Development and Evaluation of Controlled Release Bilayer Tablets of Hydrochlorothiazide and Losartan Potassium

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Abstract: The aim of the present research work was to develop bilayer tablet dosage form containing combination of immediate and sustained matrix prepared from Hydrochlorothiazide (HTZ) and Losartan Potassium (LP) respectively for the treatment of hypertension and its associated complications. Immediate release HTZ was prepared using different superdisintegrants. LP sustained layer was prepared by compression technique. Both pre-compression and post-compression parameters were analyzed for all the tablets. *In vitro* release studies were carried out as per USP in pH (1.2) and phosphate buffer pH (6.8) using USP-XXI type II. Bilayer tablet (F6) formulated using higher concentration of HPMC K 15 exhibiting higher LP release rate (83.553± 0.22) for the period of 12 h. The *In vitro* release profile of drug from sustained matrix could be best expressed by First order as the plot showed highest linearity (R^2 = 0.990) and diffusion was the dominating mechanism of drug release. The stability and FTIR studies are also indicating the absence of strong interaction between drug and polymer and compatibility among them.

Keywords: Hydrochlorothiazide, Losartan potassium, Bilayer tablets, kinetic models, controlled release.

1. INTRODUCTION

Over the past decades, pharmaceuticals have made a major contribution to improving the health status of patients. At the same time, its expenditure has increased rapidly, with spending on medicines outpacing economic growth in many countries. For many decades, treatment of acute disease or chronic illness has been mostly accomplished by delivery of drugs to patients using various pharmaceutical dosage forms including tablets, capsules, pills, suppositories, creams, ointments, liquids, aerosols and injectables as drug carriers [1]. Oral route of drug administration have wide acceptance up to 50-60% of total dosage forms and is the most convenient and preferred route for systemic effects due to its ease of dosing administration, pain avoidance, accurate dosage, patient compliance and flexibility in formulation [2, 3]. Repetitive dosing and unpredictable absorption window of Conventional dosage form led to the concept of controlled drug delivery systems. Formulation of layers from different polymers allows release of drugs with a bolus and then at a controlled rate or by targeted drug delivery in the GI tract using pH dependant polymers [4]. But often bolus drug release led to dose dumping at and failure to achieve site specific drug delivery [5]. This led to formulation of bi-layer tablets [6, 7]. Bilayer tablets provides advantages like separation of incompatible components, greatest chemical stability, lower cost, tamper resistance, improved patient compliance [8-10] and control the delivery rate of either single or two different active pharmaceutical ingredient(s) [11].

Hypertension is among the most common diseases of adults in industrialized countries and is one of the important modifiable risk factors for cardiovascular and renal diseases. The goal of Hydrochlorothiazide (HTZ) and Losartan Potassium (LP) is to prevent the complications of hypertension. Specially used for the treatment of patients with stage 1 (blood pressure 140-159 over 90-99) and stage 2 (blood pressure 160 and above over 100 and above); essential hypertension in comparison with monotherapy regimens in a calcium channel agonist or an angiotensin II receptor blocker. The two drugs in a functional combination preparation herein have different activities. Composition of HTZ (a thiazide diuretic) and LP (angiotensin-2 receptor blocker) can achieve improved preventive or therapeutic effects for cardiovascular disorders, as compared with conventional mono therapy. It may delay the progression of diabetic nephropathy and is also indicated for the reduction of renal disease progression in patients with Type II diabetics, hypertension and albuminurea [12]. The renin–angiotensin– aldosterone–system-activating effect of hydrochlorothiazide augments the efficacy of blocking the angiotensin II type 1 (AT_1) receptor with Losartan potassium [13].

The main aim of the work is to develop and evaluate a pharmaceutical equivalent, stable, robust and controlled bilayer tablet of Hydrochlorothiazide and Losartan Potassium, which is orally administered, very

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well tolerated side effect, improves depressed mood, high patient acceptability and satisfaction in recent era.

2. MATERIALS AND METHODS

Losartan Potassium (LP) and Hydrochlorothiazide (HTZ) were received from Alembic Pharma, Vadodara, India. Microcrystalline cellulose (MCC), Sodium starch glycolate (SSG), Crospovidone (CP), Crosscarmellose sodium (CCS), Hydroxypropyl methyl cellulose (HPMC) k 4, HPMC k 15 and Ethyl Cellulose (EC) were received from Mapromax, Life Sciences Pvt. Ltd., Dehradun, India. Magnesium stearate was obtained from Sun pharma, Chennai. Talc was obtained from Sanjay biological museum, Amritsar, India. As the method of preparation is direct compression technique, hence no specified reagent was required.

