CHAPTER 4
PREFORMULATION STUDIES

4.1 INTRODUCTION

Preformulation as a stage of development of a new formulation and characterise the physicochemical properties of drugs. All these parameters are studied prior to the formulation were carried out by Differential Scanning Calorimetry (DSC), X-ray diffractometry (XRD), and Fourier Transmission Infra Red (FTIR) Spectroscopy. Estimation of aceclofenac by Spectrophotometric method and Estimation of aceclofenac in plasma by HPLC method. When these studies are completed the results obtained are analysed and utilised for the development of the microparticle formulation.

4.1.1 DRUG EXCIPIENT COMPATIBILITY STUDIES

Drug polymer compatibility studies were carried out by Differential Scanning Calorimetry (DSC), X-ray diffractometry (XRD), and Fourier Transmission Infra Red (FTIR) Spectroscopy.

4.1.1.1 Differential Scanning Calorimetry

DSC thermograms of aceclofenac, individual polymers Ethyl cellulose, Eudragit RSPO, Aerosil and physical mixtures of aceclofenac and polymers were recorded in a Differential Scanning Calorimeter (Shimadzu, Model no: DSC-60).

Pure drug, pure excipients and physical mixtures of pure drug and excipients (1:1) were sealed in aluminum pan, and scanned between 25°C and 300°C with heating rate of 10°C per minute under an atmosphere of dry nitrogen. The thermograms obtained were observed for any interaction.

4.1.1.2 X-ray diffractometry (XRD)

X-ray diffractometry is one of crystallography characterization tools. It is employed by researchers for a wide variety of applications. It is used as an analytical tool in identification of the constituents of mixtures of crystalline phases and for the
measurement of lattice parameters (Shoo SK et.al, 2008). This phenomenon is used by the pharmaceutical industry to identify changes in the APIs (Active Pharmaceutical Ingredient), excipient, or methods that might alter the drug efficacy. XRD analysis, using a Philips PW 170 system (Philips USA) with Cu-K\textsubscript{2\alpha}\ radiation (40KV, 30 am; scan speed 1\textdegree/Min) to investigate the physical state of aceclofenac loaded in ethyl cellulose/Eudragit RSPO.

4.1.1.3. Fourier Transmission Infra Red Spectroscopy:

FTIR spectra were recorded for Aceclofenac, Ethyl cellulose, Eudragit RS and for 1:1 physical mixtures of aceclofenac and the individual polymers. Samples were prepared with KBr pellets (2 mg sample in 200 mg KBr) with a hydrostatic force of 5.2 N cm\textsuperscript{-2} for 3 minutes. The scanning range was 400 to 4000 cm\textsuperscript{-1} and the resolution was 4 cm\textsuperscript{-1}.
Figure 4.1.1 DSC Thermogram of Aceclofenac

Figure 4.1.2 DSC Thermogram of Ethyl Cellulose
Figure 4.1.3 DSC Thermogram of Eudragit RSPO

Figure 4.1.4 DSC Thermogram of Aerosil
Figure 4.1.5 DSC Thermogram of physical mixture (1:1) of Aceclofenac & Ethyl Cellulose

Figure 4.1.6. DSC Thermogram of physical mixture (1:1) of Aceclofenac and Eudragit RSPO
Figure 4.1.7. DSC Thermogram of physical mixture (1:1:1) of Aceclofenac, Ethyl Cellulose & Aerosil

Figure 4.1.8. DSC Thermogram of physical mixture (1:1:1) of Aceclofenac, Eudragit RSPO & Aerosil
4.2. Results and Discussion:

4.2.1. Differential Scanning Calorimetry

The DSC thermograms of pure drug aceclofenac, pure polymers [Ethyl Cellulose, Eudragit RSPO] and physical mixtures (1:1) of drug and individual polymers are shown in figures 4.1.1 to 4.1.8. Endothermic peak ($T_{\text{peak}}$) values of pure drug, excipients and drug-excipient mixtures are furnished in Table 4.1.

