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ORIGINAL ARTICLE

Comparison of *In Vitro* and *In Vivo* Dissolution of Norvir® Oral Powder: *In Vivo* Relevance of a too Rapid *In Vitro* Dissolution Test

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ABSTRACT

Objectives: Norvir® oral powder [ritonavir (RTV)] employs polyvinylpyrrolidone/vinyl acetate as the polymer to formulate an amorphous solid dispersion. Its oral absolute bioavailability is 70% in the fasted state, and it has negative food effects. The aim of this study was to perform in vitro dissolution of Norvir® powder and Wagner-Nelson deconvolution of *in vivo* data under fasted, moderate fat, and high fat conditions in order to elucidate the relevance of *in vitro* dissolution testing.

Materials and Methods: *In vitro* dissolution of Norvir® oral powder was conducted, and the human pharmacokinetic data of Norvir® powder were obtained from literature, under fasted, moderate fat, and high fat conditions. Wagner-Nelson deconvolutions were performed. The absolute fraction absorbed (F_a) profiles were compared to the *in vitro* dissolution (F_d) profiles. Levy-Polli plot analysis was also conducted. For each pharmacokinetic condition, a scale factor was estimated to approximate the extent to which *in vitro* dissolution needed to be slowed down to mimic *in vivo* dissolution.

Results: Qualitatively, there was a large difference between *in vitro* and *in vivo* dissolution. *In vitro* dissolution showed 98% release in 5 minutes. Meanwhile, from Wagner-Nelson analysis, only 5.5% of the drug dissolved (and absorbed) *in vivo* in 5 min under fasted conditions. It was not until 2 hr that 49% of the RTV dose dissolved (and was absorbed) *in vivo*. *In vivo*, moderate fat and high fat conditions were even slower in producing a certain effect. The Levy-Polli plot exhibited a "reverse-L" profile. It was concluded that such rapid *in vitro* dissolution did not mimic the *in vivo* dissolution of RTV. *In vitro* dissolution needed to be slowed by 100-fold for fasting.

Conclusion: Biopharmaceutic consideration of *in vitro* dissolution, *in vivo* pharmacokinetics, and deconvolution analysis indicated that *in vitro* dissolution was "too rapid" to adequately mimic *in vivo* dissolution. Findings suggest greater inspection of *in vitro* methods for poorly water-soluble drugs, especially those drugs where *in vivo* absorption is expected to be rate-limited by dissolution.

Keywords: IVIVC, Norvir® powder, dissolution, absorption, Wagner-Nelson

INTRODUCTION

In vitro-in vivo correlation (IVIVC) has various definitions.[1,2] We believe this reflects the frequent historical effort to relate *in vitro* dissolution with *in vivo* product performance, such that among the many potential uses of *in vitro* dissolution testing, one is to estimate or mimic *in vivo* dissolution. For example, "biorelevant media" are designed to be compositionally similar to *in vivo* gastrointestinal fluids, with the potential to then kinetically mimic *in vivo* dissolution. Interestingly, at least for immediate release (IR) oral solid dosage forms, it is uncommon to address "Is the *in vitro* dissolution profile mimicking the *in vivo* dissolution profile?". The question was investigated in this study using Norvir® oral powder. Containing 100 mg ritonavir (RTV) per packet, Norvir® oral powder was

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approved in 1996 in the United States and the European Union. RTV is a prototypical poorly water-soluble drug, requiring formulation as an amorphous solid dispersion (ASD).[3,4] The absolute oral bioavailability of each Norvir® oral powder and Norvir® tablet is about 70%.[5] Norvir® oral powder and Norvir® tablets use the same ASD intermediate, which involves hot melt extrusion of RTV and polyvinylpyrrolidone/vinyl acetate (PVPVA) as the polymer. Norvir® tablet and Norvir® oral powder are bioequivalent.[5] Although the tablet and oral powder are bioequivalent in C_{max} and area under the curve (AUC), they are not pharmaceutically equivalent and hence not therapeutically equivalent. The USP compendial dissolution method of Norvir® tablet employs 60 mM polyoxyethylene 10 lauryl ether (PE), a relatively high concentration of the surfactant. RTV drug substance solubility in 60 mM PE is 198 µg/mL, while it is only 2.338 µg/mL in media without 60 mM PE.[6] Since RTV is poorly water-soluble and its oral bioavailability is incomplete, we anticipated that RTN absorption is dissolution rate-limited.

In this study, the question pursued was "Is *in vitro* dissolution profile mimicking the *in vivo* dissolution profile?". Human *in vivo* data from Salem et al.[7] were used. Salem et al.[7] studied the pharmacokinetics of Norvir® oral powder under fasted, moderate fat, and high fat conditions. In our analysis here, absolute oral bioavailability was 70% for the fasting state, 54% for the moderate fat, and 47.8% for the high fat. The absolute oral bioavailability of each Norvir® oral powder and Norvir® tablets is about 70%.[5] From the Norvir® oral powder package insert, its oral bioavailability with a moderate fat meal is reduced 23% compared to fasted,[8] such that an absolute oral bioavailability was computed here to be 54%. Finally, according to Salem et al.,[7] the AUC ratio of moderate fat versus high fat is 1.13, such that an absolute oral bioavailability under high fat was computed here to be 47.8%. The aim of the study was to compare the *in vitro* dissolution profile to the *in vivo* dissolution profile of Norvir oral powder in fasted, moderate-fat fat and high-fat conditions.

