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# IN VIVO STUDIES & SEM OF CONTROLLED POROSITY OSMOTIC PUMP (CPOP) TABLET OF ATENOLOL

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## ABSTRACT

The objective of the present research was to explain in detail about in – vivo studies, to determine the pharmacokinetic parameters which were estimated by comparing the marketed Atenolol tablets (Texanolol-AM) with the optimized CPOP tablet of Atenolol in rabbits and by analysing in UV Spectrophotometer. The $C_{max}$, $t_{max,}$ AUC, AUMC and MRT were estimated. The animal testing is considered to be a major element of in – vivo research. This study helps in observing the overall effects of an experiment. The bioavailability study is performed to characterize the plasma concentration versus time profile. CPOP works on the principle of osmosis releasing drug at zero order kinetics so better control over drugs. In vivo performance is possible releasing the drug after an initial lag. The core tablet consists drug and osmogen in optimum quantity with other excipients. The surface morphology of coating membrane of the optimized formulation was examined by using Scanning Electron Microscopy (SEM) before and after dissolution, it was observed microporous pores are formed after dissolution.

**KEYWORDS:** Atenolol, Bioavailability, In vivo, Pharmacokinetic, SEM, Texanolol-AM,

## INTRODUCTION

A key goal for developing oral controlled dosage form is good understanding of in - vivo studies. The pharmacological responses can be related directly to the plasma levels thus bioavailability is defined as the rate and extent of absorption of unchanged drug from its

dosage form. The rate or rapidity with which a drug is absorbed is an important consideration when a rapid onset of action is considered. The aim of this work was to evaluate the bioavailability of CPOP tablet of Atenolol.

The pharmacokinetics is defined as the kinetics of drug absorption, distribution, metabolism and excretion (KADME) and their relationship with the pharmacological, therapeutic or toxicological responses. There are two aspects of pharmacokinetics studies.

1. Theoretical aspect - It involves development of pharmacokinetic models to predict drug disposition after its administration. Statistical methods are commonly applied to interpret data and assess various parameters.
2. Experimental aspect - which involves development of biological sampling techniques, analytical methods for measurement of drug and metabolites concentration in biological samples and data collection and evaluation.

## MATERIALS AND METHODS

Atenolol (API) and Mannitol was procured from SD Fine Chemicals Mumbai. Citric acid, Starch, Magnesium stearate, Colloidal silicon dioxide, Tartrazine yellow were purchased from Yarrow Chem Products, Mumbai. Eudragit (RLPO), Sodium Chloride, Sorbitol, Ethanol, Acetone and PEG 400 were obtained from Dr. Reddy's Laboratories, Hyderabad. All the chemicals and solvents were of analytical grade.

## METHODS

### 1. IN –VIVO STUDIES

**Objective**

The pharmacokinetic parameters were studied by comparing the Marketed Atenolol tablets (Texanolol - AM) with the optimized formulation in Rabbits by withdrawing blood samples and analysing in UV Spectrophotometer. The optimized formulation is provided in Table 2 and dissolution profile of optimized formulation is provided in Table 3.

**Preparation for the In vivo test**

**Selection of animals**

The healthy Male Albino Rabbits are selected for this study.

**Housing and Feeding conditions**

Animals are housed individually in the animal house, the temperature in the experimental room is maintained at 25$^{o}$ C and the relative humidity is 40%. Artificial lighting is provided for 12 hours and 12 hours dark with free water feeding.

**Table 1: Grouping of animals.**

| S. No | Grouping | Treatment |
|---|---|---|
| 1. | GROUP A (2 Rabbits) | Fed Marketed Texanolol – AM |
| 2. | GROUP B (2 Rabbits) | Fed Formulation of Cpop-Atenolol. |

**Grouping of Animals, Administrating tablets & Collection of blood samples**

The rabbits were divided into two groups and fasted with only water for 10 hours before the start of the study. The group A animals were administered with 100 mg of marketed Texanolol - AM tablet using applicator with 4 to 6 ml of water. The group B animals were fed with optimized formulation. The blood is withdrawn from the Lateral saphenous vein of rabbit through syringe with 24 gauge needle at time intervals of 1, 2, 4, 8, 12, 16, 20, 24 and 28 hours respectively. After the administration of the dose 1 ml of blood is withdrawn at the specified time intervals.

