B11 High-Throughput and Simultaneous Analysis of 12 Cannabinoids in Hemp Oil Using Liquid Chromatography With Ultraviolet (LC-UV) Detection

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Learning Overview: After attending this presentation, attendees will better understand the strategy to achieve high-throughput and simultaneous analysis of cannabinoids and appreciate a validated LC-UV method for the analysis of 12 cannabinoids in hemp oil.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by first introducing three fast LC separations of 12 cannabinoids that can be used with either UV or Mass Spectrometric (MS) detection. This presentation will further impact the forensic science community by introducing a validated LC-UV method for high-throughput and simultaneous analysis of 12 cannabinoids in hemp oil, which can be routinely used by cannabis testing labs.

In recent years, the use of products of *Cannabis sativa* L. for medicinal purposes has been in a rapid growth, although their preparation procedure has not been clearly standardized and their quality has not been well regulated. To analyze the therapeutic components (i.e., cannabinoids) in products of *Cannabis sativa* L., LC-UV has been frequently used because LC-UV is commonly available and usually appropriate for routine analysis by the cannabis growers and commercial suppliers. In the literature, a few validated LC-UV methods have been described. However, so far, all validated LC-UV methods only focused on the quantification of 11 or fewer cannabinoids. Therefore, a method able to simultaneously analyze more cannabinoids in a shorter run time is still in high demand because more and more cannabinoids have been isolated and many of them have shown medicinal properties.

In this study, the LC separation of 12 cannabinoids, namely Cannabichromene (CBC), Cannabidiolic Acid (CBDA), Cannabidiol (CBD), Cannabidivarinic Acid (CBDVA), Cannabidivarin (CBDV), Cannabigerolic Acid (CBGA), Cannabigerol (CBG), Cannabinol (CBN), Delta-8 Tetrahydrocannabinol (Δ^8-THC), Delta-9 Tetrahydrocannabinolic Acid A (Δ^9-THCA A), Delta-9 Tetrahydrocannabinol (Δ^9-THC), and Tetrahydrocannabivarin (THCV), has been systematically optimized using a Phenomenex® Luna® Omega 3µm Polar C18 150mm × 4.6mm column with regard to the effects of the type of organic solvent (i.e., methanol and acetonitrile), the content of the organic solvent, and the pH of the mobile phase. The optimization has resulted in three LC conditions at 1.0mL/minute able to separate the 12 cannabinoids: (1) a mobile phase consisting of water and methanol, both containing 0.1% formic acid (pH 2.69), with a gradient elution at 75% methanol for the first 3 minutes and then linearly increase to 100% methanol at 12.5 minutes; (2) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.1% formic acid and 20mM ammonium formate (pH 3.69), with an isocratic elution at 75% acetonitrile for 14 minutes; and (3) a mobile phase consisting of water and 90% (v/v) acetonitrile in water, both containing 0.03% formic acid and 20mM ammonium formate (pH 4.20), with an isocratic elution at 75% acetonitrile for 14 minutes.

In order to demonstrate the effectiveness of the achieved LC separations, an LC-UV method is further validated for the high-throughput and simultaneous analysis of 12 cannabinoids. The method used the mobile phase at pH 3.69, which resulted in significant improvement in throughput compared to other validated LC-UV methods published so far. The method used flurbiprofen as the internal standard. The linear calibration range of all the cannabinoids were between 0.1 to 25ppm with $R^2 \geq 0.9993$. The Limit of Quantitation (LOQ) (S/N=10) of the cannabinoids was between 17.8 and 74.2ppb. The validation used a hemp oil containing 3.2wt% CBD and no other cannabinoids, which was reported by the vendor with a certificate of analysis, as the matrix to prepare control samples: the hemp oil was first extracted using Liquid-Liquid Extraction (LLE) with methanol; cannabinoids were then spiked into the extract at both 0.5ppm and 5ppm levels. Afterward, the recovery, precision (%RSD), and accuracy (%Error) of the control samples were assessed, and the results met the requirements by the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025 and American Society for Testing and Materials (ASTM) E2549-14 guidelines.

Cannabis, Cannabinoids, LC-UV

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