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APPLICATIONS

Screening and Confirmation of Synthetic Cannabinoid Urinary Metabolites using Kinetex® Core-Shell HPLC/UHPLC Columns and a Luna Fully Porous HPLC Column

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Simon Lomas is an avid futbol fan. He once took a futbol extravaganza trip to England and saw numerous EPL, Champions League, and WC qualifying matches.

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Using Kinetex Core-Shell Technology, a fast HPLC/UHPLC screening method for synthetic cannabinoid urinary metabolites was developed. Additional comprehensive confirmatory methods for HPLC and UHPLC instrumentation were generated using Luna and Kinetex LC columns.

Introduction

Synthetic cannabinoids are widely abused compounds that are DEA listed. Following use, the metabolized synthetic cannabinoids are excreted in the user's urine. Hence, the predominant approach for testing synthetic cannabinoid use is urine analysis.

Due to the related structures of the drug metabolites, there are a limited number of unique mass-transitions for the triple-quadrupole mass-spectrometer to monitor (**Table 1**). Therefore, it is critical that analytes requiring quantification are resolved chromatographically. This limitation can affect a laboratory's ability to rapidly process samples.

Depending on lab goals and instrumentation, there are different ways to perform the chromatographic analysis. Presented here is a screening method, a low pressure confirmatory method, and a UHPLC compatible confirmatory method for the analysis of synthetic cannabinoid urinary metabolites. A lab may adopt either a single method approach or split the analysis into a screening and confirmatory assay to maximize productivity.

Materials and Methods

Standards were purchased from Cayman Chemicals, Ann Arbor, MI.

Samples were analyzed using an Agilent® 1200 series HPLC (Agilent Technologies, Palo Alto, CA, USA.) coupled with an API 5000™ triple-quadrupole mass-spectrometer (AB SCIEX, Framingham, MA, USA.).

LC/MS/MS Conditions

Table 1. Cannabinoid Peak Identification

Peak No.	Analyte	Q1	Q3	Mode
1	JWH 073 N-butanoic acid metabolite-d5	363.1	159.1/155.2	+ve
2	JWH 073 N-butanoic acid metabolite	358.2	155.1/230.1	+ve
3	JWH 018 N-pentanoic acid metabolite-d4	376.2	155.1	+ve
4	JWH 018 N-pentanoic acid metabolite	372.2	268.1/155.1	+ve
5	JWH 073 N-(4-hydroxybutyl) metabolite	344.2	155.2/241.2	+ve
6	JWH 073 N-(3-hydroxybutyl) metabolite	344.2	155.2/241.2	+ve
7	JWH 018 N-(5-hydroxypentyl) metabolite	358.3	155.2/230.2	+ve
8	JWH 018 N-(4-hydroxypentyl) metabolite	358.3	155.2/230.2	+ve
9	AM2201 N-(4-hydroxypentyl) metabolite	376.1	155	+ve
10	AM694	436.1	231/309.2	+ve
11	AM2201-d5	365.2	156.2/203.1	+ve
12	AM2201	360.2	155.1/232.2	+ve
13	JWH-073	328.2	155.2/200.2	+ve
14	JWH-018	342.2	155.1/214.2	+ve

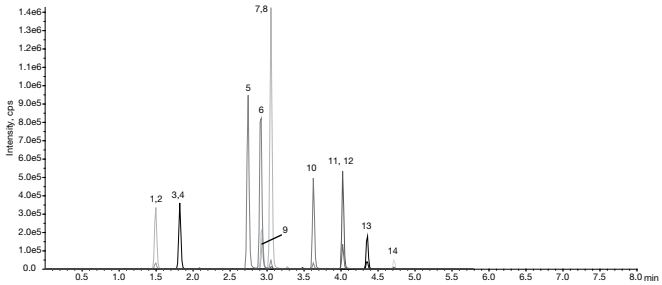
HPLC/UHPLC Screening Method

Column: Kinetex 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4462-AN
Mobile Phase: A: 10 mM Ammonium Formate
B: Acetonitrile/Methanol (50:50)
Flow Rate: 0.5 mL/min
Gradient:

Time (min)	B (%)
0	40
5	90
8	90

Backpressure: 170 bar
Inj. Volume: 0.5 µL
Temperature: Ambient
Detection: AB SCIEX API 5000 (MS/MS ESI+)
Sample: Synthetic cannabinoid urinary metabolites at 50 ng/mL (**Table 1**)

Figure 1. Synthetic cannabinoids on Kinetex 2.6 µm C18



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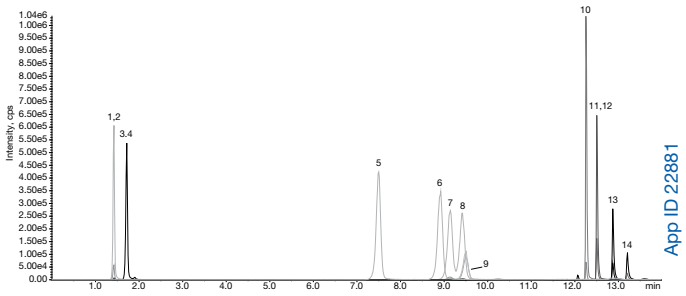
HPLC Confirmatory Method

Column: Luna 3 µm C18(2)
Dimensions: 150 x 3.0 mm
Part No.: 00F-4251-Y0
Mobile Phase: A: 10 mM Ammonium Formate
B: Acetonitrile
Flow Rate: 0.7 mL/min
Gradient:

Time (min)	B (%)
0	45
10	52
11	95
14	95

Backpressure: 267 bar
Inj. Volume: 1.0 µL
Temperature: Ambient
Detection: AB SCIEX API 5000™ (MS/MS ESI+)
Sample: Synthetic cannabinoid urinary metabolites at 50 ng/mL (**Table 1**)

Figure 2. Synthetic cannabinoids on Luna 3 µm C18(2)



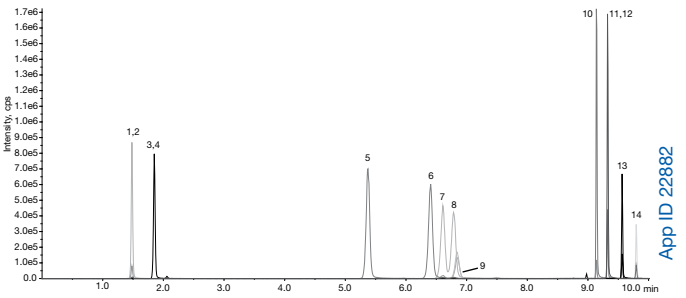
UHPLC Confirmatory Method

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
Flow Rate: 0.6 mL/min
Gradient:

Time (min)	B (%)
0	45
7	50
8	95
10	95

Backpressure: 375 bar
Inj. Volume: 1.0 µL
Temperature: Ambient
Detection: AB SCIEX API 5000 (MS/MS ESI+)
Sample: Synthetic cannabinoid urinary metabolites at 25 ng/mL (**Table 1**)

Figure 3. Synthetic cannabinoid on a Kinetex 2.6 µm C18



Recommended Sample Preparation Methodology

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/3 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

Results and Discussion

The fast screening method utilizing a Kinetex 2.6 µm C18 column allows for elution of all target compounds in less than 6 minutes. However, with a coelution of the 4- and 5-hydroxypentyl metabolites, a positive result for either of these isomers would require the use of one of the two confirmatory methods mentioned previously.

Both of these confirmatory methods obtain resolution of all compounds, including the difficult to resolve 4- and 5-hydroxypentyl metabolites. The method involving the Luna 3 µm C18(2) is suitable for HPLC instrumentation with lower pressure limitations, since it has an initial backpressure of 267 bar. Meanwhile, the second confirmatory method utilizes a Kinetex 2.6 µm C18 that's suitable for instrumentation with higher pressure limits such as UHPLC systems. By utilizing higher pressure rated instrumentation and a high efficiency Kinetex 2.6 µm core-shell HPLC column, this second confirmatory method obtains a much faster analysis time, less than 10 minutes, than the lower pressure method. Additionally, the Kinetex 5 µm C18 offers scientists another HPLC solution as selectivity would match with that of the Kinetex 2.6 µm C18, but with efficiency levels on par to the Luna 3 µm C18(2) and backpressures similar to a 5 µm fully porous media.

Conclusion

Synthetic cannabinoid urine analysis by LC/MS/MS focuses on the difficult to resolve cannabinoid metabolites. These drug-use markers can be resolved using both fully porous Luna 3 µm HPLC media and the even higher efficiency Kinetex 2.6 µm HPLC/UHPLC media. In addition to these completely resolved confirmatory methods, it can be beneficial for a lab to adopt a screening method prior to confirmation to increase productivity. In such cases, the high efficiency Kinetex 2.6 µm HPLC/UHPLC column is recommended, since it obtains excellent resolving power with limited backpressure.

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Ordering Infomation

Kinetex® Core-Shell HPLC/UHPLC Columns

5 µm Minibore Columns (mm)

Phases	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard™ ULTRA Cartridges‡ 3/pk
C18	00B-4601-AN	00D-4601-AN	00F-4601-AN	AJ0-8782 for 2.1 mm ID

5 µm MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges‡ 3/pk
C18	00B-4601-Y0	00D-4601-Y0	00F-4601-Y0	AJ0-8775 for 3.0 mm ID

5 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard ULTRA Cartridges‡ 3/pk
C18	00B-4601-E0	00D-4601-E0	00F-4601-E0	00G-4601-E0	AJ0-8768 for 4.6 mm ID

2.6 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges‡ 3/pk
C18	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)

Phases	30 x 3.0	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges‡ 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 Analytical Columns (mm)

Phases	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard ULTRA Cartridges‡ 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

Luna® Fully Porous HPLC Columns

3 µm MidBore and Analytical Columns (mm)

Phases	30 x 3.0	50 x 3.0	150 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard™ Cartridges (mm)* 4 x 2.0	4 x 3.0
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4286 for ID: 2.0-3.0 mm	AJ0-4287 3.2-8.0 mm

3 µm Microbore and Minibore Columns (mm)

Phases	50 x 1.0	150 x 1.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	SecurityGuard Cartridges (mm)* 4 x 2.0
C18(2)	00B-4251-A0	00F-4251-A0	00A-4251-B0	00B-4251-B0	00D-4251-B0	00F-4251-B0	AJ0-4286 for ID: 2.0-3.0 mm

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

*SecurityGuard analytical guard cartridges require holder, Part No.: KJ0-4282

Ordering Information

Strata™-X-Drug B SPE

Format	Sorbent Mass	Part Number	Unit
Tube			
	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

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