Separation and Characterization of Synthetic Cannabinoid Metabolite Isomers using SLIM High-Resolution Ion Mobility-Tandem Mass Spectrometry (HRIM-MS/MS)

Ralph Aderorho, Shadrack Wilson Lucas, Christopher D. Chouinard*
Department of Chemistry, Clemson University, Clemson, SC 29634, USA

***Corresponding Author:** Christopher D. Chouinard (cchouin@clemson.edu); Hunter Chemistry Laboratory, 211 S. Palmetto Blvd, Clemson, SC 29634, USA
ORCID ID: 0000-0002-1416-1812

Keywords: Synthetic cannabinoids, Isomers, SLIM, Ion Mobility-Mass Spectrometry (IM-MS)

Supporting Tables

Table S1. Instrumental parameters for the MOBILion MOBIE SLIM & Agilent 6546 QTOF.

Instrument Region	Instrumental Parameter	Experimental Value
Ionization Source	Polarity	Positive
	Gas Temp	325 °C
	Drying Gas	12 L/min
	Nebulizer	20 psi
	Sheath Gas Temp	275 °C
	Sheath Gas Flow	10 L/min
	VCap	4000 V
	Nozzle Voltage (Expt)	1000 V
	Fragmentor	400 V
	Oct 1 RF Vpp	750 V
SLIM Conditions	Funnel In	165 V
	Funnel Out	100 V
	Funnel Conductance Limit (CL)	95 V
	SLIM Bias	90 V
	SLIM Mode	HRIM (13 m)
	SLIM Wave Shape	Sine
	Fill Time	40 ms
	Trap Time	0.3 ms
	Release Time	3.2 ms
	IMS Frame Length	1000 ms
	Fill TW Frequency	15 kHz
	Fill TW Amplitude	5 V_{pp}
	Release TW Frequency	15 kHz
	Release TW Amplitude	30 V_{pp}
	Separation TW Frequency	20 kHz
	Separation TW Amplitude	35 V_{pp}
	SLIM Exit CL	50 V
	Quad Bias	45 V
	Quad Pressure (Rough Vac)	2.5 Torr
	SLIM RF Amplitude	270 V
	SLIM RF Frequency	1200 kHz

Table S2. Experimental $^{DT}CCS_{N2}$ and raw $^{SLIM}CCS_{N2}$ for the protonated species, $[M+H]^+$, for all metabolites. These $^{SLIM}CCS_{N2}$ values were derived based on a calibration using the Agilent Tune Mix ions. Chemical formulae and theoretical *m/z* are also included.

Metabolite	Formula	$[M+H]^+$ *m/z*	$^{DT}CCS_{N2}$ (Å^2)	$^{SLIM}CCS_{N2}$ (Å^2)	ΔCCS (%)
JWH 018 4-hydroxyindole			189.0 ± 0.1	187.8 ± 0.2	-0.64%
JWH 018 N-(5-hydroxypentyl)	$C_{24}H_{23}NO_2$	358.181	187.0 ± 0.1	187.0 ± 0.2	0.00%
JWH 018 6-hydroxyindole			192.4 ± 0.1	191.0 ± 0.4	-0.73%
JWH 250 N-(4-hydroxypentyl)			187.6 ± 0.1	186.4 ± 0.2	-0.64%
JWH 250 N-(5-hydroxypentyl)	$C_{22}H_{25}NO_3$	352.191	187.6 ± 0.1	186.7 ± 0.4	-0.48%
JWH 250 5-hydroxyindole			191.5 ± 0.1	189.9 ± 0.3	-0.84%
MDA-19 N-(4-hydroxybenzoyl)			196.1 ± 0.1	194.2 ± 0.1	-0.97%
MDA-19 N-(5-hydroxyhexyl)	$C_{21}H_{23}N_3O_3$	366.182	189.3 ± 0.1	188.3 ± 0.1	-0.53%
4-cyano CUMYL-BUTINACA			186.4 ± 0.1	ND	
APP-BUTINACA phenylpropanoic acid			187.3 ± 0.1	185.3 ± 0.4	-1.07%

Table S3. Experimental $^{DT}CCS_{N2}$ and raw $^{SLIM}CCS_{N2}$ for the sodiated species, $[M+Na]^+$, for all metabolites. These $^{SLIM}CCS_{N2}$ values were derived based on a calibration using the Agilent Tune Mix ions. Chemical formulae and theoretical *m/z* are also included.

