SUMMARY AND CONCLUSIONS

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 eleven 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.

Chapter - 1 mainly deals with the drug analysis. In this the chemical analysis includes classical and instrumental analysis used in the pharmaceutical drug analysis is 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 deals with the development and validation of anastrozole for the estimation in bulk samples and pharmaceutical formulations. Anastrozole is an aromatase inhibiting drug used for treatment of breast cancer after surgery, as well as for metastasis in both pre and post-menopausal women. The method development was conducted with Zodiac
C18 column (250 X 4.6 mm, 5μ) with the flow rate of 1.0mL/min. the optimized mobile phase conditions were methanol, water and acetonitrile in the ratio of 50:30:20 (v/v). Column temperature was ambient and the wavelength was 218nm. The method found to be linear, accurate, rugged and robust for validated parameters. The linearity range was determined by external standard calibration method in the concentration range of 2μg/ml to 10μg/ml (r2 = 0.999). The LOD and LOQ were found to be 0.05μg/ml and 0.165μg/ml respectively. Thus the method condition is very sensitive that can analysis in the nano gram level of concentration also. The system suitability parameters like capacity factor, asymmetric factor, tailing factor, and number of theoretical plates were tested. The amount of recovery was calculated as 99.5 to 101.2% and it was observed that all the values are within the limits. Further the precision of the method was confirmed by the repeatable analysis of formulation. The % RSD was found to be 0.588 for intraday and 0.918 for inter day. It indicated that the method has good precision. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. This method offers better turnaround of analytical values. Using the same method, assay was performed for individual samples and found that values are between good agreements. Hence this will be an excellent method for the assay determination and content uniformity of anastrozole in oral solid dosage form. The method has many advantages, e.g., simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and less flow rate, inexpensive mobile phases. Under these conditions, the retention time of anastrozole was about 7.05min, with a good peak shape (peak tailing factor < 2), and the run time was 10min.
Chapter – 3 deals with the development and validation of Cabergoline for the estimation in bulk samples and pharmaceutical formulations. Cabergoline is dopamine receptor agonist D2 receptors. Cabergoline has a direct inhibitory effect on pituitary lactotroph (prolactin) cells. The method development was conducted with Symmetry C18; 250 mm x 4.6 mm I.D; particle size 5μm with the flow rate of 1.0mL/min. The optimized mobile phase conditions were acetonitrile, 1% orthophosphoric acid and sodium dihydrogenphosphate in the ratio of 40:30:30 (v/v/v). Column temperature was ambient and the wavelength was 275nm. The method has significantly reduced runtime with better peak shape at 1.655 minutes and the runtime required for analysis is 5min. The method found to be linear, accurate, rugged and robust for validated parameters. The linearity range was determined by external standard calibration method in the concentration range of 5μg/ml to 25μg/ml ($r^2 = 0.999$). It indicated that the concentrations of cabergoline had wide linearity range for analysis. The LOD and LOQ were found to be 0.4μg/ml and 1.5μg/ml respectively. The system suitability parameters like capacity factor, asymmetric factor, tailing factor, and number of theoretical plates were tested. The amount of recovery was calculated as 99.66 to 100.16% and it was observed that all the values are within the limits. Further the precision of the method was confirmed by the repeatable analysis of formulation. The % RSD was found to be 0.946 for intraday and 0.892 for inter day. It indicated that the method has good precision. The low % RSD value indicated that there is no interference due to excipients used in formulation. Hence, the accuracy of the method was confirmed. This method offers better turnaround of analytical values. Using the same method, assay was performed for individual samples and found that values are between good agreements. Hence this will
be an excellent method for the assay determination and content uniformity of cabergoline in oral solid dosage form. The method has many advantages, e.g., simplicity, isocratic conditions, shorter run time, low injection volume, smaller particle size, and less flow rate, inexpensive mobile phases. Under these conditions, the retention time of cabergoline was about 1.655min, with a good peak shape (peak tailing factor < 2), and the run time was 5min.

Chapter – 4 deals with the development and validation of new reverse phase HPLC method for the estimation of celecoxib bulk samples and pharmaceutical formulations. The proposed RP- HPLC method has the advantages of sensitivity, simplicity, precision, accuracy and convenience for the separation and quantization of celecoxib in tablet dosage form. The method was carried out using Zodiac C18 column (100 X 4.6 mm, 5μ) with mobile phase comprised of methanol: acetonitrilein the ratio of 60:40 (v/v/v). The flow rate was set at 1.5ml/min and effluent was detected at 220nm. Under these conditions the retention time of celecoxib was found to be 3.57minute. The method was validated for specificity, accuracy, precision, linearity, and limit of detection, limit of quantification, robustness and solubility stability. The sensitivity test results of celecoxib indicated that the method was sensitive enough to detect a concentration of 1.0µg/ml and able to quantify at a concentration of above 3.3µg/ml. The RSD values of intra-day and inter day precision were very low which reveals that the proposed method was very precise. Linearity of the method was achieved at the range of 20-80µg/ml which can analysis in wide range of concentrations. Recovery and other validation results are satisfactory and the proposed method was successfully applied for quantitative determination of celecoxib in tablet dosage form.
Chapter – 5 deals with new RP-HPLC method development and validation for the estimation nebivolol in bulk samples and pharmaceutical formulations. Nebivolol is a β₁ receptor blocker with nitric oxide-potentiating vasodilatory effect used in treatment of hypertension and, in Europe, also for left ventricular failure. Drugs were determined on a chromosil C18 column (250 mm x 4.6 mm, 5μm) column. The optimized mobile phase was a methanol:water:triethyl amine in the ratio of 60:20:20 (v/v/v), pumped at a flow rate of 1 ml/min. UV detection was performed at 249nm. The developed method was validated based on ICH guidelines. Statistical analysis proves that the method is reproducible and selective for the analysis of nebivolol as bulk drug and in pharmaceutical formulations. The method was validated in the concentration ranges of 10 to 50µg/ml where it demonstrated good linearity with $r^2 = 0.999$. The percent recovery (99.1 to 100.12%), system suitability and precision (intra-day-0.51, inter day-0.21) results are found within the limit. The method was found to be robust, resisting to small deliberate changes in flow rate, column temperature and composition of the mobile phase. Due to the lack of the analytical methods this simple RP-HPLC method will be very much useful for estimation of nebivolol in bulk drug and pharmaceutical dosage form.

