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Pharmacokinetic Evaluation of Bilayered Buccoadhesive Compacts Containing Enalapril Maleate Using Rabbits

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ABSTRACT

Enalapril maleate is an angiotensin converting enzyme (ACE) inhibitor, used mainly in the treatment of hypertension and angina pectoris. It has low bioavailability (40%) due to hepatic first pass metabolism. The study was designed to develop bilayered buccoadhesive compacts of Enalapril maleate by direct compression method to reduce the dose and to improve its bioavailability. Mucoadhesive polymers such as HPMC 4KM, HPMC15KM, HPMC 15KM, HPMC 100KM, carbopol 934P were used in core layer, and ethyl cellulose used as an impermeable backing layer for the preparation of bilayered buccoadhesive compacts. Optimized formulation of buccoadhesive compacts (FEM_2) was subjected to *in vivo* bioavailability studies by using rabbits. Bilayered buccoadhesive compacts containing the mixture of Carbopol 934P and HPMC K4M in the ratio 1:1 (FEM_2) had reported the maximum percentage of *in vitro* drug release in 8 hrs. The pharmacokinetic parameters were found to be C_{max} of 46.44 ng/ml, t_{max} of 4.0 h, $AUC_{(0-24)}$ of 303.635 ng.ml/h, elimination rate constant(Ke) of 0.67 h, MRT of 4.74 h, $t_{1/2}$ of 4.95 h and absorption rate constant (Ke) of 10.994 h^{-1}. The designed buccal delivery of enalapril maleate in rabbits showed a significant improvement in the bioavailability of enalapril maleate from compacts when compared to oral route.

1. INTRODUCTION

Amongst the various routes of drug delivery, the oral route is most preferred by patient and the clinician alike because of the significant interest to their presystemic metabolism or any instability in the acidic environment associated with the oral environment[1]. The buccal mucosa offers excellent opportunities for the delivery of both local and systemically active drugs [2,3]. Compared to other absorptive mucosa, buccal mucosa is considered as potential site for drug administration, rich blood supply, lower enzymatic activity of saliva, better patient acceptance are some other prominent meritorious visage of buccoadhesive systems [4].

Buccal drug delivery provides an alternative route to the oral route of drug administration, particularly in overcoming the deficiencies confederate with the later mode of dosing [5]. Problems such as first pass metabolism and drug degradation in the GIT environment can circumvented by administering the drug via buccal route. Oral cavity is considered as the easiest accessible route of administration for self medication and the dosage form can be removed easily at any time from the buccal cavity in case of toxicity. It is also possible to administer drugs to patients who cannot be dosed orally via this route[6,7].

Enalapril maleate is angiotensin converting enzyme (ACE inhibitor) used to treat hypertension and heart failure. It inhibits angiotensin converting enzyme, which converts the angiotensin-I to angiotensin-II stimulates the synthesis and secretion of aldosterone via a potent direct vasoconstrictor effect [8]. Enalapril maleate shows low oral bioavailability of 40 % due to the extensive first pass metabolism [9,10] and pK_a of 3 which makes it a suitable candidate for oral mucosal drug delivery system[11].

In the present investigation an attempt has made to design buccoadhesive bilayered compacts of Enalapril maleate by using HPMC, Carbopol 934P and ethyl cellulose by direct compression method to reduce the dose, to achieve controlled release and improve its bioavailability.

2. MATERIALS AND METHODS

2.1 Materials

Enalapril maleate obtained gift sample from Apotex Labs Pvt Ltd. Bangalore, HPMC 4KM, obtained gift sample from Apotex Labs Pvt Ltd. Bangalore, Colorcon Pvt. Ltd. Madgoa, Goa, HPMC 15KM and HPMC 100KM obtained gift sample from Apotex Labs Pvt. Ltd. Bangalore

and Colorcon Pvt. Ltd. Madgoa, Goa, Carbopol 934P obtained gift sample from Remedex Pharma Pvt. Ltd., Bangalore/ Corel Pharma Pvt. Ltd. Ahmedabad, Ethyl Cellulose obtained a gift sample from Colorcon Pvt. Ltd. Madgoa, Goa.