EXPERIMENTAL WORK

2.1. Pre-Formulation Studies

2.1.1. Fourier Transform Infrared (FTIR) Studies of HTZ and LP

Physicochemical interaction of drug and polymer were conducted by FTIR spectroscopy (SHIMADZU, Japan) using KBr pellets at 400-4000 cm^{-1} and the spectra were recorded for pure HTZ, HTZ with polymers, pure LP and LP with polymers report was shown in Figure **1**.

2.1.2. Evaluation of Pre-Compression Parameters

Prior to the compression into tablets powders were evaluated for properties like Bulk density, tapped density, powder flow properties like angle of repose, carr's index and hausner ratio. Bulk density and tapped density were measured by using bulk density apparatus [14-16].

1. **Angle of Repose (θ)**: The frictional forces in a loose powder or granules can be measured by the angle of repose. This is the maximum angle possible between the surface of a pile of powder or granules and the horizontal plane.

$$\tan Ø = h/r$$

$$Ø = \tan^{-1}(h/r)$$

Where, θ is the angle of repose, h is the height, r is the radius.

The granules were allowed to flow through the funnel fixed to a stand at definite height. The angle of repose was then calculated by measuring the height and radius of the heap of granules formed.

2. **Bulk Density:** Both loose bulk density (LBD) and tapped bulk density (TBD) were determined. Accurately weighed amount of granules taken in a 50 ml capacity measuring cylinder was tapped for 100 times on a plane hard wooden surface and estimated the LBD and TBD, calculated by using following formula:

$$\text{LBD (Loose Bulk Density)} = \frac{\text{Mass of Powder}}{\text{Volume of Packing}}$$

$$\text{TBD (Tapped Bulk Density)} = \frac{\text{Mass of Powder}}{\text{Tapped Volume of Packing}}$$

3. **Carr's Compressibility Index**: Percent compressibility of powder mix was determined by Carr's compressibility index, calculated by using following formula:

$$\text{Carr´s Index \%} = \frac{\text{TBD} - \text{LBD}}{\text{TBD}} \times 100$$

4. **Hausner's Ratio:** It is determined by comparing tapped density to the bulk density by using following equation:

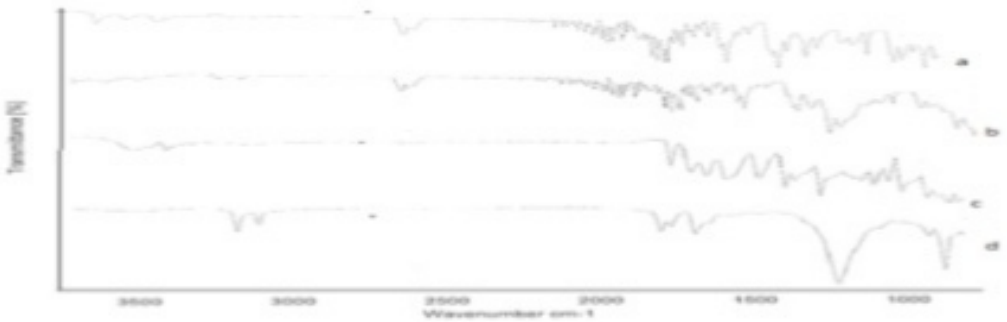

Figure 1: FTIR Spectroscopy Study on Pure Hydrochlorothiazide (a), Hydrochlorothiazide + polymers (b), Pure Losartan (c) and Losartan + polymers (d)

Hausner's ratio = TBD/ LBD

Formulation Development

Phase- I: Preparation of Instant Layer of Hydrochlorothiazide

Fast dissolving tablets of Hydrochlorothiazide were prepared by direct compression method [17] (Cadmach Machinery, Kolkata, India) after incorporating different super-disintegrants such as, CCS, CP and SSG in different concentrations. The above ingredients was weighed and mixed in geometric progression in a dry and clean mortar. Then the ingredients were passed through mesh #60.

Magnesium stearate as lubricant and talc as glidant were added in a final step and mixed. This blend was subjected to analysis of pre-compression parameters which included angle of repose, bulk density, tap density, carr's index and hausner's ratio.

The Blend was compressed on 8 mm (diameter) flat faced punch on a Rimek mini press 16 - station rotary compression machine (Cadmach, Gujrat, India). Nine formulations of Hydrochlorothiazide granules were prepared and each formulation contained one of the three superdisintegrant in different concentration. Each tablet weighing 150 mg, were obtained. Compositions of all batches are represented in Table **1**.

Evaluation of Instant Layer Tablets of Hydrochlorothiazide

Drug Content determination

Drug (Hydrochlorothiazide) equivalent to 10 mg of drug was stirred by using magnetic stirrer with appropriate volume of 0.1 N HCl (simulated gastric fluid of pH 1.2 without enzymes) for 60 min, till the entire drug leached out from complex, then the solution was filtered through 0.45 µm membrane filter. Filtered solution diluted with 0.1 N HCl prior to drug content analysis using a double beam UV-VIS Spectrophotometer (SHIMADZU, 1601, Japan) at 272nm.

