RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF FELODIPINE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

POLURI KOTESWARI [1], SISTLA RAMAKRISHNA [2], VEEREREDDY PRABHAKAR REDDY*[3], GULATI SATNAM KAUR [1], LAKSHMI M NARASU [4]

[1]S.N. Vanitha Pharmacy Mahavidyalaya, Hyderabad,
[2]Pharmacology division, Indian Institute of Chemical Technology, Hyderabad,
[3]St. Peters Institute of pharmaceutical sciences, Warangal
[4]Institute of Science and Technology, JNTUH, Hyderabad

ABSTRACT

Objective of the present study was the development and validation of a simple RP-HPLC method for the estimation of felodipine in bulk and pharmaceutical dosage forms. The analysis was carried out by using phenomenex C-8 column in isocratic mode with the mobile phase consisting of Acetonitrile and water in the ratio of 31:69 v/v at a flow rate of 1ml/min. The eluent was detected for 240 nm. The retention time of the drug was 4.28 minutes. The proposed method was statistically validated and found that it is simple, accurate, precise, robust, and suitable for the routine analysis of pharmaceutical formulations.

KEY WORDS: felodipine, HPLC, validation.

1.INTRODUCTION

Felodipine is a calcium channel blocker and of dihydropyridine class. It is used in the treatment of mild to moderate hypertension (Blychert,1990). Chemically it is 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine-carboxylic acid ethyl methyl ester. Practically it is insoluble in water and soluble in organic solvents like chloroform and methanol. Since it is a highly photosensitive drug necessary precautions were taken during the study. According to the literature very few HPLC methods (Cardoza and Amin,2002; Miglioranca,2005; Dru,1995; Margareth,1992; Basavaiah,2003; Hohyun,2003) have been reported. The present method is simple, rapid, accurate and precise for the estimation of felodipine in pharmaceutical dosage forms.

2.EXPERIMENTAL

A High performance liquid chromatograph (Shimadzu-10ATVP) equipped with two pumps (Model-10ATVP) and Shimadzu –UV-Visible detector (SPD-10ATVP), Phenomenex P/N0-00G-4274-EO C-8 column, Size: 250×4.6mm, Bath sonicator Sonica®,Ultrasonic cleaner Spincotech PVT Ltd., HPLC grade Acetonitrile, Methanol, water were purchased from SD fine Chemicals, India.

***For Correspondence**
vpreddyindia@yahoo.com

Felodipine was a gift sample from Dr. Reddys Laboratories, Hyderabad.

Preparation of the standard and sample drug solutions

To prepare standard stock solution 10mg of felodipine was accurately weighed in to a 10ml volumetric flask and dissolved in methanol and volume was made up to 10ml with the same. From this subsequent dilutions ranging from0.5 to 20µg/ml were made with phosphate buffer solution of P^H-7.4. The sample drug solution was prepared by taking not less than 20 tablets each containing 10mg of felodipine. The tablets were weighed and powdered. The quantity of powder equivalent to 10mg of felodipine was weighed, transferred to a 10ml volumetric flask and extracted with methanol. The solution was sonicated for 15min. The extracts were filtered through whatmann filter paper No:41 and residue was washed with methanol. The extracts and washings were pooled and transferred to a 10ml volumetric flasks and volume was made up to 10ml with methanol.

Determination of λ-max: From the standard dilutions 10 µg/ml solutions was scanned using UV/Visible spectrophotometer between 200 to 400nm. λ-max of 240nm was selected where drug showed maximum absorbance.

Preparation of mobile phase: Acetonitrile: water in the ratio of (31:69) mixture was selected as mobile phase. Both the solvents were filtered by using membrane filter pore size 0.45µ and degassed with bath sonicator (Sonica®, Ultrasonic cleaner).

Preparation of calibration curve: Working standard solutions of felodipine were prepared in the conc. range of 0.5 to 20 µg/ml. 20µl of this solution was injected each time into the column at a flow rate of 1ml/min. The detection of the method was monitored at 240nm. Each of the dilution was injected for 3 times into the column and corresponding chromatograms were obtained. The retention time was found to be 4.28 min. Graphs were plotted between the mean peak areas of the drug with respect to conc.

Validation of the method: The method was validated for linearity, accuracy, limit of detection, limit of quantification, inter-day and intra-day precision, and robustness.

Assay of the marketed products: 10µg/ml of the filtered sample solution was analyzed by the developed method. The analysis was repeated in triplicate. The content of the drug was calculated from the peak areas recorded.

3.RESULTS AND DISCUSSIONS

The mobile phase consisting of acetonitrile and water in the ratio of 31:69 retained a good symmetric peak at 4.28 min. A typical chromatogram was shown in figure 1. A calibration graph was plotted with concentration Vs peak area and was given in figure 2. The linear regression data (n=3) showed a good linear relationship over a concentration range of 0.5-20 µg/ml. The correlation coefficient was found to be 0.998. The limit of detection (LOD) and limit of quantification (LOQ) were determined by using the formula 3.3σ/S and 10σ/S respectively. Where σ is the standard deviation of the response and S is the slope of the calibration curve. The results were given in the table 1.

The intra-day precision was determined by analyzing standard solutions in the concentration range of 0.5 to 20 µg/ml for three times on the same day while inter-day precision was determined by analyzing corresponding standard daily for three days over a period of one weak, and percentage relative standard deviation (RSD) was calculated. The RSD was found to be less than 2 for both iner-day and intra-day precision. To confirm specificity of the proposed method the sample was injected in to the HPLC column. It was observed that excipients present in the formulation did not interfere with the drug peak. All the validation parameters were given in table 1.