2.2 Methods

2.2.1 Preparation of bilayered buccoadhesive compacts of Enalapril maleate

Bilayered buccoadhesive compacts of Enalapril maleate were formulated as mentioned in Table 1. The compacts contain two layers, i.e. core layer and backing layer. Core layer was prepared by transferring a specified quantity of lactose, microcrystalline cellulose pH 102, mannitol, carbopol 934P and HPMC with different grades to the mortar and pestle and mixed well. Enalapril maleate was added to the above mixture and mixed well. Then specified quantity of magnesium stearate was added to the above mixture and mixed well. From the above core layer mixture specified quantity of powder was transferred to the 8 mm die cavity of compression machine and compressed lightly. Then add a specified quantity of the backing layer powder containing ethyl cellulose, magnesium stearate and coloring agent to the above core layer compact and compressed [12,13].

2.2.2 Preparation of calibration curve by HPLC

Calibration curve was prepared by spiking the drug in the plasma. From this different aliquots were made. A calibration curve was prepared by transferring 0.1, 0.2, 0.4, 0.8, 1.0ml aliquots into 10 ml volumetric flask and the volume was made up to the mark. Final concentrations obtained for Enalapril maleate were 10, 20, 40, 80, and 100 ng/ml in methanol to get the linearity.

2.2.3 Pharmacokinetics study design and protocol

The study was conducted with the approved *in vivo* study design and protocol (Protocol approval number: KSHEMA/AEC/28/2011 & IAEC/ABMRCP/2012-13/43) and as per guidelines prescribed by the Institutional Animal Ethics Committee. Six New Zealand White male rabbits with a mean weight of 2.5±0.24 kg were selected for the study, (n=3). Animals were issued 6 days prior to experimentation for acclimatization and were kept on standard pellet diet and water ad libitum. Before starting the experimentation (8-10 h) food was stopped to all animals. Food and water were not given to animals up to 2 h after the start of the study. To study the oral pharmacokinetics of Enalapril maleate, 1 ml of solution containing 0.194 mg of Enalapril

maleate in 40 % v/v polyethylene glycol 400 in water was administered to rabbits (n=3) using an oral catheter. The catheter was rinsed with 5 ml of 40 % v/v polyethylene glycol 400 in water to ensure complete dosing. The formulated compact with 0.194 mg of Enalapril maleate was premoistened by dipping the compact in distilled water for 5 sec. The mouth of a rabbit (n=3) was opened using specially designed mouth restrainers and the pre-moistened compact was pressed gently against mucosal lining of the cheek using forceps for 1 min to ensure adhesion. Each rabbit was dosed with specific doses of Enalapril maleate (0.194 mg) without taking the weight of the rabbit into consideration. The blood samples (1 ml) were withdrawn from the marginal ear vein of rabbits using a 21 G needle for each study. Samples were withdrawn before administration of doses and after 1.0, 2.0, 3.0, 4.0, 8.0, 12.0, 24.0 h of dosing. The collected blood was harvested for 45 min at ambient temperature and centrifuged at 2000 rpm for 20 min. The clear supernatant serum layer was collected and stored at - 40 °C until analysis. Frozen serum samples were thawed at ambient temperature (25±2 °C) for at least 60 min. A simple and efficient one-step process was employed to isolate Enalapril maleate from rabbit serum. To aliquot of 500 µl of serum samples, 1.5 ml of methonol was added and vortexed for 1 min to ensure complete precipitation. Samples were vortexed again for 1 min and centrifuged at 10,000 rpm at 4 °C for 20 min.

The above protein-free plasma was mixed with 50 µl Enalapril maleate acting as internal standard and 20 µl was injected through syringe filter into an isocratic HPLC with UV detector. The column employed for the study was C18 (250 x 4.6mm, particle size: 5µm, High pressure gradient, Detector: UV Wavelength: 240nm). The mobile phase consisted of potassium dihydrogen orthophosphate: methanol (0.005M) pH 3(30:70) and flow rate was adjusted to 1.0 ml/min.

