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FORMULATION, OPTIMIZATION AND SIMULTANEOUS DETERMINATION OF ATORVASTATIN CALCIUM AND LOSARTAN POTASSIUM IN PURE AND BILAYER TABLETS

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ABSTRACT

The present study plans to formulate and analyze bilayer tablets of atorvastatin-Ca and losartan- K. ***In vitro*** release of atorvastatin-Ca from the fast dissolving layer was enhanced by complexation through co-grinding method with sulfobutyl ether-β-cyclodextrin (SBE7-β-CD; Captisol). The release of losartan-K from the sustained layer was obtained using two polymers, compritol 888 and carbopol 971. Quality by Design (QbD) was applied to determine material and critical quality attributes. D-optimal design was also applied for each layer to study the effect of independent variables on the chosen responses. Consequently, the two drugs in their pure form, each in its pharmaceutical formulation or both in the new formulated bilayer tablets were analytically evaluated using two simple and accurate methods. The first is an ultra-performance liquid chromatographic method (UPLC) in which separation was achieved on a C_{18} column using 1% *o*-phosphoric acid - acetonitrile (55:45 v/v) as mobile phase and a flow rate of 1 mL min^{-1}. Quantification was achieved over the concentration range1-10 µg mL^{-1} for each drug with mean recoveries of 100.59% ± 1.17 and 100.24% ± 0.90 for atorvastatin – Ca and losartan- K, respectively. The second method is a high performance- thin layer chromatographic one (HPTLC) for the simultaneous determination of both drugs. Chromatographic separation was performed on silica gel 60 F_{254} plates, with acetonitrile-chloroform-methanol-conc. ammonia (7:2:0.9:0.1 by volume) as a developing system followed by densitometric determination at 241 nm. The separation was achieved over the concentration range of 0.5-5 and 1-8 µg /spot with mean recoveries of 100.64% ± 1.29 and 98.18% ± 0.69 for atorvastatin-Ca and losartan-K, respectively. Results were statistically analyzed and found to be in accordance to those obtained by a reported method. The two methods were found to be validated according to ICH guidelines.

Keywords: bilayer tablets, atorvastatin-Ca, losartan- K, Quality by Design (QbD),

1. INTRODUCTION

In the last decade, interest in developing a combination of two or more Active Pharmaceutical Ingredients (API) in a single dosage form as a bilayer tablet has increased in the pharmaceutical industry, promoting patient convenience and compliance[1].
Aatorvastatin-Ca;([R-(R*,R*)]-(2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl–4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid calcium salt (2:1) trihydrate) is a synthetic lipid- lowering agent. While losartan –K; 1,2-n-butyl-4-chloro-1-[p-(o-1H-tetrazol-5-ylphenyl)benzyl]-imidazole-5- methanol mono potassium salt[2] is a strong antihypertensive agent. Several analytical techniques have been reported for the determination of atorvastatin-Ca and losartan –K including UPLC[3,4], HPLC[5-8], HPTLC[9-11] and spectrophotometery[12-15].

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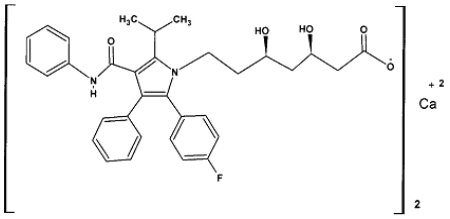
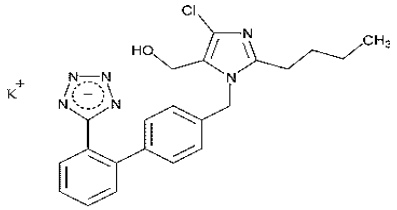
The same techniques have also been used for the simultaneous determination of the two drugs[16-20]. Due to limited oral bioavailability of atorvastatin-Ca[21], complexation with cyclodextrins as Captisol was adapted to improve its solubility and hence its bioavailability, to be used as oral fast release tablet. Whereas, losartan - K with a low elimination half life (1.5 to 2.5 hours) was suitable for oral sustained release tablet using two polymers, compritol 888 and carbopol 971 so as to maintain the plasma concentrations of the drug well above the therapeutic concentration. Captisol (SBE_7 β-CD) is a polyanionic ß-cyclodextrin derivative with a sodium sulphonate salt, it does not exhibit the nephrotoxicity associated with β-cyclodextrin[22]. Compritol 888 is composed of a well-defined mixture of mono-, di- and triglycerides of be henic acid. It is chemically known as glycerylbehenate, a hydrophobic polymer which can be used as glyceride bases for potential applications as lipidic binders to develop dosage forms with sustained-release properties [23]. Carbopol 971 is a crosslinked acrylic polymer which has the potential to

extend the release of drugs from gastroretentive delivery systems[24]. Thus, the aim of the present work is to prepare bilayer tablets of atorvastatin-Ca as a fast release layer and losartan -K as a sustained layer to reduce the frequency of administration and improve patient compliance. The new formulation was then used for the analysis of the two drugs simultaneously by UPLC and HPTLC methods.



Atorvastatin-Ca



Losartan –K

Fig.1: Chemical structure of atorvastatin-Ca and losartan –K

2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Materials

Atorvastatin- Ca, losartan- K and magnesium stearate were kindly provided from Egyptian International Pharmaceutical Co. (EIPICO) (Egypt). Sulfobutylether-β-cyclodextrin (Captisol) was provided from Ligand Pharmaceuticals, Inc. (USA). Compritol 888 was from Gattefossé (France) and Carbopol 971 from Lubrizol Advanced Materials, Inc. (USA). Anhydrous lactose, methanol, chloroform and Ortho-Phosphoric acid are obtained from Sigma -Aldrich (Germany), Acetonitrile HPLC grade is obtained from Fisher (UK).

