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Original Research Article

An Investigation on Formulation and Evaluation of Specialized Chronotherapeutical Drug Delivery System of Losartan and Hydrochlorothiazide for the Treatment of Hypertension

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Abstract: Pulsatile Drug Delivery System are the system where in there is a rapid and transient release of an active molecule within a short time period produced immediately after a predetermined off release period. In this present study efforts are made to formulate and evaluate chronotherapeutical drug delivery system of Losartan and Hydrochlorothiazide in the form of bi layered tablets for the treatment of hypertension by using various synthetic polymers. The compatibility study between drug and polymer was done by FTIR. Blend analysis of Losartan and Hydrochlorothiazide layers were studied. Based on the results these were formulated into bi layered tablets. Various evaluation tests of the tablets like weight variation, disintegration test and friability test etc were carried out. *In vitro* drug releases of the formulated tablets were carried out. Results of these studies indicated that the formulated bi layered tablets achieved a burst release after 3h-4 h lag time and hence can be used as the pulsatile drug delivery for hypertension. From this present work it can be concluded that pulsatile release of drug in the form of bi layered tablet can be achieved for newer chronotherapeutical drug delivery system.

Keywords: Pulsatile release, FTIR, Burst Release, Hypertension, Chronotherapeutical drug delivery system

INTRODUCTION

Over the past two decades, the pharmaceutical market has been demonstrating increasing demands preferably in the field of controlled and targeted drug delivery system that represents the most popular form of drug delivery systems with obvious advantages. Such system releases the drug with constant or variable release rates or targets the therapeutic agent to a specific site/tissue/organ. They showed a typical pattern of drug release in which the drug concentration is maintained in the therapeutic window for a prolonged period of time (Sustained release), thereby ensuring sustained therapeutic action. However recently there are few conditions observed for which release pattern is not suitable and requires the drug releases after a lag time. Thus such conditions lead to the requirements of a therapeutic system, which is capable of releasing the drug after predetermined time delay and maintain constant drug levels throughout the day. Thus the system is named as "Pulsatile Drug Delivery System" where in there is a rapid and transient release of an active molecule within a short time period produced immediately after a predetermined off release period [1]. Recent studies have indicated that widespread chronic diseases display time dependent symptoms for which there is a need of drug release at a predetermined time or pulses of known sequence. Thus the pulsatile drug delivery system should be designed in such a manner that it should completely release the drug as a "Pulse" after a lag time. In this field of drug release, matching of drug release to the body's circadian rhythms has been a fundamental strategy. A Circadian rhythm is an endogenously driven roughly 24 hour cycle in biochemical, physiological or behavioral processes which have been widely observed in plants, animals, fungi and cyanobacteria. Thus the pulse and amplitude of key physiological and biochemical circadian rhythms contribute to the known predictable in time pattern in the occurrence of serious and life- threatening medical events. Some typical examples of chronological behaviors are listed in the below mentioned Table 1 [2-3].

Table 1: Chronological Behaviors of some disease and drugs used.

Chronological Behaviors	Disease	Drugs used
Acid Secretion is high in afternoon and at night	Peptic ulcers	H2 Antagonists
BP is lowest at its sleep cycle and rises steeply during the early morning.	Cardiovascular disease	Nitroglycerin, Calcium channel blockers, ACE Inhibitors etc
Pain in the morning and more at the night	Arthritis	NSAIDS, Glucocorticoids
Increase in Blood sugar level after meal	Diabetes Mellitus	Sufonyl Urea. Insulin
Cholesterol synthesis is generally higher during night than day time	Hyper Cholesterolemia	HMG CoA reductase

From the literature survey, it is found that rhythmicity in these above mentioned diseases is one the basis for the development of the Chronotherapeutics. Literature survey reveals that pulsatile drug delivery systems are those systems that deliver the drug at the right time, right place and in right amount and hold good promises of benefits to the patients suffering with chronic problems.

Hence the objective of the current investigational work is to formulate and evaluate chronotherapeutical drug delivery system of Losartan and Hydrochlorothiazide in the form of bi layered tablets for the treatment of hypertension by using various synthetic polymers.

