

# The Detection of Flavonoids in Hemp Flower by LC-MS/MS

Jamie York, Dan DeLurio; Restek Corporation

## Abstract & Introduction

Hemp and cannabis are chemically complex matrices containing a wide-array of bioactive compounds, whose synergistic interactions give hemp and cannabis their therapeutic effects. Flavonoids are a group of compounds found in cannabis and account for about 2.5% of the dry weight of the flowers and leaves.<sup>1</sup> Flavonoids are antioxidant rich and can effect flavor profiles of foods. Hemp and cannabis also have some unique flavonoids that cannot be found in any other plants including cannflavin A, cannflavin B, and cannflavin C.

Routine testing in cannabis labs typically includes potency testing for phytocannabinoids and terpenes, but oftentimes does not include flavonoid testing. Flavonoids are ubiquitous in hemp and cannabis and, like terpenes, can contribute to the overall aroma and flavor of the plant. Herein, a workflow for the extraction and detection of 19 flavonoids using LC-MS/MS was developed using CBD and CBG dominant hemp flower.

## Method Conditions

<b>Column:</b>	Raptor Biphenyl			
<b>Dimensions:</b>	100 mm x 2.1 mm ID			
<b>Particle Size:</b>	2.7 µm			
<b>Pore Size:</b>	90 Å			
<b>Guard Column:</b>	Raptor Biphenyl EXP 5 mm, 2.1 mm ID, 2.7 µm			
<b>Temp.:</b>	30 °C			
<b>Diluent:</b>	60/40 Water/Methanol			
<b>Inj. Vol.:</b>	2 µL			
<b>Mobile Phase</b>				
<b>A:</b>	Water, 0.1% formic acid			
<b>B:</b>	Methanol, 0.1% formic acid			
	<b>Time (min)</b>	<b>Flow (mL/min)</b>	<b>%A</b>	<b>%B</b>
	0	0.5	60	40
	4	0.5	0	100
	5.5	0.5	0	100
	5.51	0.5	60	40
	7	0.5	60	40
<b>Detector:</b>	MS/MS			
<b>Ion Source:</b>	Electrospray			
<b>Ion Mode:</b>	ESI+/ESI-			
<b>Mode:</b>	MRM			
<b>Instrument:</b>	UHPLC			

Table 1: Method conditions for flavonoid analysis.

Analyte	Retention Time (min)	Precursor Ion	Product Ion 1	Product Ion 2	Product Ion 3	Polarity
1 Orientin	1.47	449.0	329.1	299.1	431.0	+
2 Vitexin	1.83	433.0	313.1	283.0	415.1	+
3 Rutin	1.90	609.1	300.1	301.0	271.0	-
4 Quercetin-3-D-glycopyranoside	2.01	463.1	300.0	301.0	271.0	-
5 Isovitexin	2.02	433.4	283.0	313.1	337.0	+
6 Luteolin-7-O-glucuronide	2.22	463.0	287.1	153.2	135.1	+
7 Fisetin	2.44	285.0	135.0	121.0	163.0	-
8 Apigenin-7-O-glucuronide	2.66	447.0	271.1	153.2	119.1	+
9 Quercetin	2.75	301.0	151.0	179.0	107.0	-
10 Luteolin	2.92	285.0	133.0	151.0	175.0	-
11 Kaempferol	3.14	285.0	92.9	239.0	185.0	-
12 Apigenin	3.27	271.0	153.0	119.0	69.1	+
13 Baicalin	3.36	447.1	271.1	123.1	253.1	+
14 Silymarin	3.32	481.1	301.0	125.1	152.0	-
15 Chrysin	4.14	253.0	143.2	63.1	209.0	-
16 Wogonin	4.22	285.0	270.1	151.2	179.0	+
17 Cannflavin B	4.32	369.1	313.0	298.0	165.0	+
18 Cannflavin A	4.62	437.1	313.1	165.0	298.1	+
19 B-sitosterol	5.47	397.4	161.1	135.1	147.2	+

Table 2: MRM transitions with critical pairs highlighted.

