PREFACE

The work has been taken up in view of developing new spectrophotometric and High Performance Liquid Chromatographic methods for the determination of cephalosporins and some other anti-biotics at micro gram level, which are pharmaceutically and biologically important components. New spectrophotometric (visible region) and reverse phase high performance liquid chromatographic methods for different anti biotics have been proposed in this thesis.

Chapter-I opens with prolegomenon giving a detailed view on structure, classification, effects and side-effects of cephalosporins. This chapter elaborately deals with the literature survey of several Spectrophotometric and Reverse Phase High Performance Liquid Chromatographic methods reported for the estimation of third generation cephalosporins, cefdinir, ceftriaxone and a fourth generation cephalosporin, Cefepime. Literature survey of Spectrophotometric and RP-HPLC methods for the determination of amikacin, an aminoglycoside, which is often used as a combination drug of cephalosporins is also discussed in this chapter. This chapter closes with a brief account on Spectrophotometry and reverse phase liquid chromatography.
Each Chapter consists two parts. First part focuses on spectrophotometric methods while second part deals with RP-HPLC methods.

**Chapter–II.** First part deals with the experimental details of spectrophotometric method (visible region) of Cefepime. This method is based on the formation of red colored oxidative coupling products from cefepime and catechol (C), or 4-amino acetophenone (AAP) in the presence of sodium meta periodate. The pairs of reagents C--IO$_4^-$ : AAP—IO$_4^-$ have been found to be useful in the spectrophotometric determination of cefepime.

Second part deals with RP-HPLC method for the development and validation of Cefepime. In this method mobile phase is a mixture of o-phosphoric acid, OPA, (1%) and methyl alcohol 80:20 (v/v) was prepared by diluting 800 ml of OPA and 200 ml of methyl alcohol in one litre flask. A non polar C18 column was chosen as the stationary phase for this study. A flow rate of 1.0 ml/min mobile phase was found to be suitable in the studied range of 0.5—1.5 ml/min. The retention time obtained for cefepime was 2.79 min.

**Chapter–III.** First part deals with the spectrophotometric determination of Cefdinir. Catechol is oxidized with NaIO$_4$ to form o-Benzо quinone which is coupled with Cefdinir to form a red color indo dye.
Second part is the RP-HPLC method for the development and validation of Cefdinir. In this method a mixture of 5% tetra hydro furan (THF), 15% methyl alcohol, 40% acetonitrile and 40% (0.1%) ortho phosphoric acid (OPA) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. A flow rate of 1.0 ml/min mobile phase was found to be suitable in the studied range of 0.5—1.5 ml/min. A retention time of 5.1 min. was obtained for Cefdinir.

Chapter –IV. First part consists of four spectrophotometric methods for the determination of Ceftriaxone. First method deals with the experimental details of spectrophotometric methods of determination of ceftriaxone sodium with sodium carbonate-- Folin-Ciocalteu Phenol’s reagent (FCP). FCP produces color with a purine ring containing –OH and –NH$_2$ groups, hence it produces deep blue color with Ceftriaxone. Second method is simply diazotization reaction with Nitrous acid. The –NH$_2$ group of Ceftriaxone undergoes diazotization with nitrous acid producing violet color in the presence of alkali. Third method deals with the spectrophotometric determination of Ceftriaxone with MBTH (3-methyl benzthiazolinone-2 hydrazone) reagent and Ferric Chloride. The reaction between MBTH and Ferric Chloride is an iron catalysed oxidative coupling reaction of MBTH with Ceftriaxone forming blue colored chromogen.
Fourth method deals with the spectrophotometric determination of Ceftriaxone with ferric chloride and potassium ferri cyanide. Ceftriaxone reduces ferric ion to ferrous ion which reacts with potassium ferri cyanide forming a green color complex, Ferrous ferro cyanide.

Second part is RP-HPLC method for the development and validation of Ceftriaxone. In this method mobile phase is a mixture of 0.01M Ammonium di hydrogen phosphate(ADP), acetonitrile(ACN), o-phosphoric acid, OPA, (1%) 5:50:45 (v/v/v) which was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. A flow rate of 1.5 ml/min mobile phase was found to be suitable in the studied range of 1.0—2.0 ml/min. The retention time obtained for Ceftriaxone is 5.3 min.

Chapter-V. First part deals with the experimental details of spectrophotometric method of determination of amikacin, an aminoglycoside antibiotic with catechol—sodium meta periodate. A red color indodye is formed due to coupling of amikacin with o-benzo quinone, formed due to the oxidation of catechol.

Second part is the RP-HPLC method for the development and validation of Amikacin. A mixture of acetonitrile, methyl alcohol, and o-
phosphoric acid, OPA, (0.1%) in 50:35:15 (v/v) was proved to be the most suitable of all the combinations since the chromatographic peaks obtained were well defined, resolved and free from tailing. A flow rate of 1.0 ml/min mobile phase was found to be suitable in the studied range of 0.5—1.5 ml/min and a retention time of 9.6 min was obtained for Amikacin.

Validity of the spectrophotometric methods proposed for the estimations of cephalosporins and amikacin given in chapters II to V were established from the precision (calculating % RSD, confidence limits with 0.01 and 0.05 levels from six replicate determinations) and accuracy ( % standard error, comparison of results obtained with proposed and reported methods).

In RP-HPLC methods proposed for development and validation of Cephalosporins and Amikacin, each of the sample was injected six times and the same retention times were obtained in all cases. The peak areas of samples were reproducible as indicated by low coefficient of variation. A good linear relationship was observed between the concentration of sample and the respective peak areas. High recovery values obtained from the dosage form by the proposed method indicates the method is accurate. The absence of additional peaks indicates non interference of common excipients used in the tablets. The lowest values of LOD and LOQ obtained by the proposed method
indicate the methods are sensitive. The standard solution of the drug was stable up to 24 hours as the difference in percent assay is within acceptable limit.

Part of the work reported in this thesis has already been published in two national journals, Acta Ciencia Volume XXX C,#4 and #259.