Rapid Analysis of Synthetic Cannabinoids and their Metabolites in Urine using Solid Phase Extraction and LC/MS/MS

Matthew Trass, Shahana Huq, Seyed Sadjadi, Sky Countryman and Zdravko Milanov
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

In this study, we present a method for the analysis of a group of synthetic cannabinoids and their metabolites from urine. The method utilizes a simple Solid Phase Extraction (SPE) step followed by LC/MS/MS analysis using a Kinetex 2.6 µm core-shell C18 column, providing a reliable and reproducible analytical method that is suitable for use down to levels as low as 1 ng/mL and can be transferred to clinical, forensic and toxicology labs for analytical testing.

Introduction

Synthetic cannabinoids are a group of psychoactive aminoalkylindoles compounds that are designed to mimic the effects of marijuana. They have been found to act as agonists of cannabinoid CB1 and CB2 receptors to exert their physiological effects. These compounds are typically sprayed onto natural herbs for the purpose of smoking. The herbal product itself, which is normally marketed and sold as incense, often contains several different synthetic cannabinoids.

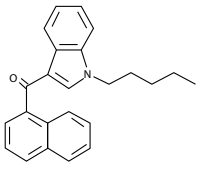
Due to the high affinity of these compounds to the cannabinoid receptors, their effective dose is lower than that of natural marijuana products, resulting in a low concentration of excreted metabolites. This, in turn, means that very sensitive analytical methods are required for the identification of these metabolites in drug testing assays (common synthetic cannabinoids and their corresponding urinary metabolites are listed in **Table 1**).

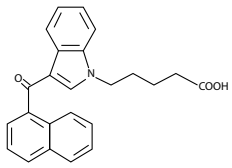
Because of their large potential for abuse and easy availability, these compounds have been listed as schedule one narcotics by the Drug Enforcement Agency (DEA). Thus, there has been an increased demand for the development of reliable, robust, and sensitive analytical methods to identify and quantify these compounds by clinical, forensic, and toxicology laboratories.

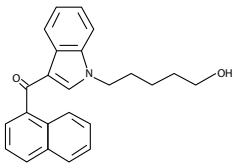
Materials and Methods

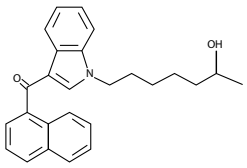
All reagents and solvents were HPLC or analytical grade. Analytical standards were purchased from Cayman Chemical. Analyses were performed using an Agilent® 1200 LC system (Agilent Technologies, Palo Alto, CA USA) equipped with an AB SCIEX API 4000™ LC/MS/MS detector (AB SCIEX, Framingham, MA, USA).

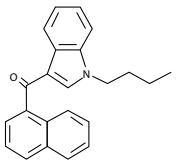
Table 1.
Synthetic cannabinoid structure

