PREFACE

Pharmaceutical analysis is important in several phases of drug development such as formulation, stability studies, dissolution studies, clinical trials and in quality control. The development of reliable analytical methods for drug determination in fast, inexpensive and sensitive way is very important.

Quality assurance and control of pharmaceutical chemicals and formulations are essential for ensuring the availability of safe and effective drug formulations to the population. The need to develop new analytical methods for assurance of quality, safety and efficacy of drugs and pharmaceuticals were quite important because of their use not only in case of health care products but also life saving substances. The analytical methods have great importance due to development of new drugs, continuous changes in manufacturing processes for existing drugs and setting up of threshold limits for individual and total impurities of drugs by regulatory authorities.

Keeping the above tasks in view, an attempt was made in the present investigation to develop new analytical methods for some of the important active pharmaceutical ingredients (API) in bulk drugs, pharmaceutical formulations, related substances and biological fluids like plasma and ocular fluids by using reverse phase high performance liquid chromatographic technique.

The thesis comprises of five chapters. In these chapters eleven new liquid chromatographic chapters were developed. The developed methods are simple, specific, robust and can be effectively applied for the analysis of drugs. A detailed account literature survey of all analytical methods existing for the selected
drugs were studied to avoid duplication of the method developed. Details about the chemical structure of the drugs and their physicochemical properties are also collected to find out the stability and homogeneity of the sample solutions. The author has made successful attempt in exploiting these features in development of new liquid chromatographic methods for some pharmaceutical ingredients.

Chapter-I comprises of introduction to chromatography, HPLC and its applications, strengths, limitations and types. This chapter gives an overview on method development by HPLC, about process related substances and brief discussion of bio-analysis, extraction of biological fluids and non compartmental analysis. The drugs selected for investigation by HPLC was also briefly discussed.

Under Chapter-II: Part-A commences the development and validation of simple HPLC method for quantitative determination of Linezolid in bulk drug and pharmaceutical formulations. A Thermo hypersil C-18 column with mobile phase consisting of mixture of acetonitrile and water in the ratio of 60:40 (v/v) at pH 6.0 adjusted with o-phosphoric acid in isocratic mode was used. The flow rate was 1.0 mL/min and the effluents were monitored at 254 nm. The retention time was 3.27 min. The detector response was linear in the concentration range of 20-60 μg/mL. The respective linear regression equation being $y = 98.543x + 180.061$. The limit of detection and limit of quantification was 0.5μg/mL and 1.7μg/mL respectively. The percentage assay of Linezolid was 99.94%. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise, accurate and robust.

Chapter-II: Part-B presents the simultaneous determination of Linezolid and its two related substances and forced degradation study of bulk drug. Two
unknown impurities in Linezolid bulk drug at levels below 0.1% (ranging from 0.05 to 0.1%) were detected by a simple RP-HPLC method. Based on the spectroscopic data (NMR, MS-MS, IR and UV) the structures of drug and impurities were characterized. Separation was achieved using Hypersil BDS-C18 column with mobile phase consisting of water: acetonitrile in the ratio of 60:40 (v/v) and 2mL each of triethylamine and glacial acetic acid (pH-adjusted to 6.0) at wave length 254 nm. The flow rate of the mobile phase was 1.0 mL/min and the total elution time, including the column re-equilibration, was approximately 30 min. The developed method was validated in terms of system suitability, selectivity, linearity, range, precision, accuracy, limits of detection and quantification for the impurities, robustness and forced degradation studies of bulk drug using acid, base, peroxide, heat and light.

