K33 Analysis of Cannabinoids in Vitreous Fluid

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Learning Overview: The goal of this presentation is to present a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method development and validation study for analyzing vitreous fluid for Tetrahydrocannabinol (THC), 11-Nor-9-Carboxy-Δ9-Tetrahydrocannabinol (THCCOOH), 11-Hydoxy-Δ9-Tetrahydrocannabinol (11-OH-THC), and Cannabidiol (CBD). Casework examples comparing blood and vitreous cannabinoids will be presented.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by showing the value of vitreous fluid as a toxicologically important matrix when other matrices are not available.

Cannabis is currently the most widely abused illicit drug in the world.[1] In the United States, recreational cannabis use is legal in 11 states and medical cannabis use is legal in 33 states. As medical and recreational uses of this substance become increasingly legal, there is a need for reliable analytical methods that can detect and analyze cannabinoids in death cases where there is a question relating to the cause and manner of death when typical matrices are not available. Vitreous humor is a gelatinous fluid located in the eyeball and is regularly drawn during autopsy and used as a matrix to test for drugs and alcohol by a postmortem toxicologist. This study utilized bovine vitreous fluid as a blank matrix for method development and validation.

THC is the primary psychoactive component in cannabis and produces the euphoric, relaxing feelings that are associated with the drug. 11-OH-THC is the primary active metabolite of THC and THCCOOH is the secondary and inactive metabolite of THC. CBD is a non-psychoactive component in cannabis that has emerged as a potential remedy for pain, anxiety, and other common health issues. Hemp-based CBD products with less than 0.3% THC content are federally legal in the United States. In this study, a method was developed and validated to quantitate THC, CBD, 11-OH-THC, and THCCOOH in bovine vitreous humor. Samples were prepared by a liquid-liquid extraction using 1 milliliter of vitreous fluid, acidified water, and organic solvents. Separations were conducted using a Phenomenex® Kinetex® C18 (2.1x100 mm, 2.6µm particle size) with VANQUISH™ High Pressure Liquid Chromatography System. Gradient elution was performed with 0.1% formic acid in water and acetonitrile. Identification and quantitation were performed with a TSQ Endura™ Triple quadrupole Mass Spectrometer (QqQ MS) operating in selective reaction monitoring mode. This LC/MS/MS technique combines low complexity sample preparation with the selectivity and sensitivity of LC/MS/MS.

This method was cross validated in whole blood and plasma matrices and used in routine, postmortem toxicology casework. Samples were screened using and Enzyme-Linked Immuno-Sorbent Assay (ELISA) and quantitatively confirmed using the developed LC/MS/MS cannabinoid assay. Limits Of Quantitation (LOQ) are typically 0.5ng/mL with an analytical measurement range reaching 250ng/mL. Casework samples testing positive by ELISA showed the presence of cannabinoid compounds. Blood samples testing positive for cannabinoids showed a positive correlation for trace levels of THCCOOH in vitreous.

This method allows for detection of CBD that can clarify the reported use of federally legal food supplements and/or products used for their reported medicinal benefit. Driving under the influence of marijuana is a major concern in society and postmortem cases involving motor vehicle fatalities need to have forensically valid testing for interpretation of marijuana usage. Furthermore, it is important to differentiate detection of CBD and metabolites versus THC and metabolites in these cases.

Reference(s):

1. United Nations Office on Drugs and Crime, *World Drug Report 2016* (United Nations publication, Sales No. E.16.XI.7).

LC/MS/MS, Cannabinoids, Vitreous