analytical chemistry

Article

pubs.acs.org/ac

Targeted Metabolomic Approach for Assessing Human Synthetic Cannabinoid Exposure and Pharmacology

Amy L. Patton,[†] Kathryn A. Seely,[†] Krishna C. Chimalakonda,[‡] Johnny P. Tran,[†] Matthew Trass,[§] Art Miranda,[§] William E. Fantegrossi,[‡] Paul D. Kennedy,[#] Paul Dobrowolski,[#] Anna Radominska-Pandya,[∥] Keith R. McCain,[⊥] Laura P. James,[¶] Gregory W. Endres,[#] and Jeffery H. Moran*[,†,‡]

[†]Arkansas Department of Health, Public Health Laboratory, Little Rock, Arkansas 72205, United States

[‡]Department of Pharmacology & Toxicology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, United States

[§]Phenomenex, Torrance, California 90501, United States

[∥]Department of Biochemistry & Molecular Biology, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, United States

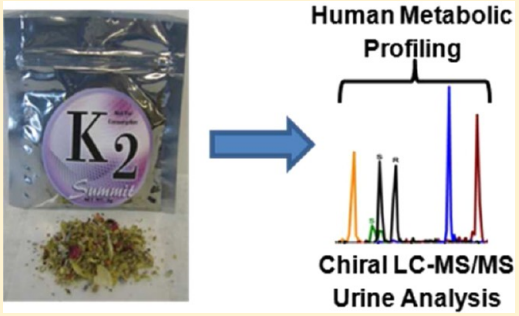
[⊥]Arkansas Poison & Drug Information Center, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205, United States

[¶]Section of Clinical Pharmacology and Toxicology, Arkansas Children's Hospital and Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, Arkansas 72205, United States

[#]Cayman Chemical Co., Ann Arbor, Michigan 48108, United States

Supporting Information

ABSTRACT: Designer synthetic cannabinoids like JWH-018 and AM2201 have unique clinical toxicity. Cytochrome-P450-mediated metabolism of each leads to the generation of pharmacologically active (ω)- and (ω-1)-monohydroxyl metabolites that retain high affinity for cannabinoid type-1 receptors, exhibit Δ^9-THC-like effects in rodents, and are conjugated with glucuronic acid prior to excretion in human urine. Previous studies have not measured the contribution of the specific (ω-1)-monohydroxyl enantiomers in human metabolism and toxicity. This study uses a chiral liquid chromatography–tandem mass spectroscopy approach (LC–MS/MS) to quantify each specific enantiomer and other nonchiral, human metabolites of JWH-018 and AM2201 in human urine. The accuracy (average % RE = 18.6) and reproducibility (average CV = 15.8%) of the method resulted in low-level quantification (average LLQ = 0.99 ng/mL) of each metabolite. Comparisons with a previously validated nonchiral method showed strong correlation between the two approaches (average r^2 = 0.89). Pilot data from human urine samples demonstrate enantiospecific excretion patterns. The (*S*)-isomer of the JWH-018-(ω-1)-monohydroxyl metabolite was predominantly excreted (>87%) in human urine as the glucuronic acid conjugate, whereas the relative abundance of the corresponding AM2201-(ω-1)-metabolite was low (<5%) and did not demonstrate enantiospecificity (approximate 50:50 ratio of each enantiomer). The new chiral method provides a comprehensive, targeted metabolomic approach for studying the human metabolism of JWH-018 and AM2201. Preliminary evaluations of specific enantiomeric contributions support the use of this approach in future studies designed to understand the pharmacokinetic properties of JWH-018 and/or AM2201.



The human cannabinoid type-1 receptor (CB1R) is the most abundant G-protein-coupled receptor in the central nervous system[1] and is responsible for the psychotropic effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive cannabinoid in cannabis.[2−4] Activation of the CB1R has therapeutic potential for a variety of medical conditions.[5−9] However, most synthetic cannabinoid drugs retain undesirable psychoactive properties and thus have not been approved for human use.[3,10,11] Synthetic cannabinoid abuse has recently emerged as a significant public health concern,[12] and one in nine high school seniors admit recent use.[13] Clinical reports show that synthetic cannabinoid users exhibit signs of central nervous system and cardiovascular toxicity including extreme agitation, hallucinations, and syncope.[14−23] Severe cases can progress to supraventricular tachycardia, generalized seizures, and death.[24−26]

The synthetic cannabinoid 1-(pentyl-1*H*-indol-3-yl)-1-naphthalenyl-methanone (JWH-018) and the fluorinated analogue

