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UV-VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF ASSAY OF BENIDIPINE HYDROCHLORIDE TABLET FORMULATION

S.S. Pendhari*, B.S Ghuge, P.A. Malode, S.P. Anantwar

Department of Quality Assurance, MVP Samaj's College of Pharmacy, Nasik, Maharashtra, India.

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ABSTRACT

Simple, rapid, sensitive, precise and specific UV spectrophotometric method for the determination of Benidipine HCl in bulk drug and pharmaceutical dosage form were developed and validated. In this method solution of Benidipine HCl were prepared in methanol. Benidipine HCl standard solution was scanned in the UV rang (400-200nm) in a 1cm quartz cell in a double beam UV spectrophotometer. The standard solution of Benidipine HCl showed maximum absorption at wavelength 237 nm. The method obeys Beer's law in the concentration range from 2-10 µg/ml. The correlation coefficient was found to be 0.991 and regression of the curve was found y = 0.0680x +0.031 with excellent recovery 99-104%. Limit of detection and limit of quantitation were found to be 0.222 µg/ml and 0.674µg/ml respectively. The method was validated for several parameters like accuracy, precision as per ICH guidelines. Statistical analysis proved that the methods are reproducible and specific for the estimation of the said drug. These methods can be adopted in routine assay analysis of Benidipine HCl in bulk or tablet dosage form.

Corresponding author
S.S. Pendhari
MVP Samaj's College of Pharmacy,
Gangapur Road, Nasik, Maharashtra, India - 422002
shilpapendhari18@gmail.com

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INTRODUCTION

The Benidipine hydrochloride (Fig.1) is a highly potent and long acting dihydropyridine (DHP) calcium channel blocker (L, N and T-type) and orally active antihypertensive agent which displace a wide range of activities in vitro and in vivo.[9] This medicine enlarges the peripheral arteries and the coronary vessels, decreases blood pressure by reducing influx of calcium ion into cells, and consequently prevents or relieves episode of angina. It is usually used for the treatment of hypertension, renal parenchymal hypertensionand angina pectoris.[3]

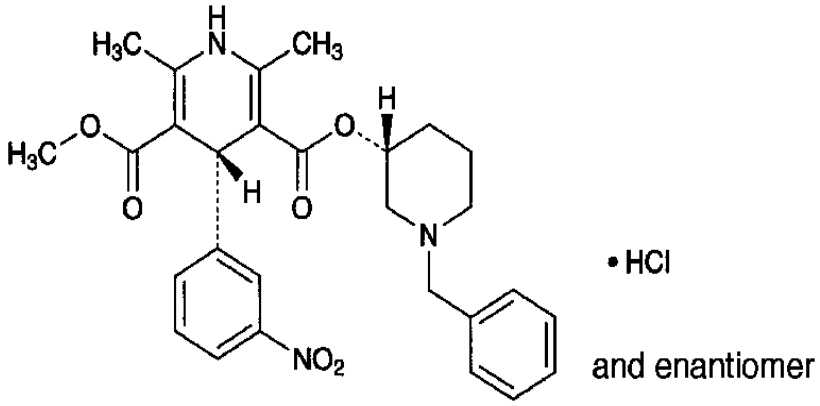

Fig. 1: Chemical Structure of Benidipine HCl.

Chemically it is known as (±) (R*)-3[(R*)-1-Benzyl-3-piperidyl] methyl 1, 4-dihydro2,6-dimethyl-4-(3-nitrophenyl)-3,5pyridine dicarboxylate hydrochloride (1:1) base . Also inhibits aldosterone-induced mineralo corticoid receptor activation and exhibits cardio protective and antiatherosclerotic effects. It has been shown electrophysiologically that benidipine inhibits calcium (Ca2+) channels, like other CCBs .[3] In addition, this drug has unique biochemical features not seen in other CCBs: 1) Strong, long-lasting action by its high affinity for the DHP binding site and the membrane approach, 2) Renalprotective effects by triple Ca2+-channel blocking, 3) Cardio- and vaso-protective effects by vascular selectivity and enhanced nitric oxide (NO) production. One characteristic of benidipine is its high affinity for the DHP binding site (i.e., the binding site in Ca2+ channels) and cell membranes.[8] BEN is listed in official monograph of Japan Pharmacopoeia which describes potentiometric titration and chromatographic procedures for its assay in tablets. BEN tablet contain not less than 95.0% and more than 105.0 of the labeled amount of Benidipine. It is a Yellow powder which is very soluble in formic acid,soluble in methanol,sparingly soluble in ethanol, and practically insoluble in water.[6]

