STATISTICAL OPTIMIZATION OF FIXED DOSE COMBINATION OF GLIMEPIRIDE AND ATORVASTATIN CALCIUM IN IMMEDIATE RELEASE TABLET FORMULATION

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ABSTRACT

Long term administration of oral hypoglycemic drugs has been reported to be associated with increased cardiovascular mortality as compared to treatment with diet alone or diet with insulin. For this complication atorvastatin calcium can be used to reduce the cardiovascular mortility. The objective of the present study was to evaluate the effects of two factors (amount of carboxymethylcellulose sodium and sodium starch glycolate) on drug release of atorvastatin calcium and glimepiride from the tablet in order to optimize the formulation by 2 factor 3 level factorial design. Two independent variables taken were carboxymethylcellulose sodium (10mg, 12.5mg and 15 mg) and sodium starch glycolate (2.5 mg, 5mg and 7.5mg). The evaluated response were percentage release of atorvastatin ca in 60 min (Y1) and percentage release of glimepiride in 60 min (Y2) was taken as the response variables. Drug release was measured in USP 2 (paddle type) apparatus using 900 ml 0.05 M phosphate buffer (pH 6.8) solution at a rotation speed 75 rpm. The dissolution data revealed that amount of carboxymethylcellulose sodium and sodium starch glycolate are very important to achieve optimum formulation. Using responses and resulted statistical equations, optimum formulation was predicted. It shows that sodium starch glycolate has more effect than carboxymethylcellulose sodium on the tablet formulation and formulation with 14.78 mg of carboxymethylcellulose sodium and 7.5 mg of sodium starch glycolate is the optimized formulation. The optimized formulation produced dissolution profiles that were closed to predicted values.

Keywords: Cardiovascular mortality, Sodium starch glycolate, Glimepiride, Atorvastatin calcium, Statistical optimization.

INTRODUCTION

Fixed dose drug combination product is defined as combination of 2 or more active drugs in a single dosage form. Fixed dose combination product (FDCs) should be formulated in such a way that it should have an advantage over single formulation product in therapeutic effect, safety or compliance. With combination therapy, it is important to model how the drugs act in combination (synergistically, additively, or antagonistically) and how the differing population pharmacokinetics will affect the outcome. It helps to reduce the pill burden in patients with chronic diseases for e.g. in diabetes, hypertension and other cardiovascular disease. Fixed dose combination products are acceptable when the dosage of ingredients meet the requirements of a defined population group [1].

For the rational formulation design of a drug information on the biopharmaceutical properties of the active ingredient is essential. Particular attention should be given to the following six factors:-1) major absorption sites of the drug, 2) absorption rate, 3) the elimination half life of the drug, 4) whether absorption is non-linear due to drug metabolism saturation or other factors, and 6) inactivation or metabolism of drug in the body, including the gastrointestinal tract. Glimepiride is an oral blood glucose-lowering drug of the sulfonylurea class and is used in the management of type 2 diabetes. Systemic availability for Glimepiride is about 100 % with protein bound of over 99.5% bound to plasma protein and elimination half life of approximately 5 hours. Maintenance dose is 1 and 4 mg daily (max, 8 mg/day) [2].

The administration of oral hypoglycemic drugs has been reported to be associated with increased cardiovascular mortality as compared to treatment with diet alone or diet with insulin. This warning is based on the study conducted by the University Group Diabetes Program (UGDP), a long-term, prospective clinical trial designed to evaluate the effectiveness of glucose-lowering drugs in preventing or delaying vascular complications in patients with non-insulin-dependent diabetes. The study involved 823 patients who were randomly assigned to one of four treatment groups [3]. To reduce the cardiovascular complication atorvastatin calcium can be used which reduces lipid cholesterol level thereby reduces cardiovascular mortality.

Atorvastatin Calcium is a selective competitive inhibitor of HMG CoA reductase with tmax 1 to 2 hours and absolute bioavailability 14%. Atorvastatin calcium undergoes high intestinal clearance and first passes metabolism which cause low systemic availability with protein binding 98% and elimination half life of 14 hours [4]. The objective of the present study is to develop an immediate release tablet formulation of glimepiride and atorvastatin calcium varying carboxymethyl cellulose and sodium starch glycolate by direct compression method.

MATERIALS AND METHODS

Glimepiride raw drug was kindly provided by Stadmed Pvt. Ltd., Kolkata. Atorvastatin calcium was gifted by Alkem Laboratory, Mumbai. Carboxymethyl cellulose sodium, MCC and magnesium stearate were purchased from Loba Chemie, Mumbai. Sodium starch glycolate and lactose were purchased from Merck India Ltd., Mumbai.

