RESTEK Pure Chromatography

Cannabis Potency Testing: Which Column Dimension is Right for You?

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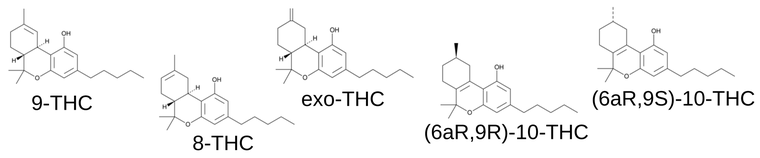
Abstract & Introduction

Potency testing cannabis products is of vital importance to the cannabis industry. This can seem straightforward on the surface, but different labs have different needs and there can be many different requirements and obstacles to overcome with regulated testing from state to state. While some labs may be interested in only a few required cannabinoids in order to meet required testing standards, others may be interested in offering as many cannabinoids as possible to outperform their competitors. In addition to the analyte list, there are a number of other factors to consider such as solvent consumption, organic solvent type (methanol vs. acetonitrile), and runtime. In this work, different column dimensions of the Raptor ARC-18 phase were utilized to develop methods to meet the needs of various labs and each method applied to hemp oil and CBD flower to show chromatographic separation in matrix.

THC Isomers

Many isomers are already present within the known, trending cannabinoids, but as more are discovered it is likely that more will be included. THC isomers share a molecular formula $C_{21}H_{30}O_2$ but vary by the location of the non-aromatic double bond. Some states, such as Colorado, are now requiring that total THC calculation include delta-8-THC, delta-9-THC, exo-THC (delta-11-THC), and delta-10-THC so it is of vital importance to be able to resolve all relevant isomers required by your state.



7 Cannabinoid Analysis on 50 x 3 mm Dimension

A 50 x 3 mm column dimension can be advantageous for cannabinoid analysis when the panel of target analytes is limited. This method is ideal for labs interested in a minimal number of cannabinoids that are required to be monitored to meet compliance regulations, such as hemp testing labs. The following method was developed using simple mobile phase additives, gradient conditions, and an overall cycle time of 8 minutes. The use of this method allows for the high throughput of samples and uses methanol as the organic modifier, which is typically more cost-effective than acetonitrile.

Column:	Raptor ARC-18 50 x 3 mm, 2.7 µm	
MPA:	Water, 0.1% CH_2O_2, 5 mM NH_4HCO_2	
MPB:	Methanol, 0.1% CH_2O_2	
Column Temp:	30 °C	
Sample:	50 ppm	
Injection Volume:	3 µL	
Flow Rate:	0.8 mL/min	
	Time (min)	**%B**
	0.00	75
	5.00	75
	5.50	95
	6.50	95
	6.51	75
	8.00	75

Table 1: Method conditions for the analysis of 7 cannabinoids.

1.	CBD
2.	CBDA
3.	9-THC
4.	8-THC
5.	(6aR, 9S)-10-THC
6.	(6aR, 9R)-10-THC
7.	THCA

Table 2: 7 Analytes monitored in Figure 1.

