The present project work was designed with following objectives.

1. Development of analytical/bio-analytical methods for simultaneous estimation of drugs used in prophylaxis of migraine (propranolol hydrochloride, flunarizine dihydrochloride, amitriptyline hydrochloride, carbamazepine and levetiracetam) & analgesics (paracetamol & aceclofenac) and validation as per ICH/USFDA guidelines.

2. Assessment of interaction of analgesics with drugs used in prophylaxis of migraine by in vitro and in vivo methods.

3. Development bio-analytical method for simultaneous estimation of drugs used in treatment of diabetic neuropathy (pregabalin, gabapentin and duloxetine hydrochloride) and validation as per USFDA guidelines.

Simple and sensitive analytical and bio-analytical methods were developed and validated for the simultaneous estimation of drugs used in prophylaxis of migraine and analgesics. The methods were based on separation of the drugs by reverse phase high performance liquid chromatography (RP-HPLC). For the separation of the selected drug combinations, C18 column was employed with isocratic elution using the inexpensive solvent system as mobile phase. The peaks obtained were well resolved and met the system suitability requirements. The chromatographic parameters and the mobile phases were same in analytical and bio-analytical method development which made the methods inexpensive. Bio-analytical method development employed a simple protein precipitation and extraction of drug from using acetonitrile. The methods were selective and sensitive. The described methods were validated as per ICH (analytical) and USFDA (bio-analytical) guidelines and were accurate, precise and robust.
One of the major risks that precipitate as a result of poly pharmacy is drug–drug interaction. To minimize drug interactions, medication safety is an important concern for the physician, pharmacist and other health care professionals.

In the present study an attempt was made to employ the developed RP-HPLC methods to assess in vitro and in vivo drug interaction of the selected drug combinations. In vitro, percentage drug dissolution of the selected anti migraine drugs were determined and their results were compared with dissolution data of the drugs in the presence of paracetamol and aceclofenac in dissolution media mimicking the gastric fluid (pH 1.2).

Although there were some changes in the percentage dissolution of anti migraine drugs in the presence of both analgesics, three combinations of drugs exhibited statistically significant changes, which are as follows.

1. Propranolol hydrochloride with paracetamol
2. Amitriptyline hydrochloride with paracetamol
3. Carbamazepine with paracetamol

Dissolution of amitriptyline hydrochloride was faster in the presence of paracetamol and paracetamol showed quicker dissolution in the presence of amitriptyline hydrochloride. A significant delay in the dissolution of propranolol hydrochloride and carbamazepine was recorded in the presence of paracetamol and the paracetamol dissolution was prolonged in the presence of carbamazepine. In order to substantiate the above results, in vivo drug interaction studies were carried out in experimental Wistar rats. The effect of one drug on the plasma concentration of another was assessed by withdrawing the blood sample from rats at different time intervals and determining their plasma concentration using the developed bio-analytical methods by RP-HPLC.
The results indicate that, there is a direct correlation between drug absorption and its bioavailability. One drug affecting the dissolution of another may also affect its bioavailability.

Propranolol hydrochloride when administered with paracetamol exhibited prolonged $T_{\text{max}}$ and extended $t_{1/2}$ which may be correlated with prolonged in vitro dissolution. Paracetamol also exhibited a delay in $T_{\text{max}}$ and shortened $t_{1/2}$ when co-administered with propranolol hydrochloride.

Plasma concentration and $C_{\text{max}}$ of paracetamol in the presence of amitriptyline hydrochloride was more until its $T_{\text{max}}$ and its elimination was also faster. Even the $C_{\text{max}}$ of amitriptyline hydrochloride when administered in the presence of paracetamol was higher and the corresponding $T_{\text{max}}$ was also considerably less. This suggests a direct correlation between in vitro dissolution and in vivo drug absorption of amitriptyline hydrochloride and paracetamol.

Carbamazepine reached its $T_{\text{max}}$ at 180 minutes with $C_{\text{max}}$ of 450 ng/mL when introduced alone and when it was administered along with paracetamol $T_{\text{max}}$ had dropped to 75 minutes. The $T_{\text{max}}$ of carbamazepine was found to decrease with extended $t_{1/2}$, while its $C_{\text{max}}$ remained same at its $T_{\text{max}}$ when carbamazepine was administered along with paracetamol. These results may be attributed to the delay in dissolution of carbamazepine in the presence of paracetamol. Pharmacokinetic parameters of paracetamol when administered in the presence and absence of carbamazepine were more or less similar even though the in vitro dissolution of paracetamol was delayed by carbamazepine.

From the in vitro and in vivo drug interaction study, paracetamol was found to interact with propranolol hydrochloride, amitriptyline hydrochloride and carbamazepine.
Chapter 7: Summary

Simple and sensitive bio-analytical method was developed for simultaneous estimation of pregabalin, gabapentin and duloxetine hydrochloride in human plasma by gas chromatography with flame ionization detection (GC-FID) method. The analytes were derivatized using ethyl chloroformate. Nitrogen was used as carrier gas. Temperature and carrier gas pressure programming was done to optimize the method. The developed method was validated as per USFDA guidelines and was selective, precise and accurate which can be employed for drug interaction assessment and pharmacokinetic determination.