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Application News

No. HPLC-018

High Performance Liquid Chromatography

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 psychotropic tetrahydrocannabinol, d9-THC and lower levels of the non- psychotropic cannabidiol, CBD. 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 CO2 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 containing 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.

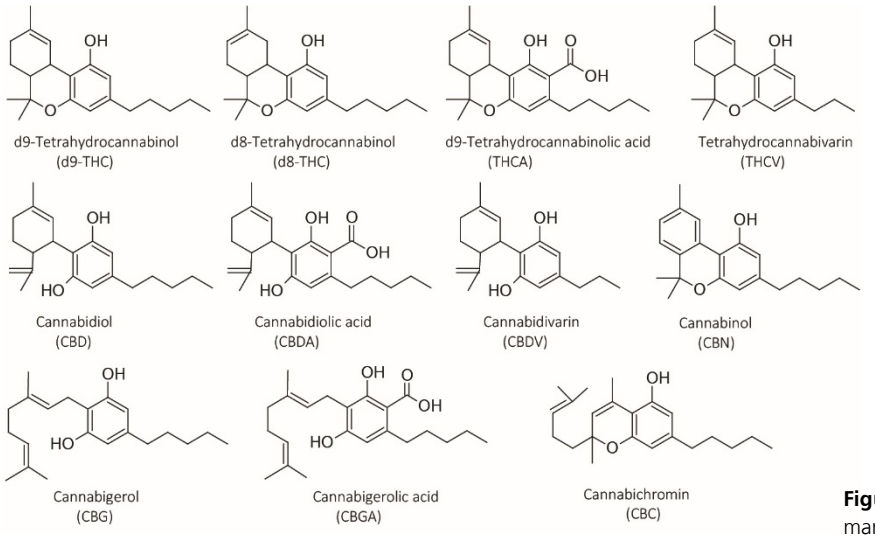


Figure 1: Cannabinoids found in hemp and marijuana

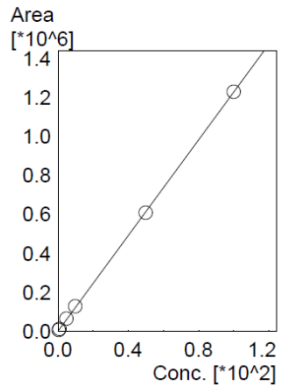
Standard Curves

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 (R2) 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.

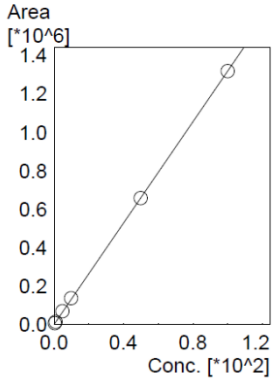
Figure 2: Standard curves

ID# : 5
Name : CBD
Quantitative Method : External Standard
Function : f(x)=12296.4*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



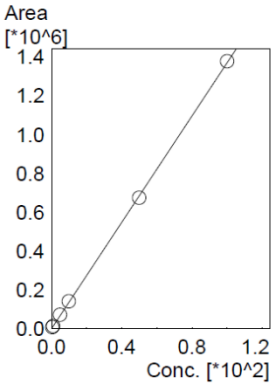
Conc.	MeanArea
0.5	6573
1	12702
5	64282
10	128639
50	609208
100	1230049

ID# : 1
Name : CBDV
Quantitative Method : External Standard
Function : f(x)=13192.4*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



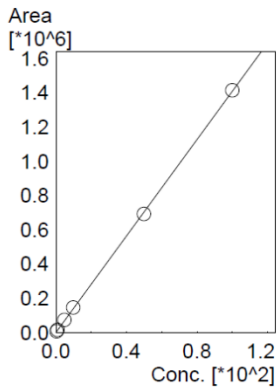
Conc.	MeanArea
0.5	6829
1	13378
5	68334
10	136051
50	656500
100	1318081

ID# : 2
Name : CBDA
Quantitative Method : External Standard
Function : f(x)=13703.4*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



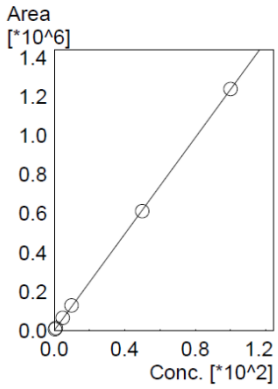
Conc.	MeanArea
0.5	7146
1	14030
5	71489
10	141043
50	674431
100	1376639

