



## APPLICATION NOTE

### Liquid Chromatography

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## Analysis of Cannabinoids in Hemp Seed Oils by HPLC Using PDA Detection

### Introduction

Cannabis sativa, from which hemp and marijuana are derived, has been a source of medicinal, industrial, and recreational commodities for centuries. The term

“hemp” refers primarily to cannabis grown as an agricultural crop and is characterized by cannabis plants that are low in delta-9 tetrahydrocannabinol (THC), the main psychoactive ingredient in marijuana. Although more than 30 nations worldwide grow hemp as an agricultural commodity, in the United States, production is strictly controlled under existing drug enforcement laws.<sup>1</sup>

As of January 2015, twenty states have passed legislation favorable to hemp cultivation. The main obstacles facing the potential U.S. market are the government drug policies and Drug Enforcement Administration (DEA) concerns that the commercial cultivation could increase the likelihood of covert production of high-THC cannabis varieties or inadvertent cross pollination, complicating DEA surveillance.

In recent years, scientific knowledge regarding the composition and health benefits of edible hemp products has significantly increased. Hemp seed oil has been promoted as a good source of nutritious omega-6 and omega-3 polyunsaturated acids, and may be a cleaner, more sustainable alternative to fish oil.<sup>3</sup> While hemp seed oil has been shown to have high nutritive value, it is generally believed that it may also afford other beneficial qualities.

Naturally occurring cannabinoids, the main biologically active component of the cannabis plant, form a complex group of closely related compounds, of which 70 are known and well described. Although no cannabinoids are metabolically produced by the hemp seed itself, contamination with resin from cannabis flowers and leaves during hemp seed oil processing can occur. Impurity reports have been primarily focused on THC for its pharmacological and toxicological relevance, upon which strict legal limits have been enforced.<sup>4</sup> However, other cannabinoids provide unique biological activities. For example, cannabidiol (CBD), typically present at higher levels than THC, shows evidence of providing anticonvulsive, anti-epileptic, and antimicrobial properties.<sup>2,3,4</sup>

In this application, we describe a technique for the monitoring of six cannabinoids, including THC and CBD, in hemp seed oil by HPLC with PDA detection. Figure 1 shows the chemical structures for the six cannabinoids analyzed in this application.

## Experimental

### Hardware/Software

A PerkinElmer HPLC system was used, including the UV detector (PerkinElmer, Shelton, CT, USA). A PDA (photodiode array) detector was also used for spectral confirmation. PerkinElmer Brownlee Analytical C18, 3.0  $\mu\text{m}$ , 4.6 x 150-mm (Part# N9303508) column was used for all analyses (PerkinElmer, Shelton, CT, USA). All instrument control, analysis, and data processing was performed via associated Chromatography Data Software (CDS).

### Method Parameters

The LC method parameters are shown in Table 1.

### Solvents, Standards and Samples

All solvents and diluents used were HPLC-grade and filtered via 0.45- $\mu\text{m}$  filters.

Table 1. LC Method Parameters.

|                       |   |            |                    |      |      |
|-----------------------|---|------------|--------------------|------|------|
| Column:               | PerkinElmer Brownlee Analytical C18, 3.0 $\mu\text{m}$ , 4.6 x 150-mm (Part# N9303508)                  |            |                    |      |      |
| Mobile Phase:         | Solvent A: 0.1% formic acid in water<br>Solvent B: 0.1% formic acid in acetonitrile<br>Solvent program: |            |                    |      |      |
|                       |   | Time (min) | Flow Rate (mL/min) | %A   | %B   |
|                       | 1   | Initial    | 1.0                | 50.0 | 50.0 |
|                       | 2   | 1.0        | 1.0                | 50.0 | 50.0 |
|                       | 3   | 1.1        | 1.0                | 20.0 | 80.0 |
|                       | 4   | 8.1        | 1.0                | 5.0  | 95.0 |
| 5                     | 8.6   | 1.0        | 50.0               | 50.0 |      |
| Analysis Time:        | 8.0 min.; wash/equilibration time = 7.0 min.  |            |                    |      |      |
| Flow Rate:            | 1.0 mL/min.   |            |                    |      |      |
| Pressure:             | ~2500 psi/172 bar maximum   |            |                    |      |      |
| Oven Temp.:           | 50 °C   |            |                    |      |      |
| PDA Detection:        | Wavelength: 210 nm  |            |                    |      |      |
| Injection Volume:     | 25 $\mu\text{L}$  |            |                    |      |      |
| Sampling (Data) Rate: | 10 pts./sec   |            |                    |      |      |
| Diluent:              | Isopropyl alcohol (IPA)   |            |                    |      |      |
| Sampling (Data) Rate: | 5 pts./sec  |            |                    |      |      |

