# AN EVOLVING ROLE OF PHARMACOKINETICS IN DRUG DISCOVERY

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***ABSTRACT-*** ***With the advancing technologies, the drug discovery and development processes are providing a wide platform for new molecular synthesis, formulations and in-vitro analysis, followed by in-vivo screening to evaluate more complex properties and processes. Preclinical and pharmacokinetic studies play a vital role in drug development processes including lead identification and optimization. The results are analyzed by a final validation in animal models, and ultimately in humans. Pharmacokinetic and metabolism characteristics provide a basis for designing appropriate human clinical trials.***

***KEY WORDS:- DMPK, drug discovery; in-vivo; in-vitro, ADME , mass spectrometry.***

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**INTRODUCTION-**

Drug discovery and development involves complex and multidisciplinary process which includes expertise from different backgrounds such as medicinal chemistry, pharmacology, preclinical development, safety assessment, clinical development and regulatory affairs and drug metabolism (and pharmacokinetics) where scientists play a crucial role in interfacing with the various disciplines (Fig. 1) [1]. In early discovery, drug metabolism input provides a platform for the selection of chemical structures and lead compounds having desirable drug metabolism and pharmacokinetic (DMPK) or safety profiles and later, preclinical data aids in the development of clinical plans with regard to human drug exposures and safety. The rejection of any NDE's(New drug entities) is due to undesirable DMPK properties. DMPK principles used for drug candidate optimization, selection and characterization during the drug discovery and development process.

Traditional drug metabolism research focused on areas such as absorption, distribution, metabolism and excretion (ADME), with particular emphasis on in vivo and in vitro metabolite identification, enzymology of DMEs, and associated metabolic pathways and reaction mechanisms. In order to fully maximize the impact of these emerging sciences on drug metabolism and the drug discovery and development process, the design and conduct of drug metabolism studies and interpretation of results must take into account these advances. Drug metabolism scientists must also in depth understanding of the discovery and development process, in order to design timely and appropriate studies that are in alignment with the traditional drug discovery and development process [2][3][4].

The main purpose of Discovery is to screen large numbers of compounds in order to select ideal candidates only for development phase, hence require technologies with high throughout capabilities. These methods can either be in vitro, in silico, in situ and isolated organs or in vivo, and vary greatly with regards to their physiological relevance, time and cost. On the other hand, development studies require more in depth analysis of a single compound, employing methods that have been thoroughly validated.

The complexity of the drug discovery process can be a contributing factor, along with a combination of specific (and interrelated) factors, including solubility, pKa, absorption, bioavailability, metabolism, formulation, pharmacokinetics, toxicity and therapeutic efficacy [5].

