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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF AZELNIDIPINE AND TELMISARTAN IN PHARMACEUTICAL DOSAGE FORM BY QBD APPROACH

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ABSTRACT: "Quality by Design" (QbD) serves as a bridge between industry and drug regulatory authorities to move towards a scientific, risk based holistic and proactive approach for development of pharmaceutical products. So, the present work describes the development of RP-HPLC method for simultaneous estimation of azelnidipine and telmisartan in pharmaceutical dosage form by QbD approach. The Box-Behnken design was used for screening where the effect of flow rate, % organic phase and temperature on retention time, resolution, number of theoretical plates (NTP) and symmetry factor (critical quality attributes) was evaluated. Chromatogram was run through Discovery C18 250 x 4.6 mm, 5µ. Mobile phase containing 0.01N Ortho Phosphoric Acid: Acetonitrile taken in the ratio of 53.8:46.2V/V was pumped through column at a flow rate of 0.9 ml/min. The developed method was validated according to guidelines of the International Conference on Harmonization (ICH). Hence, the developed method using QbD approach was better understood that, reduces the time and cost of the analysis.

INTRODUCTION: Telmisartan is an angiotensin II receptor antagonist (ARB) used to treat hypertension, diabetic nephropathy and congestive heart failure [1]. In general, angiotensin II receptor blockers (ARBs) like telmisartan bind to the angiotensin II type 1 (AT1) receptors with high affinity, causing angiotensin II to be inhibited from acting on vascular smooth muscle and ultimately resulting in a decrease in arterial blood pressure. According to recent research, telmisartan may also have PPAR-gammaagonistic characteristics that may have favourable metabolic effects [2].

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Chemically it is 2-[4-[[4-methyl -6-(1-methyl-benzimidazol – 2 - yl) - 2 – propylbenzimidazol - 1-yl] methyl] phenyl] benzoic acid **Fig. 1.** Melting point is 261-263°C, molecular weight is 514.6 g/mol, and pKa value is 3.5. It dissolves readily in formic acid, dissolves only slightly in methanol, and barely dissolves at all in ethanol. Log P value is 3.2 [3-4].

Azelnidipine is a dihydropyridine calcium channel blocker. Unlike some other calcium channel blockers, it acts gradually and results in a long-lasting decrease in blood pressure with only a slight increase in heart rate [5]. It is currently being investigated for the treatment of postischemic stroke. Azelnidipine prevents Ca2+ influx through the transmembrane of the smooth muscle voltage-dependent channels in vascular walls. L-type, T-type, N-type, P/Q-type, and R-type Ca2+ channels are among the different subcategories of Ca2+

channels. The Ca2+ L-type channels [6]. Calcium typically causes smooth muscle to contract, which increases blood pressure. Because the vascular smooth muscle does not contract when calcium channels are blocked, the smooth muscle walls relax, lowering blood pressure. Chemically it is 3-(1-Benzhydrylazetidin-3-yl) 5-isopropyl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **Fig. 2**. Molecular formula is $C_{33}H_{34}N_4O_6$. Molecular weight is 582.6g/mol, Melting point is 122-123°C, pKa value is 7.89. Azelnidipine is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF). Log P value is 5.18 [7-8].

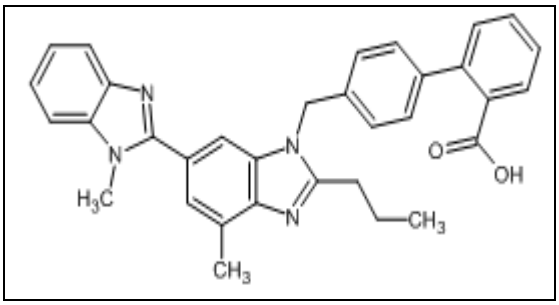


FIG. 1: STRUCTURE OF TELMISARTAN

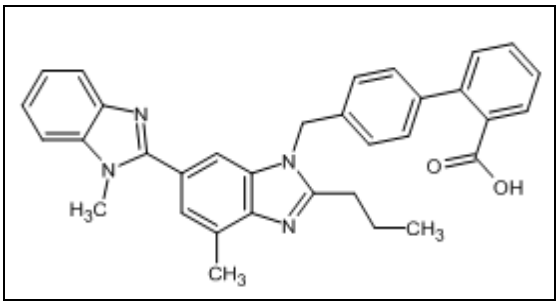


FIG. 2: STRUCTURE OF AZELNIDIPINE

Literature survey revealed that a number of methods like UV [9], HPLC [10-20], Stability indicating [21-23] that have been reported for estimation of Telmisartan and Azelnidipine individually or in combination with other drugs, but still RP-HPLC method by Quality by Design for estimation of Telmisartan and Azelnidipine is not present. To ensure process consistency throughout the product lifecycle, simple validated RP-HPLC method for the determination of telmisartan and azelnidipine in pharmaceutical dosage forms must be established using the Quality by Design (QbD) approach as per ICH Q8 (R2) guidelines.

