International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

IJAMSCR |Vol.12 | Issue 4| Oct - Dec -2024

www.ijamscr.com

DOI : https://doi.org/10.61096/ijamscr.v12.iss4.2024.506-515

ISSN: 2347-6567

Research

Experimental design approach by using rapid high performance liquid chromatographic method for the determination of telmisartan Assay in immediate release telmisartan tablets 80mg

Kasala Vinay kumar*, K. Vamsi Krishna, Mohammad Omar

Arya college of Pharmacy, Kandi, Sangareddy, Affiliated to Osmania University, Hyderabad, Sangareddy, Telangana 502285

*Author for Correspondence: Kasala Vinay kumar
Email: vinaykasala99@gmail.com

Published on: 23 Nov 2024

Published by:
DrSriram Publications

Abstract

A rapid high performance liquid dosage form. A Genesis ODS MG, 50 X 4.6mm, 5 µm or equivalent in isocratic mode, with mobile phase containing a mixture of 0.01 M potassium dihydrogen phosphate buffer (adjusted to pH 2.4 using orthophosphoric acid): methanol: Acetonitrile in the ration of 60:40 v/v) and 0.01 triethylamine : methanol (80:20%v/V) was used. The mobile phase was pumped at a flow rate of 1.0 ml/min and the eluents were monitored at 298 nm. The selected chromatographic conditions were found to effectively separate telmisartan (Rt: 4.98 min) having a resolution of 5.67. The method was validated in terms of linearity, accuracy, precision, specificity, limit of detection and limit of quantitation. Linearity for telmisartan were found okay respectively. The percentage recoveries for telmisartan ranged from 99.45-100.99%, respectively. The limit of detection and the limit of quantitation for telmisartan was found to be 1.5 µg/ml and 3.0 µg/ml, respectively. The method was found to be robust and can be successfully used to determine the drug content of marketed formulations. The method gives resolution with a short analysis time (< 10 min). The method parameter was validated and establishes to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of telmisartan in medical dosage form.

Keywords: Experimental Design, Rapid-HPLC, Telmisartan Tablets,

INTRODUCTION

Telmisartan is a non-peptide angiotensin II receptor antagonist (ARB) widely used for managing hypertension and reducing cardiovascular risk. It is chemically designated as 4'-((1,4'-dimethyl-2'-propyl(2,6'-bi-1H-benzimidazol)-1'-yl)methyl)-[1,1'-biphenyl]-2-carboxylicacid.Telmisartan's unique molecular structure allows for

high specificity and binding affinity to the angiotensin II type 1 (AT1) receptor, blocking the effects of angiotensin II and thereby reducing blood pressure.

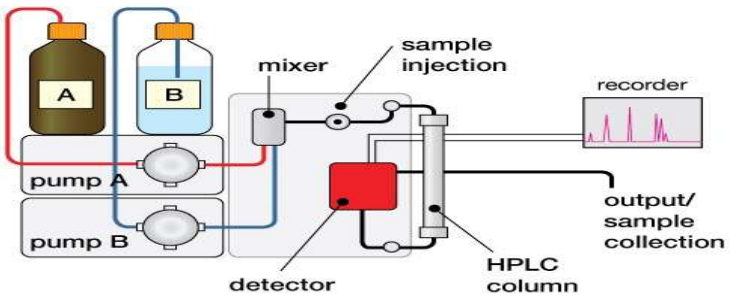
Telmisartan is notable for its high lipophilicity, which contributes to its prolonged half-life and ability to provide effective 24-hour blood pressure control. This characteristic differentiates it from other ARBs and enhances patient adherence to the medication due to its once-daily dosing regimen. The molecular structure of telmisartan includes a benzimidazole ring, which is crucial for its antagonistic activity at the AT1 receptor. This ring structure, combined with other functional groups, allows for strong and selective inhibition of the receptor, which plays a key role in the renin-angiotensin system (RAS) that regulates blood pressure and fluid balance.

