

Development and Validation of Stability Indicating RP-HPLC Method for Simultaneous Estimation of Benidipine and Telmisartan

Parimal Katolkar, Anuja Shrikhande*, Shubham Amadare, Mithali Palandurkar and Swati Vidhate

Department of Pharmaceutical Chemistry, Kamla Nehru College of Pharmacy, Rastrasant Tukadoji Maharaj Nagpur University, Butibori, Nagpur, Maharashtra, India.

<http://dx.doi.org/10.13005/bbra/3385>

(Received: 09 December 2024; accepted: 24 April 2025)

The primary goal of this work was to create and test a stability-indicating reverse-phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous detection of telmisartan (TEL) and benidipine hydrochloride (BEN) in bulk and pharmaceutical dose form. Using reverse phase chromatography, the developed method is simple, appropriate, and more efficient, stable, and reproducible. The C18 column, measuring 250 x 4.6 mm and packed with 5.0 μ m particles, was used to separate TEL and BEN. When used as a mobile phase, various solvent solutions were evaluated and improved. TEL (100 μ g/ml) and BEN (10 μ g/ml) were used for their good peak forms and resolution in Methanol: Ammonium Dihydrogen Orthophosphate Buffer, pH 3.5 (80:20 v/v). The mobile phase was flowing at 1.0 ml/min, and both analytes were detected at 237 nm using UV/Vis detectors. The method's development and validation were based on linearity, accuracy, precision, robustness, specificity, linearity, detection limit, and quantitation limit. TEL and BEN were found to be linear at doses of 5-15 and 50-150 μ g/ml. According to this study, the impact of RP-HPLC techniques included a reduction of sample quality and standardization. The fact that the percentage relative standard deviation was less than 2% proved the exceptional precision of the proposed procedure.

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

Benidipine hydrochloride is a long-acting dihydropyridine Ca^{++} channel blocker with the chemical formula 3-[(3R)-1-(phenylmethyl) piperidin-3-yl]-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine 3,5-dicarboxylate hydrochloride. (figure1).^{1,2,3} Benidipine hydrochloride (BEN) is an orally active, highly potent, long-acting dihydropyridine (DHP) calcium channel blocker (L, N, and T-type) that inhibits a wide range of in vitro and in vivo

activities. Hypertension is treated with a calcium channel blocker made up of only one enantiomer of dihydropyridine. Its anti-obesity abilities have also been confirmed. It inhibits not only L-type calcium channels but also T-type channels.^{4,5}

Telmisartan (TEL)'s chemical name is 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1-yl]].(figure 2). The synthetic counterpart of angiotensin II receptor blockers, -methyl]phenyl]benzoic acid, is used to treat

*Corresponding author E-mail: anuja282001@gmail.com



hypertension. TEL has a dissolution rate-limited absorption pattern in both humans and animals, despite being a poorly water-soluble medicine. As a result, it can be used as a model pharmaceutical to investigate how various physicochemical, physiological, and dose form aspects influence the absorption kinetics and bioavailability of hydrophobic medicines.⁶⁻¹¹

Hypertension is treated with both medication combinations that include benidipine and telmisartan. One of the few strategies on stability uncovered by a literature study was the creation and validation of an RP-HPLC method for the simultaneous assessment of Benidipine and Telmisartan.

MATERIALS AND METHODS

Instrumentation

The medications were analyzed using the HPLC Shimadzu LC 20 AD, Column Phenomenex Luna C18 (250 mm x 4.6 mm, particle size 5 μ), Acme pump, UV/vis detector, and LC Solution software.

Chemicals and reagents

The TEL standard was provided by Mylan Laboratories Limited in Telangana, India, while the BEN standard was obtained from Pure chem PVT. LTD. Benitowa®TM (Akumentis Healthcare) tablets were purchased at a local pharmacy in Ankleshwar, Gujarat, India. Benidipine (10 mg) and TEL (100 mg) were used.

Preparation of standard solution of binary mixtures of TEL (100 μ g/ml) and BEN (10 μ g/ml)

To make the stock solution, 10 mg BEN and 100.2 mg TEL were properly weighed and dissolved in the diluent, then the volume was increased by the same amount to achieve the 100 ml limit.

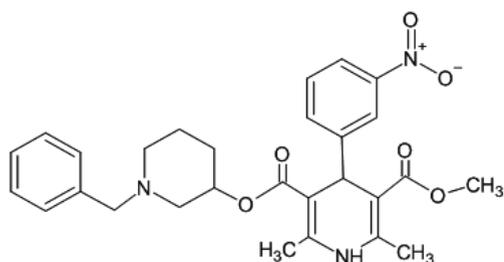


Fig. 1. Structure of benidipine hydrochloride

Method validation¹²⁻¹³

Linearity

Pipette 1.0, 1.5, 2.0, 2.5, and 3.0 ml of TEL and BEN stock solution into 20 ml volumetric flask to achieve 50, 75, 100, 125, and 150 μ g/ml and 5, 7.5, 10.0, 12.5, and 15 μ g/ml for TEL and BEN, respectively. This investigation was conducted to determine the linearity of TEL and BEN.