From the time v/s serum drug concentration data various pharmacokinetic parameters such as peak plasma concentration (C_{max}), the time at which peak occurred (t_{max}), area under the curve (AUC), elimination rate constant (Ke), biological half life ($t_{1/2}$), absorption rate constant (k_a), Clearance (Cl) and volume of distribution (V_d) were calculated as per known calculation methods.

Highest concentration of drug in plasma attained by the administrated dose is C_{max}. Time taken to reach maximum concentration of drug in plasma is t_{max}.

Area under the curve was calculated by the trapezoidal rule and formula is given by

$$AUC = \frac{(Y_2 - Y_2)(X_1 + X_2)}{2}$$

'V_d' was calculated by the following formula

$$V_d = \frac{\text{Administered dose}}{\text{Initial plasma concentration}}$$

Biological half-life ($t_{1/2}$) was calculated by the following equation

$$t_{1/2} = \frac{0.693}{K_e}$$

K_e = Elimination rate constant

Absorption rate constant was calculated by the method of residuals.

Clearance (Cl) was calculated by the following equation

$$Cl = \frac{\text{Administered dose}}{AUC}.$$

Mean residence time was calculated based on 63.2 % of drug eliminated from the body [14,15,16].

3. RESULTS AND DISCUSSION

3.1 Preparation of Standard calibration graph by HPLC

From HPLC method, standard calibration data and linearity of Enalapril maleate was shown in Table 1 & Figure 1. Standard chromatogram of the Enalapril maleate pure drug was shown in Figure 2. The system evaluation parameters of the chromatogram were shown in Table 2. Highest sharp peak at 6.065 in the chromatogram was observed.

Table 1. Standard calibration data of Enalapril maleate by HPLC

SL. No	Concentration (ng/ml)	Peak Area (Mean ± SD)
1	0	0
2	10	156120.057
3	20	377742.468
4	40	723343.54
5	80	1482691.625
6	100	1868564.416

