PROJECT REVIEW

"Detection of sulfo-conjugated anabolic steroids metabolites in antidoping initial and confirmatory analysis"

Dr. Lyris, Dr. Angelis, Dr. Tsivou, Dr. Fragkaki (Doping Control Laboratory of Greece, Greece)

Anabolic androgenic steroids (AAS) are the most frequent abused substances in sports and are included in the World Antidoping Agency (WADA) List of prohibited substances. AAS are extensively metabolized in the liver and their target tissues. The metabolic pathways are divided into phase-I and phase-II reactions. Phase-I reactions involve oxidation, hydrolysis and reduction, which introduce new functional groups for the subsequent phase-II reactions (i.e. conjugation).

For the endogenous androgens and for exogenous AAS the main phase-II reactions are conjugation with glucuronic acid (glucuronidation) or with a sulfo-moiety (sulphatation). The screening methods used by the Antidoping Laboratories usually focus on those metabolites, that are excreted unconjugated or as glucuronides into the urine. Extraction of the gluco-deconjugated steroids from the matrix and concentration of the analytes is performed by liquid-liquid extraction (with diethylether or tertbutylmethylether) or solid phase extraction followed by mass spectrometric detection either by liquid chromatography mass spectrometry or gas chromatography mass spectrometry. By using this initial screening extraction protocol the Antidoping Laboratories substantially ignore the sulfo-conjugated part of AAS. Nevertheless there are AAS for which the longer detected metabolite is a sulfo-conjugate. Sulfo-conjugated AAS are relatively easy to be detected directly since the development of instrumentation providing interfacing of liquid chromatographic (LC) separation to mass spectrometric (MS) detection, especially via electrospray ionisation (ESI), has opened up broad possibilities for the direct analysis of sulfo-conjugated substances. Moreover, sulfo-conjugated substances can be extracted from urine using ethyl acetate, instead of diethylether, as extraction solvent. The objectives of this project will be : a) t develop a screening method for the already known sulfo-conjugated metabolites of AAS, and b) To investigate the existence of not yet reported sulfo-conjugated metabolites of AAS, that can improve detectability and identification in either initial screening protocol or confirmation methods.