MATERIALS AND METHODS:

Materials: The working standard of Telmisartan and Azelnidipine was provided as a gift sample from Spectrum Pharma lab (Hyderabad). Hydrochloric acid (AR grade) and sodium hydroxide (AR grade) were purchased from Rankem, India. Hydrogen Peroxide (H_2O_2) was purchased from Qauligens. Acetic acid (AR grade), Potassium dihydrogen orthophosphate were purchased from Fisher scientific, India and S.D. Fine Chem Ltd. orthophosphoric acid andmilli-Q water (HPLC grade) were purchased from Merck India Pvt Ltd. HPLC grade Acetonitrile (ACN) and methanol (MeOH) were purchased from Fischer scientific. The marketed formulations Talma-AZ 40/4mg by Glenmark were used for assay.

Instruments and Reference Standards: The HPLC study was carried out on WATERS-2695 with a Photo diode array detector (PDA). C-18 Column (250 mm × 4.6 mm × 5µm particle size) was used at ambient temperature. Other equipments sonicator (ePEI ultrasonic generator), Analytical balance (Mettler Toledo), vortex meter (IKA Vortex), Hot air oven (Yorco scientific). pH meter (Eutech instruments pH tutor, pH meter, India) were used.

QbD Software: Design Expert® (11.0.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for generation of contour plots and 3D space.

Preparations of Solutions:

Preparation of Standard Stock Solution: Accurately weighed 40mg of Telmisartan and 4mg of Azelnidipine was transferred to 50mL volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labelled as Standard stock solution (800µg/mL of Telmisartan and 80µg/mL of Azelnidipine). 1mL from Telmisartan and Azelnidipine each stock solution was pipetted out and taken into a 10mL volumetric flask and made up with diluent. (80µg/mL of Telmisartan and 8µg/mL of Azelnidipine).

Preparation of Sample Stock Solution: 10 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to tablet was transferred into a 10 mL volumetric flask, 5mL of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (80µg/mL of Azelnidipine

and 800µg/mL of Telmisartan). 1mL of filtered sample stock solution was transferred to 10mL volumetric flask and made up with diluent.

(8µg/mL of Azelnidipine and 80µg/mL of Telmisartan)

Preparation of Buffer:

Preparation of 0.01N Potassium Dihydrogen Ortho Phosphate: Accurately weighed 1.36gm of Potassium dihydrogen Ortho phosphate in a 1000mL of Volumetric flask add about 900mL of milli-Q water and degas to sonicated and finally make up the volume with water then added 1mL of Triethylamine, P^H adjusted to 3.0 with dil. Orthophosphoric acid solution.

Preparation of 0.1% Ortho Phosphoric Acid Buffer: 1mL of ortho phosphoric acid solution in a 1000mL of volumetric flask add about 100mL of milli-Q water and final volume make up to 1000mL with milli-Q water.

Determination of Detection Wavelength: Between 200 and 400 nm, the standard solution was scanned. As shown in **Fig. 3**, the wavelength of maximum absorption for drug was determined to be 248 nm.

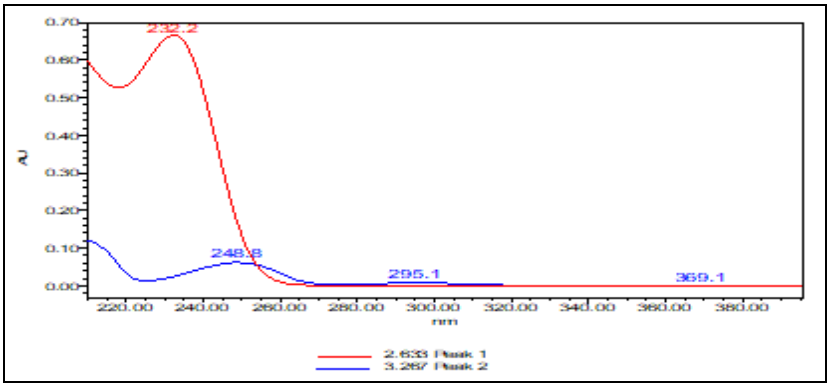


FIG. 3: OVERLAY UV SPECTRUM OF TELMISARTAN AND AZELNIDIPINE

Optimization of Mobile Phase: A variety of solvents with different compositions were screened to find out the ideal mobile phase **Table 1.**

TABLE 1: LIST OF MOBILE PHASE COMPOSITIONS SCREENED

Sr. no.	Mobile phase	Ratio(V/V)	Remark
1.	Methanol: Water	50: 50	peak was not eluted
2.	Methanol: Water	70: 30	less USP plate count was observed
3.	Acetonitrile: 0.01 N KH2PO4	60: 40	excess noise was observed
4.	0.1% OPA: Acetonitrile	60: 40	Peak was symmetric

Chromatographic Conditions: The Discovery C-18 column (250mm × 4.6 mm having 5µm particle size equilibrated with a mobile phase consisting of 0.01N Ortho phosphoric acid: Acetonitrile taken in the ratio 53.8:46.2 V/V) was used. The flow rate was kept at 0.9 mL/min, and column was set at ambient temperature. Eluents were supervised using a PDA detector at 248.0 nm.

