Asian Journal of Pharmacy and Life Science ISSN 2231 – 4423
Vol.3 (1), Jan-March, 2013

FORMULATION AND *IN VITRO* EVALUATION OF MODIFIED PULSINCAP OF A CARDIO VASCULAR DRUG

Vihar moturi*, R.S.Thakur, Arshad Bashir Khan,

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Sarjapura main road, Carmelaram post, Bangalore-560 035, Karnataka, India.

Corrosponding author's Email id: viharmoturi@gmail.com Mobile: +919000334050

ABSTRACT

The research work highlights the development and evaluation of the modified pulsincap of an cardiovascular drug to achieve the objectives such as to minimize the frequency of dosing, improve the absorption and bioavailability of the drug and improve patient compliance. Ramipril is a prodrug belonging to the class of Antihypertensive Agents, angiotensin-converting enzyme (ACE) inhibitor and it is a potent vasoconstrictor. Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, myocardial infarction and to reduce the rate of death. The modified pulsincap of cardiovascular drug containing Ramipril granules with different ratios of polymer plugs such as HPC (Hydroxy propyl cellulose), HPMC (Hydroxy propyl methyl cellulose k-15) and chitosan. The granules were prepared by wet granulation technique and evaluated for various parameters like angle of repose, carrs index, in vitro dissolution profile and content uniformity. The prepared formaldehyde treated bodies of capsules were tested for physical appeareance, visual defects, solubility studies and modified pulsincap were tested for weight variation, content uniformity, compatibility and in-vitro dissolution studies. The stability studies were carried out as per the ICH guidelines. FTIR and DSC studies show no evidence on interaction between drug, polymers and other excipients. The results revealed that all the formulated capsules had acceptable physical properties. The modified pulsincap which contains HPC, Chitosan as polymer plugs had showed the required lag time, provided the immediate release of Ramipril and frequency of dosing is reduced

Keywords: *Ramipril, modified pulsincap, immediate release, hydroxy propyl cellulose, chitosan.*

INTRODUCTION

Ramipril is a prodrug belonging to the angiotensin-converting enzyme (ACE) inhibitor class of medications [1,2]. It is metabolized to ramiprilat in the liver and, to a lesser extent, kidneys. Ramiprilat is a potent, competitive inhibitor of ACE, the enzyme responsible for the conversion of angiotensin I (ATI) to angiotensin II (ATII) [1]. ATII regulates blood pressure and is a key component of the renin-angiotensin-aldosterone system (RAAS). Ramipril may be used in the treatment of hypertension, congestive heart failure, nephropathy, and to reduce the rate of death, myocardial infarction and stroke in individuals at high risk of cardiovascular events. Nowadays Pulsatile systems are gaining a lot of interest as the drug is released completely after defined lag time. Pulsatile drug delivery system is time- and site-specific drug delivery system, thus providing special and temporal delivery [3]. Pulsatile drug delivery system is

defined as the rapid and transient release of certain amount of molecules within a short time period immediately after a predetermined off-release period, i.e., lag time[4].

A novel delivery system capable of releasing its drug contents at either predetermined time or at specific site in the GI tract known as the Modified pulsincap.It is a novel delivery system capable of releasing its drug contents at either predetermined time or at specific site in the GI tract known as the "PULSINCAP". Modified pulsincap consists of non- disintegrating body and a soluble cap. The prepared granules are filled into the body and then it is sealed with hydrogel polymer after it is again refilled with granules of drug. By this frequency of drug administration can be reduced and immediate release of drug occurs at two different intervals. The gelatin capsule bodies are to be made insoluble by cross-linking with formaldehyde vapors in closed chambers for various time intervals [5].

MATERIALS AND METHODS

Materials

Ramipril was obtained as gift from Johnson & Johnson, Mumbai, Hydroxy propyl cellulose, Hydroxyl propyl methyl cellulose, Chitosan, Lactose, Sodium Starch Glycolate and Poly vinyl pyrrolidine procured from SD Fine chemicals, Mumbai. All other materials used and received were of analytical grade.

Methods

Preparation of Modified Pulsincap of Ramipril

i) Preparation of Immediate release granules:

- Drug and excipients were passed through mesh no 33.
- Then the drug and excipients were passed into mortar and is thoroughly triturated.
- During mixing of drug and excipients 0.5% PVP solution is added as a binder so that wet mass is prepared.
- This wet mass was passed through sieve 44 and granules were collected.
- Then these granules were dried under 50^0c for 30minutes. The formula was showed in table [1].