Received: July 25, 2013
Accepted: August 29, 2013
Published: August 29, 2013

   dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390−9399

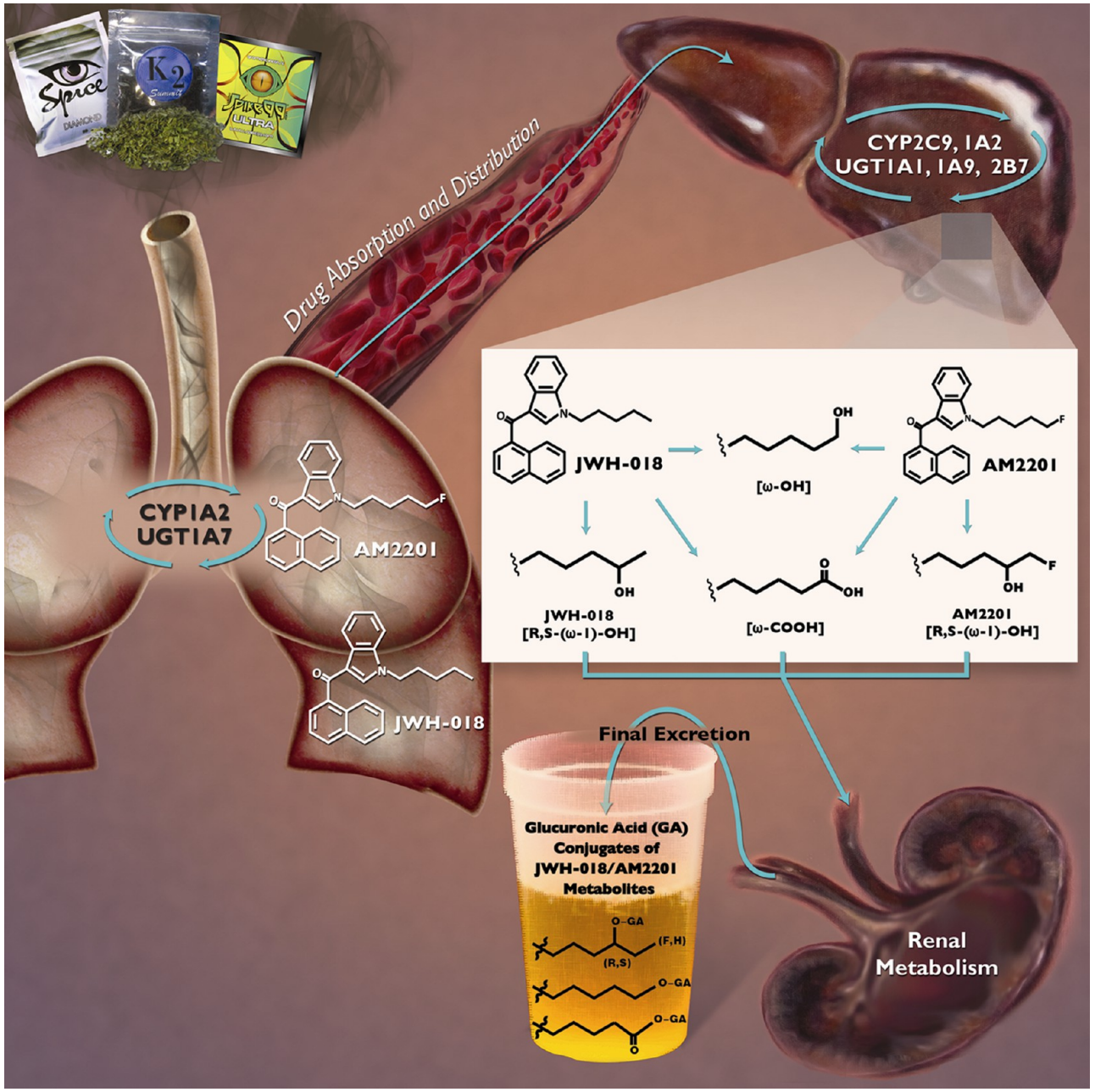


Figure 1. Schematic summary of what is known about the absorption, distribution, metabolism, and excretion of JWH-018 and AM2201, two synthetic cannabinoids in purported quasi-legal marijuana substitutes commonly branded as "K2" or "Spice". Parent drugs are absorbed by the lungs and distributed to the liver via the bloodstream where primary metabolism is thought to take place. Metabolic products are eliminated in urine as glucuronic acid conjugates.

[1-(5-fluoropentyl)-1*H*-indol-3-yl]-1-naphthalenyl-methanone (AM-2201) represent two synthetic cannabinoids contained in the purported synthetic marijuana products marketed as "K2" or "Spice". JWH-018 and AM-2201 were among the most prevalent synthetic cannabinoids found in the United States from 2010 to 2011. Thus, these cannabinoids are the subject of several recent analytical, metabolic, and toxicological investigations aimed at understanding the unique clinical toxicity of these drugs.[27−31]

Both JWH-018 and AM2201 undergo cytochrome-P450 (CYP)-mediated oxidation and uridine diphosphate glucuronyltransferase (UGT) conjugation prior to urinary excretion (Figure 1).[32−34] Lung and liver CYP2C9 and CYP1A2 isozymes are responsible for the generation of the three primary human metabolites of JWH-018 and AM2201, namely, the (ω)-monohydroxyl, (ω)-carboxyl, and (ω-1)-monohydroxyl metabolites.[32,35,36] UGT1A1, UGT1A3, UGT1A9, UGT1A10, and UGT2B7 are thought to be responsible for conjugating each metabolite with glucuronic acid.[37] Full characterization of these metabolic pathways is important because many synthetic cannabinoid metabolites retain significant pharmacological activity.[29,30,32,38,39] Since the JWH-018 and AM2201 (ω)- and (ω-1)-monohydroxyl metabolites retain nanomolar affinity for CB1R and exhibit Δ^9-THC-like effects in laboratory animals, the specific enantiomeric contributions of the chiral (ω-1)-monohydroxyl metabolites should be investigated for differences in metabolism, potency, and efficacy.

Developing a chiral analytical approach for studying specific cannabinoid enantiomers is the first step in understanding the clinical consequences of these chiral metabolites. This

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390−9399

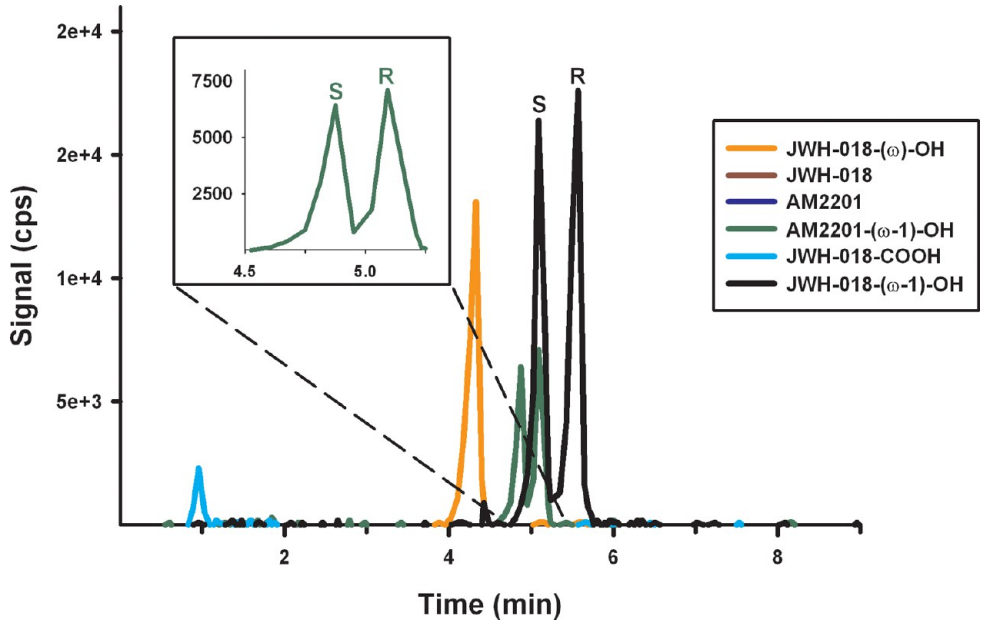


Figure 2. Representative LC−MS/MS chromatograph from a 10 ng/mL quality control sample prepared in pooled human urine. (ω-1)-Monohydroxylated metabolites are unique biomarkers for each respective cannabinoid (Figure 1). Different color tracings are representative of the Specific Reaction Monitoring (SRM) experiments used for each specific metabolite. Experimental details are provided in the Experiential Section and are listed in Table S-1.

