Chapter 6

Summary & Conclusion

6. Summary and Conclusion

A patient taking a pharmaceutical product expects the product to be safe and efficacious.

Due to abundance of pharmaceutical agents available in the pharmaceutical market in various dosage forms either as a single drug component or in combination with other drugs and also due to potency of the most of the drugs, it becomes necessary to quantitate these agents in their formulations in a precise manner.

Pharmaceutical regulatory agencies worldwide demand that the product retains its quality, purity, and potency for the time the product is commercially available and also expect to see the stability data supporting the proposed expiration date of the product in the marketing submission. Therefore there is always a need to develop validated analytical methods which are precise, accurate, selective, and sensitive and can be used for routine analysis and stability studies of the drug products.

In the present work validated analytical methods and stability indicating methods were developed as per ICH guidelines for different drugs in bulk and in their formulations.

Mainly validated Reverse Phase High Performance Liquid Chromatography (RP-HPLC),High Performance Thin LayerChromatography (HPTLC) methods were developed. After a thorough literature survey, it was realized that cream formulations are difficult to analyze, therefore it was decided to analyze certain cream formulations, which are mainly multicomponent formulations and for which no analytical method was reported at the time of development. Accordingly, validated HPLC methods were developed for three semisolid dosage forms namely cream formulation containing Butenafine hydrochloride 1% w/w and betamethasone dipropionate 0.05 % w/w(Butenaskin BM),cream formulation containing Hydrocortisone acetate 1%w/w and miconazole nitrate 2%w/w(Fumic) and an ointment containing Fluocinolone acetonide0.01%w/w and and miconazole nitrate 2% w/w (Zole –F). It was also found that for the combination of etizolam and escitalopram oxalate in a tablet dosage form no analytical method was reported, therefore a validated HPLC method for simultaneous estimation of this combination of drug was developed(Etizola Plus 5 tablet containing 0.5 mg of etizolam and 5 mg of escitalopram oxalate) .The developed method was further optimized and validated to study its application as a stability indicating method. It was also applied for dissolution testing of tablet containing etizolam and escitalopram oxalate.Racecadotril is one of the the drugs selected for method development as it was newly introduced in the Indian market and no analytical method

for its estimation was available in the literature at the time of development.Therefore a validated HPLC method was developed for its estimation in bulk and in oral powder dosage form. The method was used further, to determine racecadotril in presence of any degradation product due to its forced degradation.

A validated HPTLC method was developed for estimation of Butenafine hydrochloride and betamethasone dipropionate in a cream formulation. A validated HPTLC method was also developed for simultaneous determination of albendazole and ivermectin in a tablet dosage form.An attempt was made to quantitate albendazole and ivermectin by using IR spectroscopic technique. All these developed methods are summarized in the following section.

6.1 HPLC methods

Various developed HPLC methods are as follows

6.1.1 RP-HPLC Method for Simultaneous Determination of Butenafine Hydrochloride and Betamethasone Dipropionate in bulk and in a Cream Formulation.

A RP-HPLC method has been developed for the simultaneous determination of butenafine hydrochloride and betamethasone dipropionate on an Inertsil C18 column (250 ±4.6 mm id) using a mobile phase gradient consisting of methanol and water at a flow rate of 1 mL/min. Detection was carried out at 254 nm. Retention times of betamethasone dipropionate and butenafine hydrochloride were 4.82 (±0.80) and 16.18(±0.17) min, respectively. The method was validated with respect to specificity, linearity, precision, ruggedness, and robustness.

Linearity of the method was found to be more than 0.999, %RSD of precision,ruggedness and robustness was study was below 2% for both the drugs. System suitability parameters were within the limit.This method is simple, precise, and sensitive, and applicable for the simultaneous quantification of butenafine hydrochloride and betamethasone dipropionate in a cream formulation.

6.1.2 RP-HPLC method for simultaneous determination of Escitalopram Oxalate and Etizolam in bulk and in a Tablet Formulation.

