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Cannabidiol Does Not Convert to Δ^9-Tetrahydrocannabinol in an *In Vivo* Animal Model

Louise Wray,* Colin Stott, Nicholas Jones, and Stephen Wright

Abstract

Introduction: Cannabidiol (CBD) can convert to Δ^9-tetrahydrocannabinol (THC) *in vitro* with prolonged exposure to simulated gastric fluid; however, *in vitro* conditions may not be representative of the *in vivo* gut environment. Using the minipig, we investigated whether enteral CBD converts to THC *in vivo*.

Materials and Methods: Synthetic CBD (100 mg/mL) was administered orally in a sesame oil formulation twice daily to minipigs ($N = 3$) in 15 mg/kg doses for 5 consecutive days. Blood samples were taken before and 1, 2, 4, and 6 h after morning doses on Days 1 and 5. Six hours after the final dose on Day 5, the animals were euthanized, and samples of gastrointestinal (GI) tract contents were obtained. Liquid chromatography with tandem mass spectrometry analysis determined CBD, THC, and 11-hydroxy-THC (11-OH-THC) concentrations. Lower limits of quantification: plasma CBD = 1 ng/mL, plasma THC and 11-OH-THC = 0.5 ng/mL, GI tract CBD = 2 ng/mL, and GI tract THC and 11-OH-THC = 1 ng/mL.

Results: THC and 11-OH-THC were undetectable in all plasma samples. Maximum plasma concentrations (C_{max}) of CBD were observed between 1 and 4 h on Days 1 and 5. CBD was present in plasma 6 h after administration on Days 1 (mean 33.6 ng/mL) and 5 (mean 98.8 ng/mL). Mean C_{max} CBD values, 328 ng/mL (Day 1) and 259 ng/mL (Day 5), were within range of those achieved in clinical studies. Mean CBD exposure over 6 h was similar on Days 1 (921 h·ng/mL) and 5 (881 h·ng/mL). THC and 11-OH-THC were not detected in all GI tract samples. Mean CBD concentrations reached 84,500 ng/mL in the stomach and 43,900 ng/mL in the small intestine.

Conclusions: Findings of the present study show that orally dosed CBD, yielding clinically relevant plasma exposures, does not convert to THC in the minipig, a species predictive of human GI tract function.

Keywords: cannabidiol; conversion; minipig; tetrahydrocannabinol

Introduction

Cannabidiol (CBD), a major phytocannabinoid present in the cannabis plant, is receiving widespread attention for its potential role in the treatment of seizures in pediatric epilepsy patients. Pre-clinical[1–4] and clinical[5–7] data indicate that CBD may reduce seizure frequency in treatment-resistant childhood epilepsies, such as Dravet syndrome and Lennox-Gastaut syndrome, when dosed orally in a sesame oil formulation. In both open-label and randomized trials in patients with treatment-resistant epilepsy, oral CBD therapy reduced seizure frequency. CBD was associated with more adverse events than placebo but was generally well tolerated.[5–7]

In vitro studies have shown that CBD can be converted to Δ^9-tetrahydrocannabinol (THC) with prolonged exposure to simulated gastric fluid.[8,9] It is well known that an acid-catalyzed cyclization reaction converts CBD to THC, but this reaction has not been shown to occur in humans. In a study by Consroe et al.,[10] no THC was detected in the plasma of 14 Huntington disease patients after 6 weeks of 700 mg CBD daily doses. Human research has also shown that the adverse event profile of CBD medications is different

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Findings of this study were presented in a poster at the American Epilepsy Society Annual Meeting, December 2–6, 2016, in Houston, TX.

