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Evaluation of the impact of age-specific bile salt differences on the dissolution behavior of voriconazole using biorelevant media

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ABSTRACT: Voriconazole is a well-accepted and effective antifungal agent belonging to BCS class II. Voriconazole is a highly intriguing drug with considerably variable pharmacokinetics among adults and children primarily attributed to the drug-metabolizing enzymes. Additionally, bile salts enhance the absorption of lipophilic drugs in the GI fluids. Since voriconazole has limited solubility, age-related fluctuations in bile salts may affect the drug's pharmacokinetics. Therefore, the purpose of this study is to assess whether age-associated changes in the GI fluid composition and fluid volume may play a role in affecting the dissolution behavior of voriconazole. Based on that, the solubility and *in-vitro* dissolution studies of voriconazole were carried out in biorelevant media using 900 ml and 500 ml as GI volumes for adults and pediatrics respectively. Additionally, employing variations in the bile salt concentrations for the dissolution medium to act as a surrogate representing various age-specific cohorts. The results demonstrated that changes in GI volume had a negligible impact on the *in-vitro* dissolution profiles of voriconazole. However, as anticipated, there were some notable impacts of bile salt changes on the *in-vitro* dissolution profiles of voriconazole. Furthermore, it can be inferred that other factors, such as variations in the expression and maturation of enzymes, may have a comparatively profound impact on the disparate pharmacokinetics of voriconazole in adults and children besides bile salts alone. Since pharmacopoeial buffers are unable to mimic actual *in-vivo* conditions, leading to misinterpretation of the results. Therefore, *in-vitro* dissolution investigations carried out in biorelevant media are preferable.

KEYWORDS: Age-specific differences; bile salts; biorelevant media; *in-vitro* dissolution; voriconazole

1. INTRODUCTION

1.1. Pharmacokinetics of voriconazole

Voriconazole is a potent triazole antifungal agent which belongs to BCS Class II drugs. It is regarded as the first line of treatment against a variety of fatal invasive fungal infections, particularly Aspergillus [1-3]. However, voriconazole demonstrates substantial differences in clearance and bioavailability between adults and children, as well as vast pharmacokinetic variability [4, 5]. In addition, the oral bioavailability of voriconazole in children (45–66%) is approximately half of that in adults (96%) [6, 7]. As demonstrated in Figure 1 [8], several variables might affect a drug's pharmacokinetics, including GI tract variability, enzyme expression and ontogeny, and formulation-related features.

1.1.1. GI tract variability

Variability in the pharmacokinetics of medications is influenced by several GI tract-related parameters, including gastric pH and emptying time, intestinal transit time, immaturity of secretion, and activity of bile and pancreatic fluid; GI fluid volume and stomach capacity contribute to variability in the pharmacokinetics of drugs. Furthermore, membrane permeability, plasma protein binding, tissue binding, and total body water are some crucial variables that account for the variations in drug distribution between children and adults. Metabolism of drugs is the most important factor and responsible for variability in PK/PD of drugs in both adults and pediatrics. Additionally, certain characteristics that affect excretion as well as the GI tract have an impact on the pharmacokinetics of medications. When compared to adults, children have a lower rate of drug

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excretion due to factors such as immature glomerular filtration rate, immature renal tubular secretion, and tubular reabsorption at birth, and these factors also alter drug action [9]. In general, pediatric patients' weight-corrected medication clearance is reported to be higher than adult values [10].

