IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2020 Vol.:17, Issue:4

Application of Quality by Design Approach for Development and Validation of Analytical RP-HPLC Method for Benidipine Hydrochloride in Tablet Dosage Form

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Submission: 22 February 2020
Accepted: 29 February 2020
Published: 30 March 2020

www.ijppr.humanjournals.com

Keywords: Quality by Design, Benidipine Hydrochloride, Validation

ABSTRACT

The given study shows application of quality by design (QbD) approach to the development and validation of analytical RP HPLC method for Benidipine Hydrochloride. The optimized chromatographic method was validated based on International Conference on Harmonization (ICH) Q2 (R1) guidelines for linearity, precision, range, accuracy and robustness. The separation was carried on Chemsil ODS C18 (250mm x 4.6ID, Particle size: 5 micron) and detection was done using UV detector at 237 nm. The developed method used mobile phase Methanol: Ammonium acetate buffer (85:15), pH 3 and flow rate 1.2 ml/min, which was optimized by using of design expert software. Linearity of the developed method was confirmed over concentration range of 50 – 150 µg/mL for Benidipine Hydrochloride with correlation coefficient of 0.998. The percentage RSD for precision and accuracy of the method was found to be less than 2%. Peak was observed at retention time of 3.47min. The present method can be efficiently employed to determine the drug contents of marketed formulation.

ABBREVEATION LIST:

LONG FORM	ABBREVIATION
Quality By Design	QbD
Benidipine	BEN
Quality Risk Management	QRM
Pharmaceutical Quality System	PQS
Telmisartan	TEL
Photometric Diode Array	PDA
Not More Than	NMT
Limit Of Detection	LOD
Standard Deviation	SD
Relative Standard Deviation	RSD
Tablet	TAB
Design Of Experiment	DOE

INTRODUCTION:

Benidipine (BEN) Hydrochloride is yellowish amorphous powder having chemical formula 3-(3R)-1-benzylpiperidin-3-yl-methyl-(4R)-2, 6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5dicarboxylate Hydrochloride. Having category antihypertensive agent. BEN Hydrochloride inhibits L, N-and T-type Ca^{2+} channels. It also prevents aldosterone induced mineralocorticoid receptor activation. BEN HYDROCHLORIDE exhibits cardioprotective and anti-atherosclerotic effects (8-9). Bajaj Mohini *et. Al* review article describes the points to be considered while applying QbD to an analytical method. It also describes other features like Quality Risk Management (QRM); Pharmaceutical Quality System (PQS) and Process Analytical Technology (PAT) guidelines are also being now introduced and integrated into analytical method development processes (2). Phil Nethercote *et. al* they have studied analytical methods can be viewed as "processes" and QbD concepts applied, to improve both method validation and transfer. Its goal is to stimulate thinking and discussion on how analytical method validation and transfer could evolve as industry increasingly adopts QbD concepts (3). Majan Naim *et.al* focused on C18 column, a 250x4.6mm column of 5.0µm particle packing was selected for separation of Telmisartan (TEL) and (BEN).

Different solvent systems were tried and optimized in combinations as mobile phase TEL (40µg/ml) and BEN (4µg/ml) in buffer, pH 4.0: Methanol (50:50) was developed as it was showing good peak shapes and a significant amount of resolution. Then mobile phase was flowed at 1.0ml/min with detection of both the analyte at 210nm using photodiode array detector. This stability indicating RP-HPLC method were developed by degradation of sample and compared with standard. The percentage relative standard deviation was also < 2% showing high degree of precision of the proposed method (5). Payal G. Jain *et al* they studied RP-HPLC method was developed for the simultaneous estimation of BEN Hydrochloride and TEL in tablet. The separation was carried out using Inertsil ODS C18 column (150x4.6mm,5µm),mixture of 0.05M Potassium Dihydrogen phosphate Buffer(pH-4.5 adjusted with 1% OPA)and Acetonitrile(40:60%v/v)as a mobile phase. The flow rate was adjusted to 1ml/min and effluent was monitored at 267nm by used PDA detector. The retention time of BEN Hydrochloride and TEL were found to be 2.977 and 5.167 min respectively(7)**.** Laxman Prajapati *et al*. study was aimed to develop a novel simple, accurate, precise, robust and economic UV-spectrophotometric method for estimation of BEN 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 (6). Manish Kumar *et. al.* UV spectrophotometric method was developed on ShimadzuUV-1800 double beam spectrophotometer using methanol as solvent and wavelength of 236 nm was selected as absorbance maxima. Effect of input variables on spectrum characteristics were studied for the selection of critical parameters and proposed method was validated for various parameters like system suitability, linearity, precision, accuracy, detection limits and quantitation limits as per the ICH guidelinesQ2(R1). Linearity of the method was found to be excellent over the range 3-18µg/ml with high correlation coefficient value of 0.999. Limits of detection and quantitation were found to be 0.20µg/ml and 0.60µg/ml respectively. The mean recovery was found to be 100.35% with low percentage Relative Standard Deviation (%RSD) value (4).