The literature review revealed Development of New Visible Spectrophotometric Methods for Determination of Benidipine Hydrochloride in Bulk And Formulation Based On Oxidative coupling And Diazo Coupling Reaction, , Identification, synthesis and characterization of process related impurities of benidipine hydrochloride, stress-testing/stability studies and HPLC/UPLC method validations and First Order Derivative Spectrophotometric Method for the Determination of Benidipine Hydrochloride Pharmaceutical Preparations and Forced Degradation Study.[9,10,11]so there is need to develop simple, accurate, fast & economic method for determination of benidipine HCl in bulk drug & pharmaceutical dossage form, which can be used for routine analysis.

MATERIALS AND METHODS:

Instruments:

For Weighing, a calibrated weighing balance (Make- Shimadzu) of 1mg sensitivity was used. For analytical purpose UV spectrophotometer Shimadzu UV 2450 PC was used. All other glasswares and apparatus were made of Borosilicate and were calibrated.

Chemicals:

API-Benidipine Hcl is pure drug purchased from Pure Chem Laboratory PVT LTD, Ankhleshwar. Tablets of 4 mg strength were purchased from the local pharmacy in Nashik under commercial available brand name Benipack (koye pharmaceutical Ltd.), methanol was used in this study.

Preparation of standard stock solution:

The standard stock solution of Benidipine HCl was prepared by transferring, accurately weighed 100 mg of Benidipine HCl to 100 ml volumetric flask containing 50ml distilled water. Dissolved drug properly. Then volume was made up to the mark by using distilled water to gives concentration 1000µg/ml. From this 10 ml of the solution was transferred to a 100 ml volumetric flask and make up the volume with methanol to gives a concentration of 100µg/ml, it is standard stock solution and which is further diluted with methanol to get concentration 2-15 µg/ml.

Determination of Absorption Maxima:

The appropriate dilution of standard stock solution with methanol, solution contain 10µg/ml of Benidipine HCl was scanned in the range of 400-200nm to determine the wavelength of maximum Absorption. Drug showed Absorption maxima at 237 nm.

Preparation of Calibration Curve:

For the preparation of standard calibration curve, concentration of 2-15µg/ml were prepared by pipetting out 0.3, 0.6, 0.9, 1.2 and 1.5 ml from the 100µg/ml solution in to a 10ml volumetric flask and made up the volume with methanol. The absorbance of each solution was measured at 237 nm against methanol as blank. Calibration curve of the benidipine was plotted by taking the absorbance obtained on y-axis and the concentration of the solution on x-axis (Fig. 2). The curve showed linearity in the range of 2-15 µg/ml with correlation coefficient 0.991.

Quantitative analysis of pharmaceutical tablet dosage form:

Twenty tablets were weighed accurately and powdered. Powder equivalent to 1 mg Benidipine HCl was weighed and transferred to a 100 ml volumetric flask. It was dissolved in 100 ml methanol and sonicate for 15 minutes to get homogeneous solution. Then it was first filtered through a 0.45µ whatman filter paper. A final concentration of 100 µg/ml of benidipine HCl was prepared. This solution was filtered through filter paper to remove some un-dissolved excipients. After filtration, from this 1 ml was taken and diluted to 10 ml with methanol which gives 10 µg/ml solution and the absorbance of the solution was measured at 237nm.

Table No.1: Results obtained in the determination of benidipine HCl in tablet dosage form.

Tablet Formulation	Label claim	Amount taken	Amount found	Assay%
Benipack	4 mg	1 mg	0.98mg	98.00

METHOD VALIDATION: [4,5]

The developed method was validated as per ICH guidelines for following parameters [4,5]

Specificity :

The specificity of the method for determination of Benidipine HCl tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the excipients.

Linearity:

Aliquots of standard stock solution were further diluted with water to get the solutions of concentration within range from 2-15 µg/ml. The absorbance was measured at wavelength 237 nm. Linear calibration graph was obtained by plotting the absorbance value versus concentration of benidipine.

