PREFACE

Pharmaceutical chemistry is a science that makes use of the general laws of chemistry to study drugs i.e., their preparation, chemical nature, composition, structure, the physical and chemical properties, influence on an organism, the methods of quality control and the conditions of storage. As majority of the drugs used are of synthetic origin, the quality, safety are generally assured by monitoring and controlling the impurities effectively. The most critical aspect is the elaboration of the levels of impurities for identification and quantification. The analytical activities concerning impurities in drugs are among the most important issues in modern pharmaceutical analysis. Thus, pharmaceutical analysis occupies a vital role in statutory certification of drugs and their formulations either by the industry or by the regulatory authorities.

The various problems encountered in the pharmaceutical analysis are coupled with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, precision and accuracy which is being quickly adopted by the pharmaceutical industry and chemical laboratories depending upon the facilities available. All these salient features of a drug help a researcher not only in planning a precise experimental design but also in the interpretation of data in a scientific manner for the determination of drug in its pharmaceutical formulations.

Pharmaceutical dosage forms often contain combination of drugs for potentiating or complementing one another in therapy. In some cases, no precise
analytical methods are reported and quite often the reported methods need improvement or changes because of lack of specificity and sensitivity with existing methods.

Among the several instrumental techniques like LC-MS, GC-MS, NMR, HPLC, HPTLC, Electro-analytical, Fluorimetry, Spectrophotometry, Mass Spectroscopy, etc. available for the assay of drugs of pharmaceutical importance, the HPLC methods are simple, sensitive, specific, rapid, precise and accurate.

Keeping in view of the increasing demands on quality assurance, sincere efforts have been made to contribute, a little, to the field of pharmaceutical analysis, by the way of developing simple and economically viable HPLC methods for the determination of selected drugs of pharmaceutical importance.

In the present study, the author has developed and validated around eight reverse phase HPLC methods in all for the estimation of some selected drugs in bulk samples and pharmaceutical formulations either in single or in combined dosage forms. The following drugs were selected for present study:

1. Ceftazidime
2. Dobutamine hydrochloride
3. Efavirenz
4. Amphotericin B
5. Candesartan cilexetil
6. Irbesartan
7. Hydrochlorothiazide
8. Zidovudine
9. Lamivudine
10. Abacavir

The contents of the thesis have been divided into Ten Chapters.

Chapter - I 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.

In Chapter-II, newer, simple and accurate RP-HPLC method was developed and validated for the estimation of Ceftazidime in tablet dosage forms. Ceftazidime is chemically designated as (Z)-(7R)-7-[2-(2-Aminothiazol-4-yl)-2-(1-carboxy-1-methyl ethoxyimino) acetamido]-3-(1-pyridinomethyl)-3-cephem-4-carboxylate pentahydrate and is used in the treatment of susceptible infections especially those due to pseudomonas species. Literature survey revealed that very few analytical methods were reported for the estimation of Ceftazidime in tablet dosage forms. In the present investigation isocratic mode method with UV detection was developed for the
estimation of Ceftazidime at the 100% level. The mobile phase consisted of Acetonitrile, water and disodium hydrogen phosphate buffer in the ratio of 25:25:50 (v/v/v) yielded the best results on Luna C₁₈ column as stationary phase. The retention time was found to be 6.447 min and linearity was in the concentration range of 50-150 μg/mL. The drug solution was analyzed in UV-Visible spectrophotometer at 254 nm. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity, and precision data. It is highly specific and precise analytical procedure and its chromatographic run time 10 min allows the analysis of large number of samples in a short period of time. Hence this simple HPLC-UV method can be used as a routine sample analysis.

A new isocratic mode method with UV detection was developed for the determination of the Dobutamine hydrochloride at the 100% level was presented in Chapter - III. Dobutamine is chemically designated as (±)-4-(2-3-(p-Hydroxyphenyl)-1-methylpropyl) amino) ethyl)pyrocatechol hydrochloride which is described as sympathomimetic agent. Literature survey reveals that Dobutamine is estimated by titrimetric, HPTLC and very few HPLC methods. The mobile phase consisted of Acetonitrile, methanol and Tetrahydrofuran in the ratio of 70:20:10 (v/v/v) yielded the best results with shorter retention time (5.008 min), the drug solution was analyzed in UV-Visible spectrophotometer at 292 nm and linearity concentration range was 10-30 μg/mL. This chromatographic assay fulfilled all the
requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical procedure and its chromatographic run time 10 min allows the analysis of large number of samples in a short period of time.

