

# Application News

High Performance Liquid Chromatography

## The Determination of Cannabinoids Content Across a Large Concentration Range within Hemp Oil

No. SCA\_190\_050

### Introduction

Shimadzu's previously released application note No. HPLC-018 produced a dilution method for the determination of CBD within oil samples across a range of concentrations between 0.5 and 400 mg CBD in a 10 mL bottle.

The rise of CBD oil products in both type and concentration range has led to the production of this application note.

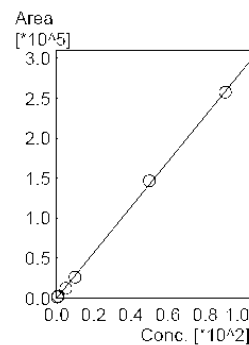
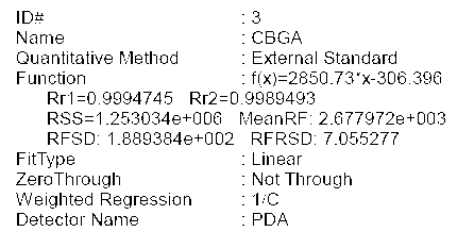
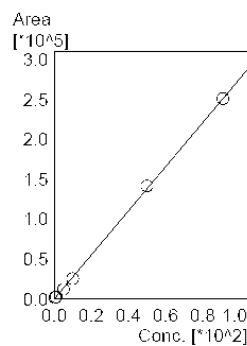
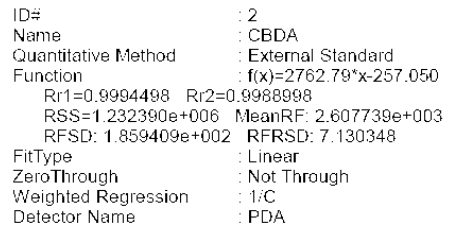
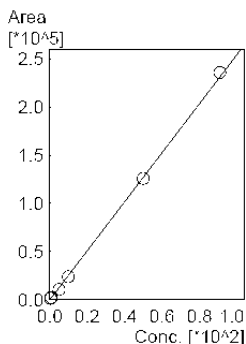
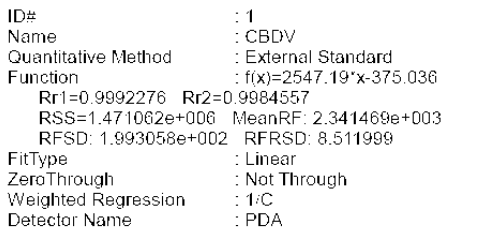
Here we have investigated a dilution method suitable for CBD oil samples across a concentration range of 20 and 3750 mg CBD per 10 mL bottle.

The measurements were performed with Shimadzu's Cannabis Analyzer with PDA. The analytical conditions were exactly adapted from application note No. HPLC-016 with slightly amended calibration curve range.

### Standard Curves

Using a comprehensive mixture of 11 cannabinoids, prepared from individual standards (supplied in methanol at 1000 µg/mL), standard curves (Figure 1) were prepared for each target analyte with a minimum acceptable correlation coefficient (R) of 0.999 over 6 standard levels (0.5 to 90.9 ppm).

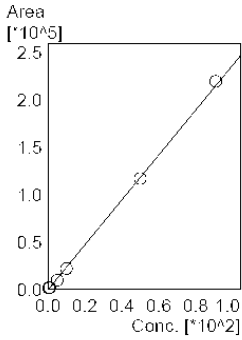
Fig. 1: Standard curves (1-11)



