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Research Article

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Validated UV-Spectrophotometric Method for Simultaneous Estimation of Benidipine Hydrochloride and Telmisartan in Bulk and Pharmaceutical Dosage Form

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ABSTRACT

A simple, economical, accurate, precise and less time consuming UV spectrophotometric method has been developed and validated for simultaneous estimation of Benidipine hydrochloride and Telmisartan in bulk and pharmaceutical dosage form. In this method Benidipine HCl and Telmisartan are exhibits maximum absorbance (λ_{max}) at 237 nm and 296 nm with methanol as the solvent. The method was validated as per the International Conference on Harmonization (ICHQ2R1) guidelines. Drugs followed the linearity in the concentration range of 5-25 µg/mL and 2-10 µg/mL with correlation coefficient (r^2) of 0.999 for Benidipine HCl and Telmisartan respectively. The validity of the proposed method was assessed by applying the standard addition technique where the percentage recovery of the added standard was found to be 98.68 and 99.6 for Benidipine HCl and Telmisartan. The limit of detection and quantification were calculated and found to be 1.529 µg/mL and 4.634 µg/mL and 0.457 µg/mL and 1.386 µg/mL for Benidipine HCl and Telmisartan respectively. The proposed method is recommended for routine analysis of Benidipine hydrochloride and Telmisartan in bulk and pharmaceutical dosage forms in regular quality control testing laboratories.

Keywords: Benidipine hydrochloride; Telmisartan; UV spectrophotometry; Beer's law; Validation

INTRODUCTION

Hypertension (HT) is a very common disorder, particularly for past middle age. It is not a disease in itself, but is an important risk factor for cardiovascular mortality and morbidity. For improved treatment of hypertension, Telmisartan and Benidipine HCl is the newer combination in market, this combination was developed to improve medication for stage II hypertension.

Benidipine Hydrochloride (BEN HCl) is a dihydropyridine type of Calcium channel blockers used for the treatment of hypertension and angina pectoris. Chemically it is 5-*O*-[(3*R*)-1-benzylpiperidin-3-yl] 3-*O*-methyl (4*R*)-2, 6-dimethyl-4-(3-nitrophenyl)-1,4 dihydropyridine-3,5-dicarboxylate; It is very soluble in formic acid, soluble in methanol, slightly

soluble in ethanol and practically insoluble in water. It acts by inhibiting transmembrane Ca^{2+} influx through the voltage dependent channels of smooth muscles in vascular walls [1].

Telmisartan (TEL) belongs to angiotensin receptor blockers with chemical name 2-[4-[[4-methyl-6-(1-methylbenzimidazol-2-yl)-2-propylbenzimidazol-1yl] methyl] phenyl] benzoic acid. It is soluble in strong base and methanol and sparingly soluble in strong acid (except HCL). It interferes with the binding of angiotensin II to the angiotensin II AT_1-receptor by binding reversibly and selectively to the receptors in vascular smooth muscle and the adrenal gland. Angiotensin II is a vasoconstrictor, which also stimulates the synthesis and release of an aldosterone blockage of its effects results in decrease in systemic vascular resistance [2].

Both combinations of Telmisartan and Benidipine drugs are used for the treatment of hypertension effectively. They work by relaxing the blood vessels and making the heart more efficient at pumping blood throughout the body. Literature survey revealed that the reported methods like UV and stability indicating RP-HPLC methods development and validation for estimation of Telmisartan and Benidipine HCl in bulk and pharmaceutical dosage form individually and in fixed dose combination.

The literature survey revealed that some UV methods [3-7] and RP-HPLC methods [8-9] are reported for the determination of BEN HCl and some UV methods [10-14] and RP-HPLC methods [15-22] are reported for the determination of TEL individually and in both combination UV method [23] and HPLC methods [24-26]. Present study involves the development and validation of a new UV Spectrophotometric method for the determination of BEN HCl and TEL in bulk and in its pharmaceutical dosage form with good accuracy and precision. The analytical method was validated according to ICHQ2R1 validation parameters [27-28]. Chemical structure of Benidipine HCl and Telmisartan are shown in Figure 1.