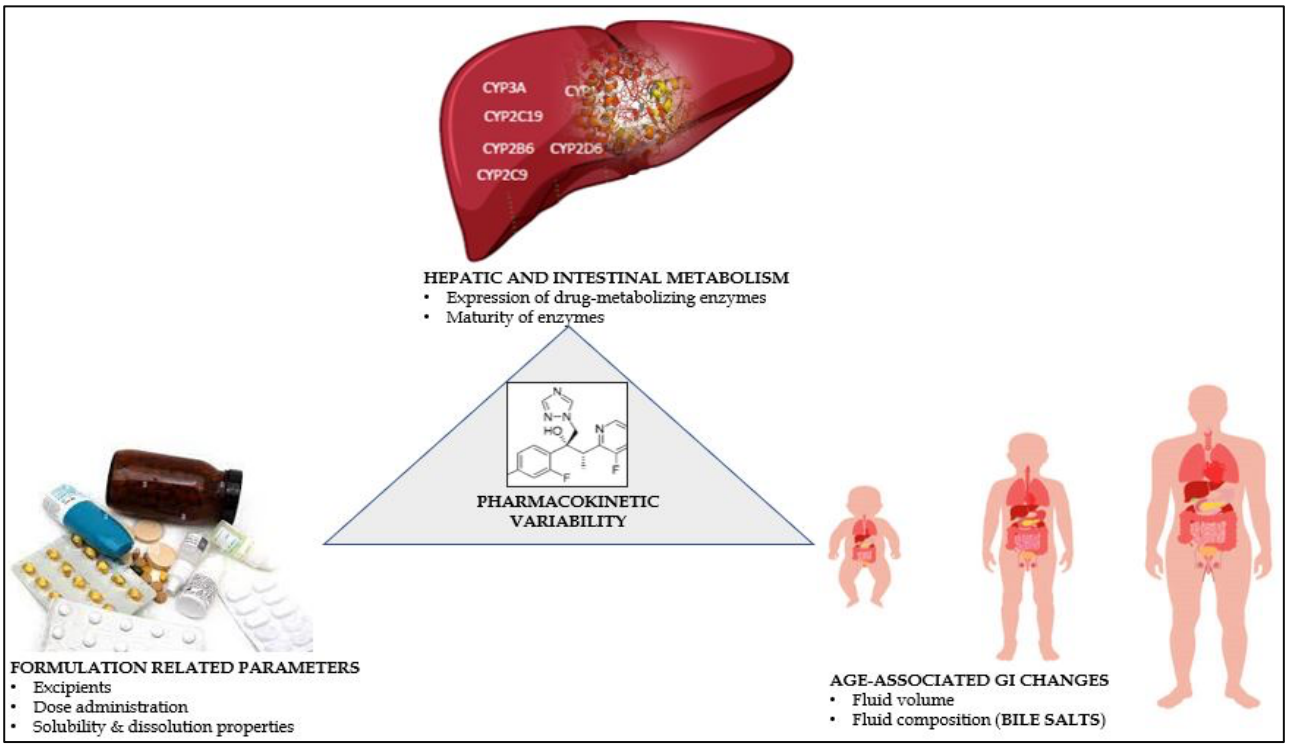

Figure 1. Various factors influencing the pharmacokinetic variations of voriconazole

1.1.2. Expression and ontogeny of enzymes

Along with drug metabolism, the development of metabolizing enzymes is crucial. Enzymatic maturation or metabolic capability, on the other hand, is entirely unrelated to body weight and it does not follow developmental growth with age. Only 2% of the voriconazole is eliminated unaltered in the urine since it undergoes extensive hepatic metabolism via numerous metabolic pathways [11]. Additionally, CYP3A suppression and capacity-limited clearance of voriconazole in adults result in nonlinear pharmacokinetics in contrast to linear pharmacokinetics in children [12], which is mostly attributable to variations in the expression and ontogeny of drug-metabolizing enzymes (DMEs). Children have higher levels of CYP2C19 and FMO expression than adults do, although CYP3A4 is more important in adults and does not affect children's higher metabolism [13].

1.1.3. Formulation-related properties

The type of formulation to be used for treating pediatric patients depends on their age group, and the most popular way to administer medication is the peroral route. Children between the ages of 5-7 and certain older children between the ages of 6 and 11 years are frequently treated with liquid formulations. Some liquid medications have disadvantages, such as an unpleasant flavor and taste, which are typically remedied by adding flavoring ingredients. Potential interactions must be taken into consideration when developing such formulations. In the case of newborns or infants, the medication is frequently blended with the baby's milk which leads to alterations in the therapeutic activity of medicament. Similarly, when the medicine is administered by opening the capsule shells, the medication's effect is altered. These procedures involve the unlicensed delivery of medications [14]. Therefore, it is important to closely supervise the administration of medication to children of different ages to ensure safe and effective drug delivery, especially for medications with high potency.

1.2. Impact of variable gastrointestinal volumes and composition (bile salts) on drug pharmacokinetics

Numerous research conducted to date has clarified the potential impact of formulation-related features and drug-metabolizing enzymes on the pharmacokinetic variation of voriconazole in adults and children. To the best of our knowledge, until recently, there are no reports on the impact of age-associated developmental changes in GI composition, specifically based on bile salt concentrations and GI fluid volumes, which could alter the disposition of voriconazole.

Bile salts are biosurfactants that are important constituents of the GI tract. They are known to enhance the absorption of some lipophilic medications by enhancing their solubility and also by enhancing cellular membrane permeability. Furthermore, bile salt enhances the chemical and enzymatic stability of hydrophobic molecules [15]. Since voriconazole is a lipophilic molecule, it may exhibit higher bioavailability in response to an increase in bile salt concentrations or vice versa [16]. Furthermore, fluctuations in gastrointestinal fluid volume based on age-related variations may alter the extent of drug absorption, which is essentially based on the concentrations of bile salts present in GI fluids along with the surface area accessible for drug absorption [17].

To assess the effect of bile salt variability on the PK profile of drugs, *in-vitro* dissolution studies conducted in a biorelevant medium serve as a surrogate indicator for predicting the *in-vivo* pharmacokinetics of the drug [17, 18]. Moreover, *in-vitro* dissolution studies conducted in biorelevant medium are ideally preferred over compendial media owing to the test conditions' close resemblance with the gut environment which further supports the better prediction of drug product performance [19].

1.3. Selection, types, and composition of biorelevant media

1.3.1. Selection of the dissolution media

The selection of a dissolution screening medium is a multi-step approach that is based on the appropriate physiological properties of the drug substance, formulation-related properties as well as interactions among its constituents. The choice of a dissolution medium and testing procedure that produces *in-vitro* results that appropriately represent the rate and extent of drug dissolution *in-vivo* is one of many complex challenges [20]. The dissolution media varies from simple buffers to biorelevant media along with the potential addition of digestive enzymes. While the variety of available buffers and simulated media makes it possible to investigate a compound's sensitivity to the composition of the medium, it can also be a challenging task for pharmaceutical scientists to choose the most practical yet biorelevant medium [21, 22]. Certain formulations show considerable dependence on GI physiology. For such formulations, conventional dissolution apparatus (a paddle or basket), and media like water, diluted HCl, or phosphate buffer should suffice. For instance, immediate-release dosage forms comprising highly soluble drugs and basic osmotic pumps can be studied using conventional dissolution media and apparatus. However, with other formulations, such as enteric-coated pellets, matrix tablets for modified release, and immediate-release dosage forms comprising poorly soluble drugs, GI physiology may play a substantial role in the release. Such formulations should take into consideration GI physiology factors that are crucial for release, and an apparatus that facilitates media change may be appropriate [18, 22, 23]. Additionally, variability in the pharmacokinetics of medications is influenced by several GI tract-related parameters, including gastric pH and emptying. Typically, buffers, surfactants, and surfactants with acid or buffers are employed as media in dissolution experiments. Biorelevant media, which contain bile salts and other physiologically relevant substances, are primarily considered for the development of *in vitro-in vivo* correlations as well as in regulatory assessments [23-25]. Given the intricacies of the human body, physiology, and chemical/biological interactions that occur, relying only on the dissolution test to predict how a medication formulation will behave *in-vivo* can be problematic. The use of biorelevant media can help with such assessments, but there is no way to know how well the dissolution test predicts *in-vivo* performance without conducting clinical investigations [26].

