INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

DESIGN AND CHARACTERIZATION OF PULSATILE TABLET IN CAPSULE DEVICE FOR HYPERTENSION THERAPY

Subashini Rajaram*, P. Aruna, Jyotsna Ramesh,

Liby Mathew and D. Shanmughapriya

Department of Pharmaceutics, Swamy Vivekananda College of pharmacy, Elayampalayam-637205, Tamilnadu, India.

ABSTRACT

The present study aimed to formulate and evaluate pulsatile tablet of Ramipril and Telmisartan in capsule device using Eudragit RL100 and Eudragit RS100 polymers for the treatment of hypertension. The core tablet of telmisartan for sustained release was prepared by direct compression method. The blend of ramipril produced immediate release to the environment (acidic). The blend and core tablet were incorporated with hard gelatin capsule with "1" in its size and the filled capsules were evaluated for its physico-chemical characteristics. The pre, post compression evaluation result complies with the standard limits with minimum standard deviations. The in-vitro drug release profile of immediate release layer shows that formulation FM_3 results 60% drug release at 20 min and it was achieved to 98% at 50 min. The in-vitro release study of core tablet such as telmisartan start its release at 3rd hour and 98% of drug release was achieved by FS_3 at 12th hour and it was linear compare to other formulations prepared. In conclusion, pulsatile drug delivery could be beneficial to deliver the drug at right time and right environment for hypertensive therapy and also it improves the patient's compliance.

Keywords: Pulsatile drug delivery system, Ramipril, Telmisartan, Immediate drug release.

1. INTRODUCTION

The oral route of drug delivery is typically considered the favored and the most user-friendly means of drug administration having the highest degree of patient's compliance[1]. Oral controlled drug delivery systems represent the most popular form of controlled drug delivery systems. Such systems release the drug with constant or variable release rate. The oral controlled release system shows a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period of time, thereby ensuring sustained therapeutic action. But there are certain conditions which demand release of drug after a lag time. In other words, it is required that the drug should not be released at all during the initial phase of dosage form administration.

Various studies conducted, suggest that pharmacokinetics, drug efficacy and side effects can be modified by following therapy matching the biological rhythm[2]. Recent studies have revealed that there are number of conditions which show predictable circadian rhythms and research is devoted to the design and evaluation of chronotherapeutic drug delivery systems that release a therapeutic agent at a rhythm to maintain the adequate drug concentration according to the needs of the physiological states of patient's body and the cardiac rhythm[3].

Nowadays, concept of chronopharmaceutics has been emerged; chronopharmacotherapry or "chronopharmaceutics" consist of two words chronobiology and pharmaceutics. chronotherapeutics, is the discipline concerned with the delivery of drugs according to inherent activities of a disease over a certain period of time. Based on these findings, diseases where a constant drug levels are not preferred, but needs a pulse of therapeutic concentration in a periodic manner acts as an initiator for the development of "Pulsatile Drug Delivery Systems" (PDDS)[4].

Pulsatile drug delivery system is time and site-specific drug delivery system, thus providing special and temporal delivery and increasing patient's compliance with rapid and transient release of certain amount of molecules within a short time period immediately after a predetermined/programmable off-release period, i.e., lag time[5].

Recent studies have revealed that diseases have a predictable cyclic rhythm and that the timing of medication regimens can improve the outcome of a desired effect. This condition demands release of drug as a "pulse" after a lag time and such systems are known as pulsatile drug delivery system (PDDS). It is well established that the physiology of the body is not uniform 24 hours a day. The delivery and therapy should be modified to achieve an effective drug level at the time required. This can be achieved by adopting a pulsatile drug delivery system of a suitable drug. Such type of formulation that are taken at bed time with preprogrammed start of drug release in early morning hours, could offer a more effective therapy than a controlled conventional release drug delivery system.

In cardiovascular diseases, bronchial asthma, and rheumatoid arthritis, remarkable efficacy, tolerability and compliance benefits could arise from modified released medications. After bedtime administration, would allow the onset of therapeutic drug concentrations to coincide with the time at which disease manifestations are more likely to occur. Performance of pulsatile delivery fulfills such goals.

Controlled releases that display a pulse release are mainly prepared by polymeric materials and systems that are made up of reservoir and covered with barrier. This barrier can be dissolved or eroded after which drug is released. The release from such formulation can be modified to get single pulse, double pulse, and multi pulse release pattern[6].

Considering the above factors, the present study aimed to formulate and evaluate a pulsatile drug release system of ramipril and telmisartan for the effective treatment of hypertension. Telmisartan is an angiotensin II receptor antagonist drug, which is mainly used to treat high blood pressure. It is well absorbed after oral administration. The bioavailability of about 50% and mean peak concentrations of telmisartan are reached in 0.5-1 hour after dosing. It decreases the chance of heart attack and stroke. It works by blocking the action of certain natural substance that tighten the blood vessels allowing the blood to flow smoothly and heart to pump efficiently.

2. MATERIALS AND METHODS

Ramipril and telmisartan were procured as a gift sample from Microlabs Pvt. Ltd, Bangalore (India). Avicel was obtained from Kemphasol, Mumbai, (India). Polyvinyl pyrrolidone, magnesium stearate and talc were purchased from Lobachemiepvt Ltd, Mumbai (India). Croscarmellose sodium was obtained from S.D. Fine chem. Ltd., Mumbai (India). Eudragit RS100 and Eudragit RL 100 were purchased from Evonik industries, Essen (Germany). All the chemicals and reagents used were of analytical grade.

