SHIMADZU
Excellence in Science

Method for Cannabinoid Analysis
- Oils, Vapes or Gummy Sweets

Cannabinoids in particular CBD are found in a range of products. The most common products are oils with soft drinks, vapes and gummy based sweets following as well.

The following application method looks at the analytical method for 11 major cannabinoids and the extraction processes for some of the major types of samples.

Reagents and Safety

Methanol	-	Highly flammable; toxic by ingestion and inhalation
Acetonitrile	-	Highly flammable; toxic by ingestion, inhalation and skin contact; may be mutagen / teratogen

Avoid skin and eye contact with reagents by wearing a lab coat, gloves and safety glasses. Do not expose phenols or methanol to a source of ignition. Avoid inhalation of acetonitrile vapour.

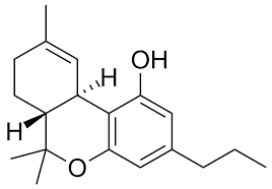
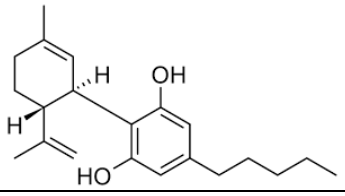
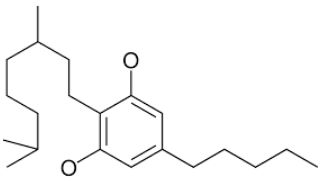
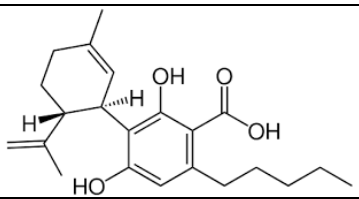
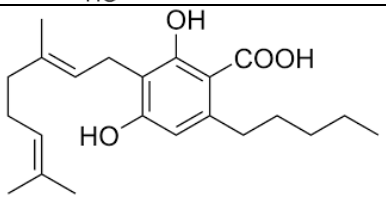
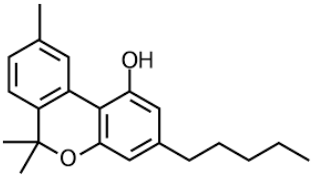
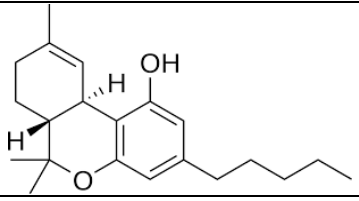
Provided the recommended precautions are adopted, the risk to operators during this procedure is minimal.

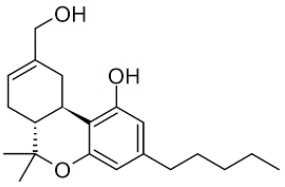
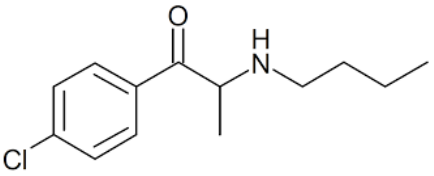
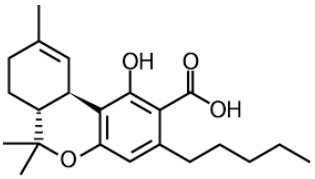
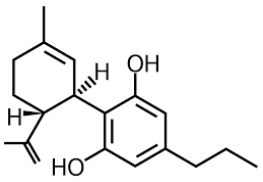
Legal Section

Shimadzu does not support or promote the use of its products or services in connection with illegal use, cultivation or trade of cannabis products. Shimadzu products are intended to be used for research use only purposes or state approved medical research. Shimadzu is not condoning the use of recreational nor medical marijuana, we are merely providing a market summary of the cannabis testing industry.

Introduction and Structures

A method for quantification of various cannabinoids within commercially available samples, by high pressure liquid chromatography (HPLC) using a reversed phase column and an external standard as described. This method can separate out all 11 major cannabinoids, however not all are required.

Compound Abbreviation	Full Name	Structure
THCV	Tetrahydrocannabivarin	
CBD	Cannabidiol	
CBG	Cannabigerol	
CBDA	Cannabidiolic Acid	
CBGA	Cannabigerolic Acid	
CBN	Cannabinol	
d9-THC	D9-THC	

Compound Abbreviation	Full Name	Structure
d8-THC	D8-THC	
CBC	(±)-Cannabichromene	
THCA	THCA-A	
CBDV	Cannabidivarin	

As all samples are shipped in solvent at a maximum concentration of 1 mg/mL no license is required to purchase these cannabinoids

Apparatus

- Balance capable of weighing 0.0001 g
- Volumetric flasks/glass vessels
- Plastic Falcon tubes for gummy analysis
- Glass or electronic pipettes
- Weighing boats

