SUMMARY
Preformulation studies and method development are an integral part of drug development process. These play a very important role in the final design of drug delivery system, deciding the route of administration and shelf life. With so many diseases of different kind, the researchers are working round the year in every part of the globe to find cures. Biologists are looking for genetic targets, chemists are synthesizing new and improved molecules and pharmacists are improving the design and bioavailability for targeted delivery for early cure. All this is supported by better techniques, which help in all phases of drug development and give fast results. Once an active compound has been identified, it is taken up for preformulation studies.

The active compound has to undergo certain stages of rigorous experiments including purity, stability and toxicity profile before it could be approved for animal studies. All these studies play a very important role in designing the formulation and deciding the route of administration of drug.

The pre-formulation studies include: -

Establishment of physical parameters -

- Purity - For every new compound, depending on its dose and toxicity, the limit of impurity is defined. Until and unless the purity of the drug is assured other studies like stability, degradation and toxicity cannot be performed. Various parameters included to establish purity are melting point, UV absorbance, HPLC, TLC and quantitative determination of ash etc.

- Thermal analysis - When a compound is subjected to temperature change, it undergoes several transitions like glassy transition, melting, polymerisation and/or decomposition. The Digital scanning calorimeter is used for studying
the thermal changes occurring in the compound due its exposure to high temperature.

- Solubility- The solubility of a compound depends upon the physical and chemical properties of the solute and the solvent as well as upon such factors as temperature, pressure, pH of the solution and to lesser extent the state of subdivision of the solute. The solubility of the compound in a particular solvent depends upon its interaction with solvent on molecular level.

- Dissociation constant- Most of the drugs are either weak acids or weak bases and according to the pH-partition hypothesis, unionized form of a drug will be absorbed preferentially in a passive manner through a membrane. This parameter is very important to know if spectra of pure species is to be recorded in solution; if effects of pH changes on physical properties are to be interpreted.

- Partition coefficient- If a liquid or solid is added to a mixture of two immiscible liquids, it gets distributed between the two phases in a definite concentration ratio. The principle of partition is important for a drug compound as it is involved in several of the applications. These include formation of emulsions of oil and water, drug action at nonspecific sites, and the absorption and distribution of drugs throughout the body.

Development of validated estimation procedure including impurity profile- ICH guidelines are available to help the analyst decide on the parameters to be taken into consideration for method validation. But the approach has to be decided by the analyst alone. A method, which depends on the accuracy and precision of measurements, can
not be validated without first being able to accurately and precisely quantitate the level of analytes present in the original samples. Various parameters are Specificity, Detection limit, Quantitation limit, Linearity, Range, Accuracy, Precision, and Robustness.

Stability studies- The stability studies are done to determine shelf-life, and co-related specifications, and it must be taken into account of the chemistry of the active ingredient and its likely vulnerability to degrade by oxidation, hydrolysis, isomerisation, polymerisation, decarboxylation, moisture, heat and light. Stability studies should not only take account of the physical state in which the compound is likely to be used, but also the immediate biological environment likely to be met on administration. These include-

- **Effect of temperature**- It is carried out during storage testing and is of particular importance for products destined to be used in tropical countries or which are subjected to heat sterilization. This tells about the shelf life of the compound and its half-life at the required temperature.

- **Effect of moisture**- During manufacture, storage and transport of the compound (drug), its protection from moisture is necessary as it may lead to decomposition of the drug and the impurity may be harmful or toxic; if taken as such. The single most important cause of loss of potency of a drug substance is the presence of moisture. It can be present as surface moisture, which dissolves the drug to the extent of its solubility S.

- **Effect of UV light on the compound**- The prediction of decomposition rate from experiments in which the effect of light is exaggerated; is conducted to
find the relation between the flux of light and the degradation rate. Degradative reactions, such as oxidation-reduction, ring rearrangement, or modification and polymerization can occur within the molecule by exposure to light especially by UV light as they have short wavelength and higher energy. This phenomenon is very common in the drugs, which have labile or photosensitive groups in them.

- Effect of change of pH- the compound tends to degrade by various reaction mechanisms when it comes in contact with buffers of different pH. The stability of a drug with respect to different pH is important in designing the formulation and route of administration. This is very important as different parts in the body have different pH environments and the compound has to be stable in that to be effective.