Table 1. Summary of Accuracy, Precision, and Quantification Limits in Urine[a]

	urine									
	quality control high (50 ng/mL)			quality control medium (10 ng/mL)			quality control low (1 ng/mL)			
analyte	concn ± SD (ng/mL)	% CV[b]	% RE[c]	concn ± SD (ng/mL)	% CV[b]	% RE[c]	concn ± SD (ng/mL)	% CV[b]	% RE[c]	LLQ[d]
(*S*)-AM2201-(ω-1)−OH	51.92 ± 4.73	9.1	3.8	9.66 ± 1.56	16.2	3.4	1.26 ± 0.22	17.7	25.9	0.67
(*R*)-AM2201-(ω-1)−OH	49.27 ± 1.29	2.6	1.5	10.19 ± 1.46	14.4	1.9	1.49 ± 0.36	23.8	49.2	1.07
JWH-018-(ω)−OH	47.75 ± 6.99	14.6	4.5	8.72 ± 1.16	13.3	12.8	1.54 ± 0.28	18.3	54.2	0.85
JWH-018-(ω)-COOH	47.82 ± 4.07	8.5	4.4	9.24 ± 1.93	20.9	7.6	1.52 ± 0.51	33.7	51.5	1.53
(*S*)-JWH-018-(ω-1)−OH	49.65 ± 3.96	8.0	0.7	9.54 ± 2.00	20.9	4.7	1.45 ± 0.32	22.2	45.2	0.97
(*R*)-JWH-018-(ω-1)−OH	46.93 ± 6.01	12.8	6.1	8.81 ± 0.59	6.7	12.0	1.45 ± 0.29	20.0	45.2	0.87

[a]Data are based on six single, independent experiments conducted on multiple, nonconsecutive days. [b]% CV, coefficient of variation, calculated as [(SD/mean) × 100] at each concentration. [c]% RE, absolute relative error, calculated as [((expected concentration − calculated concentration)/expected concentration) × 100] at each concentration. [d]LLQ, lower limit of quantification, calculated as (3 × SD) of the quality control low for each analyte.

report couples solid-phase extraction with a chiral liquid chromatography−tandem mass spectroscopy (LC−MS/MS) method to provide a comprehensive, targeted metabolomic approach for studying JWH-018 and AM2201 metabolism and toxicity. The new chiral method fully resolves each enantiomer, achieves low level quantification, is comparable to nonchiral methods, and meets or exceeds accuracy and precision requirements of most clinical and research laboratories. Analysis of 15 human urine specimens known to contain JWH-018 and AM2201 metabolites showed metabolic interindividual differences and demonstrated human enantioselectivity toward (ω-1)-monohydroxyl metabolite conjugation. Supplemental work (Supplemental Experimental Section) also validates this approach in rodent brain, liver, and lung tissue and in commercially available caprine blood.

EXPERIMENTAL SECTION

Reagents and Chemicals. All chemicals used for this study were of at least reagent grade, and unless otherwise noted, they were provided by Sigma-Aldrich (St. Louis, MO), Fisher Scientific (Pittsburgh, PA), Hemostat Laboratories (Dixon, CA), Phenomenex (Torrance, CA), or Cayman Chemical (Ann Arbor, MI). Blank, pooled human urine void of synthetic cannabinoid contamination was provided by the Arkansas Department of Health Public Health Laboratory. Blank mouse brain, liver, and lung tissue (Supplemental Experimental Section) were obtained from the University of Arkansas for Medical Sciences, Department of Pharmacology (Little Rock, AR). Absolute configuration assignments of the (+)-(*S*) enantiomer of JWH-018-(ω-1)−OH and the (+)-(*R*) enantiomer of AM2201-(ω-1)−OH were determined by the synthesis of each enantiomer through a common chiral starting material (Supplemental Figure S-1). ^{1}H NMR conformational studies have been previously published for the JWH-018 analytical metabolic standards,[35,36] and the results for the new AM2201-(ω-1)−OH metabolic standard are shown in Supplemental Figure S-2.

Equipment. Solid-phase extraction procedures were optimized for 96-well plate processing but were performed serially

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390−9399

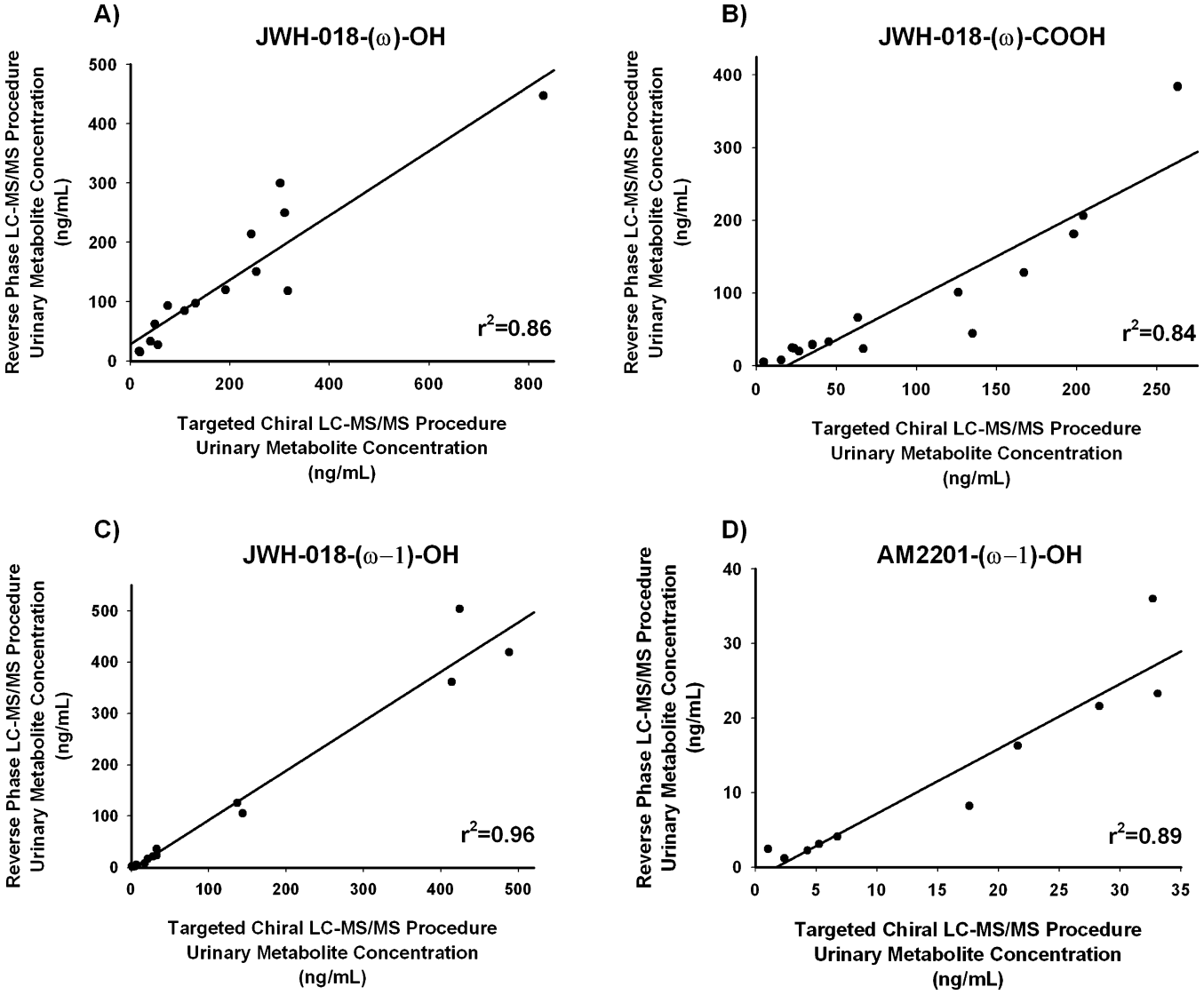


Figure 3. Correlation study results for each primary human metabolite of JWH-018 and AM2201 (A–D). Fifteen human urine samples were selected for this study. Three groups of five were chosen to represent, as described in the Experimental Section: JWH-18-only, AM2201-only, and combined JWH-018/AM2201 exposure groups. The concentration of each primary human metabolite was determined using previously validated reverse-phase LC–MS/MS applications[35,36] and compared to the results obtained with the chiral application developed for this study. Pearson correlation coefficients (r^2) are inset within each panel.

on a Gilson Nebula 215 solid-phase extraction system (Middleton, WI). Samples were analyzed using an Agilent 1200 series quaternary liquid chromatography system (Santa Clara, CA) that was interfaced with an API-4000 Q-Trap tandem mass spectrometer (AB SCIEX, Framingham, MA).