HPLC grade water was obtained from in-house Milli-Q system of Millipore (Elix 3, MilliQ A10 Academic). Acetonitrile and methanol were purchased from Spectrochem Pvt. Ltd., Mumbai. Di potassium hydrogen phosphate and Orthophosphoric acid was purchased from Merck India Ltd., Mumbai.

High pressure liquid chromatography (HPLC) method for simultaneous determination of Glimepiride and Atorvastatin calcium

SHIMADZU LC-20 AT Prominence equipped with UV/Visible detector SPD 20A & PU-2080 PLUS pump with autosampler (Smartline 3800), Soft-ware (Eurochrom 2000) was used for simultaneous analysis of Glimepiride and Atorvastatin calcium. Column used is Hypersil BDS, C_{18} (250mm X 4.6 mm, 5 μm particle sizes) with wavelength of detection at 230 nm. Mobile phase consists of acetonitrile and 10 mmole phosphate buffer (pH 3.0).

Preparation of immediate release directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

The tablet was prepared via direct compression method. Different grades of polymers are widely used for designing oral immediate drug delivery system because their flexibility to provide a desired drug release profile and cost effectiveness and broad regulatory

acceptance. Hence, in the present work, an attempt has been made to formulate the immediate release tablets of glimepiride and atorvastatin calcium using polymer (carboxymethyl cellulose sodium-Na CMC) with lactose and microcrystalline cellulose as directly compressible diluents. Sodium starch glycolate (SSG) is used as disintegrant. The formulation containing 4 mg glimepiride and 10 mg atorvastatin calcium were developed as shown in Table 1. All the raw drugs and excipients were passed through 40 mesh size sieve

separately. Then drugs, lactose, carboxymethyl cellulose sodium, microcrystalline cellulose and 50 % of the sodium starch glycolate were mixed thoroughly and passed through 20 mesh size sieve. The powder mixture was dried for 20 min at 30 °C and passed through 18 mesh size sieve. Then it was mixed with lubricating agent aerosil and magnesium stearate before compression. The tablets were prepared by 9 mm round flat punches. (Hindustan Machinary, 12 station). The weights of the tablets were 250 mg.

Table 1: Composition of 9 Tablet formulations with different ratios of Na CMC (mg) and SSG (mg)

Composition	Formulation code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Atorvastatin calcium	10	10	10	10	10	10	10	10	10
Glimepiride	4	4	4	4	4	4	4	4	4
Sod. CMC	10	10	10	12.5	12.5	12.5	15	15	15
SSG	2.5	5	7.5	2.5	5.0	7.5	2.5	5	7.5
Areosil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mg. Stearate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
MCC	10	10	10	10	10	10	10	10	10
Lactose	208.5	206	203.5	206	203.5	201	203.5	201	198.5
Total	250	250	250	250	250	250	250	250	250

Micromeritic properties of powder blend 4 mg Glimepiride and 10 mg Atorvastatin calcium formulation.

Angle of repose of powder blend (θ) was determined to check the flow ability of the powder by the funnel method [5]. Bulk density and tapped density of powder blend of tablet were calculated [6]. The compressibility index of the powder blends were determined by Carr's index [7].

Evaluation of physical properties of tablets

All prepared tablets were evaluated for uniformity of weight and drug content, as per USP 30-NF25 method. Friability was determined using Roche friabilator. Hardness was measured by using Monsanto hardness tester. Diameter and thickness were measured by digital caliper (Mitutoyo, JAPAN). The disintegration time of the tablets were measured in 0.1 N HCL at 37 ^{0}C in disintegration apparatus. Weight variation test of the tablets was carried out as per the official method of USP. For drug content, the total amount for drug for each tablet was analyzed by using the HPLC method against the calibration curve prepared from reference solution. As we have chosen the HPLC method for sample analysis, there is no chance of detection of any degradation products.

Compatibility Study of the directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Interaction of the active constituents and inactive excipients in solid state change in the may rise to change in the solubility, stability and dissolution rate and bioavailability of drugs. A number of modern techniques can be used to indicate drug/excipient reaction. Among these techniques infrared (IR), differential scanning calorimetry (DSC) study are vastly used in compatibility study.

