AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Compatibility Study of Atorvastatin Calcium and Telmisartan with Selected Excipients and Formulation of a Bilayer Tablet Using Box Behnken Design

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ABSTRACT

Present study was carried out to study compatibility of Atorvstatin Calcium (ATR) and Telmisartan (TEL) with selected generally regarded as safe (GRAS) excipients and to prepare bilayer tablet. Isothermal stress testing was performed in binary mixtures which was subjected to 50°C for 4 weeks. Isothermal stressed samples were evaluated with RP-HPLC method and FTIR analysis. A trial was conducted with single disintegrant and diluent with direct compression technique and *in-vitro* dissolution was carried out. To improve the release profile of ATR layer, multiple disintegrants were used namely croscarmellose sodium (CCS), sodium starch glycolate (SSG) and crosprovidone (CP). These disintegrants were taken as variable in Box-behnken design (BBD) in Minitab 16.2.3.0. The tablets were prepared from obtained design formulations by direct compression. Similarly, Telmisartan layer was prepared by wet granulation technique. TEL was treated with alkalizing agent, sodium hydroxide in stoichiometrical proportion. Croscarmellose sodium, sodium starch glycolate and crosprovidone were taken as variable and subjected to BBD. Intra and extra granulation was done with mixtures of disintegrants. Optimized formulation as per response surface optimizer in Minitab 16 contains be 10.0 mg of SSG, 7.36 mg of CP and 10.0 mg of CCS per tablet for ATR layer. Similarly in case of TEL tablet, it was found that as concentration of SSG, CP and CCS increases, the release of drug also increases. When multiple disintegrants were used in the combination, better release was obtained. Optimize formulation as per response surface optimizer in Minitab 16 contains 22.03 mg of SSG, 15 mg of CP and 15 mg of CCS per tablet for Telmisartan layer. Bilayer tablet was prepared with this optimized formulation in 10 station compression machine. The prepared bilayer tablet was further evaluated and *in-vitro* dissolution were compared with market products. Thus, the conversion of salt form, optimized combination of disintegrants, use of polyvinyl pyrrolidone K-30 as carrier, intra and extra granulation was observed to be effective in release of TEL from the dosage form where as only the optimized combination of disintegrants was sufficient for release of ATR from the dosage form.

Keywords: Compatibility, Isothermal stress testing, Atorvastatin Calcium, Telmisartan, Bilayer tablet.

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Received 08 April 2015, Accepted 17 April 2015

Please cite this article as: Bajracharya R *et al*., Compatibility Study of Atorvastatin Calcium and Telmisartan with Selected Excipients and Formulation of a Bilayer Tablet Using Box Behnken Design. American Journal of PharmTech Research 2015.

INTRODUCTION

High serum cholesterol in patients was reported with arterial hypertension. The statins (Atorvastatin) in combination with antihypertensive drugs (Telmisartan) can improve blood pressure control in patients with uncontrolled hypertension and high serum cholesterol levels. This combination also improves compliance, simplifies complex regimens, and might lower drug cost as well. In this back drop, the present work was undertaken to study compatibility studies of Telmisartan and Atorvastatin Calcium with selected excipients and formulate in combination as a bilayer release dosage form. As both Atorvastatin Calcium and Telmisartan belonging to BCS Class II, the dissolution enhancement of both is one of the challenging tasks.

MATERIALS AND METHOD

Telmisartan and Atorvastatin Calcium along with reference standard and other excipients, Sodium Starch Glycolate, Crospovidone, Croscarmellose Sodium, Avicel pH 102 , Sodium Lauryl Sulphate, Dicalcium Phosphate, Lactose Anhydrous, Spray Dried Lactose, Polyvinyl Pyrrolidone K 30, Calcium Carbonate, Sodium Hydroxide, Magnesium Stearate, Sodium Stearyl Sulfate, Stearic acid, Mannitol, Talcum, Colloidal Silicon Dioxide and lake sunset yellow were received from Deurali-Janta Pharmaceuticals Pvt. Ltd, Dhapasi, Kathmandu, Nepal as gift samples. Acetonitrile (HPLC grade), Methanol (HPLC grade), Potassium dihydrogen Phosphate, and Ammonium acetate were reagents used in the analysis.

Analytical Method Development

A simple, precise and accurate reversed-phase liquid chromatographic method was developed for the simultaneous estimation of ATR and TEL. The chromatographic separation was achieved on (Inertsil C18, 250 mm × 4.6 mm, 5 µ) analytical column. A mixture of ammonium acetate (0.02M, pH 4.0 adjusted with glacial acetic acid) and acetonitrile in ratio 40:60 v/v at flow rate 1.0 ml/min and detector wavelength 254 nm was found to be optimum. The retention time of ATR and TEL was found to be 4.6 and 6.1 min respectively.

PREPARATION OF THE STANDARD AND SAMPLE SOLUTION

Standard Solution Preparation

The standard stock solution of the drug was prepared by weighing accurately 50 mg ATR working standard and 40 mg TEL working standard and transferred in separate 100 ml clean dry volumetric flask marked as stock solution A and B respectively. About 70 ml of methanol was added and sonicated to dissolve it completely and cooled to room temperature and the volume was made up to the mark with the same solvent. From the above prepared stock solution, 1.0 ml and 5.0 ml was

pipette out from A and B respectively, in 50 ml clean dry volumetric flask and diluted up to the mark with methanol.

Sample Solution Preparation

The compatibility study samples solution were prepared by diluting the sample with methanol and made 0.01 µg/ml concentration of ATR sample and 0.04 µg/ml concentration of TEL samples with diluent. Similarly in case of tablet, 20 tablets were crushed and sample weighed and same concentration sample was prepared.

Standard and sample solution injected inside the column

The standard and sample solutions previously filtered through 0.2 µ filter and transferred in vials were kept in rack of HPLC with 20 µl injection volume setting along with other Chromatographic parameters. The peak areas were measured for the ATR and TEL and then assay was calculated by using suitable formulae.

Analytical Method Validation

The proposed method developed was validated. The validation was carried out for its specificity, linearity, accuracy, precision, robustness, limit of detection and quantification for both ATR and TEL.

Compatibility studies of Atorvastatin Calcium and Telmisartan

Micro-environmental pH testing

To 20 ml vial, 300 mg of ATR and 300 mg of different excipients as mentioned in Annex I were taken. This mixture was blended under vortex mixer for 4 minutes followed by addition of 60 µl of purified water using a micropipette to achieve water concentration of 20% w/w. This mixture was further mixed for 4 minutes in a vortex mixer in order to achieve consistent mixing. Vials were sealed properly and were stored in an oven set at 50°C for 4 weeks period. Drug- excipient blends, without addition of water, stored in refrigerator were taken as controls. The microenvironmental pH of drug-excipient blend was estimated by adding 3 ml of previously boiled and cooled purified water to 600 mg blend in the vial, mixing the suspension with a vortex mixer and then recording the pH with a pH meter. Microenvironmental pH at zero time was also checked. Similarly microenvironmental pH of TEL with different excipients was observed.

Isothermal stress testing

Hundred milligram of each of ATR and selected excipients in the ratio of 1:1 as mentioned in Annex were accurately weighed into 10.0 ml borosilicate glass vial (n=3) with screw capped vial followed by mixing in a vortex mixer for 4 minutes and to this mixture 20 µl of purified water was added using a micropipette to achieve water concentration of 10% w/w. This mixture was mixed

for 4 minutes in a vortex mixer in order to achieve consistent mixing. The mixture blend is further mixed using a glass capillary (both the ends of which were heat sealed). To prevent any loss of material, the capillary was broken and left inside the vial. Vials were sealed properly and were stored in an oven set at 50°C for 4 weeks period. These vials were identified as 'stability samples'. Drug- excipient blends, without addition of water, stored in refrigerator, served as controls. Similarly TEL and excipients in the ratio 1:1 as mentioned in Annex were treated. The drug excipient blends were periodically examined for any unusual color change. After completion of 4 weeks samples were analyzed as follows.

HPLC analysis

The control sample and IST samples were analyzed in HPLC as mentioned in method.

FTIR analysis

Potassium bromide was dried in moisture balance and small amount of potassium bromide was taken in an agate mortar and to this very small amount (about 2 mg) of drug excipient blend from samples was added. Proper mixing was carried out in mortar with pestle and this blend was kept in the sample plate directly in FTIR. Thus obtained powder sample was analyzed by diffuse reflection method in FTIR in the IR range of 4000 to 400 cm^{-1} taking average of 20 consecutive scans.

Software Used

For data analysis Minitab 16.2.3.0 software was used.

