

## The Determination of CBD and General Cannabinoid Content in Hemp Oils Using HPLC with UV Detection

### ■ Introduction

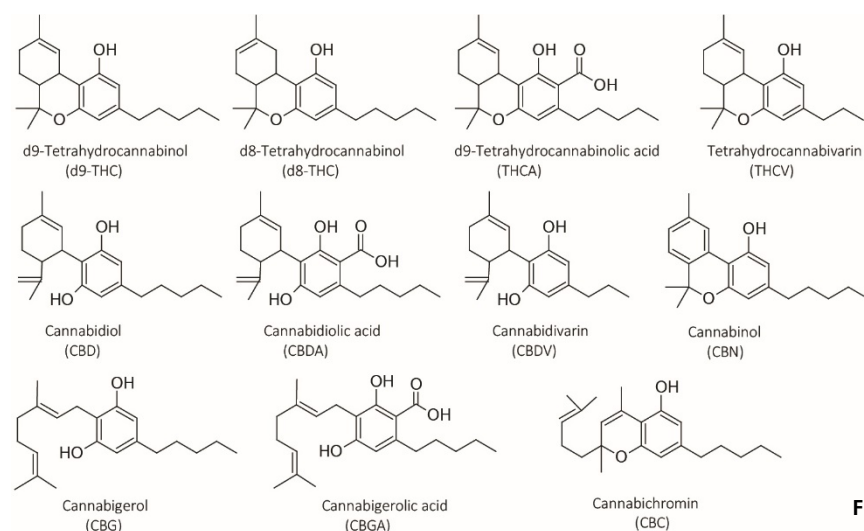
Medical marijuana generally possesses high levels of the therapeutic cannabidiol, CBD, and lower levels (generally less than 0.3%) of the psychotropic tetrahydrocannabinol, d9-THC. Pain mitigation and reduced severity of nausea and seizures are just a few of the therapeutic benefits reported by medical cannabis patients. Little has been done to better understand the chemistry of benefits from CBD. To complicate matters, there is evidence that a combination of CBD, a host of other minor cannabinoids and a complex array of terpenoids may be the most beneficial – called the “entourage effect.” CBD-rich oil has become increasingly popular and is administered via sublingual drops, gel capsules or as a topical ointment.

The main source of CBD-rich oil is industrial hemp. Hemp is considered a rustic plant as it is frost resistant, adapts to poor soil, reproduces easily, and does not require chemical fertilizers/pesticides/herbicides/fungicides to thrive. A hemp crop tends to resist mildew and requires less water than cotton. Hemp textiles are considered softer than cotton.

CBD oil is derived as concentrate from CO<sub>2</sub> or butane extraction of hemp, sometimes followed by steam distillation or ethanol distillation for purification. The Farm Bill of 2014 distinguishes hemp from marijuana, yet interpreting the law is difficult in that “CBD oil” may be classified as marijuana.

The FDA has issued warning letters to firms that market unapproved new drugs allegedly contain CBD. As part of these actions, the FDA has determined the cannabinoid content of some hemp products and many were found to contain levels of CBD that are very different from the label claim. It is important to note that such products are not approved by the FDA for the diagnosis, cure, mitigation, treatment, or prevention of any disease.

Like cannabis, hemp oil may be analyzed easily and effectively for its cannabinoid content. This application note highlights the use of a High Sensitivity HPLC method used with Shimadzu’s “Cannabis Analyzer for Potency” to determine 11 important cannabinoids, including CBD (Figure 1), in hemp oil.



**Figure 1:** Cannabinoids found in hemp and marijuana

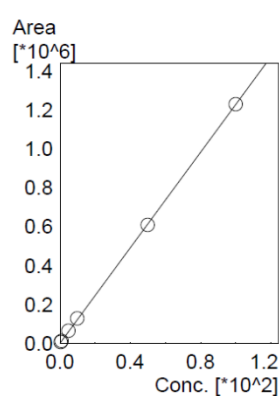
## Standard Curves

Using a comprehensive mixture of 11 cannabinoids (Shimadzu Part # 220-91239-21; 250 µg/mL), standard curves (Figure 2) were prepared for each target analyte with a minimum acceptable correlation coefficient (R<sup>2</sup>) of 0.999 over 6 standard levels.

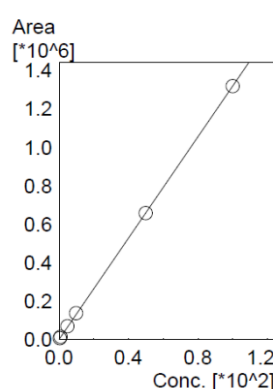
A linear dynamic range was established at 0.5 to 100 mg/L (ppm) in each analyte. This corresponds to concentrations of 0.004% to 0.8% in the original oil sample.