Preparation of Analytical Standards and Quality Control Material. Analytical calibration standards and QC standards were prepared from a common ethanol stock solution containing JWH-018 (10 μg/mL), AM2201 (10 μg/mL), JWH 018 *N*-pentanoic acid metabolite (10 μg/mL), (+)-(*S*)-JWH 018 *N*-(4-hydroxypentyl) metabolite (10 μg/mL), (−)-(*R*)-JWH 018 *N*-(4-hydroxypentyl) metabolite (10 μg/mL), JWH 018 *N*-(5-hydroxypentyl) metabolite (10 μg/mL), (+)-(*R*)-AM2201 *N*-(4-hydroxypentyl) metabolite (10 μg/mL), and (−)-(*S*)-AM2201 *N*-(4-hydroxypentyl) metabolite (10 μg/mL). A common internal standard stock solution containing JWH-018-d_9 (10 μg/mL), AM2201-d_5 (10 μg/mL), JWH 018 *N*-pentanoic acid metabolite-d_4 (10 μg/mL), (±)-(*S*/*R*)-JWH 018 *N*-(4-hydroxypentyl) metabolite-d_5 (20 μg/mL), JWH 018 *N*-(5-hydroxypentyl) metabolite-d_5 (10 μg/mL), and (±)-(*S*/*R*)-AM2201 *N*-(4-hydroxypentyl) metabolite-d_5 (20 μg/mL) was also prepared in ethanol. All analytical and internal standard solutions were stored at −40 °C until needed.

Urine calibration standards were prepared from a 1 μg/mL intermediate working standard prepared in pooled, blank human urine free of synthetic cannabinoid contamination. Final working urine standards (0.5–100 ng/mL) were prepared daily by serially diluting the intermediate working standard with pooled, blank urine. Since no second source of analytical standards is currently available, quality control (QC) material was prepared in urine at three concentrations spanning the calibration range (QCL, QCM, QCH). All QC material was prepared independent of calibration standards. Protocols for preparing blood and tissue standards are provided as Supporting Information (Supplemental Experimental Section).

Prior to sample processing and analysis, the internal standard was added identically to all analytical standards, QC material, and unknown samples. The common internal standard stock

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390–9399

Table 2. Available Clinical and Demographic Data

specimen	age	gender	clinical symptoms	duration of effects	exposure group
7	27	male	agitation, anxiety, confusion, electrolyte abnormality, fever, hallucination, hyperglycemia, hypertension, mydriasis, pallor, tachycardia, vomiting	>8 <24 h	JWH-018
10	48	male	fever, seizures, tachycardia	unknown	
14	unknown	male	no clinical information	unknown	
15	unknown	unknown	no clinical information	unknown	
57	unknown	unknown	no clinical information	unknown	
39	unknown	unknown	no clinical information	unknown	AM2201
49	19	male	agitation, anxiety, confusion, hallucination, hyperglycemia, hypertension, mydriasis, tachycardia, tremor	>8 <24 h	
59	18	male	drowsiness/lethargy, nausea, vomiting	<2 h	
61	unknown	unknown	no clinical information	unknown	
62	unknown	unknown	no clinical information	unknown	
25	unknown	male	agitation, anxiety, hypertension, vomiting	unknown	JWH-018 + AM2201
28	21	male	confusion, diaphoresis, drowsiness/lethargy, hyperglycemia, pallor	>2 <8 h	
35	18	female	confusion, drowsiness/lethargy, hyperglycemia, hypertension, pallor, tachycardia, vomiting	>2 <8 h	
37	unknown	unknown	no clinical information	unknown	
53	35	male	agitation, anxiety, dyspnea, hallucination, lacrimation, mydriasis, hyperglycemia	>2 <8 h	

solution was diluted with ethanol to yield a final 1 μg/mL spiking solution that contained all the metabolites of interest.

Solid-Phase Extraction of Standards, Quality Control Material, and Specimens. Previously optimized reaction conditions for β-glucuronidase treatment and solid-phase extraction procedures were used in this study.[35,36] All urine calibration standards, QC material, and unknown samples were processed identically. Extraction procedures used for supplemental studies are provided as Supporting Information (Supplemental Experimental Section).

Liquid Chromatography–Tandem Mass Spectrometry. The chiral LC–MS/MS method utilizes a Phenomenex Lux 3 μm Cellulose-3 analytical column (150 mm × 2 mm) heated to 40 °C. Analytes are resolved using a 20 mM ammonium bicarbonate/acetonitrile gradient starting at 60% aqueous, ramping to 5% aqueous over 10 min, and holding constant for an additional 2 min. The gradient was returned to initial conditions over 3 min and equilibrated for an additional minute. The total run time is 16 min, including the column equilibration period between injections.

Mass spectrometer methods are a modification of a method previously reported for synthetic cannabinoid analysis.[35] Supplemental Table S-1 lists the modified SRM/IDA-EPI experiment parameters for each analyte. Mass spectra obtained from EPI experiments of unknown samples were matched to EPI mass spectra obtained from previous work[26,35,36] and calibration standards to ensure interfering metabolites and other compounds were resolved. To ensure carryover was not present, matrix-matched samples containing no calibration standard material were injected, and ethanol blanks were injected following analysis of a known high-concentration sample (i.e., high standard, QCH).

Human Subject Study Design. Fifteen human urine samples collected from individuals testing positive for the unique (ω-1)-monohydroxyl metabolites of JWH-018 and/or AM2201 (Figure 1) were selected for pilot testing of the chiral assay. Three groups (n = 5 samples per group) were defined as JWH-018-only, AM2201-only, or combined JWH-018/AM2201 exposure groups. Specific biomarkers shown in Figure 1 were used to establish each exposure group. This study was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences (Little Rock, AR).

Statistical Methods. Accuracy and precision were determined using QC samples prepared during six single, independent experiments performed over several nonconsecutive days. Accuracy was calculated as the absolute percent relative error for each of the expected QC concentrations. Analytical precision was calculated as the % CV for replicate measurements at the three QC concentrations. The limit of detection was defined at less than the lowest calibrator (0.5 ng/mL), and the lower limit of quantitation (LLQ) was calculated as three times the standard deviation of six replicate analyses of the low QC standard in each matrix. Correlation studies were evaluated by calculating Pearson correlation coefficients (r^2).

