CHAPTER - 4

MATERIALS AND METHODS

Drug Profile:

Atenolol 98,99

\[
\text{H}_3\text{C}\text{CH}_3\text{N}\text{H}\text{OH}\text{O}\text{O}\text{NH}_2
\]

ATENOLOL

DRUG PROFILE Atenolol

Chemical name: 2-{4-[2-hydroxy-3-(propan-2-ylamino) propoxy] phenyl}acetamide.

Molecular formula: C_{14}H_{22}N_2O_3

Molecular weight: 266.336g/mol

Category: Anti-Hypertensive agent, beta 1 receptor antagonist.

Description: It is a colorless, not freely soluble in water, completely dissolves in acetic acid and propanol, and freely soluble in methanol and have low solubility in acetone.

Melting Point : 152-1550°C .

Dose: 25-100 mg once daily.

Mechanism of action:

Atenolol^{100} a beta adrenergic-blocking agents reduces the oxygen need of the myocardium by lowering both the rate and force of contraction of the heart. They inhibit the activation of the heart by blocking β₁ receptors, and they reduce the work of the heart by lowering heart rate, cardiac output and blood pressure. All the β
blockers are non selective at high doses and can inhibit \( \beta_2 \) receptors. The \( \beta \)-blockers reduce the frequency and the severity of angina attacks, these agents are particularly useful in the treatment of patients with myocardial infarction and have shown prolong survival.

**Pharmacokinetics**:

About 50% of administered drug is consumed after oral insertion. Maximum plasma levels are attained within 4 hours. Atenolol has less lipid solubility. It enters the placenta and distributed into breast where the concentration of atenolol is higher than in maternal plasma. Only small amounts are reported to cross the blood brain-barrier, and plasma protein binding is minimal. The plasma half life is about 6 to 7 hours. Atenolol undergoes no hepatic metabolism and is excreted mainly in urine. It is removed by heamodialysis.

**Unwanted effects**:

The main unwanted side effects of \( \beta \)-adrenoreceptor antagonist results from their recpeot-blocking action.

**Bronchoconstriction**:

Bronchoconstriction is of little importance in the absence of the airways disease, but asthmatic patients, this effect can be dramatic and life threatening. It is also of clinical importance in patients with other forms of obstructive lung disease.

**Cardiac Failure**:

Patients with heart disease may rely on a degree of sympathetic drive to the heart to maintain an adequate cardiac output, and removal of this by blocking \( \beta \)-adrenoreceptors will produce a degree of cardiac failure.
**Bradycardia:**

It can progress to life threatening heart block. It can occur in patients with coronary disease, particularly if they are being treated with antidysrhythmic drug that impair cardiac conduction.

**Effects on liver:**

Reversible cholestatic hepatitis occurred in patient getting atenolol and hepatic impairment.

**Medicinal uses:**

Atenolol is a cardioselective beta blocker, used in the maintenance of high blood pressure, chest pain, cardiac disorders and myocardial infarction. It can be useful initially in treating the migraine.
**Acelofenac**

Chemical Name:

2-[2,6-Dichlorophenyl) amino] benzeneacetic acid carboxymethyl ester

Molecular Formula : C_{16}H_{13}Cl_{2}NO_{4}

Molecular Weight : 354.19

Melting Point : 149°C

Solubility : easily dissolved in methanol, ethanol

**Therapeutic Category:** Anti-inflammatory, Analgesic.

**Pharmacokinetics:** Aceclofenac (AC) is well absorbed form the GIT peak plasma concentration are reached 1 to 3 hrs post oral ingestion. Aceclofenac is 99% bounded to plasma protein, the elimination half-life is approximately 4 hrs. A two thirds of its dose is excreted mainly as hydroxymetabolites.