Before carrying out the infra red (IR) analysis, pure drugs (atorvastatin Ca and glimepiride), polymers (Na-CMC), and tablet (drug-excipients) were kept at room temperature at 37 degree Celsius for 20 days and was scanned over a wave range of 4000 cm $^{-1}$ to 400 cm^{-1} using Perkin Elmer, Spectrum-100, UK. In Differential Scanning Calorimetry (DSC) the sample and reference material are subjected to a closely controlled programmed temperature. The differential temperature (ΔT) versus the programmed temperature (T) at which the whole system is being changed tells the temperature of transitions and whether the transition is exothermic or endothermic. The information obtained from DSC provides coupled with X-ray diffraction patterns provide a quantitative and

qualitative estimation of solid-state reactions. The methodology suggested by Van Dooren and Duphar (1983) was adopted for DSC studies of pure drugs, polymer, drug & polymer mixture, composition of glimepiride and atorvastatin calcium.

The calorimeter used DSC 821 (Parkin Elmer, Switzerland) was equipped with an intra cooler, a refrigerated cooling system. Indian standard was used to calibrate the DSC temperature and enthalpy scale. Nitrogen was used as purge gas through DSC cell at the flow rate of 50 ml/min and 100 ml/min through cooling unit. The samples (5-10 mg) were hermetically sealed in an aluminum pan and heating rate was carried out at a 100 C/min from 500C to 3000C.

Dissolution study immediate release directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Drug releases of individually 6 tablets were measured in USP 2 (paddle type) apparatus (Electrolab, TDPOGP, USPXXIII) using 900 ml 0.05 M phosphate buffer (pH 6.8) solution as dissolution media at a rotation speed 75 rpm. The dissolution medium was maintained at 37 ±0.5°C throughout the study. The samples were withdrawn at the interval of 5, 15, 30, 45 minutes. At every withdrawn, the sample was replaced with 5ml of fresh media. The release rate of tablets was evaluated in a dissolution medium 0.05 M phosphate buffer (pH 6.8, adjusted with orthophosphoric acid solution). All the solution of samples was analyzed by using high performance liquid chromatography (HPLC) method.

Optimization of immediate release formulation by Response Surface Methodology (RSM)

Experimental design

The optimization of immediate release formulation of both Glimepiride and Atorvastatin calcium layer was done by using design expert software (design expert trial version 8.0.1 state inc, Minneapolis,MN). A central composite design (CCD) with α = 1 was employed as per the standard protocol [8]. Based on prefromulation study the amounts of Na CMC (X1) and SSG (X2) were selected as the independent factors, studied at 3 levels each. The central point (0, 0) was studied in singlet. All other formulation and processing variables were kept invariant throughout the study. Table 2 summarizes an account of the 9 experimental runs studied, their factor combinations, and the translation of the coded levels to the experimental units employed during the study. Percentage release of atorvastatin ca in 60 min (Y1) and percentage release of glimepiride in 60 min (Y2) was taken as the response variables.

Table 2: Formulation trials carried out for 4 mg Glimepiride and 10 mg Atorvastatin calcium individually as per experimental design

Coded factor	Levels	
Trial No	X1	X2
I	-1	-1
II	-1	0
III	-1	1
IV	0	-1
V	0	0
VI	0	1
VII	1	-1
VIII	1	0
IX	1	1
Translation of coded levels in actual units		
Coded Level for formulation	-1	0	1
X1: Na-CMC (mg)	0	12.5	15
X2: SSG (mg)	2.5	5.0	7.5

Data analysis and validation of optimization model for matrix tablet

Various RSM computations for the current optimization study were performed employing Design Expert software (Design Expert trial version 8.0.1 State-Ease Inc, Minneapolis, MN). Polynomial models including interaction and quadratic terms were generated for all the response variables using multiple linear regression analysis (MLRA)

approach. Statistical validity of the polynomials was established on the basis of ANOVA provision in the Design expert Software. Subsequently, the feasibility and grid searches were performed to locate the composition of optimum formulations [9]. Three-dimensional (3D) response surface plots and two dimensional (2-D) contour plots were constructed based on the model polynomial functions using Design Expert software. These plots are very useful to see interaction effects on the factors on the responses. Two optimum checkpoints for the formulation were selected by intensive grid search, performed over the entire experimental domain, to validate the chosen experimental design and polynomial equations. The formulations corresponding to these checkpoints were prepared and evaluated for various response properties. Subsequently, the resultant experimental data of response properties were quantitatively compared with that of their predicted values.

