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Drug Discovery–Development Interface

Advantages of the refined developability classification system in early discovery

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ABSTRACT

Rat pharmacokinetic studies are commonly utilized in early discovery to support absorption, distribution, metabolism, and excretion optimization of active pharmaceutical ingredients (APIs). The aim of this work was to compare exposures from fit-for-purpose oral suspension and solution formulations in rats to guidance provided by the refined Developability Classification System (rDCS) with respect to identifying potential limits to oral absorption, formulation strategy selection, and to optimize oral bioavailability (BA). This investigation utilized six diverse APIs covering a large range of biorelevant solubility, metabolic stability, and oral BA in rats. While results for our model compounds acetaminophen, voriconazole, fedratinib, lemborexant, and istradefylline indicated oral BA in rats was limited by first-pass metabolism, only the results for voxelotor indicated an oral absorption limitation by intestinal dissolution/solubility. The *in vivo* studies highlighted challenges and limitations often encountered in early discovery. The rDCS analysis provided a more differentiated developability risk assessment associated with oral solid dosage form development by incorporating compound-specific physicochemical attributes and human physiology without the need of preclinical data. The rDCS results were shown to align well with the clinical/marketed formulation strategies for the investigated APIs.

Introduction

The large number of unmet medical needs across therapeutic areas creates a strong driving force for the pharmaceutical industry to continuously innovate and develop new enhanced therapies. Accelerated timelines and cost pressures further propel the need to consistently deliver new innovative drug products to the market.

Efficient fit-for-purpose strategies aimed at identifying and selecting oral drug candidates should therefore be implemented early in discovery.[1,2] Focusing on the potential need to address biopharmaceutical challenges early on with a suitable formulation approach may help lower attrition rates linked to suboptimal oral bioavailability (BA) and streamline the development process towards an adequate clinical/market formulation.[1–9]

The refined Developability Classification System (rDCS) is a powerful *in vitro* tool that has been successfully employed to assess a wide array of compounds exhibiting diverse biopharmaceutical properties.[5,10,11] In a recent study, guidance provided by the rDCS was shown to align well with literature regarding formulation selection and clinical performance of six investigated active pharmaceutical ingredients (APIs) in humans.[11]

By contrast, classical screening methods used to identify potential oral absorption risks and guide formulation selection in discovery and early development involve comparing *in vivo* exposures of suspension and solution formulations of APIs, usually in rats or beagle dogs.[2,7,9] If exposure from suspension is significantly lower than from solution (*e.g.*, ≥ two-fold difference), intestinal absorption may be limited by dissolution and/or poor solubility *in vivo*, in which case particle size reduction or enabling formulations may be required. Similar exposures from suspension and solution may suggest either (i) complete absorption from both formulations, (ii) precipitation of (solubilized) API from the solution formulation upon mixing with gastrointestinal fluids, or (iii) very low systemic exposure due to high first-pass metabolism or permeability-limited absorption.[2,3] Poor exposure from an oral solution compared to the suspension (*e.g.*, ≥ two-fold difference) may indicate chemical stability issues of dissolved API in the gastrointestinal tract or *in vivo* precipitation leading

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to a situation where the resulting precipitate does not re-dissolve during intestinal transit.[3] Implementation of animal-based methods along with guidance on formulation strategy selection are available in the form of published decision trees.[2,3,7,9]

Rodent pharmacokinetic (PK) studies are commonly utilized to support absorption, distribution, metabolism, and excretion optimization of APIs early in discovery. In this study, six diverse APIs investigated previously using the rDCS were profiled in rat PK studies by comparing oral exposures from simple solution formulations with exposures from oral suspensions. The outcomes are compared against the results from rDCS analysis to identify potential intersections in biopharmaceutical risk assessment and guidance on oral formulation strategy selection.

Materials and methods

Chemicals

The APIs investigated in this study were acetaminophen from Merck Life Science BV (Overijse, Belgium); voriconazole, voxelotor, and lemborexant from Biosynth Carbosynth (Compton, UK); and istradefylline, fedratinib, and fedratinib dihydrochloride monohydrate from MedChemExpress LLC (Monmouth Junction, NJ, USA). Key physicochemical properties highlighting the diversity within this set of APIs are shown in Fig. 1. Crystalline forms, melting points (T_m) and purities of the APIs are reported in the Supplementary Materials.

The excipients used in the formulation development studies of the APIs were hydroxypropyl methylcellulose (HPMC) 2906 4000 mPa.s (Methocel F4M Premium) sourced from Dow (Midland, MI, USA), polyethylene glycol 400 (PEG400) from Sigma Aldrich (Darmstadt, Germany), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) provided by Janssen Pharmaceutica N.V. (Beerse, Belgium), and N-methyl-2-pyrrolidone (NMP) from VWR International S.A.S (Rosny-sous-Bois, France). Purified water (MilliQ-water) was produced using a Milli-Q Advantage A10 Water Purification System (Merck KGaA, Darmstadt, Germany).

Solvents used in the UPLC studies were acetonitrile (ACN, chromatography grade), methanol (MeOH, chromatography grade), hydrochloric acid solution (0.1 N and 1 N HCl), and sodium hydroxide solution (1 N NaOH) sourced from Merck KGaA (Darmstadt, Germany). Trifluoroacetic acid (TFA) was purchased from Thermo Fisher Scientific (Rockford, IL, USA).

Formulation development & characterization

Two different formulations for dosing in rats per API were developed. These included a suspension for oral and an aqueous solution suitable for oral and intravenous dosing. The target API concentration in each formulation was 1 mg/mL, resulting in standard doses of 1 mg/kg intravenously and 5 mg/kg orally that are typically used in drug absorption studies in preclinical species.[1,2,7] Solution formulations were developed from standard vehicles often used in discovery to solubilize the compounds since the aqueous solubilities of most APIs did not support the *in vivo* study design.

The vehicle selection was driven by existing literature, with the focus on formulations that are well-tolerated and minimize potential impact on physiological and PK parameters.[1,2,7]

Oral suspension formulations

The standard vehicle for the oral suspensions was 0.5 % (w/v) hydroxypropyl methylcellulose (HPMC) 2906 4000 mPa.s (Methocel F4 M Premium) in MilliQ-water. The APIs were initially dispersed in this vehicle to create concentrated stocks at 10 mg/mL and stirred for 24 h using a magnetic stirrer (500 rpm, room temperature). This procedure was utilized to reduce particle size via stirring since particle size is often not optimized in early discovery. The stock suspensions were diluted to 1 mg/mL with blank suspension vehicle, followed by