2.2. Instrumentation

A Shimadzu thermal analyzer (Shimadzu, Kyoto, Japan) was used along with a United States Pharmacopeia (USP)-standard dissolution apparatus (Model DA-6D) (Veego, Bombay, India). Tableting was done using an EK:O tableting machine (Erweka, Frankfurt, Germany), Differential scanning calorimeter, (model Mettler DSC60, Switzerland) and pye Unicam SP 1000 IR Spectrophotometer (type pw3710, Holland) were also used. The UPLC system used was an Agilent 1290 UPLC with binary pump and UV detector, analysis was performed on a Phenomenex C $_{18}$ column (100 mm, 4.6 mm i.d., 2.6 µm) ; USA.

TLC plates used were 20 cm x 20 cm precoated with silicagel 60 F 254 (Flukachemie, Switzerland), a camag Linomate 5 sample applicator equipped with a 100 µL syringe (Hamilton,Germany) 20cm x 10 cm twin through glass chamber (Camag). The plate was scanned with a camag TLC scanner 3 with WINCATS computer software (Switzerland) using UV lamp with short wavelength (254 nm) (Desega- Germany).

2.3. Methods

Application of Quality by Design principles

Determination of material attributes, critical quality attributes (CQAs) and critical process parameters (CPP) for the preparation of fast and sustained release layers.

Materials used for the preparation of fast release layer were captisol and anhydrous lactose. Captisol was used in the preparation of drug complex to increase the solubility of atorvastatin. Anhydrous lactose was also used to increase the tablet disintegration. Critical Quality Attributes (CQAs) are a physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit, range or distribution to ensure desired product quality.

For both layers, drug release from each layer was the critical parameter to be considered. A process parameter is the process whose variability has an impact on a critical quality attribute and therefore should be monitored or controlled to ensure that process produces the desired quality[25]. The critical process parameter (CPP) in both layers was method of preparation; it was either by physical mixture or co-grinding methods in the fast release layer and by hot fusion method or simple direct compression method in the sustained release layer.

Application of design expert to study the effect of different variables

Once CQAs have been identified, the next step is to gain a comprehensive understanding of the way variation impacts the quality of output at each stage of the drug process. To evaluate the effect of material attributes and critical process parameter on the critical quality attributes, a three factors, D-optimal design was employed. All process inputs were shown in Table 1.

Table1: Experimental domains and coding of the variables

Fast release layer of Atorvastatin-Ca			*Sustained release layer of Losartan-K*		
Variables Levels			Variables Levels		
-1	0	+1	-1	0	+1
Material attributes			**Material attributes**		
Drug:Captisol ratio			Amount of compritol 888		
(X_1) 1:1	1:2	1:3	(X_1) 20	30	40
Amount of anhydrous lactose			Amount of carbabol 971		
(X_2) 0	100	200	(X_2) 0	10	20
CPPs:Critical process parameters			**CPPs: Critical process parameters**		
Method of preparation			Method of preparation		
(X_3) PM		SD	(X_3) DC		HF

Responses (CQAs) Responses (CQAs):

Y: Drug released after 30 minutes, Y_1: Drug released after 2 hours, Y_2: Drug released after 8 hours

Preparation of the fast release layer

An inclusion complexes of atorvastatin and captisol were prepared as obtained from the d-optimal design. Two methods were used; the first one is the physical mixture method in which atorvastatin and captisol were mixed in a mortar for 30 minutes. Anhydrous lactose was added, then talc and magnesium stearate were added as glidant and lubricant, respectively

to the powder blend and mixed for an additional 5 minutes. The second method is the co-grinding method, in which atorvastatin and captisol in different molar ratio were mixed and triturated in glass mortar and pestle for 20 minutes and passed through 80 mesh screen. The resultant powder blends of the two methods were compressed under constant pressure using a single punch tableting machine as shown in Table 2. In an attempt to confirm the complex formation, differential scanning calorimetry and fourier transfer Infrared spectroscopy were studied on the drug, captisol and their complex.

Table 2: Composition of atorvastatin-Ca fast-release tablet layer as obtained from D-optimal design

Ingredients (mg)	F_1	F_2*	F_3*	F_4	F_5	F_6	F_7*	F_8*	F_9*	F_{10}	F_{11}	F_{12}*	F_{13}*	F_{14}
Atorvastatin-Ca	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Captisol	17.6	17.6	17.6	17.6	17.6	17.6	35.2	35.2	35.2	35.2	35.2	52.8	52.8	52.8
Anhydrous Lactose	0	100	200	0	100	200	0	100	200	0	200	0	200	100
Talc	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Magnesium Stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Avicel 102 to	280	280	280	280	280	280	280	280	280	280	280	280	280	280

* Formulations prepared by physical mixture method

Preparation of the sustained release layer

The composition of the sustained-release tablet formulations are presented in Table 3. In the direct compression method, losartan (25 mg) was mixed with compritol 888 and carbopol 971 in glass mortar with the help of pestle for 30 minutes. Then talc and magnesium stearate were added to the powder blend and mixed for additional 5min. In the melt granulation method, compritol 888 was melted in porcelain dish on a water bath maintained at 75°C for three min. Losartan was gradually added to the melted component with continuous stirring till uniformly mixed. The molten mixture was allowed to cool and solidify at room temperature. The solidified mass was crushed in mortar and passed through a 16 mesh sieve. Carbopol 971 was then added and stirred. The resultant powder blend of each method was compressed under constant pressure using a single punch tableting machine.

Table 3: Composition of losartan-K sustained-release tablet layer as obtained from D-optimal design.

Ingredients (mg)	F_1	F_2*	F_3*	F_4	F_5	F_6	F_7*	F_8*	F_9*	F_{10}	F_{11}	F_{12}*	F_{13}*	F_{14}
Atorvastatin-Ca	25	25	25	25	25	25	25	25	25	25	25	25	25	25
Captisol	20	20	20	20	20	30	30	30	30	30	40	40	40	30
Anhydrous lactose	0	10	20	0	10	0	10	0	10	20	0	20	0	10
Talc	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Magnesium Stearate	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Avicel 102 to	100	100	100	100	100	100	100	100	100	100	100	100	100	100

* Formulations prepared by direct compression method

In vitro dissolution study of tablets for each layer

The fast release layer. *In-vitro* dissolution of atorvastatin formulations was studied using a dissolution apparatus with paddles rotating at 75 rpm. The dissolution was performed in900 mL of 0.1N HCl at 37 ± 0.5°C. At fixed time intervals, samples were withdrawn, filtered, and spectrophotometrically assayed for drug content at 245 nm. Percent drug released after 120 minutes was determined.

