BIOAVAILABILITY ENHANCEMENT OF ZIPRASIDONE: OPTIMIZATION OF CARRIERS AND METHODS EMPLOYED

Murthy S N Varanasi, John S, Srikar G, Radha Madhavi B

1. Department of Pharmaceutics, University College of Pharmaceutical sciences, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur
2. Department of Pharmaceutics, Nirmala College of Pharmacy, Atmakur, Guntur

ABSTRACT

Plan: The main objective of the present research work was to enhance the dissolution rate of Ziprasidone by preparing the solid dispersions using different carriers like PVP, PEG 4000, SSG and β-Cyclodextrin.

Preface: Ziprasidone is a class-II drug according to Biopharmaceutical Classification System. It is practically insoluble in water and it has dissolution limited bioavailability. So, the present research work it is aimed to improve the dissolution rate through solid dispersion technique which further enhances the bioavailability.

Methodology: The solid dispersions were prepared at three ratios (1:1, 1:2 & 1:3) of each carrier by three different techniques viz. Physical mixtures, Kneading method and Solvent evaporation method. The characterizations of prepared solid dispersions were done by Differential Scanning Calorimetry (DSC) and they were also characterized for their drug content and in-vitro dissolution studies.

Outcome: From DSC studies it was confirmed that the drug was dispersed in the carrier at molecular level in the obtained co-evaporates. From the results of dissolution studies, it was confirmed that the solid dispersions could enhance the bioavailability of Ziprasidone.

1. INTRODUCTION

Ziprasidone (ZPR)\textsuperscript{1,2} is a psychotropic agent belonging to the chemical class of benzisoxazole derivatives and chemically 5-{2-[4-(1, 2-benzothiazol-3-yl) piperazin-1-yl] ethyl}-6-chloro-2, 3-dihydro-1H-indol-2-one. Ziprasidone was the fifth atypical antipsychotic to gain FDA approval for the treatment of schizophrenia and acute mania and mixed states associated with bipolar disorder.
Ziprasidone antipsychotic activity is likely due to a combination of its antagonistic function at D2 receptors in the mesolimbic pathways and at 5HT2A receptors in the frontal cortex. Alleviation of positive symptoms is due to antagonism at D2 receptors while relief of negative symptoms are due to 5HT2A antagonism.1, 2

1.1. Chemical Structure of Ziprasidone

Ziprasidone is a class-II drug according to Biopharmaceutical Classification System. It has poor water solubility and high permeability. Hence, dissolution is the rate limiting step for its absorption. There are many techniques that can be used to increase the dissolution rate of poorly water soluble drug there by increasing the bioavailability of the drug. Solid dispersion is one such technique which is very widely used. Solid dispersion is said to be that the dispersion containing one or more active ingredients in an inert matrix, where the active ingredients could exist in finely crystalline, solubilized, or in amorphous states. This could lead to the enhancement of dissolution rate as well as bioavailability of the drug. The wide variety of hydrophilic carriers in solid dispersions can improve the wettability, aqueous solubility and dissolution rate of the poorly water soluble drugs.3-4

The main objective of this work is to improve the dissolution rate of Ziprasidone by preparing the solid dispersions using different carriers like PVP, SSG, PEG 4000 and β- Cyclodextrin so as to enhance bioavailability.

2. MATERIALS AND METHODS

2.1. Materials

Ziprasidone was obtained as a gift sample from P.V.S. Laboratories Ltd. A.P, India; PEG 4000, PVP, SSG and β- Cyclodextrin used were purchased from Merck Specialities, Mumbai; all other materials used were of analytical grade.

2.1.2. Methods

Solid dispersions were prepared by three methods at three different ratios. The formulation codes and their ratios were given in Table 1.


Physical mixtures were prepared by mixing ZPR and carrier in a glass mortar for ten minutes. The resulting mixture was sieved through # 100 and then stored in a desiccator at room temperature until further use.