MATERIALS AND METHODS:

Specification of drug sample-

BEN Hydrochloride Purchased from Purechem India in 10 gm quantity.

Water, Acetonitrile, Methanol, (HPLC grade), Potassium dihydrogen-ortho phosphate (Anhydrous) (AR grade).

Instruments:

For analytical purpose HPLC was performed on water 1525 containing detector water 2489 (UV-visible detector) equipped with manual injector and breeze 2 software and Chemsil ODS C18 particle size(5µm) reverse phase column was used.

Experimental work:

Method development by QbD approach and optimization of chromatographic conditions using different mobile phase like Methanol: Ammonium acetate buffer (80:20) ph-3, Methanol: Ammonium acetate buffer (85:15) ph-3, Methanol: Ammonium acetate buffer (50:50) ph-4, Methanol: Ammonium acetate buffer (70:30) ph-4, tried flow rate like1 and 1.2 ml/min. The mobile phase methanol: Ammonium Acetate buffer in the ratio 85:15% v/v at a flow rate 1.2 ml/min give good peak shape, proper plate count and stable retention time 3.4 min. The detection response measured at 237 nm and column was maintained at ambient temperature throughout study. Optimized chromatographic conditions are given in Table no.1.

Table No. 1: Optimized Chromatographic Conditions

Method parameter optimized condition
Column- Chemsil ODS C18 particle size(5µg)
Wavelength-237nm
Mobile phase composition- Ammonium Acetate buffer in the ratio 85:15% v/v
Pump mode-isocratic
Flow rate-1.2 ml/min
Injection volume-10µl
Run time-15 min

Table No. 2: Translation of coded levels in actual values

Level of variables	Concentration of factors		
	Flow rate (ml/min)	**pH**	**Mobile phase composition(methanol: buffer)**
Low level (-1)	1.2	2.8	75:25
Medium level (0)	1	3.0	85:15
High level (+1)	1.4	3.2	95:5

Design of experiments (2):

Thus, 3^3 randomized response surface designs with a Box-Behnken design were used with 17 trial runs to study the impact of three factors on the three key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all possible combinations. The A: mobile phase composition, B: pH of buffer, C: flow rate, were selected as independent variables and Retention Time (RT), Tailing, Theoretical Plate number (TPN**)** were selected as dependent variables based on risk analysis. The resulting data was processed into Design Expert 11software and analyzed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, pH and mobile phase composition on dependent variables. Table No.2 show Translation of coded levels in actual values and table No.3 show layout of actual design of DOE with the subsequent response.

Table No. 3: The layout of actual design of DOE with the subsequent response

Std	Run	Factor 1 A:Mobile Phase	Factor 2 B:pH	Factor 3 C:Flow Rate	Response 1 Retention Time	Response 2 Plate Count	Response 3 Tailing
11	1	85	2.8	1.4	2.41	4125	1.14
10	2	85	3.2	1	4.55	5126	1.21
15	3	85	3	1.2	3.41	4154	1.29
3	4	75	3.2	1.2	4.77	4872	1.27
7	5	75	3	1.4	3.01	3345	1.13
12	6	85	3.2	1.4	3.36	3809	1.17
16	7	85	3	1.2	3.44	4205	1.27
5	8	75	3	1	4.11	3989	1.3
14	9	85	3	1.2	3.55	4826	1.23
13	10	85	3	1.2	3.41	4037	1.25
1	11	75	2.8	1.2	3.39	3141	1.24
4	12	95	3.2	1.2	3.49	3336	1.14
9	13	85	2.8	1	3.42	4724	1.33
6	14	95	3	1	2.82	3317	1.11
8	15	95	3	1.4	2.25	3188	1.19
17	16	85	3	1.2	3.44	4134	1.33
2	17	95	2.8	1.2	2.63	3442	1.2

