## K40 Development and Validation of an LC/MS/MS Method for the Detection of the Metabolites of JWH-018 and JWH-073 in Human Urine

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After attending this presentation, attendees will learn the analytical method for the determination of major metabolites of JWH-018 and JWH-073 in human urine, and the quantification results of authentic urine samples.

This presentation will impact the forensic science community by presenting the fully validated analytical method for the detection of the main metabolites of new designer drugs, JWH-018 and JWH-073, in urine.

Due to their cannabis-like effect, synthetic cannabinoids have attracted much public attention since 2008. Thus, elucidation of the metabolic pattern as well as detection of the intake of these drugs has been of major concern. In the present study, a sensitive and reliable analytical method was established and validated for the simultaneous determination of the metabolites of JWH-018 and JWH-073 in human urine. For the routine screening in urine, (ω) and (ω-1)-hydroxyl, carboxyl, and hydroxyindole metabolites were selected as target drug metabolites. The samples were prepared by solid-phase extraction and analyzed using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). The LODs were 0.025ng/mL or 0.1ng/mL and the LOQs were 2.5ng/mL for all analytes. The results of the intra- and inter-day precision and accuracy were satisfactory: <10% for precision and within ±10% for accuracy at low (2.5ng/mL) and high (75ng/mL) concentrations. In this analytical method, no significant matrix effect was observed and high recoveries for all metabolites were achieved. The described method was applied to 52 authentic urine samples suspicious of JWH-018 or JWH-073 abuse and the quantification results among samples were compared. Twenty-one of the samples (40%) were found positive for at least one metabolite of JWH-018 or JWH-073. Carboxylated metabolite of JWH-073 was detected in all analyzed samples, which could be due to the metabolism of JWH-018 in humans. However, (ω) or (ω-1)-hydroxyl metabolite of JWH-073 was detected in only 12 samples. And only a small amount of these metabolites was detected compared with JWH-018 metabolites in most of the analyzed samples. In 14 samples of a total of 21 samples, (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolites, with a mean concentration ranging from 2.9 to 671.2ng/mL; however, in the rest of the samples, the relative concentration of (ω-1) hydroxyl metabolite of JWH-018 was very low (<LOQ-23.4ng/mL) compared with that of the most abundant metabolite in the respective sample. It can be assumed that herbal mixtures used by the suspects contain JWH-073 as an impurity. 6-hydroxyindole metabolite of JWH-018 was detected in samples where (ω-1) hydroxyl metabolite of JWH-018 was the most abundant metabolite. Similarly, 6-hydroxyindole metabolite of JWH-073 was detected in only two samples which contain (ω-1) hydroxyl metabolite of JWH-073 with a concentration of more than LOQ. These results suggest that at least three metabolites including (ω) and (ω-1)-hydroxyl and carboxyl metabolites should be simultaneously monitored to prove intake of JWH-018 or JWH-073. The variation in the concentrations of detected metabolites could be due to the dosage of the drug and time intervals between the use of the drug and urine collection. However, the absence of detailed information such as dosage, content of synthetic cannabinoids in herbal mixture, and urine collection time makes it difficult to interpret the variation of concentrations between metabolites in the pharmacokinetic aspects. Thus, further study for the estimation of the profiles of metabolite concentrations after JWH-018 or JWH-073 intake versus time will be essential. The developed analytical method will be useful for confirmation and quantification of the metabolites of JWH-018 and JWH-073 in urine in the field of forensic toxicology.

**JWH-018 & JWH-073, Metabolite, LC/MS/MS**

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