

RP-HPLC Method Development and Validation for Estimation of Barnidipine HCl in Bulk and *In-House* Tablets

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Abstract

A new simple, precise, accurate, sensitive and rapid chromatographic method based on RP-HPLC was developed and validated for the estimation of Barnidipine HCl in bulk and *in-house* tablet dosage form. Methanol: Water (80:20 v/v) was used as mobile phase. A gradient programming has been done, on a reverse phase C8 column (250×4.6mm×5μ) with flow rate 1 mL/min, monitored at 260 nm. The mean retention times of Barnidipine HCl were found to be 2.8 min respectively. Linearity of Barnidipine HCl was found to be 3-18 μg/mL, $R^2 = 0.999$ respectively. The developed methods have shown the best findings in terms of linearity, accuracy, precision, LOD and LOQ for API and in house tablets. The depicted method can routinely be used for the determination of Barnidipine HCl in bulk and for *in-house* tablets formulation.

Keywords: Barnidipine HCl, RP-HPLC, Antihypertensive, Validation, Gradient mode.

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INTRODUCTION

Barnidipine hydrochloride (Fig-1) [1], a long term dihydropyridine calcium channel blocker used for the treatment of hypertension, is chemically known as (3'S,4S)-1-benzyl-3-pyrrolidinyl-methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-

pyridinedicarboxylate hydrochloride. The product was originally developed by Yamanouchi Pharmaceutical (Tokyo, Japan) and is currently marketed in Japan under the trade name of Hypoca (Astellas Pharma Inc, Tokyo, Japan) [1, 2].

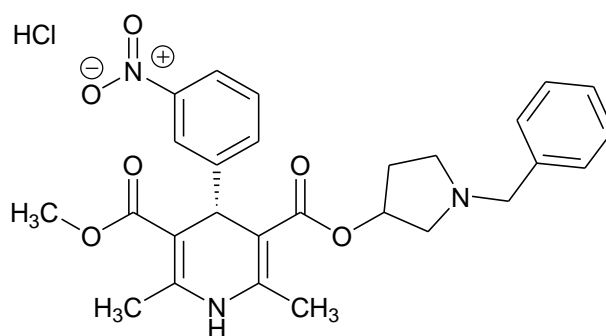


Fig-1: Structure of Barnidipine HCl

It is a new and unique dihydropyridine calcium channel blocker that having a slow onset of action and long-lasting vasodilating effect. It blocks the influx of calcium ions into both vascular smooth muscle at the level of L- type calcium channels and neuronal cells at the level of N -type calcium channels [2]. Analytical methods such as Liquid chromatographic-tandem mass spectrometric and ultra-fast Liquid

chromatography/mass spectrometry for the estimation of Barnidipine in human plasma has been reported [3]. Accelerated and natural photo-stability studies were studied for Barnidipine [4]. Liquid chromatographic, separation and thermodynamic investigation of Barnidipine enantiomers on coated and immobilized amylose tris (3, 5-dimethylphenylcarbamate) chiral stationary phases was done [5]. A Sensitive and specific

liquid chromatographic–tandem mass spectrometric method was developed and validated for Barnidipine HCl by using human plasma is also been reported [6]. Therefore, all the reported methods requires sophisticated instruments. Development of Barnidipine HCl formulation by Indian companies still under progress for overseas market. Although Chromatographic and Spectroscopic methods are reported for the quantitative estimations of Barnidipine HCl, but these methods are few. Therefore looking at the current scenario of quantitative estimation of the said drug in bulk and formulation, it was thought worthwhile to set an objective to developed and validate RP-HPLC method. The developed method has been satisfactorily applied to the estimation of Barnidipine HCl in bulk and *in-house* tablet dosage form.

Experimental

Chemicals and Reagent

The working standard of Barnidipine HCl was obtained as a gift from USV Private Ltd, Mumbai, India. Methanol (HPLC grade) was obtained from Merck Ltd Mumbai. MilliQ water was used.

