INVITRO APPROACH OF FORMULATION AND DEVELOPMENT OF BILAYER TABLET FOR ANTIHYPERTENSIVE AGENTS

J. Praveen Kumar*, Revanth Pankaj[1], K. Umasankar2, M. Kishore Babu[3]

* Associate Professor, Dept. of Pharmaceutics, Krishna Teja Pharmacy College, Tirupati.
email:jaldupraveen@gmail.com

1 .Krishna Teja Pharmacy College,Tirupati.pankajrevanth@gmail.com

2. Professor,Dept . of Pharmaceutics, Krishna Teja Pharmacy College,Tirupati.

3. Principal, Krishna Teja Pharmacy College,Tirupati.

ABSTRACT

Aim of the present study was the optimization of the immediate release (IR) layer containing hydralazine hydrochloride (HHC) 25 mg and compressed with sustained-release (SR) layer of Isosorbide dinitrate (ISDN) 40 mg to decrease the dosing frequency. Methods: In this study, Drug-excipients compatibility study was carried out by FT-IR and a preliminary trial was conducted for screening of super disintegrating agents. The amount of sodium starch glycolate (SSG) (X1) and the amount of ac-di-sol®(X2) was chosen as independent variables in 32 full factorial design while wetting time (WT) (Y1), disintegration time (DT) (Y2) and In-vitro drug release at 15 min (Q15) (Y3) were taken as dependent variables for immediate release layer. The amount of HPMC K100M (X1) and the amount of Polyoxtm WSR303 (X2) were chosen.While % cumulative drug releases at 1 h (Q1) (Y1), % cumulative drug release at 2 h (Q2) (Y2), % cumulative drug release at 4 h (Q4) (Y3) and % cumulative drug release at 6 h (Q6) (Y4), were taken as dependent variables for sustained release layer and statistically evaluation both later by using sigma plot 13.0.

Key words: Hydralazine hydrochloride, Isosorbide dinitrate, sodium starch glycolate, ac-di-sol, HPMC K100M, Polyoxtm WSR303, bilayer tablets, controlled Release.

1. INTRODUCTION

Oral Drug Delivery System

The oral drug delivery market is the largest segment of the drug delivery market and there's no sign that it is slowing down. Oral route of drug administration have wide acceptance up to 50-60% of total dosage form and is the most convenient and preferred route for systemic effect due to its ease of dosing administration, pain avoidance, accurate dosage, patient compliance and flexibility in formulation. The major aim of controlled drug delivery is to reduce dosing frequency. The design of modified release drug product are to optimize a therapeutic regimen by providing slow and continuous delivery of drug over the entire dosing interval and provide better patient compliance and patient convenience. Over 90% of the formulations manufactured today are ingested orally.[1]

All the pharmaceutical products formulated for systemic delivery via the oral route of administration, irrespective of the mode of delivery (Immediate, Extended or Controlled release) and the design of dosage forms (either solid, dispersion, or liquid), must be developed within the intrinsic characteristics of GI physiology. This shows that oral formulation is the most popular worldwide and the major attention of the researcher is towards this direction.[2, 3]

Introduction of Tablet[4, 5]

Tablets are solid preparations each containing a single dose of one or more active substances and usually obtained by compressing uniform volumes of particles. Tablets are intended for oral administration. When tablet given orally, it undergo *In- vitro* administration and dissolution followed by absorption through the gastrointestinal tract (GIT) and then the *In-vivo* bio distribution of drug which enters in to the systemic circulation then occurs.

Some are swallowed whole, some after being chewed, some are dissolved or dispersed in water before being administered and some are retained in the mouth where active substance is liberated.

The particles consist of one or more active substances with or without excipients such as diluents, binders, disintegrating agents, glidants, lubricants, substances capable of modifying the behavior of the preparation in the digestive tract, coloring matter authorized by the component authority and flavoring substances.

Types of Tablets

A) Oral Tablets for Ingestion

1. Standard compressed tablets
2. Multiple compressed tablets

a. Layered tablets
b. Compression coated tablets
c. Inlay tablets

3. Modified release tablets
4. Delayed action tablets
5. Targeted tablets

a. Floating tablets
b. Colon targeted tablets

6. Chewable tablets

B) Tablets used in the Oral Cavity

1. Buccal tablets
2. Sublingual tablets
3. Troches and lozenges
4. Dental cones

C) Tablets administered by other Routes

1. Implantation tablets
2. Vaginal tablets

D) Tablets used to prepare Solution

1. Effervescent tablets
2. Dispersible tablets
3. Hypodermic tablets
4. Tablet triturates

Layer Tablets[6]

In Layer tablet, there are two fractions i.e. loading fraction which provide loading dose and maintain fraction which provide maintenance dose by extended release. Layer tablets are composed of two or three layers of granulation compressed together. As the edges of each layer are exposed, they have the appearance of a sandwich. Fig: 1a, 1b shows various types of layered tablets. This dosage form has the advantage of separating two incompatible substances with an inert barrier between them. It makes possible sustained-release preparations with the immediate-release quantity in one layer and the slow release portion in the second. A third layer with an intermediate release might be added.

Figure 1.1(a): Single layer tablet

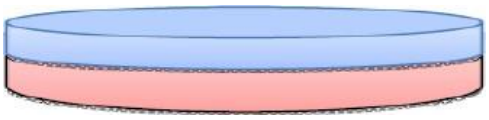


Figure 1.1(b): Bilayer tablet

Introduction to Bilayer (Multi component or Dual component) Tablet[1]

Bilayer tablet is the new era for the successful development of controlled release formulation. It is also called Dual or Multi component tablet. Bilayer tablet is better than the traditionally used dosage form. It is suitable for sequential release of two drugs in combination. It also capable of separating two types of incompatible substances and also for sustain release tablet in which one layer is immediate release as initial dose and second one is maintenance dose. Bilayer tablet contain immediate and sustained release layers. In which immediate release layer delivers the initial dose which contains superdisintegrants (promote drug release rate and attains the onset of action rapidly). It also called as a loading dose. Second layer is sustained release (maintenance dose) layer releases drug in sustained or prolonged time period.

Coronary vasodilators, antihypertensive, antihistamines, analgesics, antipyretics and antiallergenic agents are mainly suitable for this type of drug delivery. Some bilayer tablet have both the layers as the sustain release layers for example certain anti diabetic agents. Use

of bi-layer tablets is a very different aspect for anti-hypertensive, anti-diabetic, anti-inflammatory and analgesic drugs where combination therapy is often used.

Bi-layer tablets are made by compressing several different granulations fed into a die in succession, one on top of another, in layers. Each layer comes from a separate feed frame with individual weight control. Rotary tablet presses can be set up for two or three layers. More are possible but the design becomes very special. Ideally, a slight compression of each layer and individual layer ejection permits weight checking for control purposes. Figure 1.2 shows picture of bi-layer tablet.

Figure 1.2: Bilayer tablet

Table 1.1: Examples of Superdisintegrants and its mechanism of action

S. No.	Superdisintegrants	Example	Mechanism of action
1.	Crosscarmellose Ac-Di-Sol Nymce ZSX PrimellosesolutabVivasol L-HPC	ross linkedcellulose	Swells 4-8 folds in <10 seconds. Swelling and wickingboth
2.	Crosspovidone Crosspovidone MKollidon Polyplasdone	Cross linked PVP	Swells very little and returns to original sizeafter compression but act by capillary action
3.	Sodium Starch Glycolate Explotab Primogel	Cross linked Starch	Swells 7-12 folds in <30 seconds.
4.	Alginic acid NF	Cross linkedAlginic acid	Rapid swelling in ueous medium orwicking action.