MATERIAL AND METHODS

Losartan Hydrochloride and Hydrochlorothiazide and Croscaremellose sodium was obtained as gift sample from Sanofi India Limited, Goa. Rest of the Polymers were purchased from different suppliers from Bangalore.

Drug Excipient Interaction Studies [2]

FTNIR spectra of physical mixture of Losartan + Hydrochlorothiazide+ MCC+ HPMC+ Ethycellulose + Crosscaremellose+ Eudragit was carried out by using KBr pellet technique. Samples were scanned over 400-4000cm-1 and spectral region at a resolution of 4cm-1. The ratio of the sample in KBr disc was 1% (Shimadzu FTIR spectrometer)

Procedure for the preparation of the blend layers (First Layer of Losartan) and (Second layer of Hydrochlorothiazide) [3-10]

All the Raw materials, as given in the table no 2 are dispensed and are sifted through the 1.0 mm and 0.6 mm screen. Losartan potassium 50 mg, Lactose 49.08 mg, MCC 63.0 mg and Starch 21.6 mg are blended for 10 minutes in a lab scale octagonal blender at a speed of 18 rpm. Collidal Silicon dioxide 0.90 mg, Crosscaremellose sodium are added to the above blend and mixed for 5 minutes in a lab scale octagonal blender at a speed of 18 rpm. Magnesium stearate is now added to the above blend and again mixed for 10 minutes in a lab scale octagonal blender for 3 minutes at 18 rpm. Samples of the blend were now taken for the checking the blend uniformity and assay. The Composition of the blend is as follows.

Table 2: Blend Composition of Losartan Layer

SL. NO	Ingredients	Quantity per tablet
1.	Losartan Potassium	50.0 mg
2.	Lactose (Super tab)	40.9 mg
3	Microcrystalline Cellulose (AVICEL PH 102)	63.0 mg
4.	Starch (1500)	21.6 mg
5.	Colloidal Silicon dioxide	0.90 mg
6.	Croscaremellose Sodium (AC-Di-Sol [Type A])	1.80 mg
7.	Magnesium Stearate (Veg Origin)	1.80 mg
8.	Ethyl Cellulose	40.0 mg
9.	Purified Water	QS
10.	Eudragit grade used (S-100)	3.0ml
Total Quantity		**220.0mg**

Similarly all the Raw materials, as given in the table 3 are dispensed and are sifted through the 40 mesh and Hydrochlorothiazide through 50 mesh. Hydrochlorothiazide 12.500 mg, PCS starch 36.592 mg, MCC IP 30.618mg. Sodium Stearyl fumerate 0.250mg and Red ferric oxide 0.040mg. The above blend was added to the lab scale Octagonal blender, operated for 18 minutes at 18 rpm. Samples of the blend are now taken for the checking the content uniformity and assay. Based on the results, the formula and blending time will be optimised.

Table 3: Blend Composition of Hydrochlorothiazide Layer

Serial no	Ingredients	Quantity per tablet
1.	Hydrochlorothiazide	12.500 mg
2.	Pregelatinised starch (PCS Starch)	36.592 mg
3	Micro Crystalline Cellulose	30.618 mg
4.	Sodium Stearyl fumerate	0.250 mg
5.	Red ferric oxide	0.040 mg
Total Quantity		**80.0 mg**

Content Uniformity Test and Assay Test (Losartan layer)

Buffer Preparation: Dissolve about 2.72g of monobasic potassium phosphate in 2 L of water. Adjust the pH to 2.5 with phosphoric acid.

Mobile phase: Prepare mixture of buffer and acetonitrile (40:60).

Diluent: Dissolve 17.42g of dibasic potassium phosphate in 900 ml of water. Adjust pH to 8.0 with phosphoric acid, dilute with water to 1000 ml and mix. Prepare dilution in water (1:10) and mix well.

Preparation of standard: Transfer 25 mg of Losartan Potassium standard, carefully weighed, to a 50 mL volumetric flask. Dissolve and make to volume with diluent. Pipette a 1 ml aliquot to 10 mL volumetric flask and make up the volume with diluent.

