



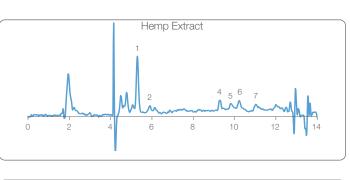
Cannabinoid's Wingman? Determination of Flavonoids in Hemp by Reversed-Phase HPLC

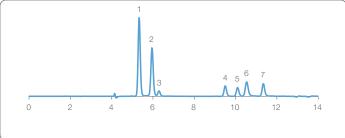
Flavonoids are found in many colorful fruits, leafy greens, vegetables, and citrus. They are generally associated with the defense system of the plant and protect the plant from hungry herbivores and disease. There are over 5,000 various forms of flavonoids identified throughout the world which are generally found in the leaves, roots, and stems. In cannabis, the highest concentrations are present in the leaves, stems, and pollen! There are approximately twenty flavonoid compounds found in cannabis including two that are wholly unique to the plant, cannflavin A, and cannflavin B. The flavonoids exhibiting the highest concentration in cannabis include kaempferol, quercetin, apigenin, luteolin, vitexin, isovitexin, and orientin. Cannabis research involving flavodoids has indicated activation of both CB1 and CB2 receptors² in addition to playing a role in the THC metabolism pathway. These discoveries indicate that flavonoids competitively bind to both the CB1 and CB2 receptors³ and provide a more therapeutic experience. Adding to the entourage effect is not the only positive attribute of flavonoids. They also contribute a wide range of overall health benefits including anti-cancer, anti-aging, DNA repair, and antiinflammatory⁴ qualities just to name a few.

As consumers of cannabis become more aware of the full capacity of the plant, analysis of the entourage compounds associated with the various strains should become commonplace. In an effort to provide the end user with a more complete experience, Hamilton Company has developed a method to confirm the 7 most common flavonoids found in cannabis utilizing the PRP-1 5 µm HPLC column. The polymeric stationary phase used in the PRP-1 column yield good peak shape while adding value to the identification. Sample preparation is kept to a minimum with only a 15 minute sonication extraction using ethanol:water 3:1. After centrifugation, the sample is injected. There is no need to filter the sample, allowing faster analysis with the dilute and shoot sampling protocol while still maintaining consistent results. This method utilizes tetrahydrofuran and formic acid as mobile phases and provides baseline separation for all the components in under twelve minutes.

- 1) Flores-Sanchez, I; Verpoorte, R. Phytochem Rev (2008) 7:615-639.
- 2) Barrett, M; Scutt, A; Evans, F. Experientia. (1986) 15;42(4):452-3.
- Pollastro, F; Minassi, A; Luigia-Grazia, F. Curr Med Chem . 2018;25(10):1160-1185. Seelinger G, Merfort I, Schempp CM. Planta Med. 2008 74(14):1667-77. 3)
- 4)

Author: Adam L. Moore, PhD





Compounds:	1. Isovitexin	2. Orientin	3. Vitexin
4. Luteolin	5. Apigenin	6. Quercetin	7. Kaempferol

Column Information

Gradient 0.0 - 1.0 min 5% B 1.0 - 1.5 min 5 - 30% B 1.5 - 8.0 min 30% B 8.0 - 13.0 min 30 - 65% B Temperature 35 °C Injection Volume 5 μL Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	oolallin illionnation			
Chromatographic Conditions Gradient 0.0 - 1.0 min 5% B 1.0 - 1.5 min 5 - 30% B 1.5 - 8.0 min 30% B 8.0 - 13.0 min 30% B 8.0 - 13.0 min 300 - 65% B Temperature 35 °C Injection Volume 5 μL Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Packing Material	PRP-1, 5 μm		
Gradient 0.0 - 1.0 min 5% B 1.0 - 1.5 min 5 - 30% B 1.5 - 8.0 min 30% B 8.0 - 13.0 min 30 - 65% B Temperature 35 °C Injection Volume 5 μL Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	P/N	79444		
Gradient 1.0 - 1.5 min 5 - 30% B 1.5 - 8.0 min 30% B 1.5 - 8.0 min 30% B 8.0 - 13.0 min 30 - 65% B 35 °C Temperature 35 °C Injection Volume 5 μL Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Chromatographic Conditions			
Injection Volume 5 μL Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Gradient	1.0 – 1.5 min 5 – 30% B 1.5 – 8.0 min 30% B		
Detection UV at 360 nm Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Temperature	35 °C		
Dimensions 150 x 4.1 mm Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Injection Volume	5 μL		
Eluent A 10 mM Formic Acid Eluent B Tetrahydrofuran	Detection	UV at 360 nm		
Eluent B Tetrahydrofuran	Dimensions	150 x 4.1 mm		
	Eluent A	10 mM Formic Acid		
	Eluent B	Tetrahydrofuran		
Flow Rate 1.0 mL/min.	Flow Rate	1.0 mL/min.		

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