Ultrafast Screen for Synthetic Cannabinoids in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Forensic Toxicology

Authors

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Abstract

Forensic drug testing has traditionally used GC/MS as the analytical method of detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times less than 15 seconds. This application note evaluated the ability of the Agilent RapidFire/MS system to analyze a panel of synthetic cannabinoid metabolites from the following Spice drugs: JWH-018, JWH-073, JWH-081, JWH-210, JWH-250, RCS-4, and AM2201, in urine with much faster sample cycle times and similar analytical results compared to GC/MS or LC/MS assays.

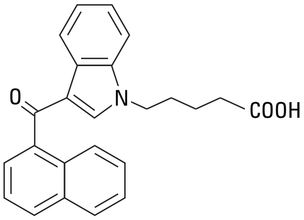
Introduction

Spice is a family of designer drugs that are synthetic cannabinoids with physiological effects similar to marijuana. These synthetic cannabinoids can be modified structurally and easily synthesized in labs. This makes measuring them in forensic drug screening a challenge. Forensic drug screening has traditionally relied on immunoassays and then analytical confirmation with a quantitative method such as GC/MS or LC/MS. The need for greater throughput, more specificity and flexibility, and faster turn-around times have placed increased demands on these traditional technologies. Mass spectrometry provides a highly selective, highly sensitive, flexible methodology but often lacks the throughput and speed that is required for screening purposes. The ability to develop a flexible, ultrafast, and selective forensic drug screen using an online SPE/MS/MS system was investigated.

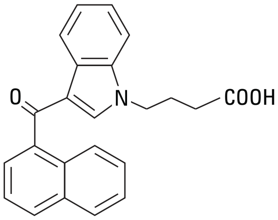
Experimental

RapidFire triple quadrupole conditions

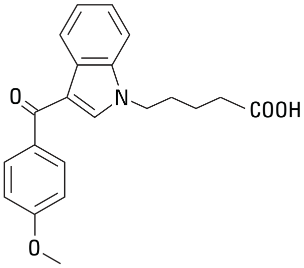
The Agilent RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6490 Triple Quadrupole Mass Spectrometer, MassHunter Qualitative Analysis B.05.00, and MassHunter Quantitative Analysis B.05.00. The samples were analyzed at a rate of 12 seconds per sample. Analyte and internal standard ions were monitored simultaneously in all experiments.



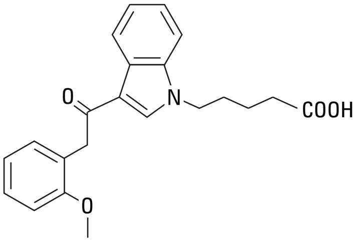
JWH-018 N-Pentanoic acid



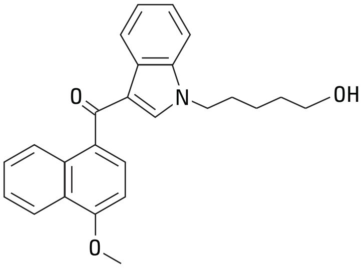
JWH-073 N-Butanoic acid



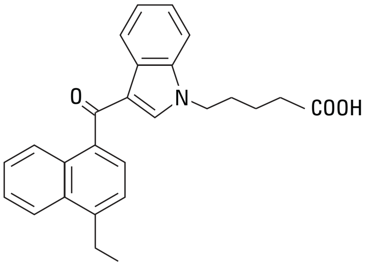
RCS-4 N-(5-carboxypentyl)



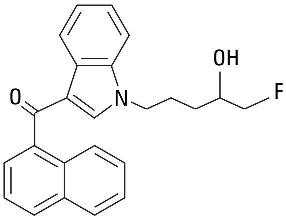
JWH-250 N-(5-carboxypentyl)



JWH-081 N-(5-hydroxypentyl)



JWH-210 N-(5-carboxypentyl)



AM2201 N-(4-hydroxypentyl)

Figure 1. Structures of the seven synthetic cannabinoid metabolite analytes in the panel.

Chemicals and reagents

All analytes were purchased from Cayman Chemical, Ann Arbor, MI. All LC/MS grade solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Sample preparation

Standard calibrators were prepared by spiking drug-free human urine with 1,000 ng/mL of each synthetic cannabinoid analyte. Serial dilutions were used to achieve the remaining standard calibrator concentrations. All samples (135 µL) were hydrolyzed with 15 µL sodium hydroxide, mixed, incubated at 65 °C for 15 minutes, and diluted with 1,350 µL of 50:50 methanol:water containing 0.1% formic acid. The samples were transferred to 96-well plates, sealed with an Agilent PlateLoc Thermal Microplate Sealer, centrifuged, and injected onto the Agilent RapidFire/MS/MS system.

