SUMMARY

The growing demand for the diseases stimulate a search for new even more effective drugs, but also calls for higher level of quality control of HIV therapeutic substances and preparations, so that they are in the highest possible degree free from any impurities that may come from the production process, as well as from decompositions products of active or auxiliary substances. Therefore, it seems appropriate to develop new analytical methods regarding their qualitative and quantitative analysis.

Several methods for the estimation of anti-HIV drugs are classified into physical, chemical, physico-chemical and biological ones. Physical methods involve the study of the physical properties such as solubility, transparency or degree of turbidity, color density, specific gravity etc. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation, redox reactions etc. Titration in non-aqueous media and complexometry are also being used in pharmaceutical analysis. Physico-chemical methods involve the study of the physical phenomena that occurs as a result of chemical reactions. These include spectrophotometric and chromatographic methods.

Visible spectrophotometric techniques are remarkable for their versatility, sensitivity and selectivity. Visible spectrophotometric methods depend only on the nature of chemical reactions involved in color development and not on the sophistication of equipment. Furthermore the basic apparatus required is a spectrophotometer, and is now fairly cheaper than most other instrument used in pharmaceutical analysis. HPLC technique has been regarded as the best among various techniques in spite of its heavy cost and proper maintenance. The

development of highly efficient micro particulate bonded phase has increased the versatility of the technique and has greatly improved the analysis of multi component mixtures.

In the view of the above facts, the author has undertaken the visible spectrophotometry and HPLC techniques for the assay of same HIV drugs in pure and their pharmaceutical formulations. The author has developed, twelve new analytical methods using visible spectrophotometry for four selected drugs such as Stavudine [STV], Lamivudine [LMV], Zidovudine [ZDV] and Efavirenz [EFZ], by exploiting their characteristics, physical and chemical properties depending upon functional groups present in each drug. In addition to the newly developed visible spectrophotometric the author also developed four HPLC methods for the assay of Stavudine [STV], Lamivudine [LMV], Zidovudine[ZDV] and Efaverinz [EFZ] in pure and their pharmaceutical formulations.

The content of the thesis has been divided into five chapters and appropriate references [Bibliography] have been placed at the end of the each chapter respectively.

Chapter-I describes a brief introduction on HIV drugs, pharmacological actions, classification, their marketed formulations and methodology involved in the development of new methods using visible spectrophotometry (Part-A) and HPLC (Part-B) methods for the assay of four selected drugs.

The manuscript given under part-A includes criteria for satisfactory spectrophotometric analysis, reactions proposed in the present investigation such as oxidative coupling reactions , redox reactions , condensation reactions , nucleophilic

substitution reaction, charge transfer reactions, diazo coupling reaction and ion association complex formation reactions. Validation of the developed methods in terms of selectivity, linearity, precision, accuracy, sensitivity, limit of detection (LOD) & limit of quantitation (LOQ) and study of robustness are explained. Classification of analytically useful functional groups in drugs, chemistry of chromogenic reagents, and the general methodology involved in developing new visible spectrophotometric methods (spectral characteristics of the colored species), optimization of experimental conditions (effect of pH, reagent concentration and order of addition, keeping time and temperature during each addition, effect of solvent, color development and stability) optical characteristics (Beer's law limits, Sandell's sensitivity, optimum photometric range and molar absorptivity useful for sensitivity), and testing of significance by F-test. Elico SL-159 model,2nm high resolution, double beam, 1cm length quartz coated optics; Wavelength range190-1100nm ; High stability, linearity, precision instrument is used for all the spectral measurements. The reagents used in the present investigation are 3- Methyl – 2 - Benza Thiazolinone Hydrazone (MBTH), Idoso benzene diacetate (IBDA), Ceric ammonium sulphate (Ce(IV)), Sodium periodate ($NaIO_4$), Phenyl Hydrazine Hydrochloride (PHH), potassium ferricyanide ($K_3Fe(CN)_6$), Brucine Solution , anhydrous ferric chloride (Fe(III)), O-Phenanthroline solution, 1,2 napthaquinone sulphonic acid sodium salt (NQS) , p-chloranilic acid (p-CA), tropaeoline ooo (TPooo), alizarin red-S(ARS).The author has developed the following new spectrophotometric methods based on different chemical reactions to determine the quantity of the drug in pure and formulations. Method M_{1a} : MBTH/IBDA/AcOH, Method M_{1b} : MBTH/Ce(IV), Method M_{1c} MBTH/$NaIO_4$, Method M_2: $NaIO_4$/PHH/$[Fe(CN)_6]^{-3}$, Method M_3 : Brucine $NaIO_4$, Method M_4: Fe(III)/O-phen, MethodM_5 :AMV/H_2SO_4, Method M_6: Fe(III)/ /$[Fe(CN)_6]^{-3}$ Method

