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Original article

Design, Development and Evaluation of Rosuvastatin Calcium and Diltiazem Hydrochloride Bilayer Tablet Using Combination Concept of Sustained Layer with Conventional Layer

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The aim was to design bilayer tablets of rosuvastatin and diltiazem hydrochloride to give conventional release of rosuvastatin calcium and sustained release of diltiazem hydrochloride. The conventional release layer comprised lactose and micro crystalline cellulose as diluents (separately and in combined form) and the sustained release layer comprised HPMC K100M and ethyl cellulose (with different ratio of HPMC K100M and ethyl cellulose) as the release retarding polymers. Direct compression method was used for formulation of the bilayer tablets. Preformulation studies were performed prior to compression. The bilayer tablets were evaluated for weight variation, dimension, hardness, friability, drug content, and disintegration time and in vitro drug release using USP dissolution apparatus type II (paddle). More than 90% of rosuvastatin calcium was released within 150 min. HPMC K100M and Ethyl cellulose sustained the release of diltiazem hydrochloride from the sustained release layer for 24 hour. After stability tests, degradation of both drugs were found but the drugs contents were found within the range. The release of diltiazem hydrochloride was found to follow a mixed pattern of Korsmeyer-Peppas, Higuchi model and zero order release models and the kinetic studies of the formulations revealed that diffusion is the predominant mechanism of drug release. The IR spectrum studies revealed that there was no disturbance in the principal peaks of pure drugs. This further confirms the integrity of pure drugs and no incompatibility of them with excipients. The stability studies were carried out for the optimized batch for three months and it showed acceptable results.

Key words: Bilayer tablet, Rosuvastatin calcium, Diltiazem hydrochloride, Conventional release, Sustained release.

Uzun Süreli ve Konvansiyonel Tabakaların Kombine Kullanımı Yaklaşımıyla Rosuvastatin Kalsiyum ve Diltiazem Hidroklorid'in İki Tabakalı Tabletinin Tasarımı, Geliştirilmesi ve Değerlendirilmesi

Amaç, rosuvastatin kalsiyumun, konvansiyonel, diltiazem hidroklorür'ün uzun sureli salımını sağlamak üzere rosuvastatin ve diltiazem hidroklorür'ün iki tabakalı tabletlerini tasarlamaktır. Konvansiyonel salım tabakası, dolgu maddesi olarak laktoz ve mikrokristal selülozdan (ayrı ayrı ve kombine olarak) oluşmaktadır ve uzun süreli salım tabakası, salım geciktiren polimerler olarak HPMC K100M ve etil selülozdan (HPMC K100M ve etil selülozun farklı oranlarıyla) oluşmaktadır. İki tabakalı tabletin formülasyonunda doğrudan basım yöntemi kullanılmıştır. Basımdan önce preformülasyon çalışmaları gerçekleştirildi. İki tabakakalı tabletler, ağırlık sapması, çap ölçümü, sertlik, friabilite, etken madde içeriği, dağılma süresi ve USP çözünme hızı cihazı tip II (palet) ile in vitro etken madde salım hızı açısından değerlendirildi.% 90'dan fazla

rosuvastatin kalsiyum 150 dakika içinde salındı. HPMC K100M ve etil selüloz uzatılmış salım tabakasından diltiazem hidroklorür'ün salımını 24 saat uzattı. Stabilite testlerinden sonra, her iki etken madde için de degredasyon tespit edildi ancak, etken madde içeriklerinin sınırlar içinde olduğu bulundu. Diltiazem hidroklorür'ün salımının Korsmeyer-Peppas modeline uyduğu bulundu. Higuchi model, sıfır derece salım modeli ve kinetik çalışmalar etken madde salımında baskın mekanizmanın difüzyon olduğunu gösterdi.IR spectrum çalışmaları etken maddenin belli başlı piklerinde bir bozulma olmadığını gösterdi. Bu daha sonra etken madde ile eksipiyanlar arasında bir geçimsizlik olmadığını da doğruladı. Stabilite çalışmaları optimize edilmiş beç için üç ay yapıldı ve uygun sonuçlar gösterdi.

Anahtar kelimeler: İki tabakalı tablet, Rosuvastatin kalsiyum, Diltiazem hidroklorür, Konvansiyonel salım, Uzatılmış salım.

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INTRODUCTION

Hypertension and hypercholesterolemia frequently coexist and may require concomitant drug treatment. Hypertension and hypercholesterolemia are major risk factors in the pathogenesis of coronary heart disease (CHD) (1,2). These two risk factors coexist in patients more frequently than would be expected by chance alone, and a syndrome of dyslipidemic hypertension has been identified. The aim of current investigation was to develop dual component bilayer tablets having different release patterns of rosuvastatin calcium (R) and diltiazem hydrochloride (D) (1,3,4). The multilayered tablet concept has been utilized to develop sustained release formulations. Such a tablet has a fast releasing layer and may contain bi- or triple layers to sustain the drug release. The pharmacokinetic advantage relies on the fact that drug release from fast releasing layer leads to a sudden rise in the blood concentration. However, the blood level is maintained at steady state as the drug is released from the sustaining layer. Thus, the developed single tablet will be sufficient instead of two to three tablets of both drugs per day, and it will also increase patient compliance and therapeutic efficacy. Bilayer tablets were prepared with one layer of drug for conventional release and second layer designed to release the drug as a sustained release. These tablets are suitable for sequential releases of two drugs in combination. Tablets are produced by applying a certain compression pressure on a bed of powder. Normally, a tablet contains a combination of two or more powders to obtain a compact with the desired properties. Direct compression is still the method of choice for the manufacture of tablets where the components are mixed together before they are compressed directly into tablets (2,5). Rosuvastatin is an antilipidemic agent that competitively inhibits hydroxymethylglutarylcoenzyme A (HMG-CoA) reductase. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonic acid, the rate-limiting step in cholesterol biosynthesis. Rosuvastatin belongs to a class of medications called statins and is used to reduce plasma cholesterol levels and prevent cardiovascular disease. Rosuvastatin acts primarily in the liver. The drug is sparingly soluble in water and has half-life of 19 hour. It is completely absorbed in GIT (3,4,6-8). Diltiazem hydrochloride, a calcium channel blocker, has been used widely for the treatment of angina pectoris and hypertension. The drug is water-soluble and has half-life of 3.4 h. The drug has low bioavailability (9-12).In the present study, an attempt was made to formulate and evaluate a bilayer system of rosuvastatin and diltiazem hydrochloride. The bilayer tablet was comprised of a conventional-release layer of rosuvastatin and a controlled-release layer of diltiazem hydrochloride. Hydroxypropyl methylcellulose (HPMC) K100 M and Ethyl cellulose (EC) were employed to control release of diltiazem hydrochloride. The release kinetics of diltiazem hydrochloride was analyzed using different models.

