RESEARCH PAPER

A Systematic QbD Approach for Developing a Robust Stability – Indicating RP-HPLC Method for Antihypertensive Formulations

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Abstract

A quality by design (QbD) approach can potentially lead to a more robust/rugged method development due to emphasis on the risk assessment and management. Aim of present investigation was to develop a new simple, rapid and sensitive RP-HPLC method by employing Box- Behnken design (BBD) approach for determination of telmisartan and azelnidipine in prepared tablets. Box-Behnken design (BBD) was employed to optimize the chromatographic conditions for HPLC method development, taking mobile phase composition (% methanol), flow rate and λmax as independent variables and retention time and tailing factor as the measured responses. The mobile phase composition was methanol: water (80:20 v/v) on a Cosmosil C18 (250mm x 4.6 ID, Particle size: 5 µm) column. In isocratic mode, it had a flow rate 0.9 mL/min and eluted analyte was detected at 256 nm. Analysis of variance (ANOVA) confirmed that the three factors were significant. Validation parameters followed the International Council for Harmonization (ICH) guidelines for the new HPLC method. Forced degradation (acid, base, oxidation, thermal and photolytic) studies were also performed according to ICH guidelines. The developed method was validated as per recommended ICH guidelines which revealed the high degree of linear, precise, accurate, sensitive and robust method over the existing RP-HPLC method. The developed BBD-based method helped in generating a design space and operating space with knowledge of all method performance characteristics, and RP-HPLC method takes less time and can be used in routine for quality control of bulk and marketed formulation of TELM and AZEL.

Keywords: RP-HPLC, Box-Behnken Design, Telmisartan, Azelnidipine, Method validation, Forced degradation
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Introduction

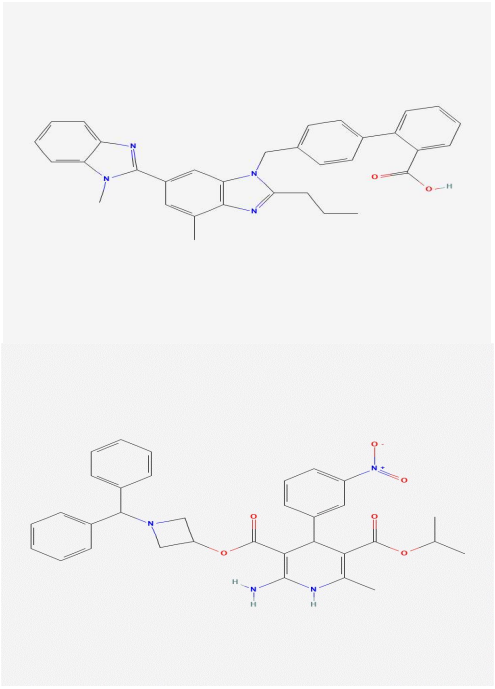
Analytical method development and validation is a very imperative technique utilized to determine the drug content in bulk and pharmaceutical dosage forms and in biological fluids like blood, serum, urine etc [1]. Method validation is the process for establishing that performance characteristics of the analytical method are suitable for the intended application. Typical parameters recommended by FDA, USP, and ICH for method validation are specificity, linearity & range, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, and system suitability test (SST) [2, 3]. Analytical techniques are developed and validated for active pharmaceutical ingredients (API), excipients, drug products, degradation products and related substances, residual solvents, etc. HPLC is a versatile analytical tool useful in identification and quantitative estimation of low concentration of drugs and metabolites in biological matrices. So, it is advantageous to develop and validate HPLC method for low dose drugs [4].

Quality by design (QbD) is used for the development of pharmaceutical processes to ensure a predefined product quality [5]. ICH Q8 (R1) guideline defines QbD as a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management [6]. for analytical methods, QbD approach involves a full understanding of how the analytical technique attributes and operating conditions affect the

analytical performance [7]. Analytical quality by design (AQbD) approach can be used in the development of a robust and cost-effective analytical method which is applicable at any stage of the lifecycle of the product. Some regulatory authorities have recently provided flexibility of changing analytical method without revalidation if the AQbD approach has been implemented during analytical method development [8, 9].