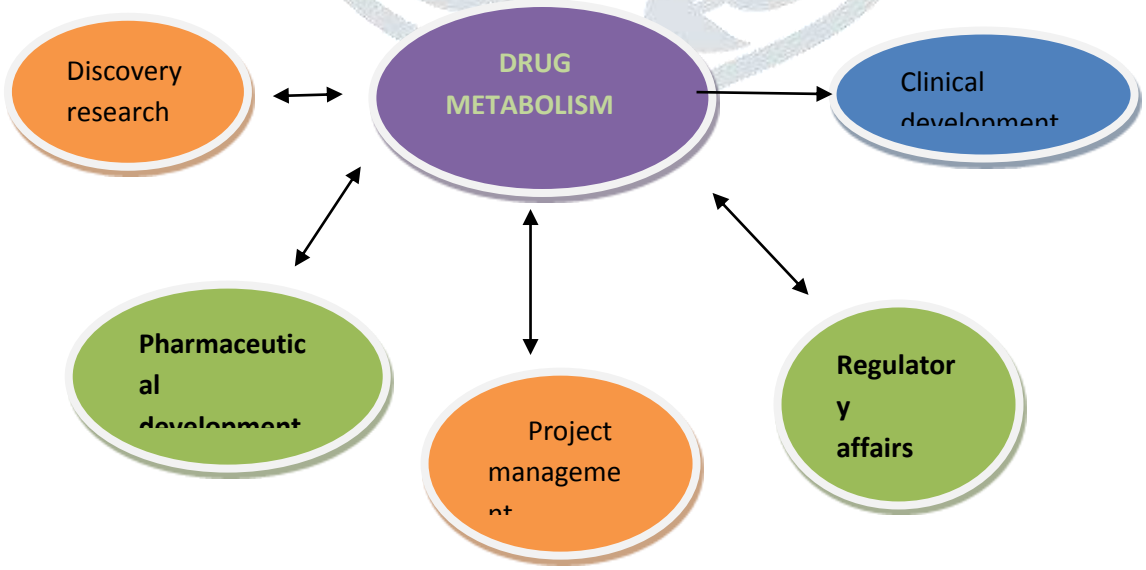


Fig 1: **Role of Pharmacokinetics**

Thus, DMPK is playing an increasingly important role in drug discovery [6][7][8][9][10]. Along with potency and selectivity, drug candidates are now selected on the basis of DMPK properties, eg, low clearance, good oral bioavailability, and an acceptable profile of metabolism in both human

and non-human tissues. This leads to greater implementation of DMPK properties into increasingly early stages of the drug discovery process. In response to this, the throughput capabilities of both pharmacokinetic (PK) and *in vitro* metabolism assays have increased substantially.