## Flavonoid Detection

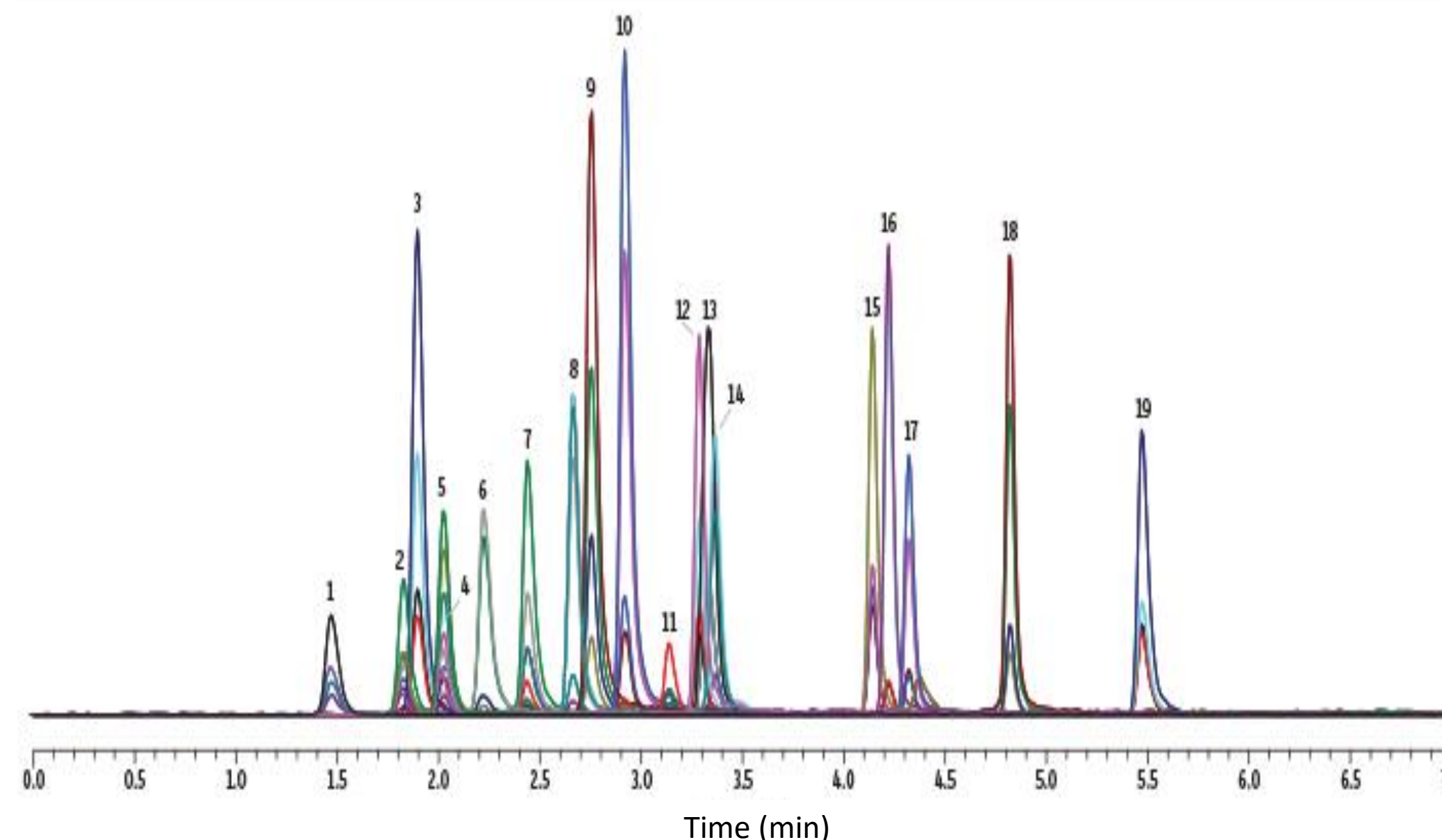


Figure 1: Chromatogram for the detection of 19 flavonoids in solvent by LC-MS/MS.

## Application to Hemp Flower Samples

A method for the extraction of endogenous flavonoids in hemp flower was developed without the need for hydrolysis. 500 mg of ground hemp flower was weighed into a 50 mL centrifuge tube. 10 mL of methanol/water 80/20 was added prior to vortexing (5 seconds) and sonicating (15 minutes). The sample was then centrifuged for 5 minutes at 4200 rpm. The supernatant was diluted 50-fold in 60:40 water:methanol, vortexed briefly, and filtered using a 0.2 µm Thomson SINGLE StEP standard filter vial prior to analysis.