1.3.2. *Types and composition of biorelevant media*

The biorelevant media was first created in 1998 by Dr. Jennifer Dressman and her team to address the need to comprehend how well poorly soluble drugs will dissolve in the GI tract [23]. Thereafter, several media have been created to simulate various GI tract segments. Biorelevant media are *in-vitro* replicas of intestinal fluids that simulate the most pertinent GI fluid factors, such as pH, osmolality, surface tension, and the solubilization capacity for drugs [25]. Biorelevant media assist in the prediction of the *in-vivo* disposition of drugs particularly poorly soluble weak bases and lipophilic drugs [27]. Biorelevant *in-vitro* dissolution testing is useful for qualitative forecasting of formulation and food effects on the dissolution and availability of orally administered drugs. It has been observed that the biorelevant media can provide a more accurate simulation

of pharmacokinetic profiles than simulated gastric fluid or simulated intestinal fluid. Additionally, the recommended biorelevant dissolution medium attributes were chosen to be in line with physiological values because other factors, including osmolality, surface tension, viscosity, and the ionic strength of GI fluids, might also impact dissolution. The approach of selecting an appropriate biorelevant dissolution media is primarily based on three key variables, including drug ionization at the stomach and small intestine pH levels, modification of superficial pH of charged drug species, and drug solubilization in mixed lipid matrix made of bile components (i.e., bile salts, phospholipids, and cholesterol) that affect dissolution [28, 29].

Various types of biorelevant media were used to simulate the fasting and fed states in gastrointestinal fluids as depicted in Table 1. Fasted State Gastric fluid (FaSSGF) simulates fasting gastric conditions in the stomach, particularly the surface tension similar to the human stomach. However, in FaSSGF, physiologically irrelevant surfactants with lower physiological pH values or very high concentrations of pepsin or bile salts, are considered. This results in overprediction of gastric dissolution. To overcome the problem, FaSSGF with lower concentrations of enzymes and bile salts was developed in order to estimate the gastric dissolution of drugs.

Table 1. Types and composition of biorelevant media

Components	**FaSSGF**	**FaSSIF**	**FeSSIF**	**FeSSGF**
Sodium Taurocholate	80 µM	3 mM	15 Mm	-
Sodium acetate	-	-	-	29.75 mM
Lecithin	20 µM	0.75 mM	3.75 mM	-
Pepsin	0.1 mg/ml	-	-	-
Acetic acid	-	-	8.65 g	17.12 mM
Sodium chloride	34.2 mM	-	11.874 g	237.02 mM
Milk/Acetate Buffer	-	-	-	1:1
Hydrochloric acid	qs ad pH 1.6			qs ad pH 5.0
Sodium hydroxide	-	qs ad pH 6.5	4.04 g	-
pH	1.6	6.5	5.0	5.0
Deionized water	1 L	1 L	1 L	-
Osmolality (mOsmol/Kg)	120.7 ± 2.5	~270	~ 670	~ 400
Buffer Capacity (mEq/pH/L)	-	~ 12	~ 72	~ 25
Surface tension (mN/m)	42.6	54	48	-

* FaSSGF- Fasted state Simulated Gastric Fluid, FeSSGF- Fed state Simulated Gastric Fluid, FaSSIF- Fasted state Simulated Intestinal Fluid, FeSSIF- Fed state Simulated Intestinal Fluid

Fasted State Small Intestinal Conditions (FaSSIF) were created to reproduce the effects of fasting in the proximal small intestine. This medium includes bile salts and phospholipids (lecithin) in addition to a stable phosphate buffer system that produces a pH indicative of measurements made from the mid-duodenum to the proximal ileum. Since cholic acid is one of the more frequent bile salts in human bile, sodium taurocholate is selected as a representative bile salt. In addition, due to the taurine conjugate's extremely low pKa, there is a minimal chance that it will precipitate or alter in micellar size with slight pH value shifts within the normal range observed in the proximal small intestine (pH 4–7). Bile salt ought to be present in the medium at a concentration of 3-5 mM. Bile salt and lecithin are found in a ratio of about 4:1 for adults [18, 22, 30-32].

The Fed State Gastric Conditions: Milk and Ensure® Plus simulate the gastric condition post ingestion of a meal. Since the meal consumed will have a significant impact on the luminal composition in the stomach in the fed state. Previously numerous attempts to mimic postprandial gastric circumstances didn't accurately represent all the variables that are crucial for understanding the effect of ingested food on the release of drugs in the stomach. They either do not take into consideration how the meal alters the composition of the stomach or display pH values that are considerably higher than average gastric pH values following meal intake, which frequently range between 3-6 or even reach neutral pH values depending on the meal's composition. The standard breakfast suggested by the US FDA to examine the effects of food in bioavailability and bioequivalence studies has minimal inter-batch variability, and is simple to prepare was chosen as the

appropriate medium representing initial gastric conditions in the fed state. Homogenized cows' milk (3.5% fat) and Ensure® Plus have a comparable composition to a breakfast meal with respect to the ratio of carbohydrate/fat/protein. In addition, their pH (6.5–6.6) and other physicochemical characteristics are comparable to those of homogenized and undigested standard breakfasts, whereas Ensure® Plus is more closely related to the characteristics of the FDA breakfast and milk is more helpful when mimicking a breakfast with reduced calorie content [18, 31, 32].