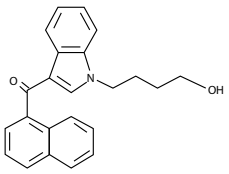

JWH018

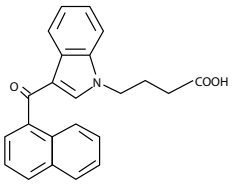

JWH018-Pentanoic acid metabolite

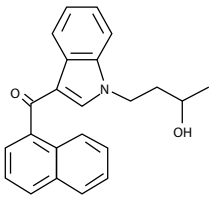

JWH018-5-Hydroxypentyl metabolite

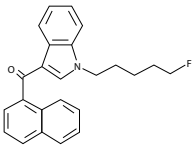

JWH018-4-Hydroxypentyl metabolite

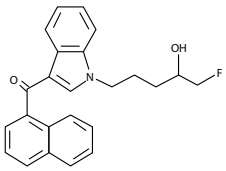

JWH073

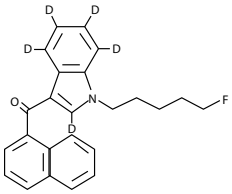

JWH073-4-Hydroxybutyl metabolite

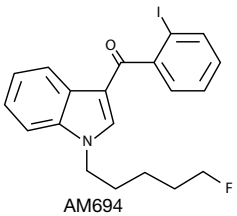

JWH073-Butanoic acid metabolite


JWH073-3-Hydroxybutyl metabolite


AM2201


AM2201-4-Hydroxypentyl metabolite


AM2201-D5


AM694

Sample Preparation

Hydrolysis: Combine 1 mL Human Urine sample (spiked with analytes at 50 ng/mL), 2 mL of 100 mM sodium acetate buffer, pH 5.0, 25 µL β-D-glucuronidase (Patella Vulgata from Sigma, 100KU). Vortex 10-15 secs, followed by incubation for 2 hours in a shaker at 55 °C to complete hydrolysis of the glucuronides.

Cartridge: Strata™-X-Drug B, 60 mg/6 mL

Part No.: 8B-S128-UBJ

Condition: Not Required

Load: Hydrolyzed sample (approx. 3 mL)

Wash 1: 2 mL 100 mM Sodium acetate buffer, pH 5.0

Wash 2: 2 mL Acetonitrile/ 100 mM Sodium acetate buffer, pH 5.0 (30:70)

Dry: >10" Hg for 5-10 minutes to remove residual water

Elute: 2 mL Ethyl acetate/Isopropanol (85:15)

Dry down: Nitrogen gas at 45 °C

Reconstitute: 0.5 mL of initial mobile phase

LC/MS/MS Parameters

Column: Kinetex 2.6 µm C18

Dimensions: 150 x 3.0 mm

Part No.: 00F-4462-Y0

Mobile Phase: A: 10 mM Ammonium formate
B: Acetonitrile

Gradient:

Time (min)	B (%)
0.00	45
7.00	50
7.01	95
10.00	95

Flow Rate: 0.6 mL/min

Injector Volume: 10 µL

Temperature: Ambient

Detection: MS/MS (AB SCIEX API 4000™)

Backpressure: 374 bar

Sample: 1 mL of urine spiked with analytes at 50 ng/mL

Ion source conditions:

Mode: Positive (+)

IS: 5500 V

TEM: 550 °C

Gas1: 50

Gas2: 50

Scan Type: MRM

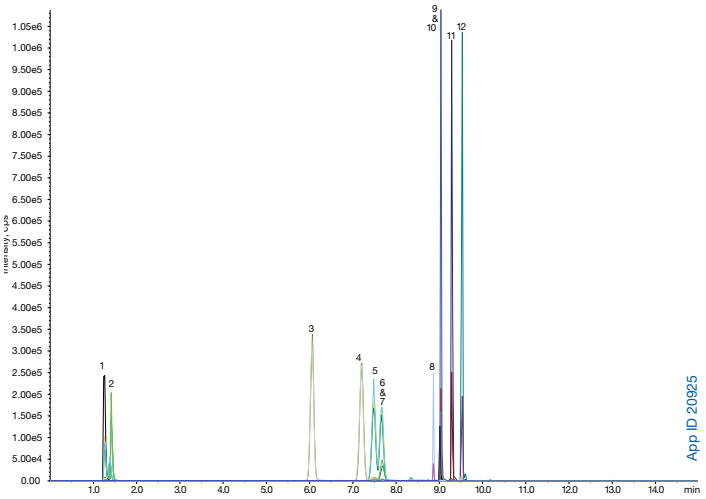
MRM Conditions

Q1	Q3	ID
360.2	155.1/232.1	AM2201
376.1	155.0	AM2201-4-hydroxypentyl
365.2	156.2	D5-AM2201
436.1	231.0/309.2	AM694
342.2	155.1/214.2	JWH018
372.2	155.1	JWH018-pentanoic acid
358.3	155.2/230.2	JWH018-5-hydroxypentyl
363.1	155.1	D5-JWH073-butanoic acid
358.3	155.2/230.2	JWH018-4-hydroxypentyl
358.3	155.2/230.2	JWH073-butanoic acid
344.2	155.2	JWH073-4-hydroxybutyl
376.2	155.1	D4-JWH018-pentanoic acid
328.2	155.2/200.2	JWH073
344.2	155.2	JWH073-3-hydroxybutyl

