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Research Article

STABILITY INDICATING REVERSE-PHASE HIGH- PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF TELMISARTAN AND BENIDIPINE HYDROCHLORIDE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: Development and validation of stability indicating reverse-phase high- performance liquid chromatography (RP-HPLC) method for simultaneous estimation of telmisartan (TEL) and benidipine hydrochloride (BND) in pharmaceutical dosage form.

Methods: Reverse phase chromatography was selected because of its suggested use for ionic and moderate to non-polar compounds. Reverse phase chromatography is simple, suitable, better regarding efficiency, stability, and reproducibility. C_{18} column, a 250×4.6 mm column of 5.0 µm particle packing, was selected for separation of TEL and BND. Different solvent systems were tried and optimized in combinations as mobile phase. TEL (40 µg/ml) and BND (4 µg/ml) in buffer, pH 4.0: Methanol (50:50) was developed as it was showing good peak shapes and a significant amount of resolution. The mobile phase was flowed at 1.0 ml/min with detection of both the analytes at 210 nm using photodiode array detector.

Result: Development of method was done, and validation was accomplished using specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantitation. The method was found linear from 20 to 60 µg/ml and 2–6 µg/ml for TEL and BND individually. The percentage recoveries of TEL 100.46% and BND100.08% were, respectively.

Conclusion: This stability indicating RP-HPLC methods were developed by degradation of sample and compared with standard. The percentage relative standard deviation was also <2 % showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of benidipine HCl and TEL in combined dosage form and quality control in bulk manufacturing.

Keywords: Telmisartan, Benidipine, Reverse-phase high- performance liquid chromatography, Stability indicating method.

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INTRODUCTION

Telmisartan (TEL) IUPAC name 2-(4-{[4-methyl-6-(1-methyl-1H-1,3-benzodiazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methyl}phenyl) benzoic acid show Fig. 1. TEL is a diabetes angiotensin receptor blocker that shows high affinity for the angiotensin II type 1 (AT1) receptors, has a long duration of action, and has the longest half-life of any angiotensin II receptor blocker (ARB). In clinical studies, TEL shows comparable antihypertensive activity to other major antihypertensive classes, such as angiotensin-converting enzyme (ACE) inhibitors, beta-blockers, and calcium antagonists [1-2]. Benidipine HCL IUPAC name 3-(3R)-1-benzylpiperidin-3-yl 5-methyl (4R)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylates show Fig. 2. Benidipine hydrochloride (BEN) is a highly potent and long-acting dihydropyridine (DHP) calcium channel blocker (L, N, and T-type) and orally active anti-anginal, antihypertensive agent which displace a wide range of activities *in vitro* and *in vivo*. It is a single enantiomers dihydropyridine calcium channel blocker for the treatment of hypertension. It has also been shown to have anti-obesity activity. It inhibits not only L-type but also T-type calcium channels [3-4]. Several studies were reported for both drugs individually using different analytical procedures for TEL like reverse-phase high-performance liquid chromatography (RP-HPLC) [5-8], potentiometry titration, Stability indicating RP-HPLC, [9,10] UV (ultraviolet)-visible spectroscopy, [11,12] absorption correction method,[13] simultaneous equation method [14] while for benidipine HPLC/UPLC,[15] UV-visible spectroscopy, etc., were available [16,17].

UTH healthcare has introduced novel fixed-dose combination (FDC) of both the examining drugs commercially as tablet formulation. Literatures resulted in the getting of few methods for this FDC product; however, no stability indicating HPLC method was found. To get simple, rapid, accurate, and economic, it was tried to develop and validate RP-HPLC method with stability indicating properties of FDC's TEL and benidipine in commercial preparations. The addition of this analytical methods in the current practice would help the pharmaceutical industries in large to preserve the excellence of their products containing these active ingredients and also the enforcement agencies in general to monitor the quality of the marketed products.

METHODS

Standard of TEL and benidipine were obtained from Yash Pharmaceuticals Ahmadabad. Tel-Benina® (UTH Healthcare) tablets were purchased from the local medical store. TEL 40 mg and benidipine 4mg were used.

TEL standard stock solution (400 µg/ml)

A sample of 40 mg of TEL was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol.

Benidipine hydrochloride (BND) standard stock solution (40 µg/ml)

A sample of 40 mg of BND was weighed and transferred to a 100 ml volumetric flask. Volume was made up to the mark with methanol, take 10 ml from this solution, and transfer to 100 ml volumetric flask and volume was made up with methanol.

Preparation of standard solution of binary mixtures of TEL (40 µg/ml) and BND (4 µg/ml)

Take 1ml from the TEL stock solution and 1ml from BND stock solution and transferred to 10 ml volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

Method validation [18-23]

Linearity

The linearity for TEL and BND were assessed by analysis of combined standard solution in range of 20–60 µg/ml and 2–6 µg/ml, respectively, 5, 7.5, 10, 12.5, and 15 ml solutions were pipette out from the stock solution of TEL (400 µg/ml) and BND (40 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to obtain 20, 30, 40, 50, and 60 µg/ml and 2, 3, 4, 5, and 6 µg/ml for TEL and BND, respectively.

