A thorough literature review was carried about the research work related to drug interaction studies on the drugs used in migraine prophylaxis. It was observed that no drug interaction assessment was done till date on drugs selected for the present work. Once the drugs were finalized for drug interaction studies, search was on the available methods for analysis of the selected drugs. Since there were no simultaneous methods published for the selected drugs, a thorough review on the analysis of individual drugs was done. Following are some of the referred articles related to drug interactions and analytical method development.

1. Review of research papers related to paracetamol

Paracetamol is a century-old drug, which, since its emergence as an agent for the treatment of adult postoperative pain in the 1980s, has found a new and important place in acute pain management. Numerous interaction studies show the existence of drug interaction when paracetamol is co-administered with other class of drugs.

Rogers SM et al.[29], in six healthy female volunteers, studied the interactions between single dose of paracetamol and oral contraceptive steroids ethinyloestradiol and levonorgestrel. They measured the changes in the plasma concentration of steroids in the presence of paracetamol. The area under the plasma concentration-time curve (AUC) was significantly increased following paracetamol administration by 22%. Between 0-3 hr maximum change in the plasma concentration of steroid was reported by them. A significant decrease in the AUC of ethinyloestradiol 2-sulphate after administration of paracetamol was observed compared with control. But paracetamol was found to unaffected the plasma concentrations of levonorgestrel.

Martin U and Prescott LF [30], reported the interaction of paracetamol with furosemide following the administration of paracetamol (1 g 4 times per day) and intra venous administration of furosemide. As per the study, paracetamol pre-
treatment has no effect on furosemide-induced diuresis or natriuresis and significant reduction in the basal output of prostaglandin E2 (PGE2) with paracetamol pre-treatment. Authors report the significant transient increase in the urinary excretion rate of PGE2 which is induced by furosemide.

Thijssen HH et al.[31], reported the interaction between paracetamol and Warfarin. Here the authors investigated and reports the effects of paracetamol and its toxic metabolite N-acetyl-para-benzoquinoneimine (NAPQI) on in vitro vitamin K-dependent gamma-carboxylase (VKD-carb) and vitamin K epoxide reductase (VKDR). Paracetamol was described to have no effect in either enzymatic reaction. NAPQI, on the other hand showed interference in the VKD carb activity. The interference is by oxidation of the cofactor vitamin K-hydroquinone and inactivation of the enzyme. The covalent binding of NAPQI with functional amino acids may be responsible for the inactivation of the enzyme. At higher concentrations NAPQI was observed to inhibit VKDR. At concentrations that were obtained during rescue therapy of paracetamol intoxication, N-acetylcysteine was found to inhibit VKDR activity. Hence authors concluded that, the potentiation of the oral anticoagulant effect by paracetamol is likely to result from NAPQI-induced inhibition of enzymes of the vitamin K cycle,

Dasgupta A et al.[32], made observations on interaction between grape fruit and paracetamol. White and pink grapefruit fluid was found to interact with paracetamol in vivo in mice. In the in vivo mouse model, the concentrations of paracetamol in sera of mice were determined by fluorescence polarization immunoassay. As summarized by the authors, the concentrations of paracetamol is increased by the white grapefruit fluid in mice both 1 hr and 2 hr after feeding
compared to controls. However, pink grapefruit fluid increases paracetamol concentrations 2 hr after feeding compared to controls.

Wahajuddin A et al.[33], proposed an analytical method for simultaneous determination of atenolol, paracetamol, hydrochlorothiazide, caffeine, cephalexin, metoprolol, propranolol hydrochloride, ketoprofen along with phenol red (a non-absorbable compound) in samples obtained from intestinal in situ single-pass perfusion studies. Here the authors developed a method by reversed phase high performance liquid chromatography. For the elution they used C18 column and the mobile phase comprising of 10 mM phosphate buffer (pH 2.5) and methanol by gradient mode. The method was found to be linear in the concentration range of 1.25–40 μg/mL. The Percentage RSD for intra and inter-day assay precision was between 0.04 and 3.08 % with the accuracy between 98.39 and 109.45 %. On stability studies they proposed the stability of all samples at different storage conditions. The method was applied for analyzing the permeability of samples obtained from in situ single pass perfusion studies. The effective permeability values obtained upon cassette administration were in close proximity to the permeability values obtained upon single administration of model compounds.

Amber S et al.[34], developed a capillary zone electrophoresis method for the determination and separation of ceftriaxone, ceftizoxime, paracetamol and diclofenac Sodium in a mixture, in pharmaceutical formulations and in human blood serum. A 50 mM sodium tetraborate background electrolyte solution (pH 9.0) and an uncoated fused-silica capillary of a total length of 57 cm were used for separation of all the drugs. Separation was achieved in 8.0 min at an applied voltage of 30 kV and detection was performed at 214 nm. The linearity of the calibration curves of
paracetamol was 5-125 mg/mL, of ceftizoxime was 5-500 mg/mL, of diclofenac was 1-125 mg/mL, and for ceftriaxone was 10-1000 mg/mL, while LOD of the paracetamol, ceftizoxime, diclofenac, and ceftriaxone was found to be 1.0, 1.0, 0.5, and 5.0 mg/mL, respectively. The method appears to be less sensitive as the concentrations for linearity, LOD and LOQ are in mg/mL.

Sushma T et al.[35], discussed the development and characterization of paracetamol complexes with hydroxypropyl-β-cyclodextrin was. The researchers have done the complexation by physical mixing, kneading and freeze drying. Characterization of the resulting complexes was done by differential scanning calorimetry and Fourier Transform infrared spectroscopy. The thermograms obtained depict an endothermic peak for paracetamol, for physical mixture to some extent for kneaded mixture. In case of freeze dried product due to formation of inclusion complex, the endothermic peak was found to disappear. This result was supported by IR data. The authors concluded that the solid inclusion complex of paracetamol prepared by freeze drying method was found to be an ideal complex in comparison with kneaded mixture. Also, the solubility of paracetamol was significantly increased (six folds of normal solubility) by complexation with hydroxypropyl-β-cyclodextrin.

Pattan SR et al.[36], estimated the paracetamol and etoricoxib simultaneously from bulk and tablets by RP- HPLC method using an inertsil C18 and C8 columns. The elution was carried out with a mobile phase consisting of methanol: acetonitrile: phosphate buffer pH 3.5.(40:20:40 v/v) at a flow rate of 1.0 mL/min with monitoring of analytes at 242 nm. The retention time of paracetamol and etoricoxib were 3.27 and 6.12 min respectively. This method was validated in terms of accuracy, precision, linearity, limit of detection and limit of quantitation.
Qiongfeng L et al.[37], developed a simultaneous LC–MS–MS method for the determination of paracetamol, pseudoephedrine and chlorpheniramine in human plasma for pharmacokinetic study by using diphenhydramine an internal standard. Liquid–liquid extraction was used for the extraction of analytes from plasma. Ethyl acetate was used as solvent for extraction. The analytes were ionized by electron impact ionization and the fragments were detected in the selected reaction monitoring mode with a triple quadrupole tandem mass spectrometer. The method was found to be linear in the concentration range of 20-10000 ng/mL for paracetamol, 1-500 ng/mL for pseudoephedrine and 0.1-50 ng/mL for chlorpheniramine. The method was found to be suitable for pharmacokinetic study as it is validated following USP guidelines.