Tablet Preparation Method

Atorvastatin Calcium layer Tablets

The composition of the ATR formulation based on BBD was taken. Spray dried lactose was sieved through mesh 20, Aerosil, Croscarmellose sodium and Crosprovidone through mesh 40 and talcum, magnesium stearate, calcium carbonate and sodium starch glycolate through mesh 100. ATR and 65% of aerosil was mixed by manual tumbling in a poly bag for 5 mins and passed through mesh 40, followed by 20 % of total calcium carbonate was passed through the same mesh 40 to wash down the adhering drug particles. Lubricant premix was prepared by alternately passing talcum, remaining aerosil and magnesium stearate. Active Premix, spray dried lactose, croscarmellose sodium, sodium starch glycolate, crosprovidone and remaining calcium carbonate was mixed manually for 20 minutes in polybag. The above mixed powder and lubricant premix was mixed for about 3 minutes. Pre-compression parameters were determined with lubricated granules. The above prepared blend was compressed using 10 stationary rotary punching machine until desired hardness was obtained.

Telmisartan layer tablets

The compositions of the formulation based on BBD were taken. TEL was sieved through mesh 100. Aerosil, Croscarmellose sodium and Crosprovidone were sieved through mesh 40 and talcum, magnesium stearate, and sodium starch glycolate through mesh 100. Sodium hydroxide was dissolved in the mixture of water and ethanol (50:50). Povidone K-30 was added and stirred till it dissolved. Lake sunset yellow was added to the solution. Crosprovidone, sodium starch glycolate and croscarmellose sodium was mixed manually in polybag. 50 % of this mixture disintegrant was mixed with Telmisartan in polybag for 5 min. The wet granulation was done manually with above granulating solution. Thus prepared granules were passed through mesh 14. The above granules were dried in oven at 60°C and screened through mesh 10. The above dried granules were mixed with remaining half mixture of disintegrants and lactose anhydrous. Lubrication was done to above mixed granules with magnesium stearate. The above prepared blend was compressed using 10 station rotary punching machine with desired hardness.

Formulation of Bilayer Tablet

After optimizing the formulation of each layer, bilayer tablets were prepared with same method of preparation. ATR optimized formulation was prepared by direct mixing with sieved excipient and TEL optimized granules was prepared by intra and extra granulation. The formulation of optimized batch was given in Annex V-A. The optimized formulations were compressed in 10 station machine by manually weighing as batch size was less. Only single punch and die were used. Two hundred milligram of ATR blend and three hundred milligram of TEL blend were weighed. ATR blend was fed into the die and aligned properly with small spatula to make uniform powder bed with smooth surface and TEL blend was fed over it and compressed.

RESULTS AND DISCUSSION

Analytical Method Validation

System suitability results

The results of tailing factor and theoretical plates obtained in system suitability (n=5) are given below in Table 1.

Table 1: Results of system suitability

	Acceptance Criteria of Tailing Factor	Tailing Factor	Acceptance Criteria of Theoretical plates	Theoretical Plates
ATR	NMT 2	1.13	NLT 2000	5137.03
TEL		1.12		5906.56

Linearity of Atorvastatin Calcium

From reference standard stock solution of ATR, samples were withdrawn and diluted with methanol (HPLC grade) in suitable volumetric flasks to get concentration of 5, 10, 20, 40 and 80 µg/ml. The solutions were transferred in the vials and subjected to HPLC analysis.

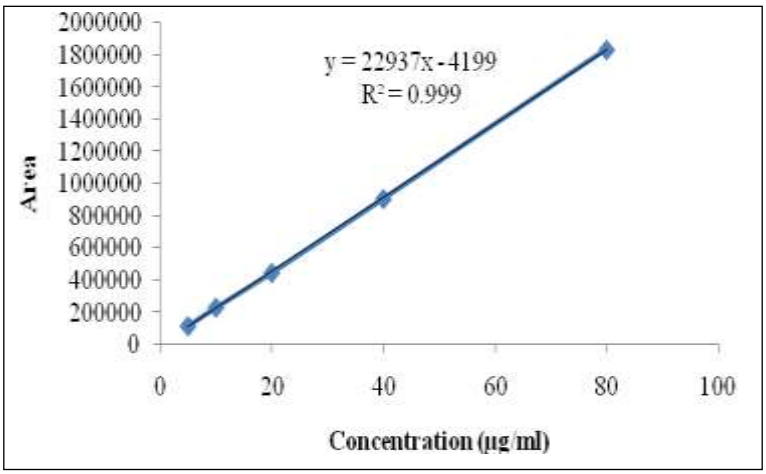

Figure 1.Areas of ATR standards and its correlation coefficient

Linearity of Telmisartan

From reference standard stock solution of TEL, sample were withdrawn and diluted with methanol (HPLC grade) in suitable volumetric flasks to get concentration of 10, 20, 40, 60 and 80 µg/ml. Vials were made and subjected to HPLC analysis.

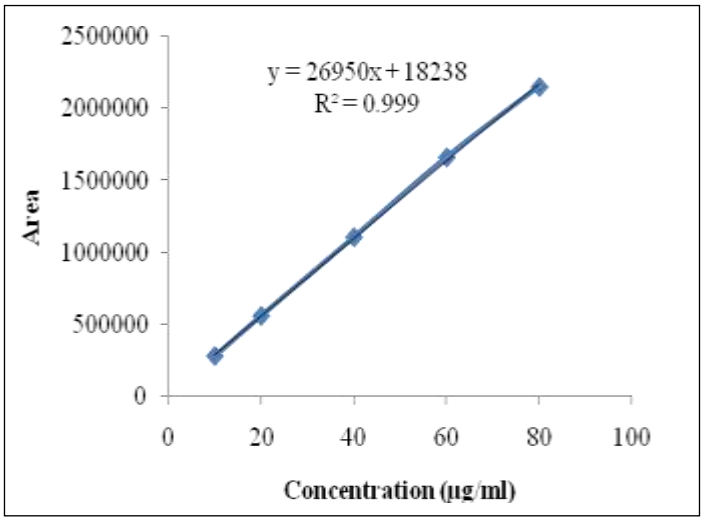

Figure 2: The area of TEL standard and its correlation coefficient

Precision

The mixed standard solution was prepared from the stock solutions A and B containing ATR and TEL respectively and injected five times. The results of five injections of mixed standard solution are given in Table 2.

Table 2: Results of five injections of mixed standard solution for precision studies

Std. injection	Retention Time of ATR	Peak Area of ATR	Retention Time of TEL	Peak Area of TEL
1	4.484	228924	6.543	1079897
2	4.483	229212	6.542	1082516
3	4.485	229834	6.543	1084864
4	4.486	229584	6.545	1084377
5	4.486	230938	6.545	1090542
Ave	4.484	229698	6.543	1084439
Std Dev	0.001	775.300	0.001	3929.337
% RSD	0.029	0.337	0.020	0.362

The % RSD for the area of five standard injections of ATR and TEL was 0.029 and 0.362 respectively which signifies that the proposed method is precise.

Intermediate Precision/Ruggedness

Intermediate precision was performed on different day. The mixed standard solution was prepared from the stock solutions A and B containing ATR and TEL respectively and injected five times. The results of five injections of mixed standard solution are given in Table 3.

Table 3: Results of five injections of mixed standard solution for intermediate precision studies

Std. injection	Retention Time of ATR	Peak Area of ATR	Retention Time of TEL	Peak Area of TEL
1	4.08	221829	6.181	1099756
2	4.08	224197	6.182	1102120
3	4.08	223562	6.181	1101941
4	4.076	223395	6.174	1101437
5	4.074	223662	6.172	1101056
Ave	4.078	223329	6.178	1101262
Std Dev	0.002	890.639	0.004	940.518
RSD	0.065	0.398	0.075	0.085

The % RSD for the area of five standard injections of ATR and TEL was 0.065 and 0.085 respectively which signifies the ruggedness of the proposed method.

Accuracy

The accuracy study was conducted by injecting triplicate standard solutions of 50%, 100% and 150%.

Limit of Detection (LOD)

By using the data from linearity curve and using the equation 2.3, the detection limit was found to be 0.11 µg/ml and 0.48 µg/ml for ATR and TEL respectively.

Limit of Quantification (LOQ)

By using the data from linearity curve and using the equation 2.4., the quantification limit was found to be 0.34 µg/ml and 1.46 µg/ml for ATR and TEL respectively.

Range

The range of concentration for the quantification of ATR in this analytical procedure is 5 - 80 µg/ml. The range of concentration for the quantification of TEL in this analytical procedure is 10-80 µg/ml.

Robustness

As part of the robustness, deliberate change in flow rate and mobile phase composition was made to evaluate the impact on the method. Variation at flow rate (0.9 ml/min to 1.1 ml/min) The standard solution of ATR (10 µg/ml) and TEL (40 µg/ml) was prepared and analyzed using various flow rates along with actual flow rate. The results of system suitability of ATR and TEL at different flow rate are given below in Table 4 and 5 respectively.