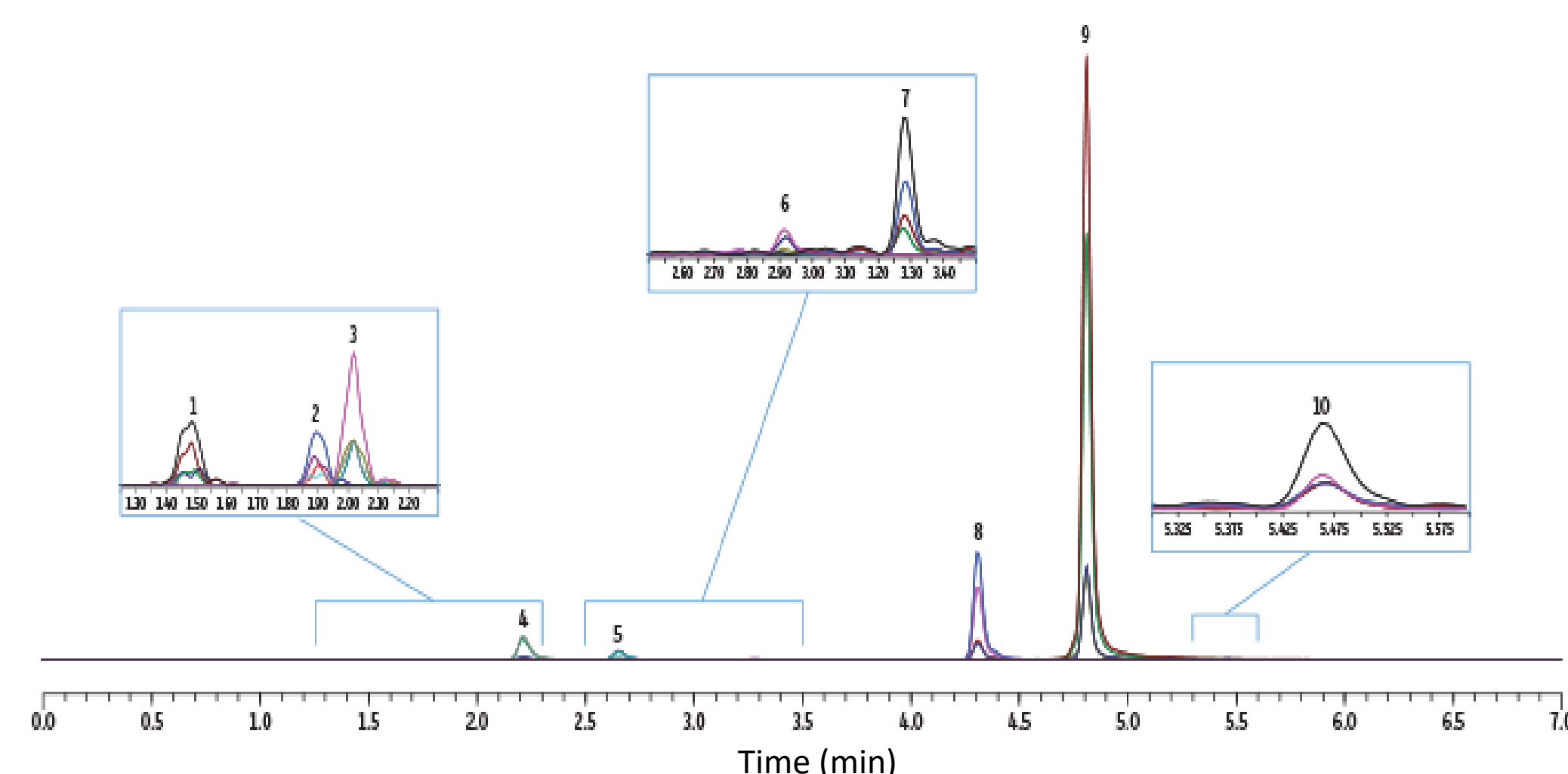


Figure 2: CBG dominant hemp flower analyzed by LC-MS/MS. 10 endogenous flavonoids were detected and are listed below.

Analyte	Retention Time (min)
1 Orientin	1.47
2 Rutin	1.90
3 Quercetin-3-D-glycopyranoside	2.01
4 Luteolin-7-O-glucuronide	2.22
5 Apigenin-7-O-glucuronide	2.66
6 Luteolin	2.92
7 Apigenin	3.27
8 Cannflavin B	4.32
9 Cannflavin A	4.62
10 B-sitosterol	5.47

Table 3: Analyte list of the endogenous flavonoids detected in CBG dominant hemp flower sample.