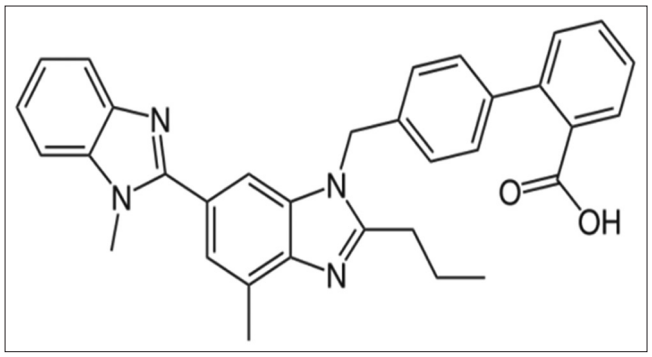

Fig. 1: Structure of telmisartan

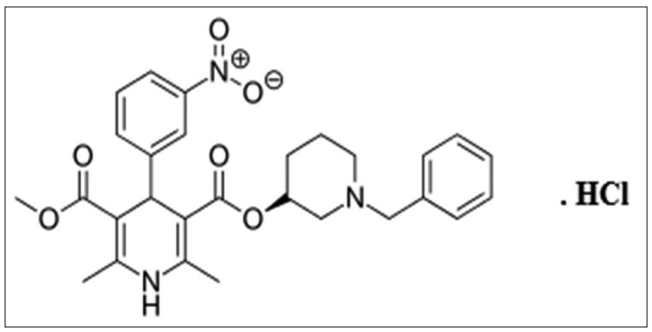

Fig. 2: Structure of benidipine hydrochloride

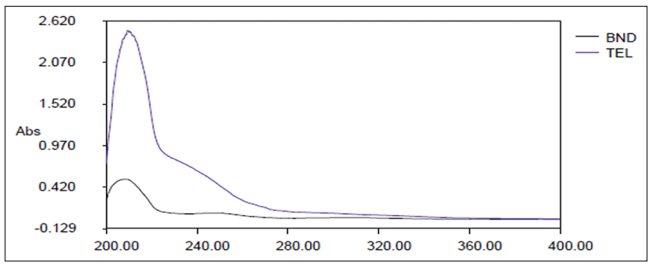

Fig. 3: Overlay ultraviolet spectrum of telmisartan and benidipine hydrochloride

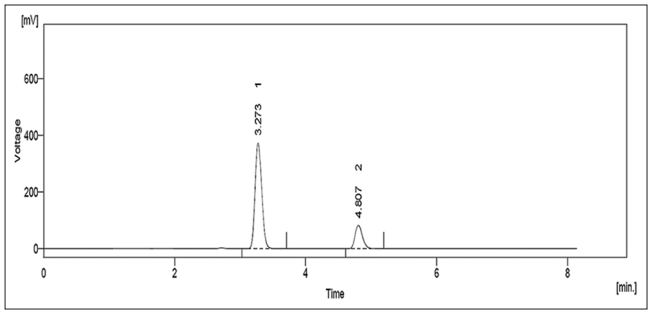

Fig. 4: Chromatogram of telmisartan and benidipine hydrochloride

Precision

Results should be expressed as relative standard deviation (RSD) or coefficient of variance. In repeatability standard solution containing TEL (40 µg/ml) and BND (4 µg/ml) was injected six times and areas of peaks were measured and % RSD was calculated. After

