ABSTRACT
Abstract

“DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC AND UPLC METHODS FOR THE DETERMINATION OF ASSAY AND RELATED IMPURITIES OF DRUG PRODUCTS”

Introduction:

Chromatography is probably the most powerful and versatile analytical technique available to the modern chemist, its power arises from its capacity to determine quantitatively many individual components present in mixture in one, single analytical procedure. Chromatographic separation techniques are multistage separation methods in which the components of a sample are distributed between two phases, of which one is stationary and the other mobile. The stationary phase may be a solid or a liquid supported on a solid or a gel. The stationary phase may be packed in a column, spread as a layer, distributed as a film or applied by other techniques. The mobile phase may be gaseous or liquid or supercritical fluid. The separation may be based on adsorption, mass distribution or ion exchange or it may be based on differences among the physicochemical properties of the molecules, such as size, mass, and volume. The sample can range in complexity from a single substance to multicomponent mixture containing widely differing chemical species.

Assay and Related impurities are two important tests of pharmaceutical drug products. Where Assay determines the content of drug, one which needs to be measured with a high degree of accuracy and precision (eg. 98-102% of label claim). Related components are the impurities in pharmaceuticals which are unwanted chemicals that remain with the active pharmaceutical ingredients (APIs), or develop during stability testing, or develop during formulation or upon aging of both API and formulated APIs to medicines. The presence of these unwanted chemicals even in small amounts may influence the efficacy and safety of the pharmaceutical products. Various analytical methodologies were employed for the determination of related components in pharmaceuticals. There is a great need for development of analytical methods for new emerging drugs. The developed methods for estimation of impurities should be stability indicating. As per the current ICH requirements the evaluation of stability sample must be carried out using stability indicating analytical methods. A review of literature reveals a large number of methods
reported over a period of 3-4 decades under the nomenclature of "Stability-indicating". However, most of the reported methods fall short in meeting the current regulatory requirement. Suitable new (UPLC/HPLC), simple, cost effective (user friendly) and stability indicating analytical methods were developed, keeping the current regulatory requirements in mind, the developed methods were extensively validated.

Chapter-I: Analytical techniques used in the research work.

1.1 Introduction to chromatography:

Chromatography was first discovered by Tswett in 1890, he isolate the plant pigment, the colored bands he produced on the adsorbent bed evoked the term, chromatography (color writing) for this type of separation. chromatography as a useful separation technique for nearly 20 years. In the late 1930s and early 1940s Martin and Synge introduced a form of liquid-liquid chromatography by supporting the stationary phase, in this case water, on silica gel in the form of a packed bed and used it to separate some acetyl amino acids. Martin and Synge suggested the use of small particles and high pressures in LC to improve the separation which proved to the critical factors that initiated the development of high performance liquid chromatography.

1.2 High performance liquid chromatography:

In pharmaceutical industry, high performance liquid chromatography is the major and integral analytical tool used in Analytical development laboratories, and quality control laboratories. HPLC is characterized by the use of high pressure to push a mobile phase solution through a column of stationary phase allowing separation of complex mixtures with high resolution. It also allows us to use a very much smaller particle size (3.5 to 5 micron (μm)). Column packing material gives a much greater surface area of interactions between the stationary phase and mobile phase, which is essential for the discrimination of different analytes in mixture. These separated components are detected after the elution of the column by a flow-through device (detector) that measures their amount. Traditional detectors for liquid chromatography include refractive index, electrochemical, fluorescence, and ultraviolet-visible (UV-Vis).
1.3 Ultra performance liquid chromatography:

Ultra performance liquid chromatography (UPLC) name itself indicates that faster separations of analytes, higher resolution and less analysis time. UPLC instrument operates at ultra high pressure (upto 15000 PSI), where stationary phase consisting of particles less than 2.5 μm. smaller particles work at increased linear velocity without a loss of efficiency, thus providing both resolution and speed. The advantage of UPLC instrumental system for liquid chromatography is that it provides better separation performance by reducing dead volumes and instrument can withstand at Ultra high pressure, therefore it is possible to increase throughput, and thus the speed of analysis increases without affecting the chromatographic performance.

Chapter-II: Stability indicating liquid chromatographic methods for the simultaneous determination of Drug Products.

2.1 Introduction:

Stability testing forms an important part of the drug product testing, it provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest period and shelf life to be established.

