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Preformulation of Immediate Release Candesartan Cilexetil Tablets Using Full Factorial Experimental Design

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***SUMMARY*. This work aimed compatibility and stability studies, five factor and two level (25) full factorial experimental design of immediate release candesartan cilexetil 32 mg tablets. Candesartan cilexetil is a drug substance, which has low solubility and chemical instability properties. Polyethylene glycol (PEG) 4000 showed stability protective property on candesartan cilexetil in tablets. Structural degradation of candesartan cilexetil was prevented by chemical reactions of terminal hydroxyl groups of PEG 4000 with carboxyl, N-H, N=N and anhydride groups of candesartan cilexetil. Stability protective mechanism was realized by converting crystalline candesartan cilexetil to amorphous form inside the amorphous region of PEG 4000. Stability evaluation showed that candesartan cilexetil could be protected ideally by PEG 4000 concentration of 2.5-10 % (w/w) in tablets. Dissolved drug from tablet could be predicted with high capability from statistical model, resulted from experimental design.Thus, stability and dissolution properties of candesartan cilexetil could be controlled in developed tablets.**

RESUMEN. Este trabajo consiste en estudios de compatibilidad, estabilidad y diseño experimental factorial completo de cinco factores y dos niveles (25) de comprimidos de candesartán 32 mg de liberación inmediata. Candesartán cilexetilo es una sustancia farmacéutica de baja solubilidad y las propiedades químicas inestables. El polietilenglicol (PEG) 4000 mostró una propiedad protectora de la estabilidad de candesartán cilexetilo en comprimidos. La degradación estructural de candesartán cilexetilo fue impedido por reacciones químicas de los grupos hidroxilo terminales de PEG 4000 con grupos carboxilo, NH, N = N y anhídrido de candesartán cilexetilo. El mecanismo de protección de la estabilidad se realizó mediante la conversión cristalina a candesartán cilexetilo en forma amorfa en el interior de la región amorfa de PEG 4000. La evaluación de la estabilidad mostró que candesartán cilexetilo puede ser protegida idealmente por PEG 4000 en concentración de 2.5-10% (w/w) en tabletas. El fármaco disuelto de la tableta podría predecirse con alta capacidad en base al modelo estadístico como resultado del diseño experimental. De este modo las propiedades de estabilidad y de disolución de candesartán cilexetilo podrían ser controlados en las tabletas desarrolladas.

INTRODUCTION

Candesartan cilexetil is a prodrug with chemical structure of benzimidazole-7-carboxylic acid derivative, demostrating angiotensin-2-antagonistic activity, serving as therapeutic agent for hypertension [1,2]. Two major challenges in development of candesartan cilexetil tablets are; chemical instability property of candesartan cilexetil [1,2] and low solubility property of candesartan cilexetil due to being biopharmaceutics classification system class II compound [3].

PEG is mentioned to exist in reference product of candesartan cilexetil 32 mg tablets [4-6]. When used in 1-10 % as co-solvent in formulation, PEG can occur as stabilizing agent for candesartan cilexetil [7]. In our study, PEG 4000 was selected as stabilizing agent due to its hydroxyl value of 25-32 [8].

In vitro dissolution test applying standard paddle method using a buffer medium with surfactant, determined 100 % dissolution and predictable bioavailability free of particle size for candesartan cilexetil 8 mg tablets containing drug particles with a mean diameter equal and less than 6 µm [3].

KEY WORDS: compatibility, dissolution, stability.

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The originality of our study relies on new trend of preformulation based on prediction and assessment of tablet stability property with chemical compatibility study. The aims of compatibility study were; to clarify the chemical mechanism of reaction of PEG 4000 with candesartan cilexetil, to determine the polymer concentration preventing drug substance degradation and to evaluate its stabilizing mechanism using thermal and structural methods. Our hypothesis was that; candesartan cilexetil could react chemically with its carboxyl, N-H, N=N and anhydride groups, by making hydrogen bonds with terminal hydroxyl groups of PEG 4000. The stabilization of candesartan cilexetil can take place when strong chemical interaction with PEG 4000 exists, herein preventing all other possible reactions.

As second step, formulation composition and manufacturing process of tablets were chosen. Preformulation series were evaluated by obtained finished product and stability results in accelerated stability study.