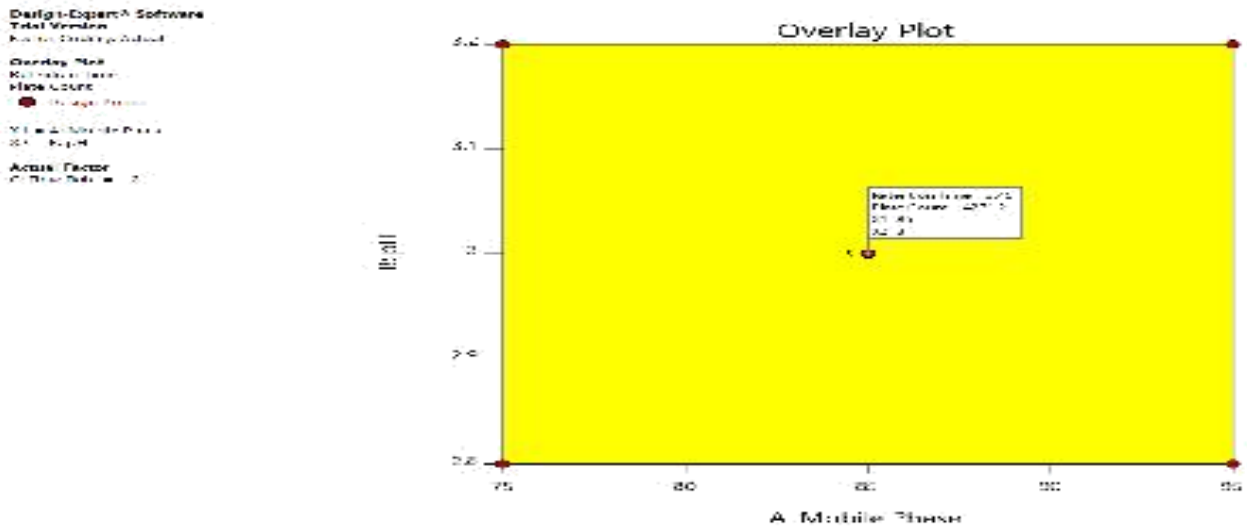

Figure No. 1: Overlay plot for mobile phase and pH

Table No. 4: Optimization solution

Number	Mobile Phase	pH	Flow Rate	Retention Time	Plate Count	Tailing	Desirability	
1	85.000	3.000	1.200	3.450	4271.200	1.274	1.000	Selected

Preparation of Standard stock solution:

Accurately weighed quantity of 25 mg of BEN Hydrochloride was transferred into 25 ml of volumetric flask, dissolved and diluted up to mark with methanol. This was a stock solution having strength of 1000 µg/ml of BEN Hydrochloride. From this solution, 1 ml of solution was pipette out and diluted up to 10 ml to get 100 µg/ml of BEN Hydrochloride.

Tablet solution preparation:

Brand name –BENEDINOL-4 Total weight of 10 tablets =3591.9mg.Average weight =3591.9 / 10 = 359.19mg.Equivalent weight for 25mg =3591.9x25/40 = 2244.93mg.Take 2.244gm in 25ml Methanol i.e. =1000 µg/ml ----TAB SOLUTION.TAKE 100µg/ml TAB ASSAY (take 1.0 ml from tab stock solution.

VALIDATION OF PROPOSED METHOD (1):

The parameters for the validation were as follows:

1. Linearity - The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration. Percentage curve fittings are calculated**.**

2. Accuracy (by Recovery method) - The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay. Mean recovery should be in the range of 98-102%. The RSD should not be more than 2.0%.

3. Precision - Prepare six different test solution of the 100% test concentration from the same sample matrix. Inject duplicate injections of each test solution. % RSD NMT 2% for test results.

4. Robustness- The robustness of an analytical method is determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but are still within the specified parameters of the assay. For example change in physical parameters like pH of mobile phase, ratio of mobile phase and wavelength.

