CHAPTER - VII

SUMMARY and CONCLUSION

CHAPTER I

INTROUCTION TO CATEGORIES OF DRUGS SELECTED, HPLC METHOD DEVELOPMENT AND VALIDATION

In this chapter a detailed description on the pharmaceutical analysis in estimation of the drug either alone or in combination and the importance of liquid chromatographic method development and validation for the simultaneous estimation of each component drug individually or in combined dosage form was presented.

Pharmaceutical analysis plays an important role in determining a drug in terms of quality and quantity because there is no second quality in drugs. To produce potent and safe drugs analytical technology is required. The analysis is concerned with an investigation of the quality control methods of synthetic drug, raw material and their preparations with definite chemical structure. The pharmaceutical analysis is a sub class of analytical chemistry that covers the analysis of drugs with respect to identification, characterization, and quantification of active pharmaceutical ingredients.

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long term stability studies. Combination of drugs are often found in pharmaceutical dosage forms for potentiating or enhancing their mode of action or complementing one another during the therapy. Hence the analytical chemistry has a challenge in developing the methods for their analysis with the help

of a number of analytical techniques which are available for the estimation of the drugs individually or and their combination.

Analytic method development and validation are key elements of any pharmaceutical development program. The RP-HPLC analysis method is developed to identify, quantity or purifying compounds of interest. This technical brief will focus on development and validation activities as applied to drug products. Methods may also support safety and characterization studies or evaluations of drug performance. Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of drug development.

Depending upon the facilities available in the pharmaceutical industries, chemical industries, and quality control laboratories adopt various RP-HPLC analytical methods to reduce the complexity of the problems occurring during pharmaceutical analysis. In my present investigation simple, precise and accurate RP-HPLC with photodiode array detector methods has been developed and validated for the analysis of selected drug combination [Alogliptin/Pioglitazone, Metformin/Benfotiamine, Dapagliflozin/Metformin, Hydrochlorothiazide/Quinapril].

CHAPTER II

RP-HPLC ESTIMATION OF ALOGLIPTIN AND PIOGLITAZONE SIMULTANEOUSLY IN COMBINED TABLET DOSAGE FORMS

In this chapter detailed description on the development and validation of stability indicating RP-HPLC with photodiode array detector method for the simultaneous quantification of alogliptin and pioglitazone in combined tablet dosage

form was reported. Alogliptin is an oral antihyperglycemic of dipeptidyl peptidase-4 inhibitor class used in the treatment of type II diabetes milletus. Pioglitazone is an anti-diabetic belonging to thiazolidinedione class of drugs prescribed to improve control of blood glucose level in adults with type II diabetes mellitus. With proper diet and exercise, alogliptin and pioglitazone combination is used in the management of high blood sugar levels caused by type II diabetes.

Optimum chromatographic conditions for separation and analysis of alogliptin and pioglitazone combination in pharmaceutical formulations have been achieved by using a Zorbax C8 column (250 mm × 4.6 mm, 5 μm) as a stationary phase. A mixture solution of 0.1 M ammonium acetate and methanol (50:50, *v/v*) with a pH of 3.5 and at a flow rate of 1 mL/min is used as mobile phase. The detection was performed at 248 nm using a photodiode array detector. Under the optimized chromatographic conditions, the retention times of the analytes were: Alogliptin – 2.883 min and Pioglitazone – 4.329 min.

The developed method was validated according to ICH guidelines for system suitability, linearity, sensitivity, selectivity, specificity, accuracy, precision and robustness. Regression analysis showed a good correlation ($R^2 \geq 0.9998$) for alogliptin and pioglitazone in the concentration range of 6.25-18.75 μg/mL and 11.25-33.75 μg/mL, respectively. The LOD and LOQ for alogliptin were found to be 0.047 μg/mL and 0.157 μg/mL, respectively. The LOD and LOQ for pioglitazone were 0.085 μg/mL and 0.284 μg/mL, respectively. The percentage recovery for alogliptin and pioglitazone were found to be in the range of 99.450-99.692 % and 100.184-100.422 %, respectively. The good percentage recovery values indicate the

accuracy of the proposed method. The method selectively determines the analytes in the sample without interference from excipients of tablet dosage forms. The %RSD values are 0.152 % and 0.234 % for alogliptin and pioglitazone, respectively. These values express the good precision of the proposed method. The results of method robustness demonstrated there were no marked changes in the system suitability parameters of the method. Different stress conditions like acid and base hydrolysis, oxidative, thermal and photo degradation were applied. Alogliptin and pioglitazone were found to degrade under all the stress conditions employed. The developed RP-HPLC method resolves the selected drug combination from their degradation products which confirms the stability indicating the power of the developed RP-HPLC method.