1 mg/ml each of the following cannabinoids was obtained from Restek® Corporation (Bellefonte, PA): cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabichromene (CBC) and tetrahydrocannabinolic acid (THC-A). 1 mg/mL of delta-9 tetrahydrocannabinol (THC) was obtained from Sigma-Aldrich®, Inc (Allentown, PA).

A 14-ppm mixture of the six standards was prepared by adding 25  $\mu\text{L}$  of each standard to 1.650 mL of isopropyl alcohol (IPA). For hemp seed oil quantitation, a standard solution containing THC and CBD was prepared by adding 25.0  $\mu\text{L}$  of each into 1.750 mL of IPA, to equal a final concentration of 14 ppm.

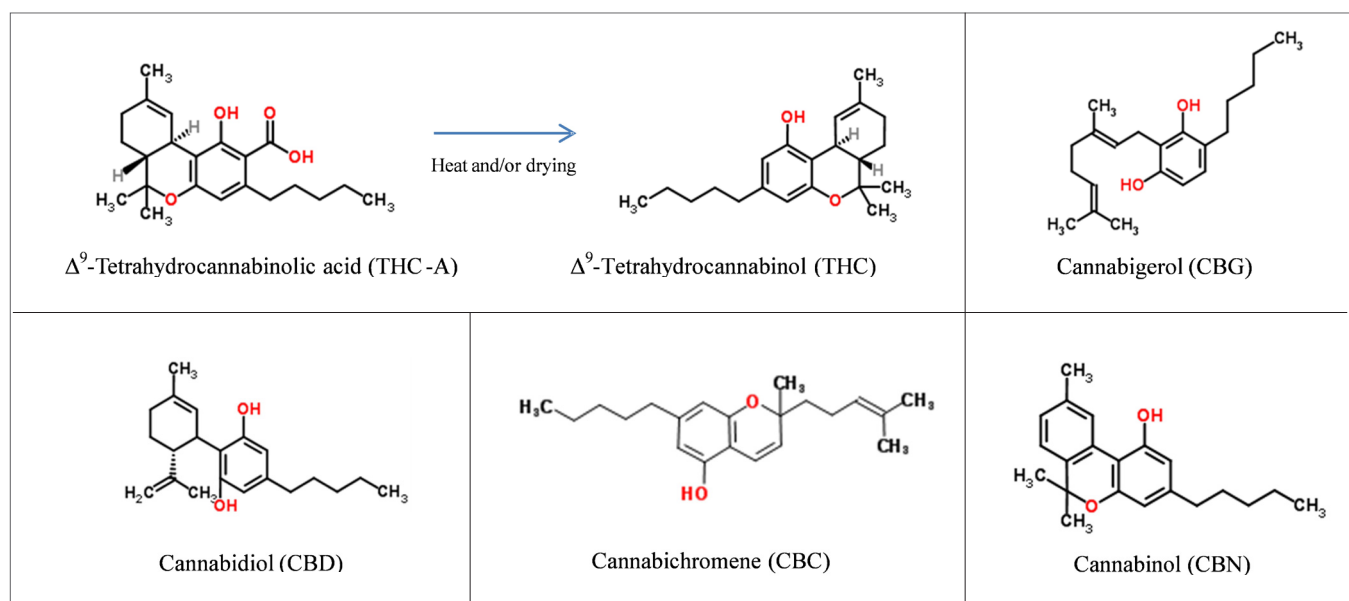


Figure 1. Chemical structure of six cannabinoids analyzed in this study.

Two brands of hemp seed oils, labelled "Brand A" and "Brand B", were obtained from a local health food store. In order to reduce the viscosity of the hemp seed oil prior to analysis, each sample was diluted nine fold in IPA by adding 0.2 mL hemp seed oil to 1.6 mL of IPA and mixing by manual shaking. A 14-ppm spiked solution of each hemp seed oil sample was prepared by adding 25.0  $\mu$ L THC standard, 25.0  $\mu$ L CBD standard, 0.2 mL hemp seed oil, and 1.55 mL IPA to a 2-mL sample vial and then mixed by manual shaking. Considering the nine fold dilution of sample, the final cannabinoid concentration corresponded to 125-ppm in undiluted hemp seed oil.