3. METHODS

Compatibility study

Compatibility study was done to analyze the interaction of drug with excipients and it was confirmed by Fourier transform infra red spectroscopy (FT-IR) and Differential scanning colorimetry (DSC).

3.1 Preparation of ramipril blend

Ramipril blend was prepared for immediate release of drug. Diluent were selected on the basis of nature of drug. Lactose monohydrate and Avicel was used as a diluent and starch paste as a binder solvent. Accurately weighed ramipril (5 mg), lactose monohydrate, croscarmellose sodium and starch paste were mixed together in mortar and pestle. Mixture was passed through #20 sieve followed by drying in hot air oven at 50 C for 15 min[7]. Different formulation batches of ramipril blend are shown in Table1.

3.2 Preparation of compressed tablets of telmisartan

The methodology adopted include

a) Preparation of granules
b) Preparation of core tablets of Telmisartan
c) Preparation of coated tablets

a) Preparation of granules

The formula for the preparation of granules was given in Table 2. The granules were prepared by dry granulation method by incorporating excipients such as microcrystalline cellulose as binder and croscarmellose sodium as superdisintegrant, Magnesium Stearate as a lubricant and purified talc as a glidant. All these ingredients were mixed together in mortar and pestle and made it as coherent mass. The prepared mass was passed through sieve No. 12 and 22. Then the granules were dried at 50ºC.

3.3Preparation of core tablet of Telmisartan

The core tablets of telmisartan were prepared by direct compression method. The dried granules were compressed as tablet by direct compression method.

- **Preparation of coated tablets**

Tablet coating was done by using5% (w/w) solutions of polymethacrylates (Eudragit RL100 and Eudragit RS100). The coating solution was prepared with the addition of various ratio of polymers Eudragit RL100: Eudragit RS100 (1:1, 1:2, 1:3). The solution was plasticized with diethyl phthalate (5%, w/w, with respect to dry polymer). Core tablets were coated by dipping method and the tablets were removed from the coating solution when the coating loads have been reached 5% (w/w). The tablets were kept in an oven for 2 h at 50ºC[7].

3.4 Preparation of tablet in capsule formulation

The first step in the formulation of tablet in capsule approach was to select the appropriate capsule size that can accommodate coated tablet and immediate release blend. For the purpose, size "1" capsule was selected according to specifications given by USP. According to USP, capsule size "1" can accommodate total weight of 500 mg. This capsule size can accommodate optimized batch of coated telmisartan tablet weighing 160 mg and optimized batch of immediate release ramipril blend weighing 340mg. Finally the filled capsules were sealed with the help of capsule hand filling machine[8,9].

3.5 Precompression parameters

Angle of repose

Flow property of a powder is usually assessed by determining angle of repose of the powders. The angle of repose is determined by the "Funnel method". The powder is allowed to fall over a paper placed on a horizontal surface through a funnel or an orifice kept at a certain convenient height. The height of the heap formed is measured by a suitable method and then the circumference of the base of heap is drawn on the paper with the help of a pencil. The radius of the circle obtained is measured. The angle of repose is given as,

$$\tan\theta = h/r$$

where,

θ = Angle of repose

h = Height of the heap

r = Radius of the base of the heap

Bulk density

It is the ratio between a given mass of a powder and its bulk volume.A given quantity of the powder is transferred to measuring cylinder and initial volume was observed. Bulk density of the powders were measured by the given equation,

$$\text{Bulk density} = w/v_0$$

Where,

W = Mass of the powder.

v_0 = Initial volume

Tapped density

A given quantity of the powder is transferred to a measuring cylinder and is tapped mechanically till a constant volume is obtained[10]. It was calculated by following equation,

$$\text{Tapped density} = w/v_f$$

Where,

W = Mass of the powder

v_f = Final volume

Hausner's ratio

It indicates the flow properties of the granules. It is measured by the ratio of tapped density to the bulk density. The formula for Hausner's ratio is[11],

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Compressibility index (Carr's index)[11]

$$C = \frac{\text{Tapped density} - \text{Bulk density} \times 100}{\text{Tapped density}}$$

3.6 Post compression parameters

Hardness test

The hardness of the tablets was determined using Pfizer tablet hardness tester. It is measured in terms of load or pressure required to crush it when placed on its edges. The force applied to the edge of the tablet is gradually increased by moving the screw knob forward until the tablet breaks. The reading is noted from the scale which indicates the pressure required in kg/sq.cm to break the tablet[10]. Hardness of 4 kg/sq.cm is considered to be a minimum requirement. The formula is as follows,

Hardness of tablet = Final value – Initial value

Friability

This test was performed using friabilator. 20 tablets were weighed and placed in the plastic chamber. The chamber is rotated for 4 minutes at 25rpm. During each revolution the tablet falls from a distance of 6 inch. The tablets were removed from the chamber after 100 revolutions and weighed. Loss in weight indicates the friability. The tablets are considered to be of good quality if the loss in weight is less than 0.8% and it was calculated using the given formula[11],

$$\text{Friability of tablets} = \frac{\text{1- wt. of loss} \times 100}{\text{Weight taken}}$$

Weight variation

The weight variations of tablets were found using analytical balance. 20 tablets were selected at random and determined their average weight. Not more than 2 of the individual weight may deviate from the average weight by more than the percentage deviation. It should fall within the prescribed limits and it was calculated by the given formula[11],