Bilayer tablet formulation was aimed to provide prolonged effect to the patient who requires concomitant drug treatment when suffering from hypertension and hypercholesterolemia together. Using the rosuvastatin drug property of long half-life (19 hours) it was formulated conventionally because there is no requirement of adding any release rate retarding polymer to make it sustained as it shows sustained effect itself due to its long half-life and also from the study of marketed product of rosuvastatin tablets (crestor) which shows 93% drug release in 1 hour. It was concluded that there is no need of add superdisintegrants as it has itself good release rate so this made the tablet cost effective. Therefore bilayer tablet is proposed to show quick onset of action of rosuvastatin after that drug remains in the body showing sustained effect (10,13-18).

MATERIALS AND METHODS

Materials

Rosuvastatin Calcium was procured from MMC Health care Pvt ltd, Baddi, India. Diltiazem Hydrochloride was a generous gift from Ranbaxy Lab. Ltd, Ghaziabad, India. HPMC K 100M were obtained as gift samples from Cadila pharmaceuticals, Ahamdabad, India. All other materials (Micro crystalline cellulose, Ethyl Cellulose Magnesium Hydroxide, Lactose Monohydrate, Polyvinyl Pyrrolidone (PVPK30), Magnesium stearate and Talc) and chemicals used were of analytical grade.

Method of preparation of bilayer tablet of rosuvastatin calcium and diltiazem hydrochloride

Bilayer tablet was prepared by direct compression method. Bilayer tablet (conventional layer of rosuvastatin and sustained layer of diltiazem) was formulated using different concentration of polymers in different ratios. After optimization by in-vitro studies bilayer tablet was prepared using optimized formula. Tablet was prepared on rotatory tablet compression single punch machine. First the sustained release layer was pre-compressed on tablet machine manually and the second layer was loaded on the top of pre-compressed layer and punched with compression machine automatically (19-24).

Preparation of bilayer tablets

Preparation of the sustained release layer

Diltiazem and other excipients were sifted through sieve no 40# and thoroughly mixed for 10 minutes in a mortar to obtain uniform distribution of the drug in formulation. Above mixture was lubricated for 2 min with magnesium stearate and mixed. Powder blend was fed into the die of single punch tablet press and weight was adjusted to obtain 400mg of tablet weight. Diltiazem layer blend was initially pre-compressed with low compression force determined by low hardness of tablets (3-4 kg/cm^2) using 5-mm flat punches. The formulation is shown in Table 1.

Preparation of conventional release layer

Rosuvastatin and other excipients were sifted through sieve no 40# and thoroughly mixed in a mortar for 10 minutes to obtain uniform distribution of the drug in formulation. The powder mixture was lubricated for 2 min with magnesium stearate.

Preparation of bilayer tablets was semi-automatic procedure as the die filling was done manually and compression automatically. First layer composed of diltiazem was pre-compressed as 400 mg of tablet then the already weighed amount of 100 mg of rosuvasatin blend was fed into the die on sustained release layer and compressed over it till the desired hardness was achieved (7-8 kg/cm^2). The formulation is shown in Table 2.

HPMC that is commonly used in hydrophilic matrix drug delivery systems was used as sustained release material in concentration between 35-50%. HPMC not only prolonged drug release but the flow property and binding

capacity of both the layers was also affected by its low and high concentration.

Combinations of drug and EC with HPMC in different ratio were tried. Although, incorporation of EC controlled drug release to some extent, the inclusion of this polymer in formulation increased the release of the drug. The reason might be that its large hydrophobic molecules imposed a discontinuity in the gel-structure leading to formation of a weaker barrier than the HPMC gel alone.

Optimization of tablet formulation using variable concentration of HPMC K100M and EC as matrixing agent

Tablets containing different excipients were prepared as per the composition given in Table 3. All the prepared tablets were evaluated for *in vitro* dissolution behavior. For preliminary trial HPMC K100M, ethyl cellulose was used, at different concentration level.

Table 1. Composition of preliminary trial batches of diltiazem hydrochloride (sustained layer)

Ingredients	**Formulation code**					
	D1 (mg)	D2 (mg)	D3 (mg)	D4 (mg)	D5 (mg)	D6 (mg)
Diltiazem HCl	120	120	120	120	120	120
HPMC K100 M	140	160	180	200	150	100
Ethyl Cellulose	-	-	-	-	50	100
Microcrystalline Cellulose (MCC)	120	105	87	68	63	55
PVPK30	10	5	3	2	7	15
Mg stearate	5	5	5	5	5	5
Talc	5	5	5	5	5	5

Table 2. Composition of preliminary trial batches of rosuvastatin calcium (conventional layer)

Ingredients	**Formulation code**			
	R1(mg)	R2(mg)	R3(mg)	R4(mg)
Rosuvastatin calcium	10	10	10	10
Microcrystalline Cellulose (MCC)	75	39	27	27
Lactose	-	39	53	56
PVP K-30	8	5	3	-
Magnessium hydroxide	5	5	5	5
Magnessium Stearate	1	1	1	1
Talc	1	1	1	1

Evaluation of bilayer tablet

Pre-compression evaluation

Prior to compression, the powder blends were evaluated for their flow and compressibility properties like angle of repose, bulk density, tapped density, compressibility Index and Hausner's ratio. The evaluation parameters were studied before and after the addition of MCC and lubricants to check and compare the inherent flow properties of the powder.