M_7:Isatin/ H_2SO_4, Method M_8: Vanillin/ H_2SO_4, Method M_9: NQS, Method $M_{10,}$:p-CA, Method M_{11a} : TPooo , Method M_{11b}: ARS, Method M_{12a}: PGNL/$NaNO_2$ and Method M_{12b}: RSNL/$NaNO_2$.

The information given under part-B, includes the introduction, classification of chromatographic techniques, instrumentation and the components of HPLC system (solvent delivery systems, solvent degassing systems, gradient elution devices, sample introduction systems liquid chromatography detectors, column packing materials inclusive of bonded phase, derivatization, gradient elution). Validation of the newly developed RP-HPLC methods (recovery, response function, sensitivity, precision and accuracy) and performance calculations (relative retention, theoretical plates, plates per meter, height equivalent to theoretical plate, capacity factor, resolution, peak asymmetry) are also presented. The author has developed four RP-HPLC methods for the determination of Stavudine [STV], Lamivudine [LMV], Zidovudine [ZDV] and Efavirenz [EFZ] which are included in their respective chapters.

Chapter-II starts with the drug profile giving brief account of chemical name, structure, and mode of action, commercially available formulations and literature on physicochemical methods reported for Stavudine [STV]. There are only few visible spectrophotometric methods for the assay of STV in pharmaceutical formulations. Existing analytical methods reveal that relatively little attention was paid in developing visible spectrophotometric methods by exploiting the useful functional groups in STV. The analytically useful functional groups in Stavudine [STV] offer a lot of scope for the development of new methods, hopefully with better sensitivity, precision and accuracy, which prompted the author to carry out investigations in this accord. The author has developed eleven visible

spectrophotometric methods. The λ_{max} for the developed methods are found to be Method M_{1a}; M_{1b}; M_{1c}; M_2; M_3; M_4; M_9; M_{10}; M_{11a} and M_{11b} with λ_{max} values of 630nm; 640nm; 650nm; 520nm; 530nm; 480nm; 464nm; 540nm; 650nm and 430nm respectively. Beer's law limits, molar absorptivity, Sandell's sensitivity and optimum photometric range for each method are calculated. The regression analysis of Beer's law data using the method of least squares was made to evaluate the slope (b), the intercept (a) and the correlation coefficient (r) for each proposed method. Commercial formulations containing stavudine were successfully analyzed by the proposed methods. The values obtained by the proposed and reference methods were compared statistically by the t-and f-test. Chemistry of the colored species is tentatively based on analogy of reactive functional moieties of stavudine with various reagents were presented in this chapter. The decreasing order of sensitivity for the proposed methods is found to be M_9 ($1.35x10^4$), M_{10} ($6.44x10^3$) M_{11a} ($5.77x10^3$), M_{11b} ($5.63x10^3$) M_2 ($4.7x10^3$), M_3 ($2.78x10^3$) M_4 ($2.68\ x10^3$), M_{1a} ($1.72x10^3$) M_{1b} ($1.6x10^3$), M_{1c} ($1.1\ x10^3$).

