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Formulation and *In-Vitro* Evaluation of Pulsatile Drug Delivery System of Trimetazidine Hydrochloride for Chronomodulated Therapy

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ABSTRACT

The main objective of the present investigation was to formulate and evaluate chronomodulated pulsatile drug delivery system of Trimetazidine Hydrochloride which was aimed to release the drug after lag time (6 hrs) in order to mimic circardian rhythm of Angina Pectoris.Preformulation studies and compatibility studies were carried out for drug and excipients. Core tablet was prepared by direct compression using sodium starch glycolate as superdisintegrant and press coated with different polymer & varying its ratio. Further prepared tablets were optimized using 32 full factorial design. Nine batches were prepared varying the amount of polymer and ratio of polumer (HPMC K4M: EC) and they were evaluated for precompressional and postcompressional tests. Optimized batch was derived statistically using desirability function (Minitab 17).The Model was validated by formulating the check point batch. Accerelated stabilitiy study was carried out of optimized batch. Preformulation and compatibility studies was carried out using FTIR , DSC which shows satisfactory results, no interaction was found between drug and excipients. Press coating of core tablet with the combination of HPMC K4M and EC was found to be providing the desired release. Results of precompressional and postcompressional was found to be within the limits. Varying the amount of coating and ratio of polymer have significant effect on lag time(Y1) as well as on time required for 90% drug release (Y2).Optimized batch shows lag time of 6 hrs followed by complete release within 1 hrs which is desiered in case of pulsatile delivery. No significant bias was found between predicted and observed value of check point batch.The data of stability study revealed that the optimized formulation is stable. Pulsatile drug delivery system of Trimetazidine Hydrochloride for chronomodulated therapy can be prepared by press coating technique using 200 mg of coating and HPMC K4M:EC(10:90) ratio of polymer which will provide lag time of 6hrs and complete release within 1 hrs.

Keywords: Chronomodulated, Pulsatile release, Lag time, Trimetazidine Hydrochloride, HPMC K4M , EC, Press Coated tablet

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INTRODUCTION

With the advancement of the technologies in the pharmaceutical field, drug delivery systems have drawn an increasing interest over the last few decades. Nowadays, the emphasis of pharmaceutical galenic research is turned towards the development of more efficacious drug delivery systems with already existing molecule rather going for new drug discovery because of the inherent hurdles posed in drug discovery and development process.

During the past several decades, conventional drug dosage forms have been widely used for treatment of various conditions. These drug dosage forms typically provide an immediate or rapid medication release, and supply a given concentration or quantity of the drug to the body's systemic circulatory system without any rate control. To maintain the effective plasma drug concentration, frequent administration is required. Due to poor drug efficacy, the incidence of side effects, frequency of administration and patient compliance of these conventional drug preparations, many traditional drug dosage forms are undergoing replacement by second-generation, modified drug-release dosage forms.

But still for many of the drugs, use of such systems is not suitable because of a number of reasons. This is particularly true in cases where the drug is subjected to large metabolic degradation. Due to first pass effect there will be reduction in the bioavailability of the drug because gradual release can result in greater degradation. Secondly drugs with short half-life need to be administered repeatedly which results in patient non-compliance. Further, in case of chronic treatment, where the drug is given in sustained release dosage form, continuous exposure of the drug to body may lead to adverse effect. Lastly, drugs which exhibit tolerance should not be delivered at a constant rate, since the drug effect decreases with time at constant drug level. In addition drug toxicity increases with time when drug levels are held constant.

Recent studies also reveal that the body's biological rhythm may affect normal physiological function, including gastrointestinal motility, gastric acid secretion, gastrointestinal blood flow, renal blood flow, hepatic blood flow, urinary pH, cardiac output, drug-protein binding, and liver enzymatic activity, and biological functions such as heart rate, blood pressure, body temperature, blood-plasma concentration, intraocular pressure, stroke volume and platelet aggregation. Most organ functions vary with the time of the day, particularly when there are rhythmic and temporal patterns in the manifestation of a given disease state. The symptoms of many diseases, such as bronchial asthma, myocardial infarction, angina pectoris, hypertension, and rheumatic disease have followed the body's biological rhythm.

Diseases where constant drug levels are not preferred, but needs a pulse of therapeutic concentration in a periodic manner acts as a push for the development of Pulsatile Drug Delivery Systems (PDDS) have developed in close connection with emerging chronotherapeutic views. This delivery is gaining much interest and attention because time specific and site-specific delivery of drugs in the right amount is obtained from this device.

The press-coating technique is one of the novel methods and has been applied for many drugs to develop the site- and/or time-controlled release preparation. This technique has many advantages such as nonsolvent process, short processing time and limited steps, and low labor and energy requirements.

Compression-coating has been used in the pharmaceutical field for different purposes:

To protect hygroscopic, light-sensitive, oxygen-labile or acid-labile drugs

To combine and separate different therapeutic drugs

To modify a drug release pattern (Delayed, Pulsatile, Programmable Drug Release)

MATERIALS AND METHODS

Preformulation Studies

For the rationale development of dosage form of a drug substance preformulation testing is the initial step and it is prerequisite. It can be defined as the process of investigating the physical and chemical properties of the drug substance alone and when combined with the excipients. The prime objective of the studies is to gather useful information about the drug to the formulator in developing stable and bioavaible dosage form.

