Cannabinoids Analysis of Hemp Derived Products: Developing Methods That are Robust and Dependable

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Introduction

- Cannabidiol (CBD) and Tetrahydrocannabinol (THC) containing cannabis products are available in various forms such as flower, vape pens, edibles, concentrates, tinctures, beverages, topicals, etc.
- Sample preparation for cannabinoid testing is very challenging due to the complexity of cannabis product matrices,.
- Here, we present simple and accurate sample preparation methods for analysis of cannabinoids from several matrices.

Materials

- MilliporeSigma Cannabinoid Certified Reference Materials (CRMs) were used to prepare 17-component calibration solutions.
- Ascentis® Express columns from MilliporeSigma (fused-core particle platform, superficially porous particle) were used.
- Commercially available hemp bud, oil, chocolate, hard candy, gummy, cream, and beverage matrices were purchased from local vendors.

Calibration Solution

A 17-mix stock solution for calibration curve from CRMs from MilliporeSigma	
CRM	Volume (µL)
C-219 (Neutrals)	250
C-218 (Acids)	250
C-171 (CBLA)	250
C-154 (CBL)	125
C-153 (CBNA)	125
Final Concentration of each cannabinoid = 125 µg/mL	
Eight-point calibration curve obtained for all seventeen cannabinoids	
Range = 0.20-125 µg/mL	
Linearity: R^2 >0.999	

Method

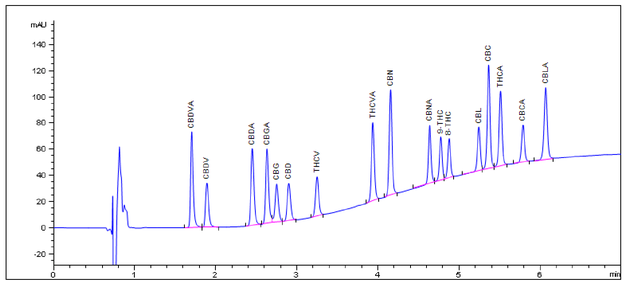


Figure 1: Chromatogram showing separation of 17 cannabinoids (5 µg/mL). Resolution of each peak was >1.0.

HPLC-DAD Parameters	
HPLC System	Agilent 1290
Mobile Phase A	5 mM Ammonium Formate + 0.1% Formic Acid in Water
Mobile Phase B	0.1% Formic Acid in Acetonitrile
Column	Ascentis Express C18 150x2.1 mm (2.0 µm)
Flow Rate	0.4 mL/min
Column Temperature	25°C
Injection	3 µL
Max pressure	560 bar
Wavelength	228 nm

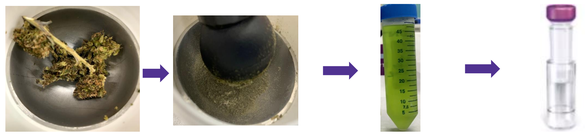
HPLC Gradient					
Time	0.0	2	7	7.1	10
% A	25	10	10	25	25
% B	75	90	90	75	75

Sample Preparation

Sample preparation for each sample type are explained below:

Hemp Bud Sample Preparation:

- Weigh sample accurately (~1 g)
- Homogenize (particle size < 1 mm)
- Extract twice with 20 mL ethanol,
- combine and dilute to 50 mL
- Filter (0.2 µm PTFE membrane)
- Dilute 1/10 and 1/100



Hemp bud sample | Homogenization in Cryo-cup | Hemp bud extract | Filter vial

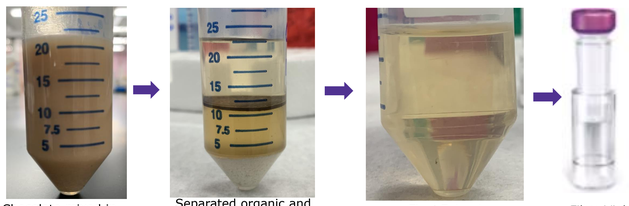
- Low temperature homogenization prevents degradation of analytes and produces uniform particle sizes. Frozen ball-milling is the preferred method for homogenization without sample degradation.
- Double extraction with ethanol maximizes cannabinoid recovery.

Hemp Oil and THC Distillate Sample Preparation:

- Weigh accurately (~30-50 mg)
- Agitate in 10 mL isopropanol for 1 minute
- Dilute to 50 mL with Methanol
- Dilute 1/10 and 1/100

- Hemp oil and THC distillate samples were prepared in a similar fashion.
- Different dilution levels were used to quantitate CBD vs. other analytes as CBD concentration was significantly higher compared to the others.

