K58 Application of Mixed-Mode Ultra High-Performance Liquid Chromatography to the Analysis of Drugs in Urine

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The goal of this presentation is to present a novel approach for the analysis of drugs in urine which utilizes a single column for both the reversed phase chromatographic and hydrophilic interaction chromatographic separation of the target solutes. Attendees will learn how the proposed methodology reduces ion suppression or ion enhancement due to solute co-elution or biological matrix effects, increases accuracy of solute identification, and minimizes sample preparation time and ample analysis time.

This presentation will impact the forensic science community by describing how the proposed methodology provides practical advantages over existing methodology in terms of rapid sample cleanup in tandem with increased resolution of target solutes without the need to change columns or instruments.

For the first time, methodology is presented for the analysis of drugs in urine employing a single column and the same elution solvents at different ratios for orthogonal separations. Depending on the elution solvent blend, separations for the basic drugs in the SAMHSA-5 panel in urine could be carried out both in the Reversed Phase Chromatographic (RPC) and Hydrophilic Interaction Liquid Chromatographic (HILIC) modes. For the analysis of the drugs in urine, all solutes could be separated using a combination of both chromatographic systems; this minimized ion suppression and allowed the unique identification of each solute using retention time. For both separations a 2.1mm x 150mm x 2.7µm superficially porous dimethylpentafluorophenylpropyl (PFP) column was employed using combinations of two acetonitrile-water-ammonium fomate elution solvents with Time-Of-Flight/Mass Spectrometric (TOF/MS) analysis.

For the separation of amphetamine, methamphetamine, MDA, MDMA, MDEA, morphine, codeine, 6-monoacetylmorphine, benzoylecgonine, and PCP, orthogonal separations were obtained using RPC and HILIC (R^2=0.0839 for relative retention time). For the RPC separation mode, a 12-minute gradient was performed, while a 6-minute isocratic separation was performed for the HILIC mode. Solid Phase Extraction (SPE) was performed on a mixed mode MM1 column. The solutes of interest were successfully separated with good recovery and allowed for minimum ion suppression or ion enhancement for the Ultra High-Performance Liquid Chromatography (UHPLC) TOF/MS analysis. For the SPE sample preparation, no evaporation and reconstitution step was required, due to the fact that the elution solvent was directly compatible with both the HILIC and RPC analysis on the PFP column. For most solutes, using both chromatographic modes linearity was observed over at least two orders of magnitude with R^2=≥0.992. For the one outlier amphetamine (HILIC), linearity is observed over two orders of magnitude using RPC with R^2=0.998. In addition, the limit of quantification for most solutes was adequate for both screening and confirmatory test cutoff concentrations, while the limit of detection was adequate for O6-monoacetylmorphine.

The applicability of the above chromatographic approach for the complementary separation of synthetic cathinones (“bath salts”) and pain management drugs will be demonstrated.

Drugs, Urine, Liquid Chromatography

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