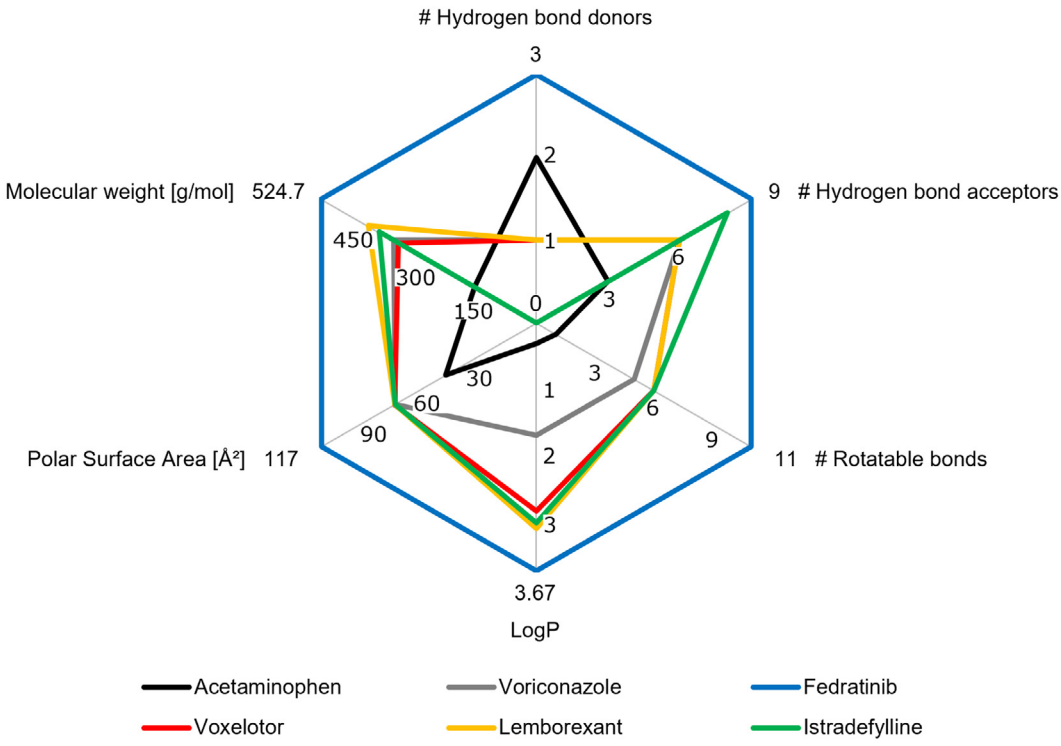

Fig. 1. Spider plot of key physicochemical properties of the six investigated APIs. All dimensions are scaled and normalized from zero to the maximum value in the dataset. Numerical values, along with references, are provided in the Supplementary Materials.

an additional 20 to 30 min of continuous stirring to generate homogenous suspensions prior to dosing.

The suspensions were visually inspected using a ZEISS Axio Vert. A1 Inverted Microscope for Advanced Routine (Carl Zeiss Microscopy GmbH, Jena, Germany) to assess morphology post suspension preparation. The pH of the suspensions was measured using a Metrohm 780 pH Meter (Metrohm AG, Herisau, Switzerland), setting the acceptance criterion within the range of pH 2 to 9. The acceptable pH range for intravenous dosing was pH 4 to 9.

The amount of API dissolved in the suspensions was determined via ultra-high performance liquid chromatography with UV detection (UPLC-UV) using a Waters Acquity UPLC System (Waters Corporation, Milford, MA, USA). Prior to analysis, the suspensions were ultracentrifuged for 20 min at 100,000 rpm (at room temperature) using a Sorvall MTX 150 Micro Ultracentrifuge (Thermo Fisher Scientific, Waltham, MA, USA). The supernatants were diluted with a 1:1 mixture of ACN and water. Information on the UPLC-UV methods can be found in previous publications.[10,11]

Solid-state properties of the dispersed APIs were evaluated using X-ray powder diffraction (PANalytical X'PertPRO MPD diffractometer, Philips, Amsterdam, The Netherlands), differential scanning calorimetry (DSC 2500, TA Instruments, New Castle, DE, USA), and, when needed, thermogravimetric analysis (TGA 5500, TA Instruments, New Castle, DE, USA). The methods applied for solid-state analysis have been described previously.[10,11] The particle size distribution (PSD) in the stock suspensions was analyzed prior to dosing with laser diffraction at Pharmaron (Beijing, China) using a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK). Further experimental details are provided in the Supplementary Materials.

Oral and intravenous solution formulations

The following standard vehicles were assessed for their ability to solubilize the APIs at 1 mg/mL: 0.9 % (w/v) NaCl solution in water, 20 % (w/v) 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) solution in water, and a 1:1 mixture of PEG400-water (PEG400/H_2O).

If the standard vehicles proved inadequate for solubilizing an API at the target concentration of 1 mg/mL, as was the case for istradefylline, potential alternative strategies involved reducing the API concentration in the formulation or incorporating additional co-solvents, such as 2 % (v/v) NMP. The selection of the co-solvent and concentration was informed by uptake studies conducted in rings from jejunal and colonic tissues of rats, which demonstrated no significant impact of 2 % NMP on the uptake of APIs with different permeabilities.[12]

The pH of the solutions was measured and adjusted within the range of 4 to 9, if necessary, and the dissolved API concentration was confirmed using UPLC-UV.

Pharmacokinetic studies in rats

The *in vivo* rat studies, including bioanalysis, were carried out at Pharmaron (Beijing, China). Male Wistar Han rats, sourced from Vital River (Beijing, China), aged 7 to 9 weeks with a body weight of 244 to 291 g were utilized. The rats had free access to food and water before and throughout the studies.

The three standard study arms for each API, as summarized in Table 1, included an intravenous (solution) and two oral arms (suspension and solution). The intravenous solution formulations were dosed via tail vein injection (n = 3) at a dose of 1 mg/kg, with a dosing volume of 1 mL/kg. The oral suspension and solution formulations were dosed via oral gavage (n = 3 per formulation) at 5 mg/kg, with a dosing volume of 5 mL/kg. Blood samples (200 μL) were collected from the jugular vein after 15 and 30 min, as well as 1, 2, 4, 8, and 24 h post-dosing. In the intravenous groups, additional early samples were taken 2 and 5 min post-dosing. Considering the long half-life of voxelotor in rats (~20 h),[13] extra samples were taken after 48 and 72 h in the respective study groups. A detailed description of the sample preparation and analytical methods for bioanalysis is provided in the Supplementary Materials.

Table 1
Overview of the conducted rat studies.

API	Route	Formulation	Vehicle	Dose [mg/kg]
Acetaminophen	i.v.	Solution	0.9 % NaCl	1
	p.o.	Solution	Water	5
	p.o.	Suspension	0.5 % HPMC F4M	-[a]
Voriconazole	i.v.	Solution	20 % HP-β-CD	1
	p.o.	Solution	20 % HP-β-CD	1[b]
	p.o.	Solution	20 % HP-β-CD	5
	p.o.	Suspension	0.5 % HPMC F4M	5
Fedratinib	i.v.	Solution	20 % HP-β-CD	1
	p.o.	Solution	20 % HP-β-CD	5
	p.o.	Suspension	0.5 % HPMC F4M	5
Fedratinib 2HCl x H_2O	i.v.	Solution	20 % HP-β-CD	1[c]
	p.o.	Solution	20 % HP-β-CD	5[c]
	p.o.	Suspension	0.5 % HPMC F4M	-[a]
Voxelotor	i.v.	Solution	20 % HP-β-CD	1
	p.o.	Solution	20 % HP-β-CD	5
	p.o.	Suspension	0.5 % HPMC F4M	5
Lemborexant	i.v.	Solution	20 % HP-β-CD	1
	p.o.	Solution	20 % HP-β-CD	5
	p.o.	Suspension	0.5 % HPMC F4M	5
Istradefylline[d,e]	i.v.	Solution	PEG400/H_2O/NMP[f]	0.25
	p.o.	Solution	PEG400/H_2O/NMP[f]	1.25
	p.o.	Suspension	0.5 % HPMC F4M	1.25

[a] A suspension formulation could not be administered because the dose dissolved fully in the standard suspension medium.
[b] An additional oral dose (1 mg/kg) was included due to nonlinear PK of voriconazole in rats.
[c] Corresponding to fedratinib free base.
[d] Formulations were prepared in amber vials due to photo-instability of the API.[14]
[e] Dosed at a reduced dose due to the API's low solubility in the solution vehicle.
[f] 1:1 PEG/H_2O with 2% NMP.

