

# Analysis of Cannabinoids in Hemp and Hemp-Containing Products Using HPLC With Post-Column Derivatization

The legalization of hemp under the 2018 Farm Bill brought new opportunities as well as new challenges to both growers of industrial hemp and manufacturers of hemp-containing products. The Farm Bill classifies hemp as the plant *Cannabis sativa L.* and all its derivatives with delta-9 tetrahydrocannabinol (THC) of not more than 0.3% concentration. To comply with Federal laws, all producers need to test their products to determine THC content as well as the concentration of other cannabinoids, particularly CBD, that are associated with pharmacological activity of *Cannabis sativa L.* plant.

A new HPLC method with post-column derivatization was developed to analyze cannabinoids in hemp and hemp-containing edible products. This post-column method is based on reaction with Fast Blue Salt reagent under basic conditions. Detection at 475 nm is performed using a UV/Vis detector.

The method utilizes a simple extraction procedure with no additional sample clean-up and is suitable for analysis of the major neutral cannabinoids as well as cannabinoid acids with high sensitivity and selectivity of detection.

## Method

### Analytical Conditions

**Analytical Column:** C<sub>18</sub> Reversed-phase Column, 4.6 x 150 mm

**Column Temperature:** 45 °C

**Flow Rate:** 1 mL/min

**Mobile Phase:** 70% acetonitrile – 30% sodium phosphate buffer (6 mM) at pH 3.5

**Injection Volume:** 20 µL

### Post-Column Conditions

**Post-Column System:** Onyx PCX, Pinnacle PCX or Vector PCX

**Heated Reactor Volume:** 1.4 mL

**Temperature:** 45 °C

**Reagent 1:** Dissolve 0.1 g of Fast Blue Salt in 240 mL of DI water. Add 40 mL of 1 N HCl and 720 mL of Acetonitrile. Protect the reagent from light and use within 3 days.

**Reagent 2:** Dissolve 8 g of NaOH in 1 L of DI water

**Detection:** UV/VIS at 475 nm

To avoid precipitation of the reagent as it ages, flush the postcolumn system regularly with water/methanol/0.1N HCl (49:49:2)

## Sample Extraction

### Dried Plant Material and Edible Products<sup>1</sup>

Weigh 0.5 g of a thoroughly homogenized sample into a 50-mL centrifuge tube. Add 20 mL of ethanol, then shake for 30 minutes using a horizontal shaker at 250 rpm. Centrifuge the tube for 5 minutes and filter the supernatant through filter paper into a 50-mL volumetric flask. Repeat extraction of the sample following the steps above. Combine both extracts in 50-mL volumetric flask and bring to volume with ethanol. Filter the extract through a 0.22 µm PTFE syringe filter into an injection vial.

### Resins, Tinctures and Oils<sup>1</sup>

Reduce the sample size to 0.05 g – 0.1 g. Extraction steps above are used except no re-extraction is needed. Dilute the extracts if needed.

### Calibration

The following cannabinoids were analyzed: delta-9 tetrahydrocannabinol (9-THC), delta-8 tetrahydrocannabinol (8-THC), cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), tetrahydrocannabivarin (THCV), delta-9 tetrahydrocannabinolic acid A (THCA-A), cannabidiolic acid (CBDA), cannabigerolic acid (CBGA).

The calibrators were prepared by diluting commercially available cannabinoids standards with methanol. The calibration range from 5 ppm to 75 ppm was used. Correlation coefficient R<sup>2</sup> exceeded 0.999 for all calibration curves.

**Analysis of Cannabinoids in Hemp and Hemp-containing Products**

Sample	CBD	9-THC	8-THC	CBG	CBN	THCV	CBDA	THCA	CBGA
Hemp Pre-Rolls	9.12 mg/g	1.53 mg/g	ND	6.63 mg/g	0.96 mg/g	ND	114.69 mg/g	3.62 mg/g	ND
CBD-Containing Tincture	14.82 mg/g	0.15 mg/g	ND	ND	0.42 mg/g	ND	ND	ND	ND
CBD-Containing Chocolate	14.89	ND	ND	ND	ND	ND	ND	ND	ND
CBD Oil	6.78 mg/g	0.32 mg/g	ND	ND	ND	ND	ND	ND	ND
CBD-Containing Chews	4.75 mg/g	ND	ND	ND	ND	ND	0.77 mg/g	ND	ND