Chapters – IV deals with the development and validation of new reverse phase HPLC method for the estimation of Efavirenz in bulk samples and pharmaceutical formulations. Efavirenz is chemically designated as (S)-6-Chloro-4-(cyclopropyl ethynyl)-1, 4dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one and is a novel antiretroviral drug. Literature revealed that very few methods are reported for the estimation of Efavirenz in bulk and tablet dosage forms. Literature survey revealed that no suitable and specific HPLC method is available for the estimation of Efavirenz in tablet dosage forms. A newer, rapid and reliable isocratic RP-HPLC-UV method for the estimation of Efavirenz was developed and validated by using mobile phase consisting a mixture of methanol, water and tetrahydrofuran in the ratio of 30:50:20 v/v/v. The retention time was found to be 3.675 min and linearity concentration range was between 20-60 μg/mL. This chromatographic assay fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical procedure and its chromatographic run time of 10 min allows the analysis of large number of samples in a short period of time. Hence this simple HPLC-UV method can be used as a routine sample analysis.
A new isocratic mode method with UV detection was developed for the determination of the Amphotericin B at the 100% level was presented in Chapter - V. Amphotericin B is chemically designated as [1R- (1R*,3S*,5R*,6R*,9R*,11R*, 15S* 16R*,17R*,18S*,19E, 21E, -23E, 25E, 27E, 29E, 31E, 33R*, 35S, 36S*,37S*)]-33-
[(3-Amino-3,6-Dideoxy-β-D-mannopyranosyl)oxy]-1,3,5,6,9,11,17,37-octahydroxy-
15, 16,18-trimethyl-13-oxo-14,39-dioxabicyclo nona - triaconta-19,21,23,25,27,29,31 - heptaene-36-carboxylic acid which is described as the Anti-fungal agent. Literature survey reveals that Amphotericin B is estimated by titrimetric, HPTLC and very few HPLC methods are reported. The mobile phase consisted of a mixture containing Acetonitrile, Tetrahydrofuran and o-phosphoric acid in the ratio of 60:30:10 (v/v/v) yielded the best results with shorter retention time, the drug solution was analyzed in UV-Visible spectrophotometer at 287 nm and the linearity was in the range of 10-30 µg/mL. This chromatography assay fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical procedure and its chromatographic run time 10 min allows the analysis of large number of samples in a short period of time.

Chapter-VI deals with the simultaneous estimation of two drugs, viz., Candesartan cilexetil and Hydrochlorothiazide in tablet dosage forms. The combination of these drugs is widely used all over the world to treat hypertension. In these cases it was found that while several methods were available for estimating Candesartan cilexetil and Hydrochlorothiazide in bulk and few methods are reported
for dosage forms and rapid, sensitive methods were not available for the present
combination. Candesartan cilexetil is chemically designated as 2-ethoxy-1-\[p-(1H-
tetrazol-5-ylphenyl)benzyl\]-7-benzimidazolecarboxylate and is used to treat
hypertension. Hydrochlorothiazide is chemically designated as (S)-6-Chloro-4-(
cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one and it
is a diuretic. A new sensitive RP-HPLC method was developed and validated for
simultaneous estimation of Candesartan cilexetil and Hydrochlorothiazide. The
mobile phase consisted of Acetonitrile and Triethyl amine in the ratio of 40:60 (v/v)
on hypersil BDS C8 column yielded the best results with short retention time of
detection at 262 nm. The retention times of Candesartan cilexetil and
Hydrochlorothiazide were found to be 2.449 and 4.895 min respectively. This
chromatographic assay method fulfilled all the requirements to be identified as a
reliable and feasible method including accuracy, linearity and precision data. It is
highly specific and precise analytical procedure, its chromatographic run time of 10
min and hence this method can be adopted for the simultaneous analysis of
Candesartan cilexetil and Hydrochlorothiazide in bulk samples and combined
pharmaceutical formulations.

