Rapid identification and quantification of performance-enhancing stimulants in human urine using high-resolution mass spectrometry

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Background and aim: The ability to accurately measure low levels of performance-enhancing substances is critical to ensuring the health and safety of the athletes, as well as maintain the integrity of competition. In recent years, the use of high-resolution mass spectrometry (HRMS) in the sports drug-testing laboratory enables toxicologists to rapidly screen for the presence of these substances by acquiring their complete chemical profile from biological samples. In this study, a comprehensive workflow combining the use of the SCIEX X500R QTOF System with a fast sample preparation procedure for the sensitive detection of a structurally-diverse panel of stimulants in human urine is described.

Methods: A series of six calibrator solutions were prepared by spiking blank urine samples with the stock standard mixture containing 15 performance-enhancing stimulants and internal standards. Spiked urine samples were diluted 10-fold with a solution of acetonitrile/methanol (80/20, v/v) followed by ultracentrifugation to give desired concentrations ranging from 1 to 1000 ng/mL. The resulting solution was directed injected into the mass spectrometer. Chromatographic separation was performed using a Phenomenex C18 column. MS and MS/MS data were collected for each sample using SWATH Acquisition on the SCIEX X500R QTOF System in positive mode. Data acquisition was TOF MS scan followed by 12 MS/MS scans using variably sized Q1 windows covering a mass range from 100 to 350 m/z. The resulting cycle time was 0.555 sec. Data was acquired using SCIEX OS Software 1.5.

Results: The assay showed excellent sensitivity and quantitative results, with limits of quantification (LOQ) and limits of detection (LOD) values ranging from 3.3 to 100 ng/mL and 1.0 to 30 nm/mL, respectively. Excellent correlation and linearity was observed, with R^2 values >0.99 for all the stimulants in the panel. Matrix effect values generated suggested no significant ion suppression due to the dilute-and-shoot sample preparation procedure. Intra-day precision and accuracy were both found to be below 20% for the calibrators at 100, 500 and 1000 ng/mL, proving the overall robustness and reproducibility of the developed workflow.

Conclusion: A comprehensive workflow for the detection of stimulants in human urine was successfully developed using the SCIEX X500R System. A rapid sample preparation procedure in combination with a highly selective MS/MS method with SWATH Acquisition enabled robust and reproducible detection of a panel of 15 stimulants in human urine with ng/mL detection limits.