Animal welfare and ethical approval

An animal care and use application was approved by the Institutional Animal Care and Use Committee (IACUC), in accordance with Pharmaron's IACUC policies and procedures.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined through non-compartmental analysis of the plasma concentration-time data using Phoenix WinNonlin software version 8.3 (Certara, Princeton, NJ, USA).[15] The plasma clearance (CL) after intravenous administration was calculated based on the dose and the area under the curve (AUC). The initial plasma API concentration immediately after intravenous administration (C_0) was estimated through back-extrapolation of the plasma concentration-time curve. The maximum plasma concentration (C_{max}) and the time to reach the maximum plasma concentration (t_{max}) after oral dosing were identified as the peak concentration and its corresponding time-point within the datasets.

The AUC was determined using the log-linear trapezoidal method. The area under the curve to infinity (AUC_{INF}) after intravenous dosing was obtained by extrapolating the terminal (log-linear) segment of the plasma concentration-time curve. The area under the curve from the time of dosing to the last measurable concentration (AUC_{LAST}) was determined for the oral groups.

The absolute oral bioavailability (BA) was calculated according to the following equation:

$$\text{Absolute BA} = \frac{D_{i.v.} \cdot AUC_{p.o.}}{D_{p.o.} \cdot AUC_{i.v.}} \cdot 100\% \quad (1)$$

where $D_{i.v.}$ is the dose administered intravenously, $D_{p.o.}$ is the dose administered orally, $AUC_{i.v}$ is the AUC_{INF} after intravenous administration and $AUC_{p.o.}$ is the AUC_{LAST} after oral administration.

All values are presented as means ± standard deviations (SD).

Estimation of oral bioavailability using systemic clearance and hepatic blood flow

An approach using intravenous and oral PK data was used to estimate contributions of oral absorption and (first-pass) metabolism to the absolute oral BA.[16] In this approach, the systemic CL is used to estimate a hepatic extraction ratio (ER_h) under the assumption that the CL is exclusively determined by hepatic metabolism.

$$ER_h = \frac{CL_{i.v.}}{Q_{liver}} \quad (2)$$

In this equation, ER_h is the estimated hepatic extraction ratio, $CL_{i.v.}$ is the systemic clearance observed after intravenous administration, and Q_{liver} is the estimated hepatic blood flow (portal vein) in rats. Q_{liver} was estimated by dividing 18.1 mL/min by the rat's body weight, following the methodology in the software Simcyp Animal Version 21 (Simcyp Ltd., Sheffield, UK).

The calculated ER_h allows for estimation of the fraction escaping the liver (f_h) according to the following equation:

$$f_h \approx 1 - ER_h \quad (3)$$

With these approximations, an absolute oral BA can be estimated (BA_{EST}) utilizing the below equation:

$$\text{Abs. } BA_{EST} = f_a \cdot f_g \cdot f_h \cdot 100\% \approx f_a \cdot f_g \cdot (1 - ER_h) \cdot 100\% \approx (1 - ER_h) \cdot 100\% \quad (4)$$

where BA_{EST} is the estimated absolute oral bioavailability, f_a is the fraction of dose absorbed (assumed to equal 1), f_g is the fraction escaping the gut (assumed to equal 1), f_h is the fraction escaping the liver, and ER_h is the estimated hepatic extraction ratio.

The BA_{EST} calculation assumes that (i) intestinal absorption is complete, (ii) metabolism of the drug in the intestinal mucosa is negligible, and (iii) the systemic CL is solely driven by hepatic metabolism. If there is a substantial difference between the BA_{EST} and the experimentally determined absolute oral BA (BA_{EXP}), the above f_a x f_g = 1 assumption may be incorrect.

Results

Formulation development & characterization

Oral suspension formulations

An overview of the oral suspension formulations, including formulation attributes, is summarized in Table 2. Images of the suspensions, including polarized light microscopic images, as well as results of the solid-state analyses can be found in the Supplementary Materials.

Suspension formulations were not obtained with acetaminophen and fedratinib dihydrochloride monohydrate due to their high solubilities in the suspension vehicle. The suspension of istradefylline was prepared from a concentrated stock at 2.5 mg/mL, followed by subsequent dilution to reach a final API concentration of 0.25 mg/mL prior to dosing. The reduced concentration was due to practical limitations in preparing the solution formulation, ensuring dosing consistency within the oral study groups at the same concentration.

The results from the particle size analysis, obtained from the concentrated stock suspensions, are presented in the Supplementary Materials. The D90 values of the PSDs were less than 20 μm for all

Table 2
Overview of the oral suspension formulations (0.5 % (w/v) HPMC F4 M vehicle) and formulation attributes.

API	pH	Target conc. [mg/mL]	Dissolved API concentration		Crystalline form dosed
			[mg/mL]	[%][a]	
Acetaminophen[b]	6.83	1	0.945	94.5	API fully dissolved[c]
Voriconazole	7.06 ± 0.04	1	0.688 ± 0.01	68.8 ± 1.0	No form change
Fedratinib	7.87 ± 0.05	1	0.0325 ± 0.0060	3.25 ± 0.60	No form change
Fedratinib 2HCl x H_2O	4.87 ± 0.05	1[d]	1.02 ± 0.00	102 ± 0	API fully dissolved[c]
Voxelotor	7.02 ± 0.07	1	0.0517 ± 0.002	5.17 ± 0.20	No form change
Lemborexant	6.96 ± 0.01	1	0.0176 ± 0.0014	1.76 ± 0.14	No form change
Istradefylline	6.74 ± 0.05	0.25	0.00130 ± 0.00002	0.520 ± 0.008	Form change

[a] Dissolved API expressed as the percentage of total API content.
[b] n = 1.
[c] Not dosed *in vivo*.
[d] Corresponding to fedratinib free base.

Table 3
Selected solution formulations for intravenous (i.v.) and oral (p.o.) administration, including formulation attributes.

