Project Review

"Quantification of 19-norandrosterone and 19-noretiocholanolone conjugates in plasma and urine samples by LC/MS/MS: metabolic studies with nandrolone."

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Nandrolone and other 19-norsteroids, potent anabolic steroids are prohibited in sports for 30 years. The detection of its main urinary metabolite, 19-norandrosterone in an amount greater than 2 ng/mL constitutes an adverse analytical finding. The presence in nutritional sport supplements of steroids not listed on the label has undoubtedly caused positive tests.
The project deals with detection and quantification in urine and blood samples of free and conjugated forms of 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE) by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS). This analytical method appears appropriate to study the phase II metabolism of nandrolone as it does not require deconjugation and derivatization steps prior to analysis. In this context, urine and blood samples collected during excretion studies of oral administration of nandrolone and also to samples containing 19-norandrosterone (19-NA) and 19-noretiocholanolone (19-NE) of endogenous origin will be analyzed to find specific biomarkers of a doping with this norsteroid. Our results for low concentrations of analyte (< 10 ng/mL) will be validated using GC/HRMS and GC/C/IRMS methods to determine 19-norandrosterone glucuronide concentrations and 13C/12C ratio, respectively.

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Results and Conclusions

The aim of the study was to develop an analytical tool using liquid chromatography ion trap mass spectrometry LC/MS/MS to determine if the presence of nandrolone metabolites originates from administration of nandrolone prohormones or are produced at the endogenous level.

In the first part of the project, a LC/MS/MS assay was validated for the quantification of the four major phase II nandrolone metabolites in human urine, 19-norandrosterone and 19-noretiocholanolone in their glucuronide and sulfate forms. This method was subsequently applied to samples collected after oral administration of 100 mg 19-norandrostenedione and samples containing nandrolone metabolites from endogenous origin.

19-norandrosterone sulfate was detected over 200 hours after precursor absorption. Based on concentrations or ratios of 19-norsteroids conjugates in urine specimens, it was not possible to discriminate between endogenous and exogenous production at the ng/mL concentration levels. However, our study showed that the concentration of 19-norandrosterone sulfate often exceeds 19-norandrosterone glucuronide. Therefore, it might be conceivable in the future to quantify 19-norandrosterone by LC/MS/MS as a routine procedure to support the positive results obtained for 19-norandrosterone glucuronide at a concentration above the 2 ng/mL threshold.

Publications

1. E. Strahm, C. Saudan, P-E. Sottas, P. Mangin, M. Saugy. Direct detection and quantification of 19-norandrosterone sulfate in urine by liquid chromatography-linear ion trap mass spectrometry. *J. Chromatogr. B.* **2007**, *852*, 491-6.
2. E. Strahm, S. Rudaz, J-L. Veuthey, M. Saugy, C. Saudan. Profiling of 19-Norsteroid sulfoconjugates in human urine by liquid chromatography mass spectrometry. *Anal. Chim. Acta.* **2008**, *613*, 228-237.
3. E. Strahm, N. Baume, P. Mangin, M. Saugy, C. Ayotte, C. Saudan. Profiling of 19-norandrosterone sulfate and glucuronide in human urine: implications in athlete's drug testing. *Steroids* **2008**, *submitted for publication.*