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Design, optimization and *in vitro - in vivo* evaluation of bilayer floating tablets of diltiazem HCl by using xanthan gum & guar gum in healthy human volunteers

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ABSTRACT

The objective of the present research work was to develop the bilayer floating tablets of Diltiazem HCl using superdisintigrants for the immediate release layer as loading dose for immediate action and HPMC K-4M, K-15M and K-100M polymers for the sustained release layer to deliver the drug at sustained or controlled manner in GIT and consequently in to systemic circulation by direct compression method. Bilayer floating tablets were punched using optimized immediate release layer and floating layer as sustained release layer. Tablets were evaluated for compatibility study, buoyancy lag time, total floating time, swelling study, in-vitro disintegration and in-vitro dissolution studies. Formulations were found uniform with respect to thickness (5.00 to 5.08 mm) and hardness (4.7 to 5.2 kg/cm^2). The friability (0.32 to 0.44%), weight variation (498 to 502 mg) and Drug content (96.34 to 99.49%) of different batch of tablets were found within prescribed limits. Formulation F7 selected as best formulation, shown buoyancy lag time of 41 sec, total floating time of more than 12 hrs and drug release of 98.12% in a period of 12 hrs. Optimized formulation (F7) followed diffusion controlled first order kinetics and fickian transport of the drug. FTIR and DSC studies revealed the absence of any chemical interaction between drug and polymers used. Approximately 37% of drug was released from optimized formulation within 1 hr and total of 98.12% of drug was released without dose dumping for extended period of 12 hr. The optimized formulation (F7) showed no significant change in physical appearance, drug content, floatability or in vitro dissolution pattern after storage at 40^{0}C/75% RH for three months. It was determined by the radiographs that floating tablets prepared by adding barium sulphate stayed in the stomach for 6.5 h. The comparison of in vivo bioavailability studies of optimized formulation F7 of bilayer floating tablet and reference formulation (DILZEM SR) in human healthy volunteers confirmed that the increase in diltiazem hydrochloride systemic exposure early after administration for immediate action and remaining drug was released in sustained manner from bilayer floating tablet by increasing the gastric residence time.

Key words: Diltiazem HCl, bilayer floating tablets, bioavailability, stability studies.

INTRODUCTION

Oral route is considered as the most promising route for drug delivery **[1]**. Development of oral controlled release systems has been a challenge to formulation scientists because of the difficulty in localizing the system in target areas of the gastrointestinal tract **[2]**. The real challenge in the development of an oral controlled-release drug

delivery system is not just to sustain the drug release but also to prolong the presence of the dosage form within the gastrointestinal tract (GIT) until all the drug is completely released at the desired period of time **[3]**. One of the novel approaches in the area of oral sustained release drug delivery is gastro retentive drug delivery system (GRDDS) **[4].** Gastroretentive drug delivery systems (GRDDS) can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability **[5].** Extended-release dosage forms with prolonged residence time in the stomach are highly desirable for drugs with i) narrow absorption windows, ii) stability problems in the intestinal or colonic environment, iii) local action in the stomach, and iv) low solubility at high pH values **[6].**

The biphasic system is used mostly when maximum relief needs to be achieved quickly and it is followed by a sustained release phase. It also avoids repeated administration of drug. Coronary vasodilator, antihypertensive, antihistaminic, analgesic, antipyretics and antiallergenic agents are mainly used for this system. The biphasic system may contain one or two drugs for immediate release and sustained release layer **[7].**

Diltiazem HCl is calcium channel blocker and used in the treatment of angina pectoris and hypertension. It is mainly absorbed from stomach and upper part of intestinal track, it has low bioavailability of 40%. It has biological half-life 3-4 h. More over with 60mg sustained dosage form time required to reach peak plasma concentration is about 3.9 h. So an administration of the loading dose also becomes beneficial to reach peak plasma concentration rapidly **[8].** Diltiazem undergoes an extensive biotransformation, mainly through the cytochrome p-450 CYP3A **[9],** which results in less than 4% of its oral dose being excreted unchanged in urine **[10].**

The present work concern with the formulation and evaluation of bilayer floating tablets of diltiazem hydrochloride having immediate and floating sustain release layer. These tablets showed the biphasic drug release means an immediate release layer releases the drug immediately as loading dose. Floating sustained release layer releases the drug for prolonged period of time as maintenance dose.

MATERIALS AND METHODS

2.1 Materials:

Dilzem SR 90mg tablet was purchased from Torrent Pharmaceuticals Limited, Ahmadabad. Diltiazem HCl was received as a gift sample from Dr. Reddy's Laboratories Limited, (Hyderabad, India), Hydroxy propyl methyl cellulose K-4M, K-15M and K-100M were obtained from Colorcon Asia Private Limited, India, Xanthan gum, Guar gum, Cross Povidone, Croscarmellose sodium, Sodium starch glycolate and Pvpk30 were gifted from MSN Labs Ltd, Hyderabad. Sodium carboxy methyl cellulose was obtained from Rubicon labs, Mumbai. All other excipients and chemicals used were of analytical grade.

2.2 Preparation of bilayer floating tablets:

2.1.1 Formulation of immediate release (IR) layer:

The immediate release granules were prepared by blending the drug with different concentration of super disintegrants like Sodium starch glycolate, Crosspovidone, Crosscarmellose sodium, PVP K30 and other excipients like microcrystalline cellulose and lactose. The powder blend was lubricated with magnesium stearate and talc. For preliminary studies to optimize the IR formulations, a weighed quantity of above lubricated drug mixture blend was fed manually into the die and directly compressed using 8 mm flat faced punch of 8 station Cadmach compression machine to get IR layer. Eight formulation batches with different super disintegrants were made in order to achieve desired disintegration time and drug release. The Composition of Diltiazem HCl immediate release tablets were shown in **Table 1.**

2.2.2 Formulation of floating sustained release (SR) layer:

The floating sustained release granules were prepared by direct compression technique. Required quantity of diltiazem HCl and polymers like HPMC K4M, HPMC K15M, HPMC K100M, Xanthan gum, and Guar gum, SCMC, alkalizing agent sodium bicarbonate, and acidifying agent citric acid were weighed and passed through sieve with mesh #40 and were mixed homogeneously in a poly-bag for about 5-10 min and was taken in a mort. The powder mass was passed through mesh #14. Finally the powder was lubricated with magnesium stearate and talc.