Precision

Results should be reported as relative standard deviation (RSD) or coefficient of variance. Six injections of TEL (100 μ g/ml) and BEN (10 μ g/ml) into a repeatability standard solution were used to measure peak areas and percent RSD. TEL and BEN were tested again on the same day using an intraday precision standard solution, and the % RSD was discovered. The ultimate inter-day precision was obtained by analyzing a standard solution made up of TEL and BEN twice over numerous days and computing the % RSD.

Accuracy

The diluent was mixed with precisely weighed volumes of 10 mg BEN and 100.2 mg TEL until the volume reached 100 ml. Pipette 2 mL of the same solution into a 20 mL volumetric flask and dilute it with diluent.

It was diluted with diluent in a 100 mL volumetric flask after being spiked with 80, 100, and 120% of the reference solution. After pipetteing 2 mL of the same solution into a 20 mL volumetric flask, diluent was added. At 237 nm, the area of each solution peak was determined. The TEL, BEN, and percentage recoveries were computed at each level.

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

The LOD was computed using the same three calibration curves that were used to determine

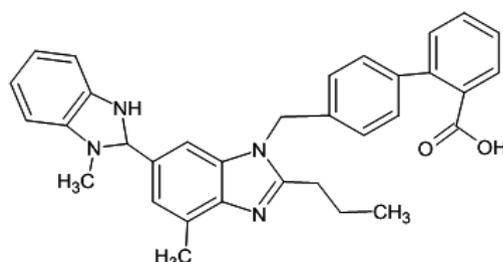


Fig. 2. Structure of telmisartan

linearity. The LOD formula is $LOD=3.3(SD/Slope)$, where SD represents the average of the three calibration curves' Y-intercepts. Slope represents the average slope of the three calibration curves. The LOQ was computed using the same three calibration curves used to demonstrate linearity. The limit of quantification is calculated

as follows: $LOQ = 10(SD/Slope)$, where SD is the sum of the three calibration curves' Y-intercepts.

Robustness

The effects of modifying each of the following factors individually on the system's fitness for standard preparation were noted.

Change in flow rate (0.9ml/min and 1.1ml/min).

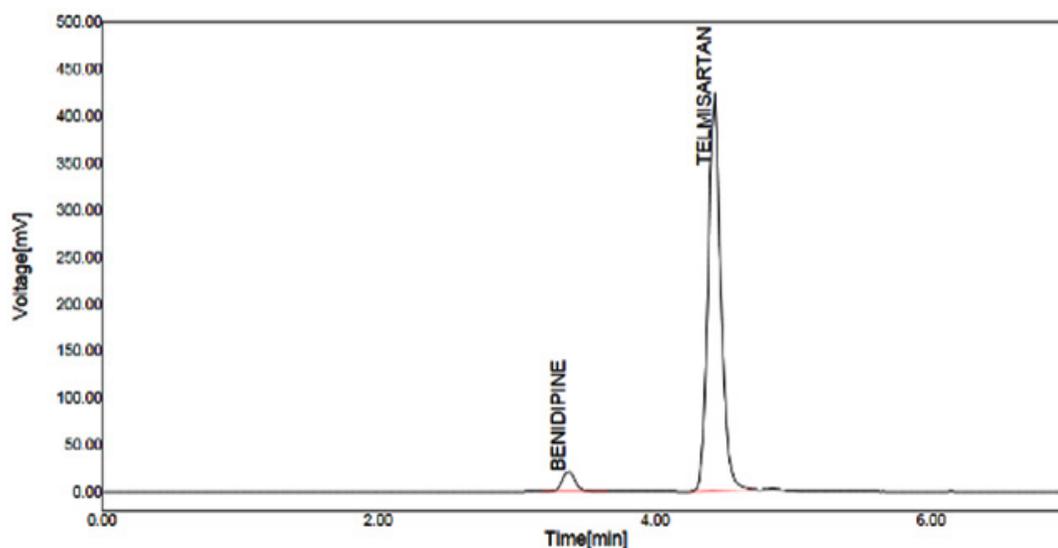


Fig. 3. Chromatogram of BEN & TEL obtained by using methanol and ammonium dihydrogen orthophosphate buffer (80:20)

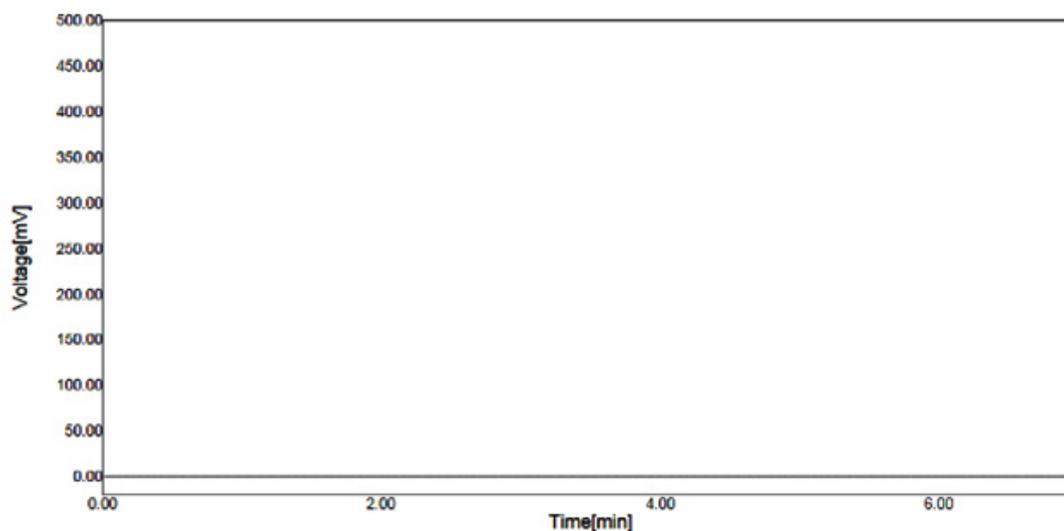


Fig. 4. Chromatogram of benidipine hydrochloride telmisartan blank

Change in wavelength (235 nm and 239 nm)
Buffer pH (3.35 & 3.65)