**Analytical Procedure**

The samples are collected and subjected to centrifugation at 3000 rpm speed for 10 minutes to separate plasma, from this separated plasma 250 (µl) was used and alkalified with 50 (µl) 0.1N sodium hydroxide and mixed. Ethyl acetate 1 ml was added to the mixture and mixed for 10 minutes for extracting the drug. The samples were again centrifuged at 3000 rpm for 5 minutes to separate the organic layer, the organic layer was separated and evaporated to dry. The remaining amount was mixed with 3 ml of phosphate buffer pH 7.4, mixed for 5 minutes and analysed at 225 nm in UV Spectrophotometer. The Atenolol in plasma was determined. The parameters that are estimated are $C_{max}$, $t_{max,}$ AUC, AUMC and MRT and the results are tabulated in Table 5. The concentration of Atenolol in plasma of both marketed product and optimized CPOP formulation is tabulated in Table 4 and curves plotted are provided in Figure 3. The formulae used to calculate the parameters are.

$$\textbf{AUC} = \frac{(t_2 - t_1)(C_1 + C_2)}{2} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \frac{(t_n - t_n)(C_n + C_n)}{2}$$

$$\textbf{AUMC} = 1/2\,(t_2 - t_1)(C_1t_1 + C_2t_2) \ldots\ldots\ldots\ldots\ldots\ldots\ldots 1/2\,(t_2 - t_1)(C_n t_n + C_n t_n)$$

**MRT** = AUMC / AUC.

The methods useful in quantitative evaluation of bioavailability are categorised by pharmacokinetic methods and pharmacodynamic methods. Pharmacokinetic methods are widely used and based on the assumption that these parameters reflects the therapeutic effectiveness of a drug. The three parameters of plasma level-time studies which are considered important for determining bioavailability are.

**$C_{max}$:** The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systematically to provide a therapeutic response. It is a function of both the rate and extent of absorption. It will increase with an increase in the dose, as well as with an increase in the absorption rate. The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration or peak height concentration or maximum drug concentration. The peak plasma level depends upon dose administered, rate of absorption and rate of elimination.

**$t_{max}$:** The peak time that gives an indication of the rate of absorption. It decreases as the rate of absorption increases. It is called as time of peak concentration. It is important in assessing the efficacy of drugs used to treat acute conditions.

**AUC:** The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation. It is important for drugs that are administered repetitively for treatment of chronic conditions. It is most important parameter in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption.

**MRT:** It is defined as the average amount of time spent by the drug in the body before being eliminated. It is Statistical moment analogy of half life $t_{1/2}$.

**AUMC:** It is obtained from a plot of product of plasma drug concentration and time (C. t) versus time "t" from zero to infinity. AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity.

**Mechanism of action of CPOP drug delivery system**

CPOP contains water soluble additives in coating membrane which after coming in contact with water, dissolves resulting in in-situ formation of microporous membrane. The resulting

membrane is substantially permeable to both water and dissolved solutes and the mechanism of drug release from these systems is primarily osmotic, with simple diffusion a minor role.