ZPR and the carrier were taken in a glass mortar and were mixed thoroughly in presence of a small quantity of water until a fine mixture was obtained. The resulting mixture was sieved through # 100 and then stored in a desiccator at room temperature until further use.
2.1.5. Solvent evaporation method \(^7\text{-}^{13}\):

The solid dispersions of ZPR were prepared as co – evaporates. In this technique, first the carrier was dissolved in methanol followed by dissolving the drug homogenously. Then the mixture was subjected to controlled evaporation at 50\(^\circ\)C until the complete evaporation of methanol. Then the obtained material was collected and sieved through # 100 and then stored in a desiccator at room temperature until further use.

Table 1: Formulations and their Codes

<table>
<thead>
<tr>
<th>Drug : Polymer</th>
<th>Physical mixing</th>
<th>Kneading method</th>
<th>Solvent-evaporation method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug : PEG4000</td>
<td>1:1 (PPE1)</td>
<td>1:1 (KPE1)</td>
<td>1:1 (SPE1)</td>
</tr>
<tr>
<td></td>
<td>1:2 (PPE2)</td>
<td>1:2 (KPE2)</td>
<td>1:2 (SPE2)</td>
</tr>
<tr>
<td></td>
<td>1:3 (PPE3)</td>
<td>1:3 (KPE3)</td>
<td>1:3 (SPE3)</td>
</tr>
<tr>
<td>Drug : PVP</td>
<td>1:1 (PPV1)</td>
<td>1:1 (KPV1)</td>
<td>1:1 (SPV1)</td>
</tr>
<tr>
<td></td>
<td>1:2 (PPV2)</td>
<td>1:2 (KPV2)</td>
<td>1:2 (SPV2)</td>
</tr>
<tr>
<td></td>
<td>1:3 (PPV3)</td>
<td>1:3 (KPV3)</td>
<td>1:3 (SPV3)</td>
</tr>
<tr>
<td>Drug : β-CD</td>
<td>1:1 (PCD1)</td>
<td>1:1 (KCD1)</td>
<td>1:1 (SCD1)</td>
</tr>
<tr>
<td></td>
<td>1:2 (PCD2)</td>
<td>1:2 (KCD2)</td>
<td>1:2 (SCD2)</td>
</tr>
<tr>
<td></td>
<td>1:3 (PCD3)</td>
<td>1:3 (KCD3)</td>
<td>1:3 (SCD3)</td>
</tr>
<tr>
<td>Drug : SSG</td>
<td>1:1 (PSG1)</td>
<td>1:1 (KSG1)</td>
<td>1:1 (SSG1)</td>
</tr>
<tr>
<td></td>
<td>1:2 (PSG2)</td>
<td>1:2 (KSG2)</td>
<td>1:2 (SSG2)</td>
</tr>
<tr>
<td></td>
<td>1:3 (PSG3)</td>
<td>1:3 (KSG3)</td>
<td>1:3 (SSG3)</td>
</tr>
</tbody>
</table>

2.2. Characterization studies

a) Solubility Determination \(^7\text{-}^{13}\):

For the determination of solubility of ZPR, excess amount was placed in 10 ml of water in sealed glass tube. The tubes were shaken on an orbital shaker and were maintained at 25 \(^\circ\)C for 24 hours. The saturated solution was centrifuged and the supernatant was filtered through 0.45-μm Whatmann filter paper. Then the filtrate was diluted suitably with water and analyzed by UV spectrophotometer at 319 nm (UV-Visible spectrophotometer, Thermo Scientific).

b) Analytical Method

Weighed accurately 100mg of ZPR pure drug and was dissolved in 100 mL of methanol (stock solution) (1000μg/mL). 10mL of solution was taken and made up to 100 mL with pH 7.5 phosphate buffer containing 2% SLS to obtain (100μg/mL). This solution was subsequently diluted with the same buffer to obtain series of dilutions containing 10, 20, 30, 40, 50 and 60μg/ml of ZPR. The absorbance of the above dilutions were measured at 319 nm by using UV-Spectrophotometer taking 7.5 phosphate buffer containing 2% SLS as blank.