Identification of Drug

Identification of drug is first step in research methodology. The sample of drug was identified by Melting point, FT-IR , DSC, Wavelength Measurements.

Melting Point Determination

Melting point of drug was determined by Capillary Method. Fine powder of Trimetazidine Hydrochloride was filled in glass capillary tube (previously sealed at one end), tube is tied with the thermometer and this thermometer is placed in liquid paraffin bath. The paraffin bath was placed on the fire and the temperature at which powder melted was noticied.

FT-IR Study

IR spectra was taken for the drug. FTIR spectra was recorded with Thermo-scientific. In the range 450-4000 cm^{-1} using a resolution of 4 $cm^{-1}$and 45 scans. Sample was diluted with KBr mixing Powder, and pressed to obtain self-supporting disks. The FT-IR spectrum of the

obtained sample of the drug was compared with the standard FT-IR spectra of the pure drug.

DSC Study

Thermal behavior of drug was examined using thermal analyzer. An accurately weighed sample (about 1 mg) was placed in sealed aluminum pans before heating under nitrogen flow (20 mL/min) at a scanning rate of 10 °C min-1 from 50 to 300 °C. An empty aluminum pan was used as reference.

Determination of λ_{max} of Trimetazidine Hydrochloride in pH 1.2 and pH 6.8

Standard stock solution of Trimetazidine Hydrochloride (100µg/mL) was prepared by dissolving 10 mg of Trimetazidine Hydrochloride in different media such as 0.1 N HCl pH 1.2, phosphate buffer pH 6.8 volumes was made up to 100 ml. These solutions were scanned in UV range between 200 to 400 nm. Wavelength at which showed maximum absorbance was selected as λ max for Trimetazidine Hydrochloride

Drug- excipients compatibility studies

Compatibility must be established between the active ingredients and other excipients to produce a stable, efficacious, attractive and safe product. FTIR of Trimetazidine Hydrochloride with different polymers and other excipients were recorded between 400 to 4000 cm–1 using FTIR spectrometer (Thermo scientific).

Preliminary Studies

Development of Trimetazidine Hydrochloride Pulsatile release tablet

Preparation of core tablet

Optimization of superdisintegrant concentration

Core tablet of Trimetazidine Hydrocholride was prepared by direct compression method. The core tablets were optimized for different concentration of superdisintegrant. Superdisintegrant used for core tablet was sodium starch glycolate as per literature review and concentration range used were 2%, 4%, 6% of total weight of core tablet. core tablets were prepared after passing all the ingredients (except glidant and lubricant) through 40# and mixing for 15 min . Then adding talc and magnesium stearate mixing it well and directly compressing in 6 mm concave punch by rotatry tablet compression machine . Total weight of core tablet was kept constant as 100mg.

Preparation of Press coated tablet

Screening of coating polymer

Optimized core tablets were compression coated with different coating polymer and varying the ratio of polymers. Powder blend for press coated tablet was prepared by dry blending together with different composition of different polymers. Half quantity of coating material

was placed in die cavity; core tablet was carefully placed in the centre of the die cavity and filled with the other half quantity of coating material. The coating material was compressed using 10 mm punch by rotary tablet compression machine.

Study effect of coating amount on lag time

Optimized ratio of polymer is being further studied for effect of coat amount on lag time. Two batches were prepared varying the amount of coating on core tablet keeping the ratio of polymer same.

***In-Vitro* disintegration time**

In-Vitro disintegration time of three tablets was determined by using digital tablet disintegration apparatus. *In-Vitro* disintegration test was carried out at 37 ± 2 ^{o}C in 900 ml phosphate buffer pH 6.8.

Drug content

Amount of the powder equivalent to 40 mg of Trimetazidine Hydrochloride was weighed and dissolved in 10 ml of Phosphate buffer pH 6.8, filtered, diluted suitably and analyzed for drug content at 231 nm using UV-spectrophotometer.

***In-Vitro* dissolution study**

In-Vitro dissolution study of optimized core tablet was performed using USP Dissolution Apparatus II (Paddle type) at rotation speed of 50 rpm using 500 mL of phosphate buffer pH 6.8 as the dissolution media maintained at 37±0.5 ^{o}C. Aliquots of 5 ml were withdrawn at different time intervals and was replaced with equal amounts of fresh release medium. The withdrawn samples was filtered through 0.45 um membrane filters and drug content in each sample was analyzed after suitable dilution by UV/VIS spectrophotometer at wavelength 231 nm. Cumulative percentage release of drug calculated and plotted against time.

Formulation optimization using 3^2 full factorial design

A 3^2 randomized full factorial design was used to quantify the significant independent variables revealed from preliminary studies. In this design 2 factors were evaluated, each at 3 levels, and experimental trials were performed at all 9 possible combinations generated by Minitab® 17.0. As shown in table in that two independent variables namely X1(Ratio of HPMC K4M: EC) & X2(Total weight of coating polymer). Y1 (Lag time) & Y2 (T90) were selected as a dependent variables.