Fed State Small Intestinal Conditions (FeSSIF) stimulate the drug dissolution in the proximal intestine. There are alterations in the hydrodynamics and intraluminal volume following a meal. After a substantial meal, the chyme's pH is lower than that of the intestinal fluid while the patient is fasting, although buffer capacity and osmolality both show a significant increase. Along with these elements, the abrupt rise in bile flow may have a significant impact on a drug's bioavailability. Additionally, unique interactions between the medication and food components that are consumed may happen. Additionally, an abrupt increase in bile salts can significantly impact the bioavailability of drugs. FeSSIF includes an acetate buffer in order to achieve greater osmolality and buffer capacity while preserving the lower pH value typical of fed state circumstances in the proximal small intestine. To reflect the biliary response to meal intake, taurocholate, and lecithin are present in significantly higher amounts than in the fasting state medium [18, 24, 30-32]. Due to wide pharmacokinetic variability among adult and children, the Pediatric Fasted State Simulated Gastric Fluid (P-FaSSGF) with 20 µM sodium taurocholate concentration for neonates (0-8 days) and 60 µM for infants (1- 12 months) were developed (as depicted in Table 2). Additionally, to define bile acids within fasted state intestine, research was carried out by varying the concentration of sodium taurocholate with 1.5 mM and 4.5 mM i.e., 50 and 150 % of adult levels in Pediatric Fasted State Simulated Intestinal Fluid (P-FaSSIF). Pediatric media were then developed by extrapolating the adult biorelevant media to maintain the same sodium taurocholate/ lecithin ratio as that of the adult reference media [5, 18, 23, 32].

Table 2. Composition of FaSSIF media for adult, child, infant, and neonate

Component	**FaSSIF Adult**	**FaSSIF Child (2-10 yr)**	**FaSSIF Infant (up to 1 yr)**	**FaSSIF Neonate (up to 28 days)**
Sodium Taurocholate	3 mM	1.5 mM	60 µM	20 µM
Lecithin	0.75 mM	0.37 mM	15 µM	5 µM
Sodium chloride	125.5 mM	68.62 mM	34.2 mM	34.2 mM
Sodium hydroxide	qs ad pH 6.5	qs ad pH 6.5	qs ad pH 6.5	qs ad pH 6.5
pH	6.5	6.5	5.0	5.0
Deionized water	1 L	1L	1 L	1 L
Osmolality (mOsmol/Kg)	~270	~180	-	-
Buffer Capacity (mEq/pH/L)	~ 12	~10	-	-

*FaSSIF- Fasted state Simulated Intestinal Fluid

Different levels of biorelevant media were introduced in order to consider the situations where less complex media can be appropriate or situations where additional factors need to be considered. Level 0 biorelevant media (pH) are considered for the approval of the immediate release of solid dosage forms comprising highly soluble drugs (BCS Class I and III drugs). Although compendial buffers have a higher buffer capacity than that of the human GI tract in the fasted state, the media are "fit for purpose" since their pH values fall within the typical range for the upper GI tract. On the other hand, Level I media (pH and buffer capacity) are considered for highly soluble drugs where the rate and extent of dissolution are governed by both pH and buffer capacity. Level II (pH, buffer capacity, and physiological solubilizing factors) is considered for poorly soluble drugs with a log P value of ≥ 2. In contrast, Level III media (special purpose) are in which the media is modified to respond to certain queries or unique formulations. Examples include adding enzymes to the media for gelatin capsules that may exhibit crosslinking or when determining the amount of release from lipid-based dosage forms [5, 18, 23, 32, 33].

The current research aims to evaluate the *in-vitro* dissolution studies of voriconazole in compendial buffers and biorelevant media with varying bile salt concentrations to forecast whether the disparities in bile

salt concentration associated with the development in pediatric age groups could possibly alter the dissolution profiles of voriconazole.