Conc.	MeanArea
0.5	1355
1	2475
5	12430
10	26485
50	146506
90.9	257615

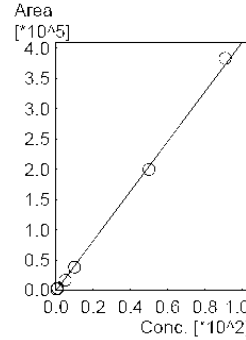
ID# : 4  
 Name : CBG  
 Quantitative Method : External Standard  
 Function :  $f(x)=2375.13 \cdot x-351.910$   
 Rr1=0.9992577 Rr2=0.9985160  
 RSS=1.229055e+006 MeanRF: 2.181245e+003  
 RFSD: 1.871405e+002 RFRSD: 8.579527  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	1082
1	2020
5	9692
10	21944
50	117220
90.9	219777



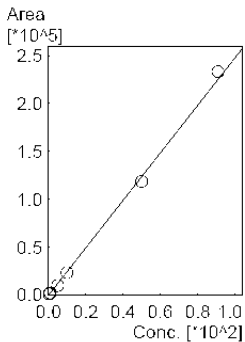
ID# : 7  
 Name : CBN  
 Quantitative Method : External Standard  
 Function :  $f(x)=4106.19 \cdot x-618.875$   
 Rr1=0.9989563 Rr2=0.9979137  
 RSS=5.167303e+006 MeanRF: 3.767049e+003  
 RFSD: 3.331585e+002 RFRSD: 8.844017  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	1857
1	3577
5	16354
10	38063
50	199671
90.9	383078



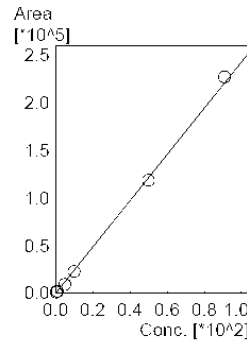
ID# : 5  
 Name : CBD  
 Quantitative Method : External Standard  
 Function :  $f(x)=2483.97 \cdot x-401.677$   
 Rr1=0.9987184 Rr2=0.9974384  
 RSS=2.322937e+006 MeanRF: 2.271154e+003  
 RFSD: 2.092815e+002 RFRSD: 9.214765  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	1074
1	2148
5	9699
10	23491
50	118644
90.9	233310



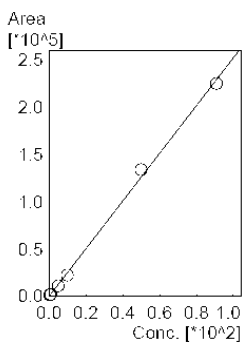
ID# : 8  
 Name : d9-THC  
 Quantitative Method : External Standard  
 Function :  $f(x)=2433.08 \cdot x-527.226$   
 Rr1=0.9986776 Rr2=0.9973569  
 RSS=2.299823e+006 MeanRF: 2.156883e+003  
 RFSD: 2.681205e+002 RFRSD: 12.430924  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	979
1	1967
5	9014
10	22486
50	118443
90.9	226914



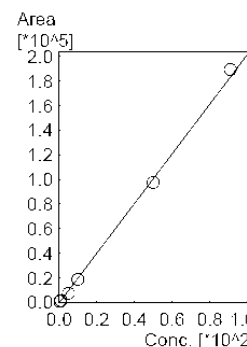
ID# : 6  
 Name : THCV  
 Quantitative Method : External Standard  
 Function :  $f(x)=2521.62 \cdot x-358.118$   
 Rr1=0.9985829 Rr2=0.9971678  
 RSS=2.647458e+006 MeanRF: 2.319178e+003  
 RFSD: 2.336080e+002 RFRSD: 10.072880  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	1181
1	2018
5	10959
10	22190
50	133610
90.9	224796

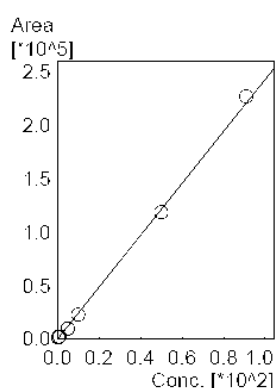


ID# : 9  
 Name : d8-THC  
 Quantitative Method : External Standard  
 Function :  $f(x)=2021.31 \cdot x-442.481$   
 Rr1=0.9985685 Rr2=0.9971390  
 RSS=1.718480e+006 MeanRF: 1.789413e+003  
 RFSD: 2.227354e+002 RFRSD: 12.447401  
 FitType : Linear  
 ZeroThrough : Not Through  
 Weighted Regression : 1/C  
 Detector Name : PDA