Based on method validation results, the proposed RP-HPLC method was demonstrated to be linear, accurate, sensitive, precise, accurate, selective, robust and specific. Therefore, the proposed method is stability indicating and can be used for routine analysis of alogliptin and pioglitazone in quality control and in stability studies.

CHAPTER III

RP-HPLC METHOD FOR THE SIMULTANEOUS ASSAY OF METFORMIN AND BENFOTIAMINE: DEVELOPMENT AND VALIDATION

Metformin, an oral anti-diabetic drug in the biguanide class of drugs, is of preference for the treatment of type II diabetes, particularly in obese persons. Benfotiamine is a synthetic fat soluble form of vitamin B_1 belonging to allithiamines family of compounds and used as a medicine or dietary supplement. It is prescribed

for the treatment of sciatica and other painful nerve conditions. The combination of benfotiamine and metformin is used in the treatment and control of Wernicke-korsakoff syndrome, diabetic neuropathy, maturity onset diabetes, type II diabetes mellitus and polycystic ovary syndrome. In this chapter, a detailed study on development and validation of a reverse phase liquid chromatographic method for the simultaneous quantification of benfotiamine and metformin in combined dosage form was presented.

Chromatographic separation and analysis of benfotiamine and metformin was performed on Hypersil BDS C18 (250 x 4.6 mm; 5 μm particle size) analytical column as the stationary phase with the temperature set at 30 ± 1 ^{o}C. Isocratic mobile phase composed of a binary system of 0.1M NaH_2PO_4 and acetonitrile in a ratio of 80:20 (*v/v*). The flow rate was set at 1 mL/min and total run time was 10 min with an injection volume of 10 μL. Evaluation of the separated drugs was performed using a photodiode array detector set at 254 nm. Both the drugs were resolved with the retention time of 2.055 min and 3.563 min for metformin and benfotiamine, respectively.

The method was validated with respect to system suitability, linearity, sensitivity, accuracy, precision and robustness in accordance with ICH guidelines. The drugs showed linearity at the selected wavelength (254 nm) in the concentration range of 100-300 μg/mL for metformin and 15-45 μg/mL for benfotiamine with regression coefficient (R^2) value of 0.9999. LOQ and LOD were 0.290 μg/mL and 0.968 μg/mL, and 0.047 μg/mL and 0.156 μg/mL for metformin and benfotiamine, respectively. The %RSD value is less than 0.1 % for both the drugs. The percentage

recovery of metformin and benfotiamine was found to be in the range of 99.510 - 99.797 % and 99.652-99.890 %, respectively.

The validation results and statistical data demonstrate that the proposed method is linear, selective, robust, accurate, sensitive and reproducible. The developed method was proved appropriate for the simultaneous analysis of metformin and benfotiamine in the pure and tablet dosage forms without the interference of excepients used commonly in tablets. Hence, the proposed method can be successfully applied for routine analysis of tablets containing the combination of metformin and benfotiamine.

CHAPTER IV

DEVELOPMENT OF STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DAPAGLIFLOZIN AND METFORMIN

Dapagliflozin is an antihyperglycemic agent belonging to the gliflozin class of drugs and approved for glycemic control in adult patients with type II diabetes. Metformin is an oral hypoglycemic agent belonging to the biguanides class of compounds and prescribed for the management of non insulin dependent diabetes mellitus. The combination of dapagliflozin and metformin, along with diet and exercise, is used to improve blood glucose control in adults with type II diabetes.

In this chapter, a detailed description on development and validation of stability indicating reverse phase liquid chromatographic method with photodiode

array detector for the simultaneous estimation of dapagliflozin and metformin in the in bulk and tablet dosage form was reported.