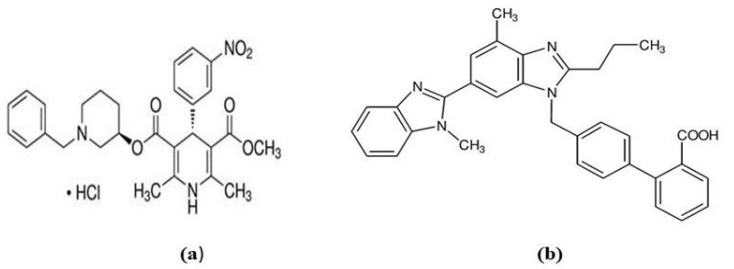

Figure 1: Chemical structure of (a) Benidipine Hydrochloride (b) Telmisartan

MATERIALS AND METHODS

Chemicals and Reagents

Benidipine hydrochloride (≥ 99%), was bought from yarrow chem products, Mumbai, India and Telmisartan (≥ 98%) standard drugs (API) were obtained as gift samples from Dr. Reddy's Laboratories, Hyderabad, India. HPLC grade Methanol was purchased from thermo fisher scientific India Pvt. limited, Mumbai, India. Water purified by the Milli-Q water purification system was used in the study. The rest of the chemicals and reagents were procured from standard commercial supplier.

Equipments

Shimadzu (UV-1780) double beam UV-visible spectrophotometer with 1 cm matched quartz cells was used for the measurement of absorbance. Shimadzu-AX-200 electronic balance was used for weighing the samples. Citizen-ultrasonicator and class 'A'volumetric glassware's were used.

Selection of Solvent for Analysis

In the present study the UV spectra of BEN HCl and TEL were obtained from different solutions (methanol, acetonitrile, distilled water, formic acid, dimethyl sulfoxide, combination of methanol and water (9:1) methanol and formic acid (5:5)) were studied. The two drugs were freely soluble in methanol, acetonitrile, formic acid, dimethyl

sulfoxide, combination of methanol and water (9:1), methanol and formic acid (5:5). At the end of these studies, Methanol was chosen as solvent for studied drugs.

Selection of Detection Wavelength for Simultaneous Estimation

In the present study the drug solutions of BEN HCl (25 µg/mL) and TEL (10 µg/mL) were prepared and scanned over a range of 200-400 nm. It was observed that the drugs showed maximum absorbance at 237 and 354 nm for BEN HCl and 296 nm for TEL. The overlay spectra of BEN HCl and TEL (25 µg/mL and 10 µg/mL) are shown in Figure 2. Which was chosen as the detection wavelength (Isosbestic wavelength 245 nm) for the determination of Benidipine HCl and Telmisartan?

Preparation of Stock and Working Standard Solutions of Analytes

Primary stock solutions of BEN HCl and TEL were prepared separately by dissolving accurately weighed 10 mg of each pure drug samples in methanol in 100 mL volumetric flasks and made the volume up to the mark using same solvent. Further, 1 mL of BEN HCl and TEL were transferred into 10 mL volumetric flasks and diluted with methanol solution to produce final concentration of 100 µg/mL each of BEN HCl and TEL respectively.

Calibration Curve

Appropriate aliquots (0.5, 1.0, 1.5, 2.0, 2.5 mL and 0.2, 0.4, 0.6, 0.8, 1.0 mL) of prepared working standard solutions of BEN HCl and TEL we transferred into series of 10 mL volumetric flasks and diluted and made up to the mark with methanol to obtain final concentration of 5-25 µg/ml of BEN HCl and 2-10 µg/ml of TEL respectively. The above solutions were scanned over the range of 200 nm to 400 nm against reagent blank. The absorbances of each solution were measured at 245 nm against methanol as blank. A calibration curve was prepared by plotting absorbance versus concentration.

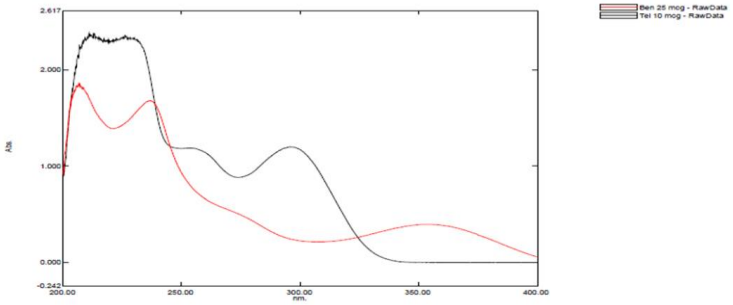

Figure 2: Overlay Spectra of Benidipine HCl and Telmisartan (25 µg/mL and 10 µg/mL)

Estimation of Benidipine HCl and Telmisartan in Tablets

For the analysis of dosage form, twenty tablets of Benidin T (Benidipine HCl and Telmisartan 4 mg and 40 mg) were ground to fine powder and mixed thoroughly. A quantity of powder equivalent to 10 mg of each of the drug was transferred to 100 mL volumetric flask and dissolved in about 20 mL methanol sonicated for 10 min and volume was made up to mark with the same solvent. The insoluble excipients were separated by filtration through Whatman filter paper. After suitable dilution, absorbance of the prepared sample solutions (25 µg/mL and 10 µg/mL) of Benidipine HCl and Telmisartan was recorded against the reagent blank at 245 nm.

