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A VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE SIMULATNEOUS ESTIAMTION OF ROSUVASTATIN AND TELMISARTAN IN BULK FORM AND MARKETED PHARMACEUTICAL FORMULATIONS

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Abstract:

Background: *A simple, accurate and precise HPLC method for simultaneous determination of Rosuvastatin and Telmisartan in pure and tablet dosage form has been developed. To develop and validate analytical method for simultaneous estimation of Rosuvastatin and Telmisartan in pharmaceutical formulation by RP-HPLC. HPLC of Waters (Model: Alliance 2695) with Phenomenex Luna C18 (4.6 mm I.D. × 250 mm, 5 µm) column was used for chromatographic separation. It contains waters injector and PDA Detector (Deuterium). Mobile phase consists of Methanol: Water (65:35% v/v) and flow rate adjusted was 1ml/min. Wavelength selected for detection was 220nm and injection volume was 10 µl. By using the developed method, retention time of Rosuvastatin and Telmisartan was found to be 3.2min and 5.4min respectively. The method has been validated for linearity, accuracy and precision. Linearity of Rosuvastatin and Telmisartan were in the range of 75–375µg/ml and 15–75µg/ml respectively. The percentage recoveries obtained for Rosuvastatin and Telmisartan were found to be in range of 99.3 – 99.6%. LOD and LOQ were found to be 12.5µg/ml and 38.1µg/ml for Rosuvastatin 3.7and 11.4µg/ml for Telmisartan. The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms. From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.*

Keywords: *Rosuvastatin, Telmisartan, Simultaneous Estimation, RP- HPLC*

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INTRODUCTION:

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components.

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance.

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry.

Chromatographic methods
Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC).

Miscellaneous Techniques
Mass Spectrometry, Thermal Analysis.

Hyphenated Techniques
GC-MS (Gas Chromatography – Mass Spectrometry), LC-MS (Liquid Chromatography – Mass Spectrometry), ICP-MS (Inductivity Coupled Plasma-Mass Spectrometry), GC-IR (Gas Chromatography – Infrared Spectroscopy), MS-MS (Mass Spectrometry – Mass Spectrometry).

MATERIALS AND METHODS:

Rosuvastatin (Pure), Telmisartan (Pure) Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

HPLC METHOD DEVELOPMENT:
TRAILS
Preparation of standard solution:
Accurately weigh and transfer 10 mg of Rosuvastatin and Telmisartan working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 2.25ml of the above Rosuvastatin and 0.45ml of the Telmisartan stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:
Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:
Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol and water in proportion 65:35 v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Luna C18 (4.6 x 150mm, 5µm) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

VALIDATION
PREPARATION OF MOBILE PHASE:
Preparation of mobile phase:
Accurately measured 650ml (65%) of HPLC Methanol and 350ml of Water (35%) were mixed and degassed in a digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Column	: Phenomenex Luna C18 (4.6×250mm) 5µ
Column temperature	: 35˚C
Wavelength	: 220nm
Mobile phase ratio (65:35 v/v)	: Methanol: Water
Flow rate	: 1ml/min
Injection volume	: 10µl
Run time	: 10minutes

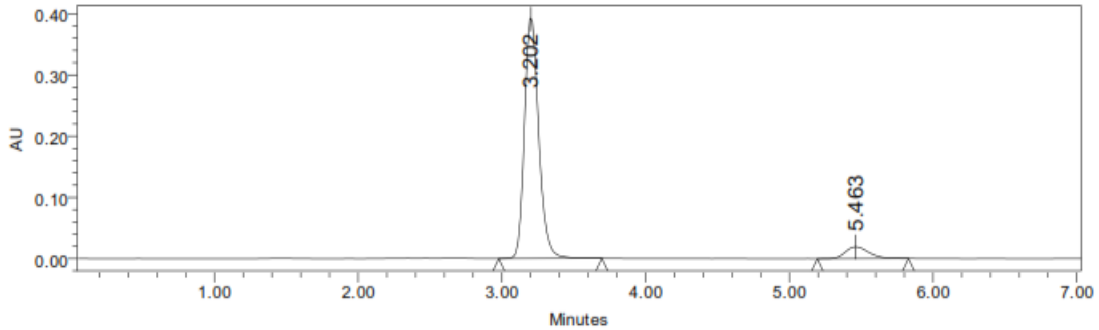


Figure: Optimized Chromatogram (Standard)

