CHAPTER – 3

Literature Review
3.1 LITERATURE REVIEW OF PREGABALIN\textsuperscript{59, 60}

3.1.1 Armagan O and Olcay S have developed two new, sensitive and selective spectrofluorimetric and spectrophotometric methods for the determination of the \(-\text{amino-}\)\(-n\)-butyric acid derivative pregabalin (PGB) in bulk drug and capsule. Pregabalin, as a primary amine compound, reacts with 7-chloro-4-nitrobenzofurazon (NBD-Cl) which is a highly sensitive fluorogenic and chromogenic reagent used in many investigations. Based on this fact, spectrophotometric and spectrofluorimetric methods for the determination of pregabalin in capsules were developed for the first time. The relation between the absorbance at 460 nm and the concentration is rectilinear over the range 0.5–7.0 ng/ml. The reaction product was measured spectrofluorimetrically at 558 nm after excitation at 460 nm. The intensity of fluorescence was directly proportional to the concentration over the range 40–400 ng/ml. The method was applied effectively to the determination of this drug in pharmaceutical dosage form. The mean recovery for the commercial capsules was 99.93\% and 99.96\% for both the study, respectively. The suggested procedures could be used for the determination of PGB in pure and capsules being sensitive, simple and selective.

3.1.2 Ramakrishna N and co-workers have developed a sensitive high-performance liquid chromatography positive ion atmospheric pressure chemical ionization tandem mass spectrometry method and validated for the quantification of pregabalin in human plasma. Following liquid–liquid extraction, the analyte was separated using an isocratic mobile phase on a reverse-phase column and analysed by MS/MS in the multiple reaction monitoring mode using the respective \([\text{M+H}]^+\) ions, m/z 160–142 for pregabalin and m/z 482–258 for the internal standard. The assay exhibited a linear dynamic range of 1–10,000 ng/ml for pregabalin in human plasma. The lower limit of quantification was 1 ng/ml with a relative standard deviation of less than 11.4\%. Precision and accuracy were acceptable for concentrations over the standard curve range. A run time of 4.0 min for each sample made it possible to analyse more than 300 human plasma samples per day. The validated method has been successfully used to analyse human plasma samples for application in pharmacokinetic studies.
3.2 LITERATURE REVIEW OF AZITHROMYCIN AND AMBROXOL

3.2.1 Chen and co-workers reported a sensitive, rapid liquid chromatographic-electrospray ionization mass spectrometric method for determination of azithromycin in human plasma and validated. Samples were separated using a thermo hypersil hipurity C18 reversed-phase column (150 mm x 2.1 mm i.d., 5 microm), together with a mobile phase containing of 20 mM ammonium acetate (pH 5.2)-acetonitrile-methanol (50:40:10, V/V/V) and was isocratically eluted at a flow rate of 0.2 ml/min. The method demonstrated that good linearity ranged from 2 to 1000 ng/ml with $r^2 = 0.9977$. The quantification limit for azithromycin in plasma was 2 ng/ml with good accuracy and precision. The higher mean extraction recovery, say 81.2% and 75.5% for azithromycin and internal standard respectively, was obtained in this work. The intra-day and inter-day precision ranged from 4.8% to 8.6% and 6.4% to 10.7% (RSD), respectively.

3.2.2 Riedel and co-workers reported two sensitive methods for the determination of the azalide antibiotic azithromycin in human serum were compared. Simultaneously High-performance liquid chromatography (HPLC) and a microbiological assay were applied to 768 serum samples obtained in a clinical study. There was outstanding agreement between the azithromycin concentrations measured by HPLC and by the bioassay. The correlation coefficient for the two methods was $r^2 = 0.96$. The precision and the sensitivity of the methods were found to be very similar.

3.2.3 Barrett and co-workers reported a validated, highly sensitive, and selective HPLC method with MS-MS detection for quantitative determination of azithromycin in human Na$_2$EDTA plasma. Roxithromycin was used as internal standard. Human plasma containing azithromycin and internal standard was ultrafiltered through centrifree micropartition devices and the concentration of AZI was determined by isocratic HPLC-MS-MS. Multiple reaction monitoring mode was used for MS-MS detection. The calibration plot was linear in the concentration range 2.55-551.43 ng/ml. Validation parameters like Inter-day and Intra-day precision and accuracy of the method were characterized by RSD and percentage deviation, respectively; both were less than 8%. Limit of quantification was 2.55 ng/ml. This method was used to determine the pharmacokinetic profile of azithromycin (250-mg tablets).
3.2.4 Taninak and co-workers reported a high-performance liquid chromatographic method for the quantitative determination of erythromycin, roxithromycin and azithromycin in rat plasma with amperometric detection under a standardized common condition using clarithromycin as an internal standard. This method was also proved to be appropriate for the determination of clarithromycin by employing roxithromycin as an internal standard. Each drug was extracted from 150 microl of plasma sample spiked with internal standard under an alkaline condition with tert.-butyl methyl ether. The detector cell potential for the oxidation of the drugs was set at +950 mV. The linearity of the calibration graphs were preserved over the concentration ranges of 0.1-10 μg/ml for erythromycin and roxithromycin 0.03-3.0 μg/ml for clarithromycin and azithromycin and. The statistical parameters like coefficients of variation and relative error were less than 9% and +/-7%, respectively. The analytical method presented here was proved to be useful for the examination of the pharmacokinetic characteristics of erythromycin, clarithromycin, roxithromycin, and azithromycin, in rats.

