

PROJECT REVIEW

“Synthesis of recently reported phase II metabolites for their inclusion in routine doping control analysis”

Dr. O. Pozo, Dr. J. Segura, Dr. R. Ventura (IMIM- Hospital del Mar Medical Research Institute)

Availability of reference materials is crucial in doping control since they are needed to confirm adverse analytical findings and to develop quantitative methods. Most of the analytes included in doping control routine methods are phase I metabolites released to urine after enzymatic hydrolysis. However, the direct detection of phase II metabolites by LCMS/ MS is a common trend in doping control analysis. Therefore, the synthesis of phase II metabolites will become more important for the field in the next years.

In previous WADA funded projects, our research group has identified several phase II metabolites which are useful for the detection of the misuse of several anabolic steroids. Among them, preliminary results indicate that 6 β -hydroxyandrosterone glucuronide and 6 β hydroxyetiocholanolone glucuronide can improve the detection of testosterone misuse. Several sulphate metabolites like epiboldenone sulphate or 17 β -methyl-5 β -androstane-3 α ,17 α -diol 3 α sulphate can help in the detection of boldenone and methyltestosterone misuse. The structure of these metabolites has been confirmed by their synthesis at small scale. This synthesis at small scale was necessary because none of the detected metabolites is commercially available.

Larger amounts of the metabolites are needed for their complete characterization by NMR techniques. Besides, the availability of these larger amounts would also allow for the development of analytical approaches able to detect and confirm their presence in urine samples.

Therefore, the goals of this project are (i) to synthesize at a larger scale several phase II metabolites previously identified as useful for the doping control field (6 β -hydroxyandrosterone glucuronide, 6 β -hydroxyetiocholanolone glucuronide, epiboldenone sulphate, 17 α -methyl-5 β androstane-3 α ,17 β -diol 3 α -sulphate, 17 β -methyl-5 β -androstane-3 α ,17 α -diol 3 α -sulphate and 17 β methyl-5 α -androstane-3 α ,17 α -diol 3 α -sulphate), (ii) to fully characterize them by well established techniques (NMR spectroscopy, mass spectrometry), (iii) to study their stability and (iv) to develop analytical approaches to quantify conjugated metabolites of testosterone (6 β hydroxyandrosterone glucuronide, 6 β -hydroxyetiocholanolone glucuronide). The synthesized standards will be distributed among WADA accredited laboratories.