Research Article

Formulation Development and Evaluation of Bi-Layered Tablet Containing Diuretic and Anti-Hypertensive Agent

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ABSTRACT

The aim of present study was to develop a Bi-layered tablet for management of hypertension using hydrochlorothiazide (HCTZ) in combination with propranolol HCl (Prop.Hcl). Bi-layered tablets were prepared using direct compression method by wet granulation technique consisting an immediate released layer and the sustained released layer. The tablets prepared were evaluated for their physico-chemical properties. Formulation (F4) prepared with 1% cross carmellose sodium (CCS), released the drug i.e. hydrochlorothiazide (HCTZ) content of the immediate layer at the end of the 45 minutes. Formulation (F8) prepared from intragranulation techniques in which combination of both the polymer concentrations in sustained layer showed the synergistic effect and the release rate of the total drug i.e. propranolol HCl content sustains till the end of 12 h respectively. The mechanism of drug release was regarded as anomalous diffusion of drug from the matrix. The optimized formulation (F8) was subjected to short term stability studies as per ICH guidelines and formulations were found to be stable after a 3 month study. It can be concluded that, the optimized formulation (F4) containing 1% CCS in Immediate layer releases the drug hydrochlorothiazide within 45 min & with combination of both polymers with an individual concentration of 7.5% in Sustained layer sustains the release of the drug up to 12 h.

Keywords: Bi-layered tablet, direct compression, hypertension, hydrochlorothiazide, propranolol HCl, anti-hypertensive, immediate layer, sustained layer, cross carmellose sodium and methocel

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INTRODUCTION

The aim of antihypertensive therapy is to abolish the risks related with elevated blood pressure (BP) without adversely affecting quality of life. Clinical trials and epidemiologic studies have been used to define individual risk and set appropriate blood pressure targets [1-3] identifying that these targets reflect expert consensus based on data available and are subject to revision as additional evidence is obtained [4]. The drug is selected based on their efficacy of lowering the BP and also in reducing cardiovascular (CV) end points including stroke, heart failure and myocardial infarction. The choice of initial drug therapy exerts some effect on long-term outcomes; it is evident that BP reduction per sec is the primary determinant of CV risk reduction. As a result, there has been a progressive lowering of BP targets in large segments of the hypertensive population, including diabetic patients and patients with established renal or vascular disease [5].

The ability to maintain constant or near-constant blood pressure in response to various stressors is central to homeostasis, and the human organism has redundant physiologic mechanisms for regulating arterial

pressure. Blood pressure is determined primarily by factors such as: renal sodium excretion and resultant plasma and total body volume, cardiac performance, and vascular tone [6]. These factors control intravascular volume, cardiac output, and systemic vascular resistance, which are the immediate hemodynamic determinants of blood pressure. Both the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) are intimately involved in adjusting these parameters on a real-time basis. In addition, genetic makeup, diet, and environmental factors influence blood pressure in individual patients. Although it is occasionally possible to identify a specific cause for hypertension in some patients, BP elevation is usually multifactorial, making it very difficult, if not impossible, to normalize pressure by interfering with only a single pressor mechanism. In addition, drug therapy directed at any one component routinely evokes compensatory (counter regulatory) responses that reduce the magnitude of response, even if it was accurately directed at the predominant pathophysiologic mechanism. In a meta-analysis by Law et al of 354 randomized, double-blind trials, the mean placebo-corrected reduction in blood pressure with monotherapy was found to be only 9.1/5.5 mm Hg [7]. There was little difference in this regard between a diuretic, b-blocker, angiotensin converting enzyme (ACE) inhibitor, angiotensin receptor blocker (ARB), or calcium channel blocker (CCB). Similar results were reported in the treatment of mild hypertension study, in which comparable blood pressure reduction was noted after long-term treatment with a diuretic, β-blocker, CCB,α-blocker, and ACE inhibitor [8]. Clinical trials suggested that a single agent usually cannot achieve blood pressure targets.

