

## D3 The Determination of Delta-9-Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), and Total THC in Seized Cannabis (Hemp and Marijuana) Samples by Liquid Chromatography With Ultraviolet Detection (LC-UV)

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**Learning Overview:** The goal of this presentation is to summarize the evaluation of an extraction and LC-UV absorbance detection method for measuring delta-9 ( $\Delta^9$ ) -THC, THCA, and total THC in seized cannabis (marijuana and hemp) samples.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing forensic scientists with an analytical procedure and quantitative method to confidently distinguish seized cannabis samples as either legal hemp or illegal marijuana.

The medicinal and recreational use of cannabis (hemp and marijuana) and cannabis-derived products continues to increase across the United States. As the industry grows, so does the need for reliable differentiation between legal and illegal products. The Agriculture Improvement Act of 2018 no longer defines hemp and its derivatives as Schedule I substances, while marijuana remains federally illegal. In addition, hemp was defined as cannabis materials having a  $\Delta^9$ -THC concentration of no more than 0.3 % on a dry weight basis. Prior to the legalization of hemp, forensic laboratories were required to only identify the presence of  $\Delta^9$ -THC through a qualitative procedure, including microscopic analysis, colorimetric test, and Gas Chromatography/Mass Spectrometry (GC/MS) detection using matching retention times and mass spectrum to pure  $\Delta^9$ -THC reference standards. Forensic laboratories are required now to distinguish seized cannabis samples as either legal hemp or illegal marijuana, leading to the need for new sample preparation protocols and standardized analytical detection methods.

The National Institute of Standards and Technology (NIST) has conducted multiple method development studies of an LC-UV detection method. Prior to analysis, cannabis samples were solvent extracted with the addition of 20mL of methanol, mixing, and centrifugation. Following a 10-fold and 100fold dilution, the extracts are filtered using a 0.45 $\mu$ m Polytetrafluoroethylene (PFTE) syringe filter into an autosampler vial. This presentation will summarize the initial evaluation of this extraction protocol and LC-UV method for the determination of  $\Delta^9$ -THC, THCA, and total THC in hemp reference samples and seized cannabis (hemp and marijuana) samples. In the case of United Kingdom reference samples, comparison was made to their Certificate of Analysis to ensure the accuracy and precise of the LC-UV methods. Overall, the NIST measurements were in good agreement for  $\Delta^9$ -THC, THCA, and total THC mass fraction (%) values in four different hemp samples in the multiple method development studies. In the case of seized cannabis samples, the LC-UV values were in good agreement with the other analytical methods developed at NIST, including GC/MS and LC couple to Tandem Mass Spectrometry (MS/MS). A Cannabis Quality Assurance Program (CannaQAP) has been developed and the development of a hemp Reference Material (RM) has been started aimed at reducing discrepancies in analytical measurements within the forensic community. The same methods summarized here are used for the initial screening of CannaQAP and RM samples.

Liquid Chromatography, Seized Samples, Cannabis