3.2.5 Fengguo and co-workers reported a sensitive and specific liquid chromatography-electrospray ionization mass spectrometry method and validated for the identification and quantification of azithromycin in human plasma. After the addition of the internal standard and 1.0M sodium hydroxide solution, plasma samples are extracted with a methylene chloride-ethyl acetate mixture (20:80, V/V). The organic layer is evaporated under a stream of nitrogen at 40°C. The residue is reconstituted with 200 μl of the mobile phase. The compounds are separated on a prepacked Shimadzu Shim-pack VP-ODS C18 (5 μm, 150 mm x 2.0 mm) column using a mixture of acetonitrile-water (65:35) (0.5% triethylamine, pH was adjusted to 6.2 with acetic acid) as the mobile phase. A single quadrupole mass spectrometer was used for detection by selected ion monitoring mode via electrospray ionization source. The method is completely validated and linear calibration curves are obtained in the concentration ranges from 5 to 2000 ng/ml. The intraday and interday batch relative standard deviations at four different concentration levels are all < 10%. The limit of detection and quantification are 2 ng/ml and 5 ng/ml, respectively.
3.2.6 Miguel and co-workers reported a LC method with UV detection for determining azithromycin impurities in tablets as pharmaceutical form. It is to be employed in routine and stability tests. A linear gradient elution was employed starting with 47% A and 53% B to reach 28% A and 72% B at 48 min. Mobile phase A was KH$_2$PO$_4$ 10 mM (H$_2$O) at pH 7.00. B was a mixture methanol:acetonitrile 1:1 (V/V). UV detection was performed at 210 nm. The chromatographic column was Phenomenex Synergi MAX-RP 4 μm 250×460 mm kept at 50 °C. Six impurities were separated and identified and it was possible to quantify five out of the six with reasonable accuracy and precision.

3.2.7 Fouda and co-workers reported a specific assay for the quantitative determination of the new antibiotic azithromycin in a low volume of human serum is described. The assay uses on-line high-performance liquid chromatography (HPLC) and atmospheric pressure chemical ionization mass spectrometry Deuterium-labeled azithromycin was synthesized and used as the internal standard of the assay. The drug and the internal standard are extracted from 50 microliters of serum, and aliquots are injected onto a standard reverse-phase HPLC column. The effluent from the HPLC column at 1 ml/min is introduced into the atmospheric pressure source of a SCIEX API III mass spectrometer. Azithromycin concentrations in serum are determined by the selected monitoring of the protonated molecular ions of the drug and the internal standard. Our assay yields accurate and precise results over the range 10 ng/ml to 250 ng/ml. The correlation between the assay and a standard HPLC-electrochemical method, requiring a larger volume of serum, has been determined. The two methods showed excellent agreement. Because of its low volume requirement, our HPLC-APCI assay can be substituted for the standard assay for the investigation of azithromycin pharmacokinetics in children.

3.2.8 Raines and co-workers reported a high-performance liquid chromatographic (HPLC) method for the measurement of azithromycin and two of its metabolites, 9a-N-desmethylazithromycin and N-desmethylazithromycin, in human tears and plasma. The drug, metabolites, and internal standard (n-propylazithromycin [IS]) were detected electrochemically after injection of the extracted sample into the HPLC system. The peak height ratio azithromycin, 9a-N-desmethylazithromycin N-desmethylazithromycin, varied linearly, with concentrations in the ranges of 0.1 mg/l to 2.0 mg/l (tears) and 0.01
mg/l to 2.0 mg/l (plasma) of azithromycin, 9a-N-desmethylazithromycin N-desmethylazithromycin, the correlation coefficient (r) was more than 0.994 mg/l for all of the compounds (n=6). The analysis of tear samples collected at different intervals within 12 hours to 144 hours after a dose of 20 mg/kg of AZI from a trachoma patient yielded concentrations ranging from 1.52 mg/l to 0.34 mg/l for AZI, 0.79 mg/l to 0.27 mg/l for 9a-N-desmethylazithromycin, and 1.99 mg/l to less than 0.20 mg/l for N-desmethylazithromycin. The concentration of AZI in plasma ranged from 0.15 mg/l to 0.01 mg/l, whereas 9a-N-desmethylazithromycin and N-desmethylazithromycin were undetectable.

3.2.9 Patricia Zubata and co-workers reported a simple liquid chromatographic method for the estimation of azithromycin raw material and in pharmaceutical forms. The sample was separated on a reverse phase C18 column and eluants monitored at a wavelength of 210 nm. The method was accurate, precise, and sufficiently selective. It is applicable for its quantification, stability, and dissolution tests.

3.2.10 Xue-Min and co-workers reported an LC-MS-MS assay for determining azithromycin in human plasma and investigating the pharmacokinetics in Chinese male volunteers following oral administration of a single dose of the capsules (0.5 g). Assay linearity was obtained in the range of 3.048-1016 µg/l (r² = 0.9995). The recovery of azithromycin in human plasma was more than 90%. The interday and intraday precision for four different concentration examined were lower than 15%.

3.2.11 Hadad and co-workers reported a high-performance liquid chromatography and multivariate spectrophotometric methods for the simultaneous determination of ambroxol hydrochloride and doxycycline in combined pharmaceutical capsules. C(18) analytical column was used to achieve the chromatographic separation mobile phase consisting of a mixture of 20mM potassium dihydrogen phosphate, pH 6-acetonitrile in ratio of (1:1, V/V) and UV detection at 245 nm. Also, the resolution has been effected by using numerical spectrophotometric methods as classical least squares principal component regression and partial least squares applied to the UV spectra of the mixture and graphical spectrophotometric method as first derivative of the ratio spectra method. Analytical figures of merit such as sensitivity, selectivity, analytical sensitivity, limit of
quantification and limit of detection were determined for classical least squares partial least squares and principal component regression methods. The proposed methods were validated and successfully applied for the analysis of pharmaceutical formulation and laboratory-prepared mixtures containing the two component combination.

