**Methods used to study drug-drug interactions: A review**

**ABSTRACT**

Drug-drug interaction is a pharmacokinetic parameter which among others determines the fate of drug in giving their therapeutic actions. Most of the drug interactions are studied by using *in vitro* methods though few *in vivo* methods are also applied. The review aimed to summarise the different aspects of methods used to study the drug interactions. In order to collect relevant data on methods used to study drug-drug interactions, different search engines such as Google Scholar, PubMed, Scopus and Research Gate were used for the literature survey. Alongside these search engines, standard textbooks were also consulted to extract relevant information. The findings revealed the different methods used to study drug-drug, drug-metal and drug-disease interactions. Nine in vitro methods used in drug-drug interaction (DDI) analysis have been thoroughly explained and discussed in this review included: UV-VIS absorption spectral analysis,Conductometric analysis, Ardon’s method, Job’s continuous variation method, Differential Scanning Calorimetry (DSC) thermogram, FTIR spectroscopic investigation, Thin Layer Chromatography technology, all of these were used to detect the interaction between two hypothetical drugs referred to as DRUG A and DRUG B,Fluorescence spectroscopy(used to examine the interaction between tannin and bovine serum albumin), and high-performance liquid chromatography (HPLC)(used to detect the interaction between tramadol and sildenafil). Additionally, in the case of the *in vivo* method, young healthy animals having different body weights are used. Lastly, three *in-silico* methods used in drug-drug interaction analysis have been discussed included: Quantitative Structure–Activity Relationship (QSAR) Models, Molecular Docking and Molecular Dynamics (MD) Simulations, and the Physiologically Based Pharmacokinetic (PBPK) Modelling. Overall, the study confirmed Organic Anion Transporting Polypeptide 1B1 (OATP1B1) plays a key role in the pharmacokinetic behaviour of the statins and serves as a useful indicator for identifying transporter mediated drug-drug interactions. Therefore, incorporating OATP1B1 assessment into DDI studies is essential to enhance prediction reliability and to improve drug safety profiles.

**Key words**: Drug-drug interaction, Spectral analysis, conductometry, Ardon’s method, Job’s method, co-morbidity and combination therapy.

**INTRODUCTION**

From a chemical, biological, and medical perspective, research on how drug molecules interact and complex with other drug species, herbs, different metal ions, and food components is essential

(Goldstein et al., 1974; Brunton et al., 2005; DGDA, n.d.; Bennett & Brown, 2008; Sinko, 2017; Hansten & Horn, 1989; Cadwallader, 1985).Patients undergoing a major surgery, hospitalised patients, patients with co-morbidities, and elderly patients are frequently prescribed multiple medications at once, a practice known as polypharmacy.Simultaneous use of more than two different drug classes may sometimes produce effects that are harmful or ineffective but in some cases,it may be beneficial(Azam et al., 2011; Mohiuddin et al., 2010). Again,various mineral supplements combined with medication therapy are a popular and effective way to treat illnesses where both medications and minerals are administered at the same time. The drugs may function on their own or may interfere or interact with one another. Such interaction can be agonistic or antagonistic.Patients may benefit from combination drug therapy in certain situations, but they may also experience severe side effects. Numerous drugs are commonly prescribed to patients with conditions such as kidney, liver, and/or heart transplantation or failure, diabetes mellitus and hypertension, anaemia, bone and lipid disorders, and so on. Drug-drug interaction might also affect the structure of the final product and cause formulation problems like tablet splitting (Shahriar et al., 2020). Drug-drug interaction is closely related with the drug safety and pharmacovigilance (Hossain & Amran, 2019). There may be a higher chance of drug interactions when taking many drugs at the same time. Drug interactions can be divided into two categories: pharmacokinetic and pharmacodynamic.Induction or inhibition of metabolising enzymes in the liver or elsewhere, drug displacement from plasma protein binding sites, changes in gastrointestinal absorption, or competition for active renal secretion are all examples of pharmacokinetic interactions.Pharmacological effects that are antagonistic or additive are examples of pharmacodynamic interactions. Therefore, pharmacological effects are undoubtedly altered by drug interactions.A moderate interaction can potentially worsen the patient's clinical condition leading to the need of further treatment, hospitalization, and/or a longer hospital stay.Major interactions can lead to life threatening conditions or cause irreversible damage to organs. Therefore, in our current review work we have summarized the different aspects of drug interactions( Bari et al., 2000; Ahsan et al., 2012; Paul Kundu et al., 2012; Ahsan et al., 2012; Afzal et al., 2013).