c) Compatibility studies by Infrared spectroscopy \(^7\text{-}^{13}\)

Fourier transform infra-red (FTIR) spectra of the pure drug and the physical mixtures were obtained by using Shimadzu FTIR-281 spectrophotometer. The samples were previously ground and mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:100 (sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 10 tons in a hydraulic press. The scanning range was 400-4000 cm\(^{-1}\) and the resolution was 1 cm\(^{-1}\).
d) Evaluation of solid dispersions

i) DSC Studies\textsuperscript{7,13}

Differential Scanning Calorimeter (DSC) thermograms of pure Ziprasidone and other solid dispersions were measured using differential scanning calorimeter (DSC 60, Shimadzu) previously calibrated using indium. The samples of 2 to 3 mg were accurately weighed into solid aluminium pans with seals and crimped. Reference pan was an empty sealed aluminium pan. The measurements were obtained at a heating rate of 10°C/min with purging of dry nitrogen at a constant rate of 20 mL/min.

ii) Drug content Analysis\textsuperscript{7,13}

Drug content was determined by dissolving solid dispersion equivalent to 5mg of the ZPR in small quantity of methanol and kept in vertex mixture for 10min. The volume was adjusted to 50mL with 2% SLS in phosphate buffer pH 7.5. The solution was filtered through Whatmann filter paper, suitably diluted and the absorbance was measured at 319nm using UV-Visible spectrophotometer.

iii) In-vitro dissolution studies\textsuperscript{2,7-13}

In-vitro dissolution studies of ziprasidone in pure drug form, solid dispersions were performed by using the 8 stage dissolution rate test apparatus (Lab India, DISSO 2000) with paddle at 50 rpm in 900 mL of distilled water. The dissolution rate was studied by placing ziprasidone pure drug and solid dispersions equivalent to 100 mg of drug.

A 5 mL aliquot was withdrawn at different time intervals, filtered (through 0.45μ) and replaced with 5 ml of fresh dissolution medium. The samples were estimated for dissolved ziprasidone by measuring absorbance at 319nm. The obtained results of the dissolution studies were subjected to kinetic analysis for better correlation.

iv) Dissolution efficiency\textsuperscript{7,13}

The dissolution efficiency (DE) of pure drug & various solid dispersions were calculated. DE is used as one of the criteria for comparing the effect of carriers and their concentrations on the dissolution rate.

Dissolution efficiency equation is defined as the area under the dissolution curve up to the time t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time.

\[
DE = \frac{\int_0^T Y \, dt}{Y_{100} \times T} \times 100\%
\]

Where Y is the percent drug release as the function of time t. T is the total time of drug release and Y 100 is 100% drug release.
v) *Flow properties*

Various flow properties like angle of repose, Carr’s index and Hausner’s ratio were determined for the pure ZPR and their selected solid dispersions.

### 3. RESULTS AND DISCUSSION

#### 3.1. Solubility determination:

The results of the solubility studies indicated that pure ZPR has very low solubility in water at 25°C i.e., 0.65µg/ml. So, this drug has dissolution limited bioavailability problems which indicated there is a need to enhance the dissolution rate to improve its bioavailability.

#### 3.2. Analytical Method:

The standard calibration curve of ZPR was constructed in pH 7.5 phosphate buffer containing 2% SLS. The results indicated that, it showed Beer’s limit within 10 – 60 µg/mL with a linearity of 0.999 (regression coefficient). The standard calibration curve was shown in Fig-2.

#### 3.3. FT-IR studies:

The obtained spectra, Fig-3 of pure drug and its solid dispersions were compared. No significant shifts in characteristic peaks of drug were observed in spectra of solid dispersion when compared with that of the pure drug. So that it was confirmed that there was no interactions between drug and carrier.

