

APPLICATIONS

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

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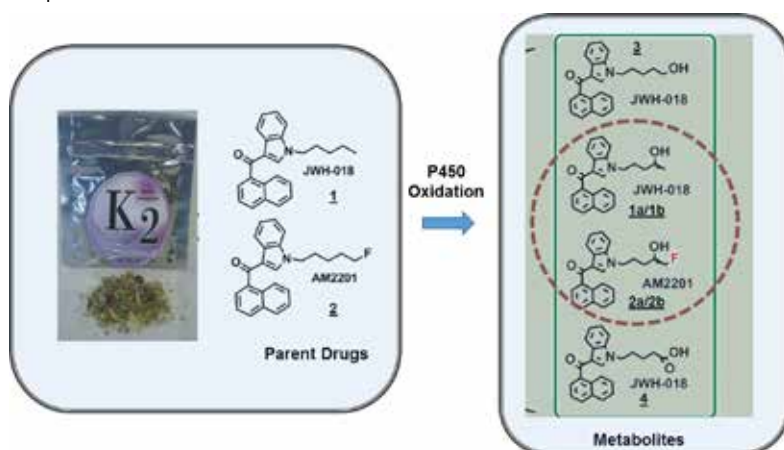
In this technote, we describe a new targeted metabolomic approach for assessing human synthetic cannabinoid exposure and pharmacology in blood and urine samples. The method utilizes a Solid Phase Extraction (SPE) step followed by chiral LC/MS/MS analysis using a Lux polysaccharide-based chiral stationary 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. With reports now indicating that 1 in 9 high school students experiment with synthetic cannabinoids and several medical reports specifically linking human injury and death to JWH-018 and AM2201, public health officials are increasingly concerned about abuse trends associated with these emerging cannabinoids.

Unfortunately, little is known about the metabolism and toxicology of these new drugs, but several clinical investigations identify the (ω -1)-hydroxyl metabolites enantiomers (**Figure 1**, Metabolites **1a/1b** or **2a/2b**), (ω)-hydroxyl (Metabolite **3**) and (ω)-carboxyl (Metabolite **4**) as primary biomarkers. These metabolites are also known to retain significant in vitro and in vivo 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 μ L of blood into 950 μ L 0.1M sodium acetate buffer (pH 5.0) and spike with 10 μ L 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/3mL
- 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 μ L Ethanol

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 clinical studies designed to understand the relationship between drug metabolism and clinical symptoms documented after JWH-018 and AM2201 use. 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 cannabinoid standards 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

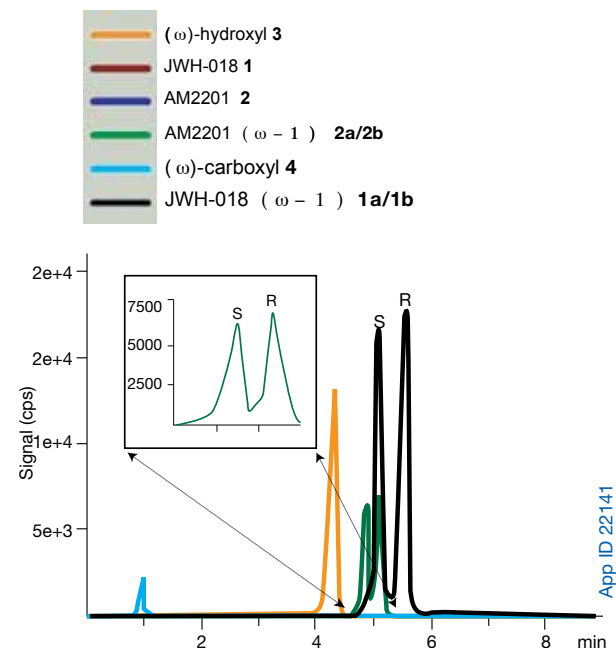
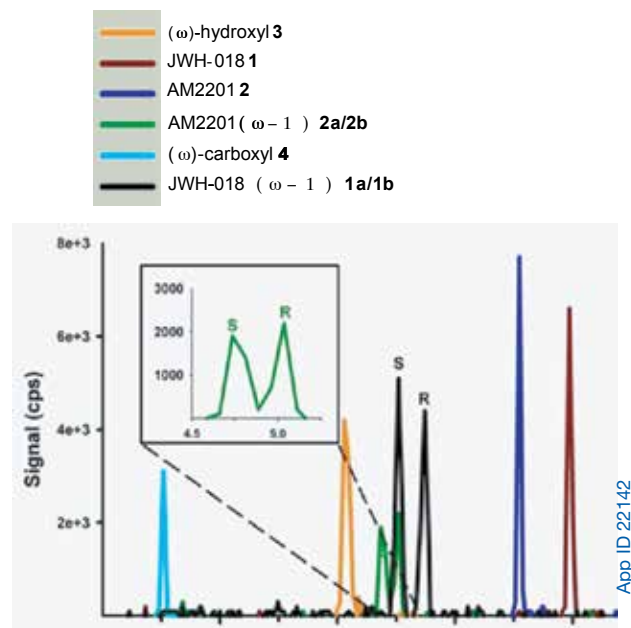


