Material and Methods

5.1 Materials

5.1.1 Drug

Tolperisone and Celecoxib were obtained from Aristo Pharma Ltd. Baddi, and Centaur Pharmaceuticals Pvt Ltd Goa, respectively.

5.1.2 Excipients

The excipients employed in formulation development are listed in table.

Table 5.1: List of excipients used in formulation

<table>
<thead>
<tr>
<th>S. No</th>
<th>Drug/Excipient</th>
<th>Manufacturer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tolperisone</td>
<td>Aristo Pharma Ltd. Baddi</td>
</tr>
<tr>
<td>2</td>
<td>Celecoxib</td>
<td>Centaur Pharmaceuticals Pvt Ltd Goa</td>
</tr>
<tr>
<td>3</td>
<td>Crospovidone</td>
<td>Micro Labs, Bangalore</td>
</tr>
<tr>
<td>4</td>
<td>Sodium starch glycolate</td>
<td>Micro Labs, Bangalore</td>
</tr>
<tr>
<td>5</td>
<td>Microcrystalline cellulose</td>
<td>Central Drug House, Mumbai</td>
</tr>
<tr>
<td>6</td>
<td>Mannitol</td>
<td>Central Drug House, Mumbai</td>
</tr>
<tr>
<td>7</td>
<td>Mg. Stearate</td>
<td>Oxford Laboratory, Mumbai</td>
</tr>
</tbody>
</table>
5.1.3 Equipments

Instruments and equipments used during the course of the project are listed in table.

**Table 5.2: List of equipments used**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Equipments</th>
<th>Manufacturer/Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UV/Visible,Spectrophotometer</td>
<td>UV-1700SHIMADZU</td>
</tr>
<tr>
<td>2</td>
<td>Analytical balance</td>
<td>DAB220</td>
</tr>
<tr>
<td>3</td>
<td>FTIR</td>
<td>Bruker corporation</td>
</tr>
<tr>
<td>4</td>
<td>Vernier Caliper</td>
<td>Global spec</td>
</tr>
<tr>
<td>5</td>
<td>Melting Point Apparatus</td>
<td>ROLEX-Digital melting point Apparatus</td>
</tr>
<tr>
<td>6</td>
<td>Tablet punching machine 12 station</td>
<td>Cadmach</td>
</tr>
<tr>
<td>7</td>
<td>Tablet Hardness Tester</td>
<td>Shankar</td>
</tr>
<tr>
<td>8</td>
<td>Friability Tester</td>
<td>Electrolab</td>
</tr>
<tr>
<td>9</td>
<td>Digital vernier caliper</td>
<td>Mitutoyo</td>
</tr>
</tbody>
</table>
5.1.4 Chemicals

Chemicals and reagents employed for the preparation of buffers, analytical solutions and other experimental purposes are listed in table.

Table 5.3: Chemicals and reagents utilize in formulation and development study

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical</th>
<th>Supplier/ Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chloroform AR</td>
<td>Qualigens Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>2</td>
<td>Methanol AR</td>
<td>Qualigens Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol AR</td>
<td>Qualigens Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>4</td>
<td>n-octanol</td>
<td>Qualigens Fine Chemicals, Mumbai</td>
</tr>
<tr>
<td>5</td>
<td>Pot. Sodium Dihydrogen Phosphate</td>
<td>Central drug house (P) Ltd Mumbai.</td>
</tr>
</tbody>
</table>
5.2 Preformulation studies of pure drug

5.2.1 Determination of $\lambda_{\text{max}}$

The purity of drug play important role in formulation of dosage forms. The efficacy of any drug depends on the purity of drugs. There are number of methods can be applied to determine the purity of drugs. Among all the methods the UV spectroscopy is best because it is cheap and produces reliable and reproducible results. In UV spectroscopy the $\lambda_{\text{max}}$ of the drug can be determined. The $\lambda_{\text{max}}$ of drug are specific and it cannot be change at specific conditions. When $\lambda_{\text{max}}$ of compound will change then it indicates that compounds have lost its actual purity.