The sustained release layer. *In-vitro* dissolution of losartan formulations was studied as previously mentioned for 2hrs followed by 6 h study in 6.8 pH phosphate buffer. The filtered samples were spectrophotometrically assayed for drug content at 206 nm. Percent drug released after 2 and 8 hours was determined.

Development of design space and determination of control strategy for the two layers

Design space can be defined as the multidimensional combination and interaction of input variables (e.g. material attributes) and process parameters that have been demonstrated to provide assurance of quality. Regardless of how a design space is developed, it is expected that operation within the design space will result in a product meeting the defined quality.

Control strategy gives us the ability to evaluate and ensure the quality of in-process and/or final product based on process data which typically include a valid combination of measured material attributes and process controls [26].

Preparation and *In vitro* dissolution of bilayer tablet formulation

The bilayer tablets of atorvastatin and losartan in ratios obtained from design space were prepared by direct compression using a single-punch tableting machine. The die was initially filled with the weighed amount of sustained release layer and compressed lightly, then the fast-release layer was added onto the obtained compressed layer and then recompressed together to combine them. The total weight of the bilayer tablet was found to be 380 mg. *In vitro* drug release studies of the bilayer tablets were performed as the same method used for losartan layer with 100 rpm.

Analytical evaluation of atorvastatin- Ca and losartan – K

Preparation of stock solutions

100 mg of each of atorvastatin- Ca and losartan – K were transferred into separate 100- mL volumetric flasks to which about 70 ml of methanol was added. The flasks were sonicated then made up to the mark with the same solvent to obtain a stock solutions of1 mg/mL of each drug.

Chromatographic conditions

UPLC method-Separation was performed on a C18 column, using a mobile phase of 1% O-phosphoric acid-acetonitrile 55:45 % (v/v) at a flow rate of 1 mL/ min. Wavelength of 254 nm was selected for detection.

HPTLC method-Analysis was performed on TLC plates. Before use, plates were washed with the mobile phase consisting of acetornitrile-cholofrom-methanol-conc. ammonium were applied to pre-washed activated plates, as 6-mm bands, 6 mm apart, by means of a Camag Linomat IV automated spray-on band applicator equipped with a 100-µL syringe. The plates were developed with 50 mL of the mobile phase, in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min. The development distance and time were 15 cm and 25 min, respectively. After development, the plates were removed and air dried. Densitometry was performed at 241 nm in reflectance mode. The slit dimensions were 6.00 mm × 0.3 mm and the scanning speed was 20 mm/s.

Linearity

UPLC method- Aliquots of working standard drug solutions (0.1 mg /mL) containing 0.01-0.1mg of either drugs were introduced into two series of 10- ml volumetric flasks and adjusted to the volume with methanol.

Triplicate 10µL injections were made for each concentration and chromatographed under the specified chromatographic conditions described previously. The Peak area of each concentration was then plotted against the corresponding drug concentration.

HPTLC method-Different volumes of stock standard solution (1 mg/mL) containing 0.5-5 mg atorvastatin or 1-8 mg losartan were introduced into a series of 10- ml volumetric flasks, then volume was completed with methanol. L0µl of each flask was applied to the TLC plates, in which separation was performed as previously stated. Peak area of each concentration was then plotted against its corresponding drug concentration.

Application of the proposed methods on formulated bilayer tablets

Ten tablets of the new bilayer tablet containing 10 mg atorvastatin- Ca and 25 mg losartan- K each were finaly powdered, mixed and weighed. An accurately weighed quantity of the powder equivalent to one tablet was introduced into a 100- ml volumetric flask, extracted and diluted to the volume with methanol. The solution was filtered off and analyzed using the proposed UPLC and HPTLC methods. The analytes peaks were identified by comparison with those of respective standard for their retention time. The peak areas were used to calculate the drugs concentrations.

3. RESULTS AND DISCUSSION

In vitro dissolution study of tablets from fast release layer

Figure (2) showed the dissolution profile of atorvastatin layer formulated according to the D-optimal design. Upon studying the effect of the chosen factors on drug release, we can conclude that the first factor which is the ratio of atorvastatin to captisol affected the dissolution rate significantly.

The maximum drug release was obtained in F_{11} (1:2 molar ratio) which showed a complete drug release in the first 45 minutes and F_{14} (1:3 molar ratio) which showed 94 % drug release in the same time. This can be explained on the bases that captisol molecule shows an exterior part with hydrophobic end, so atorvastatin would insert a portion or all of its structure into the hydrophobic cavity and the resultant non covalent association complex becomes highly water soluble.

The second factor which is the presence of anhydrous lactose, it showed an increase in drug release with increasing the amount of lactose, this is due to the high hydrophilicity of the diluent, resulting in faster movement of solvent and easier penetration of dissolution medium into the tablet matrix, leading to faster matrix erosion[27].

Results also showed that co-grinding method showed a marked increment in atorvastatin dissolution compared to the corresponding physical mixtures, probably due to the increase in drug-carrier contact surface as a consequence of the more drastic mechanical treatment during the preparation and further decrease in particle size.