	Average wt of tablet deviation	Percentage
As per IP	80 mg (or) less	10%
	80-250 mg	7.5%
	250 mg (or) more	5%
As per USP	130 mg (or) less	10%
	130-324 mg	7.5%
	324 mg (or) more	5%

$$\text{Average weight} = \frac{\text{Sum of each tablet}}{20}$$

Drug content

The telmisartan core tablets were tested for their drug content. Twenty tablets were finelypowdered; quantity equivalent to 10mg was accurately weighed and transferred to a 50ml volumetric flask. Then the volume was made up with 6.8 P^Hphosphate buffer and shaken for 10 min to ensure complete solubility of drug. The mixture was centrifuged and the solution was filtered. Concentration of 20mcg/ml was prepared and absorbance of resulting solution was determined using UV spectrophotometer at 326nm[11, 12]. The same procedure was followed for ramipril blend and determined using spectrophotometer at 237nm[13].All the experiments were repeated for three times and mean value was calculated.

3.7 In vitro drug release of ramipril blend

Prepared blend of ramipril was kept in hard gelatin capsule and dissolution studies were performed using a USP XXIII dissolution apparatus I (basket type) in 900 ml medium at 37º±0.5º C at a rotation speed of 50 rpm. In vitro release study was carried out in acidic media at pH 1.2 for 2 h. Five milliliters sample was withdrawn at specific intervals and replaced with a fresh dissolution medium. These samples were filtered using a 0.45 μm membrane filter. The concentration of samples was analyzed using UV spectrophotometer at λ max 237nm[13]. All the experiments were repeated for three times and mean value was calculated.

3.8 In vitro drug release study of telmisartan tablets

In vitro drug release studies were performed using USP XXIII dissolution apparatus II paddle type in 900 ml medium at 37.0±0.5 C, at a rotation speed of 50 rpm. Dissolution media selected was 0.1 N Hcl (pH 1.2) and phosphate buffer of pH 6.8. Dissolution test was performed for 2 h in 0.1 N Hcl (pH 1.2) and for 6 h in phosphate buffer (pH 6.8) respectively. Five milliliters sample was withdrawn at specific intervals and replaced with a fresh dissolution medium. These samples were filtered using a 0.45 μm membrane filter. The concentration of samples was analyzed using UV spectrophotometer at 326nm[12]. All the experiments were repeated for three times and mean value was calculated.

3.9 In vitro release study of tablet in capsule formulation

In vitro drug release studies were performed in USP XXIII dissolution apparatus I Basket type (TDT-08L plus, Electrolab, Mumbai, India)) in 900 ml medium at 37º±0.5º C at a rotation speed of 50 rpm. The capsule was placed in the basket. Hard gelatin capsule (500mg) containing ramipril blend and coated telmisartan tablet were placed in the dissolution medium. For simulating conditions of the GI tract, dissolution tests were carried out in media with pH 1.2 (acidic medium) for 2hrs and pH 6.8 (phosphate buffer) for 6hrs. 5 ml sample was withdrawn at predetermined time intervals and replaced with fresh dissolution media. The withdrawn samples were filtered through membrane filter 0.45μm and analyzed using UV spectrophotometer using multi component method for first 2 h at λ max 237nm[13] respectively. After 2 h dissolution sample were analyze by UV spectrophotometer at λ max 326nm[12,14,15,16,17,18,19].

Table 1: Composition of immediate release blend of ramipril

S.NO	INGREDIENTS	FI_1 (mg)	FI_2 (mg)	FI_3 (mg)
1	Ramipril	5	5	5
2	Croscarmellose sodium	150	160	170
3	Lactose monohydrate	175	165	155
4	Starch paste	10	10	10
	Total wt (mg)	**340**	**340**	**340**

Table 2: Composition of core tablet of Telmisartan

S.NO	INGREDIENTS	FS_1 (mg)	FS_2 (mg)	FS_3 (mg)
1	Telmisartan	20	20	20
2	Avicel	100	110	120
4	Polyvinylpyrolidine K30	10	10	10
5	Magnesium stearate	15	10	3
6	Talc	15	10	7
	Total weight (mg)	**160**	**160**	**160**

4. RESULTS

Preformulation studies

Compatibility studies

The results of compatability studies shown in Fig.2A-E It revealed that both drug and polymers were compatible with each other and there is no interaction between the drug and the polymer.