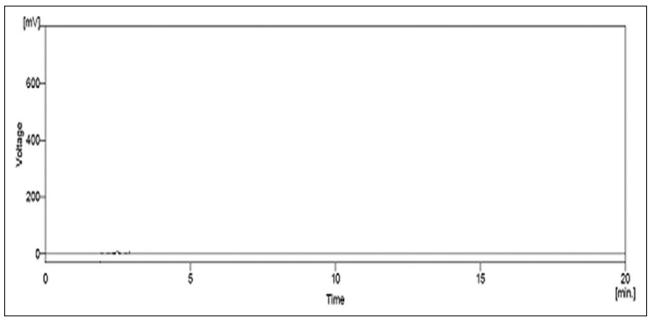

Fig. 5: Chromatogram of benidipine hydrochloride and telmisartan blank

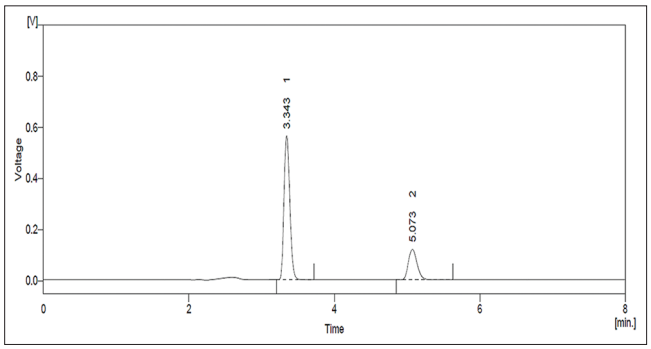

Fig. 6: Chromatogram of benidipine hydrochloride and telmisartan standard

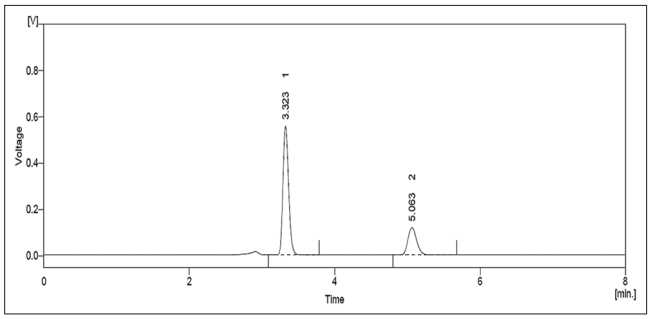

Fig. 7: Chromatogram of Benidipine hydrochloride and telmisartan sample

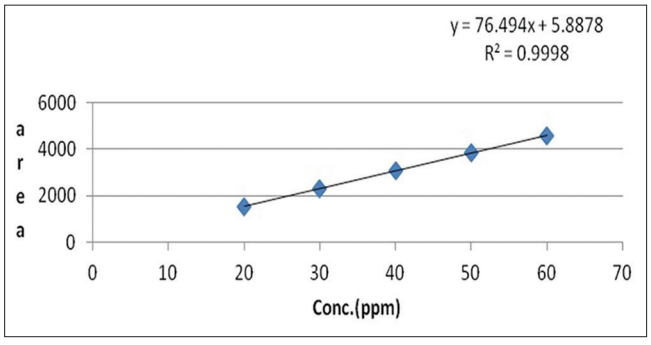

Fig. 8: Calibration curve of telmisartan

that in intraday precision standard solution containing (20, 40, and 60 µg/ml) of TEL and (2, 4, and 6 µg/ml) of BND were analyzed three times on the same day and percentage RSD was calculated. In the last inter-day precision was done by making standard solution containing (20, 40, and 60 µg/ml) of TEL and (2, 4, and 6 µg/ml) of BND were analyzed three times on the different day and percentage RSD was calculated.

Accuracy

TEL20 µg/ml and BND2 µg/ml drug solution were taken in three different flask label A, B, and C. Spiked 80%, 100%, and 120% of standard solution in it and diluted up to 10 ml. The area of each solution peak was measured at 210 nm. The amount of TEL and BND was calculated at each level and percentage recoveries were calculated.

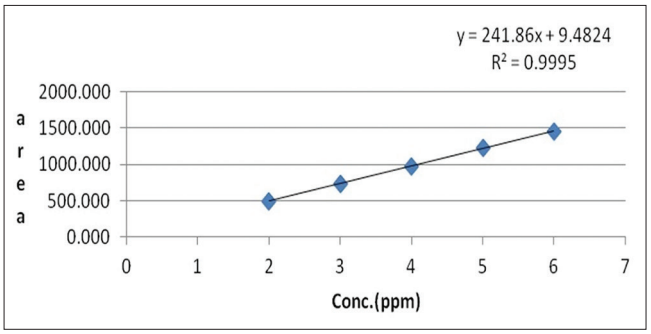

Fig. 9: Calibration curve of benidipine hydrochloride

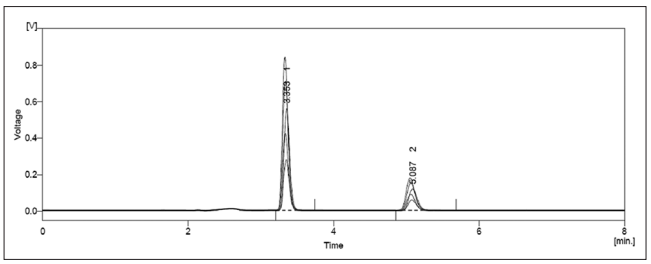

Fig. 10: Overlay chromatogram of different concentrations of binary mixtures of telmisartan and benidipine hydrochloride

Table 1: Results for system suitability test

Parameters	TEL	BEN
Theoretical plates per column	4361	7978
Symmetry factor/tailing factor	1.259	1.310
Retention time (min)	3.273 min	4.807 min
Resolution	7.416	

TEL: Telmisartan, BEN: Benidipine hydrochloride

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD was estimated from the set of three calibration curves used to determination linearity. The LOD may be calculated as, LOD=3.3×(SD/Slope) Where, SD of Y-intercepts of 3 calibration curves. Slope = Mean slope of the 3 calibration curves. The LOQ was estimated from the set of 3 calibration curves used to determine linearity. The LOQ may be calculated as, LOQ=10×(SD/Slope) where, SD of Y-intercepts of three calibration curves.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation. Flow rate of mobile phase was changed (±0.2 ml/min) 0.8 ml/min and 1.2 ml/min, ratio of mobile phase was changed (±2) Buffer: Methanol (52:48) and Buffer: Methanol (48:52) and pH of buffer was changed (±0.2) pH 4.2 and pH 3.8.

Forced degradation studies[24]

Acid degradation

Acid decomposition studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 0.1 N HCl solutions was added and mixed well and put for 3 h. After time period, the volume was adjusted with diluent to get 40 µg/ml for TEL and 4 µg/ml for BND.

Base degradation

Basic decomposition studies were performed by transferring 1ml of stock solution in to 10ml of volumetric flask. A volume of 2 ml of 0.1 N NaOH solutions was added and mixed well and put for 4h. After time period, the volume was adjusted with diluents to get 40 µg/ml for TEL and 4 µg/ml for BND.

Oxidative degradation

Oxidation decomposition studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. A volume of 2 ml of 3% H_2O_2 solutions were added and mixed well and put for 3 h. After time period, the volume was adjusted with diluents to get 40 µg/ml for TEL and 4 µg/ml for BND.

Photo degradation

Photo degradation studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was kept under UV light in UV chamber for 18 h. Then the volume was adjusted with diluents to get 40 µg/ml for TEL and 4 µg/ml for BND.

Thermal degradation

Thermal degradation studies were performed by transferring 1 ml of stock solution in to 10 ml of volumetric flask. The volumetric flask was stored in oven at 80°C for 6 h. Then, the volume was adjusted with diluents to get 40 µg/ml for TEL and 4 µg/ml for BND.