Part-B of this chapter reveals a brief note on the chemical properties and the literature survey of the HPLC methods of Stavudine [STV]. A very few HPLC methods for the assay of Stavudine [STV] in pharmaceutical formulations were reported in the literature. Taking all these views of the drug into consideration, the author has developed a simple HPLC method for the quantitative estimation of Stavudine [STV] by using a stationary phase [a stainless steel column 250mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5μm diameter YMC, Pack Pro C_{18} RS 150 x 4.6mm, 3μm and the mobile phase combination buffer [orthophsophric acid] and acetonitrile in the ratio (50:50) without internal standard. The detection was carried at 235nm. The results of

this investigation are presented in this part. Retention time, number of theoretical plates, tailing factor and area of the peak for the typical chromatogram of the standard drug are found to be 3.307min.,5324, 1.32 and 2649.204m AU respectively.

Chapter-III focuses on the introduction giving brief account of chemical name, structures, therapeutic importance, commercially available formulations and analytically useful functional groups of Lamivudine [LMV]. There are very few physicochemical methods reported in the literature, hence there is a need for sensitive, accurate and flexible visible spectrophotometric methods for its determination in pharmaceutical formulations. The author has made some attempts in this direction and succeeded in developing six visible spectrophotometric methods based on the analytically useful functional groups present in Lamivudine [LMV].Optical characteristics and regression parameters are evaluated. Correlation coefficient value which is very close to unity indicates that there is a good correlation between the concentration of the drug and absorbance. The statistical results are found to be satisfactory. Experimentally maximum absorption wavelength and linearity limits for the developed methods are as follows. Method M_{1a} (660nm, 4.0-24.0µg/mL), M_3 (530, 4.0-24.0 µg/mL), M_{10} (540nm, 5.0-30.0 µg/mL), M_{11a} (430nm, 3.33-20.0 µg/mL), M_{11b} (650nm, 3.33-20.0µg/mL) M_{12a} (530nm, 10.0-60.0 µg/mL), M_{12b} (540nm, 10.0-60.0 µg/mL)

Part-B of this chapter reveals a brief note on the literature survey of the HPLC method of lamivudine. Very few HPLC methods were reported in the literature for the assay of lamivudine in pharmaceutical formulations. Taking all these views into consideration, the author has developed a simple RP-HPLC method for the assay of lamivudine by using stationary phase [a stainless steel column 250mm long, 4.6mm

internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5μm diameter (Inertisil ODS-3V, 5μ(250mmx4.6mm)] and mobile phase combination of mixture of orthophosphoric acid and acetonitrile in the ratio of 90:10 v/v. The detection was carried at 220nm. The results of this investigation are presented in this part. Number of theoretical plates, tailing factor, Retention time and area of the peak for the typical chromatogram of the standard drug are found to be 3978, , 1.83, 3.12min., and 132023.2m AU respectively.

Chapter –IV opens with the introduction giving or brief account of chemical name, structure, therapeutic importance, analytically useful functional groups, commercially available formulations and the literature on the physicochemical methods reported so far for Zidovudine[ZDV]. Very few visible spectrophotometric methods have been reported for the assay of ZDV, which offers a scope to develop some visible spectrophotometric methods for its determination in wide pharmaceutical formulations. The author developed eight visible spectrophotometric methods, Method $M_{1a,}$ Method $M_{1c,}$ Method $M_{3,}$ Method – M_{7} , Method – $M_{8,}$ Method $M_{10,}$ Methods M_{11a} and M_{11b} by exploiting the functional groups present in Zidovudine[ZDV]. The optimum conditions for the color development of methods ($M_{1a,}$ $M_{1c,}$ $M_{3,}$ $M_{7,}$ $M_{8,}$ $M_{10,}$ M_{11a} and M_{11b}) were established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species. Regression parameters such as slope, intercept and limit of detection for the developed methods ($M_{1a,}$ $M_{1c,}$ $M_{3,}$ $M_{7,}$ $M_{8,}$ $M_{10,}$ M_{11a} and M_{11b}) have been evaluated. The proposed spectrophotometric methods for the determination of Zidovudine are simple, accurate, precise and cheap. The statistical analyses show that the data from the proposed method are in good agreement with those of the reported method.