RESULTS AND DISCUSSION

Micromeretic properties of immediate release directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

The directly compressed tablet was formulated as described in materials and methods. The properties of blended mixture which should be evaluated to ensure the proper formulation of the tablet dosage form are an important aspect in tablet formulation. Micromeritic properties of the formulation which are illustrated in table 3 were found to be within normal limit.

Table 3: Micromeretic properties of different trial formulation of immediate release directly compressed tablet

Formulation Code	Angle of repose*(Degree)	Loose bulk density* (gm/ml)	Tapped density* (gm/ml)	Carr's index* (%)	Hausner ratio*(%)
F1	32.12 ± 0.03	0.725 ± 0.02	1.005 ± 0.04	27.86 ±1.47	1.38 ± 0.16
F2	32.32 ± 0.05	0.744 ± 0.04	0.976 ± 0.06	23.77 ±1.50	1.31 ± 0.15
F3	33.48 ± 0.02	0.758 ± 0.04	0.964 ± 0.02	21.36 ±1.26	1.27 ± 0.13
F4	34.36 ± 0.04	0.744 ± 0.02	0.987 ± 0.01	24.62 ±1.42	1.32 ± 0.14
F5	33.59 ± 0.02	0.786 ± 0.03	0.938 ± 0.03	16.20 ±1.12	1.19 ± 0.15
F6	33.78 ± 0.07	0.752 ± 0.04	0.982 ± 0.06	23.42 ±1.32	1.30 ± 0.13
F7	34.46 ± 0.04	0.722 ± 0.04	0.955 ± 0.07	24.39 ±1.02	1.32 ± 0.16
F8	34.46 ± 0.04	0.701 ± 0.05	0.939 ± 0.08	25.34 ±0.06	1.33 ± 0.16
F9	33.98 ± 0.05	0.716 ± 0.06	0.946 ± 0.05	24.31 ±0.04	1.32 ± 0.18

*mean ± SD (n = 3)

Physical properties of different trial formulation of immediate release directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

All the formulated tablets containing the active drugs were evaluated to find the physical properties like hardness, thickness, friability and drug contents depicted in table 4. The friability of all the formulations was within the limit. All the formulation should uniform thickness. The property of thickness is important for packaging purpose. In a weight variation test, the pharmacopeia limit of percentage deviation for tablets whose weight is between 130-324 mg is ± 7.5%.The average percentage deviation of all the tablets were found within the limit which was less than 2%. Hardness of the tablets were found acceptable and uniform from batch to batch variation. The drug content was also found uniform and within the prescribed limit. Disintegration times of all the formulations were within 2.10 minutes.

Compatibility study of different trial formulation of immediate release directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Fourier transform infra-red (IR) study of directly compressed tablet containing 4mg Glimepiride and 10 mg Atorvastatin calcium

The drug-polymer and drug-excipient compatibility was ascertained by carrying out the IR study of - a) pure glimepiride, b) pure atorvastatin calcium, c) final combination of glimepiride and atorvastatin calcium tablet. The spectrums obtained have been illustrated in Fig.1. It has been observed that the absorption bands of both glimepiride and atorvastatin calcium in formulation and pure form remain unchanged. Also there is no drug-excipients interaction was found at the absorption bands of glimepiride and atorvastatin calcium tablet. The principle peaks of atorvastatin calcium and glimepiride are shown in table 5A and 5B.

Table 4: Physical properties and drug content of immediate release tablet

Formula-tion Code	Friability (%)	Hardness (kg/ cm²)	Crown thickness (mm)	Drug content (%)	Weight variation(mg) SD (n=20)	Disintegration Time (min)
F1	0.98 ± 0.04	6.1	3.02 ± 0.01	97.09±0.12	254.08 ± 1.58	2.06 ± 0.02
F2	1.02 ± 0.02	6.2	3.01 ± 0.02	98.03±0.03	251.02 ± 1.46	1.59 ± 0.01
F3	0.84 ± 0.12	6.8	3.05 ± 0.01	99.12±0.12	252.04 ± 1.34	1.47 ± 0.02
F4	0.95 ± 0.05	6.5	3.02 ± 0.01	98.04±0.10	251.15 ± 1.67	1.41 ± 0.02
F5	1.02± 0.04	5.9	3.01 ± 0.03	98.09±0.11	252.06 ± 1.27	1.55 ± 0.01
F6	0.96 ± 1.02	6.2	3.03 ± 0.02	97.09±0.13	249.98 ± 1.54	2.01 ± 0.03
F7	1.04 ± 0.04	6.5	3.04 ± 0.01	98.01±0.03	252.16 ± 1.49	1.39 ± 0.02
F8	1.02 ± 0.08	6.4	3.02 ± 0.01	98.01±0.12	252.46 ± 1.92	1.47 ± 0.03
F9	0.98 ± 0.03	6.6	3.01 ± 0.02	98.07±0.03	253.21 ± 1.35	1.56 ± 0.02