**Adverse effects:** GI disturbances, nausea and diarrhea, these are usually mild and reversible but in some patient peptic ulceration and sever GI bleeding may occur.
# METHODOLOGY

## Materials and Equipments

### Materials used

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Materials/ Chemicals</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Atenolol</td>
<td>Glenmark Pharmaceutical, Nasik, India</td>
</tr>
<tr>
<td>2</td>
<td>Aceclofenac</td>
<td>Glenmark Pharmaceutical, Nasik, India</td>
</tr>
<tr>
<td>3</td>
<td>PEG-400</td>
<td>SD Fine Chem Limited, Mumbai</td>
</tr>
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<td>4</td>
<td>Hydrogenated Vegetable oil</td>
<td>Research Lab, Poona</td>
</tr>
<tr>
<td>5</td>
<td>Propylene glycol</td>
<td>SD Fine Chem Limited, Mumbai</td>
</tr>
<tr>
<td>6</td>
<td>Gelatin</td>
<td>Qualigens Fine Chemicals (A Division of Glaxo India Limited) Mumbai</td>
</tr>
<tr>
<td>7</td>
<td>Methanol</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Propyl paraben</td>
<td>Merck Specialities Private Limited, Mumbai</td>
</tr>
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<td>Sl. No.</td>
<td>Equipment name</td>
<td>Source</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>1.</td>
<td>UV/ Visible spectrophotometer</td>
<td>PharmaSpec UV 1700 Shimadzu</td>
</tr>
<tr>
<td>2.</td>
<td><em>In Vitro</em> Dissolution Apparatus USP XXIII</td>
<td>Electrolab TDT-06N</td>
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<tr>
<td>3.</td>
<td>Water Bath</td>
<td>M. C. Dalal Agencies, Chennai</td>
</tr>
<tr>
<td>4.</td>
<td>Digital pH meter</td>
<td>Systronics</td>
</tr>
<tr>
<td>5.</td>
<td>Electronic Balance</td>
<td>Shimadzu BL-220H</td>
</tr>
<tr>
<td>6.</td>
<td>Refrigerator</td>
<td>Godrej</td>
</tr>
<tr>
<td>7.</td>
<td>Liquefaction temperature apparatus</td>
<td>Fabricated</td>
</tr>
</tbody>
</table>
PLAN OF WORK

► Preparation of rectal suppositories by fusion method using Hydrogenated vegetable oil, gelatin-PEG 400 as bases and propylene glycol as plasticizer.

► Evaluation of suppositories for:

   🍃 Physical characteristics (Appearance)
   🍃 Variation in weight
   🍃 Content uniformity
   🍃 Liquefaction time and temperature
   🍃 Melting range
   🍃 *In vitro* dissolution study
   🍃 Stability study for the promising formulations
   🍃 Drug-excipient interaction
   🍃 Thermal studies (DSC).
   🍃 Scanning Electron Microscopy
CALIBRATION CURVES OF ACECLOFENAC AND ATENOLOL IN pH 7.4

BUFFER:

Accurately weighed 100mg of aceclofenac was dispersed in pH 7.4 buffer to achieve a Standard solution (1mg/ml).

Subsequent dilutions were made with pH7.4 buffer to obtain concentrations (4, 8, 12, 16 & 20 µg/ml). and the absorbance was recorded at 276 nm versus reagent blank. The standard curve for aceclofenac in pH7.4 buffer was plotted against absorbance.

Similarly the standard curve for aceclofenac in methanol was carried out using the above procedure at 277 nm and the results are tabulated in table-1.

2) Accurately weighed 100 mg of atenolol was mixed in pH 7.4 buffer to achieve a standard solution of (1mg/ml).

Subsequent dilutions were made with pH7.4 buffer to attain varying concentrations of (4 to 20 µg/ml) and the absorbance were recorded at 224.5 nm against reagent blank. The standard curve for atenolol in pH 7.4 buffer was plotted against absorbance.