## Results and Discussion

Figure 2 shows the chromatogram of a standard mixture containing the six cannabinoids, all separated in under eight minutes.

As shown in Figure 3, chromatographic repeatability was confirmed via 10 injections of the 14-ppm standard mixture, demonstrating good reproducibility.

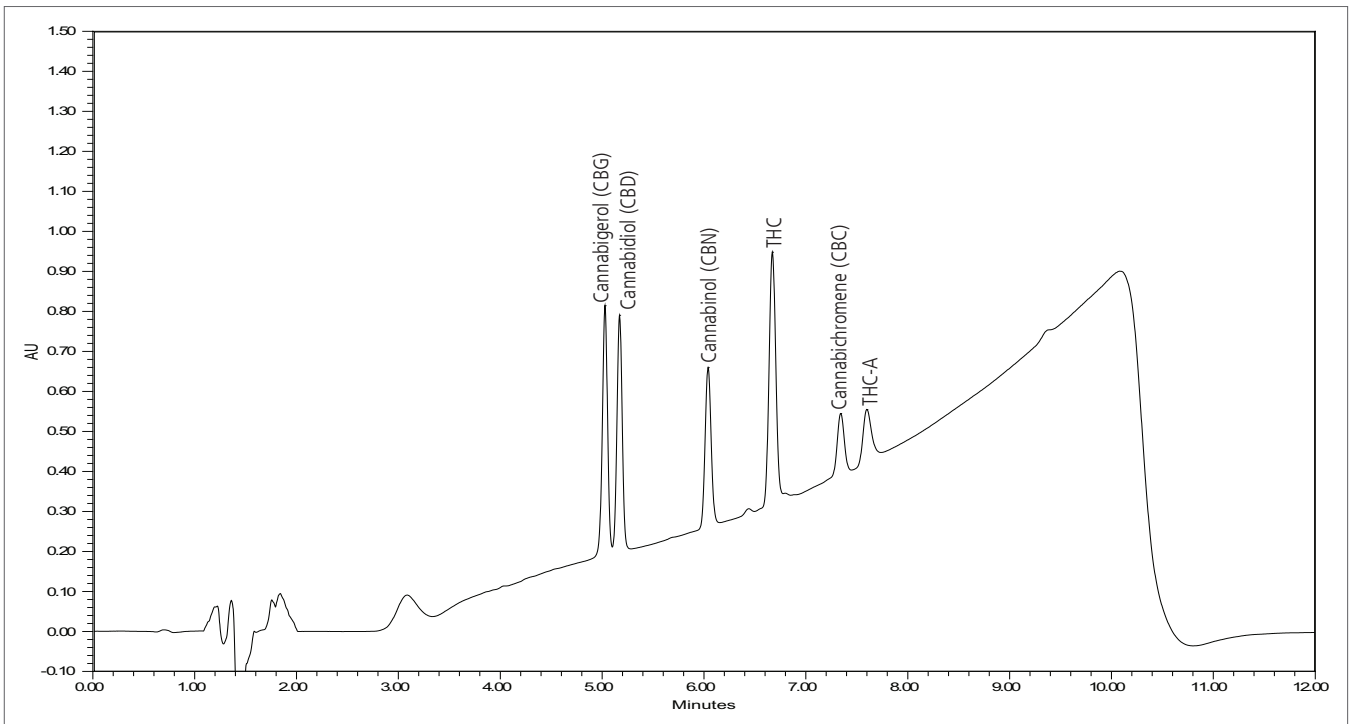


Figure 2. HPLC chromatogram showing separation of six cannabinoids in the 14-ppm standard mixture; wavelength: 210 nm.

