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Research paper

Designing biorelevant dissolution tests for lipid formulations: Case example – Lipid suspension of RZ-50

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Abstract

Biorelevant dissolution test methods for lipid formulations of RZ-50, an experimental Roche compound, were developed and compared with standard compendial methods in terms of their *in vivo* predictability. Release of RZ-50, a poorly soluble weakly acidic drug, from lipid suspensions filled in soft gelatin capsules was studied in compendial and biorelevant media using the USP Apparatus 2 (paddle method) and the USP Apparatus 3 (Bio-Dis method). Pharmacokinetic data were obtained in dogs after oral administration of a single 2.5 mg dose of RZ-50 soft gelatin capsules in the postprandial state. Level A IVIVC analysis and curve comparison of fraction drug dissolved vs. absorbed using the Weibull distribution were used to evaluate the *in vitro* methods in terms of their ability to fit the *in vivo* plasma profiles. Very low drug release was observed with the paddle method owing to poor dispersibility of the lipids in the dissolution media, whereas the Bio-Dis method hydrodynamics facilitated release of the drug by emulsifying the formulation in the medium. The best IVIVC was obtained using a dissolution medium representing fed gastric conditions in combination with the Bio-Dis method. Curve comparisons of the fraction drug absorbed and the fraction drug dissolved profiles based on Weibull distribution fits yielded similar results. The Bio-Dis/biorelevant *in vitro* method appears to be suitable for this type of lipid formulation.

Keywords: Dissolution; Lipid dosage forms; Paddle method; Bio-Dis method; Biorelevant; IVIVC; Curve comparisons

1. Introduction

It is recognized that the bioavailability of poorly soluble drugs can be markedly enhanced by meal intake and its related changes in gastrointestinal (GI) tract physiology such as secretions, digestion processes and motility [1–5]. One parameter that may play a crucial role in the dissolution improvement of poorly soluble drugs is the lipid content of the meal [4,5]. Based on this reasoning, such drugs are often formulated as lipid-based dosage forms [6–13]. Numerous lipophilic formulations have been classified and characterized for their physicochemical properties as well as their ability to improve oral bioavailability of drugs [14–22].

Dissolution testing is a widely employed method for evaluating dosage forms. Unlike for conventional immediate release pharmaceutical solid oral dosage forms, it has proven difficult to apply standard dissolution (paddle) methodology to lipid-based formulations because of their immiscibility with aqueous systems. *In vivo*, however, lipid formulations often produce good bioavailability, indicating that mixing with the GI fluids and release of the drug do occur. Dispersion of the dosage forms *in vivo* can occur via emulsification in the proximal GI tract while digestion of the lipid excipients can occur in the small intestine. These processes facilitate contact of the drug with the GI fluids and hence drug release [23]. It would be highly desirable to design an *in vitro* test that could better reflect *in vivo* release characteristics of these dosage forms, especially in terms of hydrodynamics.

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Biorelevant dissolution media have been developed over the last decade as a response to the fact that compendial media do not provide a sufficiently accurate simulation of the human GI milieu *in vivo* [24,25]. The appropriateness of predicting *in vivo* behavior of drug products using these media has been demonstrated in several *in vitro–in vivo* correlations (IVIVC) studies [26–29]. Nevertheless, for lipid-based dosage forms, preliminary studies revealed that these media (FaSSIF and FeSSIF) did not result in appropriate release from lipid-based preparations when used in conjunction with the conventional paddle method. Accordingly, new biorelevant media with various compositions were developed and results of dissolution testing compared with *in vivo* data in this study.

In summary, the present study was aimed not only at devising more appropriate media for lipid-based dosage forms, but also considered the effects of hydrodynamics on release behavior. The model compound chosen was RZ-50, a weakly acidic drug with a pK_a of 4.31. It has hydrophobic properties, with a log P of 5.94 and limited solubility in both aqueous and lipid vehicles. The compound was formulated into lipid suspensions using a mixture of medium chain triglyceride and hydrogenated vegetable oil and filled into soft gelatin capsules. RZ-50 release in the USP Apparatus 2 (paddle assembly) and the USP Apparatus 3 (reciprocating cylinder, Bio-Dis) using various biorelevant media was compared and evaluated. *In vitro–in vivo* correlation (IVIVC) analysis and curve comparisons based on Weibull distribution fitting were applied to assess the appropriateness of the various dissolution testing conditions.

