Emerging Drugs of Abuse: Updating an Existing Method with New Compounds

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Introduction

The determination of psychoactive drugs and their metabolites has become routine in many forensic toxicology laboratories. The optimization of analysis time, resolution between metabolites, method robustness, and the ability to add emerging compounds are of ultimate importance when developing an efficient method for validation. The Raptor™ Biphenyl column combines the speed of superficially porous particles (SPP) with the resolution of highly selective USLC® technology to give the analyst the ability to produce fast dilute and shoot methods while staying current with the ever-changing landscape of illegal drugs. Through this presentation we will demonstrate how emerging drugs can be easily added to an existing method while maintaining complete resolution of isobars and separation from matrix interferences in diluted human urine.

Methods

The method investigations 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). Samples were prepared in human urine and diluted 3x in a 0.2 µm PVDF Thomson SINGLE StEP® Filter Vial with 50:50 water:methanol prior to analysis.

Table 1: Analytical Conditions

Column:	Raptor™ Biphenyl 2.7 µm, 50 mm x 3.0 mm (cat# 9309A5E)		
Guard Column:	Raptor™ Biphenyl EXP® 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.)	Flow (mL/min)	%B
	0.00	0.6	25
	1.00	0.6	25
	5.00	0.6	95
	5.50	0.6	95
	5.51	0.6	25
	7.00	0.6	25
Temperature:	30°C		
Injection Vol.:	2 µL		

Discussion

Chromatographic separation is essential for analyzing synthetic cannabinoids JWH-018 and JWH-073 and their metabolites due to the presence of multiple positional isomers among the mono-hydroxylated metabolites. These isomers form because each parent compound has many sites available for hydroxylation (Figure 1). Since these positional isomers have identical molecular weights and very similar fragmentation patterns, they are indistinguishable by MS/MS detectors and chromatographic resolution is required for positive identification.

Previously, a method was presented for the comprehensive screen of 17 synthetic cannabinoids, 12 metabolites, and 5 internal standards prepared at 5 ng/mL in human urine and diluted (Tables 1 & 2). Complete resolution of isobars and separation from major matrix interferences was achieved on the Raptor™ Biphenyl column with a cycle time of 5 minutes and a total analysis time of 7 minutes (Figure 2). An extracted ion chromatogram of the isomers is shown in the inset of Figure 2.

Today, laboratories are faced with the difficult task of keeping up with the ever-growing list of synthetic cannabinoids illicit drug makers produce to avoid legal classification and detection. To determine whether the original method could keep pace, 5 new synthetic cannabinoids and salvinorin A (Table 3) were analyzed utilizing analytical conditions identical to the previous method (Table 1). These emerging drugs were prepared at 50 ng/mL in human urine and diluted. The resulting chromatogram displayed all six compounds eluting within the gradient and excellent retention and separation of the compounds from early-eluting matrix interferences (Figure 3). An overlay of the two chromatograms demonstrates that the compounds could easily be added to the methodology without the need for adjustments to the mobile phase, gradient, or analytical column (Figure 4).