RESULTS AND DISCUSSION

JWH-018 is metabolized in humans to form the (ω)-monohydroxylated, (ω)-carboxylated, and (ω-1)-monohydroxylated metabolites (Figure 1). AM2201 exposure leads to the formation of common (ω)-JWH-018 metabolites but also the distinct (ω-1)-monohydroxylated AM2201 metabolite (Figure 1).[35,36] Elucidating these metabolic pathways is important because many synthetic cannabinoid metabolites retain nanomolar affinity at CB1Rs and exhibit a range of intrinsic activities.[29,30] For example, the affinities of the (ω)- and (ω-1)-monohydroxylated metabolites of JWH-018 and AM2201 are similar to Δ^9-THC, where K_i values range between 12 and 35 nM.[32] Importantly, all of these synthetic cannabinoid metabolites are considered full agonists of the CB1R, whereas Δ^9-THC is a partial agonist. 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 chiral LC–MS/MS approach achieves baseline separation of 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 resolves isobaric metabolites and interference (Figure 2 and Supplemental Figure S-3). The chromatography of standards, QC samples, and unknown specimens was similar for all matrices evaluated (Figure 2 and Supplemental Figures S-3 and S-4). Retention times established for each analyte and isotopically labeled

dx.doi.org/10.1021/ac4024704 | Anal. Chem. 2013, 85, 9390–9399

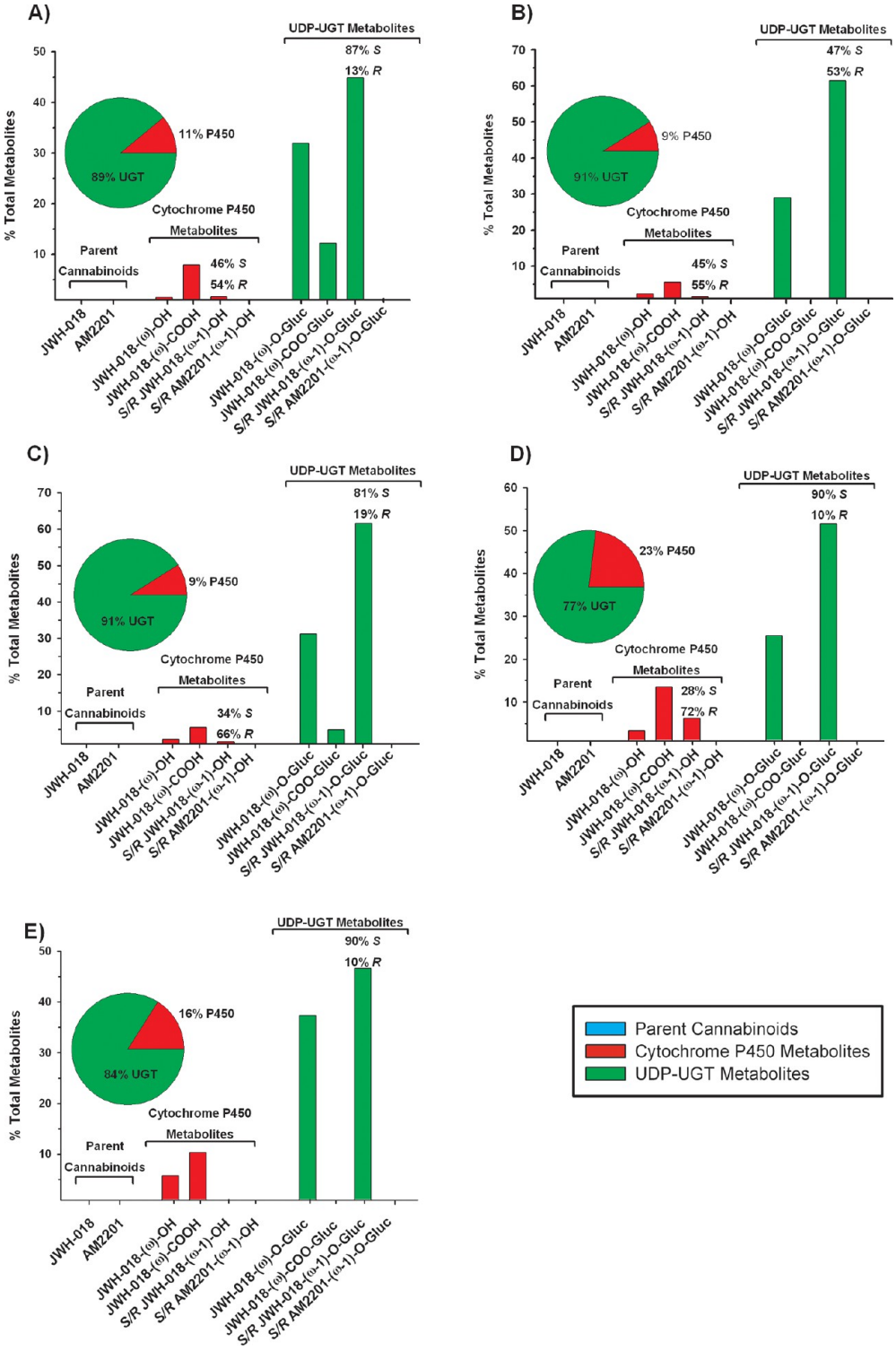


Figure 4. Metabolic profiles generated from five human urine samples representing the JWH-018-only exposure group (A–E). Selection criteria for this exposure group are provided in the Experimental Section. Values represent the relative percentage of each metabolite. Actual calculated values are provided in Supplemental Table S-6. The relative percentage of *S* or *R* enantiomers is provided above the bar representing each corresponding (ω-1)-monohydroxylated metabolite. The pie chart inset within each panel compares the total relative percentage of free cytochrome P450 metabolites versus the total relative percentage of glucuronic acid conjugates. Percent conjugation was determined as described in the Experimental Section by measuring metabolite concentrations pre- and post-β-glucuronidase treatment.

internal standard remained constant (±0.1 min). Accuracy, precision, and lower limits of quantification (LLQ) for each analyte in urine are presented in Table 1. All calibration curves were linear over the tested analytical range, where r^2 values were ≥0.99. The LLQs for each analyte are comparable to previous LLQ measurements reported with similar methods (Table 1 and Tables S-2–S-5),[35,36] and mass spectra are consistent with reference libraries previously reported.[35,36]

Most analytical applications developed for JWH-018 and AM2201 rely on reverse-phase applications.[35,40,41] To ensure that