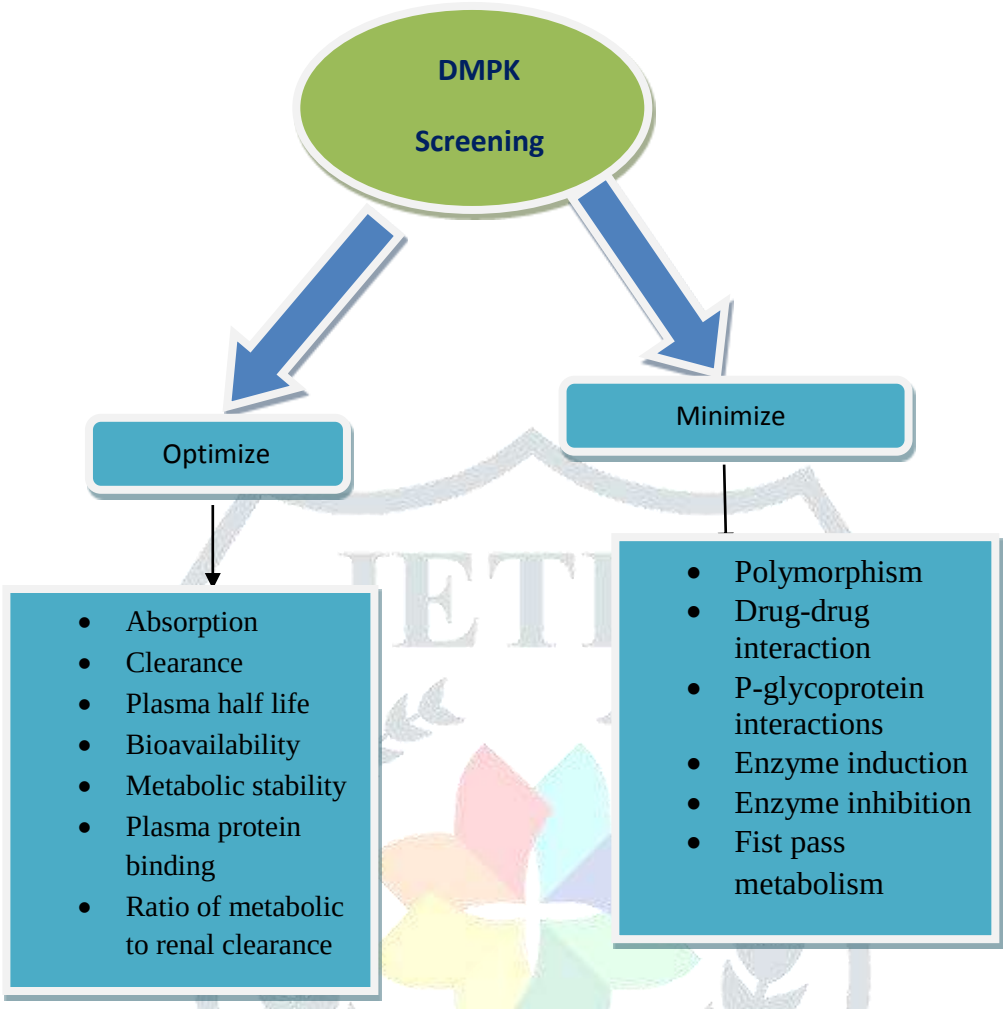


Fig 2: **Main objectives of DMPK screening**

***In-vitro* DMPK:** During the modern synthesis methods, many molecules are generated for screening purpose. Many of the molecules having high molecular weights, high log P value leads to low aqueous solubility. Many developments take place on a molecule to increase its lipophilicity by introducing a lipophilic moiety (eg. A methyl grp) to fit into a receptor is one of the best way to improve *in-vitro* potency but it will also increase lipophilicity to a greater extent which leads to face problems in its excretion from the body. New methods are used to determine solubility via Pharmacokinetic and pharmacodynamic solubility methods which forms the basis of experimental solubility data [11][12].
Oral route is the common route for drug administration, which includes number of challenges, majorly the assessment of bioavailability. *In-vitro* models to assess bioavailability includes cryopreserved pooled human liver microsomes, hepatocytes [13][14] and other liver tissue preparations (eg, S9 fractions and slices), which provide an easy to use *in vitro* system for analyzing compounds for their metabolic stability, metabolite profile, cytotoxicity. These systems can predict *in-vivo* stability.

***In-vivo* DMPK:** *In- vivo* screening is necessary to reduce the chance of toxicity before the drug administration in the body. In addition, it helps in predicting PK data prior to assessment in humans. PK studies done in different animal- models based on the type of disease to estimate the safety, effectiveness and the toxicity level related to drug. Use of both oral and intravenous routes provides general PK profiles of bioavailability, plasma elimination half-life, clearance, mean residence time and volume of distribution. The samples obtain by PK studies are analysed by mass spectrometry, which provide a idea regarding the properties of drug i.e., whether the drug is safe and effective for is use in the humans.
Another important development is the use of cannulated animals [15], which will decrease the number of animals used in the experiment. It provide information related biliary excretion of the drug to ensure the excretion of drug and its metabolites from bile in a major extent. Advances in cannulation techniques have also permitted the intermittent or continuous collection of bile over a sustained time period. Distribution of a drug in various tissues and organs is determined by the administration of drug to the animal followed by the collection of different organs and to determine that in which organ maximum amount of drug is retaining.

**DRUG DEVELOPMENT PROCESS:**
The drug discovery process emerging at a great scale due to development in technologies lead to target identification along with automation of combinatorial synthesis and high throughput screening (HTS). New chemical entities (NCE's) enter the drug discovery pipeline through

combinatorial synthesis and rational drug design where information about the target of action is used to design the lead molecule. HTS helps in the identification of the lead at its required concentration to produce desired action. In the secondary screening stage physicochemical properties like solubility, lipophilicity and stability are determined by measuring the octanol –water partition coefficient and pka. These measurements are useful in predicting the protein binding, tissue distribution and absorption in the gastrointestinal tract[16].The selected leads are further passed through the *in-vitro* and *in-vivo* screening commonly known as preclinical trials.