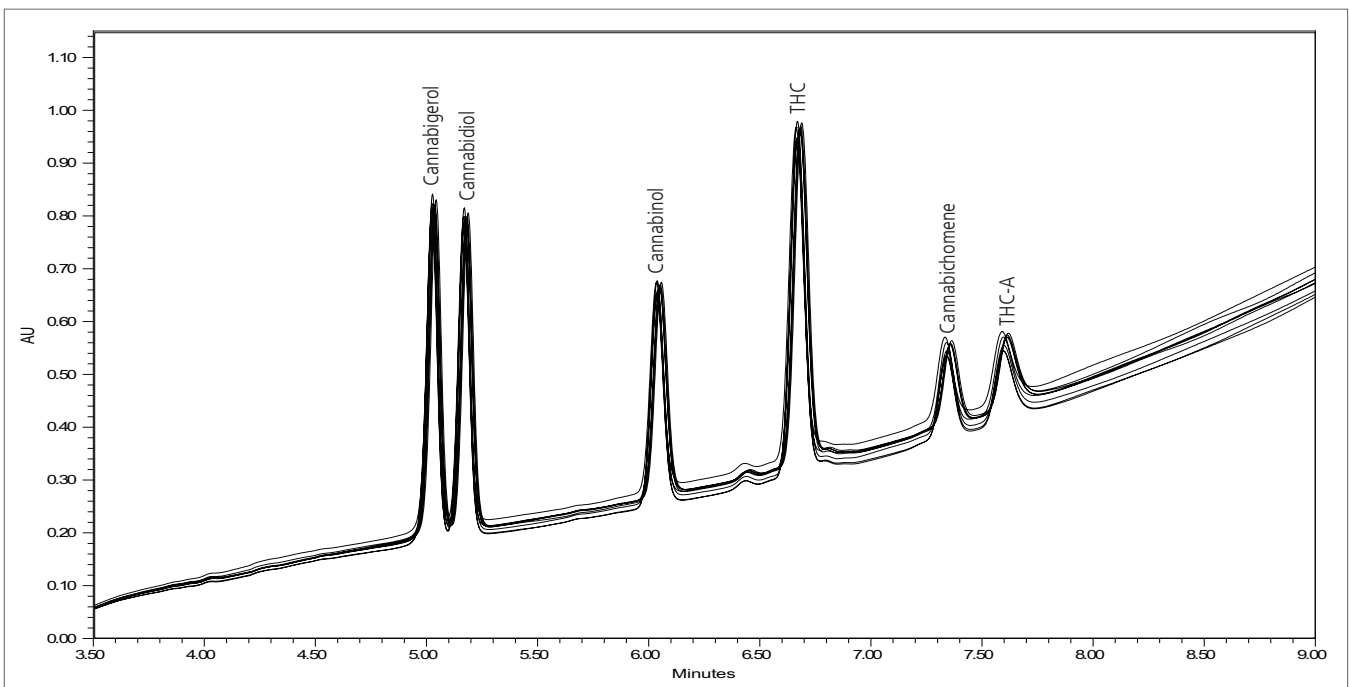


Figure 3. Overlay of 10 replicates of the 14-ppm standard mixture.

Linearity was determined for all six cannabinoids. Representative linearity plots for CBD and THC, respectively, are shown in Figure 4 and Figure 5. The linearity coefficients of determination for all six cannabinoids are listed in Table 2.

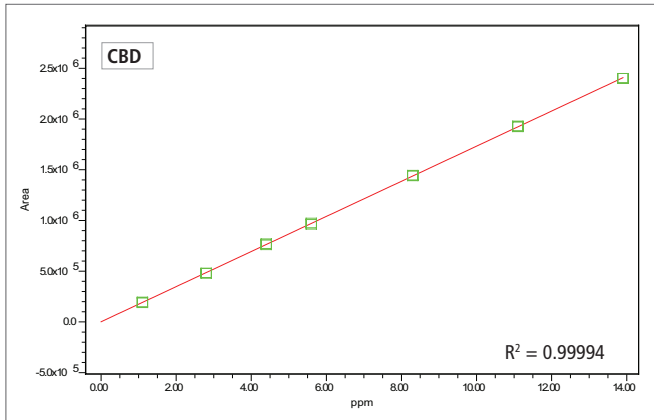


Figure 4. Linearity plot of CBD; concentration range: 1-14 ppm in IPA.

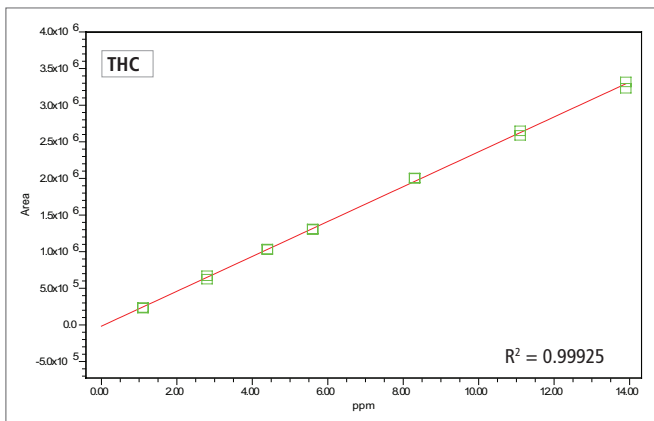


Figure 5. Linearity plot of THC; concentration range: 1-14 ppm in IPA.