Similarly the standard curve for atenolol in methanol was carried out using the aforesaid procedure at 226 nm and the result are tabulated in table-2.
The standard curves for AT and AC were plotted with absorbance of AC and AT in methanol and pH 7.4 buffer against concentration were shown in figure (2 to 5). The value of correlation coefficient (r), slope (B) and intercept (A) for each standard curve were calculated. The minimum values of RSD (coefficient of variation) for the absorbance values and the ‘r’ values which were found very close to unity indicate the accuracy and the reproducibility of the analytical method used.

**Table-1 Standard Curve of Atenolol in Methanol (λmax 226 nm)**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Conc µg/ml</th>
<th>AVG</th>
<th>± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.000</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>0.131</td>
<td>0.00103</td>
<td>0.00788</td>
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<tr>
<td>3</td>
<td>8.00</td>
<td>0.264</td>
<td>0.00075</td>
<td>0.00285</td>
</tr>
<tr>
<td>4</td>
<td>12.00</td>
<td>0.402</td>
<td>0.00084</td>
<td>0.00208</td>
</tr>
<tr>
<td>5</td>
<td>16.00</td>
<td>0.536</td>
<td>0.00121</td>
<td>0.00225</td>
</tr>
<tr>
<td>6</td>
<td>20.00</td>
<td>0.672</td>
<td>0.00084</td>
<td>0.00124</td>
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</table>

\[ A = -0.0052, \quad B = 0.03385, \quad r = 0.99998 \]
Figure-2: Calibration Curve of Atenolol in methanol
Table-2 Standard Curve of Atenolol in Phosphate Buffer pH 7.4

($\lambda_{\text{max}}$ 224.5 nm)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Conc µg/ml</th>
<th>AVG</th>
<th>± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.000</td>
<td>0.00000</td>
<td>0.00000</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>0.043</td>
<td>0.00055</td>
<td>0.01273</td>
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<tr>
<td>3</td>
<td>8.00</td>
<td>0.083</td>
<td>0.00089</td>
<td>0.01077</td>
</tr>
<tr>
<td>4</td>
<td>12.00</td>
<td>0.124</td>
<td>0.00150</td>
<td>0.01214</td>
</tr>
<tr>
<td>5</td>
<td>16.00</td>
<td>0.163</td>
<td>0.00052</td>
<td>0.00316</td>
</tr>
<tr>
<td>6</td>
<td>20.00</td>
<td>0.203</td>
<td>0.00109</td>
<td>0.00539</td>
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</table>

A= 0.001524, =0.01011, r=0.99990
Figure-3: Calibration Curve of Atenolol in pH7.4 Buffer
### Table-3 Standard Curve of Aceclofenac in Methanol (λ_{max} 276 nm)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Conc µcg/ml</th>
<th>AVG</th>
<th>± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>0.119</td>
<td>0.0052</td>
<td>0.0043</td>
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<td>3</td>
<td>8.00</td>
<td>0.239</td>
<td>0.0084</td>
<td>0.0035</td>
</tr>
<tr>
<td>4</td>
<td>12.00</td>
<td>0.361</td>
<td>0.0197</td>
<td>0.0054</td>
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<tr>
<td>5</td>
<td>16.00</td>
<td>0.483</td>
<td>0.0160</td>
<td>0.0033</td>
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<tr>
<td>6</td>
<td>20.00</td>
<td>0.601</td>
<td>0.0075</td>
<td>0.0013</td>
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A = -0.00081, B =0.03011 , r = 0.99999
Figure-4: Calibration Curve of Aceclofenac in methanol
Table-4 Standard Curve of Aceclofenac in Phosphate Buffer pH 7.4 (λ\text{max} 277 nm)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Conc µcg/ml</th>
<th>AVG</th>
<th>± SD</th>
<th>RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>0.000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>2</td>
<td>4.00</td>
<td>0.087</td>
<td>0.00082</td>
<td>0.00938</td>
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<tr>
<td>3</td>
<td>8.00</td>
<td>0.172</td>
<td>0.00052</td>
<td>0.00300</td>
</tr>
<tr>
<td>4</td>
<td>12.00</td>
<td>0.260</td>
<td>0.00252</td>
<td>0.00973</td>
</tr>
<tr>
<td>5</td>
<td>16.00</td>
<td>0.345</td>
<td>0.00075</td>
<td>0.00218</td>
</tr>
<tr>
<td>6</td>
<td>20.00</td>
<td>0.435</td>
<td>0.00122</td>
<td>0.00282</td>
</tr>
</tbody>
</table>

