CHAPTER - 12
SUMMARY & CONCLUSIONS
12. SUMMARY AND CONCLUSIONS

Analytical chemistry is the science of developing and improving methods for detection and determination of artificial and naturally occurring components in our surroundings and environment as well as within ourselves, in our tissues and body fluids, also in pharmaceutical dosage forms and in cosmetic products. Pharmaceutical analysis is important in several phases of drug development, such as formulation, stability studies, dissolution studies and quality control. The importance of reliable analytical methods for drug determination in a fast, inexpensive, sensitive and selective way is thus evident. Although there are countless works describing new analytical methods for determination of drugs that act against diseases and metabolic disorders, a review organizing these works in a systematic and complete way is lacking. In this context, the objective of this thesis is to present the main advances in the development of analytical methods for determination of drugs using liquid chromatographic techniques.

Several drugs are available in the form of pharmaceutical formulations to control diseases. Methods of assay for controlling the concentration of these chemicals in the medicines and in the living body are necessary. Pharmaceutical analysis occupies a pivotal role in statutory certification of drugs and their formulations either by the industry or by the regulatory authorities. The complexity of problems encountered in pharmaceutical analysis coupled with the importance of achieving the selectivity, speed, cost, simplicity, sensitivity, precision and accuracy results in new methods of analysis being quickly adopted by the pharmaceutical industry.

Quality assurance and control of pharmaceutical chemicals and formulations is essential for ensuring the availability of safe and effective drug formulations to the population. Quantitative estimation of the chemical entity of a drug is vital for maintaining and assuring the quality. Several distinct problems are encountered in the quantitative estimation of the drugs in API samples and formulations. The interferences caused by a number of sources such as degradation products of the drugs when they are stored for a long time, the presence of other drugs in combination products and the various additives incorporated in formulations have to be kept in view during the course of assay development for drugs in formulations.
The need to develop new analytical methods for assurance of quality, safety and
efficacy of drugs and pharmaceuticals is quite important because of their use not only as
health care products but also life saving substances. The analytical methods assume of
great importance due to i) development of new drugs ii) continuous changes in
manufacturing processes for existing drugs and iii) setting up of threshold limits for
individual and total impurities of drugs by regulatory authorities. Keeping this in view, an
attempt was made in the present investigation to develop new analytical methods for
some of the important APIs and their commercial formulations. All the methods
described in the thesis are simple, rapid, reliable and validated. The methods could be
used not only for quality control but also for process development of APIs and also in
estimation of drugs in plasma samples. The work carried out in the present investigation
was described in twelve chapters.

This Ph.D thesis comprises of ten liquid chromatographic methods in RP-HPLC
mode of method development and validation. The current developed methods are simple,
specific, robust and can effectively be applied for the analysis of drugs in characterization
and assay of API and formulations as part of routine quality control analysis in
pharmaceutical industries. It is also observed for the applicability of the developed
methods in the plasma samples for the estimation of the drug. The summarization of the
methods applications are shown in Table 12.1. A detailed account of all analytical
methods existing for the drug is made 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.
Table 12.1 - Summary of Results

<table>
<thead>
<tr>
<th>Name of the Drug</th>
<th>Application to</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>API analysis</td>
<td>Formulation analysis</td>
<td>Plasma sample analysis</td>
<td></td>
</tr>
<tr>
<td>Disopyramide</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Oxybutynin</td>
<td>√</td>
<td>√</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pioglitazone</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Ribavirin</td>
<td>√</td>
<td>√</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Pantoprazole</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Defesirox</td>
<td>√</td>
<td>√</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Paroxetine</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Metoclopramide</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Olmesartan</td>
<td>√</td>
<td>√</td>
<td>*</td>
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</tr>
</tbody>
</table>

√: Developed method can be used to successfully.
X: Developed method is not suitable.
*: Developed method requires partial modifications.