The objective of the current study is to develop and validated stability indicating reversed-phase HPLC method for the simultaneous determination of drug product. First HPLC method is developed for simultaneous determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in Tablet dosage forms, second is sensitive LC method simultaneous determination of Ciclesonide and Formoterol fumarate in dry powder inhaler where as third is Sensitive LC method for the Simultaneous determination of Diacerein and Aceclofenac in tablet dosage form.

2.2 A stability-indicating LC method for the simultaneous determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate in tablet dosage forms.
Levocetirizine dihydrochloride is \(2\text{-}\{4\text{-[}(R\text{-})(4\text{-Chlorophenyl})phenylmethyl]\text{-}1\text{-piperazinyl}\text{-}1\text{-ethoxy}\text{-}acetic acid dihydrochloride\} \) is the pharmacologically active enantiomer of cetirizine, is a potent histamine H-1 receptor antagonist. Pseudoephedrine sulfate chemically \((S\text{-}(R^*,R^*))\text{-}\alpha\text{-}(1\text{-}(Methylamino)ethyl) benzenemethanol sulfate\). Pseudoephedrine sulfate is official in USP. Levocetirizine dihydrochloride is official in IP. Levocetirizine Dihydrochloride is having antiallergic properties used once daily for the treatment of allergic rhinitis. A Fixed dose combination of 180 mg of Pseudoephedrine sulfate and 5 mg of Levocetirizine dihydrochloride is available commercially as tablets are widely used for the symptomatic treatment of allergic rhinitis. Stability testing forms an important part of the process of drug product testing is to provide evidence on how quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light and enables recommendation of storage conditions, retest periods and shelf life to be established. The objective of this work was to develop an analytical LC procedure, which would serve as stability indicating assay method for combination drug products of Pseudoephedrine sulfate and Levocetirizine dihydrochloride.

2.2.1 Results and Discussion:

2.2.1.1 Optimization of the chromatographic conditions:

In order to optimize the LC separation of Pseudoephedrine sulfate and Levocetirizine dihydrochloride initially the retention behavior of both the components was studied in the pH range of 2.5–6.8, using mobile phases of buffer (pH 2.5–6.8) and acetonitrile, methanol asorganic modifier. It was found that Pseudoephedrine sulfate eluted in void volume whereas Levocetirizine dihydrochloride elute late. Hence it was decided to work by adding ion pairing reagent (1-Octane sulphonlic acid sodium salt) in the mobile phase. To ensure that Pseudoephedrine sulfate gives better retention, resolution between Levocetirizine dihydrochloride and Pseudoephedrine sulfate not less than 5, the method was as fast as possible; a gradient run was optimized using buffer pH 3.0 and Acetonitrile. Finally The mobile phase A consisted of Potassium dihydrogen phosphate Buffer 0.05M and 1- Octane sulphonlic acid sodium salt 0.25%, pH adjusted to 3.0 with orthophospheric acid. Mobile Phase B: Acetonitrile , Gradient elution at flow rate of 1mL/min and Column temperature at 40°C. Detector wavelength
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of 242 nm using a photodiode array detector was selected as an appropriate chromatographic conditions, which gave good resolution, acceptable peak parameters for both Levocetirizine dihydrochloride and Pseudoephedrine.

2.2.2 Conclusion:

The developed simple LC method for assay determination of Levocetirizine dihydrochloride and Pseudoephedrine sulfate is linear, precise, accurate and specific. The method was validated to the requirements of ICH and the results were satisfactory.

2.3 Sensitive LC method for simultaneous determination of Ciclesonide and Formoterol fumarate in dry powder inhaler.

Formoterol fumarate, N-[2-Hydroxy-5-[(1RS)-1-hydroxy-2-[(1RS)-2-(4-methoxyphenyl)-1-methylethyl] amino] ethyl] phenyl] formamide(E)-2-butenedioate is b2-agonist with a long duration of action. Ciclesonide, (R)-11b,16a,17,21-Tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with cyclohexane carboxaldehyde 21-isobutyrate is effective novel Inhaled corticosteroids, which has very low affinity for the glucocorticoid receptor in its native form, but very high affinity when transformed to its active metabolite by esterase in the lung. Bronchodilator medications are central to the symptomatic management of chronic obstructive pulmonary disease (COPD). Long-acting inhaled bronchodilators are more convenient. Also, Systemic corticosteroids are beneficial in the management of acute exacerbations of COPD. As a result, the combination of b2-agonist and corticosteroids has been a more useful tool in the management of asthma and COPD. Fixed dose combinations of 6 mg of Formoterol Fumarate (FF) and 200 mg of Ciclesonide(CS) are available commercially as dry powder inhaler and are widely used for the treatment of COPD. Literature survey revealed that some analytical methods has been used for individual estimation of Formoterol Fumarate and Ciclesonide or combination with other drugs. The present work represents development of a simple, precise and accurate reverse phase HPLC method for simultaneous estimation of Formoterol Fumarate and Ciclesonide in dry powder inhaler.
2.3.1 Results and Discussion:

2.3.1.1 Optimization of the chromatographic conditions:

During optimization of chromatographic conditions, different mobile phase compositions, different HPLC columns, organic modifiers such as acetonitrile and methanol, and flow rate were tried to achieve acceptable system suitability parameters, as well as good separation between FF and CS. Optimum wavelength selected was 214 nm because of higher sensitivity of FF at this wavelength and also good absorption shown by CS at this wavelength. 0.1% orthophosphoric acid was used as the buffer for the mobile phase preparation. Different combinations of buffer and acetonitrile within the range of 10:90–90:10, as well as buffer and methanol within the range of 10:90–90:10 were tested. The mobile phase combination of buffer: Acetonitrile (35:65) gives a symmetric peak shape, an acceptable tailing factor, and a shorter run time up to 10 min. Finally the chromatographic separation was achieved on Hypersil BDS C8, 250 x 4.6 mm, 5 um column using a mobile phase consisting of 0.1% orthophosphoric acid and acetonitrile in the ratio of 35:65 (v/v) at a flow rate of 2.0 mL min⁻¹. The column compartment temperature was set at 40°C and the injection volume was 200 mL. The UV spectrum of both the components exhibits a relative absorption maximum at 214 nm.

2.3.2 Conclusion:

The proposed HPLC method is specific, accurate, and precise for simultaneous determination of FF and CS from its pharmaceutical dosages form. The method was validated as per ICH guidelines and the results showed an the stability-indicating property of the method.

2.4 Sensitive LC method for the simultaneous determination of Diacerein and Aceclofenac in tablet dosage form.

Diacerein, 1,8-diacetoxy-3-carboxyanthraquinone and is also known as diacetylrhein. This drug is used in the treatment of osteoarthritis. After absorption, the drug is metabolized to its active metabolite rhein. Diacerein and rhein are anthraquinone compounds that ameliorate the course of osteoarthritis. Aceclofenac is chemically (2-[(2,6-dichlorophenyl)amino] phenyl acetoxyacetic acid). It has analgesic properties and a good tolerability profile in a variety of painful conditions. A combined fixed dose of 50 mg Diacerein and 100 mg Aceclofenac is
available commercially as tablets and are widely used for the treatment of osteoarthritis. Most of reference HPLC methods are less sensitive and not stability indicating. Hence, it was felt essential to develop and validate a sensitive, accurate and stability indicating RP-HPLC method for the simultaneous determination of Aceclofenac and Diacerein in tablet dosage form.

2.4.1 Results and Discussion:

2.4.1.1 Optimization of the chromatographic conditions:

The main criteria of a successful HPLC method development for determination of Diacerein and Aceclofenac in tablet dosage form was the method should be able to determine the assay of both drugs in single run. Our objective of the chromatographic method development was to achieve a peak tailing factor that is <2, run time up to 10 min, along with a resolution that is >5 between Diacerein and Aceclofenac. In order to optimize the LC separation of Diacerein and Aceclofenac, initially, the retention behavior of both the components was studied in the pH range of 2.5-6.8 using mobile phases of buffer (pH 2.5-6.8) and acetonitrile, methanol as organic modifier. To ensure the resolution between Diacerein and Aceclofenac not to less than 5, the method was as fast as possible, an isocratic run was optimized using the mobile phase consisted of double distilled water (pH adjusted 2.7 with glacial acetic acid)-acetonitrile (45:55 v/v), isocratic elution at flow rate of 1 mL/min and column temperature at 25°C. Detector wavelength was kept at 256 nm using a photodiode array detector.

2.4.2 Conclusion:

The developed simple LC method for assay determination of Diacerein and Aceclofenac is linear, precise, accurate and specific. The method was validated to the requirements of ICH, and the results were satisfactory.