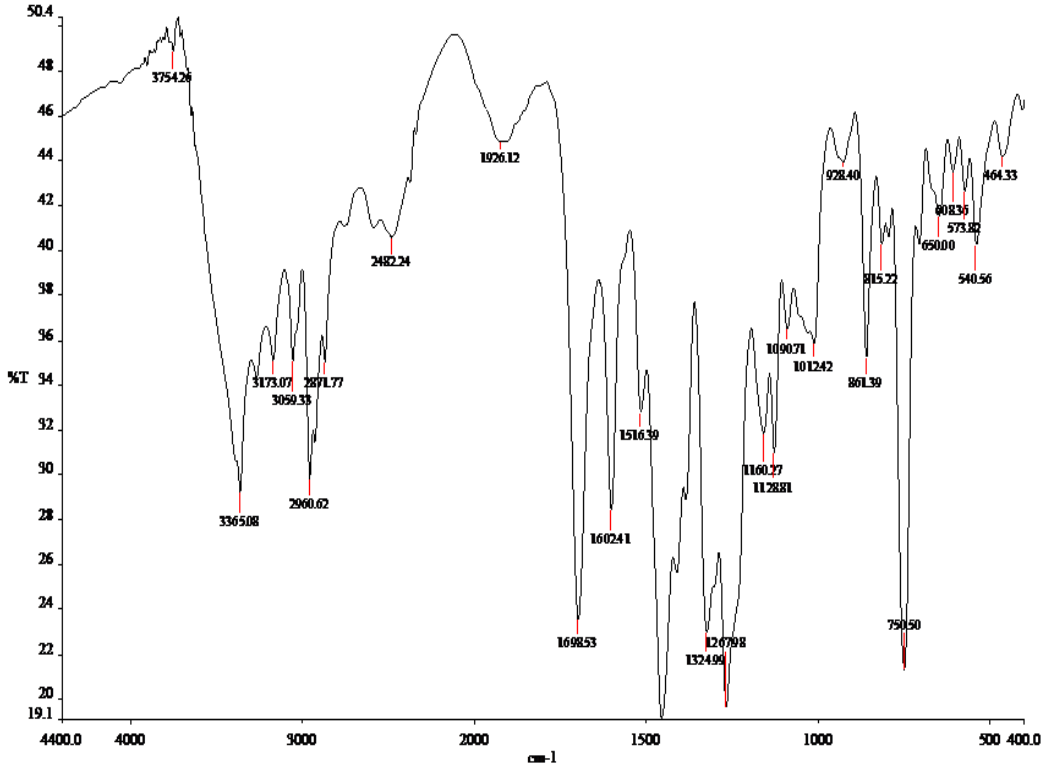

Fig. 2A: FT-IR Spectrum of Telmisartan

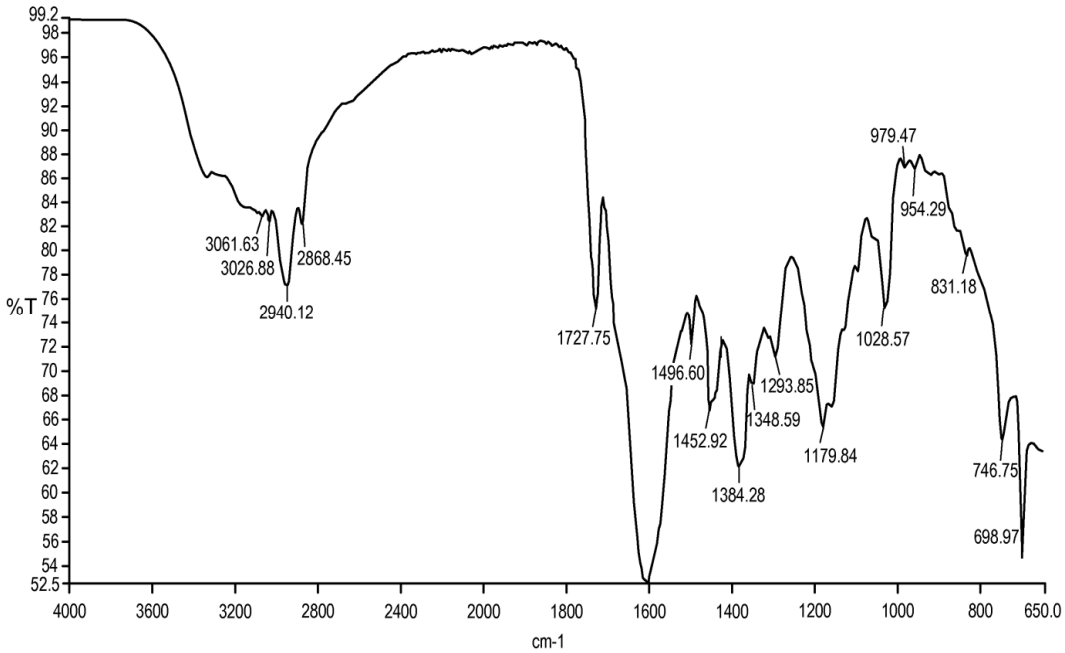

Fig. 2B: FT-IR Spectrum of ramipril

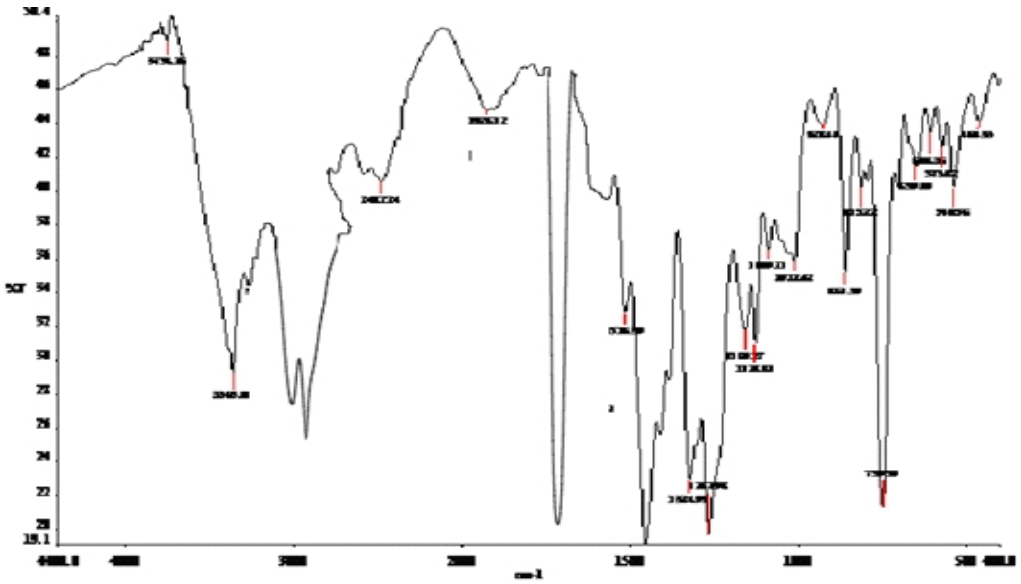

Fig. 2C: FT-IR Spectrum of physical mixture

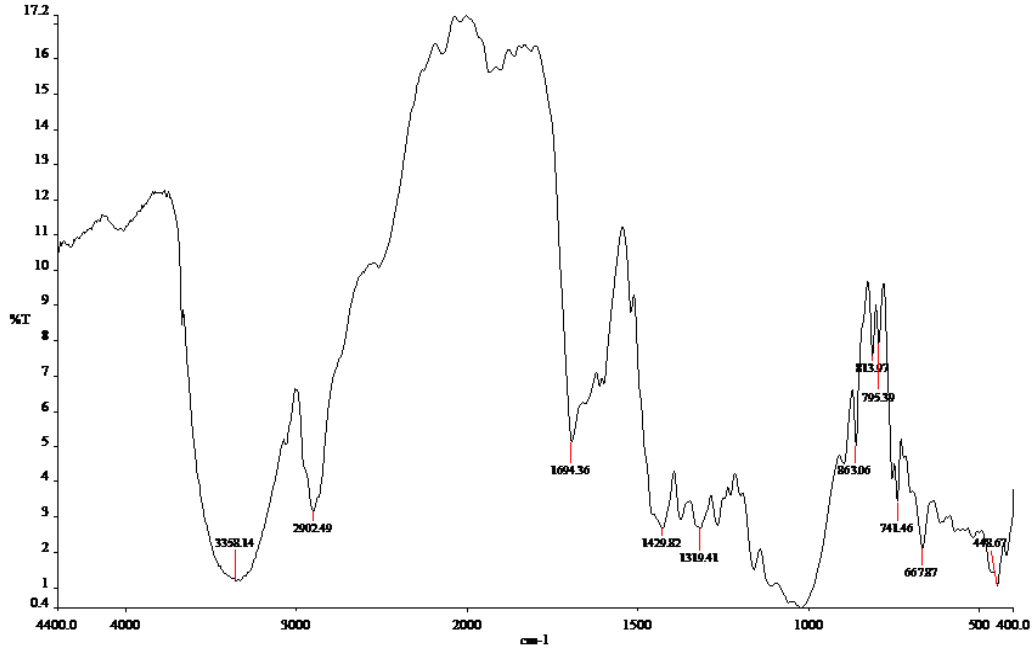

Fig. 2D: FT-IR Spectrum of FS_1

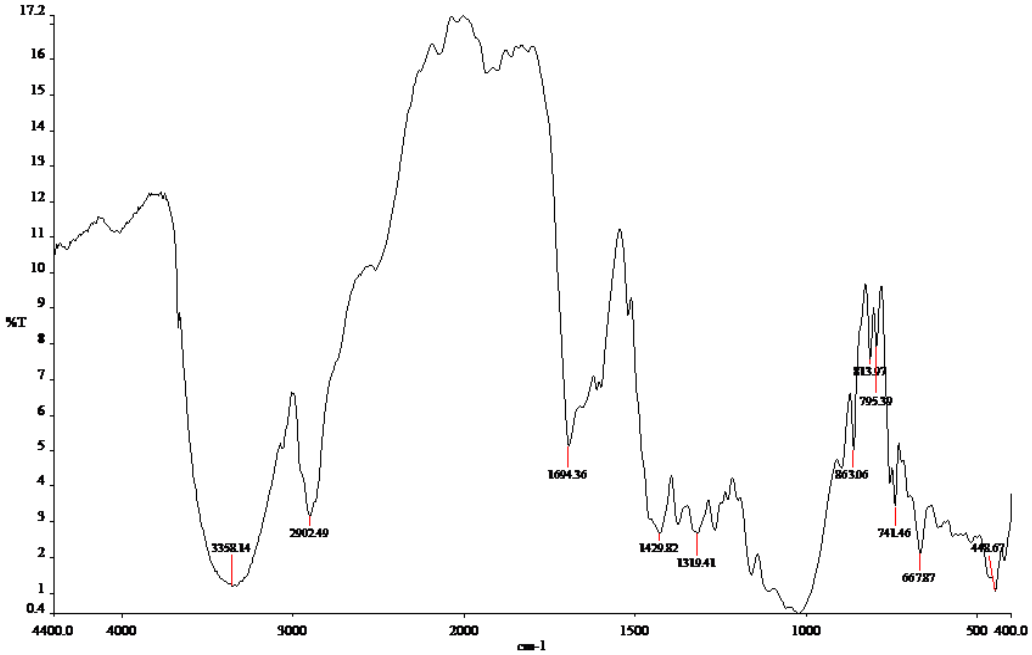

Fig. 2E: FT-IR Spectrum of FS_2

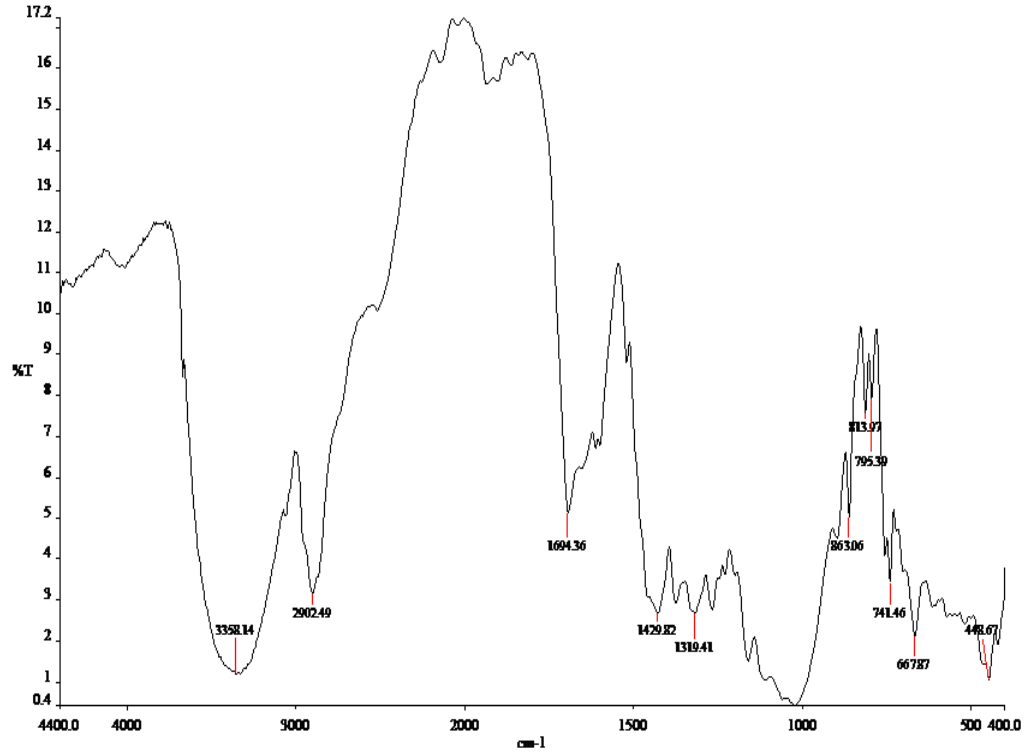

Fig. 2F: FT-IR Spectrum of FS_3

Pre compression studies

The results of precompression studies were given in Table 4, 5.

Table 4: Preformulation studies of granules of core telmisartan tablets (n=3, Mean ±SD)