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to that of THC (i.e., patients treated with oral formulations of CBD do not experience the same psychoactive adverse effects, with the exception of somnolence observed in some patient populations). A randomized, double-blind, cross-over, placebo-controlled trial revealed that, in 16 healthy male human participants, the effects of oral CBD were the same as placebo, whereas oral THC was associated with acute behavioral and physiological effects. Levels of 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC) and carboxy-THC (THC-COOH) were clearly elevated after administration of THC (but not CBD or placebo) and followed a similar time course.[11] These findings support the hypothesis that the conversion of CBD to THC reported in *in vitro* models is not representative of clinical scenarios.[12,13]

At present, a limited number of studies have been completed investigating the potential for CBD to convert to THC *in vivo* following dosing of oral formulations of CBD. Bode et al.[14] proposed that the minipig is a suitable animal model for the human gastrointestinal (GI) tract and gut metabolic capability. Both humans and pigs have omnivorous diets, and their digestive tracts have similarities with regard to pH values, transit times, and drug absorption. In addition, THC is a known substrate of human CYP2C9 and CYP3A4; both of these isoenzymes have analogs present in the minipig.[15]

Table 1. Pharmacokinetic Parameters of CBD, THC, and 11-OH-THC in Male Minipigs

Day 1[a]	Concentrations of CBD in plasma[b] (ng/mL)				
Time (h)	**Minipig 1[c]**	**Minipig 2**	**Minipig 3**	**Mean**	**SD**
0	*BLQ*	BLQ	BLQ	BLQ	NA
1	*9.80*	217	283	250	46.7
2	*2.74*	163	189	176	18.4
4	*3.21*	373	73.5	223	212
6	*3.83*	40.8	26.3	33.6	10.3
$AUC_{(0–6)}$ (h·ng/mL)	*23.4*	1130	711	921	296
$AUC_{(0–t)}$ (h·ng/mL)	*23.4*	1130	711	921	296
$AUC_{(0–inf)}$ (h·ng/mL)	*NR*	NR	764	764	NA
Cl/*F* (mL/h/kg)	*NR*	NR	19,700	19,700	NA
C_{max} (ng/mL)	*9.80*	373	283	328	63.6
T_{max} (h)	*1*	4	1	Median: 1	Range: 1–4
C_{last} (ng/mL)	*3.83*	40.8	26.3	33.6	10.3
T_{last} (h)	*6*	6	6	Median: 6	Range: 6–6
$t_{1/2}$ (h)	*NR*	NR	1.41	1.41	NA
V_z/F (mL/kg)	*NR*	NR	40,000	40,000	NA
Day 5[d]	**Concentrations of CBD in plasma (ng/mL)**				
Time (h)	**Minipig 1**	**Minipig 2**	**Minipig 3**	**Mean**	**SD**
0	25.5	17.8	94.7	46.0	42.4
1	21.4	25.4	194	80.3	98.5
2	329	234	207	257	64.1
4	98	161	215	158	58.6
6	58.4	132	106	98.8	37.3
$AUC_{(0–6)}$ (h·ng/mL)	733	834	1080	881	176
$AUC_{(0–t)}$ (h·ng/mL)	733	834	1080	881	176
$AUC_{(0–inf)}$ (h·ng/mL)	NR	NR	NR	NR	NA
Cl/*F* (mL/h/kg)	NR	NR	NR	NR	NA
C_{max} (ng/mL)	329	234	215	259	61.1
T_{max} (h)	2	2	4	Median: 2	Range: 2–4
C_{last} (ng/mL)	58.4	132	106	98.8	37.3
T_{last} (h)	6	6	6	Median: 6	Range: 6–6
$t_{1/2}$ (h)	NR	NR	NR	NR	NA
V_z/F (mL/kg)	NR	NR	NR	NR	NA

[a]Day 1: following a single 15 mg/kg oral dose of CBD.
[b]THC and 11-OH-THC were not detectable in all samples (LLOQ = 0.5 ng/mL).
[c]A partial regurgitation of minipig 1's dose was observed on the morning of Day 1. Therefore, data from this minipig have been excluded from Day 1 calculations (italicized).
[d]Day 5: following 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg).

AUC, area under the plasma concentration-time curve; BLQ, below limit of quantification; CBD, cannabidiol; Cl/*F*, total body clearance for the fraction of absorbed dose; C_{max}, maximum plasma concentration; C_{last}, time of the last measurable concentration; LLOQ, lower limit of quantification; NA, not applicable; NR, no result (no clear terminal elimination phase); 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; SD, standard deviation; THC, Δ^9-tetrahydrocannabinol; $t_{1/2}$, terminal elimination phase half-life; t_{max}, time of maximum concentration profile; t_{last}, time of the last measurable concentration; V_z/F, volume of distribution for the fraction of absorbed dose.