Table 4: Result of system suitability of ATR at different flow rate

S.No.	Flow rate (ml/min)	Theoretical plate count	Tailing Factor
1	0.9	5615.56	1.13
2	1.0	5332.60	1.13
3	1.1	4904.58	1.13

Table 5: Result of system suitability of TEL at different flow rate

S.No.	Flow rate (ml/min)	Theoretical plate count	Tailing Factor
1	0.9	6689.23	1.10
2	1.0	6345.36	1.10
3	1.1	5914.25	1.10

On evaluation of the above results, it was concluded that the slight variation in flow rate did not affect the method significantly as theoretical plates and tailing factors were within acceptable criteria. Hence it indicated that the method was robust even by slight change in the flow rate.

Variation in organic composition of the mobile phase

The standard solution of ATR (10 µg/ml) and TEL (40 µg/ml) was prepared and analyzed using the various mobile phase compositions along with the actual mobile phase composition in the method. The results of system suitability of ATR and TEL at different composition of mobile phase are given in Table 6 and 7respectively.

Table 6: Result of system suitability of ATR at different composition of mobile phase

S.No.	Change in organic composition in the mobile phase	Theoretical Plate count	Tailing factor
1	10% less	7329.27	1.07
2	Actual	5332.60	1.13
3	10% more	5076.17	1.15

Table 7: Result of system suitability of TEL at different composition of mobile phase

S.No.	Change in organic composition in the mobile phase	Theoretical Plate count	Tailing factor
1	10% less	7585.86	1.07
2	Actual	6345.36	1.10
3	10% more	6360.81	1.12

On evaluation of the above results, it was concluded the variation in 10% organic composition in the mobile phase did not affect the method significantly as theoretical plates and tailing factors were within acceptable criteria. Hence it indicated that the method was robust even by slight change in the mobile phase composition. The test for accuracy of method of assay showed mean % recovery value of 100.76 and 100.22 for ATR and TEL respectively. All these values were within the acceptable range (98.0% - 102.0%) and hence, the method holds the good accuracy.

Experimental Design

Compatibility studies

On periodic observation of IST samples and control samples, there was no any physical change in the color. There was no significant change in the potency of control samples and IST samples. In addition, no any additional peaks were appeared in the chromatographs of control samples and IST samples. In the microenvironmental pH study of binary mixture of drugs and their stability samples and control samples, the blend mixtures impart pH in the range of pH 3.6 to 9.8 at zero time. The stability samples and control samples also showed the pH in the range of pH 3.6 to 9.8. The results indicate that the microenvironmental pH did not affect the compatibility of drugs and excipients. FTIR spectra of each ingredient and IST samples were scanned. The IR spectrums of IST samples were compared with IR spectrums of control sample of binary mixtures. There were no remarkable change found in the peaks between IST samples and corresponding control samples. These confirm the stability of the drug. The spectrum of reference standard of ATR showed characteristic bands of amine (N-H) at 3364 cm^{-1}, aromatic C-H at 2972 cm^{-1}, carbonyl (C=O) group at 1650 cm^{-1}, aromatic C=C at 1579 cm^{-1}, carboxylate group at 1510 cm^{-1}, aromatic in-plane bending (C-O stretching) at 1318 cm^{-1}, C-N stretching at 1217 cm^{-1} and aromatic out of plane bend at 692 cm^{-1}. Similarly the spectrum of reference standard TEL showed characteristic bands of C=O stretching at 1696 cm^{-1}, C=N stretching at 1684 cm^{-1}, C-O stretching at 1324 cm^{-1}, C-O bend at 1410 cm^{-1}, and C-N stretching at 1304 cm^{-1}. Effect of 10% w/w moisture on ATR and TEL was studied and chromatograms revealed no significant degradation. FTIR spectra of ATR and TEL subjected to isothermal stress testing with additional 10% w/w and without moisture, retained all the peaks and intensity of the peaks were found to be intact. This suggests that ATR and TEL are

stable at 50°C with additional 10% w/w moisture and without addition of moisture. Effect of combination of ATR and TEL was also studied with and without addition of 10% w/w moisture. The chromatograms of IST samples revealed no significant degradation and FTIR spectra retained all the principal peaks with no significant change in major functional group position.

Dosage form design

Designing of bilayer tablet of fixed dosage form of TEL and ATR was done by optimizing each layer. A trial was conducted in ATR with single disintegrant and diluent with direct compression manually in 10 station with total compressed weight of 200 mg. Similarly trial conducted in TEL with single disintegrant and diluent and compressed at 300 mg. The dissolution of ATR trial tablet was found to be 70 to 75 % which complies as per Pharmacopeia where as the dissolution of trial tablet of TEL was very poor in the range of 27 to 34%. With a view to improve further dissolution of ATR, the multiple disintegrants were selected, namely SSG, CP and CCS. Other excipients include calcium carbonate as stabilizer, talc to improve sticking problem, colloidal anhydrous silica as glidant, magnesium stearate as lubricant and spray dried lactose as diluent. The quantity of disintegrant was taken as variable within the range of Pharmaceutical excipient and surface response methodology was used to optimize the ATR layer. Being poor solubility of TEL, its solubility was improved by employing following approaches: TEL was sieved and milled so that micronized TEL gives better release than powder.

1.TEL was treated with sodium hydroxide which may convert TEL into salt form, which enhance solubility.

2.Polymer PVPk-30 was used as carrier.

3.Instead of direct compression, intra and extra granulation done with disintegrants. This intra and extra granulation causes the tablet to disrupt, not only into the granules from which it was compressed, but also into powder particles from which the granulation was prepared.

The excipient selected for the formulation of TEL layer were sodium hydroxide for converting TEL into salt form, PVP-K30 as carrier, SSG, CP, and CCS as disintegrants, magnesium stearate as lubricant and lactose anhydrous as diluent. The quantity of sodium hydroxide was calculated stoichiometrically with respect to TEL. The amounts of all other excipients were kept fixed as per the limit of Pharmaceutical excipient, except the amount of disintegrants which were taken as factors in design of surface response methodology. The range of disintegrant was taken as per Pharmaceutical excipient. The optimize batch was selected with the result of the formulations given by design.

Evaluation of Tablet

Compressed tablets of all formulation had uniform weight due to uniform die fill which were within acceptable limit. The hardness, friability, DT and assay of each formulation were evaluated.

***In-vitro* Dissolution of ATR Tablets**

Results of *in-vitro* dissolution time at 15 and 30 min of all formulations of ATR are shown in Figure 3, 4 and 5 All the formulation showed similar kind of drug release pattern i.e immediate release at earlier and constant after that. All the formulations showed drug release greater than 80 % within 15 minutes. At 15 min AF-1 showed highest drug release and AF-5 showed lowest. From the figure 4, 5 and 6, Formulation AF-1, AF-3, AF-6, AF-10, AF-13 and AF-14 showed that drug released maximum within 15 min and after that remain constant. AF-2, AF-4, AF-5, AF-7, AF-8, AF-9, AF11, AF-12, AF-14 and AF-15 showed that drug released after 15 min and maximum within 30 min. AF-15 showed highest drug release within 30 min. Except AF-5, AF-6 and AF-12, all other showed drug release more than 90%.