PHASE II- Preparation of LP Matrix Tablets

Direct compression was followed to manufacture the matrix tablets of Losartan.

Firstly; the drug, polymer and other excipients selected were passed through mesh #40. Required quantity of drug, polymer and excipients were weighed properly and transferred into polyethylene bag and the blend was mixed for at least 15 min in a porcelain mortar. Secondly; the blend obtained was lubricated by adding 1% magnesium stearate and mixed again for 5min and processed further for direct compression. Compositions of all batches are represented in Table **2**.

Preparation of Bilayer Tablets of Hydrochlorothiazide and Losartan Potassium

Optimized batch of Hydrochlorothiazide (H9; the formulation which takes least time for dissolution) and LS (L5; the formulation which sustained drug release most) were selected for preparation of bilayer tablet. The quantity of powder blend for the sustained release layer was compressed lightly at 10 station Rimek tablet press using 8 mm round concave-faced punch. Over this compressed layer, required quantity of powder blend for fast release layer was placed and compressed with the hardness in the range of 8-12 $kgcm^2$ to form a bilayer matrix tablet.

Table 1: Composition of Instant Layer Tablets of HTZ

Ingredients (mg)	Formulation Code								
	H1	H2	H3	H4	H5	H6	H7	H8	H9
HTZ	25	25	25	25	25	25	25	25	25
MCC	110	108	106	110	108	106	110	108	106
CP	-	-	-	-	-	-	08	10	12
CCS	-	-	-	08	10	12	-	-	-
SSG	08	10	12	-	-	-	-	-	-
Magnesium stearate	05	05	05	05	05	05	05	05	05
Talc	02	02	02	02	02	02	02	02	02
Total weight	150	150	150	150	150	150	150	150	150

HTZ indicates Hydrochlorothiazide; MCC indicates Microcrystalline cellulose; CP indicates Crospovidone; CCS indicates Croscarmellose sodium; SSG indicates Sodium starch glycolate.

Table 2: Composition of LP Matrix Tablets

Ingredients (mg)	L1	L2	L3	L4	L5	L6	L7	L8	L9
LP	100	100	100	100	100	100	100	100	100
HPMC K 4	95	105	115	-	-	-	47.5	42.5	57.5
HPMC K 15	-	-	-	95	105	115	47.5	52.5	57.5
Lactose	45	35	25	45	35	25	45	35	25
Talc	5	5	5	5	5	5	5	5	5
Magnesium stearate	5	5	5	5	5	5	5	5	5
Total Weight	250	250	250	250	250	250	250	250	250

LP indicates Losartan Potassium; HPMC K 4 indicates Hydroxypropyl methyl cellulose K 4; HPMC K 15 indicates Hydroxypropyl methyl cellulose K 15.

Evaluation of Bilayer Tablet of HTZ and LP

Evaluation of Post-compression Parameters

1. Shape and Color of Tablets

Uncoated tablets were examined under a lens for the shape of the tablet and color was observed by keeping the tablets in light.

2. Thickness Test

Three tablets were picked from each formulation randomly and thickness was measured individually. It is expressed in mm and standard deviation was also calculated. The tablet thickness was measured using dial-caliper (Mitutoyo, Japan).

3. Weight Variation Test

20 tablets were selected randomly from each formulation and average weight was determined. The tablets were weighed individually and compared with average weight.

In all the formulations the tablets weight is more than 130 mg and less than 324 mg; hence 7.5% maximum difference allowed as per USP methods.

4. Hardness Test

The hardness of tablet was measured by Monsanto hardness tester (Electrolab Pvt. Ltd, India) and results were expressed in Kg/cm^2.

5. Friability Test

20 tablets were taken from each formulation and the friability was determined (Roche Friabilator). The equipment was run for 4min at 25 revolutions per minute. The tablets were taken out, dedusted and reweighted and % friability was calculated. The friability was determined as the mass loss in percent according to Equation:

% Friability = (Loss in weight/ Initial weight) X 100

The test complies if tablets not lose more than 1% of their weight

6. Uniformity of Drug Content

The test is mandatory for tablets with 10 mg or less weight of active ingredient. 10 randomly selected tablets from each formulation (F1 to F9) were finely powdered and powder equivalent to 10 mg of Hydrochlorothiazide was accurately weighed and transferred to 100 mL volumetric flasks containing 100 mL of 0.1N HCl. The flasks were shaken to mix the contents thoroughly. The volume was made up to the mark with 0.1 N HCl and filtered. 1mL of the filtrate was suitably diluted and HTZ content was estimated at 272nm using a double beam UV-visible spectrophotometer.

