SUMMARY AND CONCLUSIONS

Pharmaceutical analysis occupies a pivotal role to statutory certification of drugs and their dosage forms either by the industry or by the regulatory authorities. Pharmaceutical analysis plays a very vital role in the quality assurance and quality control of bulk drugs and their formulations. It is a specialized branch of analytical chemistry involves separating, identifying and determining the relative amounts of components in a sample of matter. Quality assurance and quality control of pharmaceutical dosage forms are essential for ensuring the availability of safe and effective dosage forms to consumers.

The development of HPLC methods for the determination of drugs has received great attention in analytical research because of their importance in the quality control. HPLC is the unique, versatile, universal, basic instrument and well utilized by the researchers because of its ease in the operation, availability and in terms of cost. In the present work, an attempt was made to develop a simple and rapid HPLC method for the routine analysis of two single (Levetiracetam, Lornoxicam) and four combination (Tenofovir and Emtricitabine, Candesartan and Hydrochlorothiazide, Olmesartan and Hydrochlorothiazide, Amitriptyline and Chlordiazepoxide) drugs in bulk and tablet formulations. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The developed method conditions are subjected for validation under ICH guidelines. The validation mainly includes selectivity, specificity, linearity, precision include the study of intra day and interday, accuracy, limit of detection and quantification, ruggedness, robustness etc.

Chapter – I: This chapter mainly deals with the introductory part on drug analysis. In this the chemical analysis includes, classical and instrumental analysis used in the pharmaceutical drug analysis was discussed. It concerns about methods involved for the estimation of pharmaceutical formulations using chromatic graphic techniques and also consists of brief discussion on high performance liquid chromatography and its instrumentation, method development followed by general method validation procedures and validation procedure for assay methods as per ICH guidelines.
Chapter – 2: This chapter deals with the development and validation of a new RP-HPLC method for the estimation of Levetiracetam in its pure form and tablet dosage form. Levetiracetam is an anticonvulsant medication used to treat epilepsy. Levetiracetam may selectively prevent hyper synchronization of epileptic form burst firing and propagation of seizure activity. To develop simple and economical RP-HPLC method for the estimation of Levetiracetam by using RP-HPLC in Pharmaceutical dosage forms, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with Inertsil ODS C-18 column (250 X 4.6 mm, 5 μ) column and mobile phase comprising of Methanol, Water, TEA in the ratio of 70:25:5 (ν/ν/ν) at a flow rate of 1.0ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 214nm based on peak area. The retention time was found to be 2.60 min. The optimized method was validated as per ICH guidelines. A linearity range of range 10μg/ml - 80μg/ml with correlation coefficient 0.9986 was established. The result of recovery study by standard addition method ranging from 98.13 to 101.475% suggested good accuracy. The precision of the proposed method was checked in terms of the repeatability, inter-day and intra-day time periods. The low % RSD values of inter-day (0.44) and intra-day (0.75) variations reveal that the proposed method was precise. The LOD, LOQ values were found to be 0.05μg/ml and 0.15μg/ml respectively. The absence of interference peak indicates that method can be used for routine analysis of Levetiracetam in pharmaceutical dosage form. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method. It is highly specific, precise accurate, rugged and robust analytical procedure and its retention time 2.60min allows the analysis of large number of samples in a short period of time. So this method can be used for routine analysis.

Chapter – 3: This chapter deals with the development and validation of a simple, specific, economical and precise reverse phase high performance liquid chromatographic method for determination of Lornoxicam API (active pharmaceutical ingredient) and formulation. Lornoxicam is a non-steroidal anti-inflammatory drug of the oxicam class with analgesic, anti-inflammatory and antipyretic properties. The basic chromatographic conditions were designed to be simple and easy to use and reproduce and were selected after testing the different conditions that affect HPLC analysis, for example column, aqueous and organic components of the mobile phase, proportion of mobile phase components, detection
wavelength, diluents and concentration of analyte. The on Zodiac C18 column (250 X 4.6 mm, 5μ) was used because of its advantages of high resolving capacity, better reproducibility, low-back pressure, and low tailing. Separation was achieved using a mobile phase consist of Methanol: Acetonitrile 80:20 (v/v) solutions at a flow rate of 1.0ml/min. The eluent was monitored using UV detector at a wavelength 260nm. The column was maintained at ambient temperature and injection volume of 20 μl was used. The drug was eluted in less than 10 min with good resolution and minimal tailing and without interference of excipients. The retention times of Lornoxicamwere 3.28min. To determine linearity a calibration graph was obtained by plotting Lornoxicam concentration against peak area. The method was linear in the range of 6–12 μg/ml for Lornoxicam concentration with a correlation co-efficient 0.999. Percentage relative standard deviation (%RSD) was found to be less than 2%, which was found to be 0.89 for intraday and 0.45 for interday, which proves that method was precise. The accuracy of the method was assessed by determination of recovery for three concentrations covering the range of the method. The amount of Lornoxicam was recovered, in the presence of placebo interference, was calculated. The mean recovery data obtained for each level as well as for all levels combined. The results presented good agreement with the labeled content when it is applied to for the determination of drugs in bulk and tablet dosage form. From the data obtained, the developed RP-HPLC method was found to be specific, linear, precise, accurate, rugged and robust.

