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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF FELODIPINE BY UV AND RP–HPLC METHOD

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ABSTRACT

A simple, fast, specific and accurate reverse phase high performance liquid chromatography (RP-HPLC) and UV method has been developed and subsequently validated for Felodipine. HPLC – Binary gradient system (Model 3000 Series) equipped with UV-3000-M detector, HPLC workstation software was used. Chromatographic separation was achieved isocratically with Hexon C18 (250mm x 4.6 ID, Particle size: 5 micron) using a mobile phase, Methanol and water (adjusted to pH 3 with Ortho –phosphoric acid) in the ratio of (90:10 V/V). UV detection was carried out at 238 nm. UV-Visible spectrophotometer (UV-2012) equipped with PDA detector was used for analysis. The retention time for Felodipine was found to be 3.82 min.

Keywords: *Felodipine, UV-Visible Spectrophotometric method, HPLC.*

INTRODUCTION

Felodipine is a long-acting 1,4-dihydropyridine calcium channel blocker (CCB)b. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. By inhibiting 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 exhibits fluorescent activity. In addition to binding to L-type calcium channels, Felodipine binds to a number of calcium-binding proteins, exhibits competitive antagonism of the mineral corticoid receptor, inhibits the activity of calmodulin-dependent cyclic nucleotide phosphodiesterase, and blocks calcium influx through voltage-gated T-type calcium channels. Felodipine is 3-ethyl 5-methyl-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate. Physical appearance of Felodipine is Slightly yellowish, crystalline powder solubility in Acetonitrile , methanol , ethanol & dichlororomethane. Storage in Store protected from light. The chemical structure of Felodipine is given below [10].

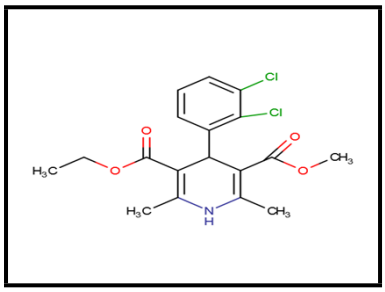

Fig. 1: Structure of Felodipine

Felodipine is used to treat mild to moderate essential hypertension. The aim of the present work is to develop analytical method and validate UV and RP-HPLC method for Felodipine drug.

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The validated method was validated in accordance with International Conference on Harmonization (ICH) guidelines.

MATERIAL AND METHODS

Felodipine was obtained from Cipla Ltd., In addition analytical reagent grade. Methanol was purchsed from Merck, (Mumbai, India). All chemicals and solvent were of analytical grade.

The chromatographic analysis was performed by an HPLC – Binary Gradient System equipped with UV -3000 -M detector , column Hexon C18 (250mm x 4.6 ID , Particle Size : 5 micron) company analytical Technologies Ltd., software : HPLC Workstation. Spectrophotometric analysis was performed by UV–Visible Spectrophotometer (Model: UV-2012) equipped with detector: PDA, wavelength: 238 nm.

A. UV Method Development: [12]

Preparation of standard stock solution: 1000 ug/mL solution of Felodipine was prepared by accurately weighing 10 mg of Felodipine. It was then transferred to 10 ml volumetric flask containing mixture of methanol: water (90:10). Mixture was prepared freshly, sonicated for 30 minutes and filtered through 0.45 um membrane filter paper.

Preparation of working solution: From stock solution 0.1 ml was diluted upto 10 mL by using solvent mixture Methanol: Water (90:10) which will give 10ug /mL.'

B. HPLC Method Development:

Preparation of stock solution: 1000 ug /mL solution of Felodipine was prepared by accurately weighing 10 mg of Felodipine. It was then transferred to 10 ml volumetric flask containing mixture of methanol: water (90:10). Mixture was prepared freshly; sonicated for 30 minutes and filtered through 0.45 um membrane filter paper. The resultant solution was scanned using UV visible spectrophotometer in the range of 200 – 400 nm.