Taking into account the similarity of the pH of the minipig stomach to that of humans, and the reported *in vitro* conversion of CBD to THC occurring in an acid medium,[9] the minipig was determined to be an appropriate *in vivo* model for the present research initiative. To assess the potential conversion of CBD to THC in the stomach, the plasma and GI tract samples of minipigs were assayed for the presence of THC and the first metabolite of THC, 11-OH-THC, after oral CBD administration. Outcomes included plasma pharmacokinetics of CBD, THC, and 11-OH-THC, and concentrations of CBD, THC, and 11-OH-THC in GI tract content samples.

Materials and Methods

The study was undertaken at Covance Laboratories in Harrogate, United Kingdom, in July and August 2016 and funded by GW Research, Ltd. The study was in full compliance with local, national, ethical, and regulatory principles, in addition to local licensing arrangements.

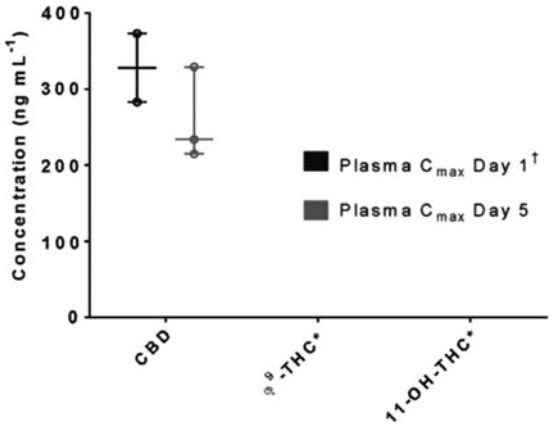

FIG. 1. Mean maximum plasma concentrations for CBD, THC, and 11-OH-THC in male minipigs after a single 15 mg/kg oral dose of CBD (Day 1), 4 days of twice-daily oral doses, and one final dose of CBD (total of nine doses, each 15 mg/kg; Day 5). Error bars represent standard deviations. *THC and 11-OH-THC levels were not detectable. †A partial regurgitation of minipig 1's Day 1 dose was observed on the morning of Day 1. Therefore, data from this minipig have been excluded from Day 1 calculations. CBD, cannabidiol; THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol.

Subjects

Three naïve male Ellegaard Göttingen minipigs (born in February 2016), without external signs of ill health, were used in the study. Each animal was kept in a separate solid floor pen, given a nominal amount of SDS SMP (E) (Special Diets Services Ltd., Witham) diet twice daily, and allowed free access to water. For 2 weeks before the first dose administration, each animal was acclimatized daily to the bleed cradle and bite bar used for blood sampling and was sham dosed with 10 or 20 mL of water each day.

CBD formulation

The CBD oral solution (GW Pharmaceuticals, London, United Kingdom) contained 100 mg/mL of synthetic CBD as the botanically occurring (–)-isomer. Purified, plant-derived CBD is known to contain small traces of THC; therefore, synthetic CBD was dosed in this study to avoid the generation of false positive results.

Protocol

CBD administration. Oral doses were administered twice per day (morning and afternoon), by gavage, for 4 days; on Day 5, a single dose was administered in the morning. The doses were administered, by weight, at ~0.15 mL/kg, to give a dose equivalent to 15 mg/kg of CBD. After each dose, the gavage was flushed with ~10 mL of water. Dosing levels were determined and justified on the basis of clinical dosing and previous findings that revealed an adequate safety margin based on toxicology studies in dogs.*

Sampling. On Days 1 and 5, blood samples were collected from a jugular vein in all animals before dose administration and 1, 2, 4, and 6 h after dose administration. Blood samples were centrifuged (3000 rpm, 10 min, 4°C) to isolate plasma.

Six hours after the final dose (Day 5), the animals were humanely euthanized and contents of the stomach and small intestine were harvested.

Plasma samples and contents of the stomach and small intestine were stored (less than –50°C) and submitted for bioanalysis. Analysis was performed using liquid chromatography with tandem mass spectrometry.

Outcome measures. Outcome measures included the following:

- Presence of THC and the first metabolite of THC (11-OH-THC) in plasma, with a lower limit of quantification (LLOQ) of 0.5 ng/mL

*GW Research Ltd., unpublished data, April 2017.