7. Wetting Time and Water Absorption Ratio

A tablet was placed in the wet tissue paper placed in a dish containing 6 mL of water and the time of complete wetting was measured and water absorption ratio calculated.

$$R = \frac{100\,(W_a - W_b)}{W_b}$$

Where, W_a = Weight after water absorption

W_b = Weight before water absorption

8. Disintegration Time

The *In vitro* disintegration time was determined by using disintegration test apparatus. One tablet was placed in each of the six tubes of the apparatus and one disc was added to each tube. The time taken for complete disintegration of the tablet with no palpable mass in the apparatus was measured in seconds.

9. *In vitro* Drug Release Studies

Bilayer tablets were subjected to *in vitro* drug release studies in simulated gastric and intestinal fluids to assess their ability in providing the desired controlled drug delivery. *In vitro* drug release of the sample was carried out using USP-XXI type II dissolution rate apparatus (model QAE 016 and NRE 002, M/S Campbell Electronics) (Paddle type) at100 rpm, 37±0.5°C, and pH 1.2 buffer (900 mL) (0.1 N HCl) for 2 h, since the average gastric emptying time is about 2 h. The dissolution medium was replaced with pH 6.8 phosphate buffer (900mL) and experiment continued for another 10 h. At predetermined time intervals, 5mL aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium. The samples withdrawn were analyzed by UV spectrophotometer using multi component mode of analysis (six tablets in each batch, and the mean values were plotted versus time ± SD).

10. Drug Release Kinetics

The dissolution data of the bilayer tablets were fitted to some kinetic models, namely, Zero order, First order, Higuchi [18] and Korsmeyer-Peppas [19, 20] in order to determine HTZ and LP release patterns and mechanisms [21, 22].

For matrix tablets, if the exponent $n < 0.5$, then the drug release mechanism is quasi-Fickian diffusion; if $n = 0.5$ then Fickian diffusion, $0.5 < n < 1$, then it is anomalous diffusion. An exponent value of 1 is indicative of case II transport or typical zero-order, and $n > 1$ is indicative of non-Fickian supercase II. The diffusion exponent is based on the Korsmeyer- Peppas equation and the erosion exponent is based on the Hixon-Crowell cube root equation.

11. Stability Studies

This is to determine for physical, chemical, therapeutics and toxicological specifications. Stability studies conducted for optimized bilayer tablets. The preliminary stability of the optimized batch as per ICH and WHO guidelines [23] and optimized formulation was sealed in aluminium packaging laminated with polyethylene. Samples were kept at $40^{o}C \pm 2^{o}C$ and 75% RH ± 5% for 3 months. At the end of the study period, the formulation was observed for change in physicochemical parameters, drug content and *in vitro* dissolution [24].

3. RESULTS AND DISCUSSION

HTZ was formulated as instant release layer using CP, CCS (Ac – Di - Sol) and SSG. Higher swelling and hydration capacity of CCS leads to faster disintegration and subsequent dissolution. This comply the sequential release of HTZ. LP is an anti-hypertensive drug and needs to release in a controlled manner. Hence LP was formulated as matrix tablet by using hydrophilic matrix polymers HPMC K4 and HPMC K15.

FTIR Spectroscopy

Identification of HTZ and LP were done by FTIR Spectroscopy as per specification. The FTIR studies proved chemical and physical compatibility between drug and polymer. FTIR spectrums are represented in Figure **1**.

The IR spectra of pure Hydrochlorothiazide showed characteristic bands at 1316/cm indicating the N = C stretch, Bands at 3545/cm (O-H broad), 3357/cm (secondary N-H), 3101/cm (=C-H and $=CH_2$ band). A prominent sharp peak was observed at 746/cm indicating O-H bending. The characteristic peaks of the Hydrochlorothiazide with excipient followed the same trajectory as that of the drug alone with minor differences. Thus there was no drug -excipient interactions found.

The IR spectra of pure of Losartan potassium showed characteristic bands at 1363/cm indicating CH_2-CH_3 deformation and band at 1260/cm (C=O) was observed. The characteristic peaks of the Losartan potassium with excipient followed the same trajectory as that of the drug alone with minor differences. Thus there was no drug -excipient interactions found.

Evaluation of Pre-Compression Parameters for Hydrochlorothiazide and Losartan Potassium

Prior to compression, the instant layer and matrix tablets of Hydrochlorothiazide and Losartan Potassium were evaluated for various pre-compression parameters such as angle of repose, bulk density (loose bulk density and tapped bulk density), carr's index and hausner' ratio. Results showed that all parameters were within limits. The results of all the batches of both Hydrochlorothiazide and Losartan Potassium were shown in Table **3**.

a) Angle of Repose (θ)

The data obtained for angle of repose for all the formulations were found to be in the range of 24.31 to 29.85 (Hydrochlorothiazide tablets) and 27.58 to 29. 97 (Losartan Potassium tablets). All the formulations prepared showed the angle of repose less than 30°, which reveals good flow property.