In our study we determined the $\lambda_{\text{max}}$ of Tolperisone and Celecoxib by using UV spectroscopy. The solution of Tolperisone and Celecoxib containing concentration of 10µg/ml was prepared in water and ethanol respectively, and UV spectrum was taken using UV spectrophotometer. The sample was scanned in the range of 200-400 cm$^{-1}$

5.2.2 Preparation of calibration curve in pH 6.8 phosphate buffer

An accurately weighed amount of Tolperisone and Celecoxib corresponding to 100mg was dissolved in a small amount of pH 6.8 Phosphate Buffer in 100ml volumetric flask and volume made up to 100ml with the same pH 6.8 Phosphate Buffer. From this stock’s solution, 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml were withdrawn and diluted up to 10ml with the pH 6.8 Phosphate Buffer in 10ml volumetric flask to get concentration of 1µg,
2µg, 3µg, 4µg, 5µg, 6µg, 7µg, 8µg, 9µg and 10µg respectively. The optical density of every solution was calculated by UV-Visible Spectrophotometer at 260 nm and 252 nm for Tolperisone and Celecoxib respectively, using pH 6.8 Phosphate Buffer as blank.

5.2.3 Preparation of calibration curve in 0.1 N NaOH

An accurately weighed amount of Tolperisone and Celecoxib equivalent to 100mg was dissolved in small amount of 0.1 N NaOH in 100ml volumetric flask and volume made up to 100ml with the same 0.1 N NaOH. From this stock’s solution, 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml were withdrawn and diluted up to 10ml with the 0.1 N NaOH in 10ml volumetric flask to get concentration of 1µg, 2µg, 3µg, 4µg, 5µg, 6µg, 7µg, 8µg, 9µg and 10µg respectively. The optical density of every solution was calculated by UV-Visible Spectrophotometer at 260 nm and 252 nm for Tolperisone and Celecoxib using 0.1 N NaOH as blank.

5.2.4 Drug excipients interaction study by FTIR

The Fourier Transform – Infrared (FT-IR) spectroscopy has numerous application in Pharmaceutical field. It is widely used in determination of identification of known and unknown compound. Apart from this it can also be used in evaluating the drug interaction. During formulation the active ingredient are used mixed with various excipients to give proper shape and appearance. Sometimes it happens after mixing the active ingredients with excipient, it produces incompatibility due to drug excipient interaction. The incompatibility of drug can alter the potency of formulation. It can also produce adverse effects to the body. Hence for pharmaceutical industries it is prime work to check the drug and excipient incompatibility.

To check the compatibility of drug and polymer in preparation of mouth dissolving tablet of Tolperisone and Celecoxib, the drug and excipients mixture of quantity 10 mg and 400 mg of KBr were acquired in a mortar and were triturated. A little volume of the triturated sample was seized and kept on to the sample holder and examined from 4000cm⁻¹ to 400cm⁻¹ in FTIR Spectrophotometer. The spectra obtained were matched with that of the
peaks obtained from the FTIR study of the pure drug sample and interpreted for the interaction of drug and excipients if any.

5.2.5 Solubility analysis

The preparation of any dosage form, it required to know the solubility of drug. The solid dosage form need particular solvent to dissolve, and produces pharmacological effect to body. Additionally, the bioavailability of drug present in solid dosage form depends upon solubility of drug. If drug is sparingly soluble in solvent then it produces minimum therapeutic response due to less availability of drug to receptors. Hence solubility of drug play important role in therapeutic effects of drug.

A little amount of sample was taken in test-tube and adding known quantity of solvent, to determine the solubility of sample. After every inclusion the system is forcefully trembled and investigated visibly for any undissolved solute particles.

5.2.6 Determination of melting point

The melting point of drugs indicates the purity of drug. Every drug has its own melting point at particular conditions. If there is any alteration in melting point of drug it inferred the change in therapeutic response of drug. When active constituents are incorporated with foreign particles or adulterants, it changes the melting point of active constituents. When drug comes in contact with moisture then it also changes the melting point of drugs. Hence melting point of drug is best indicator to check the purity of drug. If drug is pure than it will produces maximum or desired therapeutic effect to the body.