Chapter-III: Development of liquid chromatographic methods for the determination of Drug Products by using different detectors.

3.1. Introduction:

A different types of detectors has been used for the analysis of pharmaceutical drug product. UV detector, Fluorescence detector, Refractive index detector are commonly used
detectors for the determination of drug product. In this chapter isocratic reversed-phase HPLC method with combine UV and Fluorescence Detector has been developed for the simultaneous determination of Drospirenone and Ethinylestradiol in tablet dosage form. Where as another HPLC method is developed for the determination of Metoprolol and Telmisartan in the tablet dosage form by using UV detector.

3.2 A Validated LC method for the simultaneous determination of Drospirenone and Ethinylestradiol in tablet dosage form by using combine fluorescence and UV detectors.

Drospirenone is a synthetic progestin that is used in birth control, chemically $(6R,7R,8R,9S,10R,13S,14S,15S,16S,17S)-1,3',4',6,6a,7,8,9,10,11,12,13,14,15,15a,16$-hexadecahydro-10,13-dimethylspiro-[$17H$-dicyclopropa-6,7:15,16]cyclopenta [a]phenanthrene-17,2'(5H)-furan]-3,5'(2H)-dione. Ethinylestradiol is chemically 19-Nor-17α-pregna-1,3,5(10)-trien-20-yne-3,17-diol is a derivative of estradiol. Ethinyl estradiol is an orally bio-active estrogen used in almost all modern formulations of combined oral contraceptive pills. For the prevention of pregnancy 3 mg Drospirenone and 30 mcg Ethinylestradiol per tablet is used as an oral contraceptive pill in women. The present manuscript has a precise and accurate isocratic reversed-phase HPLC method for the simultaneous determination of Drospirenone and Ethinylestradiol using combine UV and Fluorescence detector.

3.2.1 Results and discussion:

3.2.1.1 Method development and optimization:

Reversed-phase columns are silica based bonded phases. C8 and C18 are the type of bonded phases most frequently used, so different brands of C8-C18 type of HPLC columns have been used. Initial method development is started with the C8 column where peak shape of the Drospirenone peak is not proper; also both the ethinylestradiol and Drospirenone peaks are closely eluted with each other. The C18 column is also unable to give better separation between the ethinylestradiol and Drospirenone peak. To improve on that front phenyl columns have been tried. Substituted aromatic ring in the phenyl column gives better selectivity and good separation between Ethinylestradiol and Drospirenone peaks due to ππ interaction between analyte and
phenyl group on the column. Endcapping of the free silanols in the phenyl column is also helpful for the improvement of peak shape for the Drospirenone peak. X Terra Phenyl 150 x 4.6 mm 5µ column will fulfill all the system suitability requirements. Ethinylestradiol is a fluorescence active compound, whereas Drospirenone is an UV active compound, so serial connection of FLD and UV Detector has been used for the simultaneous determination of both the compounds. The flow rate of the mobile phase was adjusted to obtain the expected retention time of the analyte. Finally the mobile phase was mixture of Water : Acetonitrile (35:65) v/v. at a flow rate 1 mL min$^{-1}$.

3.2.2 Conclusion:

A simple, specific liquid chromatographic method was developed for the quantification of Drospirenone and Ethinylestradiol simultaneously. This method is found to be specific, precise, accurate and linear for the detection and quantification of Drospirenone and Ethinylestradiol and useful for routine quality control analysis.

3.3 Stability-indicating RP-HPLC method for the determination of Telmisartan and Metoprolol in tablet dosage form.

Metoprolol succinate chemically is 1-(isopropyl amino)-3-[4-(2-methoxyethyl)pehnoxy] propan-2-ol is a selective β₁ receptor blocker used in treatment of several diseases of the cardiovascular system, especially hypertension. Telmisartan chemically is 2-(4-[[4-Methyl-6-(1-methyl-1H-1, 3-benzodiazol-2-yl)-2-propyl -1H-1, 3-benzodiazol-1-yl]methyl]phenyl)benzoic acid. It is angiotensin II receptor antagonist, effective in the treatment of hypertension. It is also effective when used alone or in combination with other drugs for the treatment of high blood pressure.