2. Materials and methods

2.1. Materials

RZ-50 drug substance and its lipid suspensions filled in soft gelatin capsules (2.5 mg of active ingredient per capsule) were manufactured by Hoffmann-La Roche Inc., New Jersey, USA. Acetonitrile and methanol (gradient grade) and isopropanol (extra pure, 99.5%) were obtained from Merck KGaA, Darmstadt, Germany. Egg phosphatidylcholine (Lipoid E PC®, 99.1% pure, lot 108015-1/42) was a donation from Lipoid GmbH, Ludwigshafen, Germany. Glyceryl monooleate (GMO, Rylo MG19 Pharma®, 99.5% monoglyceride, lot 173403-2202/107) was a gift from Danisco Specialities, Brabrand, Denmark. Thirty-seven percent of hydrochloric acid (fuming), 85% ortho-phosphoric acid and pepsin (Ph. Eur., 0.51 U/mg, lot 1241256) were obtained from Fluka Chemie AG, Buchs, Switzerland. Maleic acid (99% pure, lot S33471-226), and pancreatin (8 × USP specification, lot 045K0673) were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Sodium oleate (82.7% pure, lot 51110) was obtained from Riedel-de Haën, Seelze, Germany. Sodium taurocholate (NaTC, 97% pure, lot 2006040099) was used as received from Prodotti Chimici e Alimentari SpA, Basaluzzo, Italy. Long-life, heat-trated and nanogenized milk (UHT-milk) containing 3.5% fat (Milfina Hochwald, Kaiserslautern, Germany) was purchased commercially. Dichloromethane, glacial acetic acid, sodium acetate trihydrate, sodium chloride, sodium dihydrogen phosphate monohydrate and sodium hydroxide were all of analytical grade and purchased from Merck KGaA, Darmstadt, Germany.

2.2. Media preparation

The detailed compositions and the preparation methods of the media used for solubility and dissolution tests have mostly been described previously [24,30,31]. Tables 1 and 2 summarize the compositions of the simulated gastric and intestinal media used in this study. Fasted State Simulated Gastric Fluid (FaSSGF), as described by Vertzoni et al. [30], was used to represent fasted gastric conditions. A recently developed medium, Fed State Simulated Gastric Fluid (FeSSGF), which contains approximately 50% UHT-milk, was used to simulate the conditions in the fed state stomach [31]. For the upper small intestine, the media used in this study included: (1) fasted state: FaSSIF prepared according to Galia et al. [24] and its modified version, FaSSIF$_a$, and (2) fed state: FeSSIF prepared according to Galia et al. [24], and four modifications: FeSSIF$_a$, FeSSIF$_b$, FeSSIF$_c$, and FeSSIF$_d$. Simulated Intestinal Fluid without pancreatin (SIF$_{sp}$) (USP 24) was used as a control.

2.3. Quantitative analysis of RZ-50

The samples obtained from solubility and dissolution tests were filtered through 0.45 μm PTFE filters prior to quantitative analysis for RZ-50 by HPLC. For tests in FeSSGF, since simple filtration was not applicable, samples were subjected to a preparation step consisting of protein precipitation using isopropanol and subsequent centrifugation, prior to the filtration step.

Table 1
Biorelevant media simulating the gastric conditions in the preprandial and postprandial states

Fasted State Simulated Gastric Fluid (FaSSGF), pH 1.6 [30]		
Composition		
Bile salt (sodium taurocholate)	80	μM
Lecithin	20	μM
Pepsin	0.1	mg/mL
Sodium chloride	34.2	mM
Hydrochloric acid qs	pH 1.6	
Deionized water qs ad	1	L
Fed State Simulated Gastric Fluid (FeSSGF), pH 5.0 [31]; blank medium		
Composition		
Glacial acetic acid	17.12	mM
Sodium acetate	29.75	mM
Sodium chloride	237.02	mM
Deionized water qs ad	1	L

The blank medium was then mixed with UHT-milk at the ratio of 1:1

Table 2
Biorelevant media simulating the upper small intestinal conditions in the preprandial and postprandial states

Composition	FaSSIF	$FaSSIF_a$	FeSSIF	$FeSSIF_a$	$FeSSIF_b$	$FeSSIF_c$	$FeSSIF_d$
NaTC (mM)	3.0	3.0	15.0	7.5	15.0	7.5	15.0
Lecithin (mM)	0.75	0.2	3.75	2.0	3.75	2.0	3.75
GMO (mM)	—	—	—	5.0	5.0	5.0	5.0
Sodium oleate (mM)	—	—	—	0.8	0.8	0.8	0.8
Pancreatin (lipase ca. 100 U/mL)	No	Yes	No	Yes	Yes	No	No
pH	6.5	6.5	5.0	5.8	5.8	5.8	5.8
Buffer capacity (mmol $L^{-1}\Delta pH^{-1}$)	10	10	76	25	25	25	25
Osmolality (mOsm kg^{-1})	270	180	670	390	400	390	400

2.4. The high performance liquid chromatography (HPLC) system

An isocratic reversed-phase HPLC system equipped with a UV detector was used for the drug quantification. The HPLC system consisted of a Beckman 126 Pump, a Beckman 506e Autosampler, a Beckman 166 Detector, and a Zorbax RX-C8 analytical column (4.6 × 250 mm, i.d.; 5 μm) connected with a Zorbax RX-C8 guard column (4.6 × 12.5 mm, i.d.; 5 μm). The assays were operated at 40 °C. The chromatograms were evaluated with System Gold Chromatography Software (Beckman Instruments, Inc.). The mobile phase consisted of 85% acetonitrile, 14.5% ultra-purified water (Milli-Q®) and 0.5% ortho-phosphoric acid (by volume). The flow rate was set at 1.0 mL/min resulting in a run time of 10 min per sample. The injection volume was 50 μL. The detection wavelength was set at 330 nm.

