Analysis of Synthetic Cannabinoids and Metabolites: Adding New Compounds to an Existing LC-MS/MS Method

By Sharon Lupo and Frances Carroll

Abstract

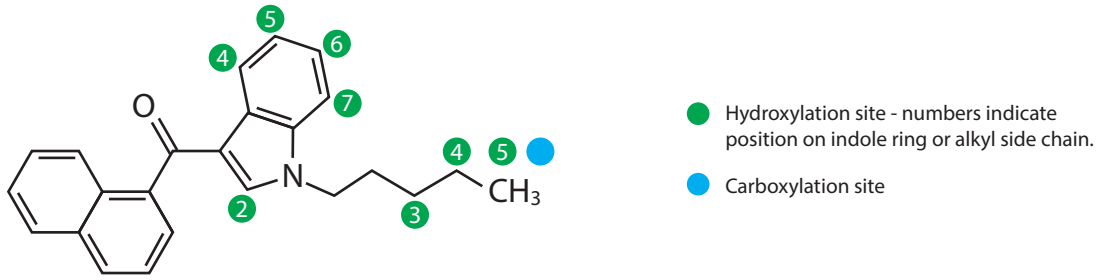
The analysis of synthetic cannabinoids and their metabolites can be a difficult and challenging task. Keeping up with the ever-growing list of synthetic cannabinoids that illicit drugmakers produce further complicates the analysis. As shown here, the retention and selectivity of the Raptor Biphenyl column allow new drugs to be added to an existing method, providing labs with an important vehicle for improving efficiency and productivity.

Introduction

The analysis of synthetic cannabinoids and their metabolites has become a routine procedure in many forensic toxicology laboratories as new drugs appear on the market. When developing methods for these compounds, optimization of analysis time, resolution between metabolites, method robustness, and the ability to add emerging compounds are of ultimate importance as they influence method effectiveness and longevity. Because the Raptor Biphenyl column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC technology, we chose it when developing a simple dilute-and-shoot method that included both synthetic cannabinoids and their metabolites in urine. Our original work produced a fast method that separated 29 target compounds in less than seven minutes.

When analyzing synthetic cannabinoids, chromatographic resolution of some target compounds that cannot be determined by MS alone is essential. For example, synthetic cannabinoids JWH-018 and JWH-073 and their metabolites must be separated chromatographically due to the presence of multiple positional isomers among the monohydroxylated metabolites. These isomers form because each parent compound has multiple sites available for hydroxylation (Figure 1). The more target compounds there are in a method, the more difficult it can be to obtain adequate resolution where needed. Here, we expand on our original method and show proof of concept for how new drugs can be added, while maintaining complete resolution of isobars and separation from matrix interferences in diluted human urine. In the ever-changing landscape of illegal drugs, the ability to add emerging drugs to existing methods allows for much more effective use of laboratory resources as analysts and instruments can focus on routine sample analysis rather than new method development.

Figure 1: Analysis of synthetic cannabinoids is complicated by the multiple hydroxylation sites for JWH-018.