For dissolution estimation of stable tablets as third step, 2^5 full factorial experimental design study was applied to obtain mathematical equations for determined target values of PEG 4000, starch maize, carboxymethylcellulose calcium (CMC Ca), hydroxypropyl cellulose (HPC) and purified water formulation factors.

All these investigations were done for ensuring a tablet composition with protected chemical stability of candesartan cilexetil and suitable dissolution property together with chosen mean particle size less than 6 µm of candesartan cilexetil.

MATERIALS AND METHODS

Materials

The ingredients provided from the following suppliers are pharmaceutical grade: Candesartan cilexetil (XunQiao, LinHai, Zhejiang, China), lactose monohydrate (Pharmatose 90M, DMV - Fonterra Excipients, Germany), PEG 4000 (Merck - Schuchardt, Germany), HPC (Klucel LF-Pharm, Ashland, USA), starch maize (Roquette, France), CMC Ca (Nichirin Chemical Ind., Japan) and magnesium stearate (Peter Greven, Germany).

The reagents provided from the following suppliers are analytical grade: Polysorbate 20 (Tween 20, Merck - Schuchardt, Germany), polysorbate 80 (Tween 80, Merck - Schuchardt, Germany), trifluoroacetic acid (Uvasol, Merck - Schuchardt, Germany), methanol (Merck, Germany), acetonitrile (Merck, Germany), sodium dihydrogenphosphate dihydrate (Emprove, Merck, Germany), potassium dihydrogenphosphate (Riedel - deHaën, Germany), sodium hydroxide (Riedel - deHaën, Germany) and hydrocloric acid (J.T. Baker, Holland).

Preparation of solid dispersions

50 mg/50 mL candesartan cilexetil solution in methanol and 50 mg/50 mL, 125 mg/50 mL, 150 mg/50 mL, 200 mg/50 mL, 250 mg/50 mL, 375 mg/50 mL, and 500 mg/50 mL PEG 4000 solutions in methanol were prepared. In order to obtain mixtures of candesartan cilexetil : PEG 4000 in 1:1, 1:2.5, 1:3, 1:4, 1:5, 1:7.5, and 1:10 ratios, equal volumes of corresponding candesartan cilexetil and PEG 4000 solutions were mixed for 30 min using Heidolph MR2002 magnetic stirrer (Germany). Candesartan cilexetil : PEG 4000 1:1, 1:2.5, 1:3, 1:4, 1:5, 1:7.5, and 1:10 (w/w) solid dispersions were prepared after evaporation of methanol in Petri dish in ambient temperature, aiming to provide an intimate contact of stabilizing agent and drug substance for compatibility study.

Ultraviolet-visible spectrophotometry

Ultraviolet-visible (UV) spectra of candesartan cilexetil, PEG 4000 and candesartan cilexetil : PEG 4000 1:1, 1:2.5, 1:5 (w/w) solutions in methanol were evaluated with Varian Cary 100 Conc UV-visible spectrophotometer (USA) between 900-190 nm.

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectra of candesartan cilexetil, PEG 4000 and solid dispersions were obtained by Nicolet 520 FT-IR spectrometer (USA) between 4000-400 cm^{-1} (scanning number: 32, resolution: 4 cm^{-1}). Transparent KBr disks were prepared using hydrolic press in compression force of 10 kN.

Differential scanning calorimetry

Candesartan cilexetil:PEG 4000 1:1 and 1:2.5 (w/w) solid dispersions were analyzed using TA Instruments Q100 DSC (USA) in nitrogen flow rate of 50 mL/min applying 10 °C/min heating rate between 25-40 °C or 25-50 °C or 25-60 °C, maintained at upper temperature for 10 min simulating stress conditions, after which cooling was done to 25 °C. Second heating was applied between 25-190 °C.

Particle size analysis

Particle size of candesartan cilexetil was determined with laser diffraction method using Malvern MasterSizer 2000 equipment having Hydro 2000 S unit (UK).