Table 1: Composition of Diltiazem HCl Immediate release layer in Bilayer tablets

Ingredients (mg)	IR1	IR2	IR3	IR4	IR5	IR6	IR7	IR8
Diltiazem HCl	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
PVP K30	7.5	4	5	7.5	5	4	5	3.5
Crosspovidone	10	12	15	10	15	12	15	12.5
Croscarmellose sodium	-	-	15	-	15	-	-	-
SSG	-	-	-	-	-	-	15	-
MCC	-	-	-	100	89.5	108.5	-	-
Lactose	100	108.5	89.5	-	-	-	-	108.5
DCP	-	-	-	-	-	-	89.5	-
Mg. Stearate	5	1.5	1.5	5	1.5	1.5	1.5	1.5
Talc	5	1.5	1.5	5	1.5	1.5	1.5	1.5
Total wt.	150	150	150	150	150	150	150	150

2.2.3 Formulation of floating bilayer tablets:
Bilayer floating tablets were prepared by direct compression method using 12 mm flat faced punch of 8 station Cadmach compression machine. First the powder of floating sustained release layer was poured in the die cavity and the powder was compressed. After the compression, the upper punch was then lifted and the immediate release powder of drug were poured in the die, containing initially compressed sustained release layer and compressed to form bilayer tablet with hardness of 5 kg/cm^2. The hardness was kept constant for all formulations and was measured using Pfizer hardness tester. The composition of different formulations of Diltiazem HCl floating bilayer tablets are depicted in the **Table 2.**

Table 2: Composition of Diltiazem HCl floating bilayer tablets

Ingredients (mg)	F1	F2	F3	F4	F5	F6	F7
IR8	**150**	**150**	**150**	**150**	**150**	**150**	**150**
Diltiazem HCl	67.5	67.5	67.5	67.5	67.5	67.5	67.5
HPMC K4M	130	-	-	-	-	130	-
HPMC K15M	-	--	140	140	-	-	-
HPMC K100M	-	87.5		-	105	-	150
Xanthan gum	35	35	35	-	17.5	17.5	10.5
Guar gum	-	--	-	35	17.5	17.5	10.5
$NaHCO_3$	70	70	70	70	70	70	70
Citric acid	-	---	-	-	--	--	25
SCMC	20	20	20	20	20	20	-
Lactose	---	60	7.5	7.5	42.5		9.5
MCC	17.5	-	-	-	-	17.5	
Mg.Sterate	5	5	5	5	5	5	3.5
Talc	5	5	5	5	5	5	3.5
Total Weight.	500	500	500	500	500	500	500

2.3 PREFORMULATION STUDIES:
2.3.1 Angle of Repose: The angle of repose was determined by using fixed funnel method. The physical mixtures of drug with different excipients were prepared and the accurately weighed drug powder or its physical mixture was taken in a funnel. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the drug powder. The powder was allowed to flow through the funnel freely onto surface. The angle of repose was calculated using the following equation **[11].**

$\theta = \tan^{-1}(h/r)$

Where, h and r are the height and radius of the powder cone respectively.

2.3.2 Bulk Density: Both loose bulk density (LBD) and tapped density (TBD) were determined were calculated using the following formulas **[12]**.

LBD = Powder weight/volume of the packing

TBD = Powder weight /tapped volume of the packing

2.3.3 Compressibility Index: The compressibility index of the granules was determined by Carr's compressibility index **[13].**

Carr's index (%) = [(TBD – LBD)/TBD] × 100.

2.3.4 Hausner's ratio: Hausner's ratio is an indirect index of ease of measuring the powder flow. It was calculated by the following formula.

Hausner's ratio = Tapped density/Bulk density.

2.4 Evaluation of drug bilayer tablets:

2.4.1 General appearance:

Morphological characters like shape and texture was determined visually.

2.4.2 Thickness:

The thickness of the prepared tablets was tested using verniercalipers. The test was done in triplicate and average was determined.

2.4.3 Hardness:

Hardness of prepared tablets was determined using Monsanto hardness tester and measured in terms of kg/cm^2.

2.4.4 Weight variation:

The weight variation test was performed as per the I.P. guidelines. Twenty randomly taken tablets were weighed together and the average weight was determined. Each tablet was then weighed individually and deviation from average weight was calculated.

2.4.5 Friability:

A sample of twenty randomly selected tablets were accurately weighed and placed in a Roche friabilator. The friabilator was operated for 4 min at a speed of 25 rpm. The tablets were removed from the friabilator, de-dusted and reweighed. The percent loss in weight due to abrasion and impact was calculated as,

%Friability= (Loss in weight/ Initial weight) x 100.

2.4.6 Drug content:

Ten tablets for each batch was taken and triturated. Powder equivalent to 100mg of drug was weighed and was transferred to breaker and 0.1N HCl was added and it was then shaken for 5 minutes and finally 0.1N HCl was added to make the volume up to 100ml and solution was then sonicated for 15 minutes and filtered through Whatman filter paper. Finally a solution was diluted suitably and the absorbance of resultant solution was measured to determine the drug content spectrophotometrically at 275nm using UV/Visible spectrophotometer Shimadzu 1800 against 0.1N HCl blank.

2.4.7 Swelling studies:

The extent of swelling was measured in terms of % of weight gained by the tablet. One tablet from each formulation was weighed and kept in petri dish containing 50 ml of 0.1N HCl buffer solution. At the end of specified time intervals tablets were withdrawn from petri dish and excess buffer blotted with tissue paper and weighed. The % of weight gained by the tablet was calculated by using the following formula:

Swelling Index (%) = M_t-M_0/M_0 X 100 **[14]**

2.4.8 Buoyancy lag time determination & total floating time:

The in vitro buoyancy was determined by the floating lag time. The tablet was placed in a 250 ml beaker containing 0.1N HCl. The time required for the tablet to rise to the surface for floating was determined as the buoyancy lag time and further total floating time of all tablets was determined by visual observation **[15]**.

2.4.9 In vitro disintegration time of immediate release tablets:
The disintegration time for all immediate release formulations was carried out using tablet disintegration test apparatus. Six tablets were placed individually in each tube of disintegration test apparatus and discs were placed. The medium, water was maintained at a temperature of 37° ± 2°C and time taken for the entire tablet to disintegrate completely was noted **[16]**.

2.4.10 In vitro dissolution studies:
In vitro drug release studies for the prepared immediate release tablets were conducted for a period of 10 min and bilayer floating tablets were conducted for a period of 24 hrs using USP XXIV type-II (Paddle) dissolution apparatus at 37±0.5oC at 50 rpm using 900 ml of 0.1N HCl as dissolution medium. At predetermined interval of time, 5 ml of sample was withdrawn from the dissolution medium and replaced with fresh medium to maintain the sink condition. After filtration and appropriate dilution, the samples were analyzed for diltiazem HCl by UV/Visible spectrophotometer Shimadzu 1800 at 238 nm.

2.5 Kinetic modeling of drug release
The dissolution profiles of all the batches were fitted to zero order, first order, Higuchi and Peppas equations **[17].** (Equation 1-4 respectively).

