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Development and Validation of Method for the Estimation of Telmisartan as Active Pharmaceutical Ingredient in Tablet Dosage form and Prepared Spherical Agglomerates by RP-HPLC

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Abstract The Present work was designed to develop and validate an accurate, precise and rapid method for the estimation of Telmisartan as Active Pharmaceutical Ingredient (API) as well as in tablet dosage form and prepared spherical agglomerates by RP-HPLC. The developed method was found to be simple, accurate, precise and sensitive. The separation was achieved on an Isocratic High Pressure Liquid Chromatography (HPLC) (Thermo Scientific) using pumps Jasco PU 2080 Plus, UV detector, column oven (Jasco), and a Reverse Phase C-18 (phenyl) Column (25 cm x 4.6 mm) i.d., particle size 5 µm. The HPLC system was run with flow rate: 0.8 ml/min Injection Volume: 10µl and run time: 10 min, Detector temp: 40 °C. The method was validated for specificity, precision, linearity, and accuracy, robustness, LOD and LOQ parameters. The recovery range was within the range of 99.0–102.0% and the method could be successfully applied for the routine analysis of the drug substance as well as the spherical agglomerates prepared by crystallo co-agglomeration technique.

Keywords: RP HPLC, Method Development, Telmisartan, tablet, Spherical agglomerates, Validation

1. INTRODUCTION

Telmisartan is an angiotensin-II AT_1 receptor antagonist with a binding affinity 3000 times greater for AT_1 than AT_2. It has the longest half-life of any ARB (24

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hours) and the largest volume of distribution. It is used to treat hypertension and prevent associated complications including stroke, heart attack, and renal failure. (15) This drug works by blocking the hormone angiotensin thereby relaxing blood vessels, causing them to (35). This drug may also be used to treat congestive heart failure and to help protect the kidneys from damage due to diabetes Prentice (1993). Telmisartan is used alone or in combination with other medications to treat high blood pressure. In addition to blocking the RAAS, telmisartan acts as a selective modulator of peroxisome proliferator-activated receptor gamma (PPAR-γ), a central regulator of insulin and glucose metabolism. It is believed that Telmisartan's dual mode of action may provide protective benefits against the vascular and renal damage caused by diabetes and cardiovascular disease (CVD) (3).

A good number of analytical methods have been developed for the determination of telmisartan in pure form and in pharmaceutical dosage forms. An UV Spectrophotometric method for the determination of telmisartan in tablet formulation has been already reported by (23) in which the absorbance maximum was found at wavelength 230nm. The developed and validated UV spectrophotometric method by using methanol as a solvent for estimation of telmisartan in API and in pharmaceutical dosage form has been reported by (9). A spectrophotometric dual wavelength method for simultaneous determination of hydrochloride and telmisartan in combined dosage form has been reported by (7). A UV spectrophotometric method for the estimation of telmisartan in bulk and tablet dosage form in which 0.1N NaOH was used as a diluent and the analysis was carried out at 234nm has been reported by (19). Several chromatographic methods were developed and reported for the estimation of telmisartan. A UV spectrophotometry method for estimation of telmisartan in bulk and pharmaceutical dosage forms in which methanol was used as a solvent and the absorbance was measured at 296 nm has been reported by (19). A spectrophotometric method for the determination of telmisartan in tablet dosage forms (18). A multi wavelength analysis for the simultaneous determination of amlodipine besylate and Telmisartan in bulk drug and dosage form by using UV spectrophotometer has been reported by (12). A UV spectrophotometric method for the simultaneous determination of telmisartan and hydrochlorothiazide in a pharmaceutical dosage form has been reported by (8). A simultaneous spectrophotometric method for the estimation of amlodipine besylate and telmisartan in a tablet dosage form has been reported by (29). A method for simultaneous estimation of telmisartan and hydrochlorothiazide using high performance thin layer chromatography (HPTLC) has been reported by (25). A method for the determination of telmisartan by HPTLC method has been reported by (5). A HPTLC method

for the simultaneous determination of telmisartan and amlodipinebesylate has been proposed by (6). A HPTLC method for the simultaneous estimation of telmisartan and ramipril in combined dosage form by (14). A RP-HPLC method was reported by for the determination of telmisartan in solid dosage forms in the concentration range 2-14µg/mL. The observed retention time of telmisartan was about 7 minutes and the theoretical plate count at lower side was about 3345 has been reported by (30).

