CHAPTER 5

SUMMARY AND CONCLUSION

Analytical chemistry may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained. In analytical chemistry, it is of prime importance to gain information about the qualitative composition of substances and chemical species, that is, to find out what a substance is composed of and exactly how much. Pharmaceutical analysis is an art and science of determining the concentration of drug constituents present in dosage formulations. It is considered as an application of procedures necessary to determine and estimate the identity, strength, quality and purity of the drugs. Purity of raw materials, quality, efficacy and safety of medicinal products can only be assured by analytical techniques. Therefore quality control is considered as the backbone of the drug industries with ever increasing need for the development of analytical techniques for the drug formulations. For pharmaceutical industries, pharmaceutical analysis is indispensable. The analyst therefore must be well versed in analytical procedure not only to apply known techniques, but also to device new better techniques wherever necessary.

New analytical techniques usually create some excitement. However, they become part of our standard methodology only if they provide some distinct advantages over existing and well established methods and permit analysts to make measurements that were hitherto impossible.
The official methods available for analysis of active ingredients of single and multicomponent formulations are few and the most of the methods are applicable only after prior separations which are tedious and time-consuming and expensive. The developed analytical methods avoid these time consuming extractions and separations and are economical in the sense that use of expensive reagents is minimized.

Hence, the work was undertaken with an aim to develop techniques for analysis of active ingredients of marketed formulations. This has developed the new methods of estimation, which serves adequately for routine drug analysis in pharmaceutical industries. In the present work, emphasis was put on to develop simple, accurate, precise and rapid methods for estimation of the components in the selected marketed formulations using spectrophotometry or high performance liquid chromatography.

Simultaneous analysis procedures are being used more frequently for estimation of drugs in multi-component pharmaceutical formulations due to their inherent advantages viz. avoid time consuming extraction and separation, economical in the sense that use of expensive regents is minimized and are equally accurate and precise. The validation of methods is carried out as per ICH guidelines.

Reversed-phase chromatography is by far the most versatile chromatographic technique in pharmaceutical analysis, and it could be said to be over-dominant; more than one method must be applied for exhaustive screening.
In the present study an attempt was made to develop spectrophotometric analytical method for estimation of drugs in bulk and marketed formulations by using simultaneous equations method, simultaneous equations method using area under the curve, graphical absorbance ratio (Q analysis) method, two-wavelength method and derivative spectroscopy. HPLC work was planned depending upon the availability of reported methods. In case a method has already been reported, attempt was made to provide alternate procedures, which are time and cost efficient. Attempt was made to develop all the best possible and applicable methods to serve as an alternate to each other. The developed methods are validated as per the ICH guidelines. Development of analytical methods were carried out by using spectrophotometric and Reverse Phase-HPLC techniques for the following.

15. Serratiopeptidase in tablet formulation.
17. Amitriptyline hydrochloride and Chlordiazepoxide in tablet formulation.
18. Amlodipine besylate and Atorvastatin calcium in tablet formulation.
19. Aspirin and Atorvastatin calcium in tablet formulation.
20. Chlordiazepoxide and Trifluoperazine hydrochloride in tablet formulation.


22. Ambroxol, Cetirizine dihydrochloride and Phenylephrine hydrochloride in tablet formulation.

23. Ambroxol, Salbutamol sulphate and Theophylline in tablet formulation.

24. Levocetirizine dihydrochloride, Phenylpropanolamine hydrochloride, Paracetamol and Ambroxol in tablet formulation.

1. Formulation containing mupirocin: A specific and sensitive high-performance liquid chromatographic method for the determination of mupirocin (MPR) from marketed formulation is developed. Attempt was made to develop derivative spectrophotometric method but reliable results were not obtained. A simple and highly selective stability indicating reverse phase high-performance liquid chromatography method (RP-HPLC) is presented for the determination of MPR from ointment formulation. The official books give the method for mupirocin but not specify its stability study. The method has been proven to be stability indicating. The chromatographic parameters are 55:45% v/v of acetonitrile and phosphate buffer (pH3.2) as mobile phase, ultraviolet detection at 223nm and 1.1ml/min flow rate. Total run time required for separation was 5 min. Linearity was expressed as a correlation
coefficient; the value is more than >0.999. Precision was expressed as percentage relative standard deviation (R.S.D.). The results are less than 2%. The lowest concentrations assayed where the signal/noise ratio was at least 10:1, this concentration was regarded as limit of quantitation (LOQ). The limit of detection (LOD) was defined as a signal/noise ratio of 3:1. The method is rapid, easy, and reliable. The peaks of the degradation products did not interfere with the analysis. The recovery of mupirocin from the marketed ointment was essentially estimated quantitatively. Based on the peak purity results, obtained from the analysis of forced degraded samples using the described method, it can be concluded that the method is specific for estimation of MPR in presence of degradants. The method has linear response in stated range and is accurate and precise. The described method can be used as stability indicating method for assay of MPR in marketed ointment formulation.