ID# : 3
Name : CBGA
Quantitative Method : External Standard
Function : f(x)=14072.8*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



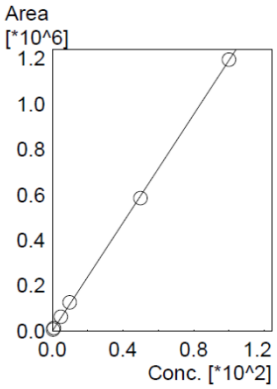
Conc.	MeanArea
0.5	7378
1	14531
5	73365
10	145769
50	691805
100	1414008

ID# : 4
Name : CBG
Quantitative Method : External Standard
Function : f(x)=12374.6*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



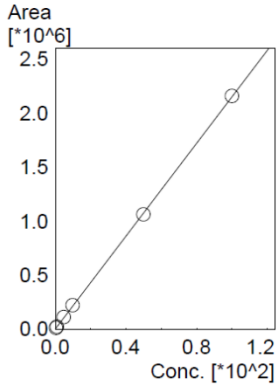
Conc.	MeanArea
0.5	6447
1	12641
5	64926
10	128170
50	612298
100	1238994

ID# : 6
Name : THCV
Quantitative Method : External Standard
Function : f(x)=11921.4*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



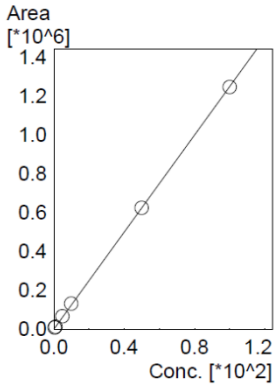
Conc.	MeanArea
0.5	6302
1	12371
5	62612
10	126309
50	585411
100	1196002

ID# : 7
Name : CBN
Quantitative Method : External Standard
Function : f(x)=21498.0*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



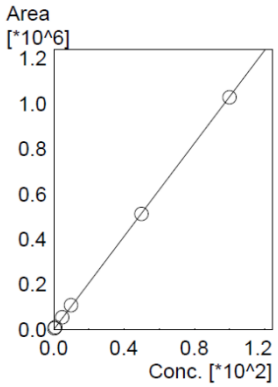
Conc.	MeanArea
0.5	11167
1	21563
5	111329
10	220512
50	1061834
100	2156664

ID# : 8
Name : d9-THC
Quantitative Method : External Standard
Function : f(x)=12474.9*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



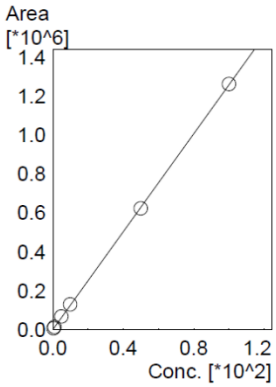
Conc.	MeanArea
0.5	7516
1	12659
5	65855
10	130498
50	623658
100	1244057

ID# : 9
Name : d8-THC
Quantitative Method : External Standard
Function : f(x)=10309.8*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



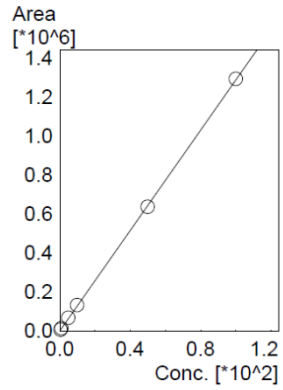
Conc.	MeanArea
0.5	5461
1	10286
5	54726
10	108013
50	512401
100	1028630

ID# : 10
Name : CBC
Quantitative Method : External Standard
Function : f(x)=12600.5*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*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 µL isopropanol to a 2 mL glass vial
Add 10 µL hemp oil sample and completely dissolve
Agitate for 30 seconds
Add 400 µL methanol to the mixture
Agitate for 30 seconds
Filter the mixture through a 0.2 µm PTFE syringe filter into an HPLC vial
(Note: Total dilution factor 81X)

Quantitative CBD Only
Add 800 uL methanol to a 2 mL glass vial
Add 200 uL 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 µg/mL or 2.3%

