Abstract:

The thesis entitled “Development and Validation of HPLC Methods for Analysis of Bioactive Compounds and Their Formulations” embodied of five chapters.

Chapter-1 deals with the current challenges of chromatographer to development analytical methods for analysis of pharmaceutical compounds, basic information of HPLC instrumentation, HPLC method development, chiral chromatography and brief review on optimization of forced degradation conditions.

Chapter-2 deals with the stability indicating HPLC method development for active pharmaceutical ingredient and its pharmaceutical dosage form. For this purpose we selected drugs namely Prasugrel hydrochloride, Dronedarone hydrochloride, Guanfacine hydrochloride and Epinastine hydrochloride. We developed HPLC method and validate as per ICH guidelines.

Stability indicating HPLC method is developed for Prasugrel hydrochloride in pharmaceutical dosage form for its quantitation. Separation of the Prasugrel and its possible impurities were analyzed on Zorbax XDB C₈ stationary phase, Agilent Technologies, USA) maintained at 30 °C using column oven, The mobile phase consists of aqueous solution of ammonium acetate (0.05 M) and acetonitrile in ratio of 40:60, v/v. pH adjusted with acetic acid to 4.5 prior to mix. The detector wavelength was kept 254 nm using UV detector. Plate counts of Prasugrel were 9351. Tailing factor for Prasugrel was 1.13. Acidic, basic hydrolysis, UV, heat and oxidation stresses were employed to prasugrel and treated samples of Prasugrel was analyzed by method. Spectral purity data of Prasugrel is analyzed using PDA in treated sample and which demonstrated the specificity for the quantitation of Prasugrel in presence of degradants. The Prasugrel was shown an extensive degradation in acidic condition, alkali hydrolysis, oxidation and employed heat condition. The hydrolytic degradation observed in both alkali and acidic condition may due to the presence of carboxyl group into the structure, resulted in the formation of polar degradants (acid). In acidic condition nitrogen containing ring may undergo protonation and by cleavage it form polar impurities. Prasugrel have linear response in range of 10 to 400 μg/ml. Good precision was observed in method and intermediate precision. Percentage RSD for six determinations was always less
Abstract

than 2.0. The recovery values for Prasugrel at 80,100 and 120% were ranging from 98.88 to 100.59%. The correlation coefficient is 0.999. LOQ of Prasugrel was 0.364 µg/ml having percentage RSD for six injections was found to be less than 5.0 and LOD of Prasugrel was 0.11 µg/ml having percentage RSD for six injections less than 10.0.

Stability indicating HPLC method is developed for Dronedarone hydrochloride and its degradants. A Base to base separation of the Dronedarone and its possible degradations were achieved with Zorbax XDB C8, (15 cm × 4.6 mm, Agilent Technologies, USA) analytical column using aqueous solution of 0.05M ammonium acetate and acetonitrile in ratio 40:60, v/v., Column oven monitored at 30 ºC . UV detector set at 220 nm using a PDA. USP plate counts for Dronedarone were 6577. USP tailing for Dronedarone were 1.09. Acidic, basic hydrolysis, UV, heat and oxidation stresses were employed to Dronedarone and treated samples of Dronedarone was analyzed by method.

Spectral purity data of Dronedarone is obtained using photodiode array detector. The Dronedarone showed extensive degradation in acid condition, be condition, oxidation and in heat condition. In all intentionally degraded samples, Dronedarone proves the specificity.

In precision study, the percentage RSD of six measurements for method precision was 0.23 and for ruggedness was 0.39 and mean of assay percentage was 99.61 and 99.91 respectively. Dronedarone have good linear response in range 10 to 300 µg/m. The correlation coefficient is 0.999. In robustness study assay percentage of Dronedarone at all deliberated conditions was 98.21 to 99.60. LOD for Dronedarone was 0.126 µg/ml and LOQ for method was 0.318 µg/ml.

