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Review Article

VIRTUAL BIOEQUIVALENCE IN PHARMACEUTICALS: CURRENT STATUS AND FUTURE PROSPECTS

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ABSTRACT

Virtual bioequivalence studies (VBE) can assess the similarity and potential differences in pharmacokinetic and clinical performance between test and reference formulations based on the translational relationship between *in vitro*, *in silico*, and *in vivo*. The crucial data from clinical trials can be delivered with the help of virtual bioequivalence research, which will speed up the creation of novel and generic medications. Virtual bioequivalence study regulation, however, has not yet reached its complete development. The current status of VBE studies in the market is booming and many pharmaceutical industries have started adapting to its benefits in submitting bioequivalence results for approval from regulatory bodies. FDA had regulated the guidelines for virtual bioequivalence, which the various regulatory agencies accept for the approval of filing ANDA. The importance of implementing VBE has benefited at present in saving cost and time; low workforce and failures can be neglected. Determining the framework for virtual bioequivalence studies for all medications and discussing the potential uses of virtual bioequivalence in the future to support the waiver and optimization of *in vivo* clinical trials are the main objectives of this review article.

Keywords: Generic drugs, Virtual bioequivalence, PBPK, Bioequivalence, IVIVC

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INTRODUCTION

Virtual bioequivalence is a pharmaceutical concept that uses computational modelling and simulation techniques to assess the equivalence of generic drugs to their reference or innovator counterparts. Bioequivalence studies are typically conducted to demonstrate that a generic drug has comparable pharmacokinetic and pharmacodynamic properties to the original branded drug [1]. Traditionally, these studies involve conducting clinical trials on human subjects, which can be time-consuming, costly, and subject to ethical considerations [2, 3]. Virtual bioequivalence offers an alternative approach that leverages computational methods to predict the bioequivalence of generic drugs without the need for extensive human trials. It involves developing and applying mathematical models that simulate the human body's drug absorption, distribution, metabolism, and excretion (ADME) processes [4, 5]. VBE, in general, deals with the pharmacokinetic parameters of the drug with the physiological process, which aids in comparing the relative bioavailability of two formulations of interest. By utilizing modelling techniques such as physiologically based pharmacokinetic (PBPK) modelling, computational fluid dynamics (CFD), and *in silico* dissolution modelling, virtual bioequivalence enables the simulation of ADME processes [6]. Because of their strength in data integration, delivery of mechanistic insights, and greater predictive capacity, PBPK models are progressively replacing more empirical approaches in pharmaceutical discovery and development [7]. These models consider drug properties, formulation characteristics, physiological parameters, and genetic variability among individuals. By integrating these factors, PBPK models can accurately predict the behavior of drugs and estimate their bioequivalence [8]. The pharmaceutical research and development field has witnessed a significant increase in the complexity of drug formulations over the years [9]. This complexity arises from various factors such as novel delivery systems, intricate drug combinations, and sophisticated manufacturing processes. As drug formulations become more complex, traditional methods of evaluating their performance and ensuring bioequivalence become less efficient and time-consuming [10, 11]. When biopharmaceutical difficulties arise during the drug development lifecycle, physiologically based absorption models are beneficial. Such mechanistic absorption models can convert measurements of the therapeutic product and *in vitro* biopharmaceutical data into anticipated *in vivo* performance [12].

Regulatory agencies such as USFDA, EMA, and other global regulatory bodies have recognized the potential of virtual bioequivalence and have started to provide guidelines and initiatives to guide its implementation. These guidelines outline the criteria for demonstrating virtual bioequivalence, including the acceptance of modelling and simulation approaches, validation strategies, and the need for robust scientific evidence [13]. Understanding the regulatory landscape is crucial for successfully adopting and accepting virtual bioequivalence in the pharmaceutical industry. While virtual bioequivalence holds immense promise, it also presents challenges that must be addressed. Model validation and the availability of accurate and comprehensive data inputs are critical factors influencing the reliability and acceptance of virtual bioequivalence studies [14]. Additionally, limited regulatory approval in specific regions and the need for further research to establish robust correlations between virtual and *in vivo* results pose obstacles that must be overcome.

