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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF BENIDIPINE HYDROCHLORIDE AND TELMISARTAN IN TABLET

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ABSTRACT

A specific, precise and accurate RP-HPLC method was developed for the simultaneous estimation of Benidipine hydrochloride and Telmisartan in tablet. The separation was carried out using Inertsil ODS $_{C18}$ column (150 x 4.6 mm, 5 µm), mixture of 0.05M Potassium Dihydrogen Phosphate Buffer (pH - 4.5 adjusted with 1% OPA) and Acetonitrile (40: 60% v/v) as a mobile phase. The flow rate was adjusted to 1 mL/min and effluent was monitored at 267 nm by used PDA detector. The retention times of Benidipine hydrochloride and Telmisartan were found to be 2.977 and 5.167 respectively. The different analytical parameters such as specificity, linearity, LOD, LOQ, precision, accuracy, robustness were determined according to ICH guidelines. The method was linear over the range 2 - 6 µg/mL and 20 - 60 µg/mL for Benidipine hydrochloride and Telmisartan. The % recoveries of Benidipine hydrochloride and Telmisartan were found to be 100.46% - 101.17% and 100.20% - 100.38% respectively. The method was found to be specific, precise and accurate and robust during study. The proposed method enables rapid quantification and simultaneous analysis of drug from commercial formulation without any interference of excipient. So, the method can be used for routine analysis of Benidipine hydrochloride and Telmisartan in tablet.

KEYWORDS: Benidipine hydrochloride, Telmisartan, RP-HPLC, Validation.

INTRODUCTION

Benidipine hydrochloride is chemically designated as 5-O-[(3R)-1-benzylpiperidin-3-yl] 3-O-methyl (4R)-2, 6-dimethyl-4-(3-nitrophenyl)-1, 4-dihydropyridine-3, 5-dicarboxylate; hydrochloride. It is not official in any pharmacopeia. It belongs to class of calcium channel blocker which is used as antihypertensive agent. It has an affinity toward dihydropyridine binding site in calcium channel.[1] So, inhibit calcium current which result minimum tachycardia or palpitation. Telmisartan is chemically designated as 2-[4-[[4-methyl-6- (1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]methyl]phenyl] benzoic acid.[2] It is official in IP and BP.[3-4] It belong Angiotensin II Type 1 Receptor Blocker and used as antihypertensive agent. As angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of aldosterone, blockage of its effects results in decreases in systemic vascular resistance.[2]

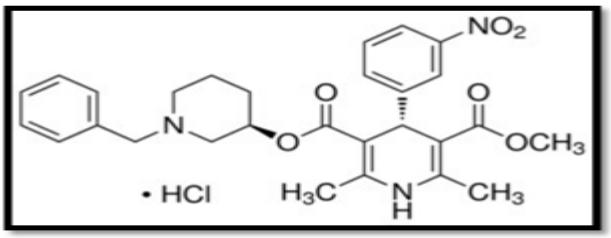

Figure 1: Chemical structure of Benidipine hydrochloride.

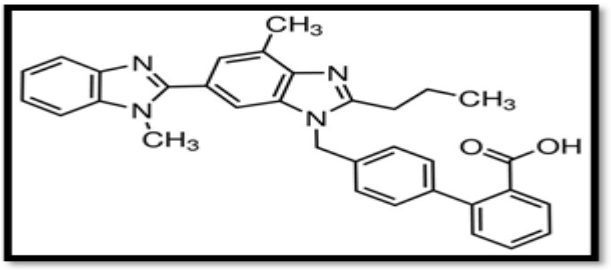

Figure 2: Chemical structure of Telmisartan.

Literature review reveals that there are various reported methods (such as RP-LC, LC-MS-MS and colorimetric)[5-7] are available for the analysis of Benidipine hydrochloride alone and with other combination. There are many officials and reported method (such as UV spectrophotometric, HPLC and HPTLC)[8-11] are available for the analysis of Telmisartan alone and with other combination. But literature survey does not reveal simultaneous estimation of Benidipine hydrochloride and Telmisartan in combination. So, Aim of present work is to develop accurate, precise, sensitive and rapid RP-HPLC method for estimation of Benidipine hydrochloride and Telmisartan in tablet.

