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Quality by Design Based Development and Quantification of Telmisartan and Rosuvastatin Calcium Using RP-HPLC

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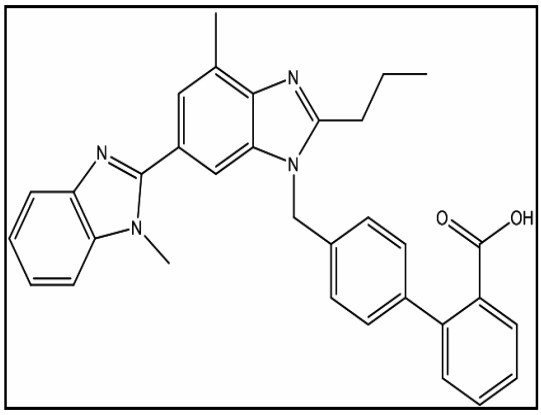
ABSTRACT

RP-HPLC technique to estimate the Telmisartan and Rosuvastatin Calcium, employing an experimental design method using response surface methodology, was developed and validated. Experimental design was used to evaluate a multivariate optimisation of experimental conditions using flow rate, buffer pH, and % of acetonitrile in the mobile phase as three independent variables. The peak symmetry and retention time of the last eluting peak were optimized employing Derringer's desirability function in which 1 ml/min flow rate, KH_2PO_4 buffer with pH 3.5 (altered with 1% ortho-phosphoric acid), and 65% v/v of acetonitrile in the mobile phase in an isocratic proportion of acetonitrile: buffer (65:35, v/v) on a C18 column. Using response surface methodology, a RP-HPLC method was developed based on DoE that resulted in a better separation of peaks with a lower retention time of less than 9 min for eluted peaks. Response of linear was reported over the range of concentration of 20-100 µg/mL for Telmisartan and 5-25 µg/ mL for Rosuvastatin Calcium.

Keywords: Chromatography, Response Surface Methodology, Telmisartan, Rosuvastatin Calcium, Validation, Design of Experiment, Pharmaceutical Analysis

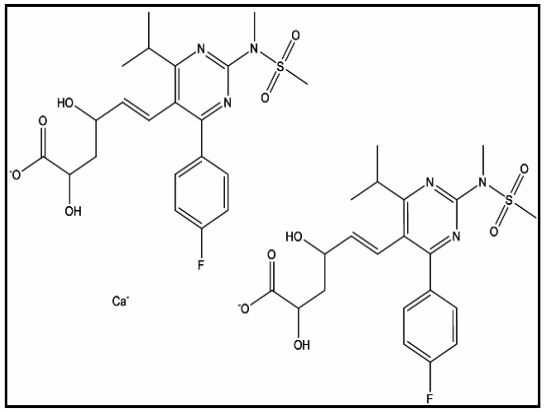
INTRODUCTION

Telmisartan (TEL), also known as 4-[[4-Methyl-6(1-methyl-1H-benzimidazole-2-yl)-2-propyl-1H-benzimidazol-1-yl]methyl]-2-biphenylcarboxylic acid (Fig. 1A). Its appearance is off-white or slightly yellowish, and crystal-like powder. It is clas2sified as an antihypertensive drug.[1,2] Rosuvastatin Calcium (ROS), also known as (E)-(3R,5S)-7[4-(4-fluorophenyl)-6-isopropyl-2 [methyl(methylsulphonylamino)]pyrimidin-5-yl]3,5-dihydroxyhepten-6-oic acid calcium (Fig. 1B). Its appearance is an off-white and creamish crystalline powder. It is classified as an antihyperlipidemic agent. Hypertension and dyslipidemia are two risk factors for cardiovascular disease that are likely to occur simultaneously. A failure to treat such multiple complications earlier may result in poor compliance, which in turn may lead to poor outcomes. As a result, the fixed dose of Telmisartan and Rosuvastatin is effective in reducing blood pressure and lowering cholesterol levels without the surge in adverse effects associated with individual drugs.[3]



(a)

Fig. 1(a). Telmisartan



(b)