Instrument used

Analysis was performed on UFLC- LC 20 AD (Shimadzu Corporation, Japan) consisting of LC -20 AD binary solvent delivery system (pump), SPD-M20A diode array detector and CTO – 10 AS vp; column oven, a Rheodyne injector with 20 µl loop and a Hamilton syringe (100 µl). Separations were achieved on a LC – GC Qualisil BDS C8 column (250×4.6mm×5µ). Data collection and analysis were performed with LC-solution (Shimadzu Corporation, Japan). All weighing operations for the present analysis were carried out with the help of SHIMADZU AUX-120 analytical balance. Ultra sonication of samples was performed using Ultrasonicator; ENERTECH Electronics Pvt. Ltd., India

Preparation of Mobile Phase

The HPLC grade methanol and water in the ratio of (80:20 v/v) was filtered through 0.4 µm membrane filter paper and mobile phase was prepared and sonicated for 15 min.

Preparation of Standard Stock Solution and Study of Calibration Curve

Stock solution of Barnidipine HCl was prepared with a concentration of 100 µg/mL in mixture of methanol and water (80:20 v/v). Determination of linearity involved analysis of six working solution having concentration range 3-18 µg/mL. Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic condition. Peak areas were recorded for all the peaks and a standard calibration curve was plotted.

Preparation of *in house* tablet

As the tablet formulation was not available in Indian market; tablet containing 10 mg of Barnidipine HCl were prepared *in-house* using direct compression technique employing SSG as super disintegrate and MCC as diluent. Prepared tablets were used as formulation for further analysis.

Preparation of Sample Solution

The sample solution was prepared from *in-house* formulated Barnidipine HCl tablets. Twenty Barnidipine HCl tablets were accurately weighed, average weighed determined and finely powered. A quantity of powered drug equivalent to 10 mg Barnidipine HCl transferred into 100 ml of volumetric flask containing 50 ml methanol, sonicated for 5 min and volume was adjusted using the same solvent. Then it was filtered through whatman filtered paper. The above prepared solution was filtered through 0.4µ membrane filter paper and was used as standard stock solution. Appropriate aliquot was pipetted out from the standard stock solution and was further diluted with the mobile phase. A 20 µl volume of each Barnidipine HCl sample was injected into sample rheodyne injector of HPLC system and the chromatograms were recorded under the chromatographic condition i.e. temperature, flow rate, mobile Phase etc. The area of each peak was determined at 237 nm and the amount of drug present in the sample mixture was determined.

Chromatographic Conditions

HPLC System	UFLC– LC 20 AD (Shimadzu Corporation, Japan)
Detector	SPD- M 20 A (Diode Array detector)
Column	LC- GC Qualisil BDS C8
Dimensions	(250×4.6mm×5µ)
Mobile- Phase	Methanol: Water (80:20 V/V)
Mode	Gradient
Flow Rate	1.0 ml/min
Temperature	Ambient temperature
Detection wavelength	237 nm
Injection Volume	20 µl

RESULT AND DISCUSSION

Optimization of Chromatographic Conditions

In method development and validation process of Barnidipine HCl, various mobile phase compositions were tested. The optimized mobile composition was found to be methanol: water (80:20 v/v) with run time 20 min. and the peak was optimized in 2.8 min. Before analysis, mobile phase and sample solutions were filtered through 0.45 μ membrane filter and ultrasonicated for 15 min. Chromatographic studies were performed at ambient temperature, with flow rate 1 mL and injection volume of 20 μ l followed by detection wavelength at 237 nm (Fig-2).

Validation of method

The analytical method was validated by various parameter such as Linearity, Precision, Limit of quantification (LOQ), Limit of detection (LOD), Accuracy, Recovery studies, Robustness and Bulk assay as per International Conference on Harmonization (ICH) guidelines [7].