Experimental parameters were set as follows: *Candesartan cilexetil refractive index* (RI): 1.665; *Absorption:* 0.1; *Dispersant RI:* 1.33 (taken for distilled water, polysorbate 80 RI was excluded); *Calculation model:* General (Calculation sensitivity: Normal, Particle size: Irregular); *Sample obscuration range:* 20 %; *Ultrasonic effect and time (sonication):* 50 % - 10 min; *Suspension stirring rate:* 2500 rpm; *Sample measurement time:* 15 s; *Measurement repetitions:* 10; *Dispersant preparation:* 1 g polysorbate 80 was dissolved in 1000 mL distilled water, mixed for 15 min. 8.5 mg candesartan cilexetil was added and suspended by mixing for 10 min using Heidolph MR2002 magnetic stirrer (Germany): prepared dispersant was filtered two times from 0.45 µm high volume filter (Merck Millipore, Germany) and afterwards once from 0.2 µm regenerated cellulose filter (Sartorius, Germany) using vacuum pump (Merck Millipore, Germany); *Sample preparation:* 100 mg candesartan cilexetil was formed into paste adding five drops of polysorbate 80. 10 mL dispersant was added to pasted sample and its suspension was prepared.

Compositions of preformulation series

Tablets contained 32 mg candesartan cilexetil corresponding to 12.31 % (w/w). PEG 4000 was included for its stability protective function on candesartan cilexetil in 1.25 % (w/w) or 2.5 % (w/w) or 3.75 % (w/w) or 5 % (w/w) or 10 % (w/w) by replacing lactose monohydrate in composition. The rest of ingredients were HPC, starch maize, CMC Ca and magnesium stearate which were kept constant. Mean tablet weight was 260 mg. Table 1 shows compositions of preformulation series.

Tablet manufacturing process

Preformulation series were manufactured with wet granulation technique in laboratory scale of 156 g (600 tablets). During preformulation studies, critical variables were tablet hardness as process parameter and PEG 4000 amount as formulation factor.

Except of hardness range in compression step, all the manufacturing steps were applied the same as follows: 1) Powder mixing was done in glass mortar by mixing candesartan cilexetil, lactose monohydrate and starch maize for 5 min. 2) Binder solution was prepared by mixing HPC, PEG 4000 and purified water for 45 min using magnetic stirrer (Heidolph MR2002, Germany). 3) Granulation was done in glass mortar by adding binder solution to powder mixture meanwhile mixing for 10 min. 4) Drying of granules was done in oven (Dedeoglu TB–5050, Turkey) at 40 °C for 2 h. 5) Sieving of granules was done with oscillator (Aymes, Turkey) through mesh screen diameter of 1.00 mm. 6) Final powder mixing and lubrication were done by mixing CMC Ca and magnesium stearate together for 2 min at 25 rpm (50 rotations) using powder mixer (Pascall Engineering Co. Ltd., UK). 7) Compression was done with single punch tabletting machine (Erweka AR400, Germany) using round, biconvex, double tablet punch having diameter of 7 mm. Tablet hardness ranges applied to preformulation series were; 45-90 N for F1 tablet, 85-135 N for F2-F5 tablets and 100-140 N for F6 tablet.

Raw materials	Functions	F1 tablet	F2 tablet	F3 tablet	F4 tablet	F5 tablet	F6 tablet
		(w/w %)					
Candesartan cilexetil	Drug substance	12.31	12.31	12.31	12.31	12.31	12.31
Lactose monohydrate	Diluent	63.69	62.44	61.19	59.94	58.69	53.69
Starch maize	Disintegrant	15.00	15.00	15.00	15.00	15.00	15.00
PEG 4000	Solubilizer / Stabilizing agent	-	1.25	2.50	3.75	5.00	10.00
HPC	Binder	3.00	3.00	3.00	3.00	3.00	3.00
CMC Ca	Disintegrant	5.50	5.50	5.50	5.50	5.50	5.50
Magnesium stearate	Lubricant	0.50	0.50	0.50	0.50	0.50	0.50

Table 1. Compositions of preformulation series.

Determination of tablet hardness

Hardness of 10 tablets were measured using Pharma Test PTB tester (Germany) [9].

