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Original Research Paper

SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF BENIDIPINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION

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ABSTRACT

The present study was aimed to develop a novel simple, accurate, precise, robust and economic UV-spectrophotometric method for estimation of benidipine hydrochloride in pharmaceutical formulation. The developed method was validated with accordance to ICH Q2 (R1) guidelines. Beer's law was obeyed in the concentration range 1-3.5µg/mL with correlation coefficient of 0.9938. The sensitivity was checked as the limit of detection and limit of quantitation which were found to be 0.04540 and 0.1375µg/mL respectively. A further study of the accuracy of the proposed method was performed using standard addition method. It can be concluded that the developed method was specific, selective, precise and robust. This method could be successfully applied for analysis of benidipine hydrochloride in its pharmaceutical formulation.

Keywords: Spectrophotometric, Benidipine hydrochloride, Calcium channel blocker, Tablet.

INTRODUCTION

Chemically benidipine hydrochloride is 3-(3R)-1-benzylpiperidin-3-yl-methyl-(4R)-2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (Figure-1). Benidipine hydrochloride is a dihydropyridine calcium channel blocker, used for the treatment of high blood pressure (hypertension). It is a triple L-, T-, and N-type calcium channel blocker. It is reno- and cardioprotective. Benidipine is a calcium channel blocker. Benidipine hydrochloride has additionally been found to act as an antagonist of the mineral corticoid receptor.[1,2]

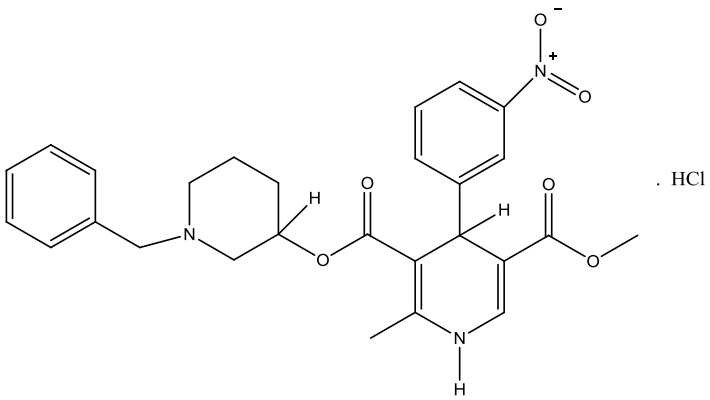

Figure 1: Structure of benidipine hydrochloride

Benidipine hydrochloride is official only in Japanese Pharmacopoeia.[2] Several methods have been reported for the analysis of benidipine hydrochloride using HPLC,[3,4] LC-MS/MS[5], UV-Visible[6,7] and colorimetric.[8] Most of these are either time consuming; involve expensive instrumentation or the use of excess organic solvents. There is no direct UV spectrophotometric method reported in the literature for the estimation of benidipine hydrochloride. The present study describes a simple, sensitive, accurate and precise spectrophotometric method for estimation of benidipine hydrochloride in tablet formulation.

MATERIAL AND METHODS

Apparatus and Instrument

Double beam UV-visible spectrophotometer (Shimadzu, model 1601) having two matched quartz cells with 1cm light path and Digital analytical balance (Shimdzu ATX 224).

Material Required

Benidipine hydrochloride received as gift sample from Prayosha Healthcare Pvt, Ltd, Akleshwar. The methanol AR grade (Astron chemical India) was purchased from market.

Selection of Solvent

The drug was very soluble in formic acid, Soluble in methanol, slightly soluble in ethanol practically insoluble in water. Therefore, methanol was selected as a solvent for analysis.

Preparation of Standard Stock Solution-

Accurately weighed benidipine hydrochloride (10mg) was transferred to a 100 ml volumetric flask, dissolved and diluted up to mark with methanol to obtain standard solution having concentration of 100µg/ml (sonicated at 37˚C for 5 min as necessary).

Working Standard Stock Solution

1ml standard stock solution was transferred to a 10 ml volumetric flask, dissolved and diluted up to mark with methanol to obtain standard solution having concentration of 10µg/ml (sonicated at 37˚C for 5min as necessary).

