"Direct urinalysis of steroids"

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Aims

Anabolic steroids are the most frequently detected prohibited substances in sports. Analysis for these substances is routinely performed using gas chromatography-mass spectrometry (GCMS) and liquid chromatography mass spectrometry (LC-MS). Although the detection capabilities of LC-MS are limited for the detection of steroids with androstanediol as a back bone to their structure, LC-MS is often more sensitive for many other steroids. Moreover, recently several publications have shown that LC-MS can detect steroids (glucuronides) directly in urine without any sample preparation.

The advantages for such a direct urinalysis are multi-fold. It reduces costs, required volume of sample and analysis time, but also it allows for the direct detection of intact conjugated steroids rather than the liberated steroid molecules after hydrolysis.

In the past several small scale attempts have been made to detect these intact steroid conjugates in urine. These attempts were quite successful, but did not meet WADA's minimum required performance limits. Recently however several scientific improvements have been made, including more sensitive instruments, optimized mobile phase compositions (e.g. methanol instead of acetonitrile), which now allow for the detection of several steroids well below WADA's MRPL. These preliminary results (e.g. 19-norandrosterone glucuronide) show that the direct urinalysis of steroid conjugates at concentrations at or below the MRPL is achievable.

Results and Conclusion

The aim of this project was to study intact conjugated anabolic steroids in urine in comparison with the traditional methods where the aglycones are detected after enzymatic hydrolysis. This approach permitted the detection of anabolic steroids misuse by liquid chromatography mass spectrometer since the glucuronide and sulfate groups are easy to ionize by ESI. Consequently, steroids with a sulphated or glucuronidated androstane framework could be detected. Research resulted in the development of a multi-target analytical method for the screening of different exogenous anabolic steroids and also for the quantification of endogenous anabolic steroids.

The use of a dilute-and-shoot strategy allowed to minimize sample preparation yielding a short turnaround time. Additionally a small volume of urine can be used. This is a great advantage compared with traditional methodologies where liquid-liquid extraction or solid phase extraction after

enzymatic hydrolysis and detection by LC-MS or GC-MS (after derivatisation step) is applied.

The use of excretion studies or synthesis *in vitro* are of great interest since the number of conjugated anabolic steroids commercially available is still very low. With this methodology new conjugated anabolic steroids were detected and included in the multitarget method.

The developed DS-LC-MS have been applied to HRMS and to QqQ-mass spectrometers. Generally, sensitivity of the high resolution mass spectrometer is higher than the triple quadrupole. However in some cases like boldenone metabolites, less interferences were detected by triple quadrupole mass spectrometer improving their detection compared with HRMS.

Due to the presence of an extra and selective transition in both positive and negative mode (loss of the glucuronide moiety) a sensitive confirmation method was developed. A triple quadrupole system was chosen due to the higher accessibility for anti-doping laboratories to these kind of detectors compared with a high resolution mass spectrometer. The dedicated sample preparation consisted in a single solid-phase extraction avoiding enzymatic hydrolysis, was developed for confirmation purposes. The confirmation method was validated and the obtained limit of detection was 50 pg/mL allowing to detect stanozolol-abuse in the low pg/mL range. In fact this sensitive method has allowed an increment of the number of positives by approximately 27% compared with previous methods.