



# Rugged Isocratic LC-UV Method for the Analysis of 16 Cannabinoids in Hemp and Cannabis Samples

## UCT Part Numbers:

### SLC-18150ID46-3UM

Selectra® C18 HPLC column  
150 × 4.6 mm, 3 μm

### SLC-18GDC46-3UM

Selectra® C18 guard cartridge  
10 × 4.6 mm, 3 μm

### SLGRDHLDR

Guard cartridge holder



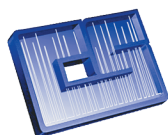
## Summary:

With the mounting interest in hemp and cannabis products for medicinal and recreational use around the world, the need for suitable analytical methods to identify and determine the concentration of cannabinoids is essential for ensuring consumer safety. Traditional analyses for measuring the potency of cannabinoids in cannabis and hemp samples have focused mainly on 5 primary analytes: THC, THC-A, CBD, CBD-A, and CBN. As the industry continues to expand and evolve, more attention is being directed toward additional, although less prevalent, cannabinoids that have been shown to exhibit physiological effects. Thus, simple yet selective analytical methodologies are needed to adequately separate and identify a wide range of cannabinoids present in the hemp and cannabis products which exist on the market, including plant products, edibles, oils, topicals and extracts.

This application note outlines a simple and robust method for the detection and quantitation of 16 cannabinoids in hemp and cannabis samples using an isocratic HPLC method coupled with UV detection. Baseline separation of all 16 cannabinoids, including that of the critical pair  $\Delta^9$ -THC and  $\Delta^8$ -THC, was successfully achieved using a Selectra® C18 column. Examples of hemp flower and oil samples analyzed using the analytical method are also presented. With the widespread legalization of hemp and cannabis, this simple method will be beneficial for any research facility wanting to implement regulatory testing without fighting the wide variation of the matrices and interfering compounds (i.e. terpenes, pigments, etc.) in the diverse products that exist in the marketplace.



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## Experimental

### Flower Sample Pretreatment:

Cannabis and hemp flower should be ground to a fine powder using a temperature-controlled method such as cryogenic milling (e.g. SPEX® 6775 Freezer/Mill®) or a Robot-Coupe® blender with dry ice to prevent conversion of the acid forms of the cannabinoids. For this work, stainless steel grinding beads were used in a 50mL centrifuge tube with cold extraction solvents.

### Extraction Procedure:

#### Plant material:

1. Weigh out 1g of homogenized plant material in a 50mL polypropylene centrifuge tube.
2. Add 15mL of acetonitrile (an alternative organic solvent and/or volume may be used depending on the testing requirements).
3. Vortex thoroughly then shake for 15-30 minutes. For this work a SPEX® 2010 GenoGrinder® (1000 rpm) was used.
4. Centrifuge the sample at  $\geq 3000 \times g$  for 5 minutes.
5. Transfer 1mL of supernatant for LC-UV analysis.

#### Concentrates:

1. Transfer 1mL/1g of CBD oil to a 15mL polypropylene centrifuge tube.
2. Add 10mL of isopropyl alcohol.
3. Vortex for 15-30 min.
4. Centrifuge the sample at  $\geq 3000 \times g$  for 5 minutes.
5. Transfer 1mL of supernatant for LC-UV analysis.

### LC-UV Analysis:

To cover the wide range of concentrations that may exist between the cannabinoids, it is recommended to run each sample undiluted and with an additional dilution protocol. This approach will allow for the accurate detection of the lower level cannabinoids that can be drowned out by higher concentration cannabinoids, most notably THC and CBD.

For this study, 1mL of sample extract was transferred into an autosampler vial for analysis (this is the undiluted fraction; x10 or x15) and an aliquot of each sample then underwent additional dilution. For oil samples, further dilution was carried out by adding 10 $\mu$ L of sample extract to 990 $\mu$ L of isopropyl alcohol (additional 100-fold dilution; x1000 total). For the flower sample, 100 $\mu$ L of sample extract was added to 900 $\mu$ L of acetonitrile (additional 10-fold dilution; x150 total).