Formulae:

Table 1: Ingredients used for the preparation of granules:

Ingredients	Quantity(mg)/1 Capsule
Ramipril	2.5
Lactose	93.75
Sodium starch glycolate	5
Polyvinylpyrollidine	Q.S

ii) Preparation of cross- linked gelatin capsules

- Hard gelatin capsule of size 00 and 50 in number were taken. Their bodies were separated from the caps. 25 ml of 15% (v/v) formaldehyde was taken into desiccators and a pinch of potassium permanganate was added to it, to generate formalin vapours.

- The wire mesh containing the empty bodies of capsule was then exposed to formaldehyde vapours. The caps were not exposed leaving them water-soluble. The desiccators were tightly closed.
- The reaction was carried out for 12 h after which the bodies were removed and dried at $50^{O}C$ for 30 min to ensure completion of reaction between gelatin and formaldehyde vapours.
- The bodies were then dried at room temperature to facilitate removal of residual formaldehyde. These capsule bodies were capped with untreated caps and stored in a polythene bag.

iii) Preparation of polymer plugs:

The polymer plugs were prepared by direct compression at 5N of pressure using double punch machine.

iv)Formulation of modified pulsincap

- Granules equivalent to 2.5mg of Ramipril (I dose) were accurately weighed and filled into previously treated bodies by manual filling.
- The bodies containing the granules were then plugged with different amounts of polymers like chitosan, hydroxyl propyl cellulose, hydroxyl propyl methyl cellulose.
- Then again the granules equivalent to 2.5mg of Ramipril (IIdose) were accurately weighed and filled into the same body. After filling, both body and cap are joined. The formula was showed in table [2].

Table 2: Preparation of polymer plugs using different ratios

Formulation code	HPC (mg)	HPMC(mg)	CHITOSAN(mg)
F1	75	-	-
F2	-	75	-
F3	-	-	75
F4	25	25	25
F5	50	25	-
F6	25	50	-
F7	-	25	50
F8	50	-	25
F9	-	50	25
F10	25	-	50

EVALUATION

Evaluation of Ramipril granules

Angle of repose [6,7]

This is the Maximum angle possible between the surface of the pile or powder and horizontal plane. Angle of repose was determined by using funnel method. The frictional forces in the lose powder can be measured by Angle of repose. The tangent of Angle of repose is equal to the coefficient friction between the particles.

$\theta = \tan^{-1} (h / r)$

Where, θ is the angle of repose, h is the height in cm and r is the radius in cm.

Compressibility index [6]

It is an important measure that can be obtained from the bulk and tapped densities. A material having values less than 20 to 30% is defined as the free flowing material, based on the apparent bulk density and tapped density, the percentage compressibility of the bulk drug was determined by using the following formula.

$I = D_T - D_b / D_T \times 100$

Where, I is the Compressibility index, D_T is the tapped density of the powder and D_b is the bulk density of the powder.

Hausner's ratio [7]

It indicates the flow properties of the powder and is measured by the ratio of tapped density to the bulk density

$$H = D_T / D_b$$

Where, H is the Hausner's ratio D_T is the tapped density of the powder and D_b is the bulk density of the powder.

Percentage yield

The measured weight was divided by total amount of all ingredients which were used for the preparation of granules.

$$\%yield=practical\ yield/Theoritical\ yield\times 100$$

Drug content uniformity

In 100 ml volumetric flask 10mg equivalent weight of granules are taken and dissolved in small quantity of methanol and the volume was made up to mark with pH 7.4 buffer and stirred for 12 hrs. After stirring the solution was filtered through whatman filter paper and from the filtrate dilutions were made and absorbance was measured spectrophotometrically at 207nm.

Tests for Formaldehyde treated empty capsules

Various tests were carried out simultaneously for formaldehyde treated and untreated capsules.

Identification attributes

The '00' capsule were one with a red cap and red colored body. They were lockable type, odourless, softy and sticky when treated with wet fingers. After formaldehyde treatment, there were no significant changes in the capsules. They were non-tacky when touched with wet fingers.

Visual defect

In about 50 capsule bodies treated with formaldehyde, about five were found to be shrunk or distorted.