MATERIALS AND METHODS

Materials

Packets of Norvir® oral powder (100 mg RTV per packet) (AbbVie; North Chicago, IL, USA) were commercially obtained. PE was purchased from Sigma Aldrich (St. Louis, MO, USA). The RTV active ingredient was from ChemShuttle (Blue Current Inc., Hayward, California). Solvents were of analytical grade and obtained from Fischer Scientific (Fischer Scientific; Hampton, NH) and Sigma-Aldrich (Sigma-Aldrich; St. Louis, MO).

In vitro dissolution profile of Norvir® oral powder

Dissolution testing was performed on Norvir® oral powder (containing 100 mg of RTV) in 900 mL of PE medium (50 mM maleic acid buffer with 60 mM polyoxyethylene 10 lauryl ether; pH 5.8) at 37 °C using 100 rpm with the USP-II apparatus (SR8PLUS, Hanson Research, Chatsworth, CA). Dissolution of a packet per vessel was performed in triplicate. 60 mM PE was used, since it is the USP compendial method for Norvir® tablets. A 2 mL sample was taken (at 0, 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, and 360 min), and replaced with 2 mL of fresh PE medium at each time point. Then, samples were filtered through a 0.45 µm membrane filter and quantified using high-performance liquid chromatography (HPLC). The concentration of RTV was determined using an HPLC method.[6] Sample analysis was conducted with a Waters 2489 HPLC system (Waters Corporation, Milford, MA) equipped with an ultraviolet-visible detector (240 nm wavelength). An isocratic mobile phase comprising 47% of acetonitrile and 53% of 0.05 M phosphoric acid was employed, with an injection volume of 25 µL and a flow rate of 1 mL/min. Separation was achieved using a 4.6×150 mm Zorbax C18 column with a 5-µm particle size. RTV exhibited a retention time of 9–10 min, and the total run time was 13 min. A calibration curve with RTV concentrations of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, and 0.098 µg/mL was generated in triplicate for each analysis, yielding an R^2 value of 0.9999.

Application of mathematical models for drug dissolution kinetics

In vitro dissolution profiles were analyzed using regression analysis with five models: zero-order, first-order, Higuchi, Hixson-Crowell, and Korsmeyer-Peppas.[9-11] Norvir® oral powder dissolution was subjected to model fitting using DDSolver®. The correlation coefficient value (R^2) of each fit was obtained.[12] The zero-order equation is % dissolved = k_o*t, where k_o is the dissolution rate constant, and t is time. The first order equation is % dissolved = 100*(1 - e^{-k1t}), where k_1 is the first order dissolution rate constant.

Higuchi's equation is % dissolved = k_H *$t^{1/2}$}, where k_H is the Higuchi dissolution rate constant.

The Hixson-Crowell equation for percentage dissolved is: % dissolved = 100 * [1 - (1 – (k_{HC} * t / 4.6416))^3], where k_{HC} is the Hixson-Crowell release rate constant. The Korsmeyer-Peppas equation is % dissolved = k_{KP} * t^n, where k_{KP} is the Korsmeyer-Peppas release rate constant, and n is the release exponent.[13-16]

Wagner-Nelson deconvolution: in vivo dissolution profiles of Norvir® oral powder

In vivo human data of Norvir® oral powder under the fasted, moderate fat, and high fat conditions were extracted from the literature.[7] The data were digitized from the graphs using a plot digitizer. The deconvolution method was utilized to obtain *in vivo* drug absorption from the fasted, moderate-fat, and high-fat plasma concentration time profiles. Assuming *in vivo* drug absorption was limited by the *in vivo* dissolution rate, deconvolution would yield that the *in vivo* drug absorption profile is also the *in vivo* dissolution profile. The Wagner-Nelson deconvolution has been applied to RTV oral pharmacokinetic profiles previously[5], and was selected here as a deconvolution method, in part because it does not require intravenous data. The Wagner-Nelson equation is:

$$F_a = (C_p+(K_{el} * AUC_{0-t})) / (K_{el} *AUC_{0-\infty}) \quad \text{Equation 1}$$

Where F_a is the fraction of the dose absorbed at time t, C_p is the plasma concentration (ng/mL) of RTV at time t, K_{el} (1/hr) is the elimination rate constant, and AUC (ng*hr/mL) is the area

under the curve. K_{el} was calculated from the least-squares fitted terminal log-linear portion of the plasma concentration–time profile. K_{el} was calculated here to be 0.223/h, 0.354/h, and 0.246/h under fasted, moderate fat, and high fat conditions, respectively. AUC_{0-t} is the integration of concentration of RTV from time "0" to "t" (time of last quantifiable drug level), *i.e.* the AUC from time "0" to "t". $AUC_{0-\infty}$ is the integration of concentration of RTV from time '0' to '∞', *i.e.* area under curve from time '0' to 'infinity'.[17] Since it concerns the relative amount of drug absorbed at time t versus the final amount of drug that was absorbed, Wagner-Nelson profile analysis always yields 100% as a terminal value (*i.e.,* F_a = 1, or 100% of the amount that was absorbed). The percent absolute absorbed profile was also calculated as the percent absorbed profile (*i.e.*, Wagner-Nelson profile) and normalized by the absolute oral bioavailability, which is 70% for fasted, 54% for moderate fat, and 47.8% for high fat (see Introduction). The *in vitro* dissolution profile of Norvir® oral powder (from *in vitro* testing using PE media) was compared to *in vivo* dissolution profiles of Norvir® oral powder under fasted, moderate fat, and high fat conditions (from Wagner-Nelson analysis of pharmacokinetic profiles, normalized for extent of absorption).