Table 2. Linearity coefficient of determination for six cannabinoids between 1 –14 ppm detected by PDA at 210 nm.

| Cannabinoid                                 | Linearity R <sup>2</sup> |
|---|--------------------------|
| Cannabigerol (CBG)                          | 0.99951                  |
| Cannabidiol (CBD)                           | 0.99994                  |
| Cannabinol (CBN)                            | 0.99996                  |
| Delta-9 tetrahydrocannabinol (THC)          | 0.99925                  |
| Cannabichromene (CBC)                       | 0.99958                  |
| Delta-9-tetrahydrocannabinolic acid (THC-A) | 0.99857                  |

As listed in Table 3, LOD (limit of detection) and LOQ (limit of quantitation) levels were established for each cannabinoid, based upon undiluted hemp seed oil and a s/n of > 3/1 for LOD and >10/1 for LOQ. The LOD and LOQ values were less than 200 ppb and 600 ppb, respectively, for all cannabinoids.

Table 3. LOQ and LOD of six cannabinoids in undiluted Hemp Seed Oil.

| Cannabinoid                                 | LOD via PDA (ppb) | LOQ via PDA (ppb) |
|---|-------------------|-------------------|
| Cannabigerol (CBG)                          | 31                | 102               |
| Cannabidiol (CBD)                           | 32                | 106               |
| Cannabinol (CBN)                            | 54                | 183               |
| Delta-9-tetrahydrocannabinol (THC)          | 34                | 112               |
| Cannabichromene (CBC)                       | 82                | 273               |
| Delta-9-tetrahydrocannabinolic acid (THC-A) | 178               | 592               |

Figures 6 and 7 show the chromatograms of un-spiked Brand A and Brand B hemp seed oils, respectively, overlaid with standard mix. CBD was present in both of the un-spiked hemp seed oils, eluting at approximately 5.1 minutes and confirmed by PDA detection (data not shown). THC was detected at very low levels (below LOQ) in both samples, while the other five analytes were not detected in either of the analyzed oils.

For comparison, an overlay of THC/CBD-spiked and un-spiked Brand B hemp seed oil is shown in Figure 8.