Fig. 1(b). Rosuvastatin Calcium

The survey includes the UV-spectrophotometric method for TEL in dosage form and Quality by design (QbD).[4,5] Some analytical procedures have been reported, like the RP-HPLC method for ROS, and other methods like stability indicating RP-HPLC for TEL and ROS.[6,7] Earlier literature survey reveals that there is no report of an optimal analytical RP-HPLC method employing experimental design techniques (QbD) for the concurrent estimation of TEL and ROS in collective pharmaceutical dosage. There are several methods that have been found in the literature for determining the TEL and ROS, either in single dosage form or in conjunction with other medications.[8–12] This study can be helpful for pharmaceutical organizations like the USFDA, ICH, etc., as they are implementing the QbD concept for the quality improvement of the product as well as the manufacturing processes. For this, ICH guidelines have been published for QbD employment in the pharmaceutical field.[13–18]

In this study, the aim was to develop an improved and optimized experimental design employing the RPHPLC technique appropriate for the repetitive quality switch of TEL and ROS in the pharmaceutical commerce and provide a description of the understanding of chromatographic factors and their interactions with separation features. The chromatographic factors optimization of like % of acetonitrile in the mobile phase, flow rate, pH of the buffer, or the wavelength were highly composite and had a substantial result on chromatographic separation. By using the Quality by Design (QbD) method, all of the independent factors were optimized easily. A Quality by Design approach uses multi-dimensional groupings and input variables to achieve the optimum conditions for quality assurance. In order to define the design space, experimental design was used to generate a region in which changes in any of the factors (e.g., pH, organic modifier percentages, etc.) do not require post-approval adjustments (ICH Q8 (R2))[19]. For optimizing more than single response (retention time and tailing factor of both drugs) simultaneously, Derringer's desirability function was the most appropriate choice. To improve separation quality, Deming used Derringer's desirability function in chromatography[20] to get improved resolution and rapid analysis. A new HPLC method for estimating TEL and ROS from a tablet formulation was developed and optimized based on the same methodology.

MATERIALS AND METHODS

Chemicals

Telmisartan (TEL) API from Cadila Pharmaceuticals Limited (Ahmedabad, Gujarat) and Rosuvastatin Calcium (ROS) API from Corona Remedies Pvt. Limited (Ahmedabad, Gujarat) were collected as a gift sample. Several HPLC-grade solvents, like acetonitrile (ACN), water, and methanol, were obtained from Thermo Fisher Scientific India Pvt. Ltd., and orthophosphoric acid and phosphate buffer were obtained from Astron Chemicals India. All the solutions were prepared with HPLC-grade acetonitrile.

Statistical analysis

Design-Expert v13.0.12.0 by Stat Ease was employed for the design of experiments (Central Composite Design), data analysis, and calculations of the desirability function. Microsoft Excel 2021 was used for the calculation of R2, standard deviation (SD), and relative standard deviation (RSD) of validated data.

Preparation of Mobile Phase

Preparation of buffer: Accurately weighed quantity of 1.36 g Potassium dihydrogen phosphate (KH_2PO_4) was transferred in 1000 mL beaker, dissolved in 200 mL HPLC grade water and sonicated for about 10 min and diluted up to the mark with HPLC grade water. It was filtered through 0.45 µm membrane filter. Buffer pH was adjusted to 3.5 using 1% ortho phosphoric acid.

Preparation of 1% ortho phosphoric acid: 1 mL of Ortho phosphoric acid was taken and dissolved in 100 mL of water.

For 100 mL of mobile phase, 65 mL of ACN and 35 mL of buffer (65:35) were taken and mixed. Then the mobile phase was degassed for 15 min with an ultrasonic bath.

Preparation of Standard stock solution

The active pharmaceutical ingredients (API) of TEL and ROS were assessed and transferred to the appropriate volumetric flask. Both APIs were liquified in adequate quantities of mobile phase (65 ACN:35 Buffer) to produce a 1 mg/mL concentration of each. Solutions for working standards were obtained by diluting standard stock solutions in the mobile phase (20-100 µg/mL for TEL and 5-25 µg/mL for ROS).

Chromatography condition

High-performance liquid chromatography was accomplished employing a Shimadzu HPLC system (Shimadzu, Model LC 201^{0}C HT Liquid Chromatograph) equipped with a serial dual plugger pump and UV detection system. Lab Solutions Lite software version 5.52 was employed for the chromatographic system operation and recording of data. The UV spectra were performed on a UV-1800 Shimadzu UV Spectrophotometer. Chromatographic separations were achieved on a Chromatopak Peerless (250mm×4.6mm, 5 µm) C18 column. The composition of the mobile phase contained ACN:10mM potassium dihydrogen phosphate (KH_2PO_4) buffer (65:35) (3.5 pH, adjusted by 1% orthophosphoric acid) with a 1.0 mL/min flow rate. Each run involved the injection of 20 µL of sample, and detection was performed at a 231nm wavelength with a run time of 13.0 minutes.