Initial Method Development by QbD Approach: A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

1. Screening Phase
2. Statistical Analysis and Final Optimization

Screening Phase: The experimental design was constructed using design expert software version 11.0.0 for the study of different variables (% organic phase, flow rate and temperature) and to verify method performances. The levels of these variables are as given in **Table 2.** The retention time, resolution, theoretical plates, asymmetry were used as a response in experimental design as controlling response, which is expected to affect and control method responses. A 3^3 factorial design consisting of three factors at three levels was considered for the experimental plan. Initially and after confirming that the process is a non-linear, Box-Behnken design was used. The experimental observations along with Design (DOE) plan are shown in **Table 3.**

TABLE 2: FACTORS AND LEVELS OF INDEPENDENT VARIABLES

CMPs	Unit	Type	Subtype	Min	Max
column temperature	°C	Numeric	Continuous	27	32.94
Flow rate	mL/min	Numeric	Continuous	0.9	0.93
%Org ratio	%	Numeric	Continuous	45	53.6

TABLE 3: BOX-BEHNKEN DESIGN PLAN AND RESPONSES

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4	Response 5
Std	Run	A:FR	B:% organic phase	C:Temparature	RT1	RT2	RS	NTP	TF
		mL/min	%	0 C	min	min	num	num	num
13	20	1.1	55	27	2.251	2.954	5.8	7132.9	1.4
3	6	1.1	45	33	2.193	2.477	2.5	7834.7	1.2
4	8	0.9	55	27	2.743	3.599	6	7229.3	1.4
2	10	1	50	30	2.429	2.876	3.6	9266	1.7
1	16	1	50	30	2.427	2.872	3.6	9263	1.7
12	17	1	50	30	2.425	2.864	3.6	9257	1.7
19	4	1	50	24.9546	2.452	3.085	4.3	8149.4	1.5
16	7	1.16818	50	30	2.079	2.525	3.7	8110.6	1.2
20	9	0.9	45	27	2.675	3.142	3.6	8416.1	1.5
18	12	1	50	30	2.424	2.865	3.6	9282	1.7
17	14	1	58.409	30	2.466	3.293	7.4	8135.2	1.7
10	15	1	50	30	2.419	2.857	3.6	9255	1.7
15	18	1	50	35.0454	2.479	3.231	6.8	8024.5	1.2
9	19	0.831821	50	30	2.912	3.584	4.1	8852	1
11	2	1	41.591	30	2.389	2.677	2.1	7425.5	1.2
7	3	1.1	55	33	2.212	2.754	5.3	7894.1	1.4
8	13	1	50	30	2.418	2.852	3.6	9257	1.7
5	1	0.9	55	33	2.834	3.185	3.5	9706.5	1.3
6	11	0.9	45	33	2.647	2.972	2.2	8003.3	1.4
14	5	1.1	45	27	2.196	2.584	3.7	8368.6	1.5

Statistical Analysis and Final Optimization: The responses obtained after carrying out the above trial runs were fed back to Design Expert software and plots like 3D-response surface plots and Graph plots were plotted. These plots revealed the influence of critical method parameters on the selected quality attributes.

The analysis of these plots was used to estimate as to which method parameter gave the most acceptable responses. Thus, based on these observations, the final critical method parameters of the method were determined and the optimized chromatographic conditions were finalized. Moreover, the evaluation of statistical analysis tool like ANOVA for each individual response was used to determine the significance of each method parameter selected for the study using the p value (probability).

Validation of the Optimized Method: Validation of analytical procedures was performed for Telmisartan and Azelnidipine using the following parameters.

Specificity: To demonstrate the method's precision, the following solutions will be prepared and injected (double-checked the peak purity).

- Blank (Na_2HPO_4:Acetonitrile 50:50 as a diluent)
- standard solution
- sample solution
- Placebo treatments

Linearity: The linearity of the method was studied over six different concentrations of Telmisartan and Azelnidipine in the range of 20-120µg/mL and 2-12µg/mL in triplicate. The calibration curve was constructed by plotting peak area on y axis versus concentration on x axis. The regression line equation and correlation coefficient values were determined.

Accuracy (% Recovery): Accuracy of the method was confirmed by a recovery study from marketed

formulation at 3-level of standard addition. Percentage recovery of Telmisartan and Azelnidipine was found out.

Precision: The Precision is reported in terms of Relative Standard deviation (RSD). There are three levels of precision: repeatability, reproducibility and intermediate precision. It is carried out on a sample API.

- Repeatability (intraday precision)
- reproducibility
- Intermediate precision (interday precision)

Limits of Detection and Quantitation: Limits of detection (LOD) and limit of quantitation (LOQ) were determined from the signal-to-noise ratio. The detection limit was refer to as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was refer to as the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

$$LOD = 3{:}3\ \delta/S,$$

$$LOQ = 10\ \delta/S$$

Robustness: For robustness studies 80 µg/mL of Telmisartan and 8 µg/mL of Azelnidipine was used. In order to demonstrate the robustness of the method, the following optimized conditions were slightly varied.

- (53.8%) 0.1% OPA: Acetonitrile (46.2%)ratio of mobile phase
- 0.90 mL/min of flow rate
- 32^0C of temperature

RESULTS AND DISCUSSION:

Statistical Analysis of Experimental Data by Design-expert Software: Analysis of variance (ANOVA) was applied to study the significance of the model generated for the five responses shown in the **Table 4-8**.

