molecular pharmaceutics

Article

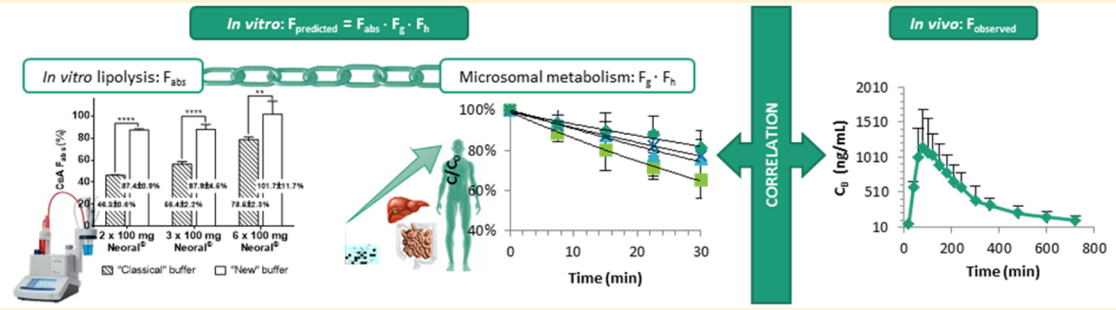
pubs.acs.org/molecularpharmaceutics

Linking *in Vitro* Lipolysis and Microsomal Metabolism for the Quantitative Prediction of Oral Bioavailability of BCS II Drugs Administered in Lipidic Formulations

Paloma Benito-Gallo,[†] Maria Marlow,[†] Vanessa Zann,[‡] Peter Scholes,[‡] and Pavel Gershkovich*[,†]

[†]School of Pharmacy, University of Nottingham, University Park, Nottingham NG7 2RD, U.K.

[‡]Quotient Clinical Ltd., Mere Way, Ruddington Fields, Nottingham NG11 6JS, U.K.



ABSTRACT: Lipidic formulations (LFs) are increasingly utilized for the delivery of drugs that belong to class II of the Biopharmaceutics Classification System (BCS). The current work proposes, for the first time, the combination of *in vitro* lipolysis and microsomal metabolism studies for the quantitative prediction of human oral bioavailability of BCS II drugs administered in LFs. Marinol and Neoral were selected as model LFs, and their observed oral bioavailabilities ($F_{observed}$) were obtained from published clinical studies in humans. Two separate lipolysis buffers, differing in the level of surfactant concentrations, were used for digestion of the LFs. The predicted fraction absorbed (F_{abs}) was calculated by measuring the drug concentration in the micellar phase after completion of the lipolysis process. To determine first-pass metabolism ($F_g \cdot F_h$), drug depletion studies with human microsomes were performed. Clearance values were determined by applying the "*in vitro* half-life" approach. The estimated F_{abs} and $F_g \cdot F_h$ values were combined for the calculation of the predicted oral bioavailability ($F_{predicted}$). Results showed that there was a strong correlation between $F_{observed}$ and $F_{predicted}$ values only when F_{abs} was calculated using a buffer with surfactant concentrations closer to physiological conditions. The general accuracy of the predicted values suggests that the novel *in vitro* lipolysis/metabolism approach could quantitatively predict the oral bioavailability of lipophilic drugs administered in LFs.

KEYWORDS: *in vitro lipolysis, microsomal metabolism, IVIVC, oral bioavailability, Δ^9-tetrahydrocannabinol, cyclosporine A*

1. INTRODUCTION

Current experimental and computational drug discovery techniques have exponentially increased the number of potential pharmacologically active drug candidates.[1] However, the majority of these new chemical compounds suffer from low aqueous solubility,[2] and therefore, their bioavailability could be limited if they are intended for oral administration. Lipid-based formulations (LFs) are increasingly utilized for the delivery of poorly water-soluble drugs. Mechanisms by which LFs may enhance the oral bioavailability of poorly water-soluble drugs include promoting drug solubilization in the intestinal milieu, delaying gastric emptying and transit time, inhibiting intestinal efflux transporters, reducing CYP-mediated gut wall metabolism, and increasing intestinal lymphatic transport of highly lipophilic compounds.[3−9] The *in vitro* assessment of LFs is challenging, since traditional dissolution testing lacks the flexibility to deal with the complicated interplay between lipid digestion products, drug-loading, and micellar solubilization.[10]

In this regard, *in vitro* lipolysis is capable of mimicking the intestinal lipid digestion process and, therefore, is a suitable technique for assessing the fate of drugs administered in LFs.[11−13] Pancreatic lipase is responsible for the *in vitro* lipolysis process. This enzyme hydrolyses the triglycerides in the formulation, releasing fatty acids and prompting a drop in pH. A pH-stat titrator is used to control this drop in pH and to keep it within the physiological range.[14] Once the lipolysis process is complete, the resulting mixture is separated into discrete layers. The most relevant of these phases is the aqueous one, since it contains micellar structures within which hydrophobic molecules are solubilized. The general assumption made by researchers working with the model is that the fraction

Received: July 1, 2016
Revised: August 18, 2016
Accepted: August 24, 2016

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

of drug dose solubilized in the micellar phase is most readily available for absorption.

Previous lipolysis studies have focused on rank-ordering the performance of LFs.[12,15−18] This was done by correlating the percentage of drug solubilized in the micellar phase with the area under the plasma concentration−time curve or the maximum concentration obtained after oral administration of the tested LF to animals (rats, mini-pigs, or dogs). As an example, Dahan and Hoffman[13,19] reported linear relationships (R^2 > 0.98, 0.99) for progesterone and griseofulvin when administered to rats in short-, medium-, and long-chain triglycerides. However, no linear *in vitro in vivo* correlation (IVIVC) was found for the other two tested drugs, vitamin D_3 and dexamethasone. To the best of our knowledge, there are no publications describing the correlation between the *in vitro* lipolysis data of LFs and drug exposure in humans.

LFs are mainly used for the delivery of drugs that belong to class II of the Biopharmaceutics Classification System (BCS), i.e. drugs characterized by high permeability and low aqueous solubility. Intestinal micellar solubilization and first-pass metabolism (rather than membrane permeability) are the main barriers to the oral bioavailability of BCS II drugs. To note, in the case of highly lipophilic compounds, the contribution of intestinal lymphatic transport to overall oral bioavailability should also be considered.[20] The intraluminal solubility of BCS II drugs in LFs can be estimated using the *in vitro* lipolysis model, and the first-pass extraction ratio can be assessed by performing microsomal stability assays. Based on this, the current work proposes, for the first time, the combination of *in vitro* lipolysis and microsomal metabolism studies for the prediction of human oral bioavailability of lipophilic drugs administered in LFs. This novel approach, if successful, would transform the lipolysis model from a qualitative tool to a quantitative one. If predictive of the *in vivo* response, this novel methodology could drastically reduce the need for animal experiments, improve accuracy and predictability for formulation design, and lead to better designed clinical trials, hence reducing the time and cost of industrial research and development.

For this investigation, Δ^9-tetrahydrocannabinol (THC) and cyclosporine A (CsA) were selected as model BCS class II drugs. THC is an orally active cannabinoid which has complex effects on the central nervous system. THC is a highly lipophilic (log P = 6.97[21]) and poorly water-soluble (S_w = 0.77−2.8 μg/mL[22]) drug marketed under the brand name Marinol. Marinol is approved for the treatment of anorexia in AIDS patients, as well as for refractory nausea and vomiting in patients undergoing chemotherapy. Marinol contains dronabinol (synthetic THC) dissolved in sesame oil.[23] CsA acts as an immunosuppressant drug and is broadly used to prevent graft rejection in organ transplantation patients, and for the treatment of severe arthritis and psoriasis, among other indications.[24] CsA is characterized by moderately high lipophilicity (log P = 3.35[21]) and very low solubility in aqueous media (S_W = 5 ± 2 μg/mL[25]), and it is commercialized mainly as Sandimmun Neoral, a lipid-based self-emulsifying drug delivery system (SEDDS) of CsA. Neoral consists of CsA dissolved in a mixture of lipids, cosolvents, and surfactant. Five published clinical studies were selected as a set of clinical data for the purpose of predicting the human oral bioavailability of THC in Marinol and of CsA in Neoral.[26−30]

The overall aim of this work was to develop a novel quantitative tool of oral bioavailability of BCS II drugs in LFs, by combining *in vitro* lipolysis and metabolism, using Marinol (THC) and Neoral (CsA) as model formulations.

2. EXPERIMENTAL SECTION

2.1. Materials. Sodium hydroxide solution (NaOH, 1 M), Trizma maleate, sodium taurocholate hydrate (98% *w/w*), L-α-lecithin (~60% pure L-α-phosphatidylcholine, from egg yolk), pancreatin powder from porcine pancreas (8 × US Pharmacopeia specifications activity), verapamil (≥99% *w/w*), dexamethasone (≥98% *w/w*), chlorpromazine (≥98% *w/w*), α-tocopherol, Kolliphor RH 40, potassium phosphate dibasic anhydrous (K_2HPO_4), potassium phosphate monobasic anhydrous (KH_2PO_4), magnesium chloride ($MgCl_2$, ≥98% *w/w*), ammonium acetate (≥99% *w/w*), and formic acid (~98% *v/v*) were all purchased from Sigma-Aldrich (Dorset, UK). Sodium chloride (99.5% *w/w*) and nicotinamide adenine dinucleotide phosphate tetrasodium salt hydrate (NADPH, 93% *w/w*) were products from Fisher Scientific (Leicester, UK). Calcium chloride anhydrous (93% *w/w*) and vitamin D_3 ($VitD_3$, 98% *w/w*) were obtained from Alfa Aesar (Heysham, UK). Sesame oil and corn oil were purchased from Acros Organics (Geel, Belgium). Dronabinol (synthetic (−)-*trans*-Δ^9-THC) and CsA were products from THC Pharm GmbH (Frankfurt, Germany) and Kemprotec Ltd. (Carnforth, UK), respectively. Cannabidiol (CBD) was kindly donated by GW Pharmaceuticals (Cambridge, UK). Propylene glycol (PG) was purchased from Amresco (Ohio, USA). Human liver microsomes were obtained from Gibco Invitrogen (Paisley, UK). Intestinal human microsomes were a product from Tebu-Bio Ltd. (Peterborough, UK). The standard buffer solutions for calibration of the pH-electrode were purchased from YSI Incorporated (Ohio, USA) and Hanna Instruments (Rhode Island, USA). All solvents were of high-performance liquid chromatography (HPLC) grade or analytical grade and were used without any further purification.

2.2. Selection of Clinical Studies and Pharmacokinetic Analysis: Calculation of the Observed Oral Bioavailability ($F_{observed}$) in Human Subjects. The selection of model formulations was done based on availability of published clinical data. An exhaustive search in the literature was performed in order to find publications which would provide the necessary information to accurately reproduce the *in vivo* digestion of the LF using the *in vitro* model. The studies were chosen based on the following criteria: (a) volunteers had to be dosed in the fasted state; (b) volunteers had to be healthy (no history of renal, hepatic, or gastrointestinal diseases) adults (18 to 55 years old); (c) clear information about the exact amount of lipidic formulation administered had to be available; and (d) relevant pharmacokinetic data for a single oral dose and intravenous (IV) administration had to be available.

More than 30 marketed LFs[31] were screened with the aim of finding published clinical trials that would meet the criteria detailed above. Some LFs were not considered because sales had been discontinued (Fortovase, saquinavir[32]), because more than one active pharmaceutical ingredient was included in the medicine (Kaletra, lopinavir and ritonavir[33]), or because they were extended-release drug products (Ketas, ibudilast;[34] MXL capsules, morphine sulfate;[35] and Detrol LA, tolterodine tartrate[36]). Most of the LFs containing antivirals and antineoplastics were rejected mainly due to available trials referring to nonhealthy volunteers, such as HIV patients (Agenerase, amprenavir;[37] Norvir, ritonavir;[38] Aptivus, tipranavir;[39] and Sustiva, efavirenz[40]) and cancer patients (Targretin, bexar-

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

otene[41]). Other LFs were discarded due to unavailable (Epadel, ethyl icosapentate; and Fenogal, fenofibrate), or very limited (Avodart, dutasteride;[42] and Infree S capsules, indomethacin farnesil[43]) available oral pharmacokinetic data for single oral doses. Some LFs were rejected because clinical trials included volunteers in the fed state, and/or it was not indicated whether the administered formulation was actually a lipidic one (Juvela N, tocopherol nicotinate;[44] Selbex, teprenone;[45] Accutane, isotretinoin;[46] and Rapamune, sirolimus[47]). If only one single valid study could be found, the LFs were not taken into account either (Depakene, valproic acid;[48] Cipro, ciprofloxacin;[49] Glakay capsules, menatetrenone;[50] Vesanoid, tretinoin;[51] Prometrium, progesterone;[52] and Hectorol, doxercalciferol[53]). The LFs of testosterone undecanoate (Andriol and Restandol) were discarded as well because the stability of this ester prodrug in the gastrointestinal tract remains unknown.