3.2.12 Heinanen and co-workers reported a method for ambroxol, trans-4-(2-amino-3, 5-dibromobenzylamino) cyclohexanol hydrochloride, and benzoic acid separation by HPLC with UV detection at 247 nm in a syrup as pharmaceutical presentation. Optimal conditions were: Column Symmetry Shield RPC8, 5 microm 250 x 4.6 mm, and methanol H₃PO₄ 8.5 mM/triethylamine pH = 2.8) 40:60 v/v. Validation was performed results from both standards and samples show suitable validation parameters.

3.2.13 Wang and co-workers reported a rapid, sensitive and specific method to estimation of ambroxol in human plasma using high performance liquid chromatography coupled with electrospray ionization mass spectrometry. Chromatographic separation was performed on a BDS HYPERSIL C18 column (250 mm x 4.6 mm, 5.0 microm, Thermo electron corporation, USA) with the mobile phase consisting of 30 mM ammonium acetate (0.4% formic acid)-acetonitrile (64:36, V/V) at a flow-rate of 1.2 ml/min. The total run time was 5.8 min for each sample. Detection and quantification was performed by the mass spectrometer using selected ion monitoring at m/z 261.9, 263.8 and 265.9 for ambroxol. The calibration curve was linear within the concentration range of 1.0-100.0 ng/ml ($r^2 = 0.9996$). The limit of quantification was 1.0 ng/ml. The extraction recovery was above 83.3%. The methodology recovery was higher than 93.8%. The intra- and inter-day precisions were less than 6.0%. The method is accurate, sensitive and simple for the study of the pharmacokinetics and metabolism of ambroxol.

3.2.14 Dincer and co-workers reported a derivative UV spectrophotometric method for the determination of ambroxol in tablets. Determination of ambroxol in tablets was conducted by using first-order derivative UV spectrophotometric method at 255 nm (n = 5). Standards for the calibration graph ranging from 5.0 to 35.0 microg/ml were prepared from stock solution. The proposed method was accurate with 98.6 +/-0.4% recovery value and precise with coefficient of variation of 1.22. These results were compared with those obtained by reference methods, zero-order UV spectrophotometric method and...
reversed-phase high-performance liquid chromatography method. A reversed-phase C(18) column with aqueous phosphate (0.01 M)-acetonitrile-glacial acetic acid (59:40:1, V/V/V) (pH 3.12) mobile phase was used and UV detector was set to 252 nm. Calibration solutions used in HPLC were ranging from 5.0 to 20.0 µg/ml. Results obtained by derivative UV spectrophotometric method were comparable to those obtained by reference methods, zero-order UV spectrophotometric method and HPLC, as far as ANOVA test, \( F(\text{calculated}) = 0.762 \) and \( F(\text{theoretical}) = 3.89 \), was concerned. Copyright 2003 Elsevier Science B.V.

3.2.15 Nobilis and co-workers described ambroxol in biological fluids using a rapid and sensitive high-performance liquid chromatographic method. The samples prepared from plasma by liquid-liquid extraction were analysed on reversed-phase silica gel by competing-ion chromatography with ultraviolet detection. The method was applied to the determination of ambroxol levels in twelve healthy volunteers after oral administration of 90 mg of ambroxol in tablets of Mucosolvan and Ambrosan.

3.2.16 Kitsos and co-workers reported a high-performance liquid chromatography method to guarantee specificity, sensitivity, precision and accuracy in analysing dipalmitoylphosphatidylcholine in rabbit eustachian tube washings, as well as to determine its varying levels after administration of ambroxol chloride. The procedure is based on a post-column derivatization with fluorescence detection using 1,6-diphenyl-1,3,5-hexatriene which exhibits increased fluorescence in a lipid environment. Dipalmitoylphosphatidylcholine was chromatographed on a Hypersil Cl8. The mobile phase for the isocratic elution consisted of 40 mmol/l choline chloride in methanol-tetrahydrofuran (97:3). Ambroxol was given to a group of New Zealand white rabbits at a dose of 30 mg/kg. A second group receiving vehicle only acted as controls.

3.2.17 Koundourellis and co-workers reported a high-performance chromatographic method for simultaneous determination of ambroxol in the presence of different preservatives in syrups. The method separates ambroxol from methyl- ethyl-, propyl- and butyl paraben and from other multi-component mixtures. The retention behaviour of ambroxol and parabens as a function of both pH and mobile phase composition was investigated. The eluents were monitored with a UV detector at 247 nm. Linear
relationships between the amount of pharmaceutical compounds and peak heights were confirmed at the concentrations of 0.74-14.08 μg/ml. The high recovery (no extraction of the samples is required and the low % RSD confirm the suitability of the proposed method for the determination of ambroxol in different pharmaceutical preparations.

