B12 The Results of an Interlaboratory Validation to Differentiate Marijuana From Hemp

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Learning Overview: After attending this presentation, attendees will gain insight regarding the benefits of interlaboratory validations, the potential for differences in analytical performance between laboratories, and the need for full, independent, and rigorous validation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by encouraging interagency collaborations and interlaboratory comparisons as part of new assay development.

Following the passage of relatively recent federal and state legislation, laboratories must now differentiate marijuana from hemp based upon the concentration of Δ-9-tetrahydrocannabinol (Δ-9-THC) in the plant. The federal 2018 Farm Bill defines hemp as *Cannabis sativa* L. containing not more than 0.3% Δ-9-THC on a dry weight basis. In order to avoid full quantitative analyses on routine plant exhibits, some organizations have adopted decision-point assays to differentiate illegal marijuana from legal or commercial hemp products.

In 2019, with support from the Texas Forensic Science Commission, Sam Houston State University, Houston Forensic Science Center, Harris County Institute of Forensic Sciences, and the Texas Department of Public Safety Crime Laboratory Service initiated a collaborative study to address the issue. Using a modification of the method first reported by the United States Drug Enforcement Administration (DEA), a qualitative decision-point assay was developed and validated. Using a 1% Δ-9-THC administrative threshold, Gas Chromatography/Mass Spectrometry (GC/MS) was used to differentiate hemp from marijuana using a qualitative assay. Deuterated internal standard and dual Selected Ion Monitoring (SIM)/scan acquisition was used to analyze methanolic extracts of suspected cannabis plant material.

The method was fully validated at all four sites in terms of limit of detection, selectivity, precision, accuracy, dilution integrity, carryover, and extract stability. Decarboxylation of Δ-9-tetrahydrocannabinolic acid to Δ-9-THC was evaluated during analysis, and potential interference from Cannabidiol (CBD) was also investigated.

Selectivity was demonstrated using more than 12 cannabinoids, including those that are known to elute in close proximity to Δ-9-THC. Selectivity and retention time stability was established over ten injections on one day and one injection over ten days at each site. Inter-assay Coefficient of Variations (CVs) (n=10) ranged from 0.0%–3-0.31% for Δ-9-THC and 0%–0.30% for all other cannabinoids. Limits of detection in methanolic extracts ranged from 0.008–0.015mg/mL (equivalent to 0.15%–0.3% Δ-9-THC by weight of plant material). Although the assay is not quantitative in nature, linearity of detector response was evaluated at each site, yielding R^2 values of 0.992–1.000. Repeatability and reproducibility of the 1% decision-point control were evaluated using the relative peak area (Δ-9-THC/Δ-9-THC-D3), yielding CVs of 1.9%–3.7% (n=10) and 2.4%–5.1% (n=50). Carryover of Δ-9-THC was observed at the equivalent of 50% or more Δ-9-THC at some sites. Secondary decision-point controls were utilized as a quality control measure. Dilution integrity was established using five- and ten-old dilutions of extracts prepared from placebo matrix fortified with 20%–50% Δ-9-THC.

Each of the collaborating laboratories selected an administrative threshold in plant material of 1% Δ-9-THC by weight, rather than the 0.3% statutory threshold. In addition to this safeguard, the analytical approach is likely to underestimate the total Δ-9-THC due to incomplete extraction efficiencies and decarboxylation rates. It should be noted that this conservative approach is designed to prevent false positive results and increase the specificity of the assay at the expense of sensitivity. Using plant materials of known Δ-9-THC content, specificity and positive predictive value were both 100%. Of the 280 analyses, a total of eight false negative results were observed, lowering the overall sensitivity and negative predictive value to 94% and 95%, respectively. Extracts were found to be stable when stored refrigerated for up to five days. Potential interferences from CBD were evaluated using negative controls and plant extracts fortified with 0, 10, 20, 30, 40, and 50% CBD. No false positives were observed. Although the assay is qualitative in nature, measurement uncertainty was estimated at 0.3% Δ-9-THC (below the administrative threshold). Expanded uncertainties using a 95.45% confidence interval (k=2) were 12.2%–21.8% among laboratories, equivalent to 0.3 ±0.04% to 0.07% Δ-9-THC by weight.

Although satisfactory performance was achieved at all sites, differences in analytical performance were also observed for some variables. Therefore, although multi-agency collaborations and interlaboratory validations are extremely beneficial, they do not supplant the need for there to be a full, independent, and rigorous validation.

Marijuana, Hemp, GC/MS

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