**Chapter-II: Part-C** describes the rapid and simple RP-HPLC method for determination of Linezolid in human plasma of feeding mothers. The method was applied to the pharmacokinetic studies in twelve feeding mothers after oral administration of one Linezolid tablet (600 mg). Efavirenz was taken as an internal standard for this analysis. Plasma sample was prepared by liquid-liquid extraction (LLE) method. Analysis of Linezolid in plasma samples were carried out using a Hypersil C18 column with UV detector (maximum absorption at 239 nm). A mixture of methanol, water and acetonitrile in the ratio of 80:10:10 (v/v/v) at pH value of 5.4 was used as a mobile phase. The RT is 2.75 for Linezolid and 4.45 for IS. The total runtime for this experiment was 10 minutes. Pharmacokinetics of Linezolid was studied through non compartmental mode and Cmax is found to be 56.3 and Tmax is 180 min.
Chapter-III: Part-A In this part the author describes the determination of Dapiprazole hydrochloride in bulk form with simple RP-HPLC method. A Thermo hypersil C-18 column with mobile phase consisting of mixture of methanol, acetonitrile and o-phosphoric acid in the ratio of 89: 9: 1 (v/v/v) at pH 5.8. The flow rate was 0.8 mL/min and the effluents were monitored at 243 nm. The retention time was 3.725 min. The detector response was linear in the concentration range of 20-120 µg/mL. The respective linear regression equation being y = 1956.4x+3409. The limit of detection and limit of quantification was 0.5µg/mL and 1.6µg/mL respectively. The % assay of Dapiprazole hydrochloride in bulk form was 99.94%.

Chapter-III: Part-B aims at simultaneous determination of Dapiprazole hydrochloride and its process related impurity-A with RP-HPLC method. Based on the spectroscopic data (NMR, MS-MS, IR and UV) the structures of drug and impurity were characterized. Separation was achieved with an Inertsil ODS C-18-3V column using the mobile phase consisting of 85:15 (v/v) buffer: acetonitrile at pH-3.2. The flow rate of the mobile phase was 1.0 mL/min and the total elution time including the column re-equilibration was approximately 30 min. The UV detection wavelength was carried at 205nm. Injection volume was 20 µL and experiments were conducted at ambient temperature. The developed method was validated in terms of system suitability, selectivity, linearity, range, precision, accuracy, limits of detection and quantification for the impurities, robustness and forced degradation studies of bulk drug were conducted under acid, alkali, peroxide, heat and light.

Chapter-IV: Part-A describes the determination of Pemetrexed disodium in bulk and pharmaceutical formulations by RP-HPLC using UV detection. Chromatographic separation was obtained using Inertsil ODS 3V C-18 column with
gradient mode of mobile phase consisting potassium dihydrogen phosphate buffer at pH 3.0 with ortho-phosphoric acid (solvent-A) and acetonitrile (solvent-B) at a flow rate of 1.0 mL/min. The eluents were monitored at 220 nm. The retention time of Pemetrexed disodium was found to be 8.13 min. A good linear relationship ($r^2=0.9998$) was observed between the concentration range of 500-1500 μg/mL. The assay of Pemetrexed disodium in sterile powder for injection (Prex 100 mg) dosage form was found to be 98.25%.

**Chapter-IV: Part-B** presents the simultaneous determination of Pemetrexed disodium and its three related substances and forced degradation study of bulk drug with RP-HPLC method. Based on the spectroscopic data (NMR, MS-MS, IR and UV) the structures of drug and its three impurities were characterized. Separation was achieved by using an Inertsil ODS C-18-3V column with an isocratic mode by the mobile phase consisting of methanol, water and o-phosphoric acid (0.5%) in the ratio of 50:45:5 (v/v/v) at pH 5.3. The flow rate of the mobile phase was 0.8 mL/min and the total elution time, including the column re-equilibration, was approximately 16 min. The UV detection wavelength was carried at 220nm. Injection volume was 20 μL and experiments were conducted at ambient temperature. The developed method was validated in terms of system suitability, selectivity, linearity, range, precision, accuracy, limits of detection and quantification and robustness. The forced degradation studies were conducted for bulk drug in acid & base hydrolysis, peroxide, thermal and photolytic conditions. The detector response was linear within the concentration range 40μg/mL to 80μg/mL for Pemetrexed disodium and its three process related impurities.
**Chapter-IV: Part-C** deals with the determination of Pemetrexed disodium in albino wistar rats’ plasma by RP-HPLC method using UV detection and its application to pharmacokinetic study. Lopinavir was used as an internal standard for this analysis. Plasma sample was prepared by LLE method. The analysis of Pemetrexed disodium in plasma samples were carried out using Hypersil BDS C-18 column with mobile phase consisting of mixture of methanol: acetonitril 90:10 (v/v) at pH value of 5.4 at 219 nm. The spiked samples were validated for specificity, precision, accuracy, sensitivity and stability. The method was applied to the pharmacokinetic studies in 12 rats after intravenous administration of Pemetrexed disodium (Prex100mg) parenteral formulation (10 mg/kg). Pharmacokinetics of Pemetrexed disodium was studied through non compartmental mode for different intervals of time.