A stability indicating method for the estimation of telmisartan related substances in tablet formulations which was well established to separate and determine about 7 related substances of telmisartan with the run time of 50 minutes has been reported by (6). A HPLC method for the assay of telmisartan in human plasma has been developed by26). Fluorescent detector was used for the detection of telmisartan and naproxen was used as internal standard in this method. A reverse phase liquid chromatographic method for quantitative estimation of telmisartan in human plasma has been reported by (30). A reverse phased high performance liquid chromatographic method for the estimation of telmisartan in serum samples has been reported by Vijay (10). which involves run time of 10 minutes. The robustness of the method was not demonstrated. An isocratic RP-HPLC method was reported for the assay of telmisartan in pharmaceutical formulation by (10). The run time of the method was 10 minutes. A HPLC method for simultaneous estimation of telmisartan and ramipril in pharmaceutical formulations has been developed by (28). The run time observed as 15 minutes with 1.5mL/minute flow rate and the detection was done at 210 nm. RP-HPLC method for the determination of telmisartan in pure and pharmaceutical formulations has been reported by (27). HPLC method for simultaneous determination of telmisartan and hydrochlorothiazide in tablets with 15 minutes run time has been reported by (11). A spectrophotometric method for simultaneous estimation of amlodipine besylate and telmisartan in tablet dosage form was reported by (20). An UPLC method for estimation of related substances in telmisartan tablet dosage form was reported by (31). RP-HPLC method for estimation of telmisartan in a tablet dosage form has been reported by (13). A RP-HPLC method for simultaneous estimation of cilnidipine and telmisartan in combined tablet dosage form has been reported by (17). A rapid stability indicating simultaneous determination of hydrochlorothiazide, ramipril and telmisartan in combined dosage form by ultra performance liquid chromatography has been reported by (31). Development of UVspectrophotometric method for estimation and validation of telmisartan as a pure API has been reported by (4). UV spectrophotometric method for estimation and validation of telmisartan in bulk and tablet dosage form has been reported by (33).

2. EXPERIMENTAL WORK

2.1 Instrumentation

HPLC with variable wavelength programmable UV-Visible detector and Rheodyne injector was employed for investigation The separation was achieved on a Isocratic HPLC (Thermo Scientific) using pumps Jasco PU 2080 Plus, UV detector, column oven (Jasco), and a Reverse Phase C-18 (phenyl) Column (25 cm x 4.6 mm) i.d., particle size 5 µm). A Sartorius analytical balance was used for weighing the materials.

2.2 Chemicals and Solvents

The reference sample of Telmisartan (API) was obtained from Meridian Medicare, Solan. The Formulation Telma (Telmisartan) was procured from the local market. The novel formulation containing Telmisartan spherical agglomerates was prepared by crystallo co- agglomeration. Preparation technique was selected from total of sixteen formulations prepared by altering the formulae and the spherical agglomerates showing improved micromeritic properties and best dissolution profile was selected for the present study. Methanol and Acetonitrile (Both HPLC grade) were purchased from Merck India Ltd. The orthophosphoric acid (AR grade) was purchased from local market.

2.3 The mobile phase

A mixture of Acetonitrile: Phosphate buffer in the ratio of 90:10 v/v was prepared and used as mobile phase. The pH of this mobile phase was adjusted to 2.4 using orthophosphoric acid

2.4 The Buffer Solution

About 2.72 g of potassium dihydrogen phosphate was diluted to 1000 mL with water containing 2ml triethylamine and sonicated. The pH was adjusted with orthophosphoric acid and then filtered through 0.45µ nylon filter.

2.5 Standard solution of the drug

For analysis 100 ppm standard solution was prepared, required concentrations were obtained from 100 ppm solution by appropriate dilution.