Sample Preparation: Transfer 1grm blend to 100ml volumetric flask. Add approximately 65 ml of diluent and sonicate for about 30 minutes. Make up the volume with diluent and mix thoroughly. Centrifuge for about 2 minutes at about 3300 rpm. Dilute 1 ml aliquot to 10 ml volumetric flask and make up the volume with diluent.

Table 4: Equipment Parameters

Requirement	**Standard**
Equipment	HPLC Detector with UV detector
Column	Purosphere Star RP 8, 4.6×250 mm.
Column temperature	25°C
Flow rate	1.4ml/minute
Detection Wavelength 230 nm	Injection Volume: 5µl and Time- 5 minutes

Procedure: Inject five replicates of standard preparation and single injection of test preparation into the chromatographic system, record the chromatograms and measure responses for the peak due to Losartan Potassium eluting at about 3.1 minutes. The relative standard deviation for peak areas of five replicate injections of standard solution should be not more than 2.0%. The Asymmetry for Losartan Potassium in standard solution and samples should be NMT 2.0. The number of theoretical plates for the peak due to Losartan Potassium in standard solution should be more than 3000.

$$\% \text{ Assay} = \frac{At \times Ws \times 1\times100\times10 \times P \times 100}{As \times 50\times10\times1\times1\times100 \times L}$$

Where,
At: Area of Losartan Potassium peak in test preparation
As: Mean area of Losartan Potassium peak in standard solution
Ws: Weight of standard (mg)
P: % Potency of standard as Losartan Potassium.
L: Label claim of Losartan Potassium in tablet (mg)

Content Uniformity Test and Assay Test (Hydrochlorothiazide layer).

Test solution:

Procedure: Test solution of about 0.125 mg/ml Hydrochlorothiazide was prepared as follows:

1 grm powder was accurately weighed in 25.0 ml volumetric flask by using 15 ml of water.

The solution was sonicated for about 10 minutes and then diluted with 0.1N HCl. The solution was cooled and centrifuged for 15 minutes. The solution thus obtained is the test solution.

Standard solution:

About 31.0 mg of Hydrochlorothiazide reference standard was dissolved in purified water. This solution was then diluted with 0.1 N HCl. The solution thus obtained is the standard solution that was used to determine the contents of the active ingredients in the blend.

Content of Hydrochlorothiazide

$$\frac{\text{Sample Area} \times \text{Std. Wt.} \times 10 \times 25 \times 20 \times \text{Purity of Std.}}{\text{Std. Area} \times 50 \times 50 \times 5 \times 100}$$

Table 5: Equipment Parameters.

Requirement	Standard
Equipment	HPLC with UV detectors
Column	Lichrospher 60 RP
Column temperature	Room temperature
Flow rate	1.0 ml/minute
Detection wavelength: 230nm	Injection volume: 3 µl and Time- 5 minutes

Particle size determination studies

Approximately 3 grms of the sample was taken. Slurry of this sample was prepared by dissolving the blend sample by dilute HNO3. Collidal stability of the slurry was determined by performing three repetitions against time. This was found to be satisfactory. Hence the equipment was switched on and measurement was taken directly from the equipment.

Determination of Angle of repose / Compressibility Index and Degree of Compression The angle of repose (q), Compressibility index (C.I.), Degree of compression (c) and the Hausner's ratio for the above blends were calculated using following equations:

0 = tan -1 (h/r) ---------- (1)

Where, q=Angle of repose

h=Height of granule above flat surface

r=Radius of circle formed by the granule pile.

C.I. = {(ρt – ρ0) / ρt} × 100 ---- (2)

Where, ρt - tapped density**,** ρ0 - bulk density

C= Ho – Hp/ Hp ---------- (3)

where c= degree of compression.

Ho – height of granule bed in the die before compression

Hp – height of granule bed in the die at a pressure p

Hausner's ratio = TBD/LBD - (4)

where TBD= Tapped bulk densities

LBD= Loose bulk densities

Optimisation and finalisation of Granulation parameters properties.

Preparation of Granulating solution: 50 ml of the water was accurately weighed to which 3 ml of eudragit solution was added. The solution was stirred throughly with the help of a stirrer. The solution was stirred continuously for 45 minutes until a uniform homogenization was obtained.

