STUDY OF FORMULATION VARIABLES ON BIOAVAILABILITY OF METFORMIN HYDROCHLORIDE

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ABSTRACT
The present study concentrates on the improving bioavailability of Metformin HCl which is a BCS-III drug. An attempt was made to enhance the intestinal permeability of Metformin by forming solid dispersions using β–CD, Pluronic F 127 and Gelucire 50/13. The optimize solid dispersion along with dependent and independent variables like Gelucire 50/13, Pluronic F 127, which are waxy non –ionic surfactants as lubricant as well as absorption enhancer with soluble and insoluble diluents were used to formulate the formulation using design expert 9. Twenty three formulations of immediate release tablet were prepared by using steam granulation method. All the formulation was evaluated for Content uniformity, Permeability coefficient, Hardness, Friability and Disintegration Time. Of which F8 formulation was optimized as it shows high permeability than other formulations. This F8 formulation on comparison with Marketed formulation showed more in-vivo bioavailability. Thus from this study it can be concluded that use of waxy non –ionic surfactants along with β-cyclodextrin played a significant role in improving the permeability and thus bioavailability of Metformin Hydrochloride.

KEYWORDS: Metformin Hydrochloride, Permeability Coefficient, immediate release, isolated goat intestine.

INTRODUCTION
Oral route is most popular for systemic effect due to its easy of ingestion, pain, avoidance, versatility and most importantly, patient compliance. Immediate Release Tablets are those tablets which are designed to disintegrate and release their medication with no special rate controlling features, such as special coatings and other techniques. The main aim in the process of drug development is to obtain a drug product with a good oral bioavailability.

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood sugar. Hyperglycemia, or raised blood sugar, is a common effect of uncontrolled diabetes and over time leads to serious damage to many of the body's systems, especially the nerves and blood vessels.

The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014. The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation.

In 2014, 8.5% of adults aged 18 years and older had diabetes. In 2012 diabetes was the direct cause of 1.5 million deaths and high blood glucose was the cause of another 2.2 million deaths.

Metformin hydrochloride (HCl) is a first line drug for Diabetes Mellitus but having low bioavailability. It is BCS class III drug, having high solubility and low permeability through intestinal mucosa, so it is necessary to increase the permeability of the drug and thus bioavailability. Metformin HCl to enhance its absorption by oral ingestion is the most convenient and commonly used route of drug delivery. Metformin HCl acts by the initial activation of AMPK a liver enzyme that plays an important role in insulin signaling, whole body energy balance and the metabolism of glucose and fats. This causes the increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors. Metformin HCl administration also increases AMPK activity in skeletal muscle. AMPK is also known to cause Glucose transporter type 4(GLUT4) deployments to the plasma membrane, resulting in insulin-independent glucose uptake.

Other antidiabetic agents lack efficacy and also have undesirable side effects. For instance, Insulin secretagogues result in weight gain, hypoglycemia and inability to protect β cells from death. Thiazolidinediones result in weight gain and kidney
toxicity. Acarbose causes gastrointestinal upset diarrhea and flatulence of Sglt clinical trial recently failed due to safety concerns. Incretin based drugs causes gastrointestinal problems such as sour stomach, belching, nausea, vomiting, indigestion and diarrhea. Insulin cannot match the natural precise timing and dosing of insulin secretion from the pancreas in response to hyperglycemia, resulting in severe complications.

Metformin having multiple uses, with few side effects. Accordingly, the drug's numerous side benefits associated with the treatment and prevention of diabetes, as well as other disorders, appear to outweigh its limited side effects.\(^{(5)}\)

All the BCS Class-III drugs show high solubility and low permeability. Due to low permeability they have less intestinal absorption. The present study concentrates on the improving bioavailability of Metformin HCl which is a BCS-III drug.

**MATERIALS AND METHOD**

Metformin Hydrochloride was obtained as gift sample from Alkem Laboratories, India, \(\beta\)-cyclodextrin was obtained as gift sample from Emcure Pharmaceuticals Pvt. Ltd., Pune while Gelucire 50/13 and Pluronic F-127 was obtained from Gattifosse India Pvt. Ltd, Mumbai. Microcrystalline cellulose and Spray Dried Lactose was purchased from Research-Lab Fine Chem, Mumbai.