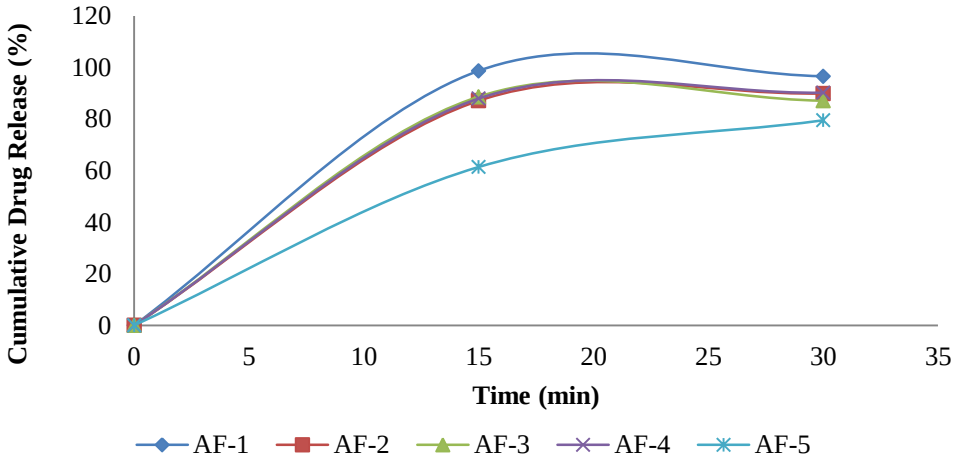

Figure 3: Dissolution profile of AF1 to AF5

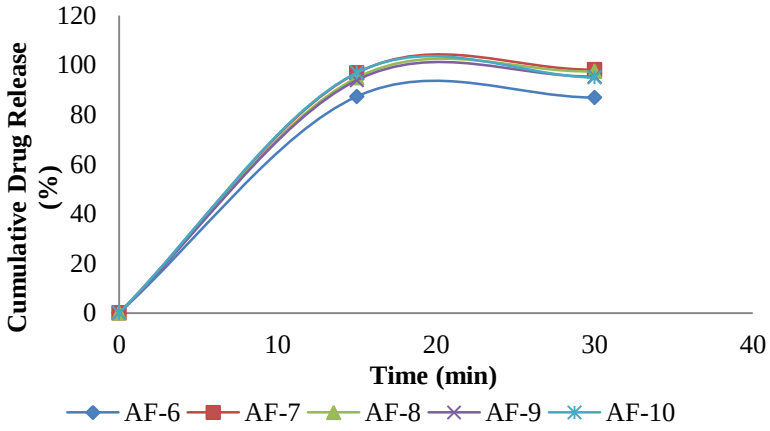

Figure 4: Dissolution profile of AF6 to AF10

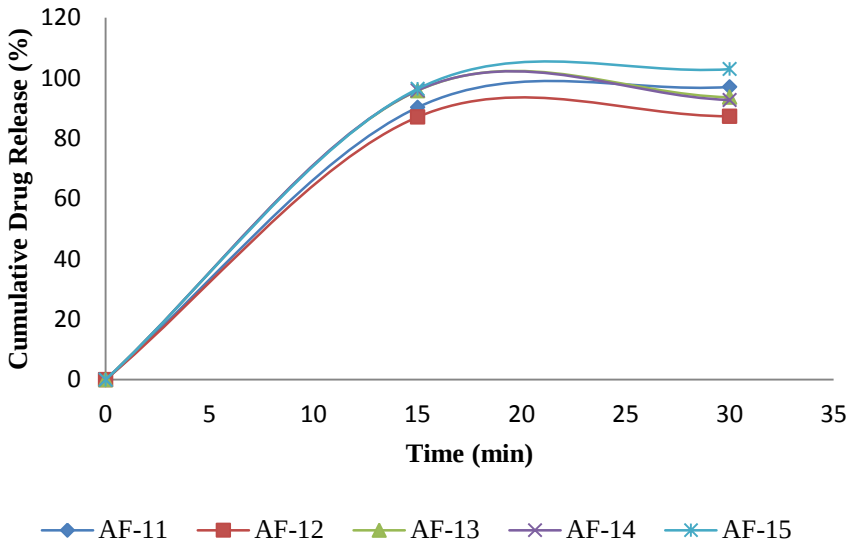

Figure 5: Dissolution profile of AF11 to AF15

***In-vitro* Dissolution of Telmisartan Tablets**

Results of *in-vitro* dissolution time at 10, 20 and 30 mins of all formulations of TEL are shown in in Figure 6, 7 and 8 All the formulation showed similar kind of drug release pattern i.e conventional release at earlier and after some lag time constant. At 10 min the lowest drug release was 60.62% of TF8 and highest was 85.39% of TF6. At 20 min the drug release is greater than 15 min with lowest of 68.52 % of TF3 and highest of 96.65% of TF15. There was an increase in release at 30 minutes compared to 20 min. There was no significant increase in drug release after 20 min except in TF2, TF4, TF7, TF9 and TF10, where there was increase in release between 4 to 8 %. The highest drug release was found to be in TF15.

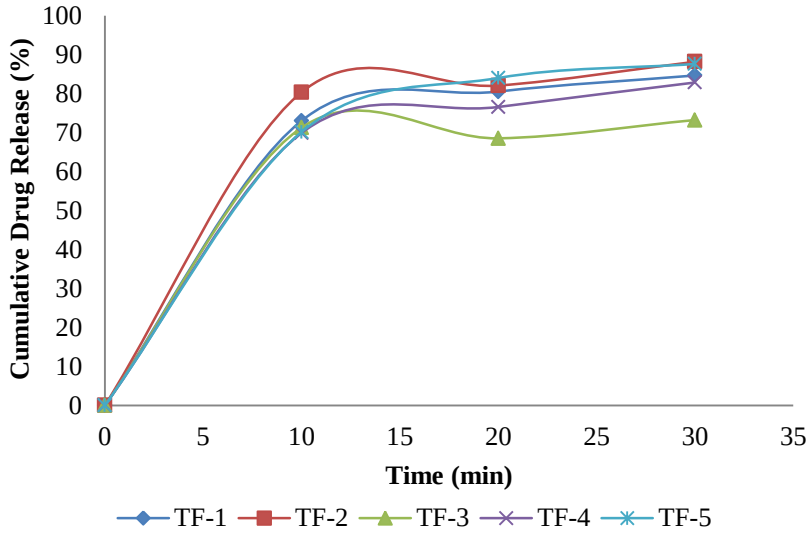

Figure 6: Dissolution profile of TF1 to TF5

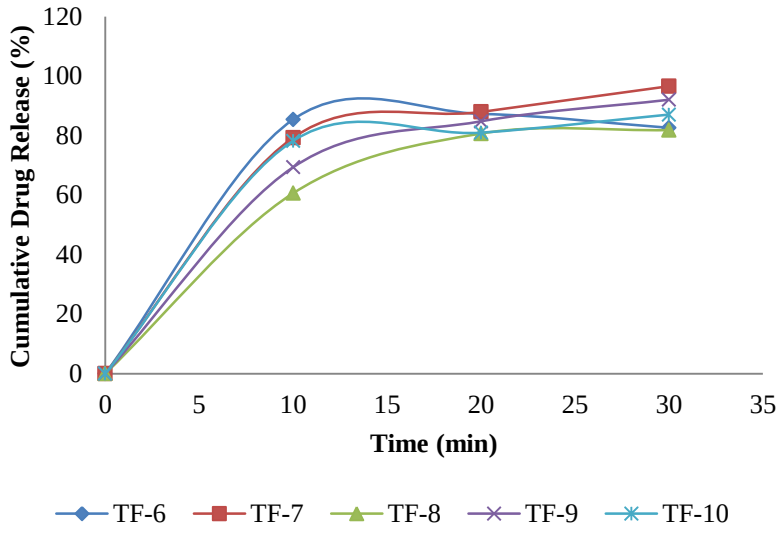

Figure 7: Dissolution profile of TF6 to TF10

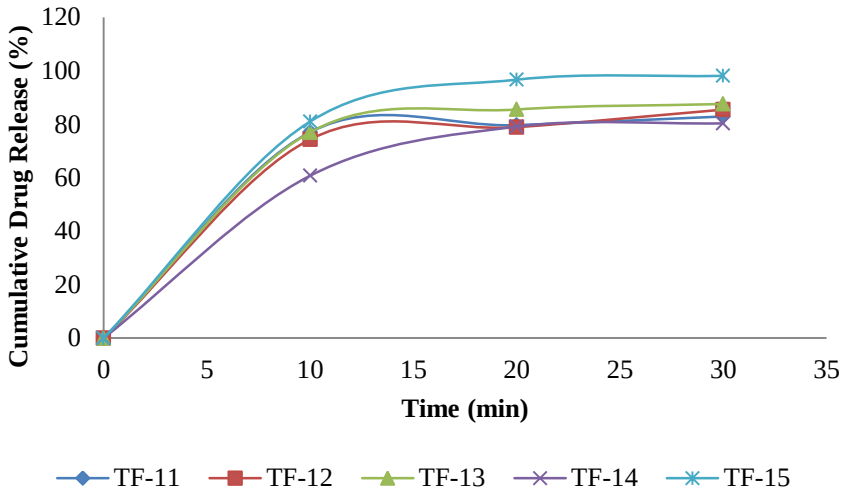

Figure8: Dissolution profile of TF11 to TF15

Regression Analysis

Regression analysis of dissolution of ATR tablet at 30 min from Minitab 16, we get equation (3.2).

