NOVEL UPLC® COLUMNS FOR THE ANALYSIS OF SYNTHETIC CANNABINOIDS AND METABOLITES IN WHOLE BLOOD FOR FORENSIC TOXICOLOGY

新規ソリッドコアパーティクル UPLC カラムを用いた全血中の合成カンナビノイド及びその代謝物分析

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INTRODUCTION

Synthetic cannabinoids, often referred to or marketed as "Spice" compounds, constitute a growing challenge for law enforcement agencies and forensic laboratories. These drugs mimic the psychoactive effects of natural cannabinoids, and their popularity and use have risen substantially in the last several years.[1, 2] While recent legislation has banned some of these compounds, minor modifications to existing structures have resulted in a proliferation of substances designed to circumvent existing laws. This current work details a strategy for the successful extraction and analysis of representatives of several different classes of synthetic cannabinoids from whole blood samples for forensic toxicology. A total of 22 synthetic cannabinoids and metabolites were extracted from whole blood samples using a rapid and universal sample preparation strategy that provides effective sample cleanup and is generic enough to use on a variety of compounds with different chemical properties. Analytical separation was achieved using Waters' newly developed CORTECS™ UPLC solid-core particle column with optimally packed 1.6 µm particles, resulting in exceptional performance and separation efficiency. Extraction recoveries ranged from 73 to 105% with an average of 92% and matrix effects were less than 20% for all compounds with only 3 greater than 15%. Calibration curves were linear from 2-500 ng/mL, with accurate and precise results from quality control samples. The analysis of several different classes of these drugs should render this method applicable to newly developed related compounds with little, if any, modification necessary.

METHODS

Chemicals and Materials

All target compounds and metabolites were obtained from Cerilliant (Round Rock, TX) and Cayman Chemical (Ann Arbor, MI).

Sample Preparation

50 µL whole blood was added to 150 µL 0.1M ZnSO4/ NH4CH3COOH in OstroTM sample preparation plate wells. Samples were vortexed for 5 sec. 600 µL ACN was then added and samples were vortexed for 3 min. Samples were then eluted under vacuum into 2 mL 96 -well collection plates. 10 µL was injected onto the UPLC/MS/MS system

Equipment

Sample Prep:	Ostro Sample Preparation Plates
UPLC System:	ACQUITY UPLC®
MS:	ACQUITY TQD
Column:	CORTECS™ UPLC C_{18} 2.1 x 100 mm 1.6 µm
Mobile Phase A:	Water + 0.1% formic acid (FA)
Mobile Phase B:	ACN + 0.1% FA

Gradient Table

Time	Flow	%A	%B
0.0	0.6	70	30
2.0	0.6	50	50
3.0	0.6	50	50
7.0	0.6	10	90
7.2	0.6	70	30
8.0	0.6	70	30

Recovery and Matrix Effects

Analyte recovery was calculated according to the following equation:

$$\%Recovery = \left(\frac{Area\ A}{Area\ B}\right) x100\%$$

Where A =the peak area of an extracted sample and B = the peak area of an extracted matrix sample in which the compounds were added post-extraction.

Matrix effects were calculated according to the following equation:

$$Matrix\ Effects = \left(\left(\frac{Peak\ area\ in\ the\ presence\ of\ matrix}{Peak\ area\ in\ the\ absence\ of\ matrix}\right) - 1\right) x\ 100\%$$

The peak area in the presence of matrix refers to the peak area of an extracted matrix sample in which the compounds were added post-extraction. The peak area in the absence of matrix refers to analytes in a neat solvent solution.

RESULTS

A representative chromatogram of all compounds from a 20 ng/mL calibration standard is shown in **Figure 1.** Peak assignments are listed in **Table 1.** Peak shape was excellent for all compounds, with no significant tailing, and all peak widths were under 3 seconds. Peaks 9 and 10, an isobaric pair of metabolites with identical precursor and product ions, were nearly baseline resolved, with a calculated resolution of 1.04, enabling unambiguous identification that would not be possible if the two compounds co-eluted. When the same mix of compounds was analyzed on an ACQUITY UPLC BEH C_{18} column, adequate separation was not achieved for these two compounds (**Figure 1B**). Co-elution of compounds 5 and 6, and 7 and 8 were also seen on the hybrid column. A comparison of all peaks was performed between the CORTECS UPLC column and two fully porous based UPLC columns (BEH C_{18} and HSS T3). This analysis revealed that peak widths on the fully porous columns ranged from equivalent to those seen on the CORTECS UPLC column to more than 2X as wide. On average, peak widths on the BEH and HSS T3 UPLC columns were 14% and 30% greater, respectively, than those seen with the CORTECS UPLC column (see **Table 2.**).