Table 6: Composition of Granulating solution

Serial no	Ingredients	Quantity
1.	Eudragit solution (S 100)	3 ml
2.	Purified Water	50 ml

Procedure for Granulating the Losartan blend for Pulsatile release

The blend (first layer) was loaded into the lab scale FBD bowl. The parameters of the FBD bowl is mentioned as mentioned in the table

Table 7: Operating requirement of the equipment

Physical Parameter	Operating range
Peristaltic pump RPM	5-30 rpm
Inlet Temperature	35-60^{0}C
Outlet temperature	35-60^{0}C
Spray rate	1.5 to 4.0 kg/cm2
Final product temperature	42-46 ^{0}C
Inlet air volume	3000- 4000m3/h
Bed Temperature	25- 30^{0}C

These coated Granules were then collected in a double lined poly bag inserted with silica gel in between two poly bags to prevent it from atmospheric humidity.

Procedure for Compression of Bilayered Tablets and their evaluation tests.

Both the Blend are now fed into the separate hopper of the double sided rotatory punching machine and compression was carried out at a speed of 10 RPM and D tooling.. Various Evaluation tests were performed on the compressed tablets which as follows.

Description: Performed by visual examination by using an optical lens.

Average weight: Average weight of 20 tablets is determined by using an analytical balance.

Uniformity of weight: Weighed individually 20 tablets taken at random.

Thickness of Tablet: Measured and recorded the thickness of 10 individual tablets using Vernier Calipers .Mean of the ten thickness values were also determined.

Hardness of the tablet: Measured and recorded the hardness of the 10 tablets by using Erweka Hardness tester

Disintegration test: One tablet was placed in each of the six tubes of the disintegration test assembly. A disc was placed over each of the tablet. The assembly was then suspended in the beaker containing purified water maintained at 37±1^{o}C. The instrument was operated and checked for the disintegration of the tablets. The time was recorded when all the six tablets have disintegrated and no solid mass is left in any of the tubes.

Friability tests: 10 tablets were weighed on a weighing balance. These are then dedusted. These tablets were then placed into the drum of the Roche friabilator. The equipment was then operated at 25 rpm for 4 minutes. The tablets were then removed and weighed again. Friability of the tablets were calculated by using the formula. Stress friability was further determined by operating the equipment for 15 minutes.

F=Initial weight- Final weight/Initial weight x100

***In Vitro* dissolution Studies of formulated Tablets**

The *invitro* dissolution was carried out using USP Dissolution testing apparatus type-II (Paddle Method. The tablets were placed in the 0.1N hydrochloric acid for first 2 hours and pH 6.8 phosphate buffers for next 8 hours respectively, then the apparatus was run at 37°C ± 0.5°C and a rotating speed of 50 rpm in a 900 ml dissolution medium. The 5 ml aliquots were withdrawn at intervals of 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10 hours and replacement was done each time with equal amounts of fresh dissolution medium maintained at same temperature. Each 5 ml aliquot was filtered through Whatman filter paper (No.41). 5 ml of sample was diluted to 10 ml 0.1N hydrochloric acid for first 2 hours and then with pH 6.8 phosphate buffers for next 8 hours and absorbance was measured at 256.0 nm using a Shimadzu-1700 UV spectrophotometer. The release data were calculated by using disso V3 software.

Note: Four point sampling was performed only for F1 trial withdrawing the samples at (1st hr, 4th hr, 8th hr and 12th hr respectively.)

Table 8: Dissolution Profiling Paramters

Apparatus	:	2 (Paddle)
Speed	:	50 RPM
Time	:	30 minutes
Temperature	:	37°C ± 0.5°C
Dissolution medium	:	0.1N HCl and pH 6.8 phosphate buffer.
Number of jars	:	6
Number of tablets per jar	:	1
Detection	:	UV at 256 nm

RESULTS AND DISCUSSIONS

Drug Excipient Interaction Studies

Drug-polymer interaction study was carried out on physical mixture of pure drug and polymers (i.e.Losartan+Hydrochlorthiazide+MCC+HPMC+Ethyl cellulose+Crosscaremellose+Eudragit) From the spectra obtained, it was observed that all the characteristic peaks of Losartan and Hydrochlorothiazide were present in the combination spectrum, thus indicating compatibility of the drug and polymer in pulsatile device.