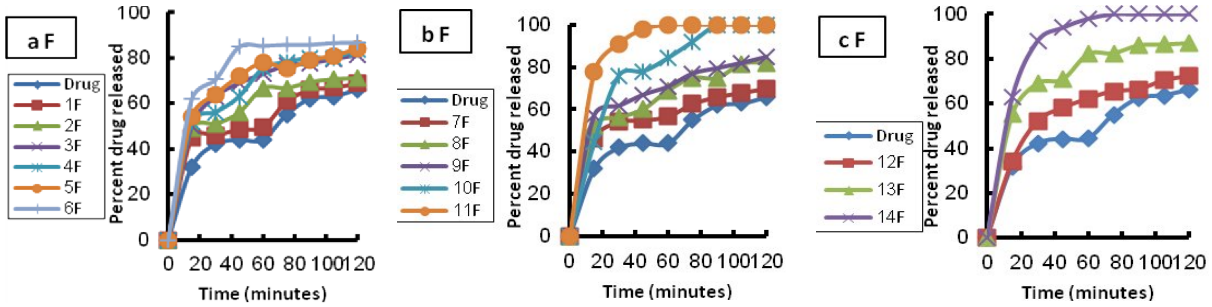


Fig. (2): % Atorvastatin- Ca released from different fast release layers, aF: formulations from 1-6, bF:formulations from 7-11 and cF: formulations from 12-14.

Differential scanning calorimetry (DSC)

The DSC thermogram of atorvastatin- Ca (Fig.3a) revealed a sharp endothermic peak at 158°C corresponding to its melting point. Another smaller and broader peak appeared at 234.3°Cwas probably due to polymorphic impurity. The thermogram of captisol (Fig. 3b) showed two endothermic peaks, the first is broad at 98.37 °C which is related to rehydration of cyclodextrin and the second one is sharp at 265.7°C indicating the beginning of decomposition events. Evidence of complexation was seen in the clear decrease of the drug peak due to its entrapment in the captisol cavity and the decrease of captisol peak at 265.7°C (Fig. 3c).

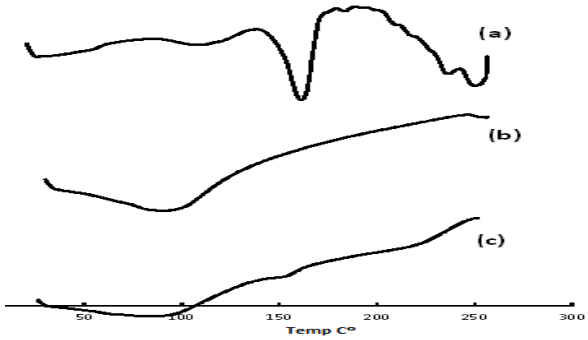


Fig 3: DSC thermogram of (a) atorvastatin- Ca, (b) captisol and (c) inclusion complex

Fourier transfer Infrared spectroscopy (FTIR)

FTIR spectrum of atorvastatin- Ca(Fig. 4a) showed the characteristic peaks at1577.77 cm^{-1}due to C=O stretching, at 3363.86 cm^{-1}due to O-H stretching, 3055.24 cm^{-1} due to aromatic C-H stretching, 1109.07 cm^{-1} due to O-H bending and 2970.38 cm^{-1} due to CH3-O stretching[28]. The spectrum of captisol (Fig.4b) is mainly characterized by intense bands at 3444.87 cm^{-1} due to O–H stretching vibration, at 2929.87 cm^{-1}due to vibration of the –CH group. The band at 1647.21cm^{-1}reflects the δ-HOH bending of water molecules attached to cyclodextrin, whereas the peaks at 1184 $cm^{-1}$for C–H stretching vibrations. In the inclusion complex spectra (Fig. 4c), evidence of strong interaction between drug and captisol was illustrated in the decrease of all peaks of the drug as well as disappearance of the bands at 3055.24 cm^{-1} and 2970.38 cm^{-1}. The alcoholic O-H stretching peak of captisol also disappeared, indicating that an interaction with the C=O group of atorvastatin- Ca might take place. All characteristic bands of captisol were slightly shifted[29].

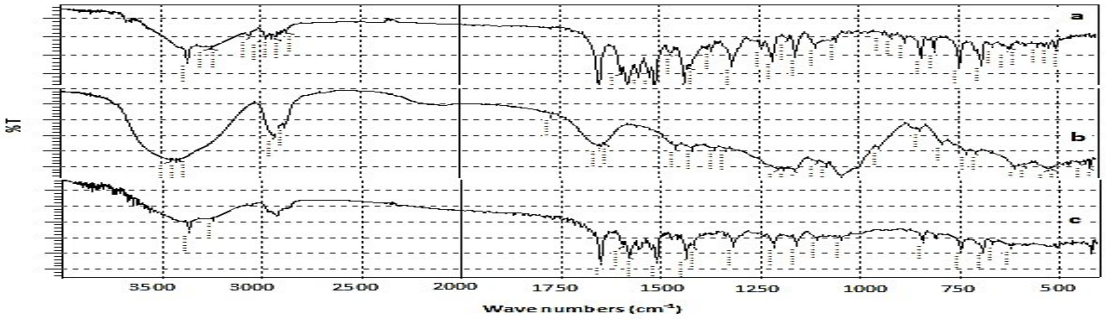


Fig 4: FT-IR spectra of (a) atorvastatin- Ca, (b) captisol and (c) inclusion complex.

In vitro dissolution study of tablets from sustained release layer.

Fig. (5) Showed the dissolution profile of losartan layer formulated according to the D-optimal design. It's clear that increasing the amount of compritol 888 led to a decrease in drug release due to the increased hydrophobicity of the matrix structure and a decrease in the total porosity of the matrices, thus decreasing the penetration of the dissolution medium into the matrix system and reducing the drug dissolution. Presence of

carbopol retarded the drug release significantly specially at pH 6.8. This is because carbopol polymers have a pKa of 6, they start to ionize at pH 4.5 and the ionization of the carboxylic acid groups causes maximum swelling thus prolonging the drug release[30]. Moreover, it is clear that drug release was greater from formulations prepared by direct compression than those from prepared by hot fusion. This could be due to the complete coating of the drug particles by the melted polymer and thus a less penetration of the dissolution medium into the matrix compared to that prepared by direct compression.