Range:

The Range of analytical procedure is interval between upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure as suitable level of precision, accuracy, linearity.

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions.

Intraday and Interday Precision (Intermediate Precision):

Intraday precision was determined by analyzing the drugs at concentrations (10µg/mL) and each concentration for three times, on the same day. Interday precision was determined similarly, but the analysis being carried out daily, for three consecutive days.

Recovery:

The accuracy for the analytical method for Benidipine was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 237 nm and results were expressed in terms of % recoveries. Standard deviation and % RSD was calculated. The results were tabulated in (Table No. 7).

$$\%\ Recovery = \frac{\text{Observed value}}{\text{True value}} \times 100$$

Repeatability:

Repeatability of the method was determined by analyzing six samples of same concentrations of drug (10µg/mL). Spectra were recorded, and the absorbance of each spectrum was measured.

Robustness:

The robustness of developed method is its capacity to remain unaffected by small changes in altered conditions. To determine the robustness of the method, the wavelength of analysis was deliberately altered and assay was evaluated. The effect of detection wavelength was studied at ± 5nm.

Solution Stability:

The stability of the solution was studied by analyzing the standard solution at 1, 2, 3, 4 and 5 days intervals.

RESULT AND DISCUSSION:

Determination of wavelength of maximum absorption:

The wavelength of maximum absorption was found to be 237.0 nm.

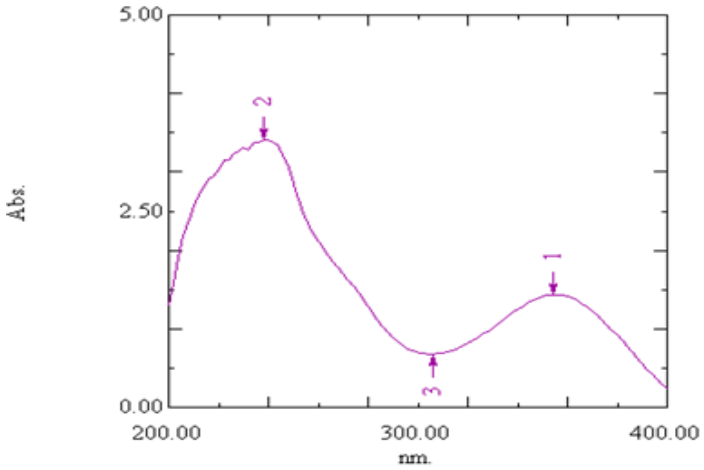

Fig 2 :Wavelengh of maximum absorption of Benidipine HCl.

Specificity:

The specificity of the method for determination of Benidipine HCl in tablet dosage form was determined by comparing the spectrum of tablet solution with that of standard solution. The sample spectrum was checked for any interference from the excipients.

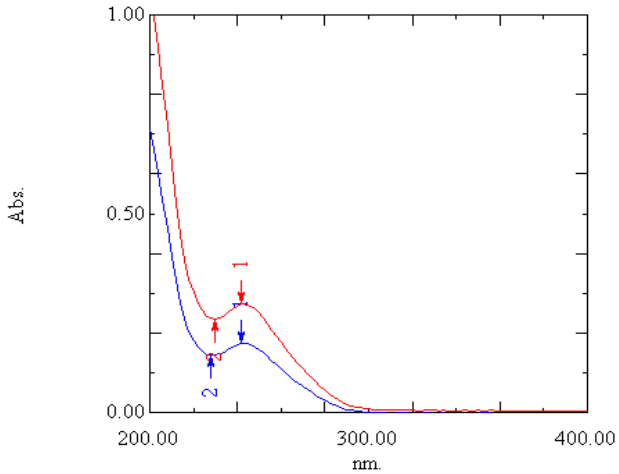

Fig3: Ultra Violet spectrum of Benidipine HCl (API) and Tablet Dosage form.

Linearity:

The linearity of this method was determined at ranging from 2-15µg/ml for Benidipine HCl. The regression equation were found to be $y = 0.0680x + 0.031$, r^2= 0.997.

Table.2: Linearity table.