2D Contour and 3D Surface plots were analyzed to visualize the effect of factors and their interactions on the Design Expert® software's responses. The regions shaded in dark blue represent lower values, and shaded in dark red represents higher values. The regions shaded in light blue, green and yellow represents intermediate values.

TABLE 4: ANOVA TABLE FOR RETENTION TIME1 USING BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.9049	9	0.1005	220.31	< 0.0001	significant
A-FR	0.8705	1	0.8705	1907.43	< 0.0001	
B-MP	0.0154	1	0.0154	33.73	0.0002	
C-Temperature	0.0003	1	0.0003	0.7076	0.4199	
AB	0.0041	1	0.0041	8.97	0.0134	
AC	0.0014	1	0.0014	3.02	0.1129	
BC	0.0009	1	0.0009	1.89	0.1996	
A^2	0.0099	1	0.0099	21.63	0.0009	
B^2	0.0001	1	0.0001	0.1431	0.7131	
C^2	0.0035	1	0.0035	7.65	0.0199	
Residual	0.0046	10	0.0005			
Lack of Fit	0.0045	5	0.0009	46.87	0.0003	significant
Pure Error	0.0001	5	0.0000			
Cor Total	0.9095	19				

The Model F-value of 220.31 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, A^2, C^2 are significant model terms.

TABLE 5: ANOVA TABLE FOR RETENTION TIME2 USING BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.56	3	0.5185	30.97	< 0.0001	significant
A-FR	1.12	1	1.12	66.88	< 0.0001	
B-MP	0.4054	1	0.4054	24.22	0.0002	
C-Temperature	0.0305	1	0.0305	1.82	0.1958	

Residual	0.2678	16	0.0167			
Lack of Fit	0.2674	11	0.0243	302.89	< 0.0001	significant
Pure Error	0.0004	5	0.0001			
Cor Total	1.82	19				

The Model F-value of 30.97 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A and B are significant model terms.

TABLE 6: ANOVA TABLE FOR RESOLUTION USING BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	22.73	3	7.58	7.57	0.0023	significant
A-FR	0.1290	1	0.1290	0.1289	0.7243	
B-MP	22.46	1	22.46	22.44	0.0002	
C-Temperature	0.1426	1	0.1426	0.1425	0.7108	
Residual	16.01	16	1.00			
Lack of Fit	16.01	11	1.46			
Pure Error	0.0000	5	0.0000			
Cor Total	38.74	19				

The Model F-value of 7.57 implies the model is significant. There is only a 0.23% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term.

TABLE 7: ANOVA TABLE FOR THEORETICAL PLATES (NTP) USING BBD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.040E+07	9	1.156E+06	12.04	0.0003	significant
A-FR	8.325E+05	1	8.325E+05	8.67	0.0147	
B-MP	20854.14	1	20854.14	0.2173	0.6511	
C-Temperature	3.173E+05	1	3.173E+05	3.31	0.0991	
AB	3.582E+05	1	3.582E+05	3.73	0.0822	
AC	4.219E+05	1	4.219E+05	4.39	0.0625	
BC	2.189E+06	1	2.189E+06	22.81	0.0008	
A^2	1.057E+06	1	1.057E+06	11.02	0.0078	
B^2	3.877E+06	1	3.877E+06	40.39	< 0.0001	
C^2	2.426E+06	1	2.426E+06	25.28	0.0005	
Residual	9.599E+05	10	95989.99			
Lack of Fit	9.594E+05	5	1.919E+05	1898.54	< 0.0001	significant
Pure Error	505.33	5	101.07			
Cor Total	1.136E+07	19				

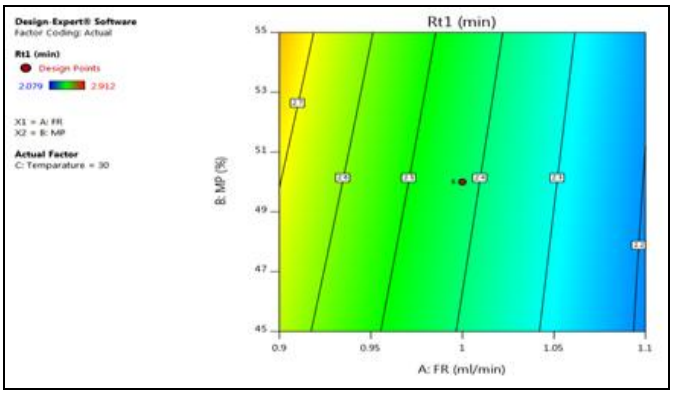
The Model F-value of 12.04 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, BC, A^2, B^2, C^2 are significant model terms.