An isocratic reversed phase HPLC method has been developed for the simultaneous determination of escitalopram oxalate and etizolam on a HiQ-silC18HS (250 x 4.6 mm) column using a mobile phase consisting of methanol:phosphate buffer pH-5 (70:30,v/v) at a flow rate of 1 mL/min and the detection was carried out at 254 nm. The retention times of escitalopram oxalate and etizolam were found to be 4.85 (± 0.31) min and 7.65 (± 0.56) min

respectively. The method was validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness.This method is simple, precise, and sensitive and is applicable for simultaneous quantification of escitalopram oxalate and etizolam in a tablet formulation. Linearity of the method was found to be more 0.999 .%RSD of precision,ruggedness and robustness was study was below 2% for both the drugs.System suitability parameters were within the limit and the method is simple, precise, and sensitive, and applicable for the simultaneous quantification of etizolam and escitalopram oxalate in a tablet dosage form.

6.1.3 Stability indicating RP-HPLC method for simultaneous estimation of Escitalopram Oxalate and Etizolam in a Tablet Formulation

The method developed in 1.2 was further optimized with respect to mobile phase composition to separate the drug peaks from the degradation product peaks.The optimized method was also validated with respect to specificity, linearity, accuracy, precision, ruggedness and robustness. Except the change in mobile phase composition from Methanol: Phosphate Buffer pH-5 (70:30 v/v) to Methanol: Phosphate Buffer pH-5 (62:38 v/v) all other experimental requirements were same. The retention times found to be 6.178 (±) 0.11 and 13.71± 0.10 for escitalopram oxalate and etizolam respectively.

This method is simple, precise, and sensitive and is applicable for simultaneous quantification of escitalopram oxalate and etizolam in a tablet formulation. Individual standard drugs ,their binary mixture and, tablets were forced to degrade under acidic(1N HCL)),basic(1NaOH),oxidative(3%H_2O_2),thermal, photolytic(UV),and humidity conditions.Escitalopram oxalate was found to be sensitive to basic and oxidative conditions,but on significant degradation was obtained for it in rest of the stress conditions.No significant degradation was obtained for etizolam under any of the above mentioned stress conditions. Degradation product were properly resolved from the escitalopram oxalate peak. All the validation parameters of the developed method are within the limit. Thus, this method was found to simple, accurate, precise, and sensitive and is applicable for simultaneous quantification of escitalopram oxalate and etizolam in a tablet formulation.

6.1.4 Application of developed RP HPLC method for the dissolution studies of etizolam and escitalopram oxalate in a tablet dosage form.

RP-HPLC method developed as **1.2** was applied for the dissolution studies of ETIZOLA PLUS-5 tablets containing etizolam 0.5 mg and escitalopram oxalate equivalent to 5 mg of

escitalopram.For this study the for dissolution studies of etizolam tablets described in Supplement I ,Japan Pharmacopoiea.XV was referred. This study was a single point technique and samples were drawn only once after 45 min.More than 70% of the drug release was obtained in 45 minutes.Thus,developed method can be applied successfully for the dissolution studies of etizolam and escitalopram oxalate in a tablet dosage form.

6.1.5 RP-HPLC Method for Simultaneous Determination Fluocinolone acetonide and Miconazole Nitrate in bulk and in an Ointment Formulation.

Ointment used for this method contain the fluocinolone acetonide and miconazole nitrate in the ratio of 1:200.This ratio was found to be a challenging one for method development. A gradient RP-HPLC method has been developed for the simultaneous determination of fluocinolone acetonide and miconazole nitrate on HiQ-silC18HS (250 x 4.6 mm) using a mobile phase gradient consisting of methanol and water and at a flow rate of 1 mL/min. Detection was carried out at 238 nm. Retention times of fluocinolone acetonide and miconazole nitrate were 3.52 (±032) and 6.55(±1.10) min, respectively. The method was validated with respect to accuracy, specificity, linearity, precision, ruggedness, and robustness.

This method is simple, precise, and sensitive, and applicable for the simultaneous quantification of miconazole nitrate and fluocinolone acetonide in an ointment formulation.