MATERIALS AND METHODS

Instruments and Apparatus

Young lin HPLC system was used for method development and validation. Data acquisition was performed on YL 9100 HPLC with PDA detector (YL-Clarity software). The separation was achieved on Inertsil ODS C_{18} column (150 × 4.6 mm, 5 µm) column. Digital balance (Sartorius CP224S, Sensitivity: 0.1 mg), Ultrasonic cleaner (PCi, 1.5L, 5H), pH meter (Systronics) and Pipettes and volumetric flask (Borosilicate) used during study.

Reagents and chemicals

Benidipine hydrochloride and Telmisartan were gifted by Advance Analytical Research and Training Institute. The commercial fixed dose combination product contained 4 mg Benidipine Hydrochloride and 40 mg Telmisartan was procured from the local market. HPLC Grade Acetonitrile, HPLC Grade Methanol, HPLC Grade Water, AR Grade Potassium dihydrogen phosphate and AR Grade Ortho-Phosphoric acid were procured from Finar chemicals ltd, India.

Chromatographic Condition

1. Stationary phase (Column) : Inertsil ODS C_{18} column (150 x 4.6mm, 5µm)
2. Mobile phase : 0.05M KH_2PO_4 Buffer (pH 4.5): Acetonitrile (40:60)
3. Detection Wavelength : 267 nm
4. Injection volume : 20 µL
5. Flow rate : 1.0 ml/min
6. Temperature : Room Temperature
7. Run time : 10 minutes

Preparation of Buffer solution

An accurately weighd 6.80 gm of Potassium dihydrogen phosphate was transferred into 1000 ml Beaker, volume was made up to mark with HPLC grade water then pH 4.5 was adjusted with diluted Ortho phosphoric acid (1%). Above solution filtered with vacuum filter using filter membrane (filter disc) (0.45µm).

Preparation of Mobile Phase (Buffer: Acetonitrile, 40:60 % v/v)

An accurately measured above prepared 400 mL of 0.05M phosphate buffer (KH_2PO_4) pH 4.5 and 600 mL of Acetonitrile. Mixed thoroughly and degassed by sonication.

Preparation of Diluent: Mobile phase was used as diluent.

Preparation of Standard Solution

Preparation of Benidipine hydrochloride standard stock solution (40 µg/mL): An accurately weighed and transferred 4 mg of Benidipine hydrochloride into 100 mL volumetric flask. Added few mL of diluent and shake to dissolved and then diluted up to the mark with diluent and mixed thoroughly.

Preparation of Telmisartan standard stock solution (400µg/mL): An accurately weighed and transferred 40 mg of Telmisartan into 100 mL volumetric flask. Added few of diluent and shake to dissolved and then diluted up to the mark with diluent and mixed thoroughly.

Working standard solution of Benidipine hydrochloride and Telmisartan (4:40 µg/mL): A 1 mL (standard stock solution of Benidipine hydrochloride 40 µg/mL) and 1 mL of (standard stock solution of Telmisartan 400 µg/mL) were pipetted out and transferred in to 10 mL volumetric flask, volume was made up to the mark with diluent and mixed thoroughly.

Preparation of Sample Solution

Preparation of sample stock Benidipine hydrochloride and Telmisartan (40:400 µg/mL): Twenty tablets were weighed and their average weight was determined then tablets were crushed into fine powder. Powder equivalent to 4 mg of Benidipine Hydrochloride and 40 mg of Telmisartan was accurately weighed and transferred to a 100 mL volumetric flask then added 60 mL diluent and Shake for 15 min and made up volume up to the mark with diluent and mixed well then sonicate. After sonication solution was filtered through Whatman filter paper No. 42. Then 1 mL (sample stock solution) was pipetted out and transferred to a 10 mL volumetric flask and made up volume up to the mark with diluent to get concentration was 4:40 µg/mL.

Method validation[12]

Specificity

Specificity of method can be termed as absence of any interference at retention times of samples. Specificity was performed by injecting blank and standard preparations. Chromatograms were recorded and retention times from sample and standard preparations were compared for identification of analytes.

