CHAPTER-9

Summary
Analytical method development and validation play important role 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 the drug products. In order to obtain a proper and suitable assay procedure for pure finished chemical compounds which are clinically proven as therapeutic agents, a systematic report of method development and validation is essential.

In the present thesis, some simple, sensitive and reasonably selective spectrophotometric methods are proposed for the determination of some active pharmaceutical drugs, i.e atazanavir, emtricitabine, eplerenone, olopatadine, pemetrexed disodium and zolmitriptan in bulk and in their pharmaceutical formulations using different reagents. The pharmaceutical drugs that are studied in the present investigations are highly used as medicaments for the treatment of viral, cancer, allergic, asthma, and migraine diseases. The significance of these drugs was discussed under the introduction part of each section.

The drugs atazanavir and emtricitabine are medications for the treatment of infection by HIV-1 viruses. Eplerenone is used in the treatment of hypertension and an adjunct in the management of chronic heart failure. Olopatadine is indicated for the treatment of allergic rhinitis urticaria and itching accompanied by skin diseases. Pemetrexed disodium belongs to a class of chemotherapeutic drugs known as folate anti metabolites. It is used for the initial treatment of patients with locally advanced or metastatic nonsquamous non-small cell lung cancer. Zolmitriptan is used for the acute treatment of migraines with or without aura in adults. All the proposed methods are
applied for the determination of the respective drug samples in their pharmaceutical formulations.

The important analytical parameters and the analytical results in the direct spectrophotometric determination of each drug are presented and discussed in each respective chapter.

*Chapter -1* gives a brief account on analytical chemistry and its applications to pharmaceutical analysis, basic principles of spectrophotometry and the types of reactions and reagents used in the present proposed analytical methods for the determination of selected drugs.

*Chapter-2* contains the drug profile and literature survey on the selected drugs, reagents and instruments employed in the present investigations and objectives of the present research work.

*In chapter-3*, the analytical results obtained in the visible spectrophotometric determination of atazanavir using some acid dyes are reported. Atazanavir forms ion-association complexes with bromothymol blue (BTB), bromophenol blue (BPB) and bromocresol green (BCG) in the presence of potassium hydrogen phthalate buffer (pH 2.4). The resultant yellow colored complexes showed $\lambda_{\text{max}}$ at 414 nm, 410 nm and 417 nm respectively. The colored species showed reasonably good molar absorptivities, $4.849 \times 10^4$ L mole$^{-1}$cm$^{-1}$, $4.581 \times 10^4$ L mole$^{-1}$cm$^{-1}$ and $5.180 \times 10^4$ L mole$^{-1}$cm$^{-1}$ respectively. The methods were able to determine the drug in the concentration range 2.0-14.0 µg mL$^{-1}$ with BTB and BPB reagents and 2.0-12.0 µg mL$^{-1}$ with BCG with Sandell’s sensitivity 0.0145 µg cm$^2$, 0.0153 µg cm$^2$, and 0.0136 µg cm$^2$ respectively. Limit of detection and limit of quantification were calculated as 0.135 µgmL$^{-1}$ and 0.405
µg mL\(^{-1}\) for BTB; 0.155 µg mL\(^{-1}\) and 0.472 µg mL\(^{-1}\) for BPB; 0.092 µg mL\(^{-1}\) and 0.28 µg mL\(^{-1}\) for BCG methods respectively.

The developed methods were applied for the determination of atazanavir in different pharmaceutical formulations with good accuracy and reproducibility. The result obtained by the proposed methods indicated that, based on molar absorptivity data and Beer’s law range, it may be concluded that among the proposed methods, AZV-BCG method is more sensitive than AZV-BTB method which in turn is more sensitive than AZV-BPB method. The proposed methods are simple, sensitive, accurate and economical for routine analysis of atazanavir sulfate in bulk and its parenteral formulations.

**Chapter-4** Discusses three visible spectrophotometric methods for the determination of emtricitabine (EMT) with two different aromatic aldehydes and a hydrazone reagent. In these methods the drug forms orange-red colored Schiff’s base complexes with p-Dimethylamino benzaldehyde (PDAB) or 4-Hydroxy-3-methoxybenzaldehyde (HMBA) in acidic medium. The resultant colored complexes showed maximum absorbance at 495 nm and 480 nm with molar absorptivities 6.099 x 10\(^3\) L mole\(^{-1}\)cm\(^{-1}\) and 6.922 x 10\(^3\) L mole\(^{-1}\)cm\(^{-1}\) respectively. These methods are applicable for the determination of the drug over the concentration range of 1.0-40 µg mL\(^{-1}\) and 2.5-35 µg mL\(^{-1}\) with Sandell’s sensitivity 0.0405 µg cm\(^{-2}\), and 0.0123 µg cm\(^{-2}\) respectively. The drug forms green colored complex with 3-Methyl 2-benzothiazolinone hydrazone (MBTH) by oxidative coupling reaction in the presence of ferric chloride as oxidizing agent. The formed colored complex showed \(\lambda_{\text{max}}\) at 630 nm with molar absorptivity 2.002 x 10\(^4\) L mole\(^{-1}\)cm\(^{-1}\). Beer’s law is obeyed in the concentration range 0.5-12.5 µg mL\(^{-1}\) with sandell’s sensitivity
0.035 µg cm⁻². The LOD and LOQ of all the methods were calculated as 0.3025 µg mL⁻¹ and 0.9166 µg mL⁻¹ (PDAB); 0.2357 µg mL⁻¹ and 0.7142 µg mL⁻¹ (HMBA); 0.072 µg mL⁻¹ and 0.215 µg mL⁻¹ (MBTH) respectively.