Telmisartan (TELM) is an angiotensin II receptor antagonist and its International Union for Pure and Applied Chemistry (IUPAC) name is 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl] methyl} phenyl) benzoic acid [10]. It is a white or slightly yellowish, odourless crystalline powder [11, 12]. It has empirical formula $C_{33}H_{30}N_4O_2$ and structural formula as shown in figure 1. Telmisartan is almost insoluble in water, slightly soluble in methanol, sparingly soluble in methylene chloride strong acid and soluble in strong base (1 M sodium hydroxide) [11, 12]. TELM is the most lipophilic compound and shows excellent oral absorption and tissue penetration [13]. Telmisartan is commercially available in particular in its free acid form, which is poorly soluble in neutral or acidic media. Thus, telmisartan is typically formulated together with a basic agent or in the form of a basic salt for improved solubility [14]. TELM, belongs to angiotensin receptor blocking (ARB) group, which are potently and selectively inhibit most of the biological effects of Ang-II, including contraction of vascular smooth muscle, rapid pressor responses, slow pressor responses, thirst, vasopressin release, aldosterone secretion, release of adrenal catecholamines, enhancement of noradrenergic neurotransmission, increases in sympathetic tone, changes in renal function, and cellular hypertrophy and hyperplasia. Telmisartan recently was found to prevent adipogenesis and weight gain through activation of PPAR-delta-dependent lipolytic pathways and energy uncoupling in several tissues [15, 16].

Azelnidipine (AZEL) is chemically (±)-3-[1-(diphenyl methyl) azetidin-3-yl] 5-propan-2-yl 2-amino-6- methyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate (Figure 1). It is a modern dihydropyridine calcium channel antagonist that is specific for the L-type calcium passages and has been approved by the FDA for the treatment of hypertension patients [17]. The antihypertensive effects of AZEL could be comparable to those of another drug, amlodipine [18]. AZEL was found more lipid-soluble and has greater selectivity for the vascular surface than older generational calcium passage antagonists, and blood flow to the brain was significantly increased in animal experiments treated with AZEL [19]. Molecular formula and molecular weight are $C_{33}H_{34}N_4O_6$ and 582.6g/mol with melting point of 122-123°C and pKa value is 7.89. Azelnidipine is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF) [20].



(a)

(b)

Figure 1: Structure of Telmisartan (a) and Azelnidipine (b)

Literature studies revealed few analytical methods for the estimation of AZEL and TELM as a single component or combine with other drug formulation. These methods were spectrophotometric [21], High Performance Liquid Chromatography (HPLC) [22-25], liquid chromatography-mass spectrometry (LC-MS) [26], high-performance liquid chromatography mass analysis capabilities of mass spectrometry (HPLC-MS-MS) [27]. So far only few HPLC method have been reported for their simultaneous estimation in their combined dosage form [28, 29]. Also, the reported method did not use any methodical approach like DoE. So, there is a need to develop HPLC method (cost effective and rapid) using DoE approach along with study of simultaneous estimation of AZEL and TELM. Subsequently, the goal of the present investigation was to develop a simple, rapid, robust, flexible and economical RP-HPLC method for the estimation of telmisartan and azelnidipine using analytical quality by design (AQbD) approach. The developed method was then validated as per ICH guidelines.

Materials and Methods

Instrumentation

HPLC studies were carried out on HPLC Binary Gradient System (Analytical Technology Ltd, HPLC 3000 Series) with UV-3000 M detector. Cosmosil C18 (250mm x 4.6 ID, Particle size: 5 µ) column and pump (P-3000-M Reciprocating) was used for study. Other equipment's like sonicator (Wenser Ultra- WUC-4L), analytical balance (Wenser High Precision Balance, PGB100), hot air oven (Yorco scientific) and pH meter (Eutech instruments) were used. Design Expert® (13.0.5.0) quadratic modelling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for generation of contour plots and 3D space.

Chemicals

Telmisartan and Azelnidipine (API) procured from Rap Analytical Laboratory, Nashik, Maharashtra, India. The marketed formulation Telma-AZ (40/8 mg) by Glenmark was used for simultaneous estimation assay. Merck (India) supplied analytical mark hydrochloric acid, phosphoric acid, hydrogen peroxide, sodium hydroxide and HPLC mark methanol along with Milli-Q system base prepared Milli-Q water was employed in the combined analysis of TELM and AZEL.

Selection of mobile phase

A variety of solvents with different compositions were screened to find out the ideal mobile phase. The final optimized conditions were determined by evaluating the effect of three factors A (mobile phase ratio) and B (Flow rate) and C (wavelength). Based on retention time and resolution, the optimized conditions selected was mobile phase methanol: water (80:20 v/v) and the flow rate of 0.9 mL/min at 256 nm wavelength.

Preparation of stock solutions

Standard stock solution of 1000 µg/ml was prepared by dissolving 10 mg of pure drug (TELM and AZEL) in 10 ml of mobile phase. Then 0.2-1 ml of stock solution of TELM was shifted into separate sets of 10 ml volumetric flasks and then diluting to 10 ml volume with the very similar solvent to made different aliquots of 20-100 µg/ml concentration. Similarly, different aliquots of 4-20 µg/ml were prepared by shifting 0.04 to 0.2 ml of stock of AZEL into separate sets of 10 ml volumetric flasks and then diluting to 10 ml volume. A total 20 tablets (Telma-AZ) were weighed, average weight was calculated and then crushed to fine powder. 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 mobile phase (10 ml) to get 1000 µg/ml solution and further dilutions were prepared as above.