Advantages of Bilayer Tablet[8, 9]

- Incompatible substance can be separated by formulating them in separate layer as a two layer tablet or separating the two layers by a third layer of an inert substance as a barrier between the two.
- Two layer tablet may be designed for sustain release; one layer for immediate release of the drug and second layer for extended release, thus maintaining a prolonged blood serum level.
- The weight of each layer can be accurately controlled, in contrast to putting one drug of a combination product in a sugar coating.
- Monograms and other distinctive markings may be impressed on the surfaces of the multi-layer tablets.
- Analytical work may be simplified by separating of the layers prior to assay.
- They are used as an extension of a conventional technology.
- Bi-layer execution with optional single layer conversion kit.
- Low cost compared to other dosage forms.
- Greatest chemical and microbial stability compared to other oral dosage forms.
- Objectionable odor and taste can be masked by coating technologies.
- Flexible concept.
- Elegance to the product.
- Offer greatest precision and the least content uniformity.
- Easy to swallow with least hang up problems.
- Fit for large scale production.
- Bi-layer tablet is suitable for preventing direct contact of two drugs and thus tomaximize the efficacy of combination of two drugs.
- Bi-layer tablets can be designed in such a manner as to modify release as either ofthe layers can be kept as extended and the other as immediate release.

Disadvantages of Bilayer Tablet[8, 9]

- Drugs with poor wetting, slow dissolution properties, optimum absorption high in GIT may be difficult to formulate or manufacture as a tablet that will still provide adequate or full drug bioavailability.
- Difficult to swallow in case of children and unconscious patients.
- Adds complexity and bilayer rotary presses are expensive.
- Some drugs resist compression into dense compacts, owing to amorphous nature,low density character.
- Cross contamination between the layers.
- Insufficient hardness, layer separation, reduced yield.
- Imprecise individual layer weight control
- Bitter testing drugs, drug with an objectionable odor or drugs that are sensitive tooxygen may require encapsulation or coating.
- It must have a chemical stability shelf life, so as not to follow alteration of themedicinal agents.

Necessitate of Bilayer Tablets[9]

For the supervision of fixed dose combinations of drugs, prolong the drug product lifecycle, buccal/mucoadhesive delivery systems, manufacture novel drug delivery systems such as chewing device and floating tablets for gastro-retentive drug deliverysystems.[7]

- For the administration of fixed dose combinations of different APIs, prolong the drug product life cycle, buccal/mucoadhesive delivery systems; fabricate novel drug delivery systems such as chewing device and floating tablets for gastro- retentive drug delivery.
- Controlling the delivery rate of either single or two different active pharmaceutical ingredient(s).

Challenges in Bilayer Tablet Manufacturing[8, 10]

Conceptually, bilayer tablets can be seen as two single-layer tablets compressed into one. In practice, there are some manufacturing challenges.

- **Delamination:** Tablet falls apart when the two halves of the tablet do not bond completely. The two granulations should adhere when compressed.
- **Cross-contamination:** When the granulation of the first layer intermingles with the granulation of the second layer or vice versa, cross-contamination occurs. It may conquer the purpose of the bilayer tablet. Proper dust collection goes a long way toward preventing cross contamination.
- **Production yields:** To prevent cross contamination, dust collection is required which leads to losses. Thus, bilayer tablets have lower yields than single-layer Tablets.
- **Cost:** Bilayer tableting is more expensive than single layer tableting for several reasons. First, the tablet press costs more. Second, the press generally runs more slowly in bilayer mode. Third, development of two compatible granulations is must, which means more time spent on formulation development, analysis and validation. These factors, if not well controlled/optimized, in one way or another will impact the bilayer compression and the quality attributes of the bilayer tablets (sufficient mechanical strength to maintain its integrity and individual layer weight control). Therefore, it is critical to obtain an insight into the root causes to enable design of a robust product and process.

Various Techniques for BiLayer Tablet

OROS® Push Pull Technology[12]

This system consists of mainly two or three layer among which the one or more layer are essential of the drug and other layer are consist of push layer. The drug layer mainly consists of drug along with two or more different agents. So this drug layer comprises of drug which is in poorly soluble form. There is further addition of suspending agent and osmotic agent. A semi permeable membrane surrounds thetablet core.

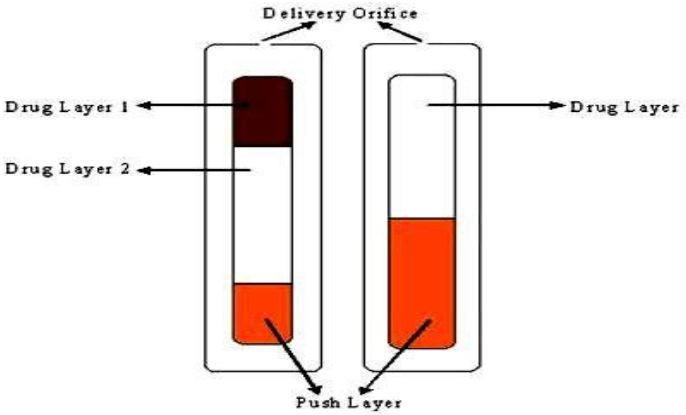


Figure 1.3: OROS® Push Pull Technology

L-OROS Technology[13]

This system used for the solubility concern. A1za developed the L-OROS system Lipid soft gel product containing drug in a dissolved state is initially manufactured, then coated with a barrier membrane, next osmotic push layer, after that a semi permeable membrane which drilled with an exit orifice.

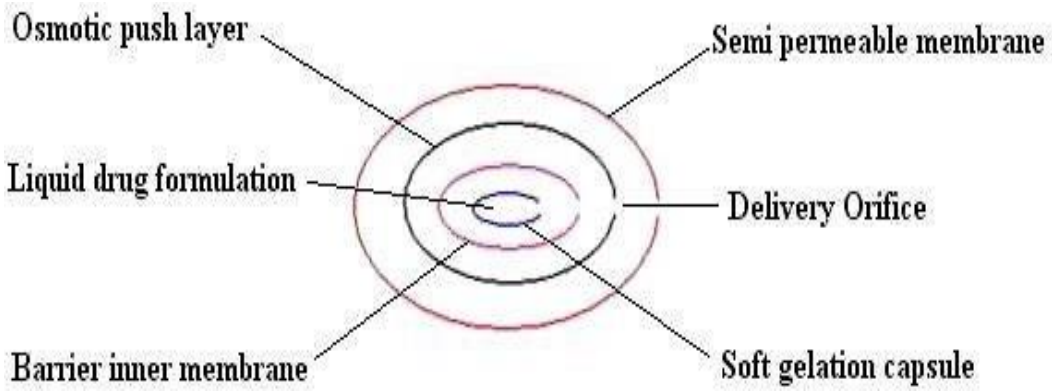


Figure 1.4: L-OROS Technology

DUROS Technology[14]

The system consists from an outer cylindrical titanium alloy reservoir (Fig.1.5).This reservoir has high impact strength and protects the drug molecules from enzymes. The DUROS technology is the miniature drug dispensing system that opposes like a miniature syringe and reglious minute quantity of concentrated form in continues and consistent from over months or Year.

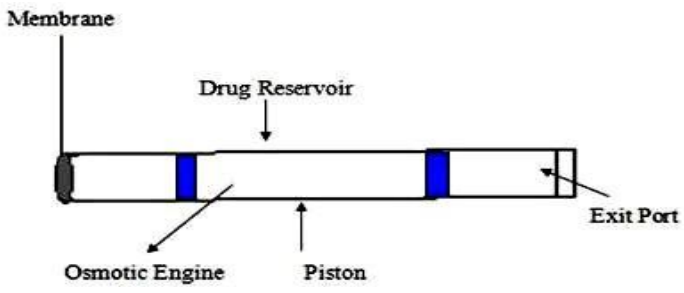


Figure 1.5: DUROS Technology

ENSOTROL Technology[12]

Solubility enhancement of an order of magnitude or to create optimized dosage form Shire laboratory use an integrated approach to drug delivery focusing on identification and incorporation of the identified enhancer into controlled release technologies

:

Figure 1.6: ENSOTROL Technology

DUREDAS™ Technology[8]

DUREDAS™ Technology is a bilayer tablet which can provide immediate or sustained release of two drugs or different release rates of the same drug in one dosage form. The tableting process can provide an immediate release granulate and a modified-release hydrophilic matrix complex as separate layers within the one tablet.

