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Research Article

DEVELOPMENT AND VALIDATION RP-HPLC METHOD FOR ANALYSIS OF FELODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT
The aim of present study was to develop and validate new simple, easy, selective, precise and accurate reverse phase high performance liquid chromatography for the estimation of Felodipine in bulk and pharmaceutical dosage form. The separation was carried on HPLC system consisting C-18 column (150 x 4.6 mm, 5 µm) at room temperature coupled with a guard column of silica with flow rate 1 ml/min. The mobile phase used was Acetonitrile: Water in the ratio 70:30. The drug was detected using UV-Vis detector at the wavelength of 238 nm and the run time was 10 minutes. The retention time was 8.29 minutes. The percentage RSD for precision and accuracy of the method was found to be less than 2 %. The method was validated as per ICH guidelines. The proposed method was found to be accurate, repeatable and consistent. It was effectively applied for the analysis of the drug in marketed formulation and could be used for the regular analysis of formulation containing the Felodipine.
Keywords: Felodipine, RP-HPLC, Validation

INTRODUCTION

Felodipine chemically, ethyl methyl-4(2, 3-dichlorophenyl)-1, 4-dihydro-2, 6-dimethyl-5methyl ester, is a calcium agonist widely used in the treatment of hypertension, heart failure and angina pectoris[1]. The molecular formula of Felodipine is $C_{18}H_{19}C_{l2}NO_4$ and Molecular weight 384.254 g/mol. A white or light yellow, crystalline powder, practically insoluble in water, freely soluble in acetone, in ethanol, in methanol and in methylene chloride, with melting point 145^0C. Felodipine is under the class of Calcium Channel Blocker used in the treatment of myocardial infarction, heart failures. Felodipine is a long-acting dihydropyridine calcium channel blocker (CCB). It acts primarily on vascular smooth muscle of voltage-gated sensitive channel cells by relaxing L-type calcium channels in their inactive conformation. By preventing the influx of calcium in smooth muscle cells, Felodipine prevents calcium-dependent myocyte contraction and vasoconstriction. Felodipine is the most potent CCB in use and is unique in that it shows fluorescent activity[1]. Felodipine binds to a number of calcium-binding proteins, demonstrates competitive antagonism of the mineral corticoid receptor. Felodipine is also used to treat mild to moderate critical hypertension[2]. Literature survey reveals that the quantitative determination of felodipine in body fluids and pharmaceutical dosage forms, were realized by titrimetry[5], spectrophotometry[6], HPLC[7-11] and gas-liquid chromatography[13]. While European Pharmacopoeia described titrimetry method for the assay of felodipine[4]. In this study, works were made to develop a simple, easy and economic HPLC method using a binary mobile phase system of acetonitrile: water (70:30) for the determination of felodipine in the raw materials as well as in the marketed dosage formulations. The developed method was validated as per the guidelines of International Conference on Harmonization (ICH)[3] and established excellent specificity, linearity, precision and accuracy for Felodipine.

MATERIALS AND METHODS

Chemicals and Reagents

Felodipine gift sample was provided by Nivedita Chemicals PVT Ltd. Andheri East, Mumbai, India. The commercial tablet dosage form was available from local market labeled FELOGARD (5 mg Felodipine) of Cipla limited. HPLC water and acetonitrile were obtained from Merck speciallities Pvt. Mumbai, India. All chemicals and reagents were of analytical grade.

HPLC Instrument and Chromatographic Conditions

The proposed method was performed on the Cyberlab LC-100B complete binary gradient HPLC system comprising UV-Vis detector and LC 100 HPLC pump. The column used for separation of analytes is phenomenex C18 column (250 x 4.6 mm 5 µm) isocratic elution with acetonitrile and water as mobile phase (70:30). The sample was injected through 772 Si Rhenodyne injector with bracket. The data was acquired, stored and analyse with Cyberlab DS 100 control data system software. The flow rate of the mobile phase 1.0 ml/min at room temperature

Mobile Phase Preparation

The mobile phase was a mixture of acetonitrile: water, 70:30 v/v solutions was filtered and sonicated for 15 minutes prior to use.

