

Ultra-Fast Liquid Chromatographic Method (Stability-Indicating) for the Determination of Felodipine

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ABSTRACT

Felodipine is a calcium channel blocker acting on vascular smooth muscle cells through stabilization of voltage-gated L-type calcium channels. A new stability-indicating ultra-fast HPLC method was proposed for the estimation of Felodipine in pharmaceutical dosage forms. The chromatographic study was accomplished on Shimadzu HPLC instrument using sodium acetate buffer: acetonitrile as mobile phase (UV detection at 237 nm). Stress degradation studies were performed and the method was validated

KEY WORDS: Felodipine; Isocratic elution; Stability-indicating RP-HPLC.

1. INTRODUCTION

Felodipine (FLD) is used for the treatment of hypertension (Nyborg and Mulvany, 1984). Felodipine (Figure1) is chemically known as 3-ethyl 5-methyl 4-(2, 3-dichlorophenyl)-2, 6-dimethyl-1, 4-dihydropyridine-3, 5-dicarboxylate. Felodipine is a potent and unique drug that shows fluorescent action (The Merck Index, 2006). In the literature survey very few analytical methods were described for the analysis of Felodipine which include spectroscopic techniques (Basavaiah, 2005), GC-MS (Dru, 1995), HPLC methods (Margareth, 1992, Lars, 1985, Fusun Gedil, 2004, Basavaiah, 2003, Nataraj, 2011, Cardoza, 2002, Mathrusri, 2013), micellar HPLC (Rapado, 1996), HPLC with amperometric detection (Lopez, 2000) and LC-MS/MS in dog and human plasma (Hohyun, 2003; Sreedevi, 2011, 2011; Yan-yan, 2006; Luis, 2005). In the current study a new stability indicating isocratic RP-HPLC method was proposed for the estimation of Felodipine for the assay of pharmaceutical formulations.

2. MATERIALS AND METHODS

Instrumentation: Chromatographic separation was achieved by using a Shimadzu HPLC system, equipped with PDA detector with C18 Zorbax column maintained at 25 °C.

Chemicals and reagents: Acetonitrile (HPLC grade), sodium hydroxide (NaOH) and hydrochloric acid (HCl), acetic acid (Spectrochem Pvt. Ltd.) and Hydrogen peroxide (H₂O₂) were bought from Merck (India). Felodipine standard was obtained from Astra Zeneca Pharma India Limited as gift sample. Felodipine is existing as PLENDIL® (Astra Zeneca Pharma India Limited, India) and FELOGARD® ER (Cipla Limited, India) tablets with label claim of 2.5, 5 and 10 mg of drug.

Felodipine stock solution was prepared using acetonitrile and dilutions were made from with mobile phase and filtered through 0.45 µm membrane filter prior to injection.

Validation:

Linearity: A set of solutions (0.1–150 µg/mL) were prepared from stock solution and peak area / peak height of the eluted chromatogram was noted. Calibration curve was obtained by plotting the concentration of the solutions on the x-axis and the corresponding peak area on the y-axis. The data was treated with linear regression analysis method (ICH guidelines, 2003).

Precision and accuracy: The intra-day and inter-day precision were studied for FLD on the same day and on three different days and the statistical analysis was performed. Accuracy of the method was computed by spiking the formulation solutions with the standard solutions and the percentage recovery was calculated.

Robustness: The robustness method was studied by incorporating small deviations in the optimized chromatographic conditions which includes wavelength (235 and 239 nm), flow rate (1.1 and 1.3 mL/min) and percentage of acetonitrile in the mobile phase (68 and 72%).

Analysis of Felodipine Tablets: 20 Tablets were bought from pharmacy medical store weighed, powdered and powder equivalent to 25 mg was transferred accurately to a volumetric flask and extracted with acetonitrile and filtered. The filtrate so obtained was diluted as per the requisite with the mobile phase and each marketed formulation (PLENDIL® and FELOGARD®) was introduced in to the HPLC system and percentage recovery was calculated.