Formulation suggestion of candidate drug- in the formulation, all constituents, active ingredients and excipients must conform to a fully stated specification, pharmacopoeial or otherwise appropriate to their use in human medicine. The choice of excipients should be carefully considered and be capable of rationale. Ideally, these should be limited to the minimum to ensure uniformity of dosage and stability throughout the period of proposed shelf-life of the product. The rationale approaches to the excipients choice as well as to their interactions with medicaments have been shown as a basis for modern modeling of pharmaceutical formulations. The importance of complexation, hydrogen bonding, ion-dipole, dipole-dipole and van der Waals attractions as the tools, which can modify the physicochemical, pharmacological or pharmacokinetical behavior of medicaments, has been emphasized. Therefore excipients are important components of pharmaceutical
formulations and they can take an active part in the improvement in the characteristics of formulations. The early prediction of drug-excipient incompatibility is vital in the pharmaceutical industry to avoid costly material wastage and time delays. In particular, the low availability of drug and the time constraints associated with the early stages of formulation development have made such predictions desirable.

There is also increasing interest in optimizing the efficacy of drug activity through the use of rationally designed drug carrier materials. Cyclodextrins (CDs) are strong candidates for a role, modifying physical, chemical and biological properties of drug molecules through the formation of inclusion complexes. The hydrophilic CDs are used to enhance the solubility and dissolution rate of poorly water-soluble drugs, whereas hydrophobic CDs are used to slow the dissolution rate of water-soluble drugs.

There are various advance instrumentation techniques available for analysis like, HPLC, HPTLC, DSC, and UV spectrophotometery. These are continuously being improved upon to facilitate the analytical chemist to get better and accurate results in less time and with less solvent consumption.

The present studies have been described under-

(i) **Sapindus saponin (Spermicidal agent)**- India and other countries across the globe are facing one major problem- *Rapid growth in Population*. The government and other agencies looking after the needs of the common man are already under pressure, thus health and family planning has been adopted as the main agenda in many countries, especially India in the 9th five year plan. Oral contraceptive, though effective are related with many side effects, thus
other forms of contraception are being explored. Vaginal contraceptives incorporating spermicides, if properly formulated and used, can be highly effective, since they are safe, free from systemic side effects and do not require any medical assistance or prescription. Traditional spermicides from plants are now being promoted and experimented upon. Neem extract has been reported to exert spermicidal action in vivo. Researchers are evaluating "Praneem polyherbal" cream and suppositories composed of three active ingredients: Neem seed extract; soapnut extract and quinine hydrochloride (for anti-HIV action), cream has exhibited high spermicidal efficacy in animal studies. Saponins isolated from many plants, which showed spermicidal action, include Bolbostemma paniculatum, Ardisia nerifolia, Madhuca butyracea, Pittosporum nilgherense, Polemonium coerulem, Schefflera capitata, and Trigonella foenum-graceum. Among the plants screened at CDRI, the most active spermicide was found to be the fruit extract of Sapindus saponin or reetha which was then taken up for further development at our institute. Spermicidal activity was found to be associated with the $\beta$-amyrin C-28 carboxylic acid type of sapogenins, such as hederagenin, oleonolic and basic acids linked to a particular sequence of sugar moieties. The active extract from the fruit of Sapindus saponin has been tested and its formulation has been developed in form of a cream. Sapindus or reetha saponin is the n-butanol soluble fraction obtained from the ethanolic extract of the fruit pericarp of the plant Sapindus mukorosii.

High performance liquid chromatography technique for saponin determination is the most-widely used method for this group of compounds.
However, the lack of chromophores allowing detection in UV, limits the choice of gradient and detection method. The pre-column derivatisation with benzoyl chloride, coumarin or 4-bromophenacyl bromide has been used successfully in some cases allowing UV detection of separation.

HPLC and HPTLC methods for estimation of active marker Sapindoside-B have been discussed here. Isolation of pure Sapindoside-B was done by column chromatography of the *sapindus saponin* and the identity of Sapindoside-B [I] and other five sapindosides was established by Electrospray Mass Spectra, and its purity was checked by TLC. Saponins were extracted from the cream formulation and then Sapindoside-B content was estimated by HPTLC and HPLC. In HPTLC method post chromatography derivatisation of TLC plate was done to visualize the spots for absorption. The HPLC method was also applied for LC-MS experiment to identify other Sapindosides.

Both methods of analysis HPLC and HPTLC give good resolution between the peaks. Other sapindosides were present before or after the marker. Estimation of Sapindoside-B was done by both the methods and the results were comparable.