***In vitro* dissolution testing**

High performance liquid chromatography (HPLC) analysis for dissolution test of 6 tablets was performed using Agilent Technologies HP 1200 series having UV detector and auto sampler (USA). Chromatographic separation was achieved on cyano column (250 mm × 4.6 mm, 5 µm particle; Clipeus, Higgins Analytical Inc., USA) with 50:50 (v/v) mixture of 1 mM phosphate (KH_2PO_4) buffer and acetonitrile as mobile phase adjusted to pH 2.0 with trifluoroacetic acid. The flow rate was 1.0 mL/min, injection volume was 20 µL, column temperature was 25 °C, detection wavelength was 254 nm, run time was 12 min. Dissolution profiles of tablets were compared in 0.05 M phosphate buffer, pH 6.5 including 0.70 % (w/v) polysorbate 20 having a volume of 900 mL at 37 ± 0.5 °C and paddle stirring rate of 50 rpm. Sample aliquots of 5 mL were withdrawn at 10, 20, 30, 45, and 60 min and replaced with equal volume of fresh medium to maintain the volume constant [10].

For dissolution profile comparison, f2 (similarity factor) of reference and developed tablets, was calculated [11].

Determination of kinetic models of drug release profiles

The best curve fit of the release data was tested with the mathematical models of zero order kinetics, first order kinetics, Hixson-Crowell model, Higuchi model and Weibull model [12]. The statistical results were obtained with linear regression analysis.

Assay and content uniformity testings

HPLC analysis for assay test of powdered 2 tablets and content uniformity test of 10 tablets was performed with Agilent Technologies HP 1200 series having UV detector and auto sampler (USA). Chromatographic separation was achieved on cyano column (250 mm × 4.6 mm, 5 µm particle; Clipeus, Higgins Analytical Inc., USA) with 40:60 (v/v) mixture of 0.05 M phosphate buffer, pH 4.5 and methanol as mobile phase adjusted to pH 4.0 with trifluoroacetic acid. The flow rate was 1.0 mL/min, injection volume was 20 µL, column temperature was 25 °C, detection wavelength was 254 nm, run time was 13 min.

Impurity testing

Total degradation products of tablets were determined by assay method. Peak areas having retention time between 6.8-7.6 min, not observed for working standard, were calculated.

Stability study

Tablets were stored for 2 months in accelerated stability condition (40 ± 2 °C, 75 ± 5 % RH) in 100 mL, white, high density polyethylene (HDPE) bottles having 80 mm height and 45 mm diameter with heat-sealed, polypropylene, child-proof caps having 42 mm diameter, each containing 60 tablets. Tablets were evaluated initially, at the end of first and second months, stored in Nüve ID 300 climatic test cabinet (Turkey).

Five factor, two level full factorial experimental design

2^5 full factorial experimental design was used to examine the effects of starch maize, PEG 4000, HPC, CMC Ca and purified water as formulation factors on responses of dissolved amount from tablet at 10 min (D_{10} %), at 20 min (D_{20} %), at 30 min (D_{30} %), at 45 min (D_{45} %), at 60 min (D_{60} %), and f_2. Each formulation factor was chosen with its lowest and highest level used in formulation, in order to explore the effects in a wider range in design space. Minitab® 16 (USA) software was used to design the study as randomized with no blocks, centers or codes, once replicated constructing 32 runs. The acceptance criteria were not less than 80 % dissolved amount at 30 min and similar dissolution profiles to reference tablet having f_2 greater than 50.

T and F tests were applied to data. Those coefficients determined as statistically significant ($p \leq 0.05$) were included in the model to generate regression equation. Effects of factors were observed with Pareto chart, normal probability and half - normal probability plots; interaction of factors were examined with contour and response surface plots [13].

Preparation of design of experiments series

Each design of experiments (DOE) serial was prepared in 52 g (200 tablets) with wet granulation manufacturing process. All DOE series were compressed at hardness range of 85-135 N, specified after preformulation study.

Estimation of tablet dissolution

A control serial was prepared as check point

of DOE study, corresponding to DOE serial number 25. *In vitro* dissolution of DOE series was detected with the method performed for preformulation series. Correlation analysis determined the relationship between predicted values obtained from mathematical equations and experimental values obtained from *in vitro* dissolution test of control serial. Prediction capability of mathematical equations was clarified with Pearson correlation and p values. f_2 was calculated to determine the closeness of predicted and experimental values.

RESULTS AND DISCUSSION

Our study was realized in order to have suitable stability and dissolution properties of immediate release candesartan cilexetil 32 mg tablets including determined candesartan cilexetil particle size [3,14,15].

