K58 Evaluation of the Long-Term Stability of Select Phenylacetylindole, Cycloalkyolindole, Quinolinyl, and Carboxamide Synthetic Cannabinoids in Human Whole Blood Using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will learn to assess the stability of synthetic cannabinoids in human whole blood using LC/MS/MS.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing long-term stability results of synthetic cannabinoids under various storage conditions.

Background/Introduction: Despite efforts to control synthetic cannabinoids, clandestine manufacturers continue to modify their structures to avoid legal consequences, creating an ever-changing analytical target for forensic laboratories. Forensic toxicology laboratories often lack the needed resources or do not have the capabilities to test for these compounds and metabolites, requiring specimens to be submitted to reference laboratories. Drug stability can be affected by long storage times, temperature, and preservatives. Although these factors can be controlled, systematic research is necessary to identify their impacts on the stability of these new synthetic cannabinoids that are continually emerging.

Objective: The purpose of this research was to assess the stability of 17 synthetic cannabinoids in human whole blood using LC/MS/MS over a 35-week period. The method was validated in accordance to the Academy Standards Board method validation guidelines for quantitative analysis and stability evaluation of the following analytes: phenylacetylindoles JWH-250 and RCS-8; cycloalkylindoles UR144 and XLR11; quinolinyls PB-22 and NM2201; and carboxamides 4-cyano CUMYL-BUTINACA, 5-fluoro-3,5-ABPFUPPYCA, 5-fluoro ADB-PINACA, 5-fluoro PY-PINACA, ADB-PINACA, APP-PICA, CUMYL-THPINACA, EMB-FUBINACA, MDMB-FUBICA, MEP-CHMICA, and MO-CHMINACA.

Methods: Stability under room temperature (20ºC), refrigerator temperature (4ºC), and freezer temperature (-20ºC) at high (10ng/mL) and low (1.5ng/mL) concentrations each in triplicate were evaluated at ten selected time points: 0-hour, 24 hours, 72 hours, 1 week, 3 weeks, 5 weeks, 9 weeks, 17 weeks, 21 weeks, and 35 weeks. Blood was preserved with sodium fluoride prior to the stability study. Extraction of analytes was conducted using Supported Liquid Extraction (SLE+) ISOLUTE® cartridges. The extracts were analyzed using a Waters® Xbridge™ reverse-phase C18 column (3.5µM, 2.1 x 50mm) by Shimadzu® HPLC with a SCIEX™ 4000 Q-Trap Electrospray Ionization/Tandem Mass Spectrometry (ESI+/MS/MS) in positive ionization mode. The total run time was eight minutes with a 0.6mL/min flow rate and 10µL injection volume.

Results: Linear calibration curves for each analyte had acceptable R^2 values >0.99 using a weighting factor of 1/x. A linear dynamic range of 0.5ng/mL to 25ng/mL was used for all analytes within acceptable ±20% calculated bias and imprecision, except NM2201 and APP-PICA had a Limit Of Quantitation (LOQ) of 0.1ng/mL and MO-CHINACA had a working range of 0.5–15ng/mL. No signs of carryover were observed. Analytes were considered stable if the average area ratio at the time point examined was within ±20% of the average area ratio response at time point zero. Phenylacetylindole, cycloalkylindole, and carboxamide analytes were stable up to 35 weeks under all temperatures, such as JWH-250, 5-fluoro PY-PINACA, and UR144, with core structures of a carbonyl substituent on a pyrazole or pyrrole with surrounding non-polar groups of hydrocarbons and heterocyclic rings. Compounds with two polar carbonyl functional groups present, such as EMB-FUBINACA, 5-fluoro ADB-PINACA, APP-PICA, and MEP-CHMICA, were found to experience degradation earlier at one week or less in room temperature and refrigerated storage conditions. 5-fluoropentyl analogs, such as XLR11 and 5-fluoro ADB-PINACA, in comparison to their counterpart analytes, UR144 and ADB-PINACA, were unstable at earlier time points of less than one week under room temperature and/or refrigeration.

Conclusion: The validated method demonstrates a sensitive and reliable way to positively identify 17 different synthetic cannabinoids in human whole blood in rapid time for stability analysis. Further, the use of SLE improved sample preparation efficiency by decreasing the extraction time from 1 hour to 30 minutes compared to traditional extraction methods, such as solid-phase extraction and liquid-liquid extraction. The select 17 synthetic cannabinoids generally degraded in the order of room temperature, refrigerator, then freezer temperature. Long-term stability results revealed that the overwhelming majority of synthetic cannabinoids were stable up to 35 weeks when kept frozen.

Synthetic Cannabinoids, Supported Liquid Extraction, Stability

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