Table: 4.1: $T_{\text{peak}}$ observed in Differential Scanning Calorimetry (DSC) thermograms of aceclofenac, Polymers and mixtures of aceclofenac polymers (1:1) and of mixtures of aceclofenac polymers and aerosil (1:1:1)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>$T_{\text{peak}}$ (°C)</th>
<th>Drug</th>
<th>$T_{\text{peak}}$ (°C)</th>
<th>Polymer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aceclofenac</td>
<td>161.94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ethyl cellulose</td>
<td>70.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Aceclofenac + Ethyl cellulose (1:1)</td>
<td>155.26</td>
<td>81.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Eudragit RSPO</td>
<td></td>
<td></td>
<td>113.80</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Aceclofenac + Eudragit RSPO (1:1)</td>
<td>162.04</td>
<td>113.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Aerosil</td>
<td></td>
<td></td>
<td>&gt; 300</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Aceclofenac + Ethyl cellulose+ Aerosil</td>
<td>158.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Aceclofenac + Eudragit RSPO+aerosil</td>
<td>158.31</td>
<td>113.27</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There were no appreciable changes in peak value of drug in the DSC thermograms of drug-excipient mixtures from that of pure drug. While the DSC thermogram of aceclofenac showed a sharp endothermic peak ($T_{\text{peak}}$) at 161.94°C in the thermograms of the drug-excipient mixtures, there were no appreciable changes in the drug $T_{\text{peak}}$ values. Though there was slight broadening or shifting towards the lower or higher temperatures, the melting endotherm of drug was well preserved. Quantity of material used in drug–excipient mixtures, resulted in lowering purity of individual component, has probably affected the peak shape and enthalpy. Similar changes are reported (Madhusudhan et al.,
2010) in the literature. Hence, it may be concluded that the slight changes observed in melting endotherm of drug were likely due to presence of excipients and not due to any significant interactions between the drug and the polymers under study.

4.2.2. X-ray diffractometry (XRD)

XRD analysis was performed by the method as mentioned in section 4.1.1.2 to find out the physical composition of the aceclofenac loaded in ethyl cellulose and eudragit RSPO. XRD patterns of drug aceclofenac produced a characteristic peak when analyzed in bulk powder form. The XRD patterns of the samples are shown in Figures 4.2.1 to 4.2.5.

The XRD runs conducted for the optimized microparticles formulations prepared with polymers ethyl cellulose and eudragit RSPO showed the characteristic peaks of aceclofenac.

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**Figure 4.2.1. The X-ray diffractogram for Aceclofenac**
Figure 4.2.2: The X-ray diffractogram for Ethyl Cellulose

Figure 4.2.3: The X-ray diffractogram for the Eudragit RSPO
4.2.3. Fourier Transmission Infra Red (FTIR) Spectroscopy

The FTIR spectra of pure drug aceclofenac, pure polymers [Ethyl cellulose, Eudragit RSPO, and 1:1 physical mixtures of drug and individual polymers are shown in the figures 4.3.1 to 4.3.8. The FTIR spectrum of pure aceclofenac showed (Figure 4.3.1) the characteristic peaks at 3319.24, 2936.04., 1717.71, 1281.36 cm$^{-1}$. The major peaks are at 3319.24 for stretching vibration of -OH of -COOH group at 2936.04 for stretching vibration >N-H group at 1717.71 for stretching vibration of >C=O of ester carbonyl group, 1281.36 for C-N stretching vibration of secondary aromatic amine. FTIR spectra are helpful to the stable nature of aceclofenac in the prepared formulation.

The FTIR spectra of the physical mixtures of drug and polymers showing characteristic peak and band values of pure aceclofenac confirming that there is no shift or change in peak pattern, all the functional groups of aceclofenac were well preserved.
Results of FTIR clearly indicate absence of any chemical interaction between the drug (aceclofenac) and the polymers [Ethylcellulose, Eudragit RSPO] and thus confirming that the drug (aceclofenac) is compatible with both the polymers and thus were selected for preparation of microparticles.

4.2.4. Methods for estimation of aceclofenac

Spectrophotometric method of USP was used for estimation of acelofenac. The method is based on the measurement of absorbance at 276nm in phosphate buffer of pH 7.4. The suitability of the method for estimation of acelofenac in micrparticle formulations containing various polymers and other excipients was confirmed through validation process.

Stock solution

100 mg aceclofenac was dissolved in methanol in a 100 ml volumetric flask and the solution was made up to volume with methanol.

Dilutions:

Stock solutions of aceclofenac was subsequently diluted with phosphate buffer of pH 7.4 to obtain a series dilutions containing, 5, 10, 15, 20, and 25 µg /ml of aceclofenac solution. The absorption of these solutions was measured in UV-VIS spectrophotometer (Lambda 25, Perkin Elmer, and Germany) at 276 nm using phosphate buffer of pH 7.4 as blank. The results are given in table 4.1.2 and figure 4.4.
Table 4.2. Concentration vs Absorbance data of aceclofenac in phosphate buffer pH 7.4 at 276 nm for calibration curve by UV spectrophotometric method.