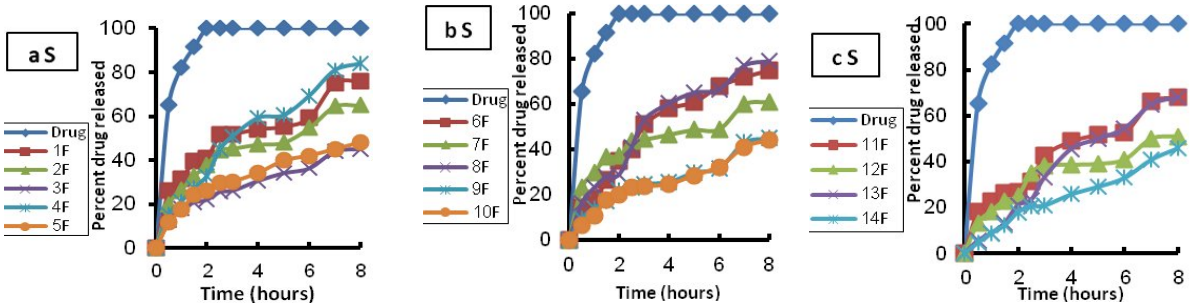


Fig. 5: % Losartan- K released from different sustained release layers, aS: formulations from 1-5, bS: formulations from 7-10 and cS:formulations from 11-14.

EXPERIMENTAL DESIGN

Fast release layer

D-optimal design was applied and the material attributes and response variables were studied and related to determine the effect of each factor on the determined responses using Design Expert-9 software[31].

Results showed that the two highest atorvastatin-Ca release formulations after 30 minutes (Y) wereF_{11} and F_{14} with percent drug released of 93% and 90%, respectively. The polynomial equation obtained in terms of coded factors for this response was:

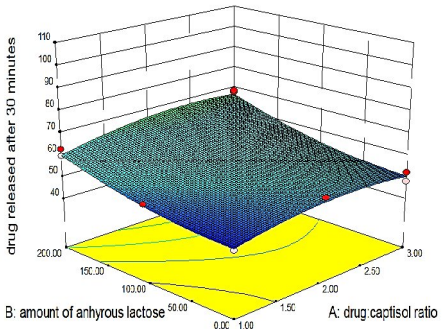
Y (drug released after 30 minutes) = 67.88 + 7.80 A + 7.92 B - 11.34 C + 0.81 AB - 5.41AC - 0.35 BC - 3.64 A^2 + 3.16 B^2

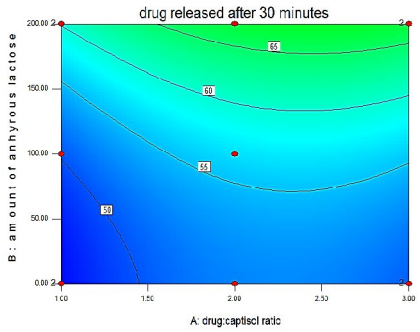
This equation can be used to make predictions about the response for given levels of each factor. A quadratic model was found to be significant for percent drug released after 30 minutes with *F* value of 51.18 *P* value< 0.0001 which implies that model and terms are significant.

The model shows a nonsignificant lack of fit of value = 3.19. The predicted R-Squared" of 0.9145 was found in reasonable agreement with the "Adjusted R-Squared" of 0.9571. These values confirm that the equations of the models are highly reliable (Table 4). Contour plots and response surface plots are shown in Fig. (6) which shows the effect of factors X_1, X_2 and X_3 on the given responses (Y).

Table 4: Analysis of variance for Y of atorvastatin- Ca as fast release layer(dissolution after 30 minutes)

Source	Mean Square	F value	p-value Prob>F
Model (Y)	450.71	51.18	< 0.0001
A-drug:captisol ratio	775.60	88.06	< 0.0001
B-amount of anhyrdrous lactose	664.81	75.49	< 0.0001
C-method of preparation	1926.41	218.73	< 0.0001
AB	5.84	0.66	0.4344
AC	314.84	35.75	0.0001
BC	1.33	0.15	0.7061
A^2	47.55	5.40	0.0425
B^2	28.38	3.22	0.1029





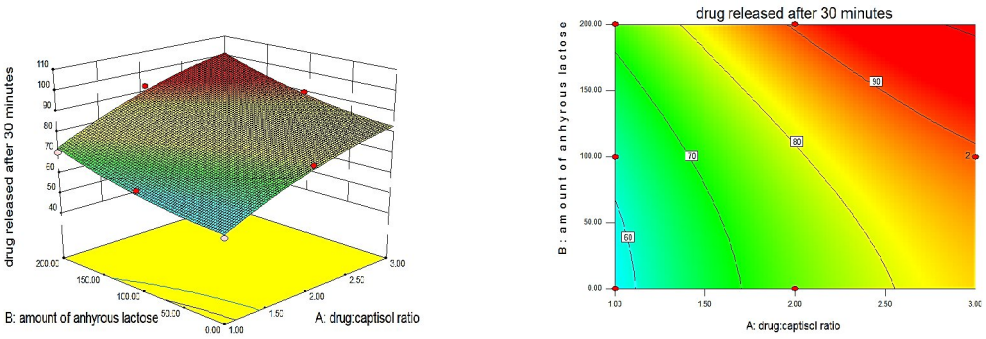


Fig.6: Response surface plots (contour and 3D) showing the effect of different independent variable on Dissolution after 30 minutes. (a) PM and (b) SD.

