K39 A Comprehensive Analysis of Synthetic Cannabinoids and Metabolites in Oral Fluid (OF) By Online Solid Phase Extraction (SPE) and Liquid Chromatography/Triple Quadruple/Mass Spectrometry (LC/QqQ/MS)

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Learning Overview: The goal of this presentation is to demonstrate the comprehensive analysis of 30 synthetic cannabinoids and metabolites in OF by LC/QqQ/MS using online SPE for sample pretreatment.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing how to detect and quantify Synthethic Cannabinoid (SC) parent drugs, as well as metabolites, in a non-invasive matrix using a relatively swift analytical technique that is sensitive enough to be able to detect low levels of structurally diverse SCs.

According to the National Institute on Drug Abuse, the emerging class of novel psychoactive substances known as SCs include man-made, mind-altering chemicals that are sprayed on dry, shredded plant material and can either be smoked or sold as liquids to be vaporized in e-cigarettes. SCs are often marketed as "synthetic marijuana" and may not be detected by traditional drug screening tests for marijuana, as they do not have the same structure as Δ^9-THC, the main active ingredient in marijuana. Due to the typically rapid metabolism of SCs and their instability in biological matrices, fast analytical methods are needed for the detection and confirmation of parent drugs, as well as metabolites, at very low levels.

SCs are transferred from the blood into OF primarily by diffusion. The detection of these drugs in an OF matrix is advantageous because there is no need for same-sex collection, it is a non-invasive technique that has little risk of adulteration, and collection can be done onsite. OF is a viscous aqueous matrix composed of electrolytes, small molecules, and higher molecular-weight compounds, including proteins and enzymes. Therefore, a sample pretreatment step, typically Liquid-Liquid Extraction (LLE) or SPE, is required prior to sample analysis. Unfortunately, both procedures are time-consuming and involve large amounts of solvents for cleanup.

This study demonstrates the comprehensive analysis of 30 synthetic cannabinoids and metabolites in OF by LC/QqQ/MS using online SPE for sample pretreatment. The aim is to be able to detect and quantify SC parent drugs, as well as metabolites, in a non-invasive matrix using a relatively swift analytical technique that is sensitive enough to be able to detect low levels of structurally diverse SCs. OF specimens are collected using a Quantisal® device consisting of a cotton pad that is placed in the mouth and turns blue upon collection of an approximate 1mL OF sample. This is transferred to a plastic collection tube containing 3mL buffer, provided by the manufacturer, for drug extraction and stability during storage. An aliquot from this sample is spiked with an internal standard SC mix and compared against a six-point calibration curve created using synthetic or authentic pooled OF and buffer (1:3, v:v). An Agilent® 6470 triple quadrupole MS equipped with Jet Stream Electrospray Ionization (ESI) technology is used in positive mode with dynamic Multiple Reaction Monitoring (MRM) (3 MRM repeats) for the characterization of the analytes (1µL injection). Binary gradient elution is performed on a ZORBAX® Eclipse Plus™ C18 Rapid Resolution HD (3.0 x 100mm x 1.8µm) column using MeOH + 0.1% formic acid as organic phase and 5mM ammonium formate + 0.1% formic acid as phase modifier with a total run time of 15min. Online SPE is carried out on an Agilent® 1290 Infinity® Flexcube using ACN, MeOH, and water for cleanup and reconditioning.

Initial results demonstrate calibration curve linearity from 0ng/mL–100ng/mL (R^2 >0.99), with Limit Of Detections (LODs) in the range of 0.5ngmL–1ng/mL. In addition, better analyte ionization was noted with authentic pooled OF as compared to synthetic OF for calibration curves. Determination of Matrix Effects (ME), Process Efficiency (PE) and Recovery (RE) showed data for most analytes to be within acceptable limits according to the Scientific Working Group for Forensic Toxicology (SWGTOX) and other relevant validation guidelines. An exception was noted in the case of PB-22 metabolites, which showed ME >198%, PE >283% and RE >135%. Following full validation and inclusion of additional SCs and metabolites, this method is expected to facilitate rapid and reliable screening of SCs in forensic toxicology applications.

Synthetic Cannabinoids, Oral Fluid, Online SPE