APPLICATIONS

Chiral LC/MS/MS Method for Analyzing Metabolites of the Synthetic Cannabinoids JWH-018 and AM2201 Contained in K2/Spice Herbal Mixtures using Strata™-X-Drug B SPE and Lux® Cellulose-3 Chiral Column

Marc Jacob, Matthew Trass, Art Miranda, Shahana Huq, Michael McCoy, JT Presley, and Erica Pike
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA

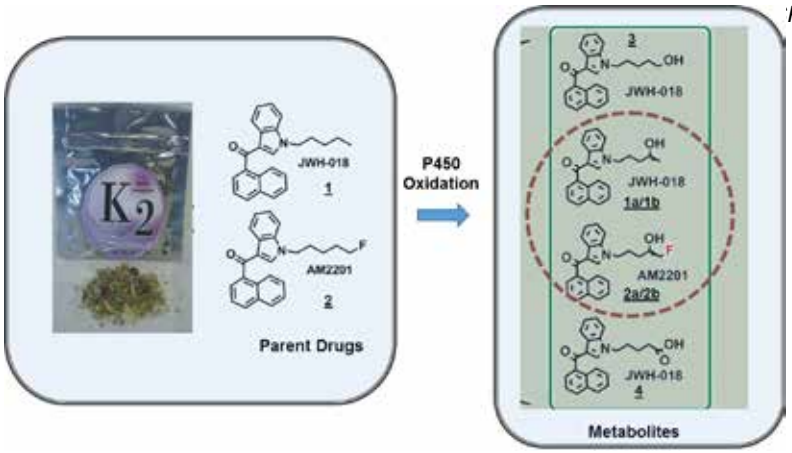
In this technote, we describe a new targeted metabolomic approach for assessing human synthetic cannabinoid exposure and pharmacology in blood and urine samples. The method utilizes a Solid Phase Extraction (SPE) step followed by chiral LC/MS/MS analysis using a Lux polysaccharide-based chiral stationary column providing a reliable and reproducible method that can be transferred to clinical research, forensic, and toxicology labs for analytical testing.

Introduction

Herbal mixtures labeled as "K2" or "Spice" are often marketed as legal marijuana substitutes to circumvent existing regulations and to avoid detection in standard drug screens. These products commonly contain the synthetic cannabinoid parent drugs JWH-018 (**Figure 1**, Parent Drug **1**) and AM2201 (**Figure 1**, Parent Drug **2**), both aminoalkylindoles and potent cannabinoid receptor agonists.

Unfortunately, little is known about the metabolism and toxicology of these new drugs, but the (ω -1)-hydroxyl metabolites enantiomers (**Figure 1**, Metabolites **1a**/**1b** or **2a**/**2b**), (ω)-hydroxyl (Metabolite **3**) and (ω)-carboxyl (Metabolite **4**) are identified as primary biomarkers. These metabolites are also known to retain significant pharmacological activity, which may offer a mechanistic explanation of the adverse effects associated with synthetic cannabinoid use. Since the (ω-1)-hydroxyl metabolites of JWH-018 and AM2201 are chiral molecules, analytical procedures capable of low level quantification of specific enantiomeric metabolites are required to further understand the metabolic and toxicological consequences of synthetic cannabinoid use.

Figure 1.
Parent drugs and metabolic oxidation compound structures. The circled compounds are chiral metabolites.



This technote describes a novel LC/MS/MS method and SPE procedure capable of simultaneously resolving enantiomers as well as parent compounds and other related metabolites.

Materials and Methods

Reagents and chemicals were obtained from Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA) and Hemostat Laboratories (Dixon, CA). All sample and analytical standards including chiral isomers of JWH-018-(ω-1)-OH and AM2201-(ω-1)-OH were synthesized and provided by Cayman Chemical (Ann Arbor, MI). Strata-X-Drug B polymeric strong cation-exchange solid phase extraction cartridges, Lux Cellulose-3 analytical column and SecurityGuard™ were obtained from Phenomenex (Torrance, CA). Samples were prepared using a Gilson Nebula 215 solid phase extraction system (Middleton, WI) and analyzed using an Agilent® 1200 Series quaternary liquid chromatography system (Santa Clara, CA) interfaced with an API 4000™ QTRAP® tandem mass spectrometer (AB SCIEX, Framingham, MA). The operation of the HPLC system and mass spectrometer was controlled by Analyst® software (version 1.5.1, AB SCIEX, Framingham, MA).

Sample Pretreatment:

Urine sample

See Reference 1 for internal standard preparation and complete experimental details.

Blood sample

Pipette 50 µL of blood into 950 µL 0.1M sodium acetate buffer (pH 5.0) and spike with 10 µL of internal standard (IS) solution. The sample was then subjected to the SPE method described below.