$M_t = M_0 + k_0t$ (1)

$\ln M_t = \ln M_0 + k_1t$ (2)

$M_t = M_0 - k_Ht^{1/2}$ (3)

$M_t/M_\alpha = K_t^n$ (4)

In these equations, M_t is the cumulative amount of drug released at any specified time (t) and M0 is the dose of the drug incorporated in the delivery system and M_t/M_α is a fraction of drug released at time (t). k_0, k_1, k_H and K are rate constants for zero order, first order, Higuchi and Korsmeyer model respectively, n is the release exponent. The n value is used to characterize different release mechanisms as given in table 1 for cylindrical shaped matrices **[18].**

The dissolution data were also fitted according to the well-known exponential Zero Order equation, which is often used to describe drug release behavior from polymeric systems.

The best fit with higher correlation ($r^2 > 98$) was found with Higuchi's equation for all the formulations.

2.6 Drug excipient compatibility studies:
2.6.1 Fourier Transform Infrared Spectroscopy (FTIR)
FTIR spectra for pure drug, physical mixture and optimized formulations were recorded using a Fourier transform Infrared spectrophotometer. The analysis was carried out in Shimadzu-IR Affinity 1 Spectrophotometer. The IR spectrum of the samples was prepared using KBr (spectroscopic grade) disks by means of hydraulic pellet press at pressure of seven to ten tons.

2.6.2 Differential Scanning Calorimetry (DSC)
Differential Scanning Calorimetry (DSC) studies were carried out using DSC 60, having TA60 software, Shimadzu, Japan. Accurately weighed samples were placed on aluminium plate, sealed with aluminium lids and heated at a constant rate of 5ºC /min, over a temperature range of 0 to 250ºC.

2.7 Stability studies:
The stability of Diltiazem HCl bilayer floating tablets to assess their stability with respect to their physical appearance, drug content and release characteristics after storing at 25°C/60% RH and 40°C/75% RH in properly closed HDPE bottles along with 1 g desiccant for 3 months **[19].**

2.8 Radiographic studies:
The study protocol was approved by the Institutional Human Ethics Committee (IHEC), bearing No. **IHEC/VGOPC/2013/017**, Vaagdevi group of pharmacy colleges, Warangal, India.

2.8.1 Determination of In-vivo gastric residence time

To make the best achieved X-ray opaque, 100 mg of the drug was replaced with barium sulfate based on the density and all other ingredients were kept constant. This amount was determined experimentally to allow X-ray visibility but not to hinder tablet buoyancy.

After overnight fasting, the volunteers were fed with a low calorie food. After half an hour, a barium sulfate-labeled tablet was given to every subject with 200 mL of water. The volunteers were asked to take 200 mL water after every 1 hour. At different time intervals (0, 0.5, 1, 2, 4 and 6 h post-administration of tablets), the volunteers were exposed to abdominal X-ray imaging in standing position.

A radiograph was made just before the administration of the tablet, at zero time, to ensure the absence of radio-opaque material in the stomach. The distance between the source of X-rays and the subject was kept constant for all images. Thus, the observation of the floating tablet movements could be easily noticed **[20].**The mean gastric retention period was estimated.

2.9 In-vivo bioavailability studies:

2.9.1 Preparation of Plasma Samples for HPLC Analysis

Human plasma (0.5 ml) was prepared for chromatography by precipitating proteins with 2.5 ml of ice-cold absolute ethanol for each 0.5 ml of plasma. After centrifugation the ethanol was transferred into a clean tube. The precipitate was resuspended with 1 ml of acetonitrile by vortexing for 1 min. After centrifugation (5000 – 6000 rpm for 10 min), the acetonitrile was added to the ethanol and the organic mixture was taken to near dryness by a steam of nitrogen at room temperature. Samples were reconstituted in 200 µ1 of 70 % of acetonitrile and 30% water was injected for HPLC analysis. The chromatographic conditions, standard chromatogram and standard calibration curves were shown in **Table 3, Figure 1 & 2** respectively.

Table 3: Chromatographic conditions:

Column	C18
Mobile Phase	Phosphate buffer (P^H 2.5): Acetonitrile(40:60)
Flow rate	1ml/min
Injection volume	20µl
Retention time	3.710

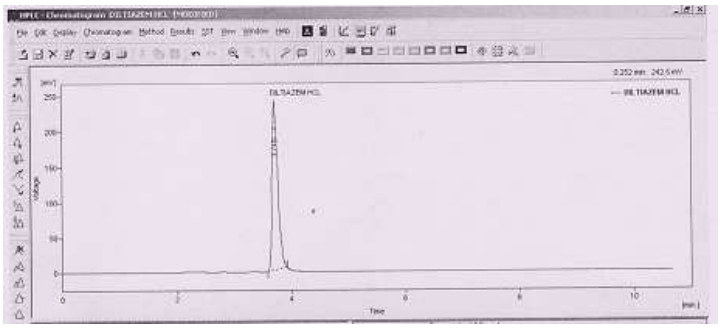

Figure 1: Standard chromatogram of Diltiazem HCL

2.10 In vivo study protocol:

Six healthy male subjects with a mean age of 28.83±3.60 years (ranging from 24 to 34 years), mean weight 69.33±7.61 Kg (ranging from 61 to 79 Kg) and a mean height of 173.17 ± 10.46 cm (ranging from 157 to 182 cm) participated in this study. Informed and signed consent and approval of the Human Ethical Committee were obtained.The volunteers were judged healthy on the basis of their previous medical history, physical examination and routine laboratory tests. None of the subjects used alcohol or tobacco. All subjects were free from drugs 15 days before and during the study. The subjects were fasted over night at least 10 hrs prior to dose. After collecting the zero hour blood sample (blank). A standardized high fat-breakfast approximately 900 K Cal was given in the morning half-an-hour before administration. Formulated Diltiazem HCl and commercial formulation was administered with 200 ml of water. All the subjects were given a glass of water for every 2 hrs (approximately 200 ml). Standardized lunch, snacks and dinner was provided to all the subjects respectively at 4, 8 and 12 hrs after the

administration of formulations. Blood sample of 3 ml was collected by cannulation method using pre-labelled 5 ml vacuutainers containing dipotassium ethylenediamine tetra acetic acid (K2EDTA). Samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12 and 24 hrs. Plasma was separated from the blood samples by centrifugation at 4000 rpm for 10 minutes. All the samples were stored in properly labelled tubes at –20º C prior to analysis. The plasma concentration of Diltiazem HCl from the experimental formulations was measured by HPLC method as described in **Table 3**.

