K4 A Validated Method for the Quantitative Determination of Anabolic Steroids in Urine by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will better understand a validated method for the quantification of anabolic steroids in urine by basic hydrolysis, liquid-liquid extraction, and LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by describing a method validation to rapidly and simultaneously confirm 12 anabolic steroids in urine.

Anabolic steroids are the synthetic analogues of testosterone. These drugs have high abuse potential and are most often used by males to increase muscle mass and enhance athletic performance. Abuse of anabolic steroids can lead to early heart attacks, strokes, liver tumors, kidney failure, and psychiatric problems. Also, stopping use of these drugs can cause depression, which in turn can lead to resumption of use. Unlike many other drugs of abuse, these drugs are often injected; therefore, users who share needles or use non-sterile injecting techniques are also at high risk for contracting dangerous infections such as viral hepatitis and HIV.

Most of the methods for the detection of steroids are currently based on Gas Chromatography/Mass Spectrometry (GC/MS) analysis. These methods involve lengthy extraction procedures followed by derivatization of steroids and metabolites. Additionally, GC/MS methods are used to detect steroid metabolites instead of the parent drug due to their lower sensitivity. An LC/MS/MS method was recently developed for the detection, identification and quantification of 12 anabolic steroids (testosterone, boldenone, fluoxymesterone, clostebol, oxandrolone, formestane, clenbuterol, dihydroepiandrosterone, epitestosterone, methandrostenolone, stanozolol, and 3-hydroxystanozolol) in urine samples. Briefly, the method involved hydrolysis of glucuronide metabolites of steroids using β-glucuronidase followed by liquid-liquid extraction. The dried extracts after reconstitution with organic/aqueous solvents mixture were injected onto an Agilent® 6460 QQQ LC/MS/MS in positive ionization mode. Separation was achieved on an Agilent® ZORBAX® Eclipse® XDB-C18 column (4.6 x 100mm, 1.8um) with a flow rate of 0.3mL/min of 0.1% formic acid in H_2O (A) and 0.1% formic acid in methanol (B). The gradient was initiated at 50% B, increased to 60% B over 2min, held at 60% B until 9min, increased to 100% B until 12min and held at 100 % B until 15min, decreased to 50% B at 15.1min, for a total run time of 19min. Method validation was conducted according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines.

Sample preparation involves basic hydrolysis followed by liquid-liquid extraction. Good linearity and reproducibility were obtained for all steroids and metabolites with a coefficient of determination R^2>0.99. Limits Of Quantification (LOQ) ranged from 1ng/mL to 5ng/mL for various steroids and metabolites. Excellent imprecision and accuracy were achieved (CV <15%) for all steroids and metabolites. Twenty-five urine samples collected from international competitors were tested using this method. Stanozolol, boldenone, testosterone, epitestosterone, and clenbuterol were the major positive steroids found in this study.

Anabolic Sterioids, LC/MS/MS, Drug

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