Experimental

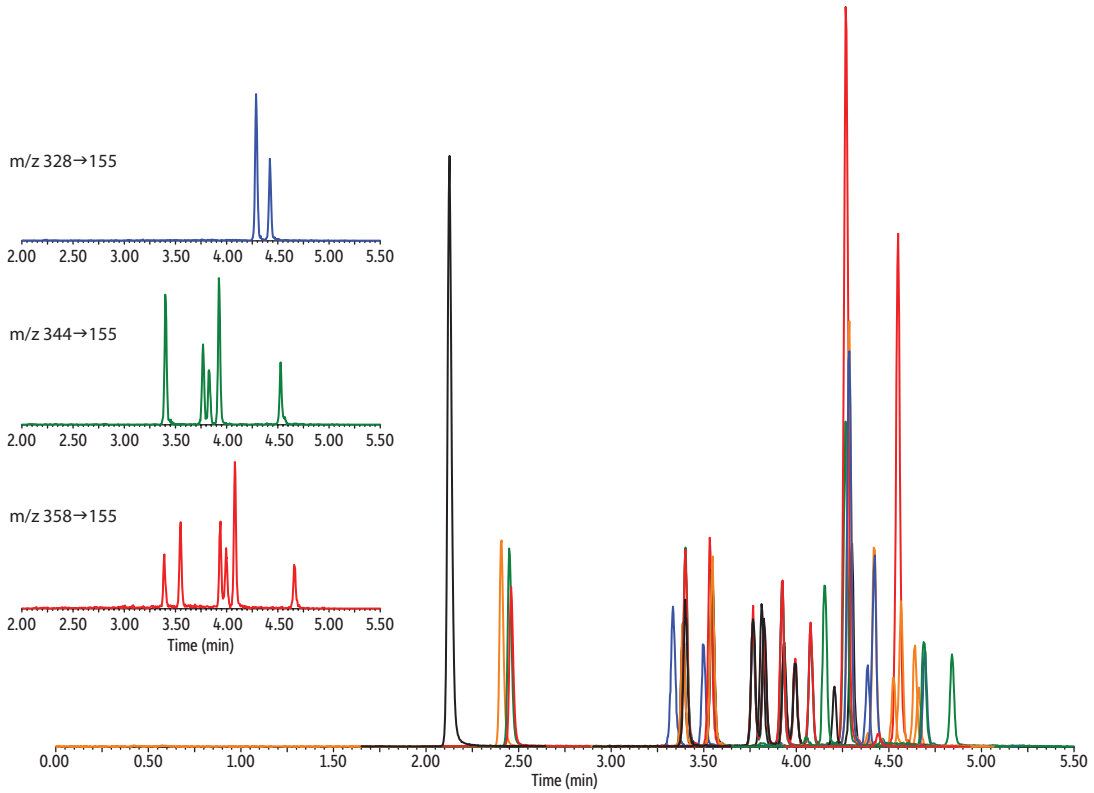
Samples were prepared in human urine and diluted 3x in a 0.2 µm PVDF Thomson SINGLE StEP filter vial with water:methanol (50:50) prior to analysis. A Raptor Biphenyl column was used as the analytical column as established in the original method. Evaluations were performed on a Waters ACQUITY UPLC I-Class equipped with a Xevo TQ-S and a Shimadzu Nexera UHPLC equipped with a SCIEX API 4500 MS/MS. Both systems utilized electrospray ionization in positive ion mode using scheduled multiple reaction monitoring (MRM). Instrument conditions were as follows and analyte transitions for the original target analytes and additional emerging drugs of interest are provided in the figures.

Analytical column:	Raptor Biphenyl (2.7 µm, 50 mm x 3.0 mm; cat.# 9309A5E)	
Guard column:	Raptor Biphenyl EXP guard column (2.7 µm, 5 mm x 3.0 mm; cat.# 9309A0253)	
Mobile phase A:	0.1% Formic acid in water	
Mobile phase B:	0.1% Formic acid in acetonitrile	
Gradient	Time (min)	%B
	0.00	25
	1.00	25
	5.00	95
	5.50	95
	5.51	25
	7.00	25
Flow rate:	0.6 mL/min	
Injection volume:	2 µL	
Column temp.:	30 °C	
Ion mode:	Positive ESI	

Results and Discussion

Today, laboratories are faced with the difficult task of keeping up with the ever-growing list of synthetic cannabinoids that illicit drugmakers produce to avoid legal classification and detection. Previously, we developed a method for the comprehensive screening of 17 synthetic cannabinoids, 12 metabolites, and five internal standards prepared at 5 ng/mL in human urine and diluted. Complete resolution of isobars and good separation from major matrix interferences were achieved with a cycle time of 5 minutes and a total analysis time of 7 minutes due to the unique selectivity and retention of the Raptor Biphenyl column (Figure 2).

Figure 2: Original Method Developed for the Combined Analysis of Synthetic Cannabinoids and Metabolites in Diluted Human Urine