Table 5A: Principle IR peaks of Atorvastatin calcium shown as follows

Peak (cm⁻¹)	Functional group	Type of vibration
3366.14	Amine (-N-H)	Stretch (medium)
2965.98	Aromatic -C-H	Stretch (medium)
1651.73	Carbonyl (C= O)	Stretch (Strong)
1576.52	Aromatic C=C	Stretch (weak, multiple)
1512.88	Carboxylate	Stretch (Strong, asymmetrical)
1315.21	Aromatic in plane bending C-H	Stretch (medium)
1220.72	Mono fluorinated benzene ring	Strong, narrow absorption band

Table 5B: Principle IR peaks of Glimepiride shown as follows

Peak (cm⁻¹)	Functional group	Type of vibration
3369.03	Amine (-N-H)	Stretch (medium)
2931.27	Aromatic -C-H	Stretch (medium)
1706.69	Amide (C=O)	Stretch (Strong)
1674.87	Methylene cyclohexane	Stretch (Scissoring)
1081.87	Sulfoxides	Stretch (Strong)
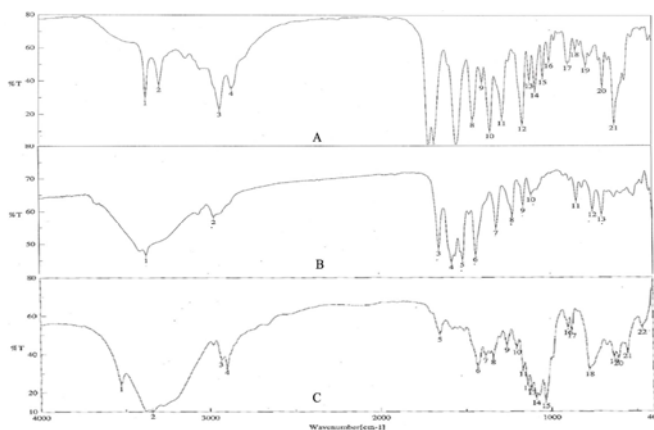
Fig. 1: Infra red spectrum (IR) of pure Glimepiride powders (A), pure Atorvastatin calcium powder (B) and Formulation (C)

Differential Scanning Calorimetry (DSC) study of directly compressed tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Interactions in the sample are derived or deduced from DSC by changes in the thermal events, such as elimination of an endotherm or exotherm peak, or appearance of a new peak. However, some broadening of peaks leading to changes in peak temperature occurs simply due to mixing of the components without indicating any significant interaction. If all thermal features more or less remain the same, compatibility can be expected as well as there is no complex formation. The differential scanning calorimetry (DSC) pattern of pure drugs (glimepiride and atorvastatin calcium) and formulation is shown in Fig. 2. It was observed that the DSC pattern of both glimepiride and atorvastatin calcium did not alter in the tablet. Also there was no drug-drug interaction between glimepiride and atorvastatin calcium as seen from Fig. 2 (C). The pure drugs displayed a single sharp endothermic peak at 160.78 °C for atorvastatin calcium & 216.49 °C for glimepiride corresponding to the melting point of the drugs and an identical peak was also observed in the tablet formulations. The thermographic result shows that the drugs retain its identity in the tablet formulations.
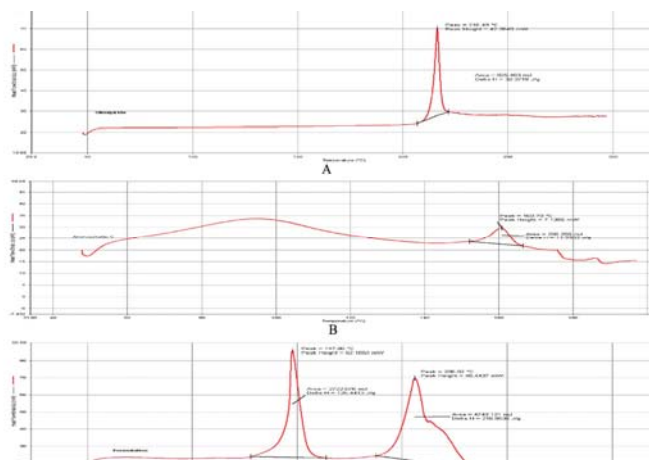
Fig. 2: Differential Scanning Calorimetry (DSC) thermogram of pure Glimepiride powder (A), **pure Atorvastatin calcium powder (B) and the tablet formulation (C)**