The melting point of Tolperisone and Celecoxib were determined by using melting point apparatus. For this, take a little amount of drug sample in capillary tube which was one sided closed and placed in a melting point apparatus and the temperature at which drug melts was noted.
5.3 Preparation of Tolperisone and Celecoxib tablets by direct compression method

The conventional solid dosage namely tablets form can be prepared by direct compression and granulation methods. The granulation methods are subdivided into two category namely dry granulation and wet granulation methods. Each method has its own specific and advantages for dosage form. The selection of technique for formulation of tablets depends on property of drugs and excipients. However, the direct compression technique is mostly preferred due to cost effective.

In direct compression technique the mixtures tablets are directly compressed without making granules. Following blends are used to prepare tablets by direct compression methods:

- Blends should free flow
- Non hygroscopic materials

Following are the advantages of direct compression method over wet granulation technique:

- Cost effective compared to other methods
- Preferably for heat sensitive active ingredients
- Preferable for moisture sensitive drugs
- Produce quick dissolution of tablets
- Tablets are minimum affected by tear and wear punches
- Minimum chances of contamination

Following are limitation of direction compression methods:

- Mostly affected by segregation
- It require 40-50% diluent or excipient
Material and Methods

- Lubricants produce adverse effect to filler

Consequently, the direct compression methods produces greater productivity and better quality of tablets compared to wet granulation technique.

Tablets of Tolperisone and Celecoxib were prepared by direct compression method. All the formulation ingredients mentioned in formulation table 1 and table 2 were weighed accordingly and mixed in a mortar and pestle. This powder blend was then allowed to dry for few moments and then again mixed well and passed through sieve no 60. Then blend were used for further processing.
### Table 5.4: Formulation of mouth dissolving Tolperisone tablets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolperisone</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
<td>7</td>
<td>9</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mannitol</td>
<td>114</td>
<td>112</td>
<td>110</td>
<td>108</td>
<td>112</td>
<td>110</td>
<td>108</td>
<td>106</td>
</tr>
<tr>
<td>Mg. Stearate</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Talc</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aspartame</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>Theoretical Weight</td>
<td>305</td>
<td>305</td>
<td>305</td>
<td>305</td>
<td>305</td>
<td>305</td>
<td>305</td>
<td>305</td>
</tr>
</tbody>
</table>
Table 5.5: Formulation of mouth dissolving Celecoxib tablets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>C1</th>
<th>C2</th>
<th>C3</th>
<th>C4</th>
<th>C5</th>
<th>C6</th>
<th>C7</th>
<th>C8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Crospovidone</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Sodium starch glycolate</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Mannitol</td>
<td>90</td>
<td>88</td>
<td>86</td>
<td>84</td>
<td>88</td>
<td>86</td>
<td>84</td>
<td>82</td>
</tr>
<tr>
<td>Mg. Stearate</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Talc</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aspartame</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Theoretical Weight</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
<td>218</td>
</tr>
</tbody>
</table>
Excipient and Drugs

Dispensing

Shifting # 60 of drug with disintegrant and fillers

Shifting of lubricant through # 60

Pre blending for 15 min

Blending with lubricants (5-6 min)

Compression process
5.4 Evaluation of pre-compression characteristics of powder blend

Powder mixture formulated was assessed for different rheological properties by using standard procedures. The evaluation was done thrice time (n=3) and mean data were reported.

5.4.1 Bulk density

The bulk density and tapped density are evaluated to determine the rate of filling of blend to die.

