K44 Updated Techniques for Characterizing Cannabis Use

Jami Reber, MSc, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902; Erin L. Karschner, PhD, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902; Joshua Seither, PhD, Armed Forces Medical Examiner System, Dover, DE 19902; Jessica L. Knittel, MS, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902; Jeff Walterscheid, PhD, Armed Forces Medical Examiner System, Dover Air Force Base, DE 19902*

Learning Overview: The goals of this presentation are to raise awareness on the emergence of several phytocannabinoids observed in current forensic casework and describe techniques to identify and confirm these compounds in human specimens using Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees that traditional analytical methods may require adjustments to achieve adequate resolution between cannabinoid isomers. It is important to monitor changes to cannabis legislation and adapt analytical methods accordingly.

Background/Introduction: Cannabis use continues to be a growing problem for forensic investigations. Although marijuana is still illegal federally, decriminalization, especially at the state-level, has permitted the widespread accessibility of cannabis. Cannabis is now available in several forms such as conventional marijuana, extracts, and edible products containing Cannabidiol (CBD) from hemp. In the military, a zero-tolerance stance remains for using cannabis products, including CBD, primarily because CBD products are unregulated. Dronabinol is the Food and Drug Administration (FDA) - approved form of pure **Δ-9-Tetrahydrocannabinol** (Δ-9-THC), which is allowed in military medical care, but also may be used to mask surreptitious use of cannabis products. Therefore, distinguishing dronabinol from cannabis use is required in some cases. Further, new cannabis strains and processing methods have led to the emergence of products containing increased **Δ-8-Tetrahydrocannabinol** (Δ-8-THC) content. These issues have motivated renovations in approaches to enhanced screening and confirmation methods regarding cannabis toxicology analyses.

Objectives: This presentation raises awareness on the emergence of several phytocannabinoids observed in current forensic casework and describes techniques to identify and confirm these compounds in human specimens using LC/MS/MS.

Methods: Blood and/or urine specimens were submitted to the laboratory as investigative cases. Routine screening encompassed a drugs of abuse immunoassay for nine drug classes, an alcohol screen by headspace gas chromatography, and a basic drug screen. If case history indicated potential CBD use, a phytocannabinoid LC/MS/MS screen was utilized to monitor for CBD, its metabolite 7-carboxy cannabidiol, cannabigerol, cannabinol, Δ-9-Tetrahydrocannabivarin (THCV), and 11-nor-9-carboxy-THCV. Presumptive positive screens were confirmed by LC/MS/MS analysis with a limit of detection at 1.0ng/mL for non-carboxylated analytes and 5.0ng/mL for carboxylated analytes. If an analyst detected possible Δ-8-THC or 11-nor-9-Carboxy-Δ-8-Tetrahydrocannabinol (Δ-8-THC-COOH) presence during confirmative LC/MS/MS analysis for Δ9-THC and its metabolites, an additional extraction was performed to confirm the presence of Δ-8-THC and Δ-8-THC-COOH by LC/MS/MS.

Results: An enzyme hydrolysis step yielded ten-fold higher signal for CBD in authentic human samples as compared to conventional alkaline hydrolysis. This improvement in CBD-glucuronide analysis has previously been attributed to enhanced cleavage of the ether glucuronide. When analyzing case specimens, the Δ-9-THC and metabolites method displayed chromatographic shouldering on the Δ-9-THC and Δ-9-THC-COOH peaks. These interfering peaks were identified with standard reference materials as Δ-8-THC and Δ-8-THC-COOH, respectively. This analytical method was adjusted to achieve adequate separation between these isomers. Among several of the cases analyzed during this time, one of them was below the limit of detection for both Δ-9-THC and Δ-9-THC-COOH, yet above the limit of detection for Δ-8-THC and Δ-8-THC-COOH. Without these improvements, this case would have been reported as negative if the additional Δ-8-isomer testing had not been pursued.

Conclusion/Discussion: Because of allowances under the 2018 Farm Bill, products containing CBD and other cannabinoids have been readily available for consumption. Standard screening procedures are unable to detect many of these phytocannabinoids; therefore, more targeted screening methods must be implemented in order to properly identify these compounds in human specimens. Traditional analytical methods may require adjustments to achieve adequate resolution between cannabinoid isomers. It is important to monitor changes to cannabis legislation and adapt analytical methods accordingly.

Cannabis, LC/MS/MS, Screening

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*Presenting Author