Metabolite	Formula	$[M+Na]^+$ *m/z*	$^{DT}CCS_{N2}$ (Å^2)	$^{SLIM}CCS_{N2}$ (Å^2)	ΔCCS (%)
JWH 018 4-hydroxyindole			206.4 ± 0.1	204.6 ± 0.1	-0.88%
JWH 018 N-(5-hydroxypentyl)	$C_{24}H_{23}NO_2$	380.163	193.9 ± 0.1	191.6 ± 0.1	-1.19%
JWH 018 6-hydroxyindole			206.3 ± 0.1	207.5 ± 0.1	0.58%
JWH 250 N-(4-hydroxypentyl)			184.3 ± 0.1	186.1 ± 0.2	0.97%
JWH 250 N-(5-hydroxypentyl)	$C_{22}H_{25}NO_3$	374.173	183.8 ± 0.1	181.1 ± 0.4	-1.48%
JWH 250 5-hydroxyindole			203.7 ± 0.1	200.8 ± 0.2	-1.43%
MDA-19 N-(4-hydroxybenzoyl)			211.1 ± 0.1	208.4 ± 0.3	-1.29%
MDA-19 N-(5-hydroxyhexyl)	$C_{21}H_{23}N_3O_3$	388.164	190.8 ± 0.1	189.4 ± 0.2	-0.74%
4-cyano CUMYL-BUTINACA			195.7 ± 0.1	193.5 ± 0.2	-1.13%
APP-BUTINACA phenylpropanoic acid			194.8 ± 0.1	192.7 ± 0.1	-1.08%

Table S4. Experimental $^{DT}CCS_{N2}$ and raw $^{SLIM}CCS_{N2}$ for the sodiated dimer species, $[2M+Na]^+$, for all metabolites. These $^{SLIM}CCS_{N2}$ values were derived based on a calibration using the Agilent Tune Mix ions. Chemical formulae and theoretical *m/z* are also included.

Metabolite	Formula	$[M+Na]^+$ *m/z*	$^{DT}CCS_{N2}$ (Å^2)	$^{SLIM}CCS_{N2}$ (Å^2)	ΔCCS (%)
JWH 018 4-hydroxyindole	$C_{24}H_{23}NO_2$	737.336	281.7 ± 0.1	289.5 ± 0.4	2.73%
JWH 018 N-(5-hydroxypentyl)			261.9 ± 0.1	267.9 ± 0.1	2.27%
JWH 018 6-hydroxyindole			290.8 ± 0.1	296.5 ± 0.2	1.94%
JWH 250 N-(4-hydroxypentyl)	$C_{22}H_{25}NO_3$	725.357	271.4 ± 0.1	278.3 ± 0.2	2.51%
JWH 250 N-(5-hydroxypentyl)			259.1 ± 0.1	266.4 ± 0.2	2.78%
JWH 250 5-hydroxyindole			267.6 ± 0.1	273.4 ± 0.2	2.14%
MDA-19 N-(4-hydroxybenzoyl)	$C_{21}H_{23}N_3O_3$	753.338	281.6 ± 0.1	285.8 ± 0.2	1.48%
MDA-19 N-(5-hydroxyhexyl)			268.4 ± 0.1	272.8 ± 0.1	1.63%
4-cyano CUMYL-BUTINACA			264.5 ± 0.1	268.2 ± 0.3	1.39%
APP-BUTINACA phenylpropanoic acid			275.0 ± 0.1	279.2 ± 0.3	1.52%