Conc.	MeanArea
0.5	812
1	1614
5	7554
10	18732
50	97461
90.9	189326

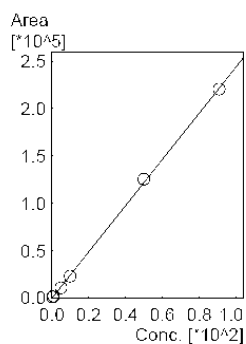


ID# : 10  
Name : CBC  
Quantitative Method : External Standard  
Function :  $f(x)=2431.79 \cdot x - 375.949$   
Rr1=0.9990080 Rr2=0.9980169  
RSS=1.722526e+006 MeanRF: 2.227153e+003  
RFSD: 1.990198e+002 RFRSD: 8.936064  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1:C  
Detector Name : PDA



Conc.	MeanArea
0.5	1089
1	2099
5	9704
10	22590
50	118397
90.9	226629

ID# : 11  
Name : THCA  
Quantitative Method : External Standard  
Function :  $f(x)=2443.94 \cdot x - 219.726$   
Rr1=0.9995418 Rr2=0.9990837  
RSS=8.029820e+005 MeanRF: 2.316215e+003  
RFSD: 1.510034e+002 RFRSD: 6.519406  
FitType : Linear  
ZeroThrough : Not Through  
Weighted Regression : 1:C  
Detector Name : PDA



Conc.	MeanArea
0.5	1193
1	2154
5	10750
10	23044
50	125560
90.9	220659

## 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 increasing 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 (Dilution A) yielded appropriate detector sensitivity to the array of minor cannabinoids.

A second, higher dilution factor (Dilution B) 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.

## Hemp Oil Sample Preparation

### Quantitative Total Cannabinoids (A)

- Add 4000  $\mu$ L isopropanol to a suitable glass vial
- Add 100  $\mu$ L hemp oil sample and completely dissolve then agitate for 30 seconds
- Add 4000  $\mu$ L methanol to the mixture then 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 (B)

- Add 980  $\mu$ L methanol to a LC vial
- Add 20  $\mu$ L of the Part A mixture
- Agitate for 30 seconds  
(Note: Total dilution factor 4050X)

Five different hemp oils were tested in this study (Table 1), purchased from various suppliers. The concentration range of the samples was 3 to 35 % (300 mg to 3500 mg) CBD per 10 mL bottle, labelled 1 to 5 in increasing concentration.

### Qualitative Analysis of Hemp Oils

Chromatograms for hemp oils appear in Figure 2-5. Peak labels appear for only those cannabinoids identified in the sample.

An addition oil sample, 500 mg CBD per 10 mL, was prepared 10 times to test variability between preparations (Table 2).

An eleventh preparation was carried out and 20 injections of this solution tested for the repeatability of the system and method (Table 3).

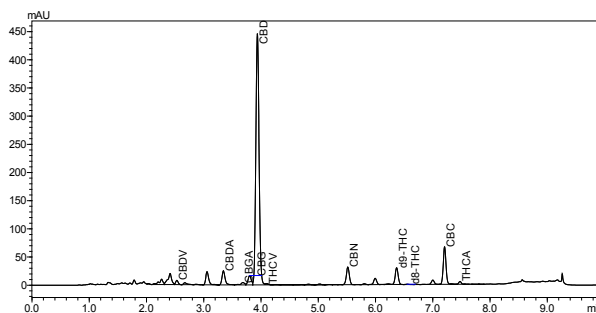


Fig. 2: Hemp oil 1 – CBD only (81X dilution)