Eventually, five medicines were short-listed: Marinol (THC),[26,27,54−58] One-Alpha capsules (alfacalcidol),[59,60] Rocaltrol (calcitriol),[60,61] Heminevrin (clomethiazole edisilate),[62,63] and Neoral (CsA).[28−30,64−69] Marinol (sesame oil), One-Alpha capsules (sesame oil and α-tocopherol), Rocaltrol (fractionated triglycerides of coconut oil or palm oil) and Heminevrin (fractionated coconut oil) are all Type I lipidic formulations, whereas Neoral is a SEDDS consisting of lipids, surfactants and cosolvents (Type IIIA). For the sake of formulation diversity, we decided to select one Type I formulation (Marinol, already available in our laboratory), and one Type III (Neoral).

The clinical studies of the selected LFs were narrowed down further according to the inclusion/exclusion criteria explained above. In the case of Neoral, numerous studies were rejected because the trials were not done with healthy volunteers, who were usually organ transplant patients,[65] due to the impossibility of confirming whether the administered formulation was actually Neoral,[66−68] and due to impossibility of purchasing the administered dose strength (i.e., 60 mg capsules are not commercially available in UK).[64,69] In the case of Marinol, some studies were not considered because of the volunteers were not healthy (cancer patients[54]), because the fed/fasted state of the subjects was not indicated,[58] and because of incomplete information about the formulation, such as the volume of coadministered oil,[55,56] or the dose strength of the capsules.[57]

Finally, it was possible to select five published clinical studies that described oral administration of THC and CsA and that fulfill the eligibility criteria described above. In the case of Marinol, the studies described the administration of: (a) 2 × 10 mg capsules (20 mg THC in ∼0.5 mL sesame oil),[26] and (b) 1 × 10 mg capsule (10 mg THC in ∼0.25 mL sesame oil).[27] Regarding Neoral, the studies described the administration of: (a) 2 × 100 mg capsules (200 mg CsA in 2 mL SEDDS),[28,29] (b) 3 × 100 mg capsules (300 mg CsA in 3 mL SEDDS),[30] and (c) 6 × 100 mg capsules (600 mg CsA in 6 mL SEDDS).[28]

Data that described intravenous administration of THC[58,70−73] (Table 1) and CsA[68,74] (Table 2) was collected as well to calculate the absolute $F_{observed}$.

2.3. *In Vitro* Lipolysis Studies. *2.3.1. Composition and Preparation of Blank Lipidic Formulations.* Blank formulations mimicking the excipient composition of Marinol and Neoral, but lacking the active pharmaceutical ingredients, were prepared and lipolysed in order to generate matrixes from which appropriate calibration curves were constructed. Marinol blank formulation consisted of plain sesame oil.[23] The exact composition of Neoral is not fully disclosed,[24] hence some

Table 1. Selected Pharmacokinetic Parameters of Tetrahydrocannabinol after Intravenous and Oral Administration, Reported in the Literature

	Intravenous					Oral		
	Wall et al.[58]		Hunt et al.[70]	Ohlsson et al.[71]	Kelly and Jones[72]	Naef et al.[73]	Naef et al.[26]	Goskonda et al.[27]
n	6 (Women)	6 (Men)	4	11	4 (Users)	8	12	18
Dose (mg)	2.2	4	2	5	5	4	20	10
AUC[a] (ng·min/mL)	12100 ± 4620	16800 ± 4000	1272 ± 90	4330 ± 620	9907 ± 3785	7028 ± 5829	1865	762 ± 527
Average AUC/dose (ng·min/mL/mg)	2277 ± 2020[c]						93	76 ± 53[b]
CL[b] (mL/min/kg)	3.60 ± 0.90	3.44 ± 0.96	10.86 ± 1.83	NR	10.64 ± 9.44	10.56 ± 3.92	11.13 ± 5.69	
Average CL[c] (mL/min/kg)	8.11 ± 5.07							

[a]AUC: Area under the concentration−time curve; CL: clearance. [b]Values are expressed as means ± SD. [c]Values expressed as weighted means ± overall SD; NR: Not reported.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 2. Selected Pharmacokinetic Parameters of Cyclosporine A after Intravenous and Oral Administration, Reported in the Literature

	Intravenous		Oral					
	Gomez et al.[74]	Hebert et al.[68]	Odeberg et al.[30]		Kim et al.[29]		Mueller et al.[28]	
n	5	5	3	6	12	24	24	
Dose (mg)	150	225	300	300	200	200	600	
AUC[a] (ng·min/mL)	400968 ± 112875	608290 ± 98073	309120 ± 61680	351480 ± 127560	322752 ± 11646	208260 ± 61740	589860 ± 142140	
Average AUC/dose (ng·min/mL/mg)	2688 ± 615[c]		1122 ± 374[c]		1232 ± 371[c]		983 ± 237[b]	
CL[b] (mL/min/kg)	5.30 ± 1.40	4.97 ± 0.80						
Average CL[c] (mL/min/kg)	5.14 ± 1.15							

[a]AUC: Area under the concentration−time curve; CL: clearance. [b]Values are expressed as means ± SD. [c]Values expressed as weighted means ± overall SD.

approximations had to be made. Based on available information, and assuming a standard amount of DL-α-tocopherol of 2.5 mg per unit dose,[75] it was calculated that one 100 mg/mL Neoral capsule contains, in addition to 100 mg CsA, the following excipients: 100 mg PG, 405 mg Kolliphor RH 40, 0.119 mL ethanol, and 0.35 mL mono-, di-, and triglycerides of corn oil. These specified amounts of excipients were mixed under constant stirring at 37 °C, and stored at room temperature until used.

2.3.2. Scaling down from in Vivo to in Vitro Conditions: Calculation of the Amount of Formulation To Disperse in the Digestion Medium of the Lipolysis Model. It has been suggested[76] that for the assessment of the mass of soluble drug in the small intestine an *in vivo* dissolution volume of 80 to 100 mL, rather than the classic 250 mL, would be more accurate. Therefore, it was decided to follow this approach in the current work.

The digestion medium of *in vitro* lipolysis model consists of approximately 40 mL. Therefore, the amount of formulation corresponding to each clinical study was scaled down accordingly to match the *in vivo* situation, as indicated in eq 1:

$$\text{Volume of LF } \textit{in vitro} = \frac{40\ \text{mL} \cdot \text{Volume of LF } \textit{in vivo}}{100\ \text{mL}} \quad (1)$$

Hence, the *in vivo* administration of 2 × 10 mg Marinol (0.5 mL), 1 × 10 mg Marinol (0.25 mL), 2 × 100 mg Neoral (2 mL), 3 × 100 mg Neoral (3 mL), and 6 × 100 mg Neoral (6 mL) *in vivo*, was mimicked in the *in vitro* lipolysis model by dispersing 200 μL of Marinol, 100 μL of Marinol, 800 μL of Neoral, 1200 μL of Neoral, and 2400 μL of Neoral, respectively.

2.3.3. Simulated Intestinal Buffers. Two different intestinal fluid compositions simulating the contents of the jejunum in the fasted state were used for lipolysis experiments. The composition of these digestion media (Table 3) differed in the concentration of sodium taurocholate (bile salt, BS) and phosphatidylcholine (phospholipid, PL). The "classical" buffer was analogous to those previously used by our and other lipolysis research groups,[12−14,16,77,78] and consisted of higher concentrations of surfactants in a proportion 4:1 BS/PL. On the other hand, the "new" buffer, was closer to Fasted-State Simulated Intestinal Fluid-version 2 and physiological conditions, and therefore contained lower surfactant concentrations in a ratio 15:1 BS/PL.[79]

Table 3. Composition of the Two Different Lipolysis Media Used for the Intraluminal Processing of Marinol and Neoral

Buffer	Concentration (mM)	
	Classical	New
Trizma maleate	50	50
Sodium chloride	150	150
Calcium chloride	2	2
Sodium taurocholate	5	3
Phosphatidylcholine	0.75	0.2

2.3.4. Experimental Procedure. *In vitro* lipolysis studies were conducted as described in previous reports.[12−14,16,77,78] Calculated formulation volumes were dispersed in 35.5 mL of biorelevant medium contained in a reaction vessel with continuous stirring and kept at 37 °C. After 15 min of equilibration, 3.5 mL of lipase/colipase extract (containing 735 tributyrin units of lipase activity per milliliter of digest) was

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

added to the mixture to initiate the enzymatic hydrolysis. A pH-stat titrator unit (T50 Graphix, Mettler Toledo Inc., Leicester, UK) coupled to a pH-electrode (DGi111-SC, Mettler Toledo Inc.) was used to keep experimental pH under control (6.80 ± 0.05) by titrating the released ionised fatty acids with 1 M NaOH solution. Each experiment was repeated six times.

Once the lipolysis process was finished, the resulting reaction mixtures were collected in ultracentrifuge tubes (Beckman Coulter, High Wycombe, UK) for subsequent density gradient ultracentrifugation. The mixtures were centrifuged (Sorvall Discovery 100SE ultracentrifuge, TH-641 rotor, Thermo Scientific, North Carolina, USA) at ∼197000 *g* and 37 °C for 90 min. After centrifugation, phases were separated, collected (sediment was resuspended in water) and stored at -80 °C until analysis.

2.3.5. Calculation of the Predicted Fraction Absorbed (F_{abs}). Since BCS II drugs are highly permeable and lipidic formulations are thought to inhibit drug efflux transporters,[5,6] it was assumed that all the mass of THC and CsA solubilized in the micellar phase (MP) would be completely absorbed. To determine F_{abs}, the concentration of drug found in the MP ($[Drug]_{MP}$) was multiplied times the *in vivo* dissolution volume (100 mL) and divided by the administered clinical dose, as indicated in eq 2:

$$F_{abs} = [Drug]_{MP}\left(\frac{mg}{mL}\right)\cdot\frac{100\ mL}{Clinical\ dose\ (mg)} \quad (2)$$

2.4. Microsomal Metabolism Stability Studies.

2.4.1. Experimental Procedure. Clearance values were determined by applying the "*in vitro* half-life" approach, which is based on the measurement of the first-order rate depletion constant of a drug substrate.[80] Microsomal incubations were conducted in a similar manner to that described previously.[81] Reaction mixtures consisting of 720 μL of 100 mM aqueous potassium phosphate buffer (KH_2PO_4/K_2HPO_4, pH 7.4), 240 μL of 2.5 mg/mL human microsomal protein in phosphate buffer, 120 μL of 100 mM aqueous $MgCl_2$, and 24 μL of 0.05−0.5 mM drug substrate were placed in a test tube under constant stirring, inside a water bath kept at 37 °C. After 3 min of preincubation, reactions were initiated by the addition of 96 μL of 125 mM NADPH in phosphate buffer. Final concentrations of each component of the reaction mixture are listed in Table 4. Experiments were performed at least five times, and the organic solvent concentration (acetonitrile) in the incubation was less than 0.5% (*v*/*v*). Verapamil and dexamethasone were used as positive and negative controls (extensive and limited hepatic metabolism), respectively. Control experiments without NADPH were carried out as well to monitor the matrix effect on THC and CsA metabolism.

Table 4. Concentrations of Microsomal Incubations Components at t = 0 min

Compound	Concentration
KH_2PO_4/K_2HPO_4	60 mM
$MgCl_2$	10 mM
Test compound	0.001, 0.0025, 0.005, 0.010 mM[a]
Human intestinal/hepatic microsomes	0.5 mg/mL
NADPH	1 mM

[a]Due to limited availability of intestinal protein, intestinal metabolism studies were performed only at a single concentration level (0.001 mM).

At five specified time points (up to 30 and 60 min, for hepatic and intestinal assays, respectively), 200 μL aliquots were removed and added to 16 × 150 mm glass tubes containing the appropriate internal standard in ice-cold methanol or acetonitrile, (to precipitate the proteins and stop the reaction).

2.4.2. Determination of in Vitro Intrinsic Clearance Values. For the determination of the depletion rate constant, k_{dep}, incubation data were fitted to a monoexponential model, as shown in eq 3:

$$\frac{C_t}{C_0} = e^{-k_{dep}\cdot t} \quad (3)$$

where C_t is the concentration of the compound remaining at each time point and C_0 is the concentration of the compound at the beginning of the incubation process. For hepatic metabolism, the depletion rate constants obtained with different initial concentrations were used to calculate the theoretical depletion constant at infinitesimally low substrate concentration ($k_{dep,[S]\to 0}$), as indicated in eq 4:[82−84]

$$k_{dep} = k_{dep,[S]\to 0}\cdot\left(1 - \frac{[S]}{[S] + K_M}\right) \quad (4)$$

where $[S]$ is the substrate concentration and K_M is the Michaelis−Menten constant.