**MATERIALS AND METHODS**

To collect relevant data on methods used to study drug-drug interactions, different search engines such as Google Scholar, Pub Med, Scopus and Research Gate were used for the literature survey. The keywords, “DDI (Drug-drug interaction)”, “Spectral analysis”, “conductometry”, “Ardon’s method”, “Job’s method”, “co-morbidity” and “combination therapy” etc. were used to find relevant research articles and gather information on different aspects of drug-drug interactions. Alongside these search engines, standard textbooks were also consulted to extract relevant information.

**FINDINGS AND DISCUSSION**

In this section, we shall discuss different methods used to study drug-drug, drug-metal and drug-disease interactions, primarily highlighting in vitro methods.

***In vitro* methods used in drug-drug interaction analysis**

The *in vitro*interaction studies (Aktar et al.,2020 )are carried out by analysing UV-VIS absorption spectra, conductometric data, Job’s method of continuous variation and Ardon’s spectrophotometric techniques. A description of each method is provided below. A few other studies are included.(Saha et al.,2015)

**(i) UV-VIS absorption spectral analysis**

Spectroscopy is defined as the interaction between matter (chemical compounds) and electromagnetic radiation (simply light). Ultraviolet and visible (UV-VIS) spectroscopy is an excellent non-destructive analytical technique useful for a large number of organic compounds and some inorganic compounds. As a function of wavelength, UV-VIS spectrophotometers measure the absorption or transmission of light that passes through a solution of compounds having very low concentrations.The normal wavelength range of UV-VIS is around 200 nm to 800 nm. From 200 to 400nm is used for UV and 400-800nm is used for visible light. The measurement is based on Lambert-Beers law(Chatten,1969) .The simple mathematical formula of this law is -

A=abC

Where,

A = Absorbance or optical density

a = Absorptivity or specific extinction co-efficient (constant).

b = Pathlengthof cell(in centimeters) or thickness of the solution through which incident light passes (constant and 1centimeter)

C = Concentration of solute (drug) in the solution(s) (gram/Liter).

This law is valid only for very dilute and transparent solutions. Absorbance is measured at different wavelengths and the obtained values are plotted by placing absorbance values at the ordinate and wavelength values at the abscissa (**Figure 1a)**.

A spectrometer is a machine that measures the optical activities of a solutions. This is performed in two ways – (i) Photometric mode and (ii) Scanning mode. The response obtained from the spectrometer is called spectrum. A schematic diagram of a spectrum and a spectrometer is shown in **Figure 1b**.

|  |  |
| --- | --- |
| (a) | (b) |
| **Figure 1**: A simple schematic diagram of (a) UV-VIS spectral analysis and (b) a spectrophotometer. | |

For drug-drug and drug-metal interaction study(Aktar et al.,2020), dilute solutions of individual drugs at different concentrations (for example, DRUG A and DRUG B) are prepared using different buffered pH solutions such as pH 1.4, 3.4, 6.4, 7.4 and 8.4. The absorbance of each drug solution is measured separately at its specific wavelength and recorded. After that, equal volumes of the two drug solutions are mixed in a 1:1 ratio, stirred thoroughly to ensure proper mixing, and left undisturbed for a certain period to allow the interaction to occur.Then, their absorbances are measured using specific wavelength. The absorbances and the spectrums of individual drugs are compared with the data that are obtained from the mixtures of two drugs. The experiments are repeated to avoid any error and biasness. The pH solutions are prepared using standard textbook(21)Works of other investigators can be consulted for further clarification ( *Evaluation of in vitro interaction*, n.d.; Amran et al., 2007; Amran et al., 2006; Amran et al., 2008; Salam et al., 2009) Other examples of the application of the UV-VIS spectral analysis include in vivo protein binding studies of drugs in rat models ( Aktar et al., 2021; Chowdhury et al., 2021; Karim et al., 2016).