Levy-Polli plot analysis

Levy-Polli plots involve the fraction of drug absorbed (F_a) versus the fraction of drug dissolved (F_d), and help to evaluate the relative contributions of drug dissolution and permeation to overall absorption kinetics.[18-21] Comparisons of F_a-F_d trajectory plots from *in vivo* PK data and from *in vitro* PE dissolution are typically conducted to assess underpinning kinetics of oral drug bioavailability, assuming the *in vitro* test profile mimics the *in vivo* dissolution profile. However, here, in light of the biopharmaceutical properties of RTV and Norvir® oral powder, it was assumed that *in vivo* drug absorption was limited by the dissolution rate, such that analysis focused on the ability of *in vitro* dissolution testing to mimic *in vivo* dissolution.

Scale factor analysis: polli equation and first-order equation

As the results show, the *in vitro* dissolution profile was much faster than the *in vivo* dissolution profile. Hence, to gauge the rate at which *in vitro* dissolution would need to be slowed to approximate *in vivo* dissolution, a scale factor was estimated. IVIVC analysis allows researchers to determine a scaling factor (SFs).[22] Two modeling approaches were taken to estimate an SF: the Polli equation and the first-order equation. For the Polli equation approach, the *in vitro* dissolution profile of Norvir® oral powder was fit to the Polli dissolution equation (Equation 2) to estimate the single fitted parameter *in vitro* k_d:[23]

$$\% \text{ dissolved} = 100 * [1 - ((M_0 - C_s*V)/(M_0 - C_s*V*e^{-kd((M0-Cs*V)/V)*t}))]$$

Equation 2

Where M_0 is the initial mass of drug in the dosage form, so it is the drug dose (100 mg), C_s is RTV solubility in PE media (0.198 mg/mL[6]), V is dissolution volume (900 mL), t is time (min), and k_d is the dissolution rate coefficient (mL/mg per min). The Polli equation is a simple, one-parameter (i.e., k_d) equation, and only requires regression. The equation can accommodate both sink and non-sink dissolution conditions. Equation 2 was fitted to % dissolved versus time data via Solver (Microsoft, Redmond, WA; version 2206) to estimate k_d.[23] Solver is a free Microsoft Excel add-in program from Microsoft and is intrinsic to Excel, although it may need to be initially loaded into Excel.[24] The initial estimate of k_d was 0.1 mL/mg per min. *In vivo* k_d values were also determined from the *in vivo* dissolution profiles of Norvir® oral powder for fasted, moderate fat, and high fat conditions (from Wagner-Nelson analysis of pharmacokinetic profiles). For all regressions, R^2 values of the fits were calculated.[10]

To summarize the rate at which *in vitro* dissolution would need to be slowed to approximate *in vivo* dissolution, an SF was estimated for each pharmacokinetic condition. For each fasted, moderate fat, and high fat condition, the SF value was taken to be the ratio of the *in vivo* k_d (from Wagner-Nelson analysis of pharmacokinetic profiles) over the *in vivo* k_d (from *in vitro* dissolution).[24] Additionally, the same analysis using the first-order equation was conducted to estimate an SF to compare *in vitro* and *in vivo* dissolution. As denoted above, the first-order equation [% dissolved = $100*(1- e^{-k1t})$] was used. Separately, it was applied to the *in vitro* dissolution profile and to each Wagner-Nelson profile (normalized for extent of absorption, under fasted, moderate fat, and high fat conditions).

Data analysis

The collected data were analyzed using SPSS Version 16 (Systat Software Inc., CA, USA). A t-test was used to compare two groups. Results were given as mean ± standard error of the mean (n=3). To compare the drug *in vivo* dissolution profiles to *in vitro* dissolution profiles, the f_2 calculation was conducted.[25]

RESULTS

In vitro dissolution profile of Norvir® oral powder

Dissolution of Norvir® oral powder in maleic acid buffer containing 60 mM polyoxyethylene10 lauryl ether (PE) (pH 5.8) is plotted in Figure 1. About 98% of RTV was dissolved in 5 min., and reached 100% in 4 hr (*i.e.*, 100 mg/900 mL or 0.111 mg/mL). Indulkar et al.[26] also observed fast *in vitro* release of RTV with PVPVA. Dissolution was conducted for 6 hours, with no evidence of drug precipitation. Because dissolution modeling can impart insight into dissolution mechanisms, Norvir® oral powder dissolution was subjected to model fitting. Among five models, the first-order and Korsmeyer-Peppas models best described RTV dissolution (R^2=0.9997 and R^2=0.9976, respectively). In the Korsmeyer-Peppas model, the value of the release exponent n characterizes the dissolution mechanism. Here, n=0.04 (*i.e.*, n≤0.45), reflecting Fickian diffusion (Case I diffusional).[27,28]