The binding capacity is the binding of both layer (conventional and sustained) was evaluated in terms of compression force required to compress the outer layer (conventional layer) to the internal layer (sustained layer). The second layer of rosuvastatin was compress on the first layer and compression force required was measured on tablet machine, if more compression force on tablet machine is required to compress means less binding capacity of second layer to the first layer.

Post-compression evaluation

After compression of powder blend the tablets were evaluated for weight variation, thickness, hardness, friability

Swelling and erosion studies

The swelling behavior of dosage form can be measured by studying its dimensional changes, weight gain or water uptake ability. The water uptake study of the dosage form was conducted by using Type II USP dissolution apparatus in 900 mL of distilled water which was maintained at 37±0.5^{0}C and rotated at 50 rpm (Figure 1). At selected intervals, the tablet was withdrawn and blotted with absorbent tissue to remove any excess dissolution medium on the surface and weighed. Percentage swelling of the tablet was expressed as percentage water uptake (%WU) calculated from following equation:

Degree of swelling (% water uptake) = [(Wt-W0)/W0] x 100

Table 3. Compositions of preliminary trials of bilayer tablet

Conventional Release Layer						
Ingredients	F1 (mg)	F2 (mg)	F3 (mg)	F4 (mg)	F5 (mg)	F6 (mg)
Rosuvastatin calcium	10	10	10	10	10	10
MCC	27	27	27	27	27	27
Lactose	56	56	56	56	56	56
Magnesium Hydroxide	5	5	5	5	5	5
Magnesium stearate	1	1	1	1	1	1
Talc	1	1	1	1	1	1
Sustained Release Layer						
Diltiazem HCl	120	120	120	120	120	120
HPMC K100 M	140	160	180	200	150	100
Ethyl Cellulose	-	-	-	-	50	100
MCC	120	105	87	68	63	55
PVPK30	10	5	3	2	7	15
Mg stearate	5	5	5	5	5	5
Talc	5	5	5	5	5	5

Where, Wo is the initial weight of the dry tablet, and Wt is the weight of the wet, swollen tablet.

Matrix erosion was determined after completion of swelling studies, on the same tablets used for the swelling determinations. After weighing, the hydrated matrices were dried in an oven at 50^0C for 24hrs and the remaining dry weight Wr, was determined. Matrix erosion was calculated according to the formula: (1, 25-26)

Erosion (% mass loss) = [(W0-Wr)/ W0]x100

Spectroscopic measurements of drugs

At 240.80 nm (zero crossing point of rosuvastatin), diltiazem gives absorbance whereas at 236.2 nm (zero crossing point of diltiazem), rosuvastatin gives absorbance hence the wavelengths 240.8 nm and 236.2 nm were selected as analytical wavelengths for determination of diltiazem and rosuvastatin, respectively. These two wavelengths were used for the estimation of diltiazem and rosuvastatin without any interference from the other drug and excipients in their combined formulation. The overlain spectra for standard rosuvastatin and diltiazem are shown in Figure 2. The overlain first derivative spectrum is shown in Figure 3.

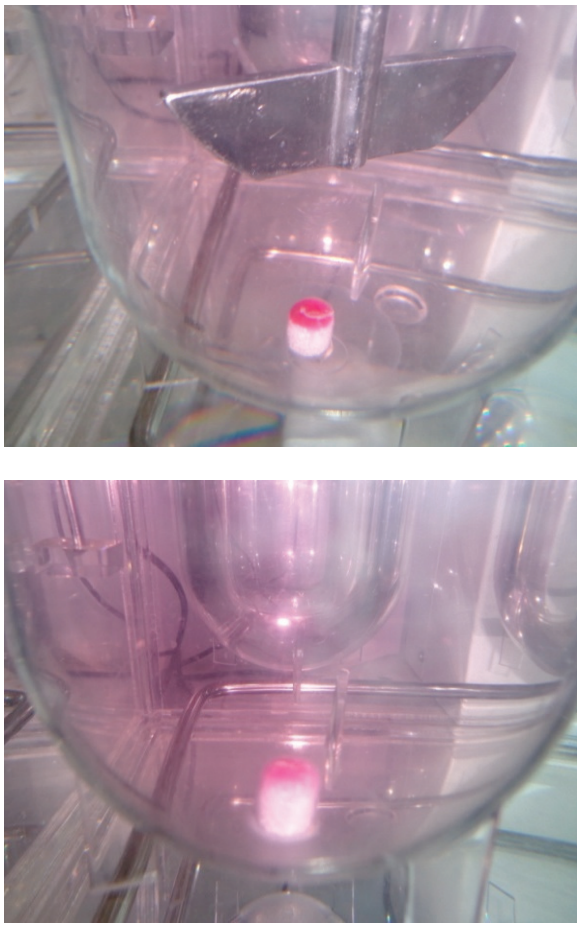

Figure 1. Axial swelling of bilayer tablet

Uniformity of drug content of diltiazem HCl

Ten tablets were selected randomly and average weight was calculated. Tablets were crushed in a mortar and accurately weighed amount of average tablet was taken from the crushed blend. Then, the samples were transferred to 100 mL volumetric flasks and were diluted up to the mark with pH 6.8 Phosphate buffer solution. The content was shaken periodically and kept for one hour to dissolve drug completely. The mixtures were filtered and appropriate dilutions were made. The drug content in each tablet was estimated at λ_{max} 236.2 nm by simultaneous method against blank reference and reported. The range of content uniformity was 90-95%.

Uniformity of drug content of rosuvastatin calcium

Ten tablets were selected randomly and average weight was calculated. Tablets were crushed in a mortar and accurately weighed amount of average tablet was taken from the crushed blend. Then, the samples were transferred to 100 mL volumetric flasks and were diluted up to the mark with methanol. The content was shaken periodically and kept for one hour to dissolve of drug completely. The mixtures were filtered and appropriate dilutions were made. The drug content in each tablet was estimated at λ_{max}=240.8 nm against blank reference and reported. The range of content uniformity is 90-100%.