Ultraviolet - visible spectrophotometry

Candesartan cilexetil showed maximum absorbance in methanol at 253 and 220 nm. PEG 4000 had no absorbance in UV region. Candesartan cilexetil:PEG 4000 1:1, 1:2.5, and 1:5 mixtures caused a new formation not available in candesartan cilexetil itself. This formation could be seen significantly in 1:1 mixture, whereas in 1:2.5 and 1:5 mixtures it was less intensely observed. The meaning of new formation is occurance of strong interaction between PEG 4000 and candesartan cilexetil, appearing at around 210 nm. Increase of PEG 4000 in mixtures caused shift and arise in absorbance at maximum wavelengths due to stronger interaction. This shows stability protective property of PEG 4000 on candesartan cilexetil.

Fourier transform infrared spectroscopy

FT-IR spectroscopy evaluation clarified that band belonging to carboxyl group of candesartan cilexetil (1716 cm^{-1}) disappeared in all mixtures. So that, candesartan cilexetil interacted with PEG 4000 mostly through carboxyl group. Besides, shifts in upper points of absorption bands were determined for N-H, N=N and anhydride groups which were reactive other than carboxyl group. When PEG 4000 ratios increased in mixtures, low absorbance values were measured indicating decrease in interaction. Thus, PEG 4000 was determined to be mostly protective on candesartan cilexetil in candesartan cilexetil : PEG 4000 1:1 mixture.

Preparation of preformulation series

The excipients were chosen for wet granulation technique in accordance to candesartan cilexetil 32 mg reference tablet composition. PEG 4000 concentration was evaluated for enhanced stabilizing effect on candesartan cilexetil. In order to increase the interaction between PEG 4000 and candesartan cilexetil, granulation phase was suitable step for their addition. Both PEG 4000 concentration and tablet hardness range were evaluated for dissolution, assay, content uniformity and total degradation products' results of preformulation series at initial, first and second months of accelerated stability condition.

Evaluation of finished product and shelf life stability

Table 2 shows stability study results of preformulation series. During evaluated shelf life, assay and content uniformity results met acceptance criteria [16].

Stability protective effect of PEG 4000 on candesartan cilexetil was determined from total degradation products' results. F1 and F2 tablets had increasing impurity profile, whereas F3-F6 tablets kept their profiles in low values near to each other. These determinations showed that PEG 4000 protected candesartan cilexetil from degradation with a concentration at least 2.5 % (w/w) in tablets.

Differential scanning calorimetry

The melting point of candesartan cilexetil disappeared in mixtures, because it incorporated inside the amorphous region of PEG 4000. PEG 4000 hindered candesartan cilexetil recrystallization. So that, PEG 4000 protects candesartan cilexetil by converting it from crystalline to amorphous form.

In vitro dissolution profiles

Mean particle size of candesartan cilexetil in tablets was determined to be 3.85 (± 2.40) µm. The particle size was ensured in order to have 100 % dissolved amount from tablets. Because of this sufficiency, particle size of drug substance was not evaluated as a formulation factor.

The dissolution profiles are presented in Fig. 1. Because of its lowest hardness value F1 tablet resulted in f_2 of 46, uncomparable to reference tablet. F2, F3, F4, F5, and F6 tablets demonstrated similar dissolution profiles to reference tablet