Sustained release layer

Results showed that the formulation with slowest losartan- K release after 8 hours were F_3,F_{10} and F_{14} with percent drug released of 45.6,44.2% and 46.6 respectively. The polynomial equation obtained in terms of coded factors for this response was: Y_1 (drug released after 2 hours) =30.04-3.34 A -4.86 B +4.49 C + 3.33 AB - 4.955E-003AC + 0.35 BC -2.72 A^2- 2.15 B^2 Y_2 (drug released after 8 hours) = 56.64 - 3.84 A - 15.98 B + 4.32 C + 7.03 AB + 2.17 BC -5.91 A^2+ 5.78 B^2

Based on the experimental design and factor combination, a quadratic model was found to be significant for percent drug released after 2 and 8 hours with *F* value of 20.46 and 39.19and *P* value < 0.0001respectively, which implies that model and terms are significant. The model shows a nonsignificant lack of fit of value = 0.1193 for Y_1 and 0.1102 for Y_2 as shown in table (5). The predicted R-Squared" of 0.9145 and 0.9031for Y_1 and Y_2 respectively was found in reasonable agreement with the "Adjusted R-Squared" of 0.9571 and 0.9395 for Y_1 and Y_2 respectively. Contour plots and response surface plots are shown in Fig. (7).

Table 5: Analysis of variance for Y_1 (drug released after 2 hours) and for Y_2 (drug released after 8 hours).

Source	Mean Square	F value	p-value Prob>F
Model (Y_1)	113.56	20.46	< 0.0001
A-compritol	113.53	20.46	0.0011
B-cabopol	282.10	50.83	< 0.0001
C-method of preparation	292.64	52.73	< 0.0001
AB	100.00	18.02	0.0017
AC	2.501E-004	4.507E-005	0.9948
BC	1.31	0.24	0.6373
A^2	22.99	4.14	0.0692
B^2	15.69	2.83	0.1236
Model (Y_2)	558.03	39.19	< 0.0001
A-compritol	101.21	7.11	0.0236
B-cabopol	3047.42	214.02	< 0.0001
C- method of preparation	263.24	18.49	0.0016
AB	506.25	35.55	0.0001
AC	27.80	1.95	0.1925
BC	50.88	3.57	0.0880
A^2	110.25	7.74	0.0194
B^2	122.76	8.62	0.0149

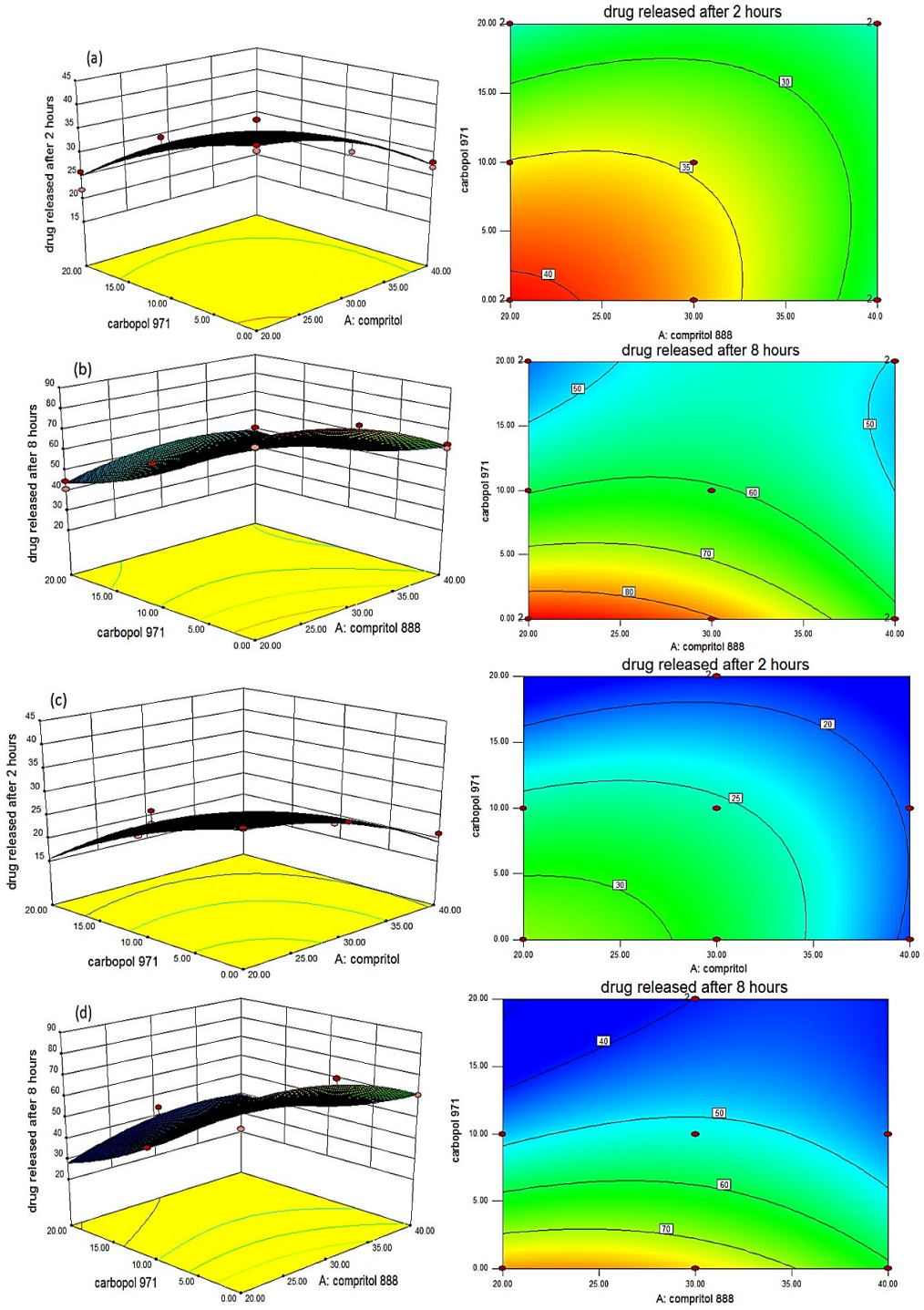


Fig. 7: Response surface plots (contour and 3D) showing the effect of different independent variable on dissolution after 2 hours (a and c: DC and MF) and 8 hours (b and d: DC and MF) respectively.