Forced degradation studies

Acid degradation

Transfer 10 ml of stock solution to a 100 ml volumetric flask, then add 10 ml of 0.1 N HCl solution and reflux for 30 minutes on a heating mantle at 60°C. It was derived from an acidic solution. After cooling, add 0.1 N NaOH solution

drop by drop and shake to neutralize. Dilute to 100 ml with diluent and filter through 0.45 μ m membrane filter for analysis.

Base Degradation

Transfer 10 ml of stock solution to a 100 ml volumetric flask, then add 10 ml of 0.1 N NaOH solution and reflux for 30 minutes on a heating mantle at 60°C. It was made using a simple solution. After cooling, add 0.1 N HCL

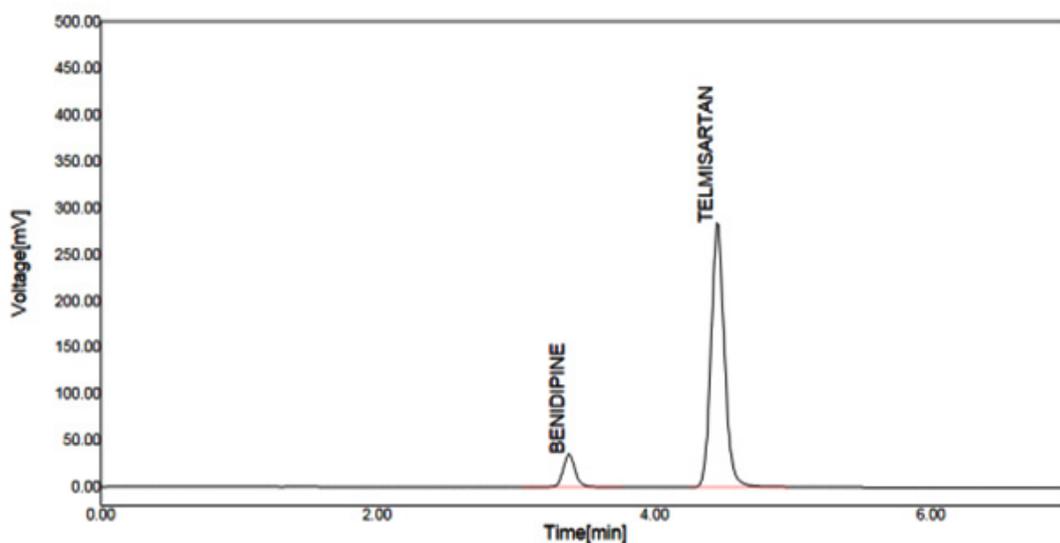


Fig. 5. Chromatogram of benidipine hydrochloride and and telmisartan sample

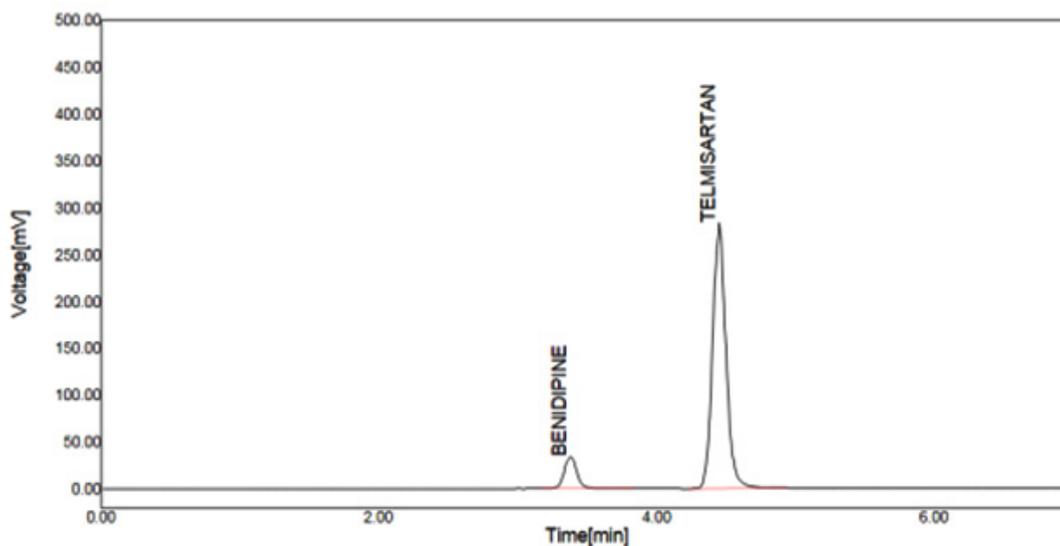


Fig. 6. Chromatogram of Benidipine hydrochloride and telmisartan standard

solution drop by drop and shake regularly to ensure neutralization. Dilute to 100 ml with diluent and filter with 0.45 μ m membrane filter before analysis.