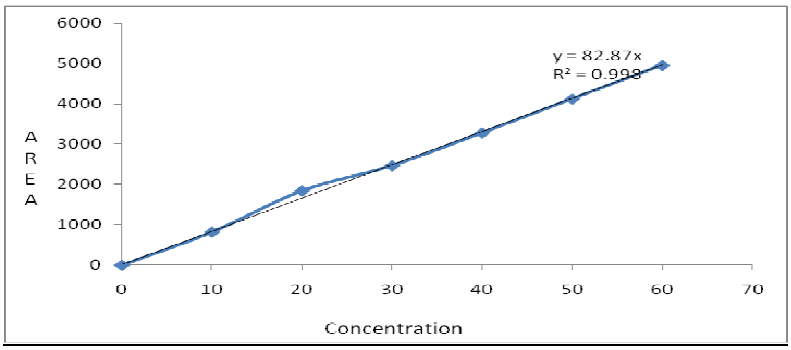

Figure 2: Standard calibration curve of Diltiazem HCL in Human plasma by HPLC

2.11 Pharmacokinetic analysis:
The pharmacokinetic parameters were determined from individual Diltiazem HCl plasma concentrations by the non compartmental approach. These parameters were: maximum plasma concentration (C_{max}), time to attain C_{max} i.e., T_{max}), area under plasma concentration–time curve from zero to the last sampling time (AUC_{0-t}), area under plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) and mean residence time (*MRT*). AUC_{0-t} was calculated by the linear trapezoidal rule and *AUC*0-• from:

$AUC_{0-t} + C_{last}/ \lambda_z$

Where C_{last} is the last quantifiable concentration and λ*z* is the apparent terminal rate constant. This constant (λ_z) was calculated by log-linear regression of the terminal segment of the plasmatic concentration versus time curve. The apparent terminal half-life ($t_{1/2}$) was calculated from:

ln_2/ λ_z

The MRT was calculated according to the following equation:

$$MRT = \frac{AUMC_{0-\infty}}{AUC_{0-\infty}}$$

Where
$AUMC_{0-\infty}$ is the area under first moment calculated by the following equation:

$$AUMC = \sum_{i=1}^{n}\left[\frac{t_i C_i + t_{i+1} C_{i+1}}{2}(t_{i+1} - t_i)\right] + \frac{C_n}{\lambda_z^2} + \frac{t_n C_n}{\lambda_z}$$

RESULTS AND DISCUSSION

3.1 Micromeritic properties of the powder blend of immediate release and sustained release floating tablets
The powdered blends of different formulations of immediate release tablets and sustained release floating tablets were evaluated for angle of repose, loose density (LBD), tapped density (TBD) and compressibility index. The results of immediate release tablets and SR floating tablets are summarized in **Table 4**. The results of SR floating tablets of LBD and TBD ranged from 0.688 to 0.812 and 0.712 to 0.872, respectively. The range of angle of repose and compressibility index was found to be 29. 23 to 34.43 and 6.2 to 7.5 respectively. The results of angle of repose

(<35) and lower compressibility index values up to 8 % indicate good flow properties of the powdered blend. The optimized formulation of immediate release tablet (IR8) prepared by using the superdisintegrants like crospovidone exhibited the LBD, TBD, angle of repose, compressibility index and Hausner's ratio of 0.692, 0.722, 31.29, 7.1 and 1.6244 respectively, which shows good flow properties of the powdered blend.

Table 4: Micromeritic properties of the powder blend of immediate release and sustained release floating tablets

Formulation Code	Angle of Repose (Degree) *	LBD (g/cm^3) *	TBD (g/cm^3) *	Compressibility Index (%)*	Hausner ratio*
IR8	31.29 ± 0.29	0.692 ± 0.42	0.722 ± 0.28	7.1 ± 0.88	1.6244
F1	32.53 ± 0.38	0.812 ± 0.18	0.842 ± 0.76	6.8 ± 0.12	1.3695
F2	34.43 ± 0.26	0.734 ± 0.28	0.732 ± 0.28	6.9 ± 0.22	1.5378
F3	31.46 ± 0.28	0.696 ± 0.38	0.842 ± 0.64	7.1 ± 0.66	1.4512
F4	32.53 ± 0.18	0.792 ± 0.56	0.712 ± 0.18	6.7 ± 0.68	1.3343
F5	32.83 ± 0.48	0.688 ± 0.42	0.742 ± 0.26	6.8 ± 0.42	1.4612
F6	33.41 ± 0.38	0.746 ± 0.45	0.872 ± 0.36	6.2 ± 0.32	1.1600
F7	29.23 ± 0.34	0.704 ± 0.24	0.854 ± 0.66	7.5 ± 0.18	1.7423

**Mean ±S.D (n=3)*

3.2 Physico-chemical parameters of Diltiazem HCl bilayer floating tablets:

The prepared tablets were evaluated for different physico-chemical properties and the results are summarized in **Table 5**. The tablets were white, circular in shape and were found to be uniform with respect to weight variation (498 to 501mg) and hardness (4.7 to 5.2 kg/cm^2). The thickness (5.00 to 5.10mm) and friability (0.32 to 0.44%) of different batch of tablets were found within acceptable range. Content uniformity of all the formulations was found to be in the range of 96.34 to 99.49 %, where the distribution of drug in all the formulations was uniform.

Table 5: Physico-chemical evaluation of Diltiazem HCl bilayer floating tablets

Formulation code	Weight variation (mg) ±SD (n=20)	Hardness (kg/cm^2) ±SD (n=3)	Thickness (mm) ±SD (n=3)	Friability (%) ±SD (n=3)	Content Uniformity (%) ±SD (n=10)
F1	499±0.5	4.9±0.13	5.00±0.01	0.32±0.01	96.34±0.18
F2	498±0.4	4.7±0.16	5.06±0.02	0.33±0.02	96.40±0.32
F3	499±0.8	5.2±0.17	5.02±0.07	0.41±0.08	98.45±0.54
F4	500±0.4	5.1±0.16	5.05±0.03	0.42±0.04	97.85±0.15
F5	501±0.6	5.0±0.04	5.10±0.07	0.44±0.02	98.10±0.66
F6	498±0.7	5.2±0.14	5.02±0.05	0.44±0.06	98.99±0.35
F7	500±0.6	5.0±0.16	5.08±0.04	0.43±0.02	99.49±0.54

3.3 Swelling study of Diltiazem HCl bilayer floating tablets:

The purpose of swelling study is to determine the water uptake capability of the polymer. Swelling study was performed on all the batches of floating tablet for 24 hours. All the bilayer floating tablets swelled but remained intact without breaking throughout the period of swelling in 0.1 N HCl. The order of swelling index observed with different polymers was HPMC K100 M > HPMC K15M > HPMC K4M. The results are summarized in **Table 6.** From the results it was concluded that increased concentration of HPMC K 100M in the formulations increases the swelling indices.