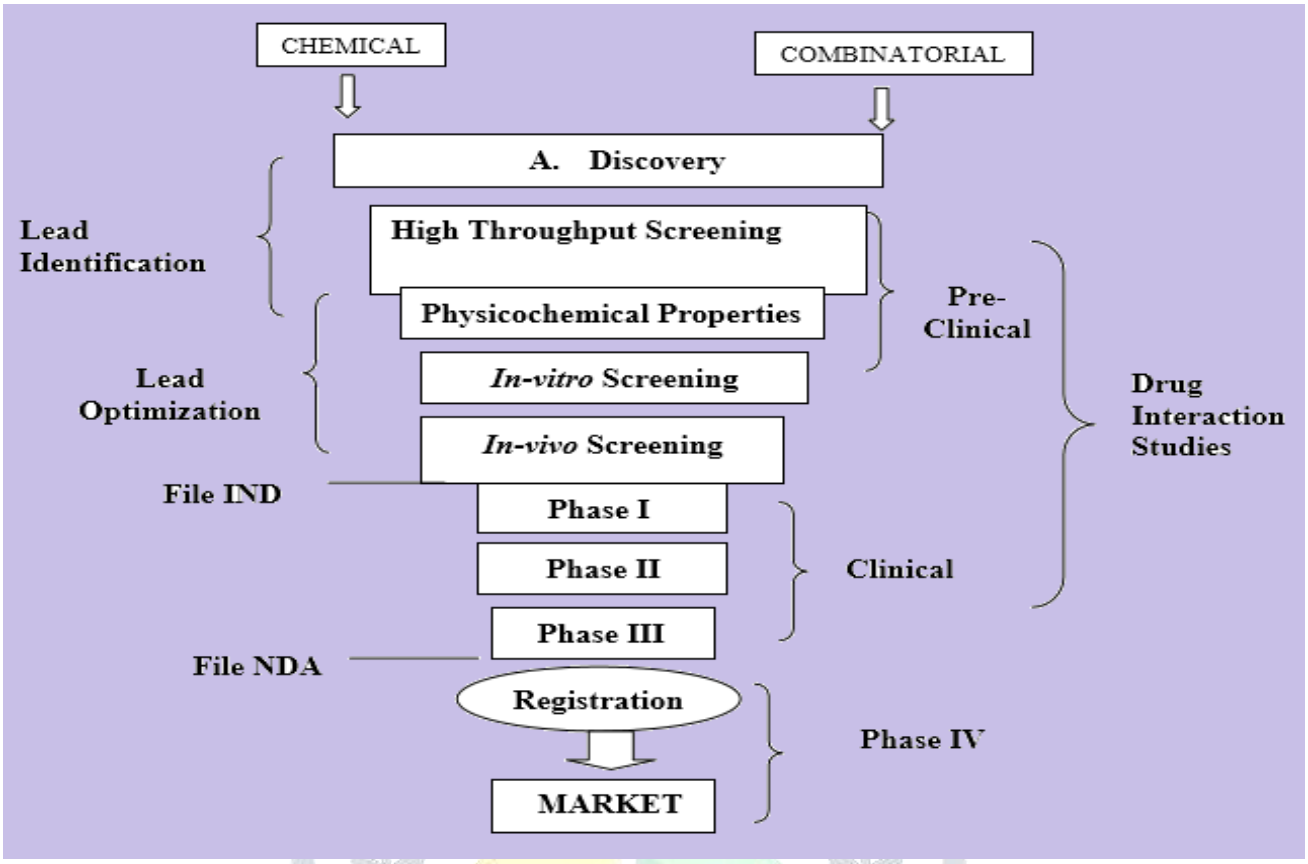


Fig 3: **Various stages of drug discovery**

*In-vitro* tests are carried out for the lead molecule in the lead optimization. The goal of lead optimization is to select the molecules which have specific biological activity in humans. Pharmacokinetic parameters like tissue penetration, intestinal absorption, stability, metabolism and elimination are estimated during *in-vitro* screening. The knowledge about the toxicity and its toxic metabolites should be well known for further drug development process. During this stage of drug development only few molecules found to be safe and efficacious to be taken for further development[17].Then, finally the lead molecule undergoes in-vitro and in-vivo studies which not only focuses the lead molecule with safety profile but the animal model also having the same toxicity profile with the humans. By the characterization of pharmacokinetic and metabolism, it helps in designing appropriate clinical trials.