Linearity Studies

Different concentration for Barnidipine HCl was prepared for linearity studies. A typical HPLC chromatogram was obtained during the determination of Barnidipine HCl. The calibration curve obtained by plotting the peak area versus concentration showed linear relationship over a concentration range of 3-18 μ g/mL respectively. The linear regression equation for Barnidipine HCl was found to be $y = 58545x + 42411$ and the regression coefficient value (R^2) = 0.9992 for drug indicating high degree of linearity. Characteristic parameter of the HPLC method are shown in Table-1 and Calibration Curve is represented in Fig-3.

Accuracy Studies

Accuracy for developed method was evaluated in terms of % recovery studies, recovery experiments were performed at three different level, 80%, 100%, and 120% drug recovered, when known amount of standard drug was added to pre-analysed sample and subjected to proposed HPLC method. Recovery studies of Barnidipine HCl were carried out by spiking three different amount of Barnidipine HCl standard 4.8, 6.0, and 7.2 μ g/mL to the developed in house tablet formulation 6 μ g/mL standard addition method. The recovery values for Barnidipine HCl in ranged from 99.83 to 99.91 are shown in Table-2.

Precision

The precision was evaluated at three levels, repeatability, reproducibility, and intermediate precision. Each level of precision was investigated by six replicate injections of concentrations 9, 12, and 15 μ g/mL respectively. The result of precision is expressed as % RSD as shown in Table-3.

Assay of in-house Barnidipine HCl tablet formulation

Assay of in-house Barnidipine HCl tablest containing 10 mg of Barnidipine HCl along with SSG and MCC was performed at concentration of 9 μ g/mL. Percent amount found for Barnidipine HCl ranged from 98.36 to 99.69 (Table-4).

Analysis of Bulk

An accurately weighed 10 mg of Barnidipine HCl was transferred into 100 mL volumetric flask; dissolved in 50 mL methanol and the volume was made with the same solvent to give 100 μ g/mL. From the filtrate, measured volume was taken and diluted with mobile phase to get the final aliquots of 9 μ g/mL and injected; the area determined for selected peak. The concentrations of the drug were determined from linear regression equations. Percent amount found value for bulk assay ranged from 100.93 to 101.83 and average value was found to be 100.53 (Table-5).

Robustness Studies

The robustness was evaluated by analyzing the sample by varying few parameters, like flow rate, temperature, and mobile phase composition (Table-6).

Limit of Quantification and Limit of Detection

The LOD and LOQ of validated method fully depends on the standard deviation of the responses and slope of constructed calibrations curve. The LOD and LOQ value was found to be 0.231 and 0.701 μ g respectively.

System Suitability Study

System suitability test is integral part of liquid chromatographic methods. Retention time, capacity factor, number of theoretical plates, and resolutions were calculated for standard solutions are summarized in Table-7.

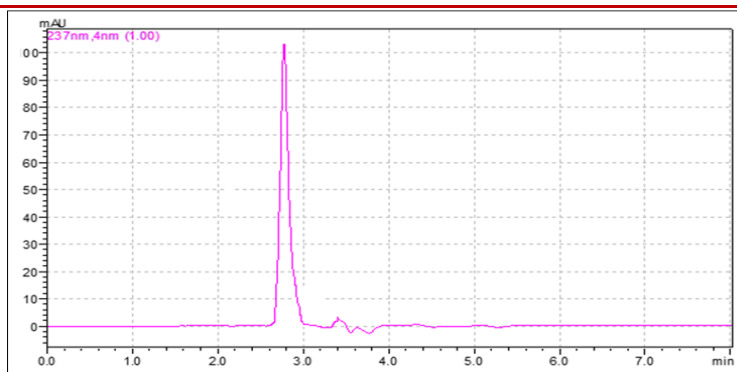


Fig-2: Optimizations of Barnidipine HCl in Methanol: Water in (80:20 v/v)

Table-1: Characteristics parameter of the HPLC method for determination of Barnidipine HCl

Parameter	Barnidipine HCl
Linearity range $\mu\text{g/mL}$	3-18 $\mu\text{g/mL}$
Slope	58545
Intercept	42411
Correlation Coefficient	0.9992