This review paper explores the current status of virtual bioequivalence in the pharmaceutical industry by examining the methodologies and tools employed, highlighting successful applications, and discussing the advantages and challenges associated with this approach. Furthermore, this review will present future perspectives and potential advancements in the field, including integrating virtual bioequivalence with emerging technologies such as artificial intelligence (AI) and machine learning (ML). The data for the review were based on the following criteria; Sources: PubMed, Scopus, Web of Science, Embase, and the websites of regulatory bodies like the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Keywords: Virtual bioequivalence; *In silico* bioequivalence; Computational modelling; Simulation; Physiologically based pharmacokinetic (PBPK) modelling; Computational fluid dynamics (CFD); *In silico* dissolution modelling; Generic drugs; Innovator drugs; Bioequivalence assessment; Pharmaceutical industry; Drug development; Regulatory guidelines. Year: 2010-2023.

Virtual bioequivalence

The VBE is an actual bioequivalence study. However, it is conducted digitally using a variety of tools. The term "bioequivalence" refers to a property in which two medications with the same active components or two dosage forms of the same drug have comparable bioavailability and have the same impact at the site of physiological

action [15]. The relative bioavailability of two preparations of the same drug is compared using the VBE. Instead of clinical investigations to establish BE, virtual bioequivalence, modelling, and simulation are used. By replicating the trials in a virtual population, we refer to the evaluation of generics' pharmacokinetic (PK) and clinical performance as "VBE," which assesses the similarities and potential differences between test and reference formulations. A formulation variable showing the distinction between a test and a reference, such as *in vitro* dissolution profiles for oral formulations, should be included in the model utilized in a VBE study [16]. In a VBE study, trials with various sample sizes and trial designs can be simulated to calculate bioequivalence's success or failure rate in a virtual population [17]. Despite the USFDA's optimistic conclusion that the VBE methodology's regulatory framework has not yet attained complete maturity, VBE simulations will be helpful in additional regulatory applications as PBPK or big data-based technologies advance quickly [18]. Indeed, the application of VBE includes, but is not limited to, generic drugs; it also serves as a valuable tool for new drugs by optimizing or wavering bioavailability/bioequivalence studies. To speed up drug development and make a regulatory review easier, the VBE technique can recreate the crucial data from clinical trials [19].

VBE concept

The stomach and intestine are two essential body parts covered by the VBE concept. The parameters to be considered are formulation qualities, physio-chemical properties, gastric residence time, and bioavailability [20]. VBE is a new strategy that can be applied to oral and non-oral dosage forms, such as transdermal, ophthalmic, topical dermatological, and oral inhalation medicines. The VBE study is still in the early stages for non-oral dosage forms but has made significant progress in oral formulations. Predicting *in vivo* performance is a crucial activity that guides the formulation and trial design strategies since the oral formulation is the most preferred dose form because of its easy, safe, and economical qualities [21]. The general concept includes the relationship between the PBPK model and the VBE model and the relationship between IVIVC and VBE Study (fig. 1) [22].

Advantages of VBE

Virtual bioequivalence offers numerous distinct advantages compared to traditional bioequivalence study methods. Firstly, it provides substantial cost savings by reducing the need for extensive and expensive clinical trials [23]. Computational modelling and simulation techniques enable efficient evaluation of generic drug equivalence, thereby minimizing resource requirements and associated financial burdens. Secondly, virtual bioequivalence significantly enhances time efficiency. The evaluation and decision-making process regarding generic drug equivalence can be expedited by leveraging computational models and simulations [24]. This acceleration results from the ability to rapidly simulate drug behaviour, absorption, distribution, metabolism, and excretion processes, allowing for quicker assessment and more prompt regulatory submissions. A noteworthy advantage of virtual bioequivalence is the reduced reliance on human trials. Utilizing computational models and available data minimizes the need for extensive clinical trials, which can be ethically sensitive and pose potential risks to participants [25, 26]. This non-invasive and less burdensome approach ensures compliance with ethical considerations and regulatory requirements, promoting a more efficient drug development process.