The bulk density was measured as reported in research paper. The mixtures were filled in a measuring cylinder, and after that the complete volume was noted. The gravity of powder mixture was concluded by utilizing digital weighing balance. The following formula was used to determine the bulk density:

\[
\text{Bulk Density} = \frac{\text{Weight of the powder}}{\text{Volume of the powder}}
\]

- Known quantity of powder
  - Poured
  - Measuring cylinder
  - Without Compacting
  - Read the unsettled apparent volume
  - Weighed powder mixture
  - Calculate Bulk Density
5.4.2 Tapped density

Tapped density was determined; the mixtures were filled in a measuring cylinder. After that the measuring cylinder was tapped 100 times. Measure the weight of the total powders. The tapped density was calculated by applying following formula:

\[ \text{Tapped Density} = \frac{\text{Weight of the powder}}{\text{Tapped Volume of the powder}} \]
5.4.3 Angle of repose

In this process the fennel was placed above graph paper at distance of 6 cm. The powder kept on fennel and slowly removed the fennel. The scale was used to measure the height of the heap. The angle of repose was calculated by applying following formula:

$$\theta = \tan^{-1}\frac{h}{r}$$

Where, $h = \text{height of heap of granular bed}$, $r = \text{radius of heap of granular bed}$. 

![Diagram showing the calculation of angle of repose](image)
5.4.4 Hausner’s ratio

Hausner’s ratio was calculated by using following formula and it was expressed in percentage

\[ H = \frac{D_t}{D_b} \]

Where \( D_t \) denoted the tapped density of the powder

\( D_b \) denoted the bulk density of the powder
5.5  Compression of powders into tablets

Before compression of powder into tablets, the Lubricant (talc) and glidant (magnesium stearate) were mixed to the prepared powders. By the help of compression the powder were punch into tablets using 10mm diameter, flat faced punches.

5.6  Evaluation of compression characteristics of tablets

After formulation of tablets it required to check the suitability of dosage form for proper therapeutic response. The various parameters are used for evaluation of compression of tablets. The thickness, friability, hardness, weight variation and dissolution test were evaluated for prepared tablets using standard procedures.

5.6.1  Weight variation test

In this process the 20 tablets were weighed separately. The average weight of one tablet was calculated by taking average mean. On I.P. it has mentioned that not more than 2 tablets produce distinctive weight. As per I.P note more than 2 of the distinctive weights from the mean weight, and none should be aberrant by longer than twice that percentage given in the monographs.
5.6.2 Thickness test

By the help of vernier caliper, we measure the thickness of the tablets in terms of micrometer. The averages of three readings were noted and the results of mean were recorded ($n = 3$)

5.6.3 Hardness test

The Monsanto hardness tester was used to determine the hardness of formulated tablets. The hardness was calculated in respect to kg/cm$^2$. Thrice readings were measured and average was noted.

5.6.4 Friability test

The Roche friabilator was used to measure the abrasion rate of formulated tablets. Measure the weight of 20 tablets and kept in the friabilator chamber. The friabilator was rotated at speed of 25 rpm for 4 min. After completion of rotation of friabilator tablets were weighted and by the help of formula the percentage weight loss was calculated.

5.6.5 Drug content

The drug content was calculated by triturating the three tablets in a mortar with pestle to get fine powder. Taken powder equivalent weight of one tablet and was dissolved in pH 6.8 phosphate. Measure the absorbance of diluted sample of Tolperisone and Celecoxib at 260 nm and 252 nm respectively, using UV-Visible Spectrophotometer. The drug content was calculated by using standard calibration curve.

5.6.6 Wetting time

The wetting time was calculated by placing the tablets in Petridish. The Petridish was consisting of 6 ml of purified water along with tissue paper folded two times. The time required for complete wetting of tablets was measured.
Wetting time = $T_t - T_0$
5.6.7 Water absorption ratio

The procedure used in wetting time was applied for the determination of water absorption ratio. Water absorption ratio R was calculated using equation.
5.6.8 *In-vitro disintegration time* \(^{215-222}\)

Rate of disintegration imparts chief role for mouth dissolving tablets. The disintegrating agents are used to enhance the disintegration of mouth dissolving tablets. The disintegrants promotes the moisture penetration into the tablets. Following are the factors which affect the rate of disintegration of mouth dissolving tablets.