Telmisartan's high lipophilicity is due to its extensive hydrocarbon content, which also accounts for its relatively low aqueous solubility. Despite this, the drug is designed to be rapidly absorbed and processed within the human body, leveraging its lipophilic nature to ensure sustained release and activity over a prolonged period. The high binding affinity to AT1 receptors ensures that telmisartan remains effective even at lower concentrations, which is particularly advantageous in managing chronic conditions like hypertension where consistent therapeutic levels need to be maintained.

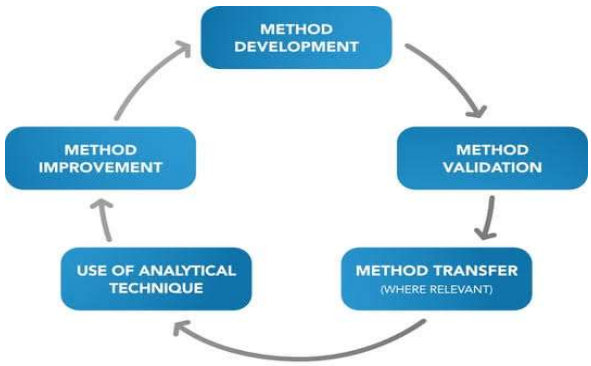
Analytical Methods for Telmisartan Assay

Quantifying telmisartan in pharmaceutical formulations requires precise and reliable analytical methods. Several techniques have been developed, each with its advantages and limitations. These include UV-spectrophotometry, High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and mass spectrometry (MS). Among these, HPLC stands out as the most widely used technique due to its high sensitivity, specificity, and ability to separate complex mixtures.

HPLC method development involves optimizing various parameters, including the choice of stationary phase, mobile phase composition, flow rate, and detection wavelength. The stationary phase is typically a reversed-phase C18 column, which provides good retention and resolution for telmisartan. The mobile phase consists of a mixture of aqueous buffer and organic solvent (e.g., acetonitrile or methanol), which is adjusted to achieve optimal separation and peak resolution. The flow rate must be carefully controlled to balance analysis time and resolution. Detection is commonly performed using UV detection at a wavelength around 296 nm, where telmisartan exhibits strong absorbance.



Method Validation: Method validation is a critical step in analytical method development, ensuring that the method produces reliable and consistent results. Validation involves assessing various parameters, including accuracy, precision, specificity, linearity, robustness, and stability. Each parameter must meet predefined criteria to confirm the method's suitability for its intended purpose.



Validation ensures that the HPLC method for telmisartan assay is reliable, accurate, and reproducible, meeting regulatory requirements and ensuring the quality of the pharmaceutical product. These optimization strategies ensure that the rapid HPLC method provides accurate, precise, and reliable results, meeting the requirements for telmisartan assay in pharmaceutical formulations.

Experimental Design in Analytical Method Development

Principles of Experimental Design

Experimental design is a systematic approach to planning experiments to obtain reliable and unbiased results. Key principles include randomization, replication, and blocking. Randomization reduces bias by randomly assigning experimental units to treatments. Replication involves repeating the experiment to ensure results are consistent. Blocking groups similar experimental units to account for variability.

Experimental design helps to identify and control sources of variability, allowing for more accurate and precise results. By systematically varying the experimental factors, researchers can determine their effects on the response variable and identify optimal conditions for the desired outcome. This approach is particularly valuable in method development and optimization, where multiple factors can influence the performance of the analytical method.

Immediate release tablets

Immediate release (IR) formulations are designed to disintegrate and release their active ingredients quickly after administration. Here are some key points about

immediate release formulations: Rapid Onset of Action: Immediate release formulations are intended to provide a quick onset of therapeutic effect. They are typically used when a fast response is required.

Disintegration and Dissolution: These formulations disintegrate and dissolve rapidly in the gastrointestinal tract, allowing the drug to be absorbed quickly into the bloodstream.