The separation and quantification of dapagliflozin and metformin combination were performed using a Supelco C18 (250 × 4.6 mm, particles 5 μm) HPLC column. Isocratic elution mode with a flow rate of 1.2 mL/min was used, and the injection volume was 10 μL. The detector was set to a wavelength of 285 nm and the column temperature was maintained at 30±1 °C. 0.1 M dipotassium hydrogen phosphate, acetonitrile, and methanol (60:30:10, *v/v/v;* pH 7.5) was used as the mobile phase. The total run time was 6 min. Under optimized chromatographic conditions, dapagliflozin and metformin were eluted with a retention time of 2.847 min and 3.804 min, respectively.

The developed RP-HPLC method was validated regarding system suitability, selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, robustness and specificity according to the International Conference on Harmonization.

A good linear relationship is obtained between the peak areas and the concentrations of the selected drug combination in the range of 2-6 μg/mL (dapagliflozin) and 200-600 μg/mL (metformin). Limits of detection were found to be 0.004 μg/mL and 0.272 μg/mL, and limits of quantification were calculated as 0.014 μg/mL and 0.907 μg/mL for dapagliflozin and metformin, respectively. The percentage RSD values for dapagliflozin and metformin are 0.098 % and 0.290 %, respectively. Mean recoveries ranged from 99.00-99.82 % and 99.70-99.82 % for dapagliflozin and metformin, respectively. In all the deliberate varied

chromatographic conditions, the system suitability parameters were not much affected. Dapagliflozin and metformin combination drug product (tablet sample solution) were exposed to thermal, photolytic, hydrolytic (acid and alkali) and oxidative stress conditions. The stressed samples were analyzed by the proposed method. There were no interfering peaks from degradation products due to stress conditions applied and the proposed method is specific for the simultaneous estimation of dapagliflozin and metformin in the presence of their degradation products.

Developed method was successfully applied for the quantification of the selected drug combination in tablet dosage form. The excipient did not interfere with drug peaks. The results of all the validation parameters were found to be within the acceptable limits. As the result, the proposed method can be used for routine quality control of tablet dosage form containing dapagliflozin and metformin.

CHAPTER V

STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF QUINAPRIL AND HYDROCHLOROTHIAZIDE IN TABLET DOSAGE FORMS

Hydrochlorothiazide is a short acting diuretic belonging to the class of compounds known as benzothiadiazines and is used to treat patients with hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and hypoparathyroidism. Quinapril is a prodrug and an angiotensin converting enzyme inhibitor used in the treatment of congestive heart failure and

hypertension. The combination of hydrochlorothiazide and quinapril is used to treat individuals with high blood pressure.

In this chapter detailed description on development and validation of stability indicating reverse phase liquid chromatographic method with photodiode array detector for the simultaneous estimation of hydrochlorothiazide and quinapril in bulk and in combined tablet dosage form was presented.

The separation and analysis of hydrochlorothiazide and quinapril was performed on Agilent C18 analytical column, 250 mm x 4.6 mm, 5 µm, using 0.1 M KH_2PO_4 and methanol in the ratio of 65:35 (*v/v*) as mobile phase at a flow rate of 1 mL/min for isocratic elution. The pH of the mobile phase was adjusted to 4.5 with dilute orthophosphoric acid. The column temperature was 25±2 ˚C and the injection volume was 10 µL. Detection of hydrochlorothiazide and quinapril was performed on a photodiode array detector at 210 nm. The retention times of hydrochlorothiazide and quinapril were 3.575 min and 4.770 min, respectively. The total run time was 6 min.

The developed method was validated according to the requirements of ICH. Linearity was obtained in the concentration range of 10-30 µg/mL and 6.25-18.75 µg/mL for quinapril and hydrochlorothiazide, respectively with a correlation coefficient of 0.9999 for both the drugs. The limit of detection was 0.045 µg/mL and 0.021 µg/mL and limit of quantification was 0.149 µg/mL and 0.071 µg/mL for quinapril and hydrochlorothiazide, respectively. For the studied drugs, recovery varied in the range of 99.70-100.21 % (quinapril) and 100.51 -100.52 % (hydrochlorothiazide) with relative standard deviation ranging

from 0.120 % (quinapril) to 0.080 % (hydrochlorothiazide). The robustness results indicated that small and deliberate variations did not have any significant effect on the measured system suitability parameters. The drugs were subjected to stress conditions of hydrolysis (oxidation, base, acid, and thermal degradation). The degradation products formed during the exposure of drugs to stress conditions applied gave peaks that are well separated from the peaks of quinapril and hydrochlorothiazide. The results of stress degradation studies proved the specificity and stability indicating abilities of the method.