Risk assessment study

The aim of risk assessment studies is to investigate the effects of numerous elements that influence the target method's quality profile (TMQP) [32]. Critical analytical attributes (CAAs) enable researchers to appraise the ties between the critical method parameters of the TMQP prior to risk assessment investigations. The data from risk assessment studies aid in determining the root causes of the problems and the sources of faults, variances, defects, or failures. Another function of risk assessment studies is to collect information on an individual's risk factors, which can then be classified into three levels and designated as low, medium, and high risk. A total of seven factors were examined for screening in this investigation. Three parameters were chosen for the systemic optimization based on their high-, medium-, and low-risk scores [33].

Method development

The experimental design was constructed using Box-Behenken design expert software version 13.0.5.0 for the study of different independent variables (methanol %, flow rate and wavelength) and to verify method performances. The levels of these variables are as given in Table 1. 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. The experimental observations along with Design (DOE) plan are shown in Table 2. In the given sets of factors and responses, seventeen trials were conducted

Table 1: Factors and levels of variables

Factors	Unit	Type	Subtype	Min	Max
Composition	%	Numeric	Continuous	70	90
Flow rate	ml/min	Numeric	Continuous	0.8	1
Wavelength	Nm	Numeric	Continuous	254	258

Table 2: Box-Behnken design based independent variables and their effect on dependant variables

Run	Factor A	Factor B	Factor C	Response 1	Response 2	Response 3	Response 4

s	A: Composition (%)	B: Flow rate (ml/min)	C: Wavelength (nm)	TELM RT (min)	TELM TF (Units)	AZEL RT (min)	AZEL TF (Units)
1	80	1	254	3.697	1.32	7.052	1.24
2*	80	0.9	256	4.161	1.35	7.948	1.29
3*	80	0.9	256	4.161	1.35	7.948	1.29
4	80	0.8	258	4.527	1.35	8.742	1.27
5	70	0.9	254	4.859	1.15	12.533	1.25
6	70	0.9	258	4.856	1.28	12.6	1.25
7*	80	0.9	256	4.161	1.35	7.948	1.29
8*	80	0.9	256	4.161	1.35	7.948	1.29
9	70	0.8	256	5.582	1.3	14.727	1.28
10	70	1	256	4.474	1.3	11.528	1.28
11	80	0.8	254	4.563	1.36	8.739	1.29
12	90	0.9	258	3.728	1.37	5.343	1.22
13	90	1	256	3.386	1.38	4.873	1.29
14	90	0.8	256	4.266	1.4	6.093	1.25
15*	80	0.9	256	4.161	1.35	7.948	1.29
16	80	1	258	3.719	1.32	7.136	1.29
17	90	0.9	254	3.703	1.43	5.278	1.25

The responses obtained after carrying out the above trial runs were fed back to Design Expert software and plots 3D-response surface plots and 2D contour plots. These plots revealed the influence of critical method parameters (CMP) 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 CMPS of the method were determined and the optimized chromatographic conditions were finalized. Moreover, the evaluation of statistical analysis tool like analysis of variance (ANOVA) for each individual response was used to determine the significance of each method parameter selected for the study using the P value.

Forced degradation

Degradation studies were carried out according to ICH guidelines [2]. The samples were subjected to different stress conditions viz acid, base, peroxide, thermal, and photolytic stress conditions separately and the solutions were analysed using HPLC. Table 3 summarize the procedure to prepare the sample solutions for forced degradation studies.

Table 3: Method of preparation of samples for forced degradation study

Stress conditions	Quantity of drug/ formulation stock solution (ml)		Volume of stress agent (ml)	Analyse after 24 h	
	TELM	AZEL		Neutralizing agent	Diluent (ml)
Acid degradation	1	1	5 ml (0.1 N HCl)	5ml (0.1 N NaOH)	10
Base degradation	1	1	5 ml (0.1 N NaOH)	5 ml (0.1 N HCl)	10
Oxidation degradation	1	1	5 ml (3% H_2O_2)	5 ml (3% sodium metabisulphite)	10
Thermal degradation	1	1	Kept in oven at 80 °C	-	10
Photolytic degradation	1	1	Sunlight for 12 h	-	10

Method validation

The optimized RP-HPLC method was validated concerning the following parameters. The validation was performed as per the ICH Q2 (R2) guidelines. The following parameters were used for validation study [1, 4].