PRODAS (Programmable Oral Drug Absorption System) [15, 16]

PRODAS is a multi-particulate drug delivery technology that is based on the encapsulation of controlled release mini tablets in the size range of 1.5 to 4 mm in diameter. This technology represents a combination of multi particulate and hydrophilic matrix tablet technologies and thus provides the desired release rates.

These considerations may include immediate release, delayed release and / or controlled release mini tablets. In addition to controlled release absorption over a specified period, PRODAS technology also enables targeted delivery of drug to specified sites of absorption throughout the GI tract. Combination products also possible by using mini tablets formulated with different ingredients.

GEMINEX Technology[17]

In this drug delivery system at different time more than one drug can be delivered. This technology basically increases the therapeutic efficacy of the drug by decreasing its side effects. It is useful both to industry as well as patient as in single tablet it provides delivery of drug at different rates.

Erodible Molded Multilayer Tablet[17, 18]

Egalet erodible molded tablets in an erosion based platform. It has the advantage of delivering zero order or delayed release with minimal impact from the gastrointestinal conditions. Egalet erodible molded multi-layered tablets are prepared by injection mouding egalet technology contains a coat and a matrix. Drug release is controlled through the gradual erosion of the matrix part.

Geomatirx Tablet[19, 20, 21]

One of the examples of bilayered tablets is geomatrix tablet. Geomatrix tablet, which is composed of different layers. The system allows the incorporation of more than one drug into the dosage form. Formulation of layers from different polymers allows manipulation over more than one rate-controlling polymer, thus enabling different types of drug delivery of one or more drugs, i.e. where the drug may be released with a bolus and then at a controlled rate or by targeted drug delivery in the GI tract using pH dependent polymers system. The biphasic system some time may contain two drugs in separate release layers. There are clearly a number of issues of concern to the production of bilayered tablets. While the mechanical strength of layered tablets has been observed not to be a controlling factor in drug release the determination of this property could be beneficial in understanding the adhesion between various layers and provide an improved characterization of the systems. Bi-layer tablets are prepared with one layer of drug for immediate release while second layer However, many floating systems reported are single-unit systems such as HBS, which are unreliable in prolonging the GRT owing to their 'all-or-nothing' emptying process. These systems thus, may result in high variability in bioavailability and local irritation due to a large amount of drug delivered at a particular site of GIT. The conventional dosage forms are retained in the stomach for 0.5-2 hrs.

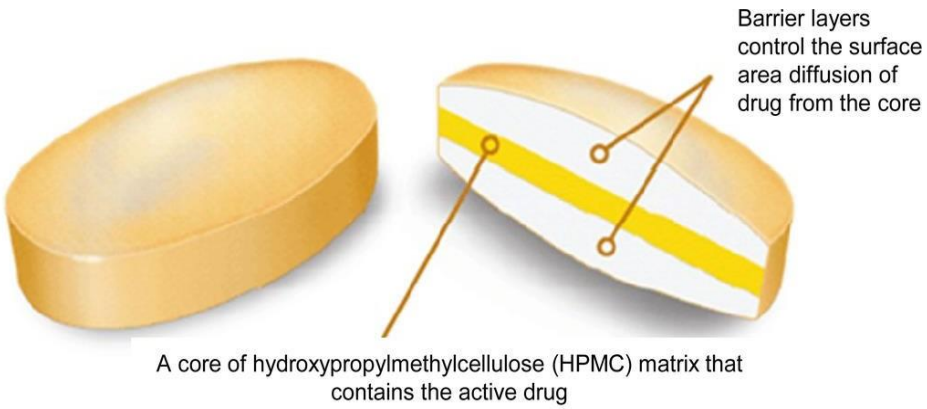


Figure 1.7: Geomatrix Tablet

Experimental work:

Preparation of Standard Calibration Curve of Hydralazine HCl

The construction of standard calibration curve of Hydralazine HCl was prepared by using mix volume of methanol and distilled water 1000ml containing 0.1ml TEA each (60:40). From the stock solution, take 50, 75, 100, 125, 150 µg/ml solutions were prepared respectively. Take the absorbance of above samples at $\lambda_{max.}$ 215 nm. The standard graph of Hydralazine HCl was constructed by taking the peak area on Y-axis and concentrations on X-axis.

Preparation of Standard Calibration Curve of Isosorbide Dinitrate

The construction of standard calibration curve of Isosorbide Dinitrate was prepared by using mix volume of methanol and distilled water 1000ml containing 0.1ml TEA each (60:40). From the stock solution, take 50, 75, 100, 125, 150 µg/ml solutions were prepared

respectively. Take the absorbance of above samples at $\lambda_{max.}$ 215nm. The standard graph of Isosorbide Dinitrate was constructed by taking the peak area on Y- axis and concentrations on X-axis.

Pre-formulation Study

Pre-formulation studies are the first step in the rational development of dosage form of a drug substance. The objective of pre-formulation studies is to develop a portfolio of information about the drug substance, so that this information useful to develop different dosage form. Pre-formulation can be defined as investigation of physical and chemical properties of drug substances alone and when combined with excipients.

Identification of Drugs

Identification of Hydralazine HCl and Isosorbide Dinitrate by FT-IR Infrared (IR) spectroscopy was conducted using a FT-IR Spectrophotometer (Shimadzu 8400S) and the spectrum was recorded in the wavelength region of 4000 to 600 cm^{-1}. The procedure consisted of dispersing a drug in KBr and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained.

Identification of Hydralazine HCl and Isosorbide Dinitrate by DSC

The DSC study was carried out using DSC-60 (Shimadzu, Tokyo, Japan). The instrument comprises of calorimeter, flow controller, thermal analyzer and operating software. The drug was heated in sealed aluminum pans under nitrogen flow (30ml/min) at a scanning rate of 5^0C/min from 50 to 300^0C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the drug.

Drug and Excipients Compatibility Study

Drug and Excipients Compatibility Study by FT-IR

The Fourier transform infrared spectrum of moisture free powdered sample of 1:1 ratio of Hydralazine HCl and Isosorbide Dinitrate with excipients was recorded on IR spectrophotometer by potassium bromide (KBr) pellet methodat Accuprec Research lab.

Drug and Excipients Compatibility Study by DSC

DSC was performed using DSC-60 (Shimadzu, Tokyo, Japan) calorimeter. The instrument comprised of calorimeter (DSC-60), flow controller (FCL-60), thermal analyser (TA-60) and operating software (TA-60). The samples (drug alone or mixture of drug and excipients) were heated in sealed aluminium pans at a scanning rate of 5ºC/min from 50 to 300ºC. Empty aluminium pan was used as a reference. The heat flow as a function of temperature was measured for the drug and drug-excipient mixture.

Physical Compatibility Study

A Pre-formulation study was carried out with potential formulation excipients to determine drug-excipients interaction/compatibility.

Protocol for drug-excipients compatibility study

(a) Drug: Excipients Ratio

Drug and excipients were taken in different ratios

(b) Pack details

USP type I Clear transparent glass vials with bromo butyl rubber stopper and aluminumseal.

(c) Storage condition

40°C/75 % RH for (Open and Close)

(d) Test to be performed Physical observation **Procedure**

Pure API, excipients and API with excipients were filled in vials; few were sealed with rubber closure and aluminum tip for 'Closed' condition, while others were remained open for 'Open' condition. Each set of 5 vials for open and closed conditions was placed at 40°C ± 2°C / 75% RH ± 5% RH in close condition and 40°C ± 2°C / 75% RH ± 5% RH in Open condition in stability chamber (Thermolab stability chamber) for time Period of 4 week. After 4 week, samples were withdrawn from stability chamber for visual analysis.