Furthermore, virtual bioequivalence enhances decision-making by providing comprehensive and detailed information on drug behaviour. Computational models can simulate drug dynamics under various scenarios, considering different formulations, physiological parameters, and patient-specific factors [27]. This allows for a deeper understanding of how these factors impact drug behaviour, enabling more informed decisions regarding generic drug equivalence and regulatory approval. Lastly, virtual bioequivalence contributes to increased access to affordable medicines. Streamlining the bioequivalence assessment process expedites the availability of generic drugs in the market. Generic drugs are crucial in improving patient access to cost-effective treatments, as they are often priced lower than their branded counterparts. The use of virtual bioequivalence methods promotes competition and drives down healthcare costs, benefiting patients and healthcare systems alike [28].

Applications of VBE

VBE offers alternatives to traditional *in vivo* bioequivalence studies, such as predicting the effect of dietary factors on drug absorption. Computational models can simulate the impact of food on drug release, dissolution, and absorption, providing insights into potential variations in bioavailability. Additionally, VBE allows for the extension of biowaivers based on the Biopharmaceutics Classification System (BCS), enabling the approval of generic drugs without conducting costly and time-consuming *in vivo* studies. VBE contributes to the development of innovative methods and techniques for assessing bioequivalence. Computational models can be used to design and optimize *in vitro* dissolution tests, which serve as surrogate measures for *in vivo* drug release [29, 30]. By simulating dissolution profiles and comparing them to reference data, VBE assists in establishing bioequivalence. VBE can be employed for quality control when there is a change in the location of pharmaceutical production. Using computational models, manufacturers can simulate the impact of production changes on drug formulation and predict potential variations in drug behaviour. It helps to ensure the consistency and equivalence of pharmaceutical products despite changes in production location. VBE aids in defending clinically relevant specifications (CRS) such as particle size standards and dissolution specifications. Computational models can simulate the impact of particle size and dissolution rates on drug performance and predict the clinical relevance of such specifications.

Furthermore, VBE allows for the risk assessment of therapeutic bioequivalence by considering factors like pharmacokinetic variability and patient characteristics, providing a comprehensive evaluation of the clinical equivalence of drugs. VBE facilitates the expansion of biowaivers by utilizing computational models to predict drug behaviour and assess bioequivalence for drugs falling within specific BCS categories. This enables the regulatory approval of generic drugs without extensive *in vivo* studies. VBE contributes to establishing dissolution specifications with clinical relevance, considering the impact of dissolution on drug performance and therapeutic outcomes. Additionally, computational models can predict the effect of food on drug absorption, aiding in understanding the potential variations in bioavailability under different dietary conditions [31-33].

Structure of the VBE study

Model construction

The model construction of virtual bioequivalence (BE) studies involves developing and implementing computational models to simulate the pharmacokinetic behaviour of drug products and assess their equivalence without conducting actual *in vivo* studies (fig. 2). By integrating experimental data and leveraging computational algorithms; these models can simulate drug behaviour in the human body and predict absorption profiles with greater precision. The model construction process typically involves data collection, formulation and drug product characterization, absorption model development, parameterization, model validation, model simulation, decision-making, and regulatory considerations. Moreover, advancements in software tools have facilitated the conduct of virtual bioequivalence studies. Tools such as GastroPlus® [34], Simcyp® [35], and PK-Sim® [36] offer comprehensive platforms for integrating physiological parameters, pharmacokinetic data, and drug properties, enabling accurate predictions and robust analyses. These software packages continue to evolve, incorporating new features and improving user interfaces to enhance usability and efficiency. Various models and software have been developed in recent years that contribute to the development of the bioequivalence field (table 1).