**Figure 2:** Standard curves

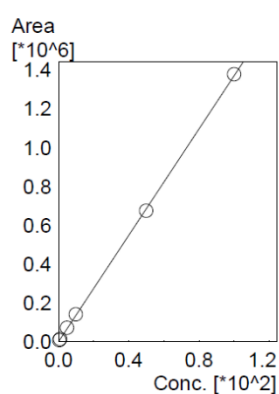
ID# : 5  
Name : CBD  
Quantitative Method : External Standard  
Function :  $f(x) = 12296.4 \cdot x + 683.111$   
Rr1=0.9999067 Rr2=0.9998135  
RSS=4.391123e+006 MeanRF: 1.267555e+004  
RFSD: 3.668508e+002 RFRSD: 2.894161  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



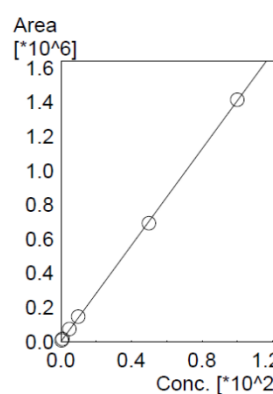
ID# : 1  
Name : CBDV  
Quantitative Method : External Standard  
Function :  $f(x) = 13192.4 \cdot x + 439.590$   
Rr1=0.9999532 Rr2=0.9999064  
RSS=2.535876e+006 MeanRF: 1.343637e+004  
RFSD: 2.422110e+002 RFRSD: 1.802652  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



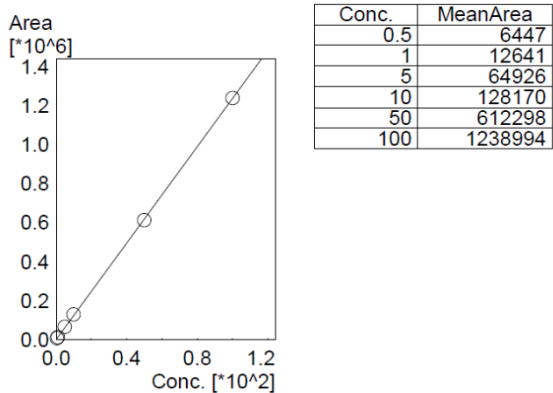
ID# : 2  
Name : CBDA  
Quantitative Method : External Standard  
Function :  $f(x) = 13703.4 \cdot x + 528.300$   
Rr1=0.9999072 Rr2=0.9998143  
RSS=5.428825e+006 MeanRF: 1.399656e+004  
RFSD: 3.167673e+002 RFRSD: 2.263179  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



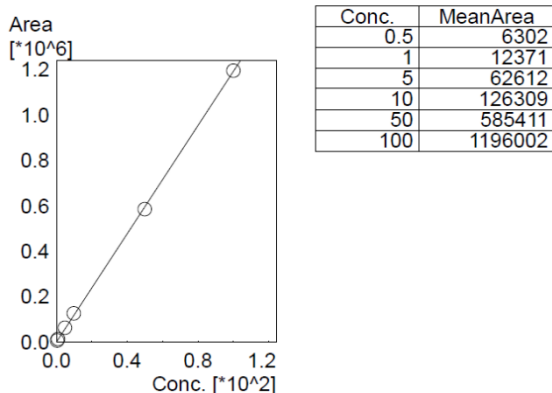
ID# : 3  
Name : CBGA  
Quantitative Method : External Standard  
Function :  $f(x) = 14072.8 \cdot x + 623.731$   
Rr1=0.9998906 Rr2=0.9997813  
RSS=6.744781e+006 MeanRF: 1.441893e+004  
RFSD: 3.558966e+002 RFRSD: 2.468260  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



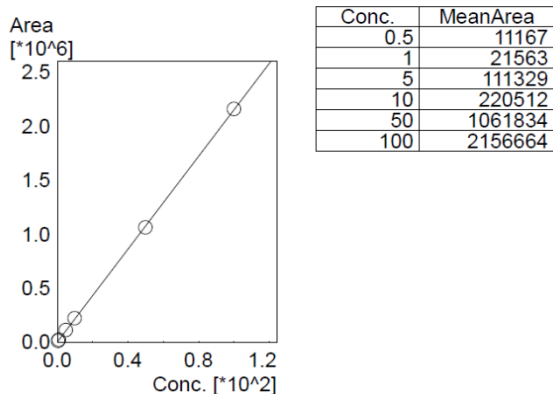
ID# : 4  
Name : CBG  
Quantitative Method : External Standard  
Function :  $f(x)=12374.6 \cdot x+518.165$   
Rr1=0.9999165 Rr2=0.9998329  
RSS=3.983632e+006 MeanRF: 1.266216e+004  
RFSD: 2.931473e+002 RFRSD: 2.315145  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



