K29 Identification of Markers of JWH-018 and JWH-073 Use in Human Urine

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The goals of this presentation are to inform the audience of the current knowledge regarding the urinary metabolites of JWH-018 and JWH-073 and to outline screening and confirmation methods for identifying the use of these compounds.

This presentation will impact the forensic science community by providing up-to-date information on the urinary metabolites of JWH-018 and JWH-073.

Recently, synthetic cannabinoids have garnered media attention as a legal alternative to cannabis. Sold as constituents of "herbal incense" under a wide variety of names including Spice, Yucatan Fire, Smoke, Sence, K2, Skunk, Space, K2 Citron, and K2 Blonde these compounds such as HU-210, JWH-018, CP 47,497, JWH-073, JWH-250, and JWH-200 are mixed with plant material and smoked. These synthetic analytes have a varying degree of selectivity and affinity for cannabinoids (CB_1 and CB_2) receptors and thus have different therapeutic and abuse potentials. As the popularity of these drugs increases, there is a developing need for analytical methods to identify and quantify the parent compounds in the herbal incense products as well as in biological matrices. On-going research will help identify metabolites of these compounds which can be used as markers of use in humans.

New drugs offer a unique challenge to the forensic toxicology community. Without authentic standard material for the multiple metabolites innovative methods of identifying the use of these compounds must be explored. Due to the lipophilic nature of these analytes, the parent compound is not excreted in urine emphasizing the

important of quickly identifying the metabolites as markers of use. Urine was collected from participants who smoked incense containing JWH-018 and JWH-073. These specimens were used to identify urine metabolites of these two compounds based on literature reports and LC- TOF analysis. Based on the literature and in-house analysis, JWH-018 and JWH-073 undergo mono-, di- and tri-hydroxylation followed by glucuronidation. Qualitative screen and confirmation methods for identifying exposure to JWH-018 and JWH-073 were developed and validated based on the presence of these urinary metabolites.

Specimens were screened for the monohydroxy glucuronide metabolites. Solid phase extraction was used to clean and concentrate unhydrolyzed urine specimens and extracts were analyzed on an LC/MS/MS for the detection of monohydroxy-glucuronide metabolites. The instrument was operated in positive ionization mode employing atmospheric pressure chemical ionization. Separation was achieved using gradient elution on a C18 HPLC analytical column. Source fragmentation of JWH-073-monohydroxy-glucuronide and JWH-018-monohydroxy-glucuronide was employed and the transitions resulting from the loss of the glucuronide moiety were monitored. Further fragmentation was then induced in the collision cell and two transitions monitored for identification purposes. The confirmation method employed is based on the presence of multiple urinary metabolites. Urine specimens underwent enzymatic hydrolysis and a liquid-liquid extraction prior to analysis. LC-MS/MS with electrospray ionization was performed on an Applied Biosystems™ API5000 system. Multiple transitions were monitored for each analyte. The following table summarizes the monitored transitions for the screening and confirmation methods:

Screening Method		
	Source Fragmentation	Collision Cell Fragmentation
JWH-018-monohydroxyglucuronide	534→358	358→155 & 358→127
JWH-073-monohydroxyglucuronide	520→344	344→155 and 344→127
Confirmation Method		
	Precursor Ion	Product Ions
JWH-018-monohydroxy	358	155 127 284 180
JWH-018-dihydroxy*	376 374	214 171 155 127
JWH-018-trihydroxy	374	189 171
JWH-073-monohydroxy	344	155 127
JWH-073-dihydroxy*	362 360	200 171 155 127
JWH-073-trihydroxy	378	189 171

*Multiple dihydroxy metabolites identified

Synthetic Cannabinoids, JWH-018, JWH-073

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