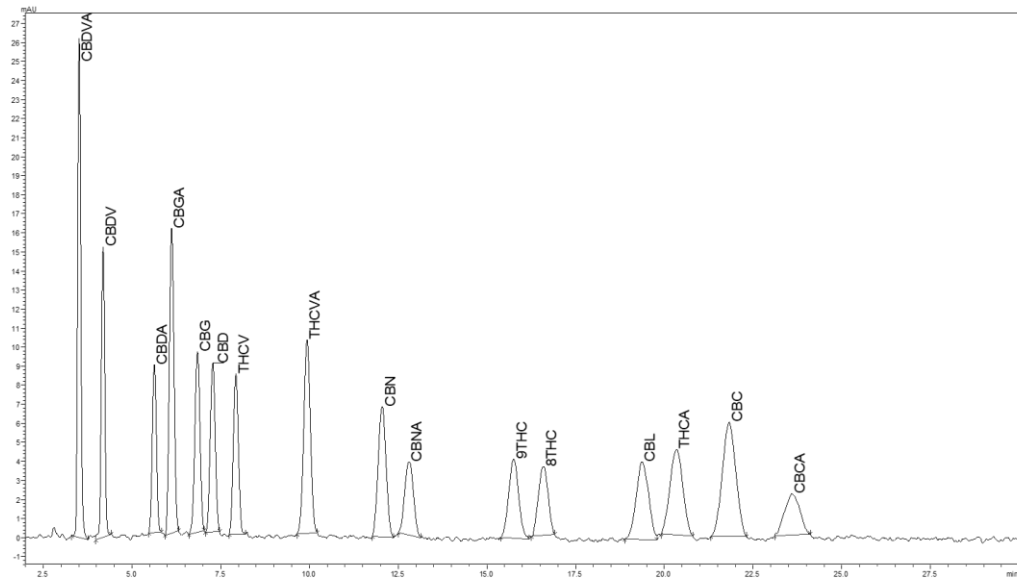


## LC-UV Parameters:

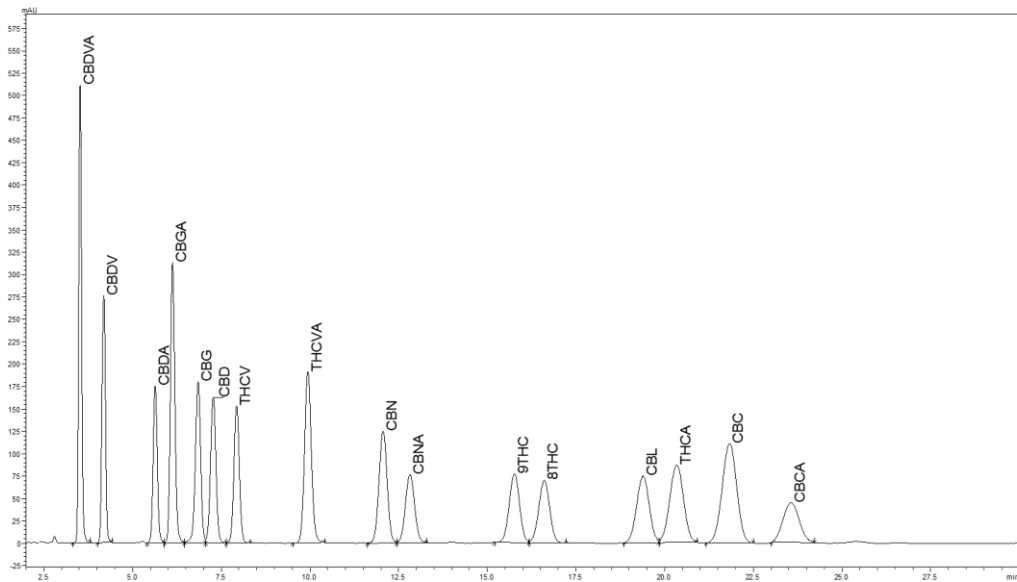
Table 1. Instrumental Conditions	
HPLC system	Shimadzu™ Nexera X2
MWD settings	230 nm
HPLC column	UCT Selectra® C18, 150 × 4.6 mm, 3 μm (p/n: SLC-18150ID46-3UM)
Guard column	UCT Selectra® C18, 10 × 4.6 mm, 3 μm (p/n: SLC-18GDC46-3UM)
Guard column holder	p/n: SLGRDHLDR
Column temperature	40°C
Flow rate	1.5 mL/min
Injection volume	20uL
Mobile phase A	Water + 0.1% formic acid + 5mM ammonium formate
Mobile phase B	Acetonitrile
Isocratic conditions	70% B