#### 3.4. DSC studies:

DSC thermograms of pure drug and its solid dispersions prepared from solvent evaporation method were shown in the Fig-4. The endotherm of the drug in its pure state was found to be at 316.7 °C, but in case of the solid dispersions with PEG-4000, PVP, β-CD and SSG, it was observed at 315.9, 288.9, 267.2 and 272.8 °C respectively. So this significant decrease in temperature of the peaks was attributed to the molecular dispersion that was taken place between the drug and carriers. So the results of the DSC studies indicated that the selected carriers were proved to be efficient for the enhancement of bioavailability.

#### 3.5. Drug content analysis:

The prepared Ziprasidone solid dispersions were tested for drug content and it was found that the drug was within the compendia limits 98-102%. All the solid dispersions were uniform in drug content. The results were shown in Table 3.
3.6. *In-vitro* dissolution studies:

The parameters of *in-vitro* drug release studies of pure ziprasidone and the prepared solid dispersions were shown in the Fig. 5. *In-vitro* drug release studies reveal that there is marked increase in dissolution rate of ziprasidone from all the solid dispersions when compared to pure ziprasidone itself.

In all the cases, the dissolution rate was found to be increased upon increase in the concentration of the carrier. The increase in dissolution rate is discussed here in two ways i.e. according to the method and according to the carrier.

3.7. Influence of method:

The four different carriers showed different orders in the increase of dissolution rate in different methods (shown in fig 6(a)). In kneading and physical mixing methods, the order of efficiency of carriers were found to be is in the order of SSG > PEG-4000 > PVP > β-CD. This might be due to the high efficiency of SSG and PEG-4000 for water to be penetrated into them and also, as there is no molecular interaction in these two methods, PVP and β-CD were found to be less effective. But in solvent evaporation method, the possible molecular interactions resulted higher dissolution rates in case of β-CD and PVP, and the order of efficiency of the carriers were found to be β-CD > PVP > PEG-4000 > SSG.

3.8. Influence of carrier:

The three different methods employed showed different order of efficiency with different carriers (shown in fig 6(b)). In case of β-CD, PVP and PEG-4000, the solvent evaporation method showed highest dissolution rates than the remaining methods and the order was found to be Solvent evaporation > Kneading > Physical mixing. But in case of SSG, the kneading method was found to be more effective than the other methods and the order was found to be Kneading > Physical mixing > Solvent evaporation. This might be because of the greater water uptake characteristics of SSG in particulate form in modified molecular arrangement form.

3.9. Dissolution Efficiency:

Dissolution efficiency is used as the criterion for comparing the effect of nature and concentration of carrier, and method on the release rate. The results were shown in Table 4.

3.10. Micromeritic properties:

The flow properties for the solid dispersions shown highest dissolution rate in each method were performed and the results were shown in Table 5. These results indicated that the solid dispersions were found to have better flowability and compressibility, which further indicated that these solid dispersions could be either compressed or filled into tablet or capsule respectively.
4. CONCLUSION

Dissolution is the rate limiting step for poorly water soluble drugs. Ziprasidone is one such drug. The use of Solid dispersion technique has increased the dissolution rate of the drug by 30-65%. The solid dispersions of Ziprasidone were successfully formulated by different methods like physical mixing, kneading and solvent evaporation methods among which solvent evaporation method was found to be better method for improving solubility of poorly soluble ziprasidone.

In-vitro release studies reveal that there is marked increase in the dissolution rate of ziprasidone from all the solid dispersions when compared to the pure ziprasidone itself. Upon comparing the dissolution rates from different methods with different carriers, the order of effectiveness of the carriers was found to be β-CD>PVP>PEG-4000>SSG and that of the methods was found to be Solvent evaporation>Kneading>Physical mixing. Thus, if we formulate poorly water soluble drugs like Ziprasidone as solid dispersions, the dissolution rate of drug can be increased markedly and hence higher bioavailability can be achieved.