Preparation of working solution: From stock solution 0.2 ml was diluted upto 20 mL by using solvent mixture Methanol: Water (90:10) which will give 20ug /mL.

Method Development:

Optimized Chromatographic conditions: Chromatographic separation was achieved using column Hexon C18 (250 mm x 4.6 ID, Particle size: 5 micron) The injection volume was 20ul, Flow rate: 1.0 ml/min. . The total run time of analysis was 3.825 min. Methanol water (90:10) pH: 3 were used as mobile phase. Run time: 15.65min.

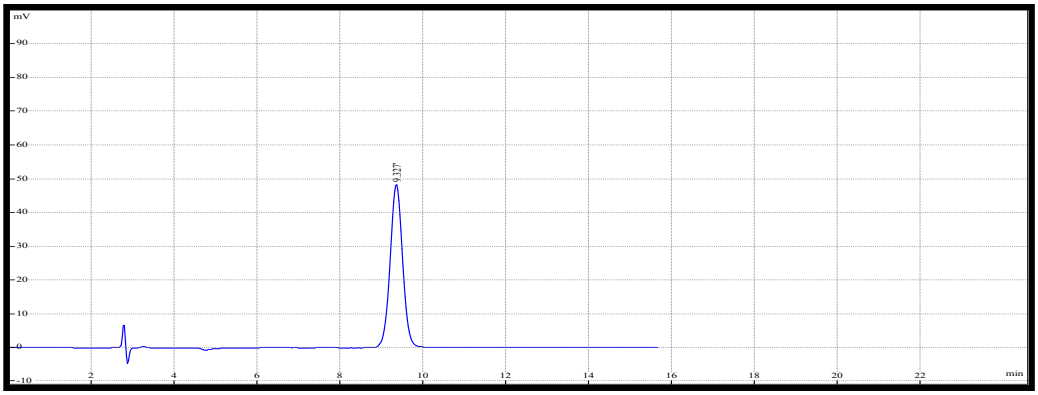

Fig. 2: Trial and error graph of Felodipine

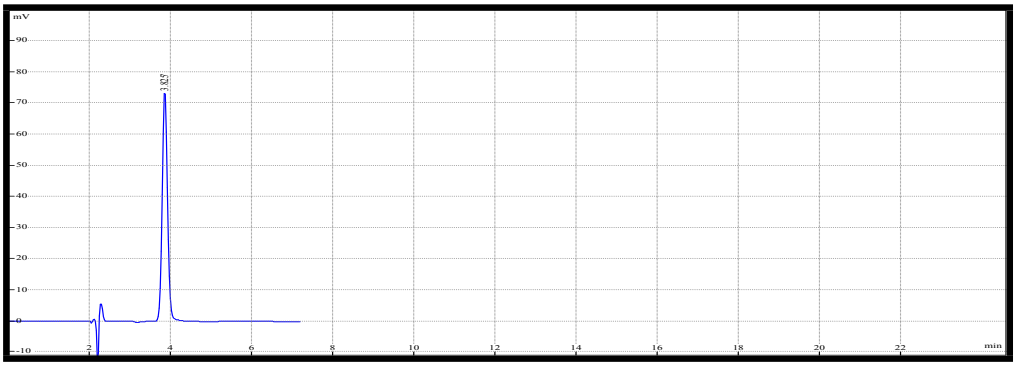

Fig. 3: Standard Chromatogram showing structure of Felodipine

Method Validation: To demonstrate the feasibility of newly developed method, validation was performed by using Felodipine according to International Conference on Harmonization (ICH) guidelines. By considering the parameters like Linearity, accuracy, precision, LOD, LOQ, Robustness, % recovery by UV and Linearity, accuracy, precision, System suitability, LOD, LOQ by using HPLC.

RESULTS

A. UV Method Development: [13-16]

Determination of maximum wavelength: From the calibration curve of UV, maximum wavelength (λmax) for Felodipine for was found to be 238 nm.