Linearity

Linearity for AZEL and TELM were assessed by analysis of combined standard solution in range of 4-20 ppm and 20-100 ppm respectively, in terms of slope, intercept and correlation coefficient value. The graph of peak area obtained v/s respective concentration was plotted.

Accuracy

The accuracy of the assay method for AZEL and TELM was evaluated in triplicate (n = 3) at concentrations of 4-20 ppm (AZEL) and 20-100 ppm (TELM) for RP-HPLC of the drug product and the recovery (50, 100 and 150 % level) was calculated for each externally spiked concentration.

Precision

The precision includes intra-day and inter-day precision studies; each of three times on the same day for intra-day and different days for inter day precision. The results were represented in the form of relative standard deviation (% RSD) values.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection limit and quantification limit were calculated using the standard equation method. The equation for

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

where, σ = standard deviation of the response and S = slope of the calibration curve.

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. For RP-HPLC, the robustness of the method was studied by deliberately varying parameters like change in wavelength and pH. The robustness of developed method was calculated in terms of % RSD.

Results and Discussion

Risk assessment study

The results of the RAS made it easy to discover the reason for the problems, and then afterwards to fix the rationale of the defects, variations, or failures. After screening and analysing the various factors in the risk assessment studies, the two responses of retention time and tailing factor were considered for the method development. The effects of each component on chromatographic conditions have been presented in Table 4.

Table 4: Risk assessment studies for method development

CAA's	Method parameters						
	Flow rat e	**Temperature of column**	**Detector wavelength**	**Vol of sam ple**	**Composition of solvent system**	**Type of flow**	**Dimension of column**
Retention time	+1	0	+1	-1	-1	0	-1
Peak area	+1	0	0	0	0	-1	0
Tailing factor	+1	-1	+1	0	+1	0	0

QbD based method optimization

Quality by design (QbD) is well established in the method development of drug product processes as described in ICH Q8, Q9 and Q11. The objective of AQbD is to design a rugged, robust method that consistently delivers the intended performance [34]. Design of Experiments (DoE) is the simple method to optimize the experimental condition with two or more variables [35, 36]. DOE for analytical methods during the development stage is needed for better improvement, a quantitative understanding of the factors that influence resolution, selectivity is an integral part of the method development.

The chromatographic method optimization was carried out employing BBD, where a total 17 experimental runs were conducted to optimize the CMAs for attaining better chromatographic separation. Table 2 shows the design matrix enlisting the experimental trial runs containing the combination of CMAs at three different levels. The analysis of coefficients indicated the prevalence of significant interactions among the critical method variables. Also, the statistical validity of the model was performed using ANOVA, which demonstrated significance of model ($P < 0.05$).

The final optimized conditions were determined by evaluating the effect of three factors A (methanol ratio), B (flow rate) and C (wavelength). The desirability plots for AZEL and TELM was generated using the Design Expert software. Based on the retention time and tailing factor the optimized conditions selected was as: mobile phase methanol: water (80:20 v/v), flow rate 0.9 ml/min and wavelength of 256 nm (Figure 2). The wavelength of 256 nm was optimized and selected as a suitable wavelength for estimation of two drugs (AZEL and TEL) as shown in Figure 2.

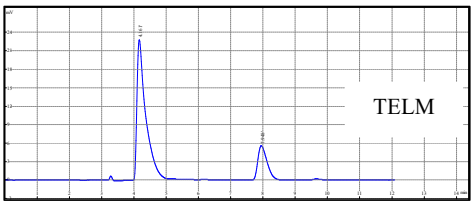
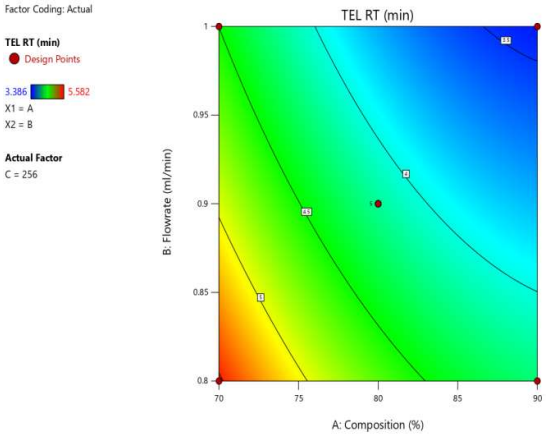
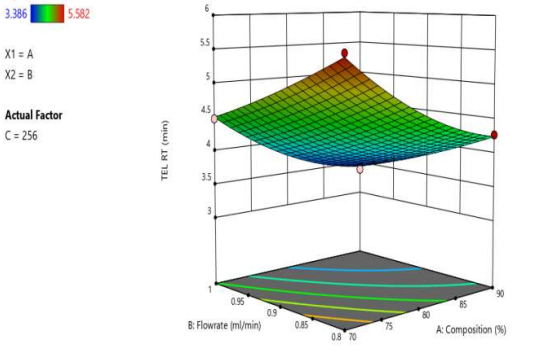


Figure 2: Chromatogram of AZEL and TELM tablet with mobile phase (methanol: water 80:20 v/v) at flow rate (0.9 ml/min) and detector wavelength of 256 nm.

