

# HPLC Application Note

## Cannabinoids



### Preparative Purification of Cannabidiol from Hemp Oil Extract

Jeffrey A. Kakaley, YMC America Inc.

This application note details a loading study for purifying cannabidiol (CBD) from a commercially available “high-CBD” hemp oil extract.

#### Background

As new research is performed to evaluate the medicinal properties of cannabis, the need to purify individual cannabinoids has increased dramatically. Products currently on the market are typically impure extracts that contain a mixture of many different cannabinoid compounds. This application note investigates the use of HPLC and YMC’s Triart C18 stationary phase to purify CBD from a hemp oil extract.

#### Sample Preparation

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

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

#### Operating Parameters

HPLC System: Agilent 1260  
Mobile Phase: 75:25 – Ethanol:Water  
Column Temp: 30°C  
Flow rate: 1.0mL/min  
Inj. Volume: 80 $\mu$ L & 100 $\mu$ L for prep  
20 $\mu$ L for analytical  
Detection  $\lambda$ : 220nm  
Columns: YMC-Triart C18, 120Å  
250x4.6mm, 10 $\mu$ m – prep  
250x4.6mm, 5 $\mu$ m – analytical  
Gradient: None, isocratic  
Runtime: 25 minutes

#### Results and Discussion

Triart C18 was chosen for its overall durability and 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, 5 $\mu$ m Triart C18 column and then scaled to a 250x4.6mm, 10 $\mu$ m Triart C18 column to perform the loading study. Loadings of 10, 40, 80, and 100 $\mu$ L of neat hemp oil were run to determine the highest sample load that could be placed on the column. Examples can be seen in Figures 1 and 2 below, with the CBD fraction highlighted in orange:

Figure 1: 10 $\mu$ L Hemp Oil on 5 $\mu$ m Triart C18

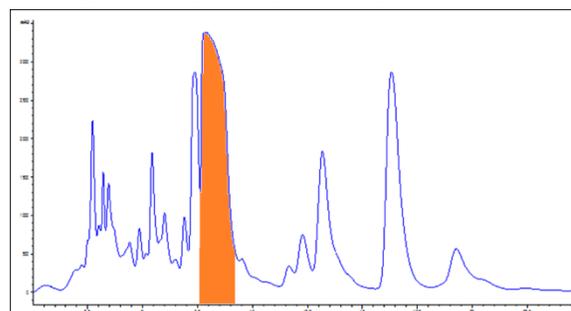
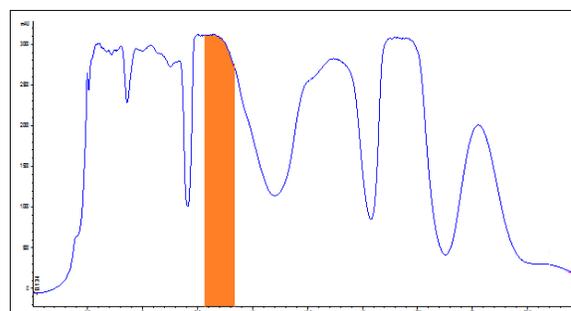


Figure 2: 100 $\mu$ L Hemp Oil on 10 $\mu$ m Triart C18



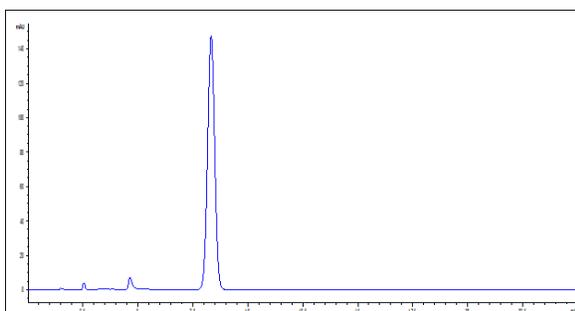
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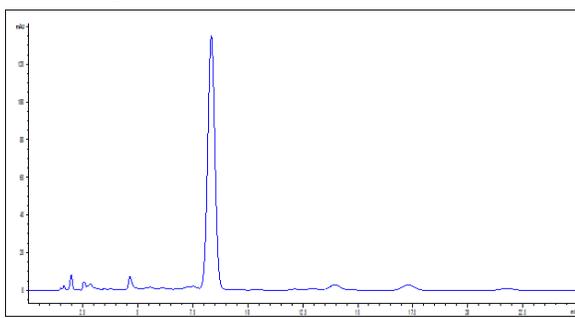


Referenced from the certification of analysis obtained from the Hemp oil manufacturer (Charlotte's Web) the concentration of CBD in the extract was 66.97mg/mL. This equates to a CBD loading of 5.358mg for the 80 $\mu$ L injection and 6.697mg for the 100 $\mu$ L injection. Fractions of the CBD peak for both loadings were taken at 10 second intervals and then reinjected to determine purity. Once fractions with the highest purity were determined, another 100 $\mu$ L 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 (in triplicate) against a standard curve. Example chromatograms of a CBD standard injection and a diluted CBD fraction can be seen in Figures 3 and 4 below:

**Figure 3: 0.1mg/mL CBD Standard**



**Figure 4: CBD Fraction after Dilution**



Final results of the 80 $\mu$ L and 100 $\mu$ L loadings can be seen in Chart 1:

**Chart 1: CBD Recovery and Purity**

Sample	CBD Recovery (mg)	CBD Loaded (mg)	Recovery (%)	Purity (by %Area)
80 $\mu$ L - Sp1e A	4.64	5.358	86.6	96.9
80 $\mu$ L - Sp1e B	4.65	5.358	86.9	96.7
80 $\mu$ L - Sp1e C	4.77	5.358	89.0	96.8
Avg:	4.69	5.358	87.5	96.8
100 $\mu$ L - Sp1e A	5.51	6.697	82.3	96.0
100 $\mu$ L - Sp1e B	5.70	6.697	85.1	96.1
100 $\mu$ L - Sp1e C	5.59	6.697	83.4	96.4
Avg:	5.60	6.697	83.6	96.2

### Conclusions

YMC-Triart C18 performed well in the CBD loading study and is shown to be a good choice for scaling up to larger particle sizes, exhibiting the same selectivity on both 5 $\mu$ m and 10 $\mu$ m materials. As expected, the lower loading (80 $\mu$ L) exhibited higher recovery and slightly higher purity as compared to higher loading (100 $\mu$ L). Additional experiments (not shown) performed with lower ethanol-containing mobile phases (<75%) indicate that higher purity and recovery can be obtained, but this is at the cost of time and productivity. Overall this data supports YMC-Triart C18 as an excellent choice for CBD purification, and could also be a viable option for purifying other cannabinoid compounds from hemp.

### YMC AMERICA, INC.

941 Marcon Blvd., Suite 201  
Allentown, PA 18109  
P: 610-266-8650  
F: 610-266-8652  
[www.ymcamerica.com](http://www.ymcamerica.com)