**Preparation of Solid Dispersion**\(^{(6)}\)

Metformin HCl with \(\beta\)-cd, Gelucire50/13 and Pluronic F-127 in different ratios were prepared by the solvent method. The drug and carriers were dissolved in ethanol (10% \(w/v\)) and the solvent was subsequently evaporated at room temperature while stirring. The products were then stored in an oven at 40°C for 24 h to ensure complete ethanol evaporation, followed by pulverization with a mortar and pestle. These Solid dispersions were used for further study of permeability enhancement.

**Evaluation of Permeability with diffusion studies using goat intestinal membrane**

The prepared solid dispersions was studied for permeability by using Franz- diffusion cell technique. The fresh goat –Intestine was taken as a membrane and 0.1 NHCl was used as diffusion Medium. The permeability coefficient was determined for further selection of proportion of permeability enhancers for final tablet formulations.\(^{(7)}\)

\[
\text{Permeability coefficient } = \text{Kp} = \frac{Q}{(A*t*(C_0-C_I))}
\]

where \(Q\) = the quantity of compound transported through the membrane in time \(t\) (min), \(C_0\) and \(C_I\) = the concentrations of the compound on the outer side (donor side) and the inner side (receptor side) of the membrane respectively. \(A\) = the area of exposed membrane in cm\(^2\). Usually \(C_0\) can be simplified as the donor concentration and \(C_I\) as 0.

The units of Kp are cm/min or cm/hr.

**Formulation of granules by steam granulation method**

All the ingredients were accurate weighed and was sifted through sieve and mixed in blender keeping 12±1 rpm for 10 minutes. The dry mix was wetted using steam from steam generator. The damp mass was passed through 24# sieve. \(^{(10,11,12)}\) All prepared granules were dried at room temperature. The dried granule was sifted through 20# sieve, and then it was compressed using rotary tablet press. Optimization was done using design expert software9.0. The Box-Behenken design gave 23 runs for the formulation (table 1 and 2).

**Table 1: Variables of the formulation**

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Level</th>
<th>+1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.of Absorbtion enhancer (X1)</td>
<td>10.00</td>
<td>15.00</td>
</tr>
<tr>
<td>Conc.of Diluent (X2)</td>
<td>35.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Type of absorption enhancer (X3)</td>
<td>Pluronic F 127</td>
<td>Gelucire50/13</td>
</tr>
<tr>
<td>Type of Diluents(X4)</td>
<td>MCC</td>
<td>Spray dried lactose</td>
</tr>
<tr>
<td>Dependant variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y1</td>
<td>Content uniformity</td>
<td></td>
</tr>
<tr>
<td>Y2</td>
<td>Permeability coefficient</td>
<td></td>
</tr>
<tr>
<td>Y3</td>
<td>Hardness</td>
<td></td>
</tr>
<tr>
<td>Y4</td>
<td>Friability</td>
<td></td>
</tr>
<tr>
<td>Y5</td>
<td>Disintegration time in minutes</td>
<td></td>
</tr>
</tbody>
</table>

*Solid dispersion of Metformin equivalent to 250mg was used in all formulations.*

**Table 2: Formulation table of Immediate release tablet as Design of expert**

<table>
<thead>
<tr>
<th>Run</th>
<th>A:Conc.of Absorbtion enhancer (mg)</th>
<th>B:Conc.of Diluents(mg)</th>
<th>C:Type of absorption enhancer</th>
<th>D:Type of diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.593</td>
<td>43.463</td>
<td>Pluronic 127</td>
<td>MCC</td>
</tr>
<tr>
<td>2</td>
<td>15.000</td>
<td>36.827</td>
<td>Gelucire</td>
<td>MCC</td>
</tr>
<tr>
<td>3</td>
<td>11.921</td>
<td>50.000</td>
<td>Pluronic 127</td>
<td>Spray dried lactose</td>
</tr>
</tbody>
</table>
EVALUATION OF IMMEDIATE RELEASE TABLETS

Pre-compression Parameters
The prepared granules were evaluated for Bulk density, tapped density cars index, Hausners ratio, angle of repose.\(^{[13,14]}\)

Post – Compression Parameters
The finished tablets were tested for Hardness, Friability, Disintegration time, Drug content, Permeability coefficient as per following standard procedure.