Stress degradation studies: These studies were conducted to evaluate the stability indicating properties and specificity of the proposed method (ICH guidelines, 2003). All solutions were prepared with an initial concentration 1 mg/mL FLD, refluxed (20 min) at 70 °C and used. For acidic degradation, FLD was refluxed using 0.1 M HCl at 70 °C. Cooled, neutralized and diluted. Alkaline and Oxidation studies were performed with 0.1 M NaOH, 30 % H₂O₂ in the same environment and these solutions were used injected and chromatograms were recorded.

3. RESULTS AND DISCUSSION

An isocratic RP-HPLC (stability indicating) method was developed for the estimation of Felodipine and Table 1 shortens various features of the previously published chromatographic methods and a clean comparison with the present established method. Mobile phase containing the mixture (30:70, v/v) of sodium acetate buffer: acetonitrile (flow rate of 1.2 mL/min) has shown a sharp peak (3.569 ± 0.07 min) with theoretical plates more than 2000 (8378.644) tailing factor less than 1.5 (1.355).

Felodipine shows linearity 0.1–150 $\mu\text{g/mL}$ with % RSD 0.423-0.726. The linear regression equation, $y = 46754x - 3566.8$ was obtained with correlation coefficient 0.9999 (Figure.2). LOQ and the LOD were 0.0789 $\mu\text{g/mL}$ 0.0261 $\mu\text{g/mL}$. The intra-day and inter-day precision studies shows the percentage RSD 0.43-0.65 and 0.89-1.02 respectively. The percentage recovery was 99.87-99.96 % in accuracy studies (0.35-0.68) (% RSD less than 2.0). The robustness was assessed by exposing the drug solution to different analytical conditions purposely changing from the original optimized conditions. The effects so obtained were summarized to calculate % RSD (0.79-1.06) specifying that the method is robust. The method was applied to the marketed formulations (PLENDIL® and FELOGARD®) and the percentage recovery was 99.26-99.68 (Table.2). The chromatograms of Felodipine were shown in Figure.3.

The capability of the method was established from the separation of FLD peak from their degradation products. The classic chromatograms of stressed samples were shown in Figure 4A-4E. Felodipine has shown a very slight decomposition during acidic (2.27 %), alkaline (9.85 %), oxidative (8.27 %), photolytic (2.5 %) and thermal (9.98 %) conditions and an extra peak was observed at 1.160 min during oxidation without interference of Felodipine peak indicating that the method is specific. The percentage drug degradation was less than 10 % in all stressed conditions indicating that Felodipine was unaffected by all degradations. The present liquid chromatographic method is specific because the drug peak was well separated even in the existence of degradation products and therefore can be useful for the estimation of Felodipine in tablets. FLD peak shows that the tailing factor was less than 1.5 and the theoretical plates were more than 2000 (Table.3).

Table.1. Comparison of the previously published methods with the present method

| S. No. | Method /Reagent | λ (nm) | Linearity ($\mu\text{g/mL}$) | Comments | Reference |
|--------|--|----------------|--------------------------------|--|-------------------|
| 1. | Methanol: phosphate buffer (0.055 M) (83:17, v/v) | 275 | 2-20 | Very narrow linearity range | Fusun Gedil, 2004 |
| 2. | Acetonitrile: ammonium acetate (80:20, v/v) | 236 | 2.49-99.60 | Very narrow linearity range | Basavaiah, 2003 |
| 3 | Phosphate buffer: Acetonitrile: Methanol (40:40:20, v/v) | 362 | (10-100) 10^3 | Mixture of solvents | Nataraj, 2011 |
| 4. | Methanol: potassium dihydrogen orthophosphate (0.01 M) (pH 3.5) (75:25, v/v) | 238 | 1-7 | Stability indicating Very narrow linearity range | Cardoza, 2002 |
| 5. | Tetra butyl ammonium hydrogen sulphate: acetonitrile (18: 82, v/v) | 237 | 0.1-350 | Stability indicating method (PDA detector) | Mathrusri, 2013 |
| 6. | Sodium acetate buffer: acetonitrile (30:70, v/v) | 237 | 0.1-150 | Stability indicating method (PDA detector) | Present work |