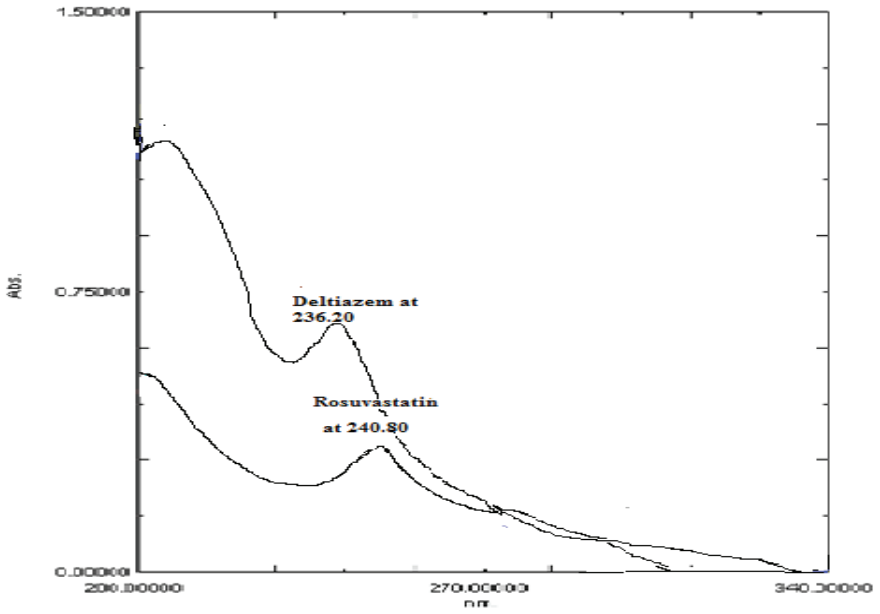

Figure 2. Overlain spectra for standard rosuvastatin and diltiazem

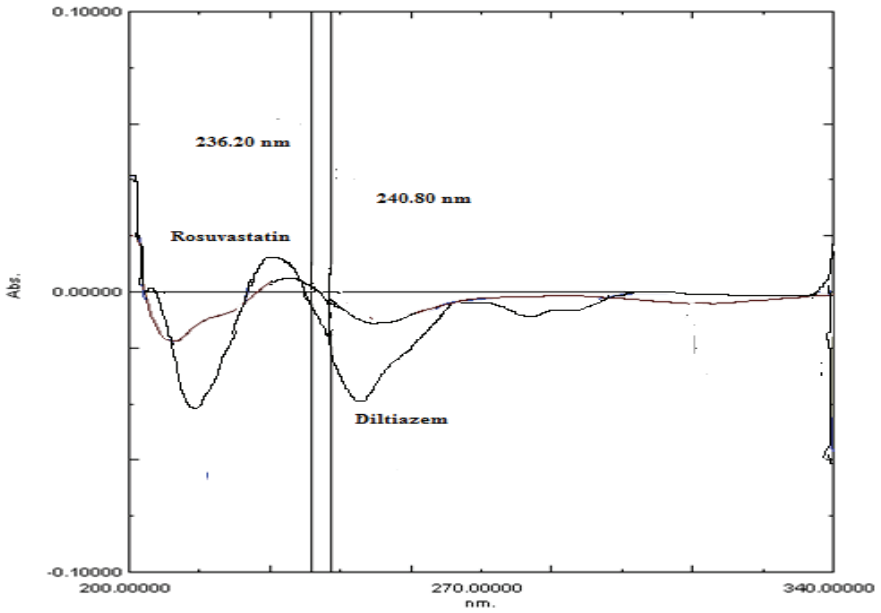

Figure 3. Overlain first derivative spectrum of rosuvastatin and diltiazem

In vitro drug release studies

The release rate of rosuvastatin calcium and diltiazem hydrochloride bilayer tablets was determined using dissolution testing apparatus II (paddle method). The dissolution test was performed using 900 mL of pH 6.8 Phosphate buffer solution, at 37 ± 0.5°C and 50 rpm. A sample (5 mL) of the solution was withdrawn

from the dissolution apparatus at intervals of 15, 30, 45, 60, 90….up-to 12 hours, and the samples were replaced with fresh dissolution medium. The samples diluted to a suitable concentration with pH 6.8 Phosphate buffer solution. Absorbance of these solutions was measured at 240.8 and 236.2 nm by simultaneous method using a Shimadzu UV-1800 UV/Visible spectrophotometer. Cumulative percentage of drug release was calculated using the equation obtained from a standard curve.

Mathematical modelling

To study the release kinetics, data obtained from in-vitro release study of diltiazem HCl was tested with Zero order release kinetic equation, First order release kinetic equation, Higuchi release model and Korsmeyer and Peppas kinetics equation (9,26-28).

Accelerated stability study of bilayer tablet

Stability is defined as the ability of a particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic, and toxicological specifications. Drug decomposition or degradation occurs during storage, because of chemical alteration of the active ingredients or due to product instability, lowering the concentration of the drug in the dosage form. The optimized formulations was stored in aluminium capped clear glass vials and were subjected to a storage condition of 40°C±2^{0}C/ 75%±5% RH for 3 months in humidity chamber. The samples were withdrawn and evaluated for hardness, friability, disintegration time, drug content and *in vitro* dissolution study (1, 28).

Similarity and difference factors

The similarity factor (f2 factor) was used to compare dissolution profiles of diltiazem before and after the stability studies. The *in vitro* release profiles of the formulations before the stability studies were considered as reference and the profiles after the stability studies were considered as test. A model independent approach was used to estimate the dissimilarity factor (f1) and similarity factor (f2) to compare the dissolution profile of optimized formulation (D1R4). The difference factor (f1) calculates the percent difference between the reference and test curve at each time point and is a measurement of the relative error between two curves.

$$f1 = \{[S_{t=1}^{n} |R_t - T_t|] / [S_{t=1}^{n} R_t]\} \times 100$$

The similarity factor (f2) is given by the following equation:

$$f2 = 50 \times \log \{[1 + (1/n) S_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\}$$

Where n is the number of pull points, Rt is the reference batch profile at time point t and Tt is the test batch profile at the same time t. The FDA suggested that two dissolution profiles were declared similar if f2 value between 50-100 and f1 was 0-15.