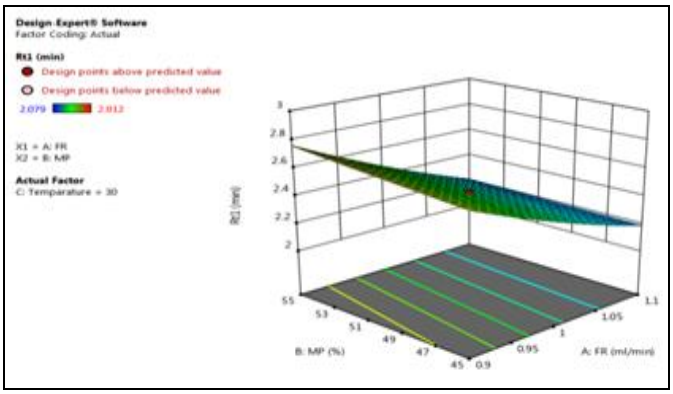
TABLE 8: ANOVA TABLE FOR ASYMMETRY USING BBD

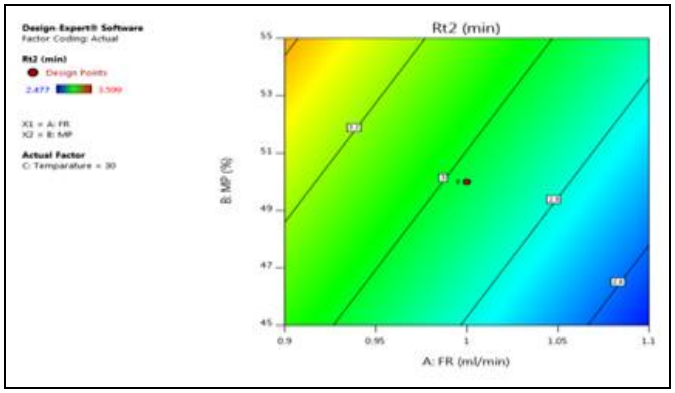
Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.7728	9	0.0859	5.48	0.0069	significant
A-FR	0.0041	1	0.0041	0.2610	0.6205	
B-MP	0.0402	1	0.0402	2.56	0.1404	
C-Temperature	0.0739	1	0.0739	4.71	0.0550	
AB	0.0112	1	0.0112	0.7178	0.4167	
AC	0.0012	1	0.0012	0.0798	0.7834	
BC	0.0112	1	0.0112	0.7178	0.4167	
A^2	0.5123	1	0.5123	32.69	0.0002	
B^2	0.0605	1	0.0605	3.86	0.0778	
C^2	0.1446	1	0.1446	9.22	0.0125	
Residual	0.1567	10	0.0157			
Lack of Fit	0.1567	5	0.0313			

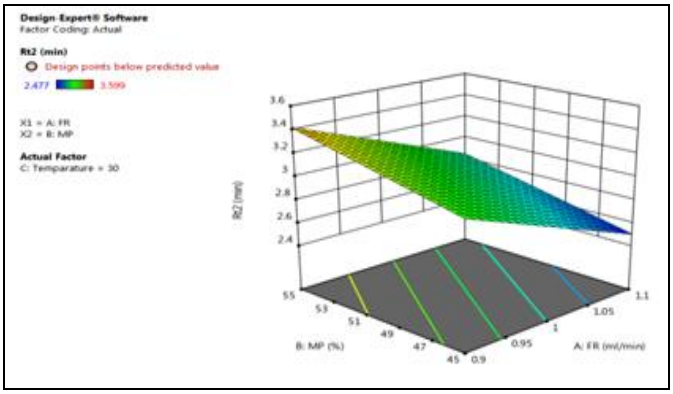
Pure Error	0.0000	5	0.0000
Cor Total	0.9295	19	

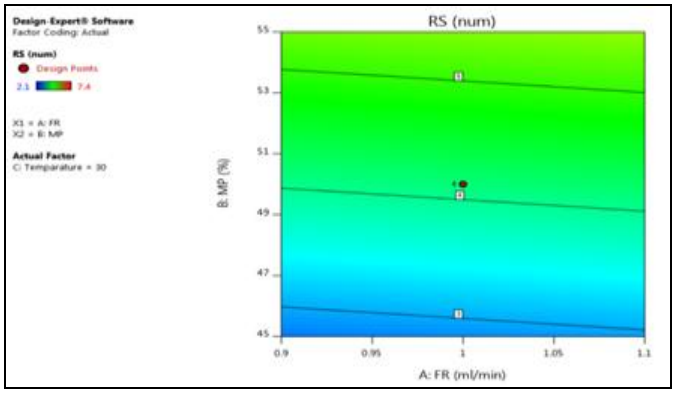
The Model F-value of 5.48 implies the model is significant. There is only a 0.69% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A^2, C^2 are significant model terms.