Stability indicating HPLC method is developed for Guanfacine hydrochloride determination. HPLC in RP mode having isocratic condition were developed to resolve Guanfacine hydrochloride from its degradants which were formed in stress conditions, using Apollo, C18 (25 cm × 4.6 mm, 5µm) column with mobile phase of ammonium acetate 50mM (volatile buffer) and acetonitrile as 65:35, v/v. UV detection has been done at wavelength 220 nm. Observed column efficiency for Guanfacine peaks were 11291 and USP tailing for Guanfacine were 1.36. The Guanfacine hydrochloride tablet kept stress degradation under of
hydrolysis (acidic, basic), oxidative, photolytic and heat condition. Proposed HPLC method was employed to stress samples analysis. In base, thermal and in oxidative conditions degradation was observed. In all other employed stress conditions Guanfacine was found to be stable. The peak purity were more than 999 for Guanfacine at 220 nm, which proves that peaks of analyte were pure at particular retention and also formulation excipients, diluent peaks and degradation peaks were not interfering with analyte peak. Percentage RSD of six measurements of method precision was 0.36. Percentage RSD of six measurements of intermediate precision was 0.42. Precision study results prove that the method is reliable and rugged. The recovery values for Guanfacine at 80, 100 and 120% were ranging 98.66 and 100.31%. The mean recovery of three concentrations (nine determinations) was 99.97% having overall percentage RSD 1.8. The calibration graph was plotted and showed good linear relationship between peak area and concentration of analyte with correlation coefficient 0.999. The linearity equation of the calibration curve is y = 13.917x + 1.8702. The LOD for Guanfacine hydrochloride 0.011 µg/ml and the LOQ was 0.038 µg/ml, having precision of 4.9 and 3.5% respectively. Assay percentage at all deliberate conditions of robustness studies are within 98.21 to 99.59, which prove the method is robust.

HPLC method is optimized for analysis of Epinastine hydrochloride in API and its tablets form in the presence of degradant which formed due to variety of stress conditions employed. HPLC in RP mode having isocratic condition were developed to resolve Epinastine hydrochloride from its degradants which were formed in stress conditions The chromatographic column YMC C\textsubscript{18} A (YMC Co. Ltd, 25 cm × 4.6 mm, 5 um,) were used. Sample and standard preparations were introduced in HPLC with injection volume 20 µl. UV detector was set at 220 nm. Observed column efficiency for Epinastine peak was 7895 and USP tailing for Epinastine were 1.33.

Stress conditions of acidic, basic hydrolysis, oxidative, thermal and photolytic condition were employed to Epinastine hydrochloride tablet. Proposed HPLC method was employed to stress samples analysis.

Degradation was observed for Epinastine in alkali, thermal and in oxidative conditions. In all other employed stress conditions Epinastine was found to be stable.
The peak purity were more than 999 for Epinastine at 220 nm, which proves that peaks of analyte were pure at particular retention and also formulation excipients, diluent peaks and degradation peaks were not interfering with analyte peak. In Repeatability study percentage RSD and mean of six measurements were 0.15, 99.69. Ruggedness study percentage RSD and mean of six measurements were 0.19, 99.52 respectively. A Recover data value for Epinastine was 99.57 to 100.25 and the percentage RSD for all measurement is 0.9.

The calibration graph was plotted and showed good linear relationship between peak area and concentration of analyte with correlation coefficient 0.999. The calibration curve of linearity equation is $y = 5.2675x - 4.5055$.

The LOD for Epinastine hydrochloride 0.05 µg/ml and the LOQ was 0.18 µg/ml, having precision less than 10.0.

Chapter-3 deals with the chiral separation of particular drugs in API and pharmaceutical dosage form by using validated liquid chromatography method. For this purpose we selected drugs namely Tolterodine tartrate and Fesoterodine fumarate.

Chiral separation method for Tolterodine tartrate was developed by using HPLC. The chromatographic column used was Chiralpak- IA (Daicel, 25 cm × 4.6 mm, 5µm,). Hexane: 2-propanol: triethylamine: trifluoroacetic acid (91:9:0.2:0.1, v/v/v/v). as mobile phase was used. Instrumental setting for flow at 1.1 ml/min. Column oven was monitored and used at 30 °C, UV detector set at 284 nm by injecting 20 µl injection volume. Resolution between S-Tolterodine tartrate and R-Tolterodine tartrate was found to be 2.9. Developed LC method was used for the quantification of S-Tolterodine tartrate as chiral impurity in R-Tolterodine tartrate. No interference was observed at retention time of both isomers which confirms the specificity. The method was found precise with percentage RSD 0.7 for S-isomer content. Good linear ship between response and concentration was observed in 0.1 to 10 µg/ml concentrations for S-Tolterodine tartrate. The calibration curve was plotted between concentration and the peak area of S-Tolterodine tartrate; correlation of 0.999 was observed with regression equation $Y= 8.472x - 0.802$. Recovery of method was concluded by recovery of added concentration of S-isomer in preanalyzed sample of R-Tolterodine tartrate tablet. Recovery was found to be in
the range of 97.30 to 101.59% for S-Tolterodine tartrate. LOD and LOQ for S-Tolterodine tartrate was determined by calibration curve method. Average peak areas of three injections were plotted against concentration. Robustness was evaluated by determining chromatographic resolution between two isomers. Obtained results indicate that the method is unaffected to small changes that made alertly. S-isomer was found to be stable in diluent as no significant change was observed up to 48 hrs.