Physical Characteristics of Hydralazine HCl and Isosorbide Dinitrate

Organoleptic Characteristics of Hydralazine HCl and Isosorbide Dinitrate This includes recording of color, odor and taste of drug using descriptive terminology. Record of color of early batches is very useful in establishing appropriate specifications for later production. Drugs generally have a characteristic odor and taste.

Solubility Study

It was performed by taking the accurate amount of the drug and then slowly addition of the solvent in small amount till the drug remained undissolved in solvent.

Melting Point

Melting Point of the drug was determined by taking small amount of drug in a capillary tube closed at one end and placed in a melting point apparatus and the temperature at which drug melts was recorded.

Formulation Development

Immediate release layer of Hydralazine HCl

Preliminary screening of super-disintegrating agent for immediate releaselayer of Hydralazine HCl

The development of the immediate release layer containing hydralazine hydrochloride 25 mg by selecting ingredients in the appropriate amount and the super-disintegrants optimized thereafter. The immediate release layer of hydralazine hydrochloride was prepared by the direct compression method. Sodium starch glycolate, croscarmellose sodium, and ac-di-sol® were used in varying amounts as shown in table 1. Batch H1 to H3 contained 2%, 3%, and 5% of sodium starch glycolate, respectively. Batch H4 to H6 contained 2%, 3%, and 4% croscarmellose sodium, respectively and batch H6 to H10 contained 2%, 3%, 4%, and 5% ac-di-sol® respectively. Prepared layer was evaluated for weight variation, thickness, hardness, friability, disintegration time, wetting time, drug content, and % cumulative drug release at 15 min.

Table 5.6: Preliminary screening of super disintegrating agent for immediate release layer of Hydralazine HCl

redients(mg)	Qty. (mg/tab)									
	H1	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**	**H10**
Hydralazine HCl	25	25	25	25	25	25	25	25	25	25
MCC PH102	51	50	48	51	50	49	51	50	49	48
Tablattose	20	20	20	20	20	20	20	20	20	20
dium starch glycolate	2	3	5	0	0	0	0	0	0	0
scamellosesodium	0	0	0	2	3	4	0	0	0	0
Ac-Di-Sol®	0	0	0	0	0	0	2	3	4	5
agnesiumstearate	1	1	1	1	1	1	1	1	1	1
Talc	1	1	1	1	1	1	1	1	1	1
Total	**100 mg/tab**									

Sustained Release Layer of Isosorbide Dinitrate

Preliminary screening of polymer for sustained release layer of IsosorbideDinitrate

The development of a sustained release layer containing isosorbide dinitrate 40 mg by selecting ingredients in the appropriate amount and polymer were optimized thereafter. The sustained release layer of isosorbide dinitrate was prepared by the direct compression method. HPMC K4M, HPMC K100M, Polyox^{tm} WSR 301 and Polyox^{tm} WSR 303 were used in various amounts as shown in table 5.11. Batch I1 was prepared with HPMC K4M and Polyox^{tm} WSR 301. Batch I2 prepared to check the effect of HPMC K4M with Polyox^{tm} WSR 301. Batch I3 and I4 was prepared with HPMC K15M with different grade of Polyox^{tm} WSR. Batch I5 and I6 was developed to check the effect of HPMC K100M different grade of Polyox^{tm} WSR. Prepared layer was evaluated for weight variation, thickness, hardness, friability, disintegration time, drugcontent, and % cumulative drug release.

Ingredients (mg)	**I1**	**I2**	**I3**	**I4**	**I5**	**I6**
Isosorbide Dinitrate	40	40	40	40	40	40
MCCPH102	88.8	88.8	88.8	88.8	88.8	88.8

HPMC K4M	60	60	-	-	-	-
HPMC K15M	-	-	60	60	-	-
HPMC K100M	-	-	-	-	60	60
Polyox™ WSR301	60	-	60	-	60	-
Polyox™ WSR303	-	60	-	60	-	60
Quinoline yellow	0.2	0.2	0.2	0.2	0.2	0.2
Magnesium stearate	1	1	1	1	1	1
Total (mg/tablet)	**250**					

Formulation of Optimized Bilayer tablets

Table 5.16: Evaluation Parameters of Optimized Formulation

HDID	
Ingredients (mg)	**Qty. (mg)**
Hydralazine HCl	25
MCC PH102	45
Tablattose	20
Sodium starch glycolate	3.99
Ac-Di-Sol®	4
Magnesium Stearate	1
Talc	1.01
	100mg
Isosorbide Dinitrate	40
MCCPH102	60.86
HPMC K100M	70
Polyox™ wsr303	77.94
Quinoline yellow	0.2
Magnesium stearate	1
	250mg
Total = 350mg	

6. RESULTS AND DISCUSSION

Determination of Drug by HPLC

Determination of Hydralazine HCl

Take about 10 mg of Standard Hydralazine HCl (25% Diluted) transfer into a clean and dry 25 ml volumetric flask, add about 15 ml of mobile phase mix to dissolve it and make up 25 ml volume with mobile phase and mix. Inject equal volume (20 µl) of the standard preparation of the five replicate injections of standard and find out following suitability and two injection of test solution. Record the chromatogram and measure thepercentage.

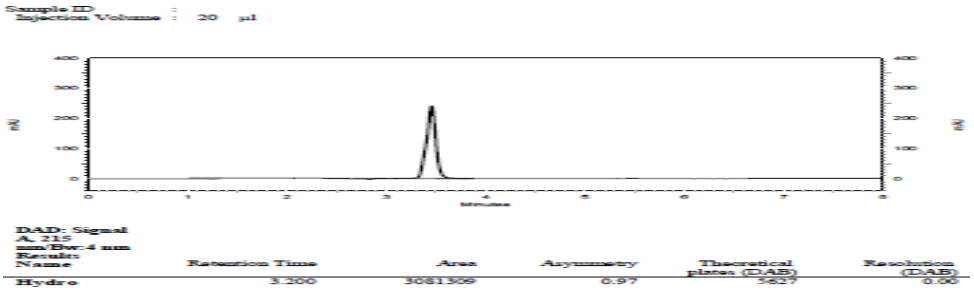


Figure 6.1: HPLC Chromatogram at 215nm of Hydralazine HCl

Determination of Isosorbide Dinitrate

Take about 10 mg of Standard Isosorbide dinitrate (25% Diluted) transfer into a clean and dry 25 ml volumetric flask, add about 15 ml of mobile phase mix to dissolve it and make up 25 ml volume with mobile phase and mix. Inject equal volume (20 µl) of the standard preparation of the five replicate injections of standard and find out following suitability and two injection of test solution. Record the chromatogram and measure thepercentage.

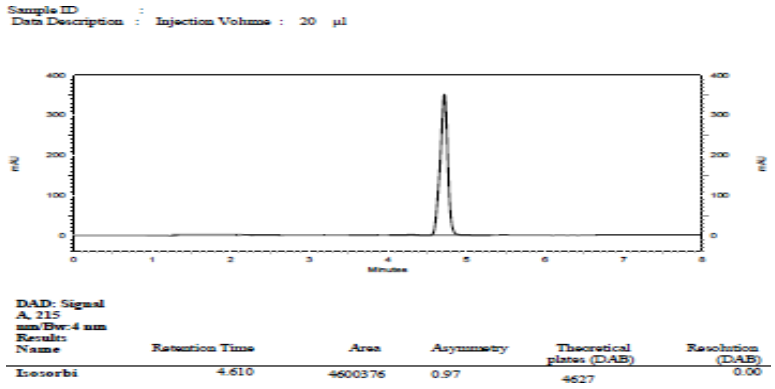


Figure 6.2: HPLC Chromatogram at 215nm of Isosorbide Dinitrate

Determine Combination of Hydralazine HCl and Isosorbide dinitrate byHPLC

Take about 10 mg of Standard Isosorbide dinitrate (25% Diluted) and about 10 mg Standard of Hydralazine HCl transfer into a clean and dry 25 ml volumetric flask, add about 15 ml of mobile phase mix to dissolve it and make up 25 ml volume with mobile phase and mix. Inject equal volume (20 µl) of the standard preparation of the five replicate injections of standard and find out following suitability and two injection of test solution. Record the chromatogram and measure the percentage.