## Reference

1. Tomko, A. M.; Whynot, E. G.; Ellis, L. D.; Dupre, D. J. Anti-Cancer Potential of Cannabinoids, Terpenes, and Flavonoids Present in Cannabis. *Cancers* 2020, 12, 1985; doi:10.3390/cancers12071985

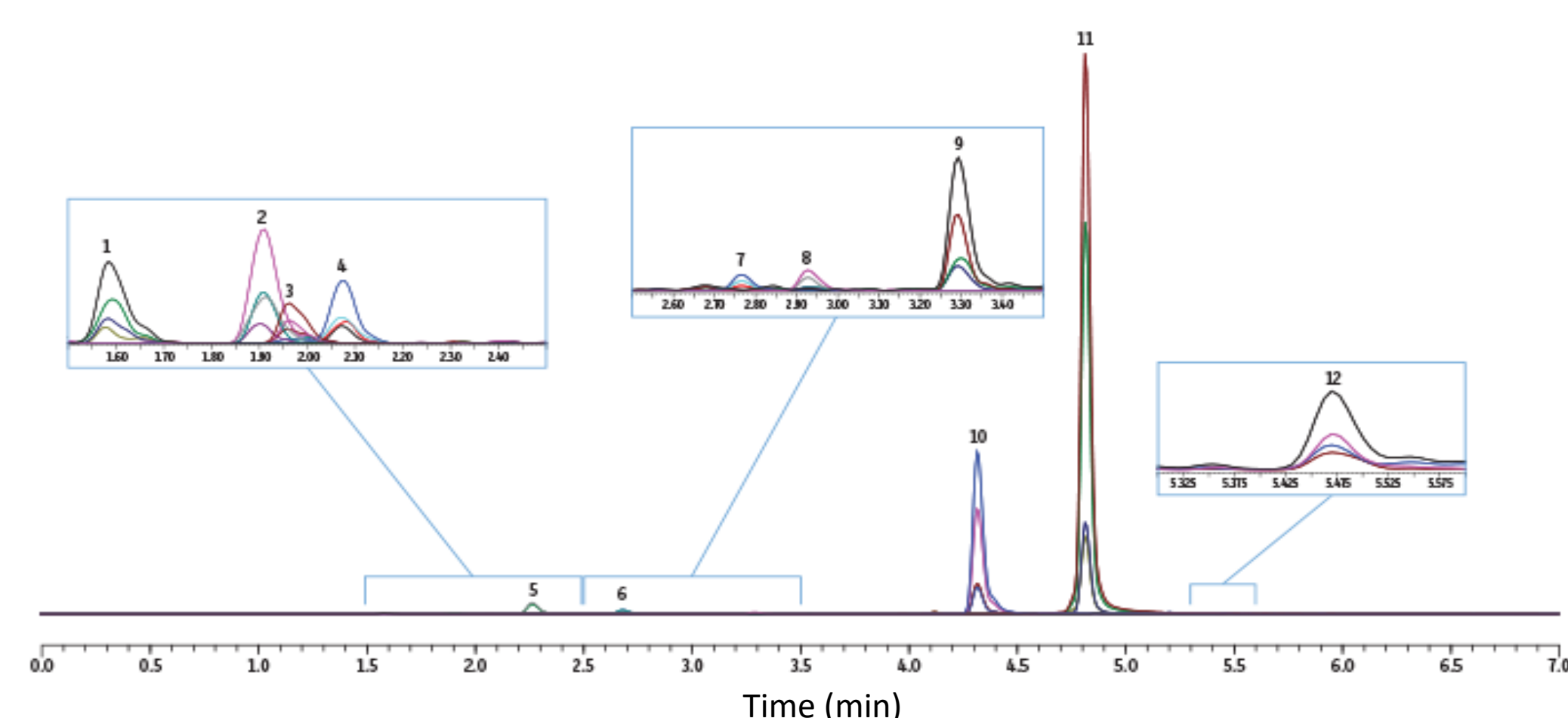


Figure 3: Chromatogram of CBD dominant hemp flower analyzed by LC-MS/MS. 12 endogenous flavonoids were detected for this sample and are listed below.

Analyte	Retention Time (min)
1 Orientin	1.47
2 Vitexin	1.83
3 Rutin	1.90
4 Quercetin-3-D-glycopyranoside	2.01
5 Luteolin-7-O-glucuronide	2.22
6 Apigenin-7-O-glucuronide	2.66
7 Quercetin	2.75
8 Luteolin	2.92
9 Apigenin	3.27
10 Cannflavin B	4.32
11 Cannflavin A	4.62
12 B-sitosterol	5.47

Table 4: Analyte list of the endogenous flavonoids detected in CBD dominant hemp flower sample.

## Recovery Experiments

Recovery experiments were performed for CBG and CBD dominant hemp flower by comparing pre-extraction spiked samples against post-extraction spiked samples using two internal standards to aid quantitation, apigenin-d5 and rutin-d5. Percent recovery ranged from 83-104% for CBD dominant hemp flower and 80-101% for CBG dominant hemp flower.