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Mean ± SD [for n=6]</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.154 ± 0.0005</td>
<td>0.35</td>
</tr>
<tr>
<td>10</td>
<td>0.301 ± 0.0020</td>
<td>0.66</td>
</tr>
<tr>
<td>15</td>
<td>0.433 ± 0.0014</td>
<td>0.33</td>
</tr>
<tr>
<td>20</td>
<td>0.594 ± 0.0043</td>
<td>0.73</td>
</tr>
<tr>
<td>25</td>
<td>0.746 ± 0.0048</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Figure 4.4 Plot of absorbance vs. concentration of aceclofenac in phosphate buffer pH 7.4 at 276 nm.

4.2.5 Validation of the Method

1. Reproducibility

Reproducibility of the above method was studied by analyzing six individually weighed samples of aceclofenac. The percentage relative standard deviations (% RSD) of the determinations were found to be less than 1.
2. Interference study of acelofenac with excipients

The interference in the above method by the excipients used in the present investigation was studied by testing their effects individually. Accurately weighed amount of acelofenac and excipients in 1:1 ratio were mixed thoroughly. From each mixture, accurately weighed powder equivalent to 100mg of acelofenac was assayed and % recovery are furnished in Table 4.3. The results indicated that none of the excipients used showed any interference and was found to be suitable for the estimation of contents used in the formulations.

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount of Aceclofenac (mg)</th>
<th>Percent Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl cellulose</td>
<td>100</td>
<td>99.91</td>
</tr>
<tr>
<td>Eudragit RSPO</td>
<td>100</td>
<td>99.95</td>
</tr>
<tr>
<td>Aerosil</td>
<td>100</td>
<td>99.60</td>
</tr>
<tr>
<td>Tween 80</td>
<td>100</td>
<td>99.98</td>
</tr>
<tr>
<td>SDS</td>
<td>100</td>
<td>99.69</td>
</tr>
</tbody>
</table>

4.3. Estimation of acelofenac in plasma

Acelofenac in plasma samples were estimated following a reported HPLC method (Mutalik. S et al, 2007) with slight modification.

4.3.1. Chromatographic conditions

The Chromatographic conditions used in the present investigation for the estimation of the acelofenac

HPLC : Shimadzu UFLC system, LC-20D
Detector : Photo Diode Array (PDA) detector, Shimadzu, SPD-W-20A.
Column : Phenomenex Luna 5µ C18 (150x4.6mm), OOF-4041-EO
Mobile Phase : 5mM sodium Phosphate buffer pH7.2: Acetonitrile :( 0.25% TEA) [buffer: acetonitril: TEA: 60:35:5].

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Flow rate : 1.0 ml/min
Pressure : 135 Kg/f
Internal Standard : Ketoprofen
Wave length : 275nm.
Sensitivity : 0.005 to 0.02 aufs

Procedure

For the estimation of aceclofenac in plasma samples, a calibration curve was constructed initially by analyzing plasma samples containing different of aceclofenac as follows:

To a series of tubes containing 0.5ml of plasma in each, 0.1ml drug solution containing 1, 2, 4, 6, 8, 10, and 20µm of aceclofenac were added and mixed. To each tube 1ml of aceclofenac was added, mixed thoroughly and centrifuged at 5000 rpm for 20 min. The organic layer (0.5ml) From each was taken into a dry tube and the acetonitrile was evaporated. To the dried residue 0.5ml of mobile phase (a mixture of 5mM sodium phosphate buffer pH 7.2, acetonitrile and Triethylamine (0.25%TEA) [60:35:5v/v] was added and mixed for reconstitution. Subsequently 20µm quantity was injected into the column for HPLC analysis.
Table 4.4. Stepwise Procedure used for estimation of aceclofenac in plasma by HPLC method.