Results and Discussion

Figure 1 shows a representative chromatogram of a spiked sample of extracted urine, obtained using a core-shell Kinetex 2.6 µm C18, column. The metabolites must be subjected to enzymatic hydrolysis prior to LC/MS analysis, and an effective sample cleanup becomes crucial because proper sample cleanup will concentrate the analytes and remove matrix interferences, resulting in increased sensitivity and extended column lifetime. Strata-X-Drug B was selected as the ideal sorbent for sample cleanup because it does not require a condition or equilibration step, saving both time and solvent, and is QC tested by extracting drug probes from urine samples, ensuring that the product performs as expected in real life analysis. As shown in **Figure 1**, the SPE method, which utilized a 30 % acetonitrile wash, yielded a chromatogram free of matrix interferences. Aside from a clean baseline, the SPE cleanup step reduces system contamination resulting in less instrument downtime.

Figure 1.
Synthetic Cannabinoids Extracted from Urine



Column:	Kinetex 2.6 µm C18
Dimensions:	150 x 3.0 mm
Part No.:	00F-4462-Y0
Mobile Phase:	A: 10 mM Ammonium formate B: Acetonitrile

Gradient:	**Time (min)**	**B (%)**
	0.00	45
	7.00	50
	7.01	95
	10.00	95

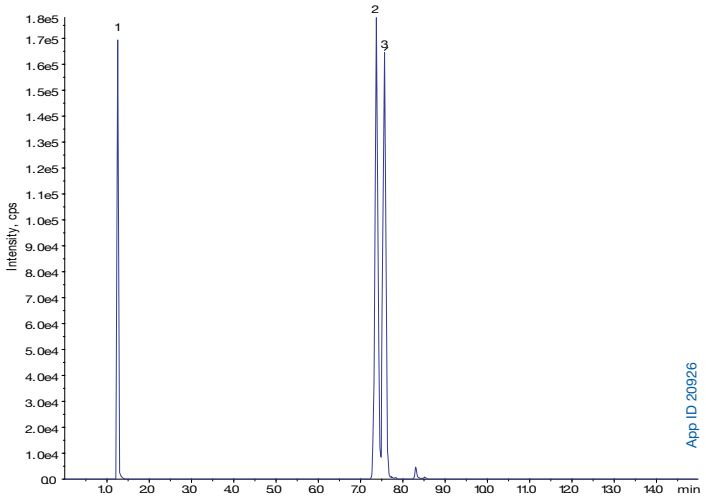
Flow Rate:	0.6 mL/min
Inj. Volume:	10 µL
Temperature:	Ambient
Detection:	MS/MS (AB SCIEX API 4000™)
Backpressure:	374 bar
Sample:	1 mL of human urine spiked with analytes at 50 ng/mL

1. JWH073-butanoic acid metabolite
2. JWH018-pentanoic acid metabolite
3. JWH073-4-hydroxybutyl metabolite
4. JWH073-3-hydroxybutyl metabolite
5. JWH018-5-hydroxypentyl metabolite
6. JWH018-4-hydroxypentyl metabolite
7. AM2201-4-hydroxypentyl metabolite
8. AM694
9. AM2201
10. D5-AM2201
11. JWH073
12. JWH018

Hydroxylation is a common way for metabolic transformation of xenobiotics as it facilitates their elimination from an organism. Based on the data reported for JWH analogs, it is natural to anticipate that the in vivo metabolism of JWH analogs should occur similarly. As shown on **Table 1**, along with the isomeric metabolites, the majority of the analytes are also structurally similar.