SPE Procedure

Cartridge: Strata-X-Drug B, 30 mg/3 mL
Part No.: 8B-S128-TBJ
Condition: NOT REQUIRED
Equilibrate: NOT REQUIRED
Load: 1 mL pretreated sample
Wash: 1 mL Sodium acetate buffer
Wash: 1 mL Sodium acetate buffer/Acetonitrile (70:30)
Elute: 5 mL Ethyl acetate/Isopropanol (85:15)
Dry: Dry down completely under a stream of nitrogen @ 60 °C
Reconstitute: 100 µL Ethanol

Revision: 0
PHEN-RUO-00027

APPLICATIONS

HPLC Conditions

Column: Lux® 3 µm Cellulose-3
Dimensions: 150 x 2.0 mm
Part No.: 00F-4492-B0
Mobile Phase: A: 20 mM Ammonium bicarbonate
B: Acetonitrile

Gradient:	**Time (min)**	**B (%)**
	0	40
	10	95
	12	95
	15	40
	16	40

Flow Rate: 0.5 mL/min
Temperature: 40 °C
Detection: Tandem Mass Spectrometer (MS/MS)
Detector: API 4000™ QTRAP® (AB SCIEX)

Table 1.
Mass Spectrometry Parameters for Selective Reaction Monitoring (SRM)

Analyte	Q1 (m/z)	Q3 (m/z)
AM2201	360	155*
		127†
(R)-(-)-AM2201-(ω-1)-OH	376	155*
		127†
(S)-(+)-AM2201-(ω-1)-OH	376	155*
		127†
JWH-018	342	155*
		127†
JWH-018-(ω)-OH	358	155*
		127†
JWH-018-(ω)-COOH	372	155*
		127†
(R)-(-)-JWH-018-(ω-1)-OH	358	155*
		127†
(S)-(+)-JWH-018-(ω-1)-OH	358	155*
		127†

*Quantification Ion † Confirmation Ion

Results and Discussion

JWH-018 is metabolized in humans to form the (ω)-monohydroxylated, (ω)-carboxylated, and (ω-1)-monohydroxylated metabolites. AM2201 exposure leads to the formation of common (ω)-JWH-018 metabolites but also the distinct (ω-1)-monohydroxylated AM2201 metabolites (**Figure 1**). A targeted metabolomic approach that simultaneously measures each primary metabolite including the enantiomeric (ω-1)-metabolites is required to facilitate future studies designed to understand the drug metabolism. This new chiral LC/MS/MS approach achieves this requirement by resolving all metabolites of interest, including the R and S enantiomers of the (ω-1)-monohydroxylated metabolites of JWH-018 and AM2201 in human urine and blood (**Figures 2** and **3**). The chromatography of standards, QC samples, and unknown urine specimens is similar for all matrices evaluated. Retention times established for each analyte internal standard remained constant (±0.1 min). All calibration curves were linear over the tested analytical range, where r^2 values were ≥0.99. The lower limits of quantification (LLOQ) for each analyte are comparable to previous LLOQ measurements reported with similar methods and mass spectra are consistent with reference libraries previously reported. [2,3]

Figures 2 and **3** represent LC/MS/MS chromatograms produced from 10 ng/mL and 5 ng/mL (respectively) synthetic cannabinoid quality control samples in human urine and blood (all synthetic cannabinoids standard were provided by Cayman Chemical). The chromatography was similar in all standards. The different color tracings are representative of the Specific Reaction Monitoring (SRM) experiments for each specific metabolite (see **Table 1** for SRM transitions).

Figure 2.
LC/MS/MS chromatograms produced from a representative 10 ng/mL quality control sample prepared in pooled human urine