The observed *in vitro* intrinsic clearance (CL_{int}) was calculated by multiplying $k_{dep,[S]\to 0}$ (or k_{dep} for intestinal metabolism) times the volume of incubation medium (V) normalized by the amount of microsomal protein (m_{inc}) as shown in eq 5:

$$CL_{int} = \frac{k_{dep,[S]\to 0}\cdot V}{m_{inc}} \quad (5)$$

Subsequently, CL_{int} values were corrected for the fraction of drug unbound in the incubation medium (CLu_{int}). For CsA the extent of nonspecific binding (fu_{inc}) was predicted using the Austin equation[85] and a logP value equal to 3.35.[86] It is known that, for highly lipophilic drugs such as THC (logP = 6.97[86]), Hallifax or Austin equations lead to poor predictions.[87] On the other hand, experimental measurement of fu_{inc} of these drugs is also extremely challenging due to nonspecific binding. Assuming that THC binds to serum proteins in the same way as to microsomal proteins, it is possible to estimate THC fu_{inc} using eq 6:

$$fu_{100\%} = \frac{fu_{X\%}}{100 - (100 - X)\cdot fu_{X\%}} \quad (6)$$

where $fu_{100\%}$ is the fraction of THC unbound in plasma (0.0102[86]), $fu_{X\%}$ is the fraction of THC unbound in the incubation media, and X is the ratio between the total concentration of proteins in human serum (approximately 70 mg/mL[88]) and the microsomal concentration in the incubations (0.5 mg/mL).

2.4.3. Calculation of the Predicted Fraction Escaping Hepatic Metabolism (F_h). Physiologically based scaling factors (standard human microsomal recovery of 32 mg microsomes/g liver,[89,90] and average liver weight of 22 g liver/kg body weight[91]) were applied to transform CLu_{int} (mL/min/mg protein) into hepatic intrinsic clearances ($CLu_{h,int}$, mL/min/kg). Hepatic clearances (CL_h) were next calculated based on the "parallel-tube" model,[92] as shown in eq 7:

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

$$CL_h = Q_h(1 - e^{-fu_b \cdot CLu_{h,int}/Q}) \quad (7)$$

where Q_h is the hepatic blood flow (21 mL/min/kg)[81,93] and fu_b is the fraction unbound in blood. For CsA the fu_b value was found in the literature (0.04[94]), whereas, for THC (0.0096), it was calculated based on the fraction of drug unbound in plasma and the blood to plasma ratio (1.603[86]). F_h was finally calculated from the CL_h, as indicated in eq 8:

$$F_h = (1 - CL_h/Q_h) \quad (8)$$

2.4.4. Calculation of the Predicted Fraction Escaping Gut Wall Metabolism (F_g). The abundance data of the relevant metabolizing enzymes in the small intestine (CYP2C9 for THC, and CYP3A for CsA) were used to transform CLu_{int} values (mL/min/mg protein) into gut intrinsic clearances ($CLu_{g,int}$, L/h). These conversion values were: 8.4 pmol CYP2C9 and 43 pmol CYP3A4 per mg of microsomal protein, and total CYP2C9 and CYP3A content in the small intestine of 12 and 70.5 nmol, respectively.[95,96]

F_g were estimated using the "Q gut" model,[97,98] as defined in eq 9:

$$F_g = \frac{Q_{gut}}{Q_{gut} + fu_g \cdot CLu_{g,int}} \quad (9)$$

The fraction of drug unbound in the enterocytes (fu_g) is commonly assumed to be 1, since this has been shown to provide the greatest accuracy of prediction when using the Q_{gut} model.[99] The gut blood flow (Q_{gut}) represents a mixture of villous blood flow (Q_{villi} = 18 L/h[100]) and the permeability across the enterocytes (CL_{perm}), as indicated in eq 10:

$$Q_{gut} = \frac{Q_{villi} \cdot CL_{perm}}{Q_{villi} + CL_{perm}} \quad (10)$$

CL_{perm} was calculated through the effective intestinal permeability ($P_{eff,CsA}$ = 1.65 μm/s,[101] and $P_{eff,THC}$ = 7.56 μm/s[86]) and the small intestine cylindrical surface area (0.66 m^2[98]).

2.5. Calculation of the Predicted Oral Bioavailability ($F_{predicted}$). To calculate the predicted oral bioavailability of THC and CsA, the estimated F_{abs}, F_g, and F_h were combined, as indicated in eq 11:

$$F_{predicted}(\%) = F_{abs} \cdot F_g \cdot F_h \cdot 100 \quad (11)$$

2.6. Analytical procedures. *2.6.1. HPLC-UV Analysis.* The quantitative determinations of THC and CsA in lipolysis phases, as well as verapamil and dexamethasone content in microsomal incubations were performed using a HPLC system (Waters Alliance 2695, Waters Corp., Milford, MA, USA) equipped with a photodiode array UV detector (Waters 996). Samples temperature was controlled by a fitted chiller at 4 °C. Data acquisitions and processing was carried out using Empower 2 software (Waters).

The analysis of THC in lipolysis samples was similar to that developed by Zgair et al.,[102] with some modifications. These changes consisted in choosing a different internal standard ($VitD_3$ instead of probucol) and using 200 μL of sample volume instead of 150 μL. Aliquots of 200 μL of MP were mixed with 60 μL of 350 μg/mL $VitD_3$ in acetonitrile, and vortex-mixed for 2 min. Subsequently, 600 μL of ice-cold acetonitrile was added, and samples were vortex-mixed for 2 min. Six hundred microliters of water was added, and samples were vortex-mixed again for another 2 min. Next, 3 mL of *n*-hexane was added, and samples were vortex-mixed for 5 min. After centrifugation at ∼1200*g* (Harrier 18/80 centrifuge, swing-out rotor, MSE, London, UK) for 15 min at room temperature, the upper organic layer was transferred to a fresh glass tube and evaporated under a gentle stream of nitrogen gas at 35 °C (Techne Dri-Block Sample Concentrator, Cambridge, UK). Residues were reconstituted in 200 μL of acetonitrile, and 10 μL was injected into the HPLC system. Both THC and $VitD_3$ were detected at 220 nm. The separation was achieved using a Sonoma C18(2) 100 × 2.1 mm, 3 μm particle size column (ES Industries, West Berlin, NJ, USA), protected by a Phenomenex C18 4 × 2 mm guard cartridge (Phenomenex, Macclesfield, UK). The mobile phase was a mixture of acetonitrile and water in a ratio of 75:25 (*v*/*v*). The flow rate was set at 0.3 mL/min for 40 min at 55 °C. The lowest validated limit of quantification was 2 μg/mL, and the linearity of the method was confirmed over a concentration range of 10−350 μg/mL based on at least 8 concentration levels, with correlation coefficient (R^2) value ≥0.99.

The sample treatment of CsA lipolysis samples was similar to that of THC samples, except for the internal standard used (20 μL of 2 mg/mL CBD in acetonitrile), the extraction solvent (methyl *tert*-butyl ether), and the volume of solvent added to reconstitute the residue (1000 μL). Both CsA and CBD were monitored at 211 nm. The separation was achieved using a Sonoma C18(2) 100 × 2.1 mm, 3 μm particle size column, protected by a Phenomex C18 4 × 2 mm guard cartridge. The mobile phase was a mixture of acetonitrile and water in a ratio of 65:35 (*v*/*v*). The flow rate was set at 0.3 mL/min for 20 min at 60 °C. The lowest validated limit of quantification was 50 μg/mL, and the linearity of the method was confirmed over a concentration range of 0.1−8 mg/mL based on at least 8 concentration levels, with correlation coefficient (R^2) value ≥0.99.

Verapamil and dexamethasone[103] microsomal incubation samples were quenched with 1000 μL 2 μM chlorpromazine in methanol (internal standard for both compounds), vortex-mixed with for 10 s, and then centrifuged at ∼1200*g* at room temperature for 10 min. The upper organic phase was carefully decanted into a fresh glass tubes and the solvent was evaporated under nitrogen gas at 35 °C. The residue was reconstituted in 150 μL of aqueous acetonitrile (50%, *v*/*v*), vortex-mixed for 5 min, and centrifuged again at ∼1200*g* for 10 min. Finally, 100 μL of the resulting solutions was transferred into HPLC vials and 20 μL was injected into the HPLC instrument. Verapamil, dexamethasone and internal standard were detected at 229, 240, and 254 nm, respectively. In both cases, the separation was achieved using a Phenomenex Gemini-NX 250 × 4 mm, 5 μm particle size column, protected by a Phenomex C18 4 × 2 mm guard cartridge. The mobile phase was a mixture of acetonitrile and aqueous ammonium acetate (10 mM, pH 4.9) in a ratio of 50:50 (*v*/*v*). The flow rate was set at 0.4 mL/min for 17 min at 40 °C.

2.6.2. LC-MS/MS Analysis. The determination of THC and CsA in microsomal incubation samples was accomplished by means of LC-MS/MS analysis since lower limits of quantification had to be achieved. Microsomal metabolism samples were treated in a similar manner to lipolysis samples. After protein precipitation using 600 μL 1 μM internal standard ($VitD_3$ for THC, CBD for CsA) in acetonitrile, the procedure of water addition, mixing, extracting solvent addition, mixing, centrifuging, decanting and evaporating, was identical to that described in the previous section. Eventually, samples were

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

Table 5. Absolute Oral Bioavailability Values Calculated from the Data Reported in Published Clinical Studies ($F_{observed}$), and Calculated with the *in Vitro* Lipolysis/Metabolism Approach ($F_{predicted}$), Using Two Different Digestion Buffers[a]

			$F_{predicted}$ (%) = F_{abs}·F_g·F_h		$F_{predicted,h}$ (%) = F_{abs}·F_h		$F_{predicted,g}$ (%) = F_{abs}·F_g	
Formulation	Dose	$F_{observed}$ (%)	Classical buffer	New buffer	Classical buffer	New buffer	Classical buffer	New buffer
Marinol	2 × 10 mg THC[26]	4.1 ± 3.6	3.4 ± 1.3	1.3 ± 0.5	9.6 ± 3.6	3.7 ± 1.3	7.0 ± 1.2	2.7 ± 0.3
	1 × 10 mg THC[27]	3.4 ± 3.8	8.3 ± 3.3	1.1 ± 0.5	23.3 ± 8.9	3.2 ± 1.3	17.1 ± 3.4	2.3 ± 0.6
Neoral	2 × 100 mg CsA[28,29]	46.5 ± 18.1	21.6 ± 1.9	40.3 ± 3.7	41.7 ± 2.6	77.9 ± 5.3	24.0 ± 1.6	44.8 ± 3.1
	3 × 100 mg CsA[30]	41.8 ± 16.9	26.3 ± 2.5	41.0 ± 4.2	50.8 ± 3.6	79.2 ± 6.3	29.0 ± 2.1	45.5 ± 3.7
	6 × 100 mg CsA[28]	36.6 ± 12.1	36.6 ± 3.5	47.4 ± 6.0	70.7 ± 4.9	91.6 ± 11.6	40.6 ± 2.9	52.6 ± 5.8

[a]Predicted values ignoring gastric metabolism ($F_{predicted,h}$), and ignoring hepatic metabolism ($F_{predicted,g}$, full lymphatic transport) are included as well. Values are expressed as means ± SD.

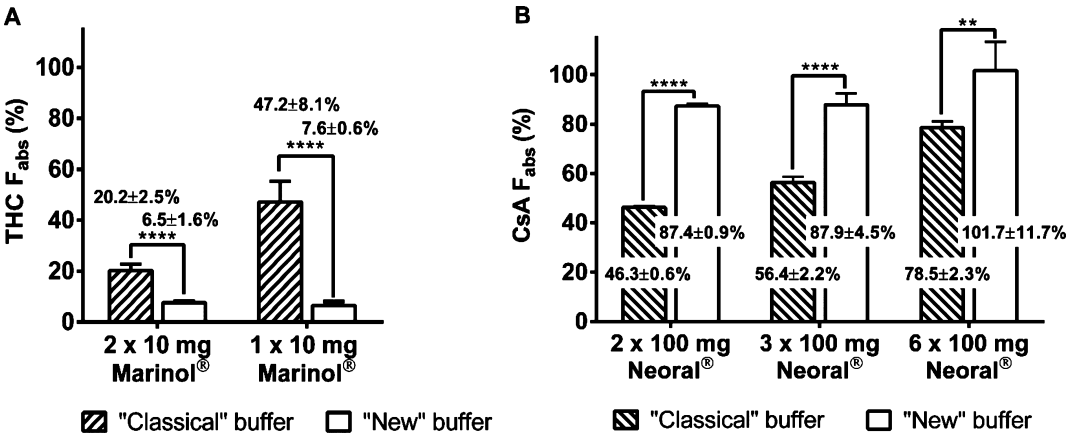


Figure 1. Fraction of absorbed dose of tetrahydrocannabinol (THC) in Marinol (A) and of cyclosporine A (CsA) in Neoral (B) estimated from lipolysis studies. Striped bars correspond to the lipolysis of the formulations using the "classical" buffer, whereas plain bars represent the "new" buffer. Values are expressed as means (n = 6) ± SD. An unpaired t-test followed by Welch's correction was used for statistical analysis. Statistically significantly different: ****, $p < 0.0001$; **, $p < 0.01$.

reconstituted in 100 μL of 0.1% (v/v) formic acid in acetonitrile, and 10 μL was injected into the HPLC instrument.