5. Limit of Detection (LOD) - a signal-to-noise ratio between 3:1 or 2:1 is generally considered acceptable for estimating the detection limit. It may be calculated based on Standard Deviation (SD) of the response and slope of the curve(S).LOD= 3.3 (SD)/ S

6. Limit of Quantitation (LOQ) - It is expressed as the conc. of analyte (e.g., percentage, parts per billion) in the sample. A typical signal-to-noise ratio is 10:1 or 20:1. It may be calculated based on SD of the response and slope of the curve(S). LOQ= 10 (SD)/S

RESULTS AND DISCUSSION

Table No.4 show Optimization solution figure No.1 & 2 gives overlay plot for mobile phase and pH and Chromatogram of BEN Hydrochloride.

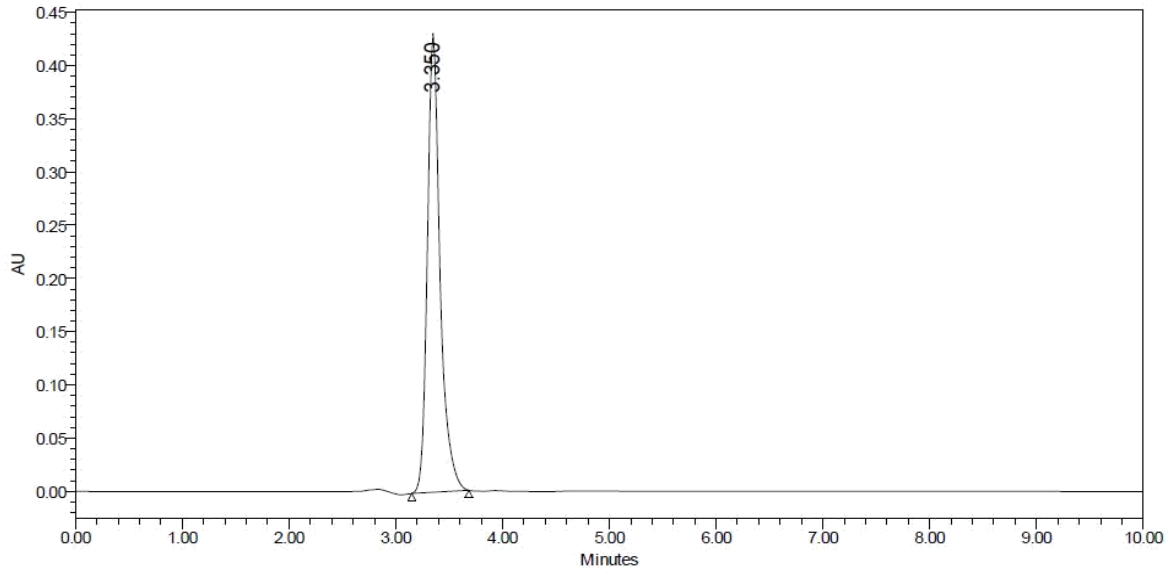

Figure No. 2: Optimized method Chromatogram of BEN Hydrochloride.

System suitability parameter for BEN Hydrochloride.

Table No. 5 USP Parameter for BEN Hydrochloride.

Sr. No.	Compound	RT (min)	Area	USP plate Count	USP tailing Factor
1	Benidipine Hydrochloride	3.441	2550214	4209	1.26

A) Linearity- Linearity was performed concentration of Benidipine Hydrochloride is in the range of 50 to 150µg/ml. results shown in Table No.6 and Figure No. 3shows linearity graph of BEN Hydrochloride.

Table No. 6: Linearity results of proposed method

BEN Hydrochloride.			
Sr. No.	**Concentration (µg/ml)**	**Area**	**RT (min)**
1	50	1869520	3.35
2	75	2329416	3.36
3	100	2895387	3.33
4	125	3493678	3.35
5	150	4042216	3.34
Correlation coefficient (R2)		0.998	
Slope		22039.61	
Intercept		722182	