Spectrofluorometry can also be used to study drug-drug interactions(Nahar et al., 2016).

**(ii) Conductometric analysis.**

Conductometry is a measurement of electrolytic conductivity to monitor a progress of chemical reaction one of which is drug-drug interaction, a very reversible and first order reaction. Conductometry has application in analytical chemistry, where conductometric titration is a standard technique. Conductometry is often applied to determine the total conductance of a solution or to analyze the end point of titrations that include ions. Conductometric titrations follows the general principle for acid-base titrations ( Deepa et al., 2016; Braun, n.d.; Khopkar, 2007) Basic and its principle is based on Ilkovic equation the simple mathematical form of which is –

Where,

id - In microamperes(µA), id is the diffusion current 607 - The constant 607 is the sum of a variety of numerical factors, including the Faraday constant (P), the density of mercury, etc.

n - In electrode reactions, n is the number of electrons involved,

D - The diffusion coefficient D in square centimeters per second (cm²/s)

m – Weighed by milligrams per second (mg/s) of the flow of Hg through the capillary,

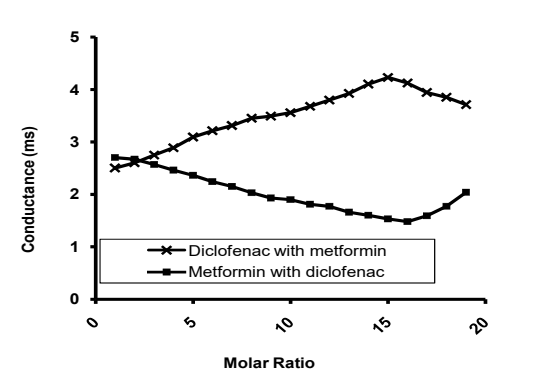
t - Time in seconds(s)(Drop time),

C - The concentration C (mmol/L) is the concentration of electroactive solute present in the solution.

d - in the subscript form indicates the term diffusion.

In simple mathematical expression it can be expressed as –

Conductometric titrations are performed to determine the molar ratios between the interacting agents and the drug molecule in the complex along with identifying the formation of complex of a drug (say DRUG A) with another drug (say DRUG B). Usually,a 100 mL beaker is filled with a diluted solution of one drug (A 0.005 M solution of DRUG A,with a volume of 40mL), and the drug is titrated separately by gradually adding another drug from a burette that has a higher concentration (0.05M,DRUG B). In reverse,40 mL volume of DRUG B at 0.05M concentration, is titrated with gradual addition of 0.005M of DRUG A. Conductance readings (expressed in mS or µS) are graphed against the molar ratios of the two DRUGS (DRUG A and DRUG B) within the system.The breaks observed in the curves obtained from the titration indicate potential site of interactions.To simulate the Ph levels in different body chambers ,conductometric titrations are carried out using solutions that have been adjusted to pH 1.4, 3.4, 6.4, 7.4 and 8.4. The pH solutions are prepared using standard textbooks ( Bates, 1964; Perrin & Boyd, 1974). Conversely, each of the drug having similar concentrations (40 mL of 0.005M) are titrated with standardized 0.05M NaOH solution and the conductance readings (expressed in mS or µS) are graphed against volume of NaOH. A conductometric titration graph (Saha et al., 2013) is shown in Figure 2.



**Figure 2.** Conductometric titration of metformin with diclofenac at pH 7.4 (Reproduced with permission from (Saha et al., 2013).

**(iii) Analysis of drug-drug interaction using Ardon’s method**

Ardon’s method is a spectroscopic method based on Lambert-Beer’s law to analyze drug-drug interaction studies.This method involves varying the concentrations of one drug (DRUG A) while concentrations of another drug (DRUG B) remain constant.By using a UV-VIS spectrophotometer, the absorbance of solutions with pH values of 1.4, 3.4, 6.4, 7.4, and 8.4 is determined at a specified wavelength for DRUG A and DRUG B.Calculations are carried out using Ardon’s equation. The Ardon equation is a spectrophotometric equation used to calculate the stability constants of 1:1 complex between drugs( John & Ost, 1974;Vogel, 1961; Ardon, 1957) The equation is -

𝐷 - mixture's absorbance.