In the antihypertensive and lipid-lowering treatment to prevent heart attack trial, only 26% of patients achieved goal BP with monotherapy despite the fact that the target BP for diabetics(36% of the patient population) was <140/90 mmHg rather than the <130/80 mm Hg mandated by current guidelines [9]. In the Hypertension Optimal Treatment trial, 33% of patients achieved their (diastolic only) BP target with mono-therapy, 45% required two drugs, and 22%needed three or more agents [10]. Systolic BP at the end of the study averaged 141 mm Hg, indicating that even a higher percentage would have required combination therapy according to current treatment standards. In the losartan intervention for endpoints trial, in which treatment to goal (<140/90 mm Hg) was aggressively pursued in patients with left ventricular hypertrophy and a mean baseline BP of 175/98 mm Hg, more than 90% required at least two antihypertensive agents [11]. The importance of blocking multiple physiologic pathways is underscored by studies using a treatment strategy known as ‘‘sequential monotherapy.’’ This approach is based on the observation that BP response to different antihypertensive medications is often quite variable, and BP control should be more readily achieved with mono-therapy if patients are exposed to multiple drugs and then treated with the most effective agent [12]. In the Strategies in Treatment of Hypertension study, treatment initiated with a low-dose combination was compared with a monotherapy arm in which patients were first treated with a β-blocker but could be switched to an ACE inhibitor or a CCB if BP remained >140/90 mm Hg. At the end of 9 months, a significantly higher percentage of patients randomized to the low-dose combination achieved target BP compared with those receiving sequential monotherapy (62% vs.49%, P = .02) [13]. The aggregate of available data suggests that at least 75% of patients will require combination therapy to achieve contemporary BP targets. This estimate reflects the results of previous studies,

the lower BP targets now in place for large segments of the hypertensive population, and the rapidly increasing prevalence of obesity. The latter is important as the presence of obesity further elevates pretreatment BP and increases the magnitude of BP reduction needed to achieve therapeutic targets [14]. The importance of achieving goal BP in individual patients cannot be overemphasized. In major clinical trials, small differences in on-treatment BP frequently translate into major differences in clinical event rates. Recent data also suggest that inadequate BP control is itself an independent risk factor for the development of diabetes in hypertensive patients [15].

Propranolol is a nonselective beta-adrenergic receptor blocking agent possessing no other autonomic nervous system activity. Propranolol specifically competes with beta-adrenergic receptor agonist agents for available receptor sites. It is extensively used as antihypertensive, antianginal, anti-arrhythmic, and in treatment of migraine [16]. Propranolol is reported to be of value in more than 20 non-cardiovascular disorders, many of which are associated with central nervous system [17]. Propranolol is highly lipophilic and almost completely absorbed after oral administration. However, it undergoes high first-pass metabolism by the liver, and on average, only about 25% of propranolol reaches the systemic circulation. Approximately 90% of circulating propranolol is bound to plasma proteins. Propranolol is extensively metabolized with most metabolites appearing in the urine. Peak plasma concentrations occur about 1 to 4 h after an oral dose. The propranolol has relatively short half-life about 3-4 h [18]. Consecutively, for an optimum effect, the dose of propranolol hydrochloride as a conventional tablet (with rapid disintegration and dissolution) must be carried out several times a day. Therapy with immediate release propranolol hydrochloride tablets typically requires 40-160 mg as daily dose given in three to four divided doses [19].

Hydrochlorothiazide is a thiazide class diuretic drug that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance [20]. It is listed on the World Health Organization's List of Essential Medicines, as the most important medications needed in a basic health system [21]. Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones [22]. Most of the research supporting the use of thiazide diuretics in hypertension was done using chlorthalidone, a different medication in the same class. Some more recent studies have reported that chlorthalidone might be the more effective thiazide diuretic [23].

MATERIALS AND METHODS

Materials:

Hydrochlorothiazide and propranolol HCl were obtained as a kind gift sample from Micro labs, Bangalore, India. Lactose monohydrate 30 GR and Croscarmellose Sodium (Ac-Di-Sol) procured from Hi-Media, Mumbai. All chemicals used in present study were of laboratory grade.