Tests	F1 tablet	F2 tablet	F3 tablet	F4 tablet	F5 tablet	F6 tablet
Finished product (initial stability) results						
Hardness (N)	62.9	93.4	112.2	113.6	99.4	120.5
Assay (w/w %)	100.27	102.08	100.98	101.14	103.05	99.98
Content uniformity	AV= 7.56 ≤ 15, RSD= 3.130 %	AV= 11.09 ≤ 15, RSD= 4.683 %	AV= 9.17 ≤ 15, RSD= 3.834 %	AV= 3.62 ≤ 15, RSD= 1.253 %	AV= 10.92 ≤ 15, RSD= 4.189 %	AV= 6.17 ≤ 15, RSD= 2.553 %
Dissolution (% at 30 min)	84.17	89.70	89.95	88.06	83.67	86.89
Total degradation products (w/w %)	0.202	0.283	0	0	0	0
Stability results at first month 40 °C / 75 % RH condition						
Hardness (N)	68.7	87.9	81.8	105.4	105.7	119.8
Assay (w/w %)	98.20	98.33	100.17	99.61	101.31	100.31
Content uniformity	AV= 3.55 ≤ 15, RSD= 1.483 %	AV= 3.90 ≤ 15, RSD= 1.615 %	AV= 4.24 ≤ 15, RSD= 1.745 %	AV= 3.97 ≤ 15, RSD= 1.494 %	AV= 2.36 ≤ 15, RSD= 0.887 %	AV= 2.13 ≤ 15, RSD= 0.877 %
Dissolution (% at 30 min)	86.35	86.81	92.40	92.87	90.44	94.03
Total degradation products (w/w %)	0.525	0.391	0	0.165	0	0
Stability results at second month 40 °C / 75 % RH condition						
Hardness (N)	66.2	86.0	82.2	99.8	112.7	106.5
Assay (w/w %)	99.94	97.97	100.92	100.27	100.03	99.36
Content uniformity	AV= 3.85 ≤ 15, RSD= 1.552 %	AV= 3.31 ≤ 15, RSD= 1.387 %	AV= 4.54 ≤ 15, RSD= 1.876 %	AV= 5.08 ≤ 15, RSD= 2.113 %	AV= 2.23 ≤ 15, RSD= 0.921 %	AV= 5.97 ≤ 15, RSD= 2.515 %
Dissolution (% at 30 min)	89.27	90.50	87.69	87.58	87.50	87.71
Total degradation products (w/w %)	0.758	0.551	0.241	0.278	0.230	0.211

Table 2. Stability study results of F1-F6 preformulation series. AV: Acceptance value, RSD: Relative standard deviation.

with f_2 values of 54, 54, 74, 58 and 69 subsequently.

F6 tablet resulted in 100 %, on the contrary F1 tablet resulted in 90 % dissolved amount at 60 min. This difference demonstrated solubilizing effect of PEG 4000 on candesartan cilexetil.

Kinetic models of drug release profiles

The drug release data were fitted to five different kinetic models, presented in Table 3. Dissolution profiles of tablets could fit to Weibull model, because of obtained higher coefficients of determination.

F1-F3 tablets showed shape parameter (b) values less than 1 meaning that, drug release was faster at initial time points and afterwards reached to plateau consistent with first order kinetics.

F4-F6 tablets showed b values greater than 1 meaning that, drug release was slower at initial time points and afterwards reached fastly to plateau demostrating S-shaped curve.

It was determined that, tablet hardness effect was dominating on drug release at initial 10 min and 20 min. Meanwhile, PEG 4000 concentration had solubilizing effect on cumulative amount of released drug.

Design of experiments study

Results of DOE study entered to Minitab® 16 (USA) statistical programme are presented in Table 4. DOE 2, DOE 3, DOE 4, DOE 5, DOE 6, DOE 7, DOE 10, DOE 12, DOE 14, DOE 17, DOE 22, DOE 25, DOE 29, and DOE 31 met acceptance criteria.

Mathematical models		F1 tablet	F2 tablet	F3 tablet	F4 tablet	F5 tablet	F6 tablet
Zero order	K_0	- 0.651	- 0.823	- 0.832	- 1.189	- 1.149	- 1.239
	R^2	0.743	0.718	0.660	0.769	0.787	0.843
First order	K_1	- 0.030	- 0.044	- 0.043	- 0.075	- 0.050	- 0.095
	R^2	0.886	0.864	0.846	0.979	0.934	0.912
Hixson - Crowell	K_S	0.027	0.037	0.037	0.057	0.045	0.082
	R^2	0.842	0.822	0.786	0.932	0.897	0.996
Higuchi	K_H	7.630	9.698	9.893	13.887	13.380	14.268
	R^2	0.842	0.822	0.770	0.865	0.879	0.922
Weibull	b	0.623	0.842	0.865	1.308	1.137	1.328
	τ_D	13.15	14.27	14.44	18.45	20.52	17.69
	R^2	0.953	0.942	0.916	0.985	0.968	0.958

Table 3. Release constants and coefficients of determination for linear relationship of drug release kinetics. K_0: The zero order release constant, K_1: The first order release constant, K_S: The constant incorporating the surface - volume relation, K_H: The Higuchi dissolution constant, R^2: The coefficient of determination, τ_D: The time interval necessary to dissolve 63.2 % of the drug present in tablet.