Godse VP et al.[38], developed a method for the determination of aceclofenac and paracetamol in tablet dosage form by reverse phase HPLC, using a mobile phase consisting mixture of methanol and water (70:30 v/v) at the flow rate of 1mL/min. C-18 (Intersil 25 cm x 4.6 mm, 10 μm.) column was used as stationary phase. The retention time of aceclofenac and paracetamol were found to be 1.8 min and 2.7 min respectively. Linearity was observed in the concentration range of 2-50 μg/mL for aceclofenac and 5-50 μg/mL for paracetamol. Percentage recoveries obtained for aceclofenac and paracetamol were 100.6 and 100.7 % respectively. The method was validated as per ICH guidelines.
2. Review of research papers related to aceclofenac

Aceclofenac is a non-steroidal anti-inflammatory agent, which is widely used as it is found to cause minimal gastric irritation compared to other NSAIDs. Many studies reveal the changes in metabolism of concomitantly administered drugs along with aceclofenac.

Anupama T et al.[39], describes the safety of aceclofenac in their sub acute toxicity studies in albino rat. Following the administration of aceclofenac at different doses, blood samples were evaluated for haematological toxicity during sustained release of aceclofenac. Their study proves the nontoxic behavior of aceclofenac.

Asia N et al.[40], in their conventional, two way, random, open label and cross over study selecting 24 healthy volunteers of either sex studied the effect of aceclofenac on the pharmacokinetics of sucralfate. The authors concluded the existence of interaction between aceclofenac and sucralfate. In their experimental design, the subjects were maintained fasting overnight and 100 mg of single oral dose of aceclofenac in the presence and absence of sucralfate was administered. At various time interval blood was withdrawn for 12 hr and they measured the pharmacokinetic parameters like C\text{max}, T\text{max} and AUC using HPLC. They observed the significant delay in the absorption of aceclofenac as the T\text{max} was delayed for 3.5 hr. The authors explained that the change in T\text{max} is may be due to formation of an unstable complex between aceclofenac and action of sucralfate.

Bikash M et al.[41], carried out a pharmacokinetic study to evaluate possible drug interaction between aceclofenac and phenytoin when concomitantly administered in rabbits. The dose regimen for phenytoin and aceclofenac was based on pilot studies so as to closely mimic human situations of use. Following the single oral administration of phenytoin and aceclofenac, plasma drug concentrations were
measured at various time intervals and authors mentioned the decrease in elimination half life of phenytoin due to concomitant administration of phenytoin and aceclofenac by 80 % and AUC of phenytoin was also decreased by 66%. This may suggest the influence of aceclofenac in decreasing the absorption of pheytoin. The result also suggests the change in metabolism pattern of phentoin due to interaction with aceclofenac.

Chandra P et al.[42], proposed a HPLC method for the simultaneous estimation of aceclofenac, paracetamol and tramadol hydrochloride in pharmaceutical dosage form. They had carried out the separation on a HiQ-Sil HS C18 column at ambient temperature. The mobile phase used was 40: 60 (v/v); phosphate buffer (pH 6.0): methanol a flow rate of 1.0 mL min with UV detection at 270 nm. But the method was found to be time consuming as the retention time of aceclofenac is 14 minutes. The linearity ranges was shown from 40-160 μg/mL for aceclofenac, 130-520 μg/mL for paracetamol and 15-60 μg/mL for tramadol. The method was validated as per ICH guidelines.

Jin Y et al.[43], developed a HPLC method for the determination of aceclofenac in human plasma collected from 18 healthy male volunteers. The authors reported that the extraction of aceclofenac was done after spiking in human plasma using ether and the extractant was redissolved in the mobile phase after dried by N\textsubscript{2} at 37 °C. Chromatography was performed on C18 column using mobile phase consisting of methanol and 0.1 mol/L ammonium acetate (pH 6.0) in the ratio 7:3 v/v at a flow rate of 1.0 mL/min and analytes were monitored at 275 nm. The linearity range was shown from 50 to 400 μg/mL. The method was validated as per ICH guidelines.

Momin MY et al.[44], developed a reverse phase HPLC method for determination of aceclofenac and paracetamol in tablet dosage form using C18
column using the mobile phase consisting of acetonitrile: 50 mM NaH$_2$PO$_4$ in a ratio of 65:35 (pH 3.0 with orthophosphoric acid) at a flow rate of 1.5 mL/min and detection at 276 nm. The retention time of aceclofenac and paracetamol was found to be 1.58 and 4.01 min respectively. The method was validated as per ICH guidelines.

Kim MJ et al.[45], reported a pharmacokinetic interactions between eperisone hydrochloride and aceclofenac. The experiment was designed as randomized, open-label, crossover study in healthy Korean men. Eperisone hydrochloride (3 doses of 50 mg each) and aceclofenac (2 doses of 100 mg each) were given to selected subjects through oral route. Blood samples were collected ≤ 24 hr after dosing, and plasma eperisone hydrochloride and aceclofenac concentrations were determined using validated LC/MS-MS. They had conducted pharmacokinetic analyses using noncompartmental methods. In their study there was no clinically significant pharmacokinetic differences exist between 150 mg eperisone hydrochloride and 200 mg aceclofenac when administrated as a monotherapy or in combination.

Medhi B et al.[46], studied the pharmacokinetic interaction between aceclofenac and phenytoin in their parallel design study by administering phenytoin 30 mg/kg/day per orally for seven days to human volunteers. The plasma concentration at various time intervals between 0-24 hr was measured on day 7. In aceclofenac group, phenytoin was administered for seven days as above. On day 8, aceclofenac 14 mg/kg along with phenytoin 30 mg/kg/day was administered and the plasma concentration at various time intervals between 0-24 hr was measured. For measuring the plasma drug concentrations the researchers have used HPLC method and they had calculated the pharmacokinetic parameters. In aceclofenac group, they have observed decrease in $t_{1/2}$ than phenytoin group. Significant changes were
observed in the pharmacokinetic parameters in aceclofenac treated group. And hence authors suggested that aceclofenac alter the pharmacokinetics of phenytoin.

3. **Review of research papers related to amitriptyline hydrochloride**

Tricylic antidepressants are another class of medication considered as first-line treatment in migraine prophylaxis. Even without the presence of depression, these agents are effective in preventing migraines, and the response is usually more rapid.

Jung-Woo C *et al.*[47], proposed a LC-MS/MS based bio-analytical method for the simultaneous quantitation of amitriptyline hydrochloride and its metabolite nortriptyline in rat plasma. Gemini C18 column with mobile phase consisting of 1% formic acid in water and methanol in the ratio of 10:90 v/v, at a flow rate of 0.2 mL/min were found to be used for elution. Concentration ranging from 0.1-500 ng/mL for amitriptyline hydrochloride and 0.08-500 ng/mL for nortriptyline were chosen for linearity in 200 μL plasma sample. The authors followed FDA guidelines for validation of bio-analytical methods.

