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ORIGINAL ARTICLE **OPEN ACCESS**

Formulation and *In Vitro- In Vivo* Evaluation of Controlled Release Bilayer Tablets Containing Combination of Antihypertensive Drugs

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ABSTRACT
Formulation and in vitro -in vivo evaluation of a once daily bilayer tablet consisting controlled release gastroretentive Verapamil Hydrochloride layer and immediate release Trandolapril for effective management of hypertension was proposed in this study. Double compression was used in the manufacturing process of the bilayer tablets. A 3^3 BBD (3-factor, 3-level Box Behnken design) was used to optimize the controlled release layer. Compritol 888 ATO, HPMC K15M, and sodium bicarbonate were chosen as factors at three levels (-1, 0, +1) and % CDR at 1.5 hr, 8 hr, 24 hr, and floating lag time as responses. Super disintegrants were used to optimize the immediate-release layer. The final bilayer tablets were assessed for in-vitro and in-vivo evaluation. During the six-month stability examination as per ICH, no major changes in the tablets' appearance and drug content were found. The optimized formulation's capacity to maintain gastroretentive ability for more than 24 hours was proven by an in-vivo gastro-retentive X-ray examination. In-vivo pharmacokinetic tests of Verapamil HCl showed considerably greater AUC, Tmax, $t_{1/2}$, and MRT values for the optimized bilayer tablets compared to the reference conventional tablets, but significantly lower Cmax values. The IVIVC coefficient (R^2) 0.983 indicates a significant correlation between the in-vitro and in-vivo profile of Verapamil HCl. As a result, it can be stated that the current study successfully developed a once daily controlled release system that contains a combination of antihypertensive drugs using an optimization technique that can effectively manage hypertension with reduced dosing frequency, and minimize side effects.
Keywords: *Gastroretentive, Controlled Release, Bilayer, In vitro-In vivo, Verapamil HCl and Trandolapril*

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INTRODUCTION

Hypertension affects a substantial number of people worldwide, with estimates ranging from sixty to seventy million individuals. This condition can impact individuals of all ages. It is essential to create a drug delivery system that boosts therapeutic efficiency and retains the intended effects for a long time to properly treat hypertension. [1, 2]

Verapamil hydrochloride (VH) faces certain challenges that hinder its absolute bioavailability. These challenges include a short half-life of 3-5 hours, pH-dependent solubility (with a pKa of 8.6), and significant first-pass intestinal wall metabolism, which is comparable to first-pass hepatic metabolism. Therefore, Verapamil hydrochloride requires the development of a controlled release drug delivery system that can be kept in the stomach. [3, 4]

The non-sulfhydryl prodrug Trandolapril (TR) (half-life 6 hours) and the pharmacokinetic characteristics of Trandolaprilat (Active form of Trandolapril), including its prolonged half-life (16-24hours), make it a suitable candidate for immediate release systems. The extended terminal elimination phase ensures a sustained presence of the active compound in the body, allowing for a prolonged therapeutic effect. Immediate release formulations of Trandolapril can rapidly deliver Trandolaprilat to the systemic circulation, providing an effective and sustained drug action over an extended period. [5, 6]

Numerous clinical trials have demonstrated that the combination of Trandolapril and Verapamil is more effective than monotherapy with either medication alone in lowering systolic blood pressure and diastolic blood pressure as well as rate pressure product (RPP). Verapamil with ACE inhibitors, according to studies, should be the first line of therapy for hypertensive people who also have diabetes or nephropathy. [7, 8]

Gastroretentive systems, which are primarily controlled-release drug delivery methods designed to stay in the gastric region for extended periods of duration, improve drug absorption and increase bioavailability while reducing the frequency of dosage. Floating devices are the fastest-growing type of gastroretentive technology, and combinations of different systems, such as floating-swelling and floating-mucoadhesive, have been formed to overcome their individual limitations. [2]

Optimization techniques, like the response surface Box Behnken Design, have successfully been used to develop gastroretentive sustained/controlled release formulations of various medications, including nicardipine hydrochloride, isosorbide mononitrate, metronidazole, guaifenesin, and loxoprofen (in bilayer tablet form). This optimization approach has proven effective with a minimal number of experimental runs. [9-13]

Based on these findings, the current study proposed the development of a controlled release system using a combination of antihypertensive drugs, optimized through Box Behnken Design. This system aims to provide better management of hypertension, reduce dosing frequency, and minimize side effects, thereby benefiting the affected population.

MATERIALS AND METHODS

Matrix Laboratories Ltd. in Hyderabad provided a gift sample of Verapamil HCl (VH), while Mylan Laboratories Limited, also in Hyderabad, supplied Trandolapril (TR). Gattefosse Pvt. Ltd., Mumbai generously offered Compritol 888 ATO as a gift sample. Other excipients were of analytical grade procured from the department. Solvents and other excipients were also of analytical / HPLC grade.

Drug- Excipient Compatibility studies

To illustrate how the drug(s) and drug(s) with excipient interact and are compatible with one another drug excipient compatibility was performed. Drug-Drug and Drug/S-excipient mixed properly and examined using an FTIR spectrophotometer in the absorbance mode (Bruker Alpha Model) using the KBr pellet technique. The 400–4000 cm-1 wave number range was used to capture the FTIR spectrums.

Preparation of optimized bilayer tablet

Firstly both the layers controlled release gastroretentive Verapamil HCl and Immediate release Trandolapril were optimized separately. As mentioned in my previous work[14] controlled release layer of Verapamil HCl was optimized using 3^3 Box- Behnken Design using Design Expert where Compritol 888ATO, HPMCK15M and Sodium bicarbonate were the factor taken at three levels -1, 0, +1 and %CDR at Q1.5Hr, Q 8Hr, Q 24Hr and FLT were dependent factors. After the numerical and graphical optimization, the formula was obtained with 0.963 desirability.