Solubility studies of treated capsules

The solubility test was carried out for normal capsules and formaldehyde treated capsules for 24 hrs. Ten capsules were randomly selected and then subjected to solubility studies at room temperatures in buffers of pH 1.2, 7.4 and 6.8. 100 ml solution and stirred for 24 hrs. The time at which the capsule dissolves or forms a soft fluffy mass was noted.

Evaluation of modified pulsincap

Weight variation

10 capsules were selected randomly from each batch and weighed individually for weight variation.

***Invitro* release profile**

Dissolution studies were carried out by using USP XXIII dissolution test apparatus (paddle) method. Capsules are tied with a cotton thread to paddle so that the capsule should be immersed completely in dissolution media but not float. In order to simulate the pH changes along the GI tract, three dissolution media with pH 1.2, 7.4 and 6.8 were sequentially used referred to as sequential pH change

method. When performing experiments, the pH 1.2 medium was first used for 2 hrs (since the average gastric emptying time is 2 hrs) then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 hrs (average small intestinal transit time is 3 hrs) the medium was removed and fresh pH 6.8 dissolution medium was added for subsequent hrs. 900ml of the dissolution medium was used at each time. Rotation speed was 50 rpm and temperature was maintained at 37±0.5^{0}C. Five millilitres of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 207 nm, by UV absorption spectroscopy.

Stability studies

The stability studies were carried out at accelerated condition of 40±2 ^{0}C, 75±5 % RH conditions, stored in desiccators; the tablets were packed in amber colour screw cap container and kept in above said condition for period of three months. The tablets were analyzed periodically for their physical appearance and *in-vitro* drug release. Results were analyzed by One-way ANOVA followed by Tukey's test. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The Modified Pulsincap of Ramipril were prepared by using different polymers such as Hydroxy propyl cellulose, Hydroxyl propyl methyl cellulose, Chitosan.The ramipril granules were evaluated for tapped density,bulk density, angle of repose,compressibility index, hausners ratio, percentage yield and drug content uniformity, bulk density, angle of repose, compressibility index, hausners ratio results were shown in table 3 & 4.

Angle of Repose:

The value of angle of repose lies between 25-28^0 indicates flow was found to be good and the value of Carr's index was found to be 9.23 which indicate that the flow was found to be excellent.

Table 3: physico chemical evaluation of granules

Trials	Height(h)	Radius(r)	□ (Degree)	SD
I	1.4	2.9	25.7º	28.06º±2.09
II	1.6	2.8	29.7º	
III	1.6	2.9	28.8º	

Carr's Index:

Table 4: physico chemical evaluation of granules

Bulk density (gm/ml)	Tapped density (gm/ml)	Compressibility index	Hausner ratio
0.413	0.455	9.23	1.101

Percentage yield:

Practical yield = 4.55gms

Theoretical yield = 5gms

% Yield = 90%

Percentage yield of the formulation was carried out and found to be 90%.

Drug content uniformity:

The drug content uniformity was found to be 92.36%w/w ± 0.53.

Tests for Formaldehyde treated capsule

Dimensions

On formaldehyde treatment, the '00' size capsule bodies showed a significant decrease in length and diameter.

Solubility studies

When the capsules were subjected to studies in different buffers, the untreated caps disintegrated within 10minutes in all the media whereas the treated bodies remained intact for about 24hrs.

Weight variation:

The weights of the capsules were found to be within the limits of IP.

***In-vitro* release studies:**

In-vitro drug release profiles of pulsatile device were found to have good immediate release and had shown required lagtime. During dissolution studies it was observed that, the first dose of the granules was released in the1.2 pH buffer and then exposed polymer plug which absorbed the surrounding fluid, swelled and forms the swollen matrix. After complete wetting of the plug, it formed a soft mass, which was then easily ejected out of the capsule body; releasing the granules into simulated intestinal fluid and colonic fluid (pH 6.8 and 7.4 phosphate buffers).

With all the formulations, there was immediate release of granules (I dose) in pH 1.2 buffer and release of II dose was observed after the release of polymer plug in pH 6.8 and 7.4 buffers. The results were shown in table 5 and figure 1.