Z = 57.30+3.375 SSG + 3.106 CP - 0.181 CCS - 0.0137 SSG × SSG - 0.098 CP × CP + 0.104 CCS × CCS + 0.001 SSG × CP + 0.017 SSG × CCS – 0.071 CP × CCS…… (3.2)

Where, Z = Drug release

In equation 3.2, SSG and CP has positive role where as CCS has negative. Based on the coefficient value, we can say that the effect of three disintegrant on release of ATR is as SSG > CP > CCS. Although SSG and CP exhibit positive role after certain concentration they exhibit negative role. CCS has negligible role however, with increase in concentration, it exhibit positive role. As per

ANOVA analysis, all the excipient shows significant effect because the p value is less than 0.05. The interaction between the excipient is not significant as the p value is less than 0.05. If the values of independent variables i.e. CCS, CP and SSG are fed in equation 3.2 predicted values of the responses are obtained. The F test between observed value and predicted value obtained from the equation 3.2, shows that there was no significant difference between observed value and predicted value at 0.05% level of significance. From this, we can say the equation 3.2 is valid equation. Regression analysis of dissolution of TEL tablet at 30 min from Minitab 16, we get equation (3.3). $Y = 71.1 – 0.343\ SSG + 0.58\ CP + 1.123\ CCS – 0.0100\ SSG \times SSG – 0.0296\ CP \times CP – 0.0395\ CCS \times CCS + 0.089\ SSG \times CP + 0.0167\ SSG \times CCS – 0.0157\ CP \times CCS$……..(3.3)

Where, Y = Drug release

In equation 3.3, CP and CCS has positive role where as SSG has negative. The ANOVA table shows that all the three disintegrant has significant effect in the dissolution, with CP having the most pronounced effect. If the values of independent variables i.e. CCS, CP and SSG are fed in equation (3.3), predicted values of the responses are obtained. The F test between observed value and predicted value obtained from the equation 3.3, shows that there was no significant difference between observed value and predicted value at 0.05% level of significance. From this, we can say the equation 3.3 is valid equation.

Optimization of Formulation

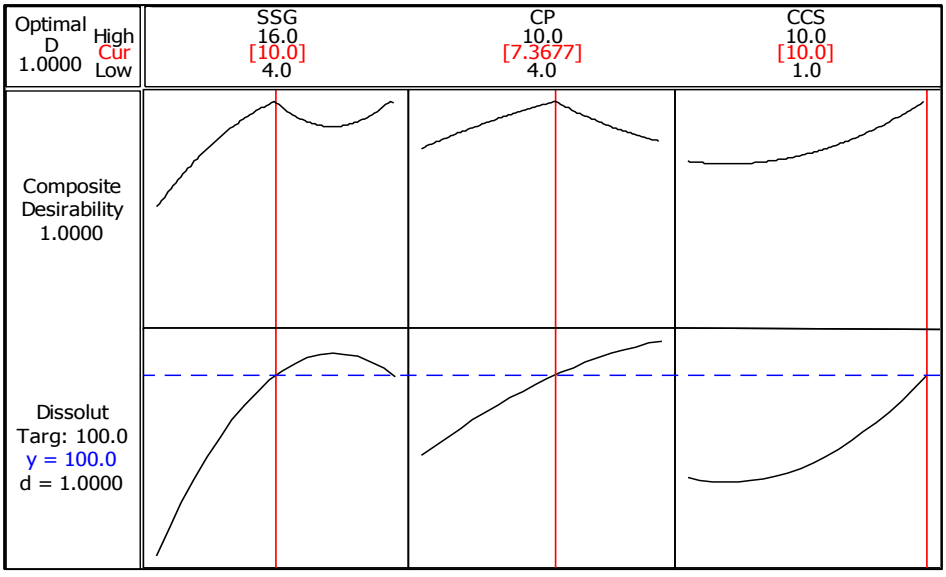

Figure 9: Optimization Plot of Dissolution at 30 mins vs. SSG, CP and CCS

For the optimization, the dissolution data of 30 min of Atorvastatin Calcium was subjected in the Minitab 16.2.3.0 software. As per response surface optimizer, given below in figure 9, with the

target value of 100 and lower and higher limit of 90 to 110 respectively, the optimize value obtained were 10.0 mg of SSG, 7.36 mg of CP and 10.0 mg of CCS per tablet with composite desirability 1.000. The contour plot shown below in figure 10, 11, 12 also favored the optimize result obtained from the response surface optimizer.

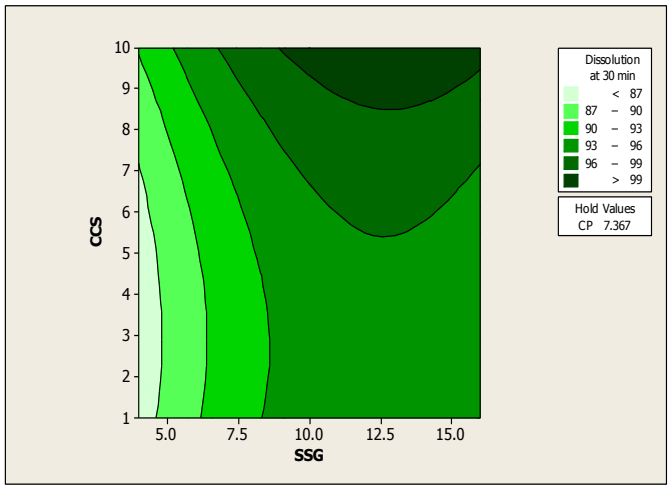

Figure 10: Contour Plot of Dissolution at 30 mins vs. CCS, SSG

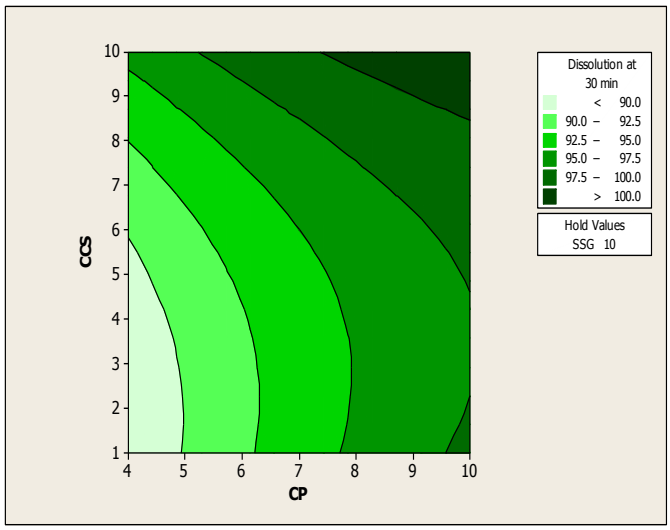

Figure 11: Contour Plot of Dissolution at 30 min Vs CCS, CP

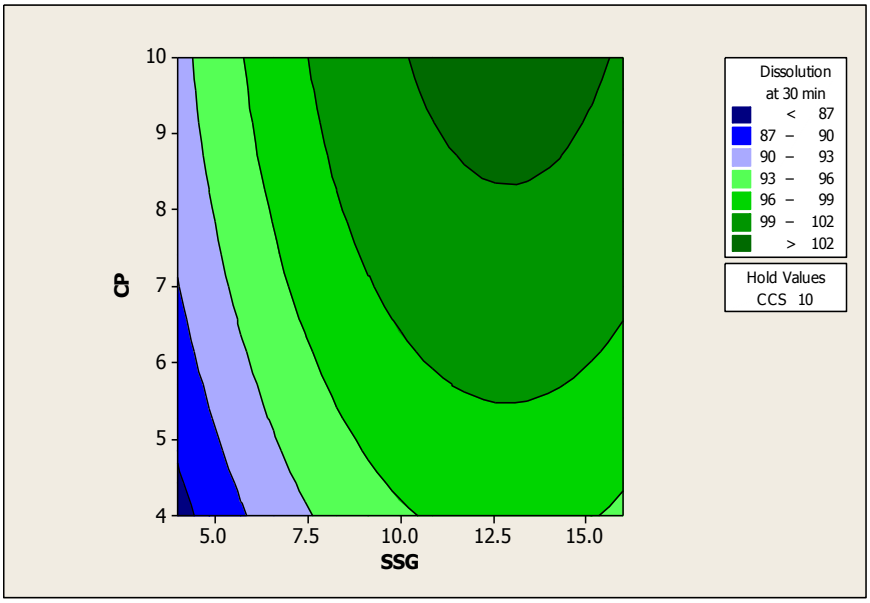

Figure 12: Contour Plot of Dissolution at 30 mins vs. CP, SSG

For the optimization of TEL layer, the dissolution data of 30 min was subjected in the Minitab 16.2.3.0 software. As per response surface optimizer, shown in Figure 3.13., with the target value of 100 and lower and higher limit of 90 to 110 respectively, the following graph appear with composite desirability 1.000. The graph below shows that all three disintegrant plays role in release of drug from the dosage form. As concentration of SSG, CP and CCS increases, the release of drug also increases. When multiple disintegrants were used in the combination, all three disintegrants shows the positive relationship with the release of drugs.