Table 2: Linearity data for TEL and BND

TEL			BND	
S. no	Concentration(µg/ml)	Area	Concentration (µg/ml)	Area
1	20	1531.035	2	489.434
2	30	2305.359	3	736.534
3	40	3054.430	4	975.650
4	50	3857.941	5	1231.962
5	60	4579.434	6	1451.016
S.D.		19.808	9.735	
Correlation coefficient		0.9998	0.9995	
Regression equation		y=76.494x+5.8878	y=241.86x+9.4824	

TEL: Telmisartan, BEN: Benidipine hydrochloride,

RESULT AND DISCUSSION

Selection of wavelength

Standard solution of TEL (40 µg/ml) and standard solution of BND (4 µg/ml) were scanned between 200 and 400 nm using UV-visible spectrophotometer. Both solutions were scanned between 200 and 400 nm. Wavelength was selected from the overlay spectra of above solutions. Both TEL and BND show reasonably good response at 210 nm show in Fig. 3.

Chromatographic separation

Standard solutions of 20–60 µg/ml of TEL and 2–6 µg/ml of BND were injected in column with 20 µl microsyringe. The chromatogram was run for appropriate minutes with mobile phase Phosphate buffer (Ph4.0): Methanol (50:50). The detection was carried out at wavelength 210 nm. The chromatogram was stopped after separation achieved completely. Data related to peak such as area, height, retention time, and resolution were recorded using software show in Fig. 4 [25].

- Column: Thermo scientific,C_{18} (25 cm × 0.46 cm) Hypersil BDS
- Mobile Phase: Phosphate buffer, pH 4.0: Methanol (50:50)
- Flow Rate: 1.0 ml/min
- Detection wavelength: 210 nm
- Runtime: 8 min
- Injection volume: 20.0 µl
- Diluents: Mobile Phase

System suitability test

It is an integral part of chromatographic method. These tests are used to verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. System suitability tests are based on the concept that the equipment, electronics, analytical operations, and samples constitute an integral system that can be evaluated as a whole. System suitability testing provides assurance that the method will provide accurate and precise data for its intended use and all parameters are respectively showed in Table 1.

Specificity

The chromatograms of BND and TEL standards and BND and TEL sample show no interference with the chromatogram of BND and TEL blank, so the developed method is specific and show response in Figs. 5-7.

Linearity and range

The linearity for TEL and BND were assessed by analysis of combined standard solution in range of 20–60 µg/ml and 2–6 µg/ml, respectively. Correlation coefficient for calibration curve TEL and BND was found to be 0.9998 and 0.9995, respectively. This response show in Figs. 8-10 and Table 2.

LOD/LOQ

Calibration curve was repeated for five times and the SD of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD=3.3*SD/slope of calibration curve, LOQ=10*SD/slope of calibration curve

For TEL
LOD = ×3.3 (SD/Slope)
= ×3.3 (19.808/76.494)
=0.855 µg/ml
=0.402 µg/ml

For BND
LOQ = 10 (SD/Slope)
= ×10 (19.808/76.494)
= 2.589 µg/ml
LOD = ×3.3 (SD/Slope)
= ×3.3 (9.735/241.86)
= 0.133 µg/ml
LOQ = ×10 (SD/Slope)
= ×10 (9.735/241.86)

Precision

Repeatability

The data for repeatability of peak area measurement for TEL and BND based on six measurements of same solution of TEL and BND are summazed in Table 3. The percentage RSD for TEL and BND was found to be 0.671 and 0.509, respectively

Intraday precision

The data for intraday precision for TEL and BND are shown in Table 4. The percentage RSD for intraday precision was found to be 0.916–1.044 for TEL and 0.649-1.331 for BND

Interday precision

The data for intraday precision for TEL and BND are shown in Table 5. The percentage RSD for interday precision was found to be 0.622-0.982 for TEL and 0.807-1.690 for BND.

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in Tables 6 and 7. Percentage recovery for TEL was 99.258%–101.413 %, while for BND, it was found to be in range of 98.982 %-101.506 %

Robustness

The effect of changes was found to be within the acceptance criteria as shown in Tables 8 and 9.The percentage RSD should be <2%.

Stability indicating method

Stability of both drugs are studied utilizing different parameter. In this study, the area of standard for stability and degradation of sample and standard were compare. The standard area of BND and TEL is 649.883 and 2649.948, respectively. Result shows BND has highest degradation in oxidation and thermal as compare to others. TEL shows highest degradation in oxidation and basic environment. The peaks of all parameters are given in Fig 11-31. The percent degradation of all parameters is given below in Tables 10 and 11 [26].

Table 3: Repeatability data for TEL and BND

S. no.	Area	
	TEL Conc. 40 µg/ml	BND Conc. 4 µg/ml
1	3072.532	981.403
2	3051.079	973.610
3	3057.200	978.335
4	3032.814	968.540
5	3093.458	980.913
6	3065.703	979.004
Mean±SD	3062.131±20.534	976.9668±4.971
%RSD	0.671	0.509

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan, BEN: Benidipine hydrochloride