(a) Retention time of TELM

The inbuilt ANOVA of quadratic model is significant, whereas the models F value was 254.15 implies that the model was significant. There was only a 0.01 % chance that an F value this large could occur due to noise. The majority of the model's term values are less than 0.05, indicating that the model as a whole found to be significant. The model summary statistic was shown in Table 5. Adequate precision measures the signal to noise ratio (S/N), and a ratio greater than 4 considered as desirable. The model S/N ration was found to be 60.2472 which indicates the signal is satisfactory. The inbuilt model graphs were presented in Figure 3.





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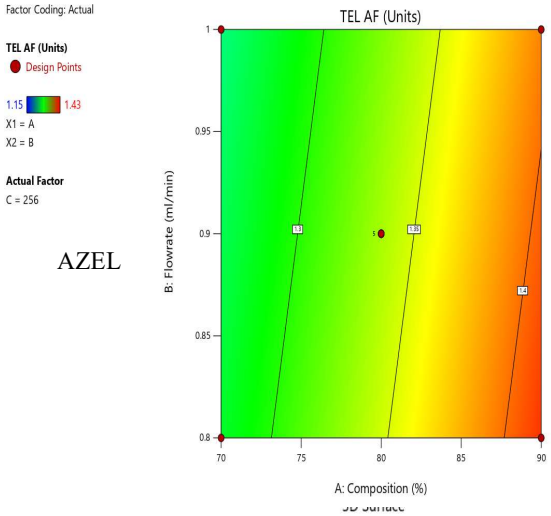
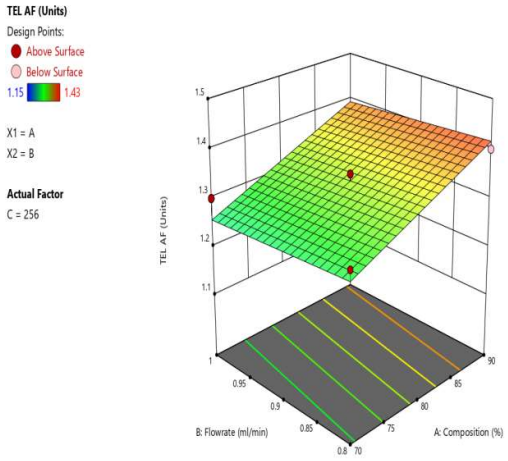




(c)

(d)

Figure 3: Inbuilt model graphs of TELM for retention time (RT) and tailing factor AF); (a) contour plot for RT, (b) 3D response plot for RT, (c) contour plot for AF and (d) 3D response plot for AF.

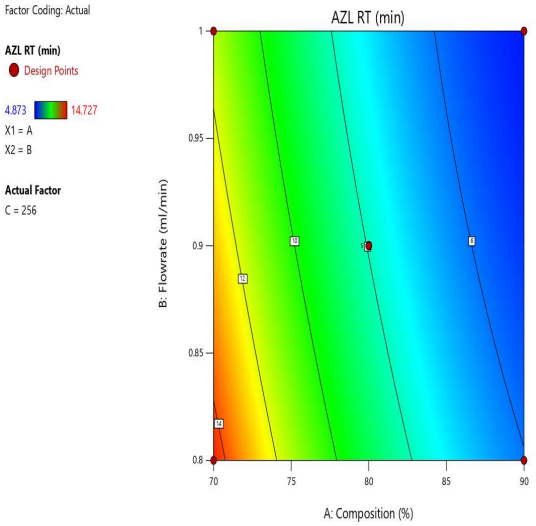
(b) Tailing factor of TELM

Symmetry and the shape of the peak indicate the developed method's accuracy. Here we optimized the tailing factor value by BBD surface response with ANOVA for the quadratic model. The inbuilt model was significant with a F value of 8.80 and there is only 0.19% chance that F value this large could occur due to noise. P values less than 0.05 indicates significance of model and greater than 0.1 indicate the model terms are not significant; here the $p < 0.001$ shown significance of present model (Table 5). An adequate signal was observed as the value of S/N ratio of 8.553 was greater than 4.

Model graphs in terms of contour plots and 3D surface response were shown in Figure 3.