No.	Compound	RT	Mol. Formula	Cone volt-age	MRM Transitions	Coll. energy
1	AM2233	1.04	$C_{22}H_{23}IN_2O$	40 40	459.2→98.05 459.2→112.1	34 22
2	RCS-4, M10	1.40	$C_{20}H_{21}NO_3$	40 40	324.2→121.0 324.2→93.0	22 46
3	RCS-4, M11	1.62	$C_{20}H_{19}NO_3$	36 36	322.2→121.0 322.2→93.0	22 46
4	AM 1248	1.87	$C_{26}H_{34}N_2O$	56 56	391.4→135.1 391.4→112.1	28 30
5	JWH-073 4-butanoic acid met.	2.54	$C_{23}H_{19}NO_3$	50 50	358.2→155.1 358.2→127.1	26 48
6	JWH-073 4-hydroxybutyl met.	2.57	$C_{23}H_{21}NO_2$	50 50	344.2→155.1 344.2→127.1	22 40
7	JWH-018 5-pentanoic acid met.	2.77	$C_{24}H_{21}NO_3$	46 46	372.2→155.1 372.2→127.1	24 50
8	JWH-073 (+/-) 3-hydroxybutyl met.	2.81	$C_{23}H_{21}NO_2$	44 44	344.2→155.1 344.2→127.1	26 46
9	JWH-018 5-hydroxypentyl met.	2.91	$C_{24}H_{23}NO_2$	40 44	358.2→155.1 358.2→127.1	24 48
10	JWH-018 (+/-) 4-hydroxypentyl met.	2.96	$C_{24}H_{23}NO_2$	40 44	358.2→155.1 358.2→127.1	24 48
11	JWH-015	5.04	$C_{23}H_{21}NO$	42 42	328.2→155.1 328.2→127.1	24 42
12	RCS-4	5.05	$C_{21}H_{23}NO_2$	44 44	322.2→135.1 322.2→92.0	26 64
14	JWH-022	5.41	$C_{24}H_{21}NO$	50 50	340.2→155.1 340.2→127.1	26 54
13	JWH-073	5.41	$C_{23}H_{21}NO$	48 48	328.2→155.1 328.2→127.1	26 48
15	XLR-11	5.52	$C_{21}H_{28}FNO$	48 48	330.3→125.1 330.3→97.1	26 32
16	JWH-203	5.66	$C_{21}H_{22}ClNO$	46 46	340.2→125.0 340.2→188.1	26 20
17	JWH-018	5.88	$C_{24}H_{23}NO$	44 44	342.2→155.1 342.2→127.1	26 42
18	RCS-8	6.30	$C_{25}H_{29}NO_2$	42 42	376.3→121.1 376.3→91.0	26 50
19	UR-144	6.43	$C_{21}H_{29}NO$	46 46	312.3→125.1 312.3→214.2	24 25
20	JWH-210	6.61	$C_{26}H_{27}NO$	48 48	370.2→183.1 370.2→155.1	26 38
21	AB 001	6.97	$C_{24}H_{31}NO$	52 52	350.3→135.1 350.3→93.0	30 46
22	AKB 48	7.13	$C_{23}H_{31}N_3O$	38 38	366.3→135.1 366.3→93.1	22 50

Table 1. *Retention times, chemical formulae and MS conditions. Compounds highlighted with the same color, have identical precursor and product ions requiring near baseline resolution.*

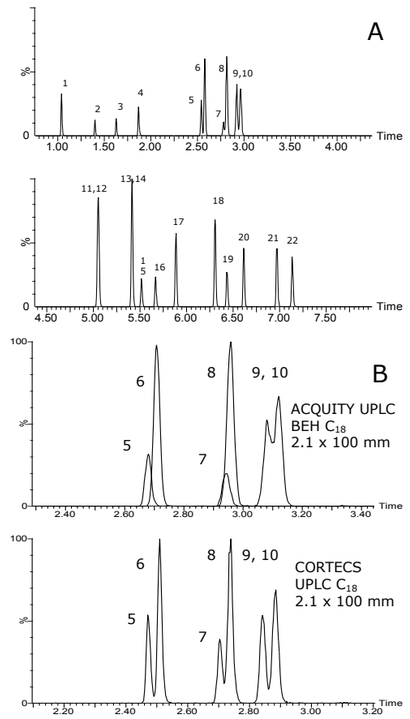

Figure 1. A. *Chromatography of synthetic cannabinoid compounds and metabolites. See Table 1 for peak assignments.* ***B.*** *Comparative chromatography for compounds 5-10 on the CORTECS UPLC C_{18} 1.6 µm and an ACQUITY UPLC BEH C_{18} 1.7 µm column is shown below the full chromatogram. See* ***Table 1****. for peak assignments.*

	Peak Width (sec)			Norm. Peak Width		
	CORTECS	BEH	HSS T3	CORTECS	BEH	HSS T3
Mean	2.30	2.59	2.96	1.00	1.14	1.30

Table 2. *Peak width comparison between a CORTECS UPLC C_{18} column, and BEH C_{18} and HSS T3 columns of equivalent dimensions*