Hardness
The hardness was determined for 10 tablets of each batch by using Monsanto tablet hardness tester. The average was determined.

Friability
Twenty tablets were weighed and placed in the Electrolab friabilator and the apparatus was rotated at 25 rpm for 4 minutes. After revolutions the tablets were deducted and weighed again. The percentage friability was measured using the formula,
\[
\% F = \frac{1-(Wt/W)}{Wt} \times 100
\]
Where, \( \% F \) = friability in percentage
\( W \) = Initial weight of the tablet
\( Wt \) = weight of tablets after revolution.

Disintegration time
Disintegration is evaluated to ensure that the drug substance is fully available for dissolution and absorption from the gastrointestinal tract. The disintegration test was done on 6 units using the apparatus described in United States pharmacopoeia method.

Drug content
The assay of Metformin HCl in tablets was estimated by UV method.

Sample preparation– 20 Tablets were weighed and finely powdered. About 10 mg equivalent of Metformin HCl was transferred in to 100 ml volumetric flask added with about 10 ml of methanol and sonicated it for 10 min and the volume was made up with distilled water. The solution was filtered and diluted1 ml of filtrate to 100 ml with distilled water and subjected for UV detection. Absorbance of sample and standard preparation were measured at 233±2 nm using distilled water as blank.

In-vitro permeability studies\(^{[15]}\)

Isolation of goat intestine
The Krebs-Ringer solution was prepared by adding 6.3g NaCl, 0.35g KCl, 0.14 g CaCl2, 0.16 g KH2PO4, 0.15 g MgSO4.7H2O, 2.1g NaHCO3 and 5g glucose to one liter of distilled water. The fresh goat intestinal lumen was carefully cleared from mucus by rinsing with a pH 7.4 buffer solutions.

An intestinal segment of the first 6–cm length was removed and transferred to oxygenated Krebs-Ringer solution. The proximal extremity of the intestine was turned back and ligated on a glass rod to form an everted bag.

Design of simultaneous Dissolution–Absorption System Using goat Intestine Segment
The in vitro continuous dissolution–absorption system design is illustrated in Figure.no.1. The system consisted of USP dissolution Apparatus 1 and a side-by-side perfusion apparatus holding isolated everted intestine segment.

The dissolution medium used was 9000 mL of 0.1 N HCl maintained at 37 ± 0.5°C. The perfusion apparatus consisted of two glass tubes, A and B, connected together. Tube B had a bent cannula at its lower end and tube A, a straight cannula at its lower end. The distance between the two cannula was kept constant. The fresh isolated intestinal segment was fixed between the ends of
tubes A and B as shown in the Figure no.1. The ends of the intestine were tied in position with a thread. The apparatus was immersed completely into the dissolution vessel. The total volume of the absorption compartment (tube A and tube B of perfusion apparatus) was 35 ml of Krebs–Ringer solution. The drug diffused from dissolution compartment to the absorption compartment. The tablet was transferred to the dissolution basket of the designed system. The tablet was rotated at 75 rpm speed. The drug was transported from dissolution compartment to absorption compartment. The transported drug from the absorption compartment was sampled with replacement (Krebs–Ringer solution) at 10min., 20min, 30min,1 hr.,1.5hrs, 2hrs and analyzed spectrophotometrically for transported Metformin HCl at 232 nm. The same procedure was preceeded for all the formulation also.

**Formula for calculation of permeability coefficient**

\[ P \text{ (cm/sec)} = \frac{Dq/dt}{A \times C_0} \]

\[ Dq/dt=\text{Amount of drug traversing the tissue in time t} \]

\[ A=\text{Exposed area of tissue} \]

\[ C_0=\text{Initial concentration of drug in donar compartment} \]

*In -vivo bioavailability study* \(^{22,23}\)

The pharmacokinetic interaction of optimized Formulated Metformin HCl tablet with Marketed Tablet was studied in normal Wistar Albino rats.

Blood samples were analyzed using a validated HPLC method to generate the pharmacokinetic profile of metformin HCl. The C max and T max, AUC and t ½ were calculated from the concentration–time data.