Figure 2 shows the extracted ion chromatogram for JWH018-4-hydroxypentyl, JWH018-5-hydroxypentyl and JWH073-butanoic acid. For accurate quantification, metabolites JWH018-4-hydroxypentyl and JWH018-5-hydroxypentyl (elute approx. 7.5 min) must be separated chromatographically because they share the same mass transitions. An analytical method for separation of these metabolites presents a significant challenge. In order to achieve separation of isobaric-pairs, we utilized the ultra-high performance of Kinetex core-shell media along with a relatively shallow gradient and relatively long run time.

Figure 2.
Chromatographic separation of JWH018-4-hydroxypentyl and JWH018-5-hydroxypentyl metabolites using a Kinetex 2.6 µm C18 column. These metabolites share the same MRM transitions and must be separated chromatographically in order to allow accurate quantitation.



Column:	Kinetex 2.6 µm C18
Dimensions:	150 x 3.0 mm
Part No.:	00F-4462-Y0
Mobile Phase:	A: 10 mM Ammonium formate B: Acetonitrile

Gradient:	**Time (min)**	**B (%)**
	0.00	45
	7.00	50
	7.01	95
	10.00	95

Flow Rate:	0.6 mL/min
Inj. Volume:	10 µL
Temperature:	Ambient
Detection:	MS/MS (AB SCIEX API 4000™)
Backpressure:	374 bar
Sample:	1 mL of urine spiked with analytes at 50 ng/mL 1. JWH073-butanoic acid metabolite 2. JWH018-5-hydroxypentyl metabolite 3. JWH018-4-hydroxypentyl metabolite

A successful cleanup step must be reproducible and yield enough recovery to achieve sensitivity requirements. **Figure 3** shows the absolute recovery for each of the synthetic cannabinoids, and the resulting CV values are contained in **Table 2** calculated for human urine samples spiked with analytes at 50 ng/mL. Both the absolute recoveries and the reproducibility produced by the SPE method are acceptable for routine testing with low limits of detection. This was achieved by utilizing Strata™-X-Drug B, a specialty SPE sorbent for extraction of basic drugs from biological matrices. The Strata-X-Drug B SPE procedures does not require conditioning or equilibration steps in order to achieve acceptable analyte recoveries, simplifying the sample preparation steps and increasing sample throughput.

Figure 3.
SPE Recovery of Synthetic Cannabinoids on Strata-X-Drug B

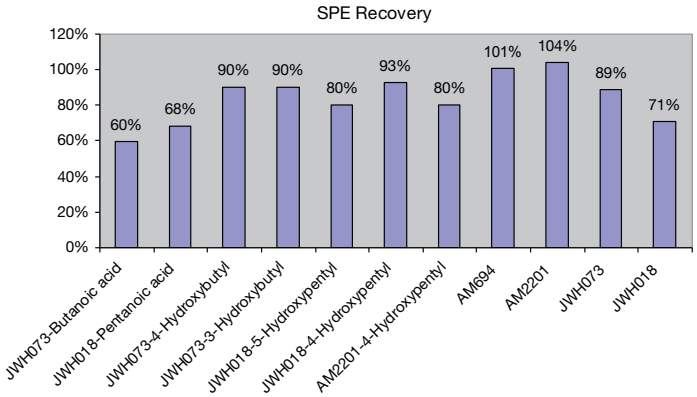


Table 2.
Percentage CV of Synthetic Cannabinoids Recovery Experiment using Strata-X-Drug B

Analyte	% CV
JWH073-Butanoic acid metabolite	13.20
JWH018-Pentanoic acid metabolit	7.60
JWH073-4-Hydroxybutyl metabolite	5.20
JWH073-3-Hydroxybutyl metabolite	3.70
JWH018-5-Hydroxypentyl metabolite	6.10
JWH018-4-Hydroxypentyl metabolite	1.60
AM2201-4-Hydroxypentyl metabolite	4.70
AM694	1.60
AM2201	5.40
JWH073	8.50
JWH018	1.90

Conclusion

In this technical note, we have described an analytical method for the analysis of synthetic cannabinoids and their metabolites. A simple and effective SPE was used to remove matrix interferences and was also required in order to achieve the necessary sensitivity to reach our target LOQ value of 1 ng/mL. The SPE step, in addition to improving method sensitivity, also minimizes system contamination, instrument downtime and greatly extends HPLC column lifetime.

