a different laboratory could have a different numerical value. Whilst decisions on an individual are based on comparative measurements made on a sample it is still highly desirable for all WADA laboratories to produce the same or similar numerical values. With the mobility of athletes it is highly likely that tribunals will be asked to consider doping cases where GC-C-IRMS measurements have been made in different laboratories and should different values be obtained the opportunity for confusion exists. This project will investigate external calibration protocols for GC-C-IRMS instruments without the use of CO_2, compare different analytical methods currently used for preconfirmation and confirmation purposes, characterize standards and provide protocols that will enable more uniformity of reporting of GC-C-IRMS results in WADA laboratories.

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Results and Conclusions

The detection of abuse of testosterone (T) or prohormones in human urine doping control samples is an analytical challenge, because the main analytical technology in use, gas chromatography/mass spectrometry (GC/MS) cannot discriminate pharmaceutical from endogenous androgens. At present, longitudinal monitoring of the steroid profile and gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS) studies are used complementary in order to prove the application of exogenous androgens. In the last years, IRMS methods have assisted the elucidation of whether an analytical finding, regarding abnormal endogenous steroids concentrations and/or ratio of testosterone to epitestosterone, is due to an individually physiological steroidal profile or has occurred as a consequence of the exogenous application of a T-like prohibited substance. The methods are based on the difference in $^{13}C/^{12}C$ ratio between pharmaceutical T and endogenous human T and similarly between precursors and metabolites, such as dehydroepiandrosterone (DHEA) and dihydrotastosterone (DHT), to endogenous reference compounds not affected by the exogenous application. Carbon isotope ratios are not absolute values, but are reported as delta values ($\delta^{13}C$) relative to a reference material, such as $CaCO_3$ obtained from the Pee Dee formation in South Caroline (Pee Dee Belemnite, PDB). Currently, calculation of the delta value of the steroids depends on the reference gas pulses, CO_2. This isotopically calibrated gas is inserted from a gas cylinder via an independent secondary capillary directly into the ion source of the IRMS instrument. In this study, funded by the World Antidoping Agency (WADA), a new external calibration approach based on a calibration curve of the ratio of m/z 45/44 responses of steroids with certified $\delta^{13}C$ values is presented. The calculation of $\delta^{13}C$ values can be achieved in conjunction with the calibration curve from the ratio of m/z 45/44 for any steroid. Among the advantages of the use of a suitable reference calibrators curve was the ability to subject the calibrators through all the processes applied to urine samples including GC injection and separation, combustion and IRMS measurement. The reference calibrators were chosen in order to cover the entire range of the expected $\delta^{13}C$ values for the exogenous and endogenous steroids as well as the range of the expected chromatographic retention times. Furthermore reference calibrators could be common for all doping control laboratories. Data of the measurement uncertainty and accuracy for a second mixture with known isotopic composition in solution as well as for surrogate internal standard with known $\delta^{13}C$ value in real urine samples have been shown. The current project enhances the harmonisation of the GC-C-IRMS analyses among the WADA Accredited Laboratories.