**NEED OF *In-vitro* RESEACH**

- By the help of *in-vitro* data we can estimate the success or failure when the drug will undergo preclinical studies.
- The degree of selectivity and interaction between the drug and the target site is established.
- To estimate the un-wanted effects on the target site and the chances of reason of drug withdrawl from the market can be determined.
- Long -term effects of the drug to be monitored and observed, as well as determining the bioequivalency, safety, dosing regimen, positive and adverse effects, and the drug-drug interactions in a living system[18][19][20].

**NEED OF *In-vivo* RESEACH**

- It provides necessary information on the mechanism of action of drugs which is useful for making hazard-based decisions and to minimize the side effects.
- To study the dispersions and permeability of drugs inside an animal model.
- Provides a detailed information on how drug substance absorbed or drug-drug interactions within a system.
- Provides a quantitative interpretation of absorption, distribution, metabolism, and excretion in animal and human models [21][22].

- *In vivo* conditions are crucial for the rapid screening and assessment of formulations

**PHARMACOKINETICS**

Pharmacokinetics provides a mathematical basis to assess the time course of drugs and their effects in the body. It involves the following processes to be quantified: Absorption, Distribution, Metabolism, and Excretion. These pharmacokinetic processes often referred to as ADME [23]; which determine the drug concentration in the body. A fundamental understanding of these parameters is required to design an appropriate drug regimen for a patient [24][25]. Clinical pharmacokinetics pertains to the application of pharmacokinetic principles to individual patients, in order to safely and effectively manage drug therapy. With an understanding of pharmacokinetics, pharmacist can increase the effectiveness, decrease the toxicity, or increase patient compliance with a therapeutic regimen. The majority of the adverse drug reactions seen in the clinic are dose-related, thus an understanding of pharmacokinetics can help minimize these problems.

**Absorption:-** Absorption is the transfer of a drug from its site of administration to the bloodstream. The rate and extent of absorption depends on the route of administration, the formulation and chemical properties of the drug, and physiologic factors that can impact the site of absorption. When a drug is administered intravenously, absorption is not required because the drug is transferred from the administration device directly into the bloodstream [26].

**Distribution:-** Protein binding plays key role in the drug interactions at the level of distribution. Competition for protein binding, especially with serum albumin , is most important potential source of drug interactions.

Clinically, the type of drug interaction are very much significant for the compounds with narrow therapeutic index, as they have plasma protein binding of about more than 90%. Furthermore, these type of interactions may be more likely to occur in those with hypoalbuminemia most commonly seen in elderly, malnutrition, patients with liver disease and chronic alcoholics. Drugs that have high affinity for, extensive binding to serum albumin are generally acidic drugs and which include warfarin, NSAID's, phenytoin, lorazepam, valproic acids etc. [27][28][29].

**Metabolism:-** Metabolism is the major concern in pharmacokinetics which play a key role in th elimination of the safety profile of a drug in combination with other commonly administerd drugs. Most interactions occurs with phase I metabolic processes. Mostly these are located primarily in the liver. This multiprotein system is responsible for the metabolism and clearance of the vast number of drugs particularly fat soluble and orally administered drugs. Interactions related to metabolism is very much difficult to predict, as it depends on many factors such as genetics, diet, age, lifestyle, health-state etc [30].