Results - Original Methodology

Figure 1: Hydroxylation and carboxylation sites for JWH-018

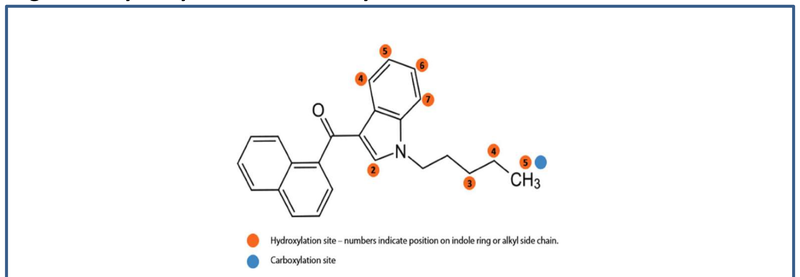
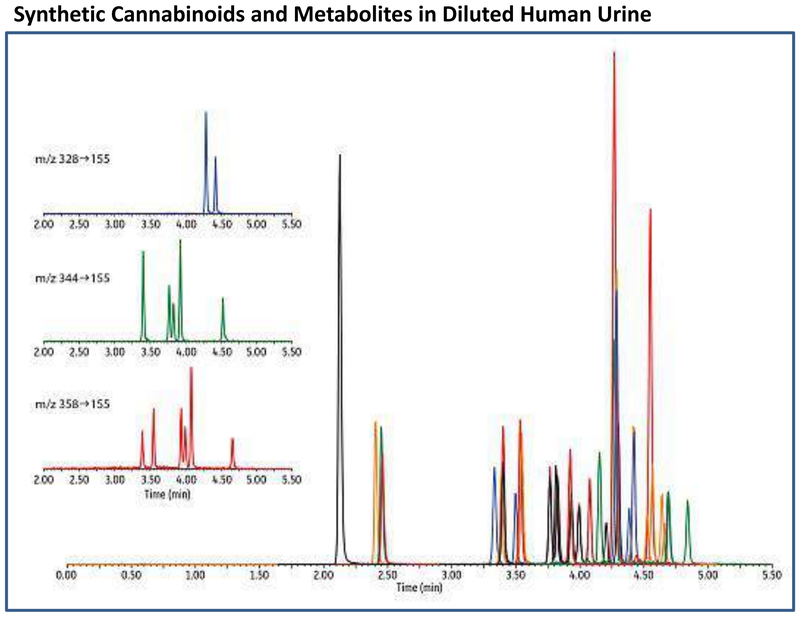


Table 2: Analyte Transitions - Originally Developed Method for the Combined Analysis of Synthetic Cannabinoids and Metabolites

Analyte	Retention Time (minutes)	Precursor Ion	Product Ion	Product Ion
Pravadoline	2.15	379.3	135.0	114.2
AM2233	2.44	459.3	112.2	98.2
JWH-200-d5	2.47	390.3	155.1	NA
JWH-200	2.48	385.3	155.1	114.2
WIN 55, 212	3.34	427.3	155.1	127.1
JWH-073 N-butanoic acid	3.39	358.3	155.1	127.1
JWH-073 4-hydroxybutyl	3.40	344.2	155.1	127.1
JWH-018 N-pentanoic acid	3.49	372.2	155.1	127.1
JWH-018 5-hydroxypentyl-d5	3.54	363.5	155.1	NA
JWH-018 5-hydroxypentyl	3.55	358.3	155.1	127.1
JWH-073 6-hydroxyindole	3.77	344.2	155.1	127.1
JWH-073 5-hydroxyindole-d7	3.81	351.2	155.1	NA
JWH-073 5-hydroxyindole	3.83	344.2	155.1	127.1
JWH-073 7-hydroxyindole	3.92	344.2	155.1	127.1
JWH-018 6-hydroxyindole	3.94	358.3	155.1	127.1
JWH-018 5-hydroxyindole	3.99	358.3	155.1	127.1
JWH-018 7-hydroxyindole	4.08	358.3	155.1	127.1
RCS-4	4.15	322.3	135.1	77.1
XLR-11	4.21	330.3	232.2	125.1
JWH-015-d7	4.27	335.3	155.1	NA
JWH-250	4.27	336.3	121.1	91.1
JWH-015	4.29	328.3	155.1	127.1
AM2201	4.30	360.3	155.1	127.1
JWH-203	4.39	340.2	188.2	125.1
JWH-073	4.42	328.3	155.1	127.1
UR-144	4.44	312.3	214.2	125.1
JWH-073 4-hydroxyindole	4.53	344.2	155.1	127.1
JWH-018-d9	4.55	351.3	155.1	NA
JWH-018	4.57	342.3	155.1	127.1
JWH-081	4.64	372.3	185.1	157.1
JWH-018 4-hydroxyindole	4.66	358.3	155.1	127.1
JWH-122	4.69	356.3	169.1	141.1
JWH-019	4.70	356.3	155.1	127.1
JWH-210	4.84	370.3	183.1	153.3