In vivo dissolution profiles of Norvir® oral powder

Figure 1 plots and Table 1 list the absolute fraction absorbed (F_a) from Wagner-Nelson analysis of human *in vivo* pharmacokinetic data, normalized for absolute oral bioavailability. The *in vitro* dissolution profile was also plotted. Of note, Wagner-Nelson

profile analysis alone always yields 100% as a terminal value (*i.e.*, F_a = 1, or 100% of the amount that was absorbed). Meanwhile, Figure 1 shows the percent absolute absorbed profile, represented by the Wagner-Nelson profile, which is normalized absolute oral bioavailability: 70% for fasted, 54% for moderate fat, and 47.8% for high fat. F_a in Figure 1 is taken to be equal to the *in vivo* dissolution profile, since RTV absorption from Norvir® oral powder is assumed to be rate-limited by *in vivo* dissolution, and not *in vivo* drug intestinal permeation or physiology such as gastric emptying.

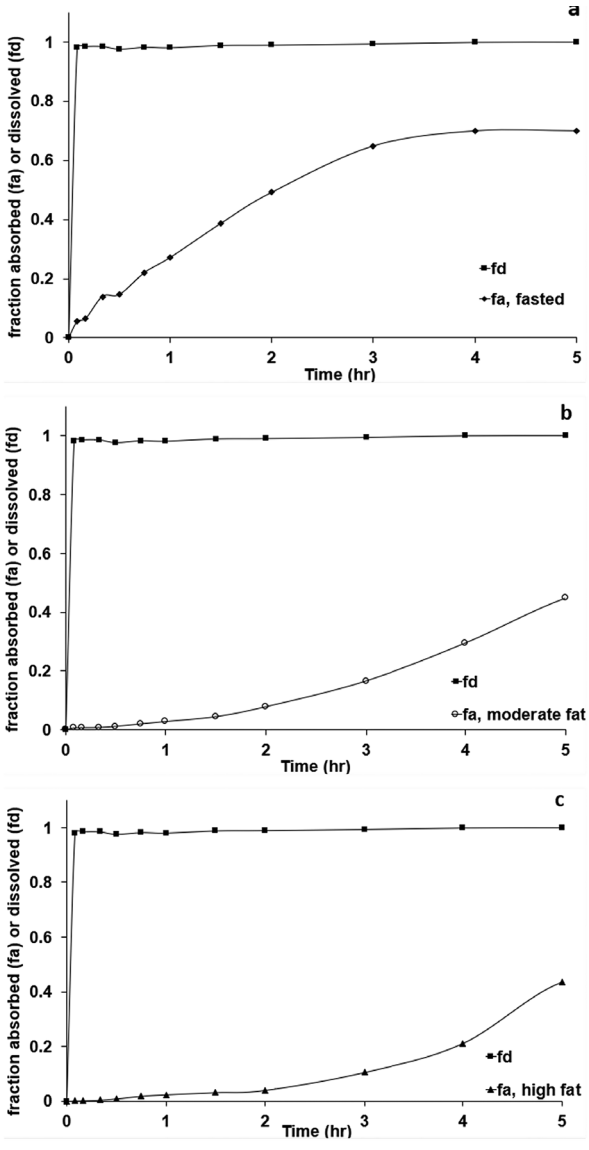

Figure 1. RTV fraction absolute absorbed (F_a) profiles from Wagner-Nelson analysis and fraction dissolved (F_d) from *in vitro* dissolution test.

*F_a was from Wagner-Nelson analysis of human *in vivo* pharmacokinetic data, under a) fasted, b) moderate fat, and c) high fat conditions.[7] F_a values are normalized for absolute oral bioavailability, which is 70% for the fasted state, 54% for a moderate-fat diet, and 47.8% for a high-fat diet. F_a is assumed to also be *in vivo* fraction dissolved, such that *in vitro* dissolution was much more rapid that *in vivo* dissolution under all conditions, including being "too rapid"

RTV: Ritonavir

In Figure 1, at 5 h, *in vitro* F_d was nearly 1 (*i.e.,* complete) while absolute F_a values were considerably lower, with 0.7 (Figure 1a for fasted), 0.447 (Figure 1b for moderate fat), and 0.436 (Figure 1c for high fat). Table 2 also lists F_d and F_a values. At 20 min, *in vitro* F_d was about 0.9998, while absolute F_a under fasted, moderate fat, and high fat conditions were only 0.140 (Figure 1a), 0.00737 (Figure 1b), and 0.00453 (Figure 1c), respectively. Pharmacokinetic inspection revealed a large difference between *in vitro* dissolution and *in vivo* dissolution. When comparing the fasted absorption profile, moderate fat absorption profile, and high fat absorption profile to the *in vitro* dissolution profile, f_2 was 8.34, 2.49, and 1.98. From Figure 1, F_a is assumed to also be the *in vivo* fraction dissolved, such that *in vitro* dissolution was much more rapid than *in vivo* dissolution under all conditions, including being "too rapid". The *in vitro* method contained a relatively high concentration of a pharmaceutical surfactant.