RESULTS AND DISCUSSION

Method validation was performed by following the International Conference on Harmonization (ICHQ2R1) guidelines.

Linearity: Fresh aliquots were prepared from standard stock solution ranging from 5-25 µg/mL and 1-10 µg/mL of Benidipine HCl and Telmisartan. The absorbance values of each concentration were recorded at 245 nm. For this study methanol was used as a blank. Results of Linearity study were shown in Table 1 and Figure 3.

Table 1: Linearity results of Benidipine HCl and Telmisartan

S. No.	Benidipine HCl		Telmisartan	
	Concentration (µg/mL)	Absorbance	Concentration (µg/mL)	Absorbance
1	5	0.213	2	0.223
2	10	0.446	4	0.421
3	15	0.639	6	0.623
4	20	0.852	8	0.832
5	25	1.045	10	1.021

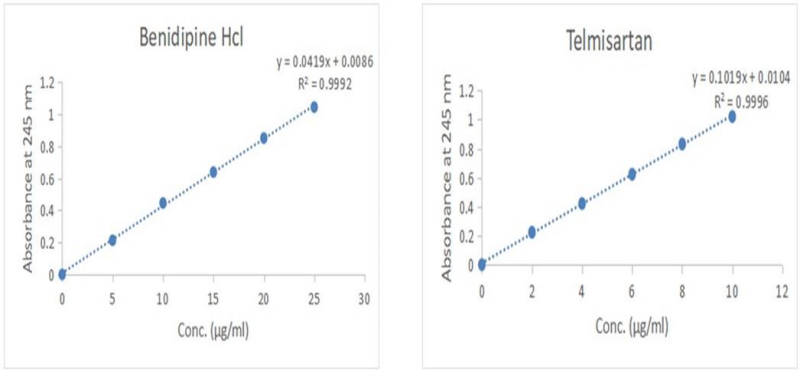

Figure 3: Linearity curves of Benidipine HCl and Telmisartan

Accuracy: Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges, each one in triplicate. From the recovery studies it was clear that the method remains very accurate for quantitative estimation of tablet as the statistical results were within the acceptance range. Results of accuracy study were shown in Table 2.

Table 2: Accuracy data of Benidipine HCl and Telmisartan.

S.No.	Benidipine HCl				Telmisartan			
	µg/mL added	µg/mL found	% Recovery	mean% recovery	µg/mL added	µg/mL found	% Recovery	Mean% recovery
1	5	5	100	99.8	2	1.98	99.1	99.9
2	5	4.97	99.5		2	2	100	
3	5	5	100		2	2	100	
1	15	15.02	100.1	100.1	6	5.97	99.5	99.9
2	15	15	100		6	6	100	
3	15	15.07	100.4		6	6.01	100.3	
1	25	25.02	100.09	100.3	10	10	100	100
2	25	25.04	100.1		10	10.01	100.1	
3	25	25.09	100.3		10	10.01	100.1	

Precision: The intraday and interday precisions were executed by analyzing six independent analyses of 15 µg/mL and 6 µg/mL of Benidipine HCl and Telmisartan. The standard deviations, relative standard deviation was calculated and results of the study are acceptable and can be considered to be very reasonable the results are summarized in Tables 3 and 4.

Table 3: Intra-day and Interday precision data of proposed method

S.No.	Conc. (µg/mL)	Benidipine HCl			Telmisartan			
		FN	AN	EN	(µg/mL)	FN	AN	EN
1	15	0.639	0.638	0.637	6	0.623	0.622	0.621
2	15	0.638	0.638	0.635	6	0.622	0.622	0.623
3	15	0.639	0.637	0.636	6	0.623	0.621	0.621
4	15	0.638	0.638	0.637	6	0.621	0.622	0.622
5	15	0.639	0.636	0.637	6	0.623	0.623	0.621
6	15	0.637	0.637	0.636	6	0.622	0.621	0.622
Mean		0.638	0.637	0.636		0.622	0.621	0.621
SD		0.0008	0.0008	0.0008		0.0008	0.0007	0.0008
%RSD		0.127	0.128	0.128		0.131	0.121	0.131