Chapter – 4: This chapter deals with the development and validation of a new RP-HPLC method for simultaneous determination of Tenofovir and Emtricitabine in formulation. Tenofovir belongs to a class of antiretroviral drugs known as nucleotide analogue reverse transcriptase inhibitors (NRTIs), used for treatment of human immunodeficiency virus 1 (HIV-1) and hepatitis B virus infections. Emtricitabine is a nucleoside reverse transcriptase inhibitor (NRTI) for the treatment of HIV infection in adults and children. The method is based on HPLC separation of the two drugs on the Chromosil C18 column (250 mm x 4.6 mm, 5μ), with isocratic conditions and simple mobile phase containing Methanol: Acetonitrile: Water in the ratio of 50:20:30 (V/V/V) at flow rate of 1mL/min using UV detection at 231nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method was selective and linear.
between concentration range 40-100µg/mL for Tenofovir and Emtricitabine, correlation coefficient was found to be 0.999 for Tenofovir and 0.998 for Emtricitabine. LOD and LOQ were found to be 0.05µg/mL and 0.15µg/mL for Tenofovir and 0.02µg/mL and 0.08µg/mL for Emtricitabine, respectively. The developed method was found to be precise as the %RSD values for repeatability and precision studies were < 2 %. % RSD for intraday precision was found to be 0.06 for Tenofovir and 0.04 for Emtricitabine and % RSD for interday precision was found to be 0.061 for Tenofovir and 1.33 for Emtricitabine, as recommended by ICH guidelines. Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.05µg/mL and 0.15 µg/mL for Tenofovir and 0.02µg/mL and 0.08µg/mL for Emtricitabine, respectively. Statistical analysis proves that the method is suitable for the analysis of Tenofovir and Emtricitabine as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Tenofovir and Emtricitabine and also for its estimation in plasma and other biological fluids.

Chapter – 5: This chapter deals with the development and validation of a new RP-HPLC method for simultaneous estimation of Candesartan and Hydrochlorothiazide from their combination product. Candesartan is an angiotensin II receptor antagonist used as a first line agent to treat uncomplicated hypertension, also used as a first line agent to delay progression of diabetic nephropathy. Hydrochlorothiazide is a diuretic drug of the thiazide class. The proposed RP-HPLC method utilizes a Inertsil ODS C18 column (250 X 4.6 mm, 5µ) i.d. column, mobile phase consisting of Methanol: Tetrahydrofuran: 0.1 % Orthophosphoric acid in the proportion of 85:05:10 (V/V/V) with apparent pH adjusted to 4.8, and UV detection at 272nm using a UV detector. The described method has been validated, apart from specificity, for response function, accuracy, system suitability and precision. The described method was linear over a range of 5-35µg/mL for both Candesartan and Hydrochlorothiazide. % recovery for each case was calculated and was found to be 98.05 to 101.76% for Hydrochlorothiazide and 98.33 to 101.28% for Candesartan. This was found to be well within the acceptance criteria of 98-102%. A signal-to-noise ratio 3:1 is generally considered acceptable for estimating the detection limit. LOD is found to be 0.06µg/ml for Candesartan and 0.09µg/ml for Hydrochlorothiazide and LOQ is found to be 0.12µg/ml for Candesartan and 0.2µg/ml
for Hydrochlorothiazide. Chromatographic peak purity data of Candesartan and Hydrochlorothiazide indicated no co-eluting peaks with the main peaks of drugs which demonstrated the specificity of assay method for their estimation in presence of degradation products. The proposed method can be useful in the quality control of combination drug products.