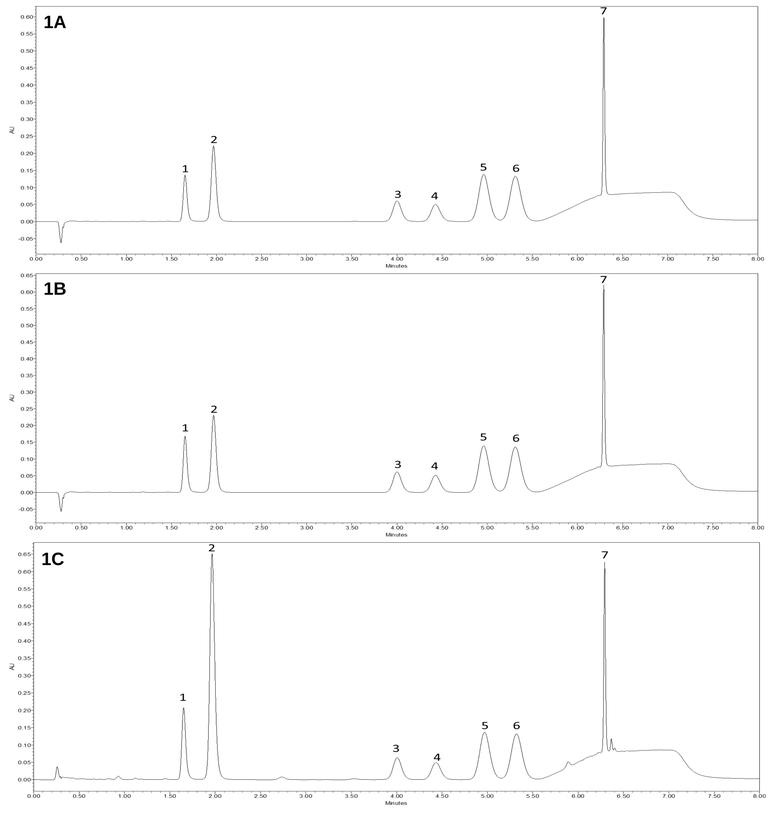


Figure 1A: Chromatogram obtained in solvent for 7 cannabinoids by the method conditions outlined in Table 1. **1B**: Chromatogram obtained from conditions outlined in Table 1 for hemp oil with analytes spiked at 50 ppm. **1C:** Chromatogram obtained from conditions outlined in Table 1 for CBD hemp flower with analytes spiked at 50 ppm (CBDA levels are endogenous).

13 Cannabinoid Analysis on 50 x 3 mm Dimension

For labs interested in using a 50 x 3 mm column dimensions, but want to monitor more cannabinoids, an additional method on this column dimension was developed. This uses the same mobile phases as the previous method, but requires a higher flow rate of 1 mL/min and two additional minutes of run time for a total cycle time of 10 minutes. This methodology is useful for labs that want to offer a rapid, extended panel using a 50 x 3 mm column dimension.

Column:	Raptor ARC-18 50 x 3 mm, 2.7 µm	
MPA:	Water, 0.1% CH_2O_2, 5 mM NH_4HCO_2	
MPB:	Methanol, 0.1% CH_2O_2	
Column Temp:	50 °C	
Sample:	50 ppm	
Injection Volume:	3 µL	
Flow Rate:	1.0 mL/min	
	Time (min)	**%B**
	0.00	65
	5.00	70
	6.50	70
	7.50	80
	8.50	80
	8.51	65
	10.00	stop

Table 3: Method conditions for the analysis of 13 cannabinoids.

1.	CBDV	**8.**	9-THC
2.	CBD	**9.**	8-THC
3.	CBG	**10**	(6aR, 9s)-10-THC
4.	THCV	**11.**	(6aR, 9R)-10-THC
5.	CBDA	**12.**	CBC
6.	CBGA	**13.**	THCA
7.	CBN		

Table 4: 13 Analytes monitored in Figure 2.

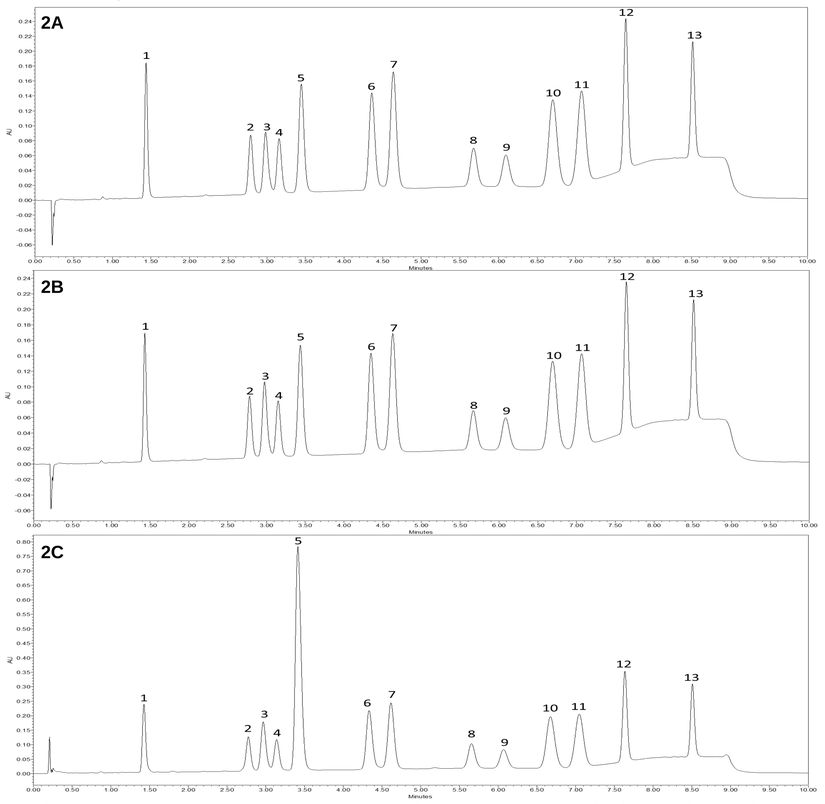


Figure 2A: Chromatogram obtained in solvent for 13 cannabinoids by the method conditions outlined in Table 3. **2B**: Chromatogram obtained from conditions outlined in Table 3 for hemp oil with analytes spiked at 50 ppm. **2C:** Chromatogram obtained from conditions outlined in Table 3 for CBD hemp flower with analytes spiked at 50 ppm (except CBDA, which is endogenous).