Table 2. Cannabinoids Tested			
Cannabinoid	Abbreviation	CAS Number	RT (min)
Cannabidivarinic acid	CBDV-A	31932-13-5	3.48
Cannabidivarin	CBDV	24274-48-4	4.14
Cannabidiolic acid	CBD-A	1244-58-2	5.69
Cannabigerolic acid	CBG-A	25555-57-1	6.16
Cannabigerol	CBG	25654-31-3	6.84
Cannabidiol	CBD	13956-29-1	7.31
Tetrahydrocannabivarin	THCV	31262-37-0	7.92
Tetrahydrocannabivarinic acid	THCV-A	39986-26-0	9.91
Cannabinol	CBN	521-35-7	12.03
Cannabinolic acid	CBN-A	2808-39-1	12.79
Δ9-Tetrahydrocannabinol	Δ9-THC	1972-08-03	15.76
Δ8-Tetrahydrocannabinol	Δ8-THC	5957-75-5	16.60
Cannabicyclol	CBL	21366-63-2	19.40
Δ9-Tetrahydrocannabinolic acid A	THC-A	20675-51-8	20.36
Cannabichromene	CBC	23978-85-0	21.85
Cannabichromenic acid	CBC-A	185505-15-1	23.62

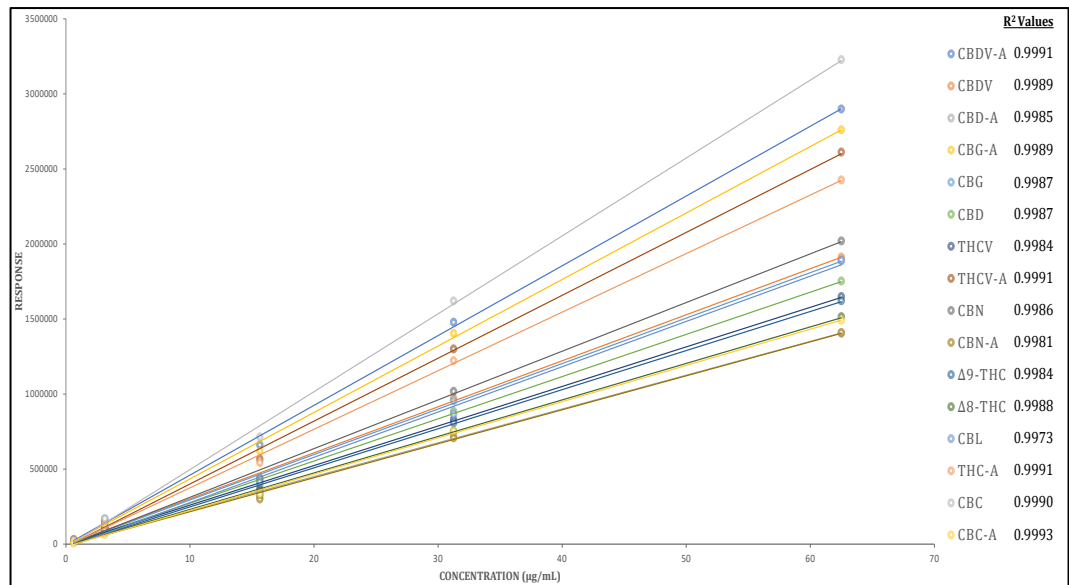




**Figure 1.** Chromatogram of the lowest calibration standard (3 µg/mL).



**Figure 2.** Chromatogram of the highest calibration standard (62.5 µg/mL).



**Figure 3.** Calibration curves for 16 cannabinoid standards and their corresponding R<sup>2</sup> values.

Note: 62.5 µg/mL is the maximum concentration that can be achieved by combining the 16 individual cannabinoid standards at a concentration of 1mg/mL (DEA cutoff) into a single mix.



## Analysis of Commercial Hemp Products:

As an example of the utility of this method, commercially available hemp oil, hemp flower and hemp protein powder samples were extracted according to the procedure outlined above and analyzed for the presence of 16 cannabinoids.