Methods:

Formula Compilation of trial no. 1-3 (F1-F3):

To take a feasibility trial of Bi-layered tablets comprising IR layer and SR layer of HCTZ and Prop HCl, using Methocel K4 M, as polymer for sustaining release of API-II. Trial taken as Capsule shaped 12.0x 6.0 mm punch with one side break line and compressed in bi-layer compression machine (**Table 1**).

Compilation of trial no. 4-6 (F4-F6):

To take a feasibility trial of Bi-layered tablets comprising IR layer and SR layer

of HCTZ and Prop HCl, using HPMC K100M as polymer for sustaining release of Prop HCl. Trial taken as Capsule shaped 12.0x 6.0 mm punch with one side break line and compressed in bi-layer compression machine (**Table 2**).

Compilation of Trial no. 7-9 (F7-F9):

To take a feasibility trial of Bi-layered tablets comprising IR layer and SR layer of HCTZ and Prop HCl, using combination of both Hypromellose K4M Hypromellose K100M as polymers for sustaining release of Prop HCl for F7 to F9. Trial taken as Capsule shaped 12.0x 6.0 mm punch with one side break line and compressed in bi-layer compression machine (**Table 3**).

Table 1: Formula for Trail 1, 2 and Trail 3 (F1-F3)

INGREDIENTS	QUANTITY USED IN FORMULATION TRIAL (mg/tablet)		
LAYER-I	F1	F2	F3
Hydrochlorothiazide	12.50	12.50	12.50
Mannitol	50.50	47.50	50.0
Lactose monohydrate 30 GR	32.50	32.50	32.50
Croscarmellose Sodium (Ac-Di-Sol)	-	2.0	0.50
Talc	3.0	3.0	3.0
Magnesium Stearate	1.50	1.50	1.50
Total	100 mg		
LAYER-II			
PropranololHCl	50.0	50.0	50.0
Methocel K 4M	10.0	15.0	20.0
Methocel K 100M	-	-	-
Microcrystalline Cellulose (Avicel pH 101)	74.0	74.0	69.0
Lactose Monohydrate (Pharmatose 200M)	55.0	50.0	50.0
Povidone K-30	5.0	5.0	5.0
Isopropyl Alcohol	Q.S	Q.S	Q.S
Purified Talc	3.0	3.0	3.0
Magnesium Stearate	3.0	3.0	3.0
Total	200 mg		

Table 2: Formula for trail 4, 5 and 6 (F4-F6)

INGREDIENTS	QUANTITY USED IN FORMULATION TRIAL (mg/tablet)		
LAYER-I	F4	F5	F6
HCTZ	12.50	12.50	12.50
Mannitol	50.50	50.50	50.50
Lactose monohydrate 30 GR	31.50	31.50	31.50
Croscarmellose Sodium (Ac-Di-Sol)	1.0	1.0	1.0
Talc	3.0	3.0	3.0
Magnesium Stearate	1.50	1.50	1.50
Total	100		
LAYER-II			
Prop HCl	50.0	50.0	50.0
Methocel K 4M	-	-	-
Methocel K 100M	10.0	15.0	20.0
Microcrystalline Cellulose (Avicel pH 101)	74.0	74.0	69.0
Lactose Monohydrate (Pharmatose 200M)	55.0	50.0	50.0

Table 3: Formula for Trail 7, 8and Trail 9 (F7-F9)

INGREDIENTS	QUANTITY USED IN FORMULATION TRIAL (mg/tablet)		
LAYER-I	F7	F8	F9
API-I	12.50	12.50	12.50
Mannitol	50.50	50.50	50.50
Lactose monohydrate 30 GR	31.50	31.50	31.50
Croscarmellose Sodium (Ac-Di-Sol)	1.0	1.0	1.0
Talc	3.0	3.0	3.0
Magnesium Stearate	1.50	1.50	1.50
Total	100	100	100
LAYER-II			
API-II	50.0	50.0	50.0
Methocel K 4M	10.0	15.0	20.0
Methocel K 100M	10.0	15.0	20.0
Microcrystalline Cellulose (Avicel pH 101)	69.0	59.0	49.0
Lactose Monohydrate (Pharmatose 200M)	50.0	50.0	50.0
Povidone K-30	5.0	5.0	5.0
Isopropyl Alcohol	Q.S	Q.S	Q.S
Purified Talc	3.0	3.0	3.0

POST COMPRESSION PARAMETERS:
THICKNESS AND DIAMETER (mm):
Control of physical dimension of the tablet such as thickness is essential for consumer acceptance and tablet uniformity. The thickness and diameter of the tablet were measured using vernier caliper. It is measured in mm.