Table 6: Swelling Index of Diltiazem HCl bilayer floating tablets

Time (hrs)	Swelling Index (%)*						
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
0	0	0	0	0	0	0	0
1	44.36	42.29	42.35	52.31	56.32	58.35	61.38
2	62.53	72.52	61.12	77.56	71.52	82.32	85.13
3	75.08	81.08	74.09	86.03	93.08	97.07	98.06
4	82.61	92.61	85.66	112.32	116.61	109.40	112.65
6	96.13	114.12	97.14	128.54	127.12	124.15	134.16
8	106.31	126.32	105.12	138.11	143.32	140.54	163.31
10	122.72	142.46	124.75	152.63	160.71	159.72	178.82
12	147.30	168.60	146.29	182.28	183.20	180.24	194.23

**Mean ±S.D (n=3)*

3.4 Buoyancy lag time determination & total floating period:

Formulations were evaluated for in vitro buoyancy lag time and all formulations. The time required for the tablet to rise to the surface (when the tablets were placed in a beaker containing 0.1 N HCl) for floating was described as the buoyancy lag time. $NaHCO_3$ induces CO_2 generation in the presence of HCl. The optimized concentration of $NaHCO_3$ was found to be 14% of total tablet weight and it was maintained constant in all the floating tablets. All the formulations had buoyancy lag time in the range of 26 to 74 sec. The total floating time was found to be more than 12 hrs, which indicates a stable gel layer formation by all polymers and that $NaHCO_3$ remains for a longer time. The results of floating lag time and total floating time was depicted in **Table 7 & Figure 3.**

Table 7: Buoyancy lag time and total floating period of Diltiazem HCl bilayer floating tablets

Formulation Code	Floating lag time(sec)*	Total Floating period (hrs)*
F1	42±1.4	>12hrs
F2	64±2.1	>12hrs
F3	38±1.8	>12hrs
F4	52±2.5	>12hrs
F5	74±1.1	>12hrs
F6	52±3.5	>12hrs
F7	26±2.6	>12hrs

**Mean ±S.D (n=3)*

After 5 sec

After 12 hour

After 26 sec

Figure 3: Pictorial presentation of in vitro floating behavior of Diltiazem HCl tablet (F7) at different time intervals

Table 8: In vitro disintegration time of immediate release tablets

Formulation code	Disintegration time (Sec)*
IR1	50
IR2	52
IR3	51
IR4	54
IR5	47
IR6	46
IR7	55
IR8	41

**Mean ±S.D (n=3)*

3.5 In vitro disintegration time of immediate release tablets:

Disintegration time is very much important parameter for immediate release tablets which is desired to be less than 60 sec. In the present investigation 8 formulations were made with different superdisintegrants like crospovidone,

croscarmellose sodium and SSG. The optimized formulation (IR8) prepared by using the superdisintegrants cross povidone disintegrated in lesser time i.e 41 sec compare with other formulations shown in **Table 8**.

3.6 In vitro release profile of immediate release tablets:

The immediate release tablets of 8 formulations (IR1-IR8) prepared by using different superdisintegrants (**Table 9**) alone and in combination showed drug release of about 96, 98, 97.5, 94.8 and 97 for IR1, IR2, IR3, IR5 and IR7 respectively at the end of 15 min. The formulations IR4 and IR6 showed the drug release of 99.5 and 100.2 at 20min and the drug release from IR8 was found to be 99.2 at the end of 10 min. The results are shown in **Table 9 & Figure 4 & 5**. Among all the formulations IR8 consisting of superdisintegrant crosspovidone considered as optimized formulation. IR8 was further used in the preparation of all bilayer floating tablets.

Table 9: In vitro release profile of immediate release tablets

Time (min)	% Cumulative drug released*							
	IR1	IR2	IR3	IR4	IR5	IR6	IR7	IR8
0	0	0	0	0	0	0	0	0
2	25	40	35	12	34	12	40	30
5	48	62	68	21	50	28	68	55
10	86	84	90	38	85	38	88.5	99.2
15	96	98	97.5	49	94.8	58	97	
20				86		88		
25				99.5		100.2		

**Mean ±S.D (n=3)*

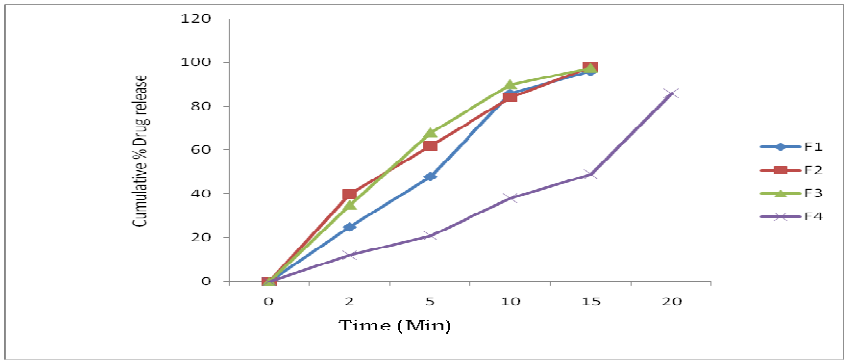

Figure 4: Comparative % drug release profiles of Diltiazem HCl IR formulations (IR1- IR4)

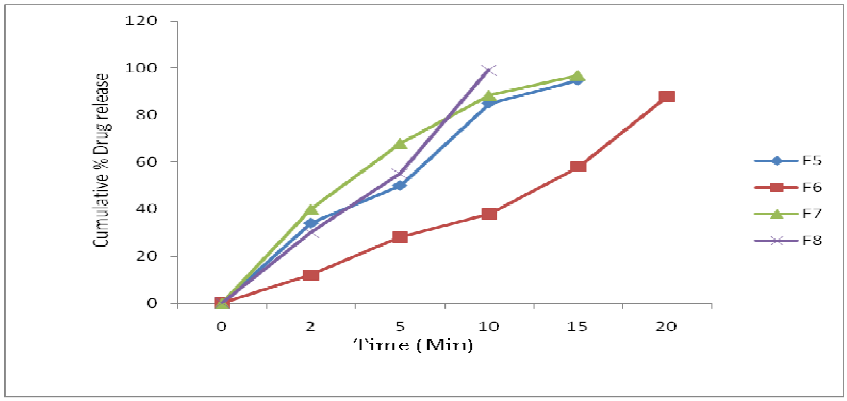

Figure 5: Comparative % drug release profiles of Diltiazem HCl IR formulations (IR5- IR8)

3.7 In vitro drug release profile of Diltiazem HCl bilayer floating tablets:

All the sustained release formulations (F1-F7) are prepared with different grades of HPMC like HPMC K4 M, HPMC K15M and HPMC K100M, natural polymers like Xanthan gum and guar gum and sodium CMC showed drug release between 98.50 to 99.25% at the end of 10-12hrs respectively. The effect of polymer on drug release

was studied by observing the release profile of sustained release formulation. In case of formulation F7 containing the polymer HPMC K100M in higher concentration, which results in strong gel strength that retards the drug release upto 12 hrs. The drug release of other formulations using HPMC K4M, HPMC K15M and sodium CMC showed drug release drastically retarded 10hrs from the prepared bilayer floating tablets. The results of drug release profiles are summarized in **Table 10 and Figure 6.**