Supporting Figures

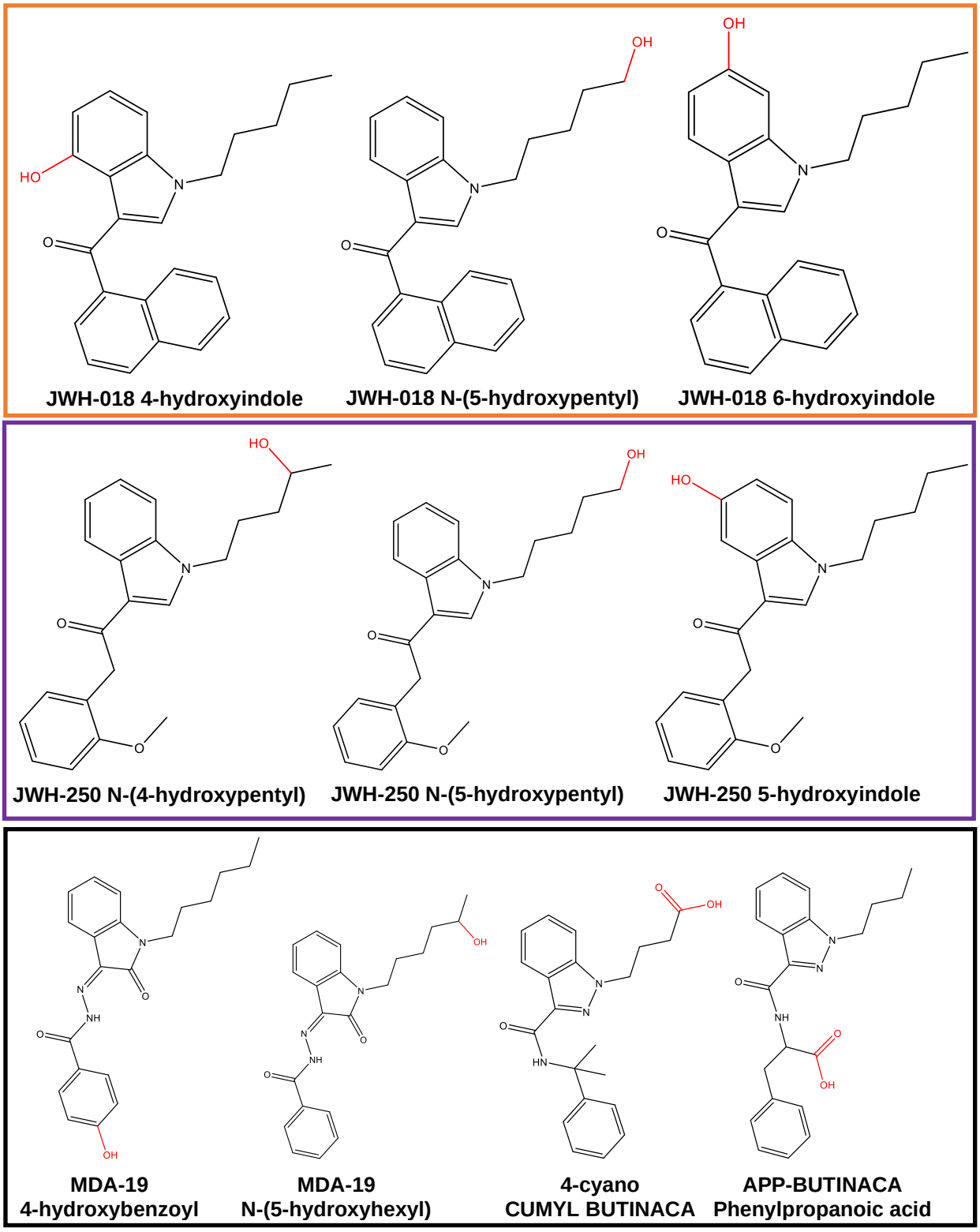

Figure S1. Structures of the synthetic cannabinoid metabolites analyzed in this study, grouped by isomers with structural differences highlighted in red.

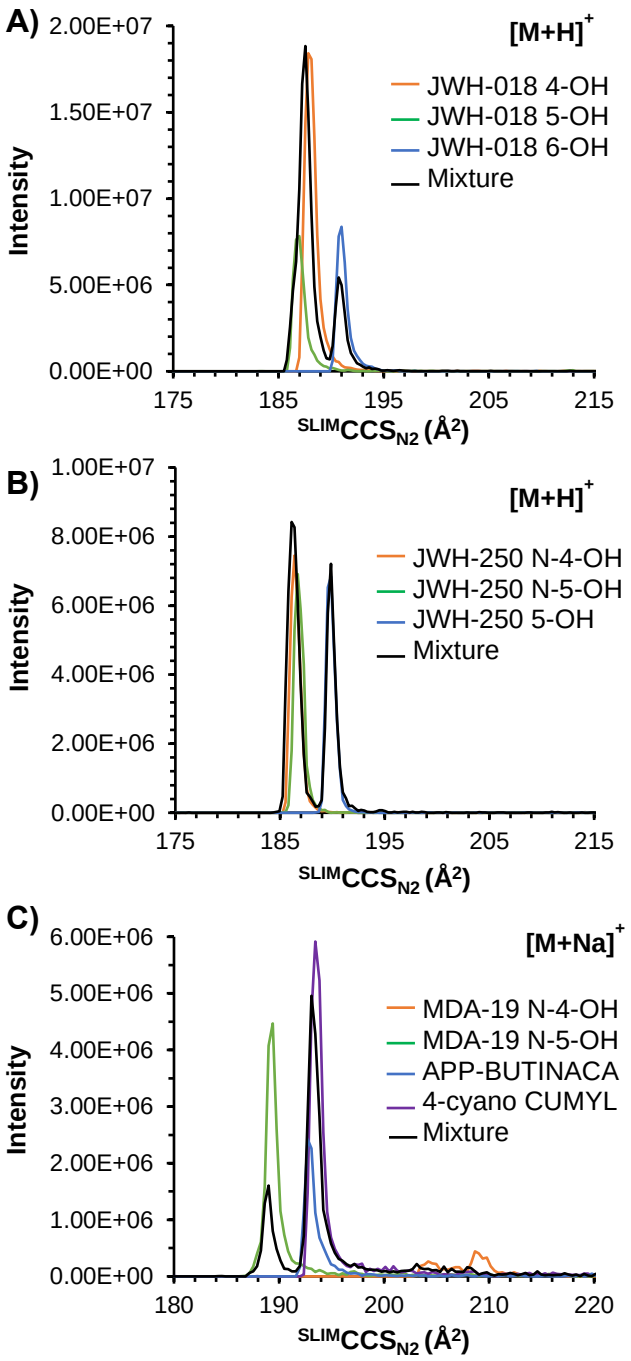

Figure S2. SLIM IM separations of (A) JWH-018 isomers as $[M+H]^+$, (B) JWH-250 isomers as $[M+H]^+$, and (C) MDA-19 isomers as $[M+Na]^+$. All groups are displayed as individual compounds and an equimolar mixture.

