SIMULTANEOUS ESTIMATION OF FIXED DOSAGE COMBINATION OF TELMISARTAN AND BENIDIPINE HYDROCHLORIDE IN HUMAN PLASMA BY HPLC-UV

Deepak Dalal[1*], Ravi Kant[2], Mohit Yadav[3]

Abstract

Objective: Simultaneous estimation of fixed dosage combination of Telmisartan and Benidipine hydrochloride in human plasma by HPLC-UV

Methods: The estimation of drugs by RP-HPLC (reverse phase chromatography) was chosen since it is advised for use with ionic and mild to non-polar compounds. Simple, specific, and superior in terms of efficiency, stability, and reproducibility is reverse phase chromatography. For the separation of TEL and BEN, the C18 column 250 x 4.6 mm, 5 µm particle size was chosen. In the mobile phase, various solvent solutions were tested and combined for optimum performance. Telmisartan (40µg/ml) and Benidipine hydrochloride (4 µg/ml) in buffer, pH 4 for Acetonitrile: Water (70:30), a concentration range (8-40 and 0.5-4 µg/ml) as it was displaying excellent peak and a sizable level of resolution. Having the mobile phase run at 1ml/min, photodiode array detectors were used to detect both analytes at 215 nm. The method was validated in different concentration ranges in human plasma.

Result: Telmisartan and Benidipine inter and intra-run precision was measured at less than 3.60% and had an accuracy of less than 1.869%. Telmisartan/benidipine in human plasma was validated by linearity, recovery matrix, and stability. The retention time of TEL and BEN is 3.4 minutes and 4.8 minutes in plasma.

Conclusion: Suggested method denotes that it is cost-effective and appropriate for the analysis of several samples due to the quick, one-step plasma preparation process and the straightforward HPLC-UV isocratic chromatographic apparatus. combination of drugs performed in human plasma by HPLC and UV method.

Keywords: Telmisartan, Benidipine hydrochloride, RP-HPLC, Human Plasma

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INTRODUCTION

Telmisartan's scientific designation is 2-(4-[4-[4-[4-[4-[4-methyl-6-(1-methyl-1H-1,3-benzo-diazol-2-yl)-2-propyl-1H-1,3-benzodiazol-1-yl]methylphenyl] benzoic acid (TEL)].[1] TEL drug also called angiotensin II receptor blocker (ARB) for hypertension has a significant binding for AT1 receptors and has the longest half-life among all ARBs.[2,3] When compared in clinical research to other main anti-hypertensive classes like beta-blockers, calcium antagonists, and ACE inhibitors, TEL has an anti-hypertensive activity that is equivalent to those other classes.[4,5] Benidipine hydrochloride having scientific designation 3-(3R).1-Benzyl-3-Piperidine-5-Methyl(4R)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyrid-ine-3,5-dicarboxylates.[6]

Benidipine hydrochloride is a dihydropyridine calcium channel blocker that is orally active, very potent, and long-acting.[7,8] It blocks a variety of processes both in vivo and in vitro. calcium channel blockers with only one enantiomer of dihydropyridine are used to treat hypertension.[9,10] Additionally, it has been demonstrated to have anti-obesity effects. For each drug separately, several studies were conducted using various analytical techniques.[11-13] For TEL, these included (RP-HPLC), UV-spectrophotometry, Stability studies, absorption correction method and simultaneous estimation equation method, etc.[14]

New fixed-dose combinations (FDC) of both medications have been made available for purchase as tablet formulations by UTH Healthcare.[15,16]

Few methods for this FDC product were found in the literature, however, there were no stability-indicating HPLC methods. To acquire something simple, rapid, accurate, and reasonably priced, an RP-HPLC method with stability indicating properties of fixed-dose combinations of TEL and BEN in pharmaceutical products was developed and validated.[17] By incorporating these analytical techniques into existing practice, the pharmaceutical industry as a whole would benefit from maintaining the quality of its products having active components.[18-20]