Determination of Analytical Wavelength

Working standard solution of benidipine hydrochloride was scanned in the range of 200-400 nm. The drug showed good absorbance at 355 nm, so it was selected as wavelength for development of spectrophotometric method [Figure-2].

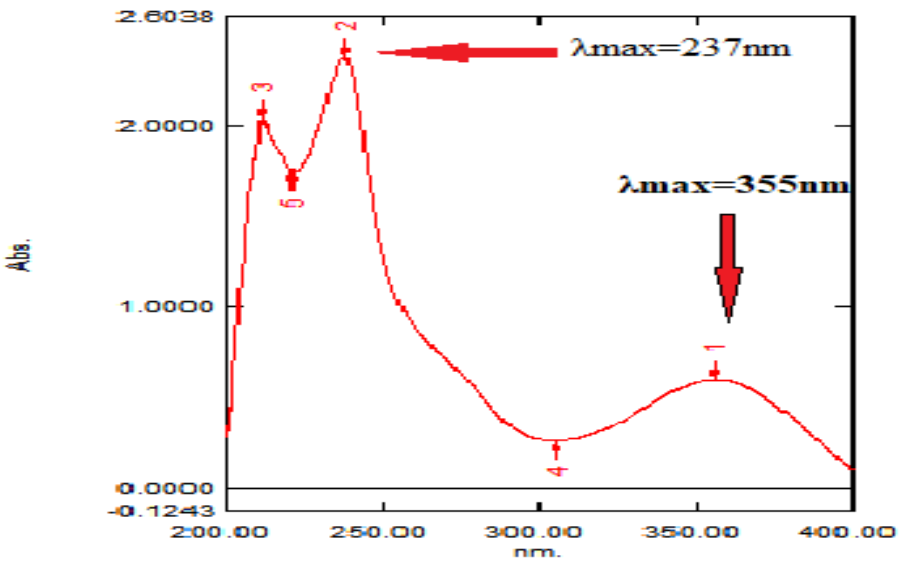

Figure 2: UV spectra of benidipine hydrochloride

Preparation of Calibration Curve

Appropriate aliquots of the working standard solutions of benidipine hydrochloride (10 µg/ml) were transferred to series of 10 ml volumetric flask and diluted up to 10 ml with methanol to give final concentration of benidipine hydrochloride in the range 1-3.5 µg/ml. The absorption spectra of above solutions were recorded and the absorbances at 355 nm were measured. Regression equation for benidipine hydrochloride was calculated from calibration curve.

Method validation

The above method was validated for various parameters such as Accuracy, Linearity, Precision, Limit of detection (LOD) and Limit of Quantitation(LOQ) according to ICH guideline.[9]

Linearity and Range

Different aliquots of benidipine hydrochloride in the range 1–3.5 ml were transferred into series of 10 ml volumetric flasks, and the volume was made up to the mark with methanol to get concentrations 1, 1.5, 2, 2.5, 3, and 3.5 µg/ml, respectively. The solutions were scanned on a spectrophotometer in the UV range 200–400 nm. The spectrum was recorded at 255 nm. The calibration plot was constructed as concentration *vs*. absorbance. The linearity was assessed in terms of slope, intercept, & correlation co-efficient values.

Repeatability

Repeatability was determined by analysing 1.5 µg/ml concentration of benidipine hydrochloride solution for five times.

Intraday & Interday Precision

Precision of the method was studied as intraday and interday variations. Intraday precision was determined by analyzing the 1, 2 and 3 µg/ml of benidipine hydrochloride solutions for five times in the same day. Interday precision was determined by analyzing the 1, 2, and 3 µg/ml of benidipine hydrochloride solutions daily for 3 days over the period of week. The result was reported in terms of relative standard deviation (RSD).

Limit of Detection and Limit of Quantitation

The limit of detection (LOD) and the limit of Quantitation (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N) using the following equations designated by international conference on harmonization (ICH) guidelines

$LOD = 3.3\times\sigma/S$

$LOQ = 10\times\sigma/S$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

Accuracy

The accuracy of the method was determined by calculating recoveries of benidipine hydrochloride (1, 1.5, 2, 2.5, 3, 3.5µg/ml) by the standard addition method. The experiment was repeated three times.

