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Development and Validation of Analytical RP HPLC Method for Benidipine Hydrochloride

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Abstract: *The aim of the present study is development and validation of analytical reverse phase (RP) high performance liquid chromatography (HPLC) from Benidipine Hydrochloride. HPLC method is used for quantification, identification and purification of particular analyte or compound. The developed HPLC chromatographic technique was validated based on international conference on harmonization (ICH) Q2 guidelines for accuracy, precision, linearity, range and robustness. High performance liquid chromatography technique is rapid and precise technique. Advance for the validated of Benidipine Hydrochloride in pure and tablet dosage form. Diethyl ether was extracted from plasma in the presence of 5M NaOH (Sodium Hydroxide) Diethyl ether is used as internal standard like Benidipine and Benidipine d5. Benidipine is dihydropyridine calcium channel blocker, which is used in the treatment of hypertension and angina pectoris and is used to examine the efficacy and safety of therapy with Benidipine in elderly hypertensive patients.*

Keywords: High performance liquid chromatography (HPLC), International conference harmonization (ICH), Benidipine hydrochloride, validation, calcium channel blocker, hypertension, angina pectoris.

I. INTRODUCTION

HPLC means high performance liquid chromatography, the Russian - Italian botanist Mikhail Tsvet is discovered by chromatographic technique.

Principle of HPLC

- High performance liquid chromatography (HPLC) principle based on Adsorption and partition chromatography.
- It important for determination of qualitative and quantitative analysis.
- It is used for determination of volatile and non- volatile compounds.
- It is used for determination of Retention time. (1)

Benidipine is a dihydropyridine derived from calcium channel blocker develop in Japan.benidipine hydrochloride having formula 3(3R)-1- benylpiperidine 3-yl methyl -2,6 dimethyl-4-(3- nitro phenyl -1-4- dihydropyridine-3-5, dicarboxylate hydrochloride having category antihypertensive agents. Benidipine is a vigorous and long lasting drugs used for the treatment of cardiovascular disease such as hypertension, Reno parenchymal, hypertension and angina pectoris. BEN HYDROCHLORIDE exhibits cardio protective and anti- atherosclerotic effect. (2) Benidipine is triple calcium channel blocker by inhibiting L, N and T type calcium channel. It having very long lasting activity, it's high affinity for cell membrane this characteristic indicated long lasting pharmacological activity of Benidipine. Benidipine is useful calcium channel blocker in the combination of hypertension therapy. In preclinical studies, the LD50 of Benidipine ranged from 83-384mg/ kg which is more 100 times needed dose to achieve a hypertensive action. (3) Calcium antagonist are mostly used for the treatment of different type of hypertension. Recently in eight JNC report, CCBs have shown good results over ACEI in the black population suffering from hypertension in terms of efficacy and prevention of stroke. (4) Benidipine exhibited no carcinogenic, antigenicity, tetra genic or mutagenic properties. In addition, CCBs may be particularly useful in elderly, isolated, systolic, hypertension, angina pectoris, peripheral vascular disease, carotid atherosclerotic, pregnancy and Raynaud syndrome. (5) Benidipine interaction with food and herbs for example goldenseal, grapefruit, ginseng reduce antihypertensive effect. Benidipine interaction with Alcohol, drinking alcohol while taking Benidipine may lead to some unwanted side effect like dizziness, drowsiness, fainting, on light-headedness.

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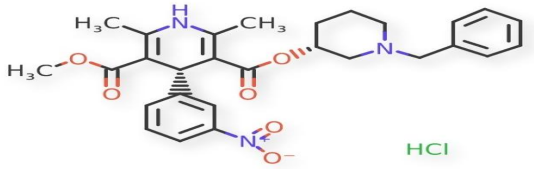
1.1 Objectives

1. The HPLC is used in the quantitative determination of plasma level of drugs and their 4metabolites.
2. The developed RP-HPLC method was validation in terms of the following parameter; specificity, accuracy, linerarity, sensitivity, precision, and stability of analytical solution.
3. The main purpose 8f the HPLC technique is to identify, quantify, and purify a particular analyte or compound both quantitative and qualified analysis can be done.
4. HPLC method main purpose is to separation compounds that are dissolve in solute.