Hoegberg LC *et al.*[48], demonstrated the adsorption behavior of amitriptyline hydrochloride and paracetamol individually and in combination to activated charcoal (AC) in order to determine the maximum adsorption capacities. 0.250 g of AC and paracetamol and/or amitriptyline hydrochloride were mixed at pH 1.2 and pH 7.2 and incubated. High-performance liquid chromatography with UV detection was used for measuring the drug concentration. They found that the tested pH differences had a minor effect on the adsorption. About 40% Q(m) reduction was shown by the mixed-drug adsorption of each drug with increasing amounts of drug/g AC. The total gram of drug adsorbed to AC was increased compared to one-drug conditions. The adsorption of the two compounds to AC seems to compete resulting in lower maximum adsorption capacity for both drugs when mixed. The authors explained that
a great adsorptive capacity might be explained by adsorption of the drugs to different AC surface sites. This might be clinically significant in terms of preventing nausea, vomiting, and subsequent aspiration.

Yuan S et al.[49], developed a HPLC method with electrospray ionization mass spectrometry for the simultaneous determination of amitriptyline hydrochloride and nortriptyline in rat plasma. The analytes were ionized by electron spray technique and were identified at \( m/z \) 278 for \([\text{amitriptyline hydrochloride} + \text{H}]^+\), 264 for \([\text{nortriptyline} + \text{H}]^+\) and 315 for \([\text{I.S.} + \text{H}]^+\) with retention times approximately 7.8, 7.4 and 10.2 min respectively. For sample preparation they used liquid–liquid extraction with methyl t-butyl ether after alkalified with 0.5 mol/L NaOH. Chromatographic separation was performed on a XB-C4 column using a mobile phase consisting of 10 mM ammonium acetate (0.6% formic acid) - acetonitrile (60:40, v/v) at a flow rate of 1.0 mL/min. Their calibration curves were linear within the ranges of 10–3200 ng/mL for amitriptyline hydrochloride and 10 -1000 ng/mL for nortriptyline. Also, they had applied this method to the pharmacokinetic study in rats after intravenous injection of amitriptyline hydrochloride.

4. Review of research papers related of carbamazepine

Carbamazepine is the second line of choice in migraine prophylaxis and is used in epileptic patients also having migraine. Many reports suggest the precipitation of drug interactions due to the co-administration of the drugs along with carbamazepine.

Spina E et al.[50], in their update about the clinically significant pharmacokinetic drug interactions with carbamazepine provided the list of most important drug interactions as follows. The drugs that accelerate the elimination of carbamazepine are phenytoin, phenobarbital and primidone accelerate. This is
probably by stimulating cytochrome P450 (CYP). Stiripentol, remacemide, acetazolamide, macrolide antibiotics, isoniazid, metronidazole, certain antidepressants, verapamil, diltiazem, cimetidine, danazol and (dextropropoxyphene) propoxyphene are the drugs inhibit the metabolism of carbamazepine. As a result, plasma concentration of carbamazepine increases to toxic level. Authors suggest the avoidance of unnecessary poly pharmacy by selecting alternative agents.

O’connors NK et al.[51], in their retrospective study on interaction between carbamazepine and clarithromycin described the change in serum levels of carbamazepine by 30 to 40 % in 5 patients. The authors reported the existence of serious drug interaction between carbamazepine and clarithromycin and suggested the requirement of dose reduction of carbamazepine by 30-50 % and monitoring of serum drug levels closely and warning the patient about the signs and symptoms of carbamazepine toxicity.

Yasui-Furukori N et al.[52], determined the impact of carbamazepine on the pharmacokinetics of paliperidone in six schizophrenic patients. The patients were initially under 6-12 mg/day dose of paliperidone alone and subsequent administration of 200 mg/day dose of carbamazepine and further rise in carbamazepine to 400 mg/day and then to 600 mg/day. Plasma concentrations of paliperidone before and after carbamazepine co-administration were quantified using liquid chromatography tandem mass spectrometry. In their study, carbamazepine significantly reduced the plasma concentration of paliperidone. The plasma concentration of paliperidone at baseline and with co-administration of 200, 400, and 600 mg/day were 45.8 ± 11.7, 26.9 ± 13.7, 17.1 ± 8.2, and 15.9 ± 7.6 ng/mL, respectively. While the concentration of paliperidone with carbamazepine co-administration at doses of 200, 400, and 600 mg/day were changed to 55.7% ± 20.7%, 36.1% ± 12.2%, and 33.6% ± 10.4%,
respectively, of baseline. Even at the carbamazepine dose of 200 mg/day this effect was seen and reached a plateau at doses higher than 400 mg/day. However, carbamazepine co-administration was found to exacerbate the psychotic symptoms in some patients. As per the observation made by the authors, they suggested the adjunctive treatment with carbamazepine reduces the concentration of paliperidone in a dose-dependent manner and it may be most likely because of the induction of several drug-metabolizing enzymes and several drug transporters.

Bernus I et al.[53], studied the interaction between carbamazepine and sodium valproate. They described the mechanism of the interaction between sodium valproate and carbamazepine which causes raised plasma concentration of carbamazepine-10,11-epoxide with unchanged plasma carbamazepine concentrations. Valproate inhibiting epoxide hydrolase, (the enzyme that catalyses the biotransformation of carbamazepine-10,11-epoxide to carbamazepine-10,11-trans-diol) may be responsible for this change in plasma concentrations as mentioned above. They measured the plasma clearance of carbamazepine, carbamazepine-epoxide and carbamazepine-diol to relevant carbamazepine metabolites present in urine under steady state conditions. The experiment was done in 17 adults receiving carbamazepine as anticonvulsant monotherapy, and in 10 adults taking the drug together with sodium valproate. They reported that the plasma carbamazepine-epoxide concentrations were higher, relative to carbamazepine dose, in the co-medicated patients. Irrespective of the administration of sodium valproate, plasma apparent clearances of carbamazepine, relative to drug dose, were similar. They concludes the involvement of sodium valproate in inhibiting the glucuronidation of carbamazepine-10,11-trans-diol, and conversion of carbamazepine-10,11-epoxide to trans-diol.
Pauwels OF [54], described the interaction between acrolide antibiotics and carbamazepine and also explained the factors contributing to this interaction. Carbamazepine-macrolide interaction lead to an increase in the level of carbamazepine in the blood, so inducing carbamazepine toxicity. He compared the extent of the interaction for each macrolide and studied the effects of age, gender, weight, the carbamazepine and macrolide dosages and the use of other antiepileptic drugs on the extent of the carbamazepine-macrolide interaction. The author in his results suggested that three macrolides (erythromycin, troleandomycin and, to a lesser extent, clarithromycin) may induce carbamazepine toxicity in clinical practice. Furthermore, the researcher observed that high dosages of carbamazepine or macrolides and the use of concurrent anticonvulsant drugs in the case of patients below 60 years of age were associated with the highest carbamazepine levels in carbamazepine-macrolide interactions. He concluded that the study may help physicians to choose a macrolide that does not interact with carbamazepine and evaluate the risk of an interaction between carbamazepine and macrolides.