Quantitative determinations were performed using a HPLC system (Agilent 1100 Series, Agilent Technologies, Waldbronn, Germany) equipped with a Quattro Ultima triple-quadrupole mass spectrometer (Waters), using electrospray ionization for ion production. Samples temperature was controlled by a fitted chiller at 4 °C. Data acquisitions and processing was carried out using MassLynx software (Waters). Separations were achieved on a Waters XBridge C18 75 × 2.1 mm, 2.5 μm particle size column, at a flow rate of 0.3 mL/min and at 60 °C. Elution was conducted with 0.1% (v/v) formic acid in water/acetonitrile 90:10 (v/v) during 7 min, and 82.5:17.5 (v/v) during 3.5 min, for THC and CsA detection, respectively. Multiple-reaction monitoring in positive mode was used to trace ions as follows (m/z precursor ion/product ion): THC (315.2/193.0), $VitD_3$ (385.3/259.3), CsA (1219.7/1202.7) and CBD (315.2/193.0). Nitrogen was used as drying and nebulization gas at flow rates of 650 L/h and 150 L/h, respectively. The desolvation and source block temperatures were and 350 and 125 °C, respectively. The capillary voltages were 3.6 kV and 4.5 kV, for THC and CsA detection, respectively. The cone voltages were 35 and 45 V for THC and $VitD_3$ analysis, and CsA and CBD analysis, respectively.

2.7. Statistical Analysis. All presented data are expressed as mean ± standard deviation (SD), unless stated otherwise. A one-way analysis of variance (ANOVA, followed by post hoc Tukey-Kramer multiple comparison test) or an unpaired t-test with Welch's correction, was used for determining statistically significant differences among the experimental groups. Pearson's correlation test[104,105] was performed for investigating the strength of the association between $F_{observed}$ and $F_{predicted}$. A p value of 0.05 was considered the minimal level of significance. Statistical analyses were performed using GraphPad Prism version 6.00 (GraphPad Software, San Diego, CA, USA).

3. RESULTS

3.1. Pharmacokinetic Analysis: Calculation of the Observed Oral Bioavailability in Human Subjects. With the aim of calculating the observed oral bioavailability in human subjects after administration of THC in Marinol and of CsA in Neoral, pharmacokinetic data were obtained from published clinical studies (Table 1 and Table 2). The ratios of the area under the curve values normalized by the dose, after oral and intravenous administration, were used to calculate the absolute oral bioavailability in the different clinical studies. The obtained $F_{observed}$ values are summarized in Table 5.

3.2. *In Vitro* Lipolysis: Prediction of the Fraction Absorbed (F_{abs}). The intraluminal processing of the selected LFs was assessed by *in vitro* lipolysis using two different digestion buffers. This was done to determine the effect of surfactant concentrations on the overall digestion process. The adequate amount of LF to be dispersed in the 40-mL-volume vessel of the digestion medium was calculated by scaling down the amount of LF orally administered to human subjects in the selected clinical studies and by assuming an *in vivo* dissolution volume of 100 mL (see eq 1). Drug solubility in the MP following the lipolysis process and ultracentrifugation was determined by means of HPLC-UV.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

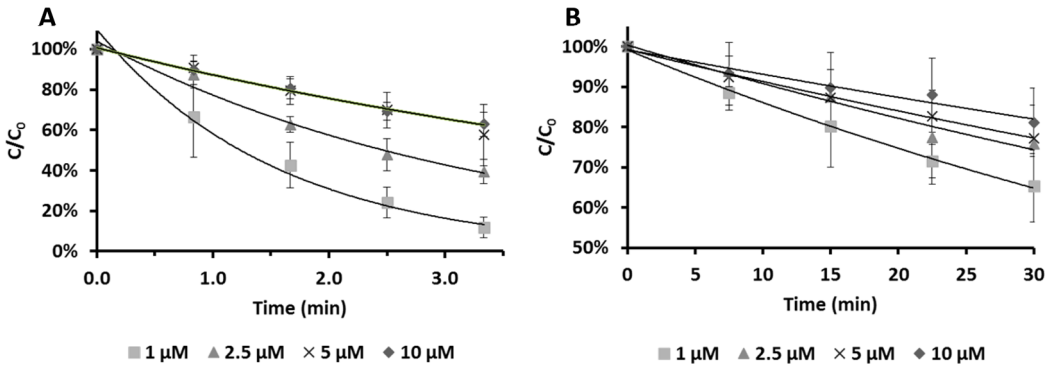


Figure 2. Depletion curves at different concentration levels derived from hepatic microsomal incubations of tetrahydrocannabinol (A) and cyclosporine A (B). The ratio between the drug concentration remaining at each time point (C) and the concentration of drug at the beginning of the incubation process (C_0) is represented versus time. Values are expressed as means (n = 6) ± SD.

When the classical buffer was used, and the proportional amounts of two and one 10-mg Marinol capsules (200 and 100 μL of 40 mg/mL THC in sesame oil) were digested, the THC concentrations in MP were 40 ± 5 μg/mL and 47 ± 8 μg/mL, respectively. Similarly, when the proportional amounts of two, three, and six Neoral capsules (800, 1200, and 2400 μL of 100 mg/mL CsA in SEDDS) were lipolysed, the concentrations of CsA in MP were 0.926 ± 0.012 mg/mL, 1.692 ± 0.066 mg/mL, and 4.709 ± 0.156 mg/mL, respectively.

When the new buffer was used instead of a classical one, the concentrations found in the MP were 6 ± 2 μg/mL THC, 15 ± 1 μg/mL THC, 1.729 ± 0.048 mg/mL CsA, 2.637 ± 0.134 mg/mL CsA, and 6.103 ± 0.703 mg/mL CsA, following the lipolysis of 200 and 100 μL of Marinol, and 800, 1200, and 2400 μL of Neoral, respectively.

As previously indicated, the working hypothesis of the *in vitro* lipolysis model is that the fraction of drug dose which is solubilized in the MP is most readily available for absorption. In addition, THC and CsA are highly permeable drugs, and LFs are thought to inhibit efflux mechanisms.[5,6] Therefore, it was assumed that all the amount of THC and CsA solubilized in the MP would completely penetrate into the enterocytes. Accordingly, the concentration values found in the MP were next introduced in eq 2 to calculate the predicted F_{abs} represented in Figure 1.

Statistical analysis (Figure 1) showed that there were significant differences (p > 0.05) in the estimated F_{abs}, when the same volume of lipidic formulation was digested using buffers differing in the level of surfactants concentrations. It was also shown that there were significant differences in the estimated F_{abs} among different doses of the same formulation when using the classical buffer, but no statistically significant differences were detected when the new buffer was used. The exception was the high-dose six Neoral capsules study, which turned up to be statistically different from the other two Neoral studies.

3.3. Hepatic Microsomal Metabolism: Prediction of the Fraction Nonmetabolized in the Liver (F_h). Apart from intraluminal solubilization, the other important factor that limits the oral bioavailability of BCS II drugs is first-pass metabolism. To determine the fraction of drug dose that is not cleared by the liver, microsomal metabolism stability studies with human hepatic microsomes were performed. The metabolism rates of THC and CsA at different initial concentrations by human liver microsomes were obtained by applying the "*in vitro* half-life" approach and fitting the data to monoexponential decay regressions (see eq 3) represented in Figure 2. These k_{dep} values were next used to determine the theoretical depletion constant at infinitesimally low substrate concentration ($k_{dep,[S]\to 0}$) and the Michaelis−Menten constant (K_M), according to eq 4. For THC, $k_{dep,[S]\to 0}$ was 0.5666 ± 0.1929 min^{-1}, and K_M was 3.76 ± 1.28 μM (R^2 = 0.91). With regard to CsA, the calculated values were 0.0158 ± 0.0010 min^{-1} and 6.59 ± 0.41 μM (R^2 = 0.99), for $k_{dep,[S]\to 0}$ and K_M, respectively.

Using the microsomal concentration and the fraction unbound in the incubation medium, $k_{dep,[S]\to 0}$ was subsequently transformed into intrinsic clearance (eq 5: $CLu_{int,THC}$ = 2.236 ± 0.761 mL/min/mg protein; $CLu_{int,CsA}$ = 0.078 ± 0.005 mL/min/mg protein). Next, physiologically based scaling factors (average microsome content in the liver, and average liver weight per kg of body weight) were applied to transformed CLu_{int} into intrinsic hepatic clearance ($CLu_{h,int}$). Subsequently, the hepatic blood flow and the fraction of drug unbound in blood were introduced in the "parallel-tube" model equation to calculate the hepatic clearance (eq 7: $CL_{h,THC}$ = 10.77 ± 3.67 mL/min/kg; $CL_{h,CsA}$ = 2.08 ± 0.13 mL/min/kg). Finally, it was estimated that approximately 49% and 90% of THC and CsA molecules, respectively, would escape first-pass metabolism in the liver (eq 8: $F_{h,THC}$ = 0.487 ± 0.166; $F_{h,CsA}$ = 0.901 ± 0.055).

3.4. Intestinal Microsomal Metabolism: Prediction of the Fraction Nonmetabolized in the Gut (F_g). First-pass metabolism can occur not only in the liver, but also within the enterocytes in the gut wall. Similarly to the hepatic metabolism experimental procedure, the calculation of the fraction of the drug dose that escapes metabolism in the gut was performed by means of microsomal metabolism stability studies with human intestinal microsomes. The metabolism rates of THC and CsA by human intestinal microsomes were obtained from the fitted monoexponential decay regressions represented in Figure 3. The obtained k_{dep} values were 0.0478 ± 0.0046 min^{-1} (R^2 = 0.999) and 0.0060 ± 0.0013 min^{-1} (R^2 = 0.963), for THC and CsA, respectively.

Again, the microsomal concentration and the fraction unbound in the incubation medium were used to transform k_{dep} into intrinsic clearance (eq 5: $CLu_{int,THC}$ = 0.188 ± 0.018 mL/min/mg protein; $CLu_{int,CsA}$ = 0.031 ± 0.002 mL/min/mg protein). Next, relative abundance data of CYP2C9 and CYP3A in the gut were used to determine the gut intrinsic clearance values ($CLu_{g,THC}$ = 16.2 ± 1.5 L/h; $CLu_{g,CsA}$ = 3.1 ± 0.2 L/h). The "Q gut" model, which accounts for mucosal blood flow and permeability across the enterocytes, was applied to finally

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

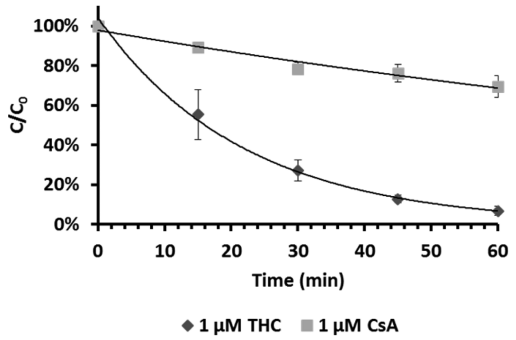


Figure 3. Depletion curves derived from intestinal microsomal incubations of 1 μM tetrahydrocannabinol (diamonds) and 1 μM cyclosporine A (squares). The ratio between the drug concentration remaining at each time point (C) and the concentration of drug at the beginning of the incubation process (C_0) is represented versus time. Values are expressed as means (n = 6) ± SD.

calculate F_g (eq 9). It was estimated that around 36% and 52% of THC and CsA molecules, respectively, would escape first-pass metabolism in the small intestine ($F_{g,THC}$ = 0.358 ± 0.034; $F_{g,CsA}$ = 0.517 ± 0.033).

3.5. Linking *in Vitro* Lipolysis and Metabolism Studies: Prediction of the Oral Bioavailability ($F_{predicted}$). The estimated absorbed (F_{abs}) and nonmetabolized ($F_g \cdot F_h$) fractions were combined for the calculation of the predicted oral bioavailability, as indicated in eq 11. Bioavailability results are summarized in Table 5. Pearson's correlation test was used for the measurement of the strength of the association between $F_{observed}$ and $F_{predicted}$. Statistical analysis showed there was significant correlation between $F_{observed}$ and $F_{predicted}$ when the new buffer was used (Pearson's r = 0.9638; p = 0.0088), but that was not the case for the classical buffer (Pearson's r = 0.8291; p = 0.0819).

4. DISCUSSION

LFs are mainly used for the oral delivery of BCS II drugs. Intestinal micellar solubilization and first-pass metabolism (rather than membrane permeability) are the main barriers to the oral bioavailability of BCS II drugs. *In vitro*, the intraluminal solubility of BCS II drugs administered in LFs can be estimated using the lipolysis model, whereas the first-pass extraction ratio can be assessed by performing microsomal stability assays.

Previous studies in the *in vitro* lipolysis field have focused on correlating the percentage of drug solubilized in the aqueous micellar phase with the drug exposure after oral administration of the tested lipidic formulation in laboratory animals.[13,15,18] So far, the obtained relationships have been qualitative in nature, and have been proven useful for rank-ordering formulations. The work presented herein proposes a novel combination of *in vitro* lipolysis and microsomal metabolism studies for the quantitative prediction of human oral bioavailability of BCS II drugs administered in LFs. This novel approach has the potential to transform the lipolysis model studies from a qualitative tool to a quantitative one.