On the bases of preliminary batches results, the low, medium and high values of independent variables were selected and the batches from F1 to F9 were formulated.

Statistical Analysis and Optimization

The experimental data obtained from all formulations were validated by ANOVA, Regression Analysis using Microsoft Excel 2010 & optimization by using Minitab®
17.0 and used to generate the study design and the response surface plots and contour plots were plotted by using Sigma Plot 11.0 . Polynomial models, including linear, interaction and quadratic terms were generated for all the response variables using the software. In addition, analysis of variance (ANOVA) was used to identify significant effects of factors on response regression coefficients.

Stability Studies

The stability testing of the optimized batches will be carried out in as per ICH (2010) guidelines i.e accelerated study at 40^{o}C ±2 and 75%±5 RH for one month. The tablets of the optimized formulation were placed in stability chamber and samples were withdrawn, visually examined and evaluated for drug content and *in-vitro* dissolution studies.

RESULT AND DISCUSSION

Identification of drug

Melting point

The observe melting point was 231-235 ^{o}C. This melting point resemble to melting point given in IP'10.

FT-IR Study

The IR spectra of pure drug shows characteristic peaks which are given below in table and depicted in figure. The obtained spectra of pure drug have been compared with standarad IR spectra of Trimetazidine Hydrochloride given in pharmacopeia(JP).The spectra matches with standard spectra of drug

DSC Study

The DSC thermogragh of Trimetazidine Hydrochloride shows endothermic peak at 240^{o}C corresponding to its melting point. The onset of melting was observed at 232 ^{o}C.Melting point is nearly same as given in IP'10 which gives purity and identity

Determination of λ_{max} of Trimetazidine Hydrochloride in pH 1.2 and pH 6.8 The spectrum of stock solution of Trimetazidine Hydrochloride shows λ_{max} at 231 nm in both pH 1.2 and pH 6.8 which was indentical to the standard spectrum of Trimetazidine Hydrochloride

Drug-Excipients compatibility study

From study of FTIR spectra of pure drug and drug – polymer mixture, it was found that functional

group peaks of pure drug remain same even in physical mixture prepared by using excipients and API. From observation and identification of peaks it can be established that API and excipients are compatible

Optimization of concentration of superdisintegrant

Table 1: formulation of core tablet

Ingredients	Quantity (mg) Batch 1	Batch 2	Batch 3
Trimetazidine Hydrochloride	40	40	40
Microcrystalline cellulose	54	52	50
Sodium starch glycolate	2	4	6
PVP K30	2	2	2
Talc	1	1	1
Magnesium stearate	1	1	1
Sunset yellow	qs	qs	qs
Total	100	100	100

Evaluation parameter for core tablet

Pre-compressional parameter

Micromeritic properties like angle of repose, bulk density, tapped density, carr's index, hausner' s ratio are shown in table 2. The prepared powder blend of core tablet shows good flow properties as indicated by low values of angle of repose, carr's index, hausner' ratio.

Table 2: Pre-compressional evaluation

Batch	Angle of repose	Bulk density(g/cm^3)	Tapped density(g/cm^3)	Carr's index(%)	Hausner's ratio
Batch 1	29.47 ± 1.71	0.43 ± 0.016	0.49 ± 0.024	12.24 ± 0.49	1.13 ± 0.015
Batch 2	28.07 ± 1.35	0.41 ± 0.012	0.48 ± 0.034	14.58 ± 0.35	1.17 ± 0.012
Batch 3	26.56 ± 1.98	0.45 ± 0.018	0.52 ± 0.028	13.46 ± 0.37	1.15 ± 0.015

Post-compressional parameter

Post –compressional evaluation of the core tablet were given in the table 3. Core tablet was characterized for weight variation, thickness, hardness, friability, drug content, *in vitro* disintegration time.

Table 3: Post-compressional evaluation

Batch	Thickness (mm)	Hardness (kg/cm^2)	% Friability	Weight uniformity	Drug Content	Disintegration time (sec)
Batch 1	2.8 ± 0.13	3.5 ± 0.054	0.79 ± 0.019	100 ± 0.11	97.48 ± 1.22	88 ± 3.28
Batch 2	3.0 ±0.15	3.7 ± 0.048	0.53 ± 0.018	100.1±0.15	96.26 ± 1.46	54 ± 5.45
Batch 3	2.9 ±0.12	3.8 ± 0.052	0.34 ± 0.015	99.9 ± 0.13	97.26 ± 1.26	30 ± 2.15

***In- vitro* dissolution of core tablets**

In- vitro drug release of core tablet was carried out in 500 ml of phosphate buffer pH 6.8 at 50 rpm in USP dissolution apparatus (type II) .