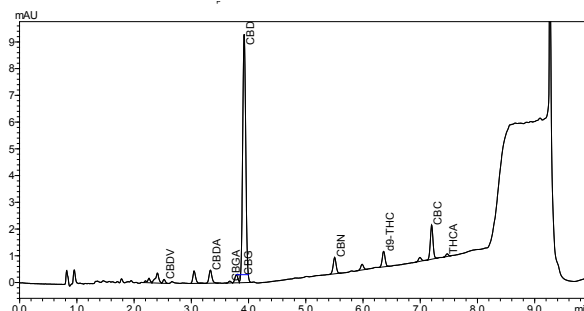


Fig. 3: Hemp oil 1 – CBD only (4050X dilution)

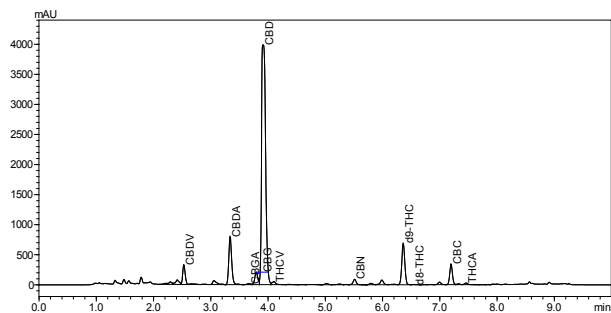


Fig. 4: Hemp oil 5 – CBD only (81x dilution)

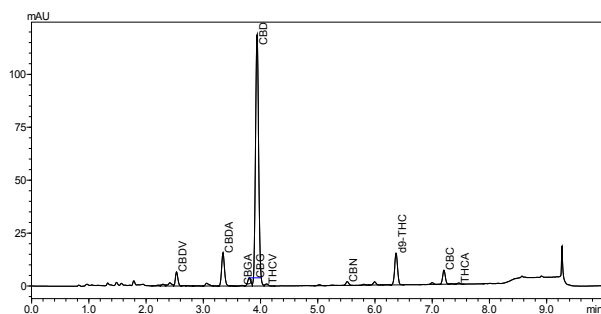


Fig. 5: Hemp oil 5 – CBD only (4050x dilution)

### Quantitative Results Summary for Hemp Oils

Table 1: Summary of cannabinoid quantitative determination for five hemp oils

ID#	Name	1		2		3		4		5	
		mg/10 mL	%	mg/10 mL	%	mg/10 mL	%	mg/10 mL	%	mg/10 mL	%
		Label Claim (CBD): 3% (300 mg/10 mL)		Label Claim (CBD): 5% (500 mg/10 mL)		Label Claim (CBD): 10% (1000 mg/10 mL)		Label Claim (CBD): 15% (1500 mg/10 mL)		Label Claim (CBD): 35% (3500 mg/10 mL)	
1	CBDV	3.52	0.04	6.06	0.06	18.83	0.19	24.98	0.25	157.36	1.57
2	CBDA	13.48	0.13	2.76	0.03	2.03	0.02	1.32	0.01	405.04	4.05
3	CBGA	2.76	0.03	0.19	0.00	0.67	0.01	0.58	0.01	13.32	0.13
4	CBG	10.34	0.10	7.55	0.08	8.11	0.08	7.83	0.08	127.90	1.28
5	CBD	282.24	2.82	432.41	4.32	1037.46	10.37	1353.24	13.53	3554.72	35.55
6	THCV	0.60	0.01	0.00	0.00	0.00	0.00	0.00	0.00	35.39	0.35
7	CBN	10.70	0.11	2.73	0.03	1.91	0.02	2.07	0.02	36.52	0.37
8	D9-THC	16.46	0.16	15.03	0.15	16.84	0.17	17.06	0.17	427.39	4.27
9	D8-THC	0.53	0.01	0.31	0.00	0.30	0.00	0.31	0.00	0.95	0.01
10	CBC	35.09	0.35	29.08	0.29	43.11	0.43	47.82	0.48	182.71	1.83
11	THCA	2.53	0.03	1.28	0.01	1.40	0.01	0.71	0.01	17.35	0.17
CBD % of label claim		94		86		104		90		102	
Total CBD % of label claim		99		87		104		90		113	