Dosage Forms: Common dosage forms include tablets, capsules, liquids, and oral disintegrating tablets.

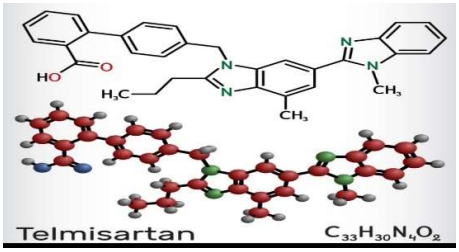
Excipients: They often contain excipients that aid in the rapid disintegration and dissolution of the drug. These include superdisintegrant, solubilizers, and permeation enhancers.

Experimental design approach by using rapid high performance liquid chromatographic method for the determination of Telmisartan assay in immediate release tablet Telmisartan tablets.

Scope of Study: Development and Validation of HPLC method for the estimation of assay in Telmisartan tablets 80 mg, the proposed method shall be used for the quantification of active material Telmisartan.

The proposed method shall be validated for Specificity, System suitability, Linearity, Accuracy, Range, Precision, and Repeatability and Robustness as per ICH guideline.

Drug profile: **Telmisartan**



A white crystalline powder, Molecular weight of 514.6, Melting point of 261 to 263°C, Solubility: The solubility of telmisartan in aqueous solutions is strongly pH-dependent, with maximum solubility observed at high and low pH.

MATERIALS AND METHODS

HPLC grade water, Potassium Di Hydrogen phosphate, Ortho Phosphoric acid, Triethlyamine, Methanol, Acetonitrile, Telmisartan, HPLC Analytical Balance pH Meter, Column, Detector.45 nylon membrane filter.

Specificity and System Suitability
Identification, Blank interference of the Experiment, System Suitability, Linearity and Range, Precision, System Precision Method Precision Accuracy Solution Stability System Stability Solution stability Robustness Change in wave length Filter variability

Methodology

Preparation of Test Sample solution: Weight and powder 20 tablets and transfer about 40 mg of Telmisartan standard into a 100 ml volumetric flask add about 70ml of diluent and sonicate to dissolve and dilute to volume with diluent. Transfer 5 ml of this solution to 50 ml volumetric flask and dilute the volume with diluent and mix.

Procedure: Equilibrate the column for not less than 30 minute with mobile phase at flow rate of 1.0ml/minute. Inject 20 µL of blank solution into the Chromatographic system, record the Chromatogram. Program the data processor to inhibit the integration of peaks due to blank.Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of test sample ample solution into the Chromatographic system, record the Chromatogram and measure the peak response. Inject 20 µL of Reference solution into the Chromatographic system, record the Chromatogram and measure the peak response. (Bracketing standard). Inject Bracketing standard after every 4 samples analysis and/or at the end of the sequence.

Evaluation of System Suitability parameters: The column efficiency as determined for the Telmisartan from standard solution ia not less than 3000 theoretical plates and tailing factor for the same peak is not more than 2.0, The relative standard deviation for Telmisartan peak area obtained from five replicate injections of standard solution is not more 2.0

Specificity and System suitability: Specificity is the ability of the analytical method to distinguish between the analyte(s) and the other components in the sample matrix [13]. In case of an HPLC method, it is assured by complete separation of peak(s) of analyte(s) from other peaks originated from the sample matrix, **The System**

Suitability Testing (SST) is used to verify that an analytical method was suitable for its intended purpose the day the analysis was done. It is an essential parameter to ensure the quality of the method for correct measurements.