Lang DG et al.[55], presented a study on interaction between lamotrigine, phenytoin and carbamazepine and explained the effect of interaction on the sodium current present in N4TG1 mouse neuroblastoma cells. The authors had investigated the effects of lamotrigine drug together with the anticonvulsant drugs phenytoin and carbamazepine on voltage-sensitive sodium channels present in N4TG1 mouse neuroblastoma clonal cells. A tonic inhibition of sodium channels was produced by lamotrigine, phenytoin and carbamazepine with IC50 values of 91, 58 and 140 µM, respectively. The drugs shifted the voltage-dependency of steady-state inactivation toward more negative potentials by 7 to 15 mV at a concentration of 100 µM, slowed the rate of recovery from inactivation and produced a use-dependent inhibition of
sodium channels. To conclude, lamotrigine inhibits sodium channels similar to that produced by phenytoin and carbamazepine.

Jickling G et al.[56], studied the toxicity of paracetamol with concomitant administration of carbamazepine. They in their case report gives information about a 34 year old man developing acute renal and liver failure on concomitant administration of paracetamol and carbamazepine. There is no evidence or information regarding the study of change in plasma concentration of the drugs but only toxicity was given by the authors. This study raised a caution, that patients on carbamazepine should avoid chronic use of paracetamol, and if required use at lower doses with vigilant monitoring for signs of liver damage.

Prashanth S et al.[57], studied the interaction between carbamazepine and antidiabetic drugs glibenclamide in healthy albino Wistar rats following single and multiple dosage treatment. They estimated the blood glucose levels and plasma glibenclamide concentrations by a RP-HPLC method to calculate pharmacokinetic parameters. When compared with glibenclamide alone treated rats, the authors observed the significant reduction in blood glucose levels and raise in glibenclamide concentrations of rats when treated along with carbamazepine. The reason of interaction may be due to inhibition of P-glycoprotein mediated transport of glibenclamide by carbamazepine, but in multiple dose study the percentage reduction of blood glucose levels and glibenclamide concentrations were reduced and it may be due to inhibition of P-glycoprotein mediated transport and induction of CYP2C9, the enzyme through which glibenclamide is metabolised.
5. **Review of research papers related to flunarizine dihydrochloride**

Flunarizine dihydrochloride is the most widely used calcium channel blocking agent for the treatment of migraine and is found to interact with many co-administered drugs.

Shivarkar NA et al.[58], described the development of UV spectrophotometric method for simultaneous estimation of flunarizine dihydrochloride & propranolol hydrochloride in bulk drug and capsule dosage form. The authors proposed two methods, one by simultaneous equation (Method I) and the other by Q-analysis/absorption ratio method (Method II). In the methods described, the linearity of flunarizine dihydrochloride & propranolol hydrochloride was in the range of 1-23 μg/mL & 4-48 μg/mL respectively at wavelengths for the method I are 253 nm and 289 nm for flunarizine dihydrochloride and propranolol hydrochloride respectively & for method II were 262.2 nm and 289 nm. The % recovery of flunarizine dihydrochloride & propranolol hydrochloride was shown as 98.49 – 101.06 % (Method I) and 99.82 – 100.86% (Method II). Authors justified that the method was sensitive, accurate and precise through their validation studies.

Ravisankar P et al.[59], developed a reverse phase high performance liquid chromatographic method for the determination of flunarizine dihydrochloride in its bulk form and pharmaceutical preparations. For the chromatographic separation they used Welchrom C18 isocratic column with mobile phase containing a mixture of methanol: acetonitrile: water 50:30:20 v/v/v (pH of 4.6) and a flow rate of 1.0 mL/min at 245 nm. The linearity of the method was shown in the range of 2 - 10 μg/mL. The limit of detection and limit of quantitation for the method were 0.101629 μg and 0.307968 μg as calculated using linearity curve. The method was described as precise, accurate and sensitive through its validation according to ICH Guidelines.
Aparicio X et al.[60], presented reversed-phase high performance liquid chromatographic method for the study of the pharmacokinetics of flunarizine dihydrochloride. The authors extracted the drugs from rat plasma by solid phase extraction method and meclizine is added as internal standard. They described that the method was suitable for bio-analysis of flunarizine dihydrochloride as the method is sensitive and they had also evaluated the parameters for a bio-equivalence study between flunarizine dihydrochloride and a liquid formulation (oral drops) with relative bioavailability of 88%.

Subramanyam K et al.[61], studied the drug interaction between sodium valproate, ethosuximide, and flunarizine dihydrochloride by administering the single dose of above said drugs to wistar rats. They had recorded Electroencephalogram (EEG) and the behavioral aspects of spike and wave discharges (SWDs) induced by gamma- hydroxyl butyrate. As per their results, behavioural changes and concomitant repetitive EEG episodes of 7 to 9 Hz SWDs, mimicking human absence seizures (AS) were showed by gamma-hydroxyl butyrate treated rats. The authors described that the method can be used as a pharmacological model. As mentioned, the valproate and ethosuccimide at 200 mg/kg, significantly reduced the SWD number and duration/hr, while flunarizine dihydrochloride showed significant reduction only at 10 but not at 5 mg/kg. To conclude, combination of flunarizine dihydrochloride, 10 mg/kg with either valproate or ethosuccimide shows significant reduction of SWD number and duration, suggesting an additive effect of flunarizine dihydrochloride.

Weizman R et al.[62], in their interaction study between calcium channel blockers verapamil and flunarizine dihydrochloride evaluated the opioid antinociceptive mechanism of the calcium channel blockers verapamil and flunarizine dihydrochloride in groups of mice by the hotplate test. Both produced a naloxone-
sensitive dose-dependent analgesia. As per their study, the role of mu receptors in the antinociceptive effect of both was reversed by beta-FNA, (mu1 and mu2 antagonists), and both enhanced the antinociceptive activity of morphine. Since the analgesic effect of flunarizine dihydrochloride, but not verapamil, was reversed by naloxonazine (mu1 antagonist). They suggested that the mu1 subtype was involved in flunarizine dihydrochloride analgesia, but not in verapamil analgesia. The authors described that, studies with the selective delta opioid agonist and the selective antagonist naltrindole indicated that the antinociceptive activity of verapamil was also mediated by delta receptor agonistic activity. But a reverse effect is exhibited by flunarizine dihydrochloride.

Wiwin FK et al.[63], developed a HPLC method for determination of flunarizine dihydrochloride in the tablet preparations and validated the method as per ICH guidelines. The method used LiChrospher C-18 column with a mobile phase consisting of methanol–ion pair solution in the ratio 8:2 v/v at a flow rate of 1.0 mL/min and the analytes were monitored at 254 nm. The HPLC method was said to be selective, precise, and accurate for routine analysis of the tablet preparations in pharmaceutical industry quality control laboratories.