Table: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP
1	Rosuvastatin	3.202	2391746	39726	1.2	9028	
2	Telmisartan	5.463	194627	8497	1.1	7398	7.4

Observation:
This trial shows improper separation sample peaks, baseline and show very less plate count in the chromatogram. So it's required more trials to obtain good peaks.
From the above chromatogram it was observed that the Rosuvastatin and Telmisartan peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

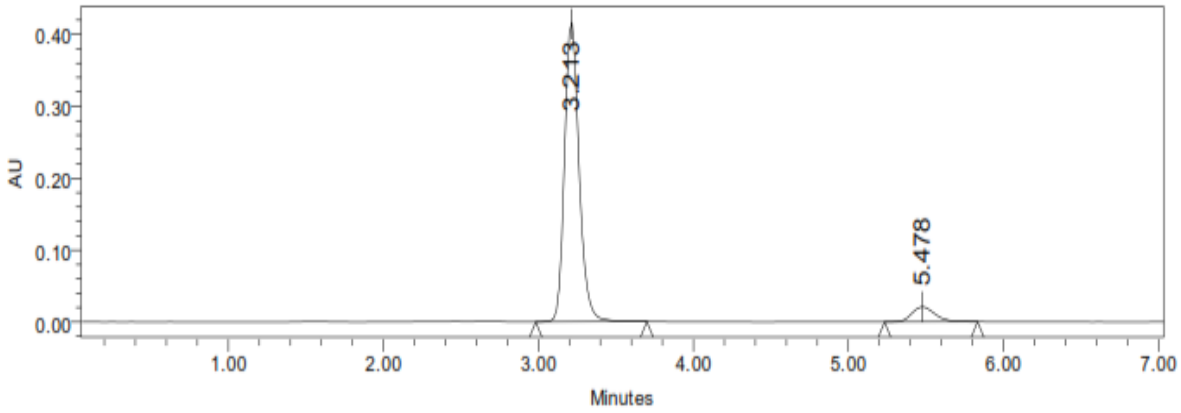


Table: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Rosuvastatin	3.213	2381649	391846	1.2	9472	
2	Telmisartan	5.478	191057	8104	1.1	8936	7.5

Table: Results of system suitability for Rosuvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosuvastatin	3.200	2391746	394171	8952	1.2
2	Rosuvastatin	3.248	2391647	381946	9561	1.2
3	Rosuvastatin	3.299	2381647	391746	6572	1.2
4	Rosuvastatin	3.297	2385631	386562	6452	1.2
5	Rosuvastatin	3.297	2385635	389164	7452	1.2
Mean			2387261			
Std. Dev.			4363.771			
% RSD			0.182794			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Telmisartan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	5.413	198362	7917	5272	1.1
2	Telmisartan	5.484	197486	7486	6291	1.1
3	Telmisartan	5.405	198354	7859	6184	1.1
4	Telmisartan	5.405	197352	7926	7145	1.1
5	Telmisartan	5.409	198453	7946	6946	1.1
Mean			198001.4			
Std. Dev.			535.1774			
% RSD			0.27029			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Standard):

Table: Peak results for assay standard

Rosuvastatin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Rosuvastatin	3.211	2397162	397161	1.2	9472
2	Rosuvastatin	3.222	2394721	389173	1.2	9745
3	Rosuvastatin	3.254	2389461	391723	1.2	8917

Telmisartan

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Telmisartan	5.414	198462	7811	1.1	8492	7.49
2	Telmisartan	5.453	198472	8193	1.1	8916	7.52
3	Telmisartan	5.424	198735	7972	1.1	9372	7.44

Assay (Sample):

Table: Peak results for Assay sample

Rosuvastatin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate
1	Rosuvastatin	3.297	2391741	381612	1.2	9472
2	Rosuvastatin	3.294	2389166	391746	1.2	8927
3	Rosuvastatin	3.295	2361731	381634	1.2	9017

Telmisartan

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Telmisartan	5.435	198641	8174	1.1	9284	7.18
2	Telmisartan	5.417	196547	8942	1.1	8974	7.44
3	Telmisartan	5.434	194027	7294	1.1	9017	7.38