**Chapter-V: Part-A** describes the estimation of Brinzolamide in bulk drug and pharmaceutical formulations by RP-HPLC with UV detector. Chromatographic separation was obtained on an Inertsil C-18 column with the mobile phase containing methanol: acetonitrile: water: ortho-phosphoric acid (0.1% v/v) in the ratio of 35: 30: 05 (v/v) at pH 4.8 at a flow rate of 1.0 mL/min. The eluents were monitored at 241 nm. The retention time of Brinzolamide was found to be 3.77 min. A good linear relationship ($r^2 = 0.9999$) was observed between the concentration range of 3-9 μg/mL. The assay of Brinzolamide in ophthalmic solution was found to be 99.26%. From the recovery studies it was found that about 99.35 % on average of Brinzolamide was recovered which indicates high accuracy of the method.

**Chapter-V: Part-B** aims at simultaneous determination of Brinzolamide and its two process related substances and forced degradation study of bulk drug. Based
on the spectroscopic data (NMR, MS-MS, IR and UV) the structures of
brinzolamide and its two impurities were characterized. The separation
was achieved by using a Chromasil ODS C-18-3V. Mobile phase is the mixture of
acetonitrile and water in the ratio of 80:20 (v/v) at pH 5.2 with flow rate of 0.8
mL/min. UV detection at 240 nm was employed to monitor the analytes. The
method was successfully validated with the parameters; system suitability,
specificity, linearity, range, precision, accuracy, limits of detection and
quantification and robustness. The detector response was linear for the Brinzolamide
and its two impurities in the concentration range at 2-12 µg/mL. The LOD for the
Brinzolamide is 0.015µg/mL and for impurity-C is 0.006µg/mL and impurity-B is
0.006µg/mL respectively. The stability study was conducted for acid & base
hydrolysis, peroxide, thermal and photolytic degradation.

**Chapter-V: Part-C** describes the determination of Brinzolamide in rabbit
aqueous humor by HPLC using UV detector and its application to pharmacokinetic
study through non compartmental analysis. Amlodipine was used as an internal
standard for this analysis. Plasma sample was prepared by LLE method. The
separation was achieved by using Kromasil BDS C-18 column with UV detector
(maximum absorption at 245 nm) for separation and quantification. A mixture of
methanol and acetonitrile in the ratio of 90:10 (v/v) was used as a mobile phase with
flow rate of 1.0mL/min. Brinzolamide and internal standard were spiked with blank
rabbit aqueous humor. The spiked samples were validated for specificity, precision,
accuracy, sensitivity and stability. The method was applied to the pharmacokinetic
study after intraocular administration of Brinzolamide eye drops (Azopt-1% (w/v))
to 12 rabbits.
The method was specific and sensitive with a limit of quantification 0.35 µg/mL. This HPLC method was validated by examining the precision and accuracy for inter and intra-day analysis in the concentration range of 6µg/mL.

The thesis is followed by summary and conclusions besides annexure consisting of research papers.