Experimental Design and Response Surface Methodology

For the three independent variables, a faced central composite design (FCCD) was considered using a partial factorial design for optimization of % of ACN in the mobile phase, pH, and flow rate for effective separation. Five replicates were taken at extreme levels, including centre points and axial points. Using Derringer's desirability function, the position of the factually optimal condition was determined by assessing the R2 coefficient of determination for the suited polynomial models. For the optimization of variable parameters in the experimental region, Response Surface Methodology (RSM) was employed.

RESULTS AND DISCUSSION

Method development and Optimization

The RP-HPLC method was developed and adopted employing Design of Experiment approach, taking into account several combinations of three independent factors. The pH, % of acetonitrile in the mobile phase, and flow rate were taken as independent factors. 231nm wavelength was selected as the optimal detection wavelength for decent detector sensitivity and response with minimal distortion based on the overlay of the both drugs in UV spectra. The best optimized chromatographic separation of both TEL and ROS was achieved by using Acetonitrile: 10mM potassium dihydrogen phosphate (KH_2PO_4) buffer (65:35) with a pH of 3.5 and a 1 mL/min flow rate, which gave good chromatographic separation. Then the final development of the design is constructed by a central composite design for a precise and accurate second-order model for the response variable.

Experimental design

The Central Composite Design (CCD), a statistical investigational design also called the Box-Wilson Design developed by G.F. Box and K.B. Wilson, is employed for the development of a second-order model that requires the least number of experiments to be performed. The axial points in this design set new figures for low and high values for all independent factors. The flow rate (X1), mobile phase pH (X2), and % of acetonitrile in the mobile phase (X3) were adopted as independent factors, while the retention time (Y1) and tailing factor (Y2) were selected as dependent factors for the central composite design. The array of CCD includes 15 optimized trials, which were developed and shown in Table (2). The optimization of % of acetonitrile in mobile phase and flow rate was selected based on the obtained responses and confirmed between 55% v/v and 75% v/v of ACN in mobile phase and 0.8 mL/min to 1.2 mL/min flow rate, respectively, and the pH range was optimized between 3.3 to 3.7 in order to achieve better peak symmetry and more accurate quantification of both drugs with a minimal

run time shown in Table (1). For Central Composite Design, the mathematical model is expressed as:

$$Y = b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X12 + b22X22 + b33X32$$

Where, Y is the estimated response, b0 is the intersection and b1, b2, b3, b12, b13, b23, b11, b22, b33 represent regression coefficients, and X1, X2, X3 represent the main effect, while X12, X22, X32 represent the quadratic terms and mistakes. The detailed experimental design matrix for Telmisartan (TEL) and Rosuvastatin Calcium (ROS) is provided in the following Table (2). For the optimization of TEL and ROS, the optimized conditions were obtained by forming and analyzing all paths. The adjusted value of R2 was achieved fine in the acceptable range of probability of P<0.05 demonstrating an optimally suitable and substantial model.

As shown in Table (3), the term interaction that has the most complete coefficient within the most suitable model was 0.02 AC for the R_t. The interaction that occurred between A and C was found to be statistically significant (P=0.001 for R_t) after the regression model was applied. Various trials were performed and it was found that by increasing the % of acetonitrile in the mobile phase, there was a swift reduction in retention time at any level of pH. A lesser level of factor A in combination with a slight surge in pH resulted in a fringe reduction in the retention time (R_t) of TEL. It was found that this interaction was coordinated because it resulted in a reduction in the analysis run time. As shown by the model T of the second response, all the parameters contributed to the ROS tailing.