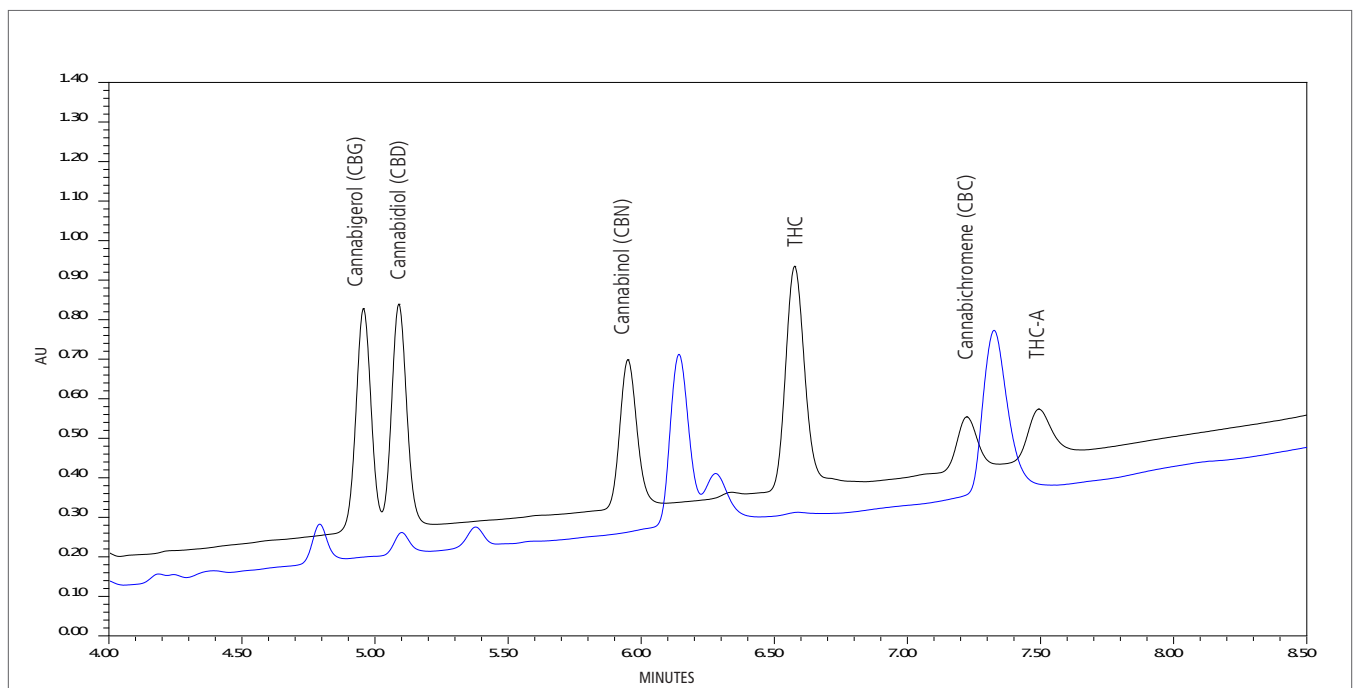


Figure 6. Overlay of a mixture of six cannabinoids (black) and Brand A of the un-spiked hemp seed oil (blue).

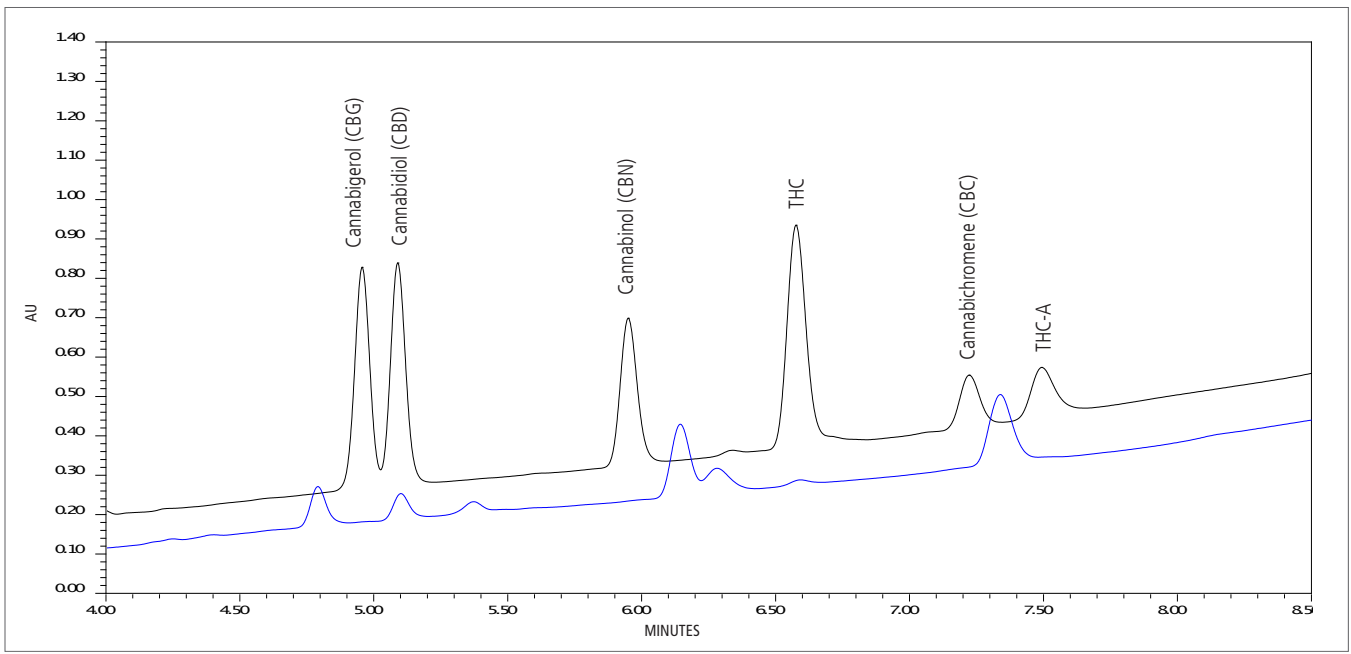


Figure 7. Overlay of a mixture of six cannabinoids (black) and Brand B of the un-spiked hemp seed oil (blue).