Figure 2: Standard Calibration Curve of Ziprasidone

Figure 3(a): FTIR Spectra of Ziprasidone
Figure 3(b): FTIR Spectra of Ziprasidone + β-CD co-evaporates

Figure 3(c): FTIR Spectra of Ziprasidone + PEG4000 co-evaporates

Figure 3(d): FTIR Spectra of Ziprasidone + PVP co-evaporates
Bioavailability enhancement of ziprasidone: optimization of carriers and methods employed

Figure 3(e): FTIR Spectra of Ziprasidone + SSG co-evaporates

Figure 4(a): DSC thermo gram of Ziprasidone (Pure)

Figure 4(b): DSC thermo gram of Ziprasidone + β-Cyclodextrin
Figure 4(c): DSC thermo gram of Ziprasidone + PEG-4000 co-evaporates

Figure 4(d): DSC thermo gram of Ziprasidone + PVP co-evaporates

Figure 4(e): DSC thermo gram of Ziprasidone + SSG co-evaporates
Bioavailability enhancement of ziprasidone: optimization of carriers and methods employed

Figure 5(a): Drug release profiles of solid dispersions with PEG-4000

Figure 5(b): Drug release profiles of solid dispersions with PVP

Figure 5(c): Drug release profiles of solid dispersions with β-CD

Figure 5(d): Drug release profiles of solid dispersions with SSG
Figure 6(a): Comparison of % drug dissolved with different carriers w. r. t. method employed

Figure 6(b): Comparison of % drug dissolved in different methods w. r. t. carrier employed

Table 2: FTIR Interpretation – Characteristic peaks

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Functional Group</th>
<th>Wave No. of characteristic peak</th>
<th>Pure Drug</th>
<th>With PVP</th>
<th>With PEG4000</th>
<th>With SSG</th>
<th>With β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>N-H stretching</td>
<td></td>
<td>3421.81</td>
<td>3420.56</td>
<td>3420.21</td>
<td>3420.17</td>
<td>3419.44</td>
</tr>
<tr>
<td>2.</td>
<td>Ketone C=O stretching</td>
<td></td>
<td>1713.88</td>
<td>1714.70</td>
<td>1713.87</td>
<td>1713.72</td>
<td>1714.28</td>
</tr>
<tr>
<td>3.</td>
<td>C-Cl stretching</td>
<td></td>
<td>744.9</td>
<td>743.53</td>
<td>743.72</td>
<td>745.20</td>
<td>744.82</td>
</tr>
</tbody>
</table>

Table 3: Results of Drug content

<table>
<thead>
<tr>
<th>Formn Code</th>
<th>Drug Content</th>
<th>Formn Code</th>
<th>Drug Content</th>
<th>Formn Code</th>
<th>Drug Content</th>
<th>Formn Code</th>
<th>Drug Content</th>
<th>Formn Code</th>
<th>Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPE1</td>
<td>99.21±0.6</td>
<td>PPV1</td>
<td>99.12±0.8</td>
<td>PCD1</td>
<td>97.31±0.3</td>
<td>PSG1</td>
<td>99.01±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPE2</td>
<td>98.23±0.7</td>
<td>PPV2</td>
<td>99.23±0.3</td>
<td>PCD2</td>
<td>99.83±0.1</td>
<td>PSG2</td>
<td>99.11±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPE3</td>
<td>99.61±0.4</td>
<td>PPV3</td>
<td>99.11±0.7</td>
<td>PCD3</td>
<td>97.91±0.6</td>
<td>PSG3</td>
<td>97.81±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE1</td>
<td>99.32±0.5</td>
<td>KPV1</td>
<td>99.42±0.3</td>
<td>KCD1</td>
<td>99.91±0.2</td>
<td>KSG1</td>
<td>98.62±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE2</td>
<td>99.51±0.1</td>
<td>KPV2</td>
<td>98.11±0.1</td>
<td>KCD2</td>
<td>99.11±0.7</td>
<td>KSG2</td>
<td>96.93±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE3</td>
<td>100.3±0.5</td>
<td>KPV3</td>
<td>99.21±0.2</td>
<td>KCD3</td>
<td>98.81±0.1</td>
<td>KSG3</td>
<td>99.03±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE1</td>
<td>99.61±0.2</td>
<td>SPV1</td>
<td>99.91±0.8</td>
<td>SCD1</td>
<td>99.71±0.4</td>
<td>SSG1</td>
<td>98.62±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE2</td>
<td>98.62±0.2</td>
<td>SPV2</td>
<td>98.32±0.2</td>
<td>SCD2</td>
<td>96.91±0.8</td>
<td>SSG2</td>
<td>99.11±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE3</td>
<td>98.84±0.5</td>
<td>SPV3</td>
<td>98.6±0.2</td>
<td>SCD3</td>
<td>100.5±0.9</td>
<td>SSG3</td>
<td>96.11±0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD (n=3)
Table 4: Results of Dissolution Efficiency