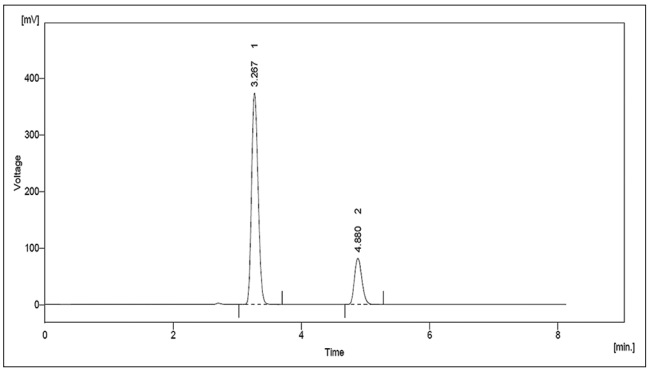

Fig. 11: Standard for stability

Table 4: Intraday precision data for estimation of TEL and BND

TEL					BND			
S. no.	Conc. (µg/ml)	Area	Mean±SD	% RSD	Conc. (µg/ml)	Area	Mean±SD	% RSD
1	20	1537.16	1535.5±16.029	1.044	2	491.406	490.0±6.526	1.331
	20	1518.737			2	482.886		
	20	1550.67			2	495.709		
2	40	3051.411	3049.9±29.626	0.971	4	974.667	975.8±6.973	0.714
	40	3078.832			4	983.395		
	40	3019.634			4	969.61		
3	60	4606.989	4643.6±42.561	0.916	6	1465.598	1476.5±9.582	0.649
	60	4633.685			6	1480.366		
	60	4690.335			6	1483.561		

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan, BEN: Benidipine hydrochloride

Table 5: Interday precision data for estimation of TEL and BND

TEL					BND			
Sr. No.	Conc. (µg/ml)	Area	Mean±SD	% RSD	Conc. (µg/ml)	Area	Mean±SD	% RSD
1	20	1529.54	1521.94±9.47	0.622	2	488.945	484.85±5.830	1.202
	20	1524.96			2	487.448		
	20	1511.33			2	478.182		
2	40	3063.62	3033.11±29.78	0.982	4	978.548	970.70±7.834	0.807
	40	3031.06			4	970.676		
	40	3004.11			4	962.88		
3	60	4597.87	4555.17±41.21	0.904	6	1464.615	1439.66±34.331	1.690
	60	4552.01			6	1416.004		
	60	4515.63			6	1438.379		

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan, BEN: Benidipine hydrochloride

Table 6: Accuracy data for TEL

S. no	Conc. Level (%)	Sample amount (µg/ml)	Amount added (µg/ml)	Amount recovered (µg/ml)	% recovery	% Mean recovery	SD	% RSD
1	80	20	16	16.093	100.582	99.258	1.168	1.177
2		20	16	15.740	98.374			
3		20	16	15.811	98.818			
4	100	20	20	20.190	100.951	101.413	0.429	0.423
5		20	20	20.360	101.799			
6		20	20	20.298	101.490			
7	120	20	24	24.088	100.367	100.733	0.483	0.480
8		20	24	24.133	100.553			
9		20	24	24.307	101.281			

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan, BEN: Benidipine hydrochloride

Table 7: Accuracy data for BND

S no.	Conc. level (%)	Sample amount	Amount added	Amount recovered (µg/ml)	% recovery	% Mean recovery	SD	% RSD
1	80	2	1.6	1.609	100.535	98.982	1.398	1.413
2		2	1.6	1.569	98.075			
3		2	1.6	1.570	98.154			
4	100	2	2	2.031	101.560	101.506	0.613	0.604
5		2	2	2.017	100.868			
6		2	2	2.042	102.090			
7	120	2	2.4	2.390	99.576	99.776	1.166	1.169
8		2	2.4	2.369	98.723			
9		2	2.4	2.425	101.030			

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan, BEN: Benidipine hydrochloride

CONCLUSION

The combined dosage form of benidipine HCl and TEL are used in the treatment of hypertension. Various methods are reported for the analysis of individual drug and in combination with other drugs but no HPLC method reported for these two drugs in combined dosage form. Therefore, a novel RP- HPLC method has been developed for the simultaneous estimation of benidipine HCl and TEL in marketed formulations. The optimized chromatogram was run for appropriate minutes with mobile phase phosphate buffer (pH 4.0): Methanol (50:50). Data related to peak such as area, height, retention time, resolution, etc., were recorded using software. Thermo scientific,C_{18} (25 cm×0.46 cm) Hypersil BDS, Mobile Phase Phosphate buffer, pH 4.0: Methanol (50:50) with flow rate 1.0 ml/min and Runtime: 8 min injection volume of 20.0 µl. The detection was carried

Table 8: Robustness data for TEL

Sr. No.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase(-2)	Area at Mobile phase(+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	3127.605	3038.758	3136.936	3060.068	3144.050	3081.693
2	3096.317	3047.785	3168.318	3054.041	3152.045	3103.322
3	3148.884	3023.195	3136.665	3072.257	3129.609	3109.673
Avg. Area	3124.269	3036.579	3147.306	3062.122	3141.901	3098.229
S.D.	26.442	12.439	18.197	9.280	11.371	14.669
% RSD	0.846	0.410	0.578	0.303	0.362	0.473

RSD: Relative standard deviation, SD: Standard deviation, TEL: Telmisartan

Table 9: Robustness data for BND

S. no.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at Mobile phase (-2)	Area at Mobile phase(+2)	Area at pH (-0.2)	Area at pH (+0.2)
1	998.946	970.575	1001.913	977.383	1004.105	984.289
2	988.943	977.370	996.071	975.437	997.674	991.177
3	990.868	965.593	1001.830	984.252	1007.707	998.113
Avg. Area	992.919	971.179	999.938	979.024	1003.162	991.193
S.D.	5.308	5.912	3.349	4.631	5.083	6.912
% RSD	0.535	0.609	0.507	0.697	0.335	0.473

RSD: Relative standard deviation, SD: Standard deviation, BEN: Benidipine hydrochloride

