## K56 Characterization of AB-FUBINACA Metabolites in Rat Urine by Liquid Chromatography/Time-of-Flight/Mass Spectrometry (LC/TOF/MS)

*Ashraf Mozayani, PharmD, PhD*, Texas Southern University, 3100 Cleburne Avenue, Houston, TX 77004; Aybike Dip, PhD, Texas Southern University, 3100 Cleburne, Houston, TX 77004; Hsinhung Chen, 2400 N Braeswood Boulevard, #204, Houston, TX 77030; Amruthesh Shivachar, PhD, Texas Southern University, 3100 Cleburne, Houston, TX 7700; Munder Zagaar, PhD, Texas Southern University, 3100 Cleburne, Houston, TX 77004; and Jeffrey Walterscheid, PhD, Harris County Institute of Forensic Sciences, 1885 Old Spanish Trail, Houston, TX 77054*

After attending this presentation, attendees will better understand the synthetic cannabinoid receptor agonist AB-FUBINACA and its metabolites in the rat model. Since it is a relatively new and potent drug that remains largely uncharacterized, this study is an important step in elucidating oxidative metabolites as targets for revised screening assays in forensic casework.

This presentation impacts the forensic science community by increasing awareness of the *in vivo* metabolites of AB-FUBINACA as determined by LC/TOF/MS analysis. The knowledge gained will help to improve detection capabilities and practices.

Synthetic cannabinoids were first produced by academic and pharmaceutical laboratories with the hope of providing therapeutic pain relief without the toxicity that accompanies chronic opiate use. While many of these drug derivatives were published in the medical literature, others were merely patented. These stale patented recipes have become the basis for a new generation of illicit drug manufacture, replacing the first generation JWH species. AB-FUBINACA is one of the newest synthetic cannabinoids introduced in the past several years, first detected in blended synthetic products in 2012.[1] Because of its potency and obscurity, little is known about the metabolism or pharmacology of these substances.

In order to study the metabolic fate of AB-FUBINACA, the Wistar rat model was utilized to deliver drugs by intraperitoneal injections and then urine was collected for analysis. Rats weighing from 160g to 200g were housed in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. After acclimatization, the rats were transferred to individual metabolic cages for the course of the experiment, to collect urine. Injections consisted of 5mg/kg of AB-FUBINACA dissolved in dimethyl sulfoxide, which were repeated daily for five days. The control group (n=3) received dimethyl sulfoxide injections, while the experimental group (n=5) received the dissolved drug. Urine samples were collected every day at the same time during injection and refrigerated until preparation for analysis.

Extractions were performed by combining 0.2mL of urine with an organic mixture of 1:1 isopropanol/1-chlorobutane, fortified with saturated magnesium sulfate and adjusted to pH 10 with ammonium hydroxide. After vortexing and centrifuging, the organic layer was removed to a fresh vial where the extract was evaporated to a residue with compressed nitrogen gas. The residue was reconstituted with mobile phase buffer and injected for LC/TOF/MS scanning. Examination of the time-of-flight data showed possible hydroxyl metabolites at 385.1687m/z, in comparison to the parent mass of 369.1721m/z. While the position of the hydroxyl addition on the molecule is unclear, the accurate mass and retention is now known for successful library matching.

Other published articles have studied metabolites of AB-FUBINACA in human liver microsomes, but this work is the first *in vivo* study to determine the metabolites of AB-FUBINACA in urine.[2] A putative hydroxyl metabolite of AB-FUBINACA was identified; however, a carboxyl metabolite has not been defined by this method.

**References:**

1. Uchiyama N, Kawamura M, Kikura-Hanajiri R, Goda Y (2012) Identification of two new-type synthetic cannabinoids, N-(1- adamantyl)-1-pentyl-1H-indole-3-carboxamide (APICA) and N-(1- adamantyl)-1-pentyl-1H-indazole-3-carboxamide (APINACA), and detection of five synthetic cannabinoids, AM-1220, AM-2233, AM- 1241, CB-13 (CRA-13), and AM-1248, as designer drugs in illegal products. *Forensic Toxicol* 30:114–25
2. Takayama, T., Suzuki, M., Todoroki, K., Inoue, K., Min, J., Kikura-Hanajiri, R., Goda, Y. and Toyo'oka, T. (2014). UPLC/ESI-MS/MS-based determination of metabolism of several new illicit drugs, ADB-FUBINACA, AB-FUBINACA, AB-PINACA, QUPIC, 5F-QUPIC α-PVT, by human liver microsome. *Biomedical Chromatography*, 28(6), pp.831--838.

**Synthetic Cannabinoids, AB-FUBINACA, LC/TOF/MS**