Formula Code	Angle of repose (θ)	Bulk density(gm/ml)	Tapped density (gm/ml)	Carr's index (%)	Hausner's ratio
FS1	38.8±0.36	0.225±0.01	0.255±0.01	12.23±0.25	1.203±0.01
FS2	39.5±0.51	0.241±0.01	0.281±0.01	14.45±0.17	1.223±0.02
FS3	36.80±0.97	0.244±0.02	0.329±0.02	14.27±0.89	1.146±0.03

Table 5: Physico-chemical evaluation of prepared core tablets (n=3, Mean ±SD)

Formula Code	Weight variation (mg)	Hardness (kg/sq.cm)	Friability (%)	Drug content (mg)
FS1	0.35±0.01	65±0.43	0.57±0.5	39.47±0.02
FS2	0.38±0.02	6.06±0.5	0.62±0.17	39.51±0.03
FS3	0.32±0.01	5.66±0.76	0.73±0.03	39.27±0.01

In vitro drug release

The in vitro percent drug release of ramipril from all formulations (FI_1, FI_2, and FI_3) was given in Table 6, Fig. 3.

Table 6: In vitro percent drug release study of Ramipril blend at 237nm in pH 1.2 (n=3, mean±SD)

S.No	Time (min)	% Drug release		
		FI_1	FI_2	FI_3
1	0	0	0	0
2	10	20.6±0.03	21.9±0.09	32.5±0.5
3	20	43.2±0.07	52.7±0.1	67.8±0.08
4	30	68.5±0.06	73.1±0.05	81.4±0.06
5	40	92.8±0.02	95.4±0.02	99.2±0.01

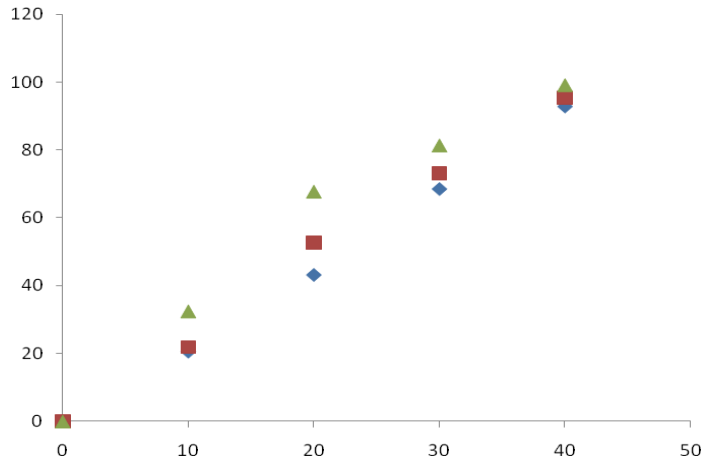

Fig. 3: In vitro percentage drug release from ramipril blend (FI_1 to FI_3)

In vitro drug release of Telmisartan

The in vitro percent drug release of telmisartan from all formulations (FS_1, FS_2, and FS_3) was given in Table 7 and Fig. 4.

