Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS/MS

UCT Part Numbers

SSHLD063
Styre Screen® HLD
60 mg, 3mL Column

SLC-18100ID21-18UM
Selectra® C18 HPLC Column
100 X 2.1 mm, 1.8 µm

SPHPHO6001-10
Select PH Buffer Pouches 100 mM
Phosphate pH 6.0

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

SLC-18GDC20-18UMOPT
Selectra® C18 Guard Column
10 X 2.0 mm, 1.8 µm

Summary:

Newly identified synthetic cannabinoids pose a significant threat to public health and safety, as their implications in drug overdose and adverse events continue to rise in the United States and around the world. The diverse chemical structures of these compounds have a great impact on their potency and side effects. These synthetic cannabinoids were previously un-reported in forensic toxicology casework in the United States. There are currently few published methods available for the analysis of these novel compounds. However, the importance of identifying and extracting these compounds from various biological matrices is becoming more critical for accurate forensic criminal investigations and clinical diagnostics.

This application note outlines a solid-phase extraction (SPE) and LC-MS/MS method for the analysis of 19 synthetic cannabinoids in urine. These specific compounds were selected based on positivity rates from several key testing labs in the area. The use of UCT's Styre Screen® HLD highly crosslinked polymeric SPE sorbent ensures efficient extraction of the synthetic cannabinoids while removing undesired matrix components and yielding clean extracts. LC separation was carried out using a Selectra® C18 UHPLC column which resulted in excellent retention and baseline separation of the critical isobaric compounds ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolite in under 10 minutes.

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SPE Procedure:

1. Sample Preparation

- To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and internal standard(s)
- Mix/vortex briefly

  Note: A hydrolysis protocol may be required if conjugated compounds are to be included into the above drug panel

2. Condition Cartridge

- 1 x 1 mL MeOH
- 1 x 1 mL DI H_2O

3. Apply Sample

- Load sample at 1- 2 mL/minute

4. Wash Cartridge

- 1 x 2 mL DI H_2O
- 1 x 2 mL ACN: H_2O (20:80, v/v) containing 1% Formic Acid
- Dry cartridges under full vacuum or pressure for 5 minutes

5. Elute Analytes

- 1 x 3 mL Ethyl Acetate
- Collect at 1-2 mL/minute

6. Dry Eluate

- Evaporate to dryness at < 40°C.

7. Reconstitute

- Reconstitute sample in 1 mL of mobile phase or other appropriate organic solvent.

LC-MS/MS Parameters	
LC-MS/MS System	Shimadzu LCMS-8050
UHPLC Column	Selectra® C18 (100 X 2.1 mm, 1.8 µm)
Guard Column	Selectra® C18 (10 X 2.0 mm, 1.8 µm)
Column Temperature	50°C
Flow Rate	0.4 mL/min
Injection volume	5 µL

Gradient Program		
Time (min)	**% Mobile Phase A: 0.1% formic acid in DI H_2O**	**% Mobile Phase B: 0.1% formic acid in MeOH**
0	70	30
3.5	55	35
9	0	100
11	0	100
11.1	70	30
14	70	30

Chromatogram:

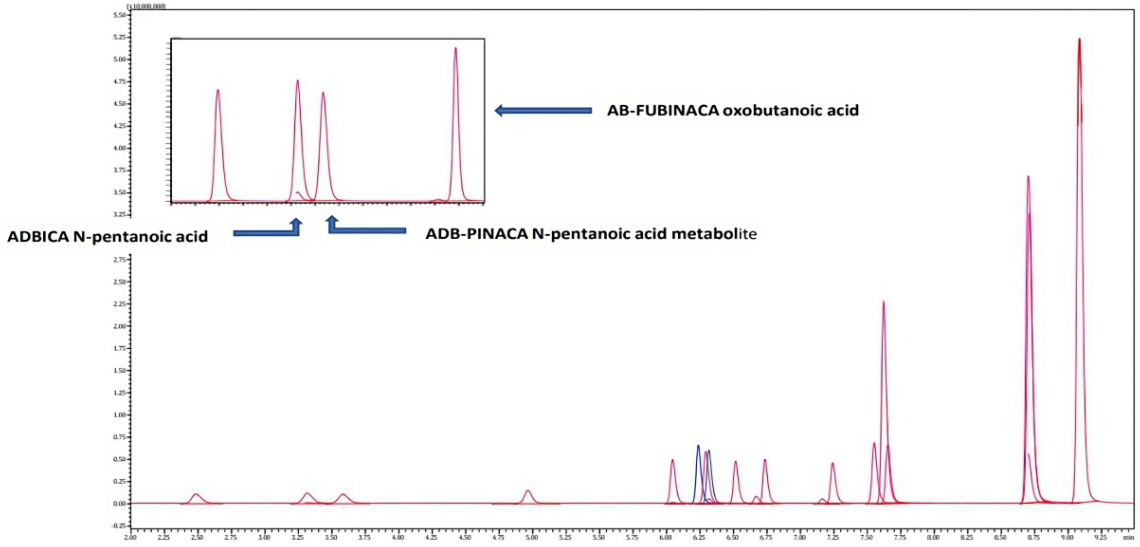

Figure 1: Chromatogram of 25 ng/mL extracted sample demonstrating the isobaric separation of ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolite.

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Results:

Recovery (%, n=5)				
Analyte	**2.5 ng/mL**	**RSD (%)**	**10 ng/mL**	**RSD (%)**
AB-PINACA N-Pentanoic Acid Metabolite	102%	0.16	110%	0.32
ADBICA N-Pentanoic Acid	115%	0.12	108%	0.71
ADB-PINACA N-Pentanoic Acid Metabolite	87%	0.12	113%	1.04
AB-FUBINACA Oxobutanoic Acid	92%	0.06	97%	0.14
5-Fluoro ADBICA	91%	0.08	106%	0.17
ADB-BICA	88%	0.09	101%	0.19
4-cyano CUMYL-BUTINACA	105%	0.09	112%	0.28
ADB-FUBICA	86%	0.12	106%	0.13
5-Fluoro MDMB-PICA	100%	0.11	109%	0.25
PB-22 3-Carboxyindole Metabolite	97%	0.12	107%	0.18
MDMB-FUBICA	98%	0.09	108%	0.19
BB-22-Carboxyindole Metabolite	103%	0.06	106%	0.18
UR-144 (XLR11) N-Pentanoic Acid Metabolite	95%	0.11	105%	0.20
AKB-48 N-Pentanoic Acid Metabolite	100%	0.09	109%	0.12
MDMB-FUBICA	93%	0.24	95%	0.30
AB-CHMINACA 3-methyl Butanoic Acid	99%	0.11	110%	0.24
BB-22	93%	0.27	103%	0.66
MA-CHMINACA	95%	0.38	97%	0.49
MDMB-CHMINACA	100%	0.43	100%	1.02