A Fixed dose combination of 40 mg Telmisartan and 50 mg Metoprolol is available commercially as tablets (Telmaxx, Glenmark pharmaceuticals), are widely used for the treatment of cardiovascular disease. The two main aspects of drug products that play an important role in shelf life determinations are assay of active drug and degradants generated during the stability study. The objective of this work was to develop an analytical LC procedure for the simultaneous determination of Metoprolol and Telmisartan in tablet dosage form. The
present study attempt was made to develop a rapid, economical, precise, accurate and stability indicating method for the simultaneous determination of Metoprolol and Telmisartan in tablet dosage form in the presence of their degradants.

3.3.1 Results and discussion:

3.3.1.1 Method development and optimization:

The main criteria for development of a successful HPLC method for determination of Telmisartan and Metoprolol in tablet was the method should be able to determine assay of both drugs in single run and should be accurate, reproducible, robust, stability indicating, free of interference from degradation products, and straightforward enough for routine use in the quality control laboratory.

The retention behavior of both the components was studied in the pH range of 2.5–6.8, using mobile phases of buffer (pH 2.5–6.8) and acetonitrile, methanol as organic modifier. To ensure resolution between Telmisartan and Metoprolol not less than 5, the method was as fast as possible, a gradient run was optimized on Inertsil ODS 3V, 150 x 4.6 mm, 5µ column by using the mobile phase consisted of mobile phase A- 0.05M sodium dihydrogen phosphate buffer pH 3.0 and mobile phase B –acetonitrile (gradient: min/A:B 0/78:22, 4/55:45, 6/55:45, 18/78:22, 20/78:22). Gradient elution at flow rate of 1.0 mL/min and Column temperature at 40°C. Detector wavelength was kept 222 nm using a photodiode array detector.

3.3.2 Conclusion:

Stress degradation study was considered as tool to check stability indicating capability of developed method and useful to establish the degradation pathways. The developed simple LC method for assay determination of Telmisartan and Metoprolol is linear, precise, accurate and specific. The method was validated to the requirements of ICH and the results were satisfactory. The developed stability-indicating analytical method can be used for the routine analysis of production samples, where sample load is higher and high throughput is essential for faster delivery of results.
Chapter-IV: stability indicating liquid chromatographic methods for the determination of related impurities of Drug products.

4.1 Introduction:

Impurities can be classified as Organic impurities, (process- and drug-related) Inorganic impurities, Residual solvents. Organic impurities can arise during the manufacturing process and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile, and include starting materials, By-products, Intermediates, Degradation products, Reagents, ligands and catalysts inorganic impurities can result from the manufacturing process. They are normally known and identified and include reagents, ligands and catalysts, Heavy metals or other residual metals, Inorganic salts. The objective of the current study is to develop and validate stability indicating reversed-phase HPLC method for the determination related impurities of drug product. First HPLC method is developed for the determination of Voriconazole along with its degradation impurities. Whereas second HPLC method is developed for the determination of Eszopiclone along with its degradation impurities.

4.2 A validated stability-indicating liquid chromatographic method for Determination of degradation impurities and diastereomers in Voriconazole tablets.

Voriconazole belongs to a class of antifungal medicines used to treat serious and invasive fungal infections, which are generally seen in immunocompromised patients. Its chemical designation is \((2R,3S)-2-(2,4\text{-difluorophenyl})-3-(5\text{-fluoropyrimidin}-4\text{-yl})-1\text{-}(1H\text{-1,2,4-triazol}-1\text{-yl})\text{butan-2-ol. Voriconazole is commercially available in two strengths, 50 mg and 200 mg, with the brand name VFend (Pfizer, Inc.). To the best of our knowledge, all available analytical methods are used only for the quantification of Voriconazole, not for the quantification of known related compounds and degradation impurities of Voriconazole in tablet dosage form. Voriconazole degrades significantly under base hydrolysis stress conditions as compared with acid hydrolysis. The major degradation impurities observed are deschloro and DFH. Chemical sameness creates difficulty in separating diastereomers, but this proposed method is capable of separating diastereomers in the Voriconazole tablet dosage form. A literature survey reveals that
the Voriconazole Tablet is not official in any pharmacopeia. The base degradation of Voriconazole may seriously affect the quality of products, and is usually associated with a reduction of the pharmacological activity and/or the occurrence of side effects. The stress conditions are useful for establishing degradation pathways, and for developing and validating suitable procedures. This present work describes analytical parameters aimed to achieve an alternative for the quantification of Voriconazole and its degradation products along with diastereomers in tablets dosage forms, in accordance with ICH recommendations.