Table 1: HPLC independent variables for CCD

Factors	Name	Level (-)	Level (0)	Level (+)
a	Flow rate (mL/min)	0.8	1	1.2
b	Buffer pH	3.3	3.5	3.7
c	Acetonitrile (%v/v)	55	65	75

Table 2: Experimental Conditions and Responses for CCD

Std.	Run	Factor 1 Flow rateml/min	Factor 2 pH	Factor 3 Acetonitrile%	1 Response Tailing Factor of ROS	2 Response Retention Time of TEL
1	14	0.8	3.3	55	0.966	9.996
2	15	1.2	3.3	55	0.99	9.95
3	13	0.8	3.7	55	1.088	10.205
4	6	1.2	3.7	55	1.099	9.998
5	9	0.8	3.3	75	0.985	8.037
6	12	1.2	3.3	75	1.007	6.393
7	8	0.8	3.7	75	1.05	9.255
8	10	1.2	3.7	75	1.069	7.729
9	2	0.66	3.5	65	1	9.826
10	4	1.34	3.5	65	1.06	8.2
11	7	1	3.16	65	0.948	8.341
12	11	1	3.84	65	1.16	9.867
13	5	1	3.5	48.18	1.066	9.835
14	3	1	3.5	81.82	1.04	7.858
15	1	1	3.5	65	1.012	9.203

Table 3: Statistical Parameter and Regression Model based on ANOVA

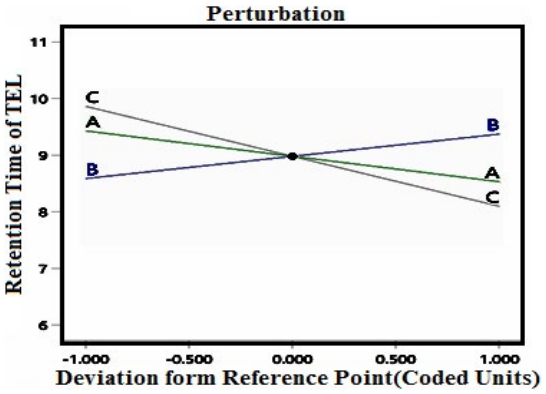
Response	Regression Model	Adjusted R^2	Model P-Value	%C.V.	Adequate Precision
R_t	21.78-0.0016A-0.0035B-0.0001C+0.96AB+0.02AC-8.34AC	0.9042	<0.0001	3.98	15.8039
T	5.51-0.0469A-0.0001B-0.3453C+0.78AB+0.91AC-0.0108AC	0.8698	<0.0004	1.97	12.6996

*P value <0.05 Significant (n=3)

Interpretation of Perturbation Plots

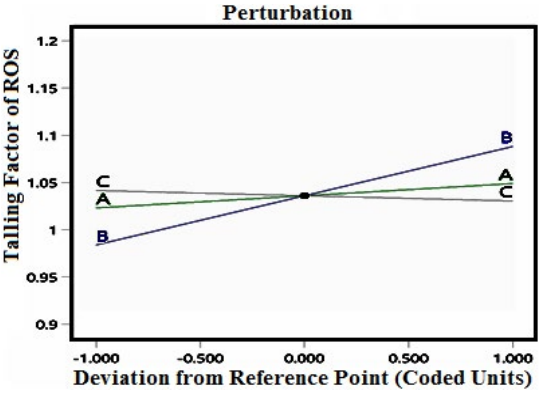
The perturbation plots illustrated in Fig. 2 deliver a better understanding of the results. With the help of the statistical software Design Expert, the experimental design was generated after all the data was processed. According to the two-dimensional coloured maps illustrated in Fig. 3, warm red colours indicate high time retention and tailing of peaks, whereas cold blue colours indicate low retention and tailing of peaks. In the created design model, the employed points were chosen by visually examining the least retention time and peaking of ROS. Fig. 3

and 4 illustrate that the TEL retention time increased to 3.7 pH, a flow rate of 0.8 mL/min, and an acetonitrile percentage of 55%. While the ROS tailing peak, reduced to acidic pH and lower acetonitrile %. As per the method's area, % of ACN had a 65% v/v content at 1mL/min flow rate and pH 3.5, resulting in quicker separation (<9 min) with adequate resolution and optimal tailing of the ROS peak (T = 1.0536).