API	Vehicle	Route	pH	Target conc. [mg/mL]	Dissolved API conc.	
					[mg/mL]	[%][a]
Acetaminophen[b]	0.9 % NaCl	i.v.	6.18	1	1.01	101.0
	Sterile water	p.o.	7.68		0.994	99.4
Voriconazole[b]	20 % HP-β-CD	i.v. & p.o.	4.00[c]	1	1.01	101
Fedratinib	20 % HP-β-CD	i.v. & p.o.	4.57 ± 0.71	1	1.02 ± 0.01	102 ± 1
Fedratinib 2HCl x H_2O	20 % HP-β-CD	i.v. & p.o.	5.27 ± 0.05	1[d]	1.02 ± 0.00	102 ± 0
Voxelotor	20 % HP-β-CD	i.v. & p.o.	4.16 ± 0.24[c]	1	1.01 ± 0.01	101 ± 1
Lemborexant	20 % HP-β-CD	i.v. & p.o.	4.21 ± 0.26[c]	1	0.996 ± 0.003	99.6 ± 0.3
Istradefylline	PEG400/H_2O/NMP	i.v. & p.o.	7.81 ± 0.02	0.25	0.262 ± 0.012	105 ± 5

[a] Dissolved API in the formulation expressed as the percentage of total API content (target value = 100 %).
[b] n = 1.
[c] Adjusted pH (the pH of the initial formulation needed adjustment as it fell outside the acceptance range of pH 4 to 9).
[d] Corresponding to fedratinib free base.

suspensions, indicating efficient particle size reduction during suspension preparation.

Oral and intravenous solution formulations

The solution formulations, including vehicle information and formulation attributes, are summarized in Table 3.

While solution formulations for most APIs could be obtained with the standard vehicles profiled, a suitable formulation for istradefylline at the target concentration of 1 mg/mL was not identified due to its poor solubility. A supersaturated solution formulation of istradefylline at a reduced concentration of 0.25 mg/mL in PEG400/H_2O/NMP was identified. This formulation was dosed immediately after preparation.

Pharmacokinetic studies in rats

The plasma concentration-time profiles of the APIs, including raw data, are provided in the Supplementary Materials. The results of the PK analyses are presented in Table 4. Absolute oral BAs, estimated using the intravenous CL and Q_{liver} (BA_{EST}), are presented in the Supplementary Materials.

Discussion

Acetaminophen

Formulation development & characterization

The analgesic and antipyretic drug acetaminophen exhibits high aqueous solubility (14.7 mg/mL at 20 °C)[17] and a solution formulation was easily obtained using either 0.9 % NaCl solution or plain water as the vehicle. A suspension formulation was not obtained with acetaminophen due to complete dissolution in the suspension vehicle. Although acetaminophen suspensions with concentrations ≥20 mg/mL have been utilized for absorption studies in rats,[18] this concentration would lead to unrealistic doses to determine PK properties in early discovery.

Pharmacokinetic studies in rats

After intravenous administration of acetaminophen at 1 mg/kg, plasma concentrations decreased rapidly and consistently across all three rats. The systemic CL (32.2 ± 3.6 mL/min/kg) was less than the hepatic blood flow (70.8 ± 0.8 mL/min/kg) and consistent with reported values in the literature (27.7 to 37.5 mL/min/kg at a 10 mg/kg dose).[19,20] Based on CL and Q_{liver}, a hepatic extraction ratio (ER_h)

Table 4
Results of the PK analysis.

API	Dose [mg/kg]	Route	Formulation	Vehicle	CL [mL/min/kg]	C_{max}[a] [ng/mL]	t_{max} [h]	AUC[b] [ng·h/mL]	Absolute BA [%]
Acetaminophen	1	i.v.	Solution	0.9 % NaCl	32.2 ± 3.6	2104.9 ± 179.5	N/A	522.9 ± 60.8	N/A
	5	p.o.	Solution	Water	N/A	1098.7 ± 101.2	0.25 ± 0.00	1034.4 ± 161.4	39.6 ± 6.2
Voriconazole	1	i.v.	Solution	20 % HP-β-CD	23.6 ± 7.3	1421.3 ± 126.3	N/A	763.9 ± 275.9	N/A
	1	p.o.	Solution	20 % HP-β-CD	N/A	146.7 ± 35.8	1.00 ± 0.00	536.7 ± 208.3	70.3 ± 27.3
	5	p.o.	Solution	20 % HP-β-CD	N/A	901.0 ± 42.5	1.33 ± 0.58	5003.9 ± 659.6	131.0 ± 17.3
	5	p.o.	Suspension	0.5 % HPMC F4M	N/A	1310.0 ± 260.6	3.33 ± 1.15	7714.8 ± 1710.3	202.0 ± 44.8
Fedratinib	1	i.v.	Solution	20 % HP-β-CD	92.3 ± 18.0	242.3 ± 29.8	N/A	185.7 ± 40.1	N/A
	5	p.o.	Solution	20 % HP-β-CD	N/A	8.7 ± 2.0	3.33 ± 1.15	45.5 ± 8.4	4.9 ± 0.9
	5	p.o.	Suspension	0.5 % HPMC F4M	N/A	13.9 ± 3.7	4.00 ± 0.00	62.0 ± 11.6	6.7 ± 1.3
Fedratinib 2HCl x H_2O	1	i.v.	Solution	20 % HP-β-CD	102.3 ± 0.6	247.9 ± 51.6	N/A	162.9 ± 0.9	N/A
	5	p.o.	Solution	20 % HP-β-CD	N/A	9.1 ± 1.0	4.00 ± 0.00	47.0 ± 4.7	5.8 ± 0.6
Voxelotor	1	i.v.	Solution	20 % HP-β-CD	1.93 ± 0.14	882.0 ± 248.7	N/A	8647.8 ± 646.7	N/A
	5	p.o.	Solution	20 % HP-β-CD	N/A	960.7 ± 112.4	3.00 ± 1.73	23,990.2 ± 5285.4	55.5 ± 12.2
	5	p.o.	Suspension	0.5 % HPMC F4M	N/A	1076.0 ± 506.7	3.00 ± 1.73	26,441.6 ± 15,272.4	61.2 ± 35.3
Lemborexant	1	i.v.	Solution	20 % HP-β-CD	58.3 ± 11.7	1385.6 ± 365.9	N/A	294.5 ± 64.6	N/A
	5	p.o.	Solution	20 % HP-β-CD	N/A	6.3 ± 2.9	0.92 ± 0.95	11.0 ± 3.0	0.8 ± 0.2
	5	p.o.	Suspension	0.5 % HPMC F4M	N/A	21.6 ± 11.7	0.33 ± 0.14	19.4 ± 8.4	1.3 ± 0.6
Istradefylline	0.25	i.v.	Solution	PEG400/H_2O/NMP	31.0 ± 4.3	206.4 ± 25.7	N/A	136.4 ± 19.8	N/A
	1.25	p.o.	Solution	PEG400/H_2O/NMP	N/A	123.0 ± 20.5	1.33 ± 0.58	408.3 ± 37.3	59.9 ± 5.5
	1.25	p.o.	Suspension	0.5 % HPMC F4M	N/A	154.7 ± 20.1	0.67 ± 0.29	447.1 ± 36.6	65.6 ± 5.4

N/A = Not applicable.
[a] In the i.v. data, the (extrapolated) C_0 is presented.
[b] AUCs are reported as the AUC_{INF} for the intravenous groups and as the AUC_{LAST} for the oral groups.

of 45.4 ± 5.2 % was estimated, indicating that the oral BA of acetaminophen may be limited by a hepatic first-pass effect (BA_{EST} = 54.6 ± 5.2 %).

