"Precursor ion scanning for the detection of new steroid markers. Routine application for the open screening of anabolic steroids and evaluation of population factors in the detectability of these markers"

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PROJECT REVIEW

This project aims at four goals:

A. Interpretation and evaluation of precursor ion scanning chromatograms (urine profile recognition)

In the field of anti-doping control, chromatograms from target steroid-analysis are generally evaluated and interpreted visually by the analyst which is trained for this purpose.

A similar approach will be adopted for evaluating the precursor scanning chromatograms. To get acquainted with the chromatograms, around 50 blank samples will be analysed. In a second step precursor ion scan chromatograms obtained from controlled administration studies (e.g. methyltestosterone, methanedienone,…) and from adverse analytical findings from routine target screening will also be investigated to get acquainted with positive samples. The applicability of instrument software for this purpose will be evaluated.

B. Application to real samples

Urine-samples will be analysed. These samples will include all out of competition samples and both out of competition samples and in competition samples

C. Study of the influence of different population factors in the detectability of different markers for several anabolic steroids

A single dose of the previously studied steroids (stanozolol and methyltestosterone) will be administered to six volunteers belonging to different population groups. Urine will be collected for three weeks and a method including all feasible markers will be applied. The best marker(s) will be selected based on the results obtained. This procedure will also be followed if promising new markers are found in the re-evaluated steroids.

D. Re-evaluation of the metabolism of additional steroids by LC-MS/MS looking for alternative markers for the detection of steroid misuse

Three or four additional steroids will be re-evaluated via the analysis by LC-MS. These steroids will be selected based on the availability of excretion studies in both laboratories.

Appropriate precursor scan and/or neutral loss scan methods will be applied for each steroid depending on their structure. Feasible metabolites will be characterized by MS techniques. The metabolic nature of these metabolites will be determined, if necessary, by the analysis of chimeric mouse urine after the drug administration. A full excretion study will be analyzed in order to determine the long term metabolites.

RESULTS AND CONCLUSIONS:

Anabolic steroids with a 3-keto-4-ene structure have in common that they fragment by LC-ESI-MS/MS at high collision energy in tree common ions: methyltropylium (m/z 105), tropylium (m/z 91) and phenyl ion (m/z 77). The use of a precursor ion scan method for these 3 ions would allow to detect new/unknown AAS and metabolites with 3-keto-4-ene structures.

In this project, the potential of approaches based on precursor ion scan for doping control purposes have been evaluated in two scenarios: (i) routine application and (ii) metabolic studies of AAS.

In the routine application, the potential use of the approach in two laboratories (Gent and Barcelona) was explored. The analysis of a common set of 12 samples allowed for the confirmation of the suitability of the method for the detection of positive samples for known steroids. The analysis of this common set of samples also revealed several limitations in the harmonization between laboratories regarding relative retention times and the precursor ions observed.

During the whole project, a total of 1911 routine-samples were analyzed using the established precursor scan protocol by the two laboratories. These samples covered both samples from athletes from high-risk sports (strength sports) and urines samples from athletes who tested positive for known steroids. Seven suspicious samples were detected. Despite the application of several analytical strategies such as product ion scan in both positive and negative ionization mode with accurate mass measurements and GC-MS, none of them could be clearly assigned to belong to the AAS family.

Regarding metabolic studies, the method allowed for the detection of several metabolites for boldione, 4-chloro-methanedienone and clostebol. Several previously unreported metabolites were detected and characterized.

Finally, the effect of population factors in the detectability of long-term metabolites was evaluated in two different scenarios: stanozolol and clostebol. Results showed that both 17-epistanozolol-*N*-Glucuroinide and 4-chloro-5-androst-3β-ol-17-one 3β-sulfate are the long-term metabolites for stanozolol and clostebol irrespective of the ethnicity of the volunteer.