PROJECT OVERVIEW

"Suitability of in competition testing in blood compared to urine matrix"

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Since the beginning of the fight against doping, urine is considered to be the preferred biological fluid in which to examine the most commonly used performance enhancing compounds. This was an obvious choice because urine acts as a waste bin where xenobiotics are deposited by the organism. The first analytical techniques used in the fight against doping were thus tailored to detect drugs or drug metabolites at concentrations that can be found in urine. The use of this biological fluid, which could be collected non-invasively during anti-doping tests, became prevalent. Samples could be easily prepared from the urine matrix, and the detection range of performance enhancing drugs was such that it was possible to demonstrate their in-competition use to enhance performance.

Nowadays, analytical techniques have significantly improved in terms of sensitivity and specificity. These improvements allow the scientists to detect forbidden drugs at very low concentration and for a longer time period in multiple different matrices. Even if the blood collection is an invasive procedure, the simplicity of the collection protocol is an important aspect to consider for athletes and doping control officers. Furthermore blood is becoming a biological matrix more and more considered within the antidoping and sport organizations and thus the detection in blood of substances included in the WADA Prohibited list is becoming evident.

The aims of this pilot study are to evaluate the sensitivity and specificity detection, in urine and blood, of 15 different compounds that are the most reported as adverse analytical findings by the WADA laboratories. Samples will be collected after a single dose administration to 3 volunteers for each substance. Finally, we will compare two analytical techniques (LC-MSMS and GC-MSMS) to appraise their use in the fight against doping in urine and blood matrices.

Results and Conclusions

During this project, analytical developments for the detection in blood of compounds like the major endogenous and exogenous steroids as well as opioids and clenbuterol were done. The quantification of endogenous steroids linked to testosterone metabolism is well documented and robust in urine matrix. The blood concentrations of these compounds are important also to give another tool to resolve the difficult topics of testosterone and related

substances abuse in sports. The future advent of the steroid module of the athlete passport as well as the establishment of reference values for the athlete population was the main motivation of this part of the project. Sample preparation was the main challenge to solve as the extraction of the investigated compound from the blood matrix is totally different than the one used for urine preparation. Different approaches were tested and a simple extraction using a solid support for a liquid extraction (SLE) was selected. Then limit of detection, linearity and first validation data are promising for a near future accomplishment of this part of the project. Collaboration with the London AntiDoping laboratory is in progress to compare the two different approaches for the quantification of testosterone and epitestosterone in blood. Results are supposed to be available in the following months.

The main exogenous steroids were detected in blood by LC-MS/MS in pg/mL ranges. For all the screenedsubstances a LOD of 100 pg/mL was reached and for few specific compounds a 10 pg/mL LOD was obtained. Comparison of the sensitivity and the time for sample preparation bet ween LC-MS/MS and GC-MS/MS revealed that the first one is more suitable and allowed to reach a lower LOD for the exogenous steroids.

Opioids quantification in blood was developed and totally validated. Comparison with immunological tests and the GC/MS quantification showed a good correlation indicating that the use of blood for the opioids detection is fit for purpose.

Clenbuterol quantification has been developed to evaluate the complementary information that blood could represent in the context of food contamination or doping abuse in case of adverse analytical finding with clenbuterol. Again, first data obtained regarding LOD, linearity and excretion study samples analyses showed that the developed sample preparation and LC-MS/MS analytical tool were adapted to this purpose.

This work gave the opportunity to the laboratory to handle with blood matrix for the detection and quantification of forbidden substances. Perspectives are to continue on this way in order to finish the ongoing validation process. Future implementation of blood analyses for the detection of drug of abuse in sports will be of high interest.