Many of the target molecules are isomeric in nature, and have to be chromatographically resolved in order to quantify them accurately as they share the same mass transitions. This challenge was resolved by utilizing the ultra-high efficiency of a Kinetex 2.6 µm C18 core-shell HPLC column combined with a relatively long, shallow gradient.

Ordering Information

Strata™-X-Drug B SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	10 mg	8B-S128-AAK	1 mL (100/box)
	10 mg	8L-S128-AAK†	1 mL (100/box)
	30 mg	8B-S128-TAK	1 mL (100/box)
	30 mg	8L-S128-TAK†	1 mL (100/box)
	30 mg	8B-S128-TBJ	3 mL (50/box)
	60 mg	8B-S128-UBJ	3 mL (50/box)
	60 mg	8B-S128-UCH	6 mL (30/box)
	60 mg	8B-S128-UCL	6 mL (200/box)
Giga™ Tube			
	100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S128-AGB	2 Plates/box
	30 mg	8E-S128-TGB	2 Plates/box
	60 mg	8E-S128-UGB	2 Plates/box

†Tab-less tube

Ordering Information

Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Columns (mm)		SecurityGuard™ ULTRA Cartridges‡					SecurityGuard ULTRA Cartridges‡
Phases	50 x 2.1	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
C18	00B-4601-AN	AJ0-8782 for 2.1 mm ID	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768 for 4.6 mm ID

2.6 µm Analytical Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	3/pk
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AJ0-8768 for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)						SecurityGuard ULTRA Cartridges‡
Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	3/pk
C18	00A-4462-Y0	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0	AJ0-8775 for 3.0 mm ID

2.6 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

1.7 µm MidBore Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	50 x 3.0	100 x 3.0	3/pk
C18	00B-4475-Y0	00D-4475-Y0	AJ0-8775 for 3.0 mm ID

1.7 µm Minibore Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4475-AN	00B-4475-AN	00D-4475-AN	00F-4475-AN	AJ0-8782 for 2.1 mm ID

1.3 µm Columns (mm)	
Phase	50 x 2.1
C18	00B-4515-AN

‡SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

Kinetex core-shell HPLC/UHPLC columns are also available in other selectivities such as XB-C18, C8, PFP, Phenyl-Hexyl, and HILIC. Please visit www.phenomenex.com/kinetex for more details.

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

APPLICATIONS

Australia
t: 02-9428-6444
f: 02-9428-6445
auinfo@phenomenex.com

Austria
t: 01-319-1301
f: 01-319-1300
anfrage@phenomenex.com

Belgium
t: 02 503 4015 (French)
t: 02 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: (800) 543-3681
f: (310) 328-7768
info@phenomenex.com

Denmark
t: 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: 09 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: 01 30 09 21 10
f: 01 30 09 21 11
franceinfo@phenomenex.com

Germany
t: 06021-58830-0
f: 06021-58830-11
anfrage@phenomenex.com

India
t: 040-3012 2400
f: 040-3012 2411
indiainfo@phenomenex.com

Ireland
t: 01 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: 051 6327511
f: 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 001-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: 030-2418700
f: 030-2383749
nlinfo@phenomenex.com

New Zealand
t: 09-4780951
f: 09-4780952
nzinfo@phenomenex.com

Norway
t: 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: (800) 541-HPLC
f: (310) 328-7768
info@phenomenex.com

Sweden
t: 08 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: 01625-501367
f: 01625-501796
ukinfo@phenomenex.com

United States
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

All other countries:
Corporate Office USA
t: (310) 212-0555
f: (310) 328-7768
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN11360213_W