3.2.18 Grzegorz Bazylak and co-workers reported a potentiometric detection of clenbuterol, ambroxol and bromhexine in marketed pharmaceuticals was described in six isocratic HPLC systems. The podant- and macrocyclic-type neutral ionophores, $N,N,N',N''$-tetracyclohexyl-oxybis $($o-phenyleneoxy$)$ diacetamide and hexakis ($2,3,6$-tri-O-octyl)$-\alpha$-cyclodextrin were applied in poly(vinyl)chloride-based liquid membrane electrodes. Both types of neutral ionophores improved the sensitivity for all mentioned drugs when compared with a tetrakis($p$-chlorophenyl)borate based electrode as well as with single wavelength UV detection. Detection Limits ($S/N = 3$) of $2.6 \times 10^{-10}$ mol/l (injected concentration) for the highly hydrophobic bromhexine were achieved with the tetracyclohexyl-oxybis $($o-phenyleneoxy$)$ diacetamide-based electrode and a cyano reversed-phase HPLC with Uptisphere UP5CN-25QS silica column ($250 \times 4.6 \text{ mm i.d.}$) eluted with acetonitrile–ethanol–perchloric acid (1.66 mM) ($60:2:38$, V/V/V) (pH 2.45). Comparable result was obtained with ($2,3,6$-tri-O-octyl)$-\alpha$-cyclodextrin-based electrodes and an XTerra RP18 hybrid silica–polymer column eluted with acetonitrile–phosphoric acid (20 mM) ($25:75$, V/V) (pH 2.60). In the mobile phases containing 60–75% V/V acetonitrile or methanol, stable and reproducible response of both types of neutral ionophore-based electrodes was observed for at least 3 days. The results of the validated procedure for reliable simultaneous determination of the drugs in fortified representative samples of pharmaceuticals were also presented.
3.3 LITERATURE REVIEW OF PRASUGREL

3.3.1 Enaksha RW and co-workers have developed a liquid chromatography-tandem mass spectrometry method to separate the four stereoisomers of the active metabolite of prasugrel, R-138727, in human plasma after derivatization with bromomethoxyacetophenone to stabilize the molecule. This technique was designed to determine the relative contribution of each stereoisomer, based on statistical analyses of each stereoisomer's chromatographic peak areas. The methodology was validated and used for the analysis of clinical samples in which R-138727 had been derivatized at the time of blood collection. This technique can be useful to determine the ratios of stereoisomers in biological samples (e.g., plasma) especially in situations in which authentic standards of each individual stereoisomer are scarce or unavailable. In humans, the metabolic formation of R-138727 from prasugrel was found to be stereoselective, where 84% of R-138727 was present as RS and RR, the two most pharmacologically potent isomers, whereas the SR and SS enantiomers accounted for 16%. The ratios of the R-138727 stereoisomers were consistent among subjects, regardless of the dose or time of sample collection or whether the blood was sampled after the first dose or after 4 weeks of therapy.

3.3.2 Lukram O and co-workers have developed a rapid and sensitive liquid chromatography tandem mass spectrometry method and validated for the determination of the active metabolite (R-138727) of prasugrel in human plasma. Because R-138727 contains a thiol group, it requires stabilization by derivatizing with N-ethyl maleimide. Commercially available trandolapril was used as the internal standard (IS). The derivatives of R-138727 and IS were extracted from human plasma using a liquid-liquid extraction technique. Chromatography was performed on a Hypurity C18, 5 μ (50 mm x 4.6 mm, i.d.) column, with the mobile phase consisting of acetonitrile and 10 mM ammonium formate (pH 3.0, 50:50 V/V), followed by detection using mass spectrometry. No significant endogenous peaks corresponding to R-138727 or IS were detected in the blank human plasma samples and no significant matrix effect was observed for R-138727 and IS in the human plasma samples. The mean recovery for R-138727 ranged from 90.1 to 104.1%, with the lower limit of quantification set at 1 ng/ml. Linearity was established.
for concentrations in the range of 1.0-500.12 ng/ml, with a coefficient of determination \((r^2)\) of 0.9958. The derivatized R-138727 was stable in human plasma for 3 months at \(-20^\circ\)C. This method increased the sensitivity and selectivity, resulting in high-throughput analysis of R-138727 using trandolapril as the IS in pharmacokinetic and bioequivalence studies, with a chromatographic run time of 3.7 min.

3.3.3 Farid NA and co-workers have developed two fast and sensitive liquid chromatography/tandem mass spectrometry (LC/MS/MS)-based bioanalytical assays and validated to quantify the active and three inactive metabolites of prasugrel. Prasugrel is a novel thienopyridine prodrug that is metabolized to the pharmacologically active metabolite in addition to three inactive metabolites, which directly relate to the formation and elimination of the active metabolite. After extraction and separation, the analytes were detected and quantified using a triple quadrupole mass spectrometer using positive electrospray ionization. The validated concentration range for the inactive metabolites assay was from 1 to 500 ng/ml for each of the three analytes. Additionally, a 5x dilution factor was validated. The interday accuracy ranged from \(-10.5\%\) to 12.5\% and the precision ranged from 2.4\% to 6.6\% for all three analytes. All results showed accuracy and precision within \(\pm 20\%\) at the lower limit of quantification and \(\pm 15\%\) at other levels. The validated concentration range for the active metabolite assay was from 0.5 to 250 ng/ml. Additionally, a 10x dilution factor was validated. The interbatch accuracy ranged from \(-7.00\%\) to 5.98\%, while the precision ranged from 0.98\% to 3.39\%. Derivatization of the active metabolite in blood with 2-bromo-3'-methoxyacetophenone immediately after collection was essential to ensure the stability of the metabolite during sample processing and storage. These methods have been applied to determine the concentrations of the active and inactive metabolites of prasugrel in human plasma.