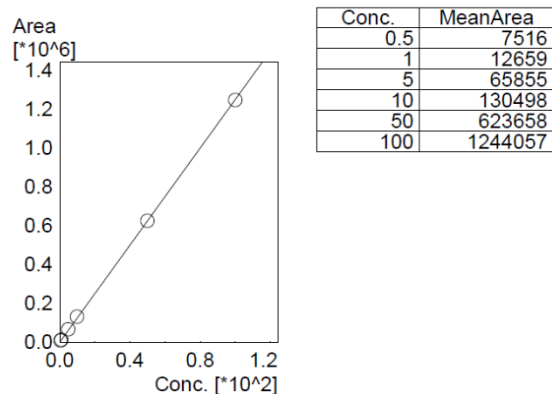
ID# : 6  
Name : THCV  
Quantitative Method : External Standard  
Function :  $f(x)=11921.4 \cdot x+681.122$   
Rr1=0.9998159 Rr2=0.9996318  
RSS=8.151028e+006 MeanRF: 1.229946e+004  
RFSD: 3.801192e+002 RFRSD: 3.090535  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



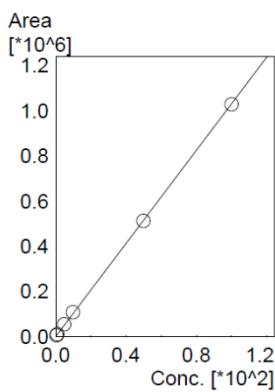
ID# : 7  
Name : CBN  
Quantitative Method : External Standard  
Function :  $f(x)=21498.0 \cdot x+609.446$   
Rr1=0.9999374 Rr2=0.9998748  
RSS=9.010927e+006 MeanRF: 2.183621e+004  
RFSD: 4.439632e+002 RFRSD: 2.033151  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



ID# : 8  
Name : d9-THC  
Quantitative Method : External Standard  
Function :  $f(x)=12474.9 \cdot x+1194.13$   
Rr1=0.9999092 Rr2=0.9998184  
RSS=4.400881e+006 MeanRF: 1.313769e+004  
RFSD: 9.750802e+002 RFRSD: 7.422007  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A

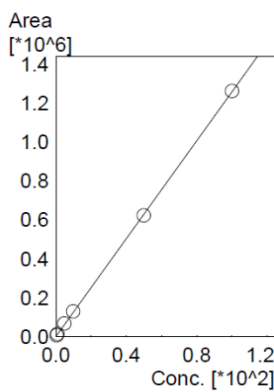


ID# : 9  
Name : d8-THC  
Quantitative Method : External Standard  
Function :  $f(x)=10309.8 \cdot x+489.425$   
Rr1=0.9998771 Rr2=0.9997541  
RSS=4.069695e+006 MeanRF: 1.058142e+004  
RFSD: 3.409993e+002 RFRSD: 3.222624  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



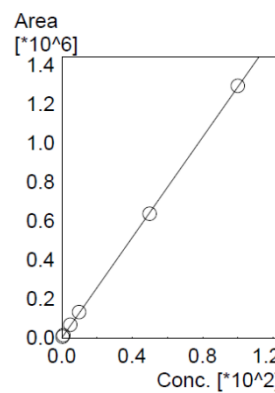
Conc.	MeanArea
0.5	5461
1	10286
5	54726
10	108013
50	512401
100	1028630

ID# : 10  
Name : CBC  
Quantitative Method : External Standard  
Function :  $f(x)=12600.5 \cdot x+615.915$   
Rr1=0.9999182 Rr2=0.9998363  
RSS=4.046390e+006 MeanRF: 1.294232e+004  
RFSD: 4.214904e+002 RFRSD: 3.256683  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



Conc.	MeanArea
0.5	6778
1	12743
5	66600
10	129427
50	623024
100	1263105

ID# : 11  
Name : THCA  
Quantitative Method : External Standard  
Function :  $f(x)=12861.3 \cdot x+425.022$   
Rr1=0.9999325 Rr2=0.9998649  
RSS=3.479378e+006 MeanRF: 1.309722e+004  
RFSD: 2.528834e+002 RFRSD: 1.930818  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1/C  
Detector Name : Detector A



Conc.	MeanArea
0.5	6595
1	13195
5	67091
10	131745
50	635235
100	1290101

### ■ Experimental

Hemp oils are typically rich in CBD, with relatively minor concentrations of other cannabinoids. All cannabinoid targets have a linear dynamic range, above which the detector response ceases to be linear with concentration. Accurate quantitation relies on the detector response to the analyte lying within the calibration range. Therefore, two dilution factors were used, depending on the quantitative goal. One dilution factor yielded appropriate detector sensitivity to the array of minor cannabinoids. A second, higher dilution factor was established for the most accurate quantitation of the major CBD component so that its response was within the established quantitative dynamic range established for that analyte.