II. STRUCTURE OF BENIDIPINE HYDROCHLORIDE



Pharmacodynamics of Benidipine hydrochloride

Benidipine reduces systolic and diastolic blood pressure as well as to present decreases in heart rate pulse after treatment. It is reported also decrease urinary protein excretion and serum triglycerides.

Pharmacokinetics of Benidipine hydrochloride

- **Absorption-** Benidipine is rapidly absorbed after oral administration reaching a maximum concentration within 2 hours.
- **Distribution-** Benidipine is highly distributed to the tissues mainly in the liver and plasma.
- **Protein binding-** Benidipine is highly bound to plasma protein and the bound from can account for even 98 percent of administered dose.
- **Metabolism-** Benidipine is almost completely metabolized in the liver.
- **Route of elimination-** The percentage of urinary excretion after oral administration is of approximate 36 percent of the administered dose.
- **Half-life-** The elimination half-life of Benidipine is registered to be approximate 1 hrs.(10)

Blood pressure is maintained by

Moments to moment regulation of cardiac output and peripheral vascular resistance exerted at three anatomic sites arterioles, postcapillary, venule and heart. Kidney. Local release of vasoactive substance. (11)

Factors influence blood pressure

- Cardiac output
- Peripheral vascular resistance
- Volume of circulating blood.
- Viscosity of blood.
- Elasticity of vessels wall.

II. MATERIALS and METHODS

2.1 Experimental Work[24]

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

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column was maintained at ambient temperature throughout study. Optimized chromatographic conditions are given in Table no.1

Table 1: Optimized Chromatographic Condition

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 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

2.2 Design of Experiments (2):

Thus, 33 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 analysed 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 3: The layout of actual design of DOE with the subsequent response

Std.	Run	Factor 1 A: Mobile Phase	Factor2 B: pH	Factor 3 C: Flow Rate	Response1 Retention Time	Response 2 Plate Count	Response 3 Tailing
11	1	84	2.7	1.3	2.40	4125	1.13
10	2	84	3.1	1	4.55	5126	1.21
15	3	84	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	3.00	3345	1.13
12	6	85	3.2	1.4	3.36	3809	1.16
16	7	85	3	1.2	3.44	4105	1.27
5	8	74	3	1	4.10	3989	1.3
14	9	84	3	1.2	3.50	4826	1.23
13	10	84	3	1.2	3.51	4037	1.25
1	11	75	2.8	1.2	3.39	3141	1.24
4	12	94	3.2	1.2	3.48	3336	1.14
9	13	84	2.8	1	3.42	4624	1.33
6	14	95	3	1	2.82	3317	1.11

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8	15	95	3	1.4	2.25	3088	1.19
17	16	84	3	1.2	3.34	4134	1.33
2	17	94	2.8	1.2	2.63	3442	1.2

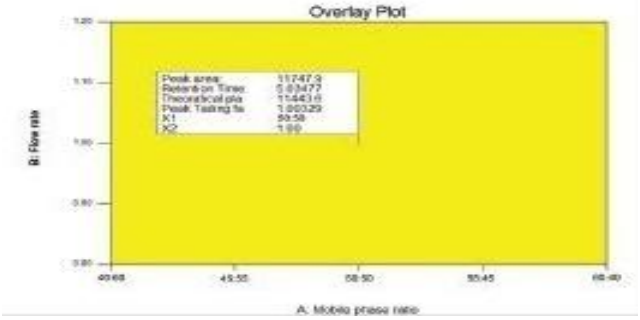

Table 4: Optimization Solution

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	1000	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. [12]

III. VALIDATION OF PROPOSED METHOD

The parameter for the validation were as follows:

1. **Linearity:** The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.
2. **Accuracy:** The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.
3. **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 may be considered at three levels: repeatability, intermediate precision and reproducibility.
4. **Robustness:** The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
5. **Detection Limit:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
6. **Quantitation Limit:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The quantitation limit is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products. [14]

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IV. CONCLUSION

HPLC method is simple, sensitive, precise and accurate RP- HPLC method was developed for quantitative estimation of Benidipine in bulk drug and pharmaceutical dosage form. (15) HPLC method can be used for the routine determination of Benidipine in bulk drug and in pharmaceutical dosage form. The proposed high performance liquid chromatographic method has also been evaluated for accuracy, precision and Robustness be convenient and effective for the quality of BEN Hydrochloride. Benidipine. Via blockade of T- type calcium channels, seems to elicit additive benefits for prevention of hypertensive cardiorenal injury.

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