K38 A Semi-Quantitative Retrospective Method Validation for Three Synthetic Cannabinoids With Analytical Confirmation in Toxicology Casework

Stephanie Kumor, MA, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; Kristopher W. Graf, BS, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090; and Sherri L. Kacinko, PhD, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will be able to discuss the importance of implementing analytical testing methods that allow for quick updates for continuously evolving synthetic cannabinoids and will be able to understand the benefits of performing a retrospective method validation to determine the concentrations of these analytes in biological fluids.

This presentation will impact the forensic science community by describing a retrospective method validation protocol used to determine semi-quantitative results of the synthetic cannabinoids 5F-AMB, 5F-ADB, and FUB-AMB, the most commonly seen analytes in toxicology casework from May 2016 through July 2017.

Since 2009, synthetic cannabinoids have presented a challenge to toxicology laboratories. As new compounds become available within the recreational drug market, labs are required 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 standard for every analyte in the panel. Development of a qualitative confirmation method allows for faster incorporation of new compounds. Since these newer drugs are not part of many laboratories' routine testing procedures, there is limited information available on their expected levels in casework, making interpretation difficult; however, observed concentrations in biological fluids can help provide insight into the toxicity of these compounds. Therefore, a retrospective method validation of a qualitative method was performed for three compounds with a high positivity rate to acquire semi-quantitative data.

The qualitative assay was developed to detect 5F-AMB, 5F-ADB, and FUB-AMB, in addition to 24 related synthetic cannabinoids. During method development, it was noted that running a calibration curve improved the precision around the cut-off concentration; however, the quantitative controls for several analytes were not meeting the stringent requirements required by quantitative validations. 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. Whole-blood samples were extracted using a liquid-liquid extraction, and analytes were detected using positive mode Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). Separation was achieved on a BEH C18, 2.1mm x 100mm column with mobile phases consisting of water containing 0.1% formic acid and an acetonitrile and methanol mixture (80:20).

A retrospective method validation was performed based on laboratory SOP and Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. The Limits Of Quantitation (LOQ) were 25pg/mL for 5F-AMB and FUB-AMB and 50pg/mL for 5F-ADB. Linearity was established across two ranges (25-200pg/mL, and 50-400pg/mL) using five calibration points (n=5) with correlation coefficients ≥0.990 for all analytes and all back-calculated calibrators within 13% of target. The three synthetic cannabinoids were measured at three different concentrations for 15 separate days to provide acceptable between-run precision (13.5% Coefficient of Variation (CV)) and accuracy (±10.6%).

Based on the results of the retrospective method validation, all data that was still available on the laboratory instruments was reprocessed to determine the concentrations of 5F-AMB, 5F-ADB, and FUB-AMB. Data was included for all runs that met the following criteria: calibration curve correlation coefficients >0.990; back-calculated calibrators within ±20% of target and controls within ±20% of target. In this data set, there were 15, 97, and 112 cases above the limit of quantification for 5F-AMB, 5F-ADB, and FUB-AMB, respectively. Of these, 47% 5F-AMB, 24% of 5F-ADB, and 38% of FUB-AMB were below the reporting limit of the qualitative assay and thus had been reported "None Detected." The concentrations of all cases that fell within the calibration range are provided in the table below.

Analyte	Concentration (pg/mL)	# Cases >ULOQ
5F-AMB	68 ± 54 (n=10)	5
5F-ADB	200 ± 90 (n=52)	45
FUB-AMB	100 ± 60 (n=74)	38

The development of a qualitative method to detect synthetic cannabinoids allows for ease of updating the scope to remain relevant within the designer drug market. The ability to obtain semi-quantitative data through a retrospective method validation offers the forensic toxicology community information concerning the toxicity and expected concentrations of 5F-AMB, 5F-ADB, and FUB-AMB in whole blood samples.

Synthetic Cannabinoids, Retrospective Validation, Semi-Quantitation

*Presenting Author