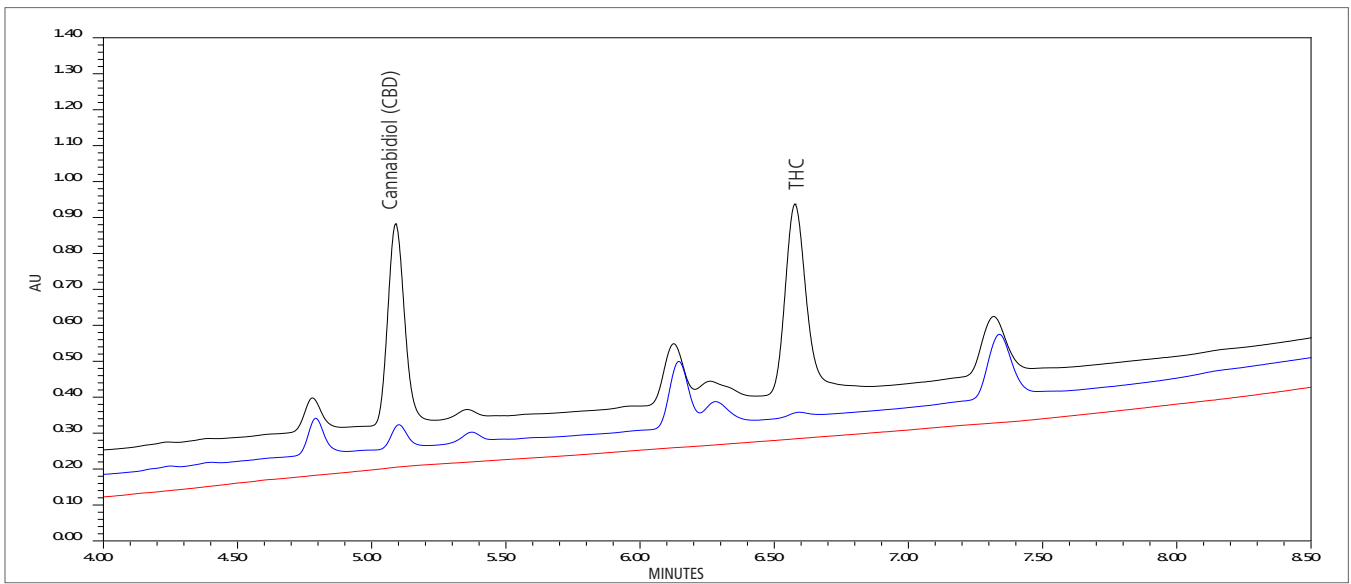


Figure 8. Overlaid chromatograms of a blank (red), un-spiked Brand B hemp seed oil (blue) and Brand B hemp seed oil spiked with 125-ppm THC and CBD (black).

The recovery of the spiked THC and CBD analytes was between 91-110%, as shown in Table 4. As CBD was observed in both brands of the un-spiked hemp seed oil, the recovery of spiked CBD was calculated by subtracting the un-spiked CBD concentration from the overall recovery.

Table 4. Recovery results for hemp seed oil spiked with THC and CBD at 125 ppm (n=2).

| Hemp Seed Oil  | CBD (%) | THC (%) |
|----------------|---------|---------|
| Brand A spiked | 91.0    | 96.6    |
| Brand B spiked | 95.6    | 110.2   |

## Conclusion

This work has demonstrated the effective chromatographic separation and quantitation of six cannabinoids, including THC and CBD, in hemp seed oils using the PerkinElmer HPLC System with UV and PDA detectors. The results exhibited exceptional linearity for each of the six cannabinoids over the concentration range tested.

## References

1. Johnson, Renee. "Hemp as an Agricultural Commodity: Congressional Research Service", CRS Report, 2015.
2. Hazekamp, Arno. "Cannabis Review", Department of Plant Metabolomics, Leiden University. 2008-2009.
3. Leizer, Cary et. al. "The Composition of Hemp Seed Oil and Its Potential as an Important Source of Nutrition", Journal of Nutraceuticals, Functional and Medicinal Food. 2000.
4. "Cannabis oil vs hemp seed oil; Cannabis oil, CBD Oil, Medical Marijuana". <http://www.cbd.org/cannabis-oil-vs-hemp-oil>.