2. Formulation containing rabeprazole: The proposed work describes a simple, selective, sensitive and reproducible difference spectrophotometric and RP-HPLC methods for the estimation of rabeprazole sodium (RPZ) in marketed tablet formulations. Attempts were made to develop first order and higher order derivative spectroscopic methods by using 0.1M methanolic HCl. The second order and higher order derivative spectra produces noisy spectra. The estimation of RPZ
by first order derivative spectroscopy produces variable results in recovery study, so not reported in the proposed methods.

Rabeprazole sodium can exhibit two different forms in basic and acidic mediums that differ in their absorption spectra in basic and acidic mediums. The maxima in difference spectrum are at 292 nm and minima at 252.5 nm. Rabeprazole sodium in µg concentration range was estimated by this method. The proposed method can be successfully used for the analysis of the drug in the marketed tablet dosage forms. The Beer Lambert's law obeyed in the range of 2-50 µg/ml. The results of analysis have been validated statistically and by recovery studies. In difference spectroscopy, accuracy was investigated by analyzing three different concentration of RPZ sample in linear range in six independent replicates on the same day (intra-day precision) and on six consecutive days (inter-day precision). Accuracy was expressed as bias (%). The bias values were close to zero. The intra-day and inter-day relative standard deviation (RSD) values and also the low RSD values obtained from the analysis of pharmaceutical formulations (Table 4.2 T4) indicated that the intermediate precision of method was good.

A simple, specific, and accurate reverse phase high performance liquid chromatographic (RP-HPLC) method for determination of rabeprazole sodium (RPZ) in bulk drug and tablets have been developed. Liquid chromatography was performed using acetonitrile, methanol mobile phase (35:65% v/v) and a flow rate of 0.5 ml /min. The effluent was monitored on a UV detector at 280 nm. Each analysis required no longer
than 3.0 min. For quantification, a calibration curve was constructed for RPZ concentration ranging between 0.5–86.0 µg/ml. Furthermore, the typical excipients included in the drug formulation do not interfere with the selectivity of the method. Data, with respect to precision and accuracy and limits of detection, are determined. The proposed chromatographic method was successfully applied to the quantitative determination of RPZ in bulk drug and marketed tablets.

The HPLC method could be utilized for more specific than the spectrophotometric methods, but it is a more costly method. However, the methods are presently considered more reliable and promising for the routine analysis of RPZ in pharmaceutical dosage forms.

3. Formulation containing serratiopeptidase: Accurate, rapid and highly sensitive first derivative spectrophotometric method has been developed for the estimation of serratiopeptidase in bulk and pharmaceutical tablet dosage forms. Other methods were attempted but no any other method was found suitable for estimation of serratiopeptidase from its pharmaceutical dosage forms by using spectrophotometry or RP-HPLC. The developed method utilizes phosphate buffer pH 7.4 as a solvent. In this solvent system, the first derivative spectrum of serratiopeptidase shows the absorbance maxima at 229.5 nm. The proposed method can be successfully used for the analysis of the drug in the marketed tablet dosage forms. The Beer Lambert’s law obeyed in the range of 2-90 µg /ml. The results of analysis have been validated statistically and by recovery studies.
4. Formulation containing loratidine and ambroxol: Different spectrophotometric methods for simultaneous estimation of loratadine (LRT) and ambroxol (AMB) in two component solid dosage forms have been developed. The graphical absorbance ratio method was attempted but exact isoabsorptive point was not found suitable after preparation of different mixed standards for estimation of formulation. The developed methods employ the application of first derivative spectrophotometric, two wavelength method, simultaneous equation and area under the curve methods. All these methods utilize 0.1M HCl as a solvent.

For the development of derivative spectrophotometry method selected linearity range according to beers law was 5-60 µg /ml and 5-70 mcg/ mL for LRT and AMB respectively. Two wavelength methods is devised in which linearity range is 5-70 µg /ml and 5-60 µg /ml for LRT and AMB respectively. For simultaneous equation method the linearity range is 2-60 µg /ml and 2-70 µg /ml for LRT and AMB respectively. Area under the curve analysis can be successfully carried out in the linearity range 10-60 µg /ml for LRT and 10-60 µg /ml for AMB.