**Selection of Animals**

Wistar Albino rats (200–280 g) of either sex were acclimatized in the animal room at least 1 day prior to the commencement of the study and were maintained on standard food and water *ad libitum*. The study protocols were prepared according to the guidelines specified by the local animal ethics committee.

**Preparation of the formulation**

The rat dose of metformin was calculated from the standard clinical human dose on the basis of surface area [mice dose =\( (\text{human dose/average body weight}) \times 7) \)].

The Formulated tablet and Standard Marketed tablet was weighed accurately equivalent to the 2.5 mg of metformin. HCl. Then it was dissolved in 2.5 ml triple-distilled water to obtain a strength of 1 mg/ml.

**Oral administration**

Eighteen rats were divided in three groups of 6 each. The overnight fasted rats received a single 10 mg/kg oral dose of metformin HCl using a rat feeding needle and syringe. The blood samples were withdrawn from each rat, through periorbital, puncture (~1.2 ml). Blood samples from the dosed rats were collected at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4 h using a heparinized syringe. It is collected in Standard EDTA Tube.

**Analytical procedures**

The HPLC method was developed and validated to determine the concentration of metformin HCl in rat plasma. Each 1.2 ml of blood sample was centrifuged for 30 minutes and plasma was separated for further analysis.

Then 100 μl of plasma was mixed with 200 μl of acetonitrile and then vortexed for 1 min. Further, these samples were allowed to stand for 30 min and then centrifugated at 1000 rpm for 10 min. 20 μL supernatant was injected onto the HPLC system. The HPLC system
was equipped with double reciprocating pump, Rheodyne injector fitted with a fixed 20μL loop, UV-VIS multiple wavelength detector set at 233 and Ezochrome, software on personal computer. Separation was obtained on a C-18 column. Maintaining optimized conditions as below: Flow rate: 1.0 ml per min, Detector wave length: 233 nm, Column temperature: 25°C, Injection volume: 20 μl, Run time: 10.0 min, Diluent: Water, Mobile phase: 30:70:1%:1% (water: acetonitrile: orthophosphoric acid: formic acid) The retention time was found to be 3.24min.

RESULTS AND DISCUSSION
Preformation studies were performed on metformin and the wavelength of maximum absorbance (λ max) of drug was found to be 232 nm The absorbance reading with standard solution containing 1-10μg/ml showed Slope of 0.0959, intercept of 0.027 and regression coefficient value of 0.9955 indicate the linear relationship between absorbance and concentration and equation obeyed linearity in range of 2-10 μg /ml. Solubility of the Metformin HCl indicated that the drug was freely soluble in water, slightly soluble in alcohol and practically insoluble in acetone and in methylene chloride. The melting point was found to 234°C.

Preliminary studies for increasing permeability of drug
Metformin being a class III drug has poor permeability, hence an attempt was made to increase the permeability by forming solid dispersions using β-CD and Pluronic F127. In this work, goat intestinal segment was used as a model for permeability studies. Drug permeation study is carried out by Franz – diffusion cell method. The permeability of Metformin hydrochloride across the excised intestinal segment is shown in table 3.

Table 3: Permeability coefficient of Metformin HCl

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Type of Dispersing agent</th>
<th>Conc.of Dispersing Agent: Drug ratio</th>
<th>Permeability coefficient (cm/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Poloxamer-407</td>
<td>1:1</td>
<td>11.2356 x 10-5</td>
</tr>
<tr>
<td>3</td>
<td>Poloxamer-407</td>
<td>1:3</td>
<td>8.1235x10-5</td>
</tr>
<tr>
<td>4</td>
<td>Poloxamer-407</td>
<td>1:4</td>
<td>4.5623x10-5</td>
</tr>
<tr>
<td>5</td>
<td>Poloxamer-407</td>
<td>1:5</td>
<td>3.6465 x 10-5</td>
</tr>
</tbody>
</table>

In the β-cd inclusion complex of drug in solution only free form of the drug, which is in equilibrium, is capable of penetrating the lipophilic barriers and thus entering systematic circulation. CDs enhance the mucosal drug permeability mainly by increasing the free drug availability at the absorptive surface.[16]

