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A Goal-Oriented Approach to the Quantitative Determination of Cannabinoids by HPLC

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Introduction

Cannabis analysis has gained new importance in the USA in light of the legalization of marijuana in several states. Cannabis contains a number of chemical alkaloids known as cannabinoids. Of primary interest for potency are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN). In marijuana plants, THC and CBD exist predominately as tetrahydrocannabinolic acid (THCA) and cannabinolic acid (CBDA). These gradually decarboxylate to THC and CBD through exposure to heat and light. There are, in fact, hundreds of cannabinoids present in extracts from the plant.

In developing HPLC methods for the quantitative analysis of cannabinoid targets, it is useful to consider various possible analysis goals. Potency determinations focus primarily on THC and CBD, but there are myriad cannabinoids closely related to those targets. Proper attention to the analysis goals should dictate the parameters guiding method development. Here, we present three variants on analysis goals and how they guide the methodology and chromatographic outcome.

Recognition of Three Analysis Goals

11 Cannabinoids of interest
THCA, d9-THC, d8-THC, CBD, CBDA, CBDV, CBC, CBN, CBG, CBGA, THCV (THCV is a difficult-to-resolve target)

Goal #1: High Throughput – The primary goal of many analysts is to run as many samples as possible per day while still achieving accurate and repeatable analytical outcomes for the primary targets. THCV is excluded.

Goal #2: Intermediate – This goal represents a compromise between sample throughput and resolving power. Quantitative analysis of THCV is mandatory.

Goal #3: High Resolution – Here, baseline resolution of the 11 cannabinoids is paramount and, with it, the ability to expand the target list as regulations change over time. Quantitative analysis of THCV is mandatory.

Method Design in Response to Goals

- Mobile phases were chosen to provide good peak shape in all cases. Solvents were also acid-modified to ensure all targets are in the neutral form.

- UV detection parameters were set in response to preliminary observations about sample targets. A detection wavelength of 276 nm yields a very flat baseline and low noise. However, while suitable for the cannabinoid acid forms, the response is weak for the non-acid forms – 220 nm was chosen as it allows for an LOQ of 1 ppm for all cannabinoid targets.

- The cannabis alkaloids are exceedingly hydrophobic. Thus, high carbon-load C18 column chemistries seemed appropriate to fully separate the targets.

- For the higher throughput methods, an SPP (Superficially Porous Particle) column with 2.7 µm particle size was chosen for increased efficiency, yet with greatly reduced back pressure. This provided reduced run times, narrow peaks and method ruggedness.

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- For the High Throughput method goal, an 8 minute cycle time was used as a guideline. Here, adequate resolution of 10 cannabinoids (minus THCV) must be achieved to support accurate quantitation.

- For the High Resolution method goal, a minimum R value of 1.5 was paramount to allow for the accurate detection and quantitation of THCV. Such resolving power demands smaller particles and longer gradient run times to achieve a higher theoretical plate count. Added benefits are the ability to resolve other minor cannabinoids in the sample and the ability to add to growing list of hydrophobic targets.

Chromatographic Outcome

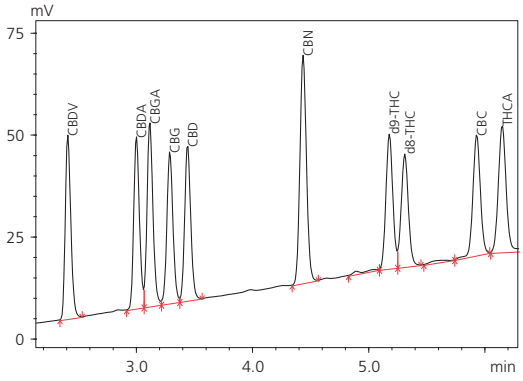


Fig. 1 a) High Throughput

Column Particle Characteristics	: SPP, 2.7 µm
Column Format	: 150X4.6mm
Column Chemistry	: C18
Mobile Phase A	: 0.1 % Formic acid in Water
Mobile Phase B	: 0.1% Formic acid in Acetonitrile
Pump Elution Mode	: Gradient
Cycle Time	: 8 min.
Detector Wavelength	: 220 nm
Estimated Sample Throughput	: 60 samples/8 hr. day