Woestenborghs R et al.[64], described a method for the determination of cinnarizine and flunarizine dihydrochloride in plasma, urine and milk samples from man and animals. The procedure involved the extraction of the drugs and their internal standard from the biological samples at alkaline pH, back-extraction into sulphuric acid and re-extraction into the organic phase (heptane-isoamyl alcohol). Gas chromatography with nitrogen-selective thermionic specific detector is described to be used for analysis of drugs. The detection limit was found to be 0.5 ng/mL in biological fluid with extraction recoveries of 87-94%. The method was applied to
plasma samples from bioavailability studies of both cinnarizine and flunarizine dihydrochloride in healthy volunteers and to plasma, urine and milk samples from flunarizine dihydrochloride treated dogs.

6. Review of research papers related to levetiracetam

Levetiracetam is the second line of choice in migraine prophylaxis and is used in epileptic patients also having migraine. Many reports indicate the existence of drug interactions due to the co-administration of the drugs along with levetiracetam.

Tomic MA et al.[65], described the synergistic interaction of levetiracetam with non-steroidal analgesics and caffeine to produce analgesic effects in rats. The authors selected ibuprofen, celecoxib, paracetamol and caffeine to suppress hyperalgesia in a model of localized inflammation by inducing rat paw inflammation induced by intraplantar carrageenan. As described in the article, a modified paw pressure test is used to examine the hyperalgesia and antihyperalgesic effects of levetiracetam, analgesics and caffeine alone and 2-drug combinations of levetiracetam with analgesics or caffeine. Isobolographic analysis was used to determine the analysis of dose-response curves for drug combination and drugs alone. A significant, dose-dependent reduction of inflammatory hyperalgesia of paracetamol, celecoxib and ibuprofen with levetiracetam was shown in the study. They suggested that analgesics or caffeine could be useful in treatment of inflammatory pain.

Ragheneau-Majlessi I et al.[66], observed that levetiracetam also exhibited the lack of effect of repeated administration on the pharmacodynamic and pharmacokinetic profiles of warfarin in 42 healthy subjects. The authors described that after a dose-finding phase and a stabilization phase, during which a warfarin treatment was introduced and the dose maintained stable for at least 5 days, 18 male and 8 female subjects were eligible and enrolled. Subjects received warfarin (2.5, 5 or
7.5 mg/day) and levetiracetam 1000 mg bid, and warfarin plus placebo in a treatment periods 7 days long. The protein binding and the pharmacokinetic profiles of R- and S-warfarin were assessed at steady state by analysis of blood samples, and the anticoagulant effect was measured using the international normalized ratio (INR). However the protein binding of warfarin is not affected by the concomitant treatment. The INR values were also not statistically altered by the concomitant administration of levetiracetam or placebo and hence they concluded that the co-administration of levetiracetam and warfarin is safe.

Bain E et al.[67], reported the drug-drug interaction between methotrexate and levetiracetam in a 46 year old man with relapsed osteosarcoma of the base of the skull receiving high-dose methotrexate and levetiracetam. The patient experienced seizures secondary to brain metastasis and was given with levetiracetam. As a result of concomitant administration of methotrexate and levetiracetam, the patient experienced delayed methotrexate elimination during cycles 2, 3 and 4. Hence lorazepam was substituted for the levetiracetam and methotrexate was eliminated to nontoxic levels within 95 hr. Consequence to this, the patient received standard supportive care and serum creatinine remained stable in all the cycles. The authors conclude that co-administration of levetiracetam and methotrexate may result in delayed elimination of methotrexate, increasing the likelihood of toxicity.

Nicolas JM et al.[68], in their in vitro evaluation of potential drug interactions with levetiracetam and 11 drugs metabolizing enzyme activities using human liver microsomes, investigated testosterone 6 beta-hydroxylation, coumarin hydroxylation, (R)-warfarin hydroxylation, (S)-mephenytoin hydroxylation, p-nitrophenol hydroxylation tolbutamide hydroxylation, dextromethorphan O-demethylation, epoxide hydrolase and UDP-glucuronyltransferase towards paracetamol,
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ethinyloestradiol, p-nitrophenol, and valproic acid. As per their results none of these activities were affected by levetiracetam.

Franzoni E et al.[69], described the absence of kinetic interaction between levetiracetam and cyclosporine in their case report. The case report was of a 14-year-old girl who underwent orthotopic heart transplantation, followed by anti rejection therapy including cyclosporine. Symptomatic occipital lobe epilepsy developed that was successfully treated with oxcarbazepine, but cyclosporine plasma levels decreased to below the anti rejection threshold. Oxcarbazepine was replaced by levetiracetam. As per the author’s conclusion, levetiracetam did not affect the metabolism of cyclosporine, and cyclosporine plasma levels have remained in the therapeutic range.

Sisodiya SM et al.[70], described the carbamazepine toxicity in a combination therapy with levetiracetam. Authors gave the proof in four patients with severe refractory epilepsy in whom introduction of levetiracetam led to disabling symptoms compatible with carbamazepine toxicity requiring either carbamazepine dose reduction or levetiracetam withdrawal. As carbamazepine and carbamazepine-epoxide blood levels were not altered during levetiracetam co-medication, a pharmacodynamic interaction was suggested by the authors and also they called for a monitoring during levetiracetam co-medication with carbamazepine for symptoms of carbamazepine toxicity.

Luszcki JJ et al.[71], reported the drug interaction between levetiracetam and numerous antiepileptic agents in their mouse maximal electroshock seizure model. Along with levetiracetam the drugs used for the study were carbamazepine, phenytoin, phenobarbital, valproate, lamotrigine, topiramate, and oxcarbazepine. The study was done following the single dose administration in combination with
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intravenous administration of levetiracetam. While authors were ascertaining the chimney test to evaluate the motor performance, they observed acute adverse effects and the step-through passive-avoidance task assessing long-term memory. Brain drug concentrations were determined to ascertain any pharmacokinetic contribution to the observed antiseizure effect. A supraadditive interaction with topiramate was reported at the fixed ratios of 1:2, 1:1, 2:1, and 4:1. Also even with carbamazepine and oxcarbazepine synergestic effect was reported but to lesser extent. In contrast, all other combinations displayed additivity. Furthermore, none of the investigated combinations were reported to alter the motor performance and long-term memory. Levetiracetam brain concentrations were unaffected by concomitant administration of antiepileptic drugs.

Tesfaye H et al. [72], demonstrated the assay of levetiracetam by enzyme multiplied immunoassay technique. It was based on competition between levetiracetam in the specimen and levetiracetam labeled with the enzyme glucose-6-phosphate dehydrogenase (G6PDH) for binding sites of antibodies (rabbit polyclonal antibodies). The activity of G6PDH grows with increasing concentration of the drug from specimen. This enzyme catalyzes conversion of nicotinamide adenine dinucleotide to NADH whose concentration was measured spectrophotometrically as a rate of change in absorbance. Samples have been analysed in 16 patients by HPLC-UV and the results were employed also for therapeutic drug monitoring. The method is sensitive but is expensive as it involves enzyme involved immune assay.

Valarmathy J et al. [73], developed a HPLC method for estimation of levetiracetam in its tablet dosage form. Separation was done by prontosil C18 column using a mobile phase consisting of buffer solution (pH 2.8) and acetonitrile in the ratio of 90:10 v/v at a flow rate of 1.2 mL/min and UV detection at 215nm. The
retention time of levetiracetam was 4 min. The method was showing its linearity in the concentration of 45μg/mL -270 μg/mL. The percentage recovery of levetiracetam was found to be 99.08%. The method was said to be validated as per ICH guidelines.