RESULTS AND DISCUSSION

Rosuvastatin calcium and diltiazem hydrochloride was identified by FT-IR study and the prominent peaks are obtained at 1543 cm^{-1}(stretching of carboxylate salt), 1378.74 cm^{-1} (presence of sulfonyl group), 1196-1150 cm^{-1} (C-O stretching of alcohol), 950 cm^{-1} , 844.29 cm^{-1} and 632.43 cm^{-1} (aromatic C-H bending) for rosuvastatin and at 1738.19 cm^{-1}, 1674.73 cm^{-1} (C=O stretching of carbonyl group), 1206.29-1173.61 cm^{-1} (C-N stretching), 833.49-635.31 cm^{-1} (aromatic C-H bending) for diltiazem. Therefore, based on the above mentioned wave numbers (cm^{-1}) different functional groups are characterized in the finger print region of IR spectrum of the pure API (Rosuvastatin and Diltiazem) and are in turn compared with the IR spectrum of the formulation blend (API+excipients) to check whether there is a shift in the prominent peaks of the API in the presence of excipients. The graph (Figures 4-6) suggests that there were no significant changes in the prominent peaks of both the drugs i.e rosuvastatin as well as diltiazem in the presence of excipients. Therefore, it may be concluded that the given list of excipients are very much compatible with both the drugs.

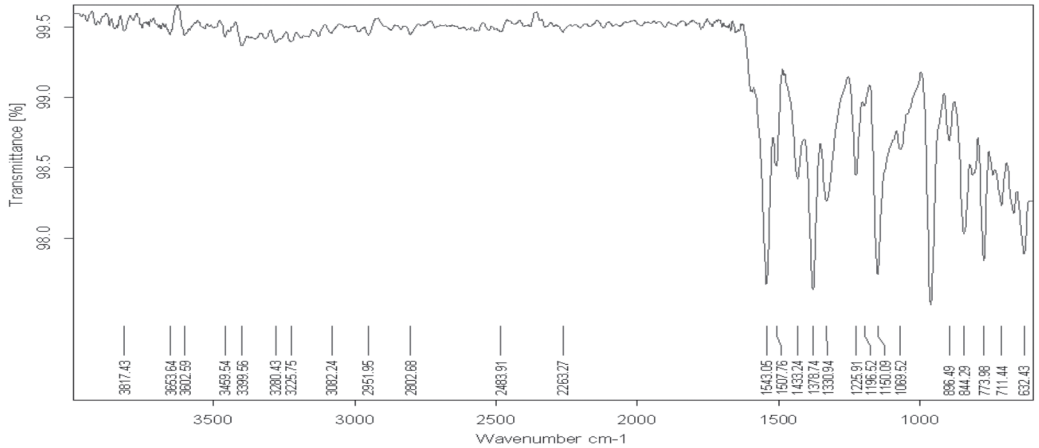

Figure 4. Fourier transform infrared spectra of rosuvastatin calcium

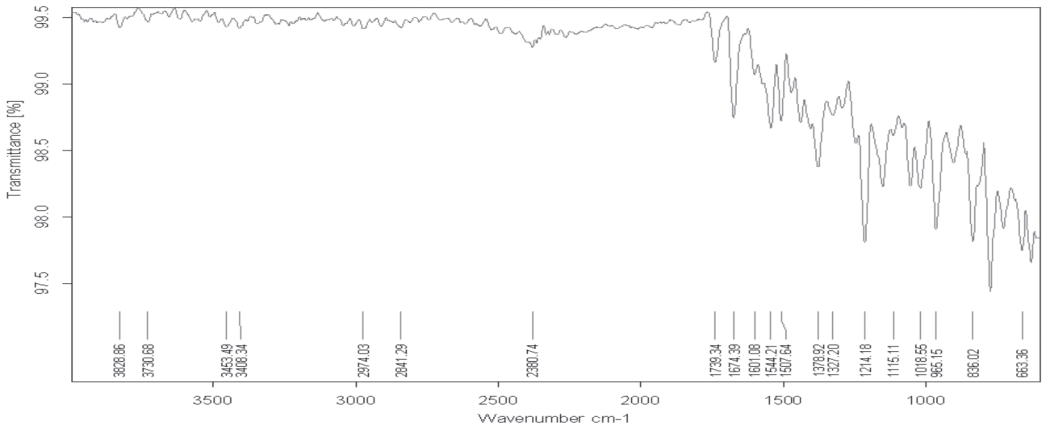

Figure 5. Fourier transform infrared spectra of diltiazem HCl

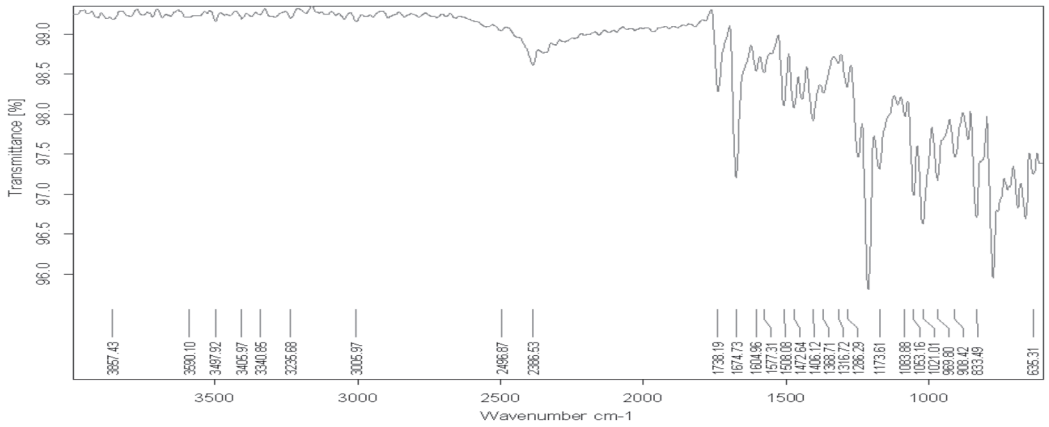

Figure 6. Fourier transform infrared spectra of rosuvastatin calcium and diltiazem HCl with excipients