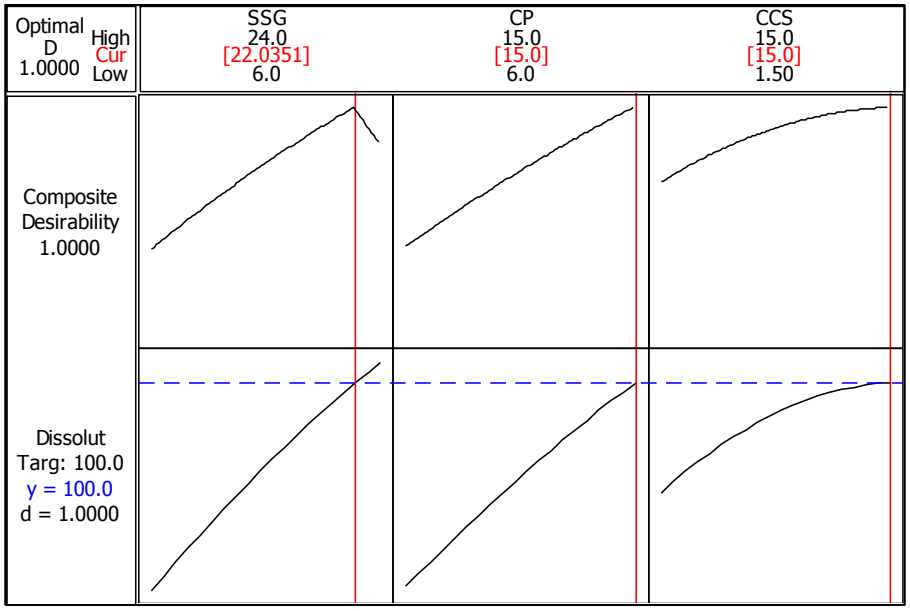

Figure 13: Optimization Plot of Dissolution of Telmisartan at 30 mins vs. SSG, CP and CCS

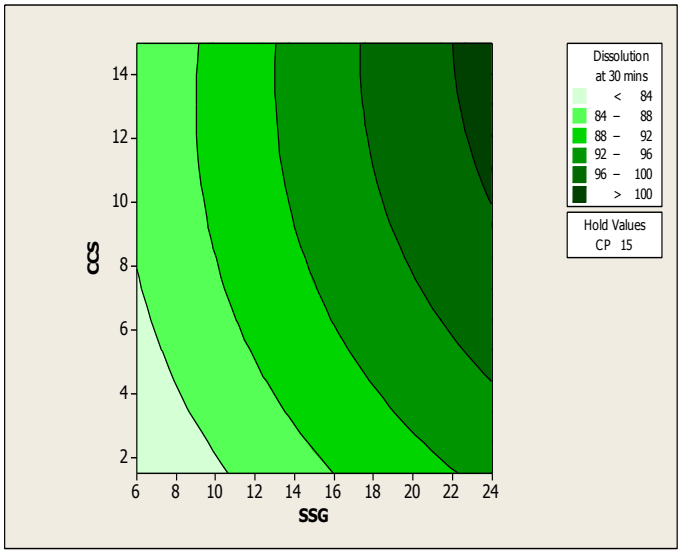

Figure 14: Contour Plot of Dissolution of Telmisartan at 30 mins vs. CCS, SSG.

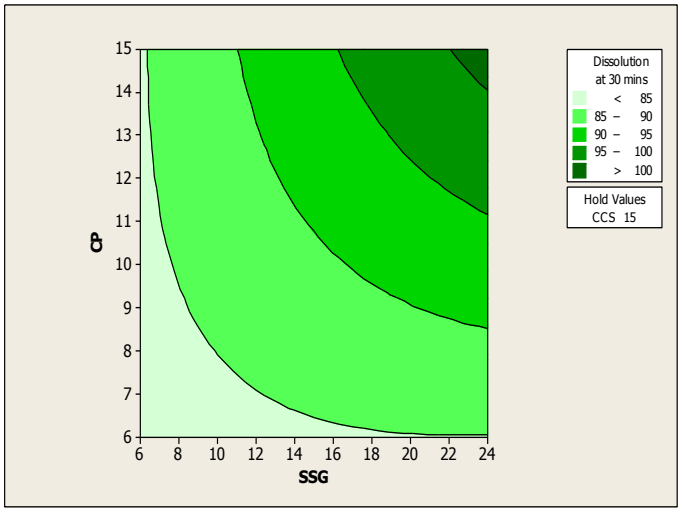

Figure 15: Contour Plot of Dissolution of Telmisartan at 30 mins vs. CP, SSG

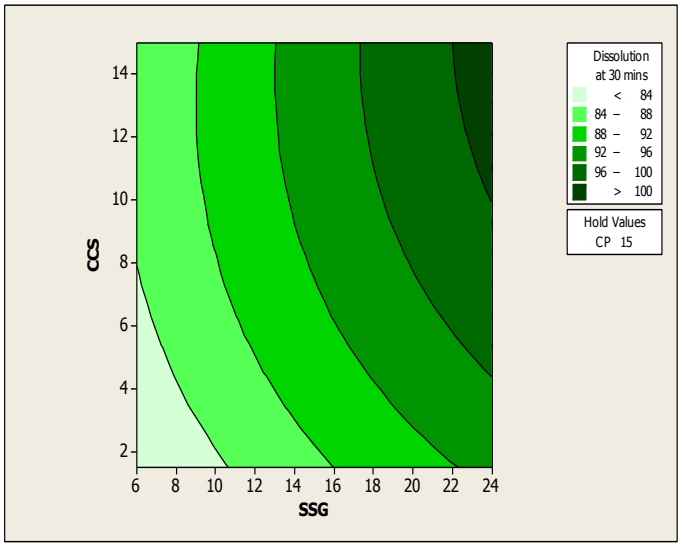

Figure 16: Contour Plot of Dissolution of Telmisartan at 30 mins vs. CCS, SSG

Designing of Bilayer tablet

The formulation of each optimized layer as per the design obtained from Minitab 16.2.3.0 was given in Annex XIII. The blend of ATR layer prepared by direct mixing and that of TEL layer was prepared by wet granulation, similarly, as mentioned above in individual layer preparation. The pre-compression parameters like bulk density, tapped density, hausner's ratio, carr's index and angle of repose were determined. As batch size was small, bilayer tablets were prepared by manually weighing each layer and compressed using single die and punch in 10 stations tablet press. The weight variation, disintegration time, hardness, assay and *in-vitro* evaluation of bilayer tablets was determined and evaluated results are given in Annex XV. Sampling was done at 10, 20 and 30 minutes in *in-vitro* dissolution analysis.

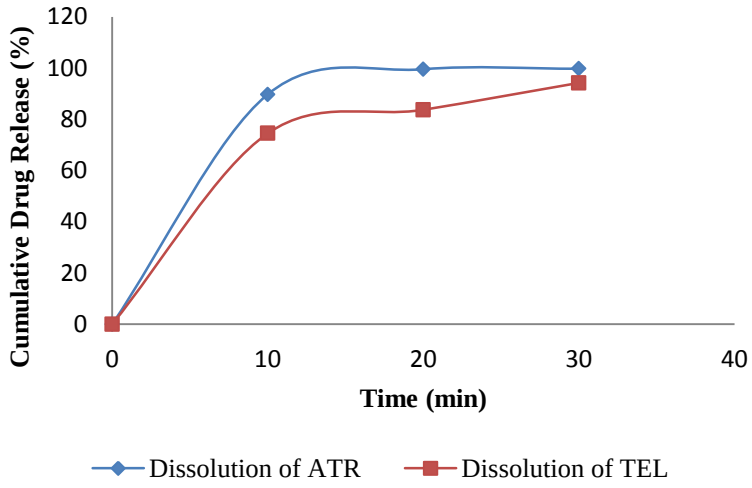

Figure 17. Dissolution profile of bilayer tablet

Comparison of Dissolution Profile with Market Product

Two Indian products labeled as M1 and M2 were purchased from local market. M1 is film coated tablet containing ATR 10 mg and M2 is film coated tablet containing TEL 40 mg. M1 and M2 were subjected to dissolution medium Phosphate buffer pH 7.5 and *in- vitro* dissolution profile was studied. As the marketed product M1 and M2 were coated tablets the initial release of both market product is slightly lower than that of bilayer tablet. The release profiles of ATR and TEL in bilayer tablet are similar to the corresponding release profile of market products containing individual content. The similarity factor and dissimilarity factor of release profile of ATR of bilayer tablet and market product M1 are 71.36 and 6.15 respectively. The similarity factor and dissimilarity factor of release profile of ATR of bilayer tablet and market product M2 are 69.40 and 5.60 respectively.

