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## **HPLC-DAD Potency Assay for the Detection and Quantitation of Flavonoids in Hemp Flower**

**Presenter: Louis P. Balas, R&D Associate, Charlotte's Web**

**Co-Authors: Alexander McCorkle, Yvonne DePorre, PhD**

**Abstract:** Flavonoids are a group of compounds found in cannabis and many other plants that are receiving increased interest for their potential antioxidant, anti-inflammatory, neuroprotective, anxiolytic, and anti-cancer properties. Over 20 different flavonoids have been identified in cannabis, two of which, cannflavin A and cannflavin B, are unique to the plant. The various flavonoids found in cannabis are thought to work synergistically with other phytocannabinoids to increase the bioactivity of those cannabinoids in a process known as the “entourage effect.” These flavonoids also contribute to the unique aroma and color of cannabis. Despite the contribution of flavonoids to the overall chemical profile of cannabis, few testing facilities have incorporated flavonoid testing into their workflow. Therefore, we developed and validated a reverse-phase HPLC-DAD method and extraction process for the quantitation of 17 different flavonoids in Charlotte’s Web Hemp. The method demonstrated good linearity (80-10 ng/ $\mu$ l,  $r^2 \geq 0.99$ ) and Precision (RSD  $\leq 1.7\%$ ). 14 of the 17 analytes tested were found in quantifiable levels within the hemp, and percent recovery ranged from 70-111% for almost all analytes, suggesting this method is effective for the extraction of endogenous flavonoids. Specificity of the method was verified via the overlay of standard and extract spectra after HPLC analysis. Determination of flavonoid content in hemp should become commonplace as consumers seek to understand the full range of benefits associated with hemp products.