Sr.No	Concentration (ppm)	Absorbnace
1	3	0.227
2	6	0.465
3	9	0.631
4	12	0.850
5	15	1.06

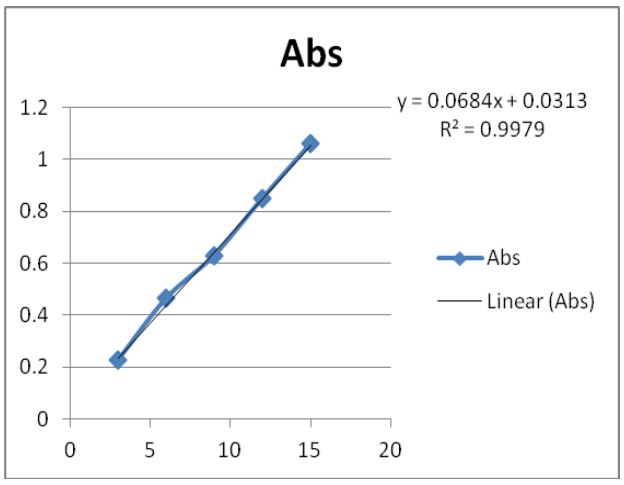

Fig 4: Linearity graph of Benidipine HCl.

The method for Benidipine HCl was found to be linear in the range of 2-15 ppm with $R^2 = 0.997$ and the straight line equation as: $y = 0.0682x + 0.031$

Precision

The precision (measurement of intraday, interday, repeatablity) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

Intraday precision:

Table.3: Intraday morning precision.

Sr. No.	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.625	-0.004	0.000016	0.0046	0.73
2	10	0.632	0.003	0.000009		
3	10	0.636	0.007	0.0001		
4	10	0.622	-0.007	0.000064		
5	10	0.629	0	0.000001		
6	10	0.631	0.002	0.000004		
		Ȳ=0.629		Σ=0.000127		

Table.4: Intraday Afternoon precision.

Sr. No.	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.625	-0.003	0.000009	0.005621	0.895
2	10	0.631	0.003	0.000009		
3	10	0.638	0.010	0.0001		
4	10	0.620	0.008	0.000064		
5	10	0.629	0.001	0.000001		
6	10	0.630	0.002	0.000004		
		Ȳ=0.628		Σ=0.000187		

Table.5: Intraday Evening precision.

Sr.No	Concentration	Evening Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.624	-0.005	0.000025	0.00497	0.791%
2	10	0.632	0.003	0.000009		
3	10	0.637	0.008	0.000064		
4	10	0.622	-0.007	0.000049		
5	10	0.630	0.001	0.000001		
6	10	0.630	0.001	0.000001		
		Ȳ=0.629		Σ=0.000149		

Interday precision:

Table.6: Interday morning precision Study.

Sr.No	Concentration	Morning absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.624	0.005	0.000025	0.00517	0.82%
2	10	0.630	0.001	0.000001		
3	10	0.638	0.009	0.000081		
4	10	0.622	-0.007	0.000049		
5	10	0.631	0.002	0.000004		
6	10	0.630	0.001	0.000001		
		Ȳ=0.629		Σ=0.000161		

Table.7: Interday Afternoon precision Study.

Sr.No	Concentration	Afternoon absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.624	0.004	0.000016	0.00488	0.777%
2	10	0.631	0.003	0.000009		
3	10	0.636	0.008	0.000064		
4	10	0.621	0.007	0.000049		
5	10	0.630	0.002	0.000004		
6	10	0.627	-0.001	0.000001		
		Ȳ=0.628		Σ=0.000143		

Table.8: Interday Evening precision.

Sr.No	Concentration	Evening absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.625	-0.004	0.000016	0.00404	0.642 %
2	10	0.635	0.006	0.000036		
3	10	0.634	0.005	0.000025		
4	10	0.619	-0.01	0.000001		
5	10	0.631	0.002	0.000004		
6	10	0.633	0.004	0.000016		
		Ȳ=0.629		Σ=0.000098		

Repeatability:

Table.9: Repeatability study.

Sr.No	Concentration	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	10	0.625	-0.004	0.000016	0.00430	0.683%
2	10	0.630	0.001	0..000001		
3	10	0.636	0.007	0.000049		
4	10	0.625	-0.004	0.000016		
5	10	0.627	-0.002	0.000004		
6	10	0.634	0.005	0.000025		
		Ȳ=0.629		Σ=0.000111		

Accuracy:

The accuracy for the analytical method for Benidipine HCl was determined at 80%, 100% and 120% levels of standard solution. Absorbance was measured at 237.0 nm and results were expressed in terms of % recoveries.