(c) Retention time of AZEL

The ANOVA used in quadratic model was significant ($p < 0.05$). the models F value was 417.37 implies that the model was significant. The model terms A, B, AB and AA^2 were less than 0.05. the predicted RA^2 of 0.9702 was in reasonable agreement with the adjusted RA^2 of 0.9957, which indicated that the difference was less than 0.2. the signal-to-noise ratio of 66.85 indicated an adequate signal. Figure 4 presented graphs for retention time of AZEL for inbuilt model.



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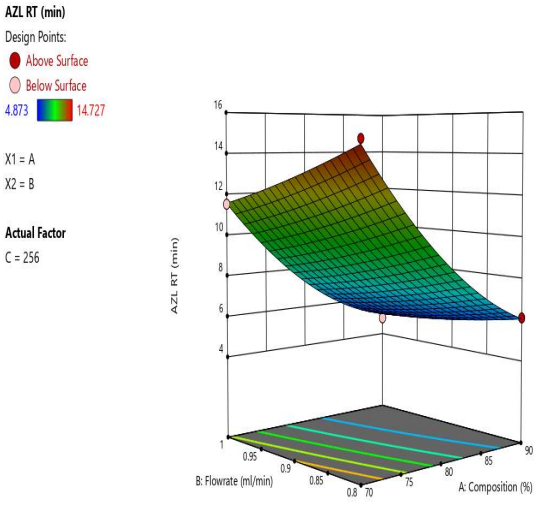

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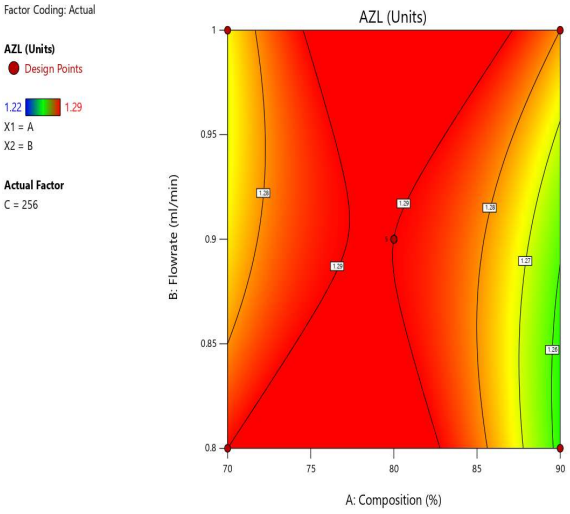
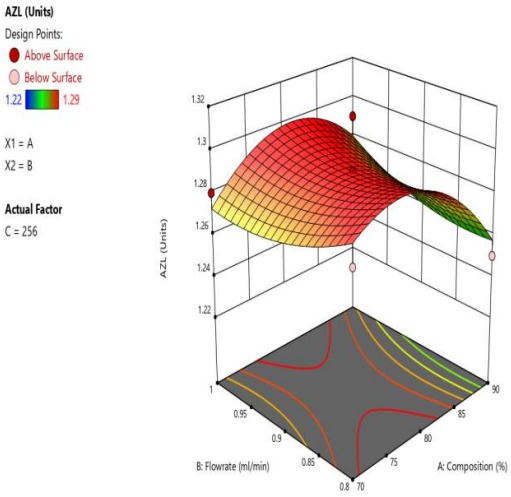




(c)

(d)

Figure 5: Model graphs for retention time (RT) and tailing factor (AF) of AZEL (a) contour plot for RT, (b) 3D surface response for RT, (c) contour plot for AF and (d) 3D surface response for AF

(d) Tailing factor of AZEL

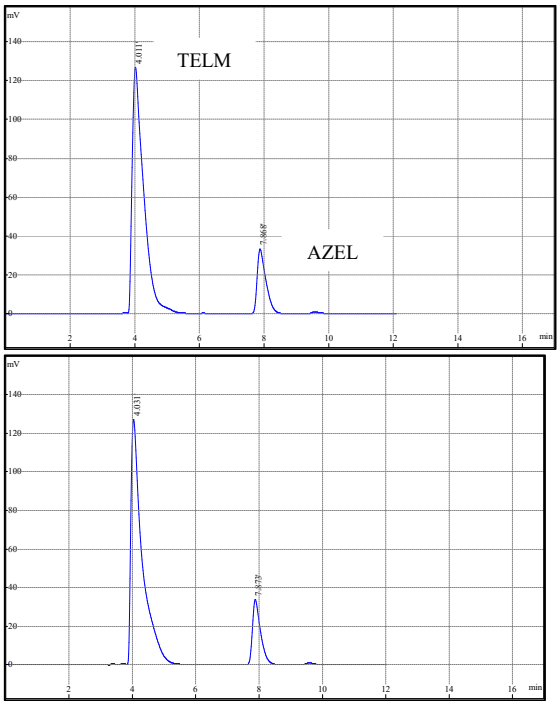
The model F value of 5.28 implied the model is significant. There was only a 1.96 % chance that an F value this large could occur due to noise. The p values less than 0.05 indicated the model was significant. In this case BC, AA^2, CA^2 was found significant model terms. A negative predicted RA^2 implied that the overall mean may be a better predictor of the response than the current model. The signal-to-noise ratio of 6.576 indicated an adequate signal (Tabe 5). The model graphs presenting contour plot and surface response plot were depicted in Figure 5.

