Detection and Quantitation of Anabolic Steroids by LC-MS/MS

Subbarao V. Kala*, Steve E. Harris, Tom D. Freijo, and Stan Gerlich
One Source Toxicology Laboratory, 1209 Genoa Red Bluff, Pasadena, TX 77504, USA

AIMS: The use of anabolic steroids in sports is prohibited by World Anti-Doping Agency. Most of the methods for the detection of steroids are currently based on GC-MS analysis. These methods involve lengthy extraction procedures followed by derivatization of steroid metabolites. Additionally, GC-MS methods are used to detect steroid metabolites instead of parent compounds due to their lower sensitivity. We have recently developed an LC-MS/MS method for the detection, identification and quantitation of anabolic steroids in urine samples.

METHODS: Using an Applied Biosystems LC-MS/MS 3200 QTRAP system, we were able to identify several anabolic steroids of abuse (boldenone, methenolone, methandienone, nandrolone, stanozol, mesterolone, norethandrolone and androstenedione) with simultaneous quantitation of testosterone and epitestosterone levels to determine the T/E ratios. Using this method, we were able to detect and quantitate parent compounds of boldenone, nandrolone and stanozol in urine samples of actual steroid users. Briefly, the method involved hydrolysis of glucuronated steroids from urine samples with β-glucuronidase (from H. pomatia) followed by liquid/liquid extraction. The extracts were injected onto a column (C18 reverse phase, 50 mm x 4.6 mm, 3µ) using a Shimadzu autosampler and the anabolic steroids were separated by gradient elution (ammonium acetate/methanol/acetic acid). Two MRM (multiple reaction mode) transitions were monitored for each steroid using positive electrospray ionization (ESI) coupled to an MS/MS detector. D3-Stanozol was used as an Internal Standard. These studies involved the determination of LOD, LOQ and ULOL. Accuracy and precision studies were also conducted.

RESULTS: The limit of quantitation (LOQ) for most of the steroids by LC-MS/MS were found to be ~0.5 ng/mL in urine on the basis of a signal to noise ratio >10. This corresponds to 250 pg on column. The ULOL for different steroids ranged from 800-1000 ng/mL. The precision at the cutoff (10 ng/mL) was found to be within a 10% coefficient of variation and the accuracy was > 90%. We did not observe any ion suppression for these steroids as a result of the urine matrix.

CONCLUSIONS: Our studies suggest that LC-MS/MS provides a unique opportunity to detect and identify both parent steroid compounds and their metabolites in urine samples with the greatest sensitivity. Presently, we are in the process of expanding the steroid panel to include other anabolic steroids and their metabolites in urine samples.

Keywords: Anabolic steroids, LC-MS/MS, Drug testing