- Note 1: CBD for samples 1-4 taken from highest dilution (4050X), all other compounds from lowest dilution (81X).
- Note 2: Sample 5 results for CBDV, CBDA, CBG, CBD, D9-THC and CBC taken from highest dilution (4050X), all other compounds from lowest dilution (81X).
- Note 3: Total CBD is the CBD and CBDA (87.7%) results combined. 87.7% is based upon the theoretical maximum CBD yield from CBDA conversion.

Table 2: Summary of CBD quantitative determination for 10 preparations of same hemp oil

Replicate	CBD Retention time (mins)	CBD Concentration (ppm)
Replicate 1	3.957	468.90
Replicate 2	3.954	456.46
Replicate 3	3.959	444.06
Replicate 4	3.956	492.59
Replicate 5	3.962	452.89
Replicate 6	3.957	473.22
Replicate 7	3.956	457.72
Replicate 8	3.959	487.40
Replicate 9	3.958	452.75
Replicate 10	3.955	451.02
Average	3.957	463.70
%RSD	0.06	3.51
Maximum	3.96	492.59
Minimum	3.95	444.06
Std. Dev.	0.0024	16.28033

Table 3: Summary of CBD quantitative determination for 20 injections of same hemp oil

Replicate	CBD Retention time (mins)	CBD Concentration (ppm)
Injection 1	3.959	450.46
Injection 2	3.955	453.64
Injection 3	3.956	452.87
Injection 4	3.959	451.05
Injection 5	3.957	451.22
Injection 6	3.952	450.84
Injection 7	3.954	450.26
Injection 8	3.957	450.88
Injection 9	3.956	449.83
Injection 10	3.953	449.81
Injection 11	3.955	449.17
Injection 12	3.956	450.63

Replicate	CBD Retention time (mins)	CBD Concentration (ppm)
Injection 13	3.956	449.35
Injection 14	3.954	448.11
Injection 15	3.950	449.44
Injection 16	3.952	448.41
Injection 17	3.954	450.24
Injection 18	3.954	448.35
Injection 19	3.955	449.57
Injection 20	3.950	448.22
Average	3.95	450.12
%RSD	0.06	0.32
Maximum	3.96	453.64
Minimum	3.95	448.11
Std. Dev.	0.00	1.45

## Discussion

Tables 1, 2 and 3 summarize the quantitative findings for the hemp oil samples.

Table 1 reflects the accurate quantitation of cannabinoids within the oil samples. These results utilize data from both dilutions.

Samples were prepared in triplicate for samples 1-3 and 5 and five times for sample 4, due to small amount of crystallization of the sample, a higher number of preparations were used to ensure accuracy of data. Samples CBD results varied by less than 1.6 %RSD, for samples 1-3 and 5. Sample 4 has a %RSD of 5.0 due to the crystallization. Across all the cannabinoids the %RSD for each compound with varying sample was less than 5 % for 97 % of all data. Only sample 2 showed samples with compounds with >5 %RSD variance, this is considered sample based and not testing based due to the accuracy across the various samples.

The nominal content of CBD was within 15 % across the samples, with only sample 5 showing a significant change in result when comparing CBD and total CBD content, this was due to the larger CBDA content within this sample.

Tables 2 and 3 reflects the accurate preparation/injection of samples using the CBD results from a single oil sample, using the 4050X dilution sample.

Samples were prepared using a positive placement pipette for the aliquot of oil samples.

The variability within the results for CBD was within 5 %RSD and 0.5 %RSD respectively for the repeated replicate dilutions /injections.



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