Linearity and Range

A series of standard solutions 2-6 µg/mL of Benidipine hydrochloride and 20-60 µg/mL of Telmisartan were prepared. An aliquot of 20 µl of each solution was injected 3 times for each standard solutions and peak area was observed. Plot of average peak area versus the concentration is plotted and from this the correlation coefficient and regression equation were generated. The calibration data of Benidipine hydrochloride and Telmisartan is given in Table 2, while Figure 4 represent overlain graph and 5 and 6 represent linearity graphs of both drugs respectively.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations as per International Conference on Harmonization (ICH) guidelines.

$$LOD = 3.3 \times \sigma/S \quad LOQ = 10 \times \sigma/S$$

Where σ = the standard deviation of the response and S = Slope of calibration curve

Method precision

The method was validated in terms of intra-day inter-day precision. The solution containing 4µg/mL of Benidipine hydrochloride and 40 µg/mL of Telmisartan was injected six times for repeatability study. Inter-day and intra-day study was performed by injecting 2, 4 and 6 µg/mL of Benidipine hydrochloride and 20, 40 and 60 µg/mL of Telmisartan solutions three times for each aliquots. The %RSD for precision study was found less than 2% as shown in Table 4.

Acuracy (% Recovery)

Accuracy was determined by calculating recovery of Benidipine hydrochloride and Telmisartan by the standard addition method. Known amounts of standard solutions of Benidipine hydrochloride (1, 2 and 3 µg/mL) and Telmisartan (10, 20 and 30 µg/mL) were added to a pre quantified test solutions of Benidipine hydrochloride (2µg/mL) and Telmisartan (20µg/mL). Each solution was injected in triplicate and the recovery was calculated by measuring peak areas. Results are shown in Table 5.

Robustness

The robustness study was performed to evaluate the influence of small but deliberate variation in the chromatographic condition. The robustness was checked by making two small

changes. Robustness of the method was studied by changing pH by ± 0.1, flow rate ± 0.02 mL/minutes and mobile phase composition ± 2 ml. After each changes sample solution was injected and system suitabilityparameters were observed. The results were shown in Table 6.

Analysis Benidipine Hydrochloride and Telmisartan in combined dosage forms

Pharmaceutical formulation of Benidipine hydrochloride and Telmisartan in combined dosage form was purchased from local pharmacy. The response of combined dosage form was measured at 267 nm for quantification of Benidipine hydrochloride and Telmisartan by using RP-HPLC. The amounts of Benidipine hydrochloride and Telmisartan present in sample solution were determined by the responses into the regression equation for Benidipine hydrochloride and Telmisartan in the method. Results are given in Table 7.

RESULT AND DISCUSION

System suitability study

To optimize the RP-HPLC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was found in a mixture of of 0.05M Potassium Dihydrogen Phosphate Buffer (pH-4.5 adjusted with 1% OPA) and Acetonitrile (40: 60% v/v) and 1.0 ml/min flow rate proved to be better than the other mixtures in terms of resolution and peak shape. The effluent was monitored at 267 nm using PDA detector. The retention times of Benidipine hydrochloride and Telmisartan were found to be 2.977 and 5.167 respectively. A chromatogram of the mixture in optimized conditions is shown Figure 3 and the system suitability parameters are shown in Table 1.

Table 1: System suitability parameter.

Parameter	Benidipine hydrochloride (n=3)	Telmisartan (n=3)
Retention time (R_t) (min)	2.977 ± 0.005	5.167 ± 0.007
Resolution (R) s	4.24 ± 0.05	
Theoretical plates (N)	3492 ± 15.33	5451 ± 46.60
Tailing factor (T) f	1.15 ± 0.01	1.12 ± 0.02

Table 2: Linearity Range study for Benidipine hydrochloride andTelmisartan

Benidipine hydrochloride			Telmisartan		
Conc. (µg/mL)	Area Mean ±SD (n=3)	% RSD	Conc. (µg/mL)	Area Mean ± SD (n=3)	% RSD
2	493.55±0.89	0.181	20	2978.30± 9.63	0.324
3	722.81±3.65	0.505	30	4365.89±29.87	0.684
4	961.26±4.23	0.441	40	5800.75±17.44	0.301
5	1227.17±6.27	0.512	50	7383.98± 54.39	0.737
6	1461.88±6.55	0.448	60	8800.64± 27.80	0.316

Table 3: Result of LOD and LOQ.

Sr. No.	Drug	LOD	LOQ
1	Benidipine Hydrochloride	0.147	0.444
2	Telmisartan	1.306	3.956

Table 4: Precision study for Benidipine hydrochloride andTelmisartan.