Table 4: In-vitro dissolution of core

Time(min)	Batch 1	Batch 2	Batch 3
0	0	0	0
2	7.84	19.47	6.63
4	16.98	26.59	78.50
6	26.79	42.31	92.37
8	34.90	58.96	96.67
10	44.89	60.03	99.08
15	66.30	69.16	97.62
20	82.15	95.76	94.20
25	96.50	91.82	--
30	95.48	90.75	--
45	92.99	--	--

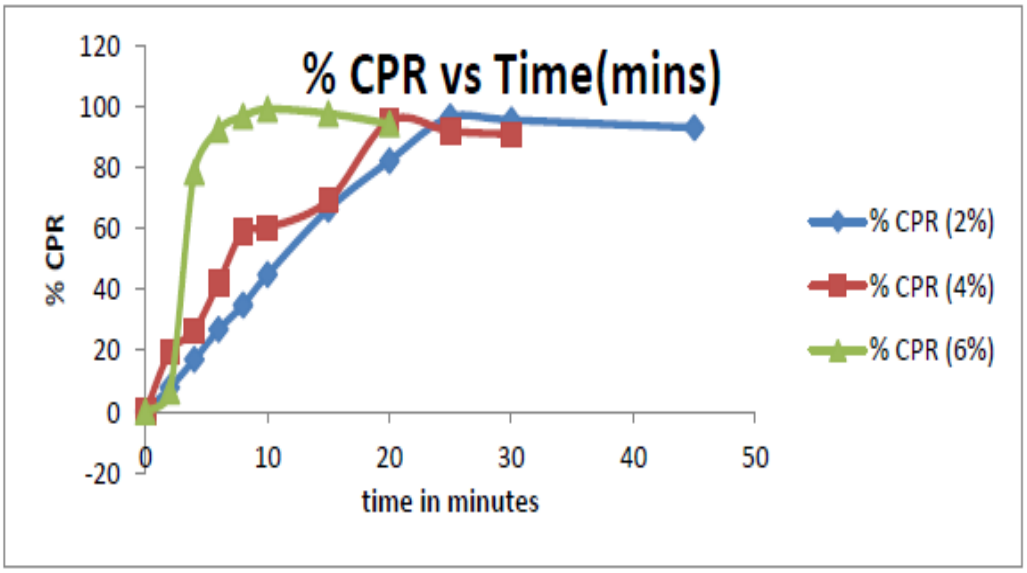

Figure 1: Dissolution data of core tablets

From study of above batches, Batch 3 was considered as optimized as it disintegrates within 30 sec and completely release the drug within 15 mins. So it is further taken for coat optimization as it required for burst release after proposed lag time

Preparation of press coated tablet

Selection of coating polymer and its ratio

Polymer screening was carried out in order to identify the independent factor that affect the lag time. Based on literature review four polymer where selected and press coated tablet were prepared by varying type and concentration of polymer as shown in table. Initially single polymer then combination was tried to study the effect on lag time.

Table 5: Formulation of preliminary batches

Batch code	Core Tablet(mg)	Klucel HXF(mg)	HPMC K4M(mg)	HPMC K100M(mg)	EC (mg)	Total (mg)
Batch C1	100	200	-	-	-	300
Batch C2	100	-	200	-	-	300
Batch C3	100	-	-	200	-	300
Batch C4	100	-	-	-	200	300
Batch C5	100	100	-	-	100	300
Batch C6	100	-	100	-	100	300
Batch C7	100	-	-	100	100	300
Batch C8	100	50	-	-	150	300
Batch C9	100	-	50	-	150	300
Batch C10	100	-	-	50	150	300

Evaluation parameter of press coated tablets

Post compressional parameter of press coated tablets

Post compressional evaluation of press coated tablet were given in table 6. Press coated tablet were characterized for weight variation, thickness, hardness, friability, drug content, *in vitro* Lag time.

Table 6: Post compressional evaluation of press coated tablet

Batch code	Thickness (mm)	Hardness (kg/cm^2)	% Friability	Uniformity of weight	Drug content (%)	Lag time (min)
Batch C1	5.04 ± 0.114	9.28 ± 0.286	0.64 ± 0.03	300.6 ± 1.67	96.26 ± 1.46	200
Batch C2	5.07 ± 0.112	9.52 ± 0.35	0.62 ± 0.07	302.8 ± 1.29	95.48 ± 1.42	240
Batch C3	5.02 ± 0.111	9.53 ± 0.52	0.67 ± 0.05	300.7 ± 1.17	95.48 ± 1.65	280
Batch C4	5.08 ± 0.123	9.42 ± 0.23	0.62 ± 0.06	304.8 ± 1.13	92.58 ± 1.23	400
Batch C5	5.05 ± 0.132	9.52 ± 0.26	0.71 ± 0.02	302.3 ± 1.52	95.54 ± 1.54	160
Batch C6	5.05 ± 0.127	9.35 ± 0.52	0.68 ± 0.05	300.8 ± 1.19	97.48 ± 1.22	210
Batch C7	5.04 ± 0.135	9.26 ± 0.42	0.65 ± 0.02	304.7 ± 1.12	93.78 ± 1.53	230
Batch C8	5.02 ± 0.111	9.50 ± 0.47	0.64 ± 0.08	303.8 ± 1.15	92.52 ± 1.24	300
Batch C9	5.03 ± 0.111	9.38 ± 0.286	0.61 ± 0.03	300.3 ± 1.76	97.26 ± 1.26	320
Batch C10	5.08 ± 0.172	9.32 ± 0.27	0.68 ± 0.06	301.3 ± 1.24	94.54 ± 1.74	340

% Cumulative release of preliminary batches (C1-C5)

In- vitro drug release of Press coated tablet was carried out in 500 ml of 0.1 N HCl (pH 1.2) for two hours and replaced by phosphate buffer pH 6.8 for subsequent hours at 50 rpm in USP dissolution apparatus (type II) .

