B11 The Differentiation of Hemp From Marijuana Using a Qualitative Decision-Point Assay

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Learning Overview: After attending this presentation, attendees will understand how qualitative decision-point assays can be used to differentiate hemp from marijuana and will gain insight regarding the analytical challenges associated with the development, optimization, and validation of these assays.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on the performance of a qualitative assay to differentiate hemp from marijuana.

C. sativa is the primary species of the *Cannabis* genus and can be broadly classified as hemp (fiber-type cannabis) and marijuana (drug-type cannabis). Regulatory changes following the passage of the 2018 Farm Bill define hemp as *C. sativa* containing no more than 0.3% Δ-9-Tetrahydrocannabinol (Δ-9-THC) on a dry weight basis. As a result, forensic laboratories must deploy methods to differentiate legal hemp from illegal marijuana. Due to the volume of submissions within the controlled substance discipline, this requirement presents a significant challenge for operational laboratories.

The United States Drug Enforcement Administration was the first to implement an analytical scheme that incorporates a decision-point approach to differentiate hemp from marijuana. Using an administratively set threshold, a qualitative assay can be used to identify Δ-9-THC in plant material.

This presentation will describe the evaluation of multiple internal standards, extraction conditions, potential interferences, and instrumental parameters. Two analytical methods using Gas Chromatography/Mass Spectrometry (GC/MS) are described and their performance is compared. Optimized methods using an administrative threshold equivalent to 1% Δ-9-THC were validated in terms of selectivity, limits of detection, repeatability, reproducibility, accuracy, dilution integrity, and carryover. *In situ* decarboxylation of Δ-9-tetrahydrocannabinolic acid and cyclization of Cannabidiol (CBD) were also assessed.

Δ-9-THC was extracted from cannabis plant material using methanol. Δ-9-THC-D3 was added post-extraction and samples were analyzed using dual Selected Ion Monitoring (SIM)/scan acquisition. Two analytical methods were developed to allow for the separation of common cannabinoids (~6mins) and a more expansive list (~12mins). Extraction efficiencies from plant matrix were 80%–92% and decarboxylation rates of 62%–67% were observed using an inlet temperature of 250ºC. Using the 12-minute assay, the limit of detection in plant material was 0.3% Δ-9-THC by weight, and linear detector responses were observed up to 10% Δ-9-THC. Dilution integrity was established for plant materials containing up to 50% Δ-9-THC. Selectivity was demonstrated using Cannabidivarin (CBDV), Cannabichromevarin (CBCV), Cannabicitran (CBT), Tetrahydrocannabivarin (THCV), Cannabivarin (CBV), Cannabicyclol (CBL), CBD, Cannabichromene (CBC), exo-THC, Δ-8-Tetrahydrocannabinol (Δ-8-THC), Δ-6a,10a-Tetrahydrocannabinol (Δ6a,10a-THC), Δ-9-THC, Δ-10-Tetrahydrocannabinol (Δ-10-THC), Cannabigerol (CBG), and Cannabinol (CBN). Repeatability and reproducibility at the decision-point yielded relative standard deviations of 3.4% (n=10) and 5.1% (n=50). Accuracy of the method was established using marijuana plant material provided by the National Institute on Drug Abuse (NIDA) Drug Supply Program (0.12% to 10.1% Δ-9-THC) and five commercial hemp samples. Replicate analyses were performed using the 15 sources of plants. Sensitivity, specificity, negative predictive value, and positive predictive value were 100% (n=70). Extracts were stable for five days when refrigerated in the dark. Potential interferences from CBD were evaluated using plant matrix fortified to the equivalent of 50% CBD by weight. Negative controls, positive controls, and plant extracts were free from interferences. Finally, measurement uncertainty was estimated at 0.3% Δ-9-THC (below the administrative threshold). The expanded uncertainty using a 95.3% confidence interval (k=2) was 12.2% (0.3±0.04% Δ-9-THC by weight of plant material).

While qualitative decision-point assays can be used to differentiate hemp from marijuana, overall assay performance can be impacted by decarboxylation rates, potential interferences, and reproducibility between instruments. Careful optimization and rigorous validation is required. Using this approach, the qualitative assay using an administrative threshold of 1% Δ,9-THC was found to be robust and reliable.

Hemp, Marijuana, GC/MS