Peaks	t_R (min)	Precursor Ion	Quantifer Product Ion	Qualifier Product Ion
1. Pravadoline	2.15	379.29	135.04	114.16
2. AM2233	2.44	459.25	112.2	98.15
3. JWH-200-d5	2.47	390.34	155.07	NA
4. JWH-200	2.48	385.28	155.07	114.16
5. WIN 55, 212	3.34	427.29	155.07	127.14
6. JWH-073 N-butanoic acid	3.39	358.27	155.08	127.11
7. JWH-073 4-hydroxybutyl	3.4	344.24	155.09	127.09
8. JWH-018 N-pentanoic acid	3.49	372.18	155.08	127.14
9. JWH-018 5-hydroxypentyl-d5	3.54	363.5	155.08	NA
10. JWH-018 5-hydroxypentyl	3.55	358.27	155.08	127.11
11. JWH-073 6-hydroxyindole	3.77	344.24	155.09	127.09
12. JWH-073 5-hydroxyindole-d7	3.81	351.21	155.07	NA
13. JWH-073 5-hydroxyindole	3.83	344.24	155.09	127.09
14. JWH-073 7-hydroxyindole	3.92	344.24	155.09	127.09
15. JWH-018 6-hydroxyindole	3.94	358.27	155.08	127.11
16. JWH-018 5-hydroxyindole	3.99	358.27	155.08	127.11
17. JWH-018 7-hydroxyindole	4.08	358.27	155.08	127.11
18. RCS-4	4.15	322.27	135.12	77.09
19. XLR-11	4.21	330.25	232.17	125.1
20. JWH-015-d7	4.27	335.28	155.07	NA
21. JWH-250	4.27	336.28	121.12	91.07
22. JWH-015	4.29	328.26	155.07	127.13
23. AM2201	4.30	360.26	155.07	127.14
24. JWH-203	4.39	340.23	188.18	125.09
25. JWH-073	4.42	328.26	155.07	127.13
26. UR-144	4.44	312.32	214.17	125.1
27. JWH-073 4-hydroxyindole	4.53	344.24	155.09	127.09
28. JWH-018-d9	4.55	351.34	155.07	NA
29. JWH-018	4.57	342.27	155.08	127.11
30. JWH-081	4.64	372.28	185.12	157.09
31. JWH-018 4-hydroxyindole	4.66	358.27	155.08	127.11
32. JWH-122	4.69	356.29	169.12	141.11
33. JWH-019	4.70	356.29	155.07	127.1
34. JWH-210	4.84	370.31	183.12	153.26

Column
Dimensions: 50 mm x 3.0 mm ID
Particle Size: 2.7 µm
Guard Column: Raptor Biphenyl EXP guard column cartridge 5.0 mm, 3.0 mm ID, 2.7 µm (cat.# 9309A0253)

Sample
Conc.: 5 ng/mL standard was prepared in urine and diluted 3x with 50:50 methanol:water
Inj. Vol.: 2 µL

Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

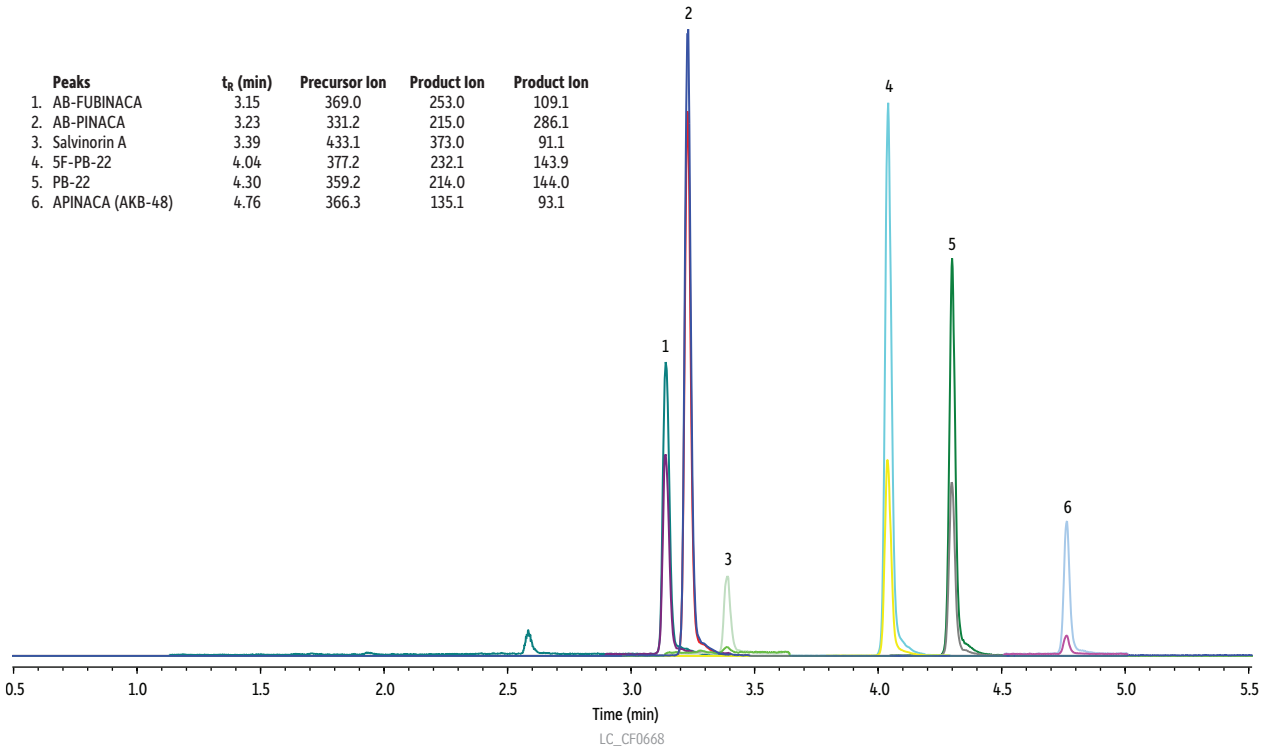
Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	75	25
1.00	0.6	75	25
5.00	0.6	5	95
5.50	0.6	5	95
5.51	0.6	75	25
7.00	0.6	75	25