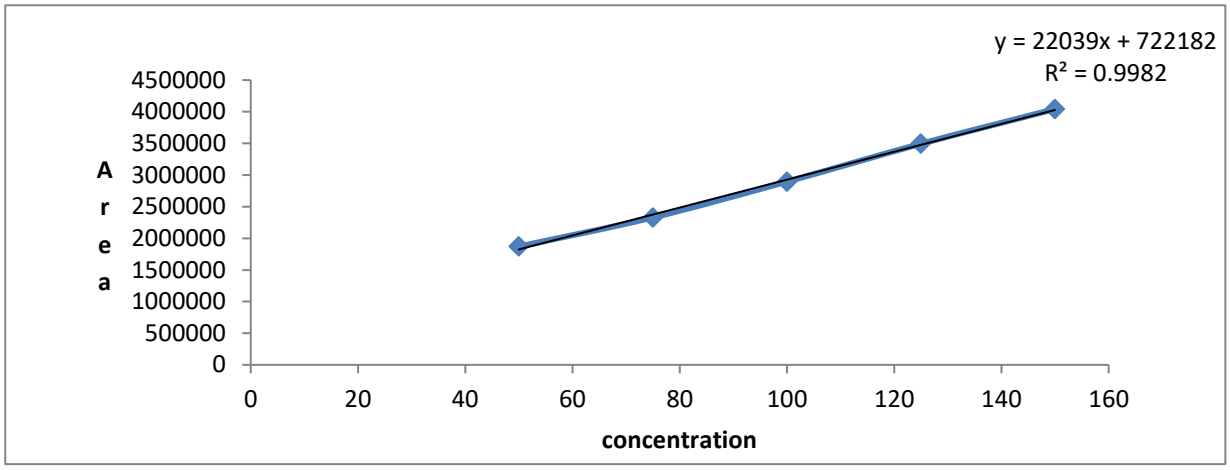

Figure No. 3: linearity graph of BEN Hydrochloride.

B) Precision- Results and Statistical Data for Precision

Precision results shown in table No. 7 of precision.

Table No. 7: Precision results of proposed method

Sr. No.	BEN Hydrochloride	
	Concentration(ug/ml)	Area
1	100	2550214
2	100	2596238
3	100	2595387
4	100	2612870
5	100	2593387
6	100	2595049
Mean	2590857.5	
S.D	21090.261	
%RSD	0.81%	

C) Accuracy (by Recovery method):

Prepare the standard solution by taking stock solution equivalent to 50%, 100%, and 150%, each in triplicate. Each concentration injected into the HPLC system. And results shown in Table No.8.

Table No. 8: results obtained by recovery method

Sr. No.	Con. Level	Conc. (µg/ml) STD Stock solution	Conc. (µg/ml)for Test stock Solution	Area	Conc. Found (µg/ml)	%Recovery	Average %Recovery	%RSD
1	50%	25	50	2368174	74.68	99.58	100.13	1.68
		25	50	2408773	76.52	102.03		
		25	50	2355248	74.09	98.79		
2	100%	50	50	2890984	98.40	98.40	98.87	0.44
		50	50	2910315	99.28	99.28		
		50	50	2902548	98.93	98.93		
3	150%	75	50	3524454	127.15	101.72	100.68	1.25
		75	50	3455412	124.01	99.93		
		75	50	3495845	125.85	100.68		

D) Robustness- Change in Flow rate

Robustness results of proposed method given in Table No. 9.

Table No. 9: Robustness results of proposed method

Sr. No.	Conc.	Area		
		As such 1.2ml/min	1.1ml/min	1.3ml/min
1	100	2550214	2543527	2550776
2	100	2596238	2563679	2579768
3	100	2595385	2543527	2545689
Mean		2580612	255244.33	2558744.33
SD		26329.1835	11634.7626	18383.8329
%RSD		1.0202	0.4562	0.7184

E) LOD and LOQ:

LOD and LOQ values of BEN Hydrochloride were determined by calibration curve. LOD and LOQ were found to be 3.15 and 9.56 µg/ml respectively.

CONCLUSION:

The RP-HPLC method was developed with mobile phase methanol: Acetate buffer pH 3 (85:15), and flow rate 1.2 ml/min, which was optimized with the help of design expert software. Before method optimization, screening studies were carried out on different mobile phases of varying composition. Based on the results obtained from these studies, suitable mobile phase with appropriate composition was selected and utilized for method development using QbD methodology. The study was done by using 3^3 Box Behnken response surface designs. In this study interaction of 3 factors; flow rate, pH and mobile phase composition vary at 3 levels. Effect of such critical process parameter on critical quality attribute of the method is studied. Responses in terms of retention times and resolution were evaluated throughout all the runs in design. The proposed high-performance liquid chromatographic method has also been evaluated for accuracy, precision and robustness and proved to be convenient and effective for the quality control of BEN Hydrochloride.

ACKNOWLEDGEMENTS:

Authors are grateful to A. D. Savkare (Associate professor) of M.V.P Samaj's college of pharmacy, for guiding us to carry out the research work. We are also thankful to Purechem pharmaceuticals, for providing API as gift sample.

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