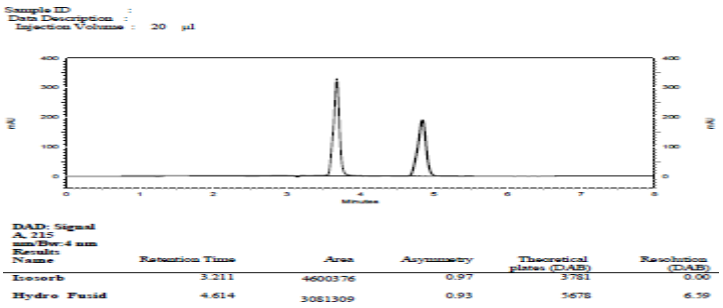


Figure 6.3: HPLC Chromatogram at 215nm Combination of Hydralazine HCl & Isosorbide dinitrate

Determination of Calibration Curve

Calibration Curve of Hydralazine HCl by HPLC

The standard calibration curve of Hydralazine HCl was obtained by plotting Area vs. concentration. Peak area of different concentrations were listed in below Table. The standard calibration curve of Hydralazine HCl was developed at λ_{max} 215nm. The calibration curve was linear between 50-150 µg/ml concentration ranges. R^2 value was obtained 0.999, it indicates the linearity of the curve.

Table 6.1: Area of different concentration of Hydralazine HCl

Concentration (µg/mL)	Peak Area
0	0
50	3355658
75	4976495
100	6593596
125	8210407
150	9843493

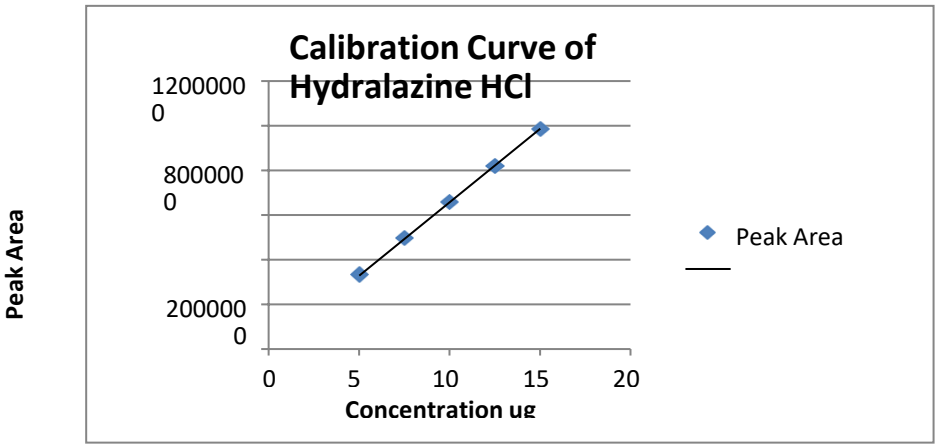


Figure 6.4: Calibration Curve of Hydralazine HCl

Calibration Curve of Isosorbide Dinitrate by HPLC

The standard calibration curve of Isosorbide Dinitratewas obtained by plotting Area vs. concentration. Peak area of different concentrations were listed in below Table. The standard calibration curve of Isosorbide Dinitrate was developed at λ_{max} 215nm. The calibration curve was linear between 50-150 µg/ml concentration ranges. R^2 value was obtained 0.999, it indicates the linearity of the curve.

Table 6.2: Area of different concentration of Isosorbide Dinitrate

Concentration (µg/mL)	Peak Area
0	0
50	6254635
75	9629461
100	12700701
125	15965898
150	18934406

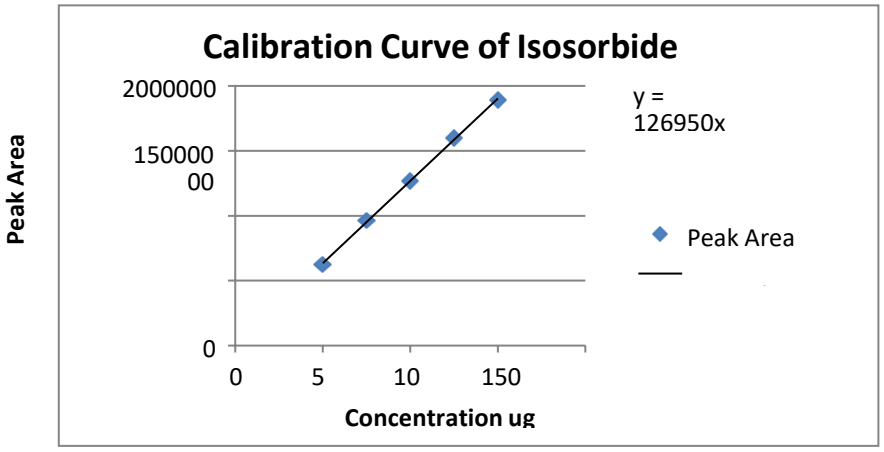
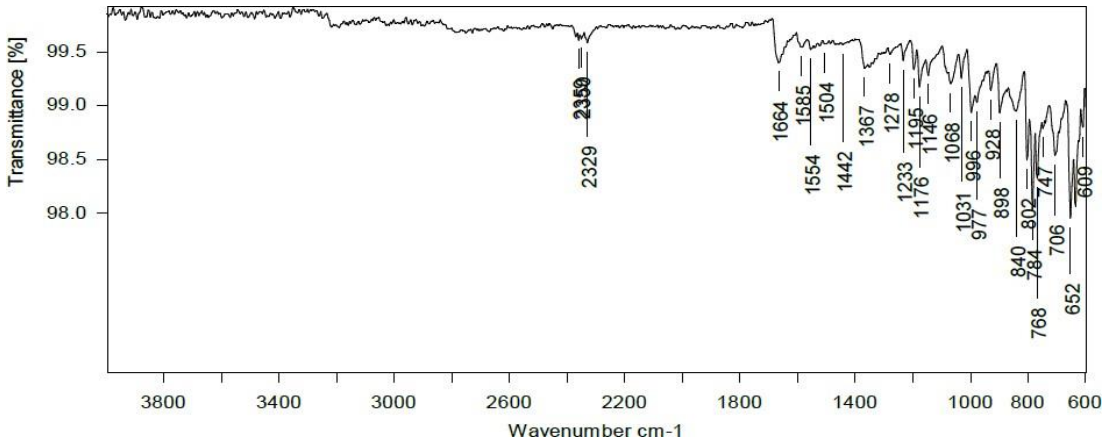


