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### **K23 Adulterants and Diluents in Urine Samples After Consumption of Cocaine: What Compounds Are Typically Found by Liquid Chromatography (LC) Combined With High-Resolution Tandem Mass Spectrometry (HRMS/MS)?**

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After attending this presentation, attendees will understand that in forensic urine samples which test positive for cocaine, in addition to metabolites of cocaine, pharmacologically active compounds are usually found. These compounds were added as adulterants to the illicit street drug. Acquisition of mass spectra in SWATH® mode on a Quadropole Time-of-Flight (QqToF) instrument allows retrospective searches for adulterants and their metabolites.

This presentation will impact the forensic science community by presenting a rapid and reliable analytical method to detect potentially toxic and hazardous adulterants in urine samples. Since this additional forensic toxicological information can be routinely collected, it can be helpful in expanding police intelligence.

**Goals:** Cocaine consumption is observed as a wide-spread phenomenon and in most cases the cocaine abusers also consume a considerable amount of adulterants, diluents, or even toxic contaminants without being aware of possible side effects, long-term adverse health effects, or acute toxicity.

In order to assess the exposure of cocaine users to such substances, an High-Performance Liquid Chromatography/Quadropole Time-of-Flight (HPLC/QqToF) method for urine samples was developed.

**Methods:** Urine samples were diluted with a mixture of water/acetonitrile/formic acid/ammonium formate (97.5/2.5/0.1%/5.0mM) and three internal standards were added (EME-D<sub>3</sub>, Tramadol-<sup>13</sup>C<sub>3</sub>, THC-D<sub>3</sub>). The diluted samples were injected onto a core shell column (C8, 50 x 2.1mm, 2.6µm) and analyzed on a QqToF instrument with typical run times of 15 minutes from injection to injection.

**Results:** In the past two years, more than 4,000 urine samples (coming from traffic controls or traffic accidents) were analyzed by LC combined with HRMS/MS and then processed against a home-built high-resolution library. This library currently contains approximately 1,000 spectra which are relevant for drug screening and also includes most of the currently observed adulterants, diluents, and contaminants. All spectra were acquired by the data independent scan mode (sequential windowed acquisition of all theoretical mass spectra) which generates a digital archive of each acquired sample. The scan parameters were optimized in order to meet the complexity of these urine samples. The following parameters were found to be most favorable for significant library hits: scan range from 50 to 950 Da, scan windows of 25 Da, scan time of 35 msec for each scan window, and collision energy of 35eV±15eV (collision energy spread).

In most of the acquired samples, if tested positive for cocaine or its metabolites, adulterants and diluents can be observed. Among the typical compounds are levamisole, phenacetin, lidocaine, and procaine. In some cases, diltiazem and benzocaine were found. Acquisition of mass spectra in sequential windowed acquisition of all theoretical mass spectra mode also allows retrospective searches for additional compounds. During the same period as the urine samples were screened, 684 cocaine hydrochloride samples were analyzed with High-Performance Liquid Chromatography/Diode Array Detection (HPLC-DAD). The following adulterants were found: Levamisole+Phenacetin+others (30%), Levamisole+Phenacetin (26.3%), Levamisole only (20.0%), Levamisole+others (10.4%), Phenacetin+others (5.1%), others only (3.2%), Phenacetin only (2.9%), and none (2.0%).

**Conclusions:** A fast and reliable analysis of urine samples for adulterants commonly found in cocaine was developed. Results from urine samples obtained with the new method confirm the results from the analysis of cocaine powder samples. The analysis of the illicit cocaine hydrochloride samples indicate that the vast majority of the cocaine contains pharmaceutically active adulterants. These adulterants are an additional health hazard for cocaine consumers.

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#### **High Resolution Tandem MS, Cocaine, Adulterants**