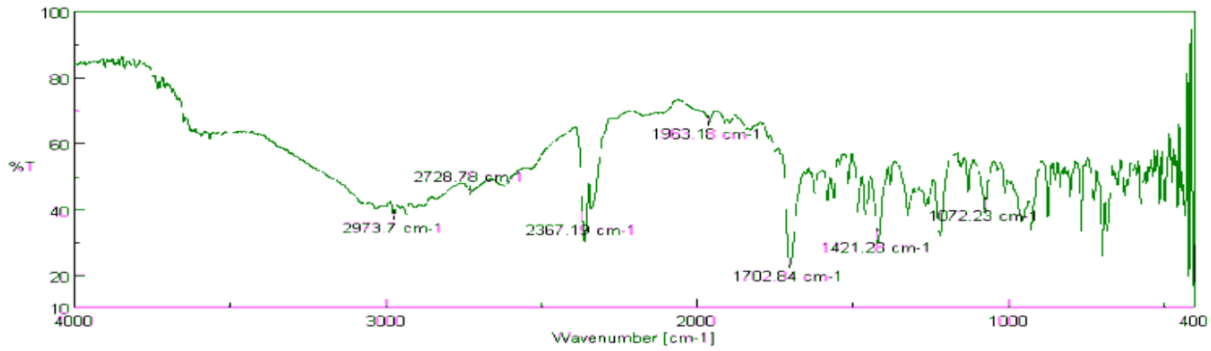

Fig-1: FTNIR Spectra For Drug + Polymers

Content uniformity and Assay results of the Blend layer

Table 9: Content Uniformity Results of Losartan Layer and Hydrochlorothiazide Layers

Sampling Locations	Losartan Content (%)	Hydrochlorothiazide Content (%)
L 1	96.0	99.0
L 2	99.0	99.0
L 3	97.0	98.0
L 4	98.0	99.0
L 5	97.0	98.0
L 6	98.0	99.0
L 7	97.0	97.0
L 8	96.0	99.0
L 9	98.0	99.0
L 10	96.0	98.0
Minimum	96.0	98.0
Maximum	99.0	99.0
Mean	97.0	98.5
% RSD	1.1	0.7
Acceptance Criteria: (Losartan Potassium) 90.0% to 110.0 % of the labelled amount of Losartan potassium. RSD: Not more than 5.0%		
Acceptance Criteria: (Hydrochlorothiazide) 85.0% to 115.0% of the labelled amount of Hydrochlorothiazide. RSD: Not more than 5.0%		

Table 10: Assay Results of Losartan Layer and Hydrochlorothiazide Layers

Blend	**Mixing time**	**Composite Assay results (%)**	**Acceptance Criteria**
Losartan Blend	12 min at 18 rpm	99.0	95% to 105% of the labeled amount of losartan potassium.
Hydrochlorothiazide Blend	18 min at 18 rpm	98.0	85% to 115% of the labeled amount of Hydrochlorothiazide

Based on the above results it was concluded that the content uniformity and blend assay are well within the acceptance criteria. Hence the same mixing time to be incorporated during the manufacturing of pilot scale batches.

Particle size determination studies

The particle size of both the blends as determined by Malvern apparatus was found to be less than 5 micron. Hence the study was found to be satisfactory.

Determination of Angle of repose / Compressibility Index and Degree of Compression

Angle of repose/ Compressibility Index and Degree of compression of these blends was studied and the results showed that blend has got a desirable flow property and can be processed further for compression operation.

Table 11: Angle of repose, Degree of compression, compression index of blend sample

Trial 1	**Angle of repose**	**Degree of compression**	**Compression index.**	**Homogenisity of mixing.**
Losartan Blend	28.36	19.25	41.80	98.87%
Hydrochrothiazide Blend	28.37	20.13	40.15	98.86%

Evaluation Test of Bilayered tablets

The tablets were subjected for various evaluation tests like Average weight, Thickness, Hardness and Disintegration test. All the results obtained were found to be satisfactory .Hence these tablets were subjected further for *in vitro* dissolution studies.