In practice, it was found that the two approaches yielded quantitative values for CBD that agreed within 0.2%.

### Hemp Oil Sample Preparation

#### Quantitative Total Cannabinoids

Add 400  $\mu$ L isopropanol to a 2 mL glass vial  
Add 10  $\mu$ L hemp oil sample and completely dissolve  
Agitate for 30 seconds  
Add 400  $\mu$ L methanol to the mixture  
Agitate for 30 seconds  
Filter the mixture through a 0.2  $\mu$ m PTFE syringe filter into an HPLC vial  
(Note: Total dilution factor 81X)

#### Quantitative CBD Only

Add 800  $\mu$ L methanol to a 2 mL glass vial  
Add 200  $\mu$ L of the Part A mixture  
Agitate for 30 seconds  
(Note: Total dilution factor 405X)

Five hemp oils were tested in this study (Figure 3), purchased from various mail-order vendors. The appearance and label information for three of the five appear below, referenced as black, blue and green. Two samples tested but not pictured are referred to as red and yellow.

**Figure 3:** Appearance and Label Information

Hemp Oil #1  
Black Label  
Label Claim: 23 mg per serving; 100 servings per 100 mL  
Calculation of Label Claim: 23000  $\mu$ g/mL or 2.3%

Hemp Oil #2  
Blue Label  
500 mg per 30 mL  
Calculation of Label Claim: 16666  $\mu$ g/mL or 1.7%

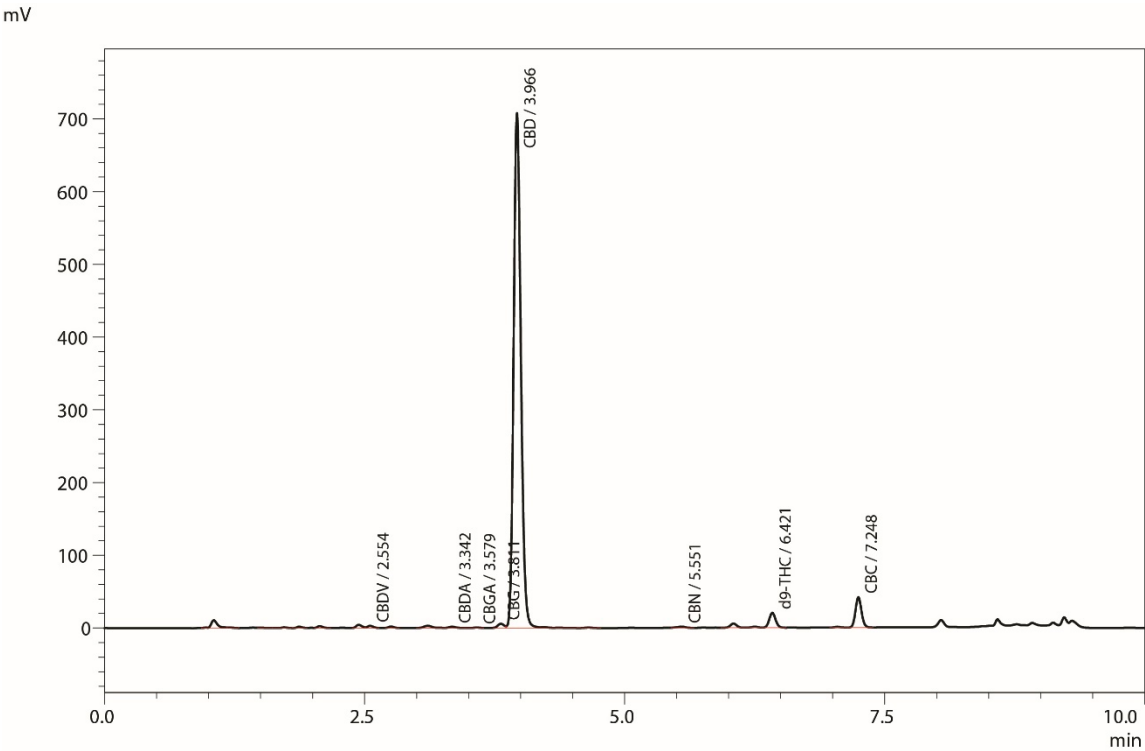
Hemp Oil #3  
Green Label  
15 mg per 1 serving per 0.5mL = 15 mg/0.5 mL  
Calculation of Label Claim: 30000  $\mu$ g/mL or 3.0%