Table 3. Commercial Hemp Product Information					
	Oil 1	Oil 2	Oil 3	Hemp Flower	Hemp Protein Powder
<b>Label Claim</b>	16.66 mg/mL CBD Oil	33.33 mg/mL Hemp Extract	50 mg/mL Hemp Extract	13.6% CBD	Nothing listed on cannabinoid content

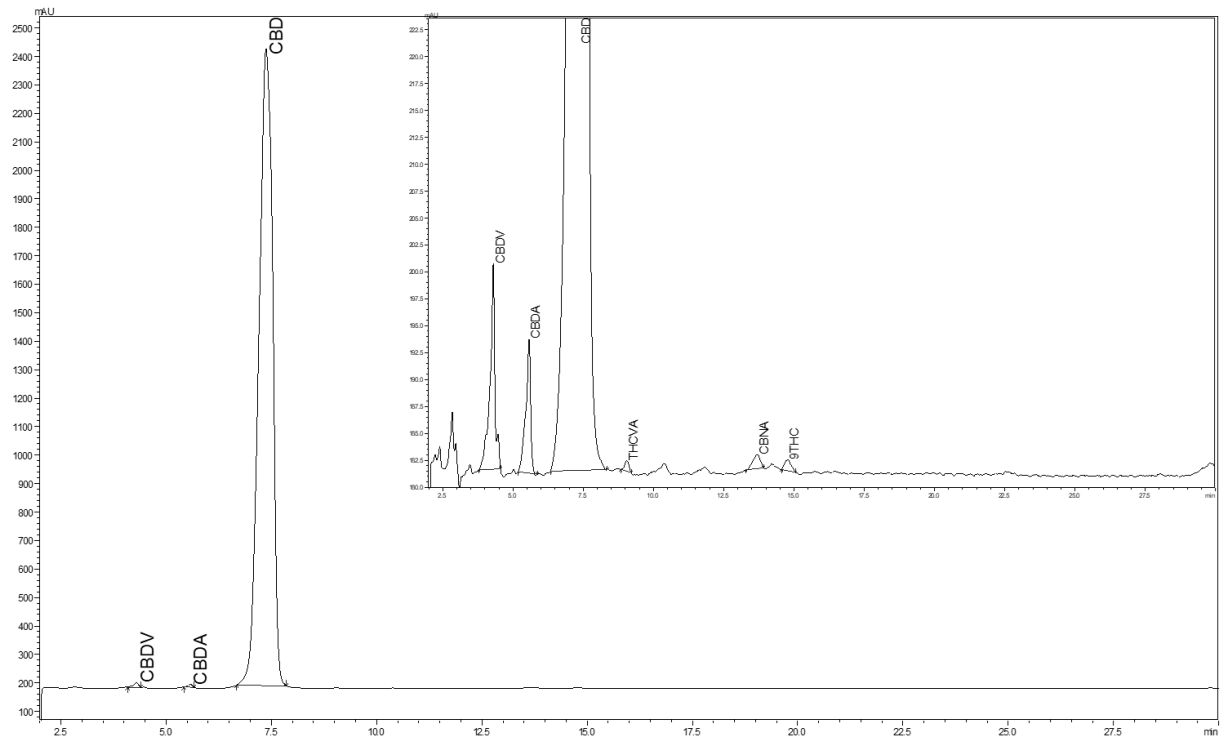
Table 4. Cannabinoid Concentrations ( $\mu\text{g/g}$ ) in Commercial Hemp Products								
Cannabinoid	Oil 1		Oil 2		Oil 3		Hemp Flower (x150)	Hemp Powder (x150)
	x10	x1000	x10	x1000	X10	x1000		
CBDV-A	-----	-----	-----	-----	-----	-----	*	-----
CBDV	74.91	-----	48.09	-----	-----	-----	254.55	-----
CBD-A	65.77	-----	76.93	-----	-----	-----	8304.00	-----
CBG-A	-----	-----	-----	-----	-----	-----	58.80	-----
CBG	-----	-----	-----	-----	-----	-----	-----	-----
CBD	Dilution required	33193	Dilution required	294.40	-----	-----	1593.01	-----
THCV	-----	-----	77.46	-----	-----	-----	-----	-----
THCV-A	*	-----	144.76	-----	-----	-----	131.70	-----
CBN	-----	-----	24.46	-----	-----	-----	-----	-----
CBN-A	15.19	-----	-----	-----	-----	-----	42.00	-----
$\Delta$ 9-THC	11.28	-----	63.16	-----	-----	-----	86.72	-----
$\Delta$ 8-THC	-----	-----	164.06	-----	-----	-----	132.07	-----
CBL	-----	-----	-----	-----	-----	-----	117.15	-----
THC-A	-----	-----	17.24	-----	-----	-----	-----	-----
CBC	-----	-----	28.82	-----	-----	-----	26.85	-----
CBC-A	-----	-----	-----	-----	-----	-----	137.55	-----

