B206 The Determination of Total Tetrahydrocannabinol (THC) Concentration in Plant Material Via High Performance Liquid Chromatography With Ultraviolet Visible Diode Array Detection (HPLC-UV DAD)

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Learning Overview: After attending this presentation, attendees will understand the process of quantitative method development and validation, the extraction of THCs from plant material for quantitation, and examples of practical application involving casework performed by the Colorado Bureau of Investigation (CBI) Forensic Science Laboratory.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by serving as a guide for laboratories seeking to develop and implement a THC quantitation procedure for plant material.

The State of Colorado, per the Colorado Revised Statutes (CRS) 35-61-101, defines industrial hemp as "a plant of the genus cannabis and any part of the plant, whether growing or not, containing a delta-9-tetrahydrocannabinol (THC) concentration of no more than three-tenths of one percent (0.3%) on a dry weight basis". Colorado statute SB17-090 indicates that the delta-9-THC concentration is determined by measuring the combined concentration of delta-9-THC and delta-9-Tetrahydrocannabinolic Acid (THCA). Colorado's legalization of medical and recreational marijuana, as well as a thriving hemp industry, has created a need for the development and utilization of scientific methods to distinguish hemp from marijuana. Primarily focused on the critical value of 0.3% THC as defined by the CRS, an HPLC-UV DAD method was developed and validated by the CBI to evaluate plant materials with total THC concentrations between 0.1% and 10%.

Samples are initially analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) prior to quantitation. The quantitative method involves the concurrent preparation of a six-point calibration curve, three positive aqueous controls, two plant process controls, and case samples (prepared in duplicate). Sample preparation consists of an initial drying step, grinding of the plant material, thermal decarboxylation of THCA to THC, and extraction in methanol. Androstenedione is used as an internal standard. Additionally, samples undergo a profiling step *prior* to decarboxylation by HPLC-UV DAD. This step allows the analyst to understand the components of the sample and, when compared with the quantitated samples, demonstrates that complete thermal decarboxylation of THCA was achieved.

Analysis of case samples utilizing this method has produced a surprising variety of results. The laboratory has analyzed samples that were quantitated below the lower quantitative limit of the method (0.1% total THCs) as well as samples above 10% THC. Perhaps most surprising has been the number of *true* hemp samples received by the CBI for analysis and the variation observed in terms of their overall cannabinoid profiles and physical appearance.

The precision of the method was determined using ten replicate injections of six THC solutions with concentrations ranging from 5 to 250µg/mL (n=60). The Relative Standard Deviation (RSD) was calculated at each concentration and all were less than 3%. The accuracy was determined by comparing one set of six calibrators with the corresponding target concentrations (n=6). Again, the RSD was calculated for each level and determined to be less than 3%. The measurement of uncertainty for the method is 8.31%, reported as a relative percentage of the total THC concentration, and was determined using replicate samplings (n=32) of a known plant sample (0.32% THC) obtained from the Colorado Department of Agriculture. The CBI has created a robust method for the determination of total THC concentration in plant material.

Tetrahydrocannabinol, Quantitation, HPLC-UV DAD

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