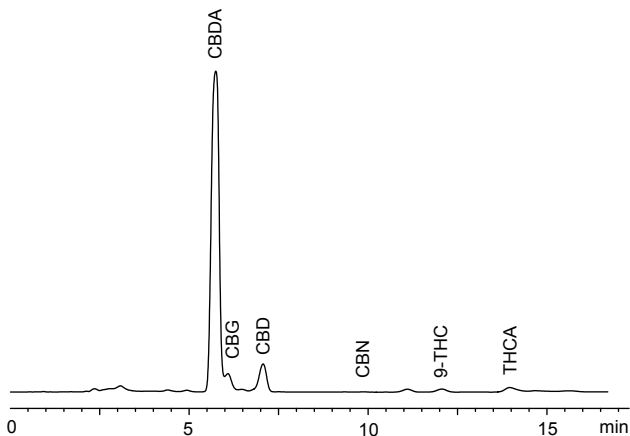


Fig 1. Chromatogram of hemp pre-roll

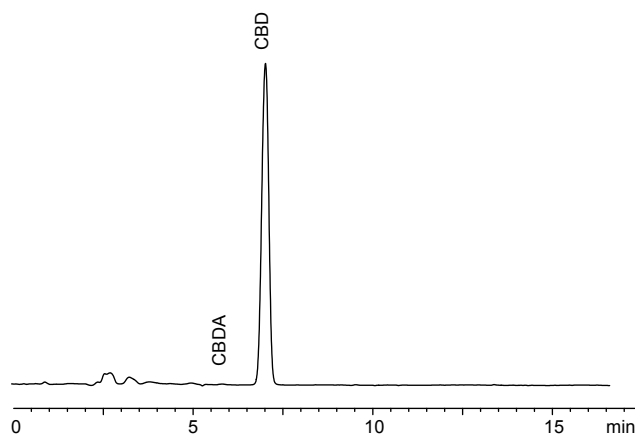


Fig 2. Chromatogram of CBD-containing chocolate

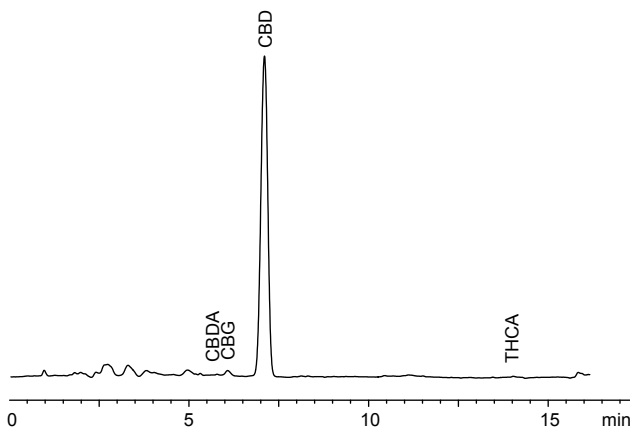


Fig 3. Chromatogram of CBD oil supplements

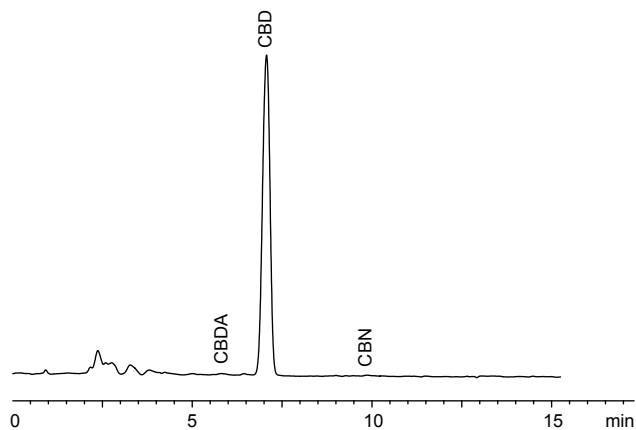


Fig 4. Chromatogram of CBD-containing chews

References

1. AOAC Official Method 2018.11