Table 10: In vitro % drug release profile of Diltiazem HCl bilayer floating tablets and reference formulation

Time (hrs)	% Cumulative drug released*							
	F1	F2	F3	F4	F5	F6	F7	DILZEM SR
0	0	0	0	0	0	0	0	18
0.5	22.45	28.15	22.50	25.05	29.78	25.76	25.1	27
1	39.66	48.46	55.42	38.74	34.97	35.46	36.5	42
2	57.52	55.55	69.40	55.40	60.69	60.55	50.1	64
4	69.52	71.70	78.64	75.48	74.49	76.73	70.16	88
8	74.43	99.41	85.85	99.99	95.45	88.69	78.26	95
10	98.85	----	99.25	----	98.50	98.00	88.38	97.5
12	----	----	----	----	----	----	98.12	0

**Mean ±S.D (n=3)*

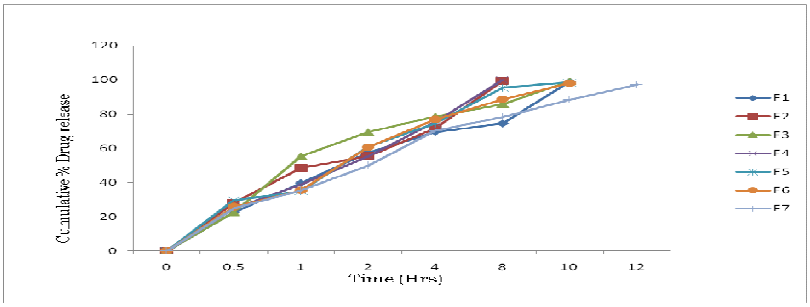

Figure 6: Comparative % drug release profiles of Diltiazem HCl bilayer floating tablets

3.8 Kinetic modeling of drug release:

To explore the mechanism of drug release from bilayer floating tablets, various kinetic models like zero order, first order, Higuchi and Korsmeyer-Peppas equations were applied to the different formulations. The release kinetics of optimized formulation (F7) was shown in **Table 11 & Figure 7-10**. From the data it was concluded that the optimized formulation followed first order kinetics. When the drug release data was fitted to Higuchi equation, linear plots were obtained with high correlation coefficient values. The drug release was proportional to square root of time indicating that the drug release was diffusion controlled.

Table 11: Release kinetics of optimized formulation (F7) of Diltiazem HCl bilayer floating tablets

Zero Order		First order		Higuchi		Korsmeyer-Peppas	
r2	*ko (h-1)*	*r2*	*k1(h-1)*	*r2*	*kH (h-1/2)*	*r2*	*n value*
0.913	5.626	0.932	-0.080	0.972	24.62	0.978	0.358

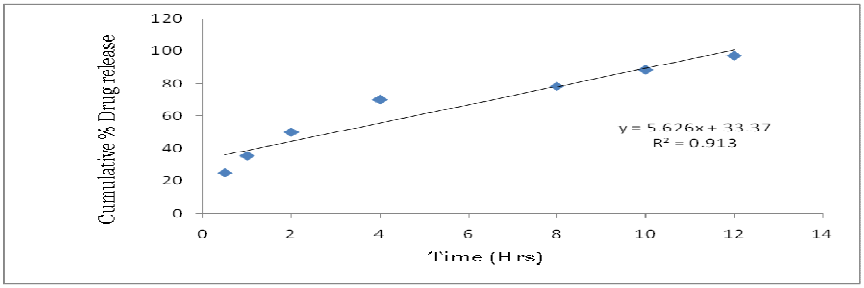

Figure 7: Zero-order release model of Diltiazem HCl optimized (F7) bilayer floating tablets

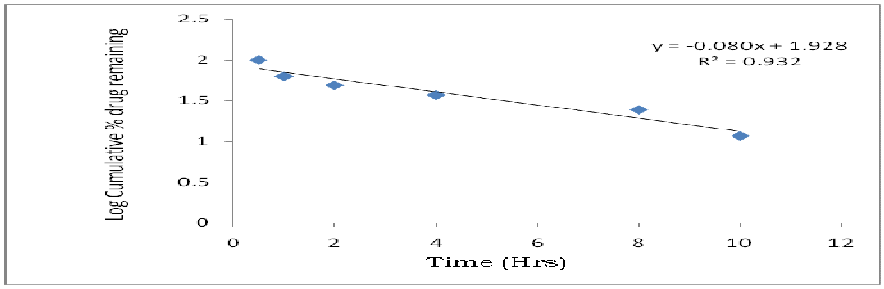

Figure 8: First-order release model of Diltiazem HCl optimized (F7) bilayer floating tablets

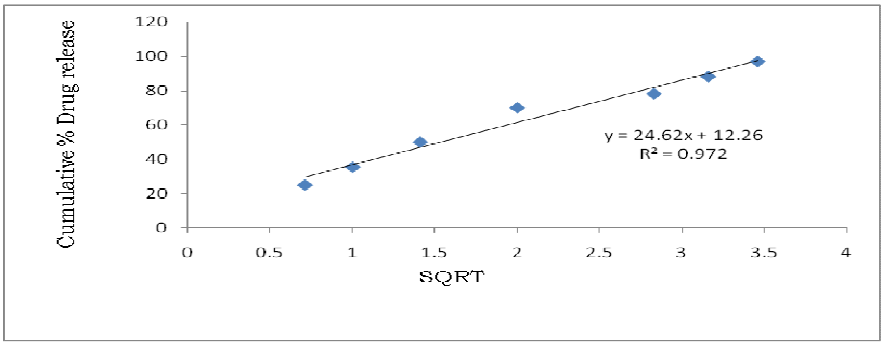

Figure 9: Higuchi plot of Diltiazem HCl optimized (F7) bilayer floating tablets

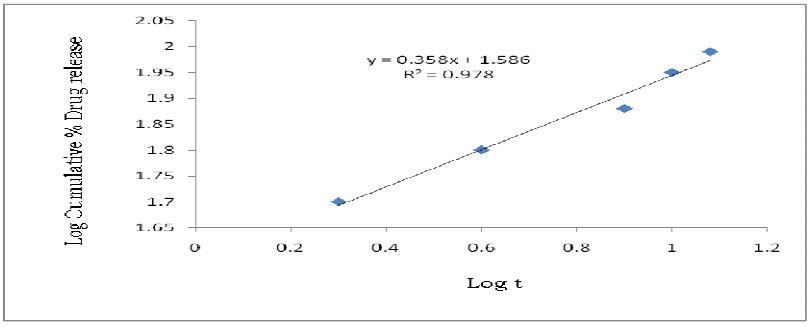

Figure 10: Korsmeyer-Peppas plot of Diltiazem HCl optimized (F7) bilayer floating tablets

3.9 Drug excipient compatibility studies:

3.9.1 Fourier Transform Infrared Spectroscopy (FTIR)

The FT-IR spectra of diltiazem HCl and its formulations were found to be identical **(Figure 11 & 12).** The characteristic IR absorption peaks of diltiazem at 2382 (amine HCl), 1680 (Lactam C=O stretch), 839 (o-substituted aromatic C-H out of plane deformation) and 781 cm^{-1} (p-substituted aromatic C-H out of plane deformation) were obtained in both the spectrum. The IR spectra indicated that no chemical interaction occurred between the drug and excipients used in the formulation.