2.5. Solubility studies

For all media, except FeSSGF, the miniaturized shake-flask method using Whatman UniPrep® was employed for RZ-50 solubility determination [32]. An excess amount of drug substance was weighed and filled into a Whatman UniPrep® filter chamber. Subsequently, 3 mL of the test media was added and the chamber was closed at one end with a plunger fitted with a filtration membrane and at the other end with a pre-attached cap. The samples were shaken at 37 °C in an orbital shaker (Heidolph Polymax 1040). At predetermined time intervals (1, 2, 4, 8 and 24 h), samples were filtered by depressing the plunger and then analyzed by HPLC.

For the FeSSGF medium, an excess amount of RZ-50 was weighed and filled into a 20 mL scintillation vial, into which the medium was subsequently added. The samples were shaken at 37 °C in an orbital shaker (Heidolph Polymax 1040). At predetermined time intervals (1, 2, 4, 8 and 24 h), protein precipitation followed by centrifugation was applied, then the samples were filtered a 0.45 μm PTFE filter and analyzed by HPLC.

2.6. In vitro dissolution studies

2.6.1. USP Apparatus 2 (paddle assembly)

The dissolution conditions consisted of 500 mL medium per vessel with a paddle revolution speed of 75 rpm and 37 °C. The sampling times were 10, 20, 30, 45, 60, 75, 90 and 120 min. Experiments were conducted in triplicate. Sampling was performed manually using a glass syringe connected with a stainless steel sampling device. The samples were withdrawn through a cylindrical polyethylene filter stick connected to the end of the sampling device. The volume withdrawn was approximately 5 mL for each sampling time point. Subsequently, with the exception of studies in FeSSGF, the samples were filtered through a 0.45 μm PTFE filter and then analyzed by HPLC.

2.6.2. USP Apparatus 3 (reciprocating cylinder, Bio-Dis)

To evaluate the effects of hydrodynamics on drug dissolution, USP Apparatus 3 was applied to the evaluation of RZ-50 dissolution from lipid suspensions. The dissolution conditions consisted of 220 mL medium per vessel with a dip rate of 15 dpm and 37 °C. The top and bottom mesh size was 840 μm. The sampling times were 10, 20, 30, 45, 60, 75, 90, 120 and 180 min. Experiments were conducted in triplicate. Sampling was performed manually using a glass syringe connected with a stainless steel sampling device. The samples were withdrawn through a cylindrical polyethylene filter stick connected to the end of the sampling device. The volume withdrawn was approximately 5 mL for each sampling time point. Subsequently, with the exception of FeSSGF, the samples were filtered through a 0.45 μm PTFE filter and then analyzed by HPLC.

The dissolution profiles from the paddle and Bio-Dis methods were constructed from the average of the percentage of cumulative drug dissolved from three vessels at each sampling time point.

2.7. Comparison of the in vitro dissolution profiles

Comparison of the dissolution profiles was performed using a model-independent approach [33]. This approach, which includes the use of the difference (f_1) and similarity factors (f_2), was applied to evaluate the dissolution profiles. Both f1 and f2 values of the dissolution of RZ-50 in the simulated fasted and fed states and the influences of bile salt, lecithin, lipolytic products and pancreatin were evaluated.

The following equations were used to calculate f_1 and f_2 values of the dissolution profiles obtained in this study:

$$f_1 = \left\{ \frac{\sum_{t=1}^{n} | R_t - T_t |}{\sum_{t=1}^{n} R_t} \right\} \times 100 \quad (1)$$

$$f_2 = 50 \log \left\{ \left[1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\} \quad (2)$$

2.8. Pharmacokinetic studies and data analysis

A single 2.5 mg oral dose of RZ-50 soft gelatin capsules was administered to four female dogs approximately 60 min after meals. Blood samples were collected at 0.5, 1, 3, 6, 12, 24 and 36 h. The plasma RZ-50 concentration was analyzed by a HPLC method provided by Hoffmann-La Roche Inc., New Jersey, USA.

Given that the pharmacokinetic data follow the one-compartment model, the Wagner–Nelson method [34] was used to deconvolute the pharmacokinetic data. Since there were no intravenous data available, the terminal elimination rate constant (λ_z) from the plasma drug concentration–time profiles following oral administration of the RZ-50 soft gelatin capsules was employed for the calculation of fraction drug absorbed (F_a), instead of the elimination rate constant from intravenous administration (k_e).

2.9. In vitro–in vivo correlations (IVIVC) analysis and curve comparisons

The deconvoluted F_a and the fraction dissolved (F_d) obtained from the *in vitro* biorelevant dissolution tests were correlated according to the Level A IVIVC approach [35] and the correlation was evaluated using linear regression analysis.

Additionally, the F_a vs. time plot was compared with the F_d profiles using the RRSBW (Weibull) distribution [33], a model-dependent approach described by the following equation:

$$W_t = W_{\max}[1 - e^{-[(t-\gamma)/\tau_d]^{\beta}}] \quad (3)$$

where W_t is the mass of drug dissolved at time t, $W_{\max}$ is the maximum cumulative mass dissolved, γ is the location parameter (the lag time before the onset of dissolution is generally assumed insignificant), τ_d is the time parameter (provides information about the overall rate of the process), and β is the shape parameter. The profiles were fit with the Weibull distribution using SigmaPlot® software, version 10.0 (Erkrath, Germany).