Data analysis

MassHunter Qualitative Analysis (B.05.00) and Quantitative Analysis (B.05.00) were used for data analysis. A $1/x^2$ weighting factor was applied during linear regression of the calibration curves. The quantitation using MassHunter Quantitative software was performed by spectral peak area ratio to a known concentration of the internal standards.

RapidFire/MS/MS conditions	
Buffer A	Water with 10 mM ammonium acetate, 0.1% formic acid
Buffer B	50% methanol with 0.1% formic acid
Buffer C	85% ethyl acetate, 15% isopropanol
Injection volume	10 µL
SPE cartridge	Agilent RapidFire cartridge A (reversed-phase C4, G9210C)
RF State 1	sip sensor
RF State 2	1,500 ms
RF State 3	3,000 ms
RF State 4	5,000 ms
RF State 5	500 ms
Triple quadruple conditions	
Gas temperature	250 °C
Gas flow	19 L/min
Nebulizer	45 psi
Sheath gas temperature	300 °C
Sheath gas flow	11 L/min
Nozzle voltage	2,000 V
Capillary voltage	3,500 V

Table 1. MRM transitions.

Analyte	Q1	Q3	Dwell	Fragmentor	CE	CAV
JWH-018 N-Pentanoic acid	372.2	155	20	380	25	3
JWH-073 N-Butanoic acid	358.1	127	20	380	45	3
JWH-081 N-(5-hydroxypentyl)	388.1	185	20	380	20	5
JWH-250 N-(5-carboxypentyl)	366.1	121	20	380	20	3
JWH-210 N-(5-carboxypentyl)	400.2	183	20	380	25	3
RCS-4 N-(5-carboxypentyl)	352.1	135	20	380	20	3
AM2201 N-(4-hydroxypentyl)	376.1	155	20	380	25	3
THCCOOH-d9 (IS)	354.1	308.2	20	380	17	2

Results and Discussion

Samples were prepared by spiking a panel of synthetic cannabinoid metabolites into drug-free human urine, performing base hydrolysis, and then diluting the samples 10-fold with an acidified methanol/water solution. The analyte panel consisted of the following synthetic cannabinoid metabolites: JWH-018 N-Pentanoic acid, JWH-073 N-Butanoic acid, JWH-081 N-(5-hydroxypentyl), JWH-210 N-(5-carboxypentyl), JWH-250 N-(5-carboxypentyl), RCS-4 N-(5-carboxypentyl), and AM2201 N-(4-hydroxypentyl). The samples were then analyzed through SPE/MS/MS using the RapidFire/MS/MS system and a C4 cartridge at 12 seconds per sample (Figure 2). This RapidFire/MS/MS methodology is capable of throughputs greater than 250 samples per hour providing a high-throughput and very efficient mode of analysis.

Prepared calibration standards were run four times a day, over a series of four days to establish both intra and interday precision and accuracy. All seven analytes had intra and interday accuracies within 15% and coefficient of variation values less than 10% for all concentrations within the linear range (Tables 2–8). This method had excellent linearity within the measured range of 5–1,000 ng/mL with an R^2 value greater than 0.9998 (Figure 3) for each analyte.

The reproducibility of the method was tested by measuring 2,000 sequential injections of all seven analytes spiked into urine at 75 ng/mL. The same cartridge was used for all 2,000 injections without deviation in pump pressures or peak shape. The instrument response was stable for all seven analytes. JWH-073 butanoic acid, for example, had a coefficient of variation < 10% and accuracy within < 5%.

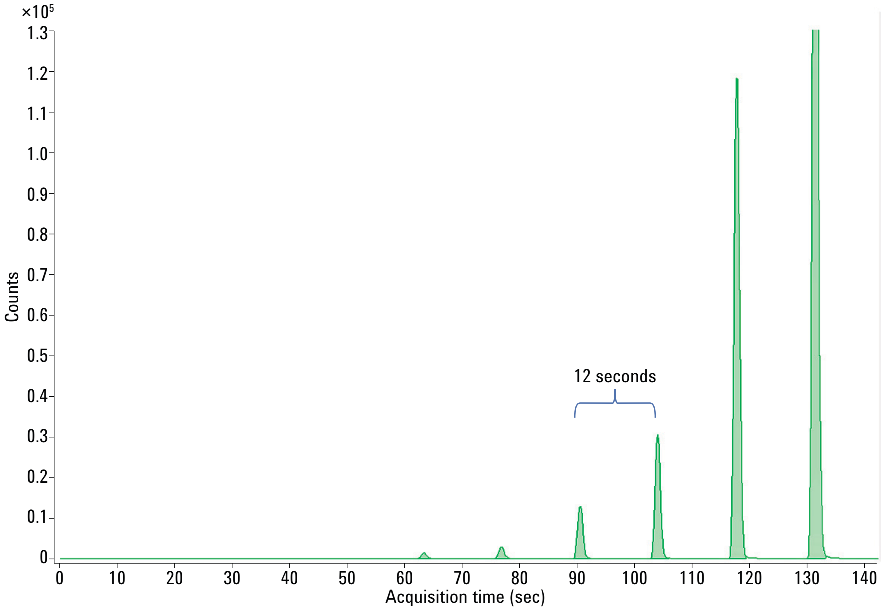


Figure 2. Representative standard curve showing injection cycle times of 12 seconds: JWH-073 N-Butanoic acid metabolite.