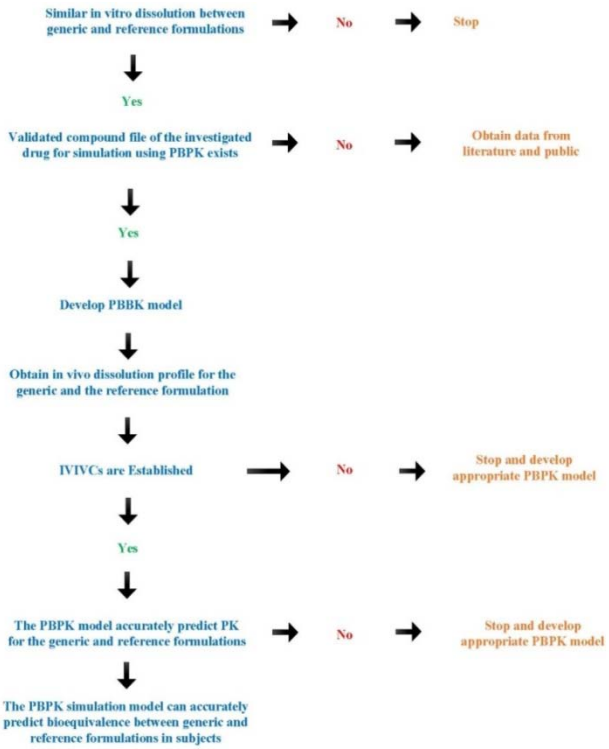

Fig. 1: Typical workflow of virtual bioequivalence studies using the PBPK model, Reproduced from "*In vitro* dissolution and *in silico* modeling shortcuts in bioequivalence testing" by Al-Tabakha MM and Alomar MJ., 2020. © 2020 by the authors. Licensee MDPI, Basel, Switzerland [4]

Table 1: Summary and recent studies of conducting virtual bioequivalence

S. No.	Drug	BSC	Dosage form	Model	Software	References
1.	Ibuprofen	2	IR suspension	ADAM	Simcyp®	[37]
2.	Dasatinib	2	IR tablet	ACAT	GastroPlus®	[38]
3.	Risperidone	2	Orodispersible film	ADAM	Simcyp®	[39]
4.	Irbesartan	2	Oral solution	ACAT	GastroPlus®	[40]
5.	Levothyroxine	1 or 3	Tablet	ADAM	Simcyp®	[41]
6.	Nifedipine	2	CR tablet	ADAM	Simcyp®	[42]
7.	Warfarin	2	IR tablet	ACAT;	GastroPlus®; Simcyp®	[43]
8.	Oseltamivir	1 or 3	Capsule	ACAT	GastroPlus®	[44]
9.	Amoxicillin	1	IR tablet	ACAT	GastroPlus®	[45]
10.	Doxycycline	1 or 2	IR tablet	ACAT	GastroPlus®	[46]
11.	Ketoconazole	2	IR tablet	ADAM	Simcyp®	[47]
12.	Tacrolimus	2	CR capsule	ACAT	GastroPlus®	[25]
13.	Naproxen	2	IR tablet	ADAM	Simcyp®	[48]
14.	Diclofenac	2	IR tablet	NA	STELLA®	[49]
15.	Isosorbide Mononitrate	1	IR tablet	NA	B^2O®	[50]