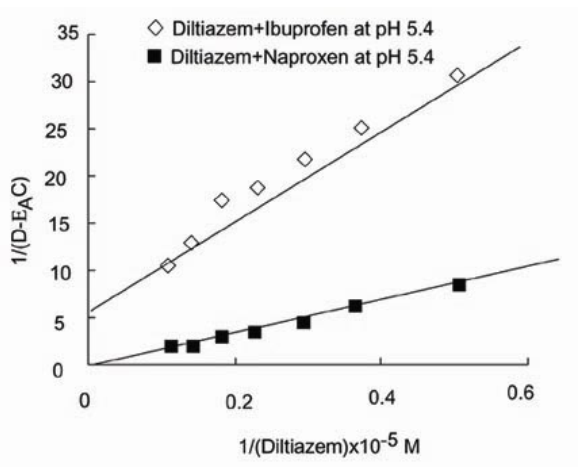
𝐵–One drug's molar concentration

𝐶 - The other drug's molar concentration

𝜖𝑐𝑜𝑚 -Complex's molar absorptivity

𝜖𝐴–Molar absorptivity of one drug

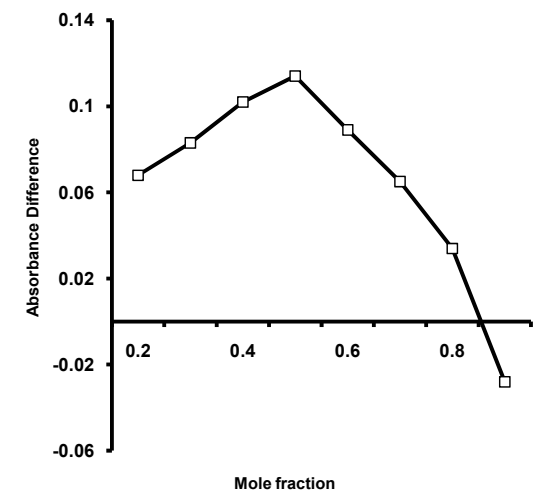
As a prerequisite for method validation, the value of n is set to 1. The satraight or linear lines are obtained by plotting the data foragainst .DRUG A's concentration, represented by the letter "C" in the equation, is maintained at 5x10-5M .In the graphs, the 1:1 complex produces a straight line with an intercept and slope.K=intercept/slope which is the complex's stability constant.This approach is suitable only for systems where a 1:1 complex mixture is formed, as illustrated in **Figure 3.**



**Figure 3**. Plots based on Ardon's method for the system of diltiazem-ibuprofen and diltiazem- naproxen  (Salam et al., 2009).

**(iv) Job’s continuous variation method**

Job’s continuous variation method is also a spectroscopic method based on Lambert-Beer’s law for analysis of drug-drug interaction study. In Job’s continuous variation method which is based on spectrophotometry, absorbance readings of series of drug mixtures with various molar ratios at different pH (pH 1.4, 2.4, 6.4, 7.4 and 8.4) are measured at specific wavelength of drugs by maintaining a constant total moles count in the mixture.Experiments of the same type using reversed drug concentrations are conducted at various pH levels(pH 1.4, 2.4, 6.4, 7.4 and 8.4) and the absorbance values of these systems are recorded at various wavelengths.The mixture's observed absorbances at different mole fractions are deducted from the total of the absorbance values measured for the free drugs under the same experimental setup.