Chocolate Sample Preparation:



Chocolate mixed in water | Separated organic and aqueous layer after centrifugation | Final Extract | Filter Vial

- Weigh accurately (~1 g)
- Dissolve in 15 mL warm water ~75 ºC
- Add 15 mL ACN
- Shake 1 min
- Add QuEChERS salt
- Centrifuge
- Draw off top Layer
- Record the volume
- Filter
- Dilute 1/10 and 1/100

Gummy And Candy Sample Preparation:

- Gummy and candy samples were prepared similar to chocolate.
- One gummy or candy was weighed, and exact weight recorded.
- Warm water volume was adjusted to ensure dissolution. (Note: sample can be cut into small pieces to help dissolution)
- Remaining process was similar to chocolate sample preparation.

Cream Sample Preparation:

- Slightly warm 1 g cream was dissolved in 5 mL MeOH by vortex for 1 min.
- Transferred entire solution to 10 mL volumetric flask, diluted to the mark with methanol and mix well.
- Filter ~ 1 mL solution with 0.2 µm PTFE filter and dilute 1:10 and 1:100.

Beverage Sample Preparation:

- Beverage was tested two ways:
  1) Dilute in half with methanol,
  2) QuEChERS extraction from 15 mL beverage to 15 mL ACN.

Results and Discussion

Hemp bud, oil and THC distillate analysis:

Table 1: Cannabinoid concentrations in hemp bud, hemp oil, and THC distillate samples.

	Hemp Bud		Hemp Oil		THC Distillate	
	Wt% (n=3)	%RSD	Wt% (n=3)	%RSD	Wt% (n=3)	%RSD
CBD	0.341	2.29	12.674	1.32	82.967	1.42
CBC	0.199	0.41	0.649	2.86	1.634	2.09
9-THC	0.097	1.27	0.269	1.65	3.911	1.32
CBDV	<LOQ	NA	0.269	1.65	0.165	4.42
CBG	1.166	2.91	0.128	1.78	1.193	0.35
CBN	0.000	0.00	0.085	5.32	0.299	4.43
CBCA	0.546	0.80	0.030	3.92	0.000	NA
CBGA	10.504	1.12	0.000	NA	0.000	NA
CBDA	4.518	0.69	0.000	NA	0.000	NA
THCA	0.192	0.88	0.000	NA	0.000	NA
CBDVA	0.025	7.58	0.000	NA	0.000	NA
CBLA	0.013	0.57	0.000	NA	0.000	NA
CBL	0.009	3.67	0.000	NA	0.000	NA
CBNA	0.005	2.29	0.000	NA	0.000	NA
Total CBD	3.971	NA	12.674	NA	82.967	NA
Total THC	0.265	NA	0.269	NA	3.911	NA

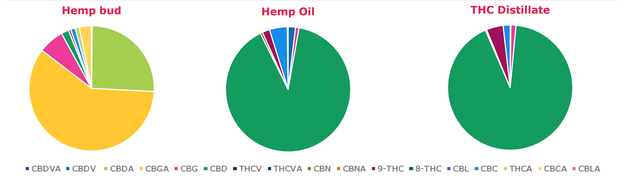


Figure 2: Cannabinoid distribution in hemp bud, hemp oil and THC distillate samples.

Chocolate analysis:

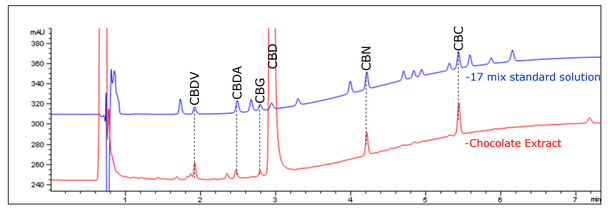


Figure 3: Overlay of chocolate extract and 17 mix cannabinoid standard.

- Acidic cannabinoids are not generally present in commercial edibles.
- The chocolate extract in Figure 3 shows a peak at the retention time of CBDA.
- However, the UV spectrum of this peak is completely different from CBDA in the standard solution (Figure 4).
- Retention time should be used with in conjunction with UV profile.