The proposed methods were applied for the determination of emtricitabine in different pharmaceutical formulations with good accuracy and reproducibility. Based on molar absorptivity data and Beer’s law range, it may be concluded that among these proposed methods, method EMT-MBTH is more sensitive than method EMT-HMBA which in turn is more sensitive than the method EMT-PDAB.

In Chapter-5 the results obtained in the assay of eplerenone by extractive visible spectrophotometry using three acid dye reagents, i.e. BTB, BCG and BPB are reported. All the developed methods were based on the formation of yellow colored ion-association complexes between the drug and the respective dye reagent. The yellow colored chromogens were quantitatively extracted in to chloroform. The extractable colored chromogens showed λₘₐₓ at 410 nm, 414 nm and 425 nm with molar absorptivities 1.036x10⁴ L mole⁻¹ cm⁻¹, 7.875x10³ L mole⁻¹ cm⁻¹ and 9.119x10³ L mole⁻¹ cm⁻¹ respectively. Beer’s law is obeyed in the concentration range of 1.25-55 µg mL⁻¹, 5-45 µg mL⁻¹, and 2.5-45 µg mL⁻¹ eplerenone for the methods BTB, BCG and BPB with Sandell’s sensitivity 0.040 µg cm⁻², 0.052 µg cm⁻², and 0.045 µg cm⁻² respectively. Limit of detection and limit of quantification values were evaluated and recorded as 0.229 µg mL⁻¹ and 0.6936 µg mL⁻¹ for BTB, 0.6778 µg mL⁻¹ and 2.054 µg mL⁻¹ for BCG, 0.3972 µg mL⁻¹ and 1.2037 µg mL⁻¹ for BPB. The optimum conditions obtained in these methods are summarized in Table-5.2.
The developed methods were applied for the estimation of eplerenone in bulk and tablet dosage forms. Based on molar absorptivity data and Beer’s law range, it may be concluded that among the proposed methods, EPL-BTB method is more sensitive than EPL-BPB method which in turn is more sensitive than EPL-BCG method.

Chapter-6 deals with the application of ion-association complex reactions for the determination of olopatadine (OLO) using three different acid dyes by extractive spectrophotometry. Olopatadine forms yellow colored ion-association complexes with bromothymol blue, bromocresol green and bromophenol blue. Which were showed maximum absorbance at 415 nm, 420 nm and 417 nm respectively. In these methods Beer’s law was obeyed in the concentration range 5.0-55 \( \mu g \) mL\(^{-1}\), 5.0-50 \( \mu g \) mL\(^{-1}\) and 5.0-45 \( \mu g \) mL\(^{-1}\) with molar absorptivities 4.109\( \times \)10\(^3\) L mole\(^{-1}\) cm\(^{-1}\), 4.493\( \times \)10\(^3\) L mole\(^{-1}\) cm\(^{-1}\) and 4.011\( \times \)10\(^3\) L mole\(^{-1}\) cm\(^{-1}\) and sandell’s sensitivity values 0.08 \( \mu g \) cm\(^{-2}\), 0.075 \( \mu g \) cm\(^{-2}\), and 0.084\( \mu g \) cm\(^{-2}\) respectively. Limit of determination and limit of quantification were calculated and reported as 1.825 \( \mu g \) mL\(^{-1}\) and 3.5833 \( \mu g \) mL\(^{-1}\) for BTB; 0.80 \( \mu g \) mL\(^{-1}\) and 2.4342 \( \mu g \) mL\(^{-1}\) for BCG; 0.9777 \( \mu g \) mL\(^{-1}\) and 2.9629 \( \mu g \) mL\(^{-1}\) for BPB methods respectively.

The optical characteristics such as absorption maxima, Beer’s law limits, molar absorptivity, sandell’s sensitivity, slope (m), intercept(c) and the correlation coefficient of the regression plots of the present methods are presented in Table 6.3. All the proposed methods are simple, sensitive, accurate and economical for routine analysis of olopatadine in bulk and its parenteral formulations. Based on molar absorptivity data and Beer’s law range, it may be concluded that among the proposed methods,
method OLO-BCG is more sensitive than method OLO-BTB which in turn is more sensitive than the method OLO-BPB.

