## K12 Identification of *In Vitro* Metabolites of PB-22 and 5-F-PB-22 by UPLC-QTOF Mass Spectrometry

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After attending this presentation, attendees will be able to identify major *in vitro* metabolites of the novel cannabimimetic compounds PB-22 and 5-F-PB-22. The goal of this presentation is to present an Ultra Performance Liquid Chromatography-Quadrupole Time-Of-Flight/Mass Spectrometry (UPLC-QTOF/MS) method for the identification of *in vitro* metabolic transformations.

This presentation will impact the forensic science community by presenting a qualitative analytical method for the detection of PB-22 and 5-F-PB-22 metabolites and identification of suitable markers for use.

Cannabimimetic compounds are sprayed onto plant material and sold for use as recreational drugs despite their adverse effects. Two compounds that have been identified in herbal products are quinolinyl carboxylates, PB-22 and 5-fluoro PB-22.

*In vitro* samples were generated by incubating the compounds at 100µM in cryopreserved human hepatocytes. At each of three distinct time points, 0, 15, and 120min, an aliquot of 100µL was removed and quenched with acetonitrile containing 0.2% acetic acid. A portion of each aliquot was hydrolyzed with 25µL of 12.3 units/µL of beta-glucuronidase in 0.1M ammonium acetate and incubated for 3.5h at 60°C.

Data were collected on both the hydrolyzed and unhydrolyzed samples using a Waters® Acquity UPLC® interfaced to a Waters® Synapt® G2 QTOF mass spectrometer. All data were acquired using a Mean Squared Error ($MS^{E}$) method, which acquires both low- and high-energy data simultaneously. Liquid chromatography was carried out using an Acquity BEH C18 column (1.7µm x 2.1 x 50mm) connected to a Vanguard BEH C18 pre-column (1.7µm x 2.1 x 5mm) and held at 30°C. A gradient elution with a flow rate of 500µL/min was used with mobile phase A consisting of water with 0.1% formic acid and mobile phase B consisting of acetonitrile with 0.1% formic acid.

In positive ion electrospray mode, PB-22 and 5-F-PB-22 parent compounds and metabolites were present as both protonated and sodiated ions. Major metabolic transformations observed for both compounds were 3-carboxyindoles and their corresponding glucuronide conjugates. Hydroxylated PB-22 and defluorinated 5-F-PB-22 followed by hydroxylation were also identified. Interestingly, no evidence for hydroxylated 5-F-PB-22 was observed. These results are in contrast to the metabolism of other currently prevalent cannabimimetic compounds in which the dominant metabolites result from monohydroxylations.

This research provides forensic practitioners with the identification of major *in vitro* metabolites of novel designer drugs PB-22 and 5-F-PB-22. Major metabolic transformations are similar for both compounds. Due to defluorination of 5-F-PB-22, several metabolites are common to both compounds, and therefore should not be used for unambiguous identification.

**PB-22, 5-F-PB-22, Metabolites**

 ** Presenting Author*