HARDNESS:
Hardness of tablets is known from the pressure applied on tablet to form a crack along its axis. It is tested by using Dr. Schleuniger8M tablet tester. It is expressed in N. 5 tablets were chosen randomly and tested for thickness. The average hardness of 5 determinations was recorded.

$1N = 22.4\ kg/cm^2$ 2

FRIABILITY:
Tablet strength was tested by using electro lab Friabilater. 20 tablets were weighed and placed in the friabilator and operated at for 100 revolutions (4min), taken out and were deducted. The percentage weight loss was calculated by reweighing the tablets. 1.0% of tablet friability is generally acceptable.

WEIGHT VARIATION:
Twenty tablets were selected at random, individually weighed in a single pan electronic balance and the average weight was calculated. The uniformity of weight was calculated. The uniformity of weight was determined according to I.P. specification. As per U.S.P not more than two of individual weights should deviate from average weight by more than 5% and none deviate more than twice that percentage.

***IN-VITRO* DISSOLUTION STUDY**
Dissolution studies become very important in characterization of the dosage forms particularly in case of sustained release dosage forms. They are also used to control the quality of dosage forms during the manufacturing process. In the *in vitro* dissolution study of the present study following parameters were employed to study the release characteristics of both the drugs from immediate release (HCTZ) and Sustained release layers (Prop HCl) as mentioned in (**Table 4**).

Data analysis:
The data obtained from the dissolution study were subjected for analysis to know the release pattern of the drug from the dosage form. To analyze the mechanism of release and release rate kinetics of the dosage form, the data obtained were fitted into Zero order, First order kinetic, Higuchi model

kinetic and Korsmeyer-Peppas model. Based on the R-value, the best-fit model was selected.

Table 4: Dissolution conditions

Dissolution Parameters	HCTZ	Prop HCl
Medium	0.1 N Hcl	6.8 Phosphate Buffer
Apparatus	USP apparatus type II	USP apparatus type II
Volume	900ml	900ml
Agitation	50 rpm	50 rpm

Stability Studies

The ICH Q1A guideline defines the stability data package for a new drug substance or drug product that is sufficient for a registration application within the three regions of the EC, Japan, and the United States. It does not seek necessarily to cover the testing for registration in or export to other areas of the world. Stability studies were carried out at 30°C ± 2°C/65% RH ± 5% RH for 12 months and at 40°C ± 2°C/75% RH ± 5% RH for 6 months for the selected formulation.

RESULTS AND DISCUSSION

Table 5: Evaluation of Physical parameters of tablets

	PHYSICAL PARAMETERS			
Formulation	**Weight**	**Hardness**	**Weight**	**Hardness**
F1	302 ± 3	78 ± 8	3.93 ± 0.05	0.07
F2	301 ± 3	76 ± 6	3.91 ± 0.05	0.02
F3	302 ± 3	79 ± 8	3.94 ± 0.05	0.09
F4	299 ± 3	74 ± 5	3.95 ± 0.05	0.17
F5	299 ± 3	76 ± 4	3.96 ± 0.05	0.25
F6	303 ± 3	79 ± 7	3.95 ± 0.05	0.37
F7	301 ± 3	77 ± 4	3.98 ± 0.05	0.19
F8	300 ± 3	76 ± 6	3.94 ± 0.05	0.31
F9	301 ± 3	77 ± 8	3.96 ± 0.05	0.32

It was found that in case of layer II as the polymer concentration increased, tablet hardness was also increased. In case of layer I as the % of crosscarmallose was increased, decrease in hardness was noticed. In all the formulations, all physical parameters of tablets within the limits. Results were showed in (**Table 5**).