The proposed procedures were successfully applied for the simultaneous determination of both drugs in laboratory prepared mixtures and in commercial tablet preparations. The validity of the proposed methods was assessed by applying the standard addition technique.

5. Formulation containing amitriptyline and chlordiazepoxide: Four methods for simultaneous estimation of amitriptyline hydrochloride (AMT)
and chlordiazepoxide (CDZ) in two component solid dosage forms have been developed. The attempts were also made to develop the analytical methods by using derivative spectroscopy, two wavelength method and graphical absorbance ratio method but not got the expected results. The developed methods employ the application of multicomponent mode of analysis, simultaneous equation, area under the curve and RP-HPLC methods. Spectrophotometric methods utilize 0.1M HCl as a solvent. These techniques have more advantages than that of the separation techniques like HPLC. The proposed four methods were found suitable for the determination of ingredients in the different tablet brands containing AMT and CDZ for routine analysis. In this work, although the individual UV absorption spectra of these two compounds overlap in the region of 210–310 nm, Vierordt’s method is more suitable than multicomponent mode of analysis for the simultaneous determination of both drugs without prior separation from each other. The LOD and LOQ are satisfactory and the S.D and RSD values are considerably low and within the acceptable limits as per ICH guidelines. The validity of the proposed methods was assessed by applying the standard addition technique.

The HPLC method could be utilized for more specific than the spectrophotometric methods, but it is a more costly method. However, the methods are presently considered more reliable and promising for the routine analysis of AMT and CDZ in pharmaceutical dosage forms. A simple, rapid, sensitive RP-HPLC method was developed and validated to
measure simultaneously the amount of AMT and CDZ at single wavelength (239 nm) in order to assess tablet formulation. The average percent recovery was found to be 100.22±0.576 and 99.85±0.39. The result of the method lies within the prescribed limit of 98-102%, showing that the method is free from interference from excipients.

The time required for the simultaneous analysis of drugs requires only about 10 minutes in addition to the time required to prepare tablet sample solution. In spectrophotometric estimation, it requires only 0.1 M HCl, thus, effective economically. In RP-HPLC method, the method is more reproducible and accurate. These methods can be used for routine simultaneous quantitation of the two drugs in combined dosage forms.

6. Formulation containing amlodipine and atorvastatin: RP-HPLC and four different spectrophotometric methods for simultaneous estimation of amlodipine (AML) and atorvastatin (ATV) in two component solid dosage forms have been developed. The attempts were also made to develop analytical methods by using simultaneous equation using area under the curve. The developed methods employ the application of first derivative spectrophotometric, absorbance ratio (Q-analysis), two wavelength method, and simultaneous equation method. Spectrophotometric methods utilize 0.1M methanolic HCl as a solvent. The first derivative method is very simple and it only requires measurement of absorbances of sample solution at 246.4nm and 236.2nm for estimation of AML and ATV from first derivative spectrum.
The proposed procedures were successfully applied for the simultaneous determination of both drugs in laboratory prepared mixtures and in commercial tablet preparations. The validity of the proposed methods was assessed by applying the standard addition technique.

Measurement of AML and atorvastatin ATV by HPLC is preferred due to its sensitivity and specificity. HPLC methods differ with respect to the mode of detection (electrochemical, fluorescence or ultraviolet) and sample preparation. Most of HPLC methods required liquid–liquid extraction with evaporation of the extract or on-line solid-phase extraction, and therefore, sample preparation is time-consuming, complex or both. This method describes a rapid and sensitive enough HPLC method, which enables the determination of AML and ATV with good accuracy at low drug concentrations in marketed formulations.

The time required for the simultaneous analysis of drugs requires only about 10 minutes in addition to the time required to prepare tablet sample solution. The correlation coefficient ("r^2") values for both the drugs were >0.999 (table 16). The proposed method was validated as per ICH guidelines. In spectrophotometric estimation, it requires only 0.1 M methanolic HCl, thus, effective economically. In RP-HPLC method, the method is more reproducible and accurate. These methods can be used for routine simultaneous quantitation of the two drugs in combined dosage forms.

7. Formulation containing aspirin and atorvastatin: The proposed spectrophotometric estimation methods of aspirin and atorvastatin consist
of simultaneous equation method, derivative spectrophotometry and graphical absorbance ratio method. Attempts were made to develop the two wavelength spectroscopic and RP-HPLC methods but not got the success. The developed methods require only 0.1 M methanolic HCl, thus, effective economically. The method is more reproducible and accurate. These methods can be used for routine simultaneous quantitation of the two drugs in combined dosage forms. The accuracy of the proposed method was ascertained by carrying out recovery studies by standard addition method. The average percent recovery was found to be 100.32±0.099 and 100.13±0.174. The result of the method lies within the prescribed limit of 98-102%, showing that the method is free from interference from excipients.

