Extraction of Synthetic and Naturally Occurring Cannabinoids in Urine Using SPE and LC-MS/MS

UCT Part Numbers

SSHLD063
Styre Screen® HLD
60 mg, 3 mL cartridge

SPHACE5001-5
Select pH Buffer Pouches
100 mM Acetate Buffer pH 5.0

BETA-GLUC-50
50mL β - glucuronidase enzyme - liquid form

SLDA100ID21-3UM
Selectra® DA HPLC
100 X 2.1 mm, 3 µm

SLDAGDC21-3UM
Selectra® DA Guard Column
10 X 2.1 mm, 3 µm

SLGRDHLDR
Guard Column Holder

Summary:

Synthetic cannabinoids (Spice) are a family of compounds that, when consumed, mimic the effects of marijuana. These products are often marketed as "legal alternatives to cannabis" or "legal highs" and have dramatically increased in popularity among different drug user populations. The biggest hurdle for testing facilities is keeping up with the ever-changing synthetic analogs produced by illicit drug makers to avoid detection. The best detection methods are liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography/mass spectrometry (GC/MS). Historically, protocols target JWH-018 and JWH-073 and their metabolites. Protocol limitations include the unavailability of reference standards and standardized testing criteria. A method targeting current and new metabolites is vital for laboratories to keep up with the constantly changing market.

While much work still needs to be done to develop standardized methods for synthetic cannabinoids, one approach some laboratories have taken is to set the limit of detection as low as analytically possible. By pairing UCT's Styre Screen® HLD polymeric solid phase extraction cartridge with the Selectra® DA HPLC column, one can produce a cleaner, more concentrated sample leading to enhanced LOD's/LOQs.

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Sample Pretreatment:

1. To 1.0 mL of urine, add 2 mL of 100Mm Acetate buffer (pH 5.0) and 50 µL of beta-glucuronidase.

2. Vortex for 30 sec and heat at 65°C for 1-2 hours.

3. Allow the sample to cool.

SPE Procedure:

1. Sample Extraction

a) Load pretreated sample directly onto SPE cartridge.

2. Wash Cartridge

a) 1 x 3 mL 100mM Acetate buffer pH 5.
b) 1 x 3 mL MeOH:100mM Acetate buffer (25:75).
c) Dry the column under full vacuum or pressure for 10 minutes.

3. Elution

a) 1 x 3 mL Ethyl Acetate.

4. Concentration

a) Evaporate the sample to dryness at 35-40°C under a gentle stream of nitrogen.
b) Reconstitute in 100 µL of mobile phase starting gradient.

LC-MS/MS Parameters:

HPLC Parameters	
HPLC	Shimadzu Nexera LC-30AD with MS-8050
Column	SelectraCore® DA Column 100 x 2.1 mm, 2.7 µm (PN: SCS27-DA1021)
Guard Column	UCT, Selectra®, DA, 10 x 2.1 mm, 3 µm
Column Temperature	40°C
Column Flow Rate	0.300 mL/min
Auto-Sampler Temperature	10 °C
Injection volume	10 µL

Gradient:

Time (min)	A% (0.1% formic acid in H_2O)	B% (0.1% formic acid in MeOH)
0	0	50
1	20	80
4	20	80
5	0	100
9.5	0	100
10	50	50
14	50	50

MS Parameters	
Instrumentation	AB Sciex 4000 Q Trap
Polarity	ESI +
Spray voltage	5000 V
Vaporizer temperature	650 °C
Collision gas	Medium
Cycle time	6.2 sec
Acquisition method	Scheduled MRM

Analyte		MRM Transitions		Rt (min)
		Q1	Q3	
1	JWH-200	385.097	154.900	5.48
2	THC-OH	331.135	313.300	6.45
3	Cannabidiol	315.142	192.900	6.56
4	JWH-073 N Butanoic Acid	358.118	155.000	6.79
5	THC-COOH	345.101	327.100	6.87
6	JWH-018 N Pentanoic Acid	372.108	154.900	6.99
7	Cannabinol	311.051	223.000	7.46
8	THC	315.200	193.000	7.73
9	JWH-250	336.113	120.800	8.09
10	JWH-073	328.082	155.000	8.47
11	JWH-018	342.113	154.900	8.73