Linearity: Linearity of method was ranging from concentrations 10 to 30 ug/ml for Felodipine. A graph is plotted with concentration on X-axis and mean absorbance on Y-axis. The $r^{2\ value}$ was found to be 0.998. Hence the developed method was found to be the linear in 10 to 30 µg/mL concentration array.

Accuracy: The mean absorbances were determined at three concentration levels. The results obtained from the below study find within the range of pharmacopoeial standard.

Precision: The precision study shown that % RSD of mean absorbance's obtained from measurements of 10, 20 & 30 µg/mL solutions were less than 2% RSD .The result shows that the method obtained is precise as per ICH guideline Q2R1.

Robustness : The absorbance of 20 ppm Felodipine solution was recorded in five replicates at three different wavelengths viz., 237, 238 (std wavelength) and 239 as given in above table. Mean, % RSD both of these statistical parameters were found in limit. All values obtained for percent assay were in agreement with pharmacopoeial standard for Felodipine .Therefore the developed method was robust for deliberate fluctuation in wavelength. % RSD for change in method parameter (i.e. wavelength ± 1) was found to be within limit (NMT 2%).

LOD & LOQ: LOD was determined by equation and found to be 0.24 µg/mL and LOQ was found to be 0.78 ug/mL.

% Recovery : Known amount of standard solution of Felodipine 5, 10 & 15 ug/mL were added to preanalysed sample solution of Felodipine (10 µg/mL). % Recovery was calculated by measuring mean absorbance. Data obtained is represented in below table. The proposed method is afforded the percent recovery as per ICH guidelines.

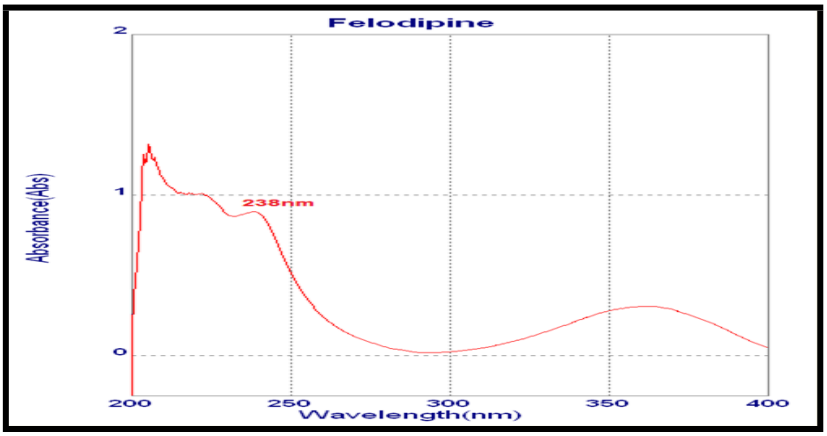

Fig. 4: Wavelength maxima for Felodipine

Table No. 1: Linearity Data

Concentration (µg/mL)	Absornance at 238 nm
10	0.2846
15	0.4926
20	0.6487
25	0.8292
30	1.13