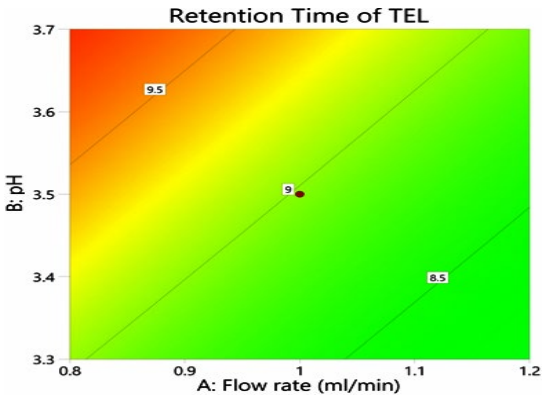
(a)

Fig. 2(a). Perturbation plot represents each factor effect on Retention time of Telmisartan



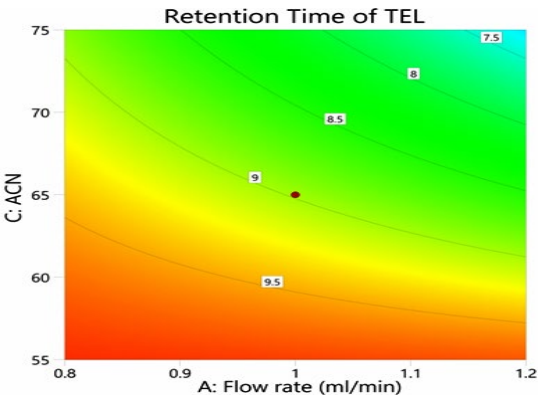
(b)

Fig. 2(b). Perturbation plot represents each factor effect on Tailing factor of Rosuvastatin



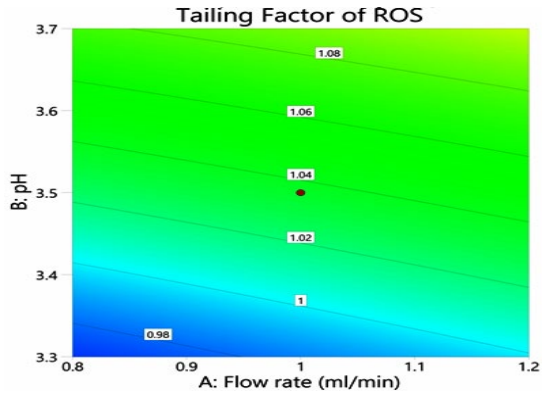
(a)

Fig. 3(a). 2-D model represents design model for Retention time of TEL in flow rate_pH



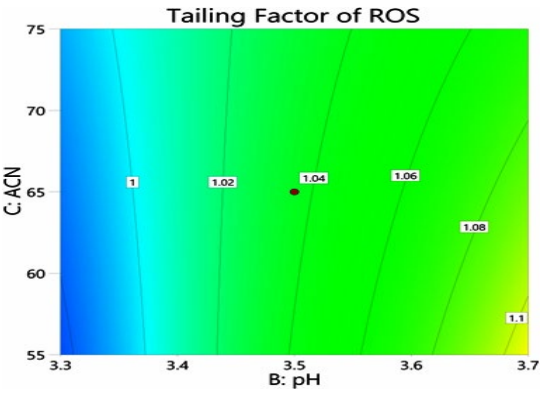
(b)

Fig. 3(b). 2-D model represents design model for Retention time of TEL in flow rate_% ACN



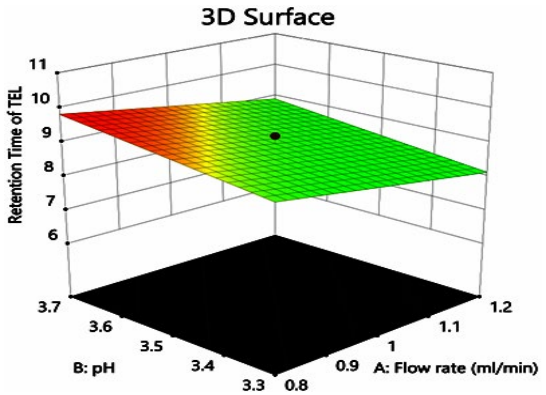
(c)

Fig. 3(c). 2-D model represents design model for Tailing factor of ROS in flow rate_pH