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



Results – Emerging Drugs Using Original Method

Table 3: Analyte Transitions for Emerging Drugs

Peak ID	Analyte	Retention Time (minutes)	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

Figure 3: Analysis of Emerging Drugs in Diluted Human Urine

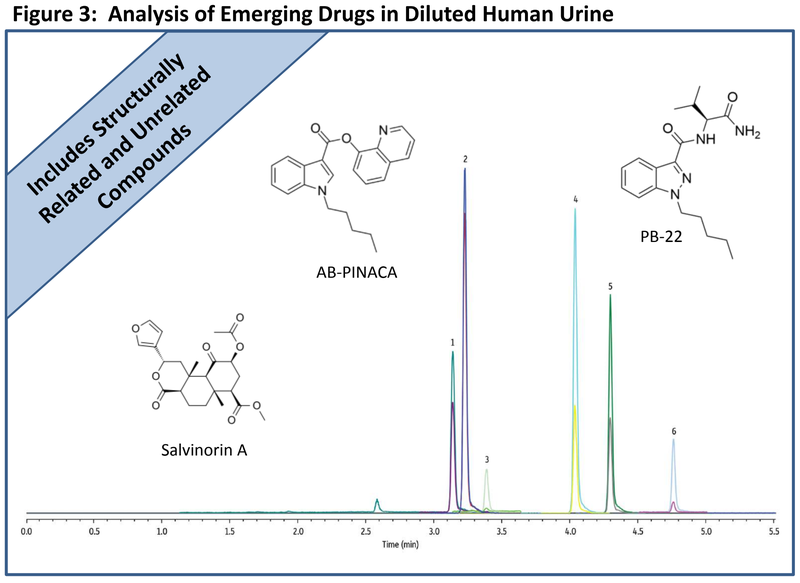
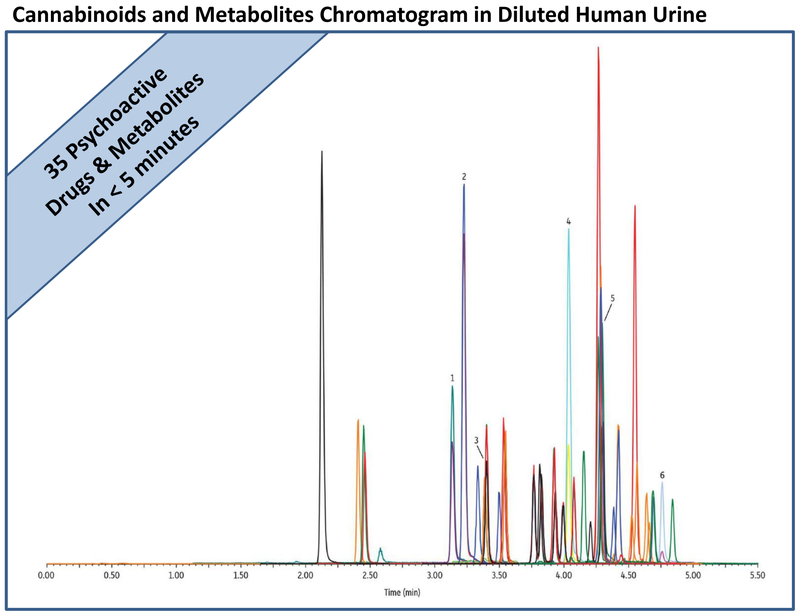


Figure 4: Overlay of Emerging Drugs Chromatogram and Synthetic Cannabinoids and Metabolites Chromatogram in Diluted Human Urine



Conclusions

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 illicit drug makers produce further complicates the analysis. The Raptor™ Biphenyl provides chromatographic solutions to your increasing list of emerging psychoactive drugs:

- Highly retentive, selective, and rugged reversed-phase separations for the simultaneous analysis of synthetic cannabinoids and metabolites.
- Easily expand analyte lists as new synthetic cannabinoids are introduced.
- Reduce analysis time with the speed of SPP.
- Quickly separate structural isomers with the unique selectivity of the Biphenyl phase.

RESTEK Pure Chromatografie

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