Effect of hydrophilic polymer (HPMC K100M)

HPMC that is commonly used in hydrophilic matrix drug delivery systems. The prepared tablets were not disintegrate, however a gel layer was formed on surface of the tablet due to swelling of HPMC in presence of water. Here concentration of HPMCK100M was kept (35-50%). Formulations containing HPMC K100M (35-45%) showed delayed release up to only 12 hours but HPMC K100M (50%) expected for delayed release up-to 24 hours. This revealed that as concentration of HPMC increased release rate of drug was sustained. HPMC K100M tablets exhibited significant effect on drug release; it is due to more viscosity and high molecular weight of HPMC K100M in addition to its slower rate of erosion and more swelling. HPMC not only prolonged drug release but the flow property and binding capacity of both the layers was also affected by its low and high concentration. In batch F4 it was found that diltiazem drug release was extended up to 24 hour with good binding capacity. Therefore from pre-compression and post-compression evaluation F4 was an optimized formula.

Effect of hydrophobic polymer (EC)

Combinations of drug and EC with HPMC in different ratio were tried. Ratio of EC: HPMC in 1:3 shown better release up to 24 hours than with EC: HPMC (1:1). Although, incorporation of EC controlled drug release to some extent, the inclusion of this polymer in formulation increased the release of the drug. The reason might be that its large hydrophobic molecules imposed a discontinuity in the gel-structure leading to formation of a weaker barrier than the HPMC gel alone. The prepared tablet is shown in Figure 7.

Pre-compression evaluation

The evaluation parameters were studied before and after the addition of MCC and lubricants to check and compare the inherent flow properties of the powder. Flow property was evaluated by measuring angle of repose, Hausner's ratio and compressibility index and it was found that blend of the different formulations (R1-R4) and (D1-D6) shown good flow property. Results are shown in Table 4, 5.

Figure 7. Bilayer tablets of rosuvastatin calcium and diltiazem HCl

Optimization of conventional and sustained release layers

Layers were optimized for final formulation on the basis of pre-compression evaluations of the blends and their binding capacity. Correct bonding is only obtained when the first layer is compressed at a low compression force so that this layer can still interact with the second layer during final compression of the tablet. Therefore compression force was optimized on trial basis in terms of hardness obtained after pre-compression (3-4 kg/cm^2) and final compression (7-8 kg/cm^2). It was found that R1, R2,R3 have good flow property but they are fail to bind with D1,D2,D3 and D6 without using binding agent, only R4 had ability to bind with D4 and D5 but it also shown variability and discontinuity in binding during the final formulation. Only R4D5 was found to have good flow property

and binding capacity at optimum compression force without the need of binding agent (shown in Table 6).

Post-compression evaluation

The mean thickness, diameter, hardness weight variation of the tablets were almost uniform for all the formulations and the friability value was below 1% indicating that tablets of all formulation having good strength. The drug content of rosuvastatin calcium in Phosphate buffer solution (pH 6.8) was in the range of 91-93% and the drug content of diltiazem hydrochloride in phosphate buffer solution (pH 6.8) was in the range of 90-95%. The results obtained are shown in Table 7.

Table 4. Pre-compression evaluation parameters for sustained layer of diltiazem HCl

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Compressibility index %	Hausner's ratio	Angle of repose
D1	0.379	0.422	10.2%	1.12	33.4
D2	0.408	0.472	13.5%	1.16	31.5
D3	0.399	0.462	13.6%	1.16	34.7
D4	0.389	0.457	14.8%	1.17	32.6
D5	0.346	0.476	27.3%	1.38	48.4
D6	0.405	0.468	13.4%	1.16	31.0

Table 5. Pre-compression evaluation parameters for conventional layer of rosuvastatin calcium

Formulation code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Compressibility index %	Hausner's ratio	Angle of repose
R1	0.536	0.595	10.0%	1.11	29.1
R2	0.520	0.592	12.2%	1.14	29.8
R3	0.516	0.587	12.1%	1.14	30.5
R4	0.507	0.579	12.4%	1.14	31.6

Table 6. Optimization of conventional and sustained release layers

Formula	MCC (%)	Lactose (%)	Binding capacity	PVP (%)	First compression force (in hardness (kg/cm^2)	Second compression force(in hardness (kg/cm^2)	Cumulative drug release (CDR) %
R1D4	75	-	low	8	4.3	10.4	87%in 300 min
R2D4	39	39	low	5	4.2	10.1	90%in 300 min
R3D4	27	53	moderate	3	4.3	9.62	90%in 270 min
R4D4	27	56	very good	-	4.2	7.42	90%in 150 min
R1D5	75	-	low	8	4.3	11.0	89%in 300 min
R2D5	39	39	low	5	4.2	10.6	92%in 300 min
R3D5	27	53	moderate	3	4.1	9.24	94%in 270 min
R4D5	27	56	good	-	4.3	7.59	90%in 135 min

Swelling index (% Water uptake study) and % erosion

The swelling index was calculated with respect to time. With increasing time, the swelling index was increased. This is because of the weight gain by tablets proportionally with increased rate of hydration. After 10 hours a gradual decrease in the swelling index was seen due to dissolution of outermost gelled layer of tablet into dissolution medium. From the results it was concluded that the release profile of the drug was influenced by this swelling. A constant release was observed due to the increase in diffusional path length caused by swelling. % Erosion was more in F5 than F4 because of the presence of ethyl cellulose with HPMC K100M in the formulation. The observations are shown in Table 8.