Table 7: % Cumulative release of preliminary batches (C1-C5)

Time (hrs)	% cumulative drug release				
	Batch C1	Batch C2	Batch C3	Batch C4	Batch C5
0	0	0	0	0	0
0.5	0.72	0.47	0.28	0.15	0.24
1	1.5	0.58	0.77	0.25	0.39
2	4.7	1.83	1.45	0.29	4.25

3	8.20	6.55	6.70	0.30	11.08
4	18.94	10.59	9.38	1.002	18.21
5	30.71	40.26	32.93	1.98	33.27
6	41.18	88.17	57.63	9.65	47.02
7	63.49	94.98	87.27	12.94	80.06
8	76.23	99.86	92.54	13.56	97.28
9	94.87	89.52	98.32	15.10	--
10	--	--	--	--	--
11	--	--	--	--	--
12	--	--	--	--	--

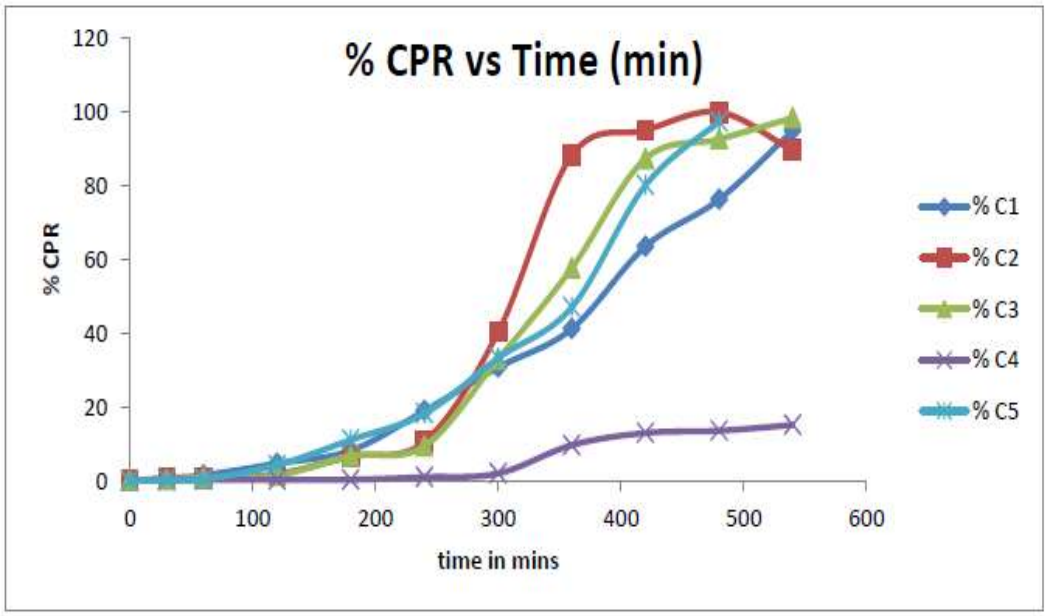

Figure 2: Dissolution data of batches C1-C5

Table 8: % Cumulative release of preliminary batches (C6-C10)

Time (hrs)	% cumulative drug release				
	Batch C6	Batch C7	Batch C8	Batch C9	Batch C10
0	0	0	0	0	0
0.5	0.34	0.57	0.10	0.188	0.10
1	0.44	2.52	0.47	1.25	0.15
2	2.45	8.31	0.96	3.11	0.28
3	6.98	10.83	4.76	6.72	0.62
4	13.76	27.09	9.79	7.13	2.07
5	16.69	34.05	13.78	11.12	2.8
6	27.85	57.01	57.61	25.48	10.2
7	36.55	72.28	73.68	31.40	12.15
8	49.42	82.43	89.43	69.11	28.11
9	67.85	89.54	97.47	82.53	35.52
10	93.25	96.25	--	96.52	47.42
11	--	--	--	--	58.54
12	--	--	--	--	--