6.1.6 RP-HPLC method for simultaneous estimation of Hydrocortisone acetate and Miconazole nitrate in bulk and in a Cream Formulation

A RP-HPLC method has been developed for the simultaneous determination of hydrocortisone acetate and miconazole nitrate on an HiQ-sil C18HS column (250 ±4.6 mm id) using a mobile phase gradient consisting of acetonitrile and 0.1 M ammonium acetate (80:20 v/v) at a flow rate of 1 mL/min. Detection was carried out at 225 nm. Retention times of hydrocortisone acetate and miconazole nitrate were 4.05(± 0.141) and 13.14 (± 0.31) min, respectively. The method was validated with respect to accuracy,specificity, linearity, precision, ruggedness, and robustness.

Linearity of the method was found to be more than 0.999, recovery of added drugs was within 98-102 %, %RSD of precision,ruggedness and robustness was study was below 2% for both the drugs.system suitability parameters were within the limit is method is simple,accurate, precise, and sensitive, and applicable for the simultaneous quantification of hydrocortisone acetate and miconazole nitrate in a cream formulation.

6.1.7 A stability indicating validated RP-HPLC Assay Method for Racecadotril in bulk and in formulation (Oral Powder).

An isocratic reversed phase HPLC method has been developed for the quantitation of Racecadotril on a Prochrome C-18 (250x4.6mm) column using a mobile phase consisting of water: acetonitrile: glacial acetic acid (49:50:1) at a flow rate of 1.2ml/min and detection at 254nm. Retention time of Racecadotril has been found to be 15.49 min. Stress degradation studies on Racecadotril were also carried out under stress testing conditions of hydrolysis, oxidation, photolysis and thermal decomposition. The method was validated with respect to linearity, precision, accuracy, specificity and ruggedness.Racecadotril was found to be susceptible to degradation mainly in basic condition.All the validations parameters were found to be within limits prescribed as per ICH guidelines.

6.2 HPTLC METHODS

6.2.1 Simultaneous HPTLC Determination of Albendazole and Ivermectin in bulk and in tablet dosage form.

A validated HPTLC method was developed for albendazole and ivermectin in a tablet dosage form.Mobile phase composition was Toluene:Diethyl ether:Ethanol:Formic acid(03:05:0.2:0.5v/v) and determination was carried out at 254nm.Linearity,recovery,precision and ruggedness study were carried out.Linearity was found to be more than 0.999 for both the drugs ,accuracy studies revealed that recovery of added standard drugs was within the limit of 98-100%.Assay values for albendazole and ivermectin in marketed formulation were within 90%-110%.

6.2.2 Simultaneous Reverse Phase HPTLC Determination of Betamethasone dipropionate and Butenafine hydrochloride in a cream formulation

A validated reverse phase HPTLC method has been developed for betamethasone dipropionate and and butenafine hydrochloride in a cream formulation. Mobile phase composition was methanol:ammonia(9:1v/v) and determination was carried out at 254nm. Rf value for butenafine hydrochloride and betamethasone dipropionate was found to be 0.26± 1.93 and 0.645± 1.62 respectively Linearity,recovery,precision and ruggedness study were carried out. Linearity was found to be more than 0.999 for both the drugs ,accuracy studies revealed that recovery of added standard drugs was within the limit of 98-100%.Assay values for butenafine hydrochloride and betamethasone dipropionate in a marketed cream formulation were within 90%-110%w/w.

6.3 IR Method

It was found that albendazole needs mineral acid or a co solvent like chloroform :methanol(70:30) to prepare a solution. Therefore sample preparation of tablet containing albendazole and ivermectin for simultaneous estimation was difficult, as ivermectin is susceptible to degradation with mineral acids.Therefore an attempt was made to take the advantage of IR spectroscopy(ATS),which requires minimum sample preparation and determinations can be carried out in the solid state of the drugs directly, to quantitate albendazole and ivermectin in a combined tablet dosage form.But as the proportion of ivermectin to albendazole in tablet is 12:400,the ivermectin peaks were masked,due to which correlation between the area of peak or height of the peak with concentration of albendazole in the tablet could not be established.

6.4 Conclusion

As discussed above all the proposed HPLC methods and HPTLC methods were successfully developed and validated as per ICH guidelies. Also their applicability in estimation of the selected drugs in their formulations was proved.