System Suitability				
S.No	**Retention Time**	**Peak Area**	**Theoretical Plates**	**Asymmetry**
1.	5.440	2478665	3711.42	1.50
2.	5.419	2390412	3724.88	1.47
3.	5.440	2301567	3716.44	1.45
4.	5.419	2479371	3684.70	1.49
5.	5.419	2388130	3735.40	1.45
6.	5.451	2478665	3395.08	3773.90
7.	5.419	2490412	3395.08	1.45
8.	5.419	2301567	3773.90	1.45
9.	5.451	2499371	3759.19	1.44
10.	5.419	2388130	3691.08	1.44
Mean	5.430	2487629		
SD	0.014	3323.51		
% RSD	0.26	0.14		

Results

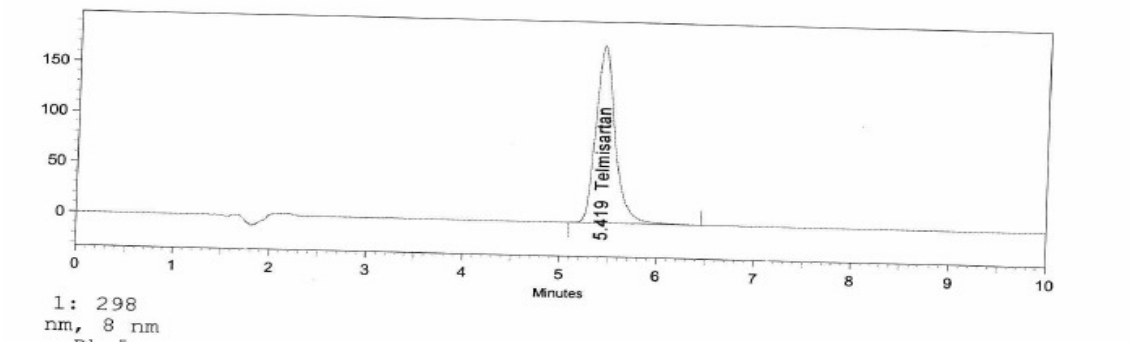
Sr.No	**Validation Parameter**	**Results**		**Acceptance Criteria**
Observed Values:	System Suitability	Theoretical plate	Observed v 3659.20	The column efficiency as determined for the Telmisartan from standard solution is not less than 3000 theoretical plates
		Tailing Factor	1.50	Tailing factor for the same peak is not more than 2.
		Retention time	0.26	The relative standard deviation for Telmisartan peak area obtained from five replicate injections of standard solution is not more 2.0
		Peak Area	0.14	

Sr.No	Validation Parameter	Results	Acceptance Criteria

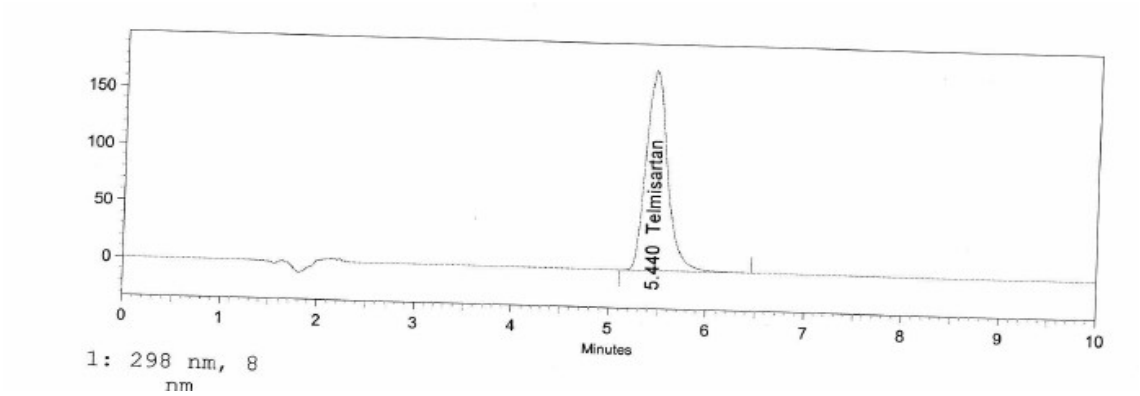
Conclusion :