Chapter – 6 deals with HPLC method for simultaneous determination of dextromethorphan and quindine in formulation. Dextromethorphan is in a class of medications called antitussives used to temporarily relieve cough caused by the common cold, the flu, or other conditions. Quinidine is a class I anti arrhythmic agent (Ia) used in the treatment of abnormal heart rhythms, such as: atrial fibrillation, atrial flutter, and ventricular arrhythmias such as paroxysmal ventricular tachycardia and also is used to treat malaria. The
method is based on HPLC separation of the two drugs on the Zodiac C18 column (250 mm x 4.6 mm, 5μ), with isocratic conditions and simple mobile phase containing methanol, acetonitrile, water and 0.1% ortho phosphoric acid in the ratio of 35:25:25:15 (v/v) at flow rate of 1mL/min using UV detection at 259nm. 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 is selective and linear between concentration range 50-100μg/ml for dextromethorphan and quindine. LOD and LOQ were found to be 0.5μg/ml and 1.6μg/ml for dextromethorphan and 0.2μg/ml and 0.6μg/ml quindine, respectively. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines. Statistical analysis proves that the method is suitable for the analysis of dextromethorphan and quindine as bulk drug and in pharmaceutical formulation without any interference from the excipients. The validated method was applied to simultaneous determination of dextromethorphan and quindine was applied for the estimation of drugs in the commercial formulation (Lastuss: 10mg of dextromethorphan and natcardine 100mg of quindine). The % assay was found to be 99.4% for dextromethorphan and 99.83% for quindine (table 6.11). This indicts that the proposed method is successfully applied for the estimation of dextromethorphan and quindine in commercial formulations. It may be extended to study the degradation kinetics of dextromethorphan and quindine and also for its estimation in plasma and other biological fluids.
Chapter – 7 deals with reversed-phase HPLC method developed and subsequently validated for simultaneous estimation of famotidine and ibuprofen from their combination product. Famotidine is a histamine H$_2$-receptor antagonist used to treat ulcers, gastro esophageal reflux disease ibuprofen is a non-steroidal analgesic, antipyretic and anti inflammatory agent used primarily for fever, pain, dysmenorrhea and inflammatory diseases such as rheumatoid arthritis, pericarditis and patent ductus arteriosus. Chromatography was carried out by isocratic technique on a reversed-phase Inertsil ODS C-18 column (250 mm x 4.6 mm, 5μ) with mobile phase water: acetonitrile: triethylamine 80:10:10 (v/v). The test method was validated for specificity, linearity, precision, accuracy, range, stability of sample solution, ruggedness and robustness were found to be meeting 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 famotidine and Ibuprofen in 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 10min run time per sample for analysis. The method was found to be accurate with percent recoveries ranging from 99.2 to 100.5% for ibuprofen and 99.4 to 101.2 for famotidine. 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 – 8 deals with reversed-phase HPLC method developed and subsequently validated for simultaneous estimation of ezetimibe and simvastatin from their combination product. Ezetimibe is in a class of medications called cholesterol-lowering medications used to reduce the amount of cholesterol and other fatty substances in the blood. Simvastatin is a hypolipidemic drug used with exercise, diet, and weight-loss to control elevated cholesterol, or hypercholesterolemia. 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: acetonitrile: 0.1% orthophosphoric acid in the ratio of 75:20:05 (V/V/V) with apparent pH adjusted to 4.8, and UV detection at 243nm using a UV detector. The described method has been validated, apart from specificity, for response function, accuracy, system suitability and precision. The nominal concentrations of standard and test solutions for ezetimibe and simvastatin were 70μg/ml. The described method was linear over a range of 50-100μg/ml for both ezetimibe and simvastatin. % recovery for each case was calculated and was found to be 98.9 to 100.38% for simvastatin and 99.1 to 100.66% for ezetimibe. 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 1.2μg/ml for ezetimibe and 0.25μg/ml for simvastatin and LOQ is found to be 4μg/ml for ezetimibe and 0.8μg/ml for simvastatin. Chromatographic peak purity data of ezetimibe and simvastatin 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.