Table 2. Individual Concentrations of CBD, THC, and 11-OH-THC in Male Minipig Gastrointestinal Tract Contents

	Concentrations of CBD (ng/mL)[a,b]				
Tissue	Minipig 1	Minipig 2	Minipig 3	Mean	SD
Stomach contents	108,000	66,700	78,900	84,500	21,200
Small intestine contents	43,100	56,600	32,000	43,900	12,300

[a]All measurements were taken after 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg).
[b]THC and 11-OH-THC were not detectable in all samples (LLOQ = 1 ng/mL).

- Plasma levels of CBD, with an LLOQ of 1 ng/mL
- Concentrations of CBD, THC, and 11-OH-THC in GI tract content samples (CBD LLOQ = 2 ng/mL, THC and 11-OH-THC LLOQ = 1 ng/mL)

LLOQs were determined by the bioanalysis laboratory as the lowest quantifiable concentrations within linear range for the analytes. The 11-OH-THC levels were quantified to allow detection of THC that has already undergone phase I metabolism.

Data analysis

Pharmacokinetic analyses were completed using Phoenix WinNonLin version 6.4 software (Pharsight Corporation, Sunnyvale, CA). Parameters calculated included area under the plasma concentration-time curve calculated from 0–t, where t is the time of the last measurable concentration (AUC_{0-t}), maximum plasma concentration (C_{max}), time of maximum concentration profile (t_{max}), the last measurable concentration (C_{last}), time of the last measurable concentration (t_{last}), terminal elimination phase half-life ($t_{1/2}$), volume of distribution for the fraction of absorbed dose (V_z/F), and total body clearance for the fraction of absorbed dose (Cl/F).

Results

Plasma samples

Individual plasma concentrations and pharmacokinetic parameters of CBD, THC, and 11-OH-THC are shown in Table 1. THC and 11-OH-THC were below LLOQ in all plasma samples (Fig. 1). On Day 1, a partial regurgitation of one minipig's dose was observed. As a result, this animal is likely to have received a reduced dose on this day, indicated by concentrations of CBD that were much lower than expected when compared with those of the other two animals. Therefore, data from this minipig have been excluded from Day 1 calculations. All subsequent doses were successfully received by all three animals, so results following the Day 5 final dose are for all three animals.

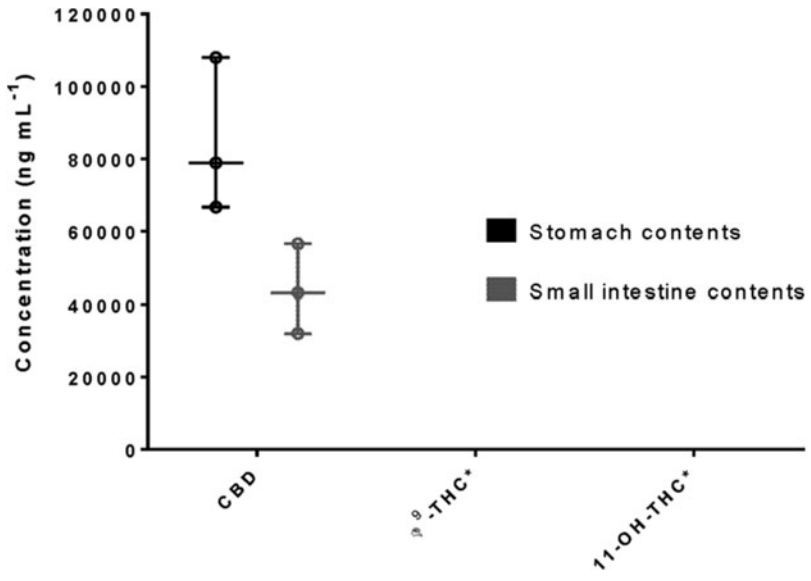

FIG. 2. Mean gastrointestinal concentrations for CBD, THC, and 11-OH-THC in male minipigs after 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg; Day 5). Error bars represent standard deviations. *THC and 11-OH-THC levels were not detectable.