2. RESULTS AND DISCUSSION

2.1. Solubility studies of voriconazole

The solubility of voriconazole was studied in different buffers such as 0.1 N HCl and FaSSIF media (3 mM, 60 µM, 20 µM, 1.5 mM). The solubility of voriconazole in 0.1 N HCl was found to be 30 ± 0.25 µg/ml. The solubility of voriconazole was observed to be significant for 0.1 N HCl attributed to its basic nature [34, 35]. However, it showed relatively low solubilities in FaSSIF media (3 mM, 60 µM, 20 µM, and 1.5 mM) in comparison to 0.1 N HCl (Figure 2). The maximum solubilities of voriconazole in FaSSIF 3 mM, FaSSIF 1.5 mM, FaSSIF 60 µM, and FaSSIF 20 µM at pH 6.5 were found to be 6.6 ± 0.25 µg/ml, 6.9 ± 0.13 µg/ml, 7.2 ± 0.03 µg/ml, and 7.56 ± 0.17 µg/ml respectively. Since bile salts are known to enhance the absorption of lipophilic drugs by increasing solubilization. In ideal conditions, voriconazole (a poorly soluble drug with a maximum aqueous solubility of 2.7 mg/ml at pH 1.2) [34] would have demonstrated higher solubilities in biorelevant media [32, 33] but on the contrary, it showed lower solubilities in the biorelevant media. However, the solubility observed in acidic media was found to be relatively higher than that of the biorelevant media which clearly indicates over prediction of the solubilities than that observed across the gastrointestinal tract. Additionally, the solubilities observed among different FaSSIF media representing age-specific cohorts, suggested that the solubility of voriconazole in different bile salt concentrations may have a trivial impact on drug absorption. Furthermore, it was observed that there are distinct variations in the luminal solubility of drugs across neonates, infants, children, and adults as a result of developmental changes in gastrointestinal fluids. The results of the solubility studies clearly illustrate that voriconazole's solubility in the FaSSIF and pharmacopeial buffers are significantly variable. However, solubility is not the only factor that might have an impact on how well a drug is absorbed orally. Other factors such as intestinal permeability, gut metabolism, luminal degradation, gastric emptying time, small intestinal transit time, intestinal permeability, and the presence of intestinal transporters may also have an impact in vivo [35-37, 38].

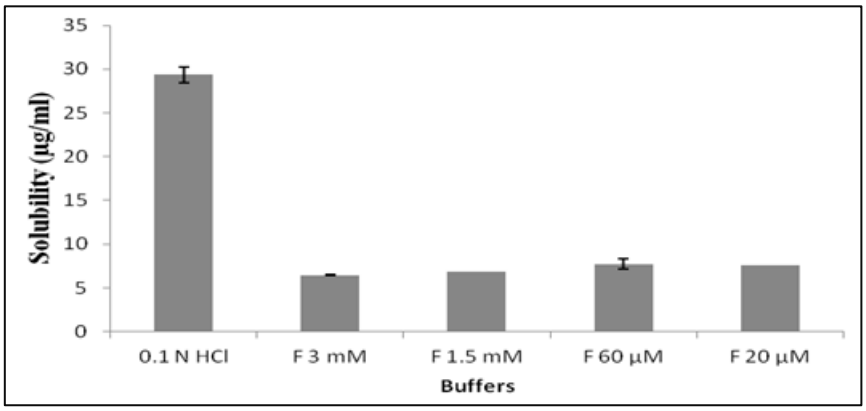

Figure 2. Solubility of voriconazole in different buffers

2.2. Calibration curves of voriconazole

The analytical method was developed and validated using various method parameters such as linearity, LOD, and LOQ. A standard stock solution of voriconazole with a concentration of 1 mg/ml was prepared using 0.1 N HCl and FaSSIF media with varying bile salt concentrations. The compendial and biorelevant stock solutions were further diluted to a concentration of 100 µg/ml. The dilution was scanned in UV- Visible range. The absorption maxima were found to be 256 nm. Further, working solutions in the concentration range of 5-30 µg/ml were prepared for both 0.1 N HCl and FaSSIF media. The absorbance of each dilution was taken at 256 nm using a UV spectrophotometer. The standard curve was plotted for each solution. Beer's law was obeyed over the concentration range of 5 to 30 µg/ml for 0.1 N HCl and for different FaSSIF media (3 mM for adults, 1.5 mM for pediatrics, 60 µM for infants, 20 µM for neonates), using regression analysis. The LOD and LOQ for voriconazole were found to be 0.1793 µg/ml and 0.5434 µg/ml for 0.1 N HCl, and 0.8071 µg/ml and 2.446 µg/ml for FaSSIF (3 mM). The linear equation of voriconazole in all buffers along with the regression

coefficients is depicted in Table 3. Voriconazole showed a linear response in all buffers within a specific concentration range [34, 38-41].

Table 3. Linearity and regression coefficient of voriconazole in various buffers

Parameter	0.1 N HCl	3 mM	1.5 mM	60 µM	20 µM
Concentration	5-30 µg/ml	5-30 µg/ml	5-30 µg/ml	5-30 µg/ml	5-30 µg/ml
Linearity Equation	y = 0.0184x + 0.0562	y = 0.0278x - 0.0122	y = 0.0253x + 0.0571	y = 0.0368x - 0.0343	y = 0.0886x - 0.0482
correlation coefficient R^2	R^2 = 0.9992	R^2 = 0.9909	R^2 = 0.998	R^2 = 0.9995	R^2 = 0.9982

2.3. Dissolution profiles of voriconazole

Dissolution of voriconazole was performed in various buffers of varying capacities. The effect of different volumes (500 and 900 ml) and different buffers (pharmacopeial and FaSSIF with different bile salt compositions) was determined and compared to assess how these developmental alterations affect the disposition of voriconazole. *In- vitro* drug dissolution rate is important to achieve high peak blood levels for a drug, the rate of drug absorption is determined by the rate of drug dissolution from the dosage form. Considering that voriconazole is a basic drug [34, 39] so the absorption peaks in acid and lowers in the fasting state, it was feasible to achieve a significant dissolution rate of voriconazole in 0.1 N HCl and 900 ml, as opposed to 500 ml and FaSSIF media (all concentrations).