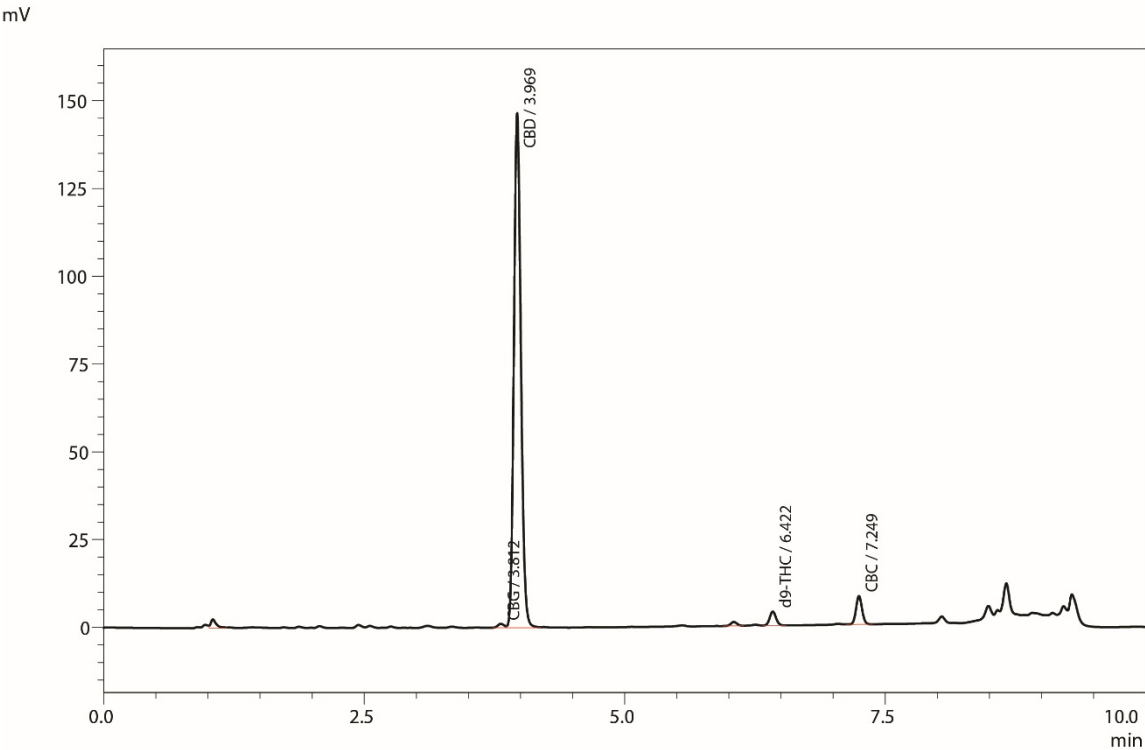
Qualitative Analysis of Hemp Oils

Chromatograms for hemp oils #1 (black), #2 (blue), and #3 (green) appear in Figure 4. Peak labels appear for only those cannabinoids identified in the sample.