Drug Content

Drug content was varied from 98.5 ±2.11 to 99.7% ± 0.97 in case of layer I. In layer II drug content was varied from 97.1 ± 0.98 to 99.32 ± 0.88 %.From the results it was found that as the concentration of polymer used was increased, entrapment of drug in to dosage form was also increased in layer II.

***IN-VITRO* DISSOLUTION STUDY**

In trial no:1 the IR layer is prepared by direct compression by using the ingredients with respect to their concentration but the release of the drug was not in a specified limit, which does not match with the release of marketed product at a time point of 45 min. So, further trail was prepared by using Ac-di-sol in order to increase the release. From the result, in SR layer Methocel K4M polymer with 5.0% Conc. not retarded the drug release that much in SR layer and then the polymer Concentration in next trail, of Methocel K4M was increased with 7.5% Conc. in the formula F2.

In IR layer, with reference to trail.no-1 in order to increase the drug release, the above trail was prepared by using Ac-di-sol with a concentration of 2%, which showed faster drug release within 45 min, when compared to marketed product. The similarity factor (F2) of both Trail-2 & Marketed was

found to be 41.41, which is not beyond the acceptance criteria i.e., 50-100. This rapid release of drug in trial.no-2 will miss lead the marketed formulation. In SR layer, from the result of F1 & F2 on compared, 7.5% concentration of Methocel K4M in formula F-2 retards more compare to F-1. But result does not meet the specified limit. Then in next trail polymer Concentration of Methocel K4M polymer used with 10.0 % Concentration in the formula F-3.In IR layer, with reference to trail.no-2 in order to decrease the drug release, the above trail was prepared by using Ac-di-sol with a concentration of 0.5 %, which showed a better drug release within 45 min, when compared to trail.no-1, but it does not match with the release of marketed product. So, further trail was developed by increasing the concentration of ac-di-sol. In SR layer, in F3, 10.0% polymer concentration of Methocel K4M retards more, but result is not under specified limit. Then next trail was prepared by changing the polymer of high viscosity grade i.e. Methocel K100M. In IR layer, with reference to trail.no-3 in order to increase the drug release, the above trail was prepared by using Ac-di-sol with a concentration of 1.0 %, which showed a better drug release within 45 min, when compared to trail.no-3, and also it matches with the release of marketed product. So, further trails were developed by considering the same formulation for IR layer. In SR layer, from the result Methocel K100M polymer with 5.0% Conc. retards more drug release compared to Methocel K4M, and then the polymer Concentration of Methocel K100M in next trail, was increased with 7.5% Conc. in the formula F5. From the result in formulation F4 & F5 on compared, 7.5 % concentration of Methocel K100M in formula F5 retards more compare to F4. But result is not under specified limits. Then in next trail polymer Concentration of Methocel K100M polymer used with 10.0 % Concentration in the formula F6. In formulation F6, 10.0% polymer concentration of Methocel K100M retards more, but result is not under specified limits. Then next trail was prepared by combining both the polymers of low viscosity i.e. Methocel K4M & of high viscosity grade i.e. Methocel K100M. On combination of both the polymers with 5% Concentration the drug release was not in specified release limit. Further trail was prepared by increasing the concentration of both the polymers to 7.5% in formulation F8.

On combination of both the polymers with 7.5% Concentration in F8 the drug release was within specified limit. Further trail was prepared by increasing the concentration of both the polymers to 10.0% in formulation F9. On combination of both the polymers with 7.5% Concentration in F8 the drug release was in specified limit. In this trail F9 by increasing the concentration of both the polymers to 10.0% the release of the drug was retained for a long period of time which is not in the specified limit.

The reproducibility trail F8 gave good result which showed the release in a specified limit. So, F8 is an optimized formula. Data of release studies in the form of graph was showed in (**Figure 1, 2 and 3**).

Data analysis

The curve fitting results of the release rate profile of the designed formulations gave an idea on the mechanism of drug release. Based on the data analysis the drug release was found to follow Zero order release kinetics, the drug release mechanism was best explained by Zero order, as the plots showed the highest linearity. This model indicates a coupling of the diffusion mechanism (Fickian diffusion) followed by erosion mechanism and indicates that the drug release was controlled by more than one process. The best fitted model for release of drug in F8 was found to be Zero order represented in figure 4 with regression co-efficient value R^2 of 0.962.