Table 3: Pre-Compression Parameters for HTZ and LP

Formulation Code	Parameters				
	Loose Bulk Density (g/mL)	Tapped Bulk Density (g/mL)	Carr's Index (%)	Hausner's Ratio	Angle of Repose (Ø) (Degree)
H1	0.347±0.004	0.415±0.004	16.3855±0.019	1.1959±0.02	26.72
H2	0.351±0.005	0.426±0.005	17.6056±0.68	1.2136±0.01	25.51
H3	0.349±0.004	0.419±0.007	16.7064±0.94	1.2005±0.01	27.54
H4	0.353±0.002	0.425±0.006	16.9411±0.76	1.2039±0.02	27.52
H5	0.354±0.006	0.422±0.006	16.1137±1.12	1.1920±0.02	24.31
H6	0.357±0.004	0.431±0.008	17.1693±0.82	1.2072±0.01	29.85
H7	0.359±0.007	0.436±0.005	17.6605±1.27	1.2144±0.03	28.77
H8	0.346±0.003	0.408±0.007	15.1960±1.09	1.1791±0.02	26.61
H9	0.348±0.003	0.412±0.004	15.5339±0.58	1.1839±0.01	27.12
L1	0.422±0.003	0.510±0.004	17.2549 ±0.63	1.2085±0.01	29.25
L2	0.429±0.004	0.522±0.007	17.8160±0.78	1.2167±0.02	28.26
L3	0.423±0.002	0.499±0.008	15.2304±1.22	1.2615±0.01	29.15
L4	0.438±0.006	0.545±0.004	19.6330±1.30	1.2442±0.01	29.26
L5	0.441±0.004	0.554±0.005	20.3971±0.59	1.2562±0.03	29.48
L6	0.436±0.007	0.524±0.006	16.7938±0.72	1.2018±0.02	27.58
L7	0.429±0.005	0.514±0.003	16.5369±0.88	1.1981±0.02	29.97
L8	0.425±0.004	0.510±0.007	16.6666±0.65	1.2±0.01	28.56
L9	0.433±0.006	0.525±0.008	17.5238±0.61	1.2124±0.01	29.25

[a]Each value represents as mean ± SD of three (n = 3) determinations.

b) Loose Bulk Density (LBD) and Tapped Bulk Density (TBD)

For the all the batches; the loose bulk density (LBD) were varied from 0.346±0.003 to 0.359±0.007 g/mL (Hydrochlorothiazide tablets) and 0.422±0.003 to 0.441±0.004 g/mL (Losartan Potassium tablets) respectively and tapped bulk density (TBD) were varied from 0.408±0.007 to 0.436±0.005 g/mL (Hydrochlorothiazide tablets) and 0.499±0.008 to 0.554±0.005 g/mL (Losartan Potassium tablets).

c) Carr's Compressibility Index and Hausner's Ratio

For the all the batches; results of Carr's index or compressibility index (%) were ranged from 15.1960±1.09 to 17.6605±1.27 (Hydrochlorothiazide tablets) and 15.2304±1.22 to 20.3971±0.59 (Losartan Potassium tablets). The directly compressible granulations had shown very good compressibility index values which result in good to excellent flow properties.

Results of Hausner's ratio were ranged 1.1791±0.02 to 1.2144±0.03 (Hydrochlorothiazide tablets) and 1.2018±0.02 to 1.2615±0.01 (Losartan Potassium tablets), which shows free flow property.

Evaluation of Post-Compression Parameters

The bilayer tablets of Hydrochlorothiazide and Losartan Potassium were evaluated for various post-compression physical properties such as shape and color, thickness, weight variation, hardness, friability, wetting and water absorption ratio, disintegration time and drug content. All the batches were produced under similar conditions to avoid processing variables. Results showed that all parameters were within limits. The results of all the batches were shown in Table **4**.

1. Shape and Color of Tablets

Formulations prepared were randomly picked from each batch examined under lens for shape and in presence of light for color. Tablets showed flat, circular shape in white color.