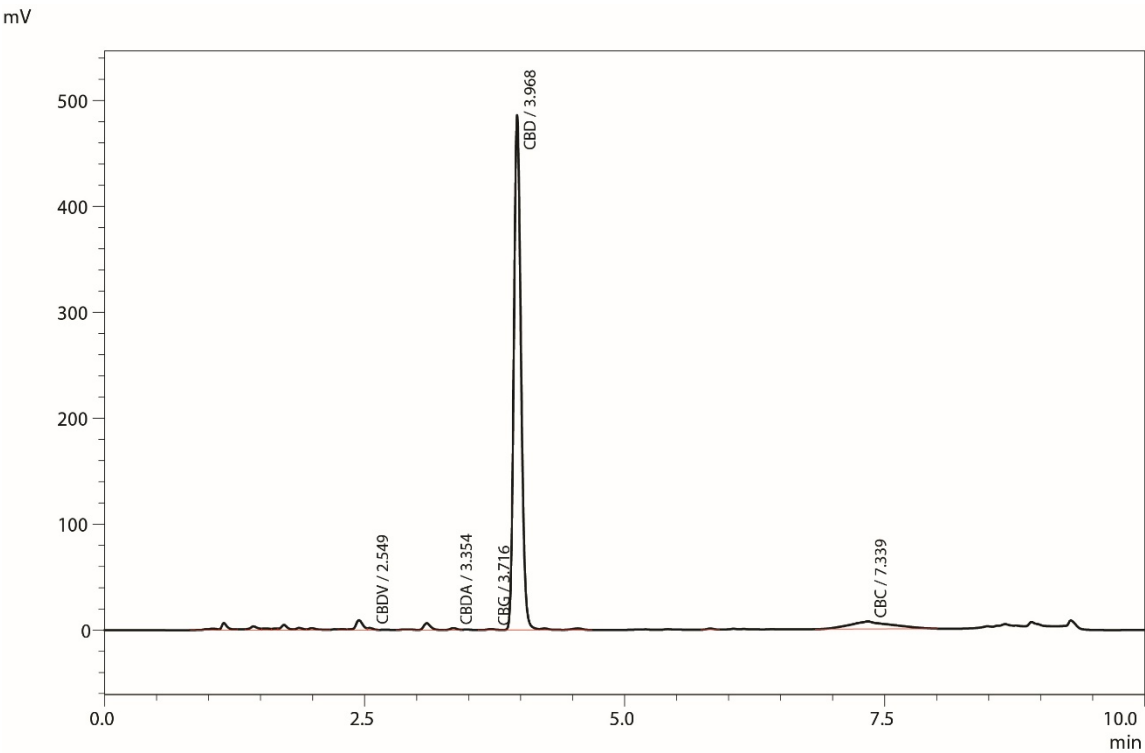
Figure 4



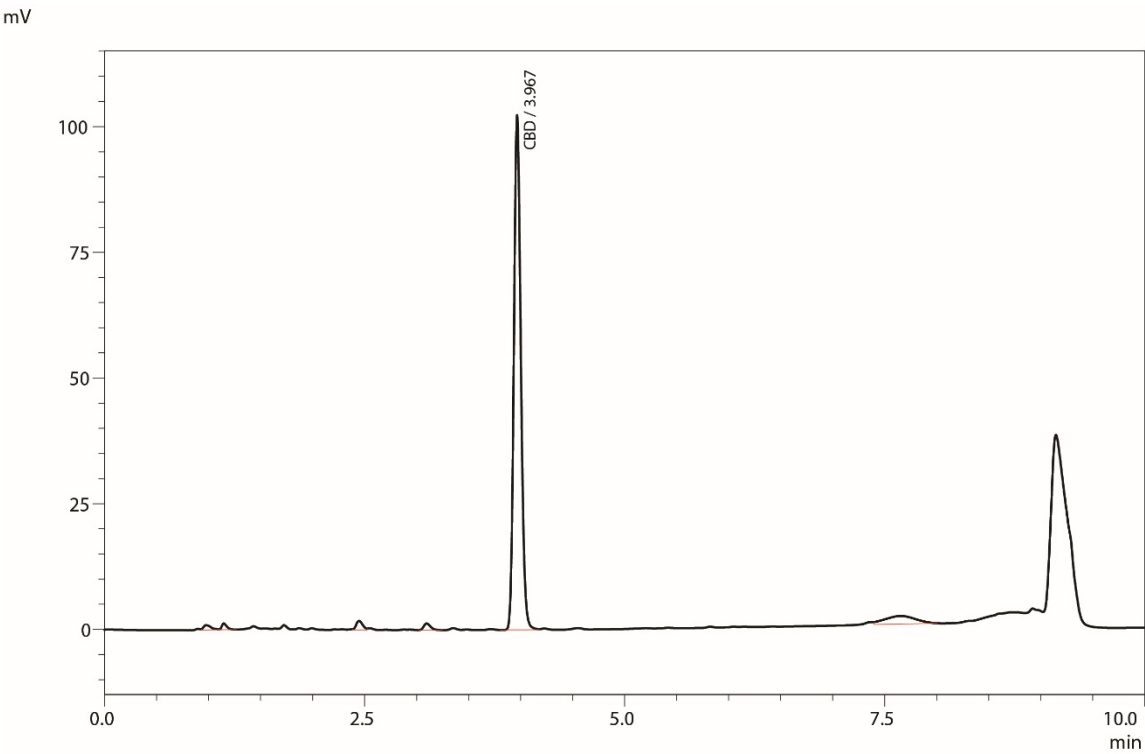
Hemp Oil #1 (Black) – Total Cannabinoids (81X dilution)