Figure 6.5: Calibration Curve of Isosorbide Dinitrate

Identification of Drugs

Identification of Hydralazine HCl by FT-IR



Identification of Isosorbide Dinitrate by FT-IR

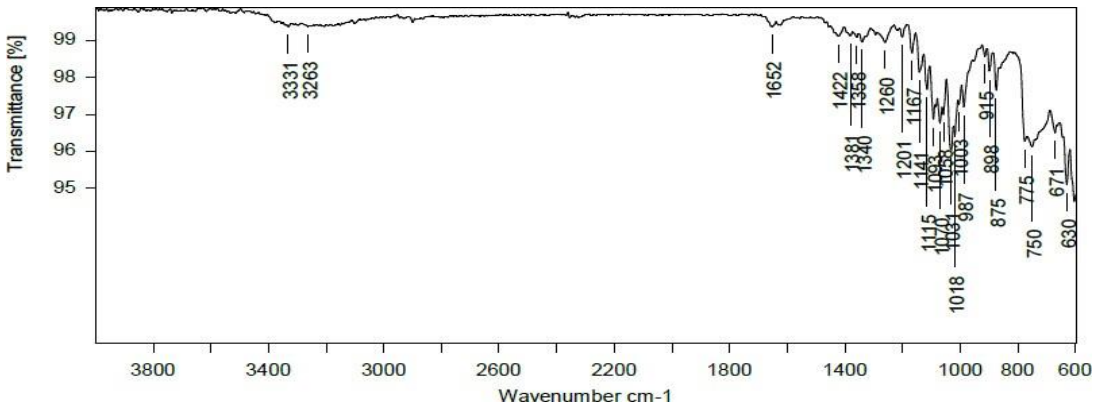


Figure 6.8: FT-IR Spectra of Isosorbide Dinitrate

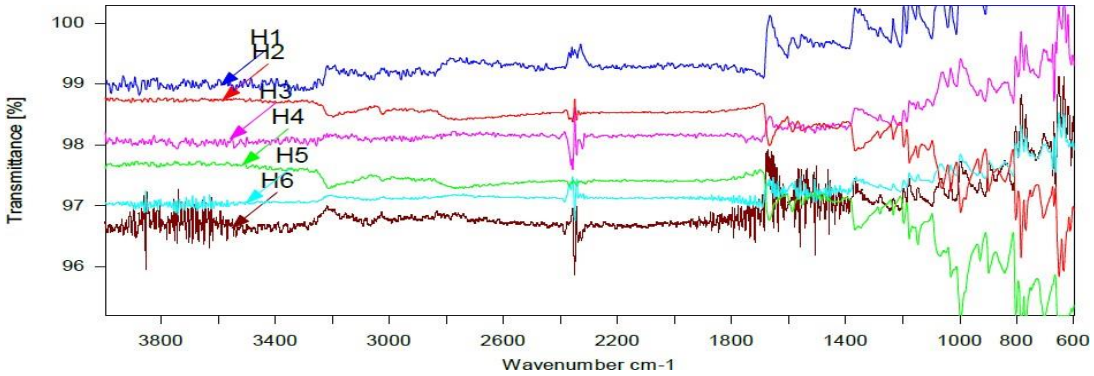
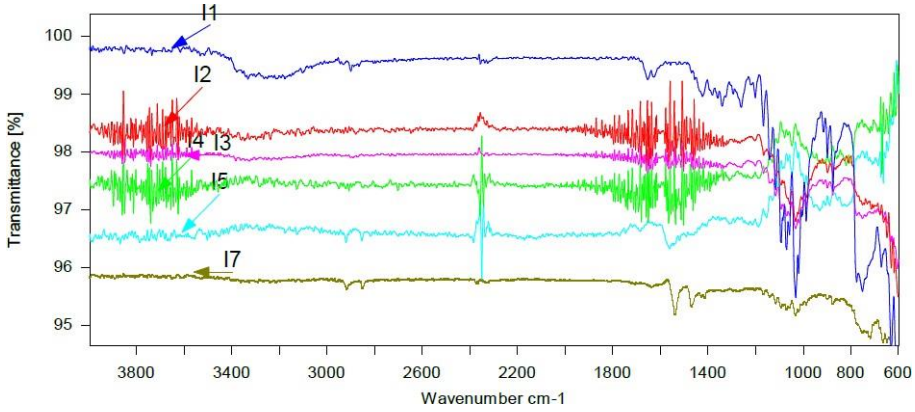


Figure 6.18: Overlay Spectra of Hydralazine HCl with all Excipients

Figure 6.24: Overlay Spectra of Isosorbide Dinitrate with all Excipients



Physical Compatibility

The API-excipients physical mixtures were analyzed visually, the results are as follow.

Table 6.7: Observation of Hydralazine HCl-Excipients Compatibility

Drug + Excipients	Ratio	Initial	After 6 month at 40 ± 2 ^{0}C / 75 ± 5 %RH
Hydralazine HCl	1	A white or almost white crystalline powder	No Change
Hydralazine HCl + MCC PH102	1:1		No Change
Hydralazine HCl + Tablattose	1:1		No Change
Hydralazine HCl + SSG	1:1		No Change
Hydralazine HCl + Acdisol	1:1		No Change
Hydralazine HCl + CrosPovidone	1:1		No Change
Hydralazine HCl + Magnesium Stearate	1:1		No Change

Table 6.8: Observation of Isosorbide Dinitrate-Excipients Compatibility

Drug + Excipients	Ratio	Initial	After 6 month at 40 ± 2 ^{0}C / 75 ± 5 %RH
Isosorbide Dinitrate	1	A white or almost white crystalline powder	No Change
Isosorbide Dinitrate + MCC PH102	1:1		No Change
Isosorbide Dinitrate + HPMCK100	1:1		No Change
sorbide Dinitrate + Polyox^{tm} wsr303	1:1		No Change
Isosorbide Dinitrate + Ethyl cellulose	1:1		No Change
sosorbide Dinitrate + Magnesium Stearate	1:1		No Change

After 6 month all samples were visually observed. Both drugs were found to be compatible with all the excipients used in formulation. The visual inspection of stored powder mixtures of Hydralazine HCl and Isosorbide Dinitrate with different tablets excipients did not show any change in color or appearance (e.g. discoloration, caking,liquefaction, formation of clumps). This represents a good preliminary indication ofphysical stability.

Flow Properties of Drug and Excipients

From the Table 6.9, it was concluded that Hydralazine HCl, Isosorbide Dinitrate, Sodium Starch Glycolate, Acdisol, Crospovidone, MCC PH102, Tablattose, HPMCK100, PolyoxWSR303, Ethyl cellulose, Magnesium Stearate have excellent flow property based on angle of repose because they all have angle of repose value between 19.52 ± 2.41 to 28.22 ± 2.73.They all have Carr's index value between 9.6 ± 0.2 to 15.0 ± 0.2% and Hauser's ratio 1.10 ± 0.3 to 1.23 ± 0.3 between showed excellentto good compressibility.

Table 6.11: Flow Properties of Drug and Excipients

Ingredients	**ılk density (gm/ml)**	**Tapped density (gm/ml)**	**Carr's index (%)**	**ıusner'sratio**	**Angle of repose (θ)**
ralazineHCl	0.57 ± 0.003	0.65 ± 0.002	12.30 ± 0.4	1.14 ± 0.5	26.6 ± 0.64
sosorbide Dinitrate	0.512 ± 0.02	0.598 ± 0.4	14.38 ± 0.3	1.16 ± 0.1	25.35 ± 2.73
dium Starch Glycolate	0.54 ± 0.01	0.63 ± 0.3	14.28 ± 0.3	1.16 ± 0.2	25.2 ± 2.94
Acdisol	0.51 ± 0.03	0.60 ± 0.3	15.0 ± 0.2	1.17 ± 0.3	23.34 ± 2.75
Crospovidone	0.56 ± 0.02	0.64 ± 0.2	12.5 ± 0.3	1.14 ± 0.3	27.21 ± 2.65
Tablattose	0.56 ± 0.03	0.62 ± 0.3	9.6 ± 0 .2	1.10 ± 0.3	26.31 ± 2.83
HPMC K100	0.57 ± 0.02	0.64 ± 0.3	10.9 ± 0.3	1.12 ± 0.2	28.22 ± 2.73
Ethyl Cellulose	0.62 ± 0.05	0.70 ± 0.03	11.1 ± 0.2	1.23 ± 0.3	25.20 ± 2.25
Polyox^{tm}wsr303	0.64 ± 0.01	0.70 ± 0.05	12.0 ± 0.3	1.20 ± 0.2	20.21 ± 2.65
MCC PH102	0.45 ± 0.04	0.52 ± 0.03	11.2 ± 0.3	1.18 ± 0.3	19.52 ± 2.41
agnesiumstearate	0.68 ± 0.02	0.75 ± 0.03	12.5 ± 0.2	1.21 ± 0.4	21.56 ± 2.41

