K57 Broad Detection of Synthetic Cannabinoids in Whole Blood Using Ultra High-Performance Liquid Chromatography-Quadrupole-Time-of-Flight (UHPLC-Q-TOF)

Robert Kronstrand, PhD, National Board of Forensic Medicine, Dept of Forensic Toxicology, Artillerigatan 12, Linkoping SE 587 58, SWEDEN; Cassandra Jaque, BSc, National Board of Forensic Medicine, Artillerigatan 12, Linkoping SE 587 58, SWEDEN; and Markus Roman, BS, National Board of Forensic Medicine, Dept of Forensic Genetics and Forensic Toxicology, Artillerigatan 12, Linkoping SE 587 58, SWEDEN*

After attending this presentation, attendees will understand the challenges associated with the analysis of synthetic cannabinoids and be able to describe the current panorama of these drugs.

This presentation will impact the forensic science community by increasing awareness of the need to analyze for synthetic cannabinoids in cases of both the living and the deceased.

Synthetic cannabinoids are the most prevalent group of new psychoactive substances emerging on the illicit market. The changes in availability from internet sites are partly a result of legislation where substances becoming scheduled are replaced by new compounds. Therefore, it is a challenge to keep methods up to date. This study was performed to create a strategy for the analysis of a broad range of synthetic cannabinoids that was flexible and to which one could easily add new compounds as they appear on the market and subsequently in the toxicological samples received.

Blood samples (1.0g) were prepared by liquid-liquid extraction with diethylether at pH 10.2. An in-house library comprising full Tandem Mass Spectrometry (MS/MS) spectra of 75 synthetic cannabinoids was built by analyzing solutions from certified standards. Identification was based on retention time, accurate mass, isotopic pattern and MS/MS spectra. Analyses was performed on an Agilent® 6550 iFunnel Q-TOF Liquid Chromatography/Mass Spectrometry (LC/MS) system combined with a 1290 Infinity® LC system. Ions were generated in positive electrospray ionization mode and were detected by data dependent acquisition MS/MS. Separation was achieved by gradient chromatography on an YMC Triart-C18 column within 8.5 minutes. Mobile phase A consisted of 0.05 % formic acid in 10mM ammonium formate and phase B of 100 % methanol.

The method was validated accordingly to guidelines for qualitative methods including selectivity, matrix effects, extraction recovery, response variation and spectra score variation at the threshold concentration, and stability in matrix. The selectivity was excellent; however, the analyte response was affected by the matrix with 28 of the 75 analytes presenting with matrix effects above 25%. Still, the variation in response and spectra scores was less than 15% at the thresholds chosen. Sixty-six analytes obtained a threshold of 100pg/g and seven analytes 200pg/g whole blood. All analytes except AM-2201 were stable when refrigerated for two weeks. After four weeks refrigeration, JWH-122 and UR-144 also showed a decrease of more than 20%.

The method was applied to cases including petty drug offences, DUID, violent crimes, and autopsies where analysis of synthetic cannabinoids was requested. In total, 442 samples were run during the first two months in routine with a positive rate of 24% resulting in the detection of 14 different parent compounds. The most prevalent compound was AB-FUBINACA (N=78) followed by AB-PINACA (N=12), BB-22 (N=10), 5F-NNEI (N=7), FUB-PB22 (N=5), 5F-PB22 (N=4), THJ2201 (N=4), 5F-AKB48 (N=3), 5F-AB-PINACA (N=2), NNEI (N=2), and one finding each of STS-135, PB22, FDU-PB22, and UR-144.

Blood as a matrix was chosen because of the better availability of parent compounds rather than metabolites from certified suppliers. A qualitative approach was chosen to simplify validation and inclusion of new analytes. High-resolution mass spectrometry with UHPLC was chosen to increase both the chromatographic and mass spectral selectivity. In conclusion, the strategy provided a sensitive, flexible, end-point method that detects parent compounds, simplifying prosecution in cases where the intake of a scheduled drug is the charge. A disadvantage of the qualitative approach is that it does not allow for a detailed interpretation; however, the pharmacodynamics of synthetic cannabinoids is largely unknown and a concentration does not necessarily increase the interpretation power.

Synthetic Cannabinoids, Whole Blood, TOF/MS