Parameters	Benidipine hydrochloride			Telmisartan		
	Conc. (µg/mL)	Mean area±SD	%RSD	Conc. (µg/mL)	Mean area±SD	%RSD
Repeatability	4	971.812±4.35	0.44	40	5856.325±32.45	0.55
Intraday	2	487.82 ± 2.69	0.55	20	2948.44 ± 19.23	0.65
	4	978.50 ± 2.51	0.25	40	5908.07 ± 29.16	0.49
	6	1467.64 ± 4.64	0.31	60	8859.85 ± 44.46	0.50
Interday	2	478.96 ± 3.81	0.79	20	2889.18 ± 22.49	0.77
	4	978.00 ± 2.96	0.30	40	5890.37± 19.00	0.32
	6	1449.53 ± 11.22	0.77	60	8721.86 ± 79.95	0.91

Table 5: Accuracy study for Benidipine hydrochloride and Telmisartan.

Drug	Conc. level (%)	Sample amount (µg/mL)	Standar d amount added (µg/mL)	Total amount found (µg/mL)	Total amount recovered (µg/mL) (n=3)	% Recovery ±SD (n=3)
Benidipine hydrochloride	50	2	1	3	3.014±0.04	100.46±1.38
	100	2	2	4	4.012±0.02	100.33±0.65
	150	2	3	5	5.058±0.03	101.17±0.69
Telmisartan	50	20	10	30	30.061±0.46	100.20±1.55
	100	20	20	40	39.781±0.35	99.45±0.88
	150	20	30	50	50.194±0.25	100.38±0.50

Table 6: Robustness study for Benidipine hydrochloride and Telmisartan.

Parameters	Change level	Area (n=3)	
		Benidipine hydrochloride	Telmisartan
pH (±0.1)	4.4	971.56	5868.59
	4.5	969.75	5847.22
	4.6	981.96	5906.78
	Mean ± SD	974.42±6.58	5874.19±30.17
	% RSD	0.67	0.513
Flow Rate (±0.02 mL/min)	0.98 mL/min	992.30	5912.25
	1.0 mL/min	969.75	5847.22
	1.02 mL/min	971.34	5736.34
	Mean ± SD	977.31±12.58	5831.93±88.94
	% RSD	1.28	1.52
Mobile Phase Composition Buffer: ACN (± 2 mL)	38:62	983.50	5943.93
	40:60	969.75	5847.22
	42:58	958.28	5777.53
	Mean ± SD	970.51±12.62	5856.22±83.56
	% RSD	1.30	1.42

Table 7: Analysis of Formulation of Benidipine hydrochloride and Telmisartan by Proposed Method.

Benidipine hydrochloride			Telmisartan		
Labelled Amount (mg)	Amount Found (mg)	% Assay	Labelled Amount (mg)	Amount Found (mg)	% Assay
4 mg	3.99	99.99	40 mg	39.97	99.93
	3.92	98.17		39.54	98.87
	3.96	99.16		39.62	99.05
Mean ± SD	3.95±0.035	99.11±0.91	Mean ± SD	39.71±0.22	99.28±0.56
% RSD	0.88	0.91	% RSD	0.57	0.56