Table 7: In vitro percent drug release study of Telmisartan coated tabletsat 326nm in pH 6.8 (n=3, mean±SD)

S.No	Time(hrs)	% Drug release		
		FS_1	FS_2	FS_3
1	1	0	0	0
2	2	0	0	0
3	3	0	0	0
4	4	0	0	0
5	5	1.86±0.01	1.51±0.21	2.58±0.19
6	6	20.74±0.48	30.67±0.46	25.30±0.62
7	8	60.69±0.32	54.04±0.54	64.50±0.70
8	10	73.41±0.67	72.73±0.73	82.07±0.09
9	12	87.23±0.45	92.08±0.76	99.43±0.83

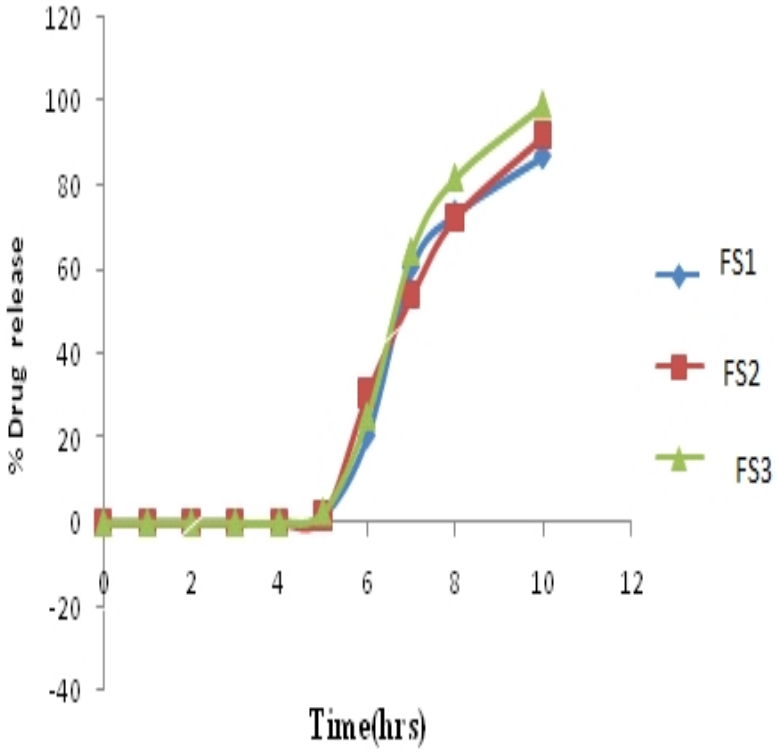

Fig. 4: In vitro percentage drug release of Telmisartan from coated formulations ($FS_1$to FS_3)

In vitro drug release from capsule device

In vitro drug release of ramipril and telmisartan tablet from capsule device was given in Fig 5.

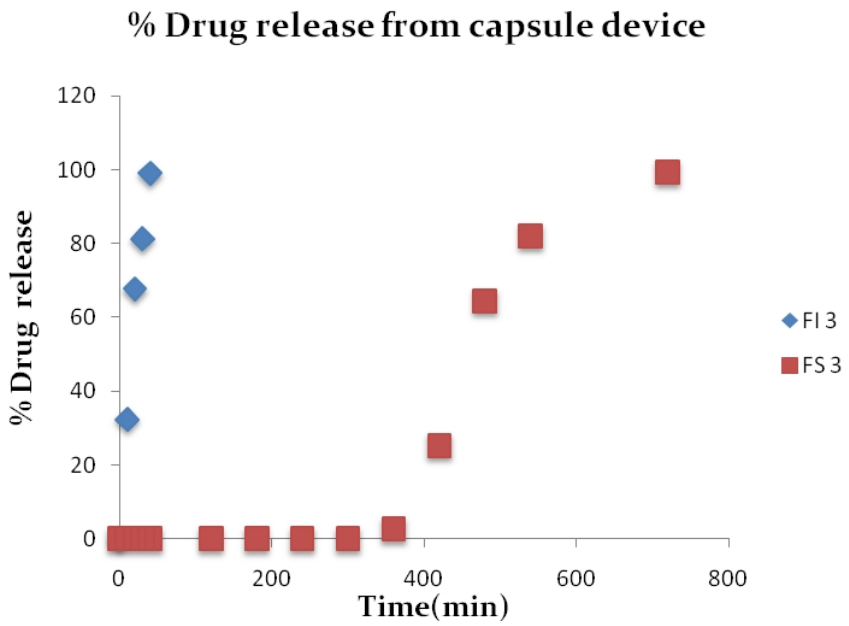

Fig. 5: In vitro percentage drug release from capsule device

5. DISCUSSION

Pulsatile delivery of telmisartan tablets were prepared by direct compression method. The prepared tablets were studied for its pre compression and post compression evaluation. The compatibility studies were done by FT-IR and the results shown that no interaction between drug and polymers.

Pre compression evaluation in tablet is most important. Evaluation of these properties will results the characteristics of powder ingredients before punch as a tablet. The observed results from pre compression evaluations shown that the prepared powders were having well in their flow properties and within the accepted limits with low standard deviation.

In general, post compression properties of tablets reflect their withstanding capacity during transportation, content uniformity and drug release. The results of post compression evaluation such as content uniformity, hardness, friability and weight variation shown within the official limits. It indicates all the prepared tablets were well in their physic-chemical properties.

The results of disintegration study on ramipril blend in capsule shown the disintegration time varied according to the concentration of superdisintegrants used. Formulation FI_3 was considered as best due to its fast release compared to other two formulations.