**Figure 4**. Job’s plot for metformin-diclofenac system at pH 7.4 (Reproduced from (Salam et al., 2009).

After that,a graph is plotted showing the absorbance differences(D) versus the mole fractions of the drugs in the mixtures generating a curve with a maximum at a specific point that reflects the molar ratios of one drug to another drug within the complex. A Job’s plot(26)is shown in **Figure 4**. Similar results were also found from other studies ( Chowdhury, 2023; Flora et al., 2023)

**(V) Differential Scanning Calorimetry (DSC) thermogram**

Differential Scanning Calorimetry (DSC) is a thermo-analytical method which is used to find the difference in heat flow between an unknown sample and a standard material as a function of temperature. It gives information about thermal transitions and reactions(Google, 2025).

DSC thermogram of DRUG A showed a long, sharp, characteristic endothermic peak at a particular temperature (in°C) with specific amount of free energy (ΔH in J/g) corresponding to the melting point of DRUG A. DRUG B shows a broad endothermic peak at another temperature (°C) representing the evaporation of water molecule from the crystal lattice and an exothermic event was observed at different temperature (°C) which could be related to acrystalline state transition. DSC curves of the physical mixture have evidenced significant shifts of the endotherm compared to the endotherm of the pure drugs ( Nabi et al., 1974; Siraji et al., 2011).

**(vi) FTIR spectroscopic investigation**

FTIR (Fourier Transform Infrared) spectroscopic investigation is an analytical technique that uses infrared light to analyze the chemical structure and composition of materials by examining their unique vibrational and rotational spectra(Google, n.d.). The FTIR imaging determines the functional groups in the studied drugs (DRUG A and DRUG B) and in their 1:1 and 1:2 mixtures. The absorption frequency corresponding to important functional groups of DRUG A and DRUG B can be obtained. The FTIR spectra of DRUG A-DRUG B complexes were judged against those of the pure drugs (Dun et al., 2013).

**(vii) Thin layer Chromatography**

Thin Layer Chromatography technology (TLC) is useful to separate a complex that has been formed as a result of interaction between two species. TLC typically employs a plate covered with silica gel, which serves as the stationary phase. The plate is“spotted” with sample solution, then placed in a jar with the appropriate solvent. Because of capillary action the solvent rises along the plate and elutes the species at different rates depending on the individual polarity. Multiple solvents are employed in order to get higher resolution of separation. The separation process is determined by the respective affinities of the components for the stationary and mobile phases. Compounds travel along the surface of the stationary phase when the TLC plate is run in solvent. Compounds with higher affinity for the stationary phase move more slowly through the plate. Furthermore, molecules with a higher affinity for the mobile phase move faster than others. Distinct spots were visualized and identified with the help of a hand-held UV light (long and short wave) as well as with the use of iodine chamber. The Rf values are calculated and used to confirm the identity of the chemical species (Aktar et al., 2021).

**(viii) Fluorescence spectroscopy**

By using variations in fluorescence intensity, wavelength shifts, or quenching effects, fluorescence spectroscopy can identify and analyze chemical interactions. It works on the basis of the idea that certain molecules produce light when excited by a particular wavelength. Drug–drug interactions (DDIs), particularly those involving protein binding or competition for biological macromolecules, are ideally studied using this approach. Fluorescence spectroscopy is frequently employed in DDI research to track the effects of one drug on another's ability to bind to proteins, such as serum albumins. Fluorescence spectroscopy was employed to examine the interaction between tannin and bovine serum albumin (BSA), identifying hydrophobic interactions and a stationary state elimination process. Such studies highlight fluorescence spectroscopy’s utility in revealing molecular-level changes and potential pharmacokinetic consequences of DDIs. In another study, Fluorescence spectroscopy was used to study the binding of gefitinib (GEF) to human serum albumin (HSA), revealing a static quenching mechanism and moderate binding affinity (Ka = 1.70 × 10⁴ M⁻¹). The decrease in Stern–Volmer constants with temperature confirmed static quenching, while thermodynamic data suggested hydrophobic and hydrogen bonding interactions. This example shows how fluorescence spectroscopy helps identify binding strength, quenching type, and interaction sites—key aspects in drug–drug interaction analysis (Kabir et al., 2016).