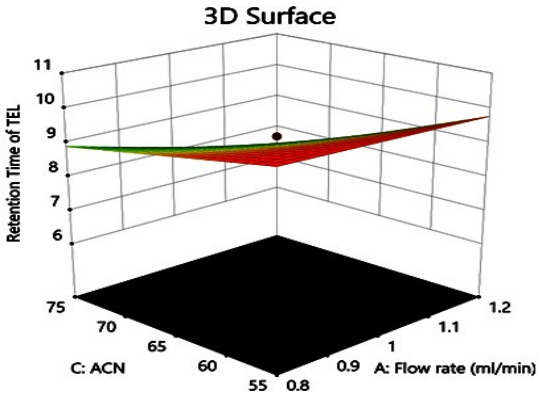
(d)

Fig. 3(d). 2-D model represents design model for Tailing factor of ROS in pH_% ACN



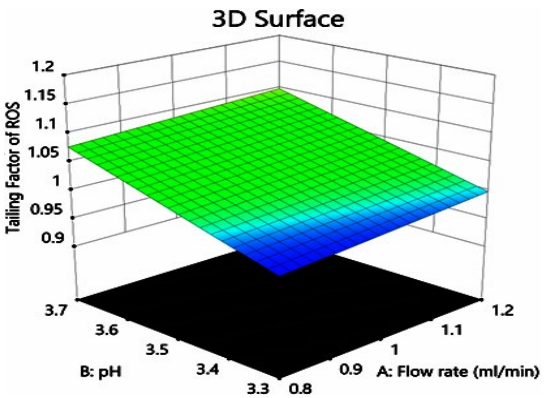
(a)

Fig. 4(a). RSM for Retention time of TEL in flow rate_pH



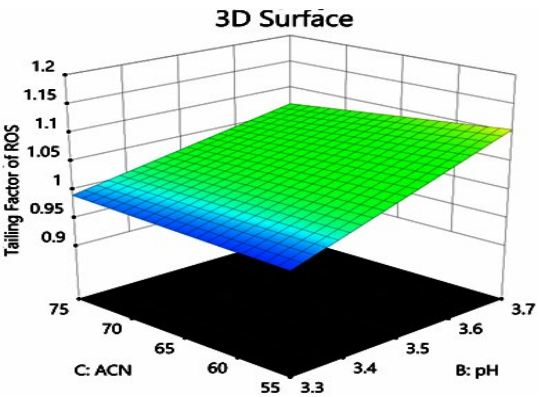
(b)

Fig. 4(b). RSM for Retention time of TEL in flow rate_% ACN



(c)

Fig. 4(c). RSM for Tailing factor of ROS in flow rate_pH



(d)

Fig. 4(d). RSM for Tailing factor of ROS in pH_% CAN

Derringer's desirability function refers to a factor optimization technique that is used for systems with multiple responses and multiple targets being optimized. Table (4) specifies standards to improve separate responses that were suggested to select the finest experimental conditions. The criteria were optimized using Design Expert 13, with retention time as a critical standard in method development. An extreme desirability value (D=1.000) was obtained with a 1 mL/min flow rate, acetonitrile at 65% v/v, and buffer pH of 3.5 as the optimal coordinates for the suggested process.

Table 4: Under optimal conditions, the experimental and predictive value of different objective functions is compared

Flow rate (mL/min)	Buffer pH	ACN (%v/v)		R_t (min)	T	Total Desirability
			Experimental value	8.8396	1.0536	
1	3.5	65	Predicted value	8.9795	1.036	1.000

Method Validation

Validation of methods involves the process of providing documentation that will satisfy the requirements of the analytical application and evaluating the developed analytical method. According to ICH and FDA guidelines, specificity, linearity, precision, accuracy, system suitability, limit of detection and quantification, robustness, and ruggedness were employed for the evaluation of an effective experimental design based on a developed analytical method that was validated.

Specificity

Specificity of the analytical technique was assessed to approve no intrusion of excipients on the retention times of TEL and ROS. An excipients mixture were prepared and injected to observe whether it is interfering with the retention times of TEL and ROS. No interference was reported on the retention times of TEL and ROS due to excipients.