Assay of Marketed Formulation

Twenty tablets were weighed and crushed to obtain fine powder. An accurately weighed tablet powder equivalent to about 10 mg of benidipine hydrochloride was transferred to 100 ml volumetric flask, dissolved in 5ml methanol and sonicated for 20 min. The volume was then made up to the mark-using methanol as solvent. The resulting solution was filtered first through Whatmann filter paper. Filtrate was appropriately diluted to get concentration of 1.5 µg/ml of benidipine hydrochloride. Absorbance of sample solutions was recorded at 255 nm and the concentration of drug in the sample was determined by using equation.

RESULTS AND DISCUSSION

Benidipine hydrochloride has the absorbance spectra maxima at 355 nm. The spectra of overlain spectra are shown in Figure 3. The polynomial regression data for the calibration plots showed good linear relationship in the concentration range of 1-3.5 µg/ml with correlation coefficient (r^2) was found to be higher than 0.9938 (Table-1, Figure-4).

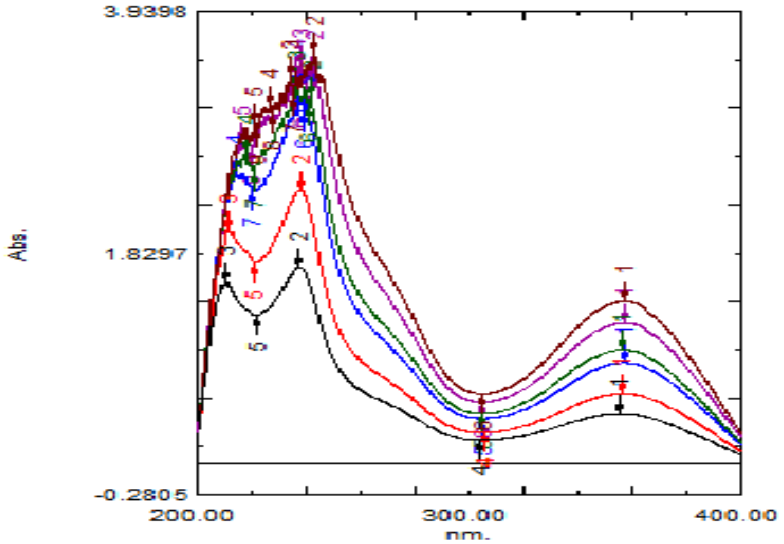

Figure 3: Overlain spectra of benidipine hydrochloride

Table 1: Linearity data of benidipine hydrochloride

Series	Absorbance
1	0.4252
1.5	0.5978
2	0.8663
2.5	0.9791
3	1.2179
3.5	1.4066

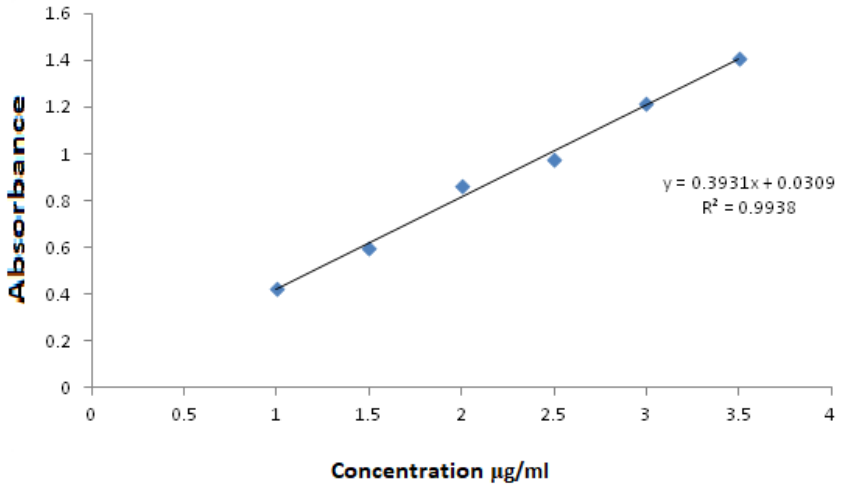

Figure 4: Calibration curve of Benidipine hydrochloride at 355 nm

Recovery studies were carried out at three different levels i.e. 80 %, 100 %, and 120 % by adding the pure drug to the previously analysed sample. Percentage recovery for benidipine hydrochloride was found between 100.74% to 100.30%, which is under acceptance criteria according to ICH guidelines (Table-2). The percentage recovery value indicates non-interference from excipients used in formulation.