\* Calculated concentration was below the lowest point on the calibration curve.

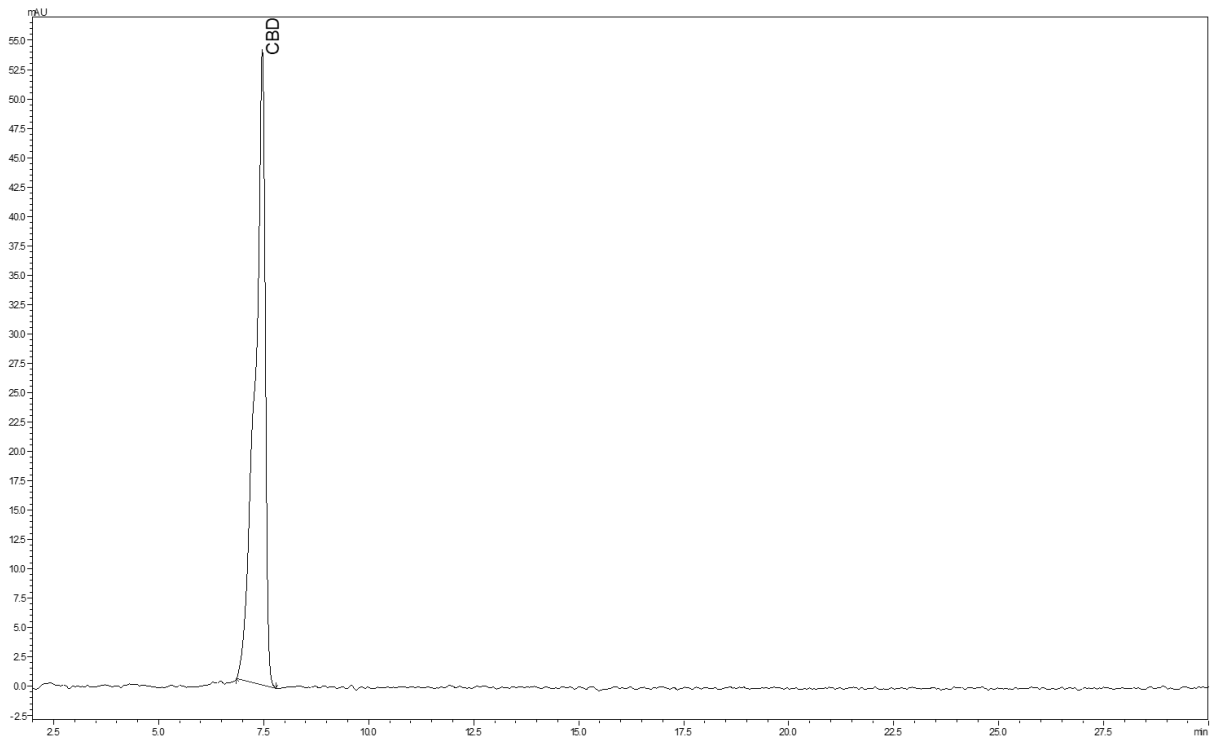
Back calculations for the final concentrations in the table were carried out by taking into account the dilution factor used during the extraction procedure (dilution factor in brackets).