A = -0.00081, B =0.03011 , r = 0.99999
Figure-5: Calibration Curve of Aceclofenac in pH7.4 Buffer
**Calculation of Displacement Value:**

The capacity of suppository from a specific mould is consistent but its volume may change due to densities of the medicaments usually distinct from the density of the base with which mould was calibrated to formulate products precisely, allowance should be made for the alteration in density of the mass due to added medicaments. The most convenient way of making this allowance is to use displacement value – the number of parts by weight of the medicament that expels one part by weight of the base.

Formula to calculate the displacement value:

1) Prepare and weigh six suppositories of base = a gm
2) Prepare and weigh six suppositories containing X % of Drug = b gm
3) Calculate the amount of base, ‘c’ gm and medicament ‘d’ gm in the six suppositories
4) Now a-c gm = weight of the base displaced by ‘d’ gm of the medicament
5) Displacement value = d/(a-c).

**Calculation of Displacement Value of Aceclofenac:**

1) Average weight of six suppositories containing only base
   
a= 6.83 gm

2) Average weight of six suppositories containing 10% of
   
Aceclofenac b = 7.09 gm

3) Amount of Vegetable present in the suppositories
4) Amount of Aceclofenac present in the suppositories

c = \frac{90 \times 7.09}{100}
\Rightarrow c = 6.381 \text{ gm}

d = \frac{10 \times 7.09}{100}
\Rightarrow d = 0.709 \text{ gm}

4) Vegetable oil displaced by aceclofenac

a-c = 6.83 - 6.381
\Rightarrow a-c = 0.449 \text{ gm}

Displacement value of Aceclofenac
\text{Displacement value of Aceclofenac} = \frac{d}{a-c}
\Rightarrow \frac{0.709}{0.449} = 1.58 \text{ gm}

1.58 \text{ gm of Aceclofenac displaces one gram of vegetable oil}

Calculation of Displacement Value of Atenolol:

1) Average weight of six suppositories containing only base

a = 6.83 \text{ gm}

2) Average weight of six suppositories containing 5% of Atenolol

b = 6.90 \text{ gm}

3) Amount of Vegetable present in the suppositories

c = \frac{95 \times 6.90}{100}
\Rightarrow c = 6.55 \text{ gm}

4) Amount of Atenolol present in the suppositories

d = \frac{5 \times 6.90}{100}
\Rightarrow d = 0.345 \text{ gm}
4) Vegetable oil displaced by aceclofenac

\[
\begin{align*}
d &= 0.345 \text{ gm} \\
a-c &= 6.83-6.55 \\
a-c &= 0.28 \text{ gm} \\
\text{Displacement value of Aceclofenac} &= \frac{d}{a-c} \\
&= \frac{0.345}{0.28} \\
&= 1.232 \text{ gm}
\end{align*}
\]

1.232 gm of Atenolol displaces 1 gm of Vegetable oil

**Calibration**: 103:

To avoid the variation in the mould capacity the moulds were calibrated before preparing the suppositories and were standardized. The softened base was poured into the mould and freezed, after freezing the suppositories were expelled from the mould and weighed individually and The total weight was considered as the capacity of the mold. This method has been repeated for different bases and the calibrated mold capacity was found in the range of 1.02-1.19 gm