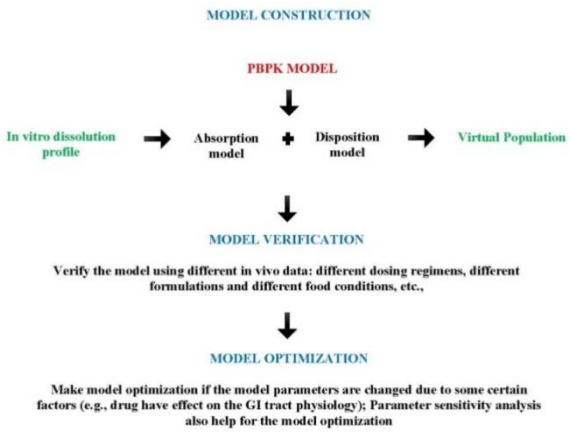

Fig. 2: Workflow of VBE study, Reproduced from "*In silico* modeling and simulation to guide bioequivalence testing for oral drugs in a virtual population" by Zhang F *et al.*, 2021. © The Author(s), under exclusive licence to Springer Nature Switzerland AG 2021 [25]

Integrating the PBPK model with *in vitro* dissolution profiles

Utilizing data from *in vitro* dissolution

Bio-predictive dissolution algorithms have been developed for the VBE study to incorporate the *in vitro* dissolution data. However, the dissolution criteria specified in each nation's pharmacopoeia typically served as quality control measures without considering physiological aspects, including pH, bile salt content, and residence times in various GI tract segments [51]. A bio-relevant medium must be created to simulate how a drug dissolves in the GI system. In the meantime, consideration should be given to how long a drug stays in each area of the GI system, especially for formulations with controlled or extended-release with a significant dissolution cross-domain [52]. Currently, the dissolution environment *in vivo* is frequently simulated using a single non-bio-relevant (e. g., pH 1.2, 4.5, or 6.8 buffer) or bio-relevant dissolution medium (e. g., simulated gastric or intestinal fluid), which is a practical when the majority of the drug is rapidly dissolved in a specific part of the GI tract under a particular pH condition [53, 54]. The are different types of approaches, such as Noyes-Whitney modification [55], fitting data to the weibull model, using lumped parameters [56], estimation of particle size [57], direct incorporation [58], and fitting of deff scales [58] already established for incorporating dissolution in PBPK models. Recently, G Pawar *et al.*, 2023 studied the PBPK modelling using *in vitro* dissolution data to demonstrate the bioequivalence of generic drugs in pediatric and adult populations. *In vitro* dissolution data of carbamazepine immediate-release tablets were used as input to determine the bio-predictive dissolution profile. The *in vitro* dissolution data showed no difference in intestinal and gastric fluid (500 ml), while a dissimilarity in dissolution was observed with a change in the bile salt concentration. The model predicted that 200 ml of media composition and the obtained data were inputted in VBE.

The VBE results demonstrated BE between the two products establishing that the PBPK model can predict the pharmacokinetic profile of carbamazepine, a poorly soluble drug, in adult and pediatric populations [59]. A two-stage dissolution technique is also used to physiologically replicate the passage from the GI tract to the gut. There is currently little knowledge about the dynamic dissolving properties of various formulations in living organisms under different conditions (fasted and fed) in distinct populations [60]. On the other hand, SM Pathak *et al.*, 2019 evaluated the mechanistic modelling of invitro dissolution data to generate drug-specific parameters required for the PBPK models using single and two-stage dissolution. This study was quite a remarkable change in the view of PBPK modelling. The mechanistic modelling approach estimated the intestinal and luminal concentration of dipyridamole. This estimation was only achievable through the two-phase dissolution experiment. Also, the author has established the importance of mechanistic modelling as these tools can simulate the impact of vital experimental parameters, such as dissolution volumes, pH, and paddle speed, on dissolution and precipitation behaviour. This helps identify critical variables that may influence the number or design of *in vitro* experiments [61].

The development of *in vitro* bio-predictive dissolving technologies currently faces several challenges. When *in vitro* dissolution profiles are not bio-predictive, it is essential to identify the underlying causes to improve the predictive capability. Here are two potential reasons and corresponding actions to address them:

Inability to replicate *in vivo* dissolution conditions: *In vitro* dissolution procedures may not accurately replicate the complex conditions of *in vivo* dissolution. Factors such as dissolving medium composition, flow rate, and rotation speed can significantly impact dissolution behaviour. To improve bio-predictiveness, it is recommended to enhance the dissolution procedures by modifying these factors to resemble the pharmaceuticals' *in vivo* dissolution properties. This may involve adjusting the composition of the dissolving medium, mimicking the physiological flow rate, or matching the rotation speed to simulate physiological conditions as closely as possible [62].