Poloxamers are triblock copolymers of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene). It consisting of 70% w/w polyoxyethylene units, is a low toxicity excipient approved by FDA for different types of preparation. They are extensively used as solubilizers, wetting agents and surface adsorption excipients.[17] Poloxamer 407 (Pluronic®F-127 [PL]) is successfully employed as a solid dispersion carrier in form of matrix dispersion used as bioavailability enhancer.[18]

The results clearly indicated solid dispersion increase the permeability of drug compared to plain drug. From results it is clear that β-cyclodextrin in 1:1 ratio shows more permeability than poloxamer and other ratios. Hence B-CD in 1: 1 ratio was optimized from preliminary work for further study.

Evaluation of Prepared granules
Table 4: Results for prepared granules

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Angle of repose (°)</th>
<th>Bulk density (gm/cc)</th>
<th>Tap density(gm/cc)</th>
<th>Hausner ratio</th>
<th>Carr’s index</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.2 ±1.11</td>
<td>0.89 ±0.02</td>
<td>0.95 ±0.02</td>
<td>1.06 ±0.06</td>
<td>6.31 ±1.23</td>
</tr>
<tr>
<td>2</td>
<td>35.2 ±1.42</td>
<td>0.83 ±0.01</td>
<td>0.93 ±0.01</td>
<td>1.12 ±0.08</td>
<td>10.49±1.46</td>
</tr>
<tr>
<td>3</td>
<td>28 ±1.02</td>
<td>0.82 ±0.02</td>
<td>0.90 ±0.02</td>
<td>1.09 ±0.05</td>
<td>8.48 ±2.23</td>
</tr>
<tr>
<td>4</td>
<td>38.6 ±0.75</td>
<td>0.82±0.01</td>
<td>0.89 ±0.01</td>
<td>1.09 ±0.01</td>
<td>7.58 ±1.89</td>
</tr>
<tr>
<td>5</td>
<td>39.1 ±0.95</td>
<td>0.85 ±0.02</td>
<td>0.97 ±0.02</td>
<td>1.13 ±0.02</td>
<td>13.37 ±1.23</td>
</tr>
<tr>
<td>6</td>
<td>29.1 ±1.23</td>
<td>0.75 ±0.01</td>
<td>0.88 ±0.01</td>
<td>1.17 ±0.06</td>
<td>14.77±1.56</td>
</tr>
<tr>
<td>7</td>
<td>35.2 ±2.06</td>
<td>0.83 ±0.01</td>
<td>0.93 ±0.02</td>
<td>1.12 ±0.05</td>
<td>10.49±1.21</td>
</tr>
<tr>
<td>8</td>
<td>30 ±2.89</td>
<td>0.83 ±0.02</td>
<td>0.89 ±0.01</td>
<td>1.07±0.08</td>
<td>6.74 ±1.36</td>
</tr>
</tbody>
</table>
### Table 5: Results for immediate release tablet