Following oral administration of an acetaminophen solution at 5 mg/kg, plasma levels exhibited rapid onset with t_{max} observed at the first sampling point. The absolute oral BA was incomplete (39.6 ± 6.2 %), consistent with the calculated BA_{EST} and in line with a literature value after administration of a ~7.5 mg/kg dose (38.9 ± 8.4 %).[20]

Due to the inability to formulate acetaminophen as a suspension, comparison of the *in vivo* performance of suspension and solution formulations was not feasible in this study. However, the inability to formulate a suspension suggests that *in vivo* dissolution and/or solubility limitations to the oral absorption of acetaminophen in rats are rather unlikely, indicating a low biopharmaceutical risk for oral acetaminophen formulation development. Since the oral BA appears to be predominantly limited by hepatic first-pass metabolism, a conventional formulation approach would be recommended for subsequent (pre-)clinical studies.[1,2,7–9]

In line with our analysis, absorption of acetaminophen occurs rapidly and efficiently from the small intestine in rats,[18,20] with oral BA limited by first-pass metabolism.[20,21] For comparison, the first-pass effect in humans was reported to be about 20 %.[17]

Comparison to rDCS analysis

The developability of acetaminophen as an oral dosage form, including formulation strategy selection, has been investigated using the rDCS in a previous study.[11] Acetaminophen exhibited high fasted state simulated intestinal fluid (FaSSIF-V1) solubility and high permeability, resulting in rDCS classification I across the entire standard dose range (5, 50 and 500 mg). This suggests complete oral absorption can be achieved from conventional solid oral dosage forms, in line with human BA values reported in the literature (*e.g.*, 89 ± 4 % at a 1000 mg dose (Panadol® tablets) and 87 ± 8 % at a supratherapeutic 2000 mg dose (Panadol® tablets)).[22]

While it was not feasible to compare exposure differences between suspension and solution formulations of acetaminophen at pharmacokinetic doses in rats, Ameer et al. conducted a comparative PK study in humans using a tablet and a solution formulation.[23] In that study, the mean relative BA for the tablet compared to the solution at a 650 mg dose was 91 %, with absolute BAs $\gtrsim$80 %.[23] This outcome supports the results from the rDCS analysis.

Voriconazole

Formulation development & characterization

The triazole antifungal voriconazole is a weak base (pK_a = 1.76)[24] with a reported aqueous solubility of 0.975 mg/mL at pH 7.2 (37 °C).[25] Voriconazole was fully solubilized in the 20 % HP-β-CD vehicle at 1 mg/mL, providing a suitable solution formulation for intravenous and oral dosing.

In the suspension formulation, utilizing 0.5 % Methocel F4 M Premium as the vehicle, 68.8 ± 1.0 % of the intended dose dissolved. XRPD analysis of the suspended solid (Supplementary Materials) indicates that there was no form change upon stirring. The D90 of the PSD in the concentrated suspension was 16.6 μm before dilution to the final suspension. Since a significant portion of the dose is solubilized, the particle size data is not fully representative of the whole dose and the exposure from the suspension should be treated with caution in this regard.

Pharmacokinetic studies in rats

PK analysis after intravenous administration of voriconazole at 1 mg/kg in rats resulted in a systemic CL of 23.6 ± 7.3 mL/min/kg with a coefficient of variation (%CV) of 31.0 %, indicating high variability. The CL of rat #1 (29.6 mL/min/kg) was nearly twice that of rat #3 (15.5 mL/min/kg). However, the mean CL is consistent with a reported literature value for a 2.5 mg/kg i.v. dose (27.7 ± 6.3 mL/min/kg).[26] The literature findings suggest nonlinear PK of voriconazole in rats, likely due to the saturation of metabolic enzymes.[26] Using CL and estimated Q_{liver}, an absolute BA_{EST} of 65.0 ± 9.8 % is obtained, indicating that oral BA in rats is limited by hepatic first-pass metabolism.

After oral administration at a 1 mg/kg dose (as solution), voriconazole plasma concentrations exhibited rapid onset, with t_{max} after 1 h, and an absolute oral BA of 70.3 ± 27.3 %. While this result indicates substantial interindividual variability, it generally agrees with the prediction of a hepatic first-pass effect limiting oral BA of voriconazole.

Following oral administration at 5 mg/kg, voriconazole plasma concentrations increased up to 1 h post-dosing and remained relatively constant up to 4 h in the solution group, while showing an upward trend up to 4 h post-dosing in the suspension group. Notably, the variability in plasma concentrations after administration of the suspension was much higher compared to that observed in the solution group. The absolute oral BAs of voriconazole from suspension and solution were 202.0 ± 44.8 % and 131.0 ± 17.3 %, respectively.

Since the model-independent approach for calculating the absolute oral BA (Eq. (1)) relies on a constant CL across varying doses, it is less suitable for drugs like voriconazole with (saturable) Michaelis-Menten elimination and dose-/concentration-dependent CL.[26] Therefore, at the 5 mg/kg dose, the "apparent" oral absolute BA of voriconazole exceeds the BA_{EST} and even surpasses 100 %.[26] The exposure (AUC) from the suspension appeared slightly elevated compared to that of the solution, noting that the difference was less than two-fold, which is often viewed as a cut-off to detect relevant differences in this type of study. Due to the nonlinear PK the exposure difference between the solution and suspension should only be viewed qualitatively, at best. Discerning any significant differences in the PK parameters of voriconazole is challenging due to the high variability in systemic CL and oral exposure in the suspension group.

In the literature, the oral BA of a voriconazole suspension in male rats (at a 30 mg/kg dose) was found to be 159 % when compared with intravenous data generated at 10 mg/kg.[27] In another study, the absolute BA of voriconazole was determined by an alternative approach.[26] Using a Michaelis-Menten elimination model, an absolute BA of 82.8 % was estimated by fitting both plasma profiles following intravenous administration of a 10 mg/kg dose and oral administration of a 40 mg/kg suspension dose with undisclosed particle size distribution.[26] These data collectively indicate reasonable absorption of voriconazole from suspension formulations in rats even at higher dose levels than tested in this investigation.

However, enabling formulation techniques may potentially result in higher systemic exposures as well as reduced variability compared to conventional formulations, as demonstrated in rats.[28] A higher intestinal dissolution rate and/or solubility of the API facilitated through an enabling formulation may increase the absorption rate, thereby raising drug concentrations at the metabolic sites. This may lead to a greater degree of metabolic saturation, resulting in higher and more reproducible systemic exposure.

Comparison to rDCS analysis

Previous assessment using the rDCS indicated a low developability risk for oral formulations of voriconazole.[11] The rDCS classifications of voriconazole fell in rDCS class I/IIa (5, 50, and 500 mg), suggesting that conventional formulations are suitable to ensure complete absorption in humans over a wide dose range. If, in addition to achieving complete absorption, rapid onset of plasma concentrations is desired, rDCS analysis recommended capping API particle

size (target particle radius (r_{target}) < 500 μm).[11] These findings were shown to align with literature information on marketed (conventional) formulations and the *in vivo* performance in humans, particularly the high oral BA of voriconazole (> 85 % at a 400 mg dose).[11,29]

While PK profiling in rats indicated saturable first-pass metabolism, direct translation of this finding to humans cannot be immediately made without further in-depth studies.