Chapter-1 commences with introduction to chromatography, HPLC, its applications, strengths, limitations and types of HPLC. This chapter gives an overview on method development by HPLC, which involves mobile phase parameters, buffer selection, choosing detector, choice of HPLC method and choosing column, optimizing separation, interpretation of chromatogram and quantitative analysis.

Chapter-2 commences with brief introduction of antiarrhythmic drug, Disopyramide used to treat certain types of serious irregular heart beats such as persistent ventricular tachycardia. This chapter describes the development and validation of simple and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method.
for quantitative determination of Disopyramide in active pharmaceutical ingredient and its tablet dosage form. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantification. Disopyramide was analyzed using Inertisil C18 column (250 mm x 4.6 mm, 5µ) at ambient temperature, with isocratic elution of Methanol: Acetonitrile: THF(50:45:5 v/v/v) at mobile pH-6. The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 265 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 265 nm enabled determination of Disopyramide with no interference from injectable solution excipients or solvents. The retention time (tR) for Disopyramide was around 2.9 min. The calibration curves were linear over a concentration range from 0.5 ppm to 3.5 ppm. Limit of detection (LOD) for Disopyramide was 0.03 ppm and Limit of quantitation (LOQ) Disopyramide was 0.2 ppm. The% assay of Deferasirox was found to be 98.96%. The developed method was successfully applied to estimate the amount of Disopyramide in APIs and tablet formulations. This method also can be applied for the estimation of the drug in plasma samples. The developed HPLC method is accurate, precise, specific, sensitive, and efficient, can be used in routine analysis in quality control laboratories.

Chapter-3 commences with a brief introduction to an anti-spasmodic drug Oxybutynin. The chapter describes a RP-HPLC method development for the drug. The chromatographic conditions required for the quantitation of the drug includes an Inertisil C18 (250X4.6 mm, 5µ) column, a mixture of Methanol: Acetonitrile: 1% Orthophosphoric acid in the ratio of 15:45:40%, v/v/v at a flow rate of 1.0 ml/min with detection at 205 nm. The retention time of the drug was 2.4 min. The detector response was linear in the concentration of 2-12 mcg/ml. A good linear relationship (r²=0.999) was observed between the concentration range of 2-12 mcg/ml. The limit of detection and limit of quantification was 0.1 and 0.25 ppm respectively. The% assay of Oxybutynin was found to be 98.8%. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the tablets. The method was validated by determining its sensitivity, accuracy and precision. The developed method is simple, fast, accurate and precise and hence can be applied for routine quality control of Oxybutynin in API sample and tablet dosage form. Thus, the developed method can be
easily used for the routine quality control of APIs and tablet dosage form of Oxybutynin within a short analysis time.

Chapter-4 commences with brief introduction of oral antidiabetic agent Pioglitazone, used to treat type II diabetes and discusses the details of the development of a RP-HPLC method. The chromatographic conditions include Zodiacsil C18 column (250 mm x 4.6 mm, 5 μ) particle sizes, with mobile phase consisting of Methanol: Acetonitrile: Water 60:20:20%, v/v/v at pH-5.5 was used. The flow rate was 1.0 ml/min and the effluents were monitored at 295 nm. The retention time was 4.5 min with chromatographic run time 8 min. The detector response was linear in the concentration of 0.5-3.5 mcg/ml. The limit of detection and limit of quantification was 0.015 mcg and 0.05 mcg/ml respectively. The % assay of Pioglitazone was found to be 98.4%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Pioglitazone in API and in its pharmaceutical dosage form. This method can also be applicable for the estimation of the drug in the plasma samples.