**Excretion:-** Elimination is the process whereby drugs and/or their metabolites are irreversibly transferred from internal to an external medium. When a drug is taken into and distributed throughout the body, it must be subsequently removed, or concentrations of the drug would continue to rise with each successive dose. The complete removal of the drug from the body is referred to as elimination. Elimination of the drug encompasses both the metabolism of the drug, and excretion of the drug through the kidneys, and to a much lesser degree into the bile. Excretion into the urine is one of the most important mechanisms of drug removal.

PK is the integral of the time coincident process of ADME and is considered as a powerful screening tool in early drug discovery process. The systemic application of pharmacokinetics can, therefore, considerably reduce the cost and time involved in the new drug development by ensuring that the candidate selected does not possess poor pharmacokinetic characteristics. The main role of DMPK in discovery is, therefore the prediction of human PK and metabolism. Reducing the rate of attrition during drug discovery and development is now considered essential, particularly as it is now possible to screen an ever-greater number of compounds.

The events following drug administration can be divided in two phases:

- Pharmacokinetic phase, in which the adjustable elements of dose, dosage form, frequency and route of administration are related to drug level-time relationships, and
- Pharmacodynamic phase, in which the concentration of drug at the site of action is related to the magnitude of the effects produced.

The ultimate goal of PK is to give patients maximal benefit of the drug by close and accurate measurements of the drug and/ or its metabolites in different biological matrices, thus offering optimum drug management and patient care [31]. The use of PK for better patient can includes:

- Individualization of patient dose and dosing regimen.
- Assessment of bioavailability and bioequivalence of the drug by the proposed routes,
- Aid in determining the mechanism of drug-drug interaction and their avoidance.
- Prediction of pharmacokinetics in man, from results obtained in animals.
- Identification of optimum methods to accelerate drug elimination from the body in the toxicity case/ over dosage.
- Identification of active metabolites of drug and quantification of their role in producing the overall response following drug administration.

**IMPORTANCE OF PHARMACOKINETICS**

Pharmacokinetics is important because:

a. The studies completed in laboratory animals may give useful indications for drug research and development. For example, less powerful molecules *in vitro* can turn out more effective *in vivo* because of their favorable kinetics (greater absorption, better distribution, etc.).

b. Pharmacokinetics supports the studies of preclinical toxicology in animals (toxicokinetics) because the drug levels in plasma or tissues are often more predictive than the dose to extrapolate the toxicity data to man. Toxicokinetics is also important to:

- verify that the animals have measurable levels of drug in plasma and that these levels are proportional to the administered dose,
- estimate the area under the curve and the maximum concentration of the drug in plasma, because these parameters can be used to represent the exposure of the body to the drug,
- evidence differences in pharmacokinetics between the various groups of treatment, the days of treatment and other factors,
- estimate the variability between animals and identify cases with abnormal levels of the drug.

c. Knowledge of the kinetics and of the effects (pharmacodynamics) of drugs in man is necessary for a correct use of drugs in therapy (choice of the best route of administration, choice of the best dose regimen, dose individualization [32].

**PHARMACOKINETIC STUDIES [33-39]:-**

| | |
|---|---|
| Study design | For 2 formulations- Two period, two sequence crossover design can be used in two different time intervals with a washing period (which is equal or more than half lives of moieties) between them. For single formulation- Single dose, randomized , cross-over study can be used.<br>Parallel design for alternative study and replicate design for highly variable drugs. |
| Study population | Number of subjects used for the study should in sufficient number depends on the type of study involved. |
| Criteria for selection of subjects | Study should be performed on healthy adult volunteers with the aim to minimize variability and should detect the differences between the study drugs. |
| Study conditions | Environment, diet, fluid intake, post dosing postures, exercise, sampling schedules etc should be standardized throughout the study. |
| Fasting and fed state | Study should be conducted after an overnight fast (at least 10 hours), with subsequent fast of 4 hours following dosing. For multiple dose fasting state studies two hours of fasting before and after the dose is considered acceptable. |
| Sampling | Atleast 3 sampling points & 4 sampling points for absorption and the elimination phase.<br>Blood should be collected from retro-sinus orbital or any other withdrawl route. |
| Tolerability | Tolerability was assessed by monitoring vital signs (blood pressure, heart rate, body temperature) at baseline, 4.5, 11.5, and 23.5 hours, and at the end of each period. |