Levy-Polli plots of Norvir® oral powder and the implication of in vitro dissolution being "too rapid"

Levy-Polli plots are helpful to understand the relationship between F_a and F_d and to assess whether overall drug absorption is dissolution rate-limited, permeation rate-limited, or mixed dissolution/permeation rate-limited, assuming *in vitro* dissolution estimates *in vivo* dissolution. Figure 2 plots the relationship between F_d from *in vitro* dissolution in PE medium and *in vivo* absolute F_a under fasted (Figure 2a), moderate fat (Figure 2b), and high fat conditions (Figure 2c). Each Levy-Polli plot exhibited the reverse "L pattern" (Hockey-Stick trajectory) of Norvir® oral powder. While F_d values increased rapidly from 0 to over 0.9, *in vivo* F_a increased by only 0.055 for the fasted condition (Figure 2a), to 0.006 for moderate fat condition (Figure 2b), and to 0.001 for the high fat condition (Figure 2c). Also, at 5 hours, F_d was 0.99, while *in vivo* absolute F_a was 0.7 under the fasted state, 0.54 under the moderate fat, and 0.478 under the high fat conditions. Results point towards *in vitro* dissolution testing being "too rapid", at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution. Hence, analysis proceeded to identify an SF to estimate the extent to which *in vitro* dissolution was too fast.

All plots exhibit a "reverse L" shape profile, reflecting the very rapid *in vitro* dissolution profile and not a "straight line" profile that would be expected from a dissolution-rate-limited absorption scenario. Results point towards *in vitro* dissolution testing being "too rapid", at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution.

Scale factor analysis: polli equation and first-order equation

The Polli equation and the first-order equation were separately used to estimate a scale factor to summarize the degree that which the observed, rapid *in vitro* dissolution would need to be slowed, for the *in vitro* dissolution profile to mimic the *in vivo* dissolution profile. The Polli model was used to fit the *in vitro* dissolution (*e.g.,* Figure 1), as well as the *in vivo* dissolution profile (*e.g.,* each panel in Figure 1 for the three conditions). Fitting involved estimating the k_d value. For the fit to the *in vitro* dissolution profile in Figure 1, k_d was 7.15 mL/mg per min, a high value reflecting the rapid dissolution.

Table 1. Percent dissolved, percent absorbed and percent absolute absorbed values of RTV oral powder under fasted, moderate fat and high fat conditions

		Fasted		Moderate fat		High fat	
Time (min)	% dissolved	% absorbed	% absolute absorbed	% absorbed	% absolute absorbed	% absorbed	% absolute absorbed
0	0.0	0	0	0	0	0	0
5	98.1	7.8	5.5	1.1	0.6	0.3	0.1
10	98.5	9.1	6.4	1.2	0.7	0.4	0.2
20	98.5	19.9	13.9	1.4	0.7	0.9	0.5
30	97.6	20.8	14.6	2.1	1.1	1.9	0.9
45	98.2	31.5	22.1	3.7	2.0	3.9	1.9
60	98.1	38.7	27.1	5.1	2.8	5.0	2.4
90	98.8	55.2	38.7	8.2	4.4	6.7	3.2
120	99.0	70.2	49.2	14.5	7.8	8.4	4.0
180	99.4	92.7	64.9	30.6	16.5	22.1	10.6
240	99.8	100	70	54.5	29.5	44.2	21.1
360	100.0	100	70	100	54	100	47.8

*Dissolution was measured during *in vitro* testing. The percent absorbed profile was obtained from Wagner-Nelson analysis, yielding 100% as a terminal value (*i.e.,* 100% of the amount that was absorbed). The percent absolute absorbed refers to the percent absorbed profile normalized by absolute oral bioavailability, which is 70% for fasted, 54% for moderate fat, and 47.8% for high fat. RTV: Ritonavir

Table 2. Fitted Polli dissolution rate coefficient (k_d) and first order dissolution rate coefficient (k_1) values to *in vivo* fraction absolute absorbed profiles

In vivo dissolution	Fitted k_d (mL/mg per min) from the Polli equation	Scaling factor (unitless) from the Polli equation	Fitted k_1 (1/min) from the first-order equation	Scaling factor (unitless) from first-order equation
Fasted	0.0324	0.00453	0.009923	0.0127
Moderate fat	0.0078	0.00109	0.002968	0.00380
High fat	0.0058	0.000773	0.002586	0.00331

*Fraction absolute absorbed profiles are plotted in Figure 1 for fasted, moderate fat, and high fat conditions. Separate fits were performed for the Polli equation (k_d) and the first-order model (k_1). Separately, SFs were calculated for each Polli equation k_d and first-order k_1 for the *in vitro* fits of k_d and k_1 to be slowed down to match the *in vivo* fits of k_d and k_1. This *in vivo* analysis assumed dissolution-rate-limited absorption

Figure 3 compares fitted and observed profiles from *in vitro* dissolution, as well as the three *in vivo* conditions.