- The retention time of standard solution and sample solution is comparable with respect to retention time
- There is no any interfering peak in the chromatogram obtained from blank solution and placebo solutic the retention time of analyte peak in the chromatogram obtained with the standard
- The column efficiency as determined for the Telmisartan from standard solution is not less than 3 theoretical plates
- Tailing factor for the same peak is not more than 2.
- The relative standard deviation for Telmisartan peak area obtained from five replicate injections of standard solution is not more 2.0

Sample Injection



Bracketing Standard

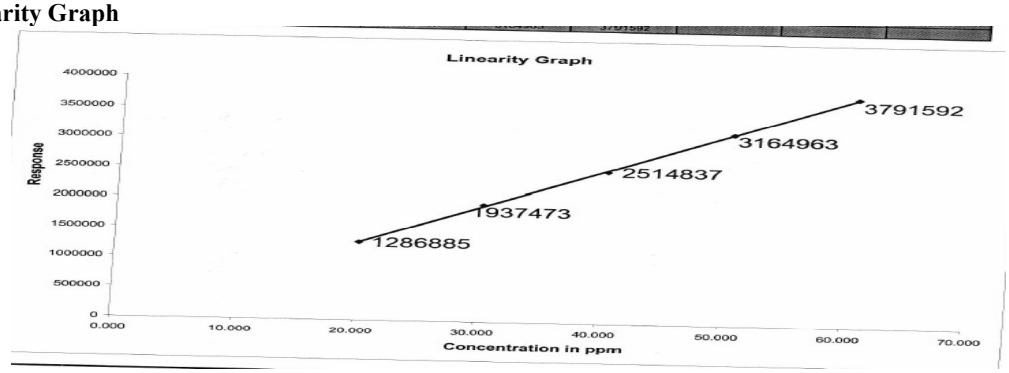


Linearity of a method: is its ability to obtain test results that are directly proportional to the sample concentration over a given range. For HPLC methods, the linear relationship between detector response (peak area and height) and sample concentration is determined. The relationship can be demonstrated directly on drug substance by dilution of standard stock or by separate weighing of the sample components, using the proposed procedures.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	**Method**	Five linearity solutions were prepared by using Telmisartan standard at concentration levels ranging from 50% to 150 % of target concentration of Telmisartan. Measured the peak area response of solution at Level 1 and Level 5 six times and other levels	
2.	**Acceptance crite**		• **Linearity :** • The co-relation is not less than 0.999

Sr.No	Validation Parameter	Results		Acceptance Criteria	
		• The % Y intercept is between -2 % to +2 % • % RSD of peak Reponses of 50 % level and 150% level should be NMT 2.0			
3.	Observed results	Correlation Coefficient	0.99985	Correlation coefficient should be not less than 0.999	
		%y-intercept	1.77	%y-intercept should be ±2.0	
		% RSD at lower level	0.16	% RSD of peak area response of 6 replicates at lower and higher levels should not be more than 2.0	
		% RSD at higher level	0.16		
Linearity level	**Concentration in ppm**	**Area-Average**	**% of RSD**	**Statistic Analysis**	
L1 (50%)	20.20	1286885	0.16	R^2	0.9998
L2 (80%)	30.180	1937473	--	Slope	61997.06
L3 (100%)	40.240	2514837	--	Y Intercept	44388.40
L4 (120%)	50.300	3164963	---	% Y Intercept	1.77
L5 (150%)	60.360	3791592	0.06	Correlation coefficient	0.99999
				Residual sum of squares	19405
Conclusion : Response of Telmisartan is linear overt the concentration range 50% to 150% target concentration					

Linearity Graph



Precision: an analytical method expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility

Sr.No	Validation Parameter	Results		Acceptance Criteria
A.	**System Suitability : Method and Procedure**			
1.	**System Suitabi**	Prepared standard solution as per the test methods and inject five times into the chromatographic system		
	Acceptance criteria	• The column efficiency as determined for the Telmisartan from standard solution ia not less than 3000 theoretical plates. • Tailing factor for the same peak is not more than 2.0 • The relative standard deviation for Telmisartan peak area • obtained from five replicate injections of standard solution is not more 2.0		
2.	**Observed Values**			
System Precision		Theoretical Plates	5546.44	The column efficiency as determined for the Telmisartan

Sr.No	Validation Parameter	Results		Acceptance Criteria
				from standard solution is not less than 3000 theoretical plates.
		Tailing Factors	1.30	Tailing factor for the same peak is not be more than 2.0
		% RSD	0.75	The % RSD of % assay from Five samples should not be more than 2.0

3. **Results :**

	Sr.No	Peak Area	Theoretical factor	Tailing Factor
System Suitability and System Precision	1	2538081	5670.41	1.29
	2	2525212	5546.21	1.28
	3	2501741	5756.52	1.27
	4	2496453	5549.72	1.29
	5	2486532	5459.23	1.28
	Mean	2512355		
	SD	18220.23		
	% RSD	0.73		

Observed Results:

- The observed theoretical plates obtained for the Telmisartan from standard solution is more than 3 theoretical plates.
- The Observed Tailing factor obtained for the Telmisartan peak from the standard solution is less than 2
- The % RSD of the peak area of Telmisartan obtained from five replica injections of the standard soluti 0.73

Conclusion :

The above data shows that the system is precise.

B. **Method Precision : Method and Procedure**

1.	**Methods Preci**	Prepared six sample solution of Telmisartan tablets 80 mg as per the test methods and inject into the chromatographic system
	Acceptance criteria	The % RSD of % assay from six samples should not be more than 2.0

2. Observed Value

Method Precision	% RSD	0.39	The % RSD of % assay from six samples should not be more than 2.0

3. **Results:**

S.No	% Assay
Injection-1	99.4
Injection-2	99.5
Injection-3	99.4
Injection-4	99.7
Injection-5	99.2
Injection-6	98.6
Mean	**99.1**
SD	**0.39**
% RSD	**0.39**

Conclusion :

The above results show that the methods is precise

Accuracy

The accuracy of an analytical method expresses the closeness of agreement between the value accepted either as a conventional true value or an accepted reference value and the value obtained.

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			

Sr.No	Validation Parameter	Results			Acceptance Criteria	
1.	Accuracy was performed by spiking the Telmisartan drugs substance to the placebo at 50%, 100 % and 50% of target concentration of Telmisartan in triplicate at each level and analyzed as per the test method					
	Acceptance Criteria	The % recovery of accuracy levels should be not less than 98.0 and not more than 102				
2. Observed Values						
Accuracy		Mean % recovery		99.8	The % recovery of accuracy levels should be not less than 98.0 and not more than 102.0	
3. **Results**						
Accuracy level	**Accuracy level in mg as Telmisartan**	**Weight of drug added in mg Telmisartan**		**% Recovery**	**Statistical Analysis**	
L1 (50 %)	Sample -1	2.49	20.25	100.2	Mean	99.8
	Sample -2	20.52	20.49	99.9		
	Sample -3	20.64	20.55	99.3		
L1 (100 %)	Sample -1	40.48	40.42	100.0	Mean	99.9
	Sample -2	40.50	40.52	99.8		
	Sample -3	40.53	40.32	99.9		
L1 (150 %)	Sample -1	60.12	59.64	98.9	Mean	99.7
	Sample -2	60.28	60.23	99.4		
	Sample -3	60.15	60.25	100.1		
Overall Statistical Analysis						
Mean	99.8	**SD**		0.30	**% RSD**	0.30
Conclusion : The % recovery of accuracy levels should be not less than 98.0 and not more than 102						

Range

Range of an analytical method is the interval between the upper and lower concentration of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy, and linearity. The range is normally derived from the linearity studies and depends on the intended application of the procedure

