

Project Review

“Precursor ion scanning for the detection of new steroid markers and the development of an LC-MS screening method for these markers”

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Anabolic steroids are amongst the most misused substances in doping control and are intensively metabolized in humans. Adequate screening for misuse of these substances therefore relies on the detection of metabolites in urine samples collected from athletes.

Most of the studies investigating the metabolism of pharmaceutically available steroids were performed in the 1980's via gas chromatography mass spectrometry (GC-MS). This research resulted in the selection of appropriate metabolites for the detection of steroid misuse. Over the years the selection of metabolites was further elaborated to include several metabolites that can result in prolonged detection times.

Over the last decade, liquid chromatography tandem mass spectrometry (LC-MS_n) was introduced as a screening technology in doping control laboratories world-wide. As a result several steroids that are difficult to detect via GC-MS became readily detectable. However, it should also be noted that several easily detectable metabolites by GC-MS are virtually undetectable via LC-MS. Indeed, both GC-MS and LC-MS are compatible techniques and the simultaneous application of both technologies is needed to cover the detection of all categories of structures.

Recently, it has been shown that precursor ion scanning via LC-MS can be used to detect new steroid metabolites and the use of this technology has resulted in the detection of previously unreported metabolites. These results have also illustrated the limitations of GC-MS for the detection of steroid metabolites. Indeed, some highly abundant metabolites in the LC-MS analysis could hardly be detected by GC-MS. This has highlighted the need for a re-investigation of steroid metabolism to allow for their adequate detection by LC-MS. Preliminary results for several steroids indicate that detection times could be prolonged considerably if more appropriate LC-MS metabolites are selected.

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Results and Conclusions

The use of LC-MS/MS in neutral loss and precursor ion scan modes allows for the detection of steroid metabolites. Several strategies based on this methodology have been applied for metabolic studies of some anabolic steroids. Previously unreported metabolites have been detected. In the case of methyltestosterone one of these metabolites increased the detectability of the steroid misuse. For stanozolol, the screening in negative ionization mode for one of the detected metabolites also improved the detection to stanozolol misuse. Several real samples have been analysed for these metabolites showing their applicability. For other steroids, additional metabolites have been found although more experiments are needed in order to prove their usefulness for doping control analysis.

Publications

Pozo OJ, Van Eenoo P, Deventer K, Lootens L, Van Thuyne W, Parr MK, Schanzer W, Sancho JV, Hernandez F, Leroux-Roels G, Meuleman P, Delbeke FT. Detection and characterization of a new metabolite of 17 α -methyltestosterone. *Drug Metab Dispos.* (2009) in press
Pozo OJ, Van Eenoo P, Deventer K, Lootens L, Grimalt S, Sancho JV, Hernández F, Meuleman P, Leroux-Roels G, Delbeke FT. Detection and structural investigation of metabolites of stanozolol in human urine by liquid chromatography tandem mass spectrometry. *Steroids.* (2009); 74(10-11):837-52.