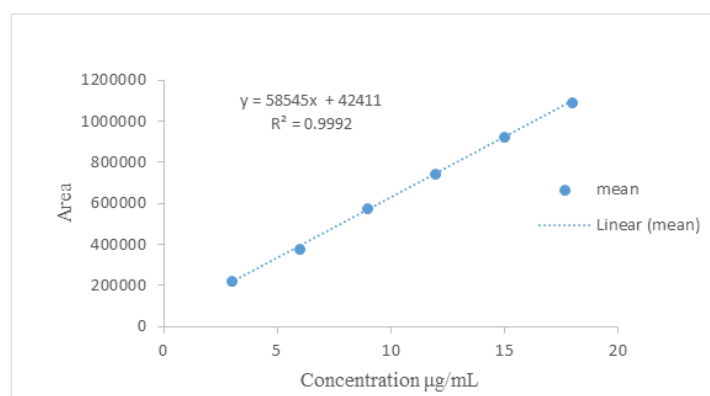


Fig-3: Calibration Curve for Barnidipine HCl

Table-2: Accuracy Studies

Drug	Initial Amount $\mu\text{g/mL}$	Amount of drug added to the analyte [%]	Total amount found \pm S.D. $\mu\text{g/mL}$	Recovery [%] [n=3]	%RSD [n = 3]
Barnidipine HCl	6	4.8	10.79 \pm 0.04	99.83	0.83
	6	6.0	11.98 \pm 0.04	99.74	0.68
	6	7.2	13.19 \pm 0.01	99.91	0.14

n- number of determinations

Table-3: Precision Studies [Intra and Inter-day]

Standard Concentration $\mu\text{g/mL}$	Amount Found $\mu\text{g/mL}$	% Amount found $\mu\text{g/mL}$ [n=3]	% RSD
Intra-day Precision			
9	8.93	99.24	0.48
12	11.85	98.76	0.50
15	14.73	98.24	0.72
Inter-day Precision			
9	9.06	100.27	0.40
12	11.73	97.26	0.49
15	14.87	99.17	1.06

n - number of determinations

Table-4: Analysis of in-house Tablets

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Barnidipine HCl	9	8.85	98.36
	9	8.89	98.78
	9	9.05	100.5
	9	8.96	99.61
	9	9.05	100.57
	9	8.97	99.69
	Mean ± SD	8.96 ± 0.08	99.60 ± 0.90
	% RSD	0.90	0.90

n - number of determinations

Table-5: Analysis of Bulk Material

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
BAR HCl	9	9.08	100.93
	9	9.05	100.58
	9	8.90	98.94
	9	9.09	101.04
	9	8.98	99.88
	6	9.16	101.83
	Mean ± SD	9.04 ± 0.090	100.53 ± 1.005
	% RSD	1.00	1.00

n- number of determinations

Table-6: Robustness study for Barnidipine HCl

Parameters	Tailing Factor	Theoretical plates	%RSD
Change in Temperature			
28	1.6	2290.8	1.68
32	1.4	2231.6	0.78
Change in mobile phase composition			
(Methanol: Water 85 :15 v/v)	1.49	2111.5	1.42
(Methanol: Water 90 : 10 v/v)	1.41	2020.4	0.31
Change in flow rate			
0.8	1.62	2304.6	1.31
1.2	1.30	2345.9	0.45

Table-7: System suitability studies

Retention time (tR)	2.8 min
Capacity factor (k0)	2.524
Theoretical plate (N)	2197.120
Resolutions	-

CONCLUSION

Proposed study describes a new RP-HPLC-PDA method for the estimation of Barnidipine HCl in bulk and in-house tablet dosage form using the simple mobile phase. The method gives good resolution with short analysis time. The method was developed and validated and found to be simple, sensitive, accurate, and precise. So the method can be used for the analysis of Barnidipine HCl in bulk and *in house* pharmaceutical dosage form.

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