Development of an alternative method to detect the exogenous origin of testosterone and its metabolites via GC/C/IRMS

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In professional sport testosterone is one of the most commonly used substances for doping. However, it is an endogenous substance and therefore, the detection of illicit intake is challenging. Since the isotopic composition of natural steroids differs from synthetic counterparts, they can be distinguished via isotopic ratio mass spectrometry. As this method is susceptible to contaminations the sample has to undergo many steps of a sophisticated extraction procedure to reduce the urine matrix.

Current methods to detect the misuse of endogenous anabolic steroids in anti-doping control rely on enzymatic hydrolysis of urinary steroid glucuronides, HPLC purification of the free steroids afterwards and final analysis via GC/C/IRMS. [1] We propose an alternative method for sample preparation that utilizes HPLC separation of conjugated steroids followed by acidic solvolysis, which is not only limited to glucuronidated moieties. Positive and negative samples can be identified according to the WADA guidelines [2] with this proof of concept method and give results similar to the currently used method for routine analysis.

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[1] Ouellet, A. , LeBerre, N. and Ayotte, C. (2013), A simplified and accurate method for the analysis of urinary metabolites of testosterone-related steroids using gas chromatography/combustion/isotope ratio mass spectrometry. Rapid Commun. Mass Spectrom., 27: 1739-1750. doi:10.1002/rcm.6620

[2] World Anti-Doping Agency (2018), Detection of Synthetic Forms of Endogenous Anabolic Androgenic Steroids by GC/C/IRMS, WADA Technical Document – TD2019IRMS, Available: https://www.wada-ama.org/sites/default/files/td2019irms_final_eng_clean.pdf