Development of design space and determination of control strategy for the two layers

Design space is the area of the parametric space within which an acceptable product can be produced[32]. Fig. (8) Shows the overlay graph of the contour plots from each response laid on top of each other. For the fast release layer of atorvastatin- ca, the suggested limits for captisol and anhydrous lactose that will give a complete drug release in 30 minutes is 1:2.82 and 200 mg respectively. For the sustained release layer of losartan-K, a set of suggested concentrations for compritol 888 and carbopol 971 were obtained. For compritol 888, the range was from 26.3 - 34.0 mg and for carbopol 971 the range was from 0.10 – 2.81mg.

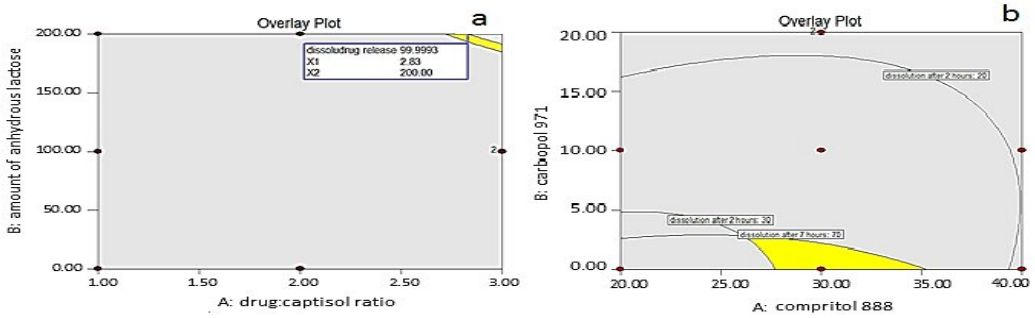


Fig. 8: Design space of the two layers prepared by solid dispersion (a: fast release layer of atorvastatin- ca and hot fusion (b: sustained release layer of losartan- K illustrating the sweet spot of overlapping region for the CQAs.

In vitro release of bilayer tablet formulation

A bilayer tablet formulation was prepared according to the results suggested by the space design. The fast release layer included atorvastatin- Ca (10 mg), captisol (49.6 mg) and 200 mg anhydrous lactose prepared by co-grinding method. While the sustained release layer of losartan- K included 27.84 mg compritol 888 and 2.37 mg carbopol 971. The bilayer tablet showed a complete atorvastatin- Ca release in 20 minutes in 0.1N HCl which was attributed to the fast disintegration of the fast release layer and rapid dissolution of the inclusion complex. Whereas losartan release was 29.3% and 70.16% after 2 and 8 hours respectively.

Analytical application

UPLC method –The proposed UPLC procedure was developed for the simultaneous determination of atorvastatin-Ca and losartan-K. Different chromatographic conditions affecting the chromatographic separation were optimized. Different mobile phases in different ratios were studied, where best peak shape and adequate separation of the two drugs was obtained by using 1% *o*-phosphoric acid - acetonitrile (55:45 v /v). Different flow rates and wavelengths were tried; good resolution with sensitive detector response was obtained at254 nm using a flow rate of 1 mL min^{-1}. Under the described parameters, the peaks of the two drugs were well resolved at retention time of 1.246 and 2.314 for losartan- K and atorvastatin-Ca, respectively, as shown in Fig.(9).

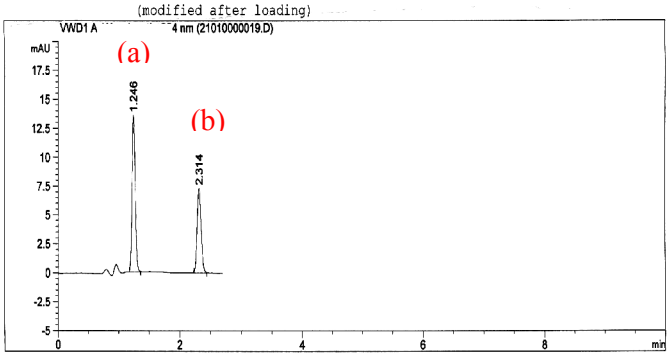


Fig. 9: UPLC chormatogram of (a) losartan-K (2 µg/ mL)and (b) atorvastatin-Ca (3 µg/ mL).

HPTLC method-Different mobile phases in different ratios and at different λ_{max} for detection were tried. It was found that acetonitrile-chloroform-methanol- conc. ammonia (7: 2: 0.9: 0.1 by volume) as a developing system followed by densitometric determination at 241 nm offered best separation and resolution. The retention factors (R_f) were approximately 0.22 and 0.63 for atorvastatin-Ca and losartan- K, respectively, as shown in Fig.(10).

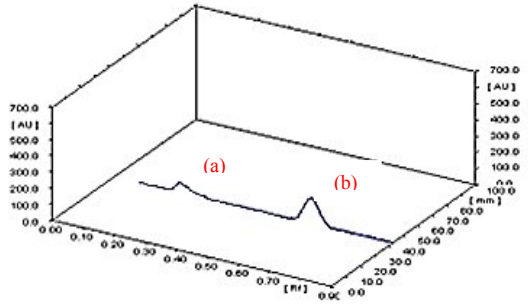


Fig. 10: Densitogram of (a) atorvastatin-Ca(2µg/ spot)and (b) losartan-K (2 µg/ spot).

Method Validation

1. System suitability- System suitability test was performed in accordance with USP [33] to ensure system performance before or during the drug analysis. Results shown in Table 6 indicate adequate resolution and reproducibility of the UPLC method.

Table 6: System suitability results of the proposed methods

Parameter	Atorvastatin-Ca	Losartan- K	Reference value
Number of theoretical plates	5830	2637	The higher the value, the more efficient the column is
Resolution factor	9.76		>2
Capacity factor (K)	3.62	1.5	1–10
Selectivity factor	2.43		≥1

2. Linearity-Under the described experimental conditions, linear calibration curves between peak areas to respective drug concentration were obtained thought the concentration ranges of 1-10 µg/ mL of both drugs using UPLC method and 0.5 -5 µg/ spot or 1-8 µg/ spot of the two drugs, respectively for HPTLC method. Regression parameters were computed and presented in Table 7.