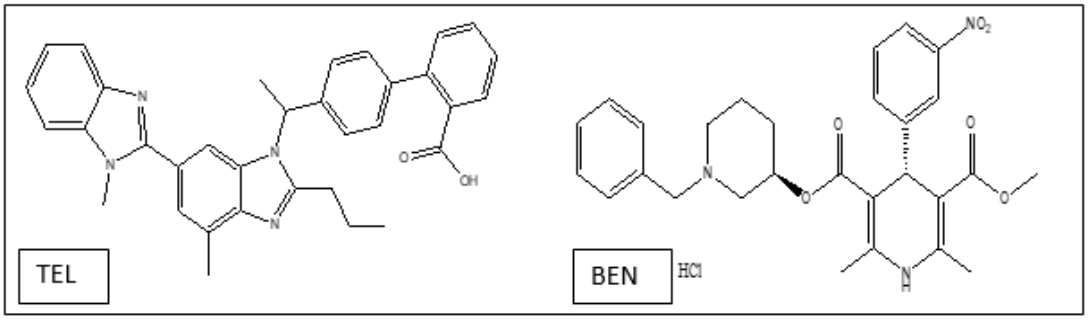

Figure 1: Chemical structure of TEL and BEN

Material and Methods

API of telmisartan and benidipine hydrochloride was obtained as a gift sample from Yash Pharmaceuti
Ahmadabad. BINASTAR-TL tablets as marketed formulation, 40 mg, and 4mg were used.

Human plasma was obtained from Testing Laboratory.

Standard stock solution of TEL (40 mg)

A TEL sample weighing 40 mg was introduced to a volumetric flask of 100 ml and for appropriate volume, methanol was used for volume makeup.

Standard stock solution of Benidipine hydrochloride (4 mg)

100 ml volumetric flask was filled with a 40 mg sample of BND after being weighed. 10 ml of solution was transferred to a volumetric flask of 100 ml, and the remaining capacity should be filled with methanol.

Standard solution of TEL and BND as (40/4 µg/ml) as binary mixtures

Take 10 ml of a volumetric flask and add 1 ml of each stock solution, and then add mobile phase that was to be used in the particular trials.

Sample preparation of Human Plasma

Frozen stored plasma samples for processing were kept at room temperature. After centrifugation for 25 min at 3000 rpm, 2.0 mL of acetonitrile and a 0.97 mL aliquot were pipetted into a 10 mL polypropylene tube. After leaving the mixture for 10 minutes at room temperature, it was centrifuged at 3000 pm for 25 minutes, resulting solution was placed in a bottle and introduced further.

Liquid chromatography/uv spectrophotometry

An HPLC (Agilent brand) equipment was used to analyze plasma samples. Agilent having column HC-18 was used for separation and quantification. To achieve improved resolution of mixed polar and non-polar samples, Water's HPLC column is bonded to C-18 ligands. The Open lab software was used to do the data acquisition. Mobile phase composition is 10mM acetonitrile(pH 4.0) and HPLC grade water in the ratio (70:30). It flowed at a rate of 1 ml per minute. 20 µl was the injection volume. With a UV-Vis detector operating at 215 nm (Fig: 2), the detection was carried out.

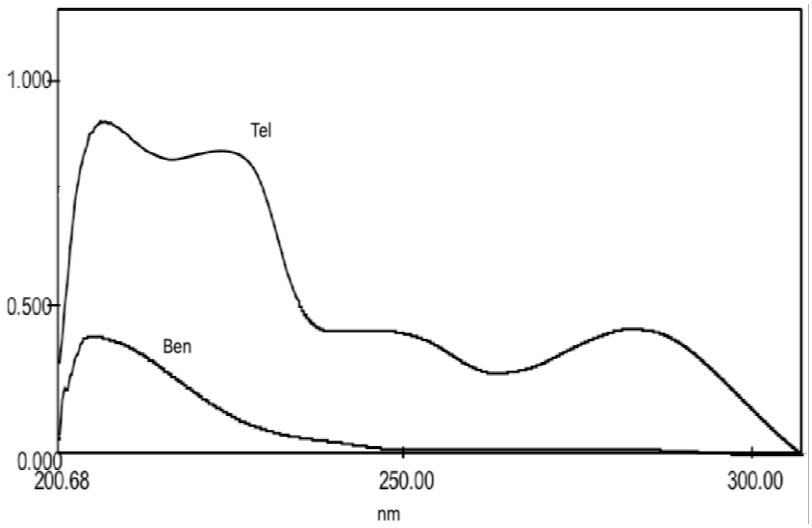

Figure 2: UV spectra of Tel (40µg/ml) and Ben (4µg/ml)

Validation of Method

The methodology was validated by USFDA specifications for validation in bio-analytical techniques, including those on specificity, accuracy, precision, linearity, recovery, and stability.[19-22]

Analyzing five distinct blank samples of human plasma allowed researchers for examining the method's selectivity. Using the current analytical technique, all blank samples were examined for involvement and comparison to a spiked sample whose analyte concentration remain at LLOQ. Three different batches of calibration standard samples of plasma were created. By measuring TEL, and BEN in five samples for three separate concentrations for three different batches of human plasma, possible to assess the precision and accuracy within and between the batches.