<table>
<thead>
<tr>
<th>Step No.</th>
<th>Stepwise process</th>
<th>Volume used</th>
<th>Amount of Aceclofenac</th>
<th>Amount of Ketoprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Plasma</td>
<td>0.5 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>5mM sodium phosphate buffer (to get PH 7.2 and (TEA 0.25%)</td>
<td>1.0 ml</td>
<td>5 µg/0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>1c</td>
<td>Ketoprofen</td>
<td>0.1 ml</td>
<td>1 µg/0.1 ml</td>
<td>5 µg/0.1 ml</td>
</tr>
<tr>
<td>1d</td>
<td>aceclofenac (10 mcg/ml)</td>
<td>0.1 ml</td>
<td>1 µg/0.1 ml</td>
<td>5 µg/0.1 ml</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>1.7 ml</strong></td>
<td><strong>1 µg/0.1 ml</strong></td>
<td><strong>5 µg/0.1 ml</strong></td>
</tr>
<tr>
<td>2</td>
<td>Extract with acetonitrile</td>
<td>1.0 ml</td>
<td>1 µg</td>
<td>5 µg</td>
</tr>
<tr>
<td>3</td>
<td>Shake 5 min (in bath sonicator)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Centrifuge 20 min (5000rpm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Separate organic layer</td>
<td>0.5 ml</td>
<td>0.5 µg (or) 500ng</td>
<td>2.5 µg (or) 2500ng</td>
</tr>
<tr>
<td>6</td>
<td>Evaporate with eppendorf vacuum concentrator at 45°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Residue – Reconstitute with mobile phase</td>
<td>0.5 ml</td>
<td>0.5 µg/0.5ml</td>
<td>2.5 µg/0.5ml</td>
</tr>
<tr>
<td>8</td>
<td>Filter with 0.22 µm nylon membrane filter (syringe filter assembly)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Inject into HPLC</td>
<td>100 µl</td>
<td>0.1 µg</td>
<td>0.5 µg</td>
</tr>
<tr>
<td>10</td>
<td>Loop volume</td>
<td>20 µl</td>
<td>20ng/20 µl</td>
<td>100ng/20 µl</td>
</tr>
<tr>
<td>11</td>
<td>Mobile: Phase Phosphate buffer (pH 7.2): acetonitrile: TEA 60:35:5</td>
<td>Conc. 1.0 µg/ml</td>
<td>Conc. 5.0 µg/ml</td>
<td></td>
</tr>
</tbody>
</table>
4.3.2. Results and Discussion

The HPLC method was used for estimation of aceclofenac in plasma samples with slight modification in ratio of mobile phase components. In this method 0.05mM sodium Phosphate buffer pH 7.2: Acetonitrile: TEA (0.25%) 60:35:5 was used as mobile phase. While, 0.05M Phosphate buffer pH 7.2: Acetonitrile: TEA: 60:35:5 was used as mobile phase in the present study. The blank plasma did not exhibit any significant peaks at the retention times of aceclofenac and internal standard (ketoprofen) (Figure 4.7.1 of blank plasma) indicating that there will not be any interference of plasma peaks with drug peaks. From the HPLC chromatogram of plasma spiked with aceclofenac and ketoprofen (Figure 4.5.1), it was observed that there was good separation of ketoprofen and aceclofenac peaks with retention times of 1.9 minutes and 4.2 minutes respectively.

HPLC chromatograms obtained by injecting 1, 2, 4, 6, 8, 10 mcg/ml of aceclofenac in mobile phase are given in the figures 4.5.2 to 4.5.7.

HPLC chromatograms of Aceclofenac & Ketoprofen (internal standard) in mobilephase in concentrations of 100, 200, 400, 600, 800, 1000, 2000, 4000, 6000, 8000, 10, 000, 15,000 and 20,000 ng/ml are given in the figures 4.6.1 to 4.6.14. HPLC chromatograms of aceclofenac in plasma spiked with various concentrations of aceclofenac (100, 200, 400, 600, 800, 1000, 2000, 4000, 6000, 8000, and 10,000 ng/ml) and 5 mcg/ml of internal standard, ketoprofen are given in the figures 4.7.2 to 4.7.12.

Peak area ratios vs. Concentration of aceclofenac in spike plasma (table 4.2) were plotted (figure 4.5.1). From the plot it is evident that there is good linear relationship ($r^2 = 0.999$) between aceclofenac concentrations and peak area ratios (of aceclofenac/ketoprofen). Low % RSD values in peak area ratios (< 7 %) indicates reproducibility of the method.
Figure 4.5.1 HPLC Calibration curve obtained from plot of peak area ratios vs. concentration of aceclofenac in spike plasma.
4.2.5. SELECTED HPLC CHROMATOGRAM OF ACECLOFENAC IN MOBILE PHASE Figure 4.5.2 to 4.5.7

Figure 4.5.2 HPLC chromatogram of 1 µg/ml aceclofenac in mobile phase

Figure 4.5.3 HPLC chromatogram of 2 µg/ml aceclofenac in mobile phase
Figure 4.5.4 HPLC chromatogram of 4μg/ml aceclofenac in mobile phase

Figure 4.5.5 HPLC chromatogram of 6μg/ml aceclofenac in mobile phase
Figure 4.5.6 HPLC chromatogram of 8µg/ml aceclofenac in mobile phase

Figure 4.5.7 HPLC chromatogram of 10 µg/ml aceclofenac in mobile phase
SELECTED HPLC CHROMATOGRAM OF ACECLOFENAC & KETOPROFEN (REFERENCE STANDARD) IN MOBILE PHASE
FIGURE 4.6.1 TO 4.6.14.