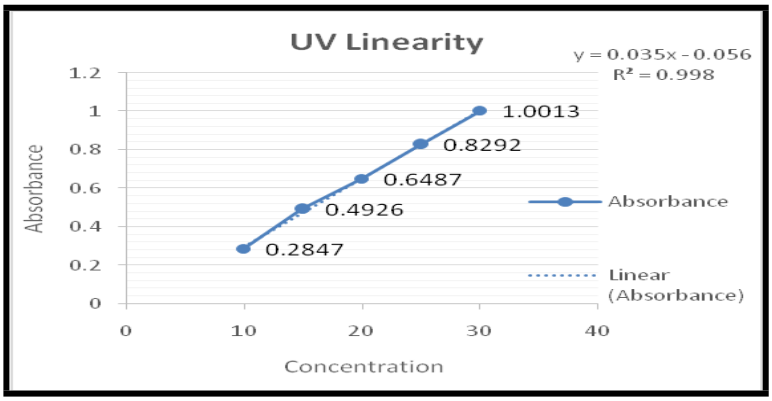

Fig. 5: UV Linearity for Felodipine

Table No. 2: Accuracy Data

Conc. (µg/ml)	Absorbance	Mean Absorbance	Mean measured conc. (µg/mL)	% Assay	Inference
10	0.2847	0.2844	9.98	99.8	Passed
10	0.2843				
10	0.2843				
20	0.6487	0.647	20.02	100.1	Passed
20	0.6488				
20	0.6488				
30	1.0013	1.0011	29.56	98.53	Passed
30	1.0012				
30	1.0008				

Table No. 3: For Interday Precision

Conc. (µg/mL)	Mean absorbance	SD	%RSD	Inference
10	0.2844	0.0003	0.0930	Passes
20	0.6487	0.0010	0.1561	Passes
30	1.0011	0.0003	0.0264	Passes

Table No. 4: for Intraday Precision

Conc. (µg/mL)	Mean absorbance	SD	%RSD	Inference
10	0.2847	0.0001	0.0203	Passed
20	0.6488	0.0001	0.0089	Passed
30	1.0012	0.0006	0.0058	Passed

Table No. 5: Robustness Data

Conc. (µg/ml)	Wavelength (nm)	Mean absorbance	SD	% RSD	Inference
20	238 (std)	0.6487	0.0002	0.0235	Passed
20	237 (-1)	0.6378	0.0003	0.0479	Passed
20	239 (+1)	0.6523	0.0001	0.0153	Passed

Table No. 6: % Recovery Data

Conc. (µg/mL)	% Added	Absorbance	% Recovery
10 + 05	50	0.4924	99.83
10 +10	100	0.6483	99.84
10 +15	150	0.8292	99.75

B. RP- High Performance Liquid Chromatography Method: [8, 9, 16]
Linearity data: A graph is plotted with concentration on X-axis and Peak area on Y-axis. The $r^{2\ value}$ was found to be 0.991. Hence the developed method was found to be the linear.

Accuracy: The mean peak areas were determined at three concentration levels. The results obtained from the below study find within the range of pharmacopoeial standard.

Precision: The precision study shown that % RSD of mean peak area obtained from measurements of 25, 75 & 125 ug/mL solutions were

less than 2% RSD .The result shows that the method obtained is precise as per ICH guideline Q2R1.

Robustness: The area of 25 ppm Felodipine solution was recorded in five replicates at three different mobile phase ratio viz., 89:11, 90:10 (std mobile phase ratio) and 91:9. % Assay of these statistical parameters was found in limit. Therefore the developed method was robust for deliberate fluctuation in wavelength. % Assay for change in parameter (wavelength ± 1) was found to be within limit (NMT 2%).

LOD and LOQ: LOD was determined by equation and found to be 0.0661 µg/mL and LOQ was found to be 0.9836 µg/mL.

% Recovery: Known amount of standard solution of Felodipine 25, 50 & 75 µg/mL were added to preanalysed sample solution of Felodipine (10 µg/mL). % Recovery was calculated by measuring mean area. Data obtained is represented in below table. The proposed method is afforded the percent recovery as per ICH guidelines.