Preparation of Standard Stock Solution

10 mg of active standard felodipine was accurately weighed and shifted into 10 ml volumetric flask and then small amount of mobile phase was added and sonicated for 5 minutes and diluted up to get stock solution of 1000 µg/ml. From this 5 ml was transferred into 50 ml volumetric flask and made up the volume with mobile phase (Concentration 100 µg/ml). From this stock solution dilutions were made to get concentrations 2-10 µg/ml and 20 µl of each solution was injected in to column.

Preparation of Sample Solution

An accurately weighed powder of equivalent to 10 mg of twenty tablets of FELOGARD (5 mg Felodipine) was weighed and was transferred to 10 ml volumetric flask and small amount of methanol was added. The solution was sonicated for 15 minutes and the final volume was made with mobile phase to achieve solution of felodipine (1000 µg/ml). The mixture was filtered through a nylon 0.45 mm membrane filter. The above was properly diluted with mobile phase to obtain final dilution of felodipine (10 µg/ml).

Table 1: Linearity of Felodipine

S. No.	Concentration	Area
1	0	0
2	2	15224.7
3	4	26820.3
4	6	41898.5
5	8	55465.5
6	10	70151.5

Table 2: Results from Accuracy Study

Concentration	Recovery level	Amount added (µg/ml)	Amount found (µg/ml)	% Recovery
10	80	8	17.8	98.88
10	100	10	20.2	101
10	120	12	21.9	99.54

*Average of three determination (n = 3)

Table 3: Assay of Felodipine

Formulation	Labeled amount (mg)	Amount Found (mg)	Assay
FELOGARD	5 mg	4.89 mg	98 %

*Average of three determination (n = 6)

Table 4: Results from the Precision Studies

Concentration (µg/ml)	Intra-day Precision		Inter-day Precision	
	SD	% RSD	SD	% RSD
2	2.44	0.005775	4.08	0.009621
6	4.13	0.009748	4.17	0.009453
10	1.65	0.003906	3.68	0.008677

*Average of three determination (n=6)

Table 5: LOD and LOQ of Felodipine

Std solution	LOD (µg/ml)	LOQ (µg/ml)
Felodipine	0.000665	0.002014

Table 6: Robustness Values of Felodipine

Parameter	Conditions	Rt	Area	% Assay
Flow rate	0.8	8.21	70346	103 %
	1.2	8.18	68465	96 %
Mobile Phase	72:28	8.26	70975	101 %
	68:32	8.32	71134	105 %

Table 7: Results from Ruggedness Studies

Sr.no	Assay Concentration	Amount Found
Analyst 1	10 µg/ml	99.73 %
Analyst 2	10 µg/ml	101.05 %

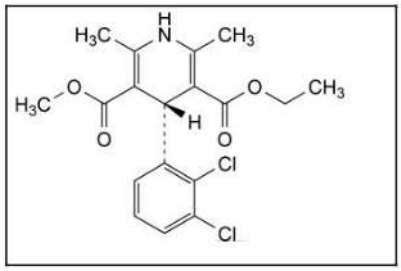

Figure 1: Structure of Felodipine

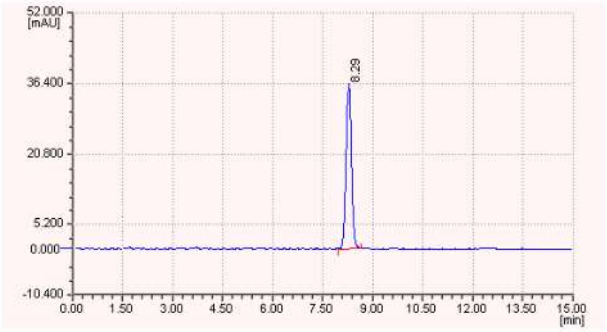

Figure 2: HPLC Chromatogram of Felodipine (Standard)

