



Introduction

As new research is performed on cannabinoids in order to investigate their individual medicinal properties, the need to purify specific components has increased dramatically. Products currently on the market are typically impure extracts containing a mixture of many different cannabinoid compounds. Purification of individual cannabinoids from these extracts/oils can be accomplished using a number of different techniques, one being liquid chromatography. This poster investigates the purification of cannabidiol (CBD) from a commercially available hemp oil extract using high pressure liquid chromatography (HPLC), complete with analysis of purity and recovery from CBD peak collections.

Experimental

Sample Preparation

Preparative injections of the hemp oil extract were made using the neat oil with no dilution. Both 80µL and 100µL injections were made and fractions collected at 10 second intervals. Fractions containing CBD were diluted 1:20 with 75:25 ethanol:water and reinjected for analysis of purity and recovery.

Standard Preparation

A CBD standard curve was constructed using 5 different standard concentrations from 0.02mg/mL to 0.20mg/mL. Dilutions were made from a 1.0mg/mL stock CBD standard (Cerilliant Corp.) using 75:25 ethanol:water as the diluent.

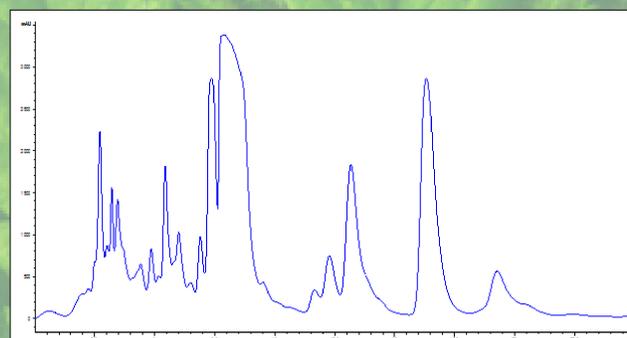
Instrument Parameters

HPLC System: Agilent 1260 HPLC
 Flowrate: 1.0mL/min
 Column Temp: 30° C
 Sample Temp: Ambient
 Detection λ: 220 nm
 Injection Vol: 80µL & 100µL for prep
 20µL for analytical
 Columns: YMC-Triart C18,
 250x4.6mm, 10µm, 120Å
 250x4.6mm, 5µm, 120Å
 Mobile Phase: 25:75 - Water:Ethanol
 Runtime: 25 minutes

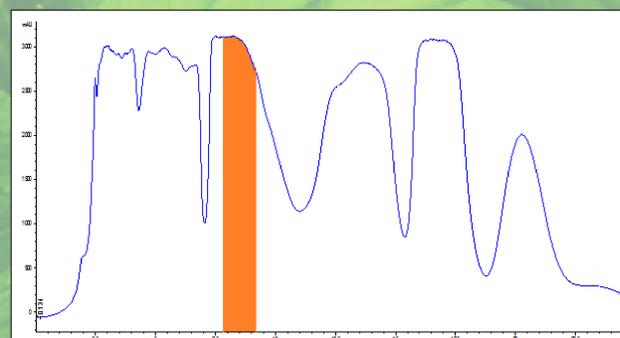
Reversed Phase LC: High Purity and Recovery for Cannabidiol

Results and Discussion

YMC's Triart C18 hybrid-silica stationary phase was evaluated for preparative purification of cannabidiol. Triart C18 was chosen for its overall chemical and thermal durability as well as its scalability to larger preparative size particles. Water and ethanol were chosen as the mobile phase solvents due to the well-understood human toxicity of ethanol, making it a better choice as compared to traditional LC solvents such as methanol or acetonitrile. A number of different isocratic conditions were evaluated before settling on the 25:75 water:ethanol configuration which gave the best compromise between resolution and overall runtime. The initial method was worked out on a 250x4.6mm, 5µm Triart C18 column and then scaled to a 250x4.6mm, 10µm Triart C18 column to perform the loading study. Loadings of 10, 40, 80, and 100µL of neat hemp oil were run to determine the highest sample load that could be placed on the column. Examples can be seen below, with the CBD fraction highlighted in orange:

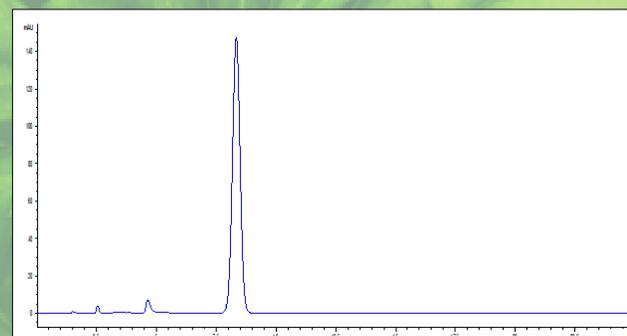


10µL Hemp Oil on 5µm Triart C18

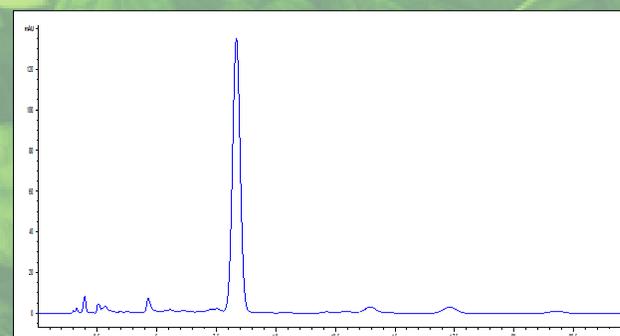


100µL Hemp Oil on 10µm Triart C18

Referenced from the certification of analysis (CofA) obtained from the Hemp oil manufacturer (Charlotte's Web) the concentration of CBD in the hemp oil was 66.97mg/mL. This equates to a CBD loading of 5.358mg for the 80µL injection and 6.697mg for the 100µL injection. Fractions of the CBD peak for both loadings were taken at 10 second intervals and then reinjected to determine purity. Once fractions with the highest purity were determined, another 100µL injection was made and one large CBD fraction was collected over the timeframe encompassing the smaller 10 second fractions. This large fraction was then diluted and analyzed for overall purity and recovery (performed in triplicate) against a standard curve. Example chromatograms of a CBD standard curve and a diluted CBD fraction are shown below:



Ex: CBD 0.1mg/mL Standard



Ex: CBD Fraction After Dilution

Conclusions

Final results of the 80µL and 100µL loadings can be seen in the chart below:

Sample	Total CBD Recovery (mg)	CBD Loaded (mg)	Recovery (%)	Purity (by % Area)
80µL-Sple A	4.64	5.358	86.6%	96.9%
80µL-Sple B	4.65	5.358	86.9%	96.7%
80µL-Sple C	4.77	5.358	89.0%	96.8%
Avg:	4.69	5.358	87.5%	96.8%
100µL-Sple A	5.51	6.697	82.3%	96.0%
100µL-Sple B	5.70	6.697	85.1%	96.1%
100µL-Sple C	5.59	6.697	83.4%	96.4%
Avg:	5.60	6.697	83.6%	96.2%

- ❖ YMC-Triart C18 performed well in the loading study and is shown to be a good choice for scaling up to larger columns for purification of CBD.
- ❖ YMC-Triart C18 scales well from 5µm (analytical) to 10µm (preparative) particle size, exhibiting the same selectivity on both materials.
- ❖ As expected, the lower loading (80µL=5.358mg) exhibited higher recovery (87.5%) and slightly higher purity (96.8%) as compared to the higher loading (100µL=6.697mg) that resulted in 83.6% recovery and 96.2% purity (average).
- ❖ From experiments performed with lower organic mobile phase conditions (<75% ethanol), higher purity and recoveries can be obtained, but this comes at a sacrifice to time/productivity.
- ❖ Overall, this data supports YMC-Triart C18 as being an excellent choice for CBD purification, and would also be a viable option for purifying other related cannabinoid compounds.



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