Mathematical equations obtained from experimental design for calculating each response are presented by Eqs. [**1**-**6**]:

$$D_{10}\ \% = 36.565 + (-1.943)A + (-9.092)C + (-0.676)D + (-4.985)E + (4.114)AC + (-2.748)AD + (-0.840)AE + (0.195)CD + (-0.520)CE + (0.955)DE + (-0.368)ACD + (-1.732)ACE + (-0.499)ADE + (1.757)CDE + (0.207)ACDE \quad [1]$$

$$D_{20}\ \% = 63.27 + (-12.58)C + (-6.08)E + (-1.91)CE \quad [2]$$

$$D_{30}\ \% = 78.633 + (-9.847)C + (-4.978)E + (-2.753)CE \quad [3]$$

$$D_{45}\ \% = 89.060 + (-4.669)C + (-2.466)E + (-1.792)CE \quad [4]$$

$$D_{60}\ \% = 92.963 \quad [5]$$

$$f2 = 46.640 + (-6.977)C \quad [6]$$

Factors affecting to dissolved amount at 10 min

The factors affecting to D_{10} % were determined to be individually HPC, purified water, interaction of PEG 4000 and CMC Ca and interaction of PEG 4000 and HPC. The following interactions were found to cause increase in D_{10} %; increase in CMC Ca level and decrease in PEG 4000 level, decrease in HPC level and decrease in PEG 4000 level, decrease in HPC level and decrease in purified water level, decrease in HPC level and nonsignificant effect of CMC Ca level, decrease in PEG 4000 level and decrease in purified water level, decrease in CMC Ca level and decrease in purified water level.

Factors affecting to dissolved amounts at 20, 30, 45 and 60 min

The factors affecting to each D_{20} %, D_{30} % and D_{45} % were obtained to be individually HPC and purified water. Their interaction both in decreasing levels, caused increase in each D_{20} %, D_{30} % and D_{45} %. There was no factor affecting to D_{60} %.

Factors affecting to similarity factor

HPC was the only factor affecting to f_2. Decrease in HPC level caused increase in f_2.

Estimation of tablet dissolution

The graphical evaluation of control serial is

DOE Serial no	Formulation factors					Responses					
	A (%)	B (%)	C (%)	D (%)	E (mg/tablet)	D10 % (n = 6)	D20 % (n = 6)	D30 % (n = 6)	D45 % (n = 6)	D60 % (n = 6)	f_2
1	2.5	20	2	8	40	68.98	90.00	92.60	93.38	93.65	32.45
2	2.5	10	6	8	60	31.50	57.05	80.34	95.98	101.00	51.50
3	10	10	2	8	40	45.80	79.14	95.17	100.68	101.92	54.40
4	10	20	2	2	40	47.61	80.15	94.79	98.32	98.51	51.66
5	10	20	6	2	40	46.92	76.81	90.93	95.58	96.30	55.60
6	10	10	6	2	40	40.56	66.74	83.19	92.79	95.11	58.58
7	10	20	2	8	40	37.06	67.09	85.70	93.49	94.39	67.94
8	2.5	10	6	8	40	26.37	51.39	73.45	90.77	95.01	41.36
9	10	10	6	8	60	17.86	38.65	56.08	77.50	88.83	29.51
10	10	20	2	2	60	34.50	63.82	83.90	95.20	97.50	61.16
11	2.5	20	6	8	40	29.36	53.70	75.02	87.80	91.14	42.91
12	2.5	10	2	8	60	42.55	74.97	85.88	90.42	91.21	59.63
13	2.5	10	2	2	40	57.06	88.08	93.54	95.26	95.44	39.41
14	2.5	20	2	2	60	43.08	76.97	91.65	96.48	97.39	60.69
15	2.5	20	6	2	60	16.42	33.57	48.70	68.14	84.22	25.13
16	2.5	10	6	2	40	21.74	42.38	59.75	80.66	90.20	32.38
17	10	10	2	2	40	42.22	72.80	88.15	93.71	94.60	65.33
18	2.5	20	2	2	40	53.87	82.76	88.08	90.81	90.70	44.01
19	10	20	6	8	40	30.38	54.77	74.37	87.92	91.54	43.26
20	2.5	20	6	8	60	24.52	47.70	68.04	85.34	92.68	36.05
21	2.5	10	6	2	60	17.04	34.27	51.06	72.63	89.26	26.77
22	2.5	10	2	2	60	46.81	78.66	87.43	89.57	89.94	52.73
23	10	20	2	8	60	26.42	54.48	75.50	90.05	93.84	43.55
24	10	10	6	8	40	33.03	57.96	76.13	89.64	91.67	46.82
25	10	10	2	8	60	36.12	66.96	87.61	97.07	97.69	73.60
26	2.5	20	6	2	40	35.47	65.71	79.32	84.03	85.20	46.03
27	10	10	6	2	60	19.62	37.63	53.67	72.84	84.93	27.89
28	10	20	6	2	60	25.88	48.24	67.22	85.23	93.50	36.11
29	10	10	2	2	60	47.07	76.16	90.30	95.53	96.04	55.82
30	2.5	20	2	8	60	52.99	81.39	87.77	90.13	90.06	45.40
31	2.5	10	2	8	40	48.37	80.15	87.62	89.57	89.73	50.10
32	10	20	6	8	60	22.92	44.39	63.33	83.42	91.63	34.71