Detector MS/MS
Ion Mode: ESI+
Mode: MRM
Instrument UHPLC

To determine whether new drugs could be added to the original method, five supplementary synthetic cannabinoids and salvinorin A (a psychotropic terpenoid) were analyzed under conditions identical to the established method. These emerging drugs were prepared at 50 ng/mL in human urine and diluted. As shown in Figure 3, the Raptor Biphenyl column provided excellent retention, which allowed separation of the target compounds from the early-eluting matrix interferences. Additionally, all six emerging drugs eluted within the gradient and were well resolved from each other. An overlay of the chromatograms for both the original and emerging drug lists demonstrates that the new drug compounds could easily be added to the existing method without the need for adjustments to the mobile phase, gradient, or analytical column (Figure 4).

Figure 3: Analysis of Emerging Drugs in Diluted Human Urine

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. AB-FUBINACA	3.15	369.0	253.0	109.1
2. AB-PINACA	3.23	331.2	215.0	286.1
3. Salvinorin A	3.39	433.1	373.0	91.1
4. 5F-PB-22	4.04	377.2	232.1	143.9
5. PB-22	4.30	359.2	214.0	144.0
6. APINACA (AKB-48)	4.76	366.3	135.1	93.1



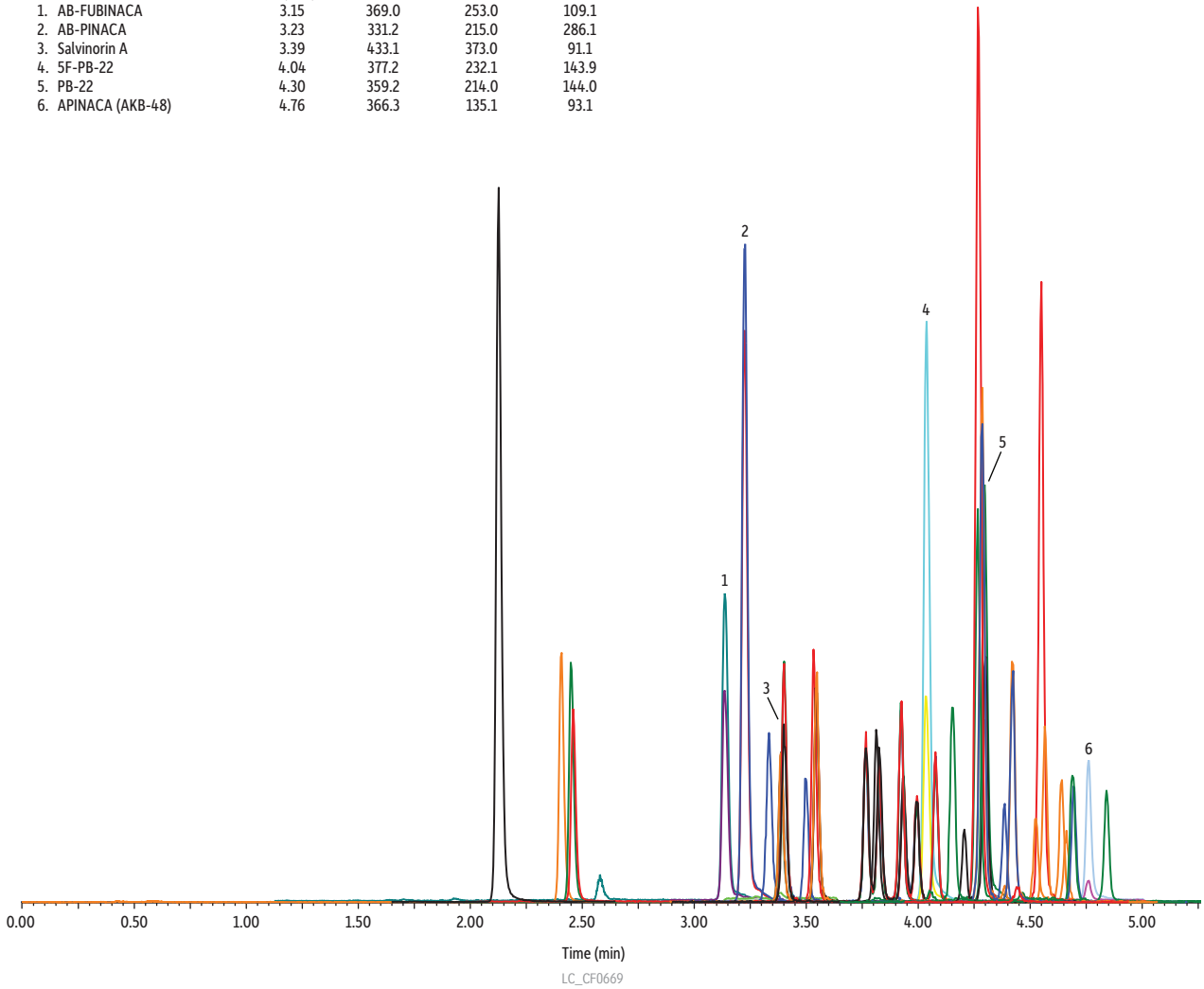
Column Raptor Biphenyl (cat.# 9309A5E)
Dimensions: 50 mm x 3.0 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 3 mm ID, 2.7 µm (cat.# 9309A0253)
Temp.: 30 °C
Sample
Conc.: 50 ng/mL in human urine and diluted 3x in a 0.2 µm PVDF Thomson SINGLE StEP filter vial with 50:50 water:methanol
Inj. Vol.: 2 µL
Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	75	25
1.00	0.6	75	25
5.00	0.6	5	95
5.50	0.6	5	95
5.51	0.6	75	25
7.00	0.6	75	25

Max Pressure: 400 bar
Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

Figure 4: Overlay of Emerging Drugs Chromatogram and Synthetic Cannabinoids and Metabolites Chromatogram in Diluted Human Urine

Peaks	t_R (min)	Precursor Ion	Product Ion	Product Ion
1. AB-FUBINACA	3.15	369.0	253.0	109.1
2. AB-PINACA	3.23	331.2	215.0	286.1
3. Salvinorin A	3.39	433.1	373.0	91.1
4. 5F-PB-22	4.04	377.2	232.1	143.9
5. PB-22	4.30	359.2	214.0	144.0
6. APINACA (AKB-48)	4.76	366.3	135.1	93.1



Column Raptor Biphenyl (cat.# 9309A5E)
Dimensions: 50 mm x 3.0 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 3 mm ID, 2.7 µm (cat.# 9309A0253)
Temp.: 30 °C
Inj. Vol.: 2 µL
Mobile Phase
A: 0.1% Formic acid in water
B: 0.1% Formic acid in acetonitrile