<table>
<thead>
<tr>
<th>Formulation No</th>
<th>Content uniformity</th>
<th>Permeability coefficient</th>
<th>Hardness</th>
<th>Friability</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Cm/sec</td>
<td>kg/cm³</td>
<td>%</td>
<td>Min.</td>
</tr>
<tr>
<td>F1</td>
<td>99.12±0.121</td>
<td>0.001328±0.0012</td>
<td>8.08±0.12</td>
<td>0.71±0.03</td>
<td>5.52±0.01</td>
</tr>
<tr>
<td>F2</td>
<td>99.23±0.231</td>
<td>0.001880±0.0016</td>
<td>8.26±0.25</td>
<td>0.086±0.03</td>
<td>5.61±0.02</td>
</tr>
<tr>
<td>F3</td>
<td>99.13±0.051</td>
<td>0.001243±0.0014</td>
<td>7.51±0.26</td>
<td>0.0066±0.03</td>
<td>5.76±0.03</td>
</tr>
<tr>
<td>F4</td>
<td>99.29±0.066</td>
<td>0.001268±0.0014</td>
<td>7.87±0.16</td>
<td>0.0056±0.04</td>
<td>5.78±0.05</td>
</tr>
<tr>
<td>F5</td>
<td>99.22±0.071</td>
<td>0.001303±0.0005</td>
<td>7.55±0.18</td>
<td>0.0062±0.05</td>
<td>5.79±0.06</td>
</tr>
<tr>
<td>F6</td>
<td>99.13±0.047</td>
<td>0.001194±0.0016</td>
<td>7.50±0.17</td>
<td>0.0071±0.05</td>
<td>5.67±0.01</td>
</tr>
<tr>
<td>F7</td>
<td>99.23±0.023</td>
<td>0.001861±0.0018</td>
<td>8.26±0.14</td>
<td>0.0073±0.08</td>
<td>5.61±0.02</td>
</tr>
<tr>
<td>F8</td>
<td>99.24±0.045</td>
<td>0.001900±0.0017</td>
<td>8.52±0.04</td>
<td>0.0074±0.02</td>
<td>5.62±0.04</td>
</tr>
<tr>
<td>F9</td>
<td>99.12±0.012</td>
<td>0.001388±0.0015</td>
<td>8.08±0.12</td>
<td>0.0072±0.04</td>
<td>5.52±0.05</td>
</tr>
<tr>
<td>F10</td>
<td>99.12±0.056</td>
<td>0.001398±0.0017</td>
<td>8.08±0.16</td>
<td>0.0061±0.04</td>
<td>5.32±0.04</td>
</tr>
<tr>
<td>F11</td>
<td>99.21±0.087</td>
<td>0.001823±0.0015</td>
<td>8.49±0.15</td>
<td>0.0074±0.06</td>
<td>5.65±0.07</td>
</tr>
<tr>
<td>F12</td>
<td>99.13±0.054</td>
<td>0.001256±0.0012</td>
<td>7.51±0.23</td>
<td>0.0062±0.09</td>
<td>5.76±0.05</td>
</tr>
<tr>
<td>F13</td>
<td>99.22±0.058</td>
<td>0.001876±0.0017</td>
<td>8.30±0.12</td>
<td>0.0072±0.08</td>
<td>5.66±0.04</td>
</tr>
<tr>
<td>F14</td>
<td>99.13±0.047</td>
<td>0.001129±0.0016</td>
<td>7.51±0.23</td>
<td>0.0059±0.07</td>
<td>5.76±0.04</td>
</tr>
<tr>
<td>F15</td>
<td>99.13±0.045</td>
<td>0.001134±0.0014</td>
<td>7.51±0.12</td>
<td>0.0061±0.02</td>
<td>5.76±0.02</td>
</tr>
<tr>
<td>F16</td>
<td>99.12±0.026</td>
<td>0.001289±0.0018</td>
<td>8.08±0.14</td>
<td>0.0072±0.02</td>
<td>5.53±0.03</td>
</tr>
<tr>
<td>F17</td>
<td>99.21±0.09</td>
<td>0.001438±0.0017</td>
<td>7.87±0.14</td>
<td>0.0064±0.03</td>
<td>5.85±0.04</td>
</tr>
<tr>
<td>F18</td>
<td>99.23±0.071</td>
<td>0.001402±0.0018</td>
<td>7.58±0.17</td>
<td>0.0063±0.04</td>
<td>5.86±0.05</td>
</tr>
<tr>
<td>F19</td>
<td>99.21±0.0251</td>
<td>0.001627±0.0012</td>
<td>8.49±0.12</td>
<td>0.0071±0.05</td>
<td>5.65±0.07</td>
</tr>
</tbody>
</table>

#### Angle of repose
Rougher and more will be irregular surface of the particles; higher will be the angle of repose. Angle of repose of all batches laid between 25.0±0.02 to 39.1±0.02 which means all batches had good or excellent flow property according to standard.

#### Bulk density
In all batches F6 had lowest bulk density while F20 had highest bulk density.

#### Tapped density
In all batches F6 had lowest tapped density while F5 had highest tap density.

#### Hausner ratio
The batches exhibited 1.05±0.01 to 1.13±0.02. Hausner ratios which was within acceptable range according to standard.

#### Carr’s index
The batches exhibited 5.31±1.01 to 14.77±1.56 Carr’s index which was within acceptable range according to standard.