Based on the result obtained minimum disintegration time (30.23±0.069 sec) and maximum %cumulative drug release (90.56±0.736 %) of Trandolapril in 30 min obtained for IRTR3 (a formulation containing 6% Crospovidone) reported in the previous work [15] finalized for the immediate release layer of Trandolapril. The bilayer tablets were fabricated via a double compaction method using BBD optimized controlled release layer formula and immediate release layer formula (Table 1) using a multi-station tablet punch machine (Rimek Minipress-II). The procedure started with a controlled release layer (CRGR-VH) powder blend being poured into the die cavity of a rotary tablet machine. The blend was then compressed once. The compressed controlled release layer was then put on top of the optimised immediate release layer (IRTR 3). The direct compression technique was then used to combine the two layers to create a bilayer tablet.

Physical Characterization and *in-vitro* evaluation of optimized bilayer tablet

Bilayer tablets were evaluated for post-compression parameters, tablet floating behavior study and swelling study.

Thickness

The standard sample size for thickness determination was 10 tablets that were picked randomly from the batch. Thickness of the tablets were measured using Vernier-caliper.

Average weight & %weight variation

Randomly twenty tablets were chosen and precisely weighed with an automated balance and checked for the %Weight variation limit as reported in Indian Pharmacopoeia. The average weights and standard deviation was calculated and reported as mean values ± SD.

Hardness

Six tablets from the batch were tested for hardness, and an average value was calculated and reported in kg/cm^2 using Monsanto hardness tester.

Drug content [16]

The instrument used for analysis was Waters 2998 with a PDA detector, and 515 HPLC Pump. Twenty tablets were weighed and finely ground to contain Verapamil hydrochloride (180 mg) and Trandolapril (2mg). The amount of powder sample transferred to a 100 ml volumetric flask equal to 180 mg of Verapamil

hydrochloride and 2 mg of Trandolapril. Then, 30ml of solvent was added and sonicated for 5 minutes. Take 10ml of the resulting solution, filter it, and then mix it with the mobile phase to make 100ml. This resulted in a solution that contained 2µg/ml of Trandolapril and 180µg/ml of Verapamil hydrochloride at its nominal concentration. A minimum of 30 minutes of mobile phase flow through the systems allowed the column to equilibrate before the injection of the drug solutions. Following that, 10µl of the standard and sample solutions were injected. Peak responses of Verapamil hydrochloride and Trandolapril were measured using chromatograms, and the percentage of drug content was determined using calibration curve.

Disintegration time of IR layer and floating lag time of CR layer

By placing the bilayer tablet inside a beaker containing 0.1 M HCl, it was possible to measure the IR layer's disintegration time as well as the floating behavior of the tablet. The length of time required by the CR layer to float on the surface and the amount of time it took for the IR layer to break and disintegrate were also measured.

Swelling study of CR layer

The technique was employed to assess the swelling behavior of the tablets in which a weighed CRGR tablet layer was placed in a dissolution basket containing 900 ml 0.1M HCl at a temperature of 37±0.5°C. The swollen tablet was periodically taken out of the solution, wiped dry with filter paper, and then reweighed. The formula was used to calculate the swelling index (SI)

$$SI = \frac{\textbf{Weight of the swollen tablet} - \textbf{Initial weight of the tablet}}{\textbf{Initial weight of the tablet}}$$

***In-vitro* drug release**

Bilayer CRGRF tablets containing Trandolapril and Verapamil HCl underwent an *in-vitro* drug release test utilizing a USP Type II dissolution apparatus (Electrolab, India), which contained 900 ml of 0.1M HCl at 37°C ± 0.5°C. The paddle's speed was set at 50 rpm. 5 ml of the sample volume was taken out at 0, 0.5, 2, 4, 6, 8 and 24 hours and replaced with 5 ml of 0.1 M HCl. Then, using RP-HPLC [16] the quantity of TR and VH were determined.

Stability studies

According to the ICH standards, stability testing of the tablets was conducted on them for six months. 25°C/60% RH and 40°C/75% RH were used as the storage temperatures. The samples were monitored for changes in physical appearance and drug content every three and six months as per protocol. [17]

***In-vivo* evaluation**

***In-vivo* gastroretentive X-ray study** [18-19]

This investigation was carried out to look at the *in-vivo* gastroretentive performance of a controlled-release Verapamil HCl (CRVH) layer. Prior approval was received by the Institutional animal ethics board, Adina Institute of Pharmaceutical Sciences, Sagar (CPCSEA registration number 1546//PO/E/S/11/CPCSEA). The investigation was conducted on 2.5 kg of healthy male New Zealand Albino rabbits. The rabbits were ordered to fast for 12 hours before to the experiment but were given access to drink. Barium sulfate (BaSO4) was included in the improved CRVH layer to enable the visualization of the tablets under X-ray without compromising their buoyancy. To examine the position of the tablet in the gastrointestinal area, X-ray pictures were acquired at various time intervals (2 hours, 8 hours, and 24 hours) following tablet administration. The experiment was conducted using the same X-ray conditions. The location of the tablets may be recognized and monitored by analyzing the X-ray pictures.