Dissolution study of immediate release tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Chromatographic analysis

Dissolution samples were analyzed by HPLC method described section 2.2.1. Figure 3 shows the representative chromatogram of a dissolution sample showing separation of 4 mg glimepiride and 10 mg atorvastatin calcium at retention time 9.247 min and 6.960 min respectively.
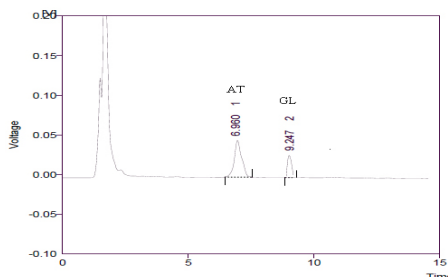

Fig. 3: Representative chromatogram of a dissolution sample

In vitro release of immediate release tablet containing 4 mg Glimepiride and 10 mg Atorvastatin calcium

Dissolution study was carried out to determine the release of 4 mg glimepiride and 10 mg atorvastatin calcium tablet as per the procedure described in section 2.2.6. Mean cumulative % release of atorvastatin calcium and glimepiride at different time intervals are illustrated in fig 4A and fig 4B respectively. The dissolution profile for both drugs was found to be different from batch to batch. But the formulation of F9 were found to be most desired release profile for both the drugs having most consistent, accurate and complete.

The response variables are % release in 60 min of atorvastatin calcium (Y1) and % release in 60 min of glimepiride (Y2). The independent variables selected are Na CMC (X1) and SSG (X2). All the in- vitro release kinetic property of 9 formulations were shown

in table 6. Three different concentration of Na CMC (X1) and SSG (X2) were used to evaluate and optimized the formulation.

Fig 4A: Invitro mean cumulative % release of Atorvastatin calcium

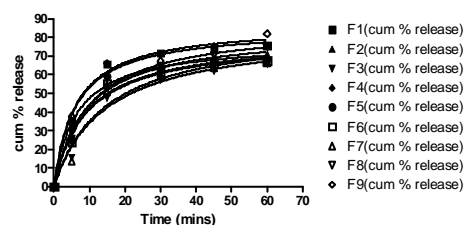
Fig 4B: In vitro mean cumulative % release of Glimepiride

Response surface methodology (RSM) optimization of the FDC of Atorvastatin calcium and Glimepiride

For optimization of the formulation we had selected central composite design. In central composite design the 2 response variable and two independence variables were taken.

Table 6: Response variables (Y1 and Y2) obtained from various trial formulations of immediate release tablet

Trial No.	Na-CMC mg (X1)	SSG mg (X2)	% Release at 60 min for Atorvastatin Ca (Y1)	% Release at 60 min for Glimepiride (Y2)
F1	10	2.5	75.512	55.175
F2	10	5.0	70.816	48.712
F3	10	7.5	65.923	42.262
F4	12.5	2.5	67.192	44.268
F5	12.5	5.0	68.347	46.274
F6	12.5	7.5	67.548	43.162
F7	15	2.5	66.465	40.066
F8	15	5.0	74.752	46.382
F9	15	7.5	81.956	52.732

Mathematical modeling for RSM optimization of FDC containing Glimepiride and Atorvastatin calcium

Mathematical relationships in the form of polynomial equation for the measured response (% Release in 60 min for atorvastatin calcium and glimepiride respectively) were obtained with the stat-ease software. The polynomial equation relating the different response and independent variable is given below:

$Y1 \ (Rel_{60 \ min \ atv}) = +67.90 + 1.70X1 + 1.18X2 + 5.96X1X2 + 5.11X1^2 - 0.33X2^2$

$Y2 \ (Rel_{60 \ min \ gle}) = +45.06 - 1.11X1 - 0.23X2 + 6.39X1X2 + 2.94 \ X1^2 - 0.75X2^2$

The above equation represents the quantitative effect of process variables and their interaction on the response. For estimation of the significance of the model, the analysis of variance (ANOVA) was determined as per the provision of design expert software as shown in Table 7A and 7B. Using 5% significance level, a model is considered significant if the p-value (significance probability value) is less than 0.05.