Figure 4.6.1 HPLC chromatogram of 100ng/ml aceclofenac and 5 μg/ml ketoprofen in mobile phase

Figure 4.6.2 HPLC chromatogram of 200ng/ml aceclofenac and 5 μg/ml ketoprofen in Mobile phase
Figure 4.6.3. HPLC chromatogram of 400ng/ml aceclofenac and 5 μg/ml Ketoprofen in mobile phase

Figure 4.6.4. HPLC chromatogram of 500ng/ml aceclofenac and 5 μg/ml ketoprofen in mobile phase
Figure 4.6.5 HPLC chromatogram of 600ng/ml aceclofenac and 5 μg/ml ketoprofen in mobile phase

Figure 4.6.6. HPLC chromatogram of 800ng/ml aceclofenac and ketoprofen 5 μg/ml in mobile Phase
Figure 4.6.7 HPLC chromatogram of 1000 ng/ml aceclofenac and ketoprofen 5 µg/ml in mobile phase.

Figure 4.6.8. HPLC chromatogram of 2000 ng/ml aceclofenac and ketoprofen 5 µg/ml in mobile phase.
Figure 4.6.9. HPLC chromatogram of 4000ng/ml aceclofenac and ketoprofen

5 µg/ml in mobile phase

Figure 4.6.10. HPLC chromatogram of 6000ng/ml aceclofenac and ketoprofen

5 µg/ml in mobile phase
Figure 4.6.11. HPLC chromatogram of 8000ng/ml aceclofenac and ketoprofen

5 µg/ml in mobile phase

Figure 4.6.12. HPLC chromatogram of 10000ng/ml aceclofenac and ketoprofen

5 µg/ml in mobile phase
Figure 4.6.13. HPLC chromatogram of 15000ng/ml aceclofenac and ketoprofen
5 µg/ml in mobile phase

Figure 4.6.14. HPLC chromatogram of 20000ng/ml aceclofenac and ketoprofen
5 µg/ml in mobile phase
4.2.7. SELECTED HPLC CHROMATOGRAMS OF ACECLOFENAC SPIKED IN PLASMA FIGURE 4.7.1 to 4.7.12.

Figure 4.7.1. HPLC chromatogram of spiked in Blank plasma

Figure 4.7.2 HPLC chromatogram of 100ng/ml aceclofenac and ketoprofen
5 μg/mlin spiked in plasma
Figure 4.7.3 HPLC chromatogram of 200ng/ml aceclofenac and ketoprofen 5 µg/ml in spiked in plasma

Figure 4.7.4. HPLC chromatogram of 400ng/ml aceclofenac and ketoprofen 5 µg/ml in spiked in plasma
Figure 4.7.5. HPLC chromatogram of 600ng/ml aceclofenac and ketoprofen 5 μg/ml in spiked in plasma

Figure 4.7.6. HPLC chromatogram of 800ng/ml aceclofenac and ketoprofen 5 μg/ml in spiked in plasma
Figure 4.7.7. HPLC chromatogram of 1000ng/ml aceclofenac and ketoprofen
5 μg/ml in spiked in plasma

Figure 4.7.8. HPLC chromatogram of 2000ng/ml aceclofenac and ketoprofen
5 μg/ml in spiked in plasma
Figure 4.7.9. HPLC chromatogram of 4000ng/ml aceclofenac and ketoprofen
5 µg/ml in spiked in plasma

Figure 4.7.10. HPLC chromatogram of 6000ng/ml aceclofenac and ketoprofen
5 µg/ml in spiked in plasma
Figure 4.7.11. HPLC chromatogram of 8000 ng/ml aceclofenac and ketoprofen 5 µg/ml in spiked in plasma

Figure 4.7.12. HPLC chromatogram of 10,000 ng/ml aceclofenac and ketoprofen 5 µg/ml in spiked in plasma
Reference