***In-vivo* pharmacokinetic study and statistical analysis** [18-20]

Treatment protocol and sample analysis: The study employed a parallel design, as outlined in Table 2. The optimized bilayer formulation consists of Verapamil HCl (VH) and Trandolapril (TR), as compared to a conventional reference tablet formulation. Three sets of three New Zealand rabbits, weighing between 2-2.5kg were employed.

Group-I was given the reference conventional tablets (RCBLT) containing Verapamil HCl and Trandolapril at dosages corresponding to 10 mg/kg of Verapamil hydrochloride and 0.1 mg/kg of Trandolapril, respectively. Group-II was administered the optimized bilayer tablet (OBCRT) formulation containing the same doses of Verapamil hydrochloride and Trandolapril as Group-II while keeping the other formulation ingredients the same.

The rabbits in groups-I and group-II fasted the night before receiving the tablet and kept fasting for an additional 8 hours thereafter but had free access to water. A bilayer tablet was equivalent to a total of 20 mg of Verapamil HCl and 0.2 mg of Trandolapril was administered to each rabbit off group-II and group-III using a pet piller.

After the tablet was administered, blood samples of 0.5 ml was drawn from the ear marginal vein at different intervals; 0, 1, 2, 3, 4, 8, 12 and 24 hours. To avoid clotting, the samples were put into tubes containing heparin. Proteins were precipitated from the samples using 10% perchloric acid, which was followed by 15 minutes of centrifugation at 4,000 rpm to extract plasma. Prior to analysis, the plasma samples were kept at -20°C. Blood samples were taken before the administration of the medicine to acquire plain plasma for the calibration curve. The RP-HPLC technique was used to calculate the levels of Verapamil HCl and Trandolapril in the test samples.

The plasma level data from the individual rabbits were used to calculate the pharmacokinetic parameters. Cmax, Tmax including (AUC0-24hr), t1/2, and MRT (mean residence time) were computed using Kinetica 5.0 PK/PD analysis software.

Statistical analysis

The Independent Sample t-test and Paired one-tail t-test were applied for the statistical analysis using SPSS Version 22 software, IBM Inc.

***In vitro-In vivo* Correlation studies**

The highest level of correlation between *in-vitro* drug release and *in-vivo* drug performance is Level A IVIVC. It shows a significant and reliable correlation between a dosage form's *in-vitro* dissolving profile and its *in-vivo* performance. The graph between the *in-vitro* VH release and the *in-vivo* VH fraction absorbed from the CRGR layer was plotted and r^2 was determined to conclude the correlation.

RESULT AND DISCUSSION

Drug- polymers Compatibility studies

The compatibility studies of both the drugs as well as the physical mixture of drug-excipients were studied using an FTIR spectrophotometer. When compared to pure drug samples spectrums Verapamil HCl (Fig. 1) and Trandolapril (Fig.2). FTIR spectrum analysis showed that comparable characteristic peaks appeared with slight changes for drug-drug mixtures (Fig. 3) and drug/s-excipient mixtures (Figs. 4 and 5). Thus, it was established that drug- drug and drug/s-excipients chemically compatible with each other. **Physical Characterization and *in-vitro* evaluation of optimized bilayer tablet**

Bilayer tablets were assessed for post-compression characteristics, tablet floating behavior study (Fig. 6) and swelling study (Fig. 7). All the parameters were within the specified limit reported in (Table 3).

***In-vitro* drug release**

In-vitro drug release study results as shown in Table 4 and Fig. 8 indicates that the %cumulative drug release of VH increases gradually. At 1 hour, approximately 18.345% of Verapamil had been released and continues to increase over time, reaching 97.89% at 24 hours in a controlled mode. Trandolapril release was faster and released approximately 99.98% within 1 hour indicating the immediate release of the drug.

Stability studies

There were no modifications distinguished in physical appearance and no major changes observed in drug content after the 6 months in the optimized formulation drug content of Verapamil HCl and Trandolapril as shown in Table 5.

***In-vivo* evaluation**

***In-vivo* gastro-retentive X-ray study**

Results from *in-vivo* gastroretentive X-ray imaging investigations revealed that the tablet was floated for 24hr or more in the gastric region of the New Zealand Albino rabbits as shown in Fig. 9.

***In-vivo* pharmacokinetic study**

The optimized bilayer tablet containing VH and TR was compared with the conventional reference tablet formulation using a parallel design-based study protocol. After the oral administration, the change in plasma concentration of Verapamil HCl and Trandolapril with time of the reference conventional bilayer and the prepared optimized controlled release floating bilayer tablet to rabbits was determined, as shown in Fig. 10. The pharmacokinetic parameters generated from the individual data analysis through PK/PD Kinetica 5.0 software for Trandolapril and Verapamil HCl and presented in table 6 and table 8 respectively. RCBLT and OBCRT show significant differences in their pharmacokinetic parameters for Verapamil HCl. Verapamil HCl from RCBLT exhibits a higher peak concentration, faster onset of action, shorter half-life, and shorter mean residence time. On the other hand, OBCRT provides a longer duration of drug exposure in the body.

Statistical analysis of *In-vivo* release data

The p-value obtained for Verapamil HCl ($p=0.038$) and Trandolapril (0.0001) was less than <0.05. It proves that there was a significant difference between plasma drug concentration data of both the drugs released from reference bilayer tablet (RCBLT) and optimized controlled release bilayer tablet (OBCRT).