15 Cannabinoid Analysis on 150 x 3 mm Dimension

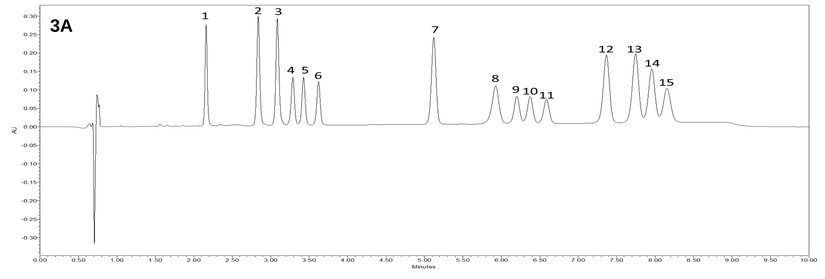
To include exo-THC and CBNA, a 150 x 3 mm, 2.7 µm column dimension was used to demonstrate the utility of a longer column dimension. The organic modifier used was 0.1% formic acid in acetonitrile, where a total of 15 cannabinoids were able to be resolved in 10 minutes.

Column:	Raptor ARC18 150x 3 mm, 2.7 µm	
MPA:	Water, 0.1% CH_2O_2, 6 mM NH_4HCO_2	
MPB:	Acetonitrile, 0.1% CH_2O_2	
Column Temp:	30 °C	
Sample:	50 ppm	
Injection Volume:	3 µL	
Flow Rate:	0.8 mL/min	
	Time (min)	**%B**
	0.00	70
	8.00	74
	8.01	70
	10.00	70

Table 5: Method conditions for the analysis of 15 cannabinoids.

1.	CBDV	**9.**	Exo-THC
2.	CBDA	**10.**	9-THC
3.	CBGA	**11.**	8-THC
4.	CBG	**12.**	(6aR, 9s)-10-THC
5.	CBD	**13.**	(6aR, 9R)-10-THC
6.	THCV	**14.**	CBC
7.	CBN	**15.**	THCA
8.	CBNA		

Table 6: 15 Analytes monitored in Figure 3.



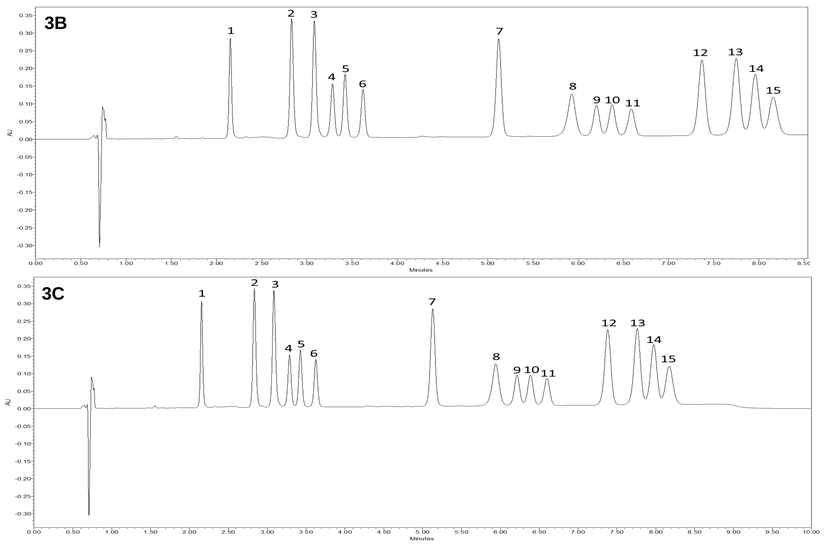


Figure 3A: Chromatogram obtained in solvent for 15 cannabinoids by the method conditions outlined in Table 5. **3B**: Chromatogram obtained from conditions outlined in Table 5 for hemp oil with analytes spiked at 50 ppm. **3C:** Chromatogram obtained from conditions outlined in Table 5 for CBD hemp flower with analytes spiked at 50 ppm.