By comparing the anticipated TEL/BEN in drug-free (A) and drug-free (B) volunteers at various levels of concentration from concentrations that were in spiked quality control (QC) specimens in six biological matrices, the matrix effect was explored. Matrix influence on the IS was also evaluated using the peak regions at their working concentration level.

The peak ratio of either concentration (A/B 100) was used to characterize the matrix effect. Each person's biological matrix was utilized only once to prepare quality control samples as well as the same concentration level of blank samples. To obtain great performance, the integrate-subject variation of the matrix effect at every single concentration range must be less than 10%. However, the stability of TEL and BEN in the biological matrix and working solutions was evaluated, and results were provided as % recoveries research on (concentration of sample under different storage conditions/theory concentration). At room temperature, six hours were used to test the stability of the working solutions for TELM and BEN.

Although stability investigations on human plasma in this medication were evaluated under several study settings, including bench top stability (standing at room temperature for 24 hours), samples at different lower, medium, and higher concentration ranges, along with long-term stability (storing at -25 °C for a month). Together, three freeze-thaw cycles of Quality control samples (freeze-thaw stability) were examined.[23-25]

RESULTS AND DISCUSSION

A variety of columns and mobile phases with different pH values and organic modifiers were tested to determine whether they could provide enough selectivity and sensitivity in short separation durations to obtain the best chromatographic conditions. The most optimal

chromatographic results are obtained using a cyan o-propyl column and acetonitrile (pH 4): HPLC grade water (70:30) mobile phase having a flow rate of 1 ml/minute with a UV spectrophotometer wavelength of 215 nm. (Fig: 2)

Careful research was done on the effects of both pH and organic modifier concentration. Due to the BEN peak's interference with endogenous biological substances, In addition to reducing run time and improving peak form, boosting the organic modifier concentration also lessens method specificity.

Less than 20% of an organic modifier concentration reduction produced great specificity for the separation of the examined drug in distinction endogenous biological components as well as more drug retention on the column, which increased peak tailing and prolonged run times.

Variation in pH is crucial to the separation process at pH (4), TEL is moreover strongly maintained on the column. In addition to superior peak shape and suitable run time, pH (4.0) was determined to be the ideal value for separating medicines from endogenous biological components. Utilizing an internal standard is important because samples that require extensive pretreatment (extraction, filtration, etc.) or preparation result in sample losses.

To correct sample losses, an internal standard should be carefully selected. Due to retention characteristics that were not significantly impacted by changes in pH, which allowed it to elute from TEL and BEN with a reasonable resolution, as well as absorbance characteristics that demonstrated high absorbance at the wavelength (215 nm).

Following oral administration of BINASTAR-TL tablets (40 mg TEL, 4 mg BEN) for 2 hours, the studied mixture can be successfully determined using the suggested method in both spiked plasma samples and actual patient plasma samples (Figs: 3&4).

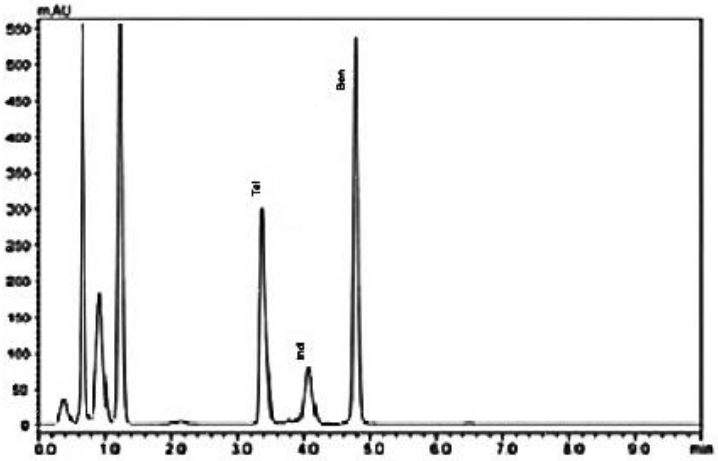

Figure 3: Shows the HPLC chromatogram of a 20 L injection of the drugs TEL, BEN, as well as indapamide (Ind) as an I.S. spiked in human plasma.