Oxidative/ Peroxide Degradation

Transfer 10 ml of stock solution to a 100 ml volumetric flask, then add 10 ml of 0.3% H₂O₂ solution and reflux for 12 hours on the heating mantle at 60°C. After cooling, the volume was increased to 100 mL and filtered through a 0.45 μ m membrane filter for analysis.

Thermal degradation

The pure medication was cooked in a 60°C oven for approximately 1 hour before being allowed to cool to room temperature. The above-mentioned medicine was correctly weighed to 9.9 mg BEN and 100.6 mg TEL and dissolved in 100 ml of diluent (mobile phase) (stock solution).

To acquire 10 μ g/ml, pipette 2 ml of a standard stock solution of medication, dilute with 20 ml diluent, filter with a 0.45 μ m membrane filter, and sonicate for 10 minutes before injecting the solution into the chromatography.

Photolytic degradation

Pure BEN and TEL medicines were placed in petri plates and subjected to UV light at 254 nm in a UV chamber for one hour. 9.9 mg BEN and 100.6 mg TEL were precisely weighed and dissolved in diluent (mobile phase) to meet the 100 ml mark (stock solution).

To obtain 10 μ g/ml of solution, pipette 2 ml from a standard stock solution of the medication and dilute it with 20 ml of diluent.

RESULTS

Selection of wavelength

A UV-visible spectrophotometer was used to scan standard solutions of TEL (100 μ g/ml) and BEN (10 μ g/ml) from 200 to 400 nm. Both solutions were scanned from 200 to 400 nm. The wavelength was determined using the overlay spectra of the aforementioned solutions. As seen in Fig. 3, TEL and BEN both exhibit a substantial response at 237 nm.

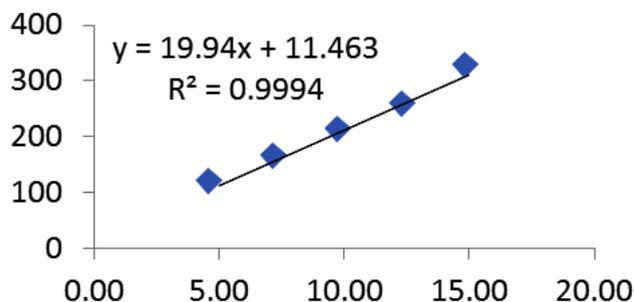


Fig. 7. Calibration curve of benidipine

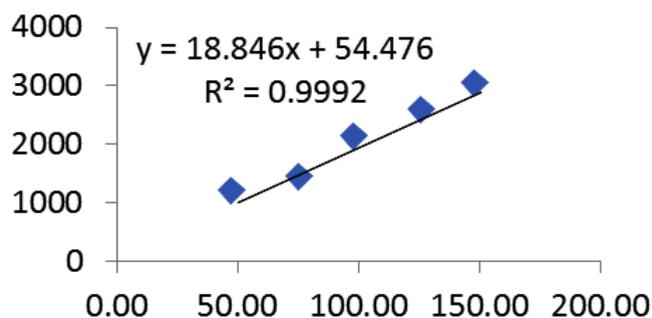


Fig. 8. Calibration curve of telmisartan hydrochloride

Chromatographic separation

Standard TEL and BEN solutions were introduced into the column via a 20 μ l micro syringe. The chromatogram was run for the required number of minutes with a methanol mobile phase and an ammonium dihydrogen orthophosphate (pH 3.5) buffer solution (80:20 v/v). The detection took place at 237 nm. After the separation was completed, the chromatogram was turned off. Figure 4 shows the program used to record the peak's size, height, retention duration, and resolution.

Chromatographic Conditions

Column : Hypersil BDS, C18,
(250 \times 4.6mm) 5 μ m
Flow Rate : 1.0 ml/min

Injection Volume : 20 μ L
Wavelength : 237 nm
Column Temp. : Ambient
Run Time : 10 min

Specificity

The BEN and TEL blank's chromatogram does not interfere with the chromatogram of the BEN and TEL standards or the BEN and TEL sample, demonstrating that the method was established with specificity, as shown in Figs. 4-6.

Linearity and range

To test the linearity of TEL and BEN, a mixed standard solution containing 50-150 μ g/ml and 5-15 μ g/ml was used. The TEL and BEN calibration curves showed correlation values of

Table 1. Intraday precision data for estimation of BEN

Name	Area	RT(min)	%Assay
Test solution	213.1641	3.38	98.67
Test solution	210.4409	3.38	98.39

Name	Preparations	% Assay
Set-1	prep-1	98.12
	prep-2	98.27
Set-2	prep-1	98.67
	prep-2	98.39
Mean		98.36
SD		0.2329
% RSD (NMT 2)		0.24

Table 3. Interday precision data for estimation of BEN

Name	Area	RT(min)	%ASSAY
Test solution	209.1348	3.37	97.78
Test solution	216.6902	3.38	99.31

Name	Preparations	% Assay
Day-1	prep-1	98.12
	prep-2	98.27
Day-2	prep-1	97.78
	prep-2	99.31
Mean		98.37
SD		0.6593
% RSD (NMT 2)		0.67