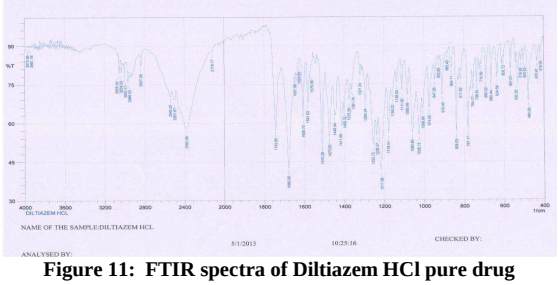

Figure 11: FTIR spectra of Diltiazem HCl pure drug

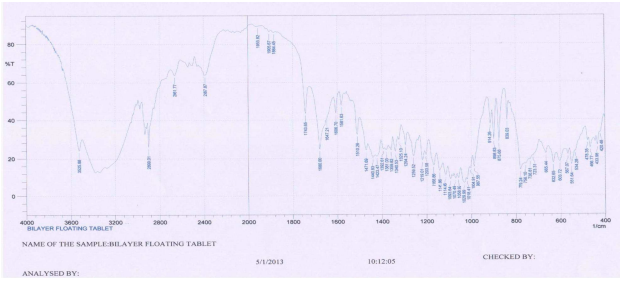

Figure 12: FTIR spectra of Diltiazem HCl floating bilayer tablet optimized formulation (F7)

3.9.2 DSC studies:

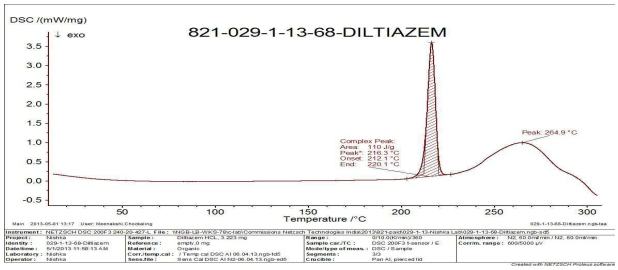

Figure 13: DSC thermogram of Diltiazem HCl pure drug

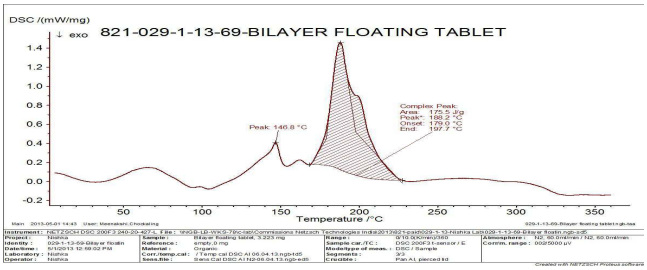

Figure 14: DSC thermogram of Diltiazem HCl optimized formulation (F7)

The DSC thermograms of the pure diltiazem **(Figure 13)** and optimized formulation (F7) **(Figure 14)** showed endothermic peaks of the pure diltiazem and the diltiazem in the formulation at 220.1 and 197.7 ^{0}C respectively. It indicates that there is no interaction takes place between drug and other excipients used in the formulation.

3.10 Stability studies:

The stability of optimized formulation (F7) of Diltiazem HCl bilayer floating tablets containing methocel K100M, natural polymers like Xanthan gum and Guar gum was tested for stability at 25°C/60% RH and 40°C/75%RH in properly closed HDPE bottles along with 1 gm desiccant for 3 months. The Diltiazem HCl release rate (**Table 12**) from the bilayer floating tablets (F7) showed no significant change during storage for 3 months, there is no significant change in floating lag time, total floating time and also in-vitro drug release profile. The formulation stored in both conditions for 3 months floated on the surface of the media (0.1N HCl) for 12 h.