Table 1: Composition of optimized bilayer tablet

Optimized CRGRF-VH Layer	
Verapamil Hcl	180.0
Compritol 888 ATO	126.0
HPMCK15M	160.0
Sodium bicarbonate	81.0
Xantham Gum	90.0
Citric acid	40.5
MCC (PH 102)	58.5
Magnesium stearate	8.0
Talc	16.0
Optimized IR-TRP Layer	
Trandolapril	2
Crosspovidone	9
MCC (PH 102)	50
Anhydrous lactose	85
Magnesium stearate	2
Talc	2
Total weight of bilayer tablet	910 mg

Table 2: *In-vivo* pharmacokinetic study protocol

Group Code	Group	Dose	No. of animal in group
G1	Reference conventional bilayer tablet containing Verapamil HCl and trandolapril (RCBLT)	Equivalent to human dose (10mg/Kg of VH & 0.1mg/kg TR)	3
G2	Optimized CRGR bilayer tablet of Verapamil HCl and trandolapril (OBCRT)		3

Table 3: Post compression characteristics of the optimized controlled release gastroretentive bilayer tablet containing Verapamil HCl and Trandolapril

Evaluation Parameter	Results
Thickness± S.D. (mm) n(=10)	4.17±0.004
Hardness± S.D.(kg/cm2) (n=5)	7.08±0.008
Friability (%) (n=3)	0.61±0.001
Average weight (n=20)	910±0.015
Drug content (%) TR	99.7±0.208
Drug content (%) VH	99.4±0.121
Disintegration time of IR layer (in min) (n=6)	3.19±0.007
Floating lag time of CR layer (in min) (n=6)	3.22±0.009
Swelling Index (%) (n=6)	105.22±0.06
Total floating time of CR layer (hr)	>24

Table 4: *In- vitro* release data of VH and TR from optimized bilayer layer tablet

Time (hr)	Cumulative % drug release (mean±SD) (n=3)	
	VERAPAMIL	TRANDOLAPRIL
0	0±000	0±000
0.25	5.13±0.314	76.45±0.687
0.5	9.04±0.123	87.56±0.737
0.75	11.08±0.342	96.89±0.456
1	18.345±0.786	99.98±0.765
1.5	21.382±0.987	-
2	27.432±0.879	-
4	35.98±0.945	-
8	52.744±0.576	-
12	73.21±0.892	-
24	97.89±0.653	-

Table 5: Stability study results of optimized bilayer tablets

Stability condition	Sampling intervals (months)	Physical appearance	Verapamil HCl % content (mean±SD) (n=3)	Trandolapril % content (mean±SD) (n=3)
25±2°C/60±5% RH	0	Good	100.01±0.046	100.09±0.062
	3	No change	99.64±0.082	99.94±0.035
	6	No change	99.39±0.074	99.73±0.086
40±2°C/75±5% RH	0	Good	100.02±0.036	100.06±0.091
	3	No change	99.38±0.059	99.57±0.044
	6	No change	98.89±0.023	98.69±0.076

Table 6: Pharmacokinetic parameters of Trandolapril in New Zealand albino rabbit (mean± SD, n = 3)

Formulation		C_{max} (ng/ml)	T_{max}	$t_{1/2}$	MRT	$AUC_{(0-t)}$
RCBLT	Trandolapril	9.17±0.325	1±0.250	5.93±4.342	9.04±0.768	83.23±3.452
OBCRT		10.45±0.341	1±0.044	6.70±0.292	10.27±0.784	101.86±2.154

Table 7: Pharmacokinetic parameters of Verapamil HCl in New Zealand albino rabbit (mean± SD, n = 3)

Formulation		C_{max} (µg/ml)	T_{max}	$t_{1/2}$	MRT	$AUC_{(0-t)}$
RCBLT	Verapamil HCl	7.5± 0.928	1±0.144	1.93±0.335	3.97±0.584	36.08±5.64
OBCRT		4.1±0.721	4±0.153	11.91±0.921	19.29±0.616	81.72±2.082