Table 4: Inter-day precision (Reproducibility) data of proposed method

S.No.	Conc. (µg/mL)	Benidipine HCl			Conc. (µg/mL)	Telmisartan		
		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3
1	15	0.636	0.630	0.634	6	0.619	0.62	0.617
2	15	0.635	0.628	0.633	6	0.618	0.62	0.615
3	15	0.634	0.629	0.632	6	0.620	0.619	0.614
4	15	0.636	0.630	0.630	6	0.619	0.620	0.616
5	15	0.634	0.629	0.632	6	0.619	0.618	0.618
6	15	0.635	0.628	0.629	6	0.618	0.619	0.617
Mean		0.635	0.629	0.631		0.618	0.619	0.616
SD		0.0008	0.0008	0.0018		0.0007	0.0008	0.0014
%RSD		0.128	0.142	0.294		0.121	0.131	0.238

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of Benidipine and Telmisartan by proposed method were determined using calibration curve. LOQ and LOD were calculated as

$LOD = 3.3 \times S.D/S$

$LOQ = 10 \times S.D/S$

Where S is the slope of the calibration curve and SD is the standard deviation of response of least concentration of calibration curve in three replicates. Results of the study were shown in Table 5.

Table 5: LOD and LOQ of Benidipine HCl and Telmisartan

S.No.	Drugs	LOD (µg/mL)	LOQ (µg/mL)
1	Benidipine HCl	1.529	4.634
2	Telmisartan	0.457	1.386

Robustness: Robustness of the method was determined by carrying out the analysis at three different wavelengths (± 2 nm). The respective absorbance was noted and the result was indicated by % RSD. Results of the study were shown in Table 6.

Table 6: Robustness studies data of the proposed method

S. No.	Conc. (µg/mL)	Benidipine HCl			Conc. (µg/mL)	Telmisartan		
		243 nm	245 nm	247 nm		243 nm	245 nm	247 nm
1	15	0.628	0.634	0.641	6	0.609	0.619	0.628
2	15	0.625	0.633	0.640	6	0.608	0.618	0.626
3	15	0.626	0.632	0.642	6	0.607	0.620	0.627
4	15	0.627	0.63	0.641	6	0.609	0.619	0.625
5	15	0.626	0.632	0.643	6	0.608	0.619	0.627
6	15	0.624	0.629	0.641	6	0.606	0.618	0.624
Mean		0.626	0.631	0.641		0.607	0.618	0.626
SD		0.0014	0.0018	0.0010		0.0011	0.0007	0.0014
% RSD		0.2259	0.2947	0.1610		0.1923	0.1216	0.2350

Application of the proposed method to tablet dosage form: The proposed methods were applied to the quantification of Benidipine HCl and Telmisartan in tablet dosage forms. The results shown in (Table 8), suggest that the method is suitable for the determination of Benidipine HCl and Telmisartan with good accuracy and precision. The excipients in the dosage forms do not interfere in the assay procedure.

Table 8: Assay results of Benidipine HCl and Telmisartan in tablet dosage form

Formulation	Label Claim (mg/tablet)	Amount Found (mg/tablet)	%Assay
Benidin T	Benidipine- 4	3.93	98.2
	Telmisartan- 40	39.8	99.5

Optical characteristics: Optical characteristics such as Beer's law limit (µg/mL)**,** Correlation coefficient, Regression equation, Slope (m) and Intercept (c) were calculated and shown in (Table 9).

Table 9: Optical characteristics

S.No.	Optical characteristics	Observed Values	
		Benidipine HCl	Telmisartan
1	Beer's law limit	5-25 µg/mL	2-10 µg/mL
2	Correlation coefficient (r^2)	0.9992	0.9996
3	Regression equation	Y=0.0419x+0.0086	Y=0.1019x+0.0104

4	Slope (a)	0.0419	0.1019
5	Intercept (b)	0.0086	0.0104
6	LOD	1.529 µg/mL	0.457 µg/mL
7	LOQ	4.634 µg/mL	1.386 µg/mL

CONCLUSION

The developed method was found to be precise as the %RSD values for intra-day and inter-day were found to be less than 2%. Good recoveries of the drugs were obtained at each added concentration, which indicates that the method was accurate. The LOD and LOQ were found to be in microgram level, which indicates the sensitivity of the method. The method was also found to be robust as indicated by the % RSD values which are less than 2%. The results of assay shows that the amount of drugs was in good agreement with the label claim of the formulation as indicated by % assay. The proposed method also can be used for the routine quality control analysis of Benidipine HCl and Telmisartan in bulk and pharmaceutical formulations.

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