“Confirmation of formestane abuse in sports: a metabolic approach”

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PROJECT REVIEW

Formestane is an anti-estrogenic drug used on the treatment of breast cancer. In humans, estrogens are strong pituitary inhibitors of gonadotrophins releasing factors. The inhibition of the estrogens synthesis produces an increase of luteinizing hormone (LH) and then a net increase of testosterone production is expected. In addition the combined administration with testosterone will reduce the side effects linked to aromatization like gynecomastia. For these reasons, anti-estrogenic substances were included in 2004 in the World Anti Doping Agency (WADA) List of Prohibited substances.

The analytical methodologies developed so far are based GC/MS or LC/MS, targeting formestane itself. Traces of formestane can be produced endogenously and detected in urine samples in low concentrations(0.5-20 ng/mL) and thus, since 2011, it is mandatory according to WADA rules to perform a confirmation based on isotope ratio mass spectrometry (IRMS) in order to assess the synthetic origin of formestane before releasing an adverse analytical finding.

The IRMS developed methods require two consecutive liquid chromatographic purifications (HPLC) before obtaining extracts of adequate purity, and not all laboratories are currently prepared to perform such IRMS analyses. The metabolism of formestane analysis has been extensively described in a single male volunteer. It appears that among the high number of metabolites described 4a-hydroxy-epiandrosterone has a longer detection window. A metabolic approach based on the detection of specific metabolites of formestane may avoid the use of IRMS for the confirmation of formestane intake, thus reducing the cost and complexity of the analyses. Simultaneously in the case IRMS should still needed, the development of a method for the detection of long term metabolites, excreted for a longer time compared to formestane and in larger amounts in the elimination phase, will certainly improve the detection capacity of formestane.

Results and Conclusions :

Formestane confirmation by IRMS is not an easy task and the method is not yet implemented in all WADA accredited laboratories. IRMS confirmations are time consuming and generate additional cost to the Testing Authorities. The aim of this work was to improve the knowledge on formestane detection and on its metabolism in order to find specific biomarkers that may reduce to the

minimum or even discard the need of IRMS. To do so, oral and transdermal excretion studies were performed and the metabolism of formestane was studied. The first observation is that if no specific method is applied (MS/MS) the estimation of formestane concentration in urine is overestimated, its detection may interfere with the detection of 2-hydroxyandrostenedione. The 4-hydroxylation of steroids is residual while the 2- hydroxylation that is much more important.

The main specific metabolites of formestane (4OH-AED), detected in different proportions depending on the administration route, are 4αOH-epiandrosterone (4OHEA) and 4αOH-androsterone (4OH-A). These metabolites have not been detected in urine samples so far. Their endogenous origin can be most probably discarded, masking their detection specific to trace back a formestane administration. The detection of these metabolites and some metabolic ratios (4OH-EA/4OH-AED, 4OHA/4OH-AED and 4OH-EA/4OH-A) may allow to detect the exogenous administration of formestane and to discriminate the route of administration without the need of using IRMS.