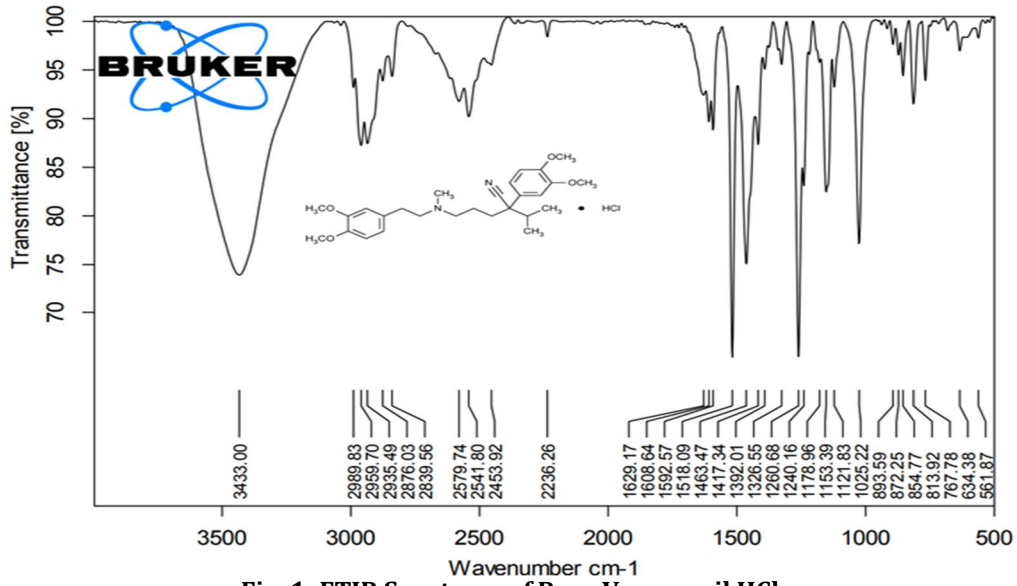

Fig. 1: FTIR Spectrum of Pure Verapamil HCl

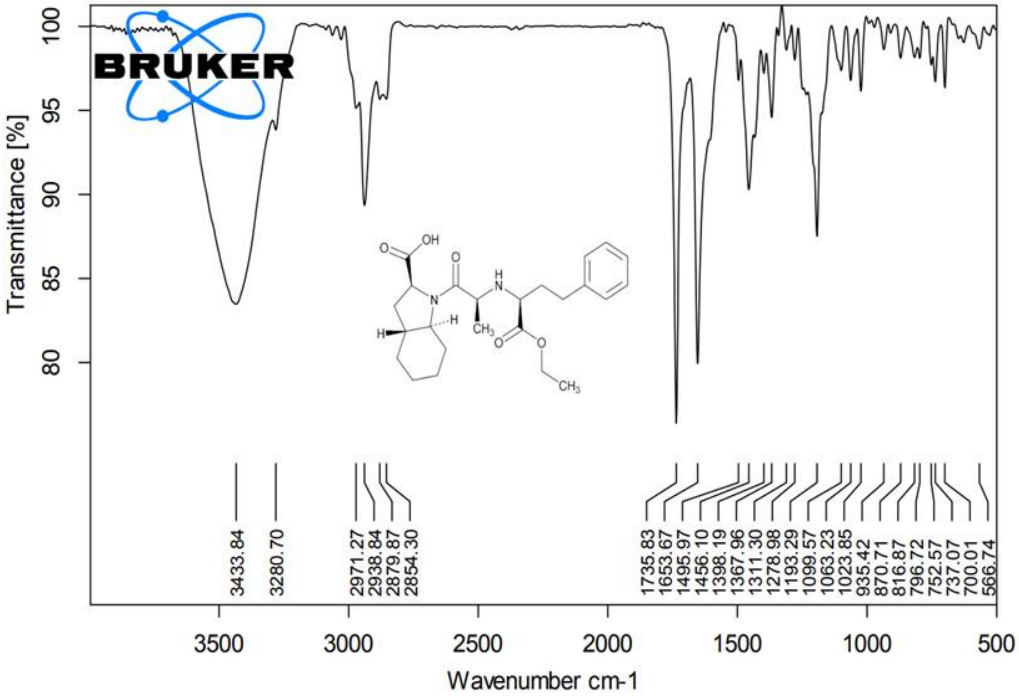

Fig.2: FTIR Spectrum of Pure Trandolapril

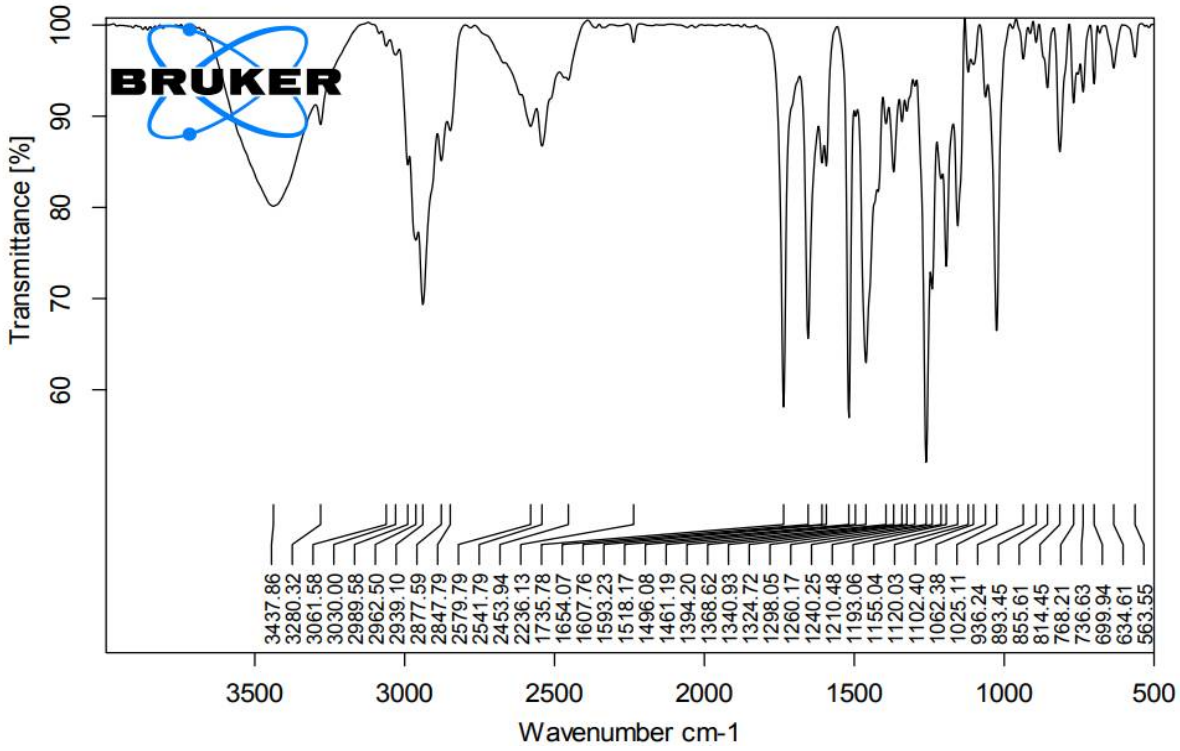

Fig.3: FTIR Spectrum Verapamil HCl-Trandolapril physical mixture

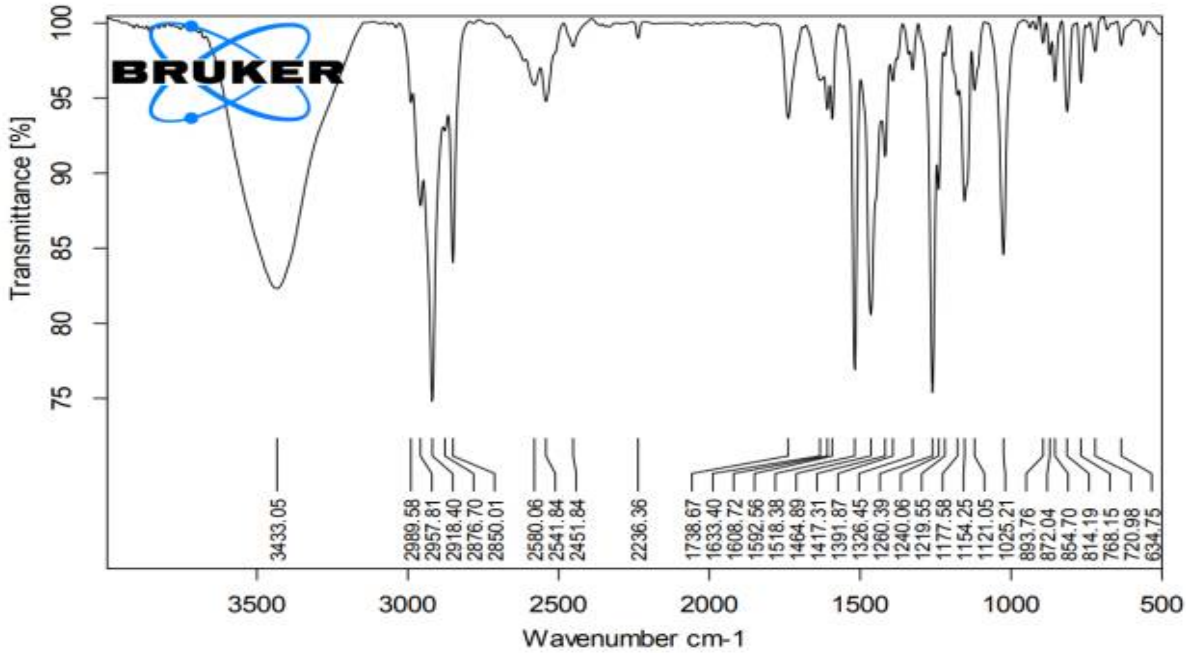

Fig. 4: FTIR Spectrum of Verapamil HCl- Excipients mixture

