K1 Analysis of Anabolic Steroids in Urine by LC/MS/MS

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After attending this presentation, attendees will learn of an LC/MS/MS technique for analyzing anabolic substances in urine.

This presentation will impact the forensic community and/or humanity by demonstrating how to quantitate steroids in urine using a non GC/MS technique, which can save additional sample preparation steps including derivatization.

This work represents the use of LC/QQQ mass spectrometry for confirmation of performance-enhancing drugs in urine, targeted for sports doping control analysis. LC/MS/MS with a high-performance 3.5 um rapid resolution column and ionization by APCI on the QQQ instrument, using MRM analysis, provides a lower-cost alternative to the current de-facto standard in international doping control, which is the EI-GC/MS high-resolution magnetic sector instrument. Additionally, increased throughput as a result of bypassing the necessary sample derivatization step, without sacrificing the sensitivity required to meet the minimum required performance levels (MRPLs) of the World Anti- Doping Agency (WADA), is also considered an advantage. Confirmation is carried out using designated quantitation ions in MRM mode. Samples were obtained from the Center for Human Toxicology (University of Utah) to generate calibration curves for quantitation.

The samples were prepared by a liquid/liquid extraction of 3 mL of control urine, spiked at specified levels. The extractions were evaporated to dryness and then reconstituted in 100 DL of liquid chromatographic (LC) mobile phase solvent. The compounds analyzed include 4D-stanozolol, 19-nor-etiocholanolone, tetrahydrogestrinone (THG), and epimetendiol, with internal standards such as methyltestosterone, and d5-etiocholanolone. Calibration curves were generated over concentrations ranging from ½ x to 10 x MRPLs with linearity coefficients (r^2 values) greater than 0.997. Reproducibility at the lowest level (½ x MRPL) was measured in terms of percent relative standard deviation (% RSD) of peak area counts for repeated injections. For triplicate injections the percentage RSDs were typically 1 – 6 %.

The signal-to-noise (S/N) was calculated by first selecting a region of the chromatogram from which to determine the root-mean-squared (RMS) noise, which was then multiplied by a factor of five. The S/N was therefore the height of the peak divided by 5 x RMS noise. This was equivalent to peak-to-peak noise. The limit of detection (LOD) was calculated by first determining the S/N for the peak at the ½ x MRPL and then scaling the concentration down to a level that corresponds to S/N = 3. For example, in the case of epimetendiol, the estimated LOD was 0.05 ng/mL in urine, or 3 pg on-column for a 2 DL injection volume.

LCMS, QQQ, Steroids

 ** Presenting Author*