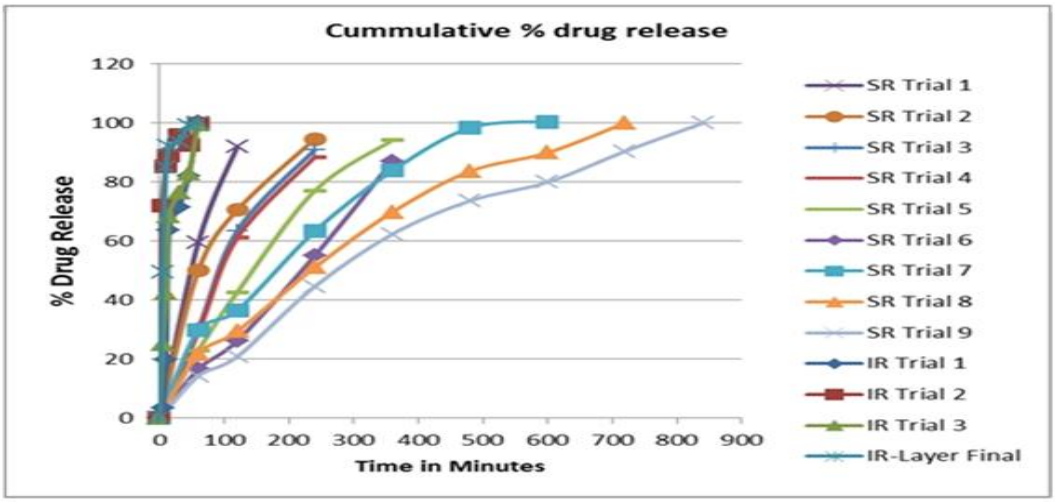

Figure 1: Compilation of F1 to F9 of both layer I (IR) &layer-II (SR)