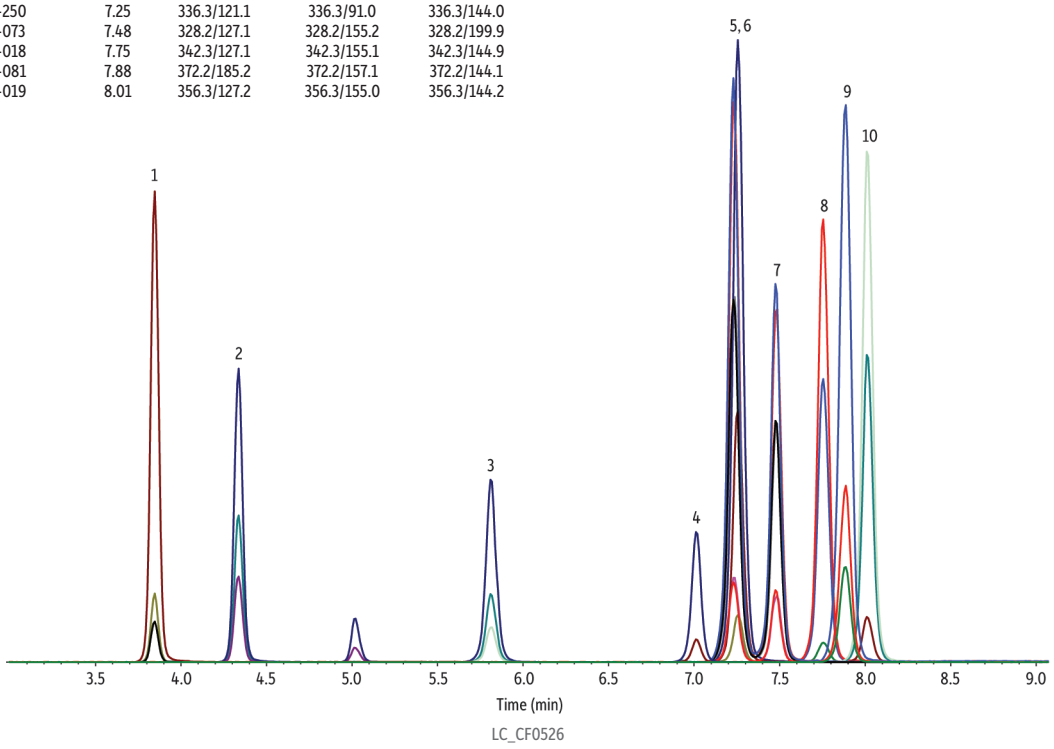
Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	75	25
1.00	0.6	75	25
5.00	0.6	5	95
5.50	0.6	5	95
5.51	0.6	75	25
7.00	0.6	75	25

Max Pressure: 400 bar
Detector MS/MS
Ion Mode: ESI+
Mode: Scheduled MRM
Instrument UHPLC

The methodology used here allows for the simultaneous analysis of emerging drugs and key existing synthetic cannabinoids, including JWH-018 and JWH-073 and their metabolites. Notably, these results were achieved with faster analysis times than were possible using a 5 µm fully porous particle (FPP) column (Figures 5 and 6). The Raptor Biphenyl column contains superficially porous particles (SPP) that significantly reduce analysis time. Using a Raptor Biphenyl SPP column provided better resolution of more compounds (including isomers and emerging compounds) in less time than traditional FPP columns, which allows more samples to be analyzed per shift.

Figure 5: Analysis of Synthetic Cannabinoids on an Ultra Biphenyl FPP Column

Peaks	RT (min)	MRM1	MRM2	MRM3
1. WIN 48098	3.85	379.2/135.2	379.2/114.3	379.2/107.3
2. JWH-200	4.34	385.3/114.0	385.3/127.0	385.3/155.0
3. WIN 55212-2	5.81	427.2/155.1	427.2/127.1	427.2/100.1
4. AM-694	7.01	436.1/309.1	436.1/231.2	--
5. JWH-015	7.23	328.3/155.1	328.3/200.1	328.3/200.1
6. JWH-250	7.25	336.3/121.1	336.3/91.0	336.3/144.0
7. JWH-073	7.48	328.2/127.1	328.2/155.2	328.2/199.9
8. JWH-018	7.75	342.3/127.1	342.3/155.1	342.3/144.9
9. JWH-081	7.88	372.2/185.2	372.2/157.1	372.2/144.1
10. JWH-019	8.01	356.3/127.2	356.3/155.0	356.3/144.2