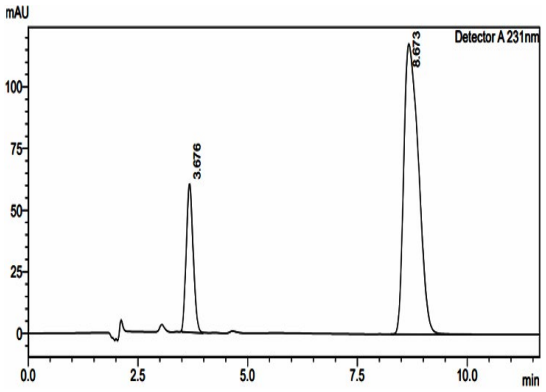


Fig. 5. Optimized Chromatogram of Rosuvastatin and Telmisartan

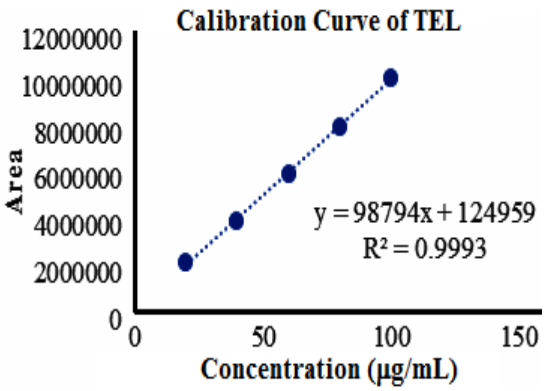
Linearity

20-100 µg/mL for TEL and 5-25 µg/mL for ROS were selected as the concentration ranges for the linearity. 100 µg/mL of standard stock of TEL was diluted with mobile phase to obtain 20, 40, 60, 80, and 100 µg/mL concentrations of TEL, and 100 µg/mL of ROS was diluted with mobile phase to get 5, 10, 15, 20, and 25 µg/mL concentrations of ROS. Six replications were performed. Results of the linearity of both drugs are shown in Table (5). A regression coefficient was calculated after plotting the calibration curve of the mean area under the curves vs. concentration. The results were R2=0.9993 for TEL and R2=0.9994 for ROS.

Table 5: Linearity data of Telmisartan and Rosuvastatin Calcium

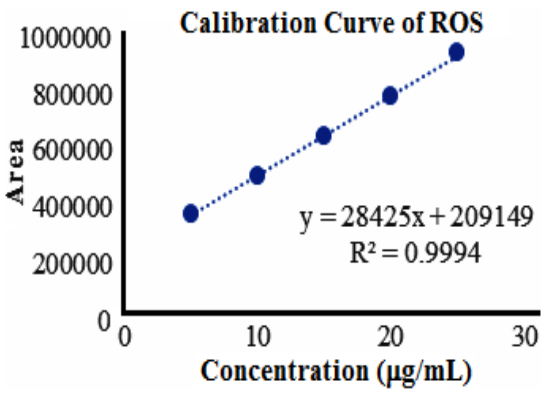
Telmisartan Concentration (µg/mL)	Rosuvastatin Calcium Mean Area ± SD	Telmisartan Concentration (µg/ml)	Rosuvastatin Calcium Mean Area ± SD	%RSD	%RSD
20	2207606 ± 7078	5	356268 ± 1000	0.32	0.28
40	3980794 ± 14096	10	491530 ± 2081	0.35	0.42
60	5988755 ± 41972	15	630546 ± 2773	0.70	0.44
80	8017022 ± 50585	20	773231 ± 4993	0.63	0.64
100	10068929 ± 36407	25	926041 ± 7077	0.36	0.76

Mean Area ± SD (n=6)



(a)

Fig. 6(a). Calibration Curve of Telmisartan (TEL)



(b)

Fig. 6(b). Calibration Curve of Rosuvastatin Calcium (ROS)

Accuracy

Accuracy was calculated by performing recovery experiments. 80%, 100%, and 120% levels were selected for the accuracy determination using the suggested HPLC technique. The percentage of recovery was calculated by adding a placebo to the standard solution. The accuracy study results are displayed in Table (6). As a result, the mean recovery ranged from 98.84% to 101.2% for both drugs.

Precision

For the precision evaluation of the suggested HPLC technique, repeatability studies with intraday and interday precisions were performed. The repeatability study was executed by injecting 40 µg/mL of TEL and 10 µg/mL of ROS (n=6). Results are shown in Table (7). The %RSD of intraday and interday precision was calculated by injecting 20, 40, and 60 g/mL for TEL and 5, 10, and 15 g/mL for ROS (n=3) to evaluate intraday and interday precision. Results are displayed in Table (8).