2.6 Sample (tablet) solution

The formulation tablets of Telmisartan (Telma - 20 mg) were crushed to give finely powdered material. From the Powder prepared a 10 µg/ml solution in mobile phase and then filtered through membrane filter paper.

3. METHOD DEVELOPMENT

For developing the method, a systematic study of the effect of various factors was undertaken by varying one parameter at a time and keeping all other conditions constant. Method development consists of selecting the appropriate wavelength and choice of stationary and mobile phases. The following studies were conducted for this purpose.

3.1 Method Optimization

The method optimization was designed using different compositions of Mobile Phases. Variations in the flow rate of the mobile phase were adjusted in such a way so as to achieve a chromatogram with theoretical plates more than 2000 and with tailing factor less than 2. The different trials were conducted to reach at an optimal chromatographical method which can be successfully validated.

3.1.1 Trial No. 1

The analysis was carried out by HPLC with the following chromatographic conditions. Consensus on chromatographic conditions was arrived on the basis of telmisartan solubility and polarity.

Mobile phase: Mixture of phosphate buffer (pH 4.0) and acetonitrile (20: 80 v/v).
Detection : UV Detector
Column : 250mm length, 4.6mm internal diameter, 5µm particle size, Aligent C18
Flow rate : 0.5 mL minute-1
Injection volume : 20 µL
Run time : 20 minutes
Column temperature : Ambient

The absorbance was found satisfactory at 296nm at which the peak response was found on higher side due to injection overload. The back pressure of the column was found significantly low and the retention time was observed as 13 minutes.

3.1.2 Trial No. 2

Based on the observed λmax, the optimum wavelength for the detection was set as 296 nm. The injection volume was reduced to 20µL to optimize the injection load and flow rate was increased to 0.8mL minute-1 to increase the desired back pressure to the column and to reduce the retention time of telmisartan. The chromatogram of trail is depicted below:

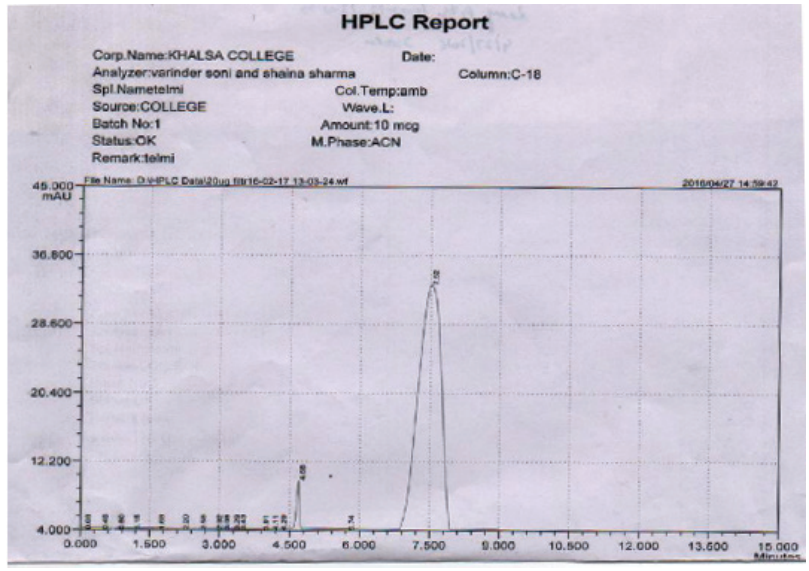

Figure 1: Chromatiogram of Trial 2

Mobile phase: Mixture of pH 4.4 phosphate buffer and acetonitrile (20: 80 v/v).
Detection : 296nm
Column : 250mm length, 4.6mm internal diameter, 5µm particle size, Aligent C18
Flow rate : 0.8 mL minute-1
Injection volume : 10µL
Run time : 15 minutes
Column temperature : Ambient

The peak symmetry of telmisartan and response were found satisfactory. The retention time of telmisartan was found about 7 minutes which need to be reduced.

3.1.3 Trial No. 3

To reduce the retention time of telmisartan, the mobile phase was optimized by increasing the acetonitrile composition in the mobile phase.