3. Results and discussion

3.1. Biorelevant dissolution media

The original compositions of biorelevant media [24,36] and newly developed media were applied to the evaluation of RZ-50 solubility and dissolution in this study. The concentrations of bile components were varied among the media representing the upper small intestine. The levels of bile salt and phospholipid in the original composition of FeSSIF are high and thought to be closer to the canine than the human GI environment [37,38]. $FeSSIF_b$ (see Table 2) was designed to reflect canine levels of bile salt and lecithin, whereas $FeSSIF_a$ reflects bile secretions in human aspirates. Pancreatin was added to these two media to assess its influence, if any, on digestion/release of RZ-50 from the formulations (compared with $FeSSIF_d$ and $FeSSIF_c$, respectively).

3.2. Quantitative analysis of RZ-50

Since the biorelevant media used in this study have rather complex compositions compared to compendial media, the possibility of interference from media components with RZ-50 quantitation had to be ruled out. It was found that the peaks of drug were satisfactorily separated from those of the media when using the mobile phase flow rate of 1 mL/min.

Recovery studies of RZ-50 in the biorelevant media were conducted prior to the dissolution tests. A recovery

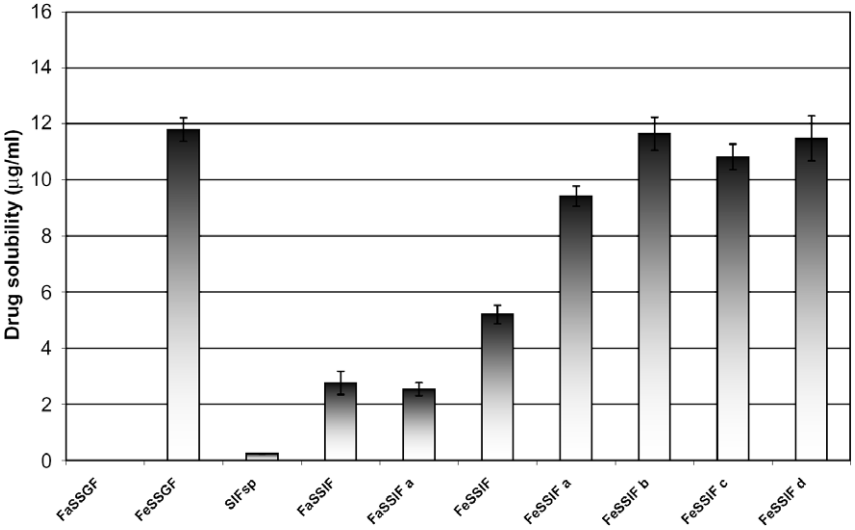

Fig. 1. Solubility of RZ-50 in various media. "ND" denotes that drug concentration was below the limit of detection.

of 95–105% was observed using the analytical methods used (data not shown).

3.3. Solubility of RZ-50 in various media

Fig. 1 shows the solubility of RZ-50 in various media. Under acidic conditions, no drug could be detected in the solubility samples. Even in FaSSGF, which contains small amounts of bile salt and lecithin, solubility of RZ-50 was under the detection limit. A positive effect on drug solubility of increasing pH was observed in SIF_{sp}, reflecting the acidic properties of the active substance. FaSSIF and FeSSIF additionally enhanced the drug solubility through the mixed-micelle effects of bile salt and lecithin. With respect to the newly designed biorelevant media, $FaSSIF_a$ gave a comparable solubility to the original FaSSIF. This reflects their similar compositions. However, in $FeSSIF_a$ the solubility of RZ-50 was nearly 2-fold higher than the original FeSSIF. This appears to be due to the effects of bile salt and lecithin combined with the lipolytic products added to the medium. The solubility of RZ-50 in $FeSSIF_b$, $FeSSIF_c$ and $FeSSIF_d$ was even better than in $FeSSIF_a$. The increase in drug solubility can be attributed to the higher amounts of bile salt and lecithin in $FeSSIF_b$ and $FeSSIF_d$. Comparison of results in these two media indicated that pancreatin has no significant effect on RZ-50 solubility. In $FeSSIF_c$, which contained no pancreatin, the solubility of RZ-50 was better than in $FeSSIF_a$, which contained pancreatin. It is possible that pancreatin interfered with the incorporation of RZ-50 into the mixed-micelles.

3.4. Previous dissolution tests used to evaluate lipid dosage forms

Several studies of release from lipid-based formulations have employed simple aqueous dissolution media for formulations containing high amounts of surfactants [10,11,22]. For more lipophilic formulations, one or more synthetic surfactants have been applied to facilitate the dispersion of dosage forms [7,13,39]. Adjunct techniques, e.g. dialysis, have also been applied with the paddle method to evaluate the release of drug from the dosage forms [13,40,41]. These approaches have been designed primarily as quality control methods. However, satisfactory predictions of performance *in vivo* with these methods have proven elusive to date.