**Suppository Preparation**: 78

Suppositories were prepared using fusion (pour moulding) method a) Fatty base (hydrogenated vegetable oil) was placed in beaker and was softened by heating, drug was thoroughly mixed with base with continuous stirring until the drug is completely dispersed in the base. Melted base along with the dispersed drug was transferred in to calibrated suppository mould (holing of suppositories is possible.
with immediate cooling hence should be avoided). b) Gelatin was taken in a beaker and small amount of water is added to it and heated on a magnetic stirrer after complete melting of the gelatin, accurately weighed amount of propylene glycol (PG), poly ethylene glycol-400 (PEG), drug along with the preservative (PG) was incorporated in the gelatin solution and was continuously stirred until a uniform mixture is obtained, the beaker is removed from the magnetic stirrer and initially cooled for few seconds to remove the air entrapment and then the solution was filled in to the calibrated moulds and immediately cooled because rapid cooling will result in good suppository if not cooled immediately the defective suppositories will be obtained with pitting and fissures. The drug loaded in each suppository is 50 and 200 mg of atenolol and aceclofenac respectively. The prepared formulations of suppositories were packed in aluminium foil and stored under refrigeration. The compositions of suppository formulation are tabulated in table-3 & 4.
Table-5: Formulation Chart of Hydrogenated vegetable oil-Atenolol Suppositories (30)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredients</th>
<th>ATVS&lt;sub&gt;0&lt;/sub&gt;</th>
<th>ATVS&lt;sub&gt;1&lt;/sub&gt;</th>
<th>ATVS&lt;sub&gt;2&lt;/sub&gt;</th>
<th>ATVS&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydrogenated vegetable oil (gm)</td>
<td>28.5</td>
<td>27.33</td>
<td>26.61</td>
<td>25.89</td>
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<td>2</td>
<td>Beeswax (gm)</td>
<td>--</td>
<td>1.44</td>
<td>2.16</td>
<td>2.88</td>
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<tr>
<td>3</td>
<td>Propyl paraben(mg).</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>Atenolol (mg)</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
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</tbody>
</table>
Table-6: Formulation Chart of Gelatin -Atenolol Suppositories (30)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredients</th>
<th>ATVS₀</th>
<th>ATVS₁</th>
<th>ATVS₂</th>
<th>ATVS₃</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Gelatin gm</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>2</td>
<td>Polyethylene Gylcol- 400 (gm)</td>
<td>---</td>
<td>03.00</td>
<td>04.5</td>
<td>06.00</td>
</tr>
<tr>
<td>3</td>
<td>Propylene glycol (gm)</td>
<td>18</td>
<td>15</td>
<td>12</td>
<td>9</td>
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<td>4</td>
<td>Propyl paraben(mg).</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>Distill Water (ml)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Atenolol (mg)</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
<td>1500</td>
</tr>
</tbody>
</table>
Table-7: Formulation Chart of Hydrogenated vegetable oil- Aceclofenac Suppositories (30)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredient</th>
<th>AVS-1</th>
<th>AVS-2</th>
<th>AVS-3</th>
<th>AVS-4</th>
<th>AVS-5</th>
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<tr>
<td>1</td>
<td>HDGN. Vegetable oil (gm)</td>
<td>23.58</td>
<td>23.91</td>
<td>24.24</td>
<td>24.6</td>
<td>8.30</td>
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<td>2</td>
<td>Beeswax (gm)</td>
<td>2.605</td>
<td>2.267</td>
<td>1.957</td>
<td>1.605</td>
<td>1.306</td>
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<tr>
<td>3</td>
<td>Propyl Paraben (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>4</td>
<td>Drug (mg)</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
<td>60000</td>
</tr>
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Table-8: Formulation Chart of Gelatin Aceclofenac Suppositories(30)

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Ingredient</th>
<th>APGS-1</th>
<th>APGS-2</th>
<th>APGS-3</th>
<th>APGS-4</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Gelatin (mg)</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
</tr>
<tr>
<td>2</td>
<td>Polyethylene Glycol 400 (mg)</td>
<td>---</td>
<td>3000</td>
<td>4500</td>
<td>6000</td>
</tr>
<tr>
<td>3</td>
<td>Propylene glycol (mg)</td>
<td>15000</td>
<td>12000</td>
<td>10500</td>
<td>9000</td>
</tr>
<tr>
<td>4</td>
<td>Propyl Paraben (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>5</td>
<td>Distill Water (ml)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Drug (mg)</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
<td>6000</td>
</tr>
</tbody>
</table>
EVALUATION OF SUPPOSITORY:

The formulated suppositories were subjected to evaluation as follows:

1. Appearance
2. Weight variation
3. Content uniformity
4. Liquefaction time/ temperature
5. Micro-melting range test
6. Disintegration test
8. Stability studies
10. Thermal analysis (DSC)

Appearance\textsuperscript{104}.

Formulated rectal suppositories were evaluated for physical appearance by visually inspecting the selected formulated suppositories by cutting along the longitudinal section and the surfaces were observed for uniform dispersion of drug within the base and for any pits or fissures by observing with the naked eye.

Weight uniformity\textsuperscript{105}.

The individual and the average weight of 20 formulated suppositories were calculated. As per I.P the prepared passes the weight variation test if no suppository deviates by 5% of average weight and not more than two suppositories should deviate by more than 7.5% of average weight.

Determination of Drug Content:
Ten suppositories were cut into tiny fragments. Tiny fragments corresponding to 100 mg drug weighed precisely and poured into 100ml volumetric flask containing 80ml pH 7.4 buffer by vigorous shaking for thirty minutes. The volumetric flask was filled up to capacity of 100ml with buffer. The absorbance was measured at 276nm for aceclofenac and 224.5nm for atenolol against the solvent blank.

The drug content was obtained from the calibration curve. Mean of three determinations was considered as average drug content of the formulated suppositories table-5 & 6.

**Liquefaction Time/ Temperature**

A pipette with narrow opening at the bottom and wide opening at the top was utilized for this test. The pipette was immersed in beaker containing hot water maintained at 37°C± 1°C in such a way that the bottom of the pipette touches the surface of hot water. The formulated suppository was inserted into the pipette from broad opening by carefully pushing the suppository downwards until it reaches the narrow part of the pipette fig-1. A plunger of glass was placed over the suppository from the broad end of the pipette. The period at which the glass rod nears the narrow end of pipette after entirely softening of suppository is termed as liquefaction time and the temperature at which the glass rod just move downwards (initially) is recorded as liquefaction temperature table-5.
Figure-1: Liquefaction Time and Temperature Apparatus (Fabricated)

Micro melting range test:
This is test is performed by using capillary tube of 10 cms long. the formulated suppository were inserted into the capillary tube upto 1cm and inserted in to beaker containing water which was heated by means of a heating mantle as the temperature is raised the solid sample will liquefy, the temperature at which the solid liquefy is recorded as micro melting range of suppository table 5 & 6.

**Dissolution Study:**

The dissolution study of aceclofenac and atenolol from the prepared suppositories was conducted using tablet dissolution tester with a basket stirrer. Phosphate Buffer pH7.4 at 37°± 0.5°Centigrade was used as the medium of dissolution and the basket was rotated at 50 rpm. Single suppository was utilized in each analysis. 5 ml of the sample were taken out with the aid of a syringe equipped with a pre-filter at convinient time interval and instantaneously replaced with same amount of medium. The absorbance of the sample was measured at 276nm for aceclofenac and 224.5nm for atenolol using spectrophotometer after appropriate dilutions. The studies were carried out in triplicate.

The results of dissolution rate data for all the prepared suppositories were evaluated according to the following equation to analyze the drug release characteristic:

2. First Order release
3. Higuchi's equation for diffusion controlled release
4. Peppa’s plot to determine the release mechanism (fickian or non fickian)

1. **Zero-Order release:**
when the rate of release of drug is unaided of the mass of drug remained in the dosage formulation and steady over a period of time, it is said to be zero-order release. This is mathematically expressed as follows,

\[ A = A_0 - K_0 t \quad \ldots 1 \]

Where

- \( A \) = amount of drug time ‘t’
- \( A_0 \) = concentration of the drug at zero minutes
- \( K_0 \) = Rate Constant per hour

When a graph of percent cumulative drug release against the time is drawn, linearity of graph represents the zero order liberation of the active material from the formulation, and the slope ‘n’ represents the rate constant.