2.3.1. In-vitro dissolution of voriconazole (200 mg)

In-vitro dissolution studies were conducted for marketed tablets using USP type-II apparatus. The dissolution test was performed using 500 ml and 900 ml of 0.1 N HCl, FaSSIF (3 mM, 1.5 mM, 60 µM, 20 µM) was taken as the dissolution medium at 50 rpm and 37°C±0.5°C. 10 ml of aliquots were periodically withdrawn and filtered. The sample volume was replaced with an equal volume of fresh dissolution medium. The samples were analyzed spectrophotometrically at 256 nm [34, 36, 40]. Sink conditions were maintained throughout the procedure. Sink conditions are where the saturation solubility of a drug in the dissolution medium is at least three times more than the drug concentration. This holds particular importance in the case of lipophilic drugs. The dissolution rate will increase when sink conditions are used, and a large sample size will make it harder to distinguish between different dissolution profiles. Since voriconazole has a saturation solubility of 2.7 mg/ml at pH 1.2, it decreases the discrimination between the dissolution profiles. In order to maintain the sink conditions, a change of medium after each withdrawal is required. Moreover, the sink condition aids in the robustness and biological relevance of the drug dissolution [27, 34, 36].

2.3.2. Effect of GI volume on the dissolution rate of voriconazole

In-vitro dissolution was performed for the marketed tablet of voriconazole in 900 ml and 500 ml of 0.1 N HCl and the impact of different volumes on the release rate was determined with the help of the similarity factor (f2). There is minimal/no effect of differential volumes on the release rate of voriconazole as the similarity factor was found to be 51.06 (Figure 3) and an f2 value greater than 50 confirms the sameness or equivalence of the two curves.

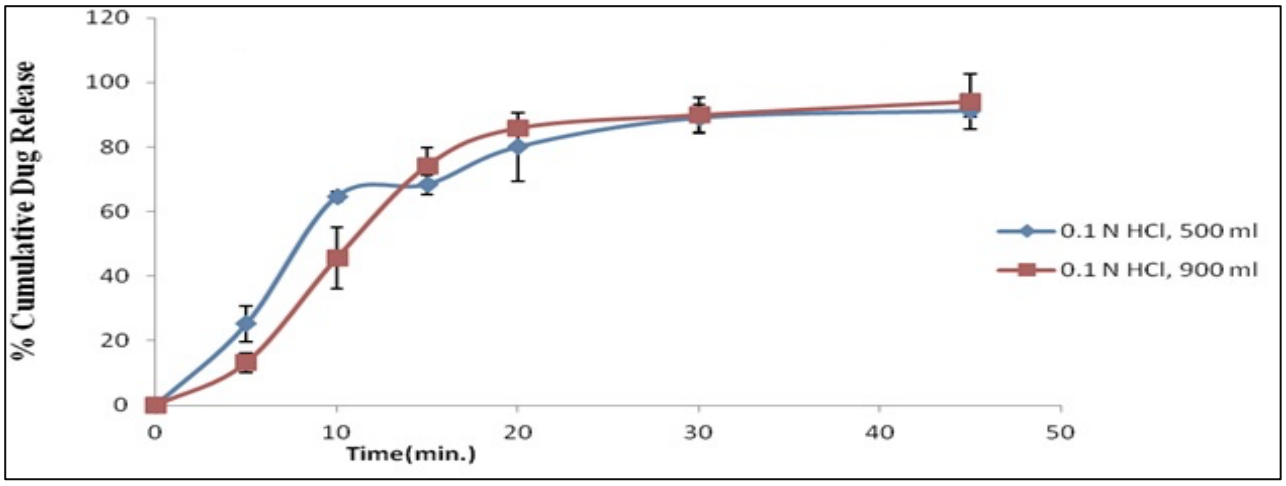

Figure 3. The release rate of voriconazole in different volumes

An *in-vitro* dissolution study was conducted for marketed tablets using USP type-II apparatus. The dissolution test was performed using 500 ml, FaSSIF (3 mM, 1.5 mM, 60 µM, 20 µM), were taken as the dissolution medium at 50 rpm and 37 °C ± 0.5 °C, and the impact of different bile salt concentration was determined. A significant release of voriconazole was obtained in 0.1 N HCl with a % drug release of 91.05 ± 1.909 %. However, the % drug release of voriconazole in FaSSIF (all concentrations) was found to be relatively very low in comparison to 0.1 N HCl. The % drug release of FaSSIF 3 mM, FaSSIF 1.5 mM, FaSSIF 60 µM, and FaSSIF 20 µM was found to be 34.81 ± 2.643, 44.58 ± 2.893, 43.91 ± 2.398, and 69.05 ± 2.504 respectively.

The similarity factor was found to be 50 for different bile salt media, FaSSIF (3 mM, 1.5 mM, 60 µM, 20 µM), indicating that could be a possible impact of different bile salt concentrations on the release rate of voriconazole (shown in Figure 4). The relatively poor release rate of voriconazole in FaSSIF media in comparison to the compendial buffer could be attributed to the fact that fasted state slows down the rate of absorption. Additionally, the results showed that the pharmacopoeial buffer (0.1 N HCl) had a higher release rate. The data produced demonstrated over-prediction of dissolution since the medium cannot accurately reflect the actual gastrointestinal condition. However, the % release of voriconazole in the biorelevant media was found to be lower, which is consistent with what is observed inside the body as per clinical findings [5,13]. The inconsistent and significantly reduced bioavailability of voriconazole in the gut could be addressed by bile salts as these are a major component of biorelevant media that more precisely forecast the GIT circumstances.

