Title	In vitro quantification of sequential metabolism kinetics of Δ^9-tetrahydrocannabinol and its metabolites
Keywords (up to 5)	Metabolism, cannabinoid
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Abstract	Use of marijuana during pregnancy has been associated with negative outcomes[1]. The most abundant and psychoactive component in cannabis, Δ^9-tetrahydrocannabinol (THC) and its active metabolite 11-OH-THC, likely perpetrate fetal risks. Determining fetal exposure to THC/11-OH-THC to assess fetal/neonatal risks from maternal consumption of marijuana is ethically and logistically challenging. An alternative is to use physiologically-based pharmacokinetic (PBPK) modeling and simulation to predict maternal cannabinoid exposure, as this is the driving force of fetal cannabinoid exposure. Here, we report metabolic kinetic parameters (V_{max} and K_m) necessary for building a linked THC/11-OH-THC maternal PBPK model. Substrate depletion studies were conducted using pooled (n=50) human liver microsomes in the presence of co-factors NADPH and UDPGA (cytochrome P450 (CYP) and UGT enzymes, respectively). Substrate depletion and metabolite formation was monitored via LC-MS/MS. Depletion and formation kinetics were analyzed and used as initial estimates for estimating V_{max} and K_m parameters using a comprehensive sequential metabolism model built in Phoenix (Certara). THC is metabolized to 11-OH-THC with K_m = 0.14±0.03 µM and V_{max} = 0.76±0.17 nmol/min/mg and to other metabolites with K_m = 5.24±9.39 µM and V_{max} = 2.30±2.82 ml/min/mg. Majority of THC is metabolized to 11-OH-THC via CYP2C9 while 7% of THC is depleted via CYP3A4. 11-OH-THC is metabolized to COOH-THC with K_m = 0.53±0.13 µM and V_{max} = 0.0056±0.0004 nmol/min/mg and to other metabolites with K_m = 19.46±26.24 µM and V_{max} = 8.92±14.24 nmol/min/mg. Formation of COOH-THC accounts for 2.3% of 11-OH-THC depletion, making COOH-THC not the major metabolite of 11-OH-THC. 11-OH-THC was depleted by UGT enzymes with K_m = 0.64±0.15 µM and V_{max} = 0.36±0.10 nmol/min/mg. UGT and CYP enzymes account for 54% and 46% depletion of 11-OH-THC, respectively. The mechanistic information presented here will be incorporated into a maternal-fetal PBPK model previously developed[2] to predict cannabinoid maternal and fetal exposure.
References	1. Grant, K.S., Petroff, R., Isoherranen, N., Stella, N. & Burbacher, T.M. Cannabis Use during Pregnancy: Pharmacokinetics and Effects on Child Development. *Pharmacol Ther*, (2017). 2. Zhang, Z., Imperial, M.Z., Patilea-Vrana, G.I., Wedagedera, J., Gaohua, L. & Unadkat, J.D. Development of a Novel Maternal-Fetal Physiologically Based Pharmacokinetic Model I: Insights into Factors that Determine Fetal Drug Exposure through Simulations and Sensitivity Analyses. *Drug Metab Dispos*, (2017).