Table 7A: ANOVA for response surface quadratic model for % release in 60 min of Atorvastatin calcium (Y1)

Table 7B: ANOVA for response surface quadratic model for release in 60 min of Glimepiride (Y2)

Response	2		Release of Gle at 60 mins			
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
	Sum of		Mean	F	p-value	
Source	Squares	df	Square	Value	Prob > F	
Model	189.65	5	37.93	31.26	0.0086	significant
A-Na CMC	7.41	1	7.41	6.11	0.0899	
B-SSG	0.31	1	0.31	0.25	0.6505	
AB	163.57	1	163.57	134.82	0.0014	
A²	17.25	1	17.25	14.22	0.0327	
B²	1.11	1	1.11	0.92	0.4093	
Residual	3.64	3	1.21			
Cor Total	193.29	8				

The Model F-value of 31.26 implies the model is significant. There is only a 0.86% chance that a "Model F-Value" this large could occur due to noise.

Response surface analysis of Atorvastatin calcium (% Release in 60 min of atorvastatin calcium)

Fig. 5 represents the contour plot and 3 dimensional analyses for the studied response properties of the % release in 60 min. From the contour plot it can be concluded that release in 60 min decrease with augmentation of both the variables. The response changes the variables in a linear and descending manner. But the contour plot shows that Na CMC has a completely greater influence on the response variables than SSG. From the contour plot it was evident that the declining trend obtained with ascending order of Na CMC. According to contour plots the best area for formulation to obtain desired responses was found.
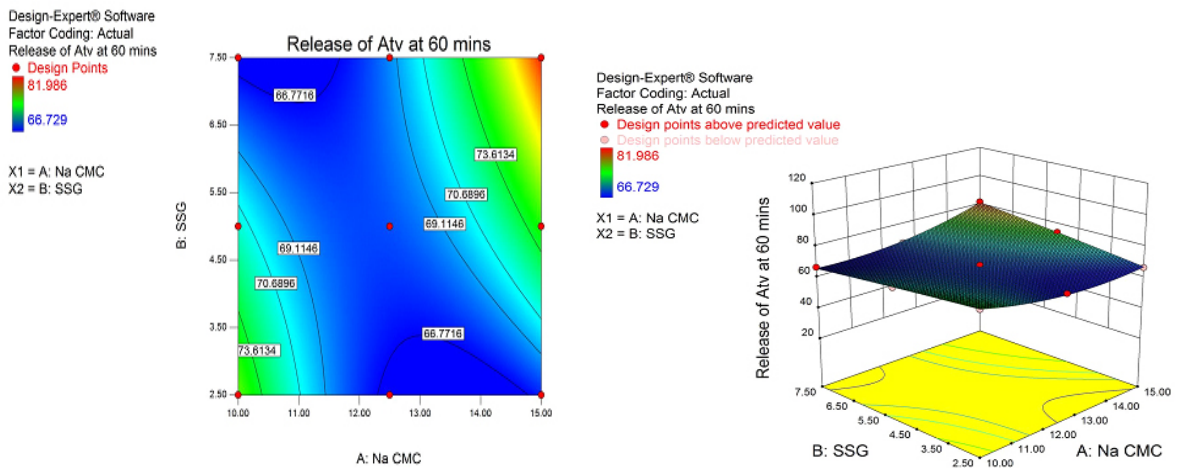

Fig. 5: Contour plot and 3D showing the effect of the amount of Na CMC (X1) and SSG (X2) on drug release at 60 min of Atorvastatin ca

Response surface analysis of Glimepiride (% Release in 60 min of glimepiride)

