**Introduction**

Medical marijuana generally possesses high levels of the psychotropic tetrahydrocannabinol, d9-THC and lower levels of the non-psychotropic cannabinoid, 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](image-url)
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.

Figure 2: Standard curves

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.
**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 µL methanol to a 2 mL glass vial
- Add 200 µL 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)
No. SSI-HPLC-018

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

<table>
<thead>
<tr>
<th>ID#</th>
<th>Name</th>
<th>Black Label (µg/mL)</th>
<th>%</th>
<th>Blue Label (µg/mL)</th>
<th>%</th>
<th>Green Label (µg/mL)</th>
<th>%</th>
<th>Red Label (µg/mL)</th>
<th>%</th>
<th>Yellow Label (µg/mL)</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CBD</td>
<td>21880.06</td>
<td>2.188</td>
<td>15242.8</td>
<td>1.524</td>
<td>24210.45</td>
<td>2.421</td>
<td>9721.64</td>
<td>0.972</td>
<td>16695</td>
<td>1.670</td>
</tr>
<tr>
<td>2</td>
<td>CBD</td>
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<td>2.188</td>
<td>15242.8</td>
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<td>24210.45</td>
<td>2.421</td>
<td>9721.64</td>
<td>0.972</td>
<td>16695</td>
<td>1.670</td>
</tr>
<tr>
<td>3</td>
<td>CBD</td>
<td>21880.06</td>
<td>2.188</td>
<td>15242.8</td>
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<td>24210.45</td>
<td>2.421</td>
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<td>0.972</td>
<td>16695</td>
<td>1.670</td>
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<tr>
<td>4</td>
<td>CBD</td>
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<td>24210.45</td>
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<td>9721.64</td>
<td>0.972</td>
<td>16695</td>
<td>1.670</td>
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</table>

Table 2: Summary of Total Cannabinoids Quantitative Determination

<table>
<thead>
<tr>
<th>ID#</th>
<th>Name</th>
<th>Black Label (µg/mL)</th>
<th>%</th>
<th>Blue Label (µg/mL)</th>
<th>%</th>
<th>Green Label (µg/mL)</th>
<th>%</th>
<th>Red Label (µg/mL)</th>
<th>%</th>
<th>Yellow Label (µg/mL)</th>
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<tbody>
<tr>
<td>1</td>
<td>CBDV</td>
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<td>2</td>
<td>CBDA</td>
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<td>0.006</td>
<td>47.04</td>
<td>0.005</td>
<td>11469.65</td>
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<td>3</td>
<td>CBGA</td>
<td>23.55</td>
<td>0.002</td>
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<td>0</td>
<td>99.49</td>
<td>0.101</td>
<td>0</td>
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<tr>
<td>4</td>
<td>CBG</td>
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<td>0.017</td>
<td>32.95</td>
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<td>243.55</td>
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<td>0.034</td>
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<td>0.055</td>
<td>0</td>
<td>0</td>
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<td>1104</td>
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<td>11</td>
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<td>1006.06</td>
<td>0.101</td>
<td>1194</td>
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<td>0.110</td>
<td>456.75</td>
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<td>809.31</td>
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</table>

<table>
<thead>
<tr>
<th>CBD % of label claim</th>
<th>Black Label</th>
<th>Blue Label</th>
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<th>Red Label</th>
<th>Yellow Label</th>
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<table>
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<th>CBD % of Total</th>
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<th>Blue Label</th>
<th>Green Label</th>
<th>Red Label</th>
<th>Yellow Label</th>
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<td>92</td>
<td>92</td>
<td>59</td>
<td>86</td>
<td>88</td>
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</table>

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.