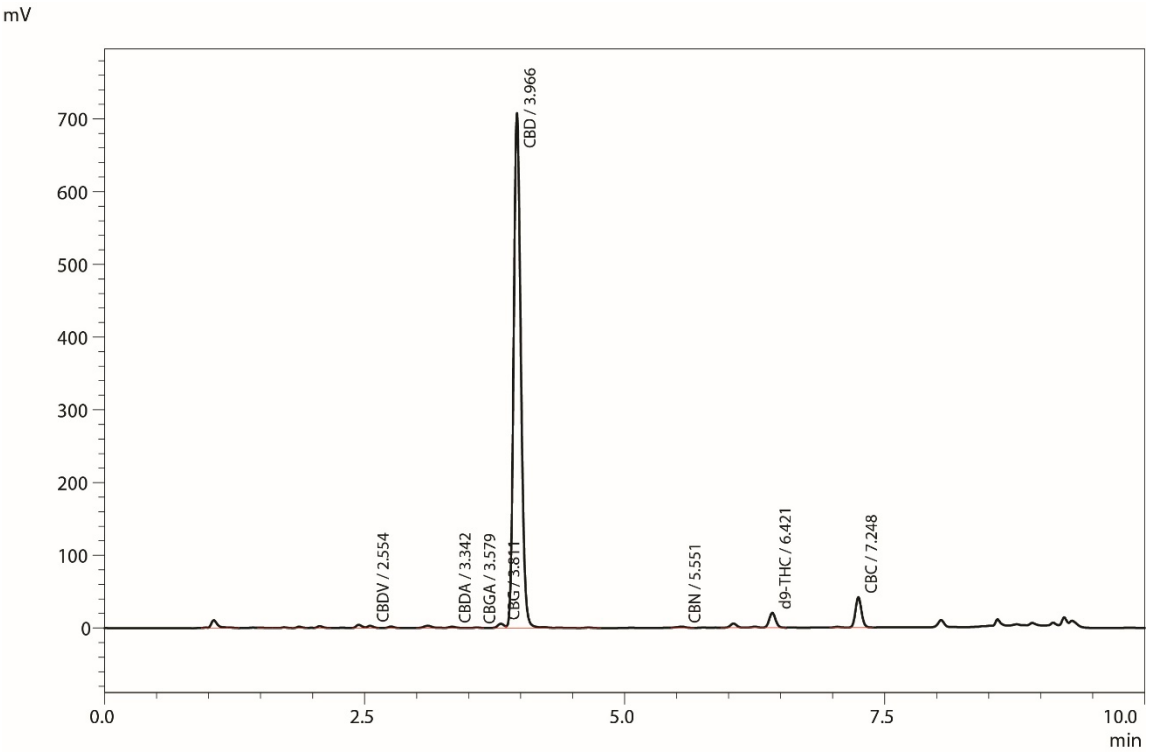
Hemp Oil #2
Blue Label
500 mg per 30 mL
Calculation of Label Claim: 16666 µg/mL or 1.7%

