K34 Tools, Techniques, and Findings for the Qualitative Analysis of Delta-9- Tetrahydrocannabinol (THC) in Oral Fluids

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After attending this presentation, attendees will gain insight into scientific methods, applications, and technologies used to test oral fluids for drugs of abuse, particularly exposure to cannabinoids.

This presentation will impact the forensic science community by providing methods and data associated with the development and validation of THC in oral fluids for two separate collection devices.

Devices and techniques used for oral fluid collection and drug screening provide both advantages and new challenges to the field of forensic toxicology. When compared to conventional biological matrices, oral fluids are easier to collect, less prone to adulteration, offer on-site specimen screening, and reflect more recent drug use often associated with impairment.

For each device, the sample collection process involves placing an adsorbent pad in one's mouth until saturated with oral fluid. Following saturation the pad is placed into a pre-packaged tube filled with a diluent buffer, which helps preserve the matrix and prevent adsorption of THC to the pad. Next, the specimen can be analyzed on-site using an immuno-assay, which identifies positive samples based on drug class. If an oral fluid specimen is found to be positive the collection process is repeated and the device will be sent for more a selective confirmation analysis.

The primary goals of this research consisted of developing and validating a more selective and sensitive confirmatory method complimenting immuno-assay field findings for the detection of cannabinoids in oral fluid. Challenges met during development included; the need to detect low concentrations with a cut-off concentration of 0.50 ng/mL (in oral fluid + diluent buffer), complexity and variability of the matrix and limited specimen volume for testing. To overcome such obstacles sample preparation involves acidic dilution of the specimen followed by a solid phase extraction (SPE) and derivatization. The derivatized extracts are then analyzed for THC by multi-dimensional GC/MS.

Validation studies involved matrix matching of synthetic oral fluid to human oral fluid, as well as, between the two collection device's diluent buffers. Each matrix was tested for and showed comparable recovery and precision and accuracy around the cutoff. All batches consisted of three spiked cutoff calibrators, three spiked positive controls (125% cutoff concentration), and three negative controls to be used for evaluating precision, accuracy, and reproducibility of the method. Stability and recovery of THC was performed by spiking positive control pools at three concentrations into each of the two collection devices containing the absorbent pad. Enough devices were spiked to compare and evaluate three concentrations, in triplicate, at nine time intervals over 21 days. Sensitivity and specificity of the assay was performed by analyzing a minimum of 40 blind controls at concentrations ranging from 0% - 1000% over four analytical batches. Testing for interfering substances involved spiking commonly encountered drugs, as well as, three possible adulterants including mouthwash, toothpaste, and denture adhesive into blank matrix for analysis.

Validation results and findings all met acceptance criteria provided by the standard operating procedure for qualitative method validation The limit of detection was calculated to be approximately 0.01ng/mL using the signal to noise ratio of a cutoff calibrator. For precision data negative controls, cutoff calibrators and positive controls were evaluated in triplicate over ten batches. All negative controls were found to be negative and the %CV values for the cutoff calibrators and positive controls were 10.2% and 6.9% respectively. Due to no false positive or false negative findings both sensitivity and specificity were calculated to be 100%. Stability studies showed a decrease in THC values over time when positive controls (125% cutoff) were prepared in bulk and frozen. To accommodate for loss of THC over time, positive controls were hand spiked with each batch. Stability studies also showed one of the collection devices to be superior regarding recovery of THC. Although interference studies showed no interferences, certain toothpaste and mouthwash brands spiked into synthetic oral fluid did show a peak very close to the retention time of THC, with ion ratios being just outside that of the acceptable range. Further investigation determined the possible identity of the interfering peak and showed that the introduction of a "disqualifier" ion to the method clearly distinguished the peak from THC without affecting sensitivity. Also, human oral fluid collected following use of the toothpaste and/or mouthwash containing the interferent lacked concentrations required to impact qualitative results.

THC, Oral Fluids, Multi-Dimensional GC/MS

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