2. Thickness Test

Thickness of the tablets was measured by dial caliper by picking randomly from all the batches. The

Table 4: Post Compression Parameters of HTZ and LP Bilayer Tablets

Formulation Code	Hardness Test (kg/cm^2) ±SD, n=3	Friability (%) ± SD, n=10	Weight Variation (%) n=10	Thickness (mm) ±SD, n=5	Disintegration Time (sec), n=6	Drug Content (%) ± SD, n=3
F1	6.73 ± 0.21	0.21± 0.04	Passes	6.00 ±0.03	248.3 ± 1.3	98.53±0.48
F2	7.51 ± 0.30	0.25 ±0.04	Passes	6.00 ±0.05	119.5 ± 2.1	99.23±0.57
F3	6.93 ± 0.50	0.22±0.08	Passes	6.05 ±0.03	78 ± 1.0	99.77±0.67
F4	6.56 ± 0.29	0.17±0.10	Passes	6.05 ±0.06	184.6 ± 2.7	99.27±0.23
F5	8.81 ± 0.51	0.19±0.04	Passes	6.05 ±0.03	96 ± 1.5	98.42±0.61
F6	6.78 ± 0.51	0.14±0.08	Passes	6.01 ±0.05	64.3 ± 1.3	99.57±0.34
F7	7.38 ± 0.47	0.22±0.10	Passes	6.06 ±0.04	162.6 ± 2.1	99.47±0.56
F8	6.83 ± 0.49	0.13 ±0.15	Passes	6.05± 0.04	84 ± 1.0	97.37±0.60
F9	6.66 ± 0.50	0.20±0.15	Passes	6.05± 0.03	36 ± 1.0	98.50±0.61

[a]Each value represents as mean ± SD.

mean thickness was (n=5) almost uniform in all the formulations and values ranged from 6.00 ±0.03 to 6.06 ±0.04 mm (bilayer tablets).

3. Weight Variation Test

The weight variation test was done to ensure the tablet contains the proper amount of drug. All the tablets passed weight variation test as the average percentage weight variation was within the limitation of USP.

4. Hardness Test

The hardness of all tablets prepared was maintained within the 2.00 to 8.00 kg/cm^2. The mean (n=3) tablet hardness were in the range 6.56 ± 0.29 to 7.51 ± 0.30 kg/cm^2 (bilayer tablets) were almost uniform.

5. Friability Test

The study results were found to be well within the approved range (<1%) in all designed formulations. The mean friability percentage (n=10) were in the range 0.13 ±0.15 to 0.25 ±0.04 (F1 to F9). F2 and F3 exhibited slightly higher friability than the others. That might be due to excess fines or improper granulation, but the values were found to be within the limit. Thus tablets possess good mechanical strength.

All batches passed the weight variation, thickness, friability and hardness, which indicated that tablet surfaces are strong enough to withstand mechanical shock or attrition during storage and transportation.

6. Uniformity of Drug Content

The drug content uniformity was performed by three trials (n=3) from each batch were analyzed spectrophotometrically. The average value and standard deviations of all the formulations were calculated. The drug content in different formulation was highly uniform and in the range of and 97.37±0.60 to 99.87±0.56% (F1 to F9). Among various formulations, F6 exhibited significantly higher Hydrochlorothiazide and Losartan Potassium content ($p < 0.05$) compared to other formulations. The results were within the acceptable range and that indicated uniformity of mixing. The cumulative percentage drug released by each tablet in the *in vitro* release studies was based on the average drug content present in the tablet.

7. Wetting Time and Water Absorption Ratio

Among the bilayer tablets (F1 to F9), wetting time and water absorption ratio for the formulation F6 prepared with HPMC K 15 (115 mg) was 35 sec and 34 respectively. From this observation HPMC K 15 provides less wetting and water absorption ratio compared with HPMC K 4 due to its higher wicking nature and fibrous structure.

8. Disintegration Time

The disintegration time of tablets (n=6) decreased with increase in the molecular weight of polymer. Disintegration time was compared among bilayer tablets (F1 to F9). The formulation F9 exhibited the fastest disintegration of 36 ± 1.0 sec with no mass left.

9. *In vitro* Drug Release Studies

In vitro dissolution studies are valuable tools to judge stability and quality of sustain release dosage forms and often used to predict the *in vivo* performance. The percentage *In vitro* drug release from

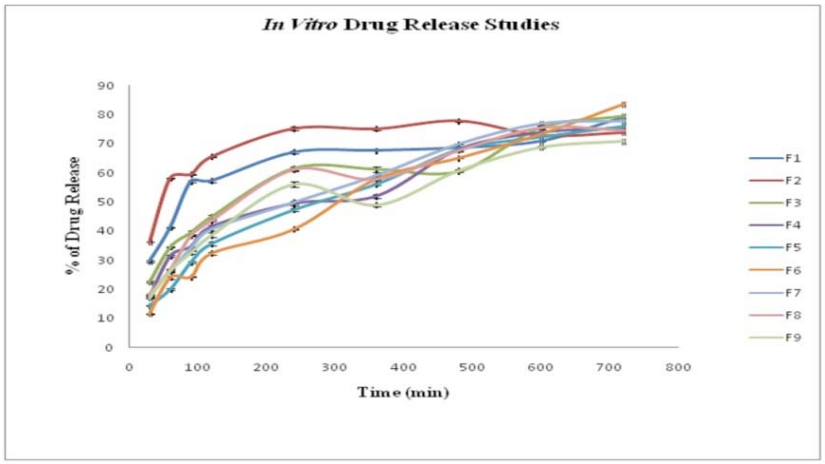

Figure 2: *In vitro* Dissolution Release Studies of Bilayer Tablets (F1 – F9).

formulations (F1-F9) was ranged from 70.860± 0.24 to 83.553± 0.22 (Figure **2**).