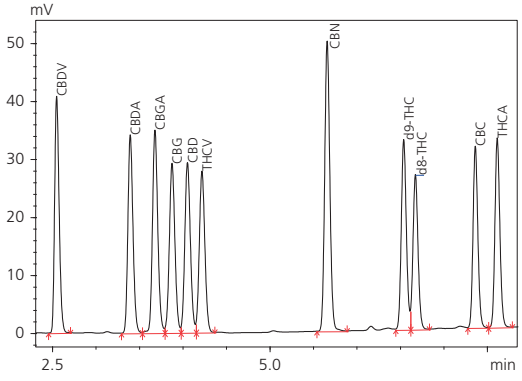


Fig. 1 b) Intermediate

Mobile Phase A, B	: Change in acid modifier
Pump Elution Mode	: Gradient modification
Cycle Time	: 10 min.
Estimated Sample Throughput	: 48 samples/8 hr. day

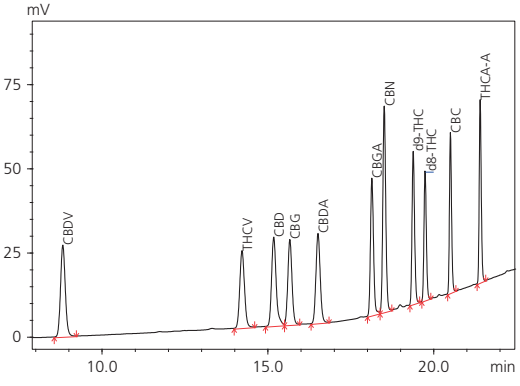


Fig. 1 c) High Resolution

Column Particle Characteristics	: sub-3 µm
Pump Elution Mode	: Gradient modification
Cycle Time	: 30 min.
Minimum Resolution Achieved	: 2.1

Standard Curves

For each method, standard curves (Fig 2) were prepared for each target analyte with a minimum acceptable correlation coefficient (R^2) of 0.999 over 5 standard levels. A linear dynamic range was established at 0.5 to 100 mg/L (corresponding to 0.05 – 10% before sample prep) in each analyte except THCA and CBDA. In many cases the natural abundance of THCA and CBDA is very high, therefore the linear dynamic range for those analytes was established from 0.5 to 250 mg/L (0.05 – 25%).

Accuracy% was calculated for a 1.0 mg/L standard level. The calculated deviations for all compounds were within ±7 % for all three methods.

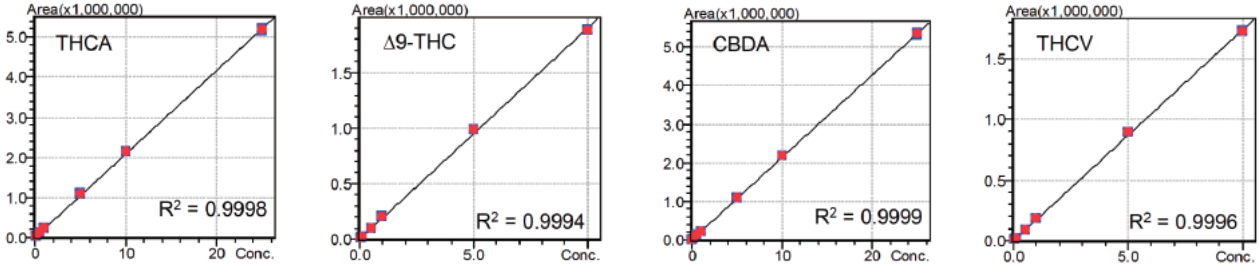
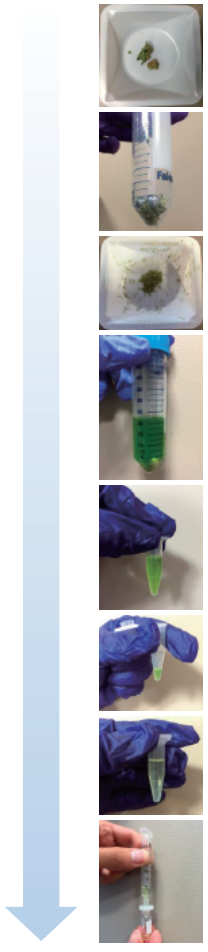


Fig. 2 Representative standard curves for selected targets according to the High Resolution method.