In vitro drug release and kinetic modeling

The release profile of sustained release layer of diltiazem HCl of two optimized formulations F4 and F5 were compared with Zero order, First order, Higuchi model, Korsmeyer-Peppas model. (Table 9, Figure 8-9).The data suggested that the release kinetics of Diltiazem from F4 follow Higuchi model because the value of regression coefficient (R^2) for Higuchi model was higher as compared to Zero order, Firstorder and Korsmeyer-Peppas Model, whereas the release kinetics of diltiazem from F5 follow Korsmeyer-Peppas Model, because the value of regression coefficient (R^2) was higher as compared to other kinetic models. It confirmed that products F4 followed Fickian kinetics n=0.57. In case of F5 exhibited non-Fickian (anomalous) diffusion behavior (n=0.61).

Table 7. Evaluation of post-compression parameters of developed formulations

Formulation code	Weight variation (mg) n=20	Thickness (mm) n=6	Diameter (mm) n=6	Hardness (kg/cm^2) n=6	% Friability n=10	Mean drug content %
F1	519±0.97	3.92±0.79	9.04±1.23	6.02±0.67	0.58±1.08	94.61±0.82(R) 91.24±0.64(D)
F2	521±1.09	3.97±0.73	9.03±1.31	6.34±0.36	0.52±1.11	95.72±0.73(R) 92.38±0.68(D)
F3	518±1.04	4.01±0.87	9.04±1.25	6.79±0.49	0.49±1.17	93.84±0.93(R) 89.93±0.85(D)
F4	511±0.98	3.99±0.93	9.05±1.19	6.82±1.03	0.43±1.23	95.49±0.69(R) 93.66±0.71(D)
F5	516±1.02	4.00±0.89	9.02±1.18	6.91±1.19	0.51±1.19	94.16±0.94(R) 93.73±0.88(D)
F6	523±1.11	3.99±0.67	9.05±1.21	7.14±0.43	0.56±1.24	95.11±0.61(R) 92.82±0.75(D)

The result shows that batch F4 and F5 are optimized batch as having good drug content.

Table 8. Swelling index (%water uptake study) and % erosion

Form. code	Swelling Index							% Erosion after 12hrs
	Time in hour							
	1	**2**	**4**	**6**	**8**	**10**	**12**	
F4	52.4	64.4	79.8	93.8	98.6	105.2	99.1	24.3%
F5	43.1	57.3	68.3	84.6	89.4	94.2	92.7	33.8%

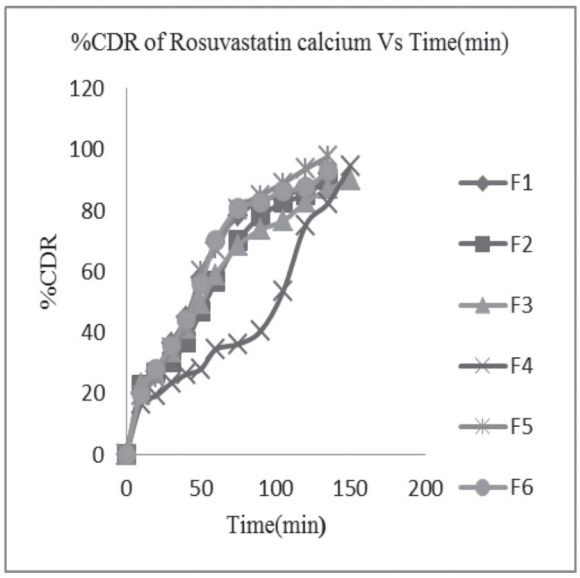

Figure 8. Comparative *in vitro* drug release profile of rosuvastatin calcium in phosphate buffer solution (pH 6.8)

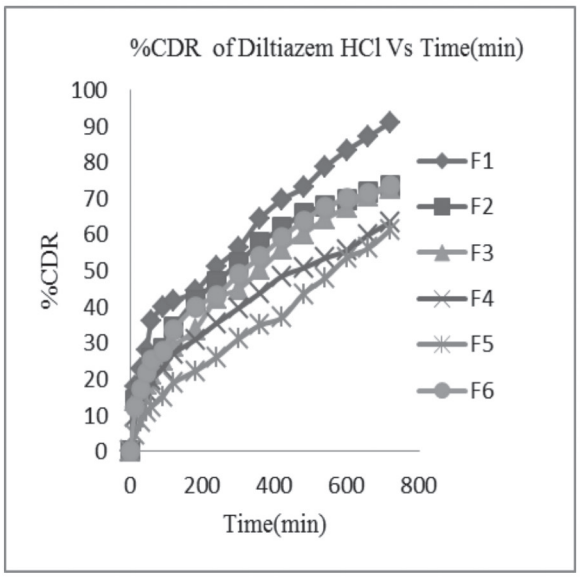

Figure 9. Comparative *in vitro* drug release profile of diltiazem HCl in phosphate buffer solution (pH 6.8)

Table 9. Kinetic modeling of optimized formulations F4 and F5

Formulation Code	Zero-order		First order		Higuchi model		Korsmeyer-Peppas			Best fit
	R^2	K_0	R^2	K_1	R^2	K_H	R^2	K_{Kp}	n	
F4	0.940	0.0757	0.963	.00094	0.998	2.29	0.974	1.53	0.579	Higuchi model
F5	0.986	0.0776	0.958	.00095	0.976	2.26	0.997	0.973	0.616	Peppas model

Stability studies of optimized formulations

The optimized formulations was stored in aluminium capped clear glass vials and were subjected to a storage condition of 40 ± 2 ^{0}C / 75 ± 5 % RH for 3 month in humidity chamber. The samples were withdrawn and evaluated for physical appearance, hardness, friability, disintegration time, drug content and *in-vitro* dissolution study all the formulations showed no significant variation in all the data after completion of stability study (Tables 10,11, Figures 10,11).

Similarity factor and difference factors

Similarity (f2) and difference factors (f1) for F4 and F5 are shown in Table 12. All formulations showed (f2) value between 50 to100 and (f1) value below 15 indicating similar release profiles of the formulations before and after stability studies.