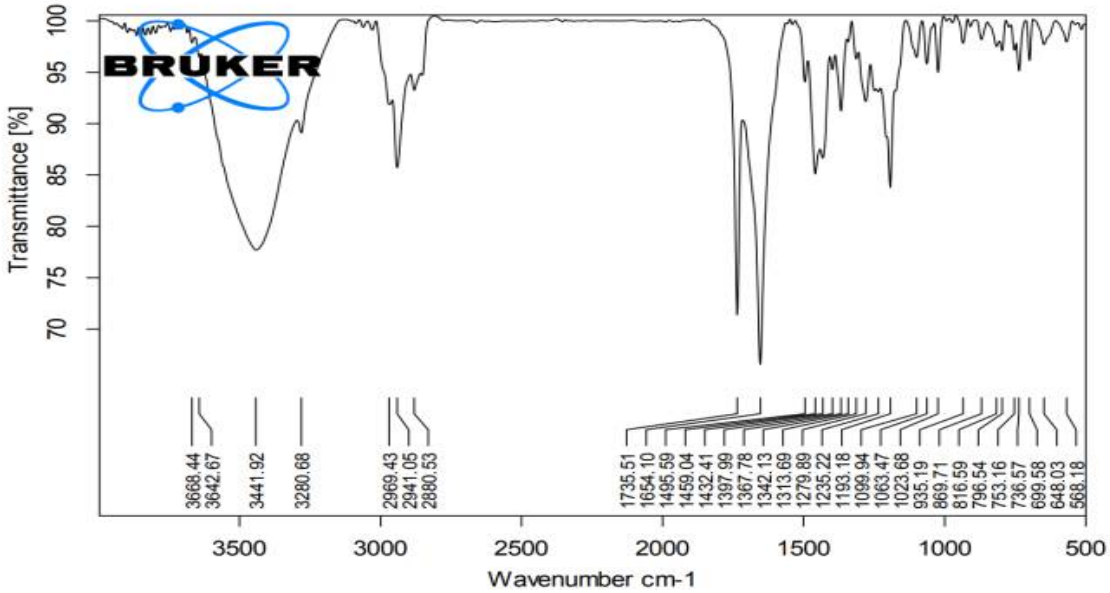

Fig. 5: FTIR Spectrum of Trandolapril- Excipient mixture

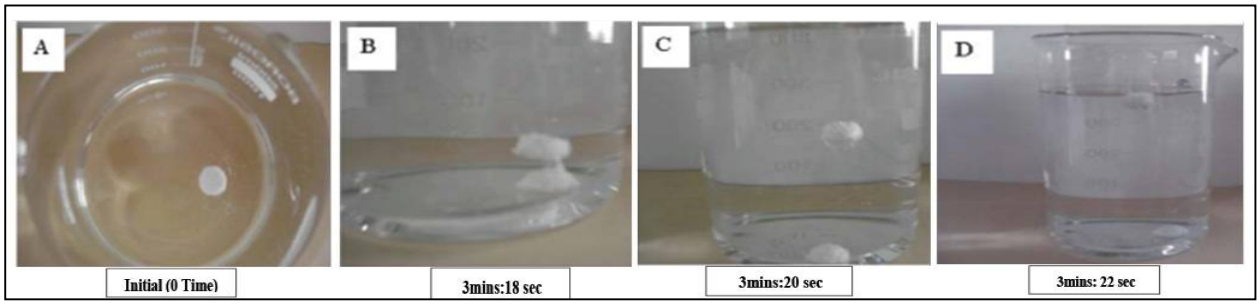

Fig.6: Photograph shows A- Tablet placed in 0.1 N HCl, B- Separation of IR and and CRGR, C- Disintegration of IR layer and upward movement of CRGR layer, D- Floating of CRGR layer on dissolution media