***Invitro* release profile:**

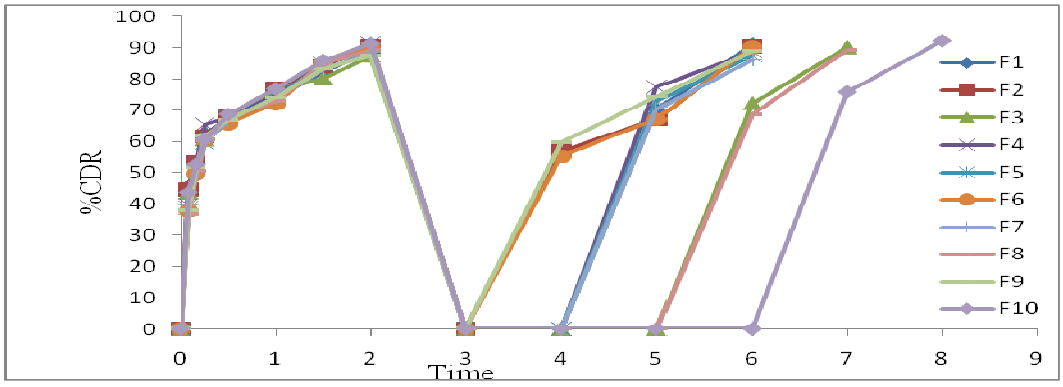

Fig1: Drug release profiles of formulations of F_1 to F_{10}

Formulations with HPC as hydrogel plug

With formulations F1(75mg),F4,F6,F10(25mg),F5,F8(50mg) at the end of 2hrs there was 89.4%,87.4%,90.3%,91.4%,89.3% and 89.3% drug release or % cdr respectively and at the end of 6hrs drug release was found to be 91.1%,88%,90.2%,92.3%,87.5% and 89.4%.The lag time showed by this plug in different ratios was 3,4,5 and 6hrs.

Formulations with HPMC as hydrogel plug:

With formulations F2(75mg),F4,F5,F7(25mg) and F6,F9(50mg) at the end of 2hrs the drug release was 90.4%,91%,89.3%,88.4%,90.3% and 87.4% respectively and at the end of 6hrs drug release or % cdr was found to be 90.5%,88%,87.5%,86.2%,90.2, and 88.9%.The lagtime showed by this plug in different ratios was found to be 3 and 4 hrs.

Formulations with chitosan as hydrogel plug:

With formulations F3 (75mg),F4,F8, F9(25mg) and F7,F10(50mg) at the end of 2hrs the drug release was 87.4%,91%,89.3%,87.4%,88.4% and 91.4% respectively and at the end of 6hrs the drug release or % cdr was found to be 90.2%,88%,89.4%,88.9%,86.2% and 92.3%. The lagtime showed by this plug in different ratios was found to be 3, 4, 5 and 6hrs.