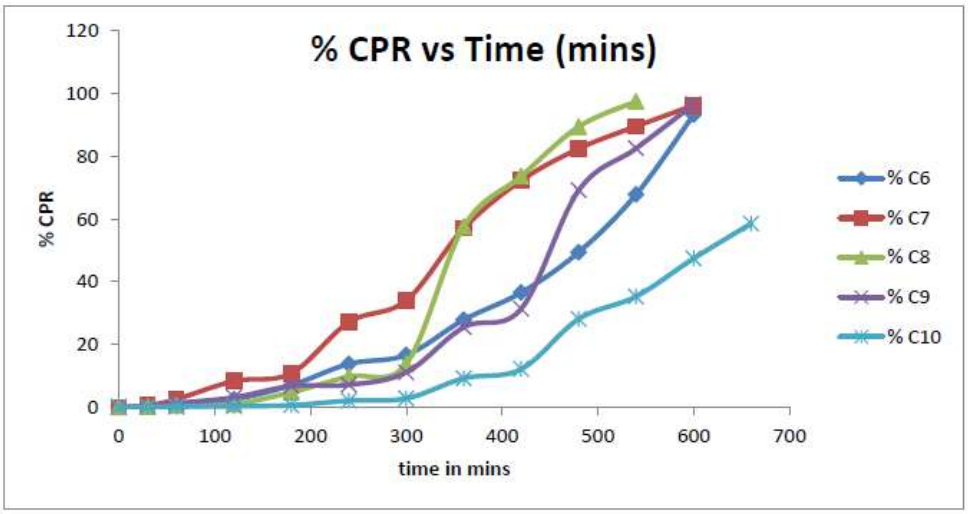

Figure 3: Dissolution data of batch C6-C10

From above study it can be said that single polymer coat is not sufficient to maintain proposed lag time. Klucel HXF is gellable polymer and also acts as disintegranting polymer so it will disintegrates quickly as compared with that of HPMC and is not capable of delaying the drug release.

It was observed that outer coat of HPMC K4M & HPMC K100M alone gets hydrated and formed the viscous gel like structure which results in retarding drug release but it was not capable to delay release of drug. While in case of EC alone used it was capable protecting the drug from being released completely in physiological environment because of its hydrophobicity. When combination of both hydrophilic polymer and hydrophobic polymer is used lag time can be maintain. HPMC K100M cannot be used as it retard the drug release to a great extent which is not advisable as it will not be able to give burst release after predetermined lag time, so HPMC K4M is used along with EC. when combination of HPMC K4M and Ethyl Cellulose is used, as a result of solubility of HPMC K4M upon contact with dissolution medium HPMC hydrated and form a compact gel with ethyl cellulose. The hydrophobicity of ethyl cellulose retard the hydration of HPMC K4M therefore dissolution did not penetrate the outer coating layer but coating erodes as time passes. So combination of two polymer are used for press coated pulsatile delivery. Polymer ratio EC: HPMC K4M (75:25) shows lag time near to proposed lag time 5-6 hrs. So it was further studied for amount of outer coat.

Selection of coat concentration

Table 9: Study effect of coat amount on lag time

Batch code	Core tablet (mg)	PMC k4M (%)	EC (%)	Total weight of coat
Batch A	100	25	75	200
Batch B	100	25	75	220

Post compressional parameter

Post compressional evaluation of Batch A & B were listed in Table 10.

Table 10: Post compressional evaluation of Batch A and Batch B

Evaluation Parameter	Batch A	Batch B
Thickness (mm)	4.94 ± 0.11	6.68 ± 0.21
Hardness (kg/cm^2)	9.06 ± 0.11	9.24 ± 0.23
% Friability	0.706 ± 0.02	0.642 ± 0.06
Weight Uniformity(mg)	302 ± 1.22	320.4 ± 1.14
Drug Content(%)	96.32 ± 1.59	96.5 ± 1.59
Lag Time(mins)	320	380

% cumulative drug release

Table 11: % cumulative drug release of batch A & B

Time (hrs)	% cumulative drug release	
	Batch A	Batch B
0	0	0
0.5	0.62	1.49
1	0.63	3.45
2	0.87	3.58
3	1.76	4.05
4	6.57	4.33
5	9.78	5.39
6	18.21	8.11
7	31.23	22.57
8	83.48	41.69
9	91.72	79.37
10	92.33	87.90

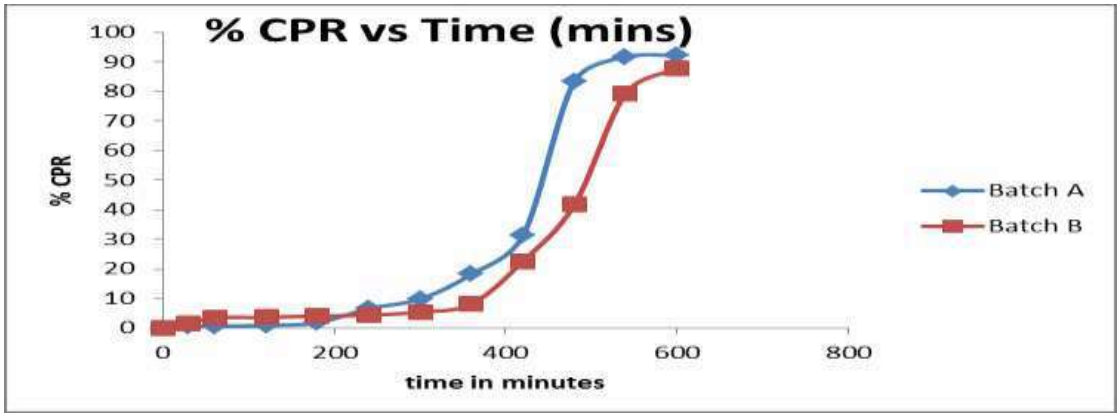

Figure 4: Dissolution data of preliminary batches A & B

From the above studied , two batches were each having different coating level means different amount of outer coating layer on core tablets. They were evaluated for the lag time and for the % Cummulative release .Batch A shows lag time of around 320 minutes and complete release in 9 -10 hrs whereas Batch B shows lag time of about 380 minutes and

release was delayed upto 12 hrs. This occurs due to more coating as compared to batch A this shows that higher the coating level more the lag time . so the amount of coaring also effects the lag time and complete drug release.