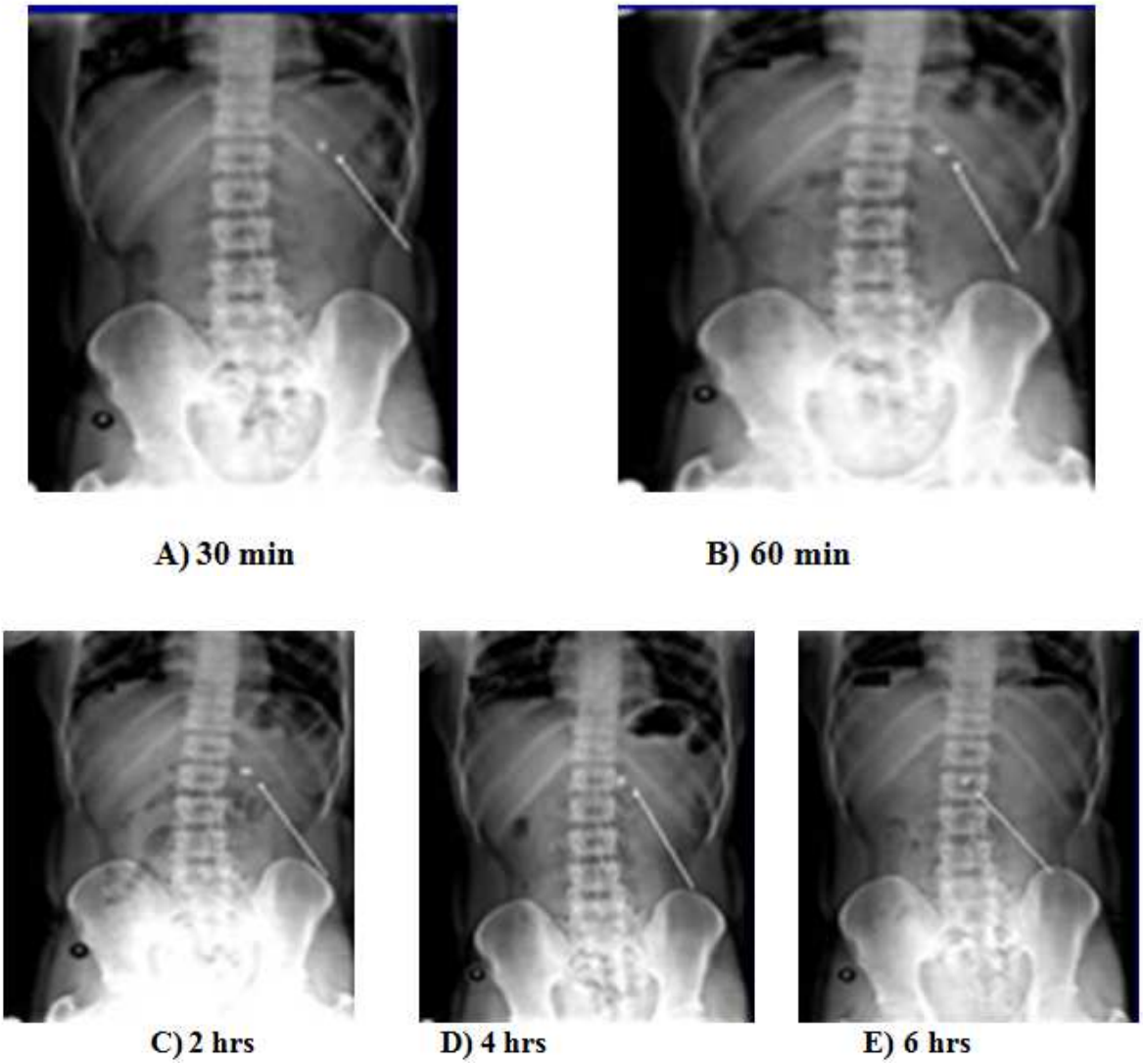

Figure 15: Radiographic images showing the presence of a $BaSO_4$ loaded bilayer floating tablet (F7) in the stomach at a different time periods

Thus, it was found that the optimized formulation of Diltiazem HCl bilayer floating tablets was stable under these storage conditions for at least 3 months.

Table 12: Physico-chemical characteristics of optimized formulation (F7) stored at 40 ±2°C /75 ±5%RH

Retest Time For F7	Floating lag time(sec)*	Total Floating time (hrs)*	*In-vitro* drug release profile (%)*
0 days	26±2.6	>12hrs	98.12
30 days	27±2.4	>12hrs	95.24
60 days	28±1.2	>12hrs	94.35
90 days	29±3.5	>12hrs	93.15

**Mean ±S.D (n=3)*

3.11 The mean gastric retention period

The radiographic images were taken at different periods post-administration of the barium sulfate-loaded tablet in three volunteers (**Figure 15**). It is clear that the tablet appears more or less at the same position for the initial 3 hrs.

This could be related to its floating ability. Later on, the tablet slightly moved downwards, yet, remained within the stomach till the end of 6 hrs. The mean gastric retention period was 6.50±0.5 h.

3.12 Bioavailability studies:

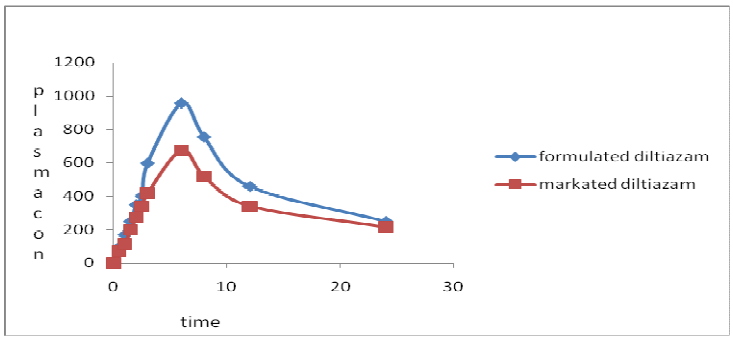

Figure 16: Plasma levels of Diltiazem HCL Bilayer floating Tablets and Marketed SR tablet (DILZEM SR 90) at different time intervals

Table 13: Comparison of pharmacokinetic parameters of Diltiazem HCL Tablets and Conventional marketed Drug in Human volunteers (Mean ± SD, n = 6)

Parameters	Diltiazem HCL Bilayer floating Tablets Mean ±SD	Reference formulation Mean ±SD
C_{max} (µg/ml)	958 ± 441	678±142
AUC $_{0-t}$ (µg.h/ml)	3845 ± 548	3046±254
AUC $_{0-\infty}$ (µg.h/ml)	4257± 453	3972±272
T_{max} (h)	6.0 ±1.0	5.8±1.8
$t_{1/2}$ (h)	3.93 ± 0.45	3.02±1.35
MRT(h)	10.00 ± 0.72	9.76±0.47

Pharmacokinetic studies were carried out in healthy human volunteers for optimized Diltiazem HCl formulation and reference formulation. C_{max} and T_{max} of optimized gastroretentive formulation was found to be 958 ± 441µg/ml and 6.0 ±1.0 h, respectively. Plasma concentrations of the optimized and reference formulations were depicted in **Figure 16**. C_{max} and T_{max} for reference formulation were estimated to be 878±142µg/ml and 5.8±1.8h, respectively. The $AUC_{0-\infty}$ for optimized gastroretentive formulation and reference product was 4257± 453 µg.h/ml and 3972±272µg.hr/ml, respectively the t1/2 for optimized gastroretentive formulation and reference formulation were 3.93 ± 0.45h and 3.02±1.35h, respectively. The MRT for optimized gastroretentive formulation and reference formulation was 10.00 ± 0.72h and 9.76±0.47h respectively. Results were showed in **(Table 13).** The comparison of in vivo bioavailability studies of optimized formulation F7 of bilayer floating tablet and reference formulation (DILZEM SR) in human healthy volunteers confirmed that the increase in diltiazem hydrochloride systemic exposure early after administration for immediate action due to immediate release layer and remaining drug was released in sustained manner from bilayer floating tablet by increasing the gastric residence time.

CONCLUSION

In the present investigation, several formulations were prepared by using different polymers for immediate layer and sustained layer separately. Based on the evaluation parameters for immediate layer, IR8 was found to be optimized formulation upon its disintegration time i.e., 41 sec. For Sustained release tablet, F7 was decided as optimized formulation, because the lag time, buoyancy period and in vitro drug release was better than other formulations.

This is due to the good sustained release properties of HPMC and other natural polymers like Xanthan gum and Guar gum. The release pattern of bilayer tablet was best fitted to First order and Higuchi kinetic model with R^2 values of 0.932 and 0.972 respectively. The value of n = 0.358 suggested that the drug is released from bilayer sustain dosage form by Fickian diffusion mechanism.

From DSC thermograms and FT-IR spectra, there was no evidence of interactions between Diltiazem HCl and the used excipients.

In vivo radiographic studies revealed that the optimized formulation (F7) remained for 6.5 h, which indicated that gastric retention time was increased by the floating principle which was considered desirable for improving the bioavailability of the drugs.

From the results of in vivo bioavailability studies of optimized formulation F7 of bilayer floating tablet and reference formulation in human healthy volunteers can conclude that the systemic concentration of the diltiazem HCl was high after administration to attain immediate action due to the immediate release layer, from sustained release layer the drug was released in controlled manner from bilayer floating tablet by increasing the gastric residence time for prolonged period of 12 h.

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