Fitted k_d values are shown in Table 2, along with fitted k_1 values. *In vivo* k_d values from fits to Figure 3 are listed in Table 2. K_d was 0.0324, 0.0078, and 0.0058 mL/mg per minute for *in vivo* F_a (*i.e., in vivo* dissolution) under fasted, moderate fat, and high fat conditions, respectively. Therefore, in comparing values, SFs were 0.00445, 0.001101, and 0.00842 for these conditions. Each scale factor was markedly less than one, indicating *in vitro* dissolution was many-fold "too rapid" (*e.g.,* about 200-fold for fasting condition), compared to *in vivo* dissolution.

A similar analysis based on fits to a first-order model provided comparable SFs and the same conclusion. k_1 was 0.782 min^{-1} for *in vitro* dissolution, indicating a notably high value reflecting rapid dissolution. *In vivo* k_1 was 0.009923, 0.002968, and 0.002586 min^{-1} for fasted, moderate fat, and high fat conditions, respectively. SF were 0.0127, 0.00380, and 0.00331 for these conditions. Again, each scale factor was markedly less than one, indicating *in vitro* dissolution was manyfold too rapid compared to *in vivo* dissolution. Regardless of whether the Polli equation or first-order model was used, the SF was always far less than unity (*i.e.*, less than 1), reflecting that the *in vivo* absolute absorption profile is much slower than the *in vitro* dissolution profile. Generally, SFs were about 0.01, reflecting that the *in vivo* drug absorption rate is 100-fold slower than *in vitro* dissolution. These SFs are qualitatively similar to the SF for a fast itraconazole ASD formulation, which was 0.0191.[24] Hence, there are ASDs with perhaps overly rapid *in vitro* dissolution testing, at least if *in vitro* dissolution is intended to mimic *in vivo* dissolution. Also, in Table 2, k_d values were about 2-4-fold larger than k_1 values. Correspondingly, the SF for k_1 was about 2-4-fold larger than the SF for k_d.

DISCUSSION

An alternative interpretation

An alternative interpretation is that the *in vitro* dissolution profile mimics the *in vivo* dissolution profile, such that *in vivo*

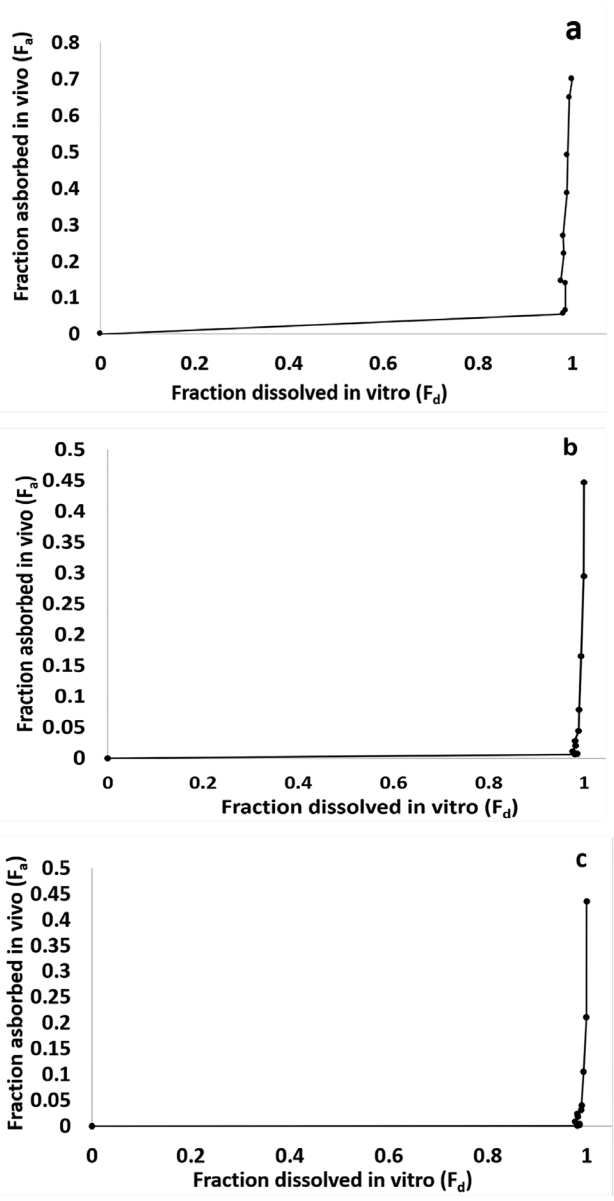

Figure 2. F_a vs. F_d relationships of Norvir® powder under a) fasted, b) moderate fat, and c) high fat conditions

dissolution is indeed nearly complete in 5-10 min, and that subsequent post-dissolution events (*e.g.*, drug permeation across the intestine) constitute the rate-limiting steps for overall RTN absorption. However, RTN has been reported to have a high intestinal permeability and be a Biopharmaceutics Classification System (BCS) Class 2 drug.[29] Of note, Karakucuk et al.[30] describe the challenges of measuring the permeability of the drug RTN with a very low solubility. Overall, however, we do accept an alternative interpretation that the very rapid *in vitro* RTN dissolution profile here mimics *in vivo* dissolution, and the incomplete and prolonged dissolution in the fasted state reflects low permeability. Some reports characterize RTN as BCS Class 2 or Class 4.[30-34] We believe the incomplete systemic availability of RTN (*i.e.*, 70%) could primarily reflect incomplete dissolution or partial (*e.g.,* 30%) first-pass metabolism. Incomplete absorption of a poorly soluble drug, can perhaps be preliminarily classed as BCS Class 4 (*i.e.,* a drug with low permeability), or it may simply reflect incomplete absorption of a Class 2 drug with very low solubility.