Table 2. Intraday precision data for estimation of TEL

Name	Area	RT(min)	%Assay
Test solution-1	1932.8728	4.45	101.15
Test solution-1	1895.3562	4.42	98.80

Name	Preparation	%Assay
Set-1	prep-1	98.53
	prep-2	99.98
Set-2	prep-1	101.15
	prep-2	98.80
Mean		99.62
SD		1.2015
%RSD (NMT 2)		1.21

Table 4. Interday precision data for estimation of TEL

Name	Area	RT(min)	%Assay
Test solution-1	1893.7627	4.40	99.30
Test solution-1	1879.0853	4.43	98.73

Name	Preparation	%Assay
Day-1	prep-1	98.53
	prep-2	99.98
Day-2	prep-1	99.30
	prep-2	98.73
Mean		99.14
SD		0.6510
%RSD (NMT 2)		0.66

0.9994 and 0.9992, respectively. Figures 7-8 and Tables 2-3 show this effect.

Precision

Intraday precision

The results for intraday precision for TEL and BEN are shown in Tables 4, 5. For intraday

precision, TEL had a percentage RSD of 1.21 and BEN had a % RSD of 0.24.

Interday precision

Tables 1 and 2 contain statistics for TEL and BEN intraday precision. The interday precision

Table 5. Results for system suitability test

Sr. No.	Name	RT (min)	Area	TP	TF	Resolution
1.	BENIDIPINE	3.38	139.5247	13290	1.04	0.00
2.	TELMISARTAN	4.43	2698.4956	12357	1.06	7.58

Table 6. Recovery Study of BEN

Name	Preparations	Area	Amount added µg/ml	Amount recovered µg/ml	Recovery (95-105) %
Accuracy at 80 %	Prep-1	172.0873	8.00	7.97	99.57
	Prep-2	175.6127	8.10	8.13	100.36
	Prep-3	169.9548	8.00	7.87	98.34
Accuracy at 100 %	Prep-1	221.3835	10.10	10.25	101.46
	Prep-2	218.4268	10.10	10.11	100.11
	Prep-3	221.0120	10.10	10.23	101.29
Accuracy at 120 %	Prep-1	259.4663	12.10	12.01	99.26
	Prep-2	255.9428	12.00	11.85	98.73
	Prep-3	264.6649	12.10	12.25	101.25

Table 7. Statistical Validation of Recovery Study

	MEAN % recovery	SD	%RSD (NMT 2)
Accuracy at 80 %	99.42	1.0179	1.02
Accuracy at 100 %	100.95	0.7381	0.73
Accuracy at 120 %	99.74	1.3287	1.33

Table 8. Recovery Study of TEL

Name	Preparations	Area	Amount added µg/ml	Amount recovered µg/ml	Recovery (95-105) %
Accuracy at 80 %	Prep-1	1539.1315	80.20	81.03	101.04
	Prep-2	1512.5122	80.00	79.63	99.54
	Prep-3	1525.4513	80.40	80.31	99.89
Accuracy at 100 %	Prep-1	1948.3221	101.20	102.57	101.36
	Prep-2	1911.5549	99.80	100.64	100.84
	Prep-3	1921.8163	100.60	101.18	100.57
Accuracy at 120 %	Prep-1	2301.8196	120.20	121.18	100.82
	Prep-2	2281.0349	120.20	120.09	99.91
	Prep-3	2249.5127	119.80	118.43	98.86

of 0.66 TEL and 0.67 BEN was discovered to have a percent RSD.

LOD/LOQ

The SD of the intercepts was computed after five calibration curve iterations. The LOD and LOQ were then determined using the following formulas:

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

For BEN

LOD = 3.3 X (SD/Slope)

= 3.3 X(3.0426/19.9403)

= 0.50 ig/ml

LOQ = 10 X (SD/Slope)

= 10 X (19.808/76.494)

= 1.53ig/ml

For TEL

LOD = 3.3 X (SD/Slope)

= 3.3 X (34.8392 / 18.8460)

= 6.10 ig/ml

LOQ = 10 X (SD/Slope)

= 10 X (34.8392 / 18.8460)

= 18.49 ig/ml

System suitability test

It's essential to the chromatographic process. The system's repeatability and resolution are evaluated to ensure that they are adequate for the analysis. System suitability assessments are based on the idea that when all of the components—equipment, electronics, analytical techniques, and sample—come together, they form a cohesive system that can be evaluated as a whole. Table 5 contains all of the features required for system compatibility testing, ensuring that the technique produces precise and accurate findings for its intended use.

Table 9. Statistical Validation of Recovery Study

	MEAN % recovery	SD	% RSD (NMT 2)
Accuracy at 80 %	100.15	0.7840	0.78
Accuracy at 100 %	100.92	0.3981	0.39
Accuracy at 120 %	99.86	0.9819	0.98

Table 10. Robustness data for BEN

Sr. No.	Area at Flow rate 0.9 ml/min	Area at Flow rate 1.1 ml/min	Area at wavelength 235 nm	Area at wavelength 239	Area at buffer pH 3.35	Area at buffer pH 3.65
1.	235.6127	199.1568	237.1048	185.0128	172.5318	255.6127
2.	231.9818	202.6159	231.5149	191.4137	175.0318	221.9818
Avg. area	233.7973	200.8864	234.31	188.213	173.782	223.797
S.D.	2.567434	2.445953	3.95266	4.52612	1.76777	2.56743
% RSD	1.098146	1.217581	1.68694	2.40478	1.01723	1.14721