The chromatogram for the standard was recorded under the above mentioned conditions. The peak symmetry was found good with the retention time of telmisartan at about 5.37 minutes.

Mobile phase: Mixture of pH 4.0 phosphate buffer and acetonitrile (10 : 90 v/v).
Detection : 296nm
Column : 250mm length, 4.6mm internal diameter, 5µm particle size, Aligent C18
Flow rate : 0.8 mL minute-1
Injection volume : 10µL
Run time : 08 minutes
Column temperature : Ambient

3.2 Detection wavelength

The spectrum of 10µg/ml solution of the Telmisartan in methanol was recorded separately on UV spectrophotometer. The peak of maximum absorbance wavelength was observed. The spectra of Telmisartan were showed maximum absorbance at 296nm.

3.3 Choice of stationary phase

Preliminary development trials have performed with octadecyl columns with different types, configurations and from different manufacturers. Finally the expected separation and peak shapes were obtained on Reverse Phase C-18 (phenyl) Column (25 cm x 4.6 mm) i.d., particle size 5 µm.

3.4 Selection of the mobile phase

In order to get sharp peak, low tailing factor and base line separation of the separation of the components, a number of experiments were carried out by varying the composition of various solvents and flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and Acetonitrile with or without different buffers indifferent combinations were tested as mobile phases using C18 column. A mixture of Acetonitrile: phosphate buffer in the ratio of 90:10 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was better defined and resolved and almost free from tailing.

3.5 Flow rate

Flow rates of the mobile phase were changed from 0.5 – 1.0 mL/min for optimum separation. A minimum flow rate as well as minimum run time gives the maximum saving on the usage of solvents. It was found from the experiments that 0.8 mL/min flow rate was ideal for the successful elution of the analyte.

4. VALIDATION OF THE PROPOSED METHOD

The proposed method was validated as per ICH guidelines. The parameters studied for validation were specificity, linearity, precision, accuracy, robustness, system suitability, limit of detection, limit of quantification, and solution stability (24).

4.1 Specificity

The specificity of method was performed by comparing the chromatograms of blank, standard and sample (Prepared from Formulation)and the spherical agglomerates. It was found that there is no interference due to excipients in the tablet formulation and also found good correlation between the retention times of standard and sample. The specificity results are shown in Table 2.

Table 2: Specificity study.

Name of the Sample	Retention Time in Min
Blank	NO PEAKS
Telmisartan	5.36

4.2 Linearity

Table 1: Optimized chromatographic conditions for estimation of Telmisartan.

Mobile phase	ACN: phosphate buffer 90:10 w/v
Pump mode	Isocratic
Mobile phase P^{H}	2.4
Diluent	Mobile phase
Column	C-18 (phenyl) Column (25 cm x 4.6 mm) i.d. 5 µm.
Column Temp	Ambient
Wavelength	296 nm
Injection Volume	10 µl
Flow rate	0.8 mL/min
Run time	10 min
Retention Time	5.36 min

Linearity was performed by preparing mixed standard solutions of Telmisartan at different concentration levels including working concentration mentioned in experimental condition i.e. 10µg/ml. Twenty micro liters of each concentration was injected in duplicate into the HPLC system. The response was read at 296 nm and the corresponding chromatograms were recorded. From these chromatograms, the mean peak areas were calculated and linearity plots of concentration over the mean peak areas were constructed individually. The regressions of the plots were computed by least square regression method. Linearity results were presented in Table 3.

Table 3: Linearity results.

S.No	Concentration of Telmisartan in µg/ml	Mean peak area
1	5	204108
2	10	407910
3	15	601120
4	20	814714
5	25	1018811
6	30	1222919
	Slope	40724
	Intercept	-2438
	Correlation coefficient	0.999
	Range: 5-30 µg/ml	

4.3 Precision

Precision is the degree of repeatability of an analytical method under normal operational conditions. The method was performed to evaluate intraday precision as well as inter day precision.

4.3.1 Intraday precision

To study the intraday precision, six replicate standard solutions (10µg/ml) of Telmisartan were injected. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.0731, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 4.