3.5. Dissolution testing using the paddle method

Biorelevant dissolution testing of RZ-50 soft gelatin capsules was initially performed using the paddle method. Comparing the dissolution of RZ-50 pure drug substance and its lipid-based formulation in FaSSIF and FeSSIF (Fig. 2), a faster dissolution rate was observed for the pure drug: 17% and 27% drug release was observed in FaSSIF and FeSSIF at 120 min, respectively, for the RZ-50 pure drug whereas no drug release from the lipid formulation

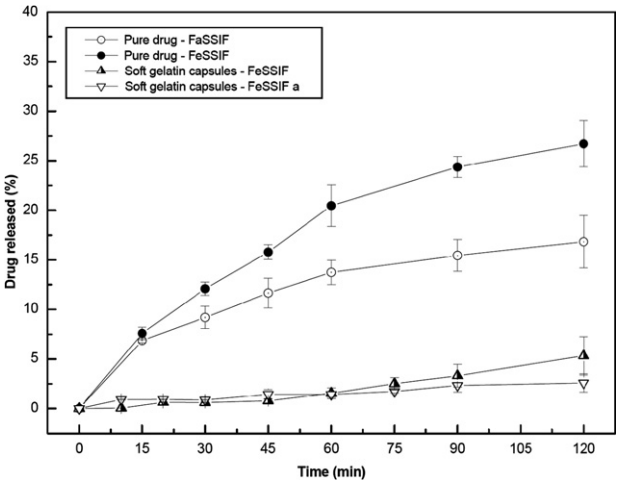

Fig. 2. Dissolution of RZ-50 pure drug substance and soft gelatin capsules in biorelevant media using the paddle method. Each symbol represents the mean ± SD of three determinations ($n = 3$).

was detected in FaSSIF and only approximately 5% drug release was observed in FeSSIF. Since oral absorption from the formulation was observed in the dogs, these test conditions are obviously inappropriate.

$FeSSIF_a$ was subsequently employed as a dissolution medium using the paddle method. Only about 3% drug release was observed, even though the medium contained lipolytic products and pancreatin. In all cases a layer of oil could be observed on the surface of dissolution medium. This problem has been encountered for other lipid formulations [23] and we hypothesized that the hydrodynamics might be an important limitation to use of the paddle method for these kinds of formulations, i.e. drug release from these types of formulation is not solely driven by dissolution, but also impacted by drug partitioning due to poor hydrodynamics and miscibility of a lipid phase with an aqueous dissolution media. As a result, the reciprocating cylinder (Bio-Dis) method was evaluated as an alternative apparatus.

3.6. Dissolution testing using the Bio-Dis method

It was observed that, for the lipid-based formulations in this study, the Bio-Dis apparatus generally achieved higher drug release and more reproducible results compared with the paddle method. The movement of the inner cylinder with the mesh inserts at the top and bottom broke up the oil layer and mixed the dosage form more homogeneously with the medium than was possible with the paddle method. On the basis of these observations, all further results were obtained with the Bio-Dis method.

3.6.1. Dissolution of RZ-50 in simulated gastric conditions

FaSSGF [30] and FeSSGF [31] were used to simulate the preprandial and postprandial gastric conditions in this study. To correspond with administration conditions in the *in vivo* pharmacokinetic study, FeSSGF, which represents

the fed gastric state during 75–165 min after the digestion of meals, was selected. Dissolution of RZ-50 from a soft gelatin capsule in FeSSGF is demonstrated in Fig. 3A. A food effect was predicted from these data, since the amount of RZ-50 released from the formulations in FaSSGF was below the limit of quantification (results not shown), whereas approximately 50% of the drug was released within the same timeframe in FeSSGF. The higher pH (5.0) together with the fat content and casein micelles in FeSSGF facilitate the release of this acidic drug *in vitro*.

3.6.2. Dissolution of RZ-50 in simulated upper small intestinal conditions

For the upper small intestine, the simulation of food effects and the differences between original and newly designed biorelevant media with respect to the release of RZ-50 from a soft gelatin capsule were evaluated.

The two fasted state media, FaSSIF and $FaSSIF_a$, are similar in terms of the amounts of bile salt and lecithin. The amount of pancreatin used in $FaSSIF_a$ was just sufficient to digest the lipid composed in the formulation and is less than the fasted state concentrations observed *in vivo* [42,43]. It was hypothesized that the pancreatin would facilitate "digestion" of the lipid formulation and hence release of RZ-50. However, addition of pancreatin actually retarded drug release, with release of RZ-50 in FaSSIF higher than in $FaSSIF_a$ ($f_1 = 37.72, f_2 = 68.86, f_1$ borderline significant), the difference being most notable after 60 min (Fig. 3A). The results are commensurate with the slightly lower solubility of RZ-50 in presence of pancreatin.

Next, the RZ-50 soft gelatin capsules were evaluated in FeSSIF and $FeSSIF_a$. As shown in Fig. 3A, the release of RZ-50 from the soft gelatin capsules in $FeSSIF_a$ was much better than in FeSSIF. The combined effects of lipolytic products and pancreatin appeared to promote the release of RZ-50 in $FeSSIF_a$ even though it contains lower amounts of bile salt and lecithin than FeSSIF. RZ-50 release was incomplete in both media due to Noyes–Whitney considerations. Based on the dose of 2.5 mg and the RZ-50 solubility of 5.2 μg/mL in FeSSIF and 9.4 μg/mL in $FeSSIF_a$, the corresponding dose to solubility (D/S) ratios in FeSSIF and $FeSSIF_a$ are 481 mL and 266 mL, respectively. As a result, sink conditions were not achieved during dissolution in either the paddle or the Bio-Dis method.