The results of validation showed that the developed method proved to be linear, sensitive, precise, accurate, robust and convenient. So, the developed method should be useful for routine quality control purpose.

CHAPTER VI

DETERMINATION OF ASPIRIN AND PRAVASTATIN IN PHARMACEUTICAL DOSAGE FORMS USING STABILITY INDICATING RP-HPLC METHOD

Pravastatin, belonging to the statins class of drug, is a synthetic lipid lowering agent used in the lowering of plasma cholesterol levels and prevention of cardiovascular disease. Aspirin is a nonsteroidal anti-inflammatory drug used in the temporary relief of various forms of pain and inflammation associated with various conditions. The aspirin and pravastatin combination is used to treat high cholesterol, lower the risk of stroke and heart attack in people with coronary heart disease.

Chapter VI contains a detailed description on development and validation of stability indicating reverse phase liquid chromatographic method with photodiode

array detector for the simultaneous estimation of aspirin and pravastatin in bulk and in the combined tablet dosage form.

The separation and quantification of aspirin and pravastatin in the presence of stress degradants was achieved by Bondapack C18 Size 250 mm × 4.6 mm, 5 µm column in an isocratic mode, with a mobile phase consisting of 0.1M orthophosphoric acid and methanol (60:40, *v/v*) with pH 4.5. The mobile phase was pumped at a rate of 1.0 mL/min and the injection volume was 10 µL. The detection was carried out at 304 nm using photodiode array detector. The retention time of aspirin and pravastatin were observed to be 3.407 min and 4.115 min, respectively.

The method was validated for system suitability, linearity, sensitivity, selectivity, specificity, robustness, accuracy, and precision. Linearity for aspirin and pravastatin in the range of 10.25-82.0 µg/mL and 5-40 µg/mL, respectively. In precision experiment relative standard deviation for aspirin is 0.044 % and pravastatin is 0.077 %. From these values, it was concluded that the method showed good repeatability. The % mean recovery of aspirin was found to be 99.55 % and for pravastatin was found to be 99.97 % which indicated that the proposed method is accurate. During method robustness testing, no significant change was observed in the system suitability parameters of aspirin and pravastatin in all the deliberately changed chromatographic conditions. The limit of detection for aspirin and pravastatin were 0.442 µg/mL and 0.123 µg/mL, respectively. The limit of quantitation for aspirin and pravastatin were 1.472 µg/mL and 0.411 µg/mL, respectively.

No peak was detected at the retention time of aspirin and pravastatin in the chromatograms of placebo blank and mobile phase blank hence proving the selectivity of the method. During force degradation, the tablet sample of aspirin and pravastatin combination was exposed to hydrolysis (acid and base hydrolysis), H_2O_2, thermal degradation and photo degradation. The proposed method specifically estimates both the drugs (aspirin and pravastatin) in presence of all the degradants generated during forced degradation study.

It can be concluded that the proposed method separates aspirin and pravastatin from their degradation products and may be employed for analysis of stability for their combined tablet dosage form.

CONCLUSION

The present research investigation presents effective stability indicating reverse phase liquid chromatographic methods with photodiode array detector were developed for the selected drug combinations [Alogliptin/Pioglitazone, Metformin/Benfotiamine, Dapagliflozin/Metformin, Hydrochlorothiazide/Quinapril, Aspirin/Pravastatin]. The developed methods were validated according to ICH guidelines by testing system suitability, linearity, accuracy, precision, selectivity, specificity, robustness, limit of detection and limit of quantification. The validation parameters were found to be within the acceptable criteria. In all the developed methods, the degradation products obtained after exposing the sample solution to stress conditions are well separated from the analyte peaks. These results demonstrated that the developed methods were specific and stability indicating. The developed and validated methods were applied to corresponding drug combinations in pharmaceutical preparation without interference from the excipients. The developed and validated methods can be successfully applied for the simultaneous estimation and for the quality control of the selected drug combinations in bulk and in pharmaceutical dosage forms.