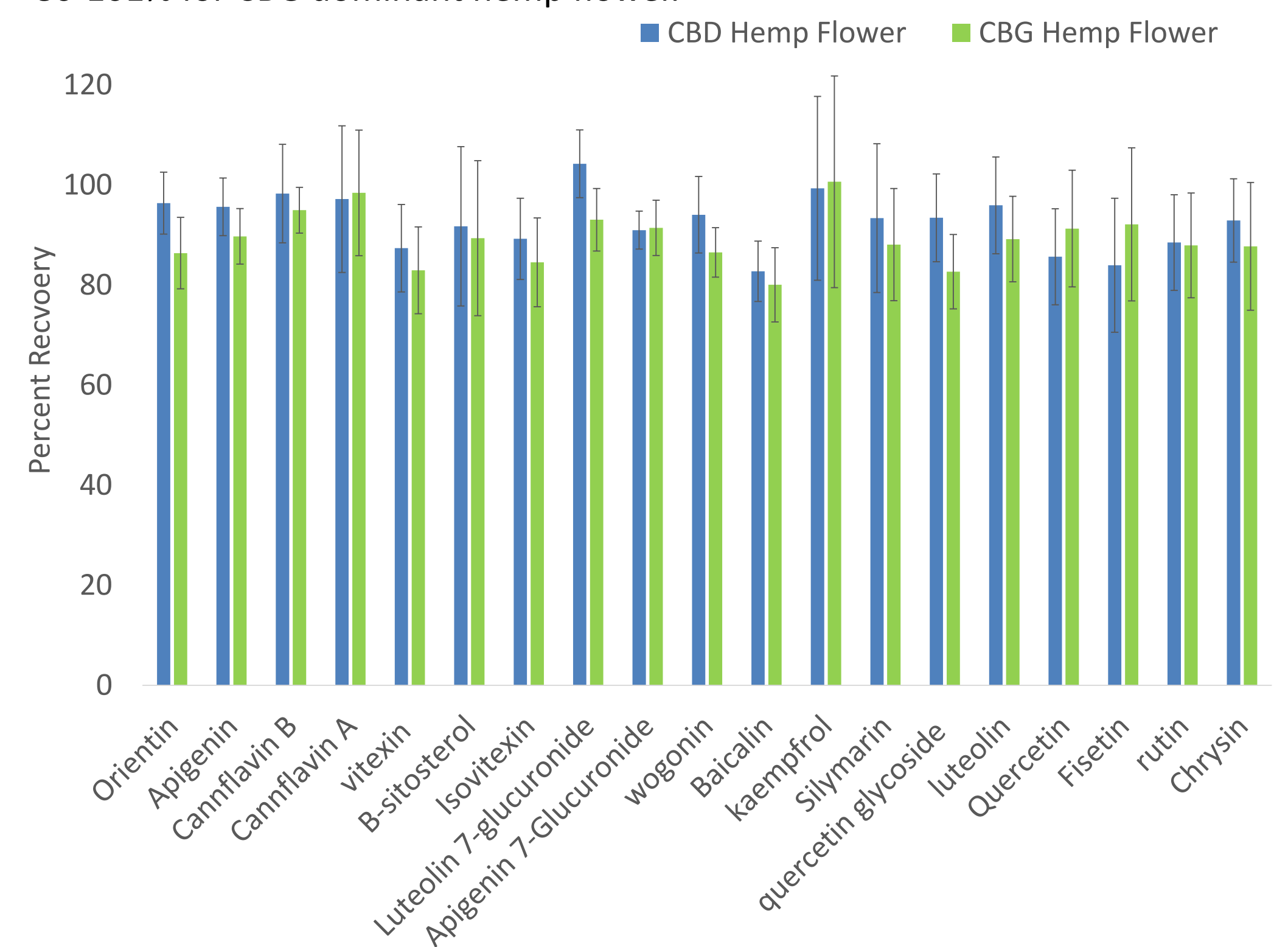


Figure 4: Recovery results spiked to a final concentration of 100 ppb.

## Conclusions

A chromatographic method was established for the analysis of 19 flavonoids using a Raptor Biphenyl 100 x 2.1 mm, 2.7 µm column. This method baseline resolves all isobars, with an overall cycle time of 7 minutes, allowing for rapid sample throughput. This methodology was applied to two different hemp flower samples to detect endogenous flavonoids in the samples. Percent recovery ranged from 83-104% for CBD hemp flower and 83-101% for CBG hemp flower, indicating this is an effective procedure for the extraction of flavonoids from hemp flower.