



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 column

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 μ m

SLDAGDC21-3UM

Selectra® DA Guard Column
10 X 2.1 mm, 3 μ m

SLGRDHDR

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 being produced by illicit drug makers in an attempt to avoid detection. Currently, the best methods for detection are liquid chromatography/ tandem mass spectrometry (LC-MS/MS) and gas chromatography/mass spectrometry (GC/MS). Historically, typical protocols target JWH-018 and JWH-073 and their metabolites. Such targeted protocols are generally limited by the availability of reference standards and lack of standardized testing criteria.

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 column with the Selectra® DA HPLC column, one can ultimately produce a cleaner more concentrated sample leading to enhanced LOD’s/LOQ’s. Having a method that can not only target current metabolites of interest, but also the new ones being created is vital for laboratories to keep up with the constantly changing market.



Sample Pretreatment:

To 1.0 mL of urine, add 2 mL of 100mM Acetate buffer (pH 5.0) and 50 µL of beta-glucuronidase, vortex for 30 sec and heat at 65°C for 1-2 hours. Allow sample to cool.

SPE Procedure:

1. Sample Extraction

- Load pretreated sample onto pre-conditioned SPE cartridge.

2. Wash Cartridge

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

3. Elution

- 1 x 3 mL Ethyl Acetate.

4. Concentration

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

LC-MS/MS Parameters:

HPLC Parameters		
HPLC: Agilent 1200 Series		
Column: UCT, Selectra [®] , DA, 100 x 2.1 mm, 3 µm		
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 program:		
Time (min)	A% (0.1% formic acid in H ₂ O)	B% (0.1% formic acid in MeOH)
0	50	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

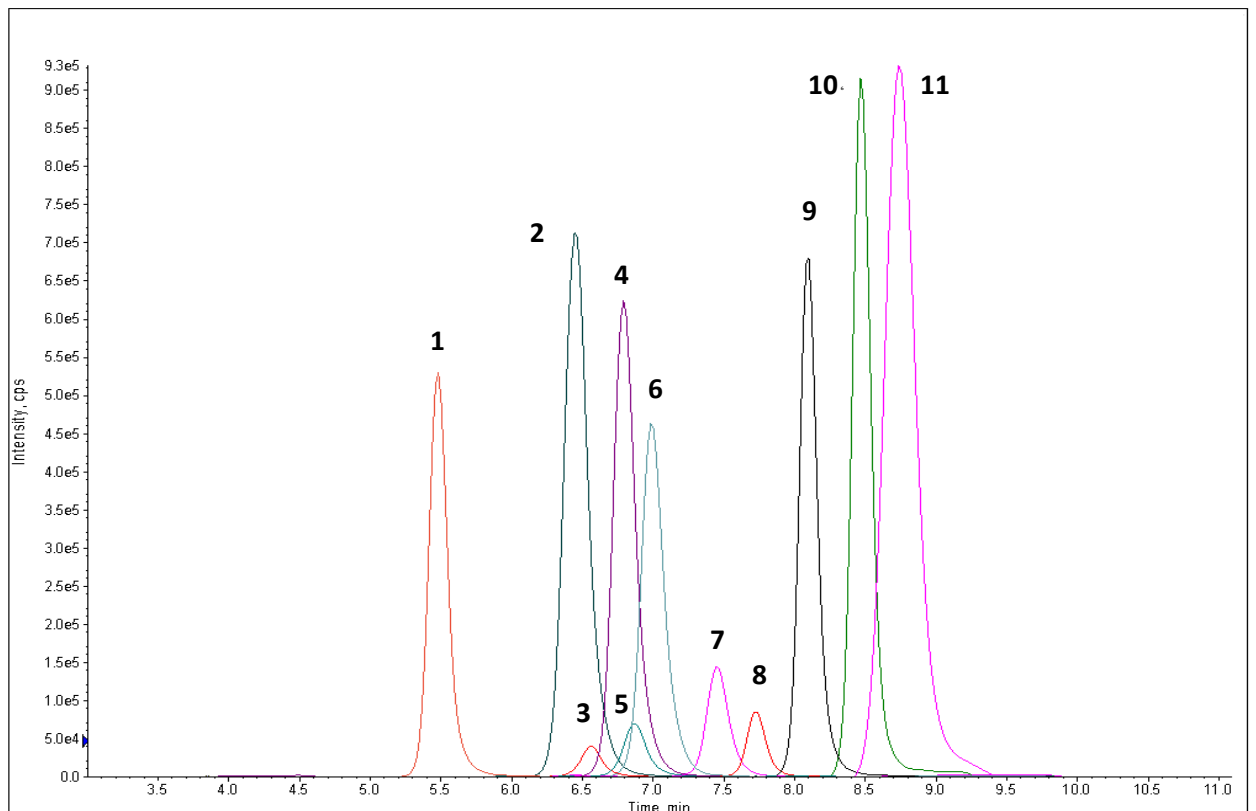


Figure 1: Chromatogram of a 100 ng/mL solvent standard

Results:

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 effects produced by synthetic cannabinoids are very similar to those induced from natural cannabinoid use. Currently, the most common way for screening individuals for recent cannabinoid usage is by immunoassay. Commercially available THC immunoassays do not cross react with synthetic cannabinoids which means labs have to develop mass-spectrometry based screening tests for these designer drugs. This simple extraction not only produces clean, concentrated extracts for spice drugs, but also THC and its metabolites.

The structures and pKa values 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 the elimination of a conditioning step which saved on time and 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. Although good recovery was achieved for most analytes under all conditions it was noted that going above 25% methanol caused the metabolites of JWH compounds to be lost 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.



Conclusions:

By utilizing UCT's SSHLD extraction columns and corresponding methodology, both THC and synthetic cannabinoid levels can be monitored simultaneously reducing both analyst time and instrument time. The universal nature of this extraction method makes it amenable to other various synthetic cannabinoids and metabolites, which is important 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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UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 •
215.781.9255 www.unitedchem.com Email: methods@unitedchem.com

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