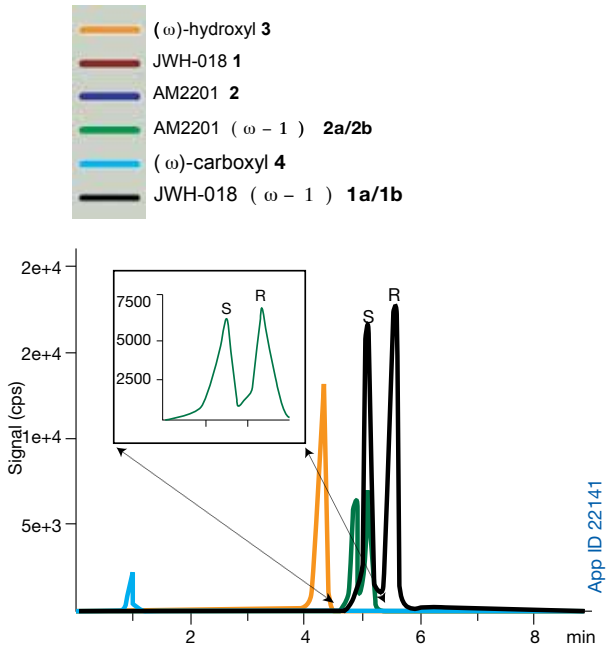
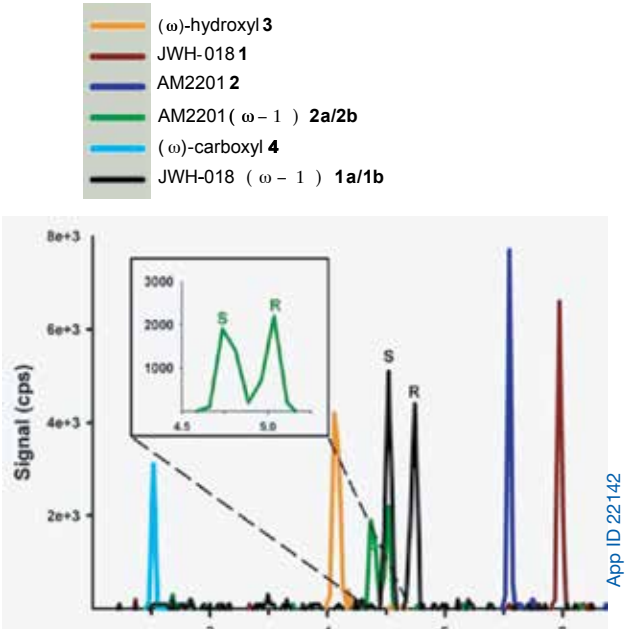
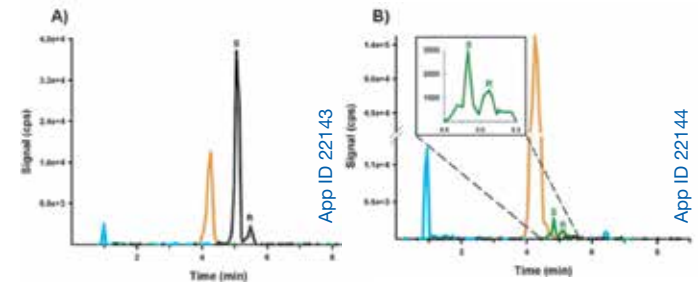


Figure 3.
LC/MS/MS chromatograms produced from a representative 5 ng/mL quality control sample prepared in blood



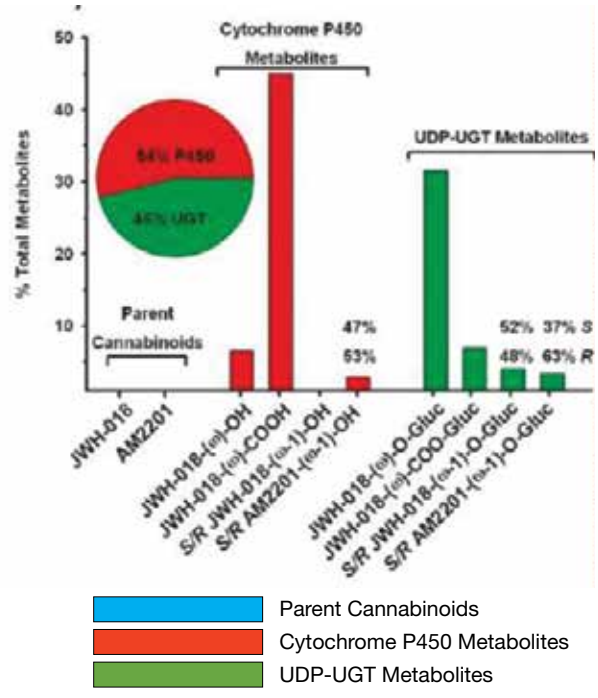
Figures 4a and **4b** show representative LC/MS/MS chromatograms of human samples which tested positive for the (ω-1)-monohydroxylated metabolite of JWH-018 (**Figure 1**, Metabolites **1a**/**1b**) and for the (ω-1)-monohydroxylated metabolite of AM2201 (**Figure 1**, Metabolites **2a**/**2b**). As shown in **Figure 1**, the (ω-1)-monohydroxylated metabolites are unique biomarkers for each respective synthetic cannabinoid.