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

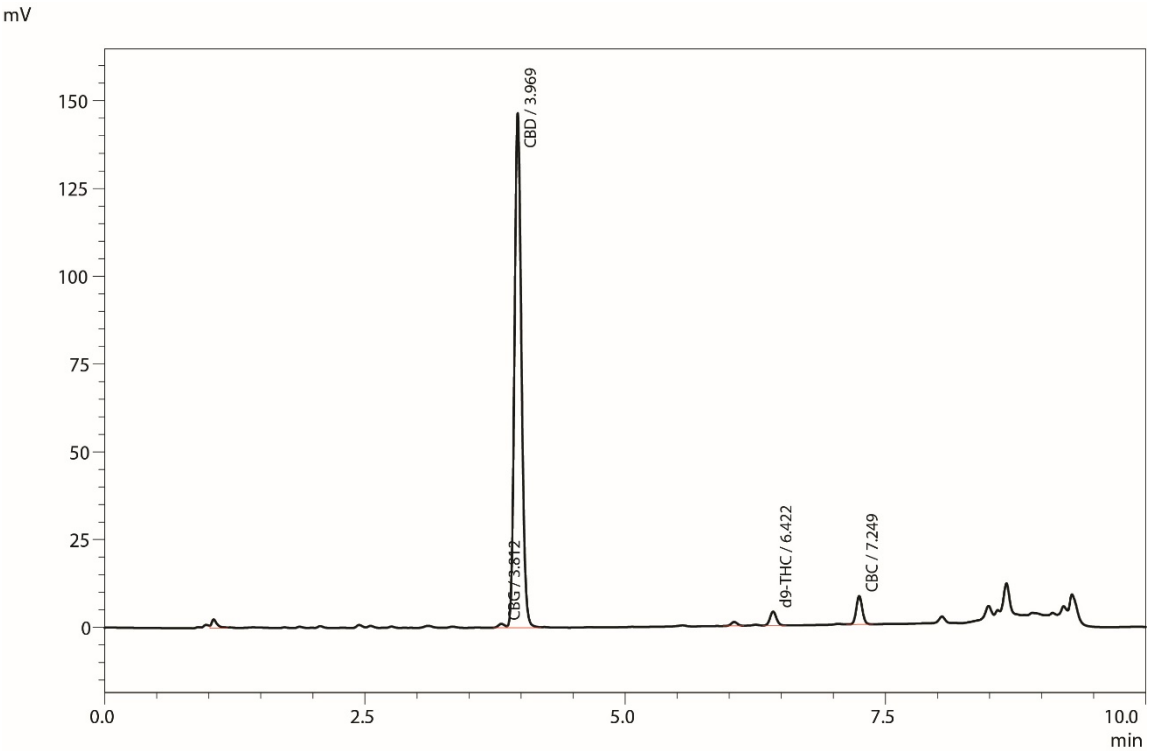
Qualitative Analysis of Hemp Oils

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.