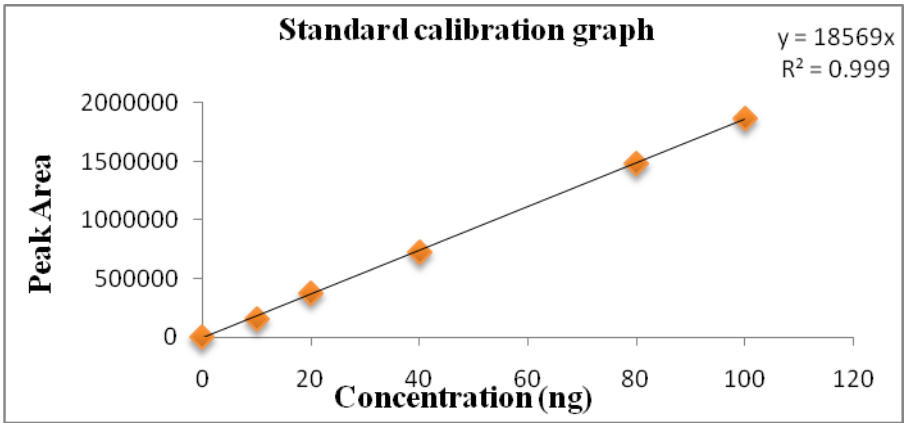

Figure 1. Standard calibration curve of Enalapril maleate pure drug by HPLC

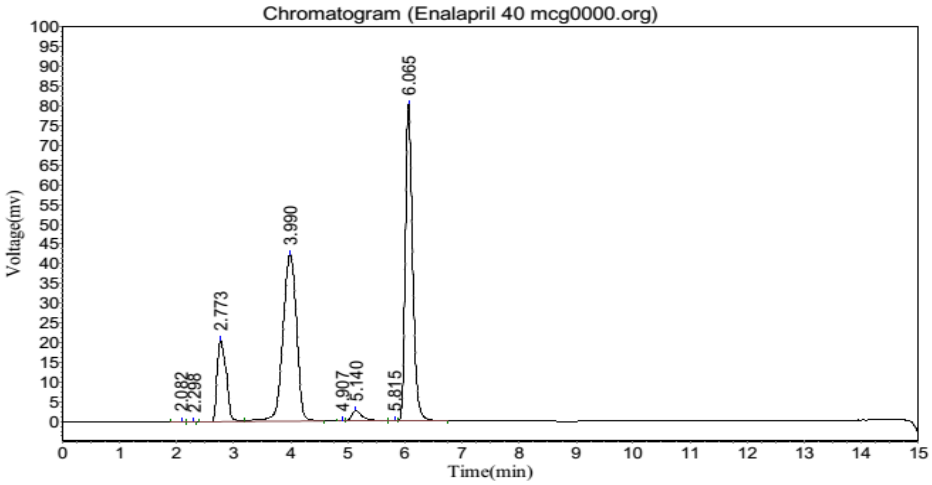

Figure 2. Standard chromatogram for Enalapril maleate by HPLC

Table 2. System evaluation parameters from chromatogram

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		2.082	161.412	1340.100	0.0802
2		2.298	58.490	394.131	0.0236
3		2.773	20535.750	227726.297	13.6274
4		3.990	42195.047	694952.813	41.5869
5		4.907	68.047	351.542	0.0210
6		5.140	2635.168	33638.164	2.0130
7		5.815	94.517	583.200	0.0349
8		6.065	80219.648	712099.125	42.6130
Total			145968.080	1671085.371	100.0000

Enalapril maleate drug peak at 5.982 was observed in rabbit plasma as shown in Figure 3. Plasma drug concentration of Enalapril maleate compacts (FEM_2) following the oral administration to Rabbits was shown in Figure 4. The system evaluation parameters from the chromatogram were shown in Table 3. Time v/s Average plasma drug concentration profiles of Enalapril maleate compacts AUC were plotted as shown in Figure 4. From AUC pharmacokinetic parameters was calculated as shown in Table 4.

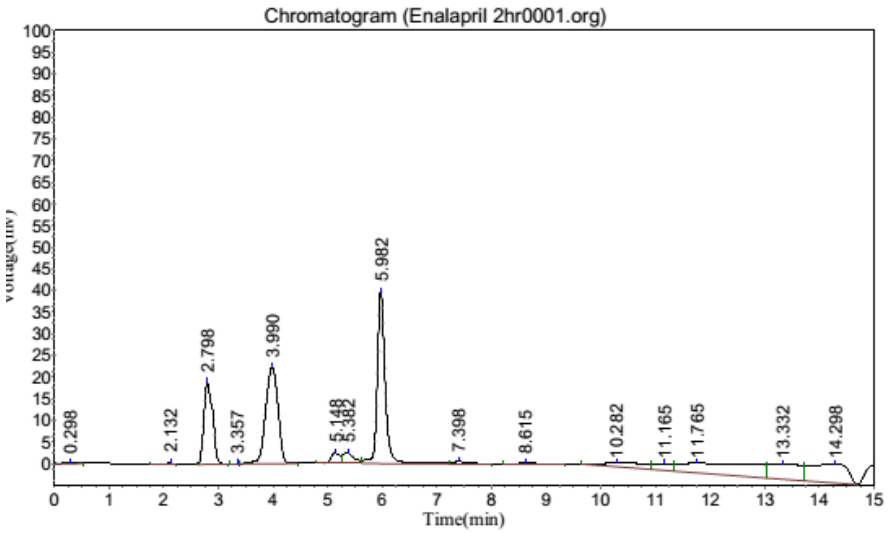

Figure 3. Determination of Enalapril maleate in rabbit Plasma by HPLC

Table 3. System evaluation parameters from chromatogram

Peak No.	Peak ID	Ret Time	Height	Area	Conc.
1		0.298	21.778	571.350	0.0334
2		2.132	283.368	2243.600	0.1312
3		2.798	18870.479	210790.500	12.3312
4		3.357	82.510	455.516	0.0266
5		3.990	22229.549	349401.719	20.4399
6		5.148	2401.977	26580.080	1.5549
7		5.382	2414.429	36337.516	2.1257
8		5.982	40048.590	403465.781	23.6026
9		7.398	511.837	7988.175	0.4673
10		8.615	98.797	2867.600	0.1678
11		10.282	745.273	52854.375	3.0920
12		11.165	1536.618	36951.859	2.1617
13		11.765	2184.947	241830.609	14.1470
14		13.332	3330.862	138290.250	8.0899