Table No. 7: Linearity data

Conc. (µg/mL)	Area *
25	1400017
50	30011240
75	4251730
100	5243410
125	6398764

****The results were mean area of three replicate injections.***

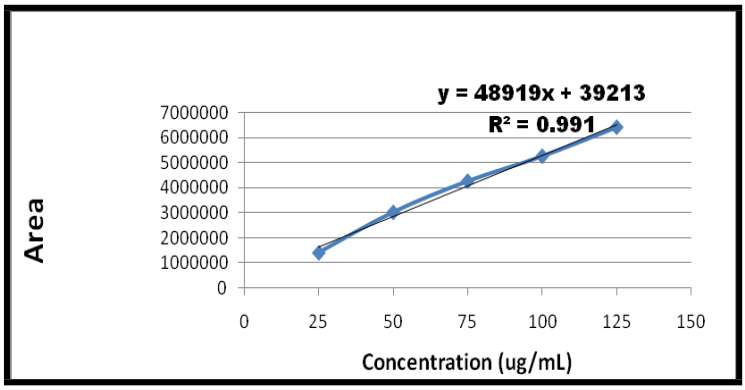

Fig. 6: HPLC Linearity for Felodipine

Table No. 8: Accuracy data

Conc. (µg/mL)	Area	Mean Area	Mean easured conc. (ug/mL)	% Assay	Inference
25	1400017	1412212	24.86	99.44	Passed
25	1424358				
25	1412262				
75	4251730	4252589	75.03	100.04	Passed
75	4241107				
75	4264932				
125	6398764	6303050	124.92	98.53	Passed
125	6269340				
125	6241046				

Table No. 9: For Intraday Precision

Conc. (µg/mL)	Mean area	%RSD	Inference
25	1400017	0.8618	Passes
75	4251730	0.2806	Passes
125	6398764	1.334	Passes

Table No. 10: For Interday Precision

Conc. (µg/mL)	Mean area	%RSD	Inference
25	1424358	0.8617	Passes
75	421107	0.2807	Passes
125	6241046	1.3210	Passes

Table No. 11: Data for Robustness Study

Conc. (µg/ml)	Mobile phase ratio	Mean RT	Mean Area	% Assay (98% to 102%)	Inference
25	90 : 10 (std)	3.825	1400017	100	Passed
25	91:9 (-1)	3.875	1390216	99.30	Passed
25	89 : 11 (+1)	3.785	1442017	103	Passed

Table No. 12: Data for % recovery

Conc.(ug/mL)	% Added	Peak Area	% Recovery
50 + 25	50	4257988	100.14
50 +50	100	5265312	100.41
50 +75	150	6254962	102.29

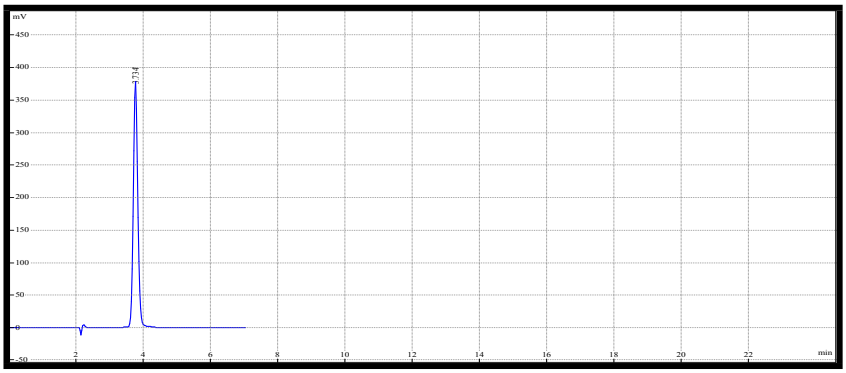

Fig. 7: 50% recovery from Felodipine

CONCLUSION

The UV–Spectrophotometric and RP–HPLC methods were developed and validated as per ICH guidelines for quantitation of Felodipine in bulk & in tablet formulation. From this study it can be concluded that, the analysis of tablet dosage formulation can be successfully performed by the UV-Spectrophotometric analysis method. The UV-Spectrophotometric and RP-HPLC methods have been evaluated for the linearity, accuracy, precision, robustness, LOD, LOQ & % Recovery in order to ascertain the suitability of the analytical method. The method was also applied to marketed sample. It has been proved that both UV-Spectrophotometric and RP-HPLC methods were selective, linear and precise. Both the methods are simple, reproducible and rapid. These methods may be adopted for routine quality control analysis of this drug.

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