

## PROJECT REVIEW

### **“Metabolism of anabolic androgenic steroids: evaluation of sulphate conjugated metabolites to improve detection capabilities”**

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There is a need for doping control laboratories to improve the detection capabilities of the administration of anabolic androgenic steroids (AAS). Most AAS are extensively metabolized. Studies on steroid metabolism have been traditionally performed using GC-MS methods. Recently, LC-MS systems have demonstrated wide possibilities for the elucidation of new phase I metabolites and the use of this technology has resulted in the detection of previously unreported metabolites. Phase II metabolic reactions of AAS have been normally studied by using specific hydrolysis of the conjugated metabolites present in urine extracts. In most cases, enzymes with  $\beta$ -glucuronidase activity were used, thus mainly conjugates with glucuronic acid have been studied up to now using both GC-MS and LC-MS/MS approaches.

Sulfate metabolites are known to be important for some endogenous steroids and they have also been described for exogenous AAS. However, the study of sulfates has been limited by the difficulties of their efficient hydrolysis to the phase I metabolites detected by GC-MS or LC-MS/MS. In spite of this, long-term sulfated metabolites have been reported for some AAS.

The objective of the project will be the evaluation of the phase II metabolism of AAS to look for sulfate conjugates of steroid metabolites that could be used as long-term markers of AAS misuse. A methodology based on the direct analysis of sulfate conjugates by LC-MS/MS will be developed. The different scan methods will be developed to identify unknown sulfate metabolites in administration study samples of different AAS. The excretion profiles of the identified sulfate metabolites will be compared with those of other steroid metabolites targeted in conventional screening procedures, in order to evaluate their interest as long-term markers of steroid misuse. Finally, a methodology addressed to the reliable detection of identified sulfate metabolites in routine antidoping analysis will be developed

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### **Result and Conclusion:**

The objective of the project was to study the phase II metabolism of anabolic androgenic steroids (AAS) to look for sulphate conjugated metabolites that could be used to improve detection capabilities of AAS misuse. A methodology based on the direct analysis of sulphate conjugates by LC-MS/MS was developed. A liquid-liquid extraction with ethyl acetate was used to extract steroid sulphates from urine samples. Based on the common mass spectrometric behavior of steroid sulphates, precursor ion and neutral loss scan methods, and selected reaction monitoring methods including theoretical transitions of potential metabolites, were used to detect new sulphate metabolites in post-administration samples. AAS studied were boldenone, boldione, methyltestosterone and metandienone. Boldenone sulphate and epiboldenone sulphate were identified as minor metabolites of boldenone in humans. These metabolites were detected in urine during the same time as the main metabolites (boldenone and  $5\beta$ -androst-1-en-17 $\beta$ -ol-3-one, BM1, detected in the glucuronide fraction). The analysis for the absence of these sulphates could be used as additional criterion of the endogenous origin of boldenone and BM1 in samples with low concentrations of these metabolites before going to GC/C/IRMS analysis. For boldione, seven metabolites conjugated with sulphate were detected. Three novel sulphate metabolites of methyltestosterone were identified and one of them was detected in urine for long time after administration, increasing the retrospectivity of the detection between two and three times with respect to other metabolites described. For metandienone, seven sulphate metabolites were detected in post-administration samples and one of them, was detected in urine for long time after administration, doubling the detection time compared to the last long-term metabolite described (the same phase I metabolite excreted in the glucuronide fraction).

The results of the project demonstrate the importance of sulphatation as a phase II metabolic pathway for AAS and the interest to study this metabolic fraction to look for new metabolites of AAS to improve the capabilities (e.g. long-term metabolites) of the detection of these compounds in doping controls.