Table 10: Degradation of BND

Condition	Area of standard	% degradation	Area of sample	% degradation
Acid	559.499	13.908	543.250	16.408
Base	577.202	11.184	570.394	12.231
Thermal	529.643	18.502	547.393	15.771
Oxidation	515.387	20.695	498.924	23.229
Photo	570.778	12.172	574.950	11.530

BEN: Benidipine hydrochloride

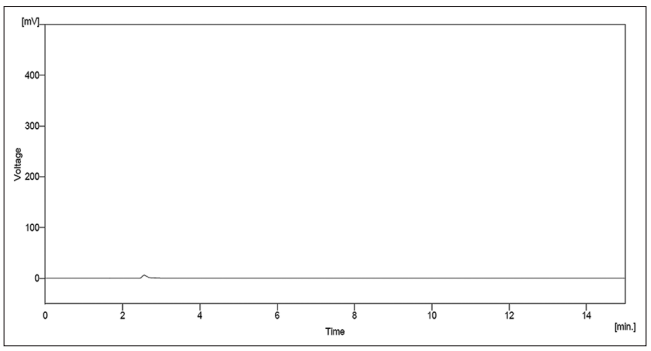

Fig. 12: Acid degradation blank

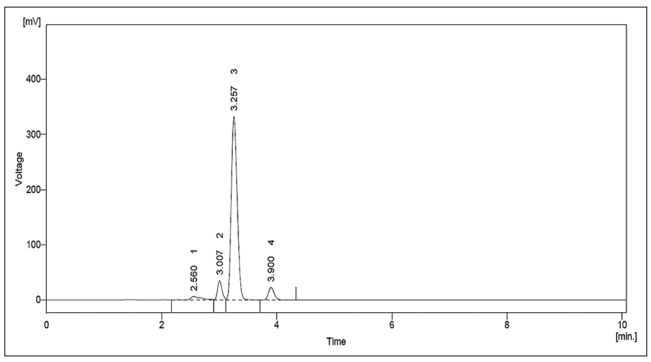

Fig. 14: Acid degradation of telmisartan

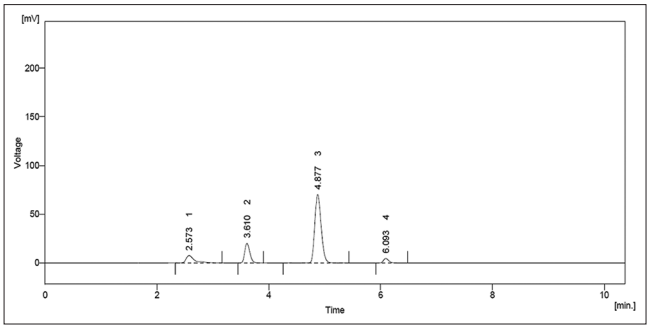

Fig. 13: Acid degradation of benidipine hydrochloride

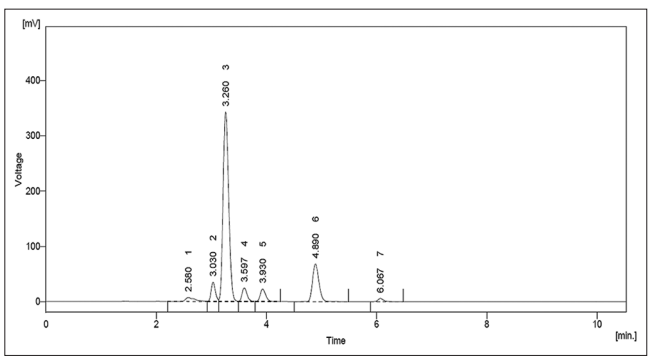

Fig. 15: Acid degradation sample

Table 11: Degradation of TEL

Condition	Area of standard	% degradation	Area of sample	% degradation
Acid	2349.042	11.355	2327.872	12.154
Base	2271.527	14.280	2299.061	13.241
Thermal	2289.502	13.602	2341.262	11.649
Oxidation	2058.846	22.306	2236.532	15.601
Photo	2278.141	14.031	2357.661	11.030