CONCLUSION

Bilayer matrix tablets of rosuvastatin calcium and diltiazem HCl was formulated successfully. HPMC K100M can be used for retarding drug release from matrix tablets. In-vitro dissolution studies showed release of rosuvastatin calcium from all the formulations (F1-F6) within 135-150 min formulated conventionally without modifying drug release time with the help of polymers or superdisintegrants. Although rosuvastatin was formulated to release conventionally but it was expected to give

Table 10. Stability data for optimized formulation batches (F4 & F5)

Formulation Batch	Parameters	Time interval (Months)							
		0		**1**		**2**		**3**	
F4	Hardness Kg/cm^2)	6.75±0.87		6.87±0.33		6.77±0.45		6.82±0.47	
	Friability (%)	0.41±0.82		0.51±0.72		0.47±0.91		0.38±0.71	
	Swelling index (%water uptake study) after 12 hr	99.2		102.4		99.5		98.9	
	% Erosion	25.4		24.4		26.8		23.8	
	% Drug content	R	D	R	D	R	D	R	D
		95.6	92.9	94.3	93.7	95.3	92.6	93.2	92.5
F5	Hardness (kg/cm2)	6.64±0.38		6.75±0.46		6.72±0.54		6.61±0.51	
	Friability (%)	0.56±0.74		0.66±0.69		0.47±0.62		0.52±0.92	
	Swelling index (%water uptake study) after 12 hr	93.1		92.7		92.4		93.2	
	% Erosion	32.6		34.7		31.2		33.0	
	% Drug content	R	D	R	D	R	D	R	D
		94.7	93.3	93.7	92.9	93.4	91.5	94.3	92.5

Table 11. Dissolution profile during stability study for optimized formulations batch (F4 & F5)

% CDR of Diltiazem hydrochloride								
Month→	0		1		2		3	
Time(hr)↓	F4	F5	F4	F5	F4	F5	F4	F5
1	13.2	15.6	14.8	14.0	13.6	15.1	14.2	14.5
2	24.8	22.8	23.6	20.6	21.7	21.7	22.8	19.8
3	29.9	28.8	27.0	24.4	25.5	26.2	25.0	25.8
5	37.4	40.6	36.5	37.0	33.7	39.4	35.4	38.6
8	49.5	55.4	48.2	54.2	44.0	55.1	47.5	54.3
12	60.1	66.8	59.0	64.7	59.5	66.2	59.2	65.1

Table 12. Similarity and difference factors of F4 and F5 before and after stability study

Time(hr)	**Drug release before stability study(Rt)**		**Drug release after stability study(Tt)**		f_2	f_1	f_2	f_1
n					**(F4)**	**(F4)**	**(F5)**	**(F5)**
	F4	F5	F4	F5				
1	19.9	12.5	14.2	14.5	70.9	7.6	61.7	10.0
2	21.6	19.0	22.8	19.8				
3	25.4	22.3	25.0	25.8				
5	39.7	31.2	35.4	38.6				
8	50.8	43.7	47.5	54.3				
12	63.5	61.4	59.2	65.1				

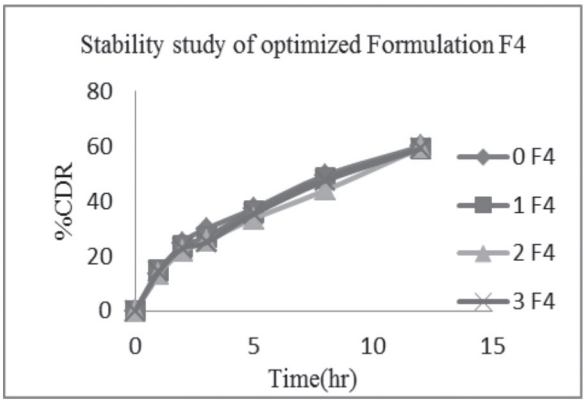

Figure 10. *In vitro* drug release profile during stability study for optimized formulations F4

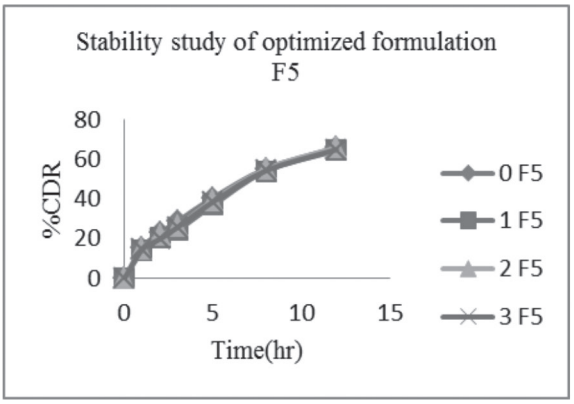

Figure 11. *In vitro* drug release profile during stability study for optimized formulations F5

sustained effect due to its long half-life (19 hrs) therefore using this pharmacokinetic property of rosuvastatin once a day Bilayer tablet was formulated with diltiazem HCl by sustaining its release for 24 hr with the help of HPMC K100M. There was no evidence of effect of one drug on the release pattern of another. The *In vitro* release study of all the formulations for 12 hours revealed that F1, F2, F3 and F6 sustained the release of drug only for 12 hour and the optimized formulations F4 and F5 can sustain the release up to 24 hour as per USP limit. The data obtained from the release kinetics of F4 fitted with Higuchi model indicated that the release of drug from the tablets was found to depend on the square root of time showed that the release mechanism was Fickian diffusion with slower erosion rates whereas F5 followed non-fickian diffusion. Stability study was conducted for 3 months. Similarity (f2) and difference factors (f1) for K4 and K5 were calculated. All formulations showed (f2) value between 50 to100 and (f1) value below 15 indicating similar release profiles of the formulations before and after stability studies. There was no significant variation in the Physical appearance, hardness, friability and *in-vitro* dissolution profiles for the optimized formulations F4 and F5. Although both the formulations shown sustained effect up to 24 hr but comparative dissolution profile of F4 and F5 shown that F5 have more sustained effect on diltiazem than F4 and also it released rosuvastatin from conventional layer only within 135 min than F4 which released the drug within 150 min. In conclusion, the objective of formulation development and evaluation of bilayer tablet of rosuvastatin calcium and diltiazem hydrochloride had been achieved.

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