4.2.1 Results and discussion:

4.2.1.1 Optimization of the chromatographic conditions:

The main objective of the chromatographic method development was to separate Voriconazole impurities from the main peak with a good amount of resolution. The initial method development started with an Isocratic mobile phase. Different combination of buffers: acetonitrile in the range of 90:10 to 10: 90 v/v were used, and it was observed that the deschloro impurity and DFH impurity are mostly polar in nature, whereas impurity A is nonpolar in nature. Then an increase in the buffer concentration of more than 50% in the mobile phase lead to more retention of impurity A on the column, which lead to an increased run time of more than 60 minutes. Also, the peak shape of impurity A is not proper. By a decrease in buffer concentration to less than 50% in the mobile phase, retention of impurity A was reduced, but the resolution between the deschloro and DFH impurities also decreased, where both peaks eluted to nearly a void volume. As a result, the gradient mobile phases were switched, where potassium dihydrogen phosphate (pH 2.5) buffer was used as mobile phase A, and mobile phase B used acetonitrile and methanol in the ratio 90:10 v/v. Different gradient programs has been attempted to improve the run time to be less than 60 minutes with good retention of the deschloro and DFH impurities on the column. The peroxide stressed sample and impurity-spiked sample were injected in the column to check for good resolution between the known and unknown impurities, and Voriconazole. During the optimization of the method, the gradient program has been finalized as time (min) / % solution B: 0/20, 20/40, 38/60, 40/20, and 45/20. The column temperature was maintained at 35 ºC and the detection was monitored at a wavelength of 256 nm. The injection volume was 20 µL.
4.2.2 Conclusion:

The gradient RP-HPLC method developed for quantitative analysis of Voriconazole and related impurities in tablet dosage form is precise, accurate, linear, robust, rugged, and specific. Satisfactory results were obtained from validation of the method.

4.3 Stability-indicating LC–UV method for the determination of Eszopiclone and degradation impurities in tablet dosage form.

Eszopiclone is a nonbenzodiazepine hypnotic drug used to treat insomnia. Eszopiclone belongs to the class of drugs known as cyclopyrrolones. Its chemical designation is (S)-6-(5-Chloro-2-pyridinyl)-7-oxo-6,7-dihydro-5H-pyrrolo[3,4-b]pyrazin-5-yl-4-methyl-1-piperazin carboxylate. Eszopiclone is commercially available in two strength 1mg, 2mg and 3mg, with brand name fulinte tablets (Sun pharmaceutical LTD.). Literature survey reveals that Eszopiclone tablet related substance method is not official in any pharmacopeia. Eszopiclone degrades significantly in Acid hydrolysis stress condition, Stress condition 5 mL of 2N HCl Heated on water bath at 60 °C for 2 Hours degrades upto 50% and convert in to unknown impurity at RRT 0.33 having m/z 129.2 which is further identified as 2 amino 5 chloropyridin. The acid degradation of Eszopiclone may seriously affect the quality of products, and is usually associated with a reduction of the pharmacological activity and/or the occurrence of side effects. The stress conditions are useful for establishing degradation pathways, developing and validating suitable procedures.

4.3.1 Results and discussion:

4.3.1.1 Identification of unknown impurity:

Stability indicating capability of method can be established by achieving the 20–80% of degradation. Acid Hydrolysis stress condition (5 mL, 2 N HCl, 60 °C for 2 Hrs) about 50% of impurity at RRT 0.33 is formed. LC/ MS analysis of same impurity has been carried out which gives the m/z 129.2. During further investigation it has been observed that pure form of 2 amino 5 chloropyridine is m/z 129.2, which is key starting material for the synthesis of Eszopiclone as well as degradation impurity formed during acid hydrolysis. Its presence in the
sample has been confirmed by injecting pure form of 2 amino 5 chloropyridine in HPLC method. Which is eluting at the same RRT of 0.33.