Limit of Detection and Limit of Quantification

Limit of detection (LOD) and quantification

(LOQ) for telmisartan and rosuvastatin were determined with ICH guidelines Q2 (R1) accordingly. LOD, the smallest analyte concentration of which stretches quantifiable response (the S/N ratio for LOD was 3.3), while LOQ, the smallest analyte concentration that can be quantified. (S/N ratio=10 for LOQ). LOD values for Telmisartan and Rosuvastatin were reported to be 0.3593 µg/ml and 0.0118 µg/mL and LOQ values for Telmisartan and Rosuvastatin were reported to be 1.0890 µg/ml and 0.0358 µg/mL. Table (9).

Table 6: Accuracy Data of Telmisartan and Rosuvastatin Calcium

Drug	Level	Amount of Sample Taken (µg/mL)	Amount of Standard Spike (µg/mL)	Total Amount of Drug	Amount of Standard Recovery Mean	%Recovery	SD*(n=3)
Telmisartan	80	40	32	72	71.54	99.36	0.06
	100		40	80	80.96	101.2	0.923
	120		48	88	86.98	98.84	0.29
Rosuvastatin	80	10	8	18	17.80	98.88	0.264
	100		10	20	19.83	99.15	0.234
	120		12	22	21.76	98.90	0.626

SD: Standard deviation

Table 7: Repeatability data of Telmisartan and Rosuvastatin (n=6)

Drug	Concentration (µg/mL)	Mean Area ± SD	%RSD
Telmisartan	40	3985351 ± 11475	0.28
Rosuvastatin	10	490558 ± 1740	0.35

(n=Number of Replications)

Table 8: Intraday and Interday Precision Data of Telmisartan and Rosuvastatin (n=3)

	Intra-day precision(n=3)			Inter-day precision(n=3)		
Drug	Concentration (µg/mL)	Mean Area ± SD	%RSD	Concentration (µg/mL)	Mean Area ± SD	%RSD
Telmisartan	20	2227606 ± 13018	0.58	20	2224272 ± 11479	0.51
	40	3980794 ± 7537	0.18	40	3981460 ± 7893	0.20
	60	5975422 ± 20936	0.35	60	5976089 ± 11273	0.18
Rosuvastatin	5	355845 ± 517	0.14	5	356511 ± 669	0.19
	10	491266 ± 617	0.12	10	490600 ± 541	0.11
	15	631346 ± 1736	027	15	631679 ± 1240	0.20

SD: Standard Deviation RSD: Relative standard deviation (n=Number of Replications)

Table 9: LOD and LOQ data of Telmisartan and Rosuvastatin (n=3)

Parameter	Telmisartan	Rosuvastatin
Mean Standard Deviation	10215	816.67
Mean Slope	93795	227517
LOD (µg/mL)	0.3593	0.0118
LOQ (µg/mL)	1.0890	0.0358

(n=Number of Replications)

Assay of Marketed Formulation

3 batches of the marketed formulation were selected for the suggested HPLC method. Solution for stock, 100 µg/mL for TEL and 100 µg/mL for ROS, was arranged by dissolving the equal quantity of tablet powder in Acetonitrile. A solution of 0.2 mL was shifted to a volumetric flask, and the mobile phase was further added to make up the volume of 10 mL. The area under the curve was compared between the sample and standard solutions after the HPLC system was employed for sample injection. The percentage of drugs and SD was calculated. Based on the results, the formulation claims are well accepted. Table (10)

Table 10: Assay Result of Marketed formulation

Formulation	Drug Name	Assay (n=3) Label claim	Amount found	% Label Claim ± SD (n=3)
Synthetic Mixture	Telmisartan	40	39.84	99.6 ± 0.1417
	Rosuvastatin	10	9.95	99.5 ± 0.0513

(n=Number of Replications)

CONCLUSION

In order to enhance the substantial parameters used to estimate the Telmisartan and Rosuvastatin in the combined dose, employing RPHPLC and Faced Central Composite Design (FCCD) methods and the response surface methodology of the D-o-E method were employed. The independent variables were optimized using Derringer's desirability function, influencing tailing factor and retention time as method responses. In accordance with the FDA and ICH guidelines, the analytical method validation proved significant in terms of linearity, accuracy, precision, specificity, and robustness. Further, the experimentally reported LOD and LOQ values of each drug were also low, thus showing high practical efficacy for estimating combination drugs in pharmaceutical dosage forms.

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Conflict of interest

It is declared that the authors have no conflict of interest.

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