When the sample extracts were initially analyzed, some of them had CBD concentrations that were significantly above the highest point on the calibration curve. Therefore, an aliquot of each hemp oil sample was diluted to fit within the calibration curve limits. Due to their low concentrations relative to CBD, the additional dilution step resulted in non-detection of the other cannabinoids (see figures 4/5 & 6/7). Ideally, the calibration curve would have a linear range that allows for a single extraction/dilution scheme to quantify low level and high concentration cannabinoids. However, this may not be feasible due to the limited concentrations of reference standard available.



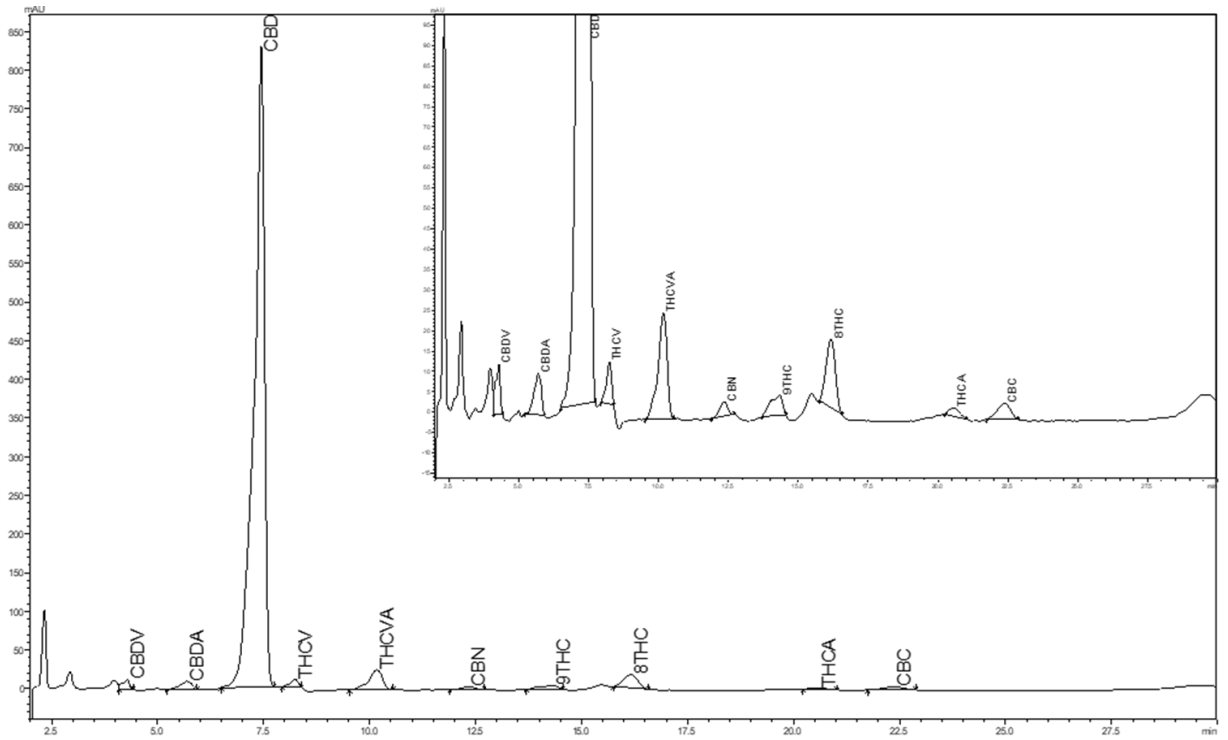


**Figure 4.** Chromatogram of Hemp Oil 1 – initial extraction (10-fold dilution).

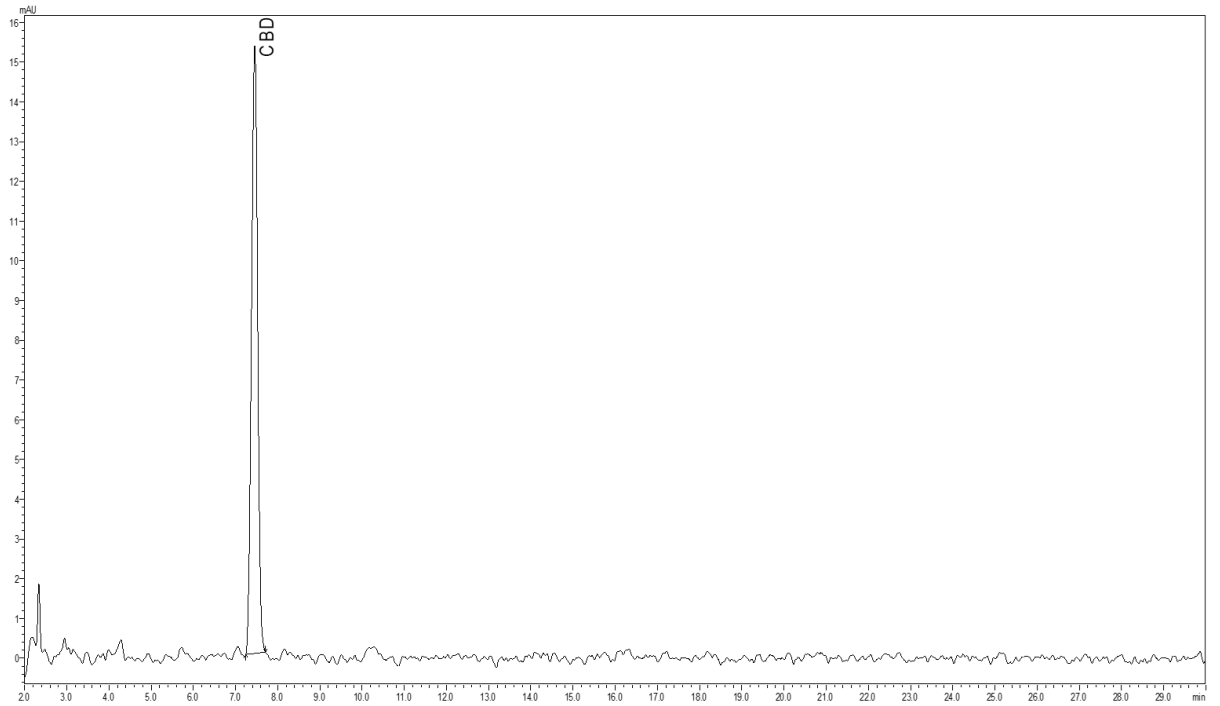


**Figure 5.** Chromatogram of Hemp Oil 1 – additional dilution (1000-fold dilution).



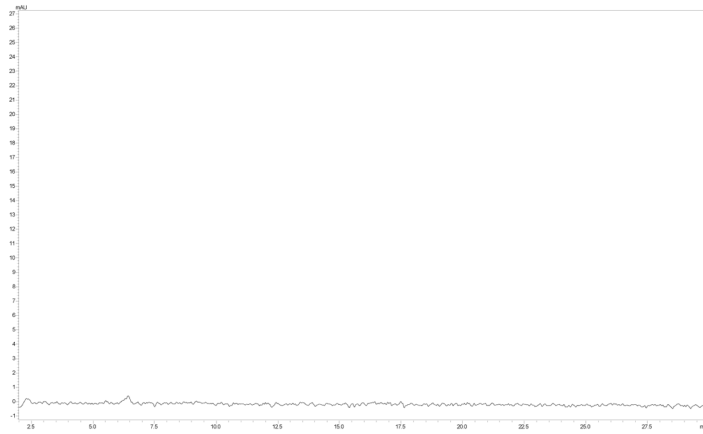