4.1. Selection of Model Lipidic Formulations and Clinical Studies. Two model LFs, Marinol (THC dissolved in sesame oil) and Neoral (CsA dissolved in a mixture of lipids, surfactants, and cosolvents, which upon mild agitation form SEDDS), were selected for evaluation based on the availability of published clinical data. Both THC and CsA belong to class II of the BCS, and are characterized by poor aqueous solubility and moderate to high lipophilicity.

As mentioned above, an extensive literature search was carried out to find articles that would provide critical information for our purposes. One of the challenges was the high variability in pharmacokinetic parameters in these clinical studies, especially in the case of THC. This variability could be partially due to the different analytical methods used, which included radioactivity. It was required that volunteers were dosed in the fasted state (the lipolysis medium consists of simulated intestinal fluids in the fasted state) and that the exact amount of orally administered formulation was clearly stated (to calculate the proportional amount of LF to digest in the lipolysis model). On the other hand, it was essential that these articles would include relevant pharmacokinetic data allowing the calculation of the absolute oral bioavailability in human subjects. Numerous studies had to be discarded from the list of relevant *in vivo* information due to the poor state of health of the volunteers (transplant,[65] and cancer[54] patients), because the fed or fasted state of the subjects was not indicated,[58] and because of incomplete and/or uncertain information about the formulation, such as the volume of coadministered oil,[55,56] or the dose strength.[28,57,69]

Eventually, based on the above criteria, five published clinical studies were selected with the aim of predicting the human oral bioavailability of THC in Marinol[26,27] (Table 1) and of CsA in Neoral[28−30] (Table 2).

4.2. *In Vitro* Lipolysis: Prediction of the Fraction Absorbed (F_{abs}). *4.2.1. Scaling down Doses from in Vivo to in Vitro Conditions.* To assess the intraluminal processing of THC and CsA lipidic formulations, and to predict F_{abs}, *in vitro* lipolysis experiments were carried out. For the estimation of the amount of drug solubilized in the small intestine, two different *in vivo* dissolution volumes were initially considered: 250 and 100 mL. The first value corresponds to the amount of water given to volunteers in clinical trials, and it has been used by the BCS as the standard volume for assessing the maximum solubility of drugs in the fasted state.[106] However, the latest publications[76] suggest that while 250 mL is the reasonable volume for the assessment of the solubility of drugs in the stomach, it might be too high for the estimation of drug solubility in the small intestine. Mudie et al. suggested in their work using a volume of 80−100 mL instead of 250 mL. Lipid digestion and absorption occur almost entirely in the upper part of the small intestine;[20] therefore, 100-mL volume was deemed appropriate in our studies. The digestion medium of the *in vitro* lipolysis model has approximately 40-mL volume; therefore, the amounts of Marinol (40 mg/mL THC) and Neoral (100 mg/mL CsA) were scaled down accordingly to match the *in vivo* situation.

4.2.2. Effect of Surfactant Levels on in Vitro Drug Solubilization. Initially, a lipolysis buffer analogous to those previously used by ours and other lipolysis research groups,[12−14,16,77,78] characterized by high concentrations of BSs and PLs (Table 3), was used for digestion of the formulations. According to literature data[23,28] and the pharmacokinetic analysis performed, both Marinol and Neoral showed approximate dose proportionality. However, F_{abs} results (Figure 1) suggested changes in the percentage absorbed dependent on dose for both formulations. Hence, it was apparent that a refinement in the lipolysis conditions was needed. Following the lead of other research groups,[107] surfactant levels were reduced down to more biorelevant

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

concentrations,[79] and lipolysis experiments were performed again. Interestingly, reduction in surfactant concentrations caused opposite effects for the two tested model drugs. The solubilized fractions of THC decreased (Figure 1A), whereas the solubilized fractions of CsA increased (Figure 1B). Marinol is a Type I lipidic formulation;[108] therefore, it highly depends on the presence of BSs and PLs to create mixed micelles within which THC and lipolytic products (fatty acids and 2-monoglycerides) are solubilized. Therefore, the lower the surfactant concentration is, the fewer the number of micelles is, and the lower the solubilized fraction would be. On the other hand, Neoral is a SEDDS (Type IIIA lipidic formulation); for this reason it does not require surfactant agents to generate solubilizing structures for CsA. The observed variable and reduced solubility of CsA when using the "classical" buffer could be explained by the inhibitory effect of BSs and PLs in SEDDS formation. An excess of BS- and PL-derived micellar structures would lead to a higher entrapment of Neoral components, thus reducing the number of SEDDS particles and decreasing the inherent solubilization capacity of Neoral. Results derived from the use of the "new" more biorelevant buffer proved to be more consistent within formulations and showed no statistically significant differences between different formulation strengths, as seems to occur *in vivo*.[28] The only exception was the study mimicking the administration of a very high-dose of CsA (six Neoral capsules), which proved to be statistically different from the other two CsA studies. Interestingly, a higher dose of digested Neoral *in vitro* led to a higher fraction of solubilized dose, whereas *in vivo* the case was the opposite. This phenomenon has already been witnessed by Berthelsen et al.[107] when working with Kolliphor RH 40 (the main surfactant component in Neoral), and it was explained by the micellar trapping hypothesis. According to this hypothesis, the reduced bioavailability *in vivo* when using very high levels of Kolliphor RH 40 might be caused by a higher amount of undigested surfactant trapping the drug, thus decreasing the amount of drug available for intestinal permeation.

After lipolysis and subsequent centrifugation, three distinct layers were obtained: an upper undigested lipid phase (only present in Marinol experiments), a middle aqueous-micellar phase, and a lower sediment phase. The general assumption made by researchers working with the *in vitro* lipolysis model is that the fraction of drug dose which is solubilized in the micellar phase is most readily available for absorption. This assumption represents an oversimplification of the absorption process, as drug contained in micelles could precipitate in the lower part of the small intestine. Arguably, the estimated fraction of absorbed drug could be slightly overestimated due to incomplete permeability and the action of efflux transporters (especially P-glycoprotein) expressed on the apical side of enterocytes. In terms of permeability, THC and CsA belong to class II of the BCS; thus, their membrane permeability is high, mainly passive, and a function of lipophilicity (P_{eff} = 7.56 and 1.65 μm/s, for THC[86] and CsA,[101] respectively). Therefore, it is expected that most of the fraction of solubilized dose would cross the apical membrane. With regard to efflux proteins, it has been suggested that high drug permeability would lead to rapid permeation into the enterocytes, making the contribution of intestinal uptake transporters generally insignificant.[109] Furthermore, Ingels et al.[5] and Konishi et al.[6] have reported the inhibitory effect of monoglycerides on P-glycoprotein activity. Based on this evidence, it can be assumed that efflux transporters do not play a role in the bioavailability of BCS II drugs delivered in LFs.

4.3. Microsomal Stability Studies: Prediction of the Fraction Escaping First-Pass Metabolism in the Liver (F_h). Since the aim of this study was to quantitatively predict the absolute bioavailability, it was evident that lipolysis results alone were not sufficient for this goal. Thus, a hepatic metabolism phase has been introduced to account for the loss of drug due to first-pass metabolism in the liver.

In humans, CYP2C9[110,111] and CYP3A4[112,113] have been identified as the main enzymes involved in THC and CsA metabolism, respectively. CYP2C9 hydroxylases THC to 11-hydroxy-THC, which may undergo further oxidation to form the carboxylic acid 11-nor-9-carboxy-THC. CYP3A4 catalyzes CsA metabolism through the monohydroxylation of amino acids 1 or 9, or the N-demethylation of amino acid 4.[114,115]

The fraction of drug that escapes first-pass metabolism in the liver was determined in this work by using human liver microsomes and applying the "*in vitro* half-life" approach. To confirm the correct experimental setup, the metabolism of positive (verapamil) and negative (dexamethasone) control compounds were assessed. The elimination rate constant values calculated for the control compounds ($k_{verapamil}$ = 0.066 ± 0.002 min^{-1}, $k_{dexamethasone}$ = 0.004 ± 0.001 min^{-1}) were in accordance with those characteristic of highly (verapamil) and poorly (dexamethasone) metabolized drugs, and were comparable to those values obtained in previous reports with similar experimental conditions.[81,94] The application of the half-life method to estimate quantitatively the metabolism rate is only valid under the assumption that C_0 is well below K_M. When K_M is unknown, and a drug is metabolized in several positions leading to an unknown number of metabolites, the rate of metabolism can still be determined by using the "multiple depletion curve method".[82−84] This approach consists in generating drug depletion curves at several concentrations and subsequently extrapolate the rate of metabolism at infinitesimally low concentration ($k_{dep,[S]\to 0}$, eq 4). Accordingly, $k_{dep,[S]\to 0}$ was calculated for THC and CsA by incubating the drugs in hepatic microsomal medium at 4 concentration levels. The obtained $k_{dep,[S]\to 0}$ values were then transformed into hepatic clearance data, through corrections for the fraction of drug unbound to microsomes and to blood proteins, physiological scaling factors, and the application of the "parallel tube" model. In the case of THC, the calculated CL_h (10.8 ± 3.7 mL/min/kg) was slightly higher, but within the same range as the total clearance reported in clinical studies after intravenous administration of THC (Table 2). For CsA, the estimated CL_h (2.1 ± 0.1 mL/min/kg) was lower than that calculated from literature pharmacokinetic data (Table 3), and this might be due to CsA being metabolized by other organs in addition to the liver when administered intravenously. Eventually, CL_h data were transformed into the fraction nonmetabolized in the liver. When F_{abs} and F_h values were combined, the *in vitro* lipolysis/hepatic metabolism approach did not sufficiently predict the *in vivo* performance of Marinol and Neoral using either of the two buffers (Table 5). CsA bioavailability was remarkably overestimated, and this fact could be explained by the extensive extraction that the drug suffers at the gut wall.[116]

4.4. Microsomal Stability Studies: Prediction of the Fraction Escaping First-Pass Metabolism in the Gut (F_g). Based on the above results, it was suggested that the accuracy of our predictions could be improved by inclusion of an intestinal

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

metabolism phase. Therefore, depletion drug assays in gut microsomal media were next performed. Due to the limited availability of intestinal protein, these studies were done at a single concentration level (1 μM). Similar to hepatic metabolism, the intrinsic clearance derived from microsomal incubations was transformed into intestinal clearance and fraction nonmetabolized, by applying the "Q gut" model.[98] Results derived from CsA experiments (CLu_{int} = 31 μL/min/mg; F_g = 0.52) were in agreement with those reported by other researchers (CLu_{int} = 27.7 μL/min/mg;[95] F_g = 0.53[98]). It is important to note that these intestinal clearance values might have been overestimated, as it has been suggested that lipidic excipients may reduce gut metabolism.[9,117] The "drug efflux-metabolism alliance"[118,119] proposes that efflux increases the time available for enterocyte-based metabolism. Accordingly, the impact of lipidic excipients on efflux proteins might reduce the time available for metabolism, and thus decrease presystemic extraction.

4.5. *In Vitro* Lipolysis/Metabolism Approach: Prediction of the Oral Bioavailability ($F_{predicted}$). Finally, by combining the fractions of drug absorbed and nonmetabolized, it was possible to propose estimated oral bioavailability values of THC in Marinol and of CsA in Neoral for different dose levels (Table 5). Pearson's correlation test showed that there was a strong correlation between $F_{observed}$ and $F_{predicted}$ values only when F_{abs} was calculated with the new buffer.

In the case of Marinol, the bioavailability was slightly underestimated, but within the range of the clinical values. This underestimation could be attributed to lymphatic transport. As mentioned before, when dealing with oral absorption of highly lipophilic drugs coadministered with long-chain triglycerides, such as THC in Marinol, the lymphatic route should be taken into consideration. Drugs absorbed via the intestinal lymphatic system are protected from hepatic first-pass metabolism since the mesenteric lymph enters the systemic circulation, bypassing the liver. However, Trevaskis et al.[120] suggested that drugs transported via intestinal lymphatics cannot avoid enterocyte-based metabolism, unless extremely large quantities of lipids are administered. Drug association with chylomicrons in the enterocyte is an essential step in the lymphatic absorption pathway.[121,122] Accordingly, because the vast majority of THC absorbed would associate with chylomicrons,[123] and be transported through the lymph, the estimated oral bioavailability of Marinol could be calculated just taking into account the fractions absorbed and not metabolized in the gut (F_{abs}·F_g). The $F_{predicted}$ values obtained ignoring the hepatic phase (2.7 ± 0.3% and 2.3 ± 0.6%, for Naef et al. and Goskonda et al. studies, respectively) were indeed closer to the average $F_{observed}$ ones, which suggests the contribution of lymphatic transport to THC oral bioavailability.

In the case of Neoral, the bioavailability estimations for Kim et al. and Odeberg et al. studies were extremely accurate. When the digestion of exceptionally high doses of formulation was mimicked (Mueller et al. study), the *in vitro* lipolysis/metabolism approach did not sufficiently predict the clinical value. As discussed before, this is most probably due to CsA micellar trapping occurring when very high amounts of Kolliphor RH 40 are used. This phenomenon cannot be accurately accounted for with the *in vitro* lipolysis model, and thus it leads to an overestimation of F_{abs} and subsequent $F_{predicted}$.