Inaccurate mathematical transformations: The dissolution model may not accurately capture the mathematical transformations required to translate *in vitro* dissolution profiles to *in vivo* behaviour. In this case, modifications to the dissolution model are necessary. This can involve refining the mathematical equations, incorporating additional parameters, or adjusting model assumptions to better align the *in vitro* data with *in vivo* observations. These modifications aim to enhance the predictive capacity of the dissolution model and improve its ability to translate *in vitro* dissolution profiles into meaningful *in vivo* predictions [63].

Absorption model

Since reference and test goods with the same active pharmaceutical component have identical disposition mechanisms, a virtual bioequivalence study focuses more on the absorption of pharmaceuticals [64]. Several commercial programs, including GastroPlus®, Simcyp®, PK-Sim®, STELLA®, and B_2O®, are accessible for undertaking VBE investigations. The compartmental absorption and transit models, such as the advanced compartmental absorption transit model and the advanced dissolution, absorption, and metabolism model, are typically used to describe oral drug absorption [65]. In truth, both absorption models' theories are similar in that they divide the GI tract into several anatomical segments based on its physiological function and use a set of differential equations to describe how drugs are translated and permeated throughout the GI tract [66]. This theoretical model was tested by Mitra A *et al.*, 2015, in etoricoxib, whose dissolution profile is crucial for absorption. The dissolution study performed between two formulations manufactured at two sites showed dissimilarity at pH 2.0. But the dissolution profile was similar at pH 4.5 and 6.8. The predictions in this dissimilarity were predicted using the absorption model developed, and the C_{max} and AUC were predicted for all formulations. The predictions made by the absorption model were validated in a bioequivalence study, which confirmed that the tablet batches were indeed bioequivalent. This study highlighted the potential of absorption modelling as a valuable tool in cases where traditional *in vitro-in vivo* correlations (IVIVC) were challenging to develop or when dissolution similarity was not achieved [67].

Similarly, the monte carlo algorithm was used by Karalis VD 2023 in a simulation to analyze the absorption rate metric in bioequivalence. The significance of the study lies in its exploration of an alternative metric, "average slope" (AS), for reflecting the absorption rate in bioequivalence assessments, compared to the traditionally used maximum plasma concentration (C_{max}). The study demonstrates that AS exhibits desirable properties as a metric for absorption rate. It is shown to be more sensitive in detecting differences in absorption rate than C_{max}, which is less suitable for accurately reflecting absorption rate. The findings suggest that using C_{max} alone may provide a false impression of bioequivalence, while AS offers a more reliable alternative [68].

Disposition model

The dispositional component is frequently described using compartmental PK models. One could derive compartmental PK parameters using intravenous data (i.e., the volume of distribution, clearance, and rate constants). When the drug's compartmental model is simple, acquiring these PK parameters can be achieved by directly fitting data to the data [69, 70]. The full PBPK model and the minimum PBPK models were used in various instances. It should be noted that a full PBPK model increases the difficulty and uncertainty of simulation, and it is challenging for the industry to collect modelling data. When human data are unavailable, it is sometimes possible to extrapolate the PK parameters for modelling from animal PBPK models. In this case, it is recommended to consider a different unique construction method called interspecies extrapolation [71].