Post-compression Evaluations of Batches H1 to H10

Batch Code	Weight variation	hickness(mm)	Hardness (kg/cm^2)	% Friability	Disintegration time (sec)	Wetting time (sec)	% Drug Content
H1	100.70 ± 0.14	2.55 ± 0.04	3.50 ± 0.05	0.43 ± 0.02	37 ± 1	28.10 ± 0.06	99.17 ± 0.07
H2	100.70 ± 0.05	2.54 ± 0.02	3.10 ± 0.04	0.46 ± 0.15	36 ± 2	27.05 ± 0.17	98.58 ± 0.04
H3	100.70 ± 1.07	2.54 ± 0.04	2.98 ± 0.14	0.46 ± 0.03	38 ± 1	28.06 ± 0.04	98.41 ± 0.08
H4	100.10 ± 0.45	2.54 ± 0.02	3.60 ± 0.11	0.46 ± 0.01	44 ± 1	35.04 ± 0.05	99.17 ± 0.06
H5	100.90 ± 0.78	2.57 ± 0.05	3.20 ± 0.09	0.47 ± 0.06	42 ± 2	34.05 ± 0.09	100.5 ± 0.05
H6	100.40 ± 0.34	2.54 ± 0.05	2.98 ± 0.07	0.47 ± 0.02	41 ± 2	34.13 ± 0.12	102.4 ± 0.09
H7	100.70 ± 1.89	2.54 ± 0.03	3.50 ± 0.03	0.46 ± 0.02	33 ± 1	27.14 ± 0.14	98.17 ± 0.04
H8	100.50 ± 0.22	2.55 ± 0.03	3.12 ± 0.05	0.49 ± 0.03	32 ± 1	26.12 ± 0.16	99.48 ± 0.17
H9	100.90 ± 0.67	2.56 ± 0.04	2.98 ± 0.08	0.49 ± 0.02	31 ± 2	23.10 ± 0.14	101.4 ± 0.11
H10	100.80 ± 1.35	2.55 ± 0.04	2.97 ± 0.08	0.40 ± 0.32	33 ± 1	24.95 ± 0.13	102.5 ± 0.13

***In-vitro* Drug Release of Batches H1 to H10**

Table 6.14: % CDR of Preliminary Batches Hydralazine HCl

Time (Min)	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10
0	0	0	0	0	0	0	0	0	0	0
5	7.4 ± 1.81	0.2 ± 2.18	1.1 ± 2.37	4.2 ± 1.74	6.8 ± 1.51	8.3 ± 2.54	5.6 ± 1.91	8.3 ± 2.14	1.7 ± 2.36	2.1 ±2.09
10	8.2 ± 2.78	0.1 ± 2.36	2.5 ± 1.84	0.5 ± 1.85	5.9 ± 2.80	9.7 ± 2.86	2.4 ± 2.78	8.6 ± 1.81	4.5 ± 2.48	4.2 ±2.35
15	5.4 ± 2.81	7.9 ± 2.51	7.3 ± 1.25	0.5 ± 1.39	72.3 ±1.15	5.3 ± 2.87	1.4 ± 2.44	3.3 ± 2.34	6.9 ± 1.46	6.9 ±1.12
20	5.3 ± 2.42	8.4 ± 2.18	9.7 ± 1.61	5.4 ± 2.15	79.6 ±1.89	4.6 ± 2.22	9.7 ± 2.28	0.1 ± 2.81	1.2 ± 2.93	1.4 ±2.65
30	8.6 ± 1.87	8.9 ± 2.59	9.2 ± 1.56	8.4 ± 2.69	0.7 ± 1.98	2.8 ± 2.29	8.6 ± 2.08	9.7 ± 2.25	9.9 ± 1.47	8.8 ±2.29

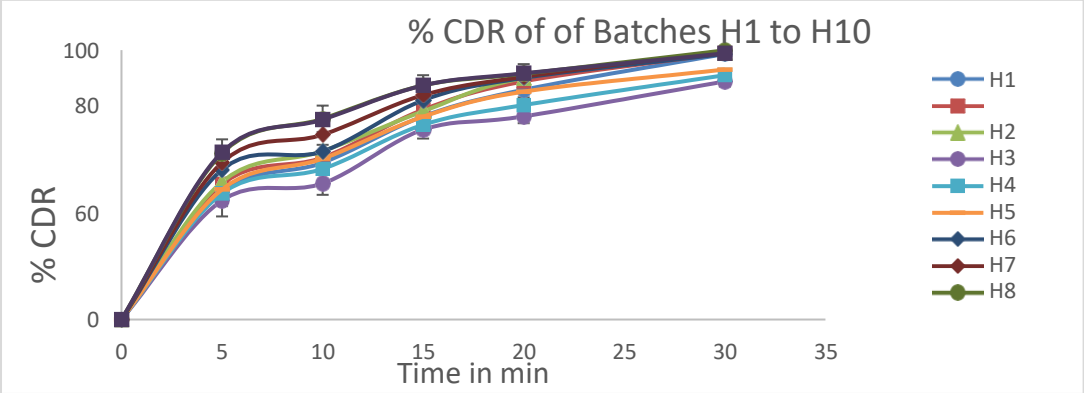


Figure 6.27: % CDR of Batches H1 to H10

Stability Study

Table 6.35: Stability Study of Optimized Formulation (HDID)

No. of Months	% Drug Content	Batch (% Drugrelease in 10hr)
0	99.8 ± 0.05	99.18 ± 0.54
6	99.77 ± 0.04	99.09 ± 0.52

All values are expressed as mean ± standard deviation, n=3

Stability study of Bilayer tablet of Hydralazine HCl and Isosorbide Dinitrate was carried out for 6 Months as per ICH guideline. All data are mentioned in table 6.35. The stability studies of the optimized formulation (HDID) showed no significant changes in the physical parameters, %drug content and % drug release in 10hr when stored at 40± 2^{o} C/ 75 ± 5 % RH. So, it was considered that formulation having good stability.

7. SUMMARY

Hypertension affects around half of the adult population worldwide, being one of the most common cardiovascular disorders (CVD). It occurs when the high cardiac output exerts pressure on the arterial wall as the blood flow increases. The conventional dosage form used in the treatment of hypertension cannot produce the desired therapeutic effect for a prolonged period. The rationale for using fixed-dose combination therapy is to obtain increased blood pressure (BP) control by employing two antihypertensive drugs with different modes of action and enhance compliance by using a single tablet. Bilayer tablet is suitable for the sequential release of two drugsin combination, separate, and sustained release.

Bilayer tablets are able to provide multiple releases kinetic of same/different drug. It is preferred to co-administer two different drugs in the same dosage form and controlling drug

release rate of two different API. It is also preferred to reduce of pill burden and safety margin of high potency drug can be increased.

The rational for combination therapy is to encourage the use of lower doses of drug to reduce the patient's blood pressure to goal to minimize dose dependent side effects and adverse reactions. When smaller doses of medication with different mechanism of action are combined, synergistic or additive effects on blood pressure are achieved and dose dependent side effects are minimized. Here in the present study two drugs Hydralazine HCl and Isosorbide dinitrate are used in the combination.

In the present study of bilayer tablet preparation Hydralazine HCl immediate release layer was prepared by the direct compression method. For primary trial SSG, CCS, and ac-di-sol® were used in varying amounts. Batch H1 to H3 contained 2%, 3%, and 5% of SSG, respectively. Batch H4 to H6 contained 2%, 3%, and 4% CCS, respectively and batch H6 to H10 contained 2%, 3%, 4%, and 5% ac-di-sol® respectively. Prepared layer was evaluated for weight variation, thickness, hardness, friability, DT, WT, drug content, and Q_{15} considered as optimization batch.