Table 4: System Precision (Intra Day).

SAMPLE	CONC (µg/ml)	INJECTION No.	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
		1	404720.2	
		2	404364.1	
Telmisartan	10	3	404981.5	0.073
		4	404732.8	
		5	404257.1	
		6	404591.5	

4.3.2 Inter Day precision

To study the interday precision, six replicate standard solution of Telmisartan was injected on third day of sample preparation. The percent relative standard deviation (% RSD) was calculated and it was found to be 0.105, which are well within the acceptable criteria of not more than 2.0. Results of system precision studies are shown in Table 5.

4.4 Accuracy

The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of the standard drug. The standard addition method was performed at 80%, 100% and 120% level of 10ppm. The solutions were analyzed in triplicate at each level as per the proposed method. The percent recovery and % RSD was calculated and results are presented in Table 6. Satisfactory recoveries ranging from 99.0 to 101.0 were obtained by the proposed method. This indicated that the proposed method was accurate.

4.5 Robustness

The robustness study was performed by slight modification in flow rate of Mobile phase, pH of the buffer and composition of the mobile phase. Telmisartan at 6 ppm concentration was analyzed under these changed experimental conditions. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature. The results of robustness study are shown in Table 7.

Table 5: System Precision (Inter Day)**.**

SAMPLE	CONC (µg/ml)	INJECTION No.	PEAKS AREA	R.S.D (Acceptance criteria ≤ 2.0%)
Telmisartan	10	1	403922.0	0.105
		2	404327.3	
		3	404457.0	
		4	403998.4	
		5	404987.8	
		6	404295.5	

Table 6: Percentage Recovery and % RSD.

Level	Amount of Telmisartan	Amount of Telmisartan	% Recovery	%RSD
80 %	6	5.97	99.5	0.118
	6	5.96	99.33	
	6	5.95	99.16	
100%	8	7.96	99.5	0.177
	8	7.98	99.75	
	8	7.97	99.62	
120%	10	9.97	99.7	0.498
	10	9.90	99.0	
	10	9.94	99.4	
			Mean % of recovery 99.44	Mean RSD =0.461

4.6 System suitability

System suitability was studied under each validation parameters by injecting six replicates of the standard solution 5 ppm). The results obtained were within acceptable limits (Tailing factor ≤2 and Theoretical plate's ≥2000) and are represented in Table 8. Thus, the system met the suitable criteria.

Table 7: Robustness.

Condition		Mean area	% assay	% difference
Unaltered		407910	100.0	0.0
Flow rate at 0.6 mL/min		403985	99.03	0.97
Flow rate at 1.0 mL/min		404398	99.13	0.87
Mobile phase:				
ACN : Buffer				
70%	30%	403587	98.9	0.10
80%	20 %	404405	99.15	0.85
pH of	mobile phase at 2.0	404698	99.25	0.75
pH of	mobile phase at 3.0	408724	100.19	0.19

Table 8: System Suitability.

Parameter	Tailing factor	Theoretical plates
Specificity study	1.71	5680
Linearity study	1.20	6694
Precision study	1.71	6705

4.7 Limit of Detection and Limit of Quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest Concentration that can be quantified reliably with a specified level of accuracy and Precision. For this sample was dissolved by using Mobile Phase and injected until peak was disappeared. After 0.86 ng/ml dilution, Peak was not clearly observed. So it confirms that 15 ng is limit of Detection and 2.62 ng dilutions is Limit of Quantification. For this study six replicates of the analyte at lowest concentration were Measured and quantified. The LOD and LOQ of Telmisartan are given in Table 9.

Table 9 : LOD and LOQ.

