## K78 Staying Relevant in an Ever-Changing Climate: The Development and Validation of a Confirmatory Qualitative Synthetic Cannabinoid Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) Method in Human Whole-Blood

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**Learning Overview:** After attending this presentation, attendees will be able to discuss the designer-drug class of synthetic cannabinoids with a focus on the latest trends and will be able to undertake the development and validation of a confirmatory analytical assay using LC/MS/MS for their detection in biological fluids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by raising awareness of the abuse and toxicity of synthetic cannabinoids as well as their detection in biological samples.

Since 2009, synthetic cannabinoids have presented a challenge to toxicology laboratories. As new compounds become available within the recreational drug market, labs need to update their analytical methods to stay relevant. The development and validation of quantitative methods can be a long and demanding process, especially when there is a lack of deuterated internal standards for every analyte in the panel. Developing a qualitative confirmation method allows faster updates to incorporate new compounds as they emerge, due to the less-stringent criteria. Synthetic cannabinoids used in abused products are continually changing with slight structural alterations to circumvent drug control regulations created for earlier generation analogs. Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors.

The proposed scope contained 27 analytes, including 10 new analytes. Concentrations range from 0.05ng/mL–1.0ng/mL.

| CURRENT | | NEW |
|---|---|---|
| CUMYL-THPINACA | FUB-JWH-018 | FDU-PB-22 |
| MDMB-FUBINACA+ | MMB-CHMINACA | 5-fluoro-NPB-22 |
| FUB-AKB48 | 5-fluoro-AMB | 5-fluoro-MDMB-PICA |
| MA-CHMINACA | FUB-JWH-018 | 5-fluoro AEB* |
| 5-fluoro-ADB* | MMB-CHMINACA | 4-cyano CUMYL BUTINACA |
| NM-2201 | AB-FUBINACA | EMB-FUBINACA+ |
| FUB-AMB | ADB-FUBINACA | ADB-FUBICA |
| MO-CHMINACA | AB-CHMINACA | MDMB-FUBICA |
| MDMB-CHMCZCA | ADB-CHMINACA | MMB-FUBICA |
| MMB-CHMICA | | 5-fluoro-EDMB-PINACA |
| * 5-fluoro-ADB and 5-fluoro AEB are reported as pairs<br>+ MDMB FUBINACA and EMB FUBINACA are reported as pairs | | |

This assay was developed to detect and qualitatively identify synthetic cannabinoid compounds in whole blood. Whole blood was fortified with internal standard, pH adjustment with 0.1 M TRIS buffer pH 10.2, and single-step liquid/liquid extractions. Separation was achieved using two different chromatographic methods run on an Acquity® UPLC BEH C18 (100mm x 2.1mm, 1.7-micron) column coupled with a VanGuard® BEH C18 1.7-micron guard column with mobile phases consisting of 0.1% formic acid in deionized water and an acetonitrile/methanol mixture. Analytes were detected using positive-ion electrospray MS/MS on a Waters® TQS MS/MS coupled with a Waters® Acquity® Ultra Performance LC System.

The methods were fully validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. During method development, it was demonstrated that running a calibration curve improved the precision around the cut-off concentration. However, the quantitative controls for several analytes did not meet the stringent requirements required for the quantitative validation, usually because of the lack of a labeled internal standard. Therefore, the method was validated qualitatively according to laboratory Standard Operating Procedure (SOP), including the evaluation of the cut-off concentration, sensitivity/specificity, carryover, matrix effect, interfering substances, and stability. Linearity was established using five calibrators. Replicates ($n$=5) at each concentration were analyzed and the correlation coefficient was >0.99 for all analytes. All ten synthetic cannabinoids were measured at three different concentrations to give precision ≤15% CV and accuracy ±15% for both within- and between-run experiments. Stability experiments ($n$=6) indicated that the synthetic cannabinoids listed above were stable in blood for up to two days at room temperature, and for at least 30 days if kept refrigerated or frozen. Exceptions were MMB FUBICA, which was not stable at room temperature, and 5-fluoro EDMB-PINACA was only stable for seven days refrigerated.

Four hundred fifty-six blood samples that had screened positive for synthetic cannabinoids using an LC/MS/MS screening method were tested using this updated method. Twenty cases tested positive for one or more of the newly added cannabinoids. Seven cases tested positive for 4-cyano CUMYL BUTINACA and three samples were confirmed positive for MMB-FUBICA in combination with other new designer cannabinoids (5-fluoro MDMB-PICA and 4-cyano CUMYL BUTINACA). Of the cases with demographic information available, the median age of the subjects was 33, and included 15 males and 1 female.

**Synthetic Cannabinoids, Novel Psychoactive Substances, Validation**