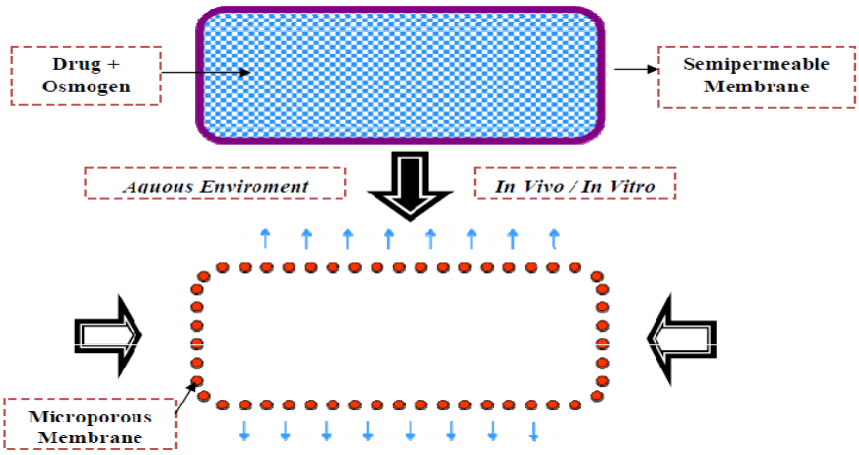



**Figure 1: Mechanism of action of CPOP.**

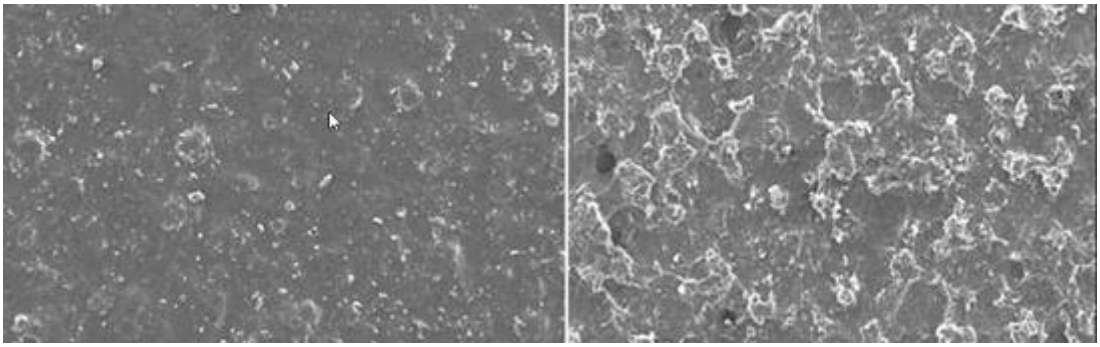
## 2. SCANNING ELECTRON MICROSCOPY (SEM)

It was invented by Manfred Von Ardenne, it is used to study Surface Morphology. It works on the principle of scattering of electrons on the sample. The electrons are released from electron gun and it heats the sample, few electrons are either absorbed or reflected or back scattered or are excited, this releases another secondary electrons which are detected by the detector and are detected by SEM. The electron beam scans object in Raster Scan pattern i.e side by side. The processing of secondary electrons is done and 3D image is obtained.

To evaluate the surface morphology of coating membrane the optimized formulation was examined by using SEM before and after dissolution. The membranes were dried at 40$^{o}$C for 10 hours and stored in wax paper sheets in desiccator for further evaluation. The membranes should be coated with gold in vacuum as it is bombarded with electrons and it has to withstand high vacuum during the scan. Before dissolution the surface is smooth until it comes into contact with water, after dissolution the water enters into due to formation of micropores which is seen in the images. The microporous pores are formed because of the pore formers or channeling agents i.e (sodium chloride and sorbitol) in the formulation. The SEM model JSM – 6380 & Jeol Manufacturer is used in this research.

## RESULTS

The images of surface morphology of the CPOP tablet before and after dissolution are provided in Figure 2.



**Before dissolution** **After dissolution**

**Figure 2: Surface morphology images of the CPOP tablet before and after dissolution.**

## RESULTS AND DISCUSSIONS

**Table 2: Composition of optimized formulation.**

| **Formulation (mg/tablet)** | **Optimized formulation** | **Coating composition** | |
|---|---|---|---|
| Atenolol | 100 | Eudragit RLPO (g) | 2.5 |
| Mannitol | 40 | Sodium chloride (mg) | 5 |
| Citric acid | 3 | Sorbitol (mg) | 1 |
| Starch | 3 | PEG 400 (g) | 2 |
| Magnesium stearate | 2.5 | Ethanol (ml) | 10 |
| Colloidal silicon di oxide | 1.5 | Acetone (ml) | 80 |
| **Total** | 150 | Tartrazine Yellow | q.s |

**Table 3: Dissolution profile of optimized formulation.**

| **S.NO** | **Time (Hours)** | **Optimized Formulation** |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 1 | 3.5 |
| 3 | 2 | 7.1 |
| 4 | 4 | 14.7 |
| 5 | 8 | 28.2 |
| 6 | 12 | 42.5 |
| 7 | 16 | 56.2 |
| 8 | 20 | 70.7 |
| 9 | 24 | 84.1 |
| 10 | 28 | 97.6% |