The general accuracy of the bioavailability predicted values, and the strong correlation shown with the clinical ones, suggests that the novel *in vitro* lipolysis/metabolism approach could satisfactory quantitatively estimate the oral bioavailability of BCS II drugs administered in LFs. However, the *in vitro* lipolysis model is not able to predict the micellar trapping of drugs caused by undigested lipidic excipients *in vivo*. Therefore, the lipolysis/metabolism approach might have limited applicability when extremely high dose levels of surfactants are ingested.

5. CONCLUSIONS

In vitro lipolysis and microsomal metabolism studies have been combined for the first time with the aim to quantitative predict the human oral bioavailability of BCS II drugs administered in LFs. This novel approach led to reasonably good predictions of the oral bioavailability of THC in Marinol and of the CsA in Neoral (model formulations) based on the similarity between the predicted bioavailability values and those reported in clinical trials after oral administration of the tested formulations to human subjects.

The use of a digestion buffer with surfactant concentrations closer to biorelevant conditions resulted in more accurate predictions of oral bioavailability in comparison to data derived from the classical buffer previously used in *in vitro* lipolysis studies.

The digestion of very high dose levels of surfactants might represent a limitation to this novel approach, since the *in vitro* lipolysis could not account for the micellar trapping phenomenon that could occur *in vivo*.

The work presented herein suggests that the novel *in vitro* lipolysis/metabolism approach has potential to transform the *in vitro* lipolysis studies from a qualitative tool to a quantitative one. Further analyses with additional BCS II drugs administered in LFs might be needed to confirm the predictive power of the *in vitro* lipolysis/metabolism approach.

AUTHOR INFORMATION

Corresponding Author

*Phone: +44 (0) 115 84 68014. Fax: +44 (0) 115 951 3412. Email: pavel.gershkovich@nottingham.ac.uk.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This paper is part of Paloma Benito-Gallo's Ph.D. dissertation. The authors would like to thank the funding support from Quotient Clinical Ltd. This work was supported by the Engineering and Physical Sciences Research Council (EPSRC grant EP/I01375X/1). The authors would like to acknowledge Jong Bong Lee and Dhiaa Taha for their help with the microsomal metabolism and HPLC-MS/MS assays, respectively. The authors are grateful for the generous donation of CBD by GW Pharmaceuticals.

ABBREVIATIONS

AUC, area under the concentration−time curve; BCS, biopharmaceutics classification system; BS, bile salt; CBD, cannabidiol; CL_g, gut clearance; CL_h, hepatic clearance; CL_{int}, intrinsic clearance; CL_{perm}, permeability across the enterocytes; CLu_{int}, intrinsic clearance accounting for the fraction of drug unbound in the incubation; CsA, cyclosporin A; *F*, bioavailability; F_{abs}, fraction absorbed; F_g, fraction escaping metabolism at the gut wall; F_h, fraction escaping metabolism in the liver; fu_b,

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

fraction unbound in blood; fu_{inc}, fraction unbound in the incubation; HPLC-UV, high-performance liquid chromatography−ultraviolet; IV, intravenous; IVIVC, *in vivo in vitro* correlation; k_{dep}, depletion rate constant; K_M, Michaelis−Menten constant; LF, lipid-based formulations; MP, micellar phase; NADPH, nicotinamide adenine dinucleotide phosphate tetrasodium salt hydrate; P_{eff}, effective intestinal permeability; PG, propylene glycol; PL, phospholipid; Q_{gut}, gut blood flow; Q_h, hepatic blood flow; Q_{villi}, villous blood flow; R^2, correlation coefficient; SEDDS, self-emulsifying drug delivery system; THC, Δ^9-tetrahydrocannabinol; $VitD_3$, vitamin D_3

REFERENCES

(1) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery Rev.* **2001**, *46* (1−3), 3−26.

(2) Lipinski, C. A. Drug-like Properties and the Causes of Poor Solubility and Poor Permeability. *J. Pharmacol. Toxicol. Methods* **2000**, *44* (1), 235−249.

(3) Di Maio, S.; Carrier, R. L. Gastrointestinal Contents in Fasted State and Post-Lipid Ingestion: In Vivo Measurements and in Vitro Models for Studying Oral Drug Delivery. *J. Controlled Release* **2011**, *151* (2), 110−122.

(4) Welling, P. Influence of Food and Diet on Gastrointestinal Drug Absorption: A Review. *J. Pharmacokinet. Biopharm.* **1977**, *5* (4), 291−334.

(5) Ingels, F.; Deferme, S.; Destexhe, E.; Oth, M.; Van den Mooter, G.; Augustijns, P. Simulated Intestinal Fluid as Transport Medium in the Caco-2 Cell Culture Model. *Int. J. Pharm.* **2002**, *232* (1−2), 183−192.

(6) Konishi, T.; Satsu, H.; Hatsugai, Y.; Aizawa, K.; Inakuma, T.; Nagata, S.; Sakuda, S. H.; Nagasawa, H.; Shimizu, M. A Bitter Melon Extract Inhibits the P-Glycoprotein Activity in Intestinal Caco-2 Cells: Monoglyceride as an Active Compound. *BioFactors* **2004**, *22* (1−4), 71−74.

(7) Charman, W. N.; Porter, C. J. H. Lipophilic Prodrugs Designed for Intestinal Lymphatic Transport. *Adv. Drug Delivery Rev.* **1996**, *19* (2), 149−169.

(8) Hunt, J. N.; Knox, M. T. A Relation between the Chain Length of Fatty Acids and the Slowing of Gastric Emptying. *J. Physiol.* **1968**, *194* (2), 327−336.

(9) Orlowski, S.; Selosse, M. A.; Boudon, C.; Micoud, C.; Mir, L. M.; Belehradek, J. J.; Garrigos, M. Effects of Detergents on P-Glycoprotein Atpase Activity: Differences in Perturbations of Basal and Verapamil-Dependent Activities. *Cancer Biochem. Biophys.* **1998**, *16* (1−2), 85−110.

(10) Porter, C. J. H.; Charman, W. N. In Vitro Assessment of Oral Lipid Based Formulations. *Adv. Drug Delivery Rev.* **2001**, *50* (Supple (0)), S127−S147.

(11) Zangenberg, N. H.; Müllertz, A.; Kristensen, H. G.; Hovgaard, L. A Dynamic in Vitro Lipolysis Model: I. Controlling the Rate of Lipolysis by Continuous Addition of Calcium. *Eur. J. Pharm. Sci.* **2001**, *14* (2), 115−122.

(12) Porter, C. J. H.; Kaukonen, A. M.; Taillardat-Bertschinger, A.; Boyd, B. J.; O'Connor, J. M.; Edwards, G. A.; Charman, W. N. Use of in Vitro Lipid Digestion Data to Explain the in Vivo Performance of Triglyceride-Based Oral Lipid Formulations of Poorly Water-Soluble Drugs: Studies with Halofantrine. *J. Pharm. Sci.* **2004**, *93* (5), 1110−1121.

(13) Dahan, A.; Hoffman, A. Use of a Dynamic in Vitro Lipolysis Model to Rationalize Oral Formulation Development for Poor Water Soluble Drugs: Correlation with in Vivo Data and the Relationship to Intra-Enterocyte Processes in Rats. *Pharm. Res.* **2006**, *23* (9), 2165−2174.

(14) Benito-Gallo, P.; Franceschetto, A.; Wong, J. C. M.; Marlow, M.; Zann, V.; Scholes, P.; Gershkovich, P. Chain Length Affects Pancreatic Lipase Activity and the Extent and pH−time Profile of Triglyceride Lipolysis. *Eur. J. Pharm. Biopharm.* **2015**, *93* (0), 353−362.

(15) Griffin, B. T.; Kuentz, M.; Vertzoni, M.; Kostewicz, E. S.; Fei, Y.; Faisal, W.; Stillhart, C.; O'Driscoll, C.; Reppas, C.; Dressman, J. B. Comparison of in Vitro Tests at Various Levels of Complexity for the Prediction of in Vivo Performance of Lipid-Based Formulations: Case Studies with Fenofibrate. *Eur. J. Pharm. Biopharm.* **2014**, *86* (3), 427−437.

(16) Han, S.; Yao, T.; Zhang, X.; Gan, L.; Zhu, C.; Yu, H.; Gan, Y. Lipid-Based Formulations to Enhance Oral Bioavailability of the Poorly Water-Soluble Drug Anethol Trithione: Effects of Lipid Composition and Formulation. *Int. J. Pharm.* **2009**, *379* (1), 18−24.

(17) Alqahtani, S.; Alayoubi, A.; Nazzal, S.; Sylvester, P.; Kaddoumi, A. Enhanced Solubility and Oral Bioavailability of γ-Tocotrienol Using a Self-Emulsifying Drug Delivery System (SEDDS). *Lipids* **2014**, *49* (8), 819−829.

(18) Larsen, A. T.; Ohlsson, A. G.; Polentarutti, B.; Barker, R. A.; Phillips, A. R.; Abu-Rmaileh, R.; Dickinson, P. A.; Abrahamsson, B.; Østergaard, J.; Müllertz, A. Oral Bioavailability of Cinnarizine in Dogs: Relation to SNEDDS Droplet Size, Drug Solubility and in Vitro Precipitation. *Eur. J. Pharm. Sci.* **2013**, *48* (1−2), 339−350.

(19) Dahan, A.; Hoffman, A. The Effect of Different Lipid Based Formulations on the Oral Absorption of Lipophilic Drugs: The Ability of in Vitro Lipolysis and Consecutive Ex Vivo Intestinal Permeability Data to Predict in Vivo Bioavailability in Rats. *Eur. J. Pharm. Biopharm.* **2007**, *67* (1), 96−105.

(20) Dahan, A.; Hoffman, A. Rationalizing the Selection of Oral Lipid Based Drug Delivery Systems by an in Vitro Dynamic Lipolysis Model for Improved Oral Bioavailability of Poorly Water Soluble Drugs. *J. Controlled Release* **2008**, *129* (1), 1−10.

(21) Advanced Chemistry Developmen Inc. ACD/I-Lab Version 12.1.0.50375. www.acdlabs.com. Toronto, ON, Canada 2014.

(22) Garrett, E. R.; Hunt, C. A. Physicochemical Properties, Solubility, and Protein Binding of Δ9 -Tetrahydrocannabinol. *J. Pharm. Sci.* **1974**, *63* (7), 1056−1064.

(23) U.S. Food and Drug Administration. Marinol® (Dronabinol Capsules). Available at: http://www.fda.gov/ohrms/dockets/dockets/05n0479/05N-0479-emc0004-04.pdf (Accessed: March 2016). 2015.

(24) European Medicines Agency. Sandimmun Neoral® (Annex III). Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Referrals_document/Sandimmun_Neoral_30/WC500144886.pdf (Accessed: March 2016). 2015.

(25) Varma, M. V. S.; Panchagnula, R. Prediction of in Vivo Intestinal Absorption Enhancement on P-Glycoprotein Inhibition, from Rat in Situ Permeability. *J. Pharm. Sci.* **2005**, *94* (8), 1694−1704.

(26) Naef, M.; Curatolo, M.; Petersen-Felix, S.; Arendt-Nielsen, L.; Zbinden, A.; Brenneisen, R. The Analgesic Effect of Oral Delta-9-Tetrahydrocannabinol (THC), Morphine, and a THC-Morphine Combination in Healthy Subjects under Experimental Pain Conditions. *Pain* **2003**, *105* (1−2), 79−88.

(27) Goskonda, V. R.; Chavan, A.; Kokate, A.; Gill, H. Liquid Cannabinoid Formulations. Patent No. US 8222292 B2, 2012.

(28) Mueller, E.; Kovarik, J.; van Bree, J.; Tetzloff, W.; Grevel, J.; Kutz, K. Improved Dose Linearity of Cyclosporine Pharmacokinetics from a Microemulsion Formulation. *Pharm. Res.* **1994**, *11* (2), 301−304.

(29) Kim, J. W.; Shin, H. J.; Yang, S. G. Cyclosporin-Containing Pharmaceutical Composition. Patent No. US 5980939 A, 1999.

(30) Odeberg, J. M.; Kaufmann, P.; Kroon, K.-G.; Höglund, P. Lipid Drug Delivery and Rational Formulation Design for Lipophilic Drugs with Low Oral Bioavailability, Applied to Cyclosporine. *Eur. J. Pharm. Sci.* **2003**, *20* (4−5), 375−382.