3. Accuracy and precision-The accuracy and precision of the proposed methods were assessed by triplicate analysis at three concentration levels covering the linearity range within one day for intraday and different three days for inter day analysis. Accuracy calculated as (R%) ranged from 99.43 to 100.86% for the two drugs using the two procedures. Intraday precision (RSD %) ranged from 0.20 to 1.50% , while inter day precision ranged from 0.49 to 1.74% for both drugs; indicating good repeatability and reproducibility of the methods, Table 7**.**

Table 7: Regression parameters for the determination of Atorvastatin-Ca and Losartan- K by the proposed analytical methods

	UPLC method		**HPTLC method**	
	Atorvastatin-Ca	**Losartan-K**	**Atorvastatin-Ca**	**Losartan-K**
λ_{max} (nm)	254	254	241	241
Linearity range	1-10 µg/ mL	1-10 µg/ mL	0.5 -5 µg/ spot	1-8 µg/ spot
Regression parameters				
Slope ± SD	7.251±0.102	5.464±0.075	2174±1.069	2906.94±0.369
Intercept ± SD	0.163±0.619	0.330±0.455	139.529±0.829	321.884±1.446
Correlation Coefficient (r^2)	0.9997	0.9997	0.9990	0.9992
Accuracy (R %)	100.25%	99.44%	99.43%	100.86%
Precision (RSD %)				
Intra day	0.79- 1.50	0.25- 1.00	0.20-0.62	0.85-1.25
Inter day	0.67-1.61	0.49-1.37	0.98-1.61	1.51-1.74
(n=9)				

4. Selectivity-It was determined by applying the proposed methods to laboratory prepared mixtures containing different ratio of the two drugs. Good recoveries of 99.23%±0.65and101.52%±0.94 were obtained for atorvastatin-Ca and losartan- K, respectively in UPLC method. While for HPTLC, recoveries were 101.54% ± 0.82 and 101.91% ± 0.91 for the two drugs, respectively, as shown in Table 8.

Table 8: Determination of atorvastatin-Ca and losartan-K in mixtures by the proposed analytical methods

UPLC method				**HPTLC method**			
Atorvastatin-Caadded (µg/mL)	**Losartan-K added (µg/mL)**	**% Recovery of Atorvastatin-Ca**	**%Recovery of Losartan-K**	**Atorvastatin-Ca added (µg/Spot)**	**Losartan-K added (µg/Spot)**	**% Recovery of Atorvastatin-Ca**	**%Recovery of Losartan-K**
1	10	100.09	101.59	1	8	101.67	102.93
3	7	98.92	101.39	2	6	102.87	101.35
5	5	99.60	101.64	3	5	101.03	102.15
7	3	99.15	100.18	4	4	101.37	100.65
10	1	98.37	102.82	5	1	100.75	102.45
Mean%±SD		99.23±0.65	101.52±0.94			101.54±0.82	101.91±0.91

5. Robustness-The average values of % RSD of response for determination of atorvastatin-Ca and losartan- K at changed conditions were less than 2 %, which reveals the robustness of both methods, Table 9.

Table 9: Robustness study for the proposedanalytical methods

Parameter	Changed condition	Atorvastatin-Ca %RSD	Losartan-K %RSD
UPLC method			
Flow rate: (1 mL/min)	± 10%	1.46	1.37
Mobile phase ratio: *o*-phosphoric acid – acetonitrile (55:45 v /v)	± 2%	0.83	1.65
Detector wavelength: (251 nm)	± 2 nm	1.46	0.99
HPTLC method			
Mobile phase ratio: acetonitrile-chloroform-methanol-conc. ammonia (7: 2: 0.9: 0.1)	±2%	1.57	0.69
Source of methanol	El Nasr- Sigma	1.40	0.72

Application of the proposed procedures to the prepared bilayer tablet

Statistical analysis of the results obtained by the suggested methods compared with a reported method[20] showed that the calculated t and F values are less than the tabulated ones indicating no significant difference between the proposed and reported methods confirming accuracy and precision at 95% confidence limit, Table 9.

Table 10: Results obtained by the proposed methods compared with reported method[20] for the determination of atorvastatin-Ca and losartan-K.

Parameter	UPLC method		HPTLC method		Reported method[2]	
	Atorvastatin-Ca	Losartan-K	Atorvastatin-Ca	Losartan-K	Atorvastatin-Ca	Losartan-K
Linearity range	1-10 µg/ mL	1-10 µg/ mL	0.5- 5 µg/spot	1-8 µg/spot	5- 20 µg/ mL	2-10 µg/ mL
N	5	5	5	5	5	5
Mean %	100.59	100.24	100.64	98.18	101.82	99.36
SD	1.17	0.90	1.29	0.69	0.35	0.46
Variance	1.08	0.95	1.13	0.83	0.59	0.68
t-	2.14	1.55	2.03	2.15	-	
F-	1.83	1.39	1.91	1.22	-	

-Reported method involved UV- measurement of atorvastatin-Ca and losartan –K in methanol at 245 and 207 nm.
-The theortical t- and f- values at p= 0.05 were 2.31 and 6.39, respectively.

4 CONCLUSIONS

Atorvastatin- Ca and losartan-K bilayer tablet formulations were prepared. The solubility and bioavailability of atorvastatin- Ca was increased by complexation with captisol, while compritol 888 and carbopol 971 were used to delay the release of losartan-K from the sustained release layer. QbD was applied for the development of bilayer tablets. D-optimal design was applied to study the effect of determined material attributes and critical process parameters on drug release from each layer. The design space was determined from which, the final ratios of polymers in each layer was determined and used to formulate a bilayer tablets. These tablets were successfully analysed by UPLC and HPTLC methods. These methods proved to be accurate and precise, thus can be effectively applied for the routine estimation of both drugs in bulk and in their combined formulations.

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