Sr.No	Validation Parameter	Results	Acceptance Criteria
Method and Procedure			
1.	Range of analytical method can be obtained from linearity, Precision and accuracy data. Report range in % with respect to sample concentration.		
Observed Values			
2.	Range	The analytical method is linear , Pr and accurate from 50% to 150% of t concentration	----
Conclusion : It was concluded from the linearity, Precision and accuracy data that the analytical method is is linear , Precise and accurate from 50% to 150% of target concentration			

Solution Stability

Stability of the analytical solution and extraction time are other parameters which are also evaluated as additional parameters during robustness study. Stability of analytical solution is determined by assessing the results obtained by subjecting the analytical solution to the method parameters for longer period of time e.g. 4 hrs, 12 hrs, 24 hrs, 48 hrs etc.

Sr.No	Validation Parame	Results	Acceptance Criteria
Method and Procedure			
1.	Standard Solution and Sample Solution was prepared as per test methods and stored at refrigerator condition. Solution Stability was evaluated at initial , 12 hours , 24 hours and 48 hours.		
A.	**Acceptance Criteri** -	The overall % RSA from initial replicate standard peaks and bracketing standards peak should be more than 2.0.	
	-	The % assay difference from initial and corresponding time intervals should be more than 2	

Sr.No	Validation Parame	Results		Acceptance Criteria
2.	**Observed Values**			
	Standard Solution	Standard Solution is stable up t hours at refrigerator condition		The overall % RSA from initial replicate standard peaks and bracketing standards peak should not be more than 2.0.
	Sample Solution	Sample solution is table up to 48 hours at refrigerator condition		The % assay difference from initial and corresponding time intervals should not be more than 2.0
3.	**Results :**			
	Standard Solution :: Over % RSD	Time Interval	Over all % RSD	
		Initial	0.19	
		12 hours	0.23	
		24 hours	0.24	
		48 Hours	0.32	
	Standard Solution :: Over % RSD	**Time Interval**	**% Assay**	**Difference of % Assay**
		Initial	98.8	--
		12 hours	99.2	0.4
		24 hours	99.1	0.3
		48 Hours	99.8	1.0
Conclusion : From the above results it is concluded that standard and sample solutions are stable up to 48 Hrs at Refrigerator				

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. It is partially evaluated during method development stages.

Sr.No	Validation Parameter	Results				Acceptance Criteria
Method and Procedure						
1.	**Filter variability**	Prepared three samples solutions as per the test method. One portion of the solution was centrigued and the other portion of sample solution was filtered through two types of filters Nylon and PVDF and calculated the difference of % assay				
	Acceptance Criteria	The difference of % assay compared from centrifuge to the filtered samples should not be more than				
2.	**Observed Values**					
Filter variability		Maximum difference (Centrifuge Vs Nylon)	0.4			The difference of % assay compared from centrifuge to the filtered samples should not be more than 2.0
		Maximum difference (Centrifuge Vs PVDF)	1.6			
3.	**Results**					
Sr.No	**% Assay**			**Difference**		
	Centrifuge	Nylon	PVDF	Centrifuge Vs Nylon	Centrifuge Vs PVDF	
1	99.5	99.5	99.2	0.1	1.3	
2	99.6	99.5	99.6	0.1	1.0	
3	99.8	99.4	99.2	0.4	1.6	
Conclusion : The Maximum difference Centrifuge Vs Nylon and PVDF membrane filter is 0.4 and 1.6.Hence it is concluded that both Nylon and PVDF filters are suitable for the filtration of the sample solutions.						

CONCLUSION

In the current study the effort has been undertaken to improve most simple, economical, sensitive and correct analytical HPLC method for the immediate valuation of these drugs without their prior separation. The method gives

resolution with a short analysis time (< 10 min). The method parameter was validated and establish to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the planned method can be used for routine analysis of telmisartan in medical dosage form.

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