(31) Strickley, R. G. Currently Marketed Oral Lipid-Based Dosage Forms: Drug Products and Excipients. In *Oral Lipid-Based Formulations: Enhancing the Bioavailability of Poorly Water-Soluble Drugs*; Hauss, D. J., Ed.; Bristol-Myers Squibb Company: Princeton, New Jersey, USA, 2007.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

(32) Roche to discontinue the sale and distribution of Fortovase (saquinavir). *News Medical*. http://www.news-medical.net/news/2005/05/18/10187.aspx.
(33) AbbVie Inc. Kaletra® (Lopinavir/ritonavir). Available at: Https://www.kaletra.com/ (Accessed: June 2016). 2015.
(34) Kyorin Pharmaceutical Co. Ketas® (Ibudilast). Available at: Http://www.kyorin-pharm.co.jp/prodinfo/medicine/pdf/KETAS_Capsules.pdf (Accessed: May 2016). 2013.
(35) Napp Pharmaceuticals. MXL® Capsules (Morphine Sulphate). Available at: http://www.chardeh.com.tw/file/Product/20130219140525268 7.pdf (Accessed: May 2016).
(36) Pfizer - Pharmacia and Upjohn Company. Detrol® LA (Tolterodine Tartrate). Available at: http://labeling.pfizer.com/showlabeling.aspx?id=719 (Accessed: May 2016). 2013.
(37) RxList Inc. AGENERASE® (Amprenavir) Capsules, Available at: Http://www.rxlist.com/agenerase-Drug.htm (Accessed on June 2016). 2014.
(38) U.S. Food and Drug Administration. Norvir® (Ritonavir), Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/020945s032lbl.pdf (Accessed: May 2016). 2016.
(39) U.S. Food and Drug Administration. APTIVUS® (Tipranavir). Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021814s011lbl.pdf (Accessed: June 2016). 2011.
(40) Csajka, C.; Marzolini, C.; Fattinger, K.; Décosterd, L. A.; Fellay, J.; Telenti, A.; Biollaz, J.; Buclin, T. Population Pharmacokinetics and Effects of Efavirenz in Patients with Human Immunodeficiency Virus Infection. *Clin. Pharmacol. Ther.* **2003**, *73* (1), 20−30.
(41) Duvic, M.; Hymes, K.; Heald, P.; Breneman, D.; Martin, A. G.; Myskowski, P.; Crowley, C.; Yocum, R. C. Group, for M. of the B. W. S. Bexarotene Is Effective and Safe for Treatment of Refractory Advanced-Stage Cutaneous T-Cell Lymphoma: Multinational Phase II-III Trial Results. *J. Clin. Oncol.* **2001**, *19* (9), 2456−2471.
(42) U.S. Food and Drug Administration. Avodart® (Dutasteride). Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2008/021319s015lbl.pdf (Accessed: March 2016). 2006.
(43) Eisai Co. Infree® S Capsules (Indometacin Farnesil). Available at: http://www.eisai.jp/medical/products/di/EPI/INF_C-SC_EPI.pdf (Accessed: May 2016). 2009.
(44) Eisai Co. Juvela N® Capsules (Tocopherol Nicotinate). Available at: http://www.eisai.jp/medical/products/di/EPI/EN_C-SC-FG_EPI.pdf (Accessed: May 2016). 2009.
(45) Eisai Co. Selbex® Capsules (Teprenone). Available at: http://www.shijiebiaopin.com/upload/product/201431323032020.pdf (Accessed: May 2016). 2014.
(46) Colburn, W. A.; Vane, F. M.; Shorter, H. J. Pharmacokinetics of Isotretinoin and Its Major Blood Metabolite Following a Single Oral Dose to Man. *Eur. J. Clin. Pharmacol.* **1983**, *24* (5), 689−694.
(47) Brattström, C.; Säwe, J.; Jansson, B.; Lönnebo, A.; Nordin, J.; Zimmerman, J. J.; Burke, J. T.; Groth, C. G. Pharmacokinetics and Safety of Single Oral Doses of Sirolimus (Rapamycin) in Healthy Male Volunteers. *Ther. Drug Monit.* **2000**, *22* (5), 537−544.
(48) Albright, P. S.; Bruni, J.; Suria, D. Pharmacokinetics of Enteric-Coated Valproic Acid. *Ther. Drug Monit.* **1984**, *6* (1), 21−23.
(49) Shah, A.; Liu, M.-C.; Vaughan, D.; Heller, A. H. Oral Bioequivalence of Three Ciprofloxacin Formulations Following Single-Dose Administration: 500 Mg Tablet Compared with 500 mg/10 mL or 500 mg/5 mL Suspension and the Effect of Food on the Absorption of Ciprofloxacin Oral Suspension. *J. Antimicrob. Chemother.* **1999**, *43* (suppl 1), 49−54.
(50) Eisai Co. Glakay® Capsules (Vitamin K2). Available at: http://www.eisai.jp/medical/products/di/EPI/GLA_SC_EPI.pdf (Accessed: May 2016). 2009.
(51) Thudi, N. R.; Shrivastav, V. K.; Monif, T.; Khuroo, A.; Gurule, S.; Partani, P. O.; Tandon, M.; Mathur, R.; Pharmacokinetic. and Bioequivalence Study of Endogenous Compound Tretinoin 10 Mg Capsules in Healthy Volunteers by Base Line Correction Approach. *Clin. Res. Regul. Aff.* **2011**, *28* (3), 68−73.
(52) Levine, H.; Watson, N. Comparison of the Pharmacokinetics of Crinone 8% Administered Vaginally versus Prometrium Administered Orally in Postmenopausal women3. *Fertil. Steril.* **2000**, *73* (3), 516−521.
(53) Upton, R. A.; Knutson, J. C.; Bishop, C. W.; LeVan, L. W. Pharmacokinetics of Doxercalciferol, a New Vitamin D Analogue That Lowers Parathyroid Hormone. *Nephrol., Dial., Transplant.* **2003**, *18* (4), 750−758.
(54) Frytak, S.; Moertel, C. G.; Rubin, J. Metabolic Studies of Delta-9-Tetrahydrocannabinol in Cancer Patients. *Cancer Treat. Rep.* **1984**, *68* (12), 1427−1431.
(55) Joerger, M.; Wilkins, J.; Fagagnini, S.; Baldinger, R.; Brenneisen, R.; Schneider, U.; Goldman, B.; Weber, M. Single-Dose Pharmacokinetics and Tolerability of Oral Delta-9- Tetrahydrocannabinol in Patients with Amyotrophic Lateral Sclerosis. *Drug Metab. Lett.* **2012**, *6* (2), 102−108.
(56) Karschner, E. L.; Darwin, W. D.; Goodwin, R. S.; Wright, S.; Huestis, M. A. Plasma Cannabinoid Pharmacokinetics Following Controlled Oral Δ9-Tetrahydrocannabinol and Oromucosal Cannabis Extract Administration. *Clin. Chem.* **2011**, *57* (1), 66−75.
(57) Schwilke, E. W.; Schwope, D. M.; Karschner, E. L.; Lowe, R. H.; Darwin, W. D.; Kelly, D. L.; Goodwin, R. S.; Gorelick, D. A.; Huestis, M. A. Δ9-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* (12), 2180−2189.
(58) Wall, M. E.; Sadler, B. M.; Brine, D.; Taylor, H. Metabolism, Disposition, and Kinetics of Delta-9-Tetrahydrocannabinol in Men and Women. *Clin. Pharmacol. Ther.* **1983**, *34* (3), 352−363.
(59) Medicines and Healthcare Products Regulatory Agency. Alfacalcidol Capsules, Available at: http://www.mhra.gov.uk/home/groups/par/documents/websiteresources/con065723.pdf (Accessed: May 2016). 2009.
(60) Brandi, L.; Egfjord, M.; Olgaard, K. Pharmacokinetics of 1,25(OH)2D3 and 1α(OH)D3 in Normal and Uraemic Men. *Nephrol. Dial. Transplant.* **2002**, *17* (5), 829−842.
(61) Jin, S.-E.; Park, J.-S.; Kim, C.-K. Pharmacokinetics of Oral Calcitriol in Healthy Human Based on the Analysis with an Enzyme Immunoassay. *Pharmacol. Res.* **2009**, *60* (1), 57−60.
(62) Jostell, K. G.; Agurell, S.; Allgén, L. G.; Kuylenstierna, B.; Lindgren, J. E.; Åberg, G.; Österlöf, G. Pharmacokinetics of Clomethiazole in Healthy Adults. *Acta Pharmacol. Toxicol.* **1978**, *43*, 180−189.
(63) Ratz, A. E.; Schlienger, R. G.; Linder, L.; Langewitz, W.; Haefeli, W. E. Pharmacokinetics and Pharmacodynamics of Clomethiazole after Oral and Rectal Administration in Healthy Subjects. *Clin. Ther.* **1999**, *21*, 829−840.
(64) Kovarik, J. M.; Mueller, E. A.; Van Bree, J. B.; Tetzloff, W.; Kutz, K. Reduced Inter- and Intraindividual Variability in Cyclosporine Pharmacokinetics from a Microemulsion Formulation. *J. Pharm. Sci.* **1994**, *83* (3), 444−446.
(65) Ptachcinski, R.; Venkataramanan, R.; Burckart, G. Clinical Pharmacokinetics of Cyclosporin. *Clin. Pharmacokinet.* **1986**, *11* (2), 107−132.
(66) Postolache, P.; Petrescu, O.; Dorneanu, V.; Zanini, A. C. Cyclosporine Bioavailability of Two Physically Different Oral Formulations. *Eur. Rev. Med. Pharmacol Sci.* **2002**, *6* (6), 127−131.
(67) Drewe, J.; Meier, R.; Vonderscher, J.; Kiss, D.; Posanski, U.; Kissel, T.; Gyr, K. Enhancement of the Oral Absorption of Cyclosporin in Man. *Br. J. Clin. Pharmacol.* **1992**, *34* (1), 60−64.
(68) Hebert, M. F.; Roberts, J. P.; Prueksaritanont, T.; Benet, L. Z. Bioavailability of Cyclosporine with Concomitant Rifampin Administrationis Markedly Less than Predicted by Hepatic Enzyme Induction. *Clin. Pharmacol. Ther.* **1992**, *52*, 453−457.
(69) Mueller, E.; Kovarik, J.; van Bree, J.; Grevel, J.; Lücker, P.; Kutz, K. Influence of a Fat-Rich Meal on the Pharmacokinetics of a New Oral Formulation of Cyclosporine in a Crossover Comparison with the Market Formulation. *Pharm. Res.* **1994**, *11* (1), 151−155.
(70) Hunt, C. A.; Jones, R. T. Tolerance and Disposition of Tetrahydrocannabinol in Man. *J. Pharmacol. Exp. Ther.* **1980**, *215* (1), 35−44.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