Post-compression parameters of 3^2full factorial design batches

Press coated tablet were characterized for weight variation, thickness, hardness, friability, and % Drug Content.

Table 12: Post Compressional Evalaution of 3^2 Full factorial design batches

Batches	Thickness (mm)	Hardness Kg/cm^2	% Friability	Weight Uniformity	% Drug Content
F1	4.13 ± 0.5	8.16 ± 0.286	0.73 ± 0.02	278.6 ± 3.05	99.71 ± 0.62
F2	4.23 ± 0.05	8.66 ± 0.35	0.75 ± 0.03	280.6 ± 1.52	99.87 ± 0.87
F3	4.2 ± 0.11	8.66 ± 0.52	0.69 ± 0.04	280.6 ± 3.05	98.67 ± 0.61
F4	5.1 ± 0.30	8.83 ± 0.27	0.70 ± 0.06	299.0 ± 3.60	97.73 ± 1.73
F5	5.1 ± 0.17	9.33 ± 0.28	0.72 ± 0.06	301.3 ± 1.96	97.31 ± 1.59
F6	5.3 ± 0.10	9.16 ± 0.57	0.66 ± 0.06	298.3 ± 2.14	96.94 ± 1.21
F7	6.03 ± 0.20	9.33 ± 0.28	0.79 ± 0.10	319.1 ± 2.64	96.54 ± 1.47
F8	6.2 ± 0.11	9.66 ± 0.57	0.77 ± 0.04	319.6 ± 3.51	97.17 ± 1.29
F9	5.93 ± 0.05	9.16 ± 0.286	0.70 ± 0.03	320. 6 ± 4.50	98.57± 1.17

***In vitro* Lag Time and *In vitro* dissolution study of press coated pulsatile tablet**

The time taken by the tablet to release the drug 10% (lag time) and remaining drug release (T90%) were evaluated in a USP XXIV dissolution apparatus II filled with 500ml of 0.1N HCl (pH 1.2) for first two hours and pH 6.8 for remaining hours at temperature 37 ± 0.5°C with paddle rotation of 50 rpm. The percentage of drug release was examined over time as shown in table & depicted in figure.

Table 13: % Cummulative Drug Release Of Factorial Design Batches

Time mins	% cumulative Drug Release								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	00	00	00	00	00	00	00	00	00
30	0.68	0.34	0.09	0.10	0.06	1.20	0.02	1.10	0.04
60	2.07	1.63	0.28	0.33	0.20	1.99	0.21	1.16	1.64
120	2.14	1.75	1.35	0.62	0.39	2.64	0.40	1.22	1.80
180	2.35	2.20	8.83	1.06	0.44	3.63	0.49	1.96	2.40
240	2.76	9.15	9.64	1.22	0.66	4.78	0.70	2.22	3.63
300	9.52	42.24	36.48	2.15	2.60	5.07	0.73	2.44	3.91
360	63.60	72.22	68.34	9.54	10.62	42.14	2.45	7.45	9.28
420	94.27	92.80	85.01	88.76	62.57	86.80	9.84	42.76	42.36
480	99.56	98.56	95.05	97.39	88.88	99.77	78.53	70.31	54.86
540	100.06	100.94	97.43	99.81	98.96	100.27	92.40	86.63	76.23
600					97.03	97.86	96.71	97.59	91.52
660								96.67	95.80
720								95.54	101.09

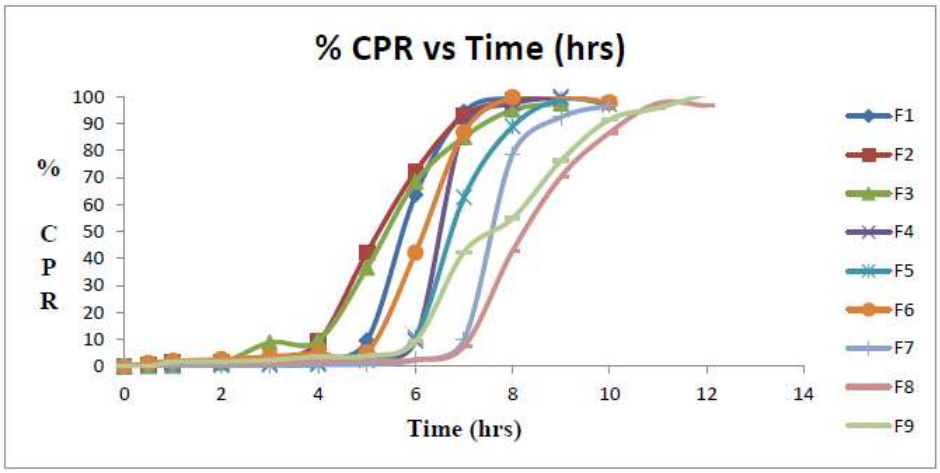

Figure 5: Dissolution Profile of Factorial Batches F1-F9

Drug release profile of press coated tablet shows sigmoid curves with lag time. For the delivery system to be pulsatile it should remain intact and shows minimal drug release in the gastric environment and triggers the drug release in intestinal region.