Chapter – 6: This chapter deals with the development and validation of a new RP-HPLC method for simultaneous estimation of Olmesartan and Hydrochlorothiazide from their combination product. Olmesartan is an angiotensin II receptor antagonist used to treat high blood pressure. Hydrochlorothiazide is a diuretic drug of the thiazide class. Chromatography was carried out by isocratic technique on a reversed-phase Chromosil C18 column (250 mm x 4.6 mm, 5μ). The test method was validated for specificity, linearity, precision, accuracy, range, stability of sample solution, ruggedness and robustness were found to be in the predetermined acceptance criteria. The results of specificity studies from peak purity curve, and peak purity index clearly suggests no interference of the excipients and mobile phase. The proposed method is accurate, selective and precise hence can be used for the routine quality-control analysis and quantitative simultaneous determination of Olmesartan and Hydrochlorothiazidein combined tablet dosage forms and API. The mobile phase is easy to prepare and economical. The sample recoveries in all formulations were in good agreement with their respective label claims. The percentage RSD for all parameters was found to be less than 2, which indicates the validity of the method is in fair agreement. The method is also fast and requires approximately 7min run time per sample for analysis. The method was found to be accurate with percent recoveries ranging from 98.1 to 101.3%. All the parameters for the two titled drugs met the criteria of ICH guidelines for method validation. The method is very simple, rapid and economic in nature as all peaks are well separated, which makes it especially suitable for routine quality control analysis work.

Chapter – 7: This chapter deals with the development and validation of a new RP-HPLC method for simultaneous determination of Amitriptylin and Chlordiazepoxide in their tablet formulations using the most commonly employed RP C-18 column with UV detection. The mobile phase was optimized with Methanol: Acetonitrile 70:30 (v/v). From the overlain spectrum of Chlordiazepoxide and Amitriptyline, wavelength was selected, at 222nm, isoabsorptive point for both the
drugs. Good resolution was carried out at 222nm and both drugs showed good absorbance at this wavelength with minimum interference of the other drug. All parameters of these proposed method was validated as per the ICH guidelines.

No peak was detected at the retention time of Chlordiazepoxide and Amitriptyline, hence proving the specificity of the method. A linearity experiment shows correlation coefficient for Chlordiazepoxide and Amitriptyline is 0.9997 and 0.9990 respectively over a range of 5-30μg/ml for Chlordiazepoxide and 12.5-75μg/ml for Amitriptyline. The regression of Chlordiazepoxide and Amitriptyline in concentrations over its peak area were found to be y = 24447x + 34475 (r²=0.999) for Amitriptyline and y = 23396x+5711.8(r² = 0.9997) for Chlordiazepoxide. The regression equation was used to estimate the amount of Chlordiazepoxide and Amitriptyline, either in tablet formulations or in validation study.

The accuracy of the method was established using recovery technique i.e. by external addition of standard in to a pre analyzed sample at three different levels. An accuracy criterion for an assay method is that the mean recovery should desirably be 100±2% at each concentration over the range of 50-150% of target concentration. Since the mean % recovery varies for both drug from 98-102% and were within the desirable confidence interval, it can be said that the proposed method was accurate.

In precision experiment, (repeatability study) relative standard deviation for Chlordiazepoxide and Amitriptyline was found to be 0.2 and 0.15 respectively. It was concluded that the analytical technique showed good repeatability. Same experiment was repeated three times in a day at three different concentrations and three different days at same concentration. These values confirmed the intraday and interday precision of the method. The intra-day and inter-day % RSD values were calculated which were found to be in the range of 0.2 and 1.80 for Chlordiazepoxide and 0.15 and 0.98 for Amitriptyline. Hence, method at selected wavelength was found to be precise.

For the robustness testing, the chromatographic conditions were changed and in all varied chromatographic conditions the resolution between Chlordiazepoxide and Amitriptyline was greater than 2.0, illustrating the robustness of the method. Ruggedness was confirmed by precision experiment at three different analysts at
standard concentration. % RSD was calculated and was found to be well within the acceptance criteria. Hence the proposed method was robust and rugged.

Under the developed conditions, the two drugs obey all the system suitable criteria. Theoretical plates are above 2500 and tailing factor is less than 2 and separation resolution is 6.25. Hence in the developed conditions more resolved peaks were observed. The two drugs Chlordiazepoxide and Amitriptyline obey all the system suitable criteria in all the validations conditions and all the validation parameters are under the acceptance criteria. Hence the developed method is valid.

LOD value was found to be 0.005µg/ml and 0.025µg/ml for Chlordiazepoxide and Amitriptyline respectively and LOQ value was found to be 0.016µg/ml and 0.0.08µg/ml for Chlordiazepoxide and Amitriptyline respectively.

The proposed method successfully applied for the estimation of Chlordiazepoxide and Amitriptyline in the marketed formulations. It was found that the proposed method is successfully applied for the estimation of Chlordiazepoxide is 99.47% and Amitriptyline is 99.05%.