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390–9399

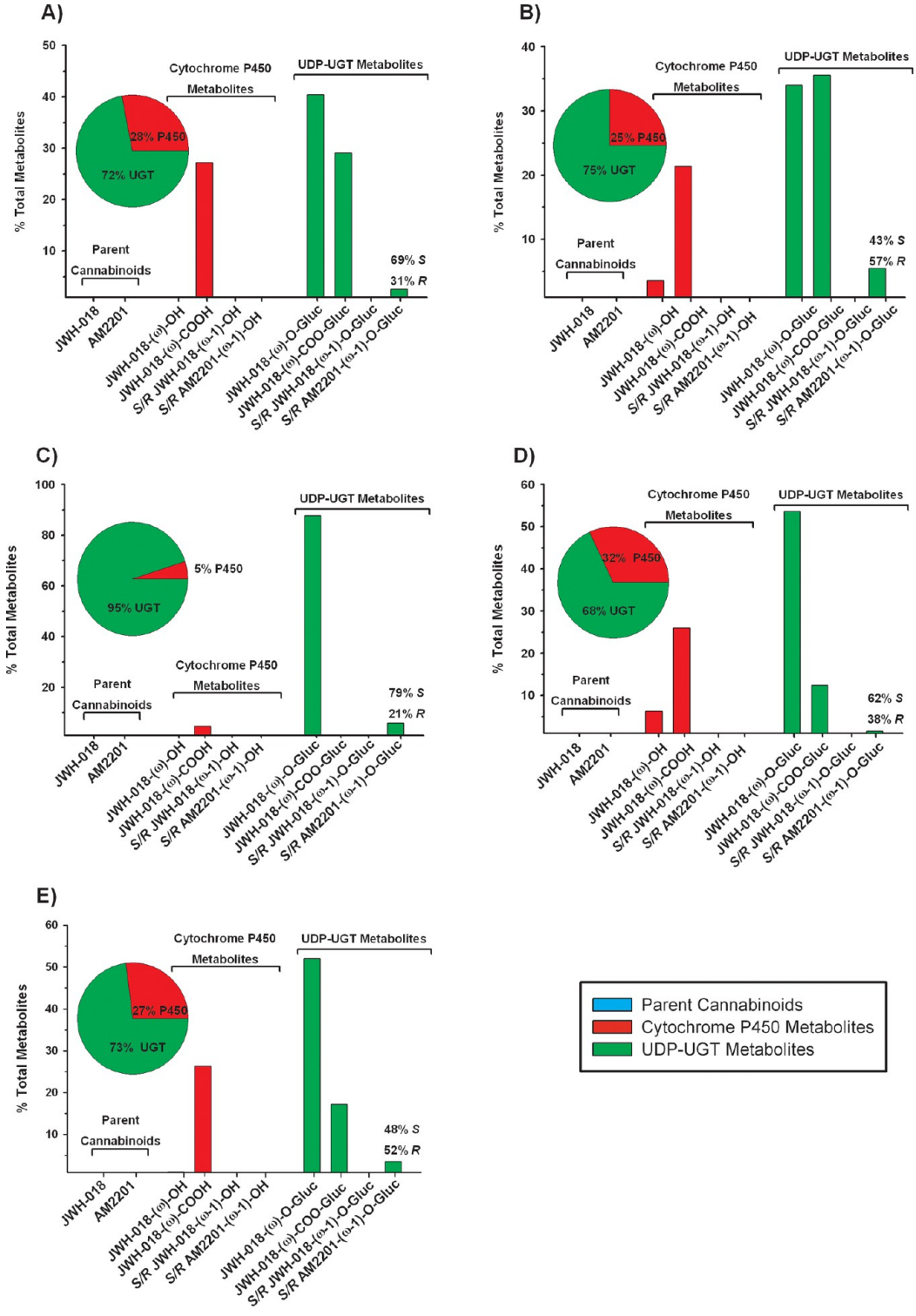


Figure 5. Metabolic profiles generated from five human urine samples representing the AM2201-only exposure group (A–E). Selection criteria for this exposure group are provided in the Experimental Section. Values represent the relative percentage of each metabolite. Actual calculated values are provided in Supplemental Table S-6. The relative percentage of *S* or *R* enantiomers is provided above the bar representing each corresponding (ω-1)-monohydroxylated metabolite. The pie chart inset within each panel compares the total relative percentage of free cytochrome P450 metabolites versus the total relative percentage of glucuronic acid conjugates. Percent conjugation was determined as described in the Experimental Section by measuring metabolite concentrations pre- and post-β-glucuronidase treatment.

the chiral application does not introduce unknown matrix effects or other analytical errors, a comparison study was completed. The results obtained from human urine samples using a previously described reverse-phase LC–MS/MS method[35,36] were compared to results obtained with the new, chiral LC–MS/MS method (Figure 3). A strong correlation between the two methods was observed for all analytes (r^2 = 0.84–0.96), demonstrating that both methods are nearly equivalent.

To begin examining the clinical utility of the chiral assay, analysis was performed on 15 human urine samples positive for