2. **First Order release:**

When the release rate is symmetrical to the first power of drug in the dosage form and expressed mathematically in the form of equation (2). Then release is said to be first order with respect to drug in the dosage form.

\[ \log C = \log C_o - \frac{K_1}{2.303} t \quad \ldots 2 \]

Where

- \( C \) = concentration of drug remaining at time ‘\( C_0 \) = Initial amount of drug.
- \( K \) = Rate Constant (per hour)

If a straight line is obtained after plotting a graph of logarithm of cumulative percent drug remained against time the drug release is said to be following first order release. By multiplying slope ‘n’ with 2.303 the rate constant ‘K’ is obtained.
3. Higuchi’s Equation (Model) for Diffusion Controlled release

The mechanism of drug release from various drug delivery system is either by diffusion or by dissolution this can be determined by using the following Higuchi’s equation.

\[
Q = \left[ \frac{D \epsilon}{\tau} (2A - \epsilon C_s) C_s t \right]^{1/2}
\] ...3

Where

\( Q \) = concentration of released drug in time \( t \)

\( D \) = diffusion constant of the drug in medium for dissolution.

\( A \) = concentration of drug in unit mass of matrix.

\( C_s \) = drug solubility in release medium.

\( \epsilon \) = Porosity of the matrix.

\( t \) = Time.

Assuming that \( D, C_3 \) and \( A \) are constant. Then equation-3 may be simplified as follows

\[
Q = k t^{1/2} \quad ............... (4)
\]

On plotting a graph of ‘Q’ the amount of released against the square root of time the linearity of the graph indicates the diffusion controlled release of the drug from the matrix devices and the slope ‘n’ is equal to the release rate constant

4. Peppa’s plot to determine the release mechanism (fickian or non fickian)

In order to understand the mode of release of drug from swellable matrices, the data were fitted to the following Peppa’s.

\[
Q = K_1 t_n 
\] ...5

\( Q \) = amount of drug released in time \( t \).

\( K \) = kinetic constant
n= slope representing release mechanism$^{83}$.

If the slope is less than or equal to 0.45 then release said to follow ficickian transport mechanism and non-fickian if slope greater than 0.45 but less than 0.89$^{108}$.

The dissolution rate data are presented in tables from 7 to 25 for the all the formulations.

**Stability Study:**

This was executed at a temperature of 25º ±3ºC/ 60± 5% Relative humidity for a span of 180 days on the suppository formulations (ATPGS3 and AVS3). 15 suppositories were separately packed in aluminum foil, placed in glass bottle and placed in a humidity chamber for 180 days. Sample is evaluated for drug content uniformity at an interval of 30 days and at the end of 180 day span dissolution test was carried to determine the drug release profile. The data is presented in table-38&39.

**Thermal Studies (DSC):**

Differential scanning calorimetry is considered to be a modern and accurate tool for exploring the process of melting, crystallization, evaporation, phase equilibria, sublimation, glassy and polymorphic transformation, dehydration, isomerization, adsorption and substance degradation. The sample crystallization is confirmed by a sharp peak on the thermogram, indicating the exothermic nature of the transformation. On the other hand, the process of melting characteristics for the sample with the crystalline structure is detected as endothermic peak on the thermogram.

**Surface Texture (SEM):**
The suppositories were viewed under scanning electron microscope. For SEM examination a part of the suppository was laminated with gold in ion sputtering machine and mounted directly on the SEM sample stub using sticking tape and the instrument was run at 20KV and then the suppository were scanned. The SEM photographs of promising formulations ATPGS-3 and AVS3 are demonstrated