Sample Preparation

This procedure was developed at a customer site

- Weigh 200 mg of flower or leaf cuttings into 50 mL centrifuge tube.
- Add two 9.5 mm steel balls into the tube.
- Shake at 1000 rpm for 1 min. with the 2010 Geno/Grinder.
- Add 20 mL of methanol to the tube.
- Shake at 1000 rpm for 1 min.
- Wait for 15 min.
- Mix using a vortex mixer for 1 min.
- Transfer 1 mL of the mixture into a 1.5 mL microtube and Centrifuge at 3000 rpm for 5 min.
- Transfer 100 µL of supernatant to a new 1.5 mL microtube.
- Add 900 µL of methanol.
- Filter the mixture through a 0.45 µm syringe filter and transfer to a 1.5 mL sample vial.



Analysis of Cannabis Flower

Figure 3 shows the chromatogram of an extract from a THC-rich flower using the Intermediate method.

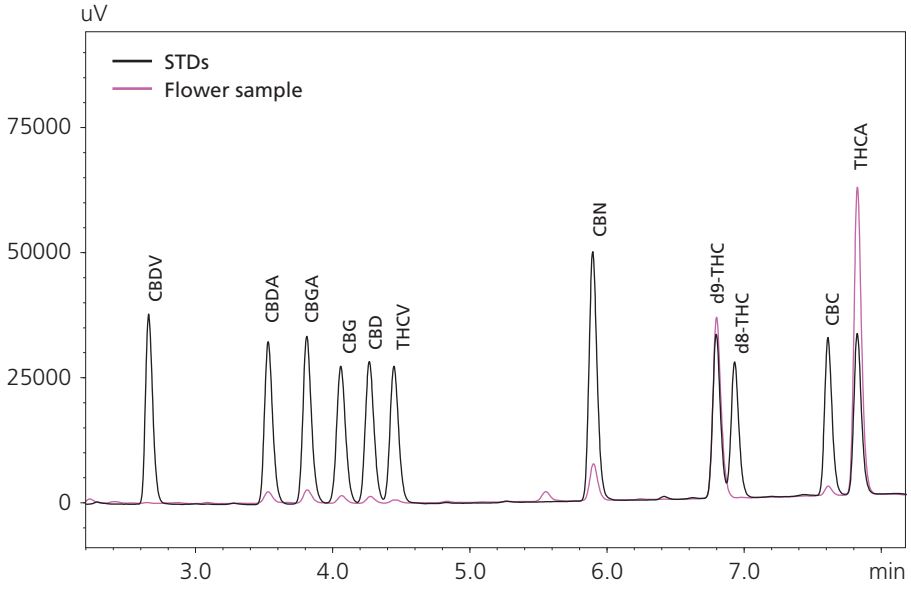


Fig. 3 Overlay of THC-rich flower extract and 10 mg/L standard mixture.

Potency Calculation

Table 4 shows the quantitative result of each compound calculated with the Intermediate method.

$$\%THCA = [THCA] \times (DIL) \times (VOL/MG) \times 100$$
$$Potency: (\%THCA \times 0.877) + \%\Delta 9\text{-}THC$$

[THCA]: Concentration of THCA, DIL = Dilution Factor, VOL = External Volume
MG = dry sample weight (mg), 0.877 = molecular weight ratio of cannabinoids to cannabinoid acids

#	Compounds	Conc (mg/L)	wt %
1	THCV	0.280	0.05
2	CBD	0.491	0.09
3	CBG	0.530	0.11
4	CBDA	0.715	0.14
5	CBGA	0.816	0.16
6	CBN	1.501	0.30
7	D9-THC	11.894	2.38
8	D8-THC	ND	ND
9	CBC	0.650	0.13
10	THCA	18.915	3.78
11	CBDV	ND	ND
Potency			5.7%

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Conclusion

This study acknowledges varied analysis goals for cannabis and illustrates some of the method considerations that can help to accomplish those goals. In each case, the analyst must compromise on questions of sample throughput and resolution of target peaks while ensuring accurate quantitation.

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