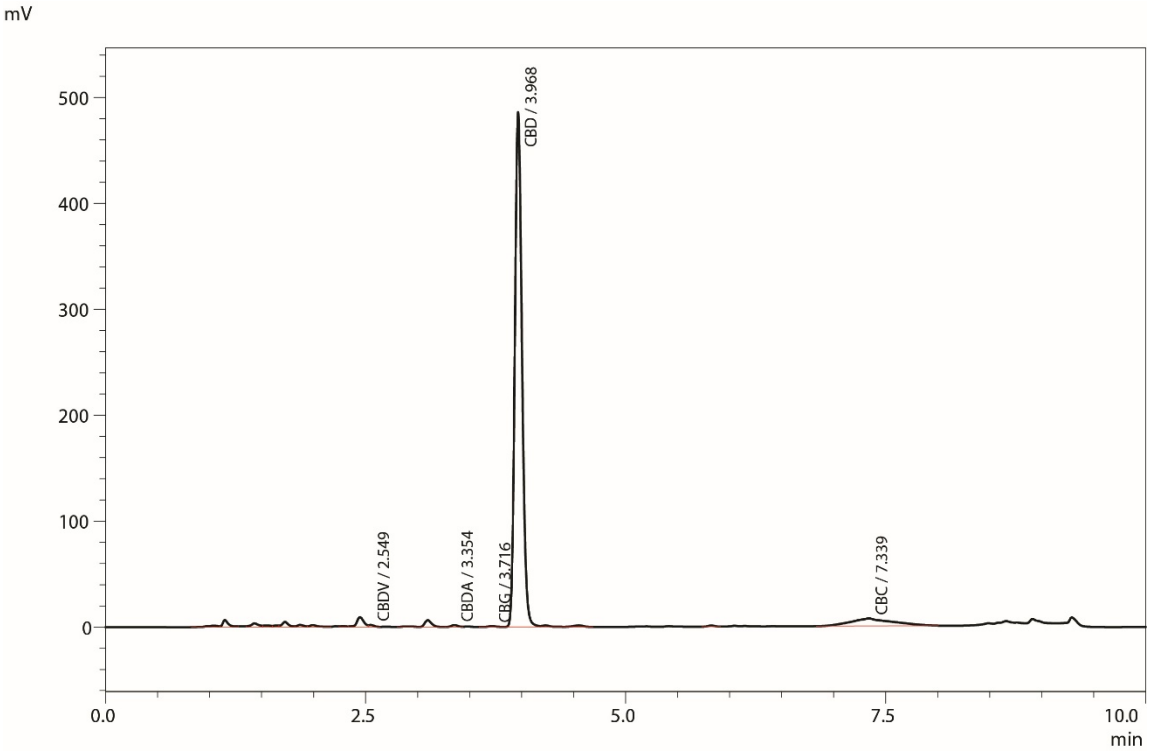
Figure 4



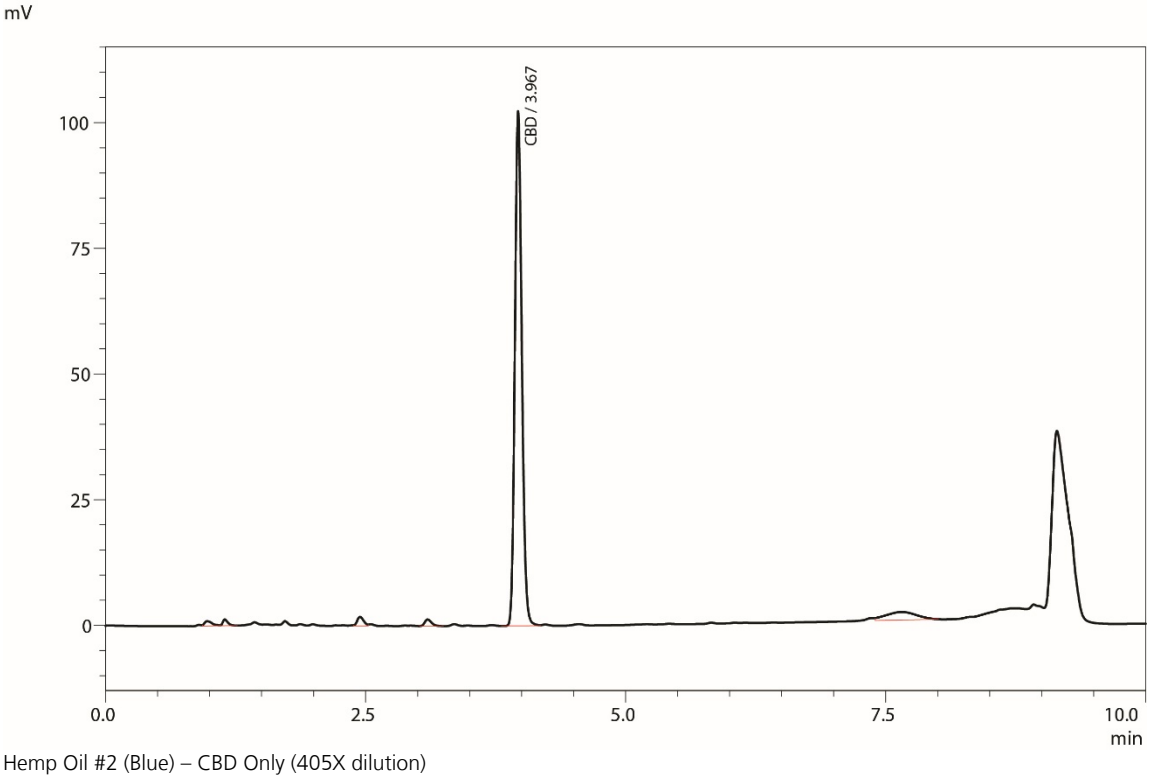
Hemp Oil #1 (Black) – Total Cannabinoids (81X dilution)



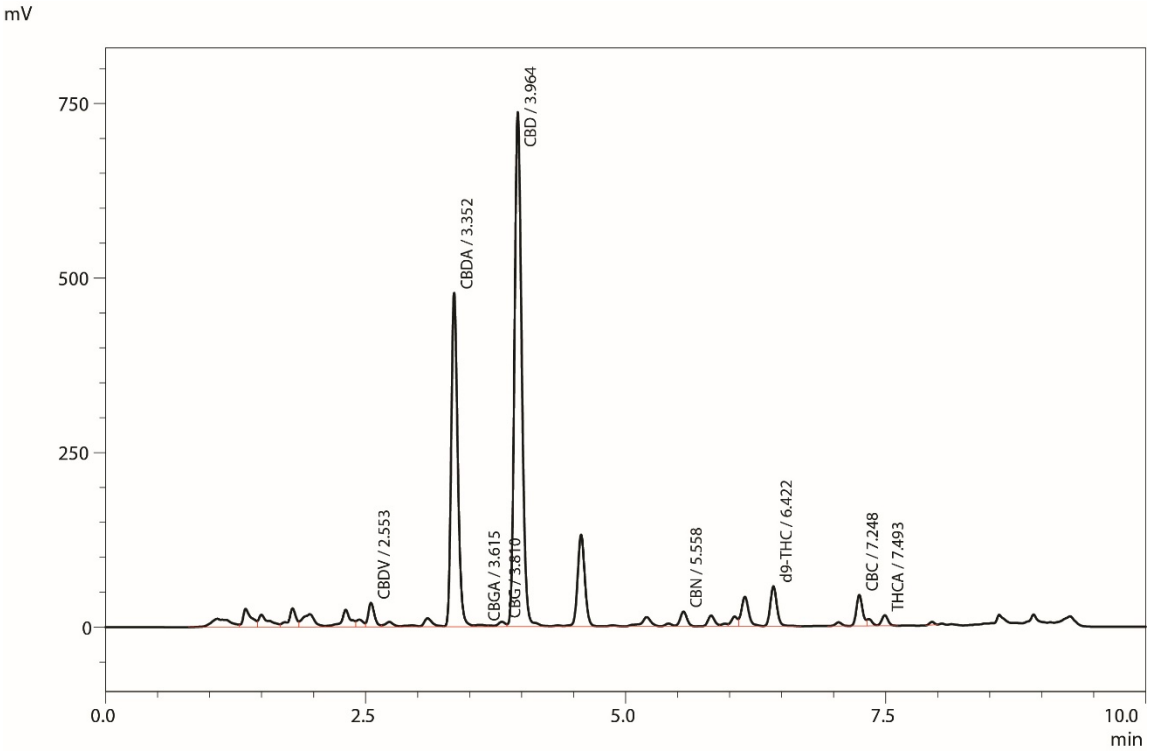
Hemp Oil #2 (Blue) – CBD Only (405X dilution)



