

The Analysis of Common Drugs of Abuse in Human Urine by LC-MS/MS

Frances Carroll, Sharon Lupo, Shun-Hsin Liang, Ty Kahler, Paul Connolly, Rick Lake, Rob Freeman, Carrie Sprout

Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823

The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) in forensic toxicology labs has increased significantly over the years. LC-MS provides sensitivity, speed, and the ability to simplify sample preparation. The Raptor™ Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles (SPP) with the resolution of Ultra Selective Liquid Chromatography™ (USLC™) technology. In this example a simple dilute and shoot method was developed for 10 common drugs of abuse and their metabolites in urine using a Raptor™ Biphenyl 5µm column. The ultra low back pressure of the 5µm particle column allows even conventional 400 bar LC systems to take advantage of this high speed separation with a total analysis time of 5 minutes.

Human urine samples were diluted 5X in mobile phase A and injected into a Shimadzu Prominence UFLCXR HPLC equipped with an AB SCIEX API 4000™ MS/MS. Detection was performed using electrospray ionization in positive ion mode using scheduled multiple reaction monitoring (MRM). The separation was performed using water and methanol mobile phases modified with 0.1% formic acid under gradient conditions on a Restek Raptor™ Biphenyl 5µm, 50 x 2.1mm column.

Preliminary linearity, precision and accuracy, and matrix effects experiments were performed during method development. Purchased human urine was fortified with 10 drug analytes and their deuterated internal standards. The calibration range for codeine and 6-MAM is from 5.00 to 750 ng/mL. The calibration range for benzoylecgonine is from 1.00 to 500 ng/mL. The calibration range for the remaining 7 analytes is from 1.00 to 750 ng/mL. Accuracy and precision were determined by fortifying human urine at a concentration of 50.0 ng/mL prior to dilution. Mean values at this level ranged from 91.9% to 103% of nominal concentrations for all

analytes. Coefficient of variation (CV) was calculated for the determination of precision and ranged from 2.90% to 6.54%. Matrix effects were assessed by infusing a solvent standard (post column) prepared at a concentration of 50 ng/mL while injecting a blank matrix sample diluted 5x in mobile phase A. Areas of ion suppression would result in dips in the response of the solvent standard. There was no evidence of ion suppression during the time period of analyte elution.