Part-B of this chapter reveals a brief note on the literature survey of the HPLC method of lamivudine. The author has developed a simple RP-HPLC method for the assay of lamivudine by using stationary phase [a stainless steel column 250mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5µm diameter][use (Inertisil ODS-3V, 5µ(250mmx4.6mm)] and mobile phase combination of mixture of Orthophosphoric acid and acetonitrile in the ratio of 90:10 v/v. The detection was carried at 220nm. The results of this investigation are presented in this part. The retention time of zidovudine was found to be 10.519min. The linearity of the method was determined at five concentration levels ranging from 5.0 to 25.0µg.mL^{-1} for zidovudine. The calibration curve was constructed by plotting response factor against concentration of drugs. The slope and intercept value for calibration curve was y = 0.0588x-0.0167 (R2=0.9913) for zidovudine. Intraday and interday precision for six repeated injections of standard and sample solutions were made for three consecutive days and response factor of drug peaks and percentage RSD were calculated and found to be 0.833 and 1.63 respectively. Recovery study carried out for the drug was performed by spiking the known standard drug in powdered formulations and the results were found to be 96.28 ± 1.120 to 100.08 ± 0.164.

Chapter-V begins with the introduction giving a brief account of chemical name(s), therapeutic importance, structure, analytically useful functional groups, commercially available pharmaceutical formulations and literature on the physicochemical methods reported for Efavirenz [EFZ]. The author developed eight visible spectrophotometric methods for the assay of Efavirenz [EFZ] in pure and dosage formulations. In order to test whether the colored species formed in the above methods, adhere to Beer's law the absorbance's at appropriate wave lengths of a set of

solutions containing varying amounts of EVZ and specified amounts of reagents (as given in the proposed procedures for each method) were recorded against the corresponding reagent blanks. The Beer's law plots of these systems are recorded against the corresponding reagent blanks are recorded graphically

Part-B of this chapter reveals a brief account on the literature survey of the HPLC methods for the assay of Efavirenz [EFZ]. A very few HPLC methods for the assay of Efavirenz [EFZ] were reported in the literature. The author has developed a simple RP -HPLC method for the quantitative detemintion of Efavirenz [EFZ] with a better sensitivity by using stationary phase [a stainless steel column 250mm long, 4.6mm internal diameter filled with octadecyl silane chemically bonded to porous silica particles of 5μm diameter] [Inertsil ODS-2 C_{18}, 5μ(250mmx4.6mm)] and mobile phase combination of buffer [Orthophosphoric acid] and acetonitrile in the ratio of 90:10 v/v. The contents of the mobile phase were acetonitrile [Orthophosphoric acid] and waterin the ratio of 70:30 v/v. They were filtered before use through a 0.45μm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 30.0min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 30min with the mobile phase flowing through the system. The eluents were monitored at 240 nm. The plot of peak area of each sample against respective concentration of efivarienz was found to be linear in the range of 10.0–60.0 $\mu g.mL^{-1}$ with correlation coefficient of 0.999.The respective linear regression equation being Y=22119.684x+6829.3428.

The data and information of selected drugs, reagents and techniques given in chapters [II-V] reveals that the proposed methods are simple, selective, sensitive and

accurate with reasonable precision and accuracy. The proposed methods developed by the author can be used as alternative methods for the routine determination of the above mentioned drugs depending upon the availability of chemicals and situation arising due to the presence of concomitants in pure and in pharmaceutical dosage formulations. Three papers were published, seven supporting papers have been published and much of the work has been communicated to reputed national and international journals.