- Quantity of disintegrants available in mouth dissolving tablets
- Nature of diluents, polymer, excipient present in mouth dissolving tablets
- Combination of various types of disintegrants
- Nature of blending of disintegrants for tablets

The natural and synthetic disintegrants are used in the preparation of mouth dissolving tablets. Following are the natural disintegrants used in the preparation of mouth dissolving tablets:

- a) Lepidus sativum
- b) Locust bean gum
- c) *Plantago ovate*
- d) *Hibiscus rosa*
- e) *Fenugreek seed* mucilage
- f) Chitosan gum Arabic
- g) Xanthan gum
- h) Gellan gum

Following are the synthetic disintegrants used in the preparation of mouth dissolving tablets:
a. Sodium starch glycolate  
b. Croscarmellose sodium  
c. Cross-linked polyvinylpyrrolidone  
d. Micro crystalline cellulose  
e. Low-substituted hydroxypropyl cellulose  
f. Cross-linked alginic acid  
g. Calcium Silicate  
h. Ion exchange resins  
i. Chitin and Chitosan  

The synthetic disintegrants are widely used comparatively natural disintegrants due to following limitations:

- Lower concentration of synthetic disintegrants are required  
- Minimum effected by compression force  
- It produces intraganularly effect to tablets  

Hence we selected synthetic disintegrants for the preparation of mouth dissolving tablets. The disintegration test apparatus was used to determine the disintegration of mouth dissolving tablets. Each tube of basket of disintegration apparatus containing formulated tablet. The pH 6.8 (simulated saliva fluid) used for the determination of disintegration of tablets at temperature 37±2 °C. The total time required to complete breakdown of the tablets was observed and noted.  

5.6.9 In-vitro drug release study  

In vitro dissolution has been properly established to develop oral dosage form. It is used to predict in vivo dissolution of tablets.
The in vitro release of mouth dissolving tablets was determined by using tablet dissolution test apparatus USP XXIII, apparatus I. The media used in dissolution apparatus was phosphate buffer pH6.8 (900 mL) and maintained it at 37 ± 1°C. The sample of 10 mL was withdrawn at different interval and volume of media was maintained by putting fresh media in chamber. The aliquots were evaluated spectrophotometrically at 260 nm and 252 nm for Tolperisone and Celecoxib.

The samples withdrawn for Celecoxib were measured spectrophotometrically at 252 nm.

5.7  Kinetic model of drug release

The release pattern of drug from tablets was determined by kinetic models. Following are the model listed below can be used to determine the release pattern of formulations.

a. Zero-Order Model

b. First-Order Model

c. Higuchi Model

d. Hixson-Crowell Cube Root Model

e. Korsmeyer-Peppas Model

There are various factors which assist in selection of kinetic studies for tablets. Some of them are:

- Nature of active constituents
- Excipients used in formulations of tablets
- Quantity of active constituent present in formulation
- Half life of drug
- Geometry of drug delivery
The different kinetics model of drug release was calculated for all the formulations:

- Zero order kinetics was calculated by plotting graph between cumulative percent drug released vs. time
- First order kinetics was calculated by plotting graph between log cumulative percent drug retained vs. time
- Higuchi’s equation was calculated by plotting graph between log cumulative percent drug released vs. square root of time
- Peppas equation was calculated by plotting graph between log of cumulative percentage release Vs log time

5.8 **Full factorial design**

The factorial designs are used to determine the impact of trial formulations. This study helps in optimizing the formulation. The formulations were optimized by characterizing the factors participating in altering the result. The full factorial design was applied for the formulated tablets. In this study the $3^2$ randomized full factorial designs was used.
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Full Factorial Design for 3 Two-level Components.

Mixture of 3 Components

Standard Mixture:
\[ x_1, x_2, x_3 \geq 0 \]
\[ x_1 + x_2 + x_3 = 1 \]

Constrained Mixture:
\[ x_{u1} \leq x_1 \leq x_{u1} \]
\[ x_{u2} \leq x_2 \leq x_{u2} \]
\[ x_{u3} \leq x_3 \leq x_{u3} \]
\[ x_1 + x_2 + x_3 = 1 \]