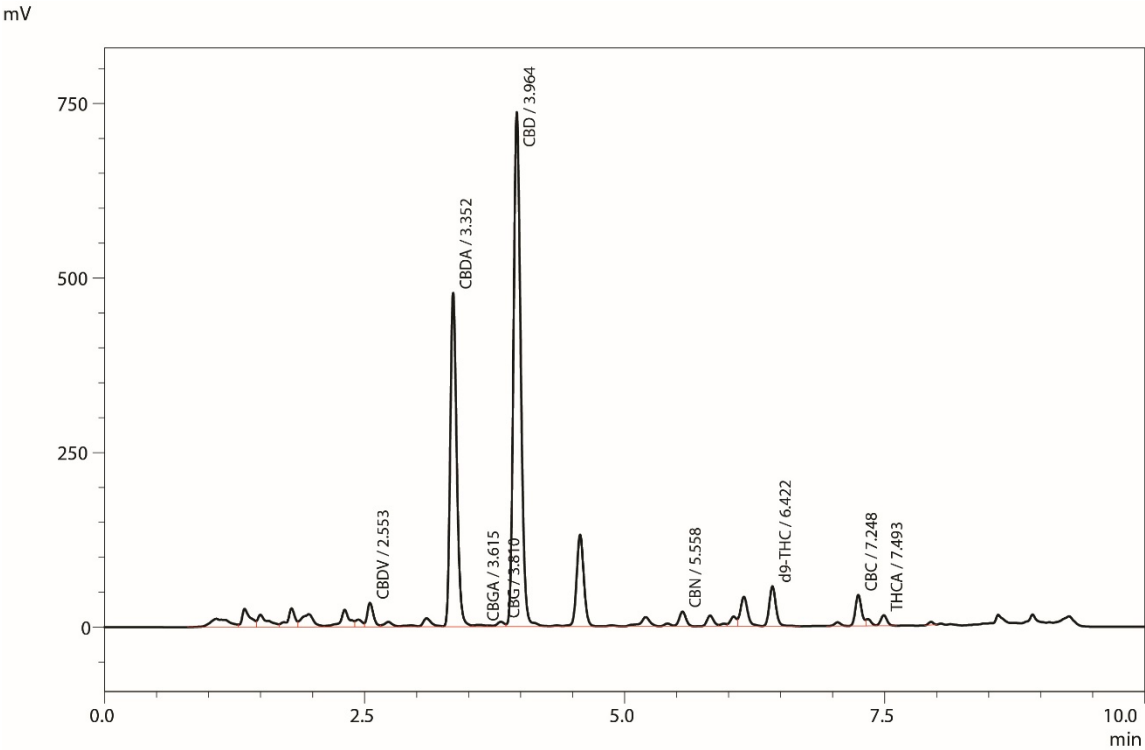
Hemp Oil #2 (Blue) – CBD Only (405X dilution)



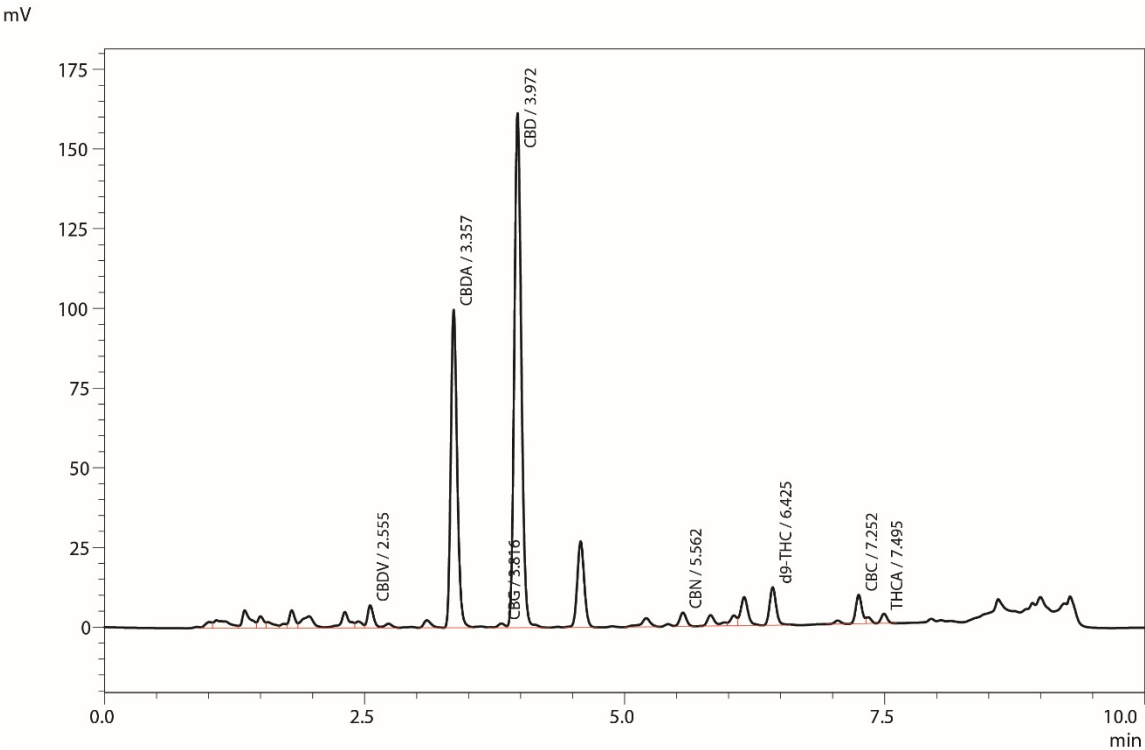
Total Cannabinoids (81X dilution)



Hemp Oil #2 (Blue) – CBD Only (405X dilution)



Hemp Oil #3 (Green) – Total Cannabinoids (81X dilution)