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>PPE1</td>
<td>PPV1</td>
<td>25.8±0.5</td>
<td>PCG1</td>
<td>28.5±0.4</td>
<td>PSG1</td>
<td>16.0±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPE2</td>
<td>PPV2</td>
<td>32.5±0.3</td>
<td>PCG2</td>
<td>31.4±0.4</td>
<td>PSG2</td>
<td>17.7±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPE3</td>
<td>PPV3</td>
<td>33.4±0.4</td>
<td>PCG3</td>
<td>34.0±0.15</td>
<td>PSG3</td>
<td>21.3±0.2#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE1</td>
<td>KPV1</td>
<td>36.2±0.3</td>
<td>KCD1</td>
<td>32.2±0.43</td>
<td>KSG1</td>
<td>30.5±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE2</td>
<td>KPV2</td>
<td>38.5±0.6</td>
<td>KCD2</td>
<td>38.9±0.45</td>
<td>KSG2</td>
<td>44.1±0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KPE3</td>
<td>KPV3</td>
<td>45.9±0.3</td>
<td>KCD3</td>
<td>44.4±0.45</td>
<td>KSG3</td>
<td>53.0±0.1*#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE1</td>
<td>SPV1</td>
<td>40.8±0.4</td>
<td>SCD1</td>
<td>45.1±0.32</td>
<td>SSG1</td>
<td>30.5±0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE2</td>
<td>SPV2</td>
<td>44.1±0.7</td>
<td>SCD2</td>
<td>55.3±0.35</td>
<td>SSG2</td>
<td>38.2±0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPE3</td>
<td>SPV3</td>
<td>48.6±0.1*</td>
<td>SCD3</td>
<td>62.9±0.9*#</td>
<td>SSG3</td>
<td>42.5±0.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results were expressed as mean ± SD (n=3), one way ANOVA followed by tukuy's post hoc test, *p<0.05, the studied four different carriers were found to statistically different, # p<0.05, the studied three methods were found to be statistically different

Table 5: Micromeritic properties of pure drug and optimized solid dispersions

<table>
<thead>
<tr>
<th>Trails</th>
<th>Angle of repose</th>
<th>Bulk Density</th>
<th>Tapped Density</th>
<th>Compressibility Index</th>
<th>Hausner’s Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>34.46</td>
<td>0.333</td>
<td>0.500</td>
<td>33.4</td>
<td>1.515</td>
</tr>
<tr>
<td>SCD3</td>
<td>24.34</td>
<td>0.612</td>
<td>0.746</td>
<td>17.96</td>
<td>1.212</td>
</tr>
<tr>
<td>KSG3</td>
<td>22.57</td>
<td>0.575</td>
<td>0.687</td>
<td>16.30</td>
<td>1.194</td>
</tr>
<tr>
<td>PSG3</td>
<td>21.64</td>
<td>0.559</td>
<td>0.658</td>
<td>15.04</td>
<td>1.177</td>
</tr>
</tbody>
</table>

ACKNOWLEDGEMENT

The authors wish to thank to the management of University College of Pharmaceutical Sciences, Acharya Nagarjuna University and Nirmala College of Pharmacy for their constant support and encouragement.

REFERENCES