**4.3.1.2 Optimization of chromatographic conditions:**

The initial method development started with an Isocratic mobile phase. Different combination of buffers: acetonitrile in the range of 90:10 to 10:90 v/v was tried. It was observed that the Impurity B and Impurity C are most polar in nature, whereas impurity A and Eszopiclone are nonpolar in nature. Increase in the acetonitrile concentration for more than 50% in the mobile phase, leads to void volume elution of impurity B and Impurity C peaks. Finally buffer and acetonitrile was adjusted in the ratio of 60:40 v/v in order to obtain better resolution between impurities and good peak shape of impurities and Eszopiclone. Inertsil ODS 3V 250mm x 4.6 mm, 5 mm column provides better retention to impurities B and C also peak shape of Eszopiclone peak is proper. By keeping the pH of the mobile phase on the acidic side, the silanol interaction with basic analyte was minimized and peak symmetry and sensitivity were improved so pH 3.5 buffer is used in the mobile phase preparation. The flow rate of the mobile phase was so adjusted to obtain the expected retention time of the analytes.

**4.3.2 Conclusion:**

The Isocratic RP-HPLC method developed for quantitative analysis of Eszopiclone and degradation impurities in tablet dosage form is precise, accurate, linear, robust, rugged and specific. Satisfactory results were obtained from validation of the method.

**Chapter-V: Development of UPLC methods for the determination of Drug products.**

**5.1 Introduction:**

High efficiency and shorter run time are the basic requirements of high-speed chromatographic separations. To fulfill these requirements, a new separation technique i.e. ultra-performance liquid chromatography (UPLC), has been developed. The objective of the current study is to develop and validated reversed-phase UPLC method for the quantitative determination of drug product. First UPLC method is developed for the determination of
Mesalamine in the presence of degradation products and its process-related impurities in tablet dosage form and second UPLC method is developed to conduct in vitro dissolution studies of two different Antihypertensive formulations in tablet dosage form such as Dronedarone tablet and Telmisartan and Hydrochlorothiazide tablet.

5.2 Stability-indicating UPLC method for the determination of Mesalamine related impurities in tablet dosage form.

Mesalamine (5-aminosalicylic acid, 5-ASA), the therapeutically active lead of sulfasalazine, which is used as a gastrointestinal anti-inflammatory drug for the treatment of inflammatory bowel diseases and active ulcerative proctitis. Mesalamine is available in tablet dosage forms (400 mg) and is an official drug of USP. Mesalamine protects against colorectal cancer in inflammatory bowel disease. For safety and quality of drug product, related substances should be known. Therefore, the aim of the present work was to develop and validate a simple, precise, accurate, short runtime and specific method for the quantification and separation of process related impurities/degradation product by reversed-phase UPLC method, in mesalamine tablet formulation.

5.2.1 Results and discussion:
5.2.1.1 Method development and optimization:

The main objective of development of RP-UPLC method was separation of Mesalamine related impurities in tablet dosage form. As the method should be able to determine all impurities of the drug product in single run with the good amount of resolution. Initial method development was started with Isocratic mobile phase. Different combination of buffer : acetonitrile in the range of 90:10 to 10:90 v/v has been tried, it has been observed that sulfanlic acid impurity is most polar in nature. Increase in organic concentration more than 5% in the buffer (95:5 buffer : acetonitrile) leads elution of sulfanlic acid impurity in the void volume. So Switch to gradient mobile phase where solvent A contains 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer) and Solvent B 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v. Different gradient programs has been tried to improves
the run time less than 60 minutes with good retention of sulfanlic acid on the column. During the optimization of chromatographic condition, solvent A contains 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 2.0 buffer). Solvent B 0.05 molar (M) dipotassium hydrogen phosphate and 0.5% octane sulphonic acid (pH 5.5 buffer) : methanol : acetonitrile in the ratio of 900:80:20 v/v. UPLC gradient program was set as time (min) / % solvent B: 0/0, 9/0, 15/15, 27/20, 30/75, 31/0 and 36/0. The column temperature was maintained at 30 °C and the detection was monitored at a wavelength 230 nm. Flow rate of mobile phase was 0.15 mL min$^{-1}$.

5.2.2 Conclusion:

A gradient RP-UPLC method was successfully developed for the estimation of mesalamine related impurities in pharmaceutical dosage form. Method is precise, accurate, linear, robust, rugged, and specific. Satisfactory results was obtained from validation of the method. Exposure of mesalamine drug product to stress conditions indicates that the drug is susceptible to acid, base hydrolysis; oxidation, photolysis and heat degradation with maximum degradation observed in base and oxidative conditions. A stability-indicating method was developed, which separates all the degradation products formed under variety of conditions.

5.3 Validated UPLC methods for in vitro dissolution studies of two different antihypertensive formulations in tablet dosage form.