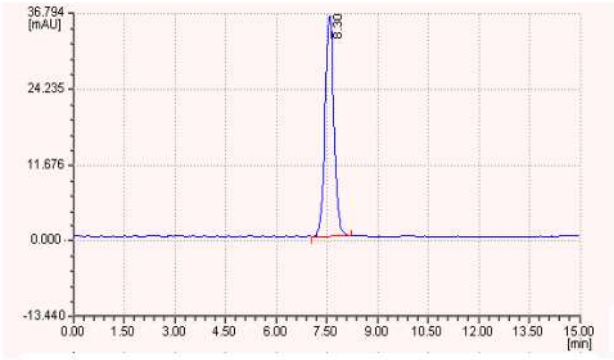

Figure 3: HPLC Chromatogram of Felodipine (Formulation)

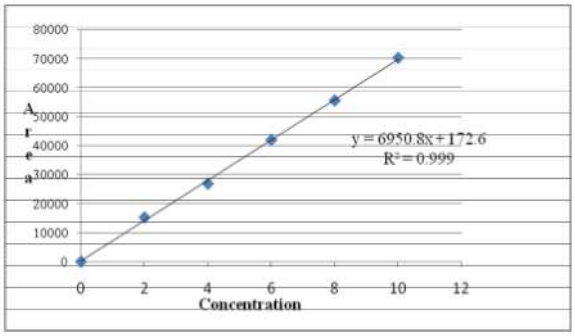

Figure 4: Calibration Graph of Felodipine

RESULTS AND DISSCUSION

HPLC Method Development

Determination of felodipine was accomplished on the Cyberlab LC-100B complete binary gradient HPLC system containing UV-Vis detector and LC 100 HPLC pump. The column used for separation of analytes was, phenomenex C18 column (250 x 4.6 mm 5 µm) using isocratic elution with acetonitrile and water as mobile phase (70:30). The sample was injected through 772 Si Rhenodyne injector with bracket.

The data was acquired, stored and analyse with Cyberlab DS 100 control data system software. The flow rate of the mobile phase was 1.0 ml/min at room temperature. The retention time of felodipine was 8.29 minutes. The peak shape of felodipine was found to be symmetrical.

Method Validation

Calibration Curve and Linearity

Calibration curve was plotted over the concentration range of 2-10 µg/ml for felodipine with regression coefficient [R^2] 0.999. Linearity was calculated by lest square linear regression analysis of calibration curve[6]. Calibration curve graph was linear over the concentration range of 2-10 µg/ml and the linear regression equation was

$$y = 6950.8x + 172.6$$

Accuracy

Accuracy of the method was confirmed by recovery studies. By adding known amount of standard to pre-analysed sample studies were carried out at three different levels (80 %, 100 % and 120 %). The proposed method is shown values within 98-102 % in Table 2.

Precision

The planned method was tested by performing intraday and interday studies. Data is elaborated in Table 4. The intraday was carried out by analyzing standard solution of 2, 4, and 6 µg/ml for three times on the same day and interday was carried out over a period of three days. Values of % RSD for interday and intraday were found to be within limits.

LOD and LOQ

Method sensitivity was estimated in the terms of Limit of Detection and Limit of Quantification by using formulae

$$LOD = 3.3*\sigma/S \quad \text{and} \quad LOQ = 10*\sigma/S$$

Where S is the slope of calibration plot, σ is standard deviation calculated using values of y intercepts of regression equation.

Obtained values were tabulated in Table 5.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. For the proposed method robustness was checked by making small deliberate changes in mobile phase and Flow rate. After making these small changes showed no any difference in peak area for estimation of Felodipine. The values are given in Table 6 point out method is robust.

Ruggedness

It is the reproducibility of a test result under operating condition from instrument to instrument and from analyst to analyst. The ruggedness of the method was performed by comparing the assay results by two analyst in the same laboratory and results given in Table 7.

CONCLUSION

An easy precise, selective and sensitive RP-HPLC assay method detection for Felodipine in pharmaceutical dosage form has been developed and validated. Thus the proposed method can be comprehensively used for general quality control analysis of Felodipine in bulk and tablet dosage form.

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