Chiral separation method for Fesoterodine fumarate enantiomers in drug substance and in solid oral dosage form by using HPLC was developed. The enantiomers were resolved on chromatographic column (Daicel, 25 cm × 4.6 mm × 5μm ID Chiralpak-IB). Hexane: 2-propanol: TFA: DEA in ratio 90:10:0.2:0.2, v/v/v/v. used as eluent. Instrumental setting for flow was 1.5 ml/min. Column oven was monitored and used at 30 °C and the UV detector was set at 220 nm by injecting 20 μl injection volume. Resolution between R-Fesoterodine fumarate and S-Fesoterodine fumarate was not less than 2.9. Role of trifluoroacetic acid and diethylamine were important in mobile phase for to avoid unwanted interaction with stationary phase. Proposed LC method was used for the quantification of S-Fesoterodine fumarate as chiral impurity in R- Fesoterodine fumarate. Proposed method was validated and found accurate, specific, robust. S-Fesoterodine fumarate showed an good linearity over the concentrations range 0.1 to 10 μg/ml. The LOD for S-Fesoterodine fumarate were 0.12 μg/ml. The LOQ for S-Fesoterodine fumarate were 0.31 μg/ml. Average recovery of S-Fesoterodine was in the range of 98.65 to 101.29%. S-Fesoterodine fumarate and R-Fesoterodine fumarate solutions in mobile phase were found to be stable for 48 hrs. The proposed method was short runtime, précised and more accurate for quantitative determination of S-Fesoterodine in R-Fesoterodine fumarate in API and its solid oral dosage form.

Chapter-4 deals with the simultaneous estimation of API’s in combine formulations. We selected drugs namely Famotidine and Ibuprofen combined tablet and Simvastatin and Sitagliptin combined tablet.

A RPLC method was optimized for the quantification of Famotidine and Ibuprofen content in their combined formulations. Chromatographic separation was achieved using column YMC Cyano,(150 × 4.6 mm, 5 μm) YMC, INC. USA, at 30
A RPLC analytical method was optimized for quantification of Sitagliptin and Simvastatin content in their formation. Chromatographic separations were achieved using YMC Cyano (150 × 4.6 mm × 5µ) YMC, INC. USA column, at 40 °C oven temperature. Buffer used was 0.01 M KH₂PO₄ solution in water adjusted pH 7.0 with 0.01M K₂HPO₄ solution in water. The mobile phase consist of acetonitrile and buffer as 35: 65, v/v. Sitagliptin and Simvastatin were quantified at a wavelength 254 nm. Twenty micro liters of sample was used as injection volume. Sitagliptin and Simvastatin tablet were kept for stress experiments of oxidation, hydrolysis (acidic, alkali), thermolysis and photolysis. The degradation was found in acidic, basic hydrolysis, oxidation, thermolysis and photolysis.

Percentage RSD in the study of method precision for Sitagliptin and Simvastatin was 1.1 and 0.9 respectively. Percentage RSD in the study of
ruggedness (intermediate precision) for Sitagliptin and Simvastatin was 0.5 and 0.8 respectively. Obtained result indicates that the developed method is précised. Accuracy data at all concentration for both analytes was within 100 ± 2%, proves the accuracy. Linear response was observed over 1.5 to 600 for Sitagliptin and 0.9 to 240 μg/ml for Simvastatin concentration with and 0.9990 respectively.

The results indicate a good linear relationship between peak areas and concentrations. Observed LOQ (μg/ml) values for Sitagliptin and Simvastatin were 1.5 and 0.9 respectively. Observed LOD (μg/ml) values for Sitagliptin and Simvastatin were 0.5 and 0.3 respectively. In robustness study at all deliberated changes the percentage assay of both analytes was 100 ± 2%. Developed HPLC method was applied for quantification of Sitagliptin and Simvastatin in the combined pharmaceutical tablet and stability studies of the same.