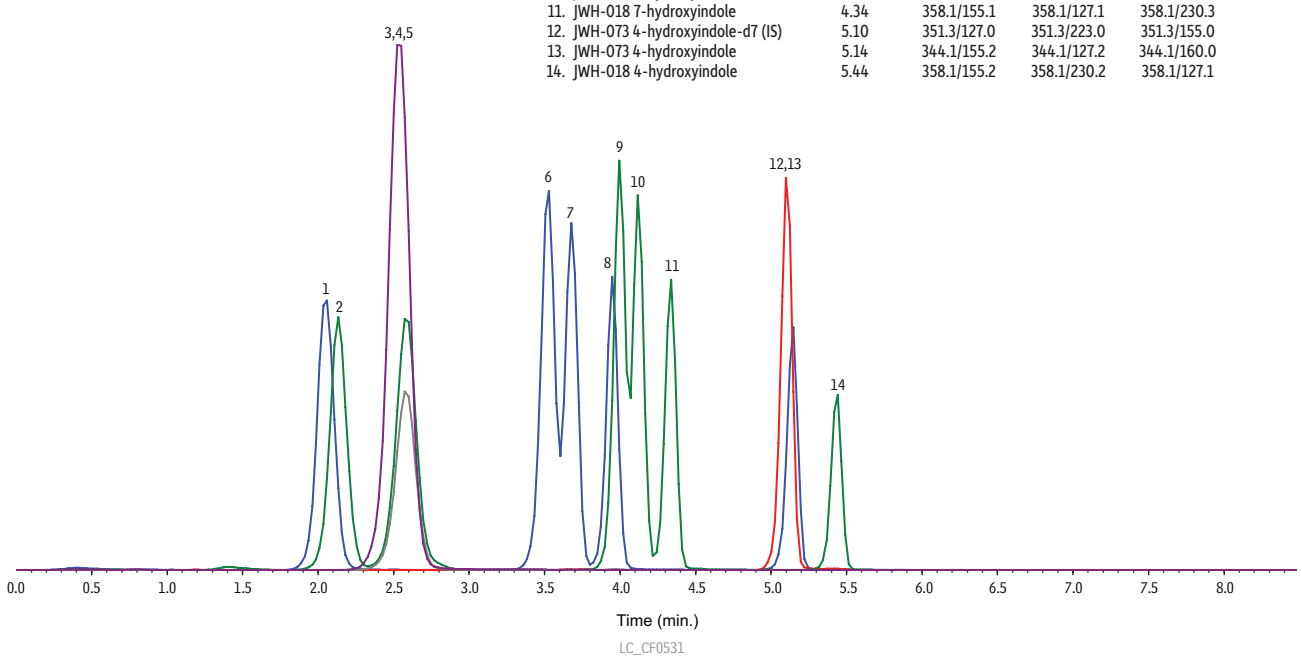
Column	Ultra Biphenyl (cat.# 9109552)
Dimensions:	50 mm x 2.1 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	40 °C
Sample	
Diluent:	Methanol
Conc.:	50 ng/mL
Inj. Vol.:	5 µL
Mobile Phase	
A:	Water + 0.1% formic acid
B:	Acetonitrile + 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0	0.5	95	5
10	0.5	5	95
10.1	0.5	95	5
12	stop		

Detector	AB SCIEX API 4000 MS/MS
Model #:	API 4000
Ion Source:	TurbolonSpray
Ion Mode:	ESI+
Ion Spray Voltage:	3000 kV
Curtain Gas:	40 psi (275.8 kPa)
Gas 1:	40 psi (275.8 kPa)
Gas 2:	40 psi (275.8 kPa)
Interface Temp.:	600 °C
Mode:	MRM
Dwell Time:	10 ms
Instrument	Applied Biosystems/MDS Sciex LC-MS/MS System
Acknowledgement	Special thanks to Paul Kennedy and Cayman Chemical for standards.

Figure 6: Analysis of Synthetic Cannabinoid Metabolites on an Ultra Biphenyl FPP Column