Table.2. Analysis of Felodipine (Tablets)

| Formulation | Labeled claim (mg) | *Amount found (mg) | *Recovery (%) |
|-------------|--------------------|--------------------|---------------|
| Brand I | 10 | 9.926 | 99.26 |
| Brand II | 10 | 9.968 | 99.68 |

*Mean of three replicates

Table.3. Stress degradation studies of Felodipine

| Stress Conditions | *Mean peak area | *Drug recovered (%) | *Drug decomposed (%) | Theoretical plates | Tailing factor |
|---------------------------|-----------------|---------------------|----------------------|--------------------|----------------|
| Standard drug (Untreated) | 2337881 | 100 | - | 8378.644 | 1.355 |
| Acidic degradation | 2284706 | 97.73 | 2.27 | 8140.15 | 1.386 |
| Alkaline degradation | 2107656 | 90.15 | 9.85 | 8867.804 | 1.373 |
| Oxidative degradation | 2144525 | 91.73 | 8.27 | 8589.393 | 1.369 |
| Thermal degradation | 2104541 | 90.02 | 9.98 | 8528.781 | 1.357 |
| Photolytic degradation | 2279327 | 97.50 | 2.5 | 7882.244 | 1.358 |

*Mean of three replicates

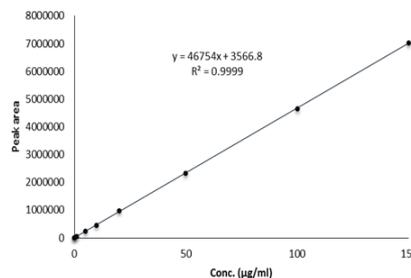
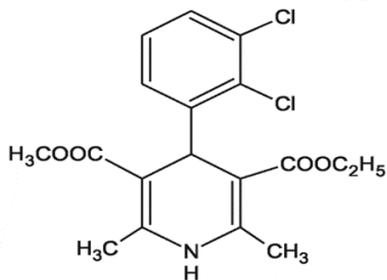
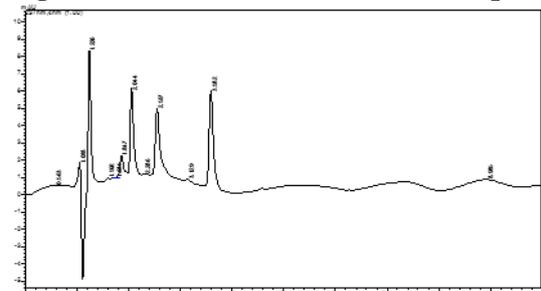


Figure.1. Chemical structure of Felodipine

Figure.2. Calibration curve of Felodipine



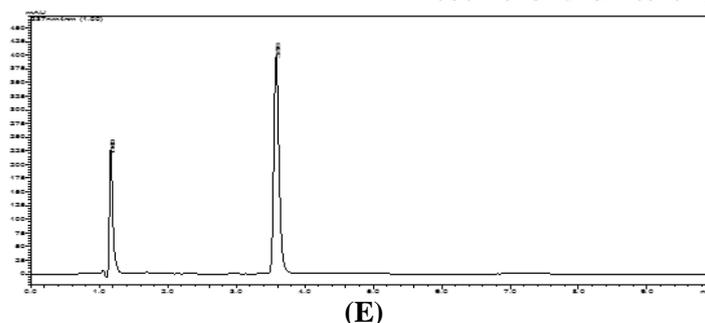


Figure.4. Typical Chromatograms of Felodipine (50 µg/ml) [A] Photolytic degradation [B] Acidic degradation [C] Thermal degradation [D] Alkaline degradation [E] Oxidative degradation

4. CONCLUSION

The proposed RP-HPLC method was validated and Felodipine is witnessed resistant towards all degradation conditions with the optimized conditions applied.

5. ACKNOWLEDGEMENT

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