%ASSAY =

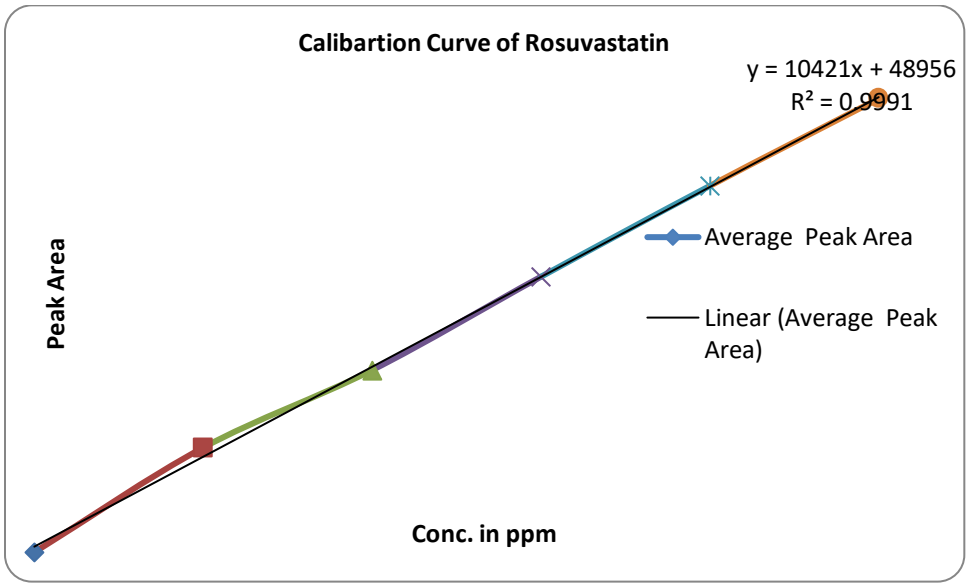
$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Rosuvastatin and Telmisartan in pharmaceutical dosage form was found to be 99.2%.

LINEARITY

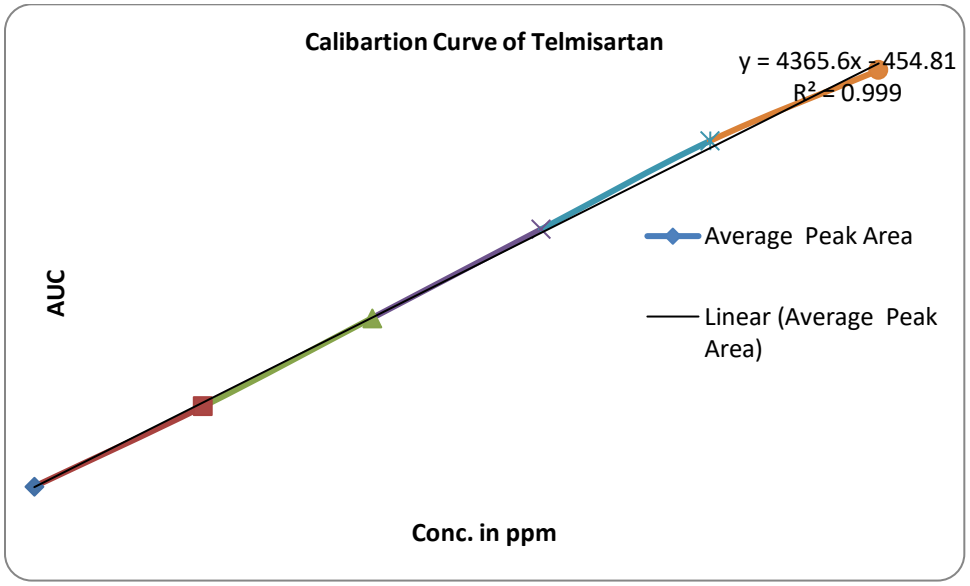
Rosuvastatin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	75	909889
80	150	1583641
100	225	2395378
120	300	3185089
140	375	3943725



Telmisartan

Concentration Level (%)	Concentration µg/ml	Average Peak Area
60	15	61953
80	30	130213
100	45	198697
120	60	267002
140	75	321658



REPEATABILITY

Table: Results of repeatability for Rosuvastatin:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosuvastatin	3.213	2397164	381741	8155	1.2
2	Rosuvastatin	3.253	2391741	371742	9174	1.2
3	Rosuvastatin	3.297	2371846	391746	7154	1.2
4	Rosuvastatin	3.215	2361748	391847	9917	1.2
5	Rosuvastatin	3.254	2371649	384622	9247	1.2
Mean			2378830			
Std.dev			14958			
%RSD			0.628797			

Acceptance criteria:

- %RSD for sample should be NMT 2
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table: Results of repeatability for Telmisartan:

S. No	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	5.441	198464	7291	6274	1.1
2	Telmisartan	5.442	193643	7219	6592	1.1
3	Telmisartan	5.409	196462	7194	6028	1.1
4	Telmisartan	5.520	194644	8174	6927	1.1
5	Telmisartan	5.424	198464	8653	5920	1.1
Mean			196335.4			
Std.dev			2190.191			
%RSD			1.115536			

Intermediate precision:

Table: Results of Intermediate precision for Rosuvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Rosuvastatin	3.211	2389572	395275	9375	1.2
2	Rosuvastatin	3.211	2391847	392175	9275	1.2
3	Rosuvastatin	3.210	2319472	312947	8265	1.2
4	Rosuvastatin	3.212	2306842	310585	6254	1.2
5	Rosuvastatin	3.211	2375972	310694	9028	1.2
6	Rosuvastatin	3.297	2396746	358373	8928	1.2
Mean			2363409			
Std. Dev.			39730.83			
% RSD			1.681082			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision for Telmisartan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Telmisartan	5.411	197284	7194	8264	1.2
2	Telmisartan	5.410	197849	7294	9174	1.2
3	Telmisartan	5.420	196572	7147	9164	1.2
4	Telmisartan	5.423	195028	7927	9733	1.2
5	Telmisartan	5.419	199474	8238	9194	1.2
6	Telmisartan	5.409	197482	7638	8973	1.2
Mean			197281.5			
Std. Dev.			1466.354			
% RSD			0.74328			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision Day 2 for Rosuvastatin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Rosuvastatin	3.211	2389562	391741	9264	1.2
2	Rosuvastatin	3.233	2381654	391047	9746	1.2
3	Rosuvastatin	3.244	2381946	391748	9816	1.2
4	Rosuvastatin	3.297	2391741	391746	9917	1.2
5	Rosuvastatin	3.297	2386452	381641	9742	1.2
6	Rosuvastatin	3.202	2374763	381645	9017	1.2
Mean			2384353			
Std. Dev.			6183.339			
% RSD			0.25933			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

Table: Results of Intermediate precision Day 2 for Telmisartan

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Telmisartan	5.411	197486	7582	6272	1.1
2	Telmisartan	5.410	197486	7184	6174	1.1
3	Telmisartan	5.420	196746	7456	5184	1.1
4	Telmisartan	5.405	195862	7814	6194	1.1
5	Telmisartan	5.409	196582	7194	6292	1.1
6	Telmisartan	5.463	198463	7745	6191	1.1
Mean			197104.2			
Std. Dev.			903.542			
% RSD			0.458408			

Acceptance criteria:

- %RSD of six different sample solutions should not more than 2

ACCURACY:

The accuracy results for Rosuvastatin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	1217218	112.5	112.4	99.6	99.3
100%	2397141	225	225	100	
150%	3514547	337.5	332.5	98.5	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

The accuracy results for Telmisartan

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	98598.67	22.5	22.4	99.9	99.6
100%	198359.7	45	45	100	
150%	291512.3	67.5	66.8	99	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table: Results for Robustness

Rosuvastatin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0mL/min	2391746	3.202	9028	1.2
Less Flow rate of 0.9mL/min	2371831	3.639	7381	1.2
More Flow rate of 1.1mL/min	2218319	2.859	9311	1.1
Less organic phase (about 5 % decrease in organic phase)	2294821	3.460	7462	1.2
More organic phase (about 5 % Increase in organic phase)	2394811	3.022	6817	1.1

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table: Results for Robustness

Telmisartan

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.1mL/min	194627	5.463	7398	1.1
Less Flow rate of 0.9mL/min	183738	6.250	6883	1.1
More Flow rate of 0.8mL/min	198373	4.863	9917	1.2
Less organic phase (about 5 % decrease in organic phase)	178471	6.196	8372	1.1
More organic phase (about 5 % Increase in organic phase)	189462	5.010	7716	1.2

Acceptance criteria:

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION:

The developed HPLC method offers several advantages such as rapidity, usage of simple mobile phase and easy sample preparation steps. Further, improved sensitivity makes it specific and reliable for its intended use. Hence, this method can be applied for the analysis of pure drug and pharmaceutical dosage forms.

From the present study it can be concluded that the proposed method is simple, sensitive, precise, specific, accurate and reproducible. Results of validation parameters demonstrated that the analytical procedure is suitable for its intended purpose and meets the criteria defined in ICH Q2R1.

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