The mean CBD C_{max} values of 328 and 259 ng/mL were observed between 1 and 4 h on both Days 1 and 5, respectively. These values are confirmed as similar to those reported in clinical studies following a 20 mg/kg daily oral dose of CBD (mean plasma concentration, 380 ng/mL).[16] CBD remained present in plasma 6 h after dosing on Days 1 and 5 at mean concentrations of 33.6 and 98.8 ng/mL, respectively. The mean exposure levels over 6 h were similar on Days 1 and 5 (921 and 881 h·ng/mL, respectively). Only one animal (minipig 3) exhibited a clear terminal phase to allow terminal parameters to be calculated from Day 1 data (Table 1). Terminal phase parameters were not calculable from the Day 5 samples, as no clear terminal phase could be identified in the data for any of the three animals. Overall, the data showed that the metabolic turnover of CBD was consistent following a single dose or multiple twice-daily doses of 100 mg/mL CBD oral formulation.

GI tract samples

Individual concentrations of CBD, THC, and 11-OH-THC in the GI tract contents of all minipigs are shown in Table 2. THC and 11-OH-THC were not detected in any of the GI tract samples. Mean CBD concentrations in GI contents reached 84,500 ng/mL (stomach) and 43,900 ng/mL (small intestine), ~326 times and 169 times the maximum day 5 plasma concentrations, respectively (Figs. 1 and 2).

Discussion

The results show that standard clinical doses of CBD in a sesame oil formulation do not convert to THC in the minipig. These data support previous reports suggesting that caution should be taken when extrapolating *in vitro* results to the *in vivo* situation.[12,13] Although *in vitro* studies are essential in drug development, absorption of a drug via the GI tract is a multistep, dynamic process that needs to account for physiologic factors, formulation, and influences from the whole organism to yield accurate predictions.[12,17]

Plasma levels of CBD found in the minipigs were similar to those measured in the clinic (average plasma concentration = 380 ng/mL),[16] supporting the notion that the CBD plasma levels in the minipig were representative of those observed in a clinical environment. High concentrations of CBD were detected in the stomach and small intestine of the minipigs. However, THC and 11-OH-THC were not found in any of the plasma or GI tract samples across all time points. These results strongly suggest that the conversion of CBD to THC observed *in vitro*[8,9] is not reproducible *in vivo*, which is in agreement with previous clinical observations from human studies.[10,11]

Schwilke et al.[18] found that after multiple high doses of THC to human subjects, the metabolites 11-OH-THC and THC-COOH increased over time, indicating accumulation, whereas THC did not. Therefore, it is likely that if CBD had converted to THC in our study, 11-OH-THC would have accumulated to a detectable level (>0.5 ng/mL) over the 5-day period. However, plasma 11-OH-THC was not detected in this study.

When the findings of the present study are set beside the data that describe the conversion of CBD to THC in simulated gastric juices *in vitro*, it is evident that the use of a complete GI system in a species predictive of the human GI tract can produce markedly different results. We have, therefore, substantially extended our understanding of the likelihood of such a conversion taking place by demonstrating its absence in an intact, *in vivo mammalian* system. We acknowledge that our study had some limitations such as group sample size and end-point analytes. Consequently, supplementary research in human subjects with the measurement of 11-COOH-THC and Δ^8-THC, in addition to validated *in silico* modeling may be required to confirm the hypothesis supported by our results that the conversion of CBD to THC does not occur *in vivo*.

Conclusion

The findings of this study suggest that, when dosed orally in a sesame oil formulation to give CBD plasma exposures similar to those known to be clinically relevant in patients, CBD does not convert to THC in the minipig. This finding significantly advances our understanding of the safety concerns regarding CBD-based therapies beyond *in vitro* systems. Given the randomized controlled clinical trials of oral CBD now published, confirmation of these findings in humans may be warranted.

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Author Disclosure Statement

All authors are employees of GW Research Ltd.

