PROJECT REVIEW

"Chemical derivatization of intact phase II metabolites of AASs for confirmatory purposes"

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The direct detection of phase II metabolites of doping substances suitable for publication on WADA's website and steroids using LC-ESI-MS/(MS) in particular, is a current trend in doping control, offering simplicity, sensitivity and time effectiveness. This approach enables the detection of a great range of phase II metabolites, as the conjugation with glucuronic and/or sulfuric acids clearly improves the ionization efficiency of steroids. Recently, the detection of abundant and/or long term sulfate metabolites has been reported for exogenous AAS using LC-ESI-MS/(MS). However, metabolite characterizations and/or confirmation procedures of sulfate metabolites rely mainly on GC-MS/(MS) methods. This is due to the fact that their analysis in positive ionization mode lacks sensitivity, whereas in negative ionization mode, Collision Induced Dissociation (CID) experiments show dominant product ions that are limited to sulfate moiety and lack structural information. Typically, steroid metabolites have additional functional groups to conjugation site, usually hydroxyls and keto groups, that are prone to derivatization. Chemical derivatization can enhance the ionization efficiency of Phase II metabolites in positive ionization mode, whereas CID product ion spectra are not limited to ions related to conjugated group, alter their fragmentation behavior and hence they can be used as a robust alternative confirmation procedure. This is a novel approach that may facilitate the confirmation of sulfate metabolites, since the laborious deconjugation step will be skipped. The simultaneous confirmation of intact sulfate and glucuronide metabolites will be examined in specific cases as the proposed methodology permits the confirmation of both sulfo- and gluco-conjugated metabolites possessing specific structural elements like keto groups with a single run. The herein proposed methodology may also used in the future as a complementary tool for the structural elucidation of newly found Phase II metabolites by LC-ESI-MS/(MS).