Table 2. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of RCS-4 N-pentanoic acid metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	100.8	3.8	100.9	2.4
10	90.4	5.8	93.8	2.8
50	100.2	5.3	99.6	2.9
100	94.1	5.3	95.6	5.9
500	102.0	8.9	98.7	3.2
1,000	104.4	4.5	103.9	2.6

Table 3. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of JWH-210 N-(5-carboxypentyl) metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	101.9	5.3	101.0	1.5
10	97.4	3.8	97.0	1.2
50	89.7	4.9	89.8	4.6
100	96.3	6.4	96.5	3.1
500	103.3	5.2	100.9	1.6
1,000	107.7	5.6	109.4	4.4

Table 4. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of AM2201 N-(4-hydroxypentyl) metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	101.1	1.7	101.5	1.2
10	101.3	9.3	99.4	1.7
50	87.3	7.7	88.7	3.2
100	91.2	4.2	96.1	4.4
500	108.0	2.4	104.1	4.8
1,000	108.3	5.4	110.0	1.9

Table 5. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of JWH-018 N-pentanoic acid metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	103.8	8.6	101.6	1.9
10	98.2	4.9	96.9	3.4
50	94.4	4.2	95.4	2.9
100	98.5	5.1	98.4	6.0
500	102.8	6.2	102.0	1.9
1,000	99.3	10.9	102.1	4.1

Table 6. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of JWH-250 N-(5-carboxypentyl) metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	104.9	7.3	102.8	1.9
10	95.6	8.1	94.6	2.5
50	95.9	5.4	97.2	2.6
100	98.5	6.1	97.2	3.1
500	100.9	5.8	98.8	3.4
1,000	105.6	4.9	105.0	1.9

Table 7. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of JWH-073 N-butanoic acid metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
10	95.1	3.7	96.8	4.4
50	101.9	4.9	102.0	2.8
100	99.7	6.6	98.6	5.9
500	101.1	8.5	99.1	3.4
1,000	97.9	6.2	99.3	5.7

Conclusions

A panel of metabolites of the following synthetic cannabinoids: JWH-018, JWH-073,JWH-081, JWH-210, JWH-250, RCS-4, and AM2201 was quickly, accurately, and precisely measured in urine using a simple sample preparation method. There is the potential to add more, or different, synthetic cannabinoid metabolite analytes to this panel on the Agilent RapidFire/MS system thus providing a quick and flexible method of measuring this fast evolving class of analytes. At a peak to peak injection cycle time of 12 seconds, this screening method is capable of throughputs greater than 250 samples per hour. Using this SPE/MS/MS methodology, increased sensitivity and specificity were achieved compared to traditional forensic screening methods without compromising throughput and speed.

Table 8. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of JWH-081 N-(5-hydroxypentyl) metabolite in urine.

(ng/mL)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
5	94.6	3.1	97.9	3.2
10	103.7	9.4	102.1	2.8
50	89.1	8.4	87.1	4.0
100	95.5	2.6	97.4	3.7
500	103.9	7.5	104.7	3.2
1,000	102.4	7.2	103.2	2.8

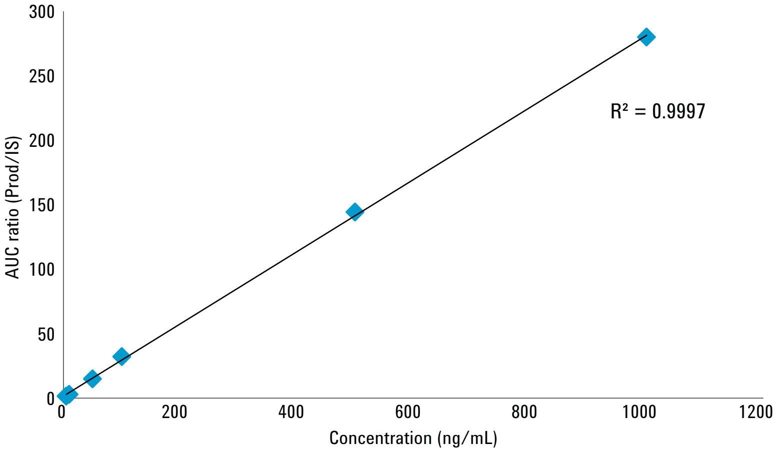


Figure 3. Representative standard curve: JWH-073 N-Butanoic acid metabolite.

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