**Figure 6.** Chromatogram of Hemp Oil 2 – initial extraction (10-fold dilution).

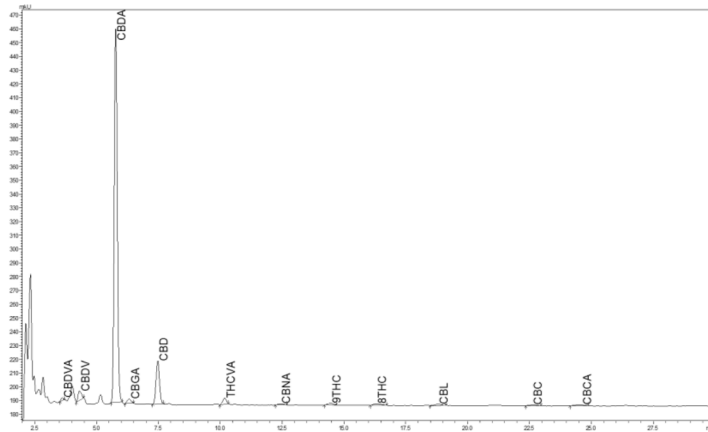


**Figure 7.** Chromatogram of Hemp Oil 2 – additional dilution (1000-fold dilution).

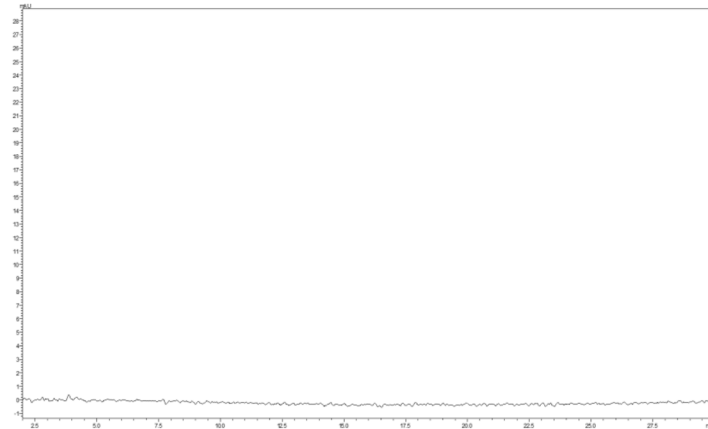




**Figure 8.** Chromatogram of Hemp Oil 3 – initial extraction (10-fold dilution).



**Figure 9.** Chromatogram of Hemp Flower (150-fold dilution).



**Figure 10.** Chromatogram of Hemp Protein Powder (150-fold dilution).

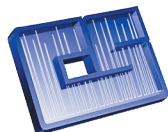
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