Appalaraju AN [74], described a HPLC method for the estimation of levetiracetam in tablet dosage form. A Sun Fire C18 column with mobile phase consisting of acetonitrile and 0.03 M potassium dihydrogen phosphate (pH adjusted to 3.0 with orthophosphoric acid) in the ratio of 15:85 v/v was used for elution. The flow rate was 1 mL /min and the effluents were monitored at 210 nm. The retention time was 5.53 min with the linearity in the concentration of 20-240 μg/mL. The limit of detection and limit of quantification was 0.16 and 0.5 μg/mL respectively. The percentage assay of levetiracetam was 99.87%. Author also described about the validation of the method using ICH guidelines

7. **Review of research papers related to propranolol hydrochloride**

Beta blockers are widely used for the prophylaxis of migraine and propranolol hydrochloride is well tolerated in patients. However the report on its interaction with other drugs indicates the need to assess drug interaction with analgesics.

Salman SA et al.[75], developed a HPLC-UV method for the quantitative determination of propranolol hydrochloride in human plasma. They had done the separation in C-18 column using a mobile phase mixture consisting of 160 mL water, 180 mL methanol, 70 mL acetonitrile, 2.5 mL acetic acid and 125 μL triethylamine at pH 3.4 and the flow rate of 0.5 mL/min. The analyte was monitored at 291 nm. The method exhibited linearity from 15-180 ng/mL. The limit of detection and limit of quantification were reported as 1 and 10 ng/mL respectively. The authors validated the method following FDA guideline but did not mention about the lower limit of quantification.
Davies JG et al. [76], studied the effect of multi dose of ibuprofen, 1600 mg/day in two groups of patients with hypertension on propranolol hydrochloride/bendrofluazide. The study was a double-blind, double-placebo, randomized crossover trial. In their study no significant difference in blood pressure was found at the end of the crossover period in either group, suggesting that the routine co-administration of ibuprofen does not attenuate the anti-hypertensive effect of thiazide diuretics or propranolol hydrochloride. A fluid retention related weight gain was observed in the bendrofluazide-treatment group by the end of the drug-free washout period. No significant change in mean weight was observed in the crossover stages in either group, although substantial weight gain was noted during ibuprofen treatment in two patients given bendrofluazide and one given propranolol hydrochloride. Even they had not noticed any biochemical variables getting affected by ibuprofen throughout the crossover period. This study suggested that ibuprofen may be administered routinely to patients receiving thiazides or propranolol hydrochloride without loss of control of the anti-hypertensive action of these drugs but it is recommended that individuals are monitored for possible weight gain or an increase in diastolic blood pressure.

Spahn-Langguth H et al.[77], developed a HPLC method for the enantiospecific assay of propranolol hydrochloride in biological medium i.e. urine and plasma using fluorescent S-flunoxaprofen. Pronethalol was reported to be used as internal standard. After extraction from human plasma or urine, they had reacted propranolol hydrochloride anhydrous organic solvents with addition of triethylamine. The diastereomeric derivatives were then resolved on an octadecylsilane column using mixtures of water and methanol with or without addition of glacial acetic acid. Conjugates were cleaved prior to analysis using beta-glucuronidase-arylsulfatase and
assayed as parent propranolol hydrochloride enantiomers. The linearity range reported was 1-20 ng/mL and they had validated the method following FDA guidelines.

Alanazi AM et al.[78], developed a stereoselective HPLC method and validated to determine S-(−)- and R-(+)-propranolol hydrochloride in rat serum. They performed the enantiomeric resolution on cellulose tris(3,5-dimethylphenylcarbamate) immobilized onto spherical porous silica chiral stationary phase, Chiralpak IB. The method was reported to use n-hexane-ethanol-triethylamine (95:5:0.4 v/v/v) as mobile phase and the flow rate was 0.6 mL/min and fluorescence detection at excitation/emission wavelengths 290/375 nm. The calibration curve was shown in the range of 10-400 ng/mL. They had validated the method by ICH guidelines in terms of linearity, accuracy, precision, limits of detection and quantitation. But other parameters such as lower limit of detection and stability studies article were not reported in the article.

Satinsky D et al.[79], described a HPLC column-switching method for the fluorescence determination of propranolol hydrochloride in urine. They developed the method by column switching method using fused core columns for sample preconcentration and determination of propranolol hydrochloride. On-line sample pretreatment and propranolol hydrochloride preconcentration were performed on an Ascentis Express RP-C18 guard column with mobile phase consisting of acetonitrile/water (5:95 v/v) at a flow rate of 2.0 mL min and at a temperature of 50 °C. pH was adjusted to 4.5 using acetic acid buffer. Fluorescence excitation/emission detection wavelengths were set at 229/338 nm. A volume of 1,500 μL of filtered urine sample solution was injected directly into the column-switching HPLC system. They described the method to be fast as the total analysis time including on-line sample
pretreatment was less than 8 min. They validated the method following ICH guidelines.

Venkatesh G et al.[80], described the development and validation of RP-HPLC-UV method for simultaneous determination of buparvaquone, atenolol, propranolol hydrochloride, quinidine and verapamil as a tool for the standardization of rat in situ intestinal permeability studies. They had used C-4 column with mobile phase consisting of ammonium acetate buffer (0.02 M, pH 3.5) and acetonitrile in the ratio of 30:70 (v/v) at a flow rate of 1.0 mL/min. The retention times of atenolol, quinidine, propranolol hydrochloride, verapamil and buparvaquone were 4.30 min, 5.96 min, 6.55 min, 7.98 min and 8.54 min respectively. The effluents were monitored by UV detection at 251 nm. The authors suggested that the method may be employed for rat in situ intestinal permeability study to assess intestinal permeability of buparvaquone, a promising lead compound for Leishmania donovani infections. The method was validated as per ICH guidelines.

Lo MW et al.[81], describes an automated HPLC method for the simultaneous determination of propranolol hydrochloride, 4-hydroxy propranolol hydrochloride and N-des isopropyl propranolol hydrochloride in plasma and urine before and after beta-glucuronidase/aryl sulfatase treatment. It involved extraction with ether at pH 10 in the presence of ascorbic acid which was added to prevent oxidation of 4-hydroxy propranolol hydrochloride. The compounds were then back extracted into dilute acid and assayed on an HPLC using a fluorescence detector. Three HPLC columns viz. phenyl, octyl and octadecyl columns were shown to be used with mobile phase consisting of methanol and phosphoric acid in the ratio 99:1 v/v. Authors explained that concentrations as low as 0.2, 1.0, and 0.2 ng/mL of propranolol hydrochloride, 4-
hydroxy propranolol hydrochloride, and N-des isopropyl propranolol hydrochloride respectively, can be measured using 1 mL of plasma.

**Review of research papers related to in vitro interaction study**

*In vitro* drug interaction studies are the important tools available and their results may be effectively correlated with the effectiveness of the drugs *in vivo*.