Figure 3.

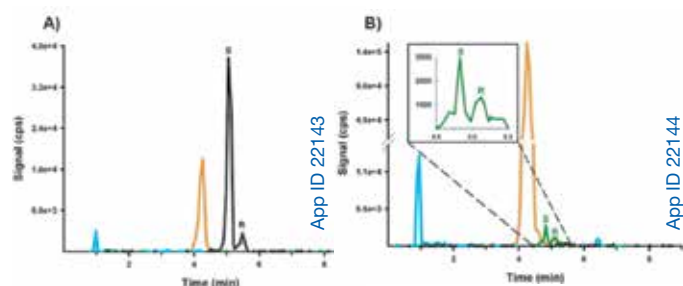
LC/MS/MS chromatograms produced from a representative 5 ng/mL quality control sample prepared in blood



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.

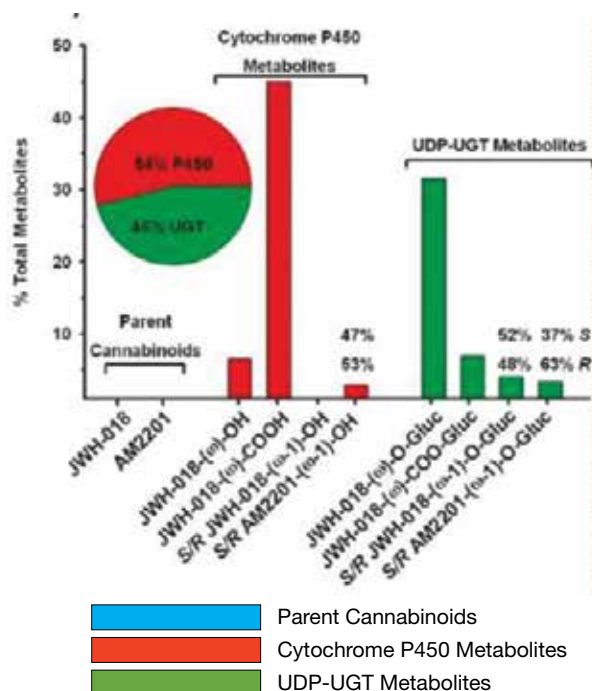
Representative LC/MS/MS chromatograms produced from (A) a human sample positive for the (ω -1)-monohydroxylated metabolite of JWH-018, and (B) a human urine sample positive for the (ω -1)-monohydroxylated metabolite of AM2201



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 for JWH-018/AM2201 metabolites



JWH-018 and AM2201 are both subjected to cytochrome-P450 mediated oxidation as well as uridine diphosphate glucuronyl-transferase (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 clinical and forensic 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

- Moran, J. H. *et al.* Anal. Chem. **2013**, 85, 9390– 9399.
- Moran, J. H. *et al.* Anal. Chem. **2011**, 83, 6381– 6388.
- 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



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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



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Strata-X is patented by Phenomenex, Inc. U.S. Patent No. 7,119,145

SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362

Caution: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP or ULTRA holders, or to any cartridges.

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