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Stable isotope ratio analysis in sports anti-doping

I thank Professor Mario Thevis for the opportunity to guest edit this issue of *Drug Testing and Analysis* dedicated to stable carbon isotope ratio ($\delta^{13}C$) analysis for sports anti-doping. Since the presentation of Southan *et al.*[1] in 1990 and the publication of Becchi *et al.*[2] in 1994, considerable efforts have been undertaken by laboratories accredited by the International Olympic Committee (IOC) and the World Anti-Doping Agency (WADA) to develop suitable methodologies in this area. WADA's requirement for all accredited laboratories to possess the capability to perform gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) within a demanding forensic environment has placed additional pressure on those groups confronting an ever-changing landscape of doping methods and technologies to combat them. During the period 1995–2001, laboratories undoubtedly considered GC-C-IRMS to be an extension of conventional gas chromatography–mass spectrometry (GC-MS) steroid profiling relying on amount-of-substance measurements. This 'plug and play' philosophy rapidly encountered problems in understanding the instrumental hardware and pharmacological variables associated with accurate determination of $\delta^{13}C$ values. At a time when much of the anti-doping focus was on developing proteomics expertise, it was not easy for laboratories to devote resources to a method that was first considered simple. Coupled with this was an expectation from national anti-doping organizations, international federations and host organizations that the 'new machine' purchased for the laboratory at considerable cost would provide immediate answers to the question of testosterone misuse. To begin this special issue Ulrich Flenker provides a timely and relevant review of these questions for GC-C-IRMS in doping control.[3]

The application of GC-C-IRMS analysis to the anti-doping arena has been put to the test in recent years with rigorous defence required of adverse analytical findings pertaining to high-profile athletes.[4–6] Such events had been anticipated by anti-doping laboratories, but each situation and the potential for it to contribute legal precedence reminds analysts of the standard required when performing complex measurements. Thankfully, frivolous claims of poor quality analytical data made by representatives outside of the anti-doping effort have proven to be unfounded and appeals made to the Court for Arbitration of Sport subsequently dismissed.[7] Nonetheless, these cases have highlighted the potential difficulties that lay audiences may have in interpreting stable isotope ratio data. To meet this challenge forensic science practitioners are increasingly using likelihood ratios.[8] To this end, Flenker *et al.*[9] provide a perspective that describes the advantages of a Bayesian approach to presenting stable isotope ratio measurements.

The confidence of laboratories to interpret $\delta^{13}C$ data has been further improved by access to certified steroid reference materials. This issue contains work by Zhang *et al.*[10] investigating calibration and data processing methods, Munton *et al.*[11] describing the certification of a fit-for-purpose matrix reference material enabling estimations of measurement uncertainty, and Yiannis *et al.*[12] investigating isotopic fractionation from the acetylation of steroids.

With much of the foundation work adapting GC-C-IRMS to the anti-doping context now completed, the present situation sees laboratories investigating the potential of stable isotope ratio technology to provide more than an answer to the question of testosterone origin. Pharmacological manipulation prescribed for legitimate medical treatment also affords rogue athletes the opportunity to attempt 'back-door' methods for increasing endogenous testosterone and dihydrotestosterone. One example presented by Piper *et al.*[13] deals with confirming administration of the aromatase inhibitor 4OH-androstenedione. Furthermore, this article describes the metabolic distinction made possible by GC-C-IRMS to differentiate the administration 4OH-androstenedione from androstenedione. Metabolic investigations are elaborated further in this issue with the review that promotes the complementary use of $\delta^{13}C$ measurements from GC-C-IRMS analysis and conventional amount of substance measurements from GC-MS steroid profiling.[14] Continuing this theme, Brooker *et al.*[15] provide a comprehensive study of the cortisone/cortisol metabolic pathways.

Hardware developments to improve GC-C-IRMS analysis have not ceased either. The integration of a GCxGC platform is presented by Brailsford *et al.*[16] to simplify sample purification for the IRMS analysis of underivatized urinary steroids.

So where will stable isotope ratio analysis lead anti-doping in the future? Proof of concept for the use of $^{2}H/^{1}H$ ($\delta^{2}H$) measurements has recently been accomplished by Piper *et al.*[17] A complementary approach using bivariate stable isotope signatures (i.e. $\delta^{13}C$ and $\delta^{2}H$) would provide additional evidence for the exogenous origin of urinary steroids and potentially increase post-administration detection periods. Stable isotope ratio measurements will also feature in the paradigm shift from population-based reference ranges towards individual subject-based monitoring. The evolution of the Athlete Steroidal Passport containing longitudinal data for a large number of urinary steroid metabolites will greatly enhance detection of steroid misuse and enable an improved intelligence-based anti-doping effort.

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