Table 2: Recovery data for benidipine hydrochloride

Conc. level (%)	Amount taken	Amount added	Total amount	Amount found	% recovery	Mean recovery	% RSD
80%	1.5	1.2	2.7	2.71	100.37%	100.74%	0.3676
				2.72	100.74%		
				2.73	101.11%		
100%	1.5	1.5	3.0	2.99	99.66%	100%	0.3333
				3.00	100%		
				3.01	100.33%		
120%	1.5	1.8	3.3	3.31	100.30%	100.30%	0.3021
				3.32	100.6%		
				3.30	100%		

Repeatability was determined by analyzing 1.5 µg/ml concentration of benidipine hydrochloride solution for five times and the % RSD was found to be < 2 (Table-3).

Table 3: Repeatability data of Benidipine hydrochloride

Sr. No.	Absorbance of benidipine hydrochloride
1	0.5885
2	0.5975
3	0.5891
4	0.5979
5	0.6001
Mean	0.59462
SD	0.005409
% RSD	0.909574

The precision of the developed method was expressed in terms of % relative standard deviation (% RSD). These results show reproducibility of the assay. The % RSD values found to be less than 2 that indicate this method precise for the determination of benidipine hydrochloride in formulation (Table-4, Table-5)).

Table 4: Interday precision data for estimation of benidipine hydrochloride

Conc. µg/ml	Abs of benidipine HCL at 355nm±SD (n=5)	%RSD
1	0.4259±0.003732	0.87617
2	0.86586±0.003955	0.45678
3	1.21528±0.004253	0.34999

Table 5: Inter-day precision data for estimation of benidipine hydrochloride

Conc. µg/ml	Abs of benidipine HCL at 355nm±SD (n=5)	%RSD
1	0.42514±0.004051	0.95278
2	0.86374±0.004013	0.46459
3	1.21324±0.004033	0.33239

The LOD and LOQ were found to be 0.04540µg/ml and 0.1375µg/ml respectively, which indicate the method is sensitive. The different validation parameters for the proposed method are summarized in Table 7

Table 6: LOD & LOQ data for benidipine hydrochloride

Parameters	Benidipine hydrochloride
LOD (µg/ml)	0.04540µg/ml
LOQ (µg/ml)	0.1375µg/ml

Table 7: Summary of Optical characteristics and Other Parameters

Parameters			Values
Linearity range(µg/ml)			1-3.5 µg/ml
Regression line equations			y = 0.3931x - 0.0309
Correlation coefficient (R^2)			0.9938
Limit of detection (µg/ml)			0.04540µg/ml
Limit of quantification (µg/ml)			0.1375µg/ml
Precision (%RSD)	Intraday precision (n=5)	1	0.87617
		2	0.45678
		3	0.34999
	Interday precision (n=5)	1	0.95278
		2	0.46459
		3	0.33239
Repeatability (n=5)			0.90957
Accuracy (% RSD, n=3)			80% = 0.36764
			100% = 0.33333
			120% = 0.30211

Quantitative determination of benidipine hydrochloride in tablets using proposed method was performed and the results were in good agreement with the labeled amount of benidipine hydrochloride (Table-8). The concentration of the drug was calculated from the linear regression equation. The % amount was found to be 100.77 %.

Table 8: Analysis of marketed formulation

Tablet	Labelled claim	Amount found	% Assay (n=6)
Bengreat-4	4 mg	4.031	100.77%

CONCLUSION

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method was fully validated and found to be simple, sensitive, accurate, precise, reproducible, and relatively inexpensive. These levels of accuracy and precision obtained indicate suitability of the developed method for the quality control analysis of the benidipine hydrochloride in its formulation. So, the developed method can be recommended for the routine of benidipine hydrochloride in pharmaceutical preparations.

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