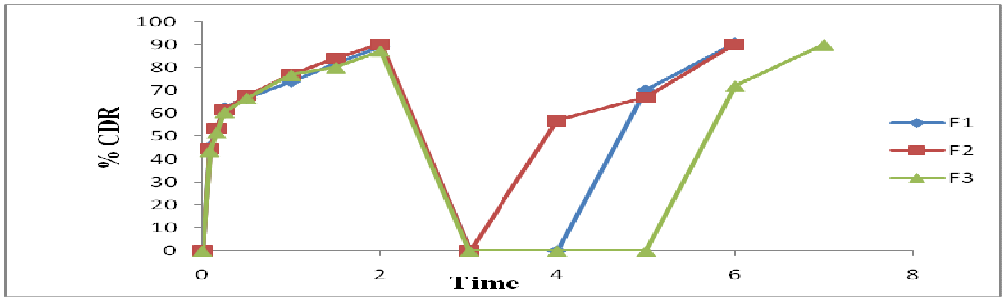

Fig 2: Drug release profiles of formulations of F_1 to F_3

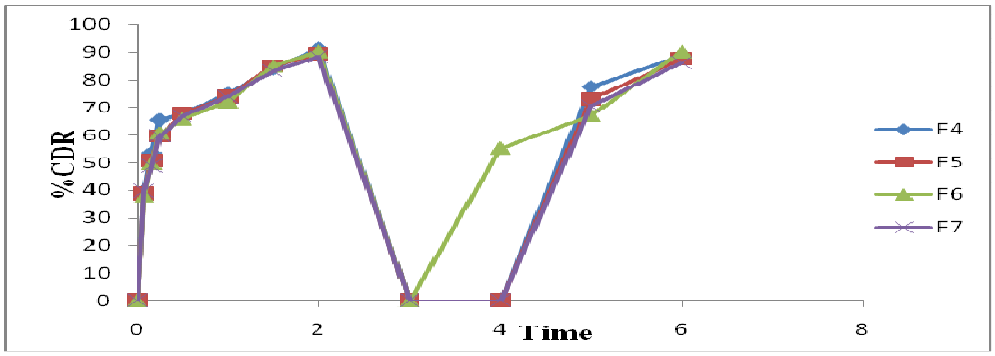

Fig 3: Drug release profiles of formulations of F4 to F7

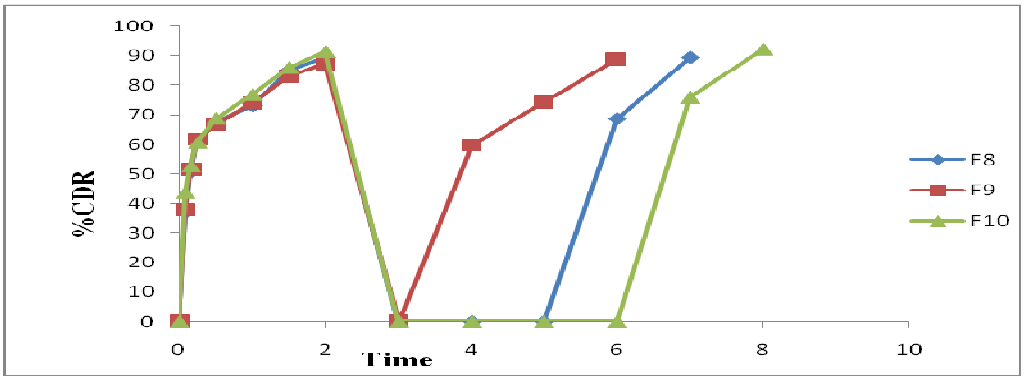

Fig 4: Drug release profiles of formulations of F_8 to F_{10}

CONCLUSION

Ramipril granules were prepared by wet granulation method using different excipients such as lactose, PVP and sodium starch glycolate.The drug polymer compatibility studies by FTIR indicates there is no possible interaction

between the drug and polymer and prepared granules were characterised by various evaluation parameters like flow properties, drug content uniformity etc.

The modified pulsincap were prepared by making the bodies of capsule insoluble by exposing to HCHO vapours, then the bodies are filled with I dose of granules manually and plugged with polymer plugs of different ratios which are prepared by direct compression. After placing of polymer plug the capsule is refilled with II dose of granules and both the body and cap are rejoined.

The lag time of formulations F2, F6, F9 was 3hrs, F1, F4, F5, F7 was 4hours, F3,F8 was 5hrs and F10 had showed 6hrs. Formulation F10 contains chitosan and HPC which had shown the required lagtime and the increased lagtime is due to the slow wettability of polymer plug.

From the above results it was found that F10 shows the required lagtime; releases the drug when its need is critical and reduces the frequency of drug administration. Formulation was stable and non-significant from P value obtained by one way ANOVA. This work demonstrated the development of modified pulsincap in order to obtain a chronotherapeutic drug delivery with desired drug profile.

ACKNOWLEDGEMENT

The authors are highly thankful to Mr. Arshad Bashir Khan, M.Pharm, (Ph.D.) and the management of Krupanidhi college of pharmacy for providing all the possible facilities to carry out the work.

REFERENCES

1. Kaplan N.M., Systemic hypertension Treatment. In: Bonow RO, Mann DL, Zipes DP, Libby P, eds. Braunwald's Heart Disease. 9th ed. Philadelphia: Saunders Elsevier; 2011**.**
2. Tripathi, K.D. Essentials of medical pharmacology. 6thed. Jaypee brothers medical publishers ,2008; 483-85.
3. Patel J. D., Aneja K., Majumdar S. H. Pulsatile Drug Delivery System. An "User-Friendly" Dosage Form. JPRHC. 2010; 2(2):204-215.
4. Kikuchi A., OkanoA.. Pulsatile drug release control using hydrogels. Advan. Drug Del. 2002; 54: 53-77.
5. Sarasija. S and Hota. A..Indian J. Pharm. Sci.2000;62:1–8.
6. Vijaya K.S., Mishra D.N.. Rapidly disintegrating oral tablets of meloxicam. Indian Drugs. 2006; 43(2): 117-121.
7. Lachman L., Libermann H.A., Kanig J.L.. The theory and practice of industrial pharmacy. Varghese Publishing House, 3 rd ed; 1991:297-301.