Fedratinib

Formulation development & characterization

The JAK2/FLT3 kinase inhibitor fedratinib is a dibasic molecule (pK_{a1} = 6.45, pK_{a2} = 9.66)[11] exhibiting pH-dependent aqueous solubility. It is freely soluble under acidic conditions (>100 mg/mL at pH 1) but practically insoluble at neutral pH (4 μg/mL at pH 7.4).[30,31] The weakly crystalline fedratinib free base was utilized as received. Multiple attempts to increase the crystallinity did not produce highly crystalline material. Weakly crystalline solids are frequently encountered in discovery when the solid form of an API is not optimized.

Both fedratinib free base and the dihydrochloride monohydrate salt were solubilized in the 20 % HP-β-CD vehicle for intravenous and oral dosing.

While the dihydrochloride monohydrate salt could not be formulated as a suspension due to very rapid and complete dissolution in the suspension vehicle, suspending the free base in the 0.5 % Methocel F4 M Premium vehicle resulted in a suspension with a dissolved fraction of 3.25 ± 0.60 % of the dose. The D90 for the concentrated stock suspension was measured to be 4.23 μm prior to diluting with vehicle and dosing. XRPD analysis of the suspended solid (Supplementary Materials) indicates that there was no form change upon stirring.

Pharmacokinetic studies in rats

Following intravenous administration of fedratinib at 1 mg/kg, the systemic CL was either 92.3 ± 18.0 mL/min/kg or 102.3 ± 0.6 mL/min/kg when the formulation was prepared using the free base or the dihydrochloride monohydrate salt, respectively. One animal in the free base group had a measurable 24 h concentration, whereas in all other rats, concentrations were only measurable up to 8 h post-dosing. In the literature, the systemic CL was reported to range between 42.0 and 45.7 mL/min/kg at a dose of 5 mg/kg in fasted or fed Sprague Dawley rats, respectively.[32]

Since the CLs exceed the estimated hepatic blood flow ($Q_{liver} \approx$ 70 mL/min/kg), this potentially suggests that pathways beyond hepatic metabolism are involved.

In line with this observation, oral BA after administration of fedratinib at 5 mg/kg was very low: 4.9 ± 0.9 % (oral solution, free base), 6.7 ± 1.3 % (oral suspension, free base), and 5.8 ± 0.6 % (oral solution, dihydrochloride monohydrate salt). Due to the systemic CL being much greater than liver blood flow, no conclusions can be made with confidence about the *in vivo* performance of the solution or suspension formulations.

Comparison to rDCS analysis

Previous rDCS analysis indicated a low developability risk for oral formulations of fedratinib in the dose range of 5 to 50 mg.[11] These doses fell into either rDCS class I or III (using solubility data generated with partially amorphous API), depending on the degree of P-gp saturation *in vivo* (since fedratinib is a P-gp substrate).[33] The 500 mg dose fell close to the predicted solubility limited absorbable dose (SLAD, *i.e.*, the class IIa/IIb boundary) in humans, indicating an increased developability risk. However, considering biorelevant *in vitro* supersaturation/precipitation analysis, classification of the 500 mg dose shifted into rDCS class I (assuming stable supersaturation in the intestine) or into class IIa (assuming precipitation as fully amorphous fedratinib). This suggests a stratified developability risk for high-dose fedratinib, depending on the relevance of *in vivo* supersaturation.[34] These risks may be mitigated with particle size reduction and/or inclusion of precipitation inhibitors in the formulation.

By contrast, profiling using the rat model did not provide useful practical guidance regarding formulation strategy selection for fedratinib.

Voxelotor

Formulation development & characterization

Voxelotor, a sickle hemoglobin polymerization inhibitor, is an ampholyte (pK_{a1} (basic) = 2.6 and pK_{a2} (acidic) = 8.3), exhibiting poor aqueous solubility (0.032 − 0.051 mg/mL).[35,36] Complete solubilization of voxelotor was achieved in the 20 % HP-β-CD vehicle, producing a solution formulation suitable for intravenous and oral dosing in rats.

When suspended in the 0.5 % Methocel F4 M Premium vehicle, approximately 5 % of the voxelotor dose was pre-dissolved. XRPD analysis of the suspended solid (Supplementary Materials) indicates that there was no form change upon stirring. The D90 for the stock suspension was measured to be 17.6 μm prior to diluting with vehicle and dosing.

Pharmacokinetic studies in rats

The PK analysis after intravenous administration of voxelotor at 1 mg/kg resulted in low systemic CL (1.93 ± 0.14 mL/min/kg), consistent with literature values in Sprague Dawley rats (1.80 mL/min/kg).[36] Considering the CL and estimated Q_{liver}, an absolute BA_{EST} of 97.0 ± 0.3 % is obtained, indicating minimal hepatic first-pass metabolism.

In both oral groups (5 mg/kg), voxelotor was absorbed with a mean t_{max} of 3.00 ± 1.73 h. In each group, one rat exhibited C_{max} within 1 h, while in the remaining rats C_{max} occurred after 4 h. It is worth noting that the variability observed in the oral suspension arm was substantially higher than in the oral solution arm. During the absorption phase, plasma curves displayed a tendency for a primary peak after 1 h, followed by a secondary peak 4 h post-dosing. Beyond the 4 h time point, plasma concentrations consistently declined. Potential reasons for the two-peak profile encompass precipitation and re-dissolution of the API in the gastrointestinal tract, or enterohepatic circulation (with re-absorption). While enterohepatic circulation of voxelotor is not specifically reported in rats, a large fraction of a 2 mg/kg i.v. dose (84.2 %) has been reported to be eliminated via bile, mostly as metabolites.[37]

The absolute oral BAs from the suspension and the solution were 61.2 ± 35.3 % and 55.5 ± 12.2 %, respectively. Considering the significant standard deviation in the suspension arm, and the less than two-fold difference in mean AUCs between suspension and solution, it appears that the formulations performed similarly *in vivo*.

Since the BA_{EST} was greater than the experimentally determined BAs for both the suspension and the solution, the underlying assumption $f_a \times f_g = 1$ is likely incorrect, suggesting $f_a \times f_g < 1$. This has been demonstrated in a portal vein study in Sprague Dawley rats, where $f_a \times f_g$ has been estimated to be 0.64.[37] These findings indicate an absorption limitation in the intestine, potentially due to low intestinal solubility of voxelotor, including potential precipitation of solubilized API from the solution formulation. This may explain why the suspension and solution performed similarly.

Since the observed intestinal absorption limitation is very likely caused by low solubility of voxelotor (the permeability of voxelotor is reported to be high),[11] a solubility-enhancing or enabling formulation approach seems most reasonable to address this limitation.

In line with our data, the absolute oral BA of voxelotor in fasted male Sprague Dawley rats, administered as a solution at 7.2 mg/kg,

was approximately 60 %.[36] When administered as a suspension with undisclosed particle size distribution, the absolute oral BA was 46 %.[36] Exposures from the suspension and solution were considered similar by the EMA.[36]

Consistent with our conclusion that oral absorption of voxelotor in rats is limited by solubility, administration of voxelotor using an enabling formulation (self-nanoemulsifying drug delivery system (SNEDDS)) resulted in a 1.7-fold increase in oral BA compared to an aqueous suspension at a 7.2 mg/kg dose.[38] While the translation of these findings to humans may appear challenging, it is important to note that the human therapeutic dose of voxelotor is extremely high (1500 mg).[39] Consequently, conventional formulation approaches seem less suitable when targeting complete absorption of high-dose voxelotor in humans.