**(ix) HPLC**

Drugs and their metabolites can be precisely separated, identified, and quantified using high-performance liquid chromatography (HPLC), an analytical method based on the differential partitioning of substances between a mobile phase and a stationary phase. When two or more medications are supplied together, it is often used in DDI studies to track changes in drug concentrations. High sensitivity and selectivity can be attained by HPLC, depending on the detection system (e.g., UV, fluorescence, or mass spectrometry). For example, HPLC techniques have been extensively used to evaluate the effects of different substances on the plasma levels of medications that are provided together, exposing quantifiable pharmacokinetic changes. These studies demonstrate how effective HPLC is in quantitatively evaluating DDIs in preclinical and clinical contexts. In a study, An HPLC method with UV detection was developed to study the drug–drug interaction between sildenafil (SDF) and tramadol (TMD) in rabbit plasma following oral co-administration. Using a C18 column and a phosphate buffer–acetonitrile mobile phase, the method enabled simultaneous quantification of both drugs with high sensitivity (LOD: 0.05 μg/mL for TMD and 0.02 μg/mL for SDF). Pharmacokinetic analysis showed that TMD altered SDF’s Cmax, Tmax, AUC, and elimination rate, indicating a significant interaction. This study demonstrates HPLC’s strength in quantifying co-administered drugs and evaluating in vivo pharmacokinetic changes in DDI research (Dahshan et al., 2019).An additional RP-HPLC Method for the Quantification of Omeprazole in Pharmaceutical Dosage Form was established and validated for the purpose of analysing drug-drug interaction(Kayesh et al., 2013).

***In vivo* methods**

In the *in vivo* studies(Amran et al., 2007) young healthy animals (mice, rats, guinea pigs, rabbits etc.)having different body weights are used.Pentobarbital sodium (50 mg/kg) is injected intraperitoneally to anesthetize the animals.Artificial ventilation through the tracheal cannula maintains respiration in order to keep pCO2, pO2 and pH at a normal level.Drug administration is carried out by inserting a polyethylene tube into the left femoral vein.To check the blood pressure,a mercury manometer is attached to the cannulated common carotid artery.After stabilising for 15 minutes, 0.5 ml of saline is administered as a vehicle or control, and normal blood pressure is noted.A dose equivalent to 1 milligram per kilogram of body weight of DRUG A, along with equal mixtures(1:1) of DRUG A + DRUG B and DRUG A+ DRUG C, are intravenously injected as bolus doses through the femoral vein into separate groups for each dose of the vehicle or drugs, and the resulting blood pressure responses are noted.Only a single dose (treatment) of either the vehicle or any of the drugs was given to each animal ( Sayeed & Rana, 2013; Kundu et al., 2015).

**Intermolecular forces involved in drug interactions**

The intermolecular forces involved in drug interactions are- (i) Van der Waals forces (dispersion, dipolar and induced dipolar), (ii) Hydrogen bonding, (iii) Coordinate covalence, (iv) Charge transfer, (v) Hydrophobic interactions (Goldstein et al.,1974). A detailed description is out of context of this article. Interested readers may consult (1,2)( Goldstein et al.,1974;Brunton et al.,2005) for further studies.

***In silico* methods used in drug-drug interaction analysis**

1. **Quantitative Structure–Activity Relationship (QSAR) Models:**

QSAR models are computational techniques that predict the biological activity of molecules based on their chemical structure. In QSAR modeling, various chemical descriptors (such as molecular weight, lipophilicity, electronic properties, hydrogen bond donors/acceptors) are mathematically correlated with biological outcomes like enzyme inhibition or induction. When applied to DDIs, QSAR models can predict whether a new drug is likely to inhibit or induce a metabolizing enzyme (e.g., CYP450 enzymes) and thus interact with other drugs. Modern QSAR models, like those built with PASS (Prediction of Activity Spectra for Substances) or PoSMNA descriptors, offer high-throughput prediction of interactions without the need for physical testing.

In a study (Dmitriev et al., 2021) the researchers developed computer models to predict drug–drug interactions (DDIs) mediated by seven major cytochrome P450 enzymes (CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, and CYP3A4). They used about 2500 known DDI records to build structure–activity relationship (SAR) models, applying the Prediction of Activity Spectra for Substances (PASS) software and Pairs of Substances Multilevel Neighbourhoods of Atoms (PoSMNA) descriptors based on molecular structures. The models achieved an average prediction accuracy of around 92%, evaluated through leave-one-out cross-validation. These SAR models are publicly available and can be used to predict potential DDIs for both existing drugs and new, not-yet-synthesized compounds.