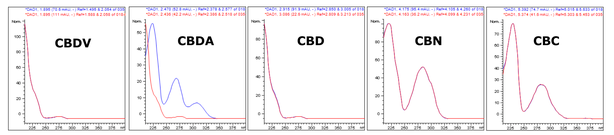


Figure 4: UV absorbance spectra of suspect peaks (red) in chocolate extract overlapped with that of standard analytes (blue).

Cream analysis:

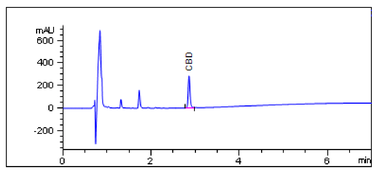


Figure 5: CBD cream extract: CBD with minor unknown early impurities.

Beverage analysis:

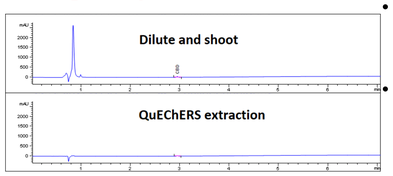


Figure 6: Beverage extracts: diluted directly to methanol (top) and extracted by QuEChERS method.

- Both analyses provided similar results. Large early peaks are most probably sugars.
- With QuEChERS, initial peaks likely separated to aqueous layer.

Chromatography

Separation and detection was reproducible and robust throughout.

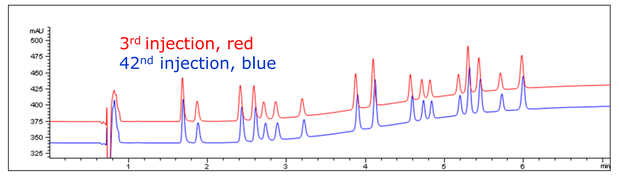


Figure 7: Retention times and resolution were stable throughout the run (3rd, red - 42nd, blue injections) for the 17-cannabinoid standard.

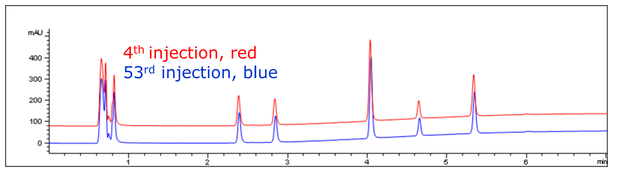


Figure 8: Chocolate extract samples showed stable retention times and separation throughout the run (4th, red - 53rd, blue injections).

Alternative Methods

Low-Cost Methanol Method:

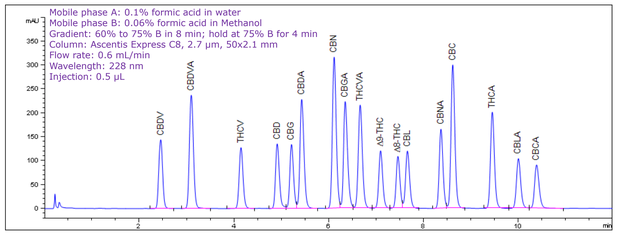


Figure 9: 17 cannabinoids separated by a low-cost methanol method. Methanol method saves ~¢40 per injection compared to ACN method.

Monolithic Silica Column Method:

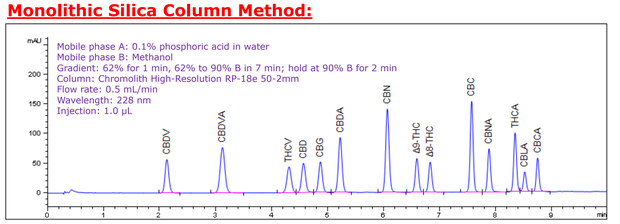


Figure 10: 14 cannabinoids separated on a monolithic silica Chromolith® column. Monolithic columns provide low back pressure, longer column life, and robustness towards complex matrices.

Summary

- In this study, we demonstrated simple sample preparation methods for various hemp product matrices.
- Cannabinoid CRM mixes can be conveniently and accurately used to prepare calibration curves.
- Fused-core Ascentis® Express column provides fast, stable and accurate resolution of cannabinoids. Chromolith® columns afford longevity and low back pressure. Resolution can be achieved with acetonitrile or low-cost methanol-based methods.
- It is demonstrated that UV absorbance spectra can be used to evaluate impurities that may overlap with cannabinoids.
- Orthogonal techniques such as LC-MS or LC-MS/MS can support identification of unknown impurities and will be the focus of future studies.

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