9.0 IN-VIVO RAT STUDY

9.1 Animal Study Protocol

Application for Permission for Animal Experiments

Part-A

Name and address of the establishments

Permission for Animal Experiments: Govt. College of Pharmacy, Karad.

1. Name and address of the establishments:
   Govt. College of Pharmacy, Karad.
   Registration no. and date of registration: 209/CPCSEA. 1st June 2000.

2. Name and address and registration no. of breeder from whom animal to be acquired for experiments mentioned in part B & C:
   Govt. College of Pharmacy, Karad

3. Place where the animals are presently kept:
   Govt. College of Pharmacy, Karad

4. Place where the experiment is to be performed:
   Govt. College of Pharmacy, Karad

5. Date on which the experiment is to be commenced: 02/04/2012

6. Duration of experiment: 1 week
Part-B

1. Project title: Engineering of Pharmaceutical Particles for Advanced Drug Delivery System

Chief investigator:
   a) Name: Dr. S. S. Patil
   b) Designation: Principal
   c) Dept. /Div: A.M. College of Pharmacy, Peth Vadgaon, Dist.-Kolhapur
   d) Mobile no.: 09421204393

2. List of names of all individuals authorized to conduct procedures under this proposal
   a) Mr. Bhokare Kuldeep Kuber

3. Funding Source: Self

4. Duration of project
   a) Number of days: 1 week
   b) Date of initiation: 02/04/2012
   c) Date of completion: 09/04/2012

5. Study objectives
   To study the some pharmacokinetic parameters of particles engineered hypolipidaemic drugs (Atorvastatin calcium & Fenofibrate).

6. Animal required
   a) Species: Rats
   b) Age/Weight: 1.0 - 1.5 yrs / 250 - 300 g
   c) Gender: Male
   d) Numbers to be used: 12
7. Rational for animal usage

a) Why animals are needed for this study?

- To reduce the cost and ethics involved in the usage of healthy human volunteers as a trial subject.
- To determine oral bioavailability of particle engineered hypolipidaemic drugs.

b) Why are particular species selected are required?

- Rat is the suitable animal model for oral pharmacokinetic studies.

c) Are similar experiments conducted in the past? If so, the number of animals and results obtained in brief?

- No

8. Description of the experiment to be used.

- Study design: Parallel
- Number of groups: Two
- Number of animals in group: Six
- Selection of subject: Randomization
- Study condition: Fast state, Oral Pharmacokinetics
- Reference product: Pure drugs
- Test product: Developed spherical crystals
- Analytical method: HPLC
- Analyte of interest: hypolipidaemic drugs
- Sampling Points: 07
- Biological matrix: Blood
- Method of sample collection: Retro orbital vein

9. Does the protocol prohibit use of anesthetic or analgesic for the conduct of painful procedure?

- No such procedure is included in protocol

10. Will the survival surgery be done?

- Not applicable
11. Method of disposal of post experimentation?

✓ Not applicable

12. Animal transportation method if extra institutional transport is envisaged.

✓ Not applicable

13. Use of hazardous agent (use of recombination DNA based agent or protocol human pathogen requires documentation approval of Institutional Biosafety Committee (IBC).

For each category, the agents and the bio safety level is required. Appropriate therapeutic measure and the mode of disposal of contaminated food animal waste carcasses must be identified.

a) Radio nuclides, b) Biological agents, c) Hazardous chemicals or drugs, d) Recombinant DNA, e) Any other

Copy of IBC to be attached as hazardous agent is used: Not applicable.
INVESTIGATOR’S DECLARATION

1. I certified that the proposed study herein is not unnecessary duplicate of previous report research.
2. I certify that all individuals working on this proposal and experimenting on the animals have been trained in animal handling procedure.
3. For the procedure listed in item 11, I certify that I have the pertinent scientific literature and have no valid alternative to any described herein, which may cause pain or distress.
4. I will obtain approval from IAECE/CPCSEA before initiating any significant changes in this study.
6. Name and address and registration no. of breeder from whom animal to be acquired for experiments mentioned in part B&C: Govt. College of Pharmacy, Karad.
7. Place where the animals are presently kept: Govt. College of Pharmacy, Karad.
8. Place where the experiment is to be performed: Govt. College of Pharmacy, Karad.
9. Date on which the experiment is to be commenced: 02/04/2012
10. Duration of experiment: 1 week

Signature

Mr. Bhokare Kuldeep Kuber (Research Student)  
Dr. S. S. Patil (Research Guide)  
A.M.College of Pharmacy, Peth Vadgaon,
CERTIFICATE

CPCSEA/IAEC Registration no- 209/GO/a/2000/ CPCSEA

This is to certify that the following protocol entitled:

"Engineering of Pharmaceutical particles for advance drug delivery system"

[Reference no.- CPCSEA-IAEC/2011-MAR/04] submitted by Mr. Kulddep K. Bhokare, under guidance of Dr. S. S. Patil [Research guide for Ph.D under Shivaji University Kolhapur] have been approved by Institutional Animal Ethics Committee of this institution in its meeting held on 16th March 2012.