3.3.4 Borole TC and co-workers have developed a sensitive, selective, precise and stability indicating (in accordance with ICH guidelines) High-Performance Thin Layer Chromatographic method of analysis for Prasugrel, to resolve drug response from that of their degradation products. The method employed TLC aluminium plates precoated with silica gel 60 F254 as the stationary phase. The solvent system consisted of Dichloromethane: Methanol (9.9:0.1 V/V). This system was found to give compact spot
for Prasugrel (Rf value 0.58±0.03). Prasugrel was subjected to stress test conditions like acid, alkali, neutral hydrolysis, oxidation, dry heat and photo degradation. The spot for product of degradation was well resolved from the drug. Densitometric analysis of drug was carried out in the absorbance mode at 254 nm. The linear regression data for the calibration plots showed good linear relationship with $r^2$ was 0.995 in the concentration range of 300-1500 ng/band. The result indicated that the drug was susceptible to degradation, to different extent in different conditions.
3.4 LITERATURE REVIEW OF SUMATRIPTAN SUCCINATE

3.4.1 Ge ZA and co-workers have developed a rapid and sensitive high performance liquid chromatography (HPLC) method with fluorescence detection for the determination of sumatriptan in human plasma. The procedure involved a liquid-liquid extraction of sumatriptan and terazosin (internal standard) from human plasma with ethyl acetate. Chromatography was performed by isocratic reverse phase separation on a C18 column. Fluorescence detection was achieved with an excitation wavelength of 225 nm and an emission wavelength of 350 nm. The standard curve was linear over a working range of 1–100 ng/ml and gave an average correlation coefficient of 0.9997 during validation. The limit of quantification (LOQ) of this method was 1 ng/ml. The absolute recovery was 92.6% for sumatriptan and 95.6% for the internal standard. The inter-day and intra-day precision and accuracy were between 0.8–3.3 and 1.1–6.3%, respectively. This method is simple, sensitive and suitable for pharmacokinetics or bioequivalence studies.

3.4.2 Nozal MJ and co-workers have developed a high performance liquid chromatographic (HPLC) method for the assay of sumatriptan succinate residues in swabs collected from manufacturing equipment surfaces and validated in order to control a cleaning procedure. The swabbing procedure using two cotton swabs moistened with water was validated applying a wipe-test and a HPLC method developed to determine low quantities of the drug. The HPLC method involves a C18 column at 25 °C, a mixture of ammonium phosphate monobasic (0.05 M)-acetonitrile (84:16, V/V) as a mobile phase and UV detection at 228 nm. Using the proposed method, the average recoveries obtained are of 88.5% for vinyl, 94.2% for glass and 95.2% for stainless steel plates with RSD of 5.5 (n=36), 2.3 (n=36), 2.2% (n=36), respectively. The method was successfully applied to the assay of real swab samples collected from the equipment surfaces.

3.4.3 Femenia FA and co-workers have developed a simple, accurate, precise and rapid HPLC method with UV detection and validated in order to determine the in vitro transdermal absorption of sumatriptan succinate. The HPLC method is a modification of that described by Nozal et al. Separation was carried out on a 250 mm Kromasil C18 column at room temperature. The detector response, at 282.7 nm, was found to be linear.
in a concentration range between 0.145 and 145 µM. The limit of detection (LOD) was 0.019 µM and the limit of quantification (LOQ) was 0.145 µM.

3.4.4 Usha Rani P and co-workers have developed a method for bio equivalence study. In this double-masked, randomized, crossover, bioequivalence study, two 166-mg tablet preparations of sumatriptan (Suminate and Imigrane) were compared in 12 healthy male subjects (mean + SD age, 25 ± 6 years). Pharmacokinetic variables-mean maximum plasma concentration, time to reach mean maximum plasma concentration, and the mean area under the plasma concentration-time curve-were not statistically significantly different for the two treatment groups. Vital variables, such as blood pressure and heart rate, also did not show any significant between-group differences. Statistical analysis revealed no difference in the two formulations. It can be concluded that the two tablet preparations of sumatriptan are likely to be bioequivalent.

3.4.5 Sonal RP and co-workers have developed a method to evaluate the transdermal delivery of the 5-HT1B/1D agonist, sumatriptan from an iontophoretic patch system, in vivo. Initial in vitro experiments were conducted to optimize formulation parameters prior to iontophoretic delivery in Yorkshire swine. It was found in vitro that increasing drug load in the patch from 9.7 to 39 mg had no statistically significant effect on cumulative delivery (cf. 305.6 ± 172.4 vs. 389.4 ± 80.4 Ig cm², respectively). However, for a given drug load (39 mg) increasing formulation pH from pH 4.7 to 6.8 significantly increased the cumulative amount of sumatriptan delivered across the skin (389.4 ± 80.4 vs. 652.4 ± 94.2 Ig cm²). A biphasic current profile comprising intensities of 1.8 mA from t = 0 to t = 180 min and 0.8 mA from t = 181 min to t = 360 min was used for the in vivo experiments. Drug levels in the blood were 13.7 ± 4.5 and 53.6 ± 10.2 ng/ml at the 30 and 60 min time-points, rising to 90–100 ng/ml during the 90–180 min time-period. The in vivo results show that the pharmacokinetics following transdermal iontophoretic delivery are comparable to those after oral, nasal or rectal administration, but do not match those upon subcutaneous injection.

3.4.6 Cheng KN and co-workers have developed a liquid chromatographic tandem mass spectrometric method for the quantitative determination of sumatriptan base in human plasma and urine and validated over the concentration range 0.2–20 ng/ml. Sumatriptan is
a 5-HT1 receptor agonist which has found widespread use in the treatment of migraine. Sumatriptan and its internal standard (D3-sumatriptan) were extracted from human matrices using C2 solid phase cartridges. The extracts were chromatographed on a C18 column, ionised using a heated nebuliser assisted atmospheric pressure ionisation (API) interface and detected by MS: MS in the multiple reaction monitoring mode. The completed validation demonstrated the method to be robust, accurate, precise and specific for the direct quantification of sumatriptan in human fluids. The method was used on a routine basis to determine the levels of sumatriptan in human volunteers following the oral administration of a 25 mg dose of sumatriptan succinate.