**Inference**: The drug release by dissolution upto 28 hours was 97.6%.

**Table 4: Concentration of Atenolol in plasma (µg/ml).**

| Time (Hours) | Marketed Texanolol-AM | Optimized Formulation |
|---|---|---|
| **0** | **0** | **0** |
| 1 | 75.22 ± *0.25* | 8.45 ± *0.24* |
| 2 | 95.43 ± *0.18* | 19.22 ± *0.78* |
| 4 | 84.92 ± *0.55* | 45.54 ± *0.61* |
| 8 | 60.47 ± *0.12* | *60.29*± *0.22* |
| 12 | *9.02* ± *0.60* | *71.09* ± *0.49* |
| 16 | *1.02* ± 0.01 | 97.22 ± *0.21* |
| 20 | 0 | 80.54 ± *0.26* |
| 24 | 0 | 50.43 ± *0.82* |
| 28 | 0 | 32.84 ± *0.01* |

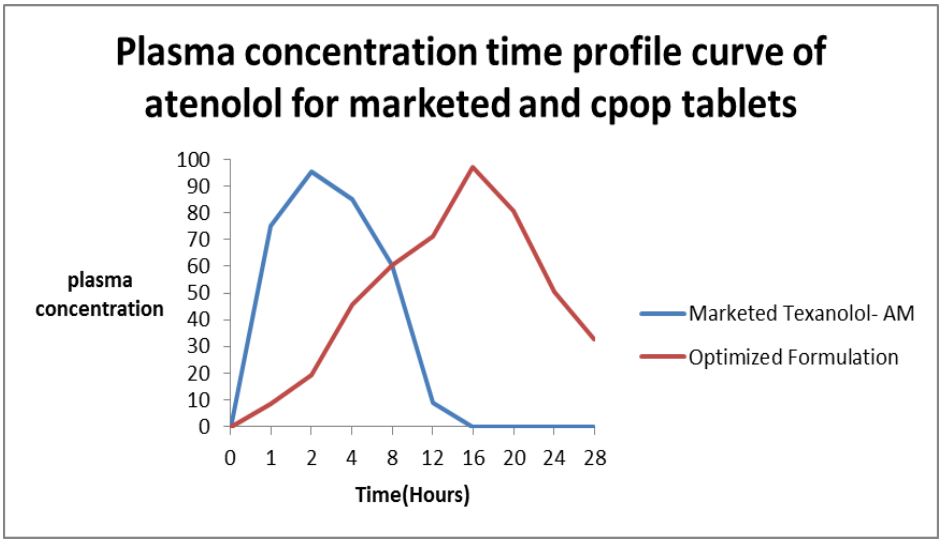



**Figure 3: Plasma concentration time profile curves of Atenolol for marketed & CPOP tablet.**

**Table 5: Results of pharmacokinetic parameters estimated.**

| S. No | Parameters | Marketed Drug Texanolol - AM | Optimized Formulation |
|---|---|---|---|
| 1. | $C_{max}$ (µg/ml) | 95.43 | 97.22 |
| 2. | $t_{max}$ (Hours) | 2 | 16 |
| 3. | AUC (h. µg/ml) | 755.16 | 1677.85 |
| 4. | AUMC (h µg/ml) | 3810.95 | 9510.8 |
| 5. | MRT (Hours) | 5.04 | 5.66 |

**OBSERVATION**

The optimized CPOP tablet of Atenolol released 97.6% of drug upto 28 hours. The in-vivo studies were carried out and the pharmacokinetic parameters estimated were $C_{max}$, $t_{max,}$ AUC,

AUMC and MRT for both marketed and optimized formulation showed good in vitro and in vivo correlation.

## CONCLUSION

The successful pharmaceutical development is the perfect understanding of the in- vivo and in – vitro performance of dosage form. In this study the drug release followed zero order kinetics independent of external factors and GIT. It can be concluded that by employing osmotic technology drug release is controlled. It is also a promising approach for the treatment of hypertension and management of cardiovascular diseases associated with hypertension. The extent of absorption is of special significance in the treatment of hypertension.

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