Table 11. Robustness data for TEL

Sr. No.	Area at Flow rate 0.9 ml/min	Area at Flow rate 1.1 ml/min	Area at wavelength 235 nm	Area at wavelength 239	Area at buffer pH 3.35	Area at buffer pH 3.65
1.	2109.4254	1724.3187	1910.3156	1841.8292	1761.8136	2027.1206
2.	2102.0468	1731.9124	1935.6418	1857.1017	1781.4218	1997.5525
Avg. area	2105.736	1728.116	1922.979	1849.465	1771.618	2012.337
S.D.	5.217458	5.369557	17.90833	10.79929	13.86509	20.9078
% RSD	0.247774	0.310717	0.931281	0.583914	0.782623	1.038981

Accuracy

The procedure's correctness was confirmed by recovery analysis on commercial formulations at three levels of standard addition. The results are shown in tables 6, 7, 8 and 9. The percentage recovery for TEL ranged from 98.86 to 101.04%, whereas the range for BEN was found to be 98.34 to 101.46%.

Robustness

The effect of changes was found to be within the acceptance criteria as shown in Tables 10 and 11. The percentage RSD should be <2%.

Forced degradation study

A variety of factors are used to assess the stability of both medications. This study compared the standard area for sample stability and deterioration. When compared to the other materials, BEN has the highest degree of oxidation and heat breakdown. TEL degrades more quickly in an oxidizing and basic atmosphere. Figure 9-13 displays the peaks of all parameters. Table 12 displays the proportion of degradation for each parameter.

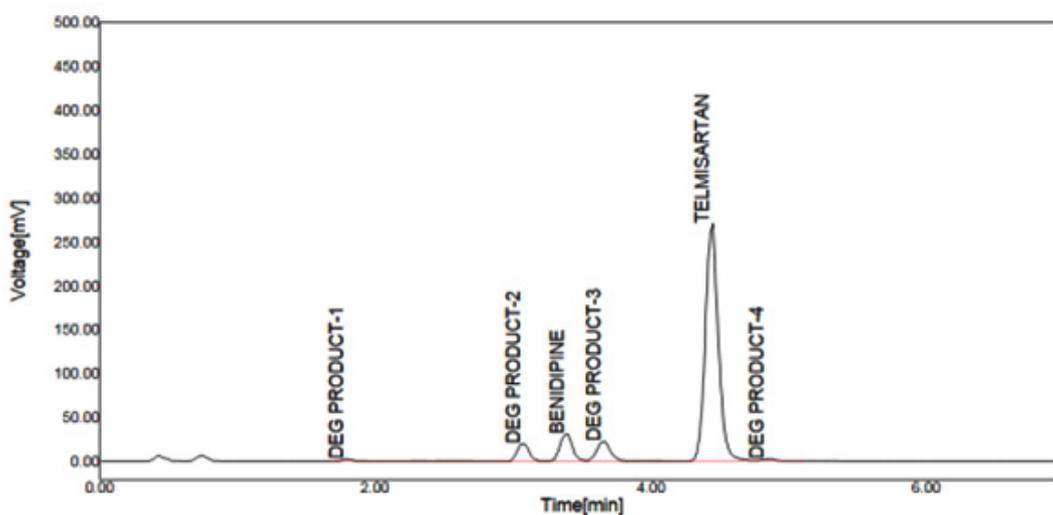


Fig. 9. Chromatogram of Acid Degradation Study

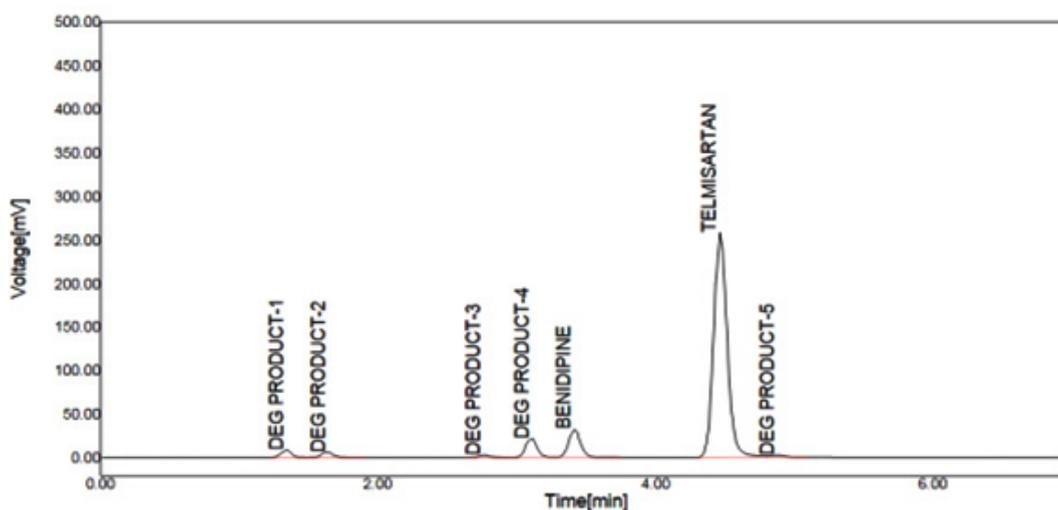


Fig.10. Chromatogram for Basic Degradation Study

Analysis of a commercial sample

It is possible to analyse BEN and TEL in their marketed tablet dose form of 1121.25 mg using the suggested methods. The accuracy of the percent assay values for telmisartan and benidipine were found to be 100.87 % and 98.74 %, respectively. Table 13 provides a summary of the work.