Table 5: Statistical summary of model

	TELM		AZEL	
	Retention Time	**Tailing Factor**	**Retention Time**	**Tailing factor**
Source model	Quadratic	Quadratic	Quadratic	Quadratic
S.D.	0.045	0.038	0.1829	0.0123
R^2	0.9969	0.6701	0.9981	0.8716
Adjusted R^2	0.9930	0.5939	0.9957	0.7766
Predicted R^2	0.9511	0.3258	0.9702	-1.05
Precision	60.2472	8.5525	66.8495	6.5758
Sequential p value	< 0.05	0.001		0.01

Forced degradation

Acid, base, thermal and photolytic stress conditions showed no any change in chromatogram of TELM and AZEL dosage form (Figure 6). Whereas, peroxide hydrolysis induced sample shown one degradation peak but it does not interfere the principal peaks as shown in figure 6 (c).



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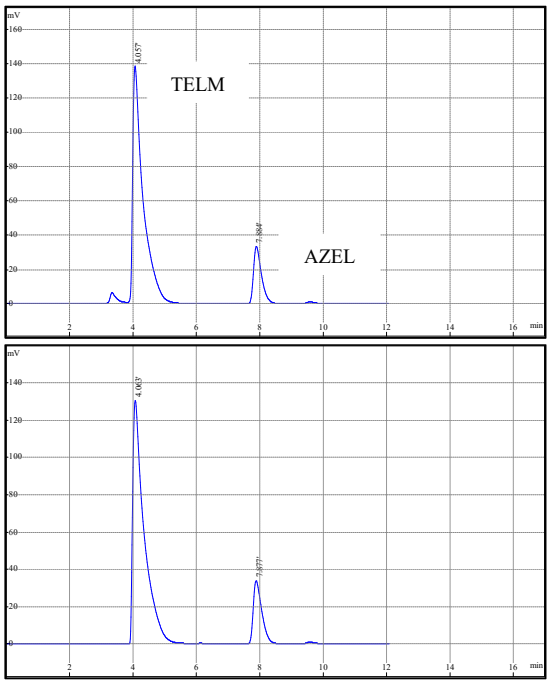

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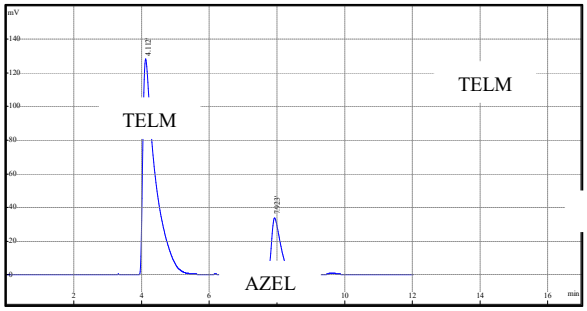


(e)

Figure 6: Chromatogram of mixture of Telmisartan and Azelnidipine under various stressed conditions as (a) acidic condition, (b) basic condition, (c) oxidation, (d) thermal condition and (e) photolytic conditions.

Method validation

System suitability test

An experiment was performed to conduct the system suitability test (n = 5). The retention time resolution, theoretical plates, area and tailing factor were considered for the calculation. The results of the experiment were founded as displayed in Table 6. The results of the experiment indicate that the device has performed admirably.