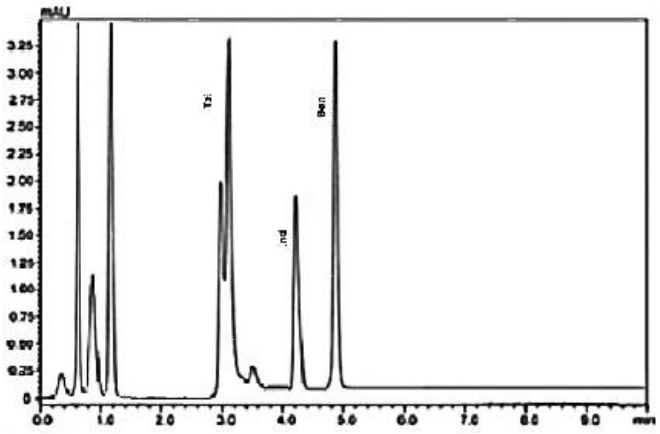

Figure 4: Shows the HPLC chromatogram of a 20 L injection of patient plasma 2.5 hours after the BINASTAR-TL tablet (40 mg TEL & 4 mg BEN) was taken orally.

Parameters for method validation

Linearity

Examining TEL and BEN with their five concentrations ranging from 8 to 40 and 0.5 to 4 µg/ml, and comparing the peak area ratio to the relevant concentration, linearity was determined. Its linearity was confirmed by the calibration graphs' intercept value and the correlation coefficient's high value (>0.98). (Table 1). The practical range was taken into account to create the calibration range for the suggested approach, and the concentration range present in pharmaceutical products for providing accurate as well as precise results.

Table 1: Binary mixture determined: Assay parameters and Regression characteristic

Parameters	Telmisartan	Benidipine
Linearity R(µg/ml)	8-40	0.5-4
Detection L (µg/ml)	0.053	0.043
N	6	6
Quantitation L(µg/ml)	0.181	0.138
Slope (b)	0.045	0.528
S.D of slope	0.001	0.009
R.S.D of slope	2.167	1.721
S.D of intercept	0.006	0.036
Intercept (a)	0.007	0.023
Correlation coefficient (r)	0.996	0.995
S.E. of regression	0.005	0.084

Regression equation: A = a + bc,
where a = absorbance, a = intercept, b = slope, c = concentration
R = Range, L = Limit

Accuracy

For accuracy, procedure was repeated three times—once for low, once for medium, and once for high concentrations of the samples. Each concentration was determined using the matching regression equation. The findings were then tabulated, and Table 2 displayed the mean recoveries, recovery percentages, and relative error.

Table 2: Results of validation parameters for the proposed technique

Drugs	Conc.(µg/ml)	Conc. Found(µg/ml) {Mean ± S.D}	Intra day % RSD	Inter day % RSD	% RE
TEL	8	6.995 ± 0.016	1.691	2.221	-11.421
	24	23.990 ± 0.068	1.351	3.156	-0.810
	40	40.100 ± 0.078	0.788	1.772	0.200
BEN	0.5	0.490 ± 0.004	0.841	0.280	-4.032
	2	1.994 ± 0.073	3.588	2.993	-0.6233
	4	4.100 ± 0.071	2.998	1.402	1.901

Table 3: Stability of TEL and BEN at varying conditions in plasma Conc. (µg/ml)

	TEL			BEN		
	8	24	40	0.5	2	4
(1) Three Freeze thaw cycles						
Mean	7.987	24.023	40.134	0.459	1.854	4.120
S.D.	0.016	0.12	0.089	0.004	0.091	0.079
% R.S.D	1.52	1.98	0.85	0.91	3.21	2.39
% RE	-1.8	0.5	0.7	-4.01	-6.41	1.98
(2) 24hr. Room temp.	7.715	23.897	40.012	0.48	1.898	4.12
Mean	0.016	0.056	0.078	0.02	0.06	0.04
S.D.	1.62	1.44	0.753	1.79	3.56	0.069
% R.S.D	-11.7	-0.9	0.2	-6.67	-0.98	-0.459
% RE						
(3) Re-injection at -10°C after 1 month						
Mean	0.798	24.321	40.12	0.491	2.03	3.92
S.D.	0.03	0.138	0.081	0.02	0.03	0.09
% R.S.D	2.18	2.71	0.803	1.659	2.31	2.62
% RE	-6	2.3	0.4	-4.89	-6.34	-2.81

Precision
The assurance of quality samples was examined six times each within the same day and over three subsequent days to gauge the degree of procedure repeatability (see Table 2).