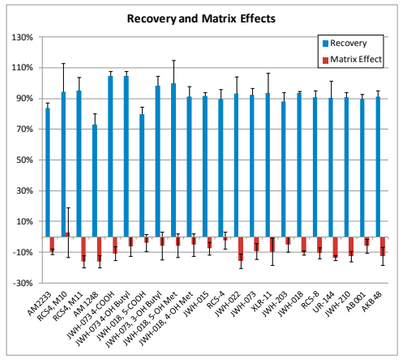

Figure 2. *Recoveries and matrix effects for synthetic cannabinoids extracted from whole blood. The blue bars indicate recovery (n=4) and the red bars indicate matrix effects (n=4) for each compound. All compounds demonstrated excellent recoveries, with all but one compound at 80% or greater and an average recovery of 92%. Matrix effects were minimal for all compounds.*

	QC concentrations (ng/mL)						
		7.5		75		300	
	R^2	%Acc.	%RSD	%Acc.	%RSD	%Acc.	%RSD	Mean % Acc.
AM2233	0.997	100.5	2.0%	103.6	3.3%	100.5	2.0%	**101.5**
RCS4, M10	0.986	97.5	3.9%	106.1	5.7%	101.7	8.4%	**101.7**
RCS4, M11	0.991	91.3	16.3%	108.8	5.1%	96.8	12.0%	**98.9**
AM 1248	0.993	83.1	10.0%	106.1	5.7%	105.4	6.4%	**98.2**
JWH-073 4-COOH	0.991	96.1	9.8%	99.3	7.4%	106.2	9.1%	**100.5**
JWH-073 4-OH Butyl	0.996	88.7	21.3%	98.1	3.5%	102.2	3.9%	**96.3**
JWH-018, 5-COOH	0.992	90.7	15.2%	97.8	3.8%	103.7	10.6%	**97.4**
JWH-073, 3-OH Butyl	0.993	79.0	8.6%	92.9	8.3%	96.6	2.9%	**89.5**
JWH-018, 5-OH Met	0.995	82.8	10.3%	100.0	10.4%	100.1	3.4%	**94.3**
JWH-018, 4-OH Met	0.992	82.3	17.9%	103.1	6.3%	96.0	1.9%	**93.8**
JWH-015	0.993	87.1	4.3%	101.8	3.9%	101.3	2.1%	**96.8**
RCS-4	0.993	92.5	8.1%	99.6	5.0%	97.3	3.6%	**96.4**
JWH-022	0.993	85.3	4.9%	100.3	4.8%	97.8	4.2%	**94.5**
JWH-073	0.994	89.6	6.5%	99.4	6.6%	97.6	4.9%	**95.5**
XLR-11	0.993	101.4	10.4%	99.6	2.8%	99.7	5.0%	**100.2**
JWH-203	0.990	82.1	12.2%	96.1	12.2%	94.6	9.3%	**91.0**
JWH-018	0.994	88.4	2.9%	97.2	3.9%	98.8	3.6%	**94.8**
RCS-8	0.992	94.3	2.6%	101.9	4.6%	99.4	4.7%	**98.5**
UR-144	0.994	85.1	5.4%	97.0	6.7%	99.2	3.7%	**93.8**
JWH-210	0.994	92.7	6.4%	96.3	4.5%	95.6	5.3%	**94.8**
AB 001	0.992	84.4	8.1%	101.0	4.7%	100.2	10.6%	**95.2**
AKB 48	0.992	92.8	9.9%	98.5	4.8%	97.7	8.4%	**96.4**
Mean % Acc.		**89.4**		**100.2**		**99.5**		

Table 3. *R^2 values for calibration curves, % accuracy and precision (%RSD) for QC samples. The far right column shows accuracy averages across all QC levels and the bottom row shows accuracy averages for all compounds at each QC level.*

Linearity, accuracy and precision

Calibration curves were extracted at concentrations ranging from 2-500 ng/mL for all components. Quality control samples (*N*=4) were prepared at 7.5, 75, and 300 ng/mL. **Table 3** summarizes R^2 values from the calibration curves and QC summary data for all compounds. All compounds showed excellent linearity over the entire calibration range with R^2 values of >0.99 for 21 of the 22 compounds. Signal-to-noise ratios were excellent with all compounds demonstrating linear responses down to 2 ng/mL. Quality control (QC) results were accurate and precise at low, medium and high concentrations. Accuracies for low level QC samples (7.5 ng/mL) ranged from 79.0-104.4% with an average of 89.4%. The results for the medium and high QC levels were excellent for all analytes, with all accuracies within 10% of expected values. Analytical precision was excellent with most % RSDs less than 10% and none greater than 13%. When QC accuracy was assessed over all levels (low, medium, and high), the means ranged from 89.5% to 101.7%.

CONCLUSIONS

- **Improved resolution on CORTECS UPLC solid -core particle column vs. fully porous particle columns**
- **Successful extraction of 22 synthetic cannabinoids and metabolites from whole blood using Ostro sample preparation plates**
- **Excellent recovery and minimal matrix effects**
- **Linear, accurate and precise performance for all compounds**
- **Resolution of critical isobaric compounds**

References

1. Seely, K.A., et al., *Spice drugs are more than harmless herbal blends: A review of the pharmacology and toxicology of synthetic cannabinoids.* Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2012. **39** (2): p. 234-243.
2. Wohlfarth, A. and W. Weinmann, *Bioanalysis of new designer drugs.* Bioanalysis, 2010. **2**(5): p. 965-979.

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