No food effect would be predicted by the release of RZ-50 lipid formulations in FaSSIF and FeSSIF: the calculated *f*-values are $f_1 = 20.20$ and $f_2 = 82.67$. By contrast, release in the new versions of the biorelevant media ($FaSSIF_a$ vs. $FeSSIF_a$) resulted in distinctly different release profiles: $f_1 = 239.62$, $f_2 = 38.14$.

Variations in bile salt and lecithin concentrations were evaluated by comparing results in $FeSSIF_a$ and $FeSSIF_b$ (Fig. 3B). The higher levels of bile salt and lecithin in $FeSSIF_b$ enhanced the dissolution of RZ-50 ($f_1 = 49.12$, $f_2 = 46.27$).

Pancreatin effects are dependent on bile salt and lecithin concentrations and are predicted by solubility behavior.

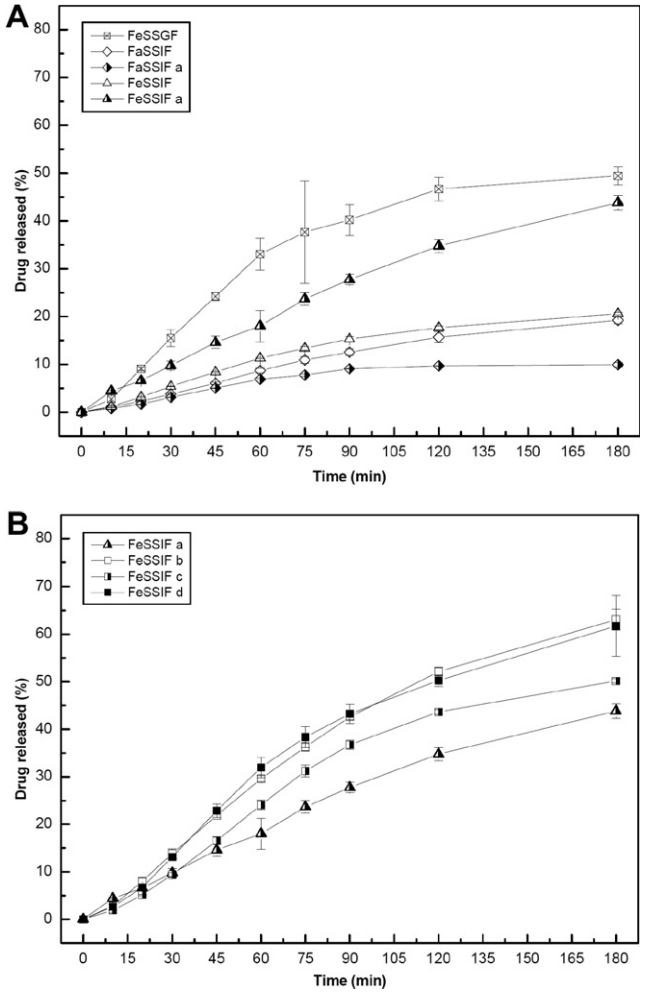

Fig. 3. Dissolution of RZ-50 soft gelatin capsules in several biorelevant media using the Bio-Dis method. (A) Comparison of RZ-50 dissolution in FeSSGF, FaSSIF, $FaSSIF_a$, FeSSIF, and $FeSSIF_a$. (B) Comparison of RZ-50 dissolution in $FeSSIF_a$, $FeSSIF_b$, $FeSSIF_c$, and $FeSSIF_d$. Each symbol represents the mean ± SD of three determinations ($n = 3$).

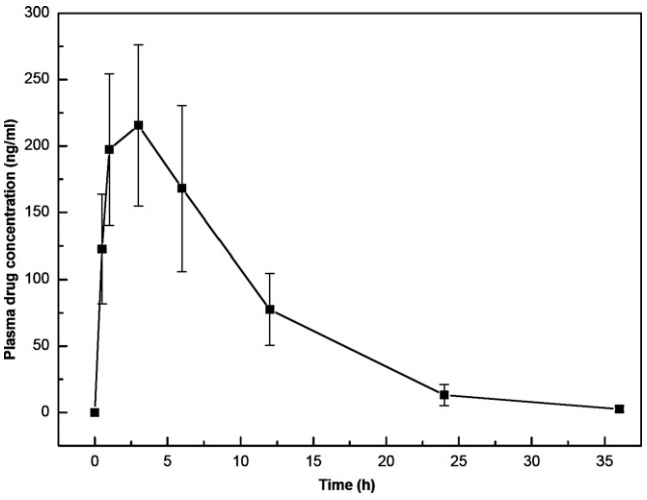

Fig. 4. Plasma drug concentration–time profile following oral administration of 2.5 mg RZ-50 lipid-based formulation to four female dogs in the fed state. Each symbol represents the mean ± SEM ($n = 4$).