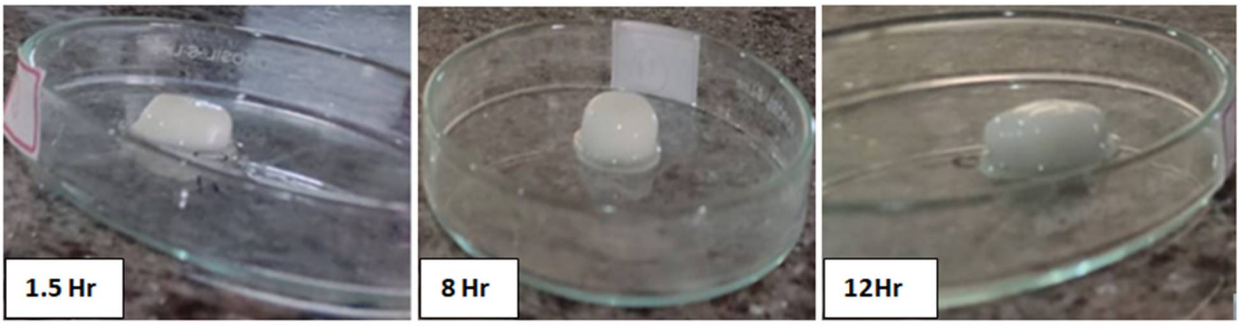

Fig.7: Picture illustrating the swelling behavior of CRVH layer of bilayer tablets subjected to dissolution testing in 1.5 hr, 8 hr and 12 hr

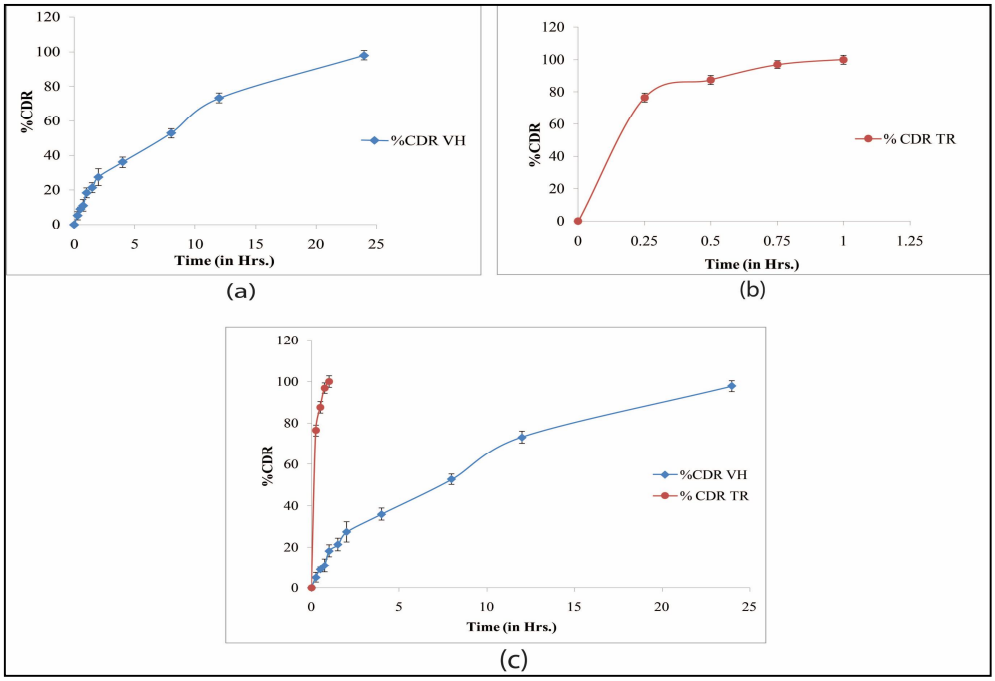

Fig. 8: In-vitro drug release profile (a) Verapamil HCl from bilayer tablet (b) Trandoalapril from bilayer tablet (c) Comparative VH & TR release from bilayer tablet