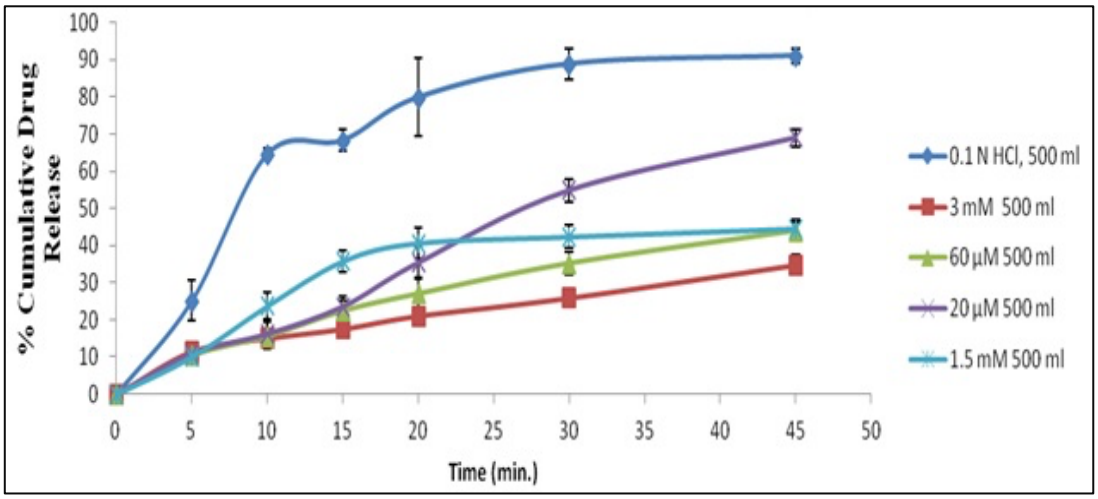

Figure 4. The release rate of voriconazole in a different medium

Overall the findings indicate that, in addition to bile salt concentrations, the lower bioavailability of voriconazole in the pediatric age groups may be primarily attributable to the expression and ontogeny of

certain enzymes, such as those in the CYP family and UGT, as well as to formulation-related properties [5,12,13]. To analyze the *in-vitro* release data various kinetic models zero-order, first-order, Higuchi model, and Korsmeyer Peppas model were used to describe release kinetics. It was found that Higuchi Model was the best-fit model for voriconazole tablets based on the R^2 values which were found to be 0.908, 0.9902, 0.9043, 0.9824, and 0.9118 for 0.1 N HCl, FaSSIF 3 mM, FaSSIF 1.5 mM, FaSSIF 60 µM, and FaSSIF 20 µM respectively.

3. CONCLUSION

The dissolution behavior of Voriconazole is influenced by variations in GI composition, especially when considered in relation to bile salt fluctuations. The results, which must be further validated with adequate clinical data of the pediatric population, suggest that, in addition to the ontogeny of metabolizing enzymes as reported through several studies, bile salt variability due to age-related differences in GI composition may play a significant role in affecting the dissolution behavior of voriconazole and its disposition within the gut. Additionally, there are notable differences between the *in-vitro* dissolution release patterns of the drugs studied in QC buffers and biorelevant media since QC buffers are not well suited to mimic real GI conditions. Biorelevant mediums are a better choice to predict drug disposition *in-vivo*. Furthermore, drug development will be more efficient and better able to predict drug responses even with sparse clinical data using *in-silico* methods like PBPK modeling. Additionally, the PBPK approach can help foresee the impact of bile salt variability on the pharmacokinetics of various BCS class II drugs. Additionally, this possibility may be helpful during the drug development process to develop safe and effective dosing, particularly in special populations, as well as lower the cost of *in-vivo* pharmacokinetic studies.

4. MATERIALS AND METHODS

4.1. Chemicals and reagents

VFEND® 200 mg tablets (Pfizer Inc., India) were purchased commercially. Voriconazole drug substance was procured from United Biotech Pvt. Ltd. (Bagbania, Himachal Pradesh, India). Acetone, hydrochloric acid solution (0.1 N HCl), glacial acetic acid, sodium dihydrogen phosphate, Sodium lauryl sulfate, sodium chloride were all analytical grade and purchased from Nice Chemicals (Gurugram, Haryana, India). Sodium hydroxide solution (1 N NaOH) was purchased from LOBA Chemie Pvt. Ltd. (Mumbai, India). Sodium taurocholate was gifted as a sample from Molychem (Mumbai, India). Lecithin was procured from HiMedia laboratories, (Mumbai, India).

4.2. Media preparation

4.2.1. Compendial buffer- 0.1 N HCl

About 800 ml of distilled water was placed in a 1000 ml volumetric flask, 0.833 ml (for 0.01 N) and 8.33 ml (for 0.1 N) of HCl was added to it dropwise and then the volume was made up with distilled water [39].

4.2.2. Biorelevant media- blank-fasted state simulated intestinal fluid (FaSSIF)

About 1.74 g of Sodium hydroxide (NaOH), 19.77 g of Sodium Dihydrogen Phosphate ($NaH_2PO_4.H_2O$), and 30.93 g of Sodium Chloride (NaCl) were dissolved in 5 L of purified water. The pH was adjusted to 6.5 using 1 N Sodium hydroxide (NaOH).

4.2.3. Biorelevant media- fasted state simulated intestinal fluid (FaSSIF) (3 mM)

About 3.3 g of sodium taurocholate was dissolved in 500 ml blank FaSSIF, about 964 mg Lecithin was dissolved in blank FaSSIF, volume was made up to 2 L with blank FaSSIF and stirred for 4-5 hrs to get a clear solution.