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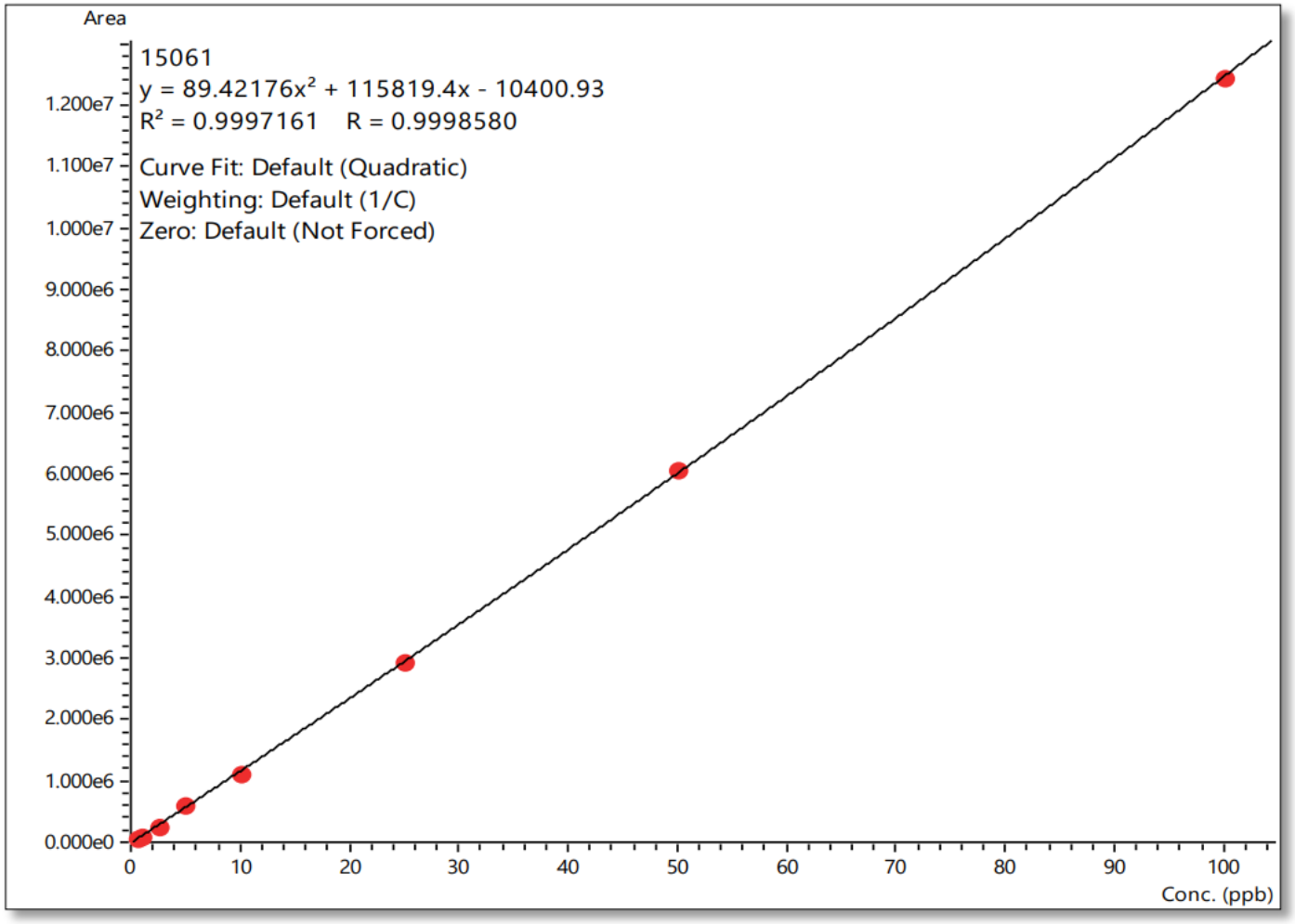
Representative Calibration Curve (ADBICA N-Pentanoic Acid):



Conclusions:

This application note outlines a simple SPE procedure for the analysis of 19 synthetic cannabinoids in urine using UCT's Styre Screen® HLD highly crosslinked polymeric SPE cartridges. All 19 compounds were analyzed in under 10 minutes using LC-MS/MS. The use of a Selectra® C18 UHPLC column resulted in excellent peak shape for all the compounds included in the method, including baseline separation of any isobaric compounds. The recoveries obtained in this research study are satisfactory for the vast majority of analytes despite their diverse chemical structures. Extracted urine samples fortified at two concentrations (2.5 and 10 ng/mL) had, on average, recoveries in the range of 85-110% and corresponding RSD values less than 5%. The quality control concentrations of 2.5 ng/mL and 10 ng/mL were chosen to ensure low-level accurate detection based on heightened potency for this class of compounds at exceptionally low biological levels. This method will be beneficial to any lab looking to implement testing of these novel synthetic cannabinoids.

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