DISCUSSION

At room temperature, the current technique was created by mixing methanol and an ammonium dihydrogen orthophosphate (pH 3.5) buffer in an 80:20 ratio. The redesigned strategy was more practical and cost-effective. The stability indicating RP-HPLC is in charge of the specific interaction

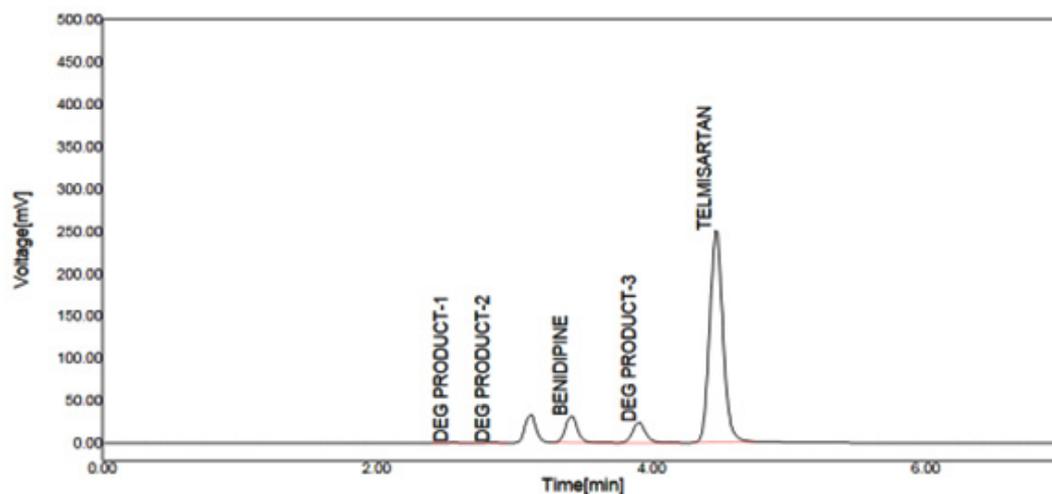


Fig. 11. Chromatogram for Peroxide Degradation Study

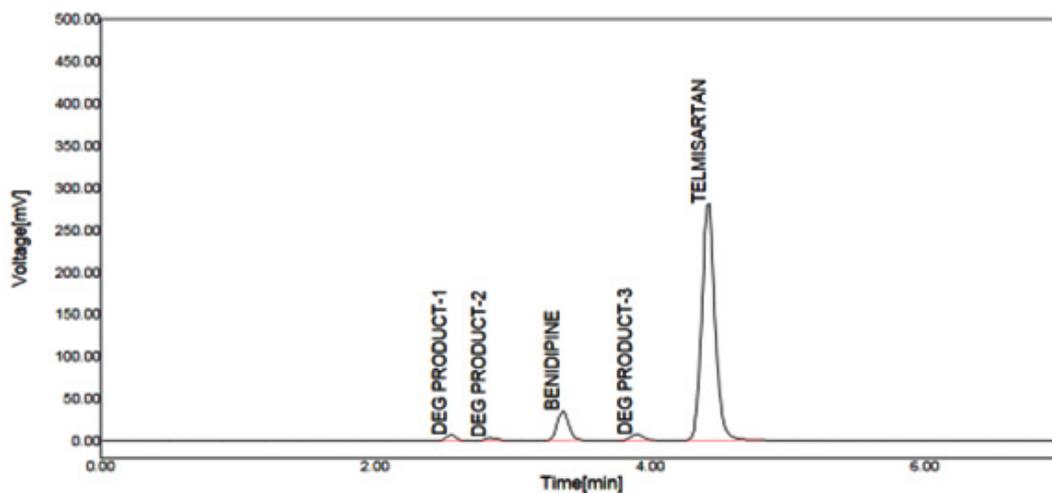


Fig. 12. Chromatogram for Thermal Degradation Study

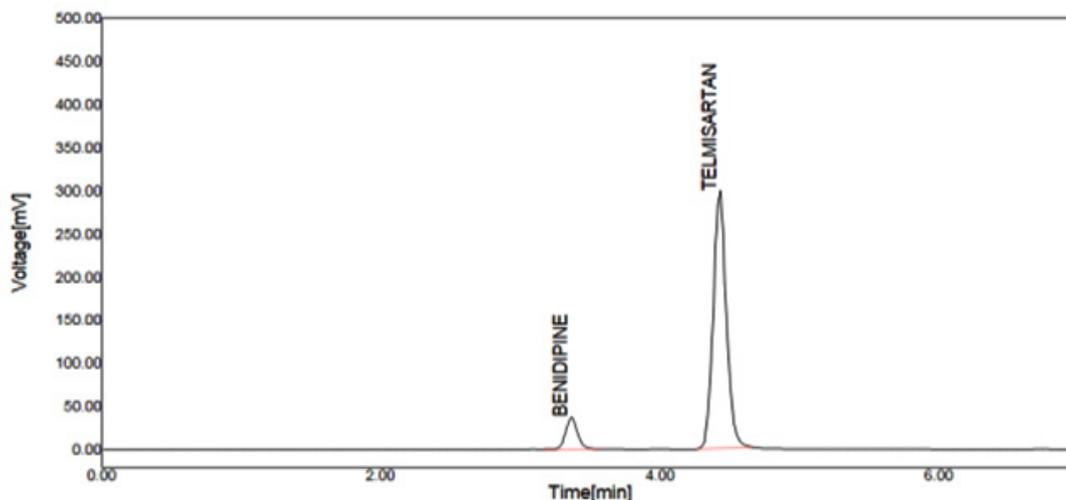


Fig. 13. Chromatogram for Photolytic Degradation Study

Table 12. Result for % degradation and % area after degradation

Stress condition	Min	BENIDIPINE		TELMISARTAN	
		% Area BEN observed after degradation	% of degradation of BEN	% Area TEL observed after degradation	% of degradation of TEL
Acidic	30 min	91.68	8.32	98.53	1.47
Basic	30 min	87.72	12.28	91.35	8.65
Peroxide	12 hr	88.11	11.89	87.98	12.02
Thermal	1 hr	91.77	8.23	97.39	2.61
Photolytic	1 hr	98.64	1.36	99.56	0.44

Table 13. The proposed method's assay results

Parameters	Benidipine	Telmisartan
Area	218.7671	1882.7360
Amount found	4.03	39.50
% Assay	100.87 %	98.74 %

between the active medicinal components and any degradation products produced at the defined conditions. The chemical and its degradants were successfully isolated using the technique outlined below.

CONCLUSION

An RP-HPLC method was developed using a UV/Vis detector to identify Telmisartan and Benidipine Hydrochloride in tablets at the same

time. The unique approach's specificity, linearity, and range, precision, accuracy, and robustness were all evaluated in accordance with ICH guidelines. The results confirmed the accuracy, speed, economy, and resilience of a method designed and validated to separate components using good chromatographic criteria. The technique's pieces are easily distinguishable, and it may be used immediately.

ACKNOWLEDGEMENT

The author would like to thank Kamla Nehru College of Pharmacy, Butibori, Nagpur for providing us the facility for carrying out the research work.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Anuja Shrikhande, Mithali Palandurkar and Swati Vidhate: carried out experiments writing – original draft, data collection and analysis and contributed to visualization and project administration, also contributed to writing, experimentation and result reporting. Parimal Katolkar, Shubham Amadare: conceptualization, RP-HPLC chromatogram data analysis of results, methodology planning, and manuscript preparation, carried out resources and research supervision.