Peaks	t_R (min)	MRM1	MRM2	MRM3
1. JWH-073 4-hydroxybutyl	2.05	344.1/155.1	344.1/127.2	344.1/144.0
2. JWH-073 N-butanoic acid	2.13	358.1/155.1	358.1/127.3	358.1/144.1
3. JWH-018 N-pentanoic acid-d4 (IS)	2.54	376.1/155.2	376.1/230.4	376.1/248.3
4. JWH-018 N-pentanoic acid	2.58	372.1/155.2	372.1/127.1	372.1/144.1
5. JWH-018 5-hydroxypentyl	2.58	358.1/155.2	358.1/127.1	358.1/230.3
6. JWH-073 6-hydroxyindole	3.52	344.1/155.2	344.1/127.1	344.1/145.1
7. JWH-073 5-hydroxyindole	3.68	344.1/155.2	344.1/127.1	344.1/160.0
8. JWH-073 7-hydroxyindole	3.95	344.2/155.1	344.2/127.1	344.2/216.3
9. JWH-018 6-hydroxyindole	4.00	358.1/155.1	358.1/127.2	358.1/145.2
10. JWH-018 5-hydroxyindole	4.12	358.1/155.1	358.1/127.2	358.1/160.2
11. JWH-018 7-hydroxyindole	4.34	358.1/155.1	358.1/127.1	358.1/230.3
12. JWH-073 4-hydroxyindole-d7 (IS)	5.10	351.3/127.0	351.3/223.0	351.3/155.0
13. JWH-073 4-hydroxyindole	5.14	344.1/155.2	344.1/127.2	344.1/160.0
14. JWH-018 4-hydroxyindole	5.44	358.1/155.2	358.1/230.2	358.1/127.1



Column Ultra Biphenyl (cat.# 9109552)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 5 µm
Pore Size: 100 Å
Temp.: 25 °C
Sample
Diluent: 50:50 mobile phase
Conc.: 50 ng/mL extracted spiked sample
Inj. Vol.: 10 µL
Mobile Phase
A: water + 0.05% acetic acid (pH approx. 3.4)
B: acetonitrile + 0.05% acetic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	55	45
2.00	0.5	55	45
6.00	0.5	15	85
6.10	0.5	5	95
7.00	0.5	5	95
7.10	0.5	55	45
8.50	stop		

Detector API 4000
Model #: API 4000
Ion Source: TurboIonSpray®
Ion Mode: ESI+
Ion Spray Voltage: 3 kV
Curtain Gas: 40 psi (275.8 kPa)
Gas 1: 40 psi (275.8 kPa)
Gas 2: 40 psi (275.8 kPa)
Interface Temp.: 600 °C
Mode: MRM
Dwell Time: 30 ms
Instrument API LC-MS/MS

Notes

Since multiple transitions are shared between analytes, only 5 transitions are shown to simplify viewing. The transitions shown are: 344.1/155.1 (blue trace), 358.1/155.1 (green trace), 372.1/155.2 (grey trace), 376.1/155.2 (purple trace - internal standard), 351.3/127.0 (red trace - internal standard).

CAD Gas was set to 4 psi.

For 1 ng/mL calibration level, see chromatogram LC_CF0530.
For 500 ng/mL calibration level, see chromatogram LC_CF0532.

Sample was prepared according to the following method:
1) Spike 1 mL blank urine sample with analytes and internal standards.

2) Hydrolyze sample:
- Add 1 mL solution of beta-glucuronidase from keyhole limpet (Sigma-Aldrich cat.# G8132). Solution is prepared at a concentration of 5,000 Fishman units/mL in 100 mM ammonium acetate buffer (pH = 5.0).
- Incubate at 60 °C for 3 hours.

3) Extract sample on 6 mL, 500 mg C18 high-load end-capped Resprep SPE cartridge (cat.# 24052):
- Add 1 mL 5 mM ammonium acetate + 0.1% acetic acid (pH = 4.2) to sample.
- Condition cartridge with 3x 1 mL acetonitrile.
- Condition cartridge with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Apply sample and allow to pass through under gravity.
- Rinse with 3x 1 mL 5 mM ammonium acetate + 0.1% acetic acid.
- Dry cartridge with vacuum for 10 minutes.
- Elute with 3 mL acetonitrile followed by 3 mL butyl chloride.

4) Concentrate sample:
- Evaporate sample to dryness under nitrogen at 40 °C.
- Reconstitute in 0.5 mL water + 0.05% acetic acid:acetonitrile + 0.05% acetic acid (50:50).

Acknowledgement Special thanks to Cayman Chemical for reference standards.

Conclusion

Labs analyzing synthetic cannabinoids and their metabolites are under increasing pressure to add new compounds to their analytical testing services. While this can be done through new method development, adding new compounds to an existing method can save time and resources. The method shown here demonstrates the advantages of the Raptor Biphenyl SPP column for this analysis; due to the column's highly retentive, selective characteristics, 35 drugs (including isomers and emerging compounds) can be analyzed in just 5 minutes.

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