(ii) CDRI no. 93/478 (Anti ischemic & Anti hypertensive agent)- Hypertension is the most common of all cardiovascular diseases afflicting about 10-20% adult population. There are different categories of medicines used for hypertension such as Diuretics- which help the body excrete superfluous fluids and salt through the kidneys and, relax blood vessels; Beta-blockers- they
block the effect of the hormone adrenaline and the sympathetic nervous system on the body; Alpha-blockers—cause the blood vessels to relax and widen; Calcium-channel blockers reduce the muscle tension in the arteries, expanding them and creating more room for the blood flow; ACE inhibitors interrupt the formation of a hormone (called angiotensin II) that makes the blood vessels contract and Angiotensin-II receptor antagonists work in a similar way to ACE inhibitors.

Ischemia refers to any condition where an organ cannot get enough oxygen or a shortage of blood and oxygen to an organ or a tissue occurs. Cardiac ischemia involves the heart muscle (myocardium). Cardiac ischemia occurs when clogged arteries prevent the heart from getting enough oxygen. Treatment may include: medication, exercise, angioplasty, and bypass surgery. Treatment Options include drugs such as beta-blockers, calcium channel blockers and nitrates, which can lower blood pressure and reduce the heart’s need for oxygen. Among the 4-piperazine oxo-pyrolidin propanes designed and synthesized for treatment of cardiovascular system (CVS) disorders, the compound 1-[4-(4-fluoro-phenyl)-piperazine-1-yl]-3-(2-oxopyrrolidin-1-yl)-propane hydrochloride (CDRI compound No.93/478) is novel and show significant anti-hypertensive and anti-ischemic activities.

HPLC method for estimation was developed and validated for all other studies. Various preformulation studies were carried out on the compound and it was found that the compound is hygroscopic, thermally stable with a shelf life of more than 4 years, it partitions preferably in water, and is susceptible to
basic pH. The oral dosage can be made by using starch soluble, HPMC, and ethyl cellulose.

(iii) **Aabulaquine [Anti-malarial combination kit containing Chloroquine and Bulaquine (CDRI no. 80/53)]** - The relapse of malaria is a major danger in the regions, which are prone to malaria, or regions where it is endemic. A combination kit [1,2] for anti-relapse treatment of *P. vivax* malaria, consisting of chloroquine tablets and bulaquine capsules has been recently developed, and marketed in India under the trade name Aablaquine. Researchers have developed method for simultaneous estimation of Primaquine and Bulaquine by TLC densitometry and UV spectrophotometry; whereas method of estimation of Bulaquine in serum and Bulaquine along with its primary metabolite Primaquine is also known, but no method for the simultaneous estimation for all the three drugs has yet been reported. Therefore, present study was undertaken to develop a simple, sensitive and reproducible HPLC as well as HPTLC assay method for chloroquine, primaquine and bulaquine. The HPLC method developed was based on reverse phase chromatography on a C8 column, with acetonitrile: sodium acetate buffer (55:45) at 254 nm. In the HPTLC method, precoated Silica plates were used with hexane: diethyl ether: methanol: diethylamine (37.5: 37.5: 25: 0.5) as the solvent system at 254 nm.

Both methods gave good resolution and separation of all the three compounds. No other constituent of the formulation interfered in the analysis. The methods were validated and the %DFA and %CV were found to be within limits. Recovery studies of the formulation were done and found to be satisfactory.
CDRI no. 97/63 (Anti malarial trioxane derivative)- The insight into structure-toxicity relationships and the progress made in the synthesis of artemisinin analogs and finally the contribution of these efforts towards rational drug design in order to access potent, non-toxic antimalarial drugs based on artemisinin have been of interest to some workers. One of the pharmacophor present in the Artemisinin is trioxane ring. Some trioxanes have been synthesized as potent antimalarials, which are simple and easy to synthesize.

For compound 97/63 several studies were conducted including dissociation constant, pH stability, UV stability and interaction with excipients. The compound was found to be very susceptible to basic pH and acidic pH but was stable near neutral pH. It degraded very fast on exposure to UV radiation but it slowed down when Cyclodextrin complexes of 97/63 were exposed to UV light. Most of the excipients did not show any interaction with the compound.

CDRI no. 97/78 (Anti malarial trioxane derivative)- HPLC method for estimation was developed and validated for all studies. The method was validated and was found to be reproducible. % DFA and %CV were within acceptable limit. Various preformulation studies were carried out on the compound and it was found that the compound is highly lypophilic, with a dissociation constant of 3.1& 7.4, thermally stable with a shelf life of more than 4 years, it partitions preferably in octanol with a coefficient of 2.3, and is susceptible to acidic pH. The oral dosage can be made by using starch soluble, HPMC, MCC, methyl cellulose and ethyl cellulose.