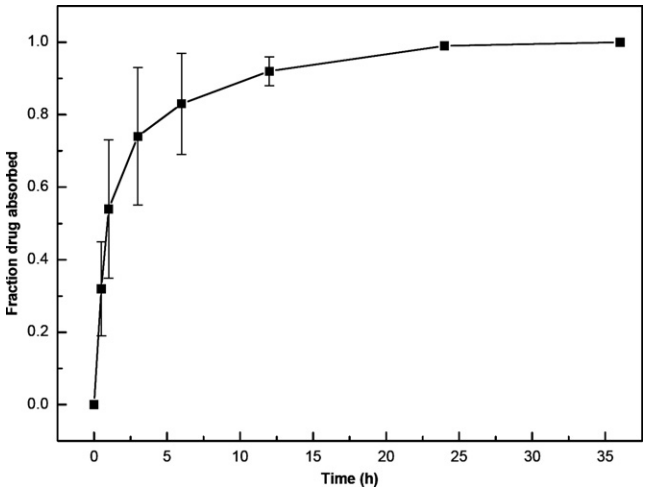

Fig. 5. Fraction drug absorbed-time profile of RZ-50 calculated using the Wagner–Nelson method. Each symbol represents the mean ± SEM ($n = 4$).

Table 3
Regression analysis parameters obtained from IVIVC analysis

Medium	Regression equation	Coefficient of determination (r^2)
FeSSGF	$y = 0.8063x - 0.1031$	0.9993
FaSSIF	$y = 0.3630x - 0.0877$	0.9381
$FaSSIF_a$	$y = 0.1629x - 0.0200$	0.9987
FeSSIF	$y = 0.3594x - 0.0671$	0.9702
$FeSSIF_a$	$y = 0.8019x - 0.1892$	0.8950
$FeSSIF_b$	$y = 1.1582x - 0.2622$	0.9389
$FeSSIF_c$	$y = 0.9612x - 0.2334$	0.9586
$FeSSIF_d$	$y = 1.1477x - 0.2555$	0.9703

Dissolution of RZ-50 in $FeSSIF_c$, which contains no pancreatin, was significantly better than in $FeSSIF_a$ ($f_1 = 24.08$, $f_2 = 61.37$). As observed with the fasted state media, FaSSIF and $FaSSIF_a$, the presence of pancreatin in the media appears to retard the release of RZ-50 from the oily suspension. At higher bile salt and lecithin levels, the adverse effects of pancreatin on dissolution rate are not discernable ($FeSSIF_b$ vs. $FeSSIF_d$). All these results are in agreement with the rank order of RZ-50 solubility in the modified FeSSIF media (Fig. 1).

Inclusion of lipolytic products in the media led to higher dissolution rate (FeSSIF vs. $FeSSIF_b$: $f_1 = 180.09$, $f_2 = 31.65$, FeSSIF vs. $FeSSIF_d$: $f_1 = 180.76$, $f_2 = 31.66$).

3.7. IVIVC analysis and curve comparisons

3.7.1. Deconvolution of pharmacokinetic data and IVIVC

Fig. 4 shows the mean plasma drug concentration–time profile of RZ-50 following oral administration of the lipid-based formulation in four female dogs approximately 60 min after meal intake. The average λ_z value was calculated to be 0.19 h^{-1}. The F_a-time profile calculated using the Wagner–Nelson method [34] is depicted in Fig. 5.

The F_a values calculated from *in vivo* data and the *in vitro* F_d values obtained from several biorelevant dissolution tests were correlated on a real-time basis. The regression analysis results are summarized in Table 3. The resulting correlations based on the Level A IVIVC analysis are shown in Fig. 6. It was revealed that the release of RZ-50 in FeSSGF correlates best with the *in vivo* pharmacokinetic data. The correlation is reasonable in view of the fact that the capsules were administered to the dogs approximately 60 min after feeding.

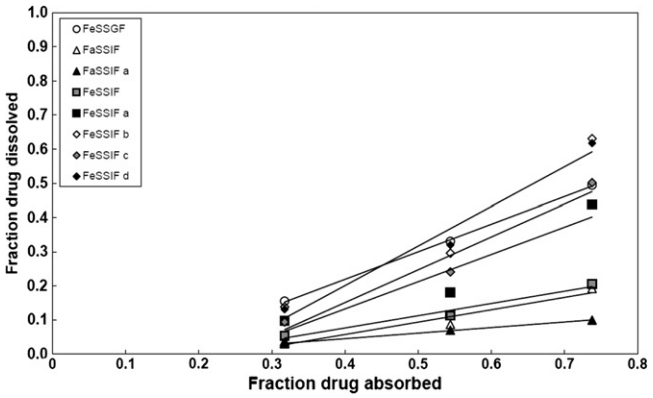

Fig. 6. *In vitro–in vivo* correlation (IVIVC) analysis of RZ-50 in various biorelevant dissolution conditions.

3.7.2. Curve comparisons

The RRSBW (Weibull) distribution was applied to compare the dissolution profiles obtained from biorelevant dissolution tests with the plot of the F_a deconvoluted by means of the Wagner–Nelson method, as illustrated in Fig. 7. Of the curves generated, only two were similar to the F_a vs. time plot for RZ-50 soft gelatin capsules: FeSSGF and $FaSSIF_a$. Of these two, IVIVC analysis indicated highest correlation for FeSSGF.

On the basis of curve comparisons and IVIVC analysis it was concluded that FeSSGF is the "best choice" for comparing future lipid-based formulations of RZ-50 to be administered in the fed state.