**BIOANALYTICAL METHODS:-**

The bioanalytical methods are employed for the quantitative determination of the analytes (drugs & their metabolites) in various biomatrices which includes blood, plasma, serum and urine samples. These are the key determinants in producing reproducible and reliable data which are well employed in the evaluation & interpretation of the bioavailability, bioequivalence and pharmacokinetic findings. It is very much important in developing the well characterized, standardized and fully validated bioanalytical method to yield reliable results for accurate data analysis[40].

Pharmacokinetics is often studied using mass spectrometry because of the complex nature of the matrix and the need for high sensitivity to observe concentrations after a low dose and a long time period. The most common instrumentation used in this application is LC-MS [41][42] with a triple quadrupole mass spectrometer and HPLC [43]. Tandem mass spectrometry is usually employed for added specificity [44]. The first step is to develop a method for the analysis of drug concentration in various biological matrices and to validate the developed method for the interpretation of results.

**Method development:-**

Method development includes the determination, evaluation and optimization of steps involved in the sample preparation, chromatographic development, detection and quantification.

Method development includes the following important steps.

- ❖ Tuning of the analyte
- ❖ Optimization of chromatographic separating condition
- ❖ Optimization of analyte extraction procedure from various biomatrices.

**Method validation:-**

Fundamental parameters to be validated are enlisted in the following table. These validation parameters are safeguarded by various regulatory bodies like ICH, USFDA, US, IUPAC, and AOAC [45][46].

Table 1.3.3: Brief definition of various validation performance characteristics

| Parameters | Activity |
|---|---|
| **Specificity** | Ability to measure desired analyte in a complex mixture |
| **Accuracy** | Agreement between measure and real value |
| **Precision** | Agreement between a series of measurement |
| **Linearity** | Proportionality of measured value to concentration |
| **Range** | Concentration interval where method is precise accurate and linear |
| **Detection limit** | Lowest amount of analyte that can be detected |
| **Quantitation limit** | Lowest amount of anayte that cn be measured or quantified |
| **Robustness** | Ability to remain unaffected by small changes in parameters |
| **Ruggedness** | Reproducibility under normal but variable laboratory conditions |

**PHARMACOKINETIC EVALUATION**:-Many of the failures of drug candidates in development are attributed to their undesirable pharmacokinetic properties in humans. Many of the new drug candidates investigated in humans were withdrawn and 77 of the 198 withdrawn candidates (40%) were due to serious pharmacokinetic problems [47] .Thus, it is very important to study the pharmacokinetics of a the drug in species, typically rats, dogs and monkeys, before it is chosen to be a development candidate. Although animal pharmacokinetics do not constitute an objective per se of drugs intended for human use, the main purpose of animal pharmacokinetic studies is to gather the appropriate information for an accurate prediction of absorption and disposition in humans to ensure the success of a drug's development.

**PHARMACOKINEIC ANALYSIS**

Pharmacokinetic analysis is performed by two methods i.e., Non-compartmental or compartmental methods. Non-compartmental methods estimate unveiling a drug by calculating the area under the curve of a concentration-time graph. Compartmental methods estimate the concentration-time graph using different kinetic models.

Analysis was done on parent drugs only not on metabolites. The rate and extent of the absorption of the drug are primarily measured by plotting the plasma concentration-time profile , the time to reach the peak concentration (tmax) reflects the rate of absorption, while the peak concentration (Cmax) reflects both the extent and the rate of absorption [48][49]. In order to have a true and accurate measurement of Cmax, adequate number of sampling points should be placed at and around the anticipated Cmax of the drug.

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