[Prof. K. B. Burade]
Chairman IAEC

PRINCIPAL
Govt. College of Pharmacy
Karad
9.2 Study procedure
The animals used for in vivo experiments were adult male rats (n=6) weighing 250-350 g. The animals were kept under standard laboratory conditions at a temperature of 25 ± 2°C and relative humidity (55 ± 5%). The animals were housed in animal cages, six per cage, with free access to standard laboratory feed (Lipton feed, Mumbai, India) and water ad libitum. 6 mg/kg body weight of the Atorvastatin Calcium and 48 mg/kg body weight of the fenofibrate were used in the study. Developed spherical crystals (AFCPLM) contains atorvastatin calcium (20 mg) & fenofibrate (160 mg) combination [Test product] and physical mixture of pure drug atorvastatin calcium (20 mg) & fenofibrate (160 mg) [Reference product] were taken and mixed with 40 mL of double distilled water. Each rat in this group was given 3.0 mL using oral feeding needle. The blood sampling was carried out for around 24 hours and 7 samples of blood were taken from each animal in the group. Blood was withdrawn from the retro orbital vein at 0, 1, 2, 4, 8, 12 & 24 hours in micro centrifuge tubes and centrifuged at 7000 rpm for 15 min. The serum was separated and stored at -20 °C until drug analysis was carried out using HPLC method. Pharmacokinetic parameters were calculated by using the different statistical software. All pharmacokinetic (PK) parameters (tmax, Cmax, AUC0-t,) were calculated individually for each subject in the group and the values were expressed as mean ±SD.

9.3 Bio-analytical Method
Instrument used: HPLC system with UV detector
Column: C 18, 250 x 4.6 mm, 5 μm (Inertsil ODS 3)
Mobile Phase: 0.1% Triethylamine: Methanol (15:85) pH adjust to 4.5 with dilute Ortho Phosphoric acid
Flow rate: 1.0 mL/min
Column temperature: 30°C
Wavelength: 246 nm
Injection volume: 20 μL
Run time: 15 minutes
Retention time: Atorvastatin - About 4.0 min, Fenofibric acid – About 10.0 min
Table 9.1: Pharmacokinetic parameters of reference and test sample of Atorvastatin & fenofibrate in rats (n=6)

<table>
<thead>
<tr>
<th>Sampling time (hours)</th>
<th>Plasma concentration (ng/mL)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference Product</td>
<td>Test Product</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATR</td>
<td>FNO</td>
<td>ATR</td>
</tr>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.5</td>
<td>4217.39 (± 145.3)</td>
<td>8147.39 (± 362.6)</td>
<td>4937.42 (± 136.8)</td>
</tr>
<tr>
<td>1.0</td>
<td>4884.91 (± 189.3)</td>
<td>23784.68 (± 263.3)</td>
<td>6782.33 (± 163.7)</td>
</tr>
<tr>
<td>2.0</td>
<td>4327.48 (± 204.6)</td>
<td>46862.17 (± 567.7)</td>
<td>5261.56 (± 189.1)</td>
</tr>
<tr>
<td>4.0</td>
<td>2667.57 (± 167.2)</td>
<td>79258.78 (± 6219.2)</td>
<td>3682.37 (± 128.9)</td>
</tr>
<tr>
<td>12.0</td>
<td>1235.43 (± 102.7)</td>
<td>47892.41 (± 1239.1)</td>
<td>2062.40 (± 93.1)</td>
</tr>
<tr>
<td>24.0</td>
<td>529.79 (± 89.3)</td>
<td>924.68 (± 125.0)</td>
<td>306.73 (± 64.8)</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>4884.91 (± 478.5)</td>
<td>79258.78 (± 2479.4)</td>
<td>6782.33 (± 392.4)</td>
</tr>
<tr>
<td>AUC 0-t (h.ng/mL)</td>
<td>149476.9 (± 4682.3)</td>
<td>3929380.8 (± 12638.5)</td>
<td>207468.8 (± 3793.7)</td>
</tr>
<tr>
<td>AUC 0-∞ (h.ng/mL)</td>
<td>154962.2 (± 6259.3)</td>
<td>3933553.7 (± 14736.4)</td>
<td>209747.1 (± 4591.2)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>1.1 (± 0.3)</td>
<td>4.3 (± 0.7)</td>
<td>0.9 (± 0.2)</td>
</tr>
</tbody>
</table>
Figure 9.1: In- vivo PK Study

Mean plasma concentration- Time profile ATR

Figure 9.2: In- vivo PK Study

Mean plasma concentration- Time profile FNO
9.4 CONCLUSION

In-vivo study in rat shows $C_{\text{max}}$, $AUC_{0-1}$ and $AUC_{0-\infty}$ of both the drugs (Atorvastatin calcium & fenofibrate) of test samples were enhanced as compared to reference samples. Inter subject variability also significantly reduced of test samples than that of reference samples. Also $T_{\text{max}}$ of both the drugs (Atorvastatin calcium & fenofibrate) of test samples were achieved faster as compared to reference samples. Improvement in PK data of developed spherical crystals (AFCPLM) was due to the spherical crystallization of drugs and incorporation of hydrophilic polymer (poloxamer) in to spherical crystallization process. Therefore in-vivo study was demonstrated that the improvement in oral bioavailability of combination of atorvastatin calcium and fenofibrate can obtain by spherical crystallization method.

9.5 REFERENCES