Saeed MA *et al.*[82], described an *in vitro* availability studies of enoxacin in presence of H₂ receptor antagonists. They had carried out *in vitro* release of enoxacin in presence of cimetidine, ranitidine and famotidine on BP 2003 dissolution test apparatus and compared with the availability of enoxacin and H2-receptor antagonists alone. The percentage dissolution of drugs was determined by UV spectroscopy at the isobestic points of the drugs. simulated gastric fluid, simulating empty stomach, simulated intestinal fluid (pH 9) and buffers of pH 7.4 simulating blood pH at 37 °C the was used to conduct the study. They had also carried out the study of the effect of H₂-receptor antagonists on the antibacterial efficacy of enoxacin turbidity method and compared with parent drug against *Staphylococcus aureus, Streptococcus pyogens, Streptococcus pneumoniae, Enterococcus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis* and *Bacillus subtilis*. On the basis of the results obtained, authors had suggested that enoxacin should be co-administered with care along with H2- receptor antagonists especially in case of ranitidine; although chances of adverse reactions are rare but decrease in MIC of enoxacin may resulted in delayed effect or require prolonged use of the drug.

Fida H *et al.*[83], carried out an interaction study between sparfloxacin and antacids by determining the dissolution and adsorption of one drug in presence of the other. The release of sparfloxacin from tablets in the presence of antacids like sodium bicarbonate, calcium hydroxide, calcium carbonate, aluminum hydroxide, magnesium...
hydroxide, magnesium carbonate, magnesium trisilicate and magaldrate was studied on BP 2003 dissolution test apparatus. The study was carried out in simulated gastric and intestinal fluids for three hr at 37 °C. As per their results the authors confirmed the decrease in dissolution rate of tablets in presence all antacids studied, whereas magaldrate and calcium carbonate exhibited relatively higher adsorption capacities in simulated gastric fluid and magnesium trisilicate and calcium hydroxide in simulated intestinal fluid, basal and histamine-stimulated acid in rabbit gastric glands. The authors described that the animal species and type of NSAID, along with the drug used to stimulate acid secretion were other factors that affect and further complicate acid

Rodriguez SS et al.[84], determined the effect of naproxen on gastric acid secretion and gastric pH by assessing the direct effects of naproxen 500 mg b.d. on basal and gastrin stimulated gastric pH, acid concentration and fluid volume by total gastric fluid collection. As per their study, naproxen resulted in a statistically significant decrease in total gastric fluid secretion with a concomitant increase in gastric acid concentration (mEq/mL) during the basal period (pre-naproxen and postnaproxen). There was no demonstrable change in serum gastrin levels shown. The total acid output before and after naproxen was shown to be not different (P > 0.25). The increase in mEq/mL from 0.04 to 0.05 was explained by a concentrating effect on acid by a decrease in non-acid volume of gastric secretion. Although statistical significance was not achieved, the decrease in mean gastric pH was consistent with these findings with a predicted change in mEq/mL from a pH of 3.3 to 3.1. The mechanisms by which NSAIDs produce their inflammatory effect in the GI tract are complicated.
Saeed MA et al.[85], reported the in vitro studies of interaction between metformin and NSAIDs, diclofenac sodium, flurbiprofen, ibuprofen, mefenamic acid, meloxicam and tiaprofenic acid. The studies were carried out using modified BP 2007 dissolution test apparatus in simulated body environments at 37°C. These drug interactions were analyzed by UV-visible spectroscopy. The effect on the availability of both metformin and NSAIDs in presence of each other was calculated by applying simultaneous equation which is derived by modifying Beers law. The concentration of the drugs were analysed by RP-HPLC with mobile phase consisting of methanol and water (80:20 v/v) maintained at a flow rate of 1 mL/min at 241 nm. As per the results tabulated, the in vitro availability of NSAIDs and metformin owing to interaction is depressed through the formation of charge transfer complexes which is found to be associated with inter- and intra-molecular rearrangement of the electronic cloud of the interacting drugs. Hence it is envisaged by the authors that concurrent administration of metformin and NSAIDs could alter the bio-availability and impair the clinical efficacy of both drugs. Effect of pH was also studied on these drug-drug interactions.

Existence of drug interactions is evident with the drugs used in migraine prophylaxis. For the selected drug combination for drug interaction assessment, there are no analytical or bio-analytical methods reported till date as per the literature survey. Literatures are also not available on the assessment of drug interactions amongst the selected drug combinations. Hence it is worthwhile to assess the drug interactions between drugs used in prophylaxis of migraine and analgesics.
8. Review of research papers related to pregabalin, gabapentin and duloxetine hydrochloride

Pain treatment during neuropathy should be multimodal including pharmacologic and non-pharmacologic techniques. Pregabalin, gabapentin and duloxetine hydrochloride are the widely used drugs in neuropathy pain management. Use of single drug may not efficiently address the need; combination of above drugs could be useful. However there are no analytical techniques available for quantitative estimation of above drugs simultaneously. Literature survey reveals the availability of several HPLC methods for estimation of above drugs individually.

Rajinder SG et al.[86], described an isocratic reversed phase HPLC method and validated for the analysis of pregabalin in bulk, pharmaceutical formulations and human urine samples, the separation was accomplished on a C18 5 μm ODS hypersil column using a methanol : acetonitrile -0.02 M dipotassium hydrogen orthophosphate (pH - 7) in the ratio 3: 1: 16 v/v/v mobile phase. pregabalin eluted isocratically at a flow rate of 1.0 mL /min. and monitored at 210 nm. The linearity of the method was in the range of 0.75 - 6.00 μg/mL. The method was validated with respect to accuracy, precision, linearity, ruggedness, limit of detection and limit of quantitation.

Jadhav S et al.[87], proposed an enantioselective high-performance liquid chromatographic method, with precolumn derivatization with Marfey’s chiral reagent, for the separation of the enantiomers pregabalin, in the bulk drug. The diastereomers of the pregabalin enantiomers were resolved to baseline on a reversed-phase C-18 column with mobile phase consisting of a mixture of 60:40 (v/v) mixture of aqueous 0.2% triethylamine (pH adjusted to 3.5 with dilute orthophosphoric acid) and acetonitrile. Resolution between the diastereomers was not less than five. The method
was found to be linear in nano gram concentration range. The method was said to be validated as per ICH guidelines and was justified in the validation results.

Mercolini M [88], developed a high performance liquid chromatographic method with fluorescence detection for the simultaneous determination of the three antiepileptic drugs gabapentin, vigabatrin and topiramate in human plasma. Separation was accomplished by using a mobile phase composed of phosphate buffer and acetonitrile on C-18 column. The method was found to be suitable for pharmacokinetic study with data describing partial validation of the method.

Chen et al.[89] developed a HPLC method for determining the optical impurity of pregabalin. The developed method was based on derivatization of pregabalin with N-5-fluro-2,4-dinitrophenyl-5-alanine amide. The derivatized compounds were separated by using Intersil C-18 column, and the mobile phase was phosphoric acid buffer and acetonitrile in the ratio 55:45 v/v. The method was found to be validated as per ICH guidelines.