1. **Molecular Docking and Molecular Dynamics (MD) Simulations:**

**Molecular Docking** simulates the binding orientation and affinity between a small molecule (drug) and a target protein (such as an enzyme or transporter). **Molecular Dynamics (MD)** simulations model the physical movement of atoms and molecules over time under defined conditions (e.g., temperature, pressure). Docking helps predict how strongly a drug can bind to an enzyme (e.g., CYP450, P-glycoprotein), which informs whether it might inhibit the enzyme and cause DDIs. It calculates binding energy and identifies interactions like hydrogen bonds, hydrophobic contacts, etc. Molecular dynamics refines this prediction by simulating the real-time behavior of the drug-protein complex, checking if the binding is stable, flexible, or transient. MD can uncover important phenomena such as conformational changes or induced fit effects that simple docking may miss. A study used molecular docking to investigate its interaction of GNF-351, which is a drug candidate that antagonizes the aryl hydrocarbon receptor, with ketoconazole. Researchers modeled the binding of GNF-351 to the human cytochrome P450 (CYP3A4) structure, generating 30 possible orientations. Docking results showed GNF-351 interacts with CYP3A4 through its aromatic rings, particularly near Phe302 and Phe304, similar to ketoconazole's binding position relative to the heme group. These findings suggest potential interactions between GNF-351 and ketoconazole, highlighting the need for careful monitoring if used together (Liu et al., 2015).

1. **Physiologically Based Pharmacokinetic (PBPK) Modeling:**

Physiologically Based Pharmacokinetic**(**PBPK) models are computational models that simulate the ADME (Absorption, Distribution, Metabolism, and Excretion) processes of drugs within a virtual organism based on physiological parameters.

PBPK models use real biological data (e.g., organ sizes, blood flow rates, enzyme expression levels) and drug-specific properties (e.g., solubility, permeability, metabolism rate) to create a "virtual human" or "virtual animal". These models predict how a drug moves through different tissues and how it is metabolized. For DDIs, PBPK models are used to simulate scenarios where one drug inhibits or induces the metabolism of another drug. Regulatory agencies (like the FDA) accept PBPK simulations as evidence for DDI risk assessment in drug development. Predicting drug–drug interactions (DDIs) with statins is challenging due to the involvement of both metabolizing enzymes and transporters. In a study (Duan et al., 2017), physiologically based pharmacokinetic (PBPK) models for pitavastatin and atorvastatin were developed using in vitro and clinical data in SimCYP®, accounting for both enzyme and transporter contributions. The models successfully predicted DDIs involving OATP1B1 polymorphism and interactions with itraconazole, erythromycin, and gemfibrozil but under-predicted DDIs with rifampin and cyclosporine. Further analysis suggested that existing models underestimate OATP1B1 inhibition by rifampin and cyclosporine. Overall, the study confirmed OATP1B1’s key role in the pharmacokinetics of these statins and identified reasons for DDI prediction discrepancies.

**CONCLUSION**

This review emphasised that DDI study is an important pharmacokinetic parameter and proper methods are needed to study this vital parameter. It may be underlined that only two drugs are able to induce the development of a DDI even if this clinical relevance is related to the pharmacology of each drug (Palleria et al., 2013). In fact, a DDI will be able to induce a clinically relevant effect in the presence of drugs with a low therapeutic index, a long half-life and a higher bound with plasma proteins. Mechanism-based pharmacokinetic modelling and simulation are useful for comparing hypothesised combination regimens and have multi-scale, translational potential (Niu et al., 2019).

Disclaimer (Artificial intelligence)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1. Name: ChatGPT,Model: GPT-3.5,Version: gpt-3.5-turbo. I used it to get idea of sentence making while editing the manuscript.I didn't directly copy and paste that. I just got idea and then adapted and rewrote the sentences in my own way.

2.

3.

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