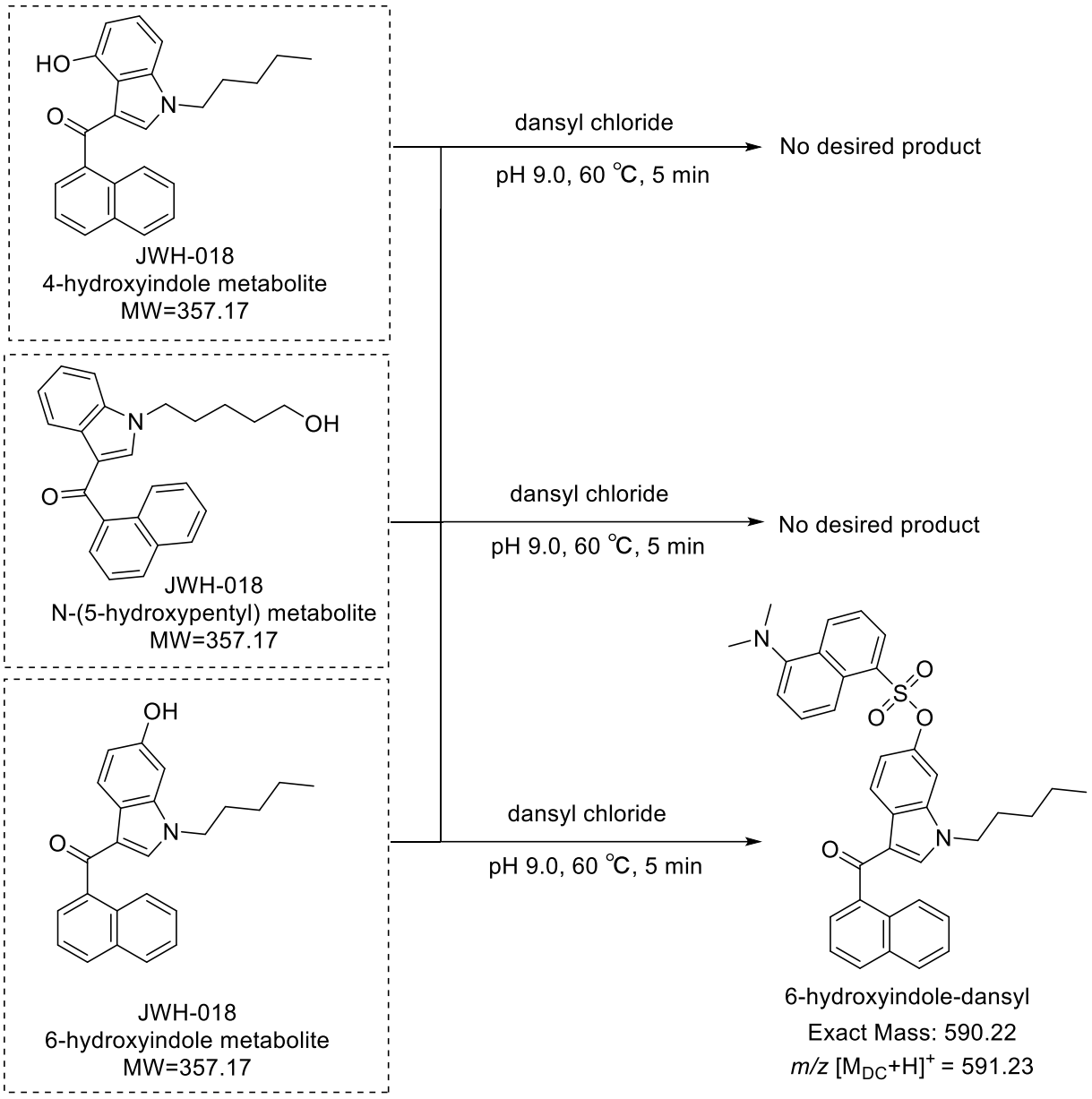

Figure S3. Reaction of dansyl chloride with JWH-018 metabolites selectively results in product formation for the 6-OH metabolite, yielding the $[M_{DC}+H]^+$ product at *m/z* 591.23.