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390–9399

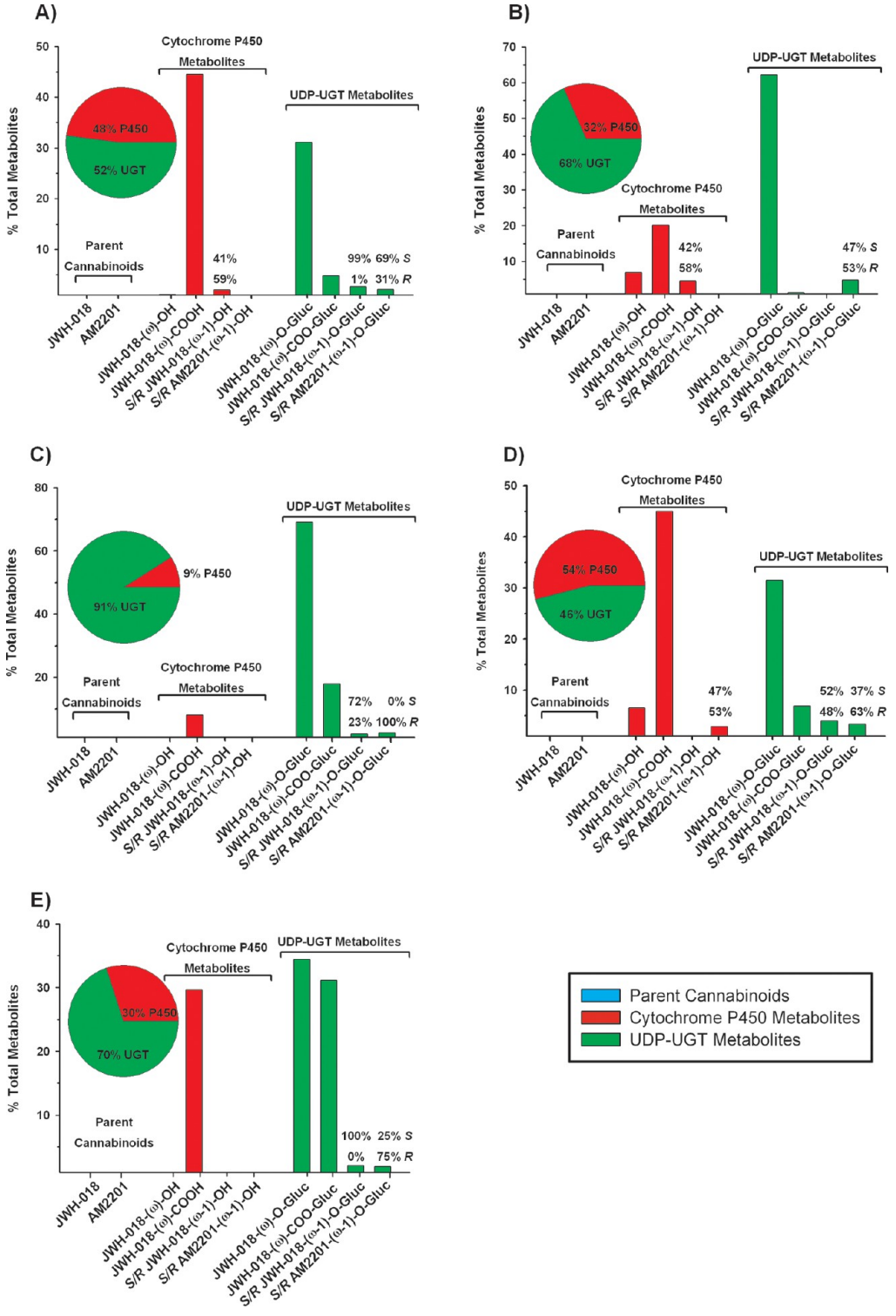


Figure 6. Metabolic profiles generated from five human urine samples representing the combined JWH-018/AM2201 exposure group (A–E). Selection criteria for this exposure group are provided in the Experimental Section. Values represent the relative percentage of each metabolite. Actual calculated values are provided in Supplemental Table S-6. The relative percentage of *S* or *R* enantiomers is provided above the bar representing each corresponding (ω-1)-monohydroxylated metabolite. The pie chart inset within each panel compares the total relative percentage of free cytochrome P450 metabolites versus the total relative percentage of glucuronic acid conjugates. Percent conjugation was determined as described in the Experimental Section by measuring metabolite concentrations pre- and post-β-glucuronidase treatment.

the (ω-1)-monohydroxyl metabolites of JWH-018 and/or AM2201 (Figure 1). Available demographic and clinical information for the JWH-018-only, AM2201-only, or combined JWH-018/AM2201 exposure groups is provided in Table 2. Chromatography of clinical samples was similar to quality controls prepared in blank urine (Figure 2 and Supplemental Figure S-3). No interfering compounds were detected, and no modifications to sample preparation procedures were introduced.

One of the most striking findings of this pilot study was that the glucuronic acid conjugate of the (*S*) enantiomer of the

dx.doi.org/10.1021/ac4024704 | *Anal. Chem.* 2013, 85, 9390–9399

JWH-018 (ω-1)-monohydroxylated metabolite was almost exclusively excreted in the JWH-018-only exposure group (81−95%; Figure 4). In contrast, specific AM2201 (ω-1)-monohydroxylated enantiomers were not preferentially excreted (Supplemental Table S-6 and Figure 5). It is intriguing to speculate that UGTs may exhibit stereospecificity toward chiral synthetic cannabinoid metabolites. The stereospecific nature of CYPs, UGTs, and drug transporters is well-known[42−46] and may explain the findings with the JWH-018 (ω-1)-monohydroxylated metabolite. Potential clinical implications of stereospecific metabolism include the increased risk of toxicity in individuals who preferentially produce the (*R*) enantiomer. Larger clinical studies are required to confirm the preliminary findings and to more closely associate the health effects with each specific enantiomer.

The relative contributions of chiral and nonchiral metabolites were also evaluated in the three exposure groups. The total metabolite concentration of P450 and UGT products varied among the three exposure groups (Figures 4−6 and Supplemental Table S-6). There are several pharmacokinetic and pharmacodynamic explanations that may account for these findings, including drug dose, the timing of specimen collection relative to drug exposure, cytochrome P450 expression, and/or genetic polymorphisms that would affect metabolism. Other confounding factors such as concomitant drug exposures may also influence the results because synthetic cannabinoid metabolism may be inhibited or induced by other xenobiotics.

CONCLUSIONS

The LC−MS/MS method presented provides low levels of quantification and a high level of accuracy and reproducibility for all the primary human metabolites of JWH-018 and AM2201 found in urine, including the chiral metabolites of each. The accuracy of this method is equivalent to previously reported methods and provides baseline resolution of each enantiomer, while resolving isobaric metabolites and interferences. This is the first study to evaluate the chirality of human metabolites of JWH-018 and AM2201 in human urine samples and to suggest potential interindividual metabolic differences, which may be relevant for understanding the clinical toxicity of synthetic cannabinoids. The high variability observed in human metabolic profiles may reflect the genetic polymorphisms and inhibition or induction of P450s and UGTs. Larger human studies using the chiral method are needed to elucidate the pharmacokinetics of these commonly used drugs of abuse and the relationship of clinical symptoms to drug clearance.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: jeffery.moran@arkansas.gov. Fax: (501) 661-2972. Tel.: (501) 661-2826.

Author Contributions

All authors contributed to the writing of this manuscript and the design and/or execution of all studies. All authors provided approval for the final version of this manuscript.

Notes

None of the authors have personal financial conflicts of interest. However, Art Miranda and Matthew Trass are employees of Phenomenex, and Paul Kennedy, Paul Dobrowolski, and Gregory Endres are employees of Cayman Chemical. Commercial products from both Phenomenex and Cayman Chemical were used in this study.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Laura Schnackenberg with the National Center for Toxicological Research (Food and Drug Administration, Jefferson, AR) for her technical assistance in peer review of the ^{1}H NMR conformational studies. The authors would also like to thank Catherine Allen for her laboratory technical assistance and Jason Truskowski and Lindsey Maples for graphic design. This work was supported by the Association of Public Health Laboratories (Grant Innovations in Quality Public Health Laboratory Practice) (J.H.M.) and by the Centers for Disease Control (Contract No. 200-2007-21729) (J.H.M.).