Comparison to rDCS analysis

Developability assessment using the rDCS resulted in a relatively low risk for the development of conventional oral formulations with low-to-medium dose voxelotor in the range of 5 to 50 mg (these doses fell into rDCS class I or IIa, respectively).[11] However, the 500 mg dose was classified in rDCS class IIb, indicating a significant developability risk due to low intestinal solubility of the compound. Customized supersaturation analysis didn't lead to a significant risk reduction as immediate precipitation was observed in a two-stage dissolution test. Therefore, rDCS analysis recommended formulating high doses of voxelotor in an enabling formulation, such as amorphous solid dispersions (ASDs) or lipid-based formulations (LBFs), in order to overcome the solubility limitation.

Notably, the literature indicates that marketed oral formulations containing voxelotor are conventional oral dosage forms, even though clinical studies in humans demonstrated incomplete absorption.[11]

Lemborexant

Formulation development & characterization

The orexin receptor antagonist lemborexant is a weak base (pK_a = 2.18 ± 0.05)[10] with a reported water solubility of 0.0148 mg/mL in phosphate buffer pH 6.8.[40] The solution formulation of lemborexant for oral and intravenous dosing in rats was produced by solubilizing the API in the 20 % HP-β-CD vehicle.

In the suspension formulation, using the 0.5 % Methocel F4 M Premium vehicle, approximately 2 % of the dose was pre-dissolved. XRPD and DSC analysis of the suspended solid (Supplementary Materials) indicates that there was no form change upon stirring. The D90 for the stock suspension was measured to be 14.0 μm prior to diluting with vehicle and dosing.

Pharmacokinetic studies in rats

Plasma concentrations after intravenous administration of lemborexant at 1 mg/kg showed a rapid decline with high systemic CL (58.3 ± 11.7 mL/min/kg). In the literature, a CL of 47.8 ± 5.5 mL/min/kg has been reported in male rats.[41] With CL and Q_{liver}, a BA_{EST} of only 10.7 ± 15.4 % is obtained, suggesting extensive hepatic first-pass metabolism.

In line with the high systemic CL and the low BA_{EST}, the absolute oral BA of lemborexant was exceptionally low: 1.3 ± 0.6 % from suspension, and 0.8 ± 0.2 % from solution both dosed at 5 mg/kg.

Consistent with our results, low oral BA of lemborexant has also been reported in the literature with only 2.5 % following administration at a 10 mg/kg dose (undescribed formulation).[41] The findings of a mass balance study at a 10 mg/kg dose suggested that absorption of orally administered [^{14}C]lemborexant occurs rapidly and completely in rats.[42] However, the associated radioactivity was excreted very rapidly via biliary excretion through feces.[42]

Because of the considerable variability of the CL in rats and the minimal oral BA of lemborexant (likely due to a very high first-pass metabolism), there is only a limited capacity for the standard suspension vs. solution approach to detect formulation differences. Although the exposures from suspension and solution differed by less than two-fold, the outcome of the *in vivo* risk profiling is inconclusive, requiring additional *in vivo* studies for further understanding.

Comparison to rDCS analysis

Previous rDCS analysis resulted in a dose-dependent developability risk classification for oral formulations of lemborexant: rDCS class $I_{5\ mg}$, IIa/$b_{50\ mg}$ and $IIb_{500\ mg}$.[11] For low doses (*e.g.*, 5 mg), rDCS analysis indicated a low developability risk, recommending a conventional formulation approach. However, customized rDCS dissolution analysis indicated the need to enhance the dissolution rate of lemborexant to ensure complete absorption, *e.g.*, through particle size reduction to a target API particle radius (r_{target}) $\lesssim$ 20 μm.

While the 50 mg dose fell close to the intestinal SLAD, the 500 mg dose was assigned to class IIb. Supersaturation analysis indicated that the risks for the 50 mg dose may be mitigated through contributions from gastric dissolution, resulting in a recommendation for particle size reduction at this dose level. On the other hand, low intestinal solubility was predicted to limit oral absorption at the 500 mg dose. Since supersaturation was not predicted to mitigate the risk of incomplete absorption, a recommendation for developing an enabling formulation resulted.

By contrast, as discussed for fedratinib, the risk profiling using the rat model didn't provide practical guidance regarding formulation strategy selection for lemborexant in this study.

Istradefylline

Formulation development & characterization

Istradefylline, an adenosine receptor antagonist, is a weak base with a reported pK_a of 0.78 and an aqueous solubility of 0.0006 mg/mL.[43] Due to the poor aqueous solubility in the standard formulations, the development of a suitable solution formulation for intravenous and oral dosing in rats required the use of a non-traditional formulation approach. Through an iterative process, a solution formulation at a reduced concentration of 0.25 mg/mL was identified in a cosolvent mixture containing PEG400/water/NMP.

In the suspension formulation (0.5 % Methocel F4 M Premium vehicle), approximately 0.5 % of the dose was pre-dissolved. The D90 for the stock suspension was measured to be 14.3 μm prior to diluting with vehicle and dosing. XRPD analysis of the suspended solid (Supplementary Materials) indicates that there was a form change upon stirring.

Pharmacokinetic studies in rats

Intravenous administration of istradefylline at 0.25 mg/kg resulted in a systemic CL of 31.0 ± 4.3 mL/min/kg, while the literature reports a CL of 17.5 mL/min/kg after administration of a 0.3 mg/kg dose.[44] The estimated ER_h (44.7 ± 4.5 %) predicts a significant hepatic first-pass effect, suggesting an absolute BA_{EST} of 55.3 ± 4.5 %.

After oral administration of suspension and solution formulations at 1.25 mg/kg, the absolute oral BAs were 65.6 ± 5.4 % and 59.9 ± 5.5 %, respectively, indicating comparable exposure driven by hepatic first-pass metabolism. This is in line with previous reported literature values of 60.8 % from a 1.5 mg/kg dose (undescribed formulation).[45] Given the reduced doses in our investigation due to poor solubility in the standard formulation vehicles, the developability risk should be viewed as higher than indicated by the *in vivo* studies, particularly if the human dose is high.

Comparison to rDCS analysis

Analysis by rDCS indicated that istradefylline has the highest biopharmaceutical risk for oral formulation development among the compounds studied in this investigation.[11] At low doses (*e.g.*, 5 mg), istradefylline fell into rDCS class IIa, with customized dissolution analysis suggesting micronization of the API as a suitable formulation approach. Medium (*e.g.*, 50 mg) and high doses (*e.g.*, 500 mg) were categorized in rDCS class IIb, indicating that solubility would limit oral absorption of istradefylline. Thus, an enabling formulation should be considered particularly for high istradefylline doses.

Risk analysis by rDCS vs. pharmacokinetic studies in rats

Results of the suspension vs. solution exposure comparisons along with the rDCS analyses of the six APIs are summarized in Table 5.

Disadvantages of biopharmaceutical profiling using the rat model

This investigation highlights various disadvantages linked to rat-based biopharmaceutical profiling of APIs in discovery. The development of a preclinical formulation can be challenging when limited by solubility in standard vehicles as exemplified by istradefylline. The use of non-traditional excipients may introduce uncertainties into the *in vivo* study read-outs.