Figure 4.
Representative LC/MS/MS chromatograms produced from (**A**) a human sample positive for the (ω-1)-monohydroxylated metabolite of JWH-018, and (**B**) a human urine sample positive for the (ω-1)-monohydroxylated metabolite of AM2201



In **Figure 5,** we demonstrate how this method was used to generate the metabolic profile of a human urine specimen which tested positive for JWH-018/AM2201 metabolites. The relative percentage of each metabolite is represented and the relative percentage of S or R enantiomers is provided above the bar of the corresponding (ω-1)-monohydroxylated metabolite.

Figure 5.
Metabolic profile generated from a human urine sample which tested positive forJWH-018/AM2201 metabolites



JWH-018 and AM2201 are both subjected to cytochrome-P450 mediated oxidation as well as uridine diphosphate glucuronyltransferase (UGT) conjugation during the metabolism process. Cytochrome-P450 metabolizes JWH-018 and AM2201 in the lung and liver while UGT is thought to be responsible for conjugating each metabolite with glucuronic acid. The pie chart inset compares the total relative percentage of free cytochrome P450 metabolites versus the total relative percentage of glucuronic acid conjugates. The conjugation percentage was determined by measuring metabolite concentrations pre- and post-ω-glucuronidase treatment (see Reference 1 for full details). These results show that when research subjects are exposed to only JWH-018 (**Figure 5**), the JWH-018 (ω-1)-monohydroxylated metabolite was excreted in a much higher concentration as compared to the other JWH-018 metabolites studied. In contrast, AM2201 (ω-1)-monohydroxylated enantiomers were not preferentially excreted. This indicates that UGTs may exhibit stereospecificity toward chiral synthetic cannabinoid metabolites.

Conclusion

The LC/MS/MS method described in this technical note is capable of fully resolving and quantifying chiral metabolites of JWH-018 and AM2201 as well as parent drugs. The precision and accuracy measurements are similar to previously developed assays which make this method easily transferrable to clinical research, forensic, and toxicology labs for analytical testing. Moreover, this chiral method can help researchers in the understanding and evaluation of the clinical toxicity, pharmacodynamics and pharmacokinetics of achiral and chiral synthetic cannabinoid metabolites produced from JWH-018 and AM2201.

References

1. Moran, J. H. *et al*. Anal. Chem. **2013**, 85, 9390– 9399.
2. Moran, J. H. *et al*. Anal. Chem. **2011**, 83, 6381– 6388.
3. Moran, J. H. *et al*. Anal. Chem. **2011**, 83, 4228– 4236.

APPLICATIONS

Ordering Information

Strata®-X-Drug B SPE

Sorbent Mass	Part No.	Unit
Tube		
10 mg	8B-S128-AAK	1 mL (100/box)
10 mg	8L-S128-AAK†	1 mL (100/box)
30 mg	8B-S128-TAK	1 mL (100/box)
30 mg	8L-S128-TAK†	1 mL (100/box)
30 mg	8B-S128-TBJ	3 mL (50/box)
60 mg	8B-S128-UBJ	3 mL (50/box)
60 mg	8B-S128-UCH	6 mL (30/box)
60 mg	8B-S128-UCL	6 mL (200/bag)
Giga™ Tube		
100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate		
10 mg	8E-S128-AGB	2 Plates/Box
30 mg	8E-S128-TGB	2 Plates/Box
60 mg	8E-S128-UGB	2 Plates/Box

† Tab-less tube

phenoLogix℠
Your Method. Our Scientists.

Free
Chiral Screening Services, provided by PhenoLogix
www.phenomenex.com/PhenoLogix

Lux® Cellulose-3 Chiral Columns

3 µm Analytical Columns (mm)							SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**150 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
Cellulose-3	00B-4492-B0	00F-4492-B0	00B-4492-E0	00D-4492-E0	00F-4492-E0	00G-4492-E0	AJ0-8621	AJ0-8622
						for ID:	2.0–3.0 mm	3.2–8.0 mm

5 µm Analytical Columns (mm)						SecurityGuard™ Cartridges (mm)	
Phases	**50 x 2.0**	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**4 x 2.0***	**4 x 3.0***
Cellulose-3	00B-4493-B0	00B-4493-E0	00D-4493-E0	00F-4493-E0	00G-4493-E0	AJ0-8621	AJ0-8622
					for ID:	2.0–3.0 mm	3.2–8.0 mm

*SecurityGuard Analytical Cartridges require holder, Part No. : KJ0-4282

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

Australia
t: +61 (0)2-9428-6444
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
info@phenomenex.com

China
t: +86 400-606-8099
cninfo@phenomenex.com

Denmark
t: +45 4824 8048
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal
t: +351 221 450 488
ptinfo@phenomenex.com

Singapore
t: +65 800-852-3944
sginfo@phenomenex.com

Spain
t: +34 91-413-8613
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland
t: +41 61 692 20 20
swissinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

TN21110718_W