REFERENCES

1. Tripathi KD. *Essentials of Medical Pharmacology*. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd. 2008:778-9.
2. Patel DM, Patel D, Patel A, Sheth A, Shah UJ. Method development and validation for simultaneous estimation of benidipine hydrochloride and metoprolol succinate in tablet. *Journal of Drug Delivery and Therapeutics*. 2019 Dec 15;9(6-s):28-33.
3. Naim M, Ahmed A, GJ KH. Stability indicating reverse-phase high-performance liquid chromatography method development and validation for simultaneous estimation of telmisartan and benidipine hydrochloride in pharmaceutical dosage form. *Asian J Pharm Clin Res*. 2018;11(5):342-50.
4. Patel JM, Dhingani AP, Garala KC, Raval MK, Sheth NR. Development and validation of bioanalytical HPLC method for estimation of telmisartan in rat plasma: application to pharmacokinetic studies. *Dhaka University Journal of Pharmaceutical Sciences*. 2012;11(2):121-7.
5. Ruilope LM. Telmisartan for the management of patients at high cardiovascular risk. *Current medical research and opinion*. 2011 Aug 1;27(8):1673-82.
6. Moffat AC, Osselton MD, Widdop B. *Clarke's Analysis of Drugs and Poisons*, 3rd edn, vol. 2. London: Royal Pharmaceutical Society. 2004:743.
7. Nakatani M, Takeshi S, Ohki T, Toyoshima K, inventors; Boehringer Ingelheim International GmbH, assignee. Solid telmisartan pharmaceutical formulations. United States patent US 8,980,870. 2015 Mar 17.
8. Martindale C. *The complete drug reference*. Sweetman SC, editor. London: Pharmaceutical press; 2009 Mar.
9. Dong MW. *Modern HPLC for practicing scientists*. John Wiley & Sons; 2006 May 19.
10. Swartz M, Krull I. *Developing and Validating Stability-Indicating Methods* by Michael Swartz and Ira Krull.
11. Naim M, Ahmed A, GJ KH. Stability indicating reverse-phase high-performance liquid chromatography method development and validation for simultaneous estimation of telmisartan and benidipine hydrochloride in pharmaceutical dosage form. *Asian J Pharm Clin Res*. 2018;11(5):342-50.
12. Snyder LR, Kirkland JJ, Dolan JW. *Introduction to modern liquid chromatography*. John Wiley & Sons; 2011 Sep 20.
13. Patel BA, Captain AD. Development and Validation of RP-HPLC Method For Estimation Of Telmisartan In Bulk And Formulation Using Fluorescence Detector. *Journal of Biomedical and Pharmaceutical Research*. 201.