Parameter	Measured Value ng/mL
Limit of Detection	0.72
Limit of Quantification	2.02

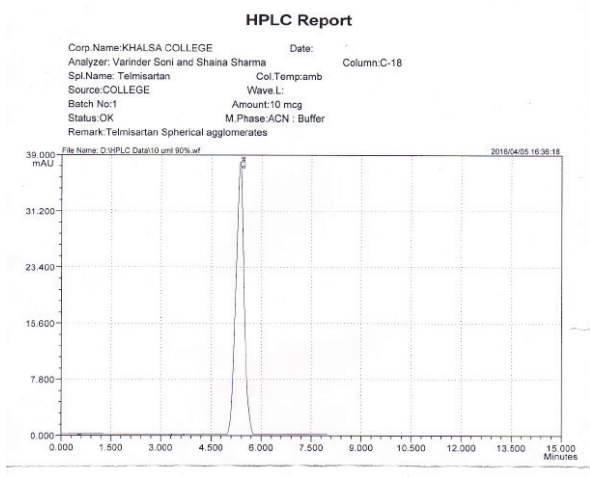

Figure 2: HPLC Chromatogram of Optimized Method.

5. ASSAY OF MARKETED TABLET FORMULATION

The proposed method was applied to the assay of commercial tablets containing Telmisartan. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. After analysis test result assay of Telmisartan in Tablet was found to be 19.27% which was very close to the labeled amount.

6. ASSAY OF PREPARED SPHERICAL AGGLOMERATES

The proposed method was applied to the assay of tablets containing Telmisartan spherical agglomerates.. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. After analysis test result assay of Telmisartan in spherical agglomerates was 18.52 and is very close to the labeled amount

DISCUSSION

To optimize the RP-HPLC parameters, several mobile phase compositions were evaluated. A satisfactory separation and good peak symmetry was found in a mixture of Acetonitrile: Buffer in the ratio of 90:10 w/v and 0.8 mL/ min flow rate proved to be better combination than the other combinations in terms of resolution and peak shape. The optimum wavelength for detection was set at 296 nm at which much better detector responses for drug were obtained. As is evident from Figure 2 the retention times were 5.36 min for Telmisartan. The number of theoretical plates was found to be 5680, which

indicates efficient performance of the column. A system suitability test was applied to representative chromatograms for various parameters. The results obtained were within acceptable limits and are represented in Table 8. Thus, the system meets suitable criteria. The calibration curve was obtained for a series of concentration in the range of 5-30µg/ml and it was found to be linear. Seven points graphs was constructed covering a concentration range 5-30µg/ml. The standard deviation of the slope and intercept were low. The data of regression analysis of the calibration curves are shown in Table. Calibration curve found to be linear with r^2=0.999, Intercept (-2438) and Slope (40802) respectively. The results obtained were within acceptable limits where capacity factor >2.0, tailing factor $\leq$2.0 and theoretical plates >2000. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%.

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Low values of standard deviation denoted very good repeatability of the measurement. Thus it was showing that the equipment used for the study was correct and hence the developed analytical method is highly repetitive. RSD of intraday precision was found to 0.0731. For the interday precision a study carried out on consecutive days indicated a RSD of 0.105. This indicates good method precision.

Standard addition method at 80%, 100% and 120% to the proposed HPLC method is carried out to find the Accuracy of the Telmisartan. The results showed good recoveries ranging from 99.00 to 101 %.

The proposed method has been applied to the assay of commercial tablets containing Telmisartan. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. The results (19.27%) presented good agreement with the labeled content. Low values of standard deviation denoted very good repeatability of the measurement.

The proposed method has been applied to the assay of tablets containing Telmisartan spherical agglomerates.. Sample was analyzed for five times after extracting the drug as mentioned in assay sample preparation of the experimental section. After analysis test result assay of Telmisartan in Tablet formulation is 18.52% and is very close to the labeled amount.

The statistical evaluation of the proposed method was revealed its good linearity, reproducibility and its validation for different parameters and let us to the conclusion that it could be used for the rapid and reliable determination of Telmisartan in tablet Marketed formulation and tablets prepared using the spherical agglomerates. All these factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive and rapid and can be applied successfully for the estimation of Telmisartan in bulk and in

pharmaceutical formulations without interference and with good sensitivity. The proposed method has opened doors to develop a bioanalytical method to detect the amount of drug using in-vivo methods. The C_{max} and T_{max} can then be calculated to determine the bioavailability of Telmisartan agglomerates prepared by CCA method using polymers.

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