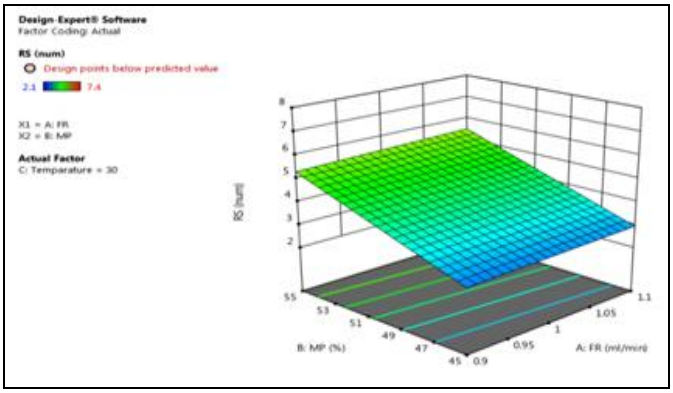


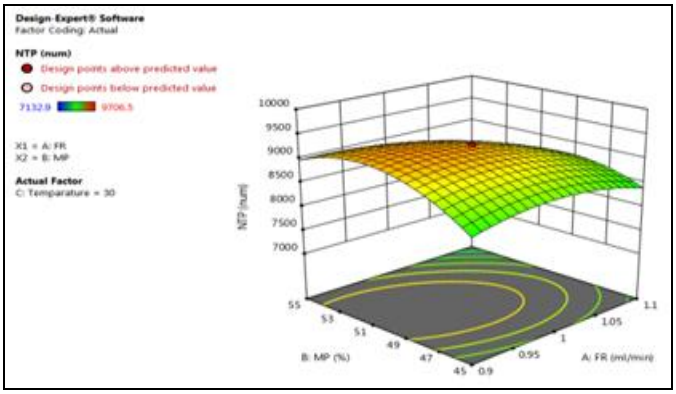


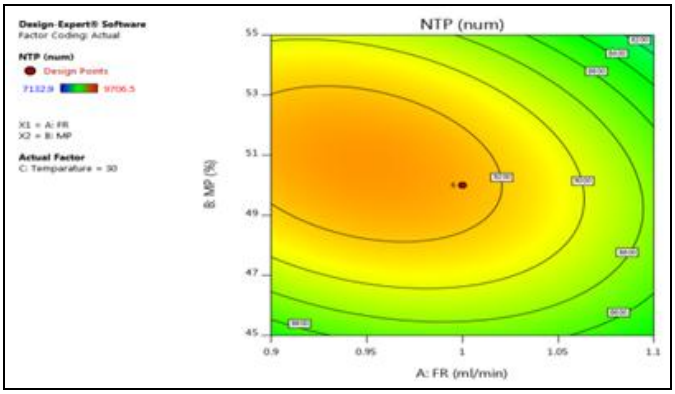












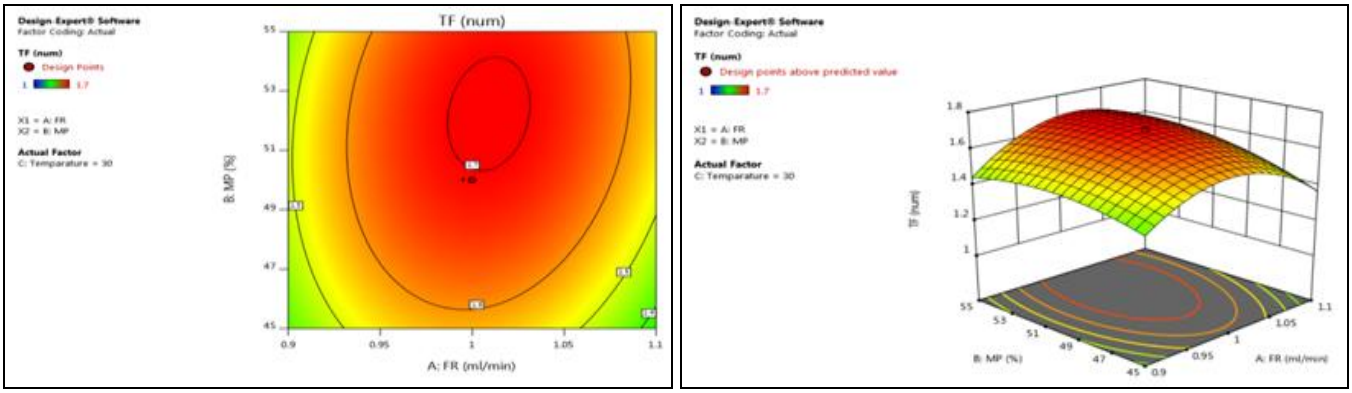


FIG. 4: 2D, 3D CONTOUR PLOT OF RETENTION TIME1, RETENTION TIME2, RESOLUTION, THEORETICAL PLATES AND ASYMMETRY

From the above 2D Contour and 3D Surface plots of retention time shown in the **Fig. 4**, It shows the two dimensional contour plot as a function of Column temperature, Flow rate and organic ratio. Based on the color code, the working region can be easily identified. Retention time maps represent the value of the retention time, with warm "red" colors indicating larger retention time, cold "blue" colors lower and light green to yellow color represent intermediate retention time.

Design Validation: From the actual versus predicted plots **Fig. 5** for the five responses, it was observed that the selected models for the respective responses were suitable for the selected design.