TEL: Telmisartan

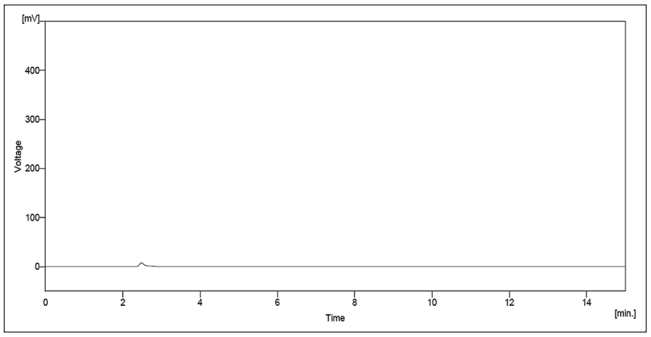

Fig. 16: Base degradation blank

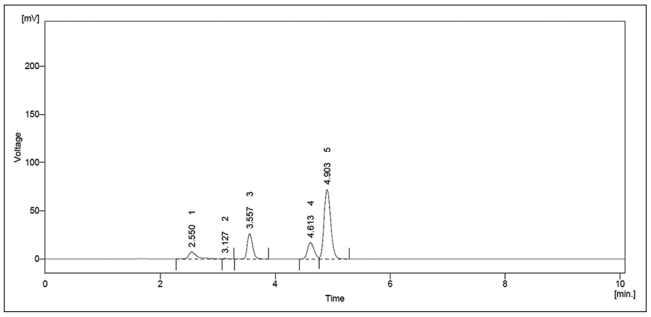

Fig. 17: Base degradation of benidipine hydrochloride

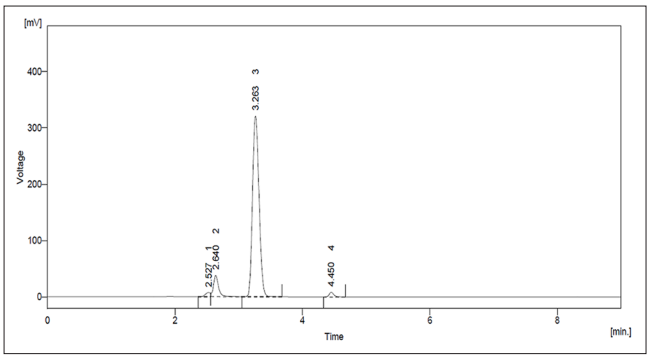

Fig. 18: Base degradation of telmisartan

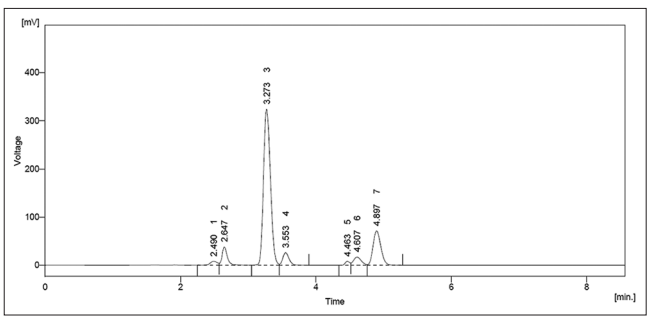

Fig. 19: Base degradation sample

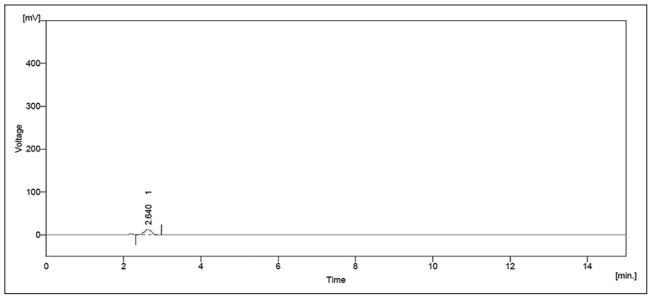

Fig. 20: Oxidation degradation blank

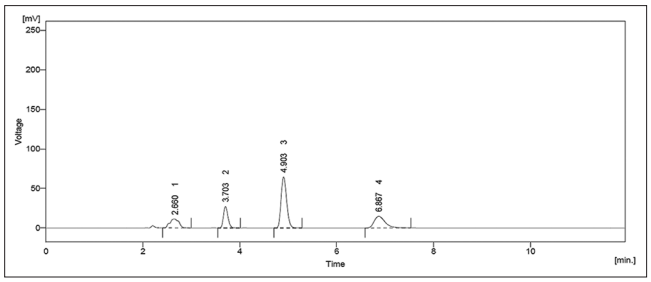

Fig. 21: Oxidation degradation of benidipine hydrochloride

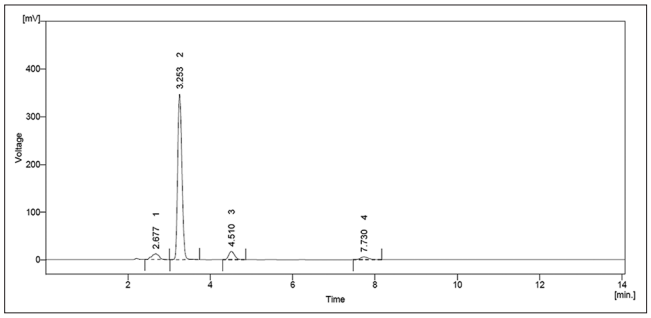

Fig. 22: Oxidation degradation of telmisartan

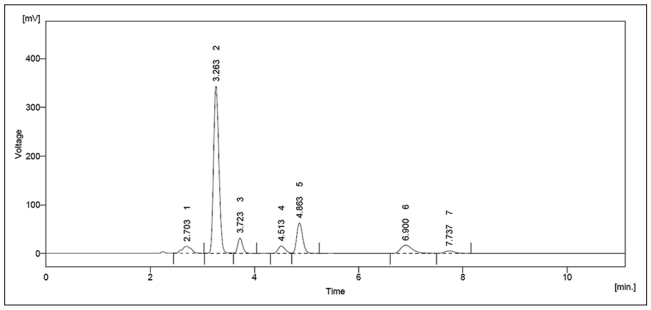

Fig. 23: Oxidation degradation sample

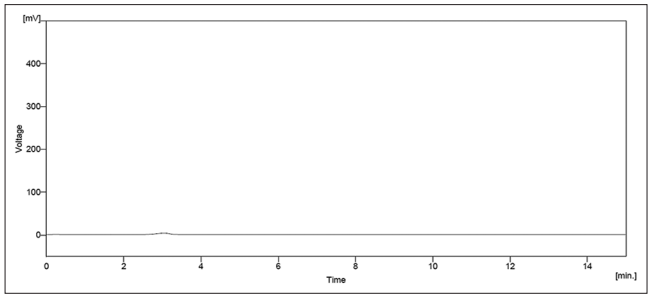

Fig. 24: Photodegradation blank

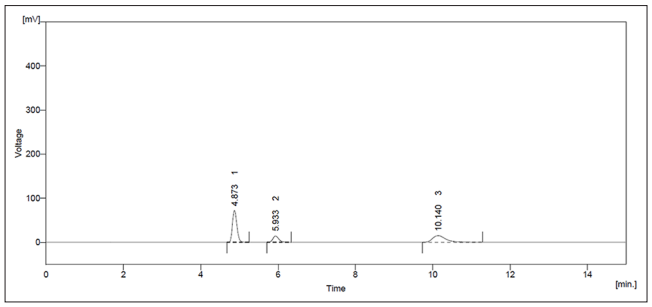

Fig. 25: Photodegradation of benidipine hydrochloride

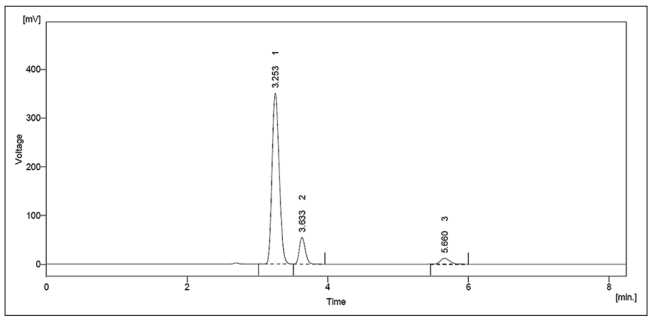

Fig. 26: Photodegradation of telmisartan

