PROJECT REVIEW

"Stable isotope ratio analysis of nandrolone and boldenone preparations"

Dr. Goebel, Dr. Brooker, (National Measurement Institute, Australia)

The World Anti-Doping Agency Prohibited List precludes the use of anabolic steroids including nandrolone and boldenone. The metabolites of nandrolone and boldenone can on occasion be found naturally at low concentration in some individuals. Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS) is the preferred method of confirming doping when a substance can both have been produced naturally by the body or through the ingestion of a prohibited substance. The use of GC-C-IRMS is recommended for samples containing metabolites of nandrolone and boldenone in the 2 ng/mL to 10 ng/mL range, as they could have been produced endogenously. GC-C-IRMS relies on the fact that synthetic versions of administered steroids are depleted in carbon-13 compared to those produced de novo. There is concern, however, that custom synthesis may produce carbon-13 enriched substrates likely to circumvent GC-C-IRMS confirmations. Analysis of commercially marketed and illegally derived ("black market") materials will provide intelligence concerning trends in steroid manufacture.

Furthermore, hydrogen is an element for which characteristic isotopic signatures may also be determined for steroids of endogenous and synthetic origin. The project proposes to measure the carbon and hydrogen isotope ratios of seized samples of nandrolone and boldenone over the course of a 12-month period to reveal any differences with values derived from legitimate pharmaceutical preparations that are known to be depleted in carbon-13. This project will build on the successful profiling of testosterone preparations (Cawley et al. 2010) by targeting an international sample population provided by WADA-accredited and law enforcement laboratories. GC-Thermal Conversion (TC)-IRMS analysis will be applied here to investigate the potential of hydrogen isotope ratios values to provide improved detection of administered nandrolone or boldenone that would otherwise be considered to be endogenous.

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Results and Conclusion

Previous studies have reported the use of chemical hydrolysis for the cleavage of testosterone esters. It was proposed that a similar reaction mechanism could be implemented for nandrolone and boldenone, however, the results revealed that a side reaction had occurred resulting in various rearrangement products. The current study presents a novel application of cholesterol esterase for the hydrolysis of steroid esters as an alternative to chemical hydrolysis.

Additional purification procedures were implemented to prepare legitimate and seized samples for CIR profiling. The validity of the complete method was thoroughly evaluated to ensure that the analysis procedures did not hinder the measurement of $\delta^{13}C$ values of the samples. The method presented herein has demonstrated its capacity to determine the $\delta^{13}C$ values of nandrolone, testosterone and boldenone esters (n=62) and is an improvement to former chemical methods. Previous methods focused on CIR profiling of only testosterone, whereas the described method allows δ13C profiling for a variety of steroid esters. However, the limiting analytical issue of the low amount of ester that could be hydrolysed in any single experiment only allowed measuring of the $\delta^{13}C$ values for nandrolone and boldenone and could not be scaled up for analysis for $\delta^{2}H$. The higher levels of analyte required for $\delta^{2}H$ analysis for nandrolone and boldenone also means that there are serious issues with measuring these steroids in urinary samples for $\delta^{2}H$ in the low ranges needed (2 ng/mL to 10 ng/mL for nandrolone and 5 ng/mL to 30 ng/mL for boldenone Stable isotope ratio analysis of Nandrolone and Boldenone preparations.

This research was the first comprehensive $\delta^{13}C$ profiling study of synthetic nandrolone and boldenone preparations. The results demonstrate that CIR analysis enables the detection of administered nandrolone and boldenone since available data for current preparations shows $\delta^{13}C$ values differ significantly from values that can be considered endogenous. Most importantly for doping control, proof of concept was accomplished. The finding that there were no nandrolone or boldenone preparations in this study were found to possess $\delta^{13}C$ values within the endogenous zone strengthens current protocols for the confirmation of anti-doping violations. The results from this study provide intelligence for the field of sports drug testing and will be made available to WADA accredited laboratories.