The granules of 23 formulations were prepared by Steam granulation method. These Granules were evaluated for precompression parameters. This method is having advantage like higher binder distribution uniformity and diffusion rate into powders, more spherical granule with large surface area were formed thereby increases dissolution rate of the drug from granules, favorable thermal balance results in rapid drying, time efficient as processing time was shorter and environment friendly as no organic solvent is used. 

Content uniformity
Content uniformity of all the formulations were in range of 99.0 - 99.5%. Showing uniformity of mixing and compression.

Graphs of content uniformity

Final Equation in Terms of Coded Factors
Content uniformity= +99.17 +0.020X1 +0.055X2 +0.082X3 -4.845E-003X4

Figure 2: 3 D Surface plot of effect of conc. of diluents and absorption enhancer on content uniformity( A- PluronicF127 in combination with MCC, B- PluronicF127 in combination with Spray dried Lac, C- Gelucire 50/13 in combination with Spray dried lactose and D- Gelucire 50/13 in combination with MCC)
From the above fig no. 2 it was cleared that with increase in conc. of diluent and conc. of absorption enhancer drug content increases. As such there is negligible difference in drug content of tablet containing gelucire 50/13 and pluronic F 127. But as results show that tablets containing combination of Gelucire 50/13 with MCC and Spray dried lactose showing good content of uniformity when Compared with tablets containing Pluronic F 127 with the combination of MCC and Spray dried lactose showing less content of uniformity.

**Permeability coefficient**
Permeability coefficient of all the formulations were in range of 0.11-0.19 showing remarkable increase in the permeability coefficient.

**Final Equation in Terms of Coded Factors**
Permeability through goat intestine = $+1.510E-003 - 4.878E-005 \times X_1 + 1.975E-005 \times X_2 + 1.440E-004 \times X_3 - 1.791E-004 \times X_4$.

From the graph below in Fig.no.3 it was cleared that the tablets containing gelucire 50/13 showed more permeability than tablets containing Pluronic F127.

When the concentration of Gelucire 50/13 was increased from 5% to 7.5% the Permeability coefficient was increased. The tablet containing 7.5% concentration of Gelucire 50/13 showed more Permeability coefficient than Pluronic F127. Concentration of Absorption enhancer affect on Permeability coefficient of drug. Concentration of Gelucire 50/13 has positive effect might be due to surfactant carrier family that has been described as a being penetration enhancer, a characteristic attributed to its surfactant and lipid nature. Lipid surfactants such as Gelucire reportedly cause cell membrane polar defects, thus changing their physical properties and making them more permeable than Poloxamer 407, which is a polymeric surfactant that has been described as a permeability enhancer. Poloxamer 407 in SDs did not improve the bioavailability, most likely because of the low intrinsic ability of Poloxamer 407 to favor cell entry. Gelucire enhances biological membrane penetration and could thus increase the bioavailability of drugs.[21] The another reason might be Pluronic F127 is having viscosity around 5800 mPas while pure Gelucire 50/13 exhibited a viscosity of 50mPas. The Pluronic F127 is having a property of forming gel in the aqueous media. This might be causing the effect on less drug release and thus less content uniformity of tablets containing Pluronic F127.[24]
Figure 3: 3D Surface plot of effect of conc.of diluents and absorption enhancer on Permeability (A-PluronicF127 in combination with MCC,B- PluronicF127 in combination with Spray dried Lactose, C- Gelucire 50/13 in combination with Spray dried lactose and D- Gelucire 50/13 in combination with MCC) Hardness

Hardness of all the formulations is In the range of 7.04-8.72 kg/cm³

Final Equation in Terms of Coded Factors
Hardness = +7.82+0.097* X1+0.26* X2+0.20* X3-0.35* X4.
Figure 4: 3D Surface plot of effect of conc. of diluents and absorption enhancer on Hardness (A- Pluronic F127 in combination with MCC, B- Pluronic F127 in combination with spray dried lactose, C- Gelucire 50/13 in combination with spray dried lactose and D- Gelucire 50/13 in combination with MCC)

From the above fig. 4 it was cleared that addition of MCC as a diluent in Tablets containing Pluronic F127 as well as Gelucire 50/13 causes increased Hardness. While addition of spray dried lactose as diluents causes a slight decrease in hardness.