Based on the results (Table **5**), formulation F6 exhibited higher drug release (83.553± 0.22) which was significantly different ($p < 0.05$) from other formulations respectively. This is due to faster dissolution of the highly water soluble of drug from the superficial layers of matrix and its diffusion out of matrix which leads to the entry of dissolution media through the pores. Hence, formulation F6 was confirmed as an optimized matrix tablet. Due to highest drug release F6 was chosen for stability studies.

F6 exhibited an increased release over 12 h, due to increase in the concentration of HPMC K 15 in the formulation. A sufficient polymer concentration in the hydrophilic matrix system is required to form a uniform gel barrier around the tablet upon hydration. This barrier is expected to prevent the drug from immediate release into the dissolution medium. If the polymer concentration is low, a complete gel layer may not form resulting in a significant amount of drug being released too quickly. This effect of slower Losartan Potassium release for higher polymer level is due to the longer period of time required to reach the polymer chain

Table 5: ***In vitro*** **Drug Release Studies of HTZ and LP Bilayer Tablet**

Time	% of Drug Release, n=6								
(min)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
30	29.611± 0.12	36.224± 0.08	22.504± 0.12	17.174± 0.09	14.312± 0.08	11.548± 0.15	17.964± 0.11	18.162± 0.17	17.076± 0.09
60	41.225± 0.24	58.217± 0.13	34.498± 0.26	31.327± 0.23	19.871± 0.12	24.011± 0.26	26.196± 0.23	26.690± 0.26	26.490± 0.18
90	57.011± 0.21	59.432± 0.19	39.509± 0.22	34.555± 0.30	29.194± 0.28	24.163± 0.41	34.052± 0.36	38.397± 0.23	32.570± 0.36
120	57.236± 0.31	65.486± 0.23	44.927± 0.56	41.442± 0.15	35.575± 0.39	32.310± 0.18	40.642± 0.67	43.614± 0.19	38.560± 0.49
240	67.035± 0.44	75.106± 0.26	61.510± 0.42	49.430± 0.43	47.301± 0.61	40.574± 0.53	49.813± 0.55	61.182± 0.41	56.024± 0.74
360	67.612± 0.37	75.015± 0.65	61.196± 0.68	51.918± 0.68	56.163± 0.54	57.858± 0.29	59.025± 0.46	57.840± 0.63	48.957± 0.85
480	68.552± 0.10	77.748± 0.47	60.444± 0.87	68.024± 0.74	68.033± 0.33	65.035± 0.47	69.816± 0.27	67.741± 0.28	60.812± 0.48
600	70.876± 0.51	72.783± 0.30	75.482± 0.35	73.801± 0.35	72.428± 0.84	73.285± 0.17	76.782± 0.19	75.590± 0.32	68.744± 0.43
720	79.075± 0.28	73.756± 0.18	79.363± 0.21	75.231± 0.17	76.116± 0.26	83.553± 0.22	77.610± 0.20	74.642± 0.62	70.860± 0.24

[a]Each value represents as mean ± SD of six (n = 6) determinations.

Table 6: Kinetic Data of HTZ and LP Bilayer Tablets

Formulation Code	Regression Value	Zero Order	First Order	Higuchi	Korsmayer Peppas
F1	r^2	0.596	0.822	0.819	0.930
F2	r^2	0.541	0.856	0.816	0.973
F3	r^2	0.784	0.925	0.935	0.885
F4	r^2	0.853	0.987	0.965	0.893
F5	r^2	0.906	0.987	0.988	0.920
F6	r^2	0.917	0.990	0.989	0.903
F7	r^2	0.879	0.981	0.986	0.913
F8	r^2	0.797	0.919	0.932	0.945
F9	r^2	0.821	0.927	0.940	0.916

disentanglement concentration at the tablet surface, which in turn equates to greater resistance of the matrix to surface erosion.

10. Drug Release Kinetics

To ascertain the mechanism of drug release, *in-vitro* release data were fitted into various kinetic models such as First order, zero order, Higuchi and Korsmayer Peppas. The *In vitro* profile of bilayer tablets could be best expressed by first order as the plot showed highest linearity (R^2 = 0.990) as compared to zero-order plots (Table **6**).

