



## B83 Method Development for Separation and Quantitation of 17 Cannabinoids Using Gas Chromatography/ Mass Spectrometry (GC/MS) and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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Learning Overview: After attending this presentation, attendees will learn about the different methods used to quantitate cannabinoids using GC/MS and LC/MS/MS.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by providing insight into GC/MS and LC/MS/MS method development focused on the separation of cannabinoids for quantitation.

Cannabis has been considered a Schedule I controlled substance since the 1970s. Federal, state, and local forensic labs have traditionally used qualitative methods to identify the presence or absence of Tetrahydrocannabinol (THC) in confiscated cannabis samples. However, the 2018 Farm Bill removed hemp from the controlled substances list and defined it as cannabis containing less than 0.3% total THC content. By defining a concentration threshold, the 2018 Farm Bill created a need for THC quantitation and, with that, an inherent requirement for quantitative methods. A cannabis research program has been established to assist the forensic community in making quantitative measurements in various cannabis samples through the development of analytical methods, a quality assurance program, and cannabis reference materials. The work presented will include LC/MS/MS and GC/MS method development for the quantitation of 17 cannabinoids in multiple cannabis materials.

The LC/MS/MS method development utilized MS/MS selectivity to reduce necessity of LC separation of cannabinoids with distinct ion transitions, allowing for focused chromatographic separation of the isomeric cannabinoids. To determine the most effective chemistry for cannabinoid separation, reversed phase C18 and biphenyl columns were evaluated. Separation was achieved using gradient elution, with 0.1 % formic acid in water and 0.1 % formic acid in methanol as mobile phases. Data acquisition was carried out in multiple reaction monitoring mode, using positive electrospray ionization for the neutral cannabinoids and negative electrospray ionization for the acidic cannabinoids.

The GC/MS method described here focuses on the baseline separation of nine neutral cannabinoids while limiting the total run time to less than ten minutes, permitting a higher sample throughput in forensic laboratories. As part of the method development, a detailed comparison of GC column stationary phase and oven temperatures were evaluated with an emphasis placed on optimizing separation and sensitivity for delta-9-THC. One of the GC stationary phases evaluated included the non-polar 5% phenyl phase that is currently utilized in forensic laboratories for measuring delta-9-THC in cannabis samples. However, a second stationary phase consisting of 35% phenyl was found to provide a superior separation. Additionally, the initial oven temperature was optimized to identify the highest temperature that provided the earliest elution of the cannabinoids without sacrificing chromatographic separation. As a result, a quick ten-minute quantitative method using a 35% phenyl stationary phase was developed and evaluated for the analysis of various cannabis plant and oil samples.

Chromatography, Cannabis, Mass Spectrometry