3.4.7 Mclaughlin DA and co-workers have developed an assay method of sumatriptan was based on HPLC with tandem MS-MS detection. MK-462 and sumatriptan were extracted using an automated solid-phas extraction technique on a C2 Varian Bond-Elut cartridge. The n-diethyl analogues of MK-462 and sumatriptan were used as internal standards. The analytes were chromatographed using reversed-phase (nitrile) columns coupled via a heated nebulizer interface to an atmospheric pressure chemical ionization source. The chromatographic run times were less than 7 min. Both methods were precise, accurate and selective down to plasma concentrations of 0.5 ng /ml. The assay for MK-462 was adapted to separately monitor the unlabeled and 14C-labeled species of the drug following intravenous administration of radiolabeled material to man.

3.4.8 Oxford J and Lant MS and co-workers have developed a method to determine sumatriptan in plasma by thermospray liquid chromatography-mass spectrometry combined with the advanced automated sample processor was selected to achieve this. Although the assay was required quickly criteria for intra- and inter-assay accuracies and precisions of ± 10% had to be achieved. These were obtained only by using a co-eluting deutenum-labelled internal standard.

3.4.9 Dulery BD and co-workers have developed liquid chromatographic-electrospray-mass spectrometric (LC-ESI-MS) method to compare the pharmacokinetics of antimigraine compounds. The concentration of each parent drug was determined using a solid-phase extraction method and LC-ESI-MS analysis demonstrating the high sensitivity and specificity of the methods down to subnanogram levels in rabbit plasma.
samples. Pharmacokinetic parameters evaluated after administration of single intravenous and oral doses was very similar and the ANOVA analysis did not show any statistically significant differences for $t_{1/2}$, $C_{\text{max}}$, $V$ or $AUC$ (normalised). The pharmacokinetic parameters showed short $t_{1/2}$ (range 1.14-1.9 h) either after intravenous (iv) or oral (po) administration and high total body clearance (CL) after the po dose both probably due to extensive and rapid metabolism of the parent drugs as suggested by the low values for bioavailability (range 13.4-22.8%).

3.4.10 Trinath M and co-workers have developed two new simple UV spectrophotometric methods and validated for the simultaneous determination of sumatriptan (SUM) and naproxen sodium (NAP) in their combined dosage forms. First method is $Q_1$ Absorption Ratio Method using two wavelengths, 272 nm ($\lambda_{\text{max}}$ of NAP) and 284 nm (Isoabsorptive point). The second method is the First order derivative technique. In this method the zero crossing point of naproxen sodium was selected at 298 nm and for sumatriptan it was 335 nm. The solvent used was methanol in both the above mentioned methods. The linearity range for $Q_1$ Absorption Ratio was 10-90 $\mu$g/ml and for derivative method it was 20-190 $\mu$g/ml. All methods were validated statistically and recovery studies were carried out. All methods were found to be accurate, precise and reproducible. These methods were applied to the assay of the drugs in marketed formulation, which were found in the range of 98.0% to 102.0% of the labeled value for both sumatriptan and naproxen sodium. Hence, the methods herein described can be successfully applied in quality control of combined pharmaceutical dosage forms.
3.5 LITERATURE REVIEW OF IMATINIB MESYLATE

3.5.1 Velpandian T and co-workers have developed a rapid and sensitive HPLC method with UV detection for the estimation of imatinib from the plasma of patients with chronic myeloid leukemia (CML). The robustness of the method was checked by conducting first dose pharmacokinetics on blood samples from four patients who had been administered Gleevec (100 mg) in an oral dose. Samples were prepared in a simple and single step by precipitating the plasma proteins with methanol and injecting 50 µl aliquot from supernatant for analysis. Assay was conducted using a C8 column under isocratic elution with 0.02M potassium dihydrogen phosphate-acetonitrile (7: 3, V/V) at a flow rate of 1 ml/min and detected using photodiode array at 265 nm. Calibration plots in spiked plasma were linear in a concentration range of 0.05-25 µg/ml. The inter and intra-day variation of standard curve was <4% (RSD). This method could be a simple and quick method for the estimation of imatinib from the patient's plasma.

3.5.2 Olivia R and co-workers have developed a high-performance liquid chromatography (HPLC) method with UV/Diode Array Detection (DAD) for trough imatinib concentration determination in human plasma. Imatinib trough levels were measured in plasma from 65 CML patients using our method and LC-MS/MS as the reference method. Results with these two methods were compared using Deming regression, chi-square test, and sign test. Results: The calibration curve was prepared in blank human plasma. HPLC-UV/DAD calibration curves were linear from 80 to 4000 ng/mL, and the limit of quantification was set at 80 ng/mL. The between-day variation was 6.1% with greater than 96% recovery after direct plasma deproteinization and greater than 98% recovery from the column. No significant differences in imatinib plasma levels were found between HPLC-UV/DAD and LC-MS/MS.

3.5.3 Robert AP and co-workers have developed a liquid chromatographic–electrospray ionization mass spectrometric (LC–MS) method for quantifying imatinib and its main metabolite (CGP 74588) in plasma. The assay uses deuterated imatinib as the internal standard; acetonitrile deproteination; a phenomenex luna C 18 (2) (5 mm, 50 X 4.6 mm) reversed-phase analytical column; a gradient mobile phase of 0.1% formic acid in methanol and water; and mass spectrometric detection using electrospray positive mode.
electron ionization. The assay has a lower limit of quantification (LLOQ) of 30 ng/ml and is linear between 30 and 10000 ng/ml for both imatinib and CGP 74588. We demonstrated the suitability of this assay for imatinib using it to quantify the concentrations of imatinib and CGP 74588 in plasma of a patient given a 200-mg dose of imatinib orally. This LC-MS assay should be an important tool for future pharmacokinetic studies of imatinib.