(71) Ohlsson, A.; Lindgren, J.-E.; Wahlen, A.; Agurell, S.; Hollister, L. E.; Gillespie, H. K. Plasma Delta-9-Tetrahydrocannabinol Concentrations and Clinical Effects after Oral and Intravenous Administration and Smoking. *Clin. Pharmacol. Ther.* **1980**, *28* (3), 409−416.
(72) Kelly, P.; Jones, R. T. Metabolism of Tetrahydrocannabinol in Frequent and Infrequent Marijuana Users. *J. Anal. Toxicol.* **1992**, *16* (4), 228−235.
(73) Naef, M.; Russmann, S.; Petersen-Felix, S.; Brenneisen, R. Development and Pharmacokinetic Characterization of Pulmonal and Intravenous Delta-9-Tetrahydrocannabinol (THC) in Humans. *J. Pharm. Sci.* **2004**, *93* (5), 1176−1184.
(74) Gomez, D. Y.; Wacher, V. J.; Tomlanovich, S. J.; Hebert, M. F.; Benet, L. Z. The Effects of Ketoconazole on the Intestinal Metabolism and Bioavailability of Cyclosporine. *Clin. Pharmacol. Ther.* **1995**, *58*, 15−19.
(75) Joint FAO/WHO Expert Commitee on Food Additives. Evaluation of Certain Food Additives and Contaminants. Available at: http://apps.who.int/iris/bitstream/10665/44515/1/WHO_TRS_960_eng.pdf (Accessed: May 2016). 1987.
(76) Mudie, D. M.; Murray, K.; Hoad, C. L.; Pritchard, S. E.; Garnett, M. C.; Amidon, G. L.; Gowland, P. A.; Spiller, R. C.; Amidon, G. E.; Marciani, L. Quantification of Gastrointestinal Liquid Volumes and Distribution Following a 240 mL Dose of Water in the Fasted State. *Mol. Pharmaceutics* **2014**, *11* (9), 3039−3047.
(77) Kaukonen, A.; Boyd, B.; Porter, C. H.; Charman, W. Drug Solubilization Behavior During in Vitro Digestion of Simple Triglyceride Lipid Solution Formulations. *Pharm. Res.* **2004**, *21* (2), 245−253.
(78) Williams, H. D.; Sassene, P.; Kleberg, K.; Calderone, M.; Igonin, A.; Jule, E.; Vertommen, J.; Blundell, R.; Benameur, H.; Müllertz, A.; Porter, C. J. H.; Pouton, C. W. Communicated on Behalf of the, L. C. Toward the Establishment of Standardized In Vitro Tests for Lipid-Based Formulations, Part 4: Proposing a New Lipid Formulation Performance Classification System. *J. Pharm. Sci.* **2014**, *103* (3), 2441−2455.
(79) Bergström, C. A. S.; Holm, R.; Jørgensen, S. A.; Andersson, S. B. E.; Artursson, P.; Beato, S.; Borde, A.; Box, K.; Brewster, M.; Dressman, J.; Feng, K.-I.; Halbert, G.; Kostewicz, E.; McAllister, M.; Muenster, U.; Thinnes, J.; Taylor, R.; Mullertz, A. Early Pharmaceutical Profiling to Predict Oral Drug Absorption: Current Status and Unmet Needs. *Eur. J. Pharm. Sci.* **2014**, *57*, 173−199.
(80) Nath, A.; Atkins, W. M. A Theoretical Validation of the Substrate Depletion Approach to Determining Kinetic Parameters. *Drug Metab. Dispos.* **2006**, *34* (9), 1433−1435.
(81) Obach, R. S. Prediction of Human Clearance of Twenty-Nine Drugs from Hepatic Microsomal Intrinsic Clearance Data: An Examination of In Vitro Half-Life Approach and Nonspecific Binding to Microsomes. *Drug Metab. Dispos.* **1999**, *27* (11), 1350−1359.
(82) Jigorel, E.; Houston, J. B. Utility of Drug Depletion-Time Profiles in Isolated Hepatocytes for Accessing Hepatic Uptake Clearance: Identifying Rate-Limiting Steps and Role of Passive Processes. *Drug Metab. Dispos.* **2012**, *40* (8), 1596−1602.
(83) Obach, R. S.; Reed-Hagen, A. E. Measurement of Michaelis Constants for Cytochrome P450-Mediated Biotransformation Reactions Using a Substrate Depletion Approach. *Drug Metab. Dispos.* **2002**, *30* (7), 831−837.
(84) Sjögren, E.; Lennernäs, H.; Andersson, T. B.; Gråsjö, J.; Bredberg, U. The Multiple Depletion Curves Method Provides Accurate Estimates of Intrinsic Clearance (CLint), Maximum Velocity of the Metabolic Reaction (Vmax), and Michaelis Constant (Km): Accuracy and Robustness Evaluated through Experimental Data and Monte Carlo Si. *Drug Metab. Dispos.* **2009**, *37* (1), 47−58.
(85) Austin, R. P.; Barton, P.; Cockroft, S. L.; Wenlock, M. C.; Riley, R. J. The Influence of Nonspecific Microsomal Binding on Apparent Intrinsic Clearance, and Its Prediction from Physicochemical Properties. *Drug Metab. Dispos.* **2002**, *30* (12), 1497−1503.
(86) GastroPlus Software. Lancaster, CA, 2016.
(87) Gertz, M.; Kilford, P. J.; Houston, J. B.; Galetin, A. Drug Lipophilicity and Microsomal Protein Concentration as Determinants in the Prediction of the Fraction Unbound in Microsomal Incubations. *Drug Metab. Dispos.* **2008**, *36* (3), 535−542.
(88) Yang, C. X.; Li, Y. F.; Huang, C. Z. Determination of Total Protein Content in Human Serum Samples with Fast Red Vr by Resonance Light Scattering Technique. *Anal. Lett.* **2002**, *35* (12), 1945−1957.
(89) Wilson, Z. E.; Rostami-Hodjegan, A.; Burn, J. L.; Tooley, A.; Boyle, J.; Ellis, S. W.; Tucker, G. T. Inter-Individual Variability in Levels of Human Microsomal Protein and Hepatocellularity per Gram of Liver. *Br. J. Clin. Pharmacol.* **2003**, *56* (4), 433−440.
(90) Barter, Z. E.; Bayliss, M. K.; Beaune, P. H.; Boobis, A. R.; Carlile, D. J.; Edwards, R. J.; Houston, J. B.; Lake, B. G.; Lipscomb, J. C.; Pelkonen, O. R.; Tucker, G. T.; Rostami-Hodjegan, A. Scaling Factors for the Extrapolation of in Vivo Metabolic Drug Clearance from in Vitro Data: Reaching a Consensus on Values of Human Microsomal Protein and Hepatocellularity per Gram of Liver. *Curr. Drug Metab.* **2007**, *8* (1), 33−45.
(91) McGinnity, D. F.; Soars, M. G.; Urbanowicz, R. A.; Riley, R. J. Evaluation Of Fresh And Cryopreserved Hepatocytes As In Vitro Drug Metabolism Tools For The Prediction Of Metabolic Clearance. *Drug Metab. Dispos.* **2004**, *32* (11), 1247−1253.
(92) Kwon, Y. Clearance. In *Handbook of Essential Pharmacokinetics, Pharmacodynamics and Drug Metabolism for Industrial Scientists*; Kwon, Y., Ed.; Kluwer Academic Publishers Publishers: Hingham, MA, USA, 2001; pp 83−103.
(93) Davies, B.; Morris, T. Physiological Parameters in Laboratory Animals and Humans. *Pharm. Res.* **1993**, *10* (7), 1093−1095.
(94) Riley, R. J.; McGinnity, D. F.; Austin, R. P. A Unified Model For Predicting Human Hepatic, Metabolic Clearance From In Vitro Intrinsic Clearance Data In Hepatocytes And Microsomes. *Drug Metab. Dispos.* **2005**, *33* (9), 1304−1311.
(95) Gertz, M.; Houston, J. B.; Galetin, A. Physiologically Based Pharmacokinetic Modeling of Intestinal First-Pass Metabolism of CYP3A Substrates with High Intestinal Extraction. *Drug Metab. Dispos.* **2011**, *39* (9), 1633−1642.
(96) Paine, M. F.; Hart, H. L.; Ludington, S. S.; Haining, R. L.; Rettie, A. E.; Zeldin, D. C. The Human Intestinal Cytochrome P450 "Pie. *Drug Metab. Dispos.* **2006**, *34* (5), 880−886.
(97) Gertz, M.; Harrison, A.; Houston, J. B.; Galetin, A. Prediction of Human Intestinal First-Pass Metabolism of 25 CYP3A Substrates from In Vitro Clearance and Permeability Data. *Drug Metab. Dispos.* **2010**, *38* (7), 1147−1158.
(98) Yang, J.; Jamei, M.; Yeo, K. R.; Tucker, G. T.; Rostami-Hodjegan, A. Prediction of Intestinal First-Pass Drug Metabolism. *Curr. Drug Metab.* **2007**, *8* (7), 676−684.
(99) Hatley, O. *Mechanistic Prediction of Intestinal First-Pass Metabolism Using in Vitro Data and Preclinical Species and in Man*; The University of Manchester: Manchester, 2013.
(100) Granger, D. N.; Richardson, P. D.; Kvietys, P. R.; Mortillaro, N. A. Intestinal Blood Flow. *Gastroenterology* **1980**, *78*, 837−863.
(101) Chiu, Y.-Y.; Higaki, K.; Neudeck, B. L.; Barnett, J. L.; Welage, L. S.; Amidon, G. L. Human Jejunal Permeability ofCyclosporin A: Influence of Surfactants on P-Glycoprotein Efflux in Caco-2 Cells. *Pharm. Res.* **2003**, *20* (5), 749−756.
(102) Zgair, A.; Wong, J. C. M.; Sabri, A.; Fischer, P. M.; Barrett, D. A.; Constantinescu, C. S.; Gershkovich, P. Development of a Simple and Sensitive HPLC-UV Method for the Simultaneous Determination of Cannabidiol and Δ9-Tetrahydrocannabinol in Rat Plasma. *J. Pharm. Biomed. Anal.* **2015**, *114* (0), 145−151.
(103) Lee, J. B. *HPLC-UV Assays for Verapamil and Dexamethasone (Unpublished Work)*; 2015.
(104) Frank, L. D.; Andresen, M. A.; Schmid, T. L. Obesity Relationships with Community Design, Physical Activity, and Time Spent in Cars. *Am. J. Prev. Med.* **2004**, *27* (2), 87−96.
(105) Dutilleul, P.; Stockwell, J. D.; Frigon, D.; Legendre, P. The Mantel Test versus Pearson's Correlation Analysis: Assessment of the Differences for Biological and Environmental Studies. *J. Agric. Biol. Environ. Stat.* **2000**, *5* (2), 131−150.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

(106) U.S. Food and Drug Administration. Guidance for Industry: Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. 2000.
(107) Berthelsen, R.; Holm, R.; Jacobsen, J.; Kristensen, J.; Abrahamsson, B.; Müllertz, A. Kolliphor Surfactants Affect Solubilization and Bioavailability of Fenofibrate. Studies of in Vitro Digestion and Absorption in Rats. *Mol. Pharmaceutics* **2015**, *12* (4), 1062−1071.
(108) Pouton, C. W. Lipid Formulations for Oral Administration of Drugs: Non-Emulsifying, Self-Emulsifying and "self-Microemulsifying" Drug Delivery Systems. *Eur. J. Pharm. Sci.* **2000**, *11* (Supple (0), S93−S98.
(109) Custodio, J. M.; Wu, C.-Y.; Benet, L. Z. Predicting Drug Disposition, Absorption/Elimination/Transporter Interplay And The Role Of Food On Drug Absorption. *Adv. Drug Delivery Rev.* **2008**, *60* (6), 717−733.
(110) Mazur, A.; Lichti, C. F.; Prather, P. L.; Zielinska, A. K.; Bratton, S. M.; Gallus-Zawada, A.; Finel, M.; Miller, G. P.; Radomińska-Pandya, A.; Moran, J. H. Characterization of Human Hepatic and Extrahepatic UDP-Glucuronosyltransferase Enzymes Involved in the Metabolism of Classic Cannabinoids. *Drug Metab. Dispos.* **2009**, *37* (7), 1496−1504.
(111) Bland, T. M.; Haining, R. L.; Tracy, T. S.; Callery, P. S. CYP2C-Catalyzed delta(9)-Tetrahydrocannabinol Metabolism: Kinetics, Pharmacogenetics and Interaction with Phenytoin. *Biochem. Pharmacol.* **2005**, *70* (7), 1096−1103.
(112) Kolars, J. C.; Schmiedlin-Ren, P.; Schuetz, J. D.; Fang, C.; Watkins, P. B. Identification of Rifampin-Inducible P450IIIA4 (CYP3A4) in Human Small Bowel Enterocytes. *J. Clin. Invest.* **1992**, *90* (5), 1871−1878.
(113) Kronbach, T.; Fischer, V.; Meyer, U. A. Cyclosporine Metabolism in Human Liver: Identification of a Cytochrome P-450III Gene Family as the Major Cyclosporine-Metabolizing Enzyme Explains Interactions of Cyclosporine with Other Drugs. *Clin. Pharmacol. Ther.* **1988**, *43* (6), 630−635.
(114) Yatscoff, R. W.; Rosano, T. G.; Bowers, L. D. The Clinical Significance of Cyclosporine Metabolites. *Clin. Biochem.* **1991**, *24* (1), 23−25.
(115) Dunn, C.; Wagstaff, A.; Perry, C.; Plosker, G.; Goa, K. Cyclosporin. *Drugs* **2001**, *61* (13), 1957−2016.
(116) Kolars, J. C.; Watkins, P. B.; Merion, R. M.; Awni, W. M. First-Pass Metabolism of Cyclosporin by the Gut. *Lancet* **1991**, *338* (8781), 1488−1490.
(117) Wade, A. E. Effects of Dietary Fat on Drug Metabolism. *J. Env. Pathol Toxicol Oncol.* **1986**, *6* (3−4), 161−189.
(118) Benet, L. Z.; Cummins, C. L. The Drug Efflux−metabolism Alliance: Biochemical Aspects. *Adv. Drug Delivery Rev.* **2001**, *50* (Supple), S3−S11.
(119) Benet, L. Z.; Cummins, C. L.; Wu, C. Y. Unmasking the Dynamic Interplay between Efflux Transporters and Metabolic Enzymes. *Int. J. Pharm.* **2004**, *277* (1−2), 3−9.
(120) Trevaskis, N. L.; Porter, C. J. H.; Charman, W. N. An Examination Of The Interplay Between Enterocyte-Based Metabolism And Lymphatic Drug Transport In The Rat. *Drug Metab. Dispos.* **2006**, *34* (5), 729−733.
(121) Dahan, A.; Hoffman, A. Evaluation of a Chylomicron Flow Blocking Approach to Investigate the Intestinal Lymphatic Transport of Lipophilic Drugs. *Eur. J. Pharm. Sci.* **2005**, *24* (4), 381−388.
(122) Gershkovich, P.; Hoffman, A. Uptake of Lipophilic Drugs by Plasma Derived Isolated Chylomicrons: Linear Correlation with Intestinal Lymphatic Bioavailability. *Eur. J. Pharm. Sci.* **2005**, *26* (5), 394−404.
(123) Wong, J.; Sivak, O.; Wasan, K. M.; Fischer, P. M.; Gershkovich, P. The Role of Lymphatic Transport in the Intestinal Absorption of Lipophilic Cannabinoids. AAPS, San Antonio, TX, USA, *AAPS*2013.

DOI: 10.1021/acs.molpharmaceut.6b00597
Mol. Pharmaceutics XXXX, XXX, XXX−XXX