Bostjan M et al.[90], proposed a HPLC method for the evaluation of pregabalin in a pharmaceutical dosage form using fluorescamine as a derivatization agent. After a precolumn derivatization (5 min, room temperature), the reaction mixture was separated on a C18 column with isocratic elution using 0.2% of triethylamine in a mixture of methanol and water (10 : 90 v/v). 3-Aminopentanoic acid was used as the internal standard. Using fluorescent detection (λ<sub>ex</sub> 395 nm, λ<sub>em</sub> 476 nm), a low detection limit of 0.02 μg/mL was reached. The method was linear in the concentration range 0.125–25 μg/mL. The method was found to be validated as per ICH guidelines.

Ramakrishna N et al.[91], developed high-performance liquid chromatography tandem mass spectrometry method and validated for the quantification of pregabalin.
in human plasma. In this method the sample was separated by liquid–liquid extraction, the analyte was separated using an isocratic mobile phase on a reverse-phase column and analyzed by MS/MS in the multiple reaction monitoring mode using the respective [M+H]^+ ions, m/z 160–142 for pregabalin and m/z 482–258 for the internal standard. The method was found to be linear in the concentration range range of 1–10,000 ng/mL for pregabalin in human plasma. The method was found to be validated as per FDA guidelines.

Vikas VV et al. [92], developed a HPLC method for estimation of pregabalin in human plasma using mefaxalone as the internal standard. Sample preparation involved simple protein precipitation by using acetonitrile as solvent. The extract was analyzed by high-performance liquid chromatography coupled to electrospray tandem mass spectrometry (LC-MS–MS). Chromatography was performed isocratically on Thermo Hypurity C18 column. The method was found to be linear in the concentration range of 10–10000 ng/mL. The method was found to be validated as per FDA guidelines.

Vermeij TAC and Edelbroek PM [93], developed a simultaneous method for determination of the γ-amino-n-butyric acid derivatives pregabalin, gabapentin and vigabatrin in human serum by high-performance liquid chromatography. The protein precipitation of the serum was done using trichloroacetic acid and aliquots of the supernatant were precolumn derivatized with o-phtaldialdehyde and 3-mercaptopropionic acid. Separation was achieved on an Altima 3 C18 column using isocratic elution; the drugs were monitored using fluorescence detection. The method was found to be linear in the concentration range up to 63mg/L for pregabalin, 40 mg/L for gabapentin and 62mg/L for vigabatrin. The method was found to be validated as per FDA guidelines.
Berry X et al.[94], describes a selective and sensitive HPLC method for the assay of the new antiepileptic drug pregabalin in serum/plasma. Protein precipitation was done using acetone, followed by the pre-column derivatization of pregabalin with picryl sulfonic acid. C-8 column was used for chromatography of pregabalin. The method was found to be linear in the concentration range of 0.5 mg/L to 8 mg/L. The method was found to be validated as per FDA guidelines.

Anthony B et al.[95], described a reversed-phase HPLC method for the determination of gabapentin and its major degradation impurity, 3,3-pentamethylene-4-butyrolactam and validated in pharmaceutical tablets and capsules. Separation was achieved on a Brown Lee Spheri-5 Cyano column using an acetonitrile–10 mM KH\(_2\)PO\(_4\)/10 mM K\(_2\)HPO\(_4\) (pH 6.2) (8:92, v/v) mobile phase. The method was found to be linear in the concentration range of 1 mg/L to 50 mg/L. The compounds were eluted isocratically at a flow rate of 1 mL/min at 210 nm. The method was validated according to USP guidelines.

Danielle CR et al.[96], developed a quantitative method for vigabatrin and gabapentin in human serum by gas chromatography and tandem mass spectrometry. After protein precipitation using acetonitrile, the compounds were pre-column derivatized by methylation and analysed on a polydimethylsiloxane column using splitless injection mode. The linearity of the method was found to be in the range from 5 to 80 µg/mL for vigabatrin and from 5 to 30 µg/mL for gabapentin.

Gambelunghe C et al.[97], developed an analytical method for determining gabapentin in serum using gas chromatography/tandem mass spectrometry. In this method serum gabapentin level in mice at 1 and 6 hr after oral or intraperitoneal treatment (300 mg/kg) was determined. The concentrations of gabapentin after 1 hr were 4.02 and 4.32 µg/mL in mice treated orally and intraperitoneally, respectively.
At the end of 6<sup>th</sup> hr gabapentin level was found to decrease to 66% in both groups. The validation of the method was done as per FDA guidelines.

Jason TJ <i>et al.</i>[98], developed a high performance liquid chromatographic method and validated for the analysis of duloxetine hydrochloride and desmethyl duloxetine hydrochloride in human plasma. Protein precipitation was done by using acetonitrile and by liquid - liquid extraction the drugs were extracted. Plasma was adjusted to pH 10 with 1.0 M sodium carbonate and extracted with hexane which contained 2% isopropyl alcohol. The analytes were pre-column derivatized with dansyl chloride (500 μg/mL). A Phenomenex Primesphere 5 C18 HC column provided chromatographic separation of the analytes followed by fluorescence detection with excitation and emission wavelengths at 285 nm and 525 nm respectively. The method showed its linearity in the concentration range from 500 ng/mL to 1200 ng/mL. The validation of the method was done as per FDA guidelines.

Srinivasulu P <i>et al.</i> [99], developed a reversed phase liquid chromatographic analytical method for duloxetine hydrochloride in the presence of its impurities and degradation products generated from forced decomposition studies. The degradation of duloxetine hydrochloride was observed under acid hydrolysis. The drug was found to be stable in other stress conditions studied. Separation of the drug from the synthetic impurities and degradation products formed under stress conditions was achieved on a Zorabax XDB C18 column using a mixture of aqueous 0.1% trifluoroacetic acid, methanol, tetrahydrofuran (60:20:20, v/v/v) as mobile phase. The method showed its linearity in the concentration range from 650 ng/mL to 900 ng/mL. The validation of the method was done as per FDA guidelines.

Mohammed Y <i>et al.</i>[100], described the application of ethyl chloroformate as a precolumn derivatizing reagent for the GC determination of isoniazid and hydrazine.
As per the reference, ethyl chloroformate can be used to derivatize the drugs containing amine and carboxylic group by converting them to ester.

Hiroyuki K [101], in his review on derivatization reactions for the determination of amines by GC provides detailed description about the various derivatization techniques available for amines respect to reactivity, selectivity and sensitivity. Their applications to the determination of individual amines, ammonia and N-nitrosoamines in various environmental samples are also described. The article was very much useful for selection of suitable derivatization technique for our project.

Qiu Y et al.[102], in their article have discussed the application of ethyl chloroformate derivatization for gas chromatography-mass spectrometry based metabonomic profiling. Here the authors give a detailed description about the use of ethyl chloroformate as derivatizing agents and is used for the optimization of derivatization of selected drugs in our study.

Numbers of RP-HPLC methods are reported for estimation of pregabalin, gabapentin and duloxetine hydrochloride individually. However there are no literatures available for their simultaneous analysis. Hence there is a scope for development of bio-analytical techniques for the simultaneous estimation of pregabalin, gabapentin and duloxetine hydrochloride.