In chapter-7 pemetrexed disodium forms Schiff’s base complexes with aromatic aldehydes i.e PDAC or HMBA in acidic medium and electrophilic coupling complex with MBTH in presence of ceric ammonium sulfate as oxidative reagent. The formed colored complexes showed $\lambda_{\text{max}}$ at 515 nm, 470 nm and 720 nm with molar absorptivity $1.296 \times 10^4 \text{ L mole}^{-1}\text{cm}^{-1}$, $1.511 \times 10^4 \text{ L mole}^{-1}\text{cm}^{-1}$, $8.3891 \times 10^3 \text{ L mole}^{-1}\text{cm}^{-1}$; and Sandell’s sensitivity 0.046µg cm$^{-2}$, 0.0395µg cm$^{-2}$, 0.0712µg cm$^{-2}$ respectively. Beer’s law is obeyed in the concentration range 2.5-40 µg mL$^{-1}$, 2.5-35 µg mL$^{-1}$ and 10-70 µg mL$^{-1}$ of pemetrexed disodium for methods PDAC, HMBA and MBTH respectively. Limit of detection and limit of quantification were calculated as 0.33 µg mL$^{-1}$ and 1.00 µg mL$^{-1}$ for PDAC; 0.232 µg mL$^{-1}$ and 0.703 µg mL$^{-1}$ for HMBA; 0.825 µg mL$^{-1}$ and 2.50 µg mL$^{-1}$ for MBTH methods respectively.

The optical characteristics such as absorption maxima, Beer’s law limits, molar absorptivity, sandell’s sensitivity, slope (m), intercept(c) and the correlation coefficient of the regression plots of the present methods are presented in Table 7.3.

Based on molar absorptivity data and Beer’s law range, it may be concluded that among the proposed methods, method PEM-HMBA is more sensitive than method PEM-PDAC which in turn is more sensitive than the method PEM-MBTH. The proposed methods are applied for the determination of pemetrexed disodium in its pharmaceutical formulations with good accuracy and precision.
Chapter-8 deals with the development and validation of three spectrophotometric methods for the determination of zolmitriptan (ZOL) with P-Dimethylamino cinnamaldehyde (PDAC), P-Dimethylamino benzaldehyde (PDAB) and 1,2-Napthoquinone-4-Sulphonic acid (NQS) (Folin’s reagent). The drug forms red colored chromogens based on Schiff’s base reactions with PDAC and PDAB. The formed colored complexes showed maximum absorbance at 560 nm and 540 nm with reasonably good molar absorptivities \(4.668 \times 10^3\) L mole\(^{-1}\)cm\(^{-1}\) and \(4.237 \times 10^3\) L mole\(^{-1}\)cm\(^{-1}\) respectively, obeying Beer’s law in the concentration range of 5-55 µg mL\(^{-1}\) and 10-60 µg mL\(^{-1}\) with Sandell’s sensitivity 0.0615µg cm\(^{-2}\), 0.0677µg cm\(^{-2}\) respectively. Limit of detection and limit of quantification values were calculated as 0.91 µgmL\(^{-1}\), 2.76 µg mL\(^{-1}\) for PDAC, 1.21 µg mL\(^{-1}\), 3.66 µg mL\(^{-1}\) for PDAB and 1.95 µg mL\(^{-1}\), 5.90 µg mL\(^{-1}\) for NQS methods. Zolmitriptan forms orange colored complex using Folin’s reagent in alkaline medium by derivatize reaction. The formed orange colored chromogen was quantitatively analyzed at 485 nm in the concentration range of 10-80 µg mL\(^{-1}\) with molar absorptivity of \(3.103 \times 10^3\) L mole\(^{-1}\)cm\(^{-1}\) and Sandell’s sensitivity 0.0925µg cm\(^{-2}\).

The proposed methods are simple, extraction-free, sensitive, accurate and economical for routine analysis of zolmitriptan in bulk and its pharmaceutical formulation. Based on molar absorptivity data and Beer’s law range, it may be concluded that among the proposed methods, method ZOL-PDAC is more sensitive than method ZOL-PDAB which in turn is more sensitive than the method ZOL-NQS.
Chapter IX

Chapter IX comprises the summary on the present investigations for the development of spectrophotometric methods for the determination of the selected drugs, atazanavir, emtricitabine, eplerenone, olopatadine, pemetrexed disodium, and zolmitriptan in bulk and their formulations.

All the proposed methods for the determination of various selected pharmaceutical drugs using different acid dyes, aromatic aldehydes, hydrazone with different oxidants and Folin’s reagent as chromogenic reagents are quite simple, sensitive, and cost effective methods for the accurate analysis of respective pharmaceutical formulations.