Table.10: Accuracy Study.

Sr. No.	%concentration	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
1	80	8	3	5	0.570 0.572 0.570	0.570

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.068x+ 0.031

$$x = (y - c)/m$$

$$x = \frac{0.570 - 0.031}{0.0680}$$

X= 7.926 ppm

% Recovery from 8 ppm solution: $\frac{x}{8} * 100$

= (7.926/8)* 100

= 99.08%

Table.11: Recovery Study.

Sr. No.	%concentration	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
1	100	10	5	5	0.715 0.713 0.716	0.714

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.068x+ 0.031

$$x = (y - c)/m$$

$$x = \frac{0.714 - 0.031}{0.0680}$$

X= 10.05 ppm

% Recovery from 10 ppm solution: $\frac{x}{10} * 100$

= (10.05/10)* 100

= 100.53%

Table.12:Recovery Study.

Sr. No.	%concentration	Concentration in ppm	Volume of API stock (ml)	Volume of Tablet stock (ml)	Absorbance (nm)	Mean
1	120	12	7	5	0.882 0.881 0.885	0.882

The concentration is calculated by using straight line equation:

$$y = mx + c$$

y = 0.068x +0.031

$$x = (y - c)/m$$

$$x = \frac{0.882 - 0.031}{0.0680}$$

X= 12.51ppm

% Recovery from 12 ppm solution: $\frac{x}{12} * 100$

= (12.51/10)* 100

= 104.28%

Limit of Detection and Limit of Quantification:

It is calculated by using slope and standard deviation from linearity and precision respectively:

Limit of detection (LOD):

$$LOD = 3.3 \times SD/Slope$$

$$LOD = 3.3 \times 0.0046/0.068$$

$$LOD = 0.222\ ppm$$

Limit of quantification (LOQ):

$$LOQ = 10 \times SD/Slope$$

$$LOQ = 10 \times 0.00046/0.068$$

$$LOQ = 0.674\ ppm$$

Robustness:

Table.12:Robustness Study.

Sr.No	Wavelength(nm)	Absorbance	(Y-Ȳ)	$(Y-\bar{Y})^2$	SD	%RSD
1	232	0.528	-0.001	0.000121	0.0031	0.550
2	233	0.541	0.002	0.000004		
3	234	0.552	0.013	0.000164		
4	235	0.560	0.021	0.000441		
5	236	0.564	0.025	0.000625		
6	237	0.565	0.026	0.000676		
7	238	0.558	0.019	0.000361		
8	239	0.547	0.008	0.000064		
9	240	0.528	-0.011	0.000121		
10	241	0.506	-0.033	0.001089		
11	242	0.480	-0.059	0.003481		
		Ȳ=0.539		Σ=0.007216		

RESULT AND DISCUSSION

Sr No.	Validation Parameters	Results
1	Absorption maxima(nm)	237nm
2	Beers range (µg/ml)	2-15µg/ml
4	Standard Regression Equation	y = 0.0680x + 0.031
5	Correlation Coefficient (r2)	0.997
6	Accuracy(8,10&12 ppm)	99.08%, 100.53% & 104.28%
7	Precision (%RSD)	0.8053
8	LOD &LOQ(µg/ml)	0.222 &0.674
9	Robustness(%RSD)	0.550
10	Assay (%)	99.25

CONCLUSION

The UV-spectrophotometric method was developed and it is found to be simple, accurate, precise, highly sensitive, reproducible and inexpensive. The proposed method was found suitable for determination of Benidipine HCl in in API and its bulk dosage form without any interference from the excipients. The validation procedure confirms that this is a workable method for their quantification in the raw material and also in the formulations. Hence it can be effectively applied for the routine analysis of Benidipine HCl in bulk drug. Its advantages are low cost of reagents, speed and simplicity of sample treatment, satisfactory precision and accuracy.

ABBREVIATIONS

UV : Ultra Violet
µm : micrometre
nm : nanometre
ml : millilitre
UV- Vis : Ultraviolet-Visible
API : Active Pharmaceutical Ingredient
% : Percentage
Ppm : Parts per million
API : Active Pharmaceutical Ingredient
HCl : Hydrochloride

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CONFLICT OF INTEREST:

The authors do not report any conflict of interest.

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