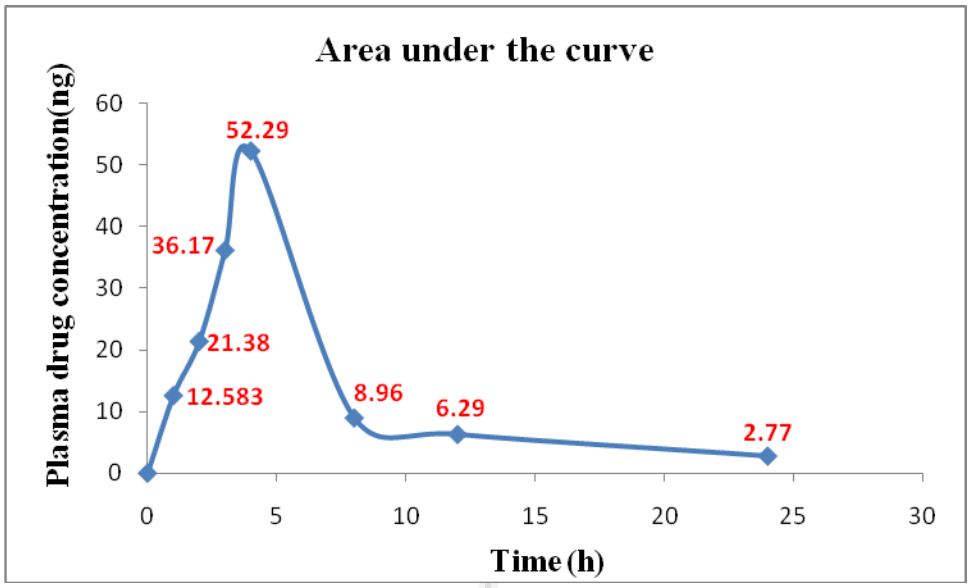

Figure 4. Time v/s Plasma drug concentration area under the curve of Enalapril maleate compacts (FEM2)

From Area under the curve, the extent of drug absorption after 24 h was calculated. The C_{max} and t_{max} obtained after application of Enalapril maleate compact was found to be 52.29 ng/ml and 4 h. The elimination rate constant Ke and the biological half life obtained after application of enalapril maleate compact for was found to be 0.67 h and 1.03 h. The $AUC_{(0-24)}$ and mean residence time obtained after application of Enalapril maleate compact was found to be 303.635 ng.h/ml and 11 h 06 min. The absorption rate constant Ka was found to be 10.994 h^{-1}. The results were shown in Table 4 and Figure 4.

3.2 Parameters for HPLC method development

- Mobile phase: Potassium dihydrogen orthophosphate (0.01M): Methanol(0.005M) pH3(30:70)
- Detector : UV detector, nm: 209
- Flow rate: 1ml/minute
- Injection volume; 20µl
- Diluent: Methanol
- Column dimension: 250x4.6 mm, 5µ

Table 4. Pharmacokinetic parameters

Pharmacokinetic Parameters	Observation
C_{max}	52.29 ng/ml
t_{max}	4 h
$AUC_{(0-24)}$	303.635 ng.h/ml
Elimination rate contant(Ke)	0.67 h
Biological half life	1.03 h
Vd	0.015 L
Absorption rate constant(Ka)	10.994 h^{-1}
Mean residence time (MRT)	11 h 06 min
Clearance (Cl)	0.0006

4. CONCLUSION

From the results it was concluded that the Enalapril maleate compacts were released the drug prolonged period of time. The mean residence time was found to be 11 h 06 min, indicates that more residence time of compacts was observed. From the pharmacokinetic evaluation, it was concluded that Enalapril maleate compacts was released and absorbed slowly over a prolonged period of time. It was also concluded that the designed buccoadhesive compacts can overcome the disadvantage of poor and erratic oral bioavailability of Enalapril maleate associated with currently marketed formulations.

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