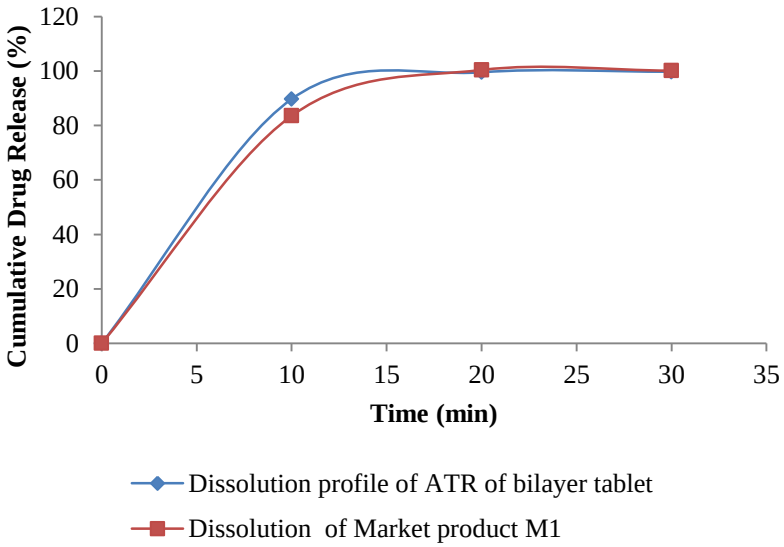

Figure 18: Comparison of Dissolution profile of ATR of bilayer tablet and market product M1

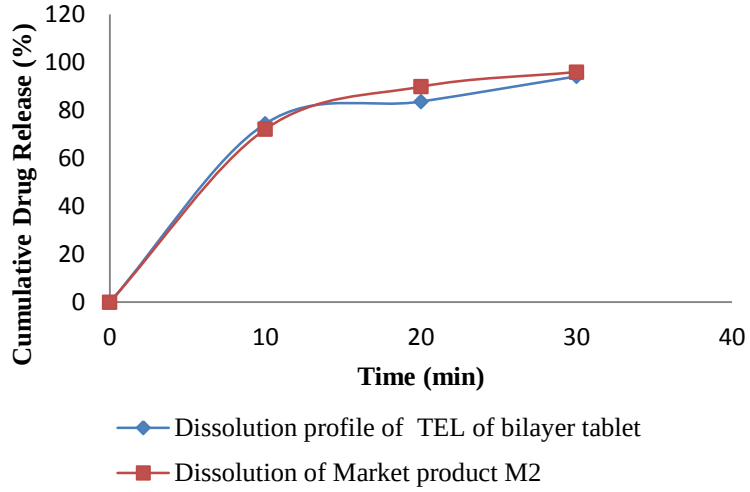

Figure 19: Comparison of Dissolution profile of TEL of bilayer tablet and market product M2

Annex- I

Atorvastatin Calcium layer formulation using Box Behnken design having 3 factors

Batch Size: 500 Tablets Compression Weight: 200 mg

Formulation No.	ATR (g)	CAC (g)	SSG (g)	CP (g)	CCS (g)	TALC (g)	CAS (g)	MS (g)	SDL (g)	Total (g)
AF-1	5.42	2.50	5.00	2.00	5.00	2.00	2.00	3.00	73.10	100.00
AF-2	5.42	2.50	2.00	3.50	5.00	2.00	2.00	3.00	74.60	100.00
AF-3	5.42	2.50	2.00	3.50	0.50	2.00	2.00	3.00	79.10	100.00
AF-4	5.42	2.50	8.00	2.00	2.75	2.00	2.00	3.00	72.30	100.00
AF-5	5.42	2.50	2.00	2.00	2.75	2.00	2.00	3.00	78.30	100.00

AF-6	5.42	2.50	5.00	2.00	0.50	2.00	2.00	3.00	77.60	100.00
AF-7	5.42	2.50	8.00	5.00	2.75	2.00	2.00	3.00	69.30	100.00
AF-8	5.42	2.50	8.00	3.50	5.00	2.00	2.00	3.00	68.60	100.00
AF-9	5.42	2.50	5.00	3.50	2.75	2.00	2.00	3.00	73.80	100.00
AF-10	5.42	2.50	5.00	3.50	2.75	2.00	2.00	3.00	73.80	100.00
AF-11	5.42	2.50	5.00	5.00	0.50	2.00	2.00	3.00	74.60	100.00
AF-12	5.42	2.50	2.00	5.00	2.75	2.00	2.00	3.00	75.30	100.00
AF-13	5.42	2.50	5.00	3.50	2.75	2.00	2.00	3.00	73.80	100.00
AF-14	5.42	2.50	8.00	3.50	0.50	2.00	2.00	3.00	73.10	100.00
AF-15	5.42	2.50	5.00	5.00	5.00	2.00	2.00	3.00	70.10	100.00

Abbreviations: AF- Atorvastatin Calcium Formulation, TALC- Talcum, CAS- Colloidal Silicon Dioxide, MS- Magnesium stearate, SDL- Spray Dried Lactose, g- gram

Annex- II

Telmisartan layer formulation using Box Behnken design having 3 factors

Batch Size: 500 Tablets Compression Weight: 300 mg

Formulation No:	**TEL (g)**	**NaOH (g)**	**SSG (g)**	**CP (g)**	**CCS (g)**	**PVPK-30 (g)**	**MS (g)**	**LSY (g)**	**LAC (g)**	**Total (g)**
TF-1	20.30	1.60	7.50	3.00	7.5	6.00	4.50	0.01_2	99.58_8	150.00
TF-2	20.30	1.60	7.50	5.20	4.12_5	6.00	4.50	0.01_2	100.71_3	150.00
TF-3	20.30	1.60	7.50	3.00	0.75	6.00	4.50	0.01_2	106.33_8	150.00
TF-4	20.30	1.60	3.00	5.25	7.50	6.00	4.50	0.01_2	101.83_8	150.00
TF-5	20.30	1.60	7.50	5.25	4.12_5	6.00	4.50	0.01_2	100.71_3	150.00
TF-6	20.30	1.60	3.00	7.50	4.12_5	6.00	4.50	0.01_2	102.96_3	150.00
TF-7	20.30	1.60	7.50	7.50	7.50	6.00	4.50	0.01_2	95.08_8	150.00
TF-8	20.30	1.60	3.00	3.00	4.12_5	6.00	4.50	0.01_2	107.46_3	150.00
TF-9	20.30	1.60	12.00	5.25	7.50	6.00	4.50	0.01_2	92.83_8	150.00
TF-10	20.30	1.60	7.50	7.50	0.75	6.00	4.50	0.01_2	101.83_8	150.00
TF-11	20.30	1.60	12.00	3.00	4.12_5	6.00	4.50	0.01_2	98.46_3	150.00
TF-12	20.30	1.60	12.00	5.25	0.75	6.00	4.50	0.01_2	99.58_8	150.00
TF-13	20.30	1.60	7.50	5.25	4.12_5	6.00	4.50	0.01_2	100.71_3	150.00
TF-14	20.30	1.60	3.00	5.25	0.75	6.00	4.50	0.01_2	108.58_8	150.00
TF-15	20.30	1.60	12.00	7.50	4.12_5	6.00	4.50	0.01_2	93.96_3	150.00

Abbreviations: TF- Telmisartan formulation , NaOH- Sodium Hydroxide, PVPK-30-Povidone30, LSY- Lake Sunset Yellow, LAC- Lactose

Annex- III

Evaluation of tablets of Atorvastatin Calcium layer

Experiments	Average Weight (mg) (n=20)	±SD	Average Thickness (mm) (n=10)	±SD	Average Diameter (mm) (n=10)	±SD	Average Hardness (Kg/cm^2) (n=10)	±SD	Friability (%)	Assay (%)	DT(s	Dissolution % (n=6)
AF-1	209.0	± 2.68	1.96	± 0.01	11.3	± 0.01	5.15	± 0.39	0.72	103.78	40	96.58
AF-2	198.8	± 4.44	1.96	± 0.02	11.29	± 0.01	5.95	± 0.87	0.64	101.85	60	89.90
AF-3	205.3	± 3.35	1.96	± 0.02	11.28	± 0.01	6.68	± 0.91	0.36	97.07	60	87.10
AF-4	203.4	± 2.84	1.95	± 0.02	11.28	± 0.02	4.96	± 0.58	0.14	99.43	60	90.20
AF-5	202.7	± 3.72	1.93	± 0.02	11.29	± 0.01	6.34	± 0.68	0.32	101.87	70	79.50
AF-6	202.8	± 3.16	1.96	± 0.01	11.3	± 0.01	6.46	± 0.87	0.01	103.22	60	86.84
AF-7	202.0	± 2.60	1.95	± 0.02	11.3	± 0.01	4.15	± 0.66	0.104	102.71	30	98.10
AF-8	192.1	± 4.28	1.93	± 0.03	11.3	± 0.01	4.94	± 1.69	0.02	101.77	38	97.40
AF-9	203.0	± 4.03	1.91	± 0.03	11.29	± 0.01	5.74	± 1.19	0.15	99.58	50	95.40
AF-10	203.0	± 3.62	1.96	± 0.02	11.3	± 0.01	5.35	± 1.17	0.10	103.88	55	94.91
AF-11	202.2	± 2.14	1.95	± 0.02	11.3	± 0.01	5.93	± 0.33	0.06	102.53	50	97.07
AF-12	206.3	± 3.65	1.94	± 0.02	11.3	± 0.01	6.02	± 0.45	0.13	103.47	50	87.30
AF-13	201.6	± 5.00	1.96	± 0.02	11.29	± 0.01	5.77	± 1.22	0.12	101.44	50	93.60
AF-14	200.1	± 3.40	1.96	± 0.02	11.28	± 0.01	5.30	± 1.45	0.05	97.07	45	92.70
AF-15	203.8	± 2.69	1.95	± 0.02	11.29	± 0.01	4.57	± 0.95	0.08	102.90	25	103.00