4.2.4. Biorelevant media- fasted state simulated intestinal fluid (FaSSIF) (1.5 mM)

About 1.65 g of sodium taurocholate was dissolved in 500 ml blank FaSSIF, about 128 mg Lecithin was dissolved in blank FaSSIF, and volume was made up to 2 L with blank FaSSIF and stirred for 4-5 hrs to get a clear solution.

4.2.5. Biorelevant media- fasted state simulated intestinal fluid (FaSSIF) (60 µM)

About 64 mg of sodium taurocholate was dissolved in 500 ml blank FaSSIF, about 19.316 mg Lecithin was dissolved in blank FaSSIF, volume was made up to 2 L with blank FaSSIF, and stirred for 4 hrs to get a clear solution (time 4-5 hrs).

4.2.6. Biorelevant media- fasted state simulated intestinal fluid (FaSSIF) (20 µM)

About 21.506 mg of sodium taurocholate was dissolved in 500 ml blank FaSSIF, about 6.42 mg Lecithin was dissolved in blank FaSSIF, volume was made up to 2 L with blank FaSSIF, and stirred for 4 hrs to get a clear solution (time 4-5 hrs) [5,18,23,25,32].

Different types of biorelevant media and their composition are shown in Table 1. Additionally, the biorelevant media with varying bile salt concentrations representing age-specific cohorts are depicted in Table 2.

4.3. Solubility measurements

To determine the solubility of voriconazole in all buffers, the shake-flask method was used. The solubility of voriconazole was assessed in pharmacopoeial buffers and biorelevant media.

4.3.1. Preparation of stock solution

For both adults and pediatrics with specific bile salt concentrations, the standard stock solution of voriconazole was prepared by transferring 100 mg of the drug into a 100 ml of volumetric flask and made up to the mark with 0.1 N HCl and FaSSIF media to get a solution of 1 mg/ml. The solution is shaken for 24 hours at room temperature. The content of each flask was then filtered through a Whatman filter paper.

4.3.2. Preparation of working solution

From the above standard stock solution, 10 ml of the sample solution was transferred to a 100 ml volumetric flask and made up to the mark in compendial and biorelevant media to get a concentration of 100 µg/ml. The above solution was further diluted to get working solutions of 5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, and 30 µg/ml in 0.1 N HCl and FaSSIF media. The working solutions are then assayed spectrophotometrically at 256 nm. The solubility of each aliquot was determined in triplicate (n=3) [38, 40, 41].

4.3.3. Spectrophotometric analysis and method validation

The UV-1800®, a UV double-beam spectrophotometer (Shimadzu, Japan), was used to analyze the prepared solutions at 256 nm [40]. The method validation was performed in terms of linearity, precision, accuracy, LOD, and LOQ. To estimate linearity, different aliquots of voriconazole in the range of 5-30 µg/ml were prepared. The solutions were analyzed at 256 nm. A Calibration curve was plotted by taking concentration on the x-axis and absorbance on the y-axis (data not shown here). The limit of Detection (LOD) is the lowest amount of the drug in the sample that can be detected, but not necessarily quantified. The limit of Quantification (LOQ) is an amount of analyte that can be quantified with a specified limit of accuracy and precision. Both the LOD and LOQ were quantified for 0.1 N HCl and FaSSIF (3 mM) [40, 42].

4.4. *In-vitro* dissolution studies of voriconazole

In- vitro dissolution studies of voriconazole were carried out according to the pharmacopeial method [34, 39]. *In-vitro* dissolution studies of voriconazole were conducted as a function of changing the pH (1.1-6.8), GI volume (900 ml for adults; 500 ml for pediatrics), and composition of FaSSIF media (3 mM for adults, 1.5 mM for pediatrics, 60 µM for infants, 20 µM for neonates) as a function of variable bile salt concentration. The composition of different biorelevant media as well as the volumes of GI fluids chosen to conduct the dissolution studies were based on the research studies conducted by Dr. Jennifer Dressman and the team who developed the biorelevant media with varying bile concentrations. Additionally, elaborated research was carried out by Maharaj et al. 2016, employing a biorelevant medium with different bile salt and lecithin concentrations to mimic the real gut environments. Additionally, Thakkar & Thakkar conducted research to determine how intestinal bile salts affect the oral bioavailability of voriconazole. The selection of the biorelevant media for the current research was largely based on the studies that have been conducted to date [8, 18, 31]. *In-vitro* dissolution studies were conducted in the following media- compendial media (0.1 N HCl) and FaSSIF (1.5 mM, 3 mM, 60 µM, and 20 µM). All the samples were withdrawn at 0, 5, 10, 15, 20, 25, 20, and 45 min time points and analyzed at 256 nm. The calibration curves were drawn. Voriconazole dissolution release profiles were generated for changes in volume and change in composition to media. The similarity

factor was calculated based on the released amount of drug at each time point to understand the effect of these parameters (volume and composition of media) on the dissolution profile of voriconazole.

4.5. USP apparatus II (paddle assembly)

The dissolution conditions consisted of a medium volume of 500 ml and 900 ml and biorelevant media (FaSSIF- 1.5 mM, 3 mM, 60 µM, and 20 µM) per vessel with a paddle revolution speed of 50 rpm. The temperature in the vessels was 37 ± 0.5 °C throughout each dissolution run. Experiments were conducted in triplicate. Sampling was performed manually using glass syringes connected with the stainless-steel sampling devices. 10 ml of aliquots were periodically withdrawn, filtered using Whatman filter paper, and the withdrawn sample volume was replaced with an equal volume of fresh dissolution medium. The samples were analyzed spectrophotometrically at 256 nm [36, 38, 40, 43].

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