Chapter-VII deals with the simultaneous estimation of two drugs, viz.,
Irbesartan and Hydrochlorothiazide in tablet dosage forms. The combination of these
drugs is widely used all over the world to treat hypertension. Several analytical
methods were reported in the literature for the estimation of these two drugs either in
single or in combined form and rapid, sensitive and specific method was not available for this combination. Irbesartan is chemically designated as 2-Butyl-3-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one and is used to treat hypertension. Hydrochlorothiazide is chemically designated as (S)-6-Chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one and it is a diuretic. A new sensitive RP-HPLC method was developed and validated for simultaneous estimation of Irbesartan and Hydrochlorothiazide. The mobile phase consisted of Acetonitrile, methanol and Triethyl amine in the ratio of 30:15:55 (v/v/v) on hypersil BDS C$_8$ column yielded the best results with shorter retention time of detection at 274 nm. The retention times of Irbesartan and Hydrochlorothiazide were found to be 5.903 and 7.040 min respectively. The total chromatographic run time was only 10 min allows the analysis of large number of samples in short period of time. This chromatographic assay method fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical method and hence this method can be adopted for the simultaneous analysis of Irbesartan and Hydrochlorothiazide in bulk samples and combined pharmaceutical formulations.

Chapter-VIII deals with the simultaneous estimation of three drugs, viz., Efavirenz, Lamivudine and Zidovudine in tablet dosage forms. The combination of these drugs is widely used all over the world to treat AIDS. Several analytical methods were reported in the literature for the estimation of these three drugs either
in single or in combined form and rapid, sensitive and specific method was not available for this combination. Efavirenz is chemically designated as (S)-6-Chloro-4-(cyclopropylethynyl) -1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoazin-2-one.

Lamivudine is chemically designated as (-)-1-[(2R, 5S)-2-(Hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine. Zidovudine is chemically designated as 3'-Azido-3'-deoxythymidine and it is an Anti-viral drug. A new sensitive RP-HPLC method was developed and validated for simultaneous estimation of Efavirenz, Lamivudine and Zidovudine in combined dosage form. The mobile phase consisted of Acetonitrile, Methanol and dipotassium hydrogen orthophosphate in the ratio of 40:40:20 (v/v/v) on Luna C18 column yielded the best results with shorter retention time of detection at 259 nm. The retention times of Efavirenz, Lamivudine and Zidovudine were found to be 5.915, 8.407 and 11.361 min respectively. This chromatographic assay method fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical method and hence this method can be adopted for the simultaneous analysis of Efavirenz, Lamivudine and Zidovudine in bulk samples and combined pharmaceutical formulations.

Chapter-IX deals with the simultaneous estimation of three drugs, viz., Abacavir, Lamivudine and Zidovudine in tablet dosage forms. The combination of these drugs is widely used all over the world to treat AIDS. A few analytical methods were reported in the literature for the estimation of these three drugs either in single
or in combined form and rapid, sensitive and specific method was not available for this combination. Abacavir is chemically designated as \{(IS,4R)-4-[2-Amino-6-(cyclopropylamino)-9H-purin-9-yl]cyclopent-2-enyl\}methanol. Lamivudine is chemically designated as \((-\)-1-\[(2R, 5S)-2-(Hydroxymethyl)-1, 3-oxathiolan-5-yl]\)cytosine. Zidovudine is chemically designated as 3'-Azido-3'-deoxythymidine. A new sensitive RP-HPLC method was developed and validated for simultaneous estimation of Abacavir, Lamivudine and Zidovudine. The mobile phase consisted of acetonitrile: methanol: ammonium dihydrogen phosphate buffer in the ratio of 70:20:10 (v/v/v) on Luna C\textsubscript{18} column yielded the best results with shorter retention time of detection at 266 nm. The retention times of Abacavir, Lamivudine and Zidovudine were found to be 2.623, 6.190 and 8.933 min respectively. The total chromatographic run time was only 10 min allows the analysis of large number of samples in short period of time. This chromatographic assay method fulfilled all the requirements to be identified as a reliable and feasible method including accuracy, linearity and precision data. It is highly specific and precise analytical method and hence this method can be adopted for the simultaneous analysis of Abacavir, Lamivudine and Zidovudine in bulk samples and combined pharmaceutical formulations.

Finally, a brief summary of the investigations and conclusions drawn by the author is presented at the end of the thesis under Chapter-X.

In all the above methods a full scale validation to assess the viability of the method has been performed. The validation includes selectivity, specificity, linearity,
limit of detection and quantification, linear dynamic range, accuracy, precision etc. Statistical evaluation of the data is also included and has been performed by the method of least-squares using regression factors, slopes and intercepts and statistical errors in the parameters. These methods can be easily adopted for routine analysis for the assay of pharmaceutical and related products.