References

1. Jones NA, Hill AJ, Smith I, et al. Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo [published online November 11, 2009]. J Pharmacol Exp Ther. 2010;332:569–577.
2. Jones NA, Glyn SE, Akiyama S, et al. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure. 2012;21:344–352.
3. Jones N, Hill T, Stott C, et al. Assessment of the anticonvulsant effects and tolerability of GW Pharmaceuticals' cannabidiol in the anticonvulsant screening program [Abstract No. 3.034]. Presented at: American Epilepsy Society Annual Meeting; December 4–8, 2015; Philadelphia, PA. www.aesnet.org/meetings_events/annual_meeting_abstracts/view/2325855 Accessed May 8, 2017.
4. Jones N, Hill A, Hill T, et al. Assessment of the anticonvulsant effects and tolerability profile of cannabidiol: GW Pharmaceuticals' preclinical program [Abstract No. 2.405]. Presented at: American Epilepsy Society Annual Meeting; December 5–9, 2015; Seattle, WA. www.aesnet.org/meetings_events/annual_meeting_abstracts/view/1868957 Accessed May 8, 2017.
5. Devinsky O, Marsh E, Friedman D, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open label interventional trial. Lancet Neurol. 2016;15:270–278.
6. Devinsky O, Cross JH, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med 2017;376:2011–2020.
7. Thiele E, Mazurkiewicz-Beldzinska M, Benbadis S, et al. Cannabidiol (CBD) significantly reduces drop seizure frequency in Lennox Gastaut syndrome: results of a multicenter, randomized, double blind, placebo-controlled trial (GWPCARE4) [Abstract No. 1.377]. Presented at: American Epilepsy Society Annual Meeting; December 2–6, 2016; Houston, TX.
8. Watanabe K, Itokawa Y, Yamaori S, et al. Conversion of cannabidiol to D9-tetrahydrocannabinol and related cannabinoids in artificial gastric juice, and their pharmacological effects in mice. Forensic Toxicol. 2007;25:16–21.
9. Merrick J, Lane B, Sebree T, et al. Identification of psychoactive degradants of cannabidiol in simulated gastric and physiological fluid. Cannabis Cannabinoid Res. 2016;1:102–112.
10. Consroe P, Kennedy K, Schram K. Assay of plasma cannabidiol by capillary gas chromatography/ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. Pharmacol Biochem Behav. 1991;40:517–522.
11. Martin-Santos R, Crippa JA, Batalla A, et al. Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. Curr Pharm Des. 2012;18:4966–4979.
12. Antunes F, Andrade F, Ferreira D, et al. Models to predict intestinal absorption of therapeutic peptides and proteins. Curr Drug Metab. 2013;14:4–20.
13. Pelkonin O, Boobis AR, Gundert-Remy U. In vitro prediction of gastrointestinal absorption and bioavailability: an experts' meeting report. Eur J Clin Pharmacol. 2001;57:621–629.
14. Bode G, Clausing P, Gervais F, et al. The utility of the minipig as an animal model in regulatory toxicology. J Pharmacol Toxicol Methods. 2010;62:196–220.
15. Soucek P, Zuber R, Anzenbacher P, et al. Minipig cytochrome P450 3A, 2A and 2C enzymes have similar properties to human analogs. BMC Pharmacol. 2001;1:1471–2210.
16. Wong M, Devinsky O, Thiele E, et al. A dose ranging safety and pharmacokinetic study of cannabidiol (CBD) in children with Dravet syndrome (GWPCARE1) [Abstract No 2.361]. Presented at: American Epilepsy Society Annual Meeting; December 2–6, 2016; Houston, TX. www.aesnet.org/meetings_events/annual_meeting_abstracts/view/2422093 Accessed May 8, 2017.
17. Zgair A, Lee J, Wong JC, et al. Effect of long-chain triglyceride formulation on the oral bioavailability of cannabidiol. Abstract presented at: American Association of Pharmaceutical Scientists 2015 Annual Meeting and Exposition; October 25–29, 2015; Orlando, FL. http://abstracts.aaps.org/Verify/AAPS2015/PosterSubmissions/T2331.pdf Accessed March 24, 2017.
18. Schwilke EW, Schwope DM, Karschner EL, et al. Delta9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC plasma pharmacokinetics during and after continuous high-dose oral THC. Clin Chem. 2009;55:2180–2189.

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Abbreviations Used

CBD = cannabidiol
GI = gastrointestinal
LC-MS/MS = liquid chromatography with tandem mass spectrometry
LLOQ = lower level of quantification
THC = Δ^9-tetrahydrocannabinol
11-OH-THC = 11-hydroxy-Δ^9-tetrahydrocannabinol