Table 4. Results of experimental design. A: Content of PEG 4000, B: Content of starch maize, C: Content of HPC, D: Content of CMC Ca, E: Content of purified water.

shown in Fig. 2. Correlation analysis demonstrated that experimental values and predicted values obtained from mathematical equations had Pearson correlation value of 0.981 and p value of 0.003. f_2 was found to be 61.57. These results indicated good model fitting.

CONCLUSIONS

The framework of this preformulation study consisted of compatibility study, preformulation development and experimental design study of immediate release candesartan cilexetil 32 mg tablets.

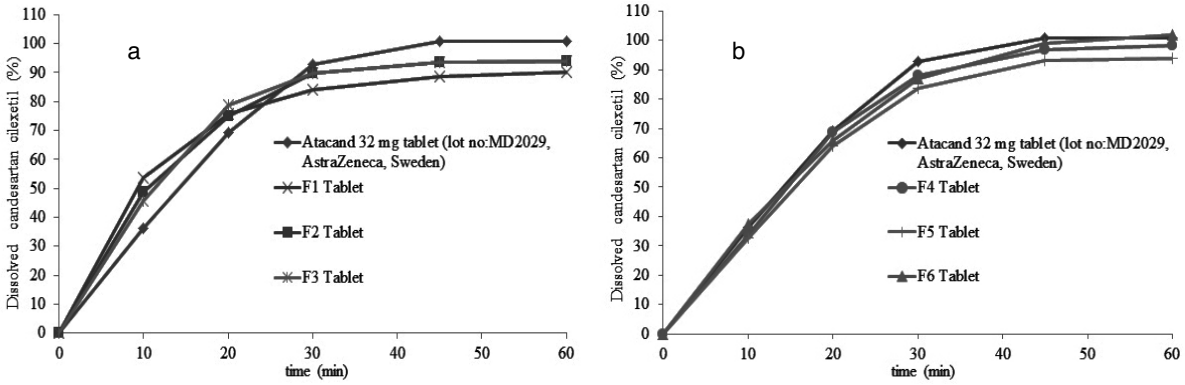

Figure 1. Comparative dissolution profiles of (**a**) reference and F1-F3 tablets and (**b**) reference and F4-F6 tablets.

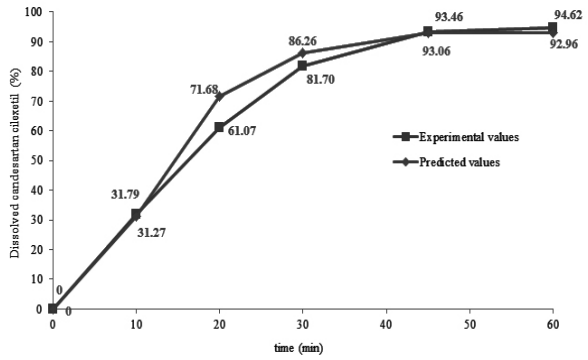

Figure 2. Comparative dissolution profiles of experimental and predicted values of control serial.

Stable immediate release candesartan cilexetil 32 mg tablets can be developed including PEG 4000 concentration of 2.5-10 % (w/w). Tablet dissolution can be determined from mathematical equations, obtained from experimental design study [17].

The key conclusion is that; dissolution and total degradation products of these tablets are two critical quality attributes, necessary to be evaluated [18].

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