16 Cannabinoid Analysis on 150 x 3 mm Dimension

Tetrahydrocannabinol acetate, or THCO, is a popular cannabinoid but can be tricky to add into methods due to its affinity for the stationary phase and typically requires high organic to elute. A method was developed using the previous analyte list to add THCO acetate and has an overall cycle time of 12 minutes.

Column:	Raptor ARC-18 150 x 3mm, 2.7 µm	
MPA:	Water, 0.1% CH_2O_2, 6 mM NH_4HCO_2	
MPB:	Acetonitrile, 0.1% CH_2O_2	
Column Temp:	30 °C	
Sample:	50 ppm	
Injection Volume:	3 µL	
Flow Rate:	0.8mL/min	
	Time (min)	**%B**
	0.00	70
	8.00	74
	8.01	100
	10.00	100
	10.01	70
	12.00	70

Table 7: Method conditions for the analysis of 16 cannabinoids.

1.	CBDV	**9.**	Exo-THC
2.	CBDA	**10.**	9-THC
3.	CBGA	**11.**	8-THC
4.	CBG	**12.**	(6aR, 9s)-10-THC
5.	CBD	**13.**	(6aR, 9R)-10-THC
6.	THCV	**14.**	CBC
7.	CBN	**15.**	THCA
8.	CBNA	**16.**	THCO acetate

Table 2: 16 Analytes monitored in Figure 4.

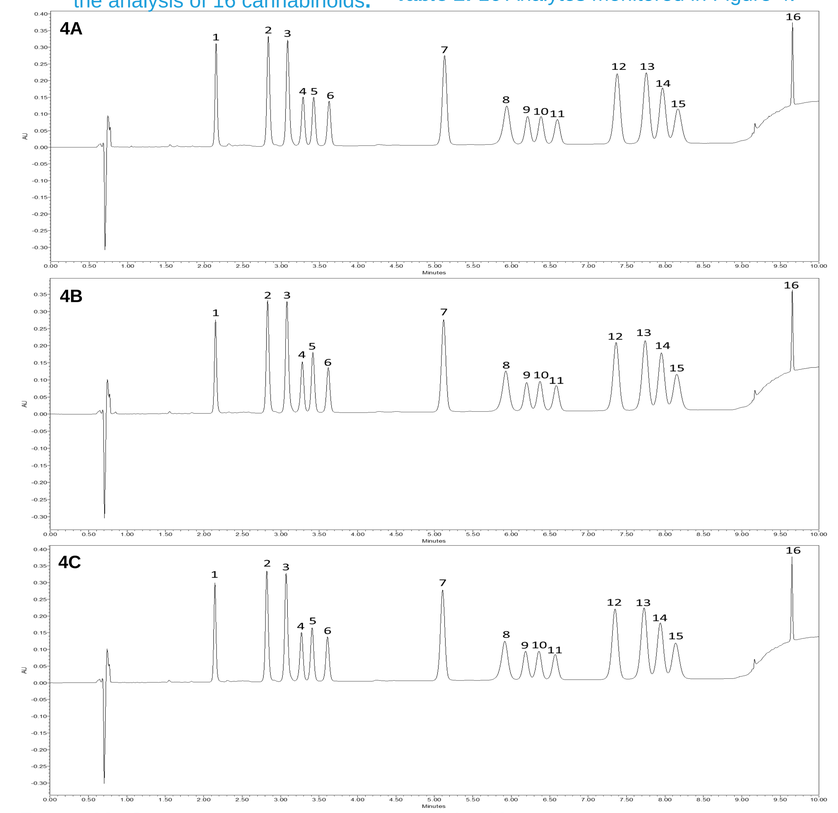


Figure 4A: Chromatogram obtained in solvent for 16 cannabinoids by the method conditions outlined in Table 7. **4B**: Chromatogram obtained from conditions outlined in Table 7 for hemp oil with analytes spiked at 50 ppm. **4C:** Chromatogram obtained from conditions outlined in Table 7 for CBD hemp flower with analytes spiked at 50 ppm.

Conclusions

Choosing the right column dimension for the needs of your lab has a number of considerations. It is important to remember that shorter columns do not always mean shorter methods, and sometimes it is necessary to use larger column dimensions. In cases where there is a need to resolve challenging compounds, there are many tools at your disposal to help achieve the desired result. Larger column dimensions can give more resolving power than smaller column dimensions, but it is also good to consider buffer concentration and organic modifier to help alter selectivity.