Fig. 6 represents the contour plot and 3 dimensional analyses for the studied response properties of the % release in 60 min of glimepiride. From the contour plot it can be concluded that release in 60 min decrease with augmentation of both the variables. The response changes the variables in a linear and descending manner. But the contour plot shows that Na CMC has a completely greater influence on the response variables than SSG. From the contour plot it was evident that the declining trend obtained with ascending order of Na CMC. At the lowest level of Na CMC the percentage of drug release at 60 min decreases with an increase in the level of SSG.
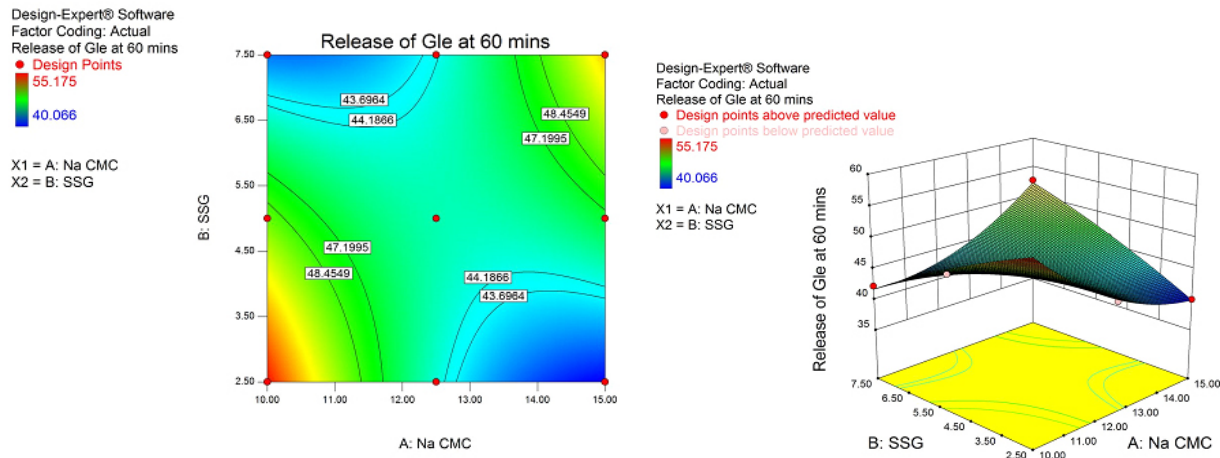

Fig. 6: Contour plot and 3D showing the effect of the amount of Na CMC (X1) and SSG (X2) on drug release at 60 min of Glimepiride

Optimization of the independent variables (Y1 and Y2) of the formulation

This was the most important part of response surface methodology. The % release at 60 min (Y1 and Y2) was targeted and the process variables were kept within the range. The criteria for optimum formulation are shown in table 8:

Table 8: Release Property of optimum formulation

Release property	Target
% Release at 60 min of atorvastatin Ca	80
% Release at 60 min of glimepiride	55

After generating the model polynomial equation to relate the dependant and independent variable, the combination was optimized for all 2 responses. The final optimal experimental parameter were calculated using the optimization technique in this design expert software which allows to compromise among various responses and searches for a combination of factor level that jointly optimize a set of responses by satisfying the requirement of each responses in the set. In the study the optimization was performed with constraints for the responses, present in table 8. The design expert software gives 2 checkpoint solutions of which 1st one has high desirability value of 0.870.

Solutions

Number	Na CMC	SSG	Release of A	Release of G	Desirability	
1	14.78	7.50	80.0002	51.3645	0.870	Selected
2	10.00	2.50	75.7556	54.9882	0.824	

The optimal level and amount of two independent variable is given below –

Independent Factor	Optimum level	Optimum amount (mg)
Na CMC	0.913	14.78
SSG	1.000	7.5

The optimum formula is:

Composition	Amount(mg)
Atorvastatin calcium	10
Glimepiride	4
Sod. CMC	14.78
SSG	7.5
Areosil	2.5
Mg. Stearate	2.5
MCC	10
Lactose	198.72
Total	250

Table 9 summarized the observed & predicted responses & the residual values for the drug release. Test performed at optimal values of the analytical parameters are investigated in the study. From the result presented in the table 9, it can be concluded that the optimized formulations ensured the release profile, which was much closed to the predicted values with low residual value and therefore factorial design is valid for predicting the optimum concentration. Table 10 shows the invitro dissolution profile of the optimized formulation.

Table 9: Observed and predicted responses (Y1 and Y2) and residual values for the drug test performed at optimal values of factors investigated

Response	Observed response	Predicted response	Residual value
Y1	81.526	80.00	1.526
Y2	53.732	51.364	2.368

Table 10: Invitro cumulative % release of atorvastatin calcium and glimepiride from the optimized formulation Release (min)

Formulation	0	5	15	30	45
% release of atorvastatin	0	38.697	66.465	67.221	76.728
% release of glimepiride	0	8.262	21.967	34.621	42.561

CONCLUSION

The immediate release formulation of FDC containing glimepiride and atorvastatin calcium was prepared by direct compression. It was concluded that the Response Surface Methodology (R.S.M.) & multiple response optimization utilizing a polynomial equation can be successfully use to design an immediate released formulation for predetermined release profile in a very short time period & with a small no. of experimental runs. The optimum amount obtained were 14.78 mg and 7.5 mg for Na CMC and SSG respectively. From DSC and FTIR study it may be concluded that no interaction occurs between the two API (glimepiride and atorvastatin calcium).

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