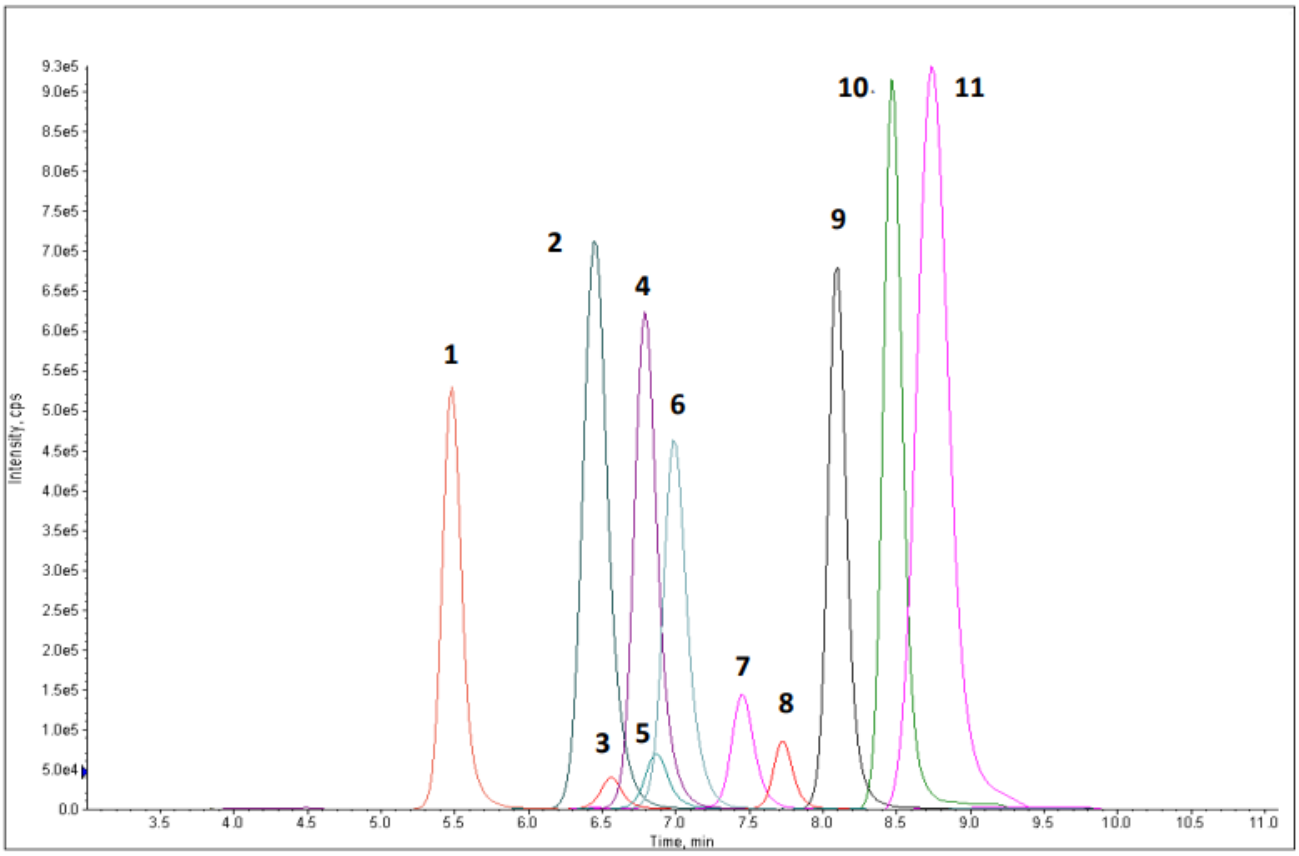

Figure 1: Chromatogram of a 100 ng/mL solvent standard

Compound Name	2.5 ng/mL		7.5 ng/mL		75 ng/mL		300 ng/mL	
	Avg. Recovery %	RSD% (n=3)	Avg. Recovery %	RSD% (n=3)	Avg. Recovery %	RSD% (n=3)	Avg. Recovery %	RSD% (n=3)
THC	81	4.7	82	5.8	74	6.2	70	5.4
JWH200	94	5.2	102	7.7	94	6.5	95	5.5
JWH073	81	5.5	93	6.3	89	7.5	89	6.4
JWH250	98	6.7	103	5.2	93	5.2	94	4.1
JWH018	77	4.3	93	4.5	83	4.1	81	3.8
CBN	81	6.8	81	6.7	69	4.8	66	6.8
CBD	86	5.5	91	6.9	78	5.4	76	3.4
THC-COOH	97	6.2	114	4.9	115	6.3	109	6.9
THC-OH	97	7.8	103	5.8	91	7.6	95	7.4
JWH073 Butanoic Acid	89	6.1	96	5.5	91	6.5	93	5.2
JWH018 Pentanoic Acid	98	4.8	99	3.2	92	8.1	91	5.1
Overall Mean	**89**	**5.7**	**96**	**5.6**	**88**	**6.2**	**87**	**5.4**

Discussion:

The properties of synthetic cannabinoids and their metabolites make them ideal candidates for clean-up via solid phase extraction (SPE). Opting to go with a polymeric resin allowed for eliminating conditioning steps, saving time and overall solvent usage. Several combinations of buffer/methanol washes were evaluated for optimal cleanliness and recovery, ranging from 75% buffer: 25% methanol to 50% buffer: 50% methanol. We achieved good recovery for most analytes under all conditions. Going above 25% methanol caused us to lose the metabolites of JWH compounds in the wash. 100% Ethyl Acetate was determined to be the best elution solvent after also evaluating 50% Ethyl Acetate: 50% Hexane, and 85% Ethyl Acetate: 15% IPA solvent combinations.

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Conclusions:

Using UCT's SSHLD extraction cartridge and methodology, both THC and synthetic cannabinoid levels can be monitored while simultaneously reducing analyst time and instrument time. The universal nature of this extraction method makes it amenable to other synthetic cannabinoids and metabolites, which is vital due to the continuous evolution of newly synthesized chemical analogs.

References:

[1] Arntson, Amanda. "Journal of Analytical Toxicology." Validation of a Novel Immunoassay for the Detection of Synthetic Cannabinoids and Metabolites in Urine Specimens. N.p., 26 Apr. 2013. Web. 10 July 2015.

[2] Crews, Bridgit O. "Synthetic Cannabinoids." - AACC.org. N.p., Feb. 2013. Web. 10 July 2015.

[3] "Synthetic Cannabinoids." Encyclopedia of Cancer (2009): 2891. Web

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