3.5.4 Katerina M and co-workers have developed, validated and compared flow injection analysis (FIA) and ultra-high-performance liquid chromatography (LC)/tandem mass spectrometry methods for the determination of imatinib in plasma from patients with chronic myeloid leukemia. The plasma for analysis by both methods was deproteinized by methanol containing d8-imatinib. The separation was achieved on a 1.7 μm C18 column with a linear gradient (4 mM ammonium formiate and acetonitrile, pH 3.2). FIA was performed at flow rate of 0.03 ml/min (0.1% formic acid in methanol). Multiple reaction monitoring mode on the tandem mass spectrometer (API 4000, AB Sciex) in positive ESI were used for detection.

3.5.5 Silvia DF and co-workers have developed a new method using high performance liquid chromatography coupled with electrospray mass spectrometry for the quantification of plasma concentration of tyrosine kinase inhibitors imatinib, dasatinib and nilotinib. A simple protein precipitation extraction procedure was applied on 250 μl of plasma aliquots. Chromatographic separation of drugs and internal standard (quinoxaline) was achieved with a gradient (acetonitrile and water + formic acid 0.05%) on a C18 reverse phase analytical column with 20 min of analytical run, at flow rate of 1 ml/min. Mean intra-day and inter-day precision for all compounds were 4.3 and 11.4%; mean accuracy was 1.5%; extraction recovery ranged within 95 and 114%. Calibration curves ranged from 10,000 to 62.5 ng /ml. The limit of quantification was set at 78.1 ng /ml for imatinib and at 62.5 ng /ml for dasatinib and nilotinib. This novel developed methodology allows a specific, sensitive and reliable simultaneous determination of the three tyrosine kinase inhibitors imatinib, dasatinib and nilotinib in a single chromatographic run, useful for drugs estimation in plasma of patients affected by chronic myeloid leukemia.
3.5.6 Abdalla A and co-workers have developed a method to validate and compare HPLC and LC/MS/MS analytical methods and their applicability for the quantification of imatinib in human plasma. A total of 50 patients with chronic myeloid leukemia (CML) in chronic phase (CP) receiving 400 mg/day imatinib were enrolled in the study. Drug levels were determined by HPLC–UV and LCMSMS. HPLC intra-day accuracy ranged from 100.51 to 103.19%. LCMSMS accuracy ranged from 89.72 to 106.29%. The correlation coefficient between both methods was $r^2 = 0.96$. HPLC can be used for imatinib levels’ determinations in patients accurately and precisely.

3.5.7 Teoh M and co-workers have developed HPLC method for quantification of imatinib in tissues. Methanol (1:1 V/V plasma) and pH 4, 40:30:30 (V/V/V) water methanol- acetonitrile at 5 ml/g (brain) and 10 ml/g (spleen, kidney, liver) ratio was added to the samples, homogenized, sonicated, centrifuged (15,000 rpm, 5 min, 2°C) and the supernatant injected into an Inertsil CN-3 column (4.6 mm x 150 mm, 5 μm) using 64:35:1 (V/V/V) water-methanol-triethylamine (pH 4.8), flow rate 1 ml/min, 25°C. Imatinib eluted at 7.5 min (268 nm). Linearity: 0.1-50 μg/ml; precision, accuracy, inter- and intra-day variability was within 15%. Recovery was above 95% (plasma), 80% (brain) and 90% (kidney, liver, spleen).
3.6 LITERATURE REVIEW OF CLEBOPRIDE

3.6.1 Philip RR and co-workers have developed a procedure for the analysis of clebopride in plasma using capillary gas chromatography-negative-ion chemical ionization mass spectrometry. Employing an ethoxy analogue as internal standard, the two compounds were extracted from basified plasma using dichloromethane. Subsequent reaction with hepta fluoro butyryl imidazole produced volatile mono hepta fluoro butyryl derivatives whose ammonia negative-ion mass spectra proved ideal for selected-ion monitoring. The recovery of clebopride from plasma at 0.536 nmol/l was found to be 85.5 ± 0.9 % (n = 3) whilst measurement down to 0.268 nmol/l was possible with a coefficient of variation of 7.9%. Plasma levels of the compound are reported in two volunteers following ingestion of 1 mg of clebopride as the malate salt.

3.6.2 Yano M and co-workers have developed a radio immune assay to determine clebopride. An antiserum was raised in albino rabbits by injection with a clebopride [4-amino-N-(1-benzylpiperidin-4-yl)-5-chloro-o-anisamide] hemisuccinyl derivative -bovine serum albumin conjugate; the [3H] immunogen used was clebopride hemisuccinyl-[3H] tyrosine methyl ester. The antiserum, [3H] immunogen and human serum (or urine) samples, or standard clebopride solution, were incubated together at room temperature for the assay; the unchanged drug could be separated from its glucuronide conjugate by ether extraction of samples before the assay. The method was specific; only clebopride and its glucuronide were found to be susceptible to the antiserum. Free and bound forms of the [3H] immunogen were separated with use of bovine γ-globulin. The limit of sensitivity of the method is 0.1 ng ml⁻¹ of clebopride.