The statistical parameters determined for estimation of each drug are satisfactory, taking into account the fact that all methods developed are for simultaneous analysis. The quantitative, statistical and recovery study results obtained with each method indicate the accuracy and reproducibility of respective methods for analysis of respective drug formulations. Thus, the methods can be employed as part of the quality control procedures for the formulations covered in this work.

8. Formulation containing trifluperazine and chlordiazepoxide: RP-HPLC and four spectrophotometric methods for simultaneous estimation of trifluperazine hydrochloride and chlordiazepoxide in two component solid dosage forms have been developed. Attempts were also made to
develop simultaneous equation using area under the curve. Reproducible results were not obtained. Spectrophotometric methods employ the application of simultaneous equation, the absorbance ratio (Q-analysis), first derivative spectrophotometric and two wavelength methods. All these methods utilize 0.1M HCl as a solvent. The spectrophotometric simultaneous equation method was developed, because there is an intense peak of both drugs in the range of 200-400 nm. The time required for the simultaneous analysis of drugs requires only about 10 minutes in addition to the time required to prepare tablet sample solution. First derivative method, the method is very simple and it only requires measurement of absorbances of sample solution at 223.5 nm and 245.5 nm for estimation of TFP and CDZ from first derivative spectrum. As there was no spectral overlap amongst TFP and CDZ with sufficient difference in absorbance maximum of TFP and CDZ by more than 10nm, derivative spectroscopy was a better option to resolve the spectra of the two drugs. The average percent recovery was found to be 100.02±0.299 and 100.06±0.240. The low values of SD and RSD indicated that the proposed method has good precision. Linearity was determined for all spectrophotometric methods for TFP in the range of 1-50 µg/ml and for CDZ, 1-80 µg/ml. The correlation coefficient (‘r²’) values for both the drugs were >0.999. After several trials mobile phase was selected as 0.010 millimol ammonium acetate buffer and methanol in the ratio 10:90 v/v at a flow rate 1 ml/min for RP-HPLC. Quantitation was achieved with UV detection
at 239 nm based on peak area, with linear calibration curves at concentration ranges from 1-100 µg/ml for TFP and CDZ respectively. The method has successfully been applied to pharmaceutical dosage forms. No chromatographic interference from the tablet excipients was found. The method was validated using ICH guidelines and was found to be highly precise, accurate. Comparison between developed HPLC method and reported spectrophotometric methods indicated that there is improvement in accuracy and precision in case of HPLC method. It has advantage of being specific and highly sensitive.

The proposed methods are suitable for routine determination of both the drugs in their formulations, but they can not be considered as stability indicating assays. The laboratory prepared mixtures as well as commercial tablet formulations were studied with excellence recoveries in all the methods, which show that there is no interference between the two drugs and the excipients used in the tablet formulations. The LOD and LOQ of the methods are satisfactory. All the methods are accurate, reproducible, precise and economic.

9. Formulations containing rabeprazole and domperidone: Simultaneous equation method, derivative spectroscopy, area under the curve and RP-HPLC are developed for simultaneous determination of rabeprazole sodium and domperidone maleate in tablets. Attempts were also made to develop two wavelength and graphical absorbance ratio method by spectrophotometry. The proposed first order derivative method is more rapid and simple than the other spectrophotometric methods, while the
RP-HPLC method has greater sensitivity and accuracy. The proposed methods are suitable for routine determination of both the drugs in their formulations, but they can not be considered as stability indicating assays. The laboratory prepared mixtures as well as commercial tablet formulations were studied with excellence recoveries in all the methods, which show that there is no interference between the two drugs and the excipients used in the tablet formulations. All the methods are accurate, reproducible, precise and economic.

The LOD and LOQ of the methods are satisfactory. The S.D and RSD values considerably low and within acceptable limits as per ICH guidelines.