Recovery studies of the drug were carried out for the accuracy parameter. These studies were carried out at three levels i.e., multiple level recovery studies to the sample solution 50%, 100% and 150% of the standard drug solutions were added, dilutions were made and analyzed by the method. The percentage recovery and the percentage RSD was calculated and found to be within the limits. The assay value of the marketed formulation was found to be within the limits. The low RSD value indicated suitability of this method for routine analysis of felodipine in pharmaceutical dosage forms. The results were shown in table 2 and table 3.

Robustness of the method was studied by making small deliberate variations in method parameters. Sample dilutions were made in phosphate buffer of pH 7.4 containing 1% tween-80 and injected in to HPLC. Results were shown in table 4.

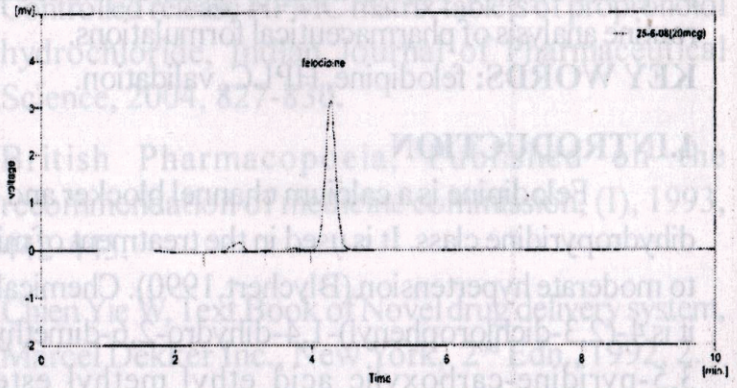

Figure 1 Typical chromatogram of felodipine

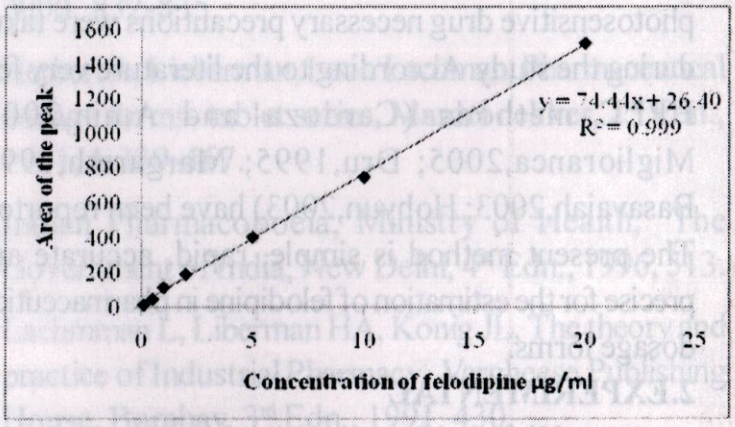

Figure 2 Standard graph of felodipine in PBS (pH 7.4)

Table 1: Validation parameters

parameter	value
Retention time	4.28
Linearity	0.5 – 20 µg/ml
Correlation coefficient	0.9998
LOD	0.13 µg/ml
LOQ	0.38 µg/ml
Precision intra-day	1.19
Precision inter- day	1.2
No of theoretical plates	3912

Table 2: Recovery studies of felodipine

Drug	%Level	n	Conc. µg/ml	Amt. recovered µg/ml	% recovery	%RSD
felodipine	50	3	5	4.95	99	1.2
	100	3	10	9.82	98.2	0.9
	150	3	15	14.98	99.8	0.75

Table 3: Assay of the marketed formulation

drug	n	Label claim mg/tablet	Amt found mg/tablet	Mean% recovery	%RSD
felodipine	3	10	10.13	101.3	1.25

Table 4: Robustness of the method

solvent	Retention time	Tailing factor	Theoretical plates
Buffer	4.28	1.17	3912
Tween-buffer	4.3	1.12	4014
methanol	4.49	1.14	2992

REFERENCES

Basavaiah K, Chandrashekar U and Prameela CH, Determination of felodipine in bulk drug and in tablets by high performance liquid chromatography, Indian Journal of Chemical technology, 10, 2003, 454-456.

Blychert E, Wingstrand K, Edgar B and Lidman K, Plasma concentration profiles and antihypertensive effect of conventional and extended-release felodipine tablets, Br.J. clin.Pharmac., 29, 1990, 39-45.

Cardoza R M and Amin P.D, A stability indicating LC method for felodipine, Journal Pharmaceutical and biomedical analysis, 27, 2002, 711-718.

Dru J.D, Hsieh J.Y, Matuszewski B.K, Dobrinska M.R, Determination of felodipine, its enantiomers and a pyridine metabolite in human plasma by capillary gas chromatography with mass spectrometric detection, Journal of chromatogram.B, Biomedical appl., 666, 1995, 259-67.

Hohyun K, Hyeongjin R, Seung Y, Hee J and Sang H, Sensitive Determination of Felodipine in Human and Dog Plasma by Use of Liquid–Liquid Extraction and LC–ESI–MS–MS, Chromatographia, 58, 2003, 235-240.

Margareth G, Kurt JH and Carl GR, Determination of four carboxylic acid metabolites of felodipine in plasma by high-performance liquid chromatography, Journal of chromatogram.B, Biomedical appl., 573, 1992, 265-274.

Miglioranca LH, Rafael E, Barrientos A, Schug BS, Blume HH, Pereira AS, De Nucci G, Felodipine quantification in human plasma by high-performance liquid chromatography coupled to tandem mass spectrometry, Journal of chromatogr.B, 814, 2005, 217-223.