Generally, systemic exposure after oral administration is impacted by species-specific factors *in vivo*, including intestinal solubility, dissolution, luminal stability, permeation across the intestinal epithelium as well as gut and hepatic metabolism. Separating the contribution of solubility/dissolution, permeability and metabolic stability to oral BA is complex, requiring in-depth *in vitro* and *in vivo* studies in preclinical species. Additionally, a lack of predictive correlation between oral BA in classical preclinical animal models (whether in mice, rats, dogs or non-human primates) and humans has been well documented.[46]

In this investigation, high hepatic CL (fedratinib and lemborexant) or nonlinear PK due to saturation of metabolism (voriconazole) were encountered, complicating the readouts for biopharmaceutical risk assessment *in vivo*.

In early discovery phases the most stable crystalline form may not be available or may not have been identified yet. This means that any such *in vivo* based biopharmaceutical risk assessment would have to be repeated in rats and/or possibly higher species once the development form is identified. The repeat of these studies would, however, result in increased costs and potentially longer development timelines. Another factor that complicates the scenario and cannot be overlooked is the lack of an early reliable predicted human dose.

Profiling in preclinical species cannot currently be completely avoided since the *in vivo* studies are also used to assess first-pass effect, understand metabolic fate, characterize and predict preclinical drug exposure-pharmacology response relationships, prepare for toxicology studies, and estimate safety margins of new molecular entities. However, the results from such *in vivo* studies may not translate well into humans, as exemplified by the significant first pass effect observed with acetaminophen in rats but not observed in published human data.

Advantages of the rDCS approach for biopharmaceutical profiling

The rDCS provides several advantages in early-stage developability assessment, effectively addressing above-mentioned challenges associated with animal-based methods by:

Table 5
Comparison of the results from the pharmacokinetic studies in rats with rDCS analysis. The therapeutic doses are indicated in **boldface.** The results and conclusions of the rDCS analyses were summarized from previous work. .[11]

API	Pharmacokinetic studies in rats				rDCS analysis			
	Rat oral dose [mg/kg]	Formulation development		Limitation for oral BA (f_a x f_g x f_h)	Human dose [mg]	rDCS Class	Customized t ests	Limitation for oral absorption (f_a)
Acetaminophen	5	Suspension	☒[a]	Hepatic metabolism	5	I	Not triggered	–
					50	I		
		Solution	☑		**500**	I		
					1000	I		
Voriconazole	1 and 5	Suspension	☑	Hepatic metabolism (at lower doses)	5	I	Not triggered	–
					50	I		
		Solution	☑		**200/400**	I		
					500	IIa	Dissolution rate	
Fedratinib	5	Suspension	☑[b] ☒[a,c]	Inconclusive (metabolism strongly limiting oral BA)	5	I or III	Not triggered	(Permeability[d])
					50	I or III		
		Solution	☑		**400**	I, IIa or IIb	Supersaturation	(Solubility[e])
					500	I, IIa or IIb		
Voxelotor	5	Suspension	☑	Intestinal dissolution and/or solubility	5	I	Dissolution rate	Particle size
					50	I/IIa		
		Solution	☑		500	IIb	Supersaturation	Solubility
					1500	IIb		
Lemborexant	5	Suspension	☑	Inconclusive (metabolism strongly limiting oral BA)	**5**	I	Dissolution rate	Particle size
					10	I		
		Solution	☑		50	IIa/IIb		
					500	IIb	Supersaturation	Solubility
Istradefylline	1.25	Suspension	☑	Hepatic metabolism and potentially solubility[f]	5	IIa	Dissolution rate	Particle size
					20/40	IIb		Particle size & Solubility
		Solution	☑[f]		50	IIb		
					500	IIb	–	Solubility

☑ Development of a suitable preclinical formulation was feasible. ☒ Development of a suitable preclinical formulation was not feasible.
[a] Complete dissolution of the dose in the suspension vehicle.
[b] Fedratinib free base.
[c] Fedratinib dihydrochloride monohydrate.
[d] If the API concentration is too low to saturate P-gp *in vivo*.
[e] If intestinal precipitation is relevant *in vivo*.
[f] A solution formulation could only be obtained when the API concentration was reduced, and an additional co-solvent was included.

(i) Considering human gastrointestinal physiology with relevant cut-offs for human BA,
(ii) Separately assessing the key drivers for intestinal drug absorption: human small intestinal solubility and permeability (standard investigations), as well as small intestinal dissolution rate and supersaturation (customized investigations),
(iii) Allowing for easy adjustment of the dose for risk classification, with a standard dose range of 5, 50, and 500 mg when a reliable predicted human dose is not yet available,
(iv) Supporting development efforts on a shorter turnaround time as new and more stable polymorphs are identified,
(v) Identifying biopharmaceutical risks related to the pure API, with the option to incorporate specific excipient/formulation effects into the risk classification,[34]
(vi) Providing easy interpretation and visual presentation of the results,
(vii) Providing specific guidance on formulation efforts necessary to achieve complete absorption,
(viii) Allowing for high throughput in API screening.

The results of the rDCS analyses for the six compounds investigated in this study were found to align well with literature information. While the rDCS provides an ethical framework adhering to the principles intended to replace, reduce and refine animal experimentation (the so-called "3R" principles) in pharmaceutical research and development,[47,48] it also carries significant practical advantages in terms of time and cost, setting the stage for streamlined drug development.

Comparison of rDCS and rat suspension vs. solution profiling for early developability assessment

This investigation is the first head-to-head comparison of rDCS and rat suspension vs. solution profiling for developability assessment and formulation selection across a diverse set of APIs.

Initial developability assessment and formulation selection based only on rat suspension vs. solution exposure may lead to suboptimal first in human (FiH) formulation selection. The oral BA differences observed between suspension and solution formulations for all six APIs do not exceed the commonly used two-fold threshold, which is often employed as a signal to pursue conventional formulation approaches. This conclusion deviates from literature data, particularly for voxelotor and istradefylline. The *in vivo* comparison is further complicated by extensive first-pass metabolism.

Comparison of these findings with rDCS analysis and data from the literature on marketed formulations and their pharmacokinetic behavior suggests that the rDCS approach offers a more reliable and predictive tool for formulation selection.

Conclusions

In this work rats were utilized for early biopharmaceutical risk assessment and the results were compared to rDCS analysis.

The oral suspension vs. solution exposure comparison did not indicate substantial (*e.g.*, ≥ two-fold) differences for any of the six APIs used in this investigation. The results for acetaminophen, voriconazole, fedratinib, lemborexant and istradefylline indicated oral BA in rats is limited by first-pass metabolism. For voxelotor, the rat studies suggested that dissolution and/or solubility were limiting oral absorption. The complexity observed with the model compounds in this investigation highlighted the disadvantages of utilizing the rat for biopharmaceutical risk assessment in discovery. The lack of the most stable crystalline form and reliable human dose prediction further complicate the scenario.

In contrast, rDCS analysis provided a highly differentiated biopharmaceutical risk assessment for the six APIs, which has been shown to correlate well with their clinical formulation strategy. As the rDCS presents an animal-free approach to biopharmaceutical profiling, this investigation holds particular value within the context of promoting ethical frameworks such as the 3R principles, aiming to replace, reduce, and refine animal experimentation in pharmaceutical research and development.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.xphs.2024.12.021.

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