Chapter-5 describes a brief introduction to an antiviral agent Ribavirin, indicated for severe respiratory syncytial virus infection, hepatitis C infection (in combination with peginterferon α2a or α2b). This chapter describes a RP-HPLC method development for the drug. The chromatographic conditions required for the quantitation of the drug includes an Chromosil C18 column (250 X 4.6 mm, 5 μ) with mobile phase consisting of Methanol: Acetonitrile : 0.01M NH₄H₂PO₄ 20:35:45%, v/v/v (pH adjusted to 3.0) was used. The flow rate was 1 ml/min with injection volume of 20 micro liters and the effluents were monitored at 225 nm. The retention time was 1.52 min and chromatographic run time has been extended to 6 min. The detector response was linear in the concentration of 2-14 μg/ml. The limit of detection and limit of quantification was 0.025 and 0.1 μg/ml respectively. The % assay of Ribavirin was found to be 100.1%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise
and accurate, which is useful for the routine/regular determination of Ribavirin in APIs and in its pharmaceutical dosage form.

Chapter-6 commences with the introduction of a proton pump inhibitor drug, Pantoprazole used for short term treatment of erosion and ulceration of the esophagus caused by gastro esophageal reflux disease. A simple and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for quantitative determination of Pantoprazole in API samples and formulations. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. Pantoprazole was analyzed by using chromosil C18 column (250 mm x 4.6 mm, 5µ) at ambient temperature, with isocratic elution of Acetonitrile: 0.01M KH2PO4 (40:60%, v/v) with mobile phase pH-6.5. The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 270 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 270 nm enabled determination of Pantoprazole without any interference from injectable solution, common excipients used in tablets or solvents. The retention time (tR) for Pantoprazole was around 9.4 min. The calibration curves were linear over a concentration range from 3 mcg to 18 mcg/ml. Limit of detection (LOD) for Pantoprazole was 0.2 mcg/ml and Limit of quantitation (LOQ) Pantoprazole was 1.0 mg/ml. The % assay of Pantoprazole was found to be 98.8%. The developed method was successfully applied to estimate the amount of Pantoprazole in APIs and formulations. This method may also be suitable the for the estimation of the drug in plasma samples. The developed HPLC method is precise, sensitive, accurate, specific and efficient and can be used in routine analysis in quality control laboratories.

Chapter-7 opens with a brief introduction to an iron-chelating agent Deferasirox, generally used to treat iron over load caused by blood transfusions in adults and describes a validated, RP-HPLC method for the estimation of Deferasirox in pharmaceutical formulations and API samples. The chromatographic conditions used are a Chromosil C18 column (250 mm x 4.6 mm, 5 µ), with mobile phase consisting of Methanol: Acetonitrile: 1% OPA (80:15: 5%, v/v/v) with pH 3.9) was used. The flow rate was 1.0 ml/min and the effluents were monitored at 250 nm. The retention time was 2.58 min. The detector response was linear in the concentration of 2-14 mcg/ml, with the regression
coefficient of 0.9999. The % assay of Deferasirox is found to be 99.9%. Quantification was done by calculating area of the peak and the detection and quantitation limits were 0.05 and 0.5 mcg/ml respectively. The method was validated by determining its accuracy, precision and system suitability. The developed HPLC method is accurate, precise, specific, sensitive, and efficient and can be used in routine analysis in quality control laboratories.

Chapter-8 records the details of the development of a RP-HPLC method for the estimation of antidepressant agent Paroxetine HCl. The chromatographic conditions includes a Chromosil C18 column (250 mm x 4.6 mm, 5µ) analytical column, a mixture of Methanol: Acetonitrile: 1% Orthophosphoric acid (25:40:35%, v/v/v) as the mobile phase at a flow rate of 1.0 ml/min with detection at 235 nm. The retention time of the drug was 2.911 min. The detector response was linear in the concentration of 1-7 mcg/ml. The percentage assay of Paroxetine HCl was 97.65%. The limit of detection and limit of quantification was 0.02 and 0.3 mcg/ml respectively. The method was validated by determining its sensitivity, accuracy and precision. The results of the study showed that the developed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Paroxetine HCl in APIs and in its pharmaceutical dosage forms. This method may also be suitable for the estimation of the drug in the plasma sample analysis.