Hemp Oil #3 (Green) – CBD Only (405X dilution)



## Quantitative Results Summary for Hemp Oils

**Table 1:** Summary of CBD Quantitative Determination for five hemp oils

		1		2		3		4		5	
		Label Claim (CBD): 23000 µg/mL		Label Claim (CBD): 16666 µg/mL		Label Claim (CBD): 30000 µg/mL		Label Claim (CBD): 8000 µg/mL		Label Claim (CBD): 8333 µg/mL	
		Black Label		Blue Label		Green Label		Red Label		Yellow Label	
ID#	Name	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%
5	CBD	21880.06	2.188	15242.8	1.524	24210.45	2.421	9721	0.972	16695	1.670
CBD % of label claim			95		92		81		122		200

**Table 2:** Summary of Total Cannabinoids Quantitative Determination

		1		2		3		4		5	
		Black Label		Blue Label		Green Label		Red Label		Yellow Label	
ID#	Name	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%	µg/mL (ppm)	%
1	CBDV	83.02	0.008	46.99	0.005	841.57	0.084	215.89	0.022	284.54	0.028
2	CBDA	55.07	0.006	47.04	0.005	11469.65	1.147	311.73	0.031	155.95	0.016
3	CBGA	23.55	0.002	0	0.000	99.49	0.010	0	0.000	0	0.000
4	CBG	165.92	0.017	32.95	0.003	243.55	0.024	187.81	0.019	288.51	0.029
5	CBD	21855.56	2.186	14955.69	1.496	22872.62	2.287	9721.64	0.972	16695	1.670
6	THCV	0	0.000	0	0.000	0	0.000	0	0.000	0	0.000
7	CBN	37.19	0.004	0	0.000	335.23	0.034	17.12	0.002	41.09	0.004
8	d9-THC	554.46	0.055	0	0.000	1621	0.162	387.34	0.039	625.2	0.063
9	d8-THC	0	0.000	0	0.000	0	0.000	29.94	0.003	51.83	0.005
10	CBC	1006.06	0.101	1194	0.119	1104	0.110	456.75	0.046	809.31	0.081
11	THCA	0	0.000	0	0.000	379.59	0.038	38.31	0.004	112.66	0.011
Total Cannabinoids %			2.38		1.63		3.90		1.14		1.91
CBD % of Total			92		92		59		86		88

### ■ Discussion

Tables 1 and 2 summarize the quantitative findings for the hemp oil samples. Table 1 reflects the accurate quantitation of CBD using the higher dilution factor (405X). Table 2 reflects the quantitation of the comprehensive target list.

As a general sample observation, hemp oils #1 (black) and #2 (blue) exhibited a transparent, weak-yellow/green coloration. Our assumption was that each of these is a product of multi-step purification after extraction; for example, CO<sub>2</sub> or butane extraction followed by steam distillation. Notably, hemp oil #3 (green) was opaque brown/green and gritty in appearance. It also had the most intense smell – a distinctly “earthy” odor. Accordingly, our assumption was that the sample was the result of crude extraction only, with no further refinement.

It is important to note that it has been reported in the literature that the whole plant can be more beneficial to the consumer because it contains not only the cannabinoids, but also an array of terpenes providing a synergistic “Entourage Effect”. The whole plant can also provide essential fatty acids, plant sterols for lowering cholesterol, and antioxidants chlorophyll and Vitamin E.

Hemp oils #1 (black) and #2 (blue) showed high ratios of CBD to total cannabinoids, both at 92%, and the lowest quantity of other cannabinoids. This finding supported the assumption, along with transparency and color, that these oils were the more highly purified samples. Both samples also tested close to label claim at 95% and 92%, respectively.

Hemp oil #3 (green) revealed the highest content of CBD and total cannabinoids, yet exhibited the lowest ratio of CBD to total cannabinoids (59%). This observation is consistent with the assumption that its crude appearance reflected the least amount of post-extraction purification. Although its CBD % of label claim tested the lowest (81%), this sample did contain the highest level of CDB compared to all other oils tested.

Hemp oils #4 (red) and #5 (yellow) tested higher than label claim at 122% and 200%, respectively. The observation is consistent with FDA findings for CBD products, perhaps calling into question the type and accuracy of testing used to justify label claim.

In summary, all samples contained less d9-THC than the amount allowed by law (0.3%). Also, all samples showed an array of other cannabinoids, but the minor component, THC-V, was not detected in any of the hemp oil samples. From a quality control point of view two samples were within a reasonable range of the label claim at +/-10%. One sample was well below label claim and two other samples were well above the label claims, one by as much as 200%. When purchasing CBD oils, one should consider 1) label claim, 2) actual concentration, 3) the quality control from batch to batch, 4) other cannabinoids of importance, 5) whole plant complexity, and 6) the selling price.

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