Mobile Phase Preparation

MOBILE PHASE A – Water + 0.085% Phosphoric Acid

- 1 L of HPLC Grade water + 1 mL Phosphoric acid

MOBILE PHASE B Acetonitrile + 0.085% Phosphoric Acid

- 1L of HPLC or Gradient Grade Acetonitrile + 1 mL Phosphoric acid

Standard Preparation

Standard Solution (1000 mg/L)

- Solutions are shipped from the supplier in either methanol or acetonitrile at a maximum concentration of 1000 mg/L

Calibration Standards

- Prepare the highest concentration calibration standard by combining 100 µL of each of the 11 cannabinoid standards (90.9 mg/L).
- If less than 11 cannabinoids are being used add 100 µL of Methanol as an alternative
- Prepare the rest of the calibration standards from the highest concentration 90.9 mg/L standard in the following concentrations 0.5, 1.0, 5.0, 10.0 and 50.0 mg/L in methanol
- Example of preparation of 5.0 mg/L solution:
  100 µL of the 50.0 mg/L standard plus 900 µL of methanol

Sample Types

CBD samples are sometimes referred to as Isolate, Broad Spectrum of Full Spectrum.

Isolate samples should only contain CBD.

Broad Spectrum typically have no THC, CBC or THCA.

Full spectrum should contain most of the 11 cannabinoids

No sample should have more than 0.2% D9-THC

Sample Preparation – CBD Containing Oils

1. Pipette 4 mL of propan-2-ol (IPA) into a suitable clean glass container
2. Pipette 100 µL oil sample
   a. note that positive displacement pipettes are recommended due to the oil samples adhesion to air displacement pipettes nibs
3. Agitate for approximately 30 seconds
4. Pipette 4 mL of methanol into the solution
5. Agitate for approximately 30 seconds
6. Syringe filter through a 0.2 µm PTFE filter

This forms Dilution A (Dilution Factor 81)

1. Take a 20 µL volume of Dilution A
2. Add 980 µL of Methanol

This forms Dilution B (Dilution Factor 4050)

Dilution A is to quantify all cannabinoids excluding CBD
Dilution B is to quantify CBD

Sample Preparation – CBD Containing Vape Samples

1. Pipette 7 mL of propan-2-ol (IPA) into a suitable clean glass container
2. Pipette 50 µL oil sample
    a. note that positive displacement pipettes are recommended due to the oil samples adhesion to air displacement pipettes nibs
3. Agitate for approximately 30 seconds
4. Pipette 7 mL of methanol into the solution
5. Agitate for approximately 30 seconds
6. Syringe filter through a 0.2 µm PTFE filter

This forms Dilution A (Dilution Factor 81)

3. Take a 100 µL volume of Dilution A
4. Add 900 µL of Methanol

This forms Dilution B (Dilution Factor 4050)

Dilution A is to quantify all cannabinoids excluding CBD
Dilution B is to quantify CBD

Sample Preparation – CBD Containing Soft Drinks

Carbonated Drinks

1. Take the whole sample and add to a vessel with twice the volume
2. Sonicate to remove the carbonation
3. Add the sample volume of sample in Methanol
4. Filter through a 0.2 μm PTFE filter

Non-Carbonated Drinks

1. Add the sample volume of sample in Methanol
2. Filter through a 0.2 μm PTFE filter

In both cases if the drink is 250 mL then 250 mL Methanol will be required

Sample Preparation – CBD Containing Gummy Sweets

1. Cut up several gummy sweets into small pieces
2. Weigh 2.0 g ± 0.2 g
3. Add 20 mL of HPLC Grade water
4. Warm in a water bath to 50 °C
5. Agitate occasionally until all of the sweets are dissolved
6. Add 20 mL of Acetonitrile
7. Agitate the sample for 5 minutes (manually or mechanically)
8. Add 8.0 g ± 0.25 g Magnesium Sulfate (Anhydrous – dried e.g. Fisher M/1100/60)
9. Agitate the sample for 5 minutes (manually or mechanically)
10. Allow sample to naturally separate into two separate layers

    SAMPLE A

11. Filter an aliquot of the top layer of each sample through a 0.2 mm PTFE syringe filter

    SAMPLE B

12. Take 0.25 mL of Sample A and 0.75 mL of Methanol

Dilution A is to quantify all cannabinoids excluding CBD

Dilution B is to quantify CBD

Analytical Conditions

- Column : Shimadzu NexLeaf CBX Potency 2.7 mm, 150x 4.6 mm i.d.