Comparison of scale factors

In a IVIVC study of itraconazole ASDs, a similar analysis was conducted. Itraconazole tablets were denoted to be Fast, Medium, and Slow dissolving, and this rank order was observed for both the *in vitro* dissolution and *in vivo*. However, *in vivo* dissolution for Fast, Medium, and Slow was only 0.0178 times, 0.213 times, and 0.217 times that of *in vitro* dissolution, respectively. That is, fast was about 50-fold slower *in vivo* than *in vitro*. Medium and Slow were each about 5-fold slower *in vivo* than *in vitro*.[24] Here, *in vitro* dissolution of Norvir® oral powder using a scale factor of 0.00453 based on k_d parameterization into a highly pharmaceutical surfactant was significantly more rapid, being about 200-fold faster than *in vivo* dissolution in the fasted state.

Evaluation of in vitro dissolution modeling to fit in vivo dissolution profiles

The Polli equation is a single-parameter dissolution equation, which means only the dissolution rate coefficient (k_d) is fitted in regressing the equation to the dissolution data, without the need for a fitted extent of dissolution parameter. The fitted k_d values were calculated to match those predicted by the Polli equation and the observed dissolution profiles. It was found to be 7.15 (mL/mg per min), which was a high value due to the IR profile. A similar fast release profile was observed in Itraconazole ASD, and k_d values were calculated as 30.12 (mL/mg per min).[24] Moreover, k_d values were calculated to fit the *in vitro* dissolution profiles to the *in vivo* absorption profiles. The units of k_d in Equation 2 were mL/mg per min, similar to those for the z-factor dissolution model.[23] After the comparison of the observed *in vitro* dissolution profiles of Norvir® powder with fitted *in vitro* dissolution profiles *via* the Polli equation, k_d values were calculated to align *in vitro* PE dissolution with *in vivo* dissolution in fasted, moderate fat, and high fat conditions. As shown in Table 2, the k_d values were calculated as 0.03, 0.008, and 0.006 mL/mg per min for fasted, moderate fat, and high fat conditions to match the dissolution profiles. While there was no significant difference (p=0.333>0.05) between the k_d values of the moderate fat and high fat conditions, the k_d value was lower than that of the fasted conditions. As perhaps expected, the dissolution rate coefficient, k_d was generally smaller when drug solubility (C_s) was larger.[23] It was confirmed by the literature.[35,36] Xu et al.[5] found that RTN solubility was 7.4±1.1 µg/mL in FaSSIF-V2 and 18.5±1.9 µg/mL in FeSSIF-V2. Kokott et al.[36] reported RTN solubility in FaSSIF was 5.4±0.6 µg/mL. Similar results were observed with the ketoconazole and itraconazole tablets. The k_d value of ibuprofen in FeSSIF-V2 (0.0780 mL/mg per min) was higher than ketoconazole in FaSSGF (0.0154 mL/mg per min)[23] due to the ibuprofen solubility in FeSSIF-V2 (1.76 mg/ mL) being lower than the solubility of ketoconazole in FaSSGF (11.2 mg/ mL).[37] Moreover, the reason for the lower k_d values of RTV in the fed state compared to the fasted state can be related to the