8. CONCLUSION

The present study was undertaken with an aim formulation and evaluation of Bilayer Tablets containing Hydralazine HCl and Isosorbide Dinitrate by Direct compression technology was to formulate a stable, safe and convenience dosage form for the better management of most common cardiovascular disorders or blood pressure. The formulations of bilayer tablets showed good results in case of Hydralazine HCl immediate release layer physicochemical parameters and prepared using concentration of superdisintegrants sodium starch glycolate and ac-di-sol® for the fast release layer and sustained release layer of isosorbide dinitrate containing HPMC K100 M and polyox^{tm} WSR 303 for the delay the drug release up to 10-12 hrs. The FTIR and DSC analysis indicates that there were no drug-drug and drug-excipients interactions. Pre-compression and post compression parameters were found to be within the satisfactory limits and hence suitable to formulate Bilayer tablets. Formulation batch HDID was finally optimize in which HD9 (Hydralazine HCl) batch is selected as immediate release layer and ID9 (Isosorbide Dinitrate) batch is selected as sustained release layer as final selected formulation. Batch HDID provides better drug release profile.

REFERENCES

1. Ravali, M., Prathyusha, A., & Rao, V. U. M. (2015) 'An overview on Bilayer Tablet'. *International Journal of Innovative Pharmaceutical Sciences and Research,* vol. 3, no. 5, pp. 451-469.
2. Yie, W. C. (1992) *Novel drug delivery systems,* Marcel Dekker Inc, 2nd ed.
3. Chien, Y. W. (1992) *Novel drug delivery systems*. Marcel Dekker Inc, 2nd ed New York, 139-140.
4. Aulton, M E. (2002) *Pharmceutics, The Science of dosage form design (Bilayer Tablets),* 2nd ed, Churchill livingstone, pp. 414-418.

5. Lieberman, H. A., Lachman, L., Schwartz, J. B. *Pharmaceutical Dosage forms: tablets*, volume 3, 2nd edition.
6. Aggarwal, S., Syan, N., & Mathur, P. (2013) 'Bi-layer tablet technology— opening new ways in drug delivery systems: an overview'. *International journal of research in pharmaceutical and biomedical sciences*, vol.4, pp. 2229-3701.
7. Ashok, P. H., & Kumar, T. A. (2012). 'A Novel Approach of Bi-layer Tablet Technology-A review'. *International research journal of pharmacy*, vol. 3, no. 5, pp. 44-49.
8. Rishikesh, G., Paul, T. R., Mohiuddin, A. A. (2014) 'Bilayered Tablet Technology: An Overview'. *World Journal of Pharmaceutical Research*, vol. 3, no. 4, pp. 150-163.
9. Gopinath, C., Bindu, V. H., & Nischala, M. (2013) 'An overview on bilayered tablet technology'. *Journal of global trends in pharmaceutical sciences*, vol. 4, no. 2, pp. 1077-1085.
10. Barthwal, P., Ganarajan, G., Kothiyal, P. (2013). 'Bilayer a review', *International Journal of chemical and pharmaceutical sciences*, vol. 2, no. 4, pp. 1788-1797.
11. Jha, M. K., Rahman, M. H., & Rahman, M. M. (2011) 'Biphasic oral solid drug delivery system: A review'. *International Journal of Pharmaceutical Sciences and Research*, vol. 2, no. 5, pp. 1108.
12. www.durect.com Verma, R., Devre, K., Gangrade, T. (2014) 'Bi-layer tablets for various drugs: A review'. *Scholars Academic Journal of Pharmacy*, vol. 3, no. 3, pp. 271-279.
13. http://www.port/technology.com
14. Verma, R. K., & Garg, S. (2001) 'Drug delivery technologies and future directions'. *Pharmaceutical Technology*, vol. 25, no. 2, pp. 1-14.
15. Kaur, P., Dhiram, S., & Arora, S. (2013) 'Floating Bilayer Tablet Technology: A Review'. *International Journal Pharmaceutical Sciences Review Research*, vol. 19, no. 1, pp. 112-122.
16. Arun, D., Venu Gopal, N., Shekar, L., Ramarav, B., Karunakar, K., & Surendra, Y. (2012) 'A review of novel approach in bilayer tablet technology'. *International Journal of Pharmaceutical, Biological and Chemical Sciences*, vol. 1, no. 1, pp. 1-8.
17. Kale, S. S., Saste, V. S., Ughade, P. L., & Baviskar, D. T. (2011) 'Bilayer tablet'. *International Journal of Pharmaceutical Sciences Review and Research*, vol. 9, no. 1, pp. 25-30.
18. Ravula, A. N., & Goud, B. A. (2011) 'Recent advances in oral pulsatile drug delivery'. *Journal of Advanced Pharmaceutical Sciences*, vol. 1, no. 1, pp. 57-62.
19. Bingi, M., Gudas, G., Debnath, S., Sreekanth, C. B. V., Raguveer, P., & Srikanth, T. (2011) 'Design and evaluation of buccoadhesive bi-layer tablet of paroxetine hydrochloride'. *International Journal of Pharma Professional's Research*, vol. 2, no. 1, pp. 195-197.
20. Natarajan, R., Vaishnani, R., & Rajendran, N. N. (2011) 'Formulation and evaluation of immediate release tablets of paroxetine HCl using different superdisintegrants'. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, vol. 2, no. 3, pp. 1095-1099.
21. Timmins, P., Dennis, A. B., & Vyas, K. A. (2002) *U.S. Patent No. 6,475,521*. Washington, DC: U.S. Patent and Trademark Office.

22. Patel, M., Sockan, G. N., & Tamizh, M. (2010) 'Challenges in the formulation of bilayered tablets: A review'. *International Journal of Pharmaceutical Research & Development*, vol. 2, no. 10, pp. 005.
23. Jan Vogeleer, Bialyer Tablet- Why special Technique required. The Courtoy- R292F Tablet Press designed for Quality Bilayer Tablet, Niro Pharma System.
24. Abshagen, U., & Spörl-Radun, S. (1981) 'First data on effects and pharmacokinetics of isosorbide-5-mononitrate in normal man'. *European journal of clinical pharmacology*, vol. 19, no. 6, pp. 423-429.
25. Namrata, M., Sirisha, V. N. L., Sruthi, B., Harika, I. B., Kirankumar, P., Rao, Y. K. K., & Rao, O. U. (2013) 'A Review on Bi-layer Tablets'. *International Journal of Pharmaceutical and Phytopharmacological Research*, vol. 1, no. 2, pp. 240- 246.
26. Joseph, R. R. (1996) In; Banker, GS and Rhodes, CT, Eds., Modern Pharmaceutics, 3rd Edn., Vol. 72.New York, Marcel Dekker Inc.369.
27. Li, S. P., Karth, M. G., Feld, K. M., Di Paolo, L. C., Pendharkar, C. M., & Williams, R. O. (1995) 'Evaluation of bilayer tablet machines—a case study'. *Drug development and industrial pharmacy*, vol. 21, no. 5, pp. 571-590.
28. Patel, J. S. (2013) 'A review on bilayer tablets'. *Journal of drug discovery and therapeutics*, vol. 1, no. 03, pp. 40-48.
29. Nilawar, P. S., Wankhade, V. P., & Badnag, D. B. (2013) 'An emerging trend on bilayer tablets'. *International journal of pharmacy and pharmaceutical science research*, vol. 3, no. 1, pp. 15-21.
30. Mandal, U., & Pal, T. K. (2008) 'Formulation and in vitro studies of a fixed-dose combination of a bilayer matrix tablet containing metformin HCl as sustained release and glipizide as immediate release'. *Drug development and industrial pharmacy*, vol. 34, no. 3, pp. 305-313.
31. Purohit, R., Naruka, P. S., Chauhan, C. S., & Bhatt, D. (2013) 'Formulation, development and evaluation of bilayer tablet of Nebivolol and Indapamide'. *Indo American Journal of Pharmaceutical Research*, vol. 3, no. 7, pp. 5105-5117.
32. Vishal, M., Anuj, K., Pankaj, P., Deepti, P., Shraddha, S., Mansee, S., & Dutta, M. (2012) 'Formulation development and evaluation of Bilayer tablets of Lornoxicam'. *International Journal of Drug Development and Research,* vol. 4, no. 2.