3^2 full factorial design with its responses

From the preliminary batches it was seen that two polymer and coat weight have mark effect on response variable, to investigate their effect a 32 full factorial design was employed. All the nine possible runs with their decoded values and response is given in the table 14.

Table 14: 3^2 Full factorial design with their response

Independent Variables				Dependent Variables	
Coded Value		Decoded Value			
X1	X2	X1	X2	Y1(T10%)	Y2(T90%)
-1	-1	10:90	180	5	6.45
0	-1	20:80	180	4	7
1	-1	30:70	180	4	7.45
-1	0	10:90	200	6	7.45
0	0	20:80	200	5.45	8.1
1	0	30:70	200	5	8.3
-1	1	10:90	220	7	8.45
0	1	20:80	220	6.15	9.15
1	1	30:70	220	6	10

Statistical analysis & optimization

Regression Analysis of Factorial batches Response

Regression coefficient and p-value of full model was calculated for lag time, drug release. P value less than 0.05 indicate significance of factor.

Coefficient value obtained from the regression analysis is shown with their p- value in the table 15.

Table 15: Regresssion Coefficient and their p-value

Coefficient	β0	β1	β2	β12	β11	β22	R^2
Lag Time (hrs) Y1							
FM	5.28	-0.5	1.025	4.02E-17	0.33	-0.125	0.995
p- value	2.2E-05	0.00405	0.00049	1	0.069	0.33	
Time Required for 90 % Drug Release(hrs) Y2							
FM	7.927	0.61	1.11	0.137	-0.116	0.233	0.996
p- value	7.5E-06	0.00253	0.00043	0.184	0.379	0.131	

From the regression analysis as shown in table R^2 values for Lag time (Y1) and Drug Release (Y2) was found to be 0.995 and 0.996 which shows that response are best fitted in Model, so there is good correlation between independent variables and dependent variables. P- values for some interactive and quadratic terms have higher value than the significance p- value ($p < 0.05$) so need to use the reduced model.

Optimized Batch Formulation:

Table 16: Formulation of Optimized Batch

Core Tablet Ingredients	Quantity (mg)
Trimetazidine Hydrochloride	40
Microcrystalline Cellulose	50
Sodium Starch Glycolate	6
PVP K30	2
Talc	1
Magnesium Stearate	1
Sunset Yellow	Qs
Coating Layer	
HPMC K4M:EC	10:90
Coating Level (mg)	200

Check Point Batch Validation

To validate evolved model, a checkpoint batch was prepared at $X_1 = 0.5$ and $X_2 = -0.5$ levels means X_1=25:75 ratio of polymer (HPMC K4M:EC) and X_2 = 190 mg of coating. Dependent variables were determined and compared with predicted values as shown in Table.

Table 17: Observed Value and Predicted Value along with % Error

Dependent Variables	Predicted Value	Observed Value	% Error
Y_1	4.51	4.45	-1.34
Y_2	7.67	8.0	4.125

When the batch was prepared using defined level of Amount of polymer and HPMC:EC polymer ratio using Minitab 17, the results obtained with check point batch were very close to predicted values. Thus, it can be concluded that the statistical model is mathematically valid.

Stability Studies

From the result of stability study, it was observed that optimized formulation was stable for one month at 40^{o}C ± 2 ^{o}C and 75%RH ± 5% RH as per the ICH guidelines(2010).

When similarity factor (f2) and dissimilarity factor (f1) where calculated from CPR of drug release, it was found to be 84.50 & 3.90 which were within optimum range. From that it was concluded that prepared optimized batch was stable.

Table 18: % CPR data for Stability Studies

Time (hrs)	Before Stability	After Stability
0	0	0
0.5	0.12	0.14
1	0.35	0.23
2	0.43	0.38
3	0.88	0.72
4	1.16	0.97
5	1.27	2.15
6	9.46	7.70
7	90.46	88.27
8	95.76	91.58
9	97.06	95.88

CONCLUSION

Now a days various routes of administration are developed for delivery of drug, among them oral route is the best route of drug administration. over past two decades there has been a growing appreciation on importance of circardian rhythm on GIT physiology and on the diseases states, together with realization of the significance of time of administration. Time specific delivery of drug via pulsatile delivery is desirable for chronotherapy of disease such as hypertension, asthma, arthritis, angina pectoris, hypercholestremia which are being affected by circardian rhythm.

Pulsatile drug delivery system of Trimetazidine Hydrochloride for chronotherapy can be prepared by press coating technique. Rapid release of drug after lag time consistent with the requirement of pulsatile dosage form was completed with the developed formulation. From the prepared formulation it can be concluded that ratio of polymer (HPMC K4M: EC) and the amount of coating are responsible for lag time and the drug release. Developed formulation shows satisfactory results of lag time and drug release. Developed formulation containing 200 mg of coating having 10:90 % ratio of polymer (HPMC K4M:EC) provide desired lag time(6 hrs) and drug release. We can concluded that the novel press coated tablet developed for Trimetazidine Hydrochloride could be a promising alternative for chronomodulated therapy of Angina Pectoris

which is being taken at night time for patients suffering from Angina Pectoris.

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