10. Formulation containing ambroxol, cetirizine and phenylephrine: Simultaneous estimation of active ingredients from multicomponent pharmaceutical products normally requires the freedom of sample solution from absorbing excipients matrix. The problem becomes rigorous when the excipients matrix absorbs over the wavelength region of interest. Examples that can be cited for absorbing excipients are methyl paraben, propyl paraben, polyvinyl pyrrolidone, colors, etc. Considering the facts, a generalized approach to overcome such type of interference using multiwavelength spectroscopy has been discussed. Multicomponent spectroscopy, derivative spectroscopy and RP-HPLC methods permit simple, rapid and direct determination of ambroxol, cetirizine and phenylephrine as nasal decongestant in allergy with cough. In cases where more number of sampling wavelengths are to be used for
improving accuracy, more number of mixed standards are necessary, which affects time efficiency of analysis. Hence, an alternate derivative spectroscopy approach to the multicomponent analysis has been discussed taking examples of tablet formulations containing phenyl ephrine, cetrizine and ambroxol. Spectrophotometry methods have more advantages than that of the separation techniques like HPLC or GC. Attempts were also made to develop simultaneous equation and graphical absorbance ratio method but no reproducible results were found. The proposed first derivative method is simple and it only requires measurement of absorbances of sample solution at 243.5, 261.5 and 323 nm for estimation of PEP, CTZ and AMB. Recovery studies ranging between 99-100% were suggestive of accuracy of the method.

For RP-HPLC method, the retention time of PEP, CTZ and AMB was found to be 2.2 min, 2.81 min and 3.59 min, respectively. The percent estimation of drug in laboratory mixture with ±SD was found to be 99.8±2.128, 100.08±0.820 and 100.15±0.723 for PEP, CTZ and AMB, respectively. The average recovery of PEP, CTZ and AMB was 99.96±0.148, 100.24±0.187 and 100.16±0.264 % respectively. The standard deviation values were less than 1.60 for phenyl ephrine and cetrizine and less than 1.10 for ambroxol. Recovery studies were between 98-102 percent. The method was found to be comparable in accuracy, precision and time economy to the multiwavelength spectroscopy method.

11. Formulations containing ambroxol, salbutamol and theophylline: The proposed simultaneous equation and derivative spectroscopy methods

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provide simple, accurate and reproducible quantitative analysis for the
determination of ambroxol, salbutamol and theophylline in tablet dosage
forms, without any interference from the excipients. Attempts were made
to develop RP-HPLC method for the estimation of drug formulation. The
proper chromatographic separation and resolution was not achieved in
the selected solvent system and at selected wavelength. The developed
spectrophotometric detection proposed for determination is an alternative
to other methodologies. This one, besides being simple, accurate and
precise, is free from many disadvantages that are common in other
spectrophotometric methods: complex sample treatment, critical working
conditions, heating of the reaction mixture, expensive chemicals and
instrumentation, high time consuming, etc. Moreover, the system
presents a high sensitivity, good quality results (R.S.D., 2%) and wide
range of linear response. The most important advantages is use of 0.1 M
HCl which allows the selective and sensible determination of the analytes,
Hence, the proposed method can be recommended for the routine
determination of these drugs in their pure form or their preparations.
The proposed method is simple as there is no need for solvent extraction
and direct as it estimates each drug independent of the other. The
method has the advantage of lower cost, rapid and environmental
protecting. The proposed method was completely validated and suitable
for quality control laboratories, where economy and time are essential.
The values of SD or RSD and coefficient of correlation are within the
prescribed limit of 2%, showing high precision of the method
12. Formulations containing Levocetirizine phenylpropanolamine, paracetamol and ambroxol: The marketed combination is indicated for seasonal allergic rhinitis, acute allergic reactions due to drugs, food or insect bites. The UV absorption spectra of the studied drugs show severe overlap. Hence, the spectrophotometric simultaneous determination of drugs was difficult, therefore RP-HPLC techniques was developed for the estimation of drugs from marketed formulation. All the analytical methods developed are simple, rapid, precise and accurate. In these methods, due care has been taken to eliminate interference from all possible excipients in the formulations. The methods have been developed with special attention to the cost and time efficiency. The statistical parameters determined for estimation of each drug are satisfactory, taking into account the fact that all methods developed are for simultaneous analysis. The quantitative, statistical and recovery study results obtained with each method indicate the accuracy and reproducibility of respective methods for analysis of respective drug formulations. The time required for the simultaneous analysis of drugs requires only about 10 minutes in addition to the time required to prepare tablet sample solution. High percentage of recovery shows that the compounds are completely extracted from tablet formulations and free from the interference of the excipients. In conclusion, the developed HPLC method allows the quantitation of three compounds in pharmaceutical formulations using the same dilution and the same injection volume in a short analytical time.
Thus, the methods can be employed as part of the quality control procedures for the formulations covered in this work.

The compilation of above research work will help the pharmaceutical industry sector by providing methods for routine economical analysis.