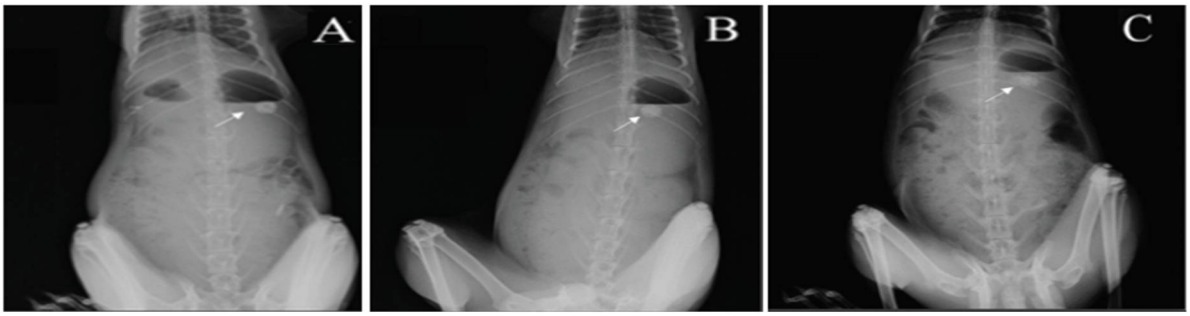

Fig.9: X-ray pictures (A–C) showing the position of the floating barium sulfate-labeled tablet in the stomach of a New Zealand albino rabbit at various times. A) 2 hours, B) 8 hours, and C) 24 hours

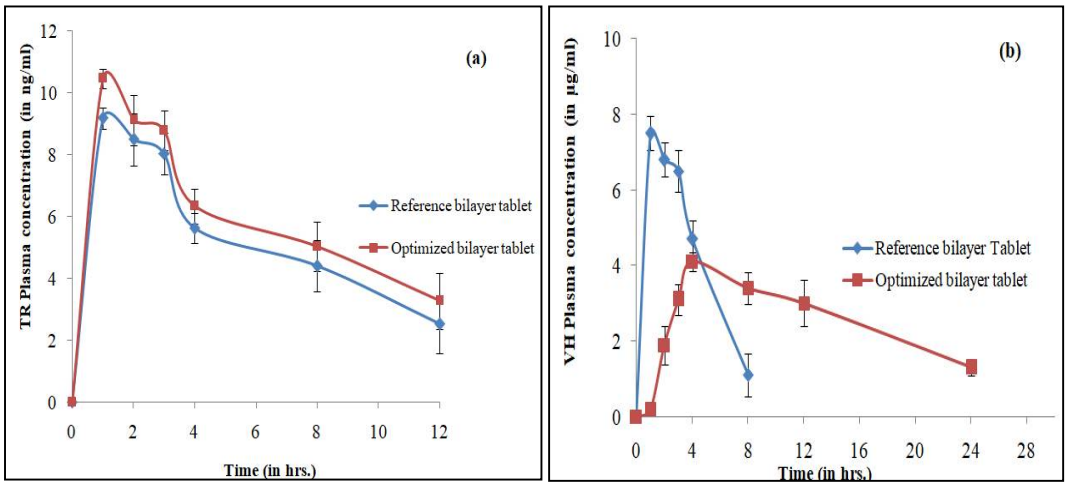

Fig.10: Plasma concentration-time curve in New Zealand albino rabbit from reference bilayer tablet and Optimized bilayer tablet (a) Trandolapril (b) Verapamil HCl

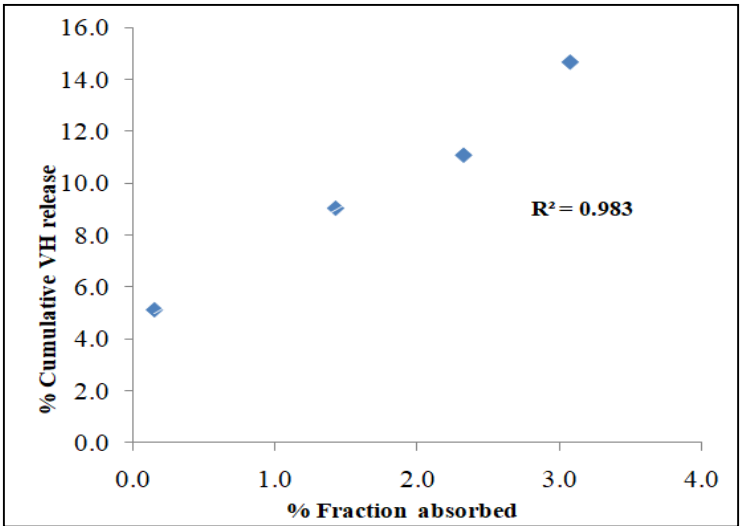

Fig. 11: Graph illustrating *IVIVC* correlation of Verapamil HCl in vitro release and in vivo fraction absorbed from CRGR layer of bilayer tablet

***In vitro-In vivo correlation* studies**

The correlation coefficient value for the *IVIVC* graph was found to be 0.983 (Fig. 11) which can be considered very high and indicates a strong correlation between the *in-vitro* and *in-vivo* data. This suggests that the *in-vitro* release study is a reliable surrogate for predicting the *in-vivo* behavior of the Verapamil HCl from controlled release gastroretentive bilayer tablet, providing valuable information for formulation development, optimization, and regulatory purposes.

CONCLUSION

The once-daily bilayer tablet containing a controlled release gastroretentive (floating) layer of Verapamil HCl (Calcium channel blocker) and an immediate release layer of Trandolapril (ACE inhibitor) was developed successfully by optimization technique (Box –Behnken Design) that will releases drugs continuously for more than 24 hours in controlled manner. Based on pharmacokinetics result it can also be concluded that the optimized formulation will be more acceptable to patient due to reduced dosing frequency, and minimum side effects as compared to conventional tablet. The study also proved to be a simple that can easily adopted for the development of new bilayer tablets that can be easily scaled up for successful commercialization.

CONFLICT OF INTEREST- Declared None

ETHICAL APPROVAL: For *in-vivo* study on experimental animal, approval was taken from Institutional animal ethics board, Adina Institute of Pharmaceutical Sciences, Sagar, (CPCSEA registration number: 1546//PO/E/S/11/CPCSEA).

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