Virtual population considerations

A virtual population is a necessary component of a VBE study, but essential points must be remembered. First, though most clinical BE studies are typically conducted on healthy adult volunteers, some drugs must be tested on patients with specific conditions (such as cancer) or populations (such as children or older adults). The population database currently included in commercially available PBPK software (such as Simcyp® and GastroPlus®) can be modified

and used for a specific population of the VBE study. Second, estimating the sample size for VBE studies using the same techniques as for clinical BE research is advised. Different study designs employ various methods for calculating sample sizes. Third, the population variability setting is essential to obtaining accurate estimates in VBE investigations. However, previous papers did not consider intra-and inter-subject variability; some VBE research ignored it entirely [72, 73]. In short, either physiological or PK parameters might be increased by the intra-and inter-subject variability. There has been a recent trend toward the idea that some physiological characteristics are connected. Such inter-correlations are also anticipated in modelling, even though it is not geared toward GI physiology. Therefore, when introducing variability, it is essential to consider the interactions between physiological parameters. Adding inter-subject and intra-subject variability improves the model's predictive ability, particularly for visualizing highly variable compounds and calculating the sample size for BE studies, even though it is challenging to determine which approach is more reasonable [74].

Verification of model

PBPK model verification is emphasized in recent FDA and EMA guidelines. The sponsor must submit verification procedures and findings for regulatory assessment to ensure the models' robustness. If the model were tested using simulations of various dose concentrations and administration routes, more confidence would be achieved. Nevertheless, there isn't a single standard for model validation in VBE studies [75]. The FDA guideline's criteria for the internal and external verifications of level A IVIVC were frequently employed in assessing VBE studies. The average absolute percent prediction error for C_{max} and AUC must be less than 10%, and each formulation's percent prediction error must not be higher than 15%, according to the evaluation standard for internal predictability. The 10% prediction error for C_{max} and AUC is the evaluation criterion for external predictability [76-78]. The accurate forecast was likewise determined by a fold error of less than two. In other research, a stricter technique known as the average bioequivalence criterion (90% confidence interval within 80-125%) was also used [79, 80]. It is important to note that the intended usage and regulatory implications should strongly influence the scope of model validation and verification. It is advised that the trial designs of the VBE study be consistent with the clinical bioequivalence trial during model validation, including the type of trial design (e.g., crossover or parallel design), subject information (e.g., numbers, sex ratio, weight, age), and drinking water volume. This will eliminate the impact of trial design variation on the simulations [81].

Optimization of model

Since every drug has different absorption, distribution, metabolism, and excretion characteristics, model optimization should be done on a case-by-case basis. The VBE study offers an overall result incorporating other contributing elements but cannot establish a quantitative link between the modelling result and a particular aspect. A parameter sensitivity analysis (PSA) can be an effective method for model parameter optimization in this situation. A PSA enables the relationship to be quantified and further identifies the parameter that most significantly affects the outcome variation, allowing for model optimization [82]. A PSA contains local and global sensitivity assessments, which permit changing one or more parameters at once to see how the changes may affect the model's output locally or globally. As input parameters for PSA, the formulation factors (such as diffusion coefficient, dose volume, and dissolution), as well as the physiological conditions (such as GI pH, GI transit time, and volume of fluid in the stomach) and active pharmaceutical ingredient (such as particle size, particle density, solubility, and permeability), can be used. It is generally advised against changing several components of the primary analysis at once because it could be challenging to determine which assumptions are to blame for any potential discrepancies observed.

The global PSA can also overcome this problem by considering the inter-correlations between factors. It is challenging to distil the PSA law into a single statement that applies to all medications because it may be affected by various conditions. Conducting the PSA should be done case by case [83].