4. Conclusions

Biorelevant dissolution test methods for liquid lipid dosage forms were designed and evaluated in this study using a lipid suspension of RZ-50 as a model drug product. The paddle method appeared to be inappropriate for the *in vitro* dissolution test of lipid-based dosage forms. By contrast, the Bio-Dis method provided a higher release and more reproducible results. Of the various media employed in the Bio-Dis, FeSSGF, a dissolution medium representing the fed state stomach, correlated best with the pharmacokinetic data. This seems reasonable in view of the fact that the formulations were administered to the dogs about 60 min after feeding. As a result, the Bio-Dis apparatus, together with media representing the stomach in the fed state, appears to be suitable for the evaluation

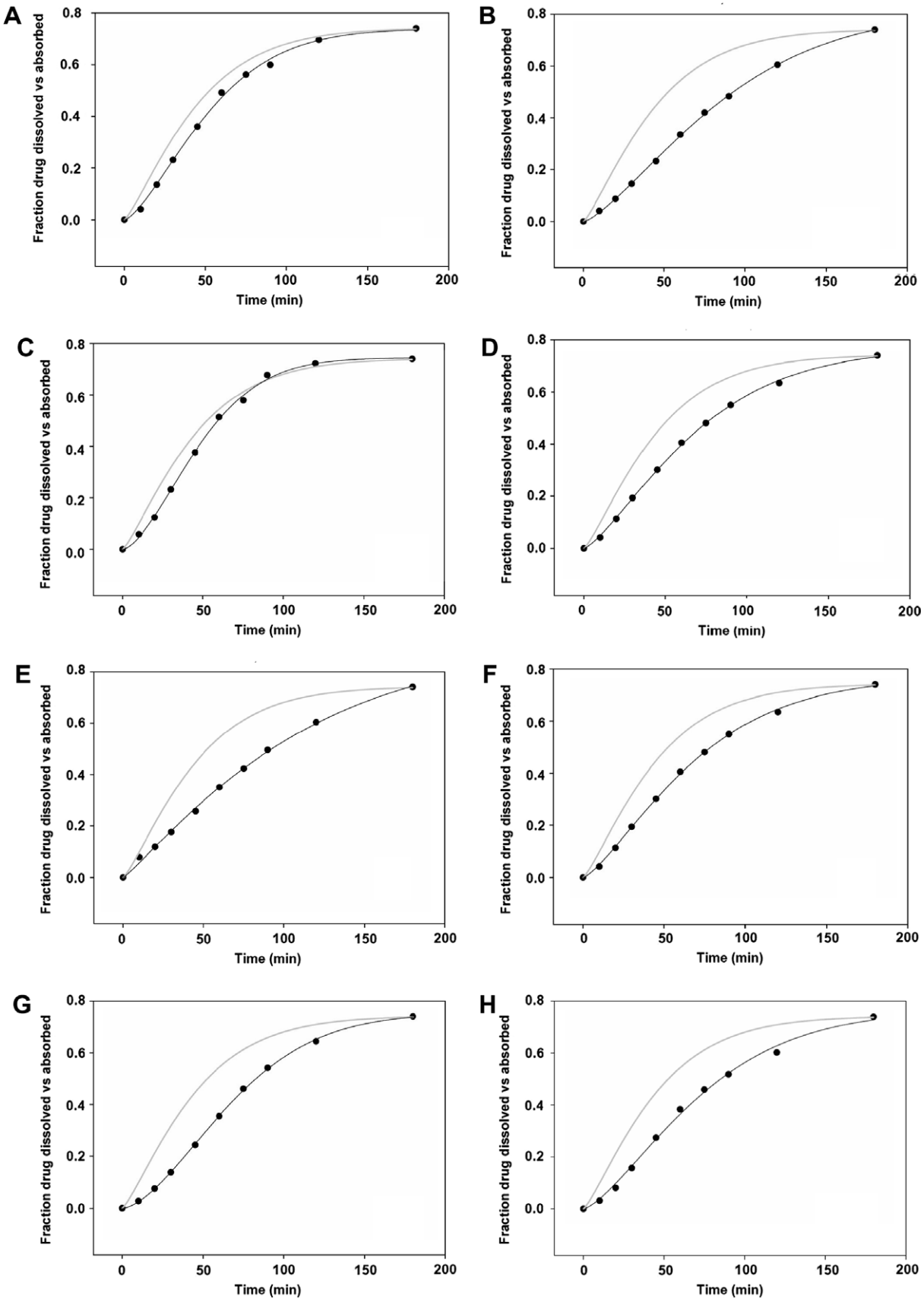

Fig. 7. The fraction drug dissolved (F_d) of RZ-50 soft gelatin capsules in various media, fitted with the Weibull distribution. The graphs compare fitted (black lines) and observed (dot) F_d values with the F_a vs. time plots (gray line in each group). (A) FeSSGF, (B) FaSSIF, (C) $FaSSIF_a$, (D) FeSSIF, (E) $FeSSIF_a$, (F) $FeSSIF_b$, (G) $FeSSIF_c$, and (H) $FeSSIF_d$.

of drug release from liquid lipid dosage forms in the post-prandial state.

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