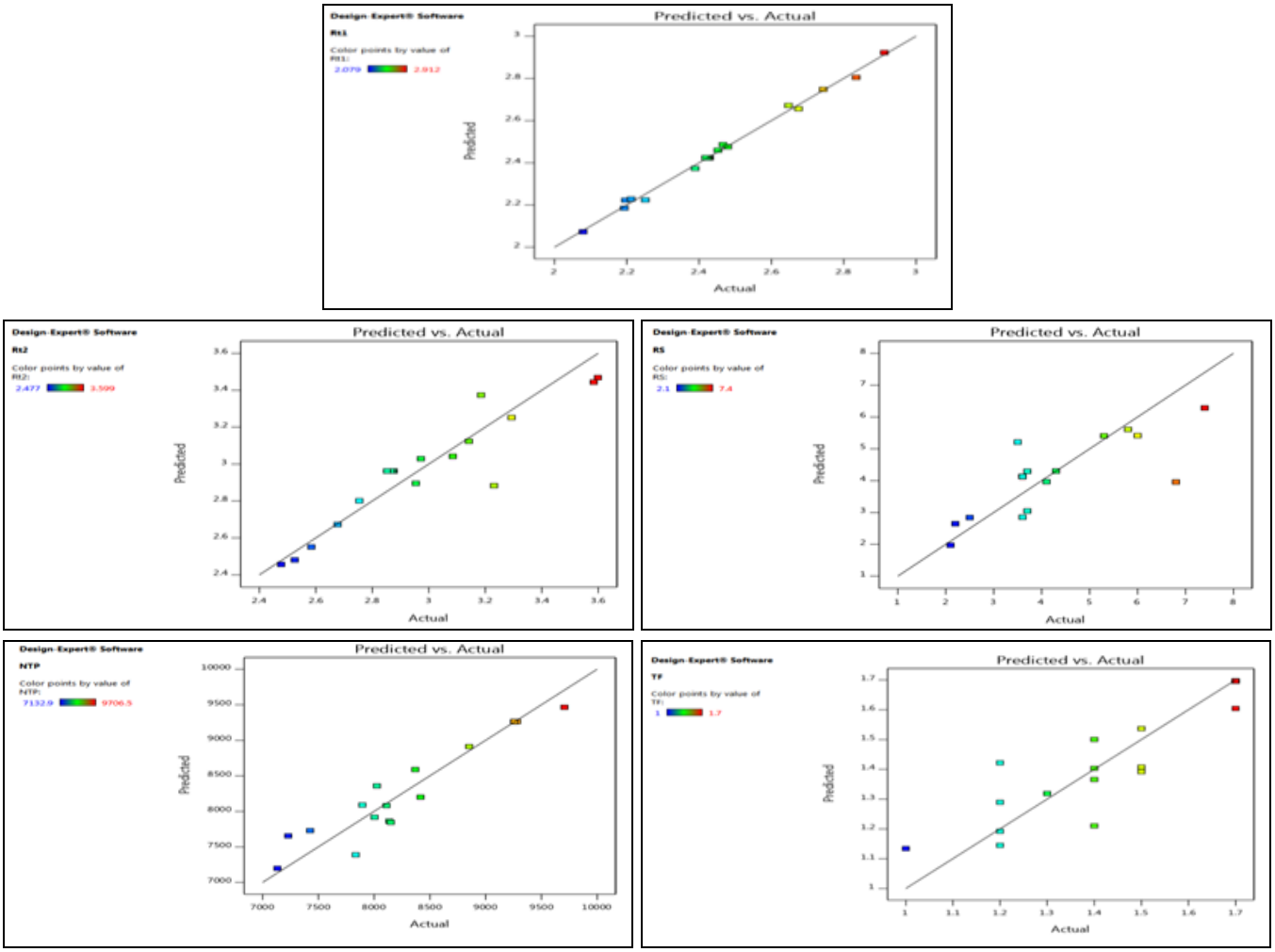


FIG. 5: ACTUAL VERSUS PREDICTED PLOTS FOR RETENTION TIME1, RETENTION TIME 2, RESOLUTION, THEORETICAL PLATES AND ASYMMETRY

It was further evidenced from the ANOVA **Table 4-8** that the selected models were significant with $p < 0.05$. Hence, the selected models were suitable for the design employed in this work.

Optimization by Desirability Function: In order to understand the results, 3D space was obtained after processing all data using the software. A composite desirability was applied to get an optimum set of conditions based on the specified goals and boundaries for the each response. This desirability function was depends on a scale of desirability function ranges between d = 0, for a completely undesirable response, to d = 1 for a fully desirable response Based on the specified goals and boundaries for the retention time, area, Asymmetry and a composite desirability (D) of 1 was obtained as shown in **Fig. 6**, which gave the optimal flow rate of 1.0 mL/min. To confirm these optimum set of conditions, three replicate injections of 80µg/mL and 8µg/mL of Telmisartan and Azelnidipine was analyzed to determine if their observed retention time, asymmetry and theoretical plates were within the predicted ranges.

It was observed that the differences between the observed and predicted peak response were less than 5% and the corresponding optimized chromatogram of optimized method was shown in the **Fig. 7.**