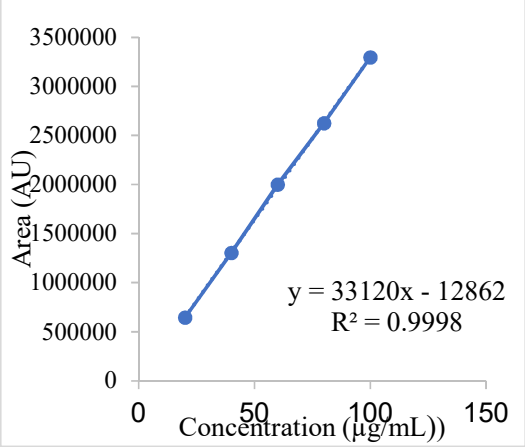
Table 6: System suitability parameters

Sr.	Parameters	TELM	AZEL

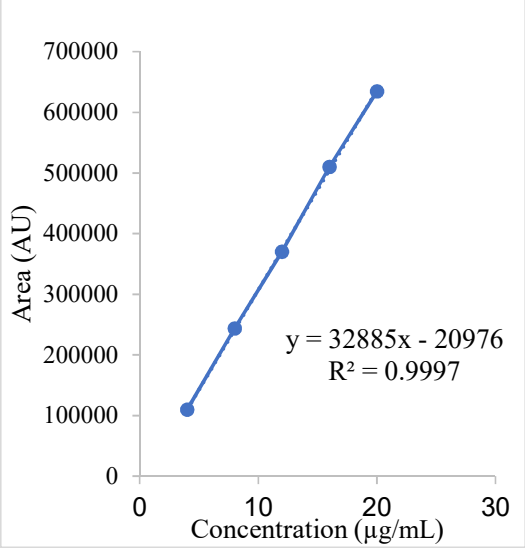
No.			
1	Retention time (RT in min)	4.161	7.948
2	Resolution (RS)	7.17	0.00
3	Theoretical plates (NTP)	7064	8602
4	Area (AU)	446804	113809
5	Asymmetry factor (AF)	1.35	1.29

Linearity

The results of calibration curve (n = 5) as depicted in Figure 7 a & b, deciphered that the developed method for formulation of TELM and AZEL was linear with a decent regression coefficient (R^2) of 0.9998 and 0.9997 respectively.



(a)



(b)

Figure 7: Calibration curve of combination (a) TELM (20-100 µg/mL) and (b) AZEL (4-20 µg/mL). It indicates that TELM and AZEL has obeyed good linearity with the working standard solutions (n = 5).

Accuracy and precision

The accuracy was determined in terms of percentage recovery. For this, a predetermined concentration was considered and an amount of 50%, 100% and 150% of the drug was added. The results of accuracy and precision were presented in Table 7 & 8. The recovery was found in the range of 99-100 % for TELM and for AZEL 100 %. The % RSD was found below the precision limit of 2% (Table 8).

Table 7: Accuracy (% recovery) data

% Composition	TELM			AZEL		
	Conc. Taken (µg/mL)	Conc. Found (µg/mL)	% Recovery	Conc. Taken (µg/mL)	Conc. Given (µg/mL)	% recovery
50 %	60	60.2928	100.4880	12	12.0161	100.1348
100 %	80	79.9763	99.9704	16	16.0832	100.5205
150 %	100	100.0725	100.0725	20	20.0474	100.2370

Tabe 8: Precision data

Samples (µg/mL)	TELM				Samples µg/mL)	AZEL			
	Intraday		Interday			Intraday		Interday	
	%SD	%RSD	%SD	%RSD		%SD	%RSD	%SD	%RSD
20	0.16	0.02	0.20	0.03	4	0.53	0.10	0.39	0.20
60	0.20	0.03	0.20	0.03	12	0.61	0.11	0.45	0.20
100	0.07	0.03	0.24	0.04	20	0.68	0.11	068	0.21

Robustness

The retention time, tailing factor and plate count of the TELM and AZEL were not significantly affected by changes in the developed method, such as changes in mobile phase pH and detector wavelength. Table 8 summarizes the findings.

LOD and LOQ

LOD and LOQ indicated the high sensitivity of developed method. LOD for TELM and AZEL was found to be 0.17 and 0.11 respectively. Whereas, LOQ of TELM was 0.52 and 0.35 for AZEL. The LOD and LOQ was found to be in standard limit (NMT 3 and NMT 10 respectively)

Table 8: Robustness study data

Changes	Retention Time		Plate count		Tailing factor	
	TELM	AZEL	TELM	AZEL	TELM	AZEL
mobile phase pH 2.8	4.12	7.92	7766	8554	1.35	1.30
mobile phase pH 3.2	4.08	7.95	7886	8428	1.37	1.28
Wavelength 254 nm	4.11	7.91	7856	8419	1.33	1.28
Wavelength 258 nm	4.08	7.79	7809	8738	1.33	1.27

Conclusion

The new simple, rapid, accurate, and precise method was developed for RP-HPLC analysis and has been validated according to ICH guidelines. The new method is economical, the mobile phase is easily available, and it employs a simple UV detector. BBD approach is applied to the optimization of chromatographic conditions by studying the interaction and quadratic effects of the significant factors on the two selected responses. The models used for screening and optimization steps were significant and confirmed method predictability. The validation of the study results revealed that the developed method could be adapted for the routine analysis of tablet dosage form.

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