Hence the order of release for formulations followed first order kinetics. Release of the drug from the bilayer tablets containing hydrophilic polymers generally involves factors of diffusion and release was governed by Fickian transport and it indicates the delivery of drug from the tablet through diffusion dominated mechanism.

One-way ANOVA Post-Hoc analysis (Duncan and Turkey) was used for regression analysis of drug release data derived from model equations with the aid of SPSS package, version 12.0. Statistical significance was set at $p < 0.05$. Taking into account the number of independent variables, R^2 was used to determine how well a regression model describes the release data. Mean ± SD for drug loading tablets was calculated by using Microsoft Office Excel 2003.

11. Stability Studies

According to ICH and WHO guidelines, after 3 months accelerated stability study at 40°C ± 2°C and 75% RH ± 5% (Yorco Scientific Industries, India), optimized formulation (F6) showed negligible change over time for parameters like appearance, drug content and dissolution. No significant difference in the drug content between initial and formulation stored at 40°C ± 2°C and 75% RH ± 5% for 3 months.

Table 7: *In Vitro* Dissolution of F6 in Stability Study

Time (min)	% LP released in F6 (before storage)[b]	% LP released in F6 (after storage)[a, b]
0	0	0
30	11.548± 0.15	11.142 ± 0.22
60	24.011± 0.26	22.458 ± 0.30
90	24.163± 0.41	22. 704 ± 0.15
120	32.310± 0.18	29. 988 ± 0.24
240	40.574± 0.53	38. 052 ± 0.78
360	57.858± 0.29	56. 475 ± 0.85
480	65.035± 0.47	63. 216 ± 0.66
600	73.285± 0.17	71. 871 ± 0. 73
720	83.553± 0.22	82. 594 ± 0.32

[a]Storage at 40°C ± 2°C /75% RH ±5% for three months.
[b]Mean ± SD, n=3.

The protocols of stability studies were in compliance with the guidelines in the WHO document for stability testing of products intended for the global market.

The statistical analysis of the parameter dissolution efficiency [25] of dissolution data (Table **7**) after storage at 40°C ± 2°C and 75% RH ± 5% for 3 months as showed in Figure **3** depicts no significant change by Student's t -test indicating that F6 could provide a minimum shelf life of 2 years.

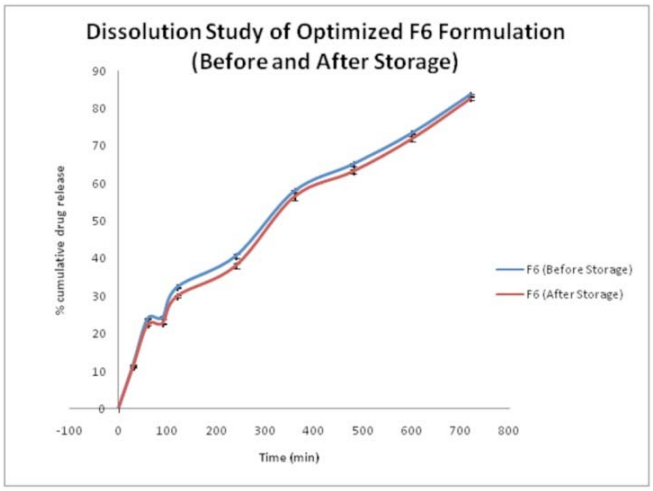

Figure 3: *In-Vitro* Dissolution Study of Optimized Bilayer Tablet (F6) (Before and After Storage)

4. CONCLUSION

Losartan readily dissolves in purified water or very well released at a relatively high pH (pH= 6.8) but it is very slowly released at a low pH (pH =1.2). So an instant coating layer of Hydrochlorothiazide was provided to overcome the acidic medium of stomach (pH of stomach is 1.2 for complete dissolution). Hence we have developed a bilayer tablet with an optimized instant release layer of HTZ prepared by microcrystalline cellulose and sodium starch glycolate as superdisintegrant and a controlled release matrix tablet of LP prepared by HPMC K 15. Prepared bilayer tablets were film coated in a conventional coating pan. The tablet exhibits satisfactory pre- and post-compression parameters. Our data represents that the formulated bilayer tablet provides an excellent drug release data (optimized F6), hence it concluded that bilayer tablets showed an immediate release effect to provide the loading dose of the drug, followed by sustained release for 24 h, indicating a promising potential of the Hydrochlorothiazide and Losartan bi-layer tablet as an alternative to the conventional dosage form for the better treatment of hypertension.

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DECLARATION OF CONFLICT OF INTEREST

The authors report no conflict of financial interest. The alone are responsible for the content and writing of the paper.

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