3.6.3 Philip RR and co-workers have developed a procedure for the simultaneous assay of clebopride and its major metabolite, N-desbenzylclebopride in plasma. The method utilizes capillary gas chromatography—negative-ion chemical ionization mass spectrometry with selected-ion monitoring of characteristic ions. Employing 2-ethoxy analogues as internal standards, the benzamides were extracted from basified plasma using dichloromethane. Subsequent reaction with heptafluorobutyric anhydride produced volatile mono- and diheptafluorobutyryl derivatives of clebopride and N-desbenzylclebopride, respectively. The methane negative-ion mass spectra of these
derivatives exhibited intense high-mass ions ideal for specific quantification of low levels in biological fluids. Using this procedure the recovery of the drug and metabolite from human plasma was found to be $84.4 \pm 1.5\%$ ($n = 3$) and $77.4\pm4.7\%$ ($n = 3$), respectively, at 0.5 ng/ml. Measurement of both compounds down to 0.10 ng/ml with a coefficient of variation of less than 10.5% is described. Plasma levels are reported in four volunteers up to 24 h following oral administration of 1 mg of clebopride malate salt.

3.6.4 Huizing G and co-workers have developed a method to quantize metoclopramide and its newly developed analogue clebopride, together with some of their metabolic products, following extraction from biological tissues and fluids, and subsequent separation on silica gel thin-layer chromatographic plates. Diazotisation, followed by coupling with N-(1-naphthyl)ethylenediammonium dichloride, carried out on the thin-layer plate, is utilised for visualisation. The intensity of the spots is measured by photodensitometric analysis. The effect of variation of various experimental conditions is studied. The method has proven to be satisfactory for the measurement of 20 ng /ml of these compounds in biological material; the results are well within the accepted limits of deviation.

3.6.5 Zhirong T and co-workers have developed a sensitive and specific liquid chromatography–electrospray ionization-mass spectrometry (LC–ESI-MS/MS) method and validated for the identification and quantification of clebopride in human plasma using itopride as an internal standard. The method involves a simple liquid–liquid extraction. The analytes were separated by isocratic gradient elution on a CAPCELL MG-III C$_{18}$ (5 µm, 150 mm × 2.1 mm i.d.) column and analysed in multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI) interface using the respective [M+H]$^+$ ions, $m/z$ 373.9 → $m/z$ 184.0 for clebopride, $m/z$ 359.9 → $m/z$ 71.5 for itopride. The method was validated over the concentration range of 69.530–4450.0 pg/ml for clebopride. Within- and between-batch precision (RSD %) was all within 6.83% and accuracy ranged from −8.16 to 1.88%. The LLOQ was 69.530 pg /ml. The extraction recovery was on an average 77% for clebopride. The validated method was used to study the pharmacokinetics profile of clebopride in human plasma after oral administration of clebopride.
3.7 AIM AND PLAN OF WORK

3.7.1 AIM

Literature review revealed that

- Only spectrofluorimetric, spectrophotometric and high-performance liquid chromatography positive ion atmospheric pressure chemical ionization tandem mass spectrometry methods were there for the estimation of pregabalin, this method involve the extraction of pregabalin by liquid–liquid extraction. But no HPLC method was reported for its analysis with impurities. So the study was aimed to develop and validate a simultaneous method for the estimation of pregabalin and its impurities in pure by RP-HPLC method.

- Several methods reported for the determination of azithromycin and ambroxol individually and also in combination with other drugs by UV spectrophotometry, HPLC, HPTLC, and LC-MS in pure and pharmaceutical dosage forms. But, no method has been reported for simultaneous estimation of azithromycin and ambroxol in combination. So the study was aimed to develop and validate a simultaneous method for the estimation of azithromycin and ambroxol in pure and marketed sample by RP-HPLC method.

- A few analytical methods like LC-MS and HPTLC methods were there for the estimation of prasugrel, but no HPLC method was reported for its analysis. So the study was aimed to develop and validate a new method for the estimation of prasugrel in pure and marketed formulation by RP-HPLC method.

- A few analytical methods like HPLC, LC-MS, thermospray liquid chromatography-mass spectrometry and liquid chromatographic-electrospray-mass spectrometric (LC-ESI-MS) method were there for the estimation of sumatriptan. Two UV spectrophotometric methods were also reported, first method was Q₁ absorption ratio method and the second method was the first order derivative technique. So the study was aimed to develop and validate a
new method for the estimation of sumatriptan succinate in pure and marketed formulation by UV spectrophotometric method.

- A few analytical methods like HPLC, LC-MS, high performance liquid chromatography coupled with electrospray mass spectrometry, ultra-high-performance liquid chromatography (LC)/tandem mass spectrometry and LC/MS/MS methods were there for the estimation of imatinib mesylate. There was no UV spectrophotometric method reported for the estimation of imatinib mesylate. So the study was aimed to develop and validate a new method for the estimation of imatinib mesylate in pure and marketed formulation by UV spectrophotometric method.

- A few analytical methods like capillary gas chromatography-negative-ion chemical ionization mass spectrometry, radio immune assay, liquid chromatography electrospray ionization-mass spectrometry (LC-ESI-MS/MS) and HPTLC methods were there for the estimation of clebopride. There was no UV spectrophotometric method reported for the estimation of clebopride. So the study was aimed to develop and validate a new method for the estimation of clebopride in pure and marketed formulation by UV spectrophotometric method.
3.7.2 PLAN OF WORK

The study was planned to develop a new method and validate the same as per the ICH guidelines.

➤ Primarily the study was planned to develop a new, simple, selective, sensitive and economical RP-HPLC methods for drugs (a) pregabalin and its impurities (b) azithromycin and ambroxol (c) prasugrel and to validate the developed methods as per ICH guidelines including the parameters like
  1. System suitability
  2. Specificity
  3. LOQ (Limit of Quantification)
  4. LOD (Limit of Detection)
  5. Linearity
  6. Precision
  7. Accuracy
  8. Robustness
  9. Solution stability

➤ Further the study was planned to develop a new, simple and economical UV spectrophotometric methods for drugs (a) sumatriptan succinate (b) imatinib mesylate (c) clebopride and to validate the developed methods with the same parameters.