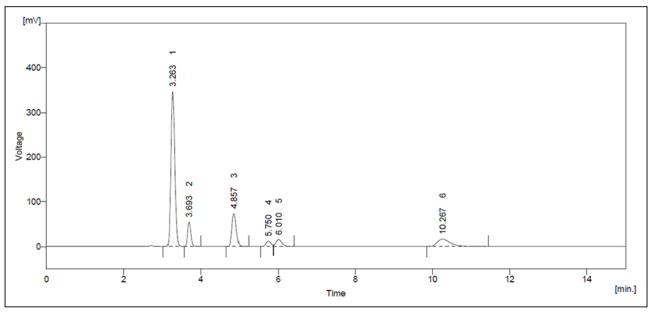

Fig. 27: Photodegradation sample

out at wavelength 210 nm. It was found to be simple, precise and accurate. In this stability indicating RP-HPLC methods were developed by degradation of sample and compared with standard. The percentage RSD was also <2% showing high degree of precision of the proposed method. The proposed method can be used for routine analysis of benidipine HCl and TEL in combined dosage form. It can be also used in the quality control in bulk manufacturing.

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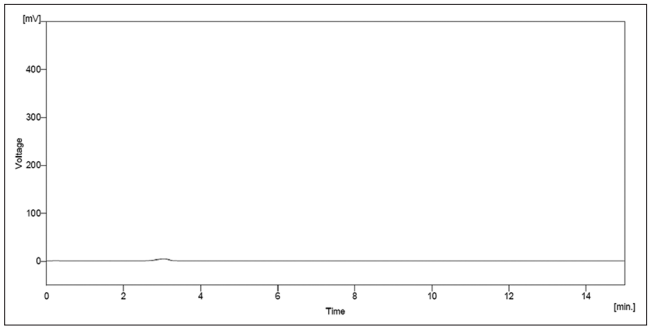

Fig. 28: Thermal degradation blank

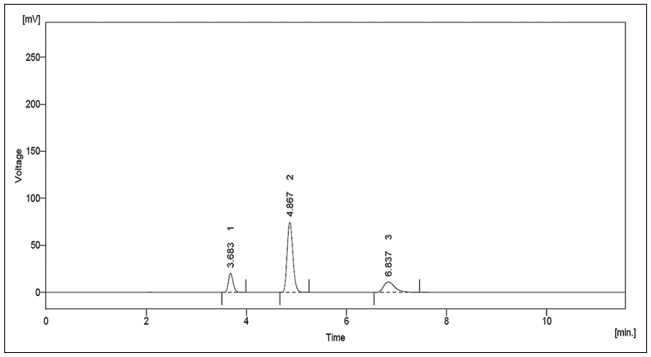

Fig. 29: Thermal degradation of benidipine hydrochloride

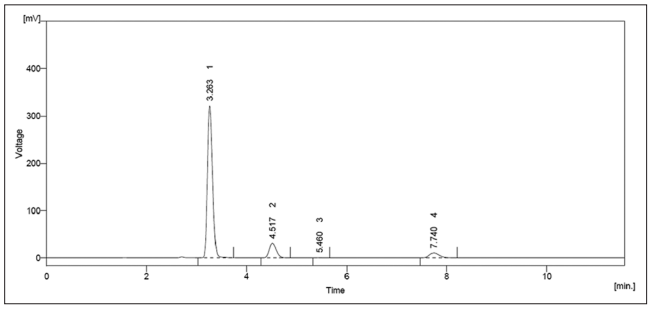

Fig. 30: Thermal degradation of telmisartan

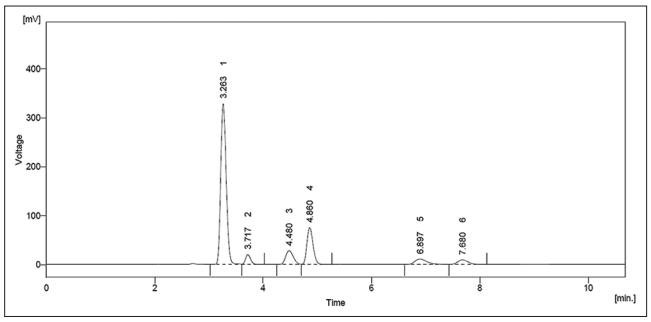

Fig. 31: Thermal degradation sample

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