REFERENCES

(1) Eggan, S. M.; Lewis, D. A. *Cereb. Cortex* **2007**, *17*, 175−191.
(2) Gaoni, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1964**, *86*, 1646−1647.
(3) Howlett, A. C.; Breivogel, C. S.; Childers, S. R.; Deadwyler, S. A.; Hampson, R. E.; Porrino, L. J. *Neuropharmacology* **2004**, *47* (Suppl 1), 345−358.
(4) Devane, W. A.; Dysarz, F. A., III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605−613.
(5) Lee, H. K.; Choi, E. B.; Pak, C. S. *Curr. Top. Med. Chem.* **2009**, *9*, 482−503.
(6) Turcotte, D.; Le Dorze, J. A.; Esfahani, F.; Frost, E.; Gomori, A.; Namaka, M. *Expert Opin. Pharmacother.* **2010**, *11*, 17−31.
(7) Vemuri, V. K.; Janero, D. R.; Makriyannis, A. *Physiol. Behav.* **2008**, *93*, 671−686.
(8) Graham, E. S.; Ashton, J. C.; Glass, M. *Front. Biosci.* **2009**, *14*, 944−957.
(9) Pertwee, R. G. *Br. J. Pharmacol.* **2009**, *156*, 397−411.
(10) Aggarwal, S. K.; Carter, G. T.; Sullivan, M. D.; ZumBrunnen, C.; Morrill, R.; Mayer, J. D. *J. Opioid Manage.* **2009**, *5*, 153−168.
(11) Moreira, F. A.; Grieb, M.; Lutz, B. *Best Pract. Res., Clin. Endocrinol. Metab.* **2009**, *23*, 133−144.
(12) EMCDDA. *Spice and Related Synthetic Cannabinoids*; European Monitoring Centre for Drugs and Drug Addiction: Lisbon, Portugal, 2009.
(13) Vandrey, R.; Dunn, K. E.; Fry, J. A.; Girling, E. R. *Drug Alcohol Depend.* **2012**, *120*, 238−241.
(14) Muller, H.; Sperling, W.; Kohrmann, M.; Huttner, H. B.; Kornhuber, J.; Maler, J. M. *Schizophr. Res. Treat.* **2010**, *118*, 309−310.
(15) Vearrier, D.; Osterhoudt, K. C. *Pediatr. Emerg. Care* **2010**, *26*, 462−465.
(16) Every-Palmer, S. *Drug Alcohol Depend.* **2011**, *117*, 152−157.
(17) Every-Palmer, S. *Addiction* **2010**, *105*, 1859−1860.
(18) Schneir, A. B.; Cullen, J.; Ly, B. T. *J. Emerg. Med.* **2010**, *40*, 296−299.
(19) Lapoint, J.; James, L. P.; Moran, C. L.; Nelson, L. S.; Hoffman, R. S.; Moran, J. H. *Clin. Toxicol.* **2011**, *49*, 760−764.
(20) Simmons, J. R.; Skinner, C. G.; Williams, J.; Kang, C. S.; Schwartz, M. D.; Wills, B. K. *Ann. Emerg. Med.* **2011**, *57*, 187−188.
(21) Young, A. C.; Schwarz, E.; Medina, G.; Obafemi, A.; Feng, S. Y.; Kane, C.; Kleinschmidt, K. *Am. J. Emerg. Med.* **2011**, *30*, 1320.e5−1320.e7.
(22) Gunderson, E. W.; Haughey, H. M.; Ait-Daoud, N.; Joshi, A. S.; Hart, C. L. *Am. J. Addict.* **2012**, *21*, 320−326.
(23) Mir, A.; Obafemi, A.; Young, A.; Kane, C. *Pediatrics* **2011**, *128*, e1622−e1627.

(24) Simmons, J.; Cookman, L.; Kang, C.; Skinner, C. *Clin. Toxicol.* **2011**, *49*, 431−433.
(25) Tofighi, B.; Lee, J. D. *J. Addict. Med.* **2012**, *6*, 240−241.
(26) Patton, A. L.; Chimalakonda, K. C.; Moran, C. L.; McCain, K.; Radominska-Pandya, A.; James, L.; Moran, J. H. *J. Forensic Sci.* **2013**, epub ahead of print.
(27) Wells, D. L.; Ott, C. A. *Ann. Pharmacother.* **2011**, *45*, 414−417.
(28) Fattore, L.; Fratta, W. *Front. Behav. Neurosci.* **2011**, *5*, 60.
(29) Brents, L. K.; Gallus-Zawada, A.; Radominska-Pandya, A.; Vasiljevik, T.; Prisinzano, T. E.; Fantegrossi, W. E.; Moran, J. H.; Prather, P. L. *Biochem. Pharmacol.* **2012**, *83*, 952−961.
(30) Brents, L. K.; Reichard, E. E.; Zimmerman, S. M.; Moran, J. H.; Fantegrossi, W. E.; Prather, P. L. *PLoS One* **2011**, *6*, e21917.
(31) Seely, K. A.; Brents, L. K.; Radominska-Pandya, A.; Endres, G. W.; Keyes, G. S.; Moran, J. H.; Prather, P. L. *Chem. Res. Toxicol.* **2012**, *25*, 825−827.
(32) Chimalakonda, K. C.; Seely, K. A.; Bratton, S. M.; Brents, L. K.; Moran, C. L.; Endres, G. W.; James, L. P.; Hollenberg, P. F.; Prather, P. L.; Radominska-Pandya, A.; Moran, J. H. *Drug Metab. Dispos.* **2012**, *40*, 2174−2184.
(33) Wintermeyer, A.; Möller, I.; Thevis, M.; Jübner, M.; Beike, J.; Rothschild, M.; Bender, K. *Anal. Biol. Chem.* **2010**, *398*, 2141−2153.
(34) Gronewold, A.; Skopp, G. *Forensic Sci. Int.* **2011**, *210*, e7−e11.
(35) Chimalakonda, K. C.; Moran, C. L.; Kennedy, P. D.; Endres, G. W.; Uzieblo, A.; Dobrowolski, P. J.; Fifer, E. K.; Lapoint, J.; Nelson, L. S.; Hoffman, R. S.; James, L. P.; Radominska-Pandya, A.; Moran, J. H. *Anal. Chem.* **2011**, *83*, 6381−6388.
(36) Moran, C. L.; Le, V. H.; Chimalakonda, K. C.; Smedley, A. L.; Lackey, F. D.; Owen, S. N.; Kennedy, P. D.; Endres, G. W.; Ciske, F. L.; Kramer, J. B.; Kornilov, A. M.; Bratton, L. D.; Dobrowolski, P. J.; Wessinger, W. D.; Fantegrossi, W. E.; Prather, P. L.; James, L. P.; Radominska-Pandya, A.; Moran, J. H. *Anal. Chem.* **2011**, *83*, 4228−4236.
(37) Chimalakonda, K. C.; Bratton, S. M.; Le, V. H.; Yiew, K. H.; Dineva, A.; Moran, C. L.; James, L. P.; Moran, J. H.; Radominska-Pandya, A. *Drug Metab. Dispos.* **2011**, *39*, 1967−1976.
(38) Hruba, L.; Ginsburg, B. C.; McMahon, L. R. *J. Pharmacol. Exp. Ther.* **2012**, *342*, 843−849.
(39) Ginsburg, B. C.; Schulze, D. R.; Hruba, L.; McMahon, L. R. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 37−45.
(40) Wohlfarth, A.; Scheidweiler, K. B.; Chen, X.; Liu, H. F.; Huestis, M. A. *Anal. Chem.* **2013**, *85*, 3730−3738.
(41) Hutter, M.; Broecker, S.; Kneisel, S.; Auwarter, V. *J. Mass Spectrom.* **2012**, *47*, 54−65.
(42) Bratton, S. M.; Mosher, C. M.; Khallouki, F.; Finel, M.; Court, M. H.; Moran, J. H.; Radominska-Pandya, A. *J. Pharmacol. Exp. Ther.* **2011**, *340*, 46−55.
(43) Haritos, V. S.; Ghabrial, H.; Ahokas, J. T.; Ching, M. S. *Pharmacogenetics* **2000**, *10*, 591−603.
(44) Roy, P.; Tretyakov, O.; Wright, J.; Waxman, D. J. *Drug Metab. Dispos.* **1999**, *27*, 1309−1318.
(45) Campo, V. L.; Bernardes, L. S.; Carvalho, I. *Curr. Drug Metab.* **2009**, *10*, 188−205.
(46) Brocks, D. R. *Biopharm. Drug Dispos.* **2006**, *27*, 387−406.