MCC has more free hydroxyl group and thus the interaction forces in a contact point may be stronger because of stronger hydrogen bond of hydroxyl groups, which can cause increase in hardness. This interlocking phenomenon can have effect of increasing hardness than that of spray dried lactose.²³

Friability
The friability of all the formulations is in the range of 0.0054-0.0078%.

Final Equation in Terms of Actual Factors
From the above fig.no.5 it is cleared that the tablets containing spray dried lactose shows less friability than the tablets containing MCC as diluents. The friability is within the limits of all the formulations that is < 1%. As spray dried lactose and Microcrystalline cellulose both enhances rheological properties of wetted mass resulting in good sphericity and low friability, high density. Both show friability less than 1%. It might be during steam granulation slight melting of spray dried lactose might have taken place which increase density of spray dried lactose than MCC. Hence this slight difference in friability might be the reason between friability of tablets containing MCC and spray dried lactose.\(^{(26)}\)

**Disintegration**

Disintegration time for all the formulations is in the range of 5.46-6.00 minutes.

**Final Equation in Terms of Coded Factors**

\[
\text{Disintegration} = +5.78 - 0.11\times X_1 + 0.046\times X_2 + 0.072\times X_3 + 0.091\times X_4
\]
From the above figure no. 6 it was cleared that when the tablets containing the pluronic F 127 and MCC get disintegrated rapidly than other tablets. While the tablets containing gelucire50/13 and Spray dried lactose get disintegrated slowly.

Microcrystalline cellulose is partially depolymerised cellulose prepared from alpha cellulose. When compressed, the MCC particles are deformed plastically due to the presence of slip planes & dislocation. A strong compact is formed due to the extremely large number of clean surfaces brought in contact during plastic deformation & the strength of hydrogen bonds formed. The mechanism involves is interlocking. MCC has a very high intraparticle porosity with approximately 90–95% of the surface area being internal. Therefore surface area is not directly influenced by the nominal particle size. This high porosity promotes swelling and disintegration of MCC tablets, which is attributed to the penetration of water into the hydrophilic tablet matrix by means of capillary action of the pores and by a subsequent disruption of the hydrogen bonds MCC allowing water to enter the tablet matrix by means of capillary pores, which break the hydrogen bonding between adjacent bundles of cellulose microcrystals and exhibit very good disintegrant property. The particle size of MCC is small. The decrease in particle size increases binding strength and decreases disintegration time. MCC is found in the concentration of 10-25% as a filler, binder, disintegrant. MCC is useful as a disintegrant when used in proportion of at least 5-15%.[27]

While the Tablets containing spray dried lactose exhibited increase in crushing strength with decrease in the particle size. The disintegration time of spray-dried lactose tablets was essentially independent of compaction force. Due to compaction pressure, tablets containing spray dried lactose disintegrated before gel or precipitate could block the pores. This gelling and precipitation dominated the disintegration time. Spray-dried lactose exhibited strong increase in disintegration time.[28]

**In-vivo bioavailability study**

HPLC method showed the good peak with good symmetry. Hence this method was finalized for the development of metformin.

The Albino rats treated with both Optimized Formulated Metformin Tablet and Marketed Tablet. Glycomet- 250 mg (USV LIMITED) was used as the marketed formulation. The result showed that, the C max of Formulated tablet was found to be higher than that of marketed formulation.

**Table 5: Pharmacokinetic parameters for In-vivo bioavailability study.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Formulated tablet</th>
<th>Marketed tablet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C max (ng)</td>
<td>70.74</td>
<td>16.78</td>
</tr>
<tr>
<td>T max (min)</td>
<td>9.12</td>
<td>11.24</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-4hr&lt;/sub&gt; (ng/min/ml)</td>
<td>2.1676</td>
<td>1.468</td>
</tr>
<tr>
<td>t 1/2 (min.)</td>
<td>12.38</td>
<td>13.58</td>
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CONCLUSION
The in-vivo evaluation of optimized formulation with marketed formulation showed improvement in bioavailability than Marketed formulation. Hence, it can be concluded that use of waxy non-ionic surfactants played a significant role in improving the permeability and thus bioavailability of Metformin Hydrochloride.

REFERENCES