K76 The Identification of Drug Metabolites in Adulterated Urine Samples Using Direct Analysis in Real Time-Time Of Flight/Mass Spectrometry (DART®-TOF/MS)

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Learning Overview: The goal of this presentation is to discuss complications that arise with adulteration of urine samples in drug analysis and how to analyze the samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by introducing a new analytical technique to screen for drugs and their metabolites as well as detecting the presence of adulterants in urinalysis.

With the increased technology to identify drugs and their metabolites in urine screenings, more individuals are using forms of adulterants to evade positive results.[1] In a previous study, it was concluded that certain adulterants (e.g., bleach, Drano®, and eye drops) were able to create false-negatives when using a common screening procedure comprised of Enzyme-Linked Immunosorbent Assay (ELISA) and adulterant test strips (Intect® 7 and AdultaCheck® 6).[2] To circumvent false negatives using immunoassay techniques and to avoid subjectivity through color detection, this study proposes using DART®-TOF/MS to identify not only the drug of interest, but also any adulterants present within the sample. DART®-TOF/MS is an ambient ionization technique that has been shown through previous literature to have a high-resolution mass spectra and rapid analysis with minimal sample preparation.[3] DART®-TOF/MS has been used previously with bodily fluids and has shown to identify endogenous substances (e.g., creatinine) as well as exogenous substances (e.g., prescription drugs).[3]

For this study urine samples were collected anonymously by volunteers under Institutional Review Board (IRB) NO: SBE-16-12568. Volunteers also provided information on the study survey regarding drug use within the past week, which was then used to identify samples containing the drugs of interest and their metabolites. Samples collected contained tetrahydrocannabinol (11-nor-carboxy-delta-9-THC), cocaine (benzoyl ecgonine), amphetamines (d-amphetamine), and benzodiazepines (lorazepam, lorazepam glucuronide). These samples were then adulterated with bleach, vinegar, eye drops, Drano®, nitrite, table salt, and hand sanitizer at different concentrations (i.e., 5, 10, 25, 50% v/v or w/v). Adulterated samples were prepared for analysis using IonSense® SPE-it™ fiber kits, which uses a solid-phase micro extraction technique to isolate the analytes from components in the urine matrix. After sample preparation, Solid-Phase Microextraction (SPME) fiber strips were analyzed in the positive ionization mode on DART®-TOF/MS by placing the fiber into the ionization stream.

Initial data supports the use of DART®-TOF/MS as an analytical screening technique to identify drug metabolites and the presence of adulterants. The peaks observed during DART®-TOF/MS analysis indicated adulteration at low concentrations (i.e., 5% v/v) does not mask the presence of the drug/metabolite, unlike the combined immunoassay and adulterant test strip screening techniques, which were unable to detect adulteration at these low concentrations, as well the target drug/metabolite in some instances. Bleach was one adulterant that was easily observed in analysis, due to the presence of chlorinated peaks. A urine sample containing amphetamines and its metabolites was adulterated with bleach at the same five concentrations. As the concentration of bleach increased from the unadulterated amphetamine sample to a 50% v/v adulterant concentration, it was more difficult to observe the signal from the protonated amphetamine. However, the adulterant was still observed. Amphetamine was readily observed up to 10% v/v adulteration. Additional peaks, such as metabolites and fragments of the parent, were also observed in the resulting spectra. This trend was observed for the drugs in other urine samples that were exposed by different adulterants. Tampering of urine samples by adulteration requires the sample to be flagged and can lead to consequences for the individual; however, this cannot be achieved by current screening methods at low adulterant concentrations.

Reference(s):

1. Joel B. Bennett. Introduction. In: *Preventing Workplace Substance Abuse: Beyond Drug Testing to Wellness.* Ed.: Joel B. Bennett et al. (Washington, DC: American Psychological Association, 2003).
2. Olivieri, Bianca, Mark Marić, and Candice Bridge. Determining the Effects of Adulterants on Drug Detection Via ELISA and Adulterant Tests Strips. *Drug Testing and Analysis* (2018).
3. Cody, Robert B., James A. Laramée, and H. Dupont Durst. Versatile New Ion Source for the Analysis of Materials in Open Air Under Ambient Conditions. *Analytical Chemistry* 77, no. 8 (2005): 2297-2302.

DART®-TOF/MS, SPME, Urine Adulterants