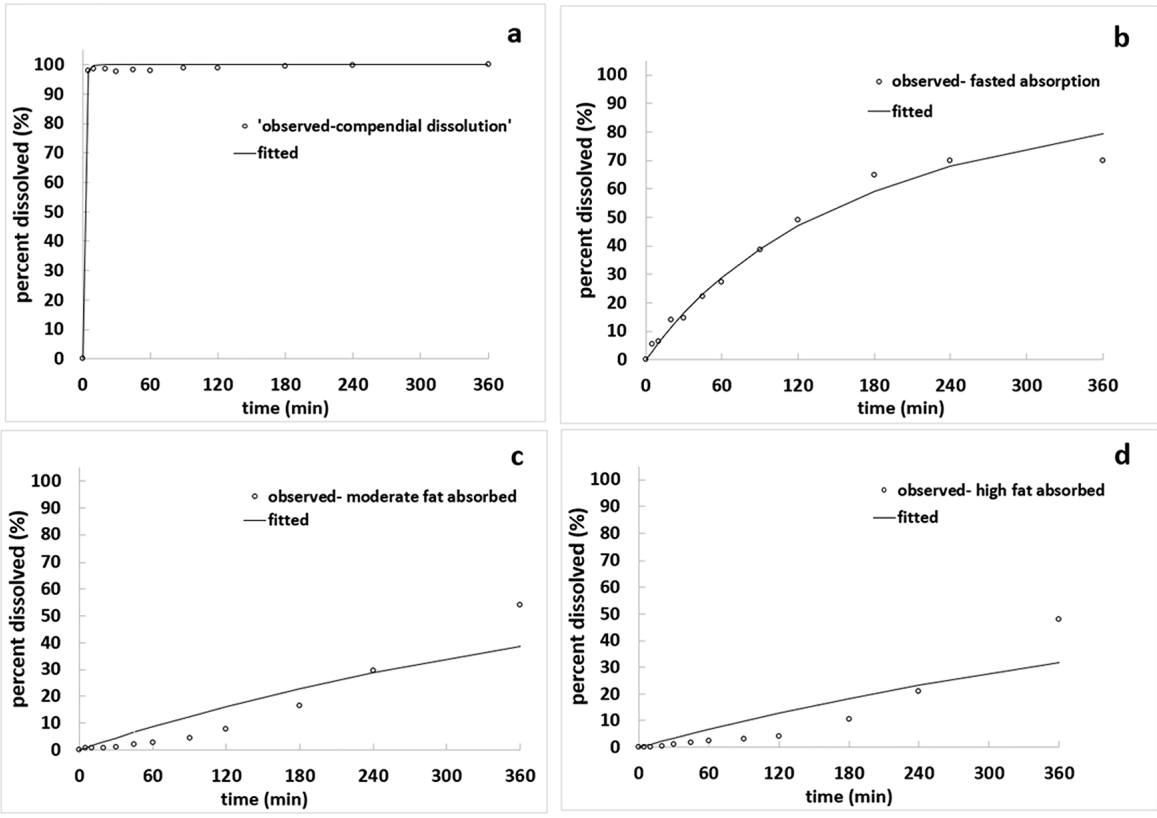

Figure 3. Fitted dissolution profiles *via* Polli equation and observed dissolution profiles for each food condition. Panels are *in vitro* dissolution in PE medium (a), and *in vivo* dissolution under fasted (b), moderate fat (c), and high fat (d) conditions

fact that the Fed state colloids are large and slowly diffusing relative to other biorelevant media, colloids.[38-40] In addition to the k_d values, S_f and first-order dissolution constants (k_1) were also calculated and given in Table 2. S_f is unitless and reflects a single algorithm for *in vitro* dissolution scaling. It is a multiplier with a value less than 1 that slows *in vivo* dissolution relative to *in vitro* dissolution. The k_d values from *in vivo* were 0.4% lower for the fasted condition, while they were 0.1% lower for the moderate fat and high fat conditions, compared to the k_d from the *in vitro* dissolution. These results were similar to the literature.[24] For example, *in vitro* dissolution of itraconazole was faster than its *in vivo* dissolution, similar to the results obtained here. Moreover, the k_1 values were calculated from the first-order dissolution equation, which is a common differential equation for fitting percent dissolved versus time profiles. Compared to the Polli equation, the first-order equation, [% dissolved = $100*(1 - e^{-k1t})$], has a limitation due to its solution not accommodating a solubility limit impact on the percentage dissolved. As it is shown in Table 2, the rank order of the k_1 values was similar to the k_d values. The k_1 values under the fasted state were higher than those under the fed states due to the presence of high solubility and slow diffusion of large colloids under the fed conditions.[23]

CONCLUSION

The present investigation performed *in vitro* dissolution of Norvir® oral powder and Wagner-Nelson deconvolution of *in vivo* data to elucidate the relevance of *in vitro* dissolution testing. Qualitatively, there was a large difference between *in vitro* dissolution and *in vivo* dissolution. *In vitro* dissolution showed 98% release in 5 min. Meanwhile, from Wagner-Nelson analysis, only 5.5% of the drug dissolved (and was absorbed) *in vivo* in 5 min under fasted conditions. 49% of the RTV dose dissolves (and is absorbed) *in vivo* after 2 hours. It was concluded that such rapid *in vitro* dissolution was not mimicking *in vivo* dissolution of this poorly water-soluble drug, as it has been reported to have high intestinal permeability. Rather, *in vitro* dissolution, which involved a high surfactant concentration, was "too rapid,", while *in vivo* dissolution was better estimated by Wagner-Nelson analysis. For each pharmacokinetic condition (*i.e.,* fast, moderate fat, and high fat), a scale factor was estimated to approximate the degree to which *in vitro* dissolution needed to be slowed to mimic *in vivo* dissolution. For fasting, *in vitro* dissolution needed to be slowed by about 100-fold. Findings suggest greater inspection of *in vitro* methods of poorly water-soluble drugs, or at least those drugs where *in vivo* absorption is expected to be rate-limited by *in vivo* dissolution.

Ethics

Ethics Committee Approval: Not required.

Informed Consent: Not required.

Footnotes

Authorship Contributions

Concept: A.N.O., J.E.P., Design: A.N.O., Data Collection or Processing: A.N.O., Analysis or Interpretation: A.N.O., J.E.P., Literature Search: A.N.O., J.E.P., Writing: A.N.O., J.E.P.

Conflict of Interest: The authors declare no conflicts of interest.

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