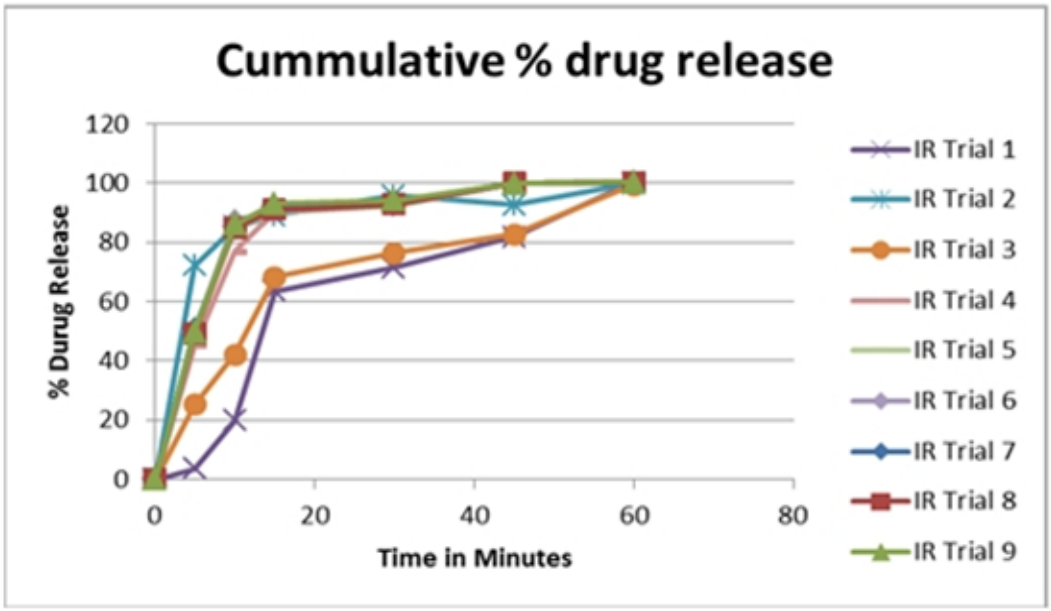

Figure 2: Dissolution profile of hydrochlorothiazide in all formulations

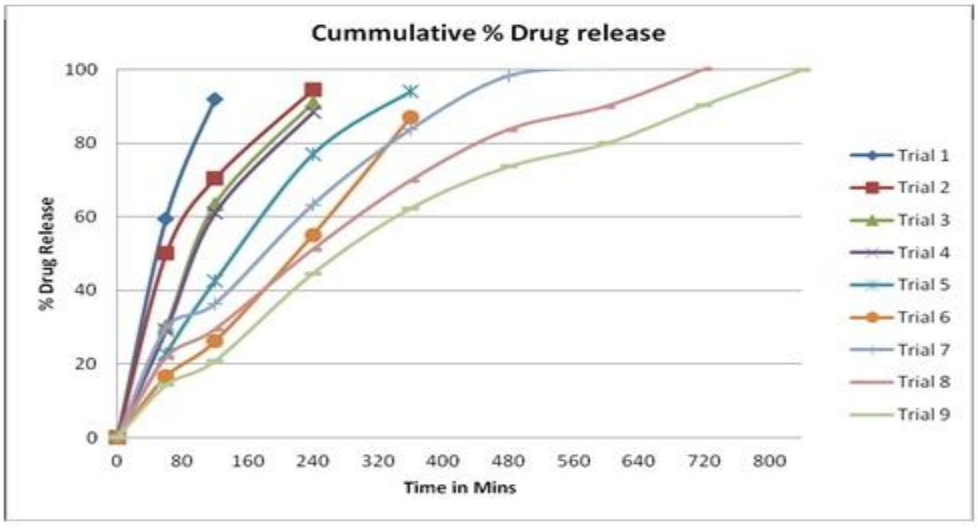

Figure 3: Dissolution profile of propranolol HCl in all formulations

Figure 4: Zero order release graph of formulation F8

Stability Studies

The stability studies were done for trial 8 (F8) at different conditions as per ICH guidelines and from the results it was found that formulation was stable throughout the period.

SUMMARY

From all the prepared formulations F1 to F9,the immediate layer was prepared with direct compression method, among these formulations F4 prepared with 1% CCS, releases the drug content of the immediate layer at the end of the 45mins, the formula of F4 for immediate layer was selected for remaining formulations (F5toF9). This concludes that the super disintegrant played a good role in disintegrating the Layer-I in formulation F4.

The Sustained layer was prepared by using wet-granulation technique. In the initial trials both the polymers Methocel K4M, Methocel K100M were taken individually to check the feasibility of the polymer to sustain the release of anti-hypertensive. These viscous polymers did not sustain the release of anti-hypertensive to the desired level individually in formulations F1toF6. So, as that was not an optimum profile, a combination of both low viscous and high viscous polymers i.e. Methocel K4M and Methocel K100M were used in the formulations of F7 to F9. Among all these formulations, formulation F8 was optimized to obtain the release of API-II for a sustained period of 12h (within specification). The incorporation of both the polymers intra-granularly at individual concentrations of 7.5% optimizes the release profile within specifications in formulation F8. From this it can be concluded that with the combination of both the polymer concentrations showed the synergistic effect and the release rate of the drug sustains.

It can be concluded that, the optimized formulation (F8) Containing 1% CCS in Immediate layer releases the drug within 45 minutes & combination of both polymers with an individual concentration of 7.5% in sustained layer sustains the release profile of the drug up to 12 h. From graphs plotted for various Kinetic models, it can be concluded thatF8 is following Zero-order kinetics showed a regression value (R^2=0.962) and which is fitted to Korsmeyer-Peppas equation as the plots of that model had shown similar regression values (R^2=0.9358). The release mechanism can be concluded by diffusion mechanism. From the stability studies completed for 1 month& 3 months, it can be concluded that the formulationF8 is the stable one, as all the parameters like appearance, assay of the drugs and consistency in dissolution studies were found to be intact, even after simulating extreme conditions during their storage.

CONCLUSION

From the above study it can be concluded that, Bi-layered matrix tablets comprising diuretic agent in immediate release layer and anti-hypertensive agent in sustained release layer are a good means in promising a combination therapy, while achieving patient compliance for Hypertension.

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