Abbreviations: SD- Standard Deviation, DT- Disintegration Time, Sec- Seconds, mm- millimeter

Annex- IV

Evaluation of tablets of Telmisartan layer

Experiments	Average Weight (mg) (n=20)	±SD	Average Thickness (mm) (n=10)	±SD	Average Diameter (mm) (n=10)	±SD	Average Hardness (Kg/cm^2) (n=10)	±SD	Friability (%)	Assay (%)	DT (min)	Dissolution %
TF-1	310.3	± 2.92	2.71	± 0.02	11.30	± 0.01	5.80	± 0.29	0.114	98.81	6	84.64
TF-2	307.7	± 5.95	2.71	± 0.02	11.29	± 0.01	6.09	± 0.21	0.193	99.50	6	88.15
TF-3	305.2	± 6.06	2.71	± 0.02	11.30	± 0.01	6.03	± 0.27	0.044	99.80	7	73.21

TF-4	311.0	± 3.36	2.71	± 0.02	11.30	±0.03	5.99	± 0.12	0.093	97.30	6	82.84
TF-5	304.2	± 6.09	2.72	± 0.01	11.29	± 0.01	6.64	± 0.39	0.107	96.38	7	87.60
TF-6	305.2	± 5.49	2.72	± 0.01	11.30	± 0.01	6.28	± 0.51	0.153	96.60	7	82.61
TF-7	307.6	± 4.82	2.71	± 0.01	11.29	± 0.01	5.50	± 0.30	0.122	97.86	6	96.58
TF-8	303.2	± 3.59	2.72	± 0.01	11.29	± 0.01	5.26	± 0.28	0.187	96.05	5	81.84
TF-9	308.6	± 3.55	2.71	± 0.01	11.29	± 0.01	5.83	± 0.25	0.286	103.68	6	92.04
TF-10	308.4	± 3.86	2.72	± 0.02	11.29	± 0.01	6.47	± 0.37	0.15	99.05	7	87.05
TF-11	316.7	± 4.27	2.73	± 0.01	11.29	± 0.01	5.22	± 0.45	0.094	99.86	6	82.89
TF-12	306.0	± 3.77	2.72	± 0.02	11.29	± 0.01	5.73	± 0.21	0.127	98.00	5	85.44
TF-13	312.2	± 3.56	2.72	± 0.02	11.29	± 0.01	6.08	± 0.15	0.161	104.70	6	87.55
TF-14	306.1	± 6.16	2.71	± 0.07	11.29	± 0.01	6.29	± 0.33	0.155	97.15	7	80.30
TF-15	313.5	± 3.97	2.72	± 0.02	11.30	± 0.01	6.06	± 0.50	0.176	105.31	7	98.08

Annex- V

A. Optimized formulation

Atorvastatin Calcium layer					Batch Size: 500 tablets					
Formulation	**ATR (g)**	**CAC(g)**	**SSG (g)**	**CP (g)**	**CCS (g)**	**TALC (g)**	**CAS (g)**	**MS (g)**	**SDL (g)**	**Total(g)**
OF	5.42	2.5	2.41	5.0	0.5	2.0	2.0	3.0	77.17	100

Telmisartan Layer					Batch Size: 500 tablets					
Formulation	**TEL (g)**	**NaOH (g)**	**SSG (g)**	**CP (g)**	**CCS (g)**	**PVPK-30 (g)**	**MS (g)**	**LSY (g)**	**LAC (g)**	**Total (g)**
OF	20.30	1.60	11.02	7.50	7.50	6.0	4.50	0.01_2	91.57	150

B. Evaluation of Bilayer tablets

Experiment	**Average Weight (mg) (n=20)**	**±SD**	**Average Thickness (mm) (n=10)**	**±SD**	**Average Diameter (mm) (n=10)**	**±SD**	**Average Hardness (Kg/cm2) (n=10)**	**±SD**	**Friability (%)**	**Assay (%)**	**DT (min)**	**Dissolution %**
BT	509.45	± 4.33	4.27	± 0.01	11.27	± 0.010	6.79	± 0.93	0.01	98.70 (ATR)	6.0	99.73 (ATR)
										100.64 (TEL)		94.20 (TEL)

Abbreviations: OF- Optimized formulation, BT- Bilayer Tablet

CONCLUSION

Compatibility studies of Atorvastatin Calcium and Telmisartan was conducted with selected GRAS excipients and found to be compatible as per the result of micro environmental pH, HPLC and FTIR analysis of Isothermal Stress Testing samples. The micro environmental pH study indicates that Atorvastatin Calcium and Telmisartan did not undergo pH sensitive degradation. There was no significant change in potency remained in IST samples and control samples which indicates that Atorvastatin Calcium and Telmisartan did not undergo degradation. The results of FTIR analysis also favored the compatibility as there was no significant change in principal peaks in IR spectra of binary mixtures of IST samples when compared with corresponding control samples of binary mixtures. The IR spectrum of IST samples and control samples of some mixtures does not match with the IR spectra of corresponding reference standard of active which might be due to physical or chemical interaction. The result of trial conducted with single disintegrant and diluent showed that Atorvastatin Calcium exhibit release within the marginal Pharmacopeia Limit whereas Telmisartan exhibit very poor release. In order to enhance the release of Telmisartan, it was treated with sodium hydroxide to form salt form and PVPK-30 was used as carrier and multiple disintegrants were used with intra and extra granulation technique. For better release of Atorvastatin Calcium multiple disintegrants were used and direct compression was done. Simultaneous method was used in RP-HPLC for determination of Atorvastatin Calcium and Telmisartan. The method was validated and found to be simple, accurate, precise and robust. The mobile phase was prepared and the optimum concentration of its constituent was found to be in the ratio of 40:60 of buffer and acetonitrile respectively. The detection was carried out at 254 nm. The linearity range for Atorvastatin Calcium was 5 µg/ml to 80 µg/ml and that of Telmisartan was 10 µg/ml to 80 µg/ml. The mean % recoveries of Atorvastatin Calcium and Telmisartan were found to be 100.76% and 100.22% respectively. The LOD for the drug Atorvastatin Calcium was found to be 0.11 µg/ml, LOQ for the drug Atorvastatin Calcium was found to be 0.34 µg/ml. Similarly, the LOD of Telmisartan was found to be 0.48 µg/ml and LOQ was found to be 1.46 µg/ml. Study was concentrated on design, characterization, optimization and evaluation of each layer of tablet and preparation of bilayer from optimized formulation. Box Behnken design was used in Minitab 16 with SSG, CP and CCS as three independent variables. Fifteen formulations were obtained from the Box Behnken design and Atorvastatin Calcium tablet was prepared by direct compression method and Telmisartan Tablet was prepared by wet granulation with intra and extra granulation technique. Optimize formulation as per Response surface optimizer in Minitab 16.2.3.0 was found to be 10.0 mg of SSG, 7.36 mg of CP and 10.0 mg of CCS per tablet for

Atorvastatin Calcium layer. Similarly in case of Telmisartan tablet, it was found that as concentration of SSG, CP and CCS increases, the release of drug also increases. When multiple disintegrants were used in the combination, better release was obtained. Optimize formulation as per Response surface optimizer in Minitab was found to be 22.03 mg of SSG, 15 mg of CP and 15 mg of CCS per tablet for Telmisartan layer. The contour plot showed that the formulation was in the optimum zone. Bilayer tablet was prepared with optimized formulation obtained from the response surface optimizer by manually weighing and compressed using single die and punch in 10 station compression machine. The bilayer tablet was evaluated for hardness, friability, drug content, *in-vitro* disintegration and *in-vitro* dissolution. The *in-vitro* release profiles were compared with release profile of market products and found to be almost similar. Selecting appropriate formulation excipients and manufacturing technology is the design feature of any dosage form. Screening of incompatible excipient and selection of compatible excipient helps to reduce instability problem. The present study shows that Atorvastatin Calcium and Telmisartan are compatible with selected GRAS excipients. The individual layer tablet of Atorvastatin Calcium showed better release of drug content by direct compression using optimized combination of disintegrants namely Sodium Starch Glycolate, Croscarmellose Sodium and Crosprovidone. Telmisartan showed better release with sodium salt and povidone K-30 as carrier. Optimized combination of disintegrants, intra and extra granulation proven to be effective in better release of Telmisartan. Bilayer tablet prepared by using optimize formulation of each layer, showed better release profile of both drug contents.

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