VBE study challenges and prospects

Compared to other delivery routes, the application of VBE studies in oral medication formulations is more advanced, although numerous difficulties remain to overcome. With only a few applications to BCS class 4 medications, the successful VBE applications primarily concentrate on oral formulations containing BCS classes 1, 2, and 3. Modelling becomes challenging for BCS class 4 medicines with low solubility and low permeability because they are more responsive to GI physiology. While this is the case, patients and particular populations (such as children and older adults) are rarely the subject of VBE studies. It should be noted that under some circumstances, the model simulation cannot be supported by human clinical outcomes [84]. Human bioequivalence data in certain situations led to the conclusion that the test product was not bioequivalent to the reference regarding AUC; however, the model simulation did not recapitulate this conclusion. It was hypothesized that the discrepancy between the simulated and observed results for AUC was due to the simulation's lack of propagating clearance-related inter-occasion variability. There are additional hurdles for VBE studies at the moment, which can be broken down into three categories:

- Limited *in vitro* biopharmaceutical tools with reliable bio-predictive capabilities;
- Limited modelling methodologies with the ability to precisely replicate *in vivo* behaviour using *in vitro* data;
- Limited *in vivo* information (e.g., GI physiology and dynamic body interaction with medications).

Fortunately, new technologies can offer unique solutions to these problems. The advancement of molecular dynamics (e.g., micro-Raman spectroscopy), cutting-edge imaging techniques (e.g., capsule endoscopy, magnetic resonance), and drug makers can lead to a thorough understanding of the drug-body interaction mechanism (e. g., bile acids and their conjugates). Additionally, the accuracy of the models will be substantially improved by the development of bio-predictive tools *in vitro* and model algorithms [85]. This development may help to establish a solid basis for VBE trials of more complex oral medications in various virtual populations. The VBE trials, however, are still in the initial stages for non-oral dose forms. The development of PBPK models for non-oral dose forms such as orally inhaled, ophthalmic, topical dermatological, and transdermal medicines is currently subject to a paucity of research. Several significant problems must be resolved concerning non-oral dose forms:

- Creating bio-predictive *in vitro* techniques that can spot discrepancies between the test and reference formulations;
- Modelling more mechanistic PBPK models with reliable data.
- Establishing a relationship between *in vivo* drug behaviour and *in vitro* data.

Given the inapplicability of conventional evaluation methods, the VBE study is anticipated to grow into a valuable tool for assessing non-oral dosage forms and significantly accelerate the development of novel and generic medications [86].

Also, AI algorithms can automate the integration and analysis of diverse data sources relevant to virtual bioequivalence studies. AI techniques such as natural language processing (NLP) can extract information from scientific literature, clinical trial data, and regulatory documents, enabling comprehensive data synthesis. This assists in enhancing the accuracy and reliability of computational models used in virtual bioequivalence. Machine learning and deep learning can be employed to develop predictive models that simulate drug behaviour, absorption, and distribution. By analyzing large datasets, AI algorithms can identify patterns, relationships, and predictive factors, allowing for more accurate predictions of pharmacokinetic and pharmacodynamic properties. AI can suggest optimized formulations that meet bioequivalence criteria by considering various formulation parameters such as excipients, dosage forms, and release rates. This streamlines the formulation development process and enhances the efficiency of virtual

bioequivalence assessments. AI can facilitate real-time monitoring and surveillance of virtual bioequivalence. By analyzing data from diverse sources, including electronic health records, adverse event databases, and post-marketing surveillance, AI algorithms can identify potential signals and safety issues related to generic drugs. This enables timely interventions, risk mitigation, and continuous evaluation of virtual bioequivalence [87-89].

CONCLUSION

This article reviews the current situation, difficulties, potential for the future, and process of the VBE research for pharmaceuticals. The discussion is primarily centered on all types of drugs; By waiving or improving *in vivo* clinical investigations, it is hoped that VBE studies will advance significantly and address additional concerns for drug applicants and regulatory bodies. This is because we have a thorough grasp of how medications interact with the body and the body's physiology, and we have access to entirely mechanistic *in vitro* and *in silico* technologies. This article concludes the use and importance of virtual bioequivalence in pharmaceuticals and it has been emerging, and many Industries started using it for bioequivalence prediction. In the prospects, to overcome the difficulties in conventional bioequivalence studies, the usage of virtual bioequivalence studies will be, and it will impact great importance in filing ANDA dossier.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest in this article.

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