Figures

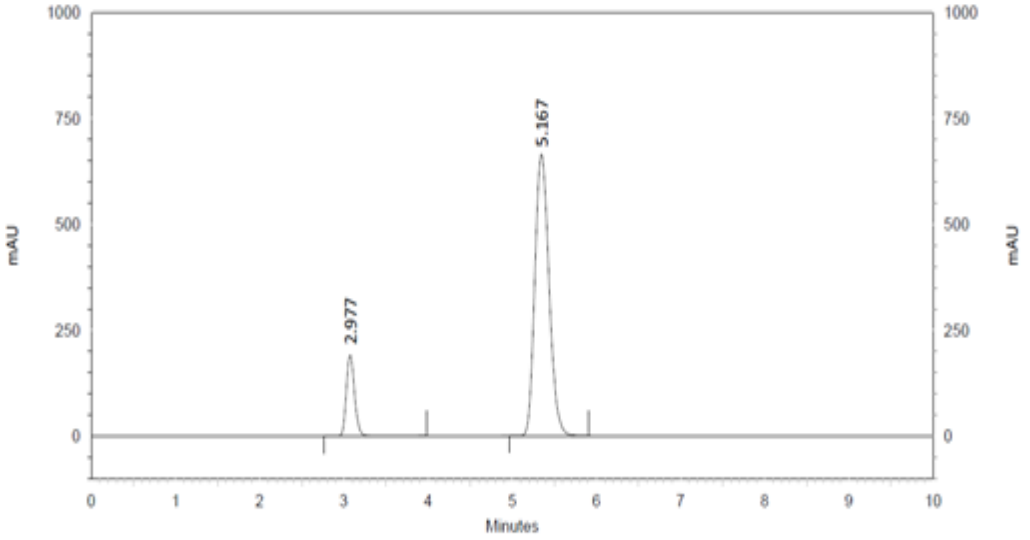

Figure 3: Optimized chromatogram of Benidipine hydrochloride and Telmisartan.

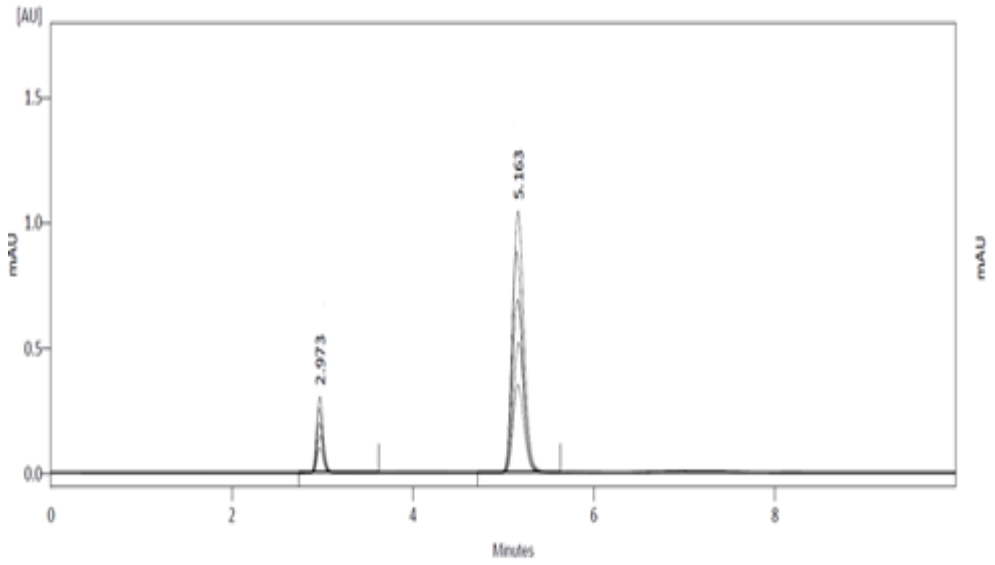

Figure 4: Overlain chromatogram of linearity study.

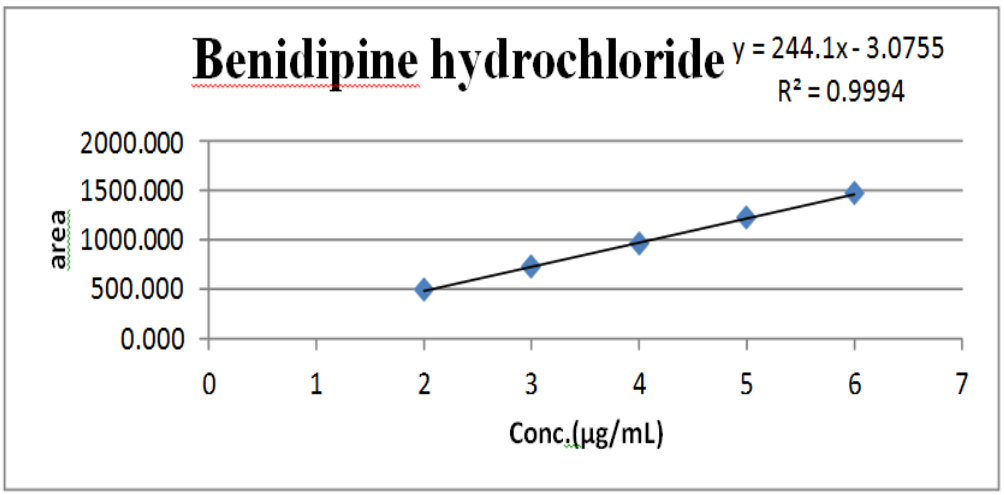

Figure 5: Calibration Curve of Benidipine hydrochloride.