Determination of in-vitro drug release profile gives the exact bioavailability of drug in the medium. This might be correlated with in-vivo bioavailability of drugs. In this study in-vitro drug release was performed on all three formulations (FI_1, FI_2, and FI_3) in pH 1.2 (acidic medium) for 2hrs and FS_1, FS_2, and FS_3 in pH 6.8 (phosphate buffer) for 6hrs to evaluate the percentage of drug release from all the formulations. The percentage drug release was varied according to the concentration of superdisintegrants as well as polymers used.

In general, telmisartan is freely soluble in phosphate buffer such as pH 6.8. Eudragit is a polymer, enable to achieve the desired drug release profile with the drug being released at the right place and time or, if necessary, over a desired period of time. In our study we have prepared pulsatile release tablet of telmisartan with combination of different grades of eudragit as polymer for pulsatile release with different concentration[20].

The in-vitro drug release was performed for 12 hours and the release was occurred from the formulations only at 5th hour and the highest release was occurred at 12th hour from FS_3 (1:3) formulation (99.43±0.83). This result reveals that FS_3 produced higher release profile from among all the formulations due to its composition and also the concentration of polymers coated over the drug. In general the combination of polymers such as eudragit RS100 and RL 100 produces customized drug release profile with different grades and ratios.

CONCLUSION

Pulsatile drug delivery is an new drug delivery approach to deliver the drug from such system at the right time, right place and in right amounts, holds good promises of benefit to the patients suffering from chronic problems like arthritis, asthma, hypertension etc. Designing of proper pulsatile drug delivery will enhances the patient compliance, optimum drug delivery to the target site and minimizes the undesired effects. In conclusion this approach could be beneficial to the patients with hypertension.

REFERENCES

1. Aithal KB, Harish NM, Rathnanand M and Shirwaikar A. Chronotherapeutics and disease management. Treatment in synchrony with body's rhythms- An emerging science. Pharma Times. 2006;38(10):15-18.
2. Lemmer B. Chronopharmacokinetics implications for drug treatment. J Pharm Pharmacology. 1999;51:887-890.
3. Youan BC. Chronopharmaceutics: Gimmick or clinically relevant approach to drug delivery. J Controlled Release. 2004;98:337-353.
4. Skloot G. Nocturnal Asthma Mechanisms and Management. The Mount Sinai J of Med. 2002;69:140-147.
5. Kikuchi A and Okano T. Pulsatile drug release control using hydrogels. Adv Drug Deliv Rev. 2002;54:53-77.
6. Mallikarjuna R. A review on current scenario of pulsatile drug delivery system. IJAPR. 2012;3:890-900.
7. Prasanth VV, Mitesh P Modi, Sam T Mathew and Abin Abraham. Formulation and evaluation of enteric coated time release press coated tablets of Theophylline for chronopharmacotherapy. Der Pharmacia Lettre. 2012;2:599-606.
8. Arora S, Ali J, Ahuja A, Baboota S and Qureshi J. Pulsatile drug delivery system: an approach to controlled drug delivery. Indian J Pharm Sci. 2006;68(3):295-300.
9. Krogel I and Bodmeier R. Pulsatile drug release from an insoluble capsule body controlled by an erodible plug. Pharm Res. 1998;15(3):474-481.
10. Anal AK. Time - Controlled Pulsatile Delivery Systems for Bioactive Compounds. Recent Patents on Drug Delivery and Formulation. 2007;1:73-79.
11. Banker GS and Anderson NR. In: The Theory and Practice of Industrial Pharmacy, Lachman L, Liberman HA, Kanig JL, 3rdedition, Vanghese publishing house, 293-345.
12. Ajit Pandey, Sawarkar H, Mukesh Singh, Kashyap P and Priyanka Ghosh. UV-Spectrophotometric Method for estimation of Telmisartan in Bulk and Tablet Dosage Form. Int J Chem Tech Res. 2011;3(2):657-660.
13. Syed Iftequar, Lahoti Swaroop, Zahid Zaheer, Mirza Shahid, Sayad Imran and Dehghan MH. UV Spectrophotometric methods for estimation of Ramipril in Pharmaceutical dosage form by absorption maxima method and area under curve. Int J Drug Dev and Res. 2012;4(1):286-290.
14. Ying-Huan, L and Zhu J. Modulation of combined-release behaviours from a novel tablets-in-capsule system. Journal of controlled release. 2004;95:381–389.
15. Siegel RA and Pitt CG. A strategy for oscillatory drug release general scheme and simplified theory. Journal of controlled release. 1995;46:59-73.
16. Saslawski O, Weigarten C and Benoit JP. Magnetically responsive microspheres for the pulsed delivery of insulin. Life Sciences. 1988;(42):1521.
17. Rogel I and Bodmeiera R. Pulsatile drug release from an Insoluble Capsule Body Controlled by an Erodible Plug. Pharmaceutical research. 1998;15:3.
18. Qui M, Wang P and Wu D. A Novel pH-and Time-Dependent System for Colonic Drug Delivery. Drug Development and Industrial Pharmacy. 2003;29:661–667.
19. Zou H and Jiang X. Design and gamma-scintigraphic evaluation of a floating and pulsatile drug delivery system based on an impermeable cylinder. Chem Pharm Bull. 2007;55:580–585.
20. Pozzi F, Furlani P, Gazzaniga A and Davis S. The Time Clock system: A new oraldosage form for fast and complete release of drug after predetermined lag time. Journal of controlled release. 1995;31:99-108.