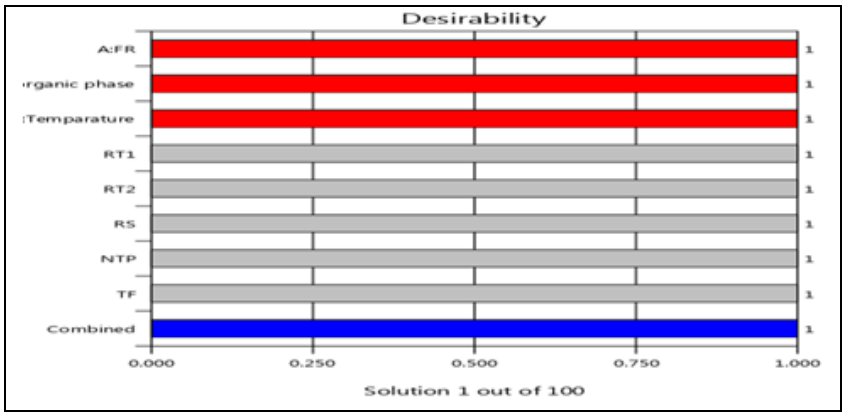


FIG. 6: OVERALL DESIRABILITY OF FINAL METHOD

TABLE 9: FINAL OPTIMIZED HPLC CHROMATO-GRAPHIC CONDITIONS

Property	Value
Mobile phase	(53.8%) 0.1% OPA: Acetonitrile (46.2%)
Temp	32 ^{0}C
Flow rate	0.90mL/min

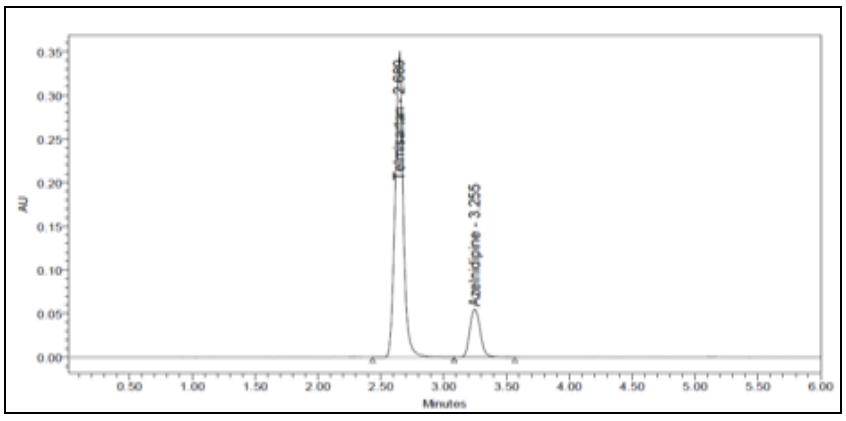


FIG. 7: CHROMATOGRAM OF FINAL OPTIMIZED METHOD

Method Validation: The developed method was linear over the concentration range with 20-120 µg/Ml (Telmisartan) and 2-12 µg/mL (Azelnidipine) with a correlation coefficient of 0.996 and 0.9998 respectively. For the accuracy studies at 50,100 and 150% levels, the % recovery of the drug was to be within 98-102%. Intermediate precision, reproducibility and repeatability were carried out, and the % RSD values were found to be less than 2%. LOD value was found to be 0.57

µg/mL of telmisartan and 0.06 µg/mL of Azelnidipine. LOQ value was found to be 1.37 µg/mL of telmisartan and 0.19 µg/mL of Azelnidipine. The robustness of the developed method was checked by making minor changes in the experimental conditions like flow rate, % organic composition and temperature, and %RSD values for the peak area were found to be less than 2%. The summary of the method validation parameters was shown in **Table 10.**

TABLE 10: RESULTS OF THE VALIDATION PARAMETERS

Parameter		Telmisartan	Azelnidipine	Limit
Linearity Range (µg/mL)		20-120(µg/mL)	2-12(µg/mL)	R< 1
Regression coefficient		0.9996	0.9998	
Slope(m)		20390	42739	
Intercept(c)		7997.2	3247.6	
Regression equation (Y=mx+c)		y = 20390x + 7997.2	y = 42739x + 3247.6	
Assay (% mean assay)		100.12%	99.45%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	0.4	NMT 2.0%
Accuracy % recovery		99.76%	99.59%	98-102%
LOD		0.57	0.06	NMT 3
LOQ		1.73	0.19	NMT 10
Robust-ness	FM	1.1	0.9	%RSD NMT 2.0
	FP	0.6	0.3	
	MM	1.2	0.6	
	MP	0.5	0.4	
	TM	1.2	0.6	
	TP	0.6	0.4	

CONCLUSION: The Pharmaceutical industry and its regulators are strongly focused on all quality issues because at the end of the day, drugs often make the difference between life and death. Hence, Quality by design is an approach that aims to ensure the quality of medicine by employing statistical, analytical and risk management methodology in the design, development and manufacturing of medicines. The analytical QbD concepts were extended to the RP-HPLC method development for Telmisartan and Azelnidipine, and to determine the best performing system and the final design space, a multivariant study of several important process parameters such as the combination of 3 factors namely the flow rate, mobile phase composition and temperature at 3 different levels was performed. Their interrelationships were studied and optimized at different levels using central composite design. Here, a better understanding of the factors influencing chromatographic separation in the ability of the methods to meet their intended purposes is done. All the validated parameters were found within the acceptance criteria. The QbD approach to method development has helped to better understand the method variables hence leading to less chance of failure during method validation and transfer. The automated QbD method development approach using the Design Expert software has provided a better performing more robust method in less time compared to manual method development. This approach offers a practical knowledge understanding that help for the development of a chromatographic optimization that can be used in the future. The statistical analysis of data indicates that the method is reproducible, selective, accurate, and robust. This method will be used further for routine analysis for quality control in Pharmaceutical industry.

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