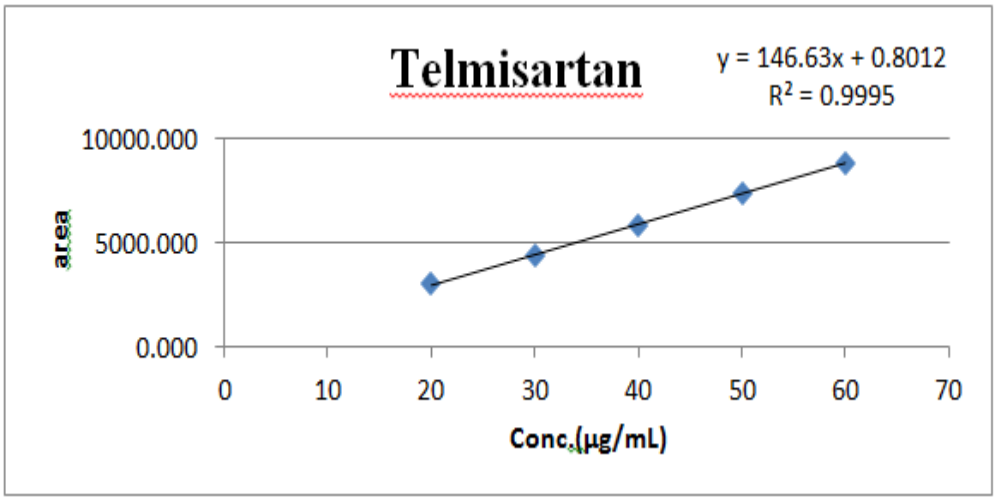

Figure 6: Calibration Curve of Telmisartan.

DISCUSSION

System suitability study

System suitability sown in Table 1.

Specificity

The method was found to be specific as there was no interference observed in any of the parameters under observation.

Linearity and Range

The linearity of Benidipine hydrochloride and Telmisartan were found between 2-6 µg/mL and 20-60 µg/mL respectively. The results are shown in Table 2.

LOD and LOQ

The LOD was found to be 0.147 µg/mL for Benidipine hydrochloride and 1.306 µg/mL for Telmisartan, while the LOQ was found to be 0.444 µg/mL for Benidipine Hydrochloride and 3.956 µg/mL for Telmisartan shown in Table 3.

Precision

The % RSD for repeatability study for Benidipine hydrochloride and Telmisartan was found to be 0.44 and 0.55 respectively. The Inter-day and Intra-day study also show % RSD value for Benidipine hydrochloride and Telmisartan within the acceptable limit. Results for precision study are shown in Table 4.

Accuracy

Accuracy of the method was confirmed by recovery study at three levels (50%, 100% and 150%) of standard addition. Percentage recovery for Benidipine hydrochloride was found to be 100.46%-101.17%, while for Telmisartan it was found to be 100.20%-100.38% as shown in Table 5.

Robustness

The typical variations studied under this parameter were pH, flow rate and mobile phase composition. Overall % RSD was found to be less than 2% for all the variations which indicates that the proposed method is robust. Robustness data are shown in Table 6.

Analysis of sample tablets by proposed method

Applicability of the proposed method was tested by analyzing the commercially available marketed formulation. The percentage of Benidipine hydrochloride and Telmisartan were found to be 99.11% and 99.28% respectively. Results as % Assay are shown in Table 7.

CONCLUSION

A RP-HPLC method has been developed for simultaneous estimation of Benidipine hydrochloride and Telmisartan in tablet using PDA detector. The developed method was validated as per ICH guideline and specificity, linearity and range, precision, accuracy and robustness was performed. The result of study reveals that, a developed and validated method was found to be specific, precise, accurate, rapid, economical and robust, as it separated components with good chromatographic criteria. Method has short run time and components were well separated. All results were found satisfactory. So, this newly developed method

can be suitable for the routine analysis of combined dosage forms.

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