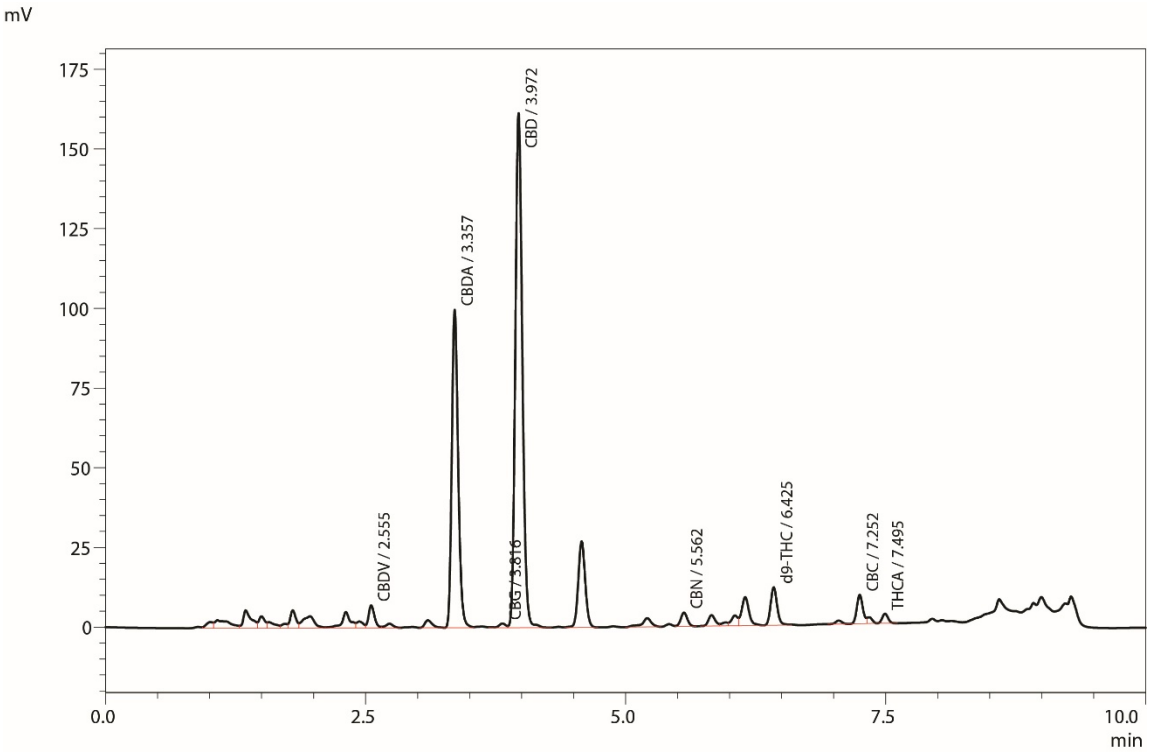
Total Cannabinoids (81X dilution)



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 Lable		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 Lable		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, CO2 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 CBD 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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