Table 12: Physical parameter test results of the tablet

Trial	Description	Average weight	Thickness	Hardness	Disintegration Test	Friability
Losartan layer	Complies	220.0 mg	6.9 - 7.0mm	55 N	-	0.25%
Hydrochlorothiazide layer	Complies	80.0 mg	6.8 - 6.9 mm	45 N	1 minute	0.11%

Dissolution profiling results

The results showed that the ethyl cellulose produced an increased lag time prior to drug release. When the dissolution medium reached the tablet after eroding or rupturing the barrier layer, rapid drug release was observed. Burst release of the drug was observed in the formulated tablet Hence from the below data it can be concluded that the Ethylcellulose showed highest lag time which is based upon the hydration of outer barrier layer or water penetration through outer barrier layer. Ethylcellulose is semipermeable in nature, although it is naturally insoluble in water. The results indicate that Formulated bilayered tablets achieved a burst release after 3 h-4 h lag time and hence can be used as the pulsatile drug delivery for hypertension.

Table 12: Dissolution test results of the tablet

Time (Hrs)	% drug released
0.5	0
2.0	0
3.0	4.0± 0.059
4.0	17.0± 0.051
5.0	33.0± 0.033
6.0	53.0± 0.056
7.0	68.0± 0.021
8.0	72.0± 0.024
9.0	84.0± 0.033
10.0	97.0± 0.034

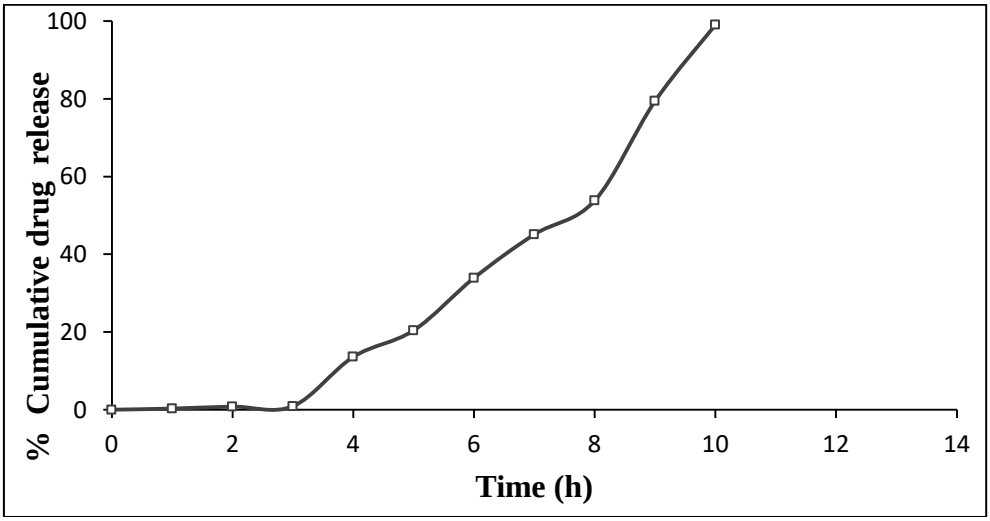

Fig-2: *In vitro* Dissolution profiling of formulated tablet

SUMMARY AND CONCLUSION

From the above work it can be concluded that the drug polymer compatibility studies were found to be satisfactory, Hence Losartan blend was prepared by using granulation method. Blend samples passed the content uniformity and assay tests. All the results of the micrometrics tests were found to be satisfactory. Physical parameter of Fluidized Bed Dryer equipment was finalized and Granulation of the Losartan blend was taken successfully. Evaluation tests carried out on compressed tablets were well within the acceptance criteria. Hence the tablets can be subjected for further chemical analysis. *In vitro* Dissolution studies results indicate that Formulated bilayered tablets achieved a burst release after 3 h-4 h lag time and hence can be used as the pulsatile drug delivery for hypertension.

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