Chapter-9 commences with a brief introduction to an anti-epileptic drug Lamotrigine. A simple and isocratic RP-HPLC method was developed and validated for quantitative determination of Lamotrigine in API samples and formulations. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. Lamotrigine was analyzed by using Chromosil C18 column (250 mm x 4.6 mm, 5µ) at ambient temperature, with isocratic elution of Methanol: Acetonitrile : 0.01M NaH₂PO₄ 15:35:50%, v/v/v with mobile phase pH-6.5. The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 295 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 295 nm enabled determination of Lamotrigine with no interference from injectable solution, excipients or solvents. The retention time (t_R) for Lamotrigine was around 5.35 min. The calibration curves were linear over a
concentration range from 2.0 mcg to 14 mcg/ml. The percentage assay of Lamotrigine was 99.2%. Limit of detection (LOD) for Lamotrigine was 0.05 mcg/ml and Limit of quantitation (LOQ) Lamotrigine was 0.5 mcg/ml. The developed method was successfully applied to estimate the amount of Lamotrigine in injection formulations. The developed RP-HPLC method is accurate, precise, specific, sensitive, and efficient and can be used in routine analysis in quality control laboratories. This method may also be suitable for the estimation of the drug in the plasma sample analysis.

Chapter-10 commences with a brief introduction to an anti-emetic drug Metoclopramide. A simple and isocratic RP-HPLC method was developed and validated for quantitative determination of Metoclopramide in API samples and formulations. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. Metoclopramide was analyzed by using Chromosil C18 column (250 mm x 4.6 mm, 5µ) at ambient temperature, with isocratic elution of Acetonitrile: 1% Triethylamine 50:50%, v/v with mobile phase pH-6.8. The flow rate was set 1.0 ml/min and the analysis was performed at a wavelength of 270 nm using Photo Diode Array (PDA) detector. Efficient UV detection at 270 nm enabled determination of Metoclopramide with no interference from injectable solution, excipients or solvents. The retention time (t_R) for Metoclopramide was around 2.56 min. The calibration curves were linear over a concentration range from 0.5 mcg to 3.5 mcg/ml. The percentage assay of Metoclopramide was 98.9%. Limit of detection (LOD) for Metoclopramide was 0.01 mcg/ml and Limit of quantitation (LOQ) Metoclopramide was 0.05 mcg/ml. The developed method was successfully applied to estimate the amount of Metoclopramide in injection formulations. The developed RP-HPLC method is accurate, precise, specific, sensitive, and efficient and can be used in routine analysis in quality control laboratories. This method may also be suitable for the estimation of the drug in the plasma sample analysis.

Chapter-11 opens with a brief introduction to an anti-hypertensive agent Olmesartan. This chapter describes a validated, reversed phase High Performance Liquid Chromatography Method for the estimation of Olmesartan in pharmaceutical formulations. The chromatographic conditions used are chromosil C18 (250 x 4.6 mm, 5
μ) column with mobile phase consisting of Methanol: Water 80:20%, v/v was used. The flow rate was 1.0 ml/min and the effluents were monitored at 265 nm. The retention time was 2.28 min. The Chromatographic run time was 6 min. The detector response was linear in the concentration of 10-100 mcg/ml, with the regression coefficient of 0.997. Quantification was done by calculating area of the peak and the detection and quantification limits were 0.25 and 1.0 mcg/ml respectively. The percentage assay of Olmesartan was 98.5%. The method was validated by determining its accuracy, precision and system suitability. The results of the study showed that the developed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Olmesartan in API and in its pharmaceutical dosage forms.

Analytical methods development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity, potency and performance of drug products. The efficient development and validation of analytical methods are critical elements in the development of pharmaceuticals. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance. Experience is one of these factors—both the experience level of the individual scientists and the collective experience level of the development and validation department. A strong mentoring and training program is another important factor for ensuring successful methods development and validation. A survey of literature showed that there are very few visible HPLC methods of analysis for the selected drugs reported at the time of the commencement of this investigation. Efforts were made hoping to fill this gap, as majority of these are very frequently used in current clinical practice, and succeeded in developing analytical methods using HPLC methods.