Stability
The stability studies for the analytes were made to account for typical circumstances involving the processing of clinical samples.

Under varied processing and storage circumstances, the stability of the analytes in plasma was examined. Table 3 provides an overview of the outcomes. According to the findings, TEL and BEN remained constant throughout the experiment.

Selectivity and Sensitivity
At the analyte retention times, there was no evidence of an endogenous source of interference. The typical chromatogram of spiked plasma samples with BEN and TEL is shown in (Fig. 3).

Table 4: A statistical evaluation of the variables needed for the presented method's system suitability assessment.

Parameters	Value obtained		Reference
	TEL	BEN	
1. Resolution [Rs]	4.653	5.001	R > 0.9
2. Tailing factor [T]	1.001	1.088	T = 1
3. Relative retention	2.250	2.645	>1
4. No.of theoretical plates[N]	2300	3655	Efficiency Increases with separation
5. Capacity factor [K]	8.000	2.300	1-10
6. HETP [cm/plate]	0.012	0.007	Smaller value, higher column efficiency
7. Retention time	3.4	4.8	

LOD and LOQ
A regression line's residual standard deviation and slope were used to discover (LOD) and (LOQ), Using samples that contained analytes in range, a specific calibration curve was built for the calculations of LOD and LOQ, For telmisartan and benidipine, a ratio of signal-to-noise of 3:1 and 10:1 was calculated. Telmisartan and Benidipine had respective LODs of 0.017 and 0.023 µg/ml and LOQs of 0.058 and 0.071µg/ml.
To determine LOD and LOQ, the following formulae were used.
LOD = Ca x Rv/b
LOQ = Cg × Rv/b
where Rv is the residual variance from the regression, b is the slope, and Ca/Cg is the coefficient for LOD/LOQ.

Ruggedness
The suggested HPLC procedure proved to be reliable on the HPLC instrument (Shimadzu, Japan) while analyzing a different colleague analyst as well. The robustness of the procedure was shown by the identical results and retention times (#0.01 min), which showed there were no substantial differences from one lab to another, one instrument to another, one analyst to another, and from one time to another.

Robustness
When it was attempted to purposely change the organic strength (2.5%) and pH (1.0 unit) for the mobile phase, the suggested HPLC method showed to be dependable because the duration of retention of the peaks was not considerably influenced (#0.01 min).

Studies on system suitability
For the operational standard solutions, (Table 4) displays the resolution, theoretical plate count, and peak asymmetry. The results showed that the technique was appropriate for analyzing these medications in combination with human plasma.

Comparing the proposed technique to the existing method
It was found that: When comparing the suggested method with the existing method for a concurrent estimate of the binary mixture.

1. No work done on this combination on human plasma by HPLC.
2. The suggested approach is very delicate. (linearity range: 8-40 and 0.5-4 µg/ml for TEL and BEN) while comparing with the established method (linearity range: 1.0-0.700 g/ml for both analytes).
3. The only sample preparation step before RP-HPLC in the described technique is acetonitrile-based precipitation of proteins, as opposed to

other methods, which rely on liquid-liquid extraction and require extensive sample preparation before injection, which increases the risk of drug loss during preparation.
4. The published method, however, does pharmacokinetic research and has a shorter run time.
5. The suggested method uses isocratic elution, which is easier to use than gradient elution in the other method. Additionally, Compared to LC-MS/MS, HPLC/UV is less expensive.

CONCLUSION

TEL/BEN in human plasma is quantitatively determined using an efficient, straightforward, and quick HPLC-UV technique. The suggested HPLC methodology is an efficient method for simultaneously determining the drugs in the human plasma in combined dosage form, a precise and accurate procedure using RP-HPLC. The chromatographic method using HPLC is economical and ideal for the routine analysis of samples. The method can be used for therapeutic drug monitoring in humans. This technique has been validated to satisfy ICH standards for sensitivity, accuracy, precision and other parameters as per ICH Q2R1 guidelines.

Abbreviations

HPLC: High performance liquid chromatography; UV: Ultraviolet; ICH: International Conference on Harmonization; LOQ: Limit of quantitation; LOD: Limit of detection; RSD: Relative standard deviation; TEL: Telmisartan; BEN: Benidipine hydrochloride; IND: Indapamide

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