Cannabinoids

HPLC Cold Column Cannabinoid Analysis

Cannabinoid method developed by **Dr. Korey Brownstein (October 2023)**.
Last edited by **Kevin Hernandez (October 2025).**

Targeted cannabinoids (6): CBDA, CBG, CBD, THCA, CBGA, Δ9-THC

Cannabinoid content is evaluated by Agilent HPLC using Korey's Cold Column Method. This low-temperature method preserves cannabinoid acid forms by maintaining the column at 10 °C. Each sample is analyzed in triplicate to ensure reproducibility and minimize extraction variability.

🧪 **HPLC cold column/ethanol cannabinoid evaluation** 🧪

Extraction time: Overnight (about 18 hrs.)

Sample run time: 20 min

Equipment
• Sonicator
• Analytical balance
• Agilent HPLC-UV 1220 Infinity II system controlled by Agilent Control Panel software
• Ascentis® Express C18 column, 2.7 µm (10 °C column chiller)
• 0.45 µm membrane filters (nylon or PTFE)
• 20 mL glass scintillation vials
• 1 mL autosampler vials
• Mortar and pestle or Geno grinder
• No. 10 and No. 35 sieves

Reagents
• 200-proof ethanol
• 18 MΩ HPLC-grade water
• 5 mM ammonium formate
• 0.1 % formic acid
• 95 % formic acid
• Acetonitrile (HPLC grade)

Standards
Cannabidiolic Acid (CBDA) (CAS #: 1244-58-2)
Cannabigerolic Acid (CBGA) (CAS #: 25555-57-1)
Cannabigerol (CBG) (CAS #: 25654-31-3)
Cannabidiol (CBD) (CAS #: 13956-29-1)
Δ9-Tetrahydrocannabinolic acid A (THCA) (CAS #: 23978-85-0)
Δ9-Tetrahydrocannabinol (Δ9-THC) (CAS #: 1972-08-3)

Standard Preparation
All standard solutions are prepared at 1000 µg/mL.
Acidic cannabinoids are prepared in acetonitrile; neutral cannabinoids are prepared in methanol.

Reagent Preparation

Mobile Phase A – Water (HPLC) with 5 mM Ammonium Formate + 0.1 % Formic Acid

- Add 1.0 L HPLC water to the amber bottle.
- Add 210 µL formic acid (95 %).
- Add 0.3153 g ammonium formate.
- Add 1.0 mL formic acid (95 %).
- Mix gently, allow to stand 10 min, label with prep date and initials.

Mobile Phase B – Acetonitrile (HPLC grade) + 0.1 % Formic Acid

- Add 1 L acetonitrile to 1 L bottle.
- Add 1 mL formic acid (95 %).
- Mix and label with prep date and initials.

Protocol

1. Grind plant material using a non-heat method (mortar and pestle or Geno grinder).
2. Sieve through No. 35 (nested below No. 10).
3. Weigh 0.1000–0.1050 g into 20 mL glass vials.
4. Add 20 mL 200-proof ethanol.
5. Sonicate for 30 min at room temperature.
6. Extract overnight (~18 h) in the dark.
7. Filter through 0.45 µm nylon/PTFE filter into autosampler vials.
8. Prepare triplicate vials (A, B, C) for each sample.
9. Combine residual extracts to make three QC vials (1 mL each).
10. Maintain column temperature at 10 °C.
11. Set flow rate to 0.350 mL/min.
12. HPLC gradient program: 0-2 min, 90% B; 2-5 min, 90% B; 5.0-5.1 min, 100% B; 5.1-11.10 min, 100% B; 11.10-11.20 min, 75% B; and 11.20-20 min, 75% B.
13. UV- DAD wavelength is set 228 nm for detection.

Methods developed by Dr. Korey Brownstein (October 2023) and modified by Kevin Hernandez (October 2025)

HPLC Quechers Cannabinoid Analysis (Water's Edible Method)

Cannabinoid methods developed or adapted by Kevin Hernandez at USDA-ARS PGRU.

Targeted cannabinoids (6): CBDA, CBG, CBD, THCA, CBGA, Δ9-THC

Cannabinoid content is evaluated by Agilent HPLC-UV using a modified version of Water's Quecher Method.This low-temperature method preserves cannabinoid acid forms by maintaining the column at 10 °C. Each sample is analyzed in triplicate to ensure reproducibility and minimize extraction variability.

🧪 **HPLC Quechers cannabinoid evaluation** 🧪

Extraction time: 30 mins

Sample run time: 20 min

Materials and Equipment:

- Homogenized or freeze-ground plant sample
- Analytical balance
- 15 mL centrifuge tubes
- Vortex mixer
- Ultrasonic bath (sonicator)
- Centrifuge capable of 3000 RCF
- Pipettes and pipette tips
- Water (HPLC grade)
- Acetonitrile (HPLC grade)
- CEN QuEChERS extraction salts (p/n 186006813)

Reagents and Solutions:

- HPLC-grade water
- HPLC-grade acetonitrile
- CEN QuEChERS salts (p/n 186006813)

Procedure:

1. Weigh 0.05 g of freeze-ground or homogenized sample into a 15 mL centrifuge tube.
2. Add 1 mL of water to the tube.
3. Vortex the sample for 1 minute to ensure thorough mixing.

4. Sonicate the tube for 20 minutes to facilitate extraction.
5. Add 1 mL of acetonitrile to the tube.
6. Vortex again for 1 minute.
7. Add 0.5 g of the CEN QuEChERS salts (p/n 186006813) to the 15 ml test tube.
8. Shake the mixture vigorously for 1 minute to allow partitioning.
9. Add 9 mL of Acetonitrile to dilute
10. Centrifuge the sample at 3000 RCF for 5 minutes.
11. Carefully collect the top acetonitrile layer.
12. Maintain column temperature at 10 °C.
13. Set flow rate to 0.350 mL/min.
14. HPLC gradient program: 0-2 min, 90% B; 2-5 min, 90% B; 5.0-5.1 min, 100% B; 5.1-11.10 min, 100% B; 11.10-11.20 min, 75% B; and 11.20-20 min, 75% B.
15. UV- DAD wavelength is set 228 nm for detection.
16. The extract can be injected directly for analysis or diluted with acetonitrile depending on the expected concentration of cannabinoids.

Method provided by Tran,Twohig, and Hudalla (2021) and modified by Kevin Hernandez (October 2025).

- Van Tran, Kim, et al. “Analysis of Cannabinoids in Cannabis Plant Materials and Edible Products Using Ultraperformance Liquid Chromatography (UPLC) with PDA and Mass Detection.” *Waters*, Waters Corporation, 1 Mar. 2021, www.waters.com/nextgen/us/en/library/application-notes/2021/analysis-of-cannabinoids-in-cannabis-plant-materials-and-edible-products-using-ultraperformance-liquid-chromatography-uplc-with-pda-and-mass-detection.html.