Dronedarone HCl (DRO) mainly used for cardiac arrhythmias, it was recommended as an alternative to amiodarone for the treatment of atrial fibrillation and atrial flutter. Its chemical designation is N-(2-Butyl-3-(p-(3-dibutylamino)propoxy)benzoyl)-5-benzofuranyl)methane sulfonamide. Dronedarone tablets were obtained from India market. Each tablet was labeled contain 400 mg of Dronedarone. Telmisartan is an angiotensin II receptor antagonist used in the management of hypertension. Its chemical design 4’-[(1, 4’-Dimethyl-2’-propyl-[2, 6’-bi-1H-benzimidazol]-1’-yl) methyl]-[1, 1’-biphenyl]-2-carboxylic acid. Hydrochlorothiazide is a thiazide type diuretic which reduces reabsorption of electrolytes from the renal tubules. Its chemical designation is 6-Chloro-3, 4-dihydro-2H-1, 2, 4-benzothiazidin-7-sulfonamide-1,1-dioxide. Telmisartan and Hydrochlorothiazide are indicated
for the treatment of hypertension. Telmisartan and Hydrochlorothiazide tablets were obtained from India market. Each tablet was labeled as Telmisartan 40 mg and Hydrochlorothiazide 12.5 mg, or Telmisartan 80 mg and hydrochlorothiazide 12.5 mg.

Literature survey reveals that Dronedarone HCl tablet is not official in any pharmacopeia, where as Telmisartan and Hydrochlorothiazide tablet is official in USP. In this method run time is 15 minutes. For in vitro dissolution study of any drug product, numbers of analyzed samples are large in number, So there is need of lesser run time method for the analysis of these samples. There is no one UPLC method is reported for in vitro dissolution study of these two formulations. The present work describes analytical parameters aimed to achieve an alternative method for the quantification of Dronedarone HCl in tablet formulation and Telmisartan and hydrochlorothiazide in tablet formulation by UPLC method with shorter run time i.e. 1 minutes and 3 minutes respectively for in vitro dissolution study in tablet dosage form.

5.3.1 Results and Discussion:

5.3.1.1 Method Development and Optimization:

To work on the cost effectiveness of the method it has been tried to use same column and mobile phase for the analysis of these two formulations. So different columns with different particle size has been tried i.e. 2.5µm, 2.0µm and 1.7 µm. It has been observed that 2.5µm, 2.0µm particle size column are unable to provide the shorter run time compare to 1.7 µm particle size column. Different type of stationary phases such as C8 and C18 has been tried. On C8 type of stationary phase Dronedarone is elutes in void volume where as in C18 type of stationary phase retention of Dronedarone has been observed. Buffer pH 2.0 to 4.5 has been tried and it has observed that higher pH provides the more retention of Telmisartan compare to lower pH, so finally 2.5 pH gives expected result. Different mobile phase combination of buffer (pH 2.5) : acetonitrile in the range of 90:10 to 10: 90 v/v has been tried. Mobile phase ratio buffer : acetonitrile 45:55 v/v provides the expected retention time for Dronedarone. Whereas gradient mobile phase in the ratio of time (min) / % solution B: 0/10, 1/10, 2.0/90, 2.5/10 and 3.0/10 the expected retention time for Telmisartan and Hydrochlorothiazide.

During the optimization of the method. The chromatographic column used was an Acquity UPLC BEH C18 50 x 2.1mm, 1.7µm. with Mobile phase used as purified water adjusted
pH 2.5 with Orthophospheric acid (solution A) and Acetonitrile (solution B) in the ratio 45:55 (v/v) for dronedarone whereas gradient mobile phase in the ratio of time (min) / % solution B: 0/10, 1/10, 2.0/90, 2.5/10 and 3.0/10 for Telmisartan and Hydrochlorothiazide. The flow rate was 0.5 mL min\(^{-1}\). The column temperature was maintained at 40 °C and the detection was monitored at a wavelength 290 nm for Dronedarone whereas 265 nm for Telmisartan and Hydrochlorothiazide.

5.3.2 Conclusion:

The RP-UPLC method developed for quantitative analysis of Dronedarone HCl in tablet dosage form, Telmisartan and Hydrochlorothiazide tablet dosage form is precise, accurate, linear, robust, rugged and specific. Satisfactory results were obtained from validation of the method.