  : With Shimadzu NexLeaf CBX column guards 2.7 mm, 5 x 4.6 mm
- Temperature : 35°C
- Injected volume : 5 µL
- Mobile phases : A : Water + 0.085% Phosphoric Acid

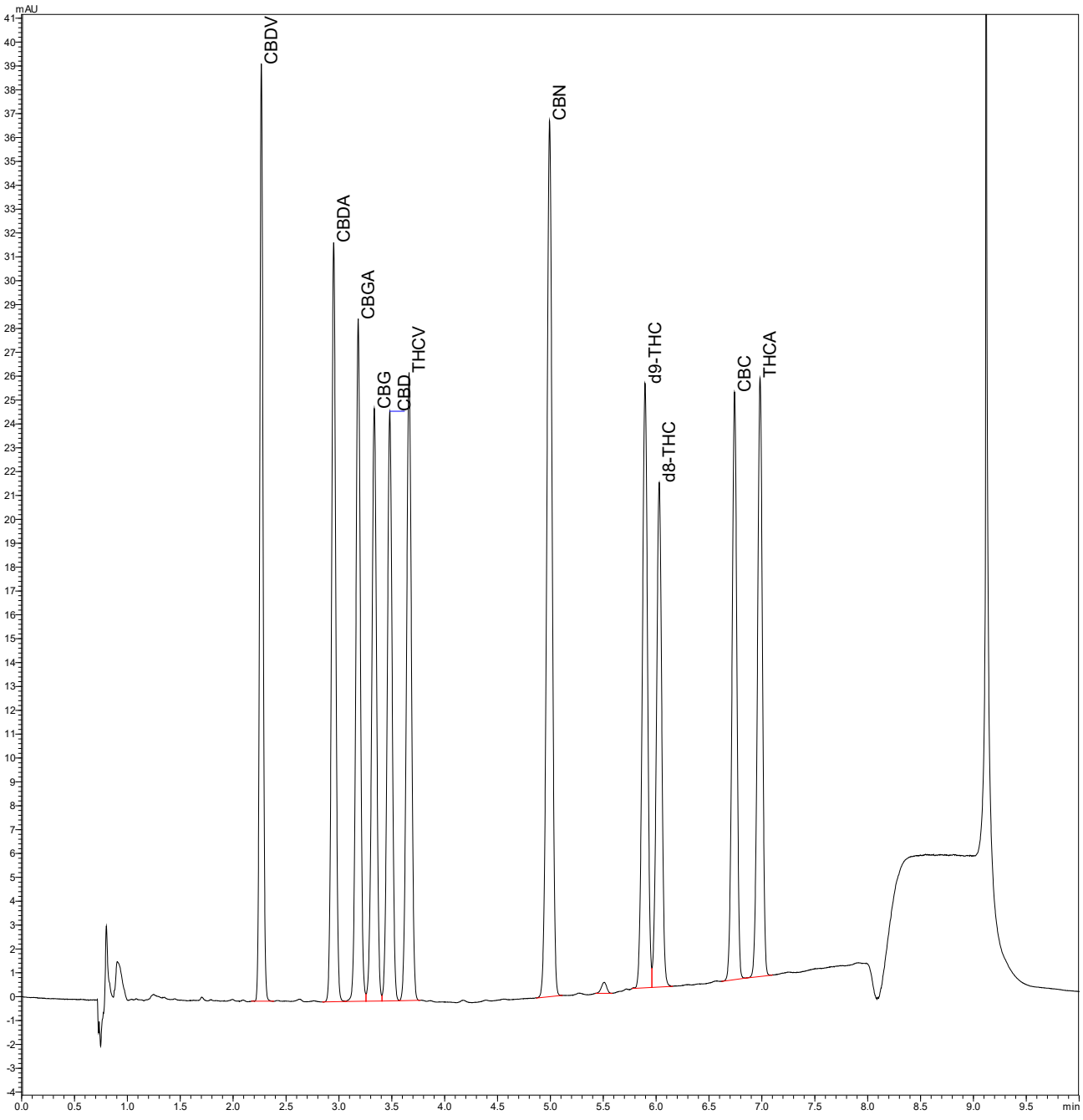
  : B : Acetonitrile + 0.085%

  : C: Acetonitrile: Water 50:50
- Gradient :

Time (mins)	%A	%B
0.00	30	70
3.00	30	70
7.00	15	85
7.01	5	95
8.00	5	95
8.01	30	70
10.00	30	70

- Flow rate : 1.6 mL/min
- Wavelength : 220 nm (cell at 40 °C)
- Runtime : 10 minutes
- Column Wash : 50:50 Acetonitrile: Water
- Injection Rinse : Mobile Phase B

Typical Chromatography



Information to be recorded

- Weight and Volume of Standard used
- How calibration curve solutions were prepared

- Preparation of each sample solution with all weights/volume

- Set up of Instrumentation
- Vial positions
- How long column was equilibrated

- R and R^2 values of calibration curve
- Results for samples, included each cannabinoid present and its quantity.
- Are your results the same as the company have stated are present.