RESEARCH ARTICLE

A New Stability Indicating Liquid Chromatographic Method for the Quantification of Felodipine

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Abstract

Introduction: Felodipine is used for the treatment of hypertension. In the present study a new stability-indicating RP-HPLC method has been established for the determination of Felodipine.in pharmaceutical dosage forms and the method was validated. **Material and Methods:** Shimadzu Model CBM-20A/20 Alite, UFLC system was used for the assay of Felodipine using a mixture of phosphate buffer (pH 7.0) and acetonitrile (20:80, v/v) as mobile phase with a flow rate of 1.2 ml/min (UV detection at 234 nm). Forced degradation studies were performed by exposing Felodipine to different stress conditions and the method was validated as per ICH guidelines. **Results and Discussion:** Felodipine is found to be more sensitive towards oxidative degradation. Conclusion: The proposed method is simple and economical and can be used for the determination of Felodipine in pharmaceutical formulations.

Key words: Felodipine, isocratic mode, reversed-phase high-performance liquid chromatography, stability indicating, validation.

INTRODUCTION

Felodipine (FLD) [Figure 1] acts by blocking calcium channels and thereby widens blood vessels to facilitate the blood flow easily. It is a dihydropyridine derivative. It inhibits the influx of extracellular calcium ions into myocardial and vascular smooth muscle cells, causes dilatation coronary and systemic arteries and thereby decreases myocardial contractility.[1] It is used to treat mild to moderate essential hypertension, FLD is used to treat mild to moderate essential hypertension.[2] In the literature survey very few analytical methods have been reported for the determination of FLD which include spectroscopic techniques,[3] gas chromatography–mass spectrometry (MS),[4] high-performance liquid chromatography (HPLC) biological fluids[5,6] and HPLC,[7-12] micellar HPLC,[13] and HPLC with amperometric detection[14] and also in the biological fluids such as rat plasma and other dosage forms[15-19] and LC-tandem MS/MS in dog and human plasma.[20-24] In the present study, the authors have proposed new stability indicating LC method for the determination of FLD in pharmaceutical dosage forms.

MATERIALS AND METHODS

Instrumentation

Chromatographic separation was achieved using a Shimadzu Mode.000l CBM-20A/20 Alite HPLC system, equipped with SPD M20A prominence photodiode array detector with C18 Zorbax column (250 mm × 4.6 mm i.d., 5 µm particle size) maintained at 25°C.

Chemicals and reagents

FLD is available as tablets with brand names FELOGARD® ER (Cipla Ltd., India) and PLENDIL® (Astra Zeneca Pharma India Ltd., India) with label claim of 2.5, 5, and 10 mg of the drug. All chemicals were of analytical grade and used as received. FLD standard was obtained from Cipla Ltd. (India).

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Acetonitrile (HPLC grade), sodium hydroxide (NaOH), and hydrochloric acid (HCl), phosphate buffer 7.0 (Spectrochem Pvt., Ltd.), and hydrogen peroxide (H2O2) were purchased from Merck (India).

Phosphate buffer (pH 7.0) can be prepared by mixing 0.2 M potassium dihydrogen phosphate and 29.1 ml of 0.2 M of NaOH in a 1000 mL volumetric flask with the help of HLC grade water. FLD stock solution (1000 µg/mL) was prepared by weighing accurately 25 mg of FLD in a 25 mL volumetric flask with acetonitrile, and further dilutions were made from the stock solution with mobile phase and filtered through 0.45 µm membrane filter before injection.

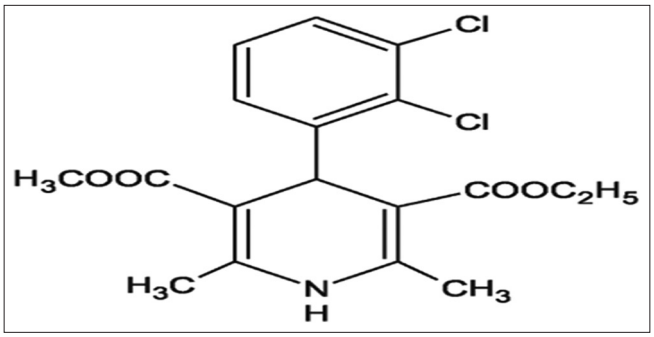

Figure 1: Structure of Felodipine

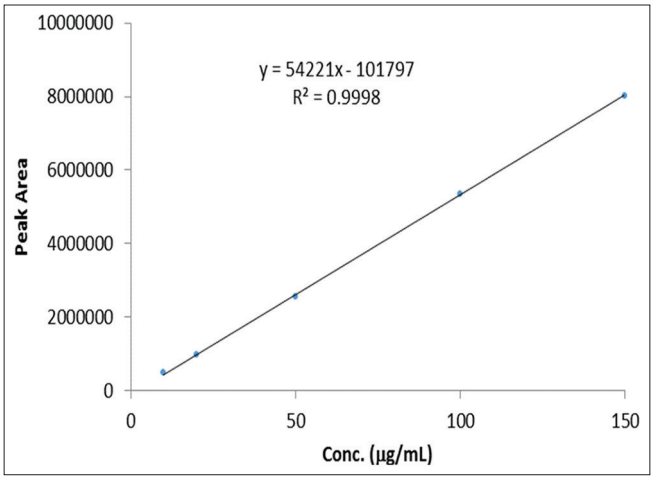

Figure 2: Calibration curve of Felodipine

Method validation[25]

Dilutions were made from the stock solution (0.1–150 µg/mL), and 20 µL of each solution was injected into the HPLC system, and the peak area of the chromatogram was obtained. Calibration curve was plotted plotting concentration on the X-axis and the corresponding peak area on the Y-axis. The precision of the assay method was evaluated at three concentration levels (10, 20, and 50 µg mL) and the percentage relative standard deviation (% RSD) was calculated. The accuracy of the assay method was evaluated using standard addition and recovery. Robustness of the method was studied for 50 µg/mL of FLD.

Assay of marketed formulations (Tablets)

FLD tablets are with brand names FELOGARD® and PLENDIL®. Tablets were procured and extracted with mobile phase and filtered. The filtrate was obtained and was diluted as per the requirement, and 20 µL solution was injected into the HPLC system, and the percentage recovery was calculated.

Stability studies

Forced degradation studies were performed to evaluate the stability indicating properties and specificity of the method.[26] All solutions for stress studies were prepared at an initial concentration of 1 mg/mL of FLD

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

Method/reagent	λ (nm)	Linearity (µg/mL)	Comments	Reference
Methanol: Phosphate buffer (0.055 M) (83:17, v/v)	275	2–20	Very narrow linearity range	[7]
Acetonitrile: Ammonium acetate (80:20, v/v)	236	2.49–99.60	Very narrow linearity range	[8]
Phosphate buffer: Acetonitrile: Methanol (40:40:20, v/v)	362	(10–100) 10^3	Mixture of solvents	[9]
Methanol: Potassium dihydrogen orthophosphate (0.01 M) (pH 3.5) (75:25, v/v)	238	1–7	Stability indicating. Very narrow linearity range	[10]
Tetra butyl ammonium hydrogen sulfate: Acetonitrile (18: 82, v/v)	237	0.1–350	Stability indicating method (PDA detector)	[11]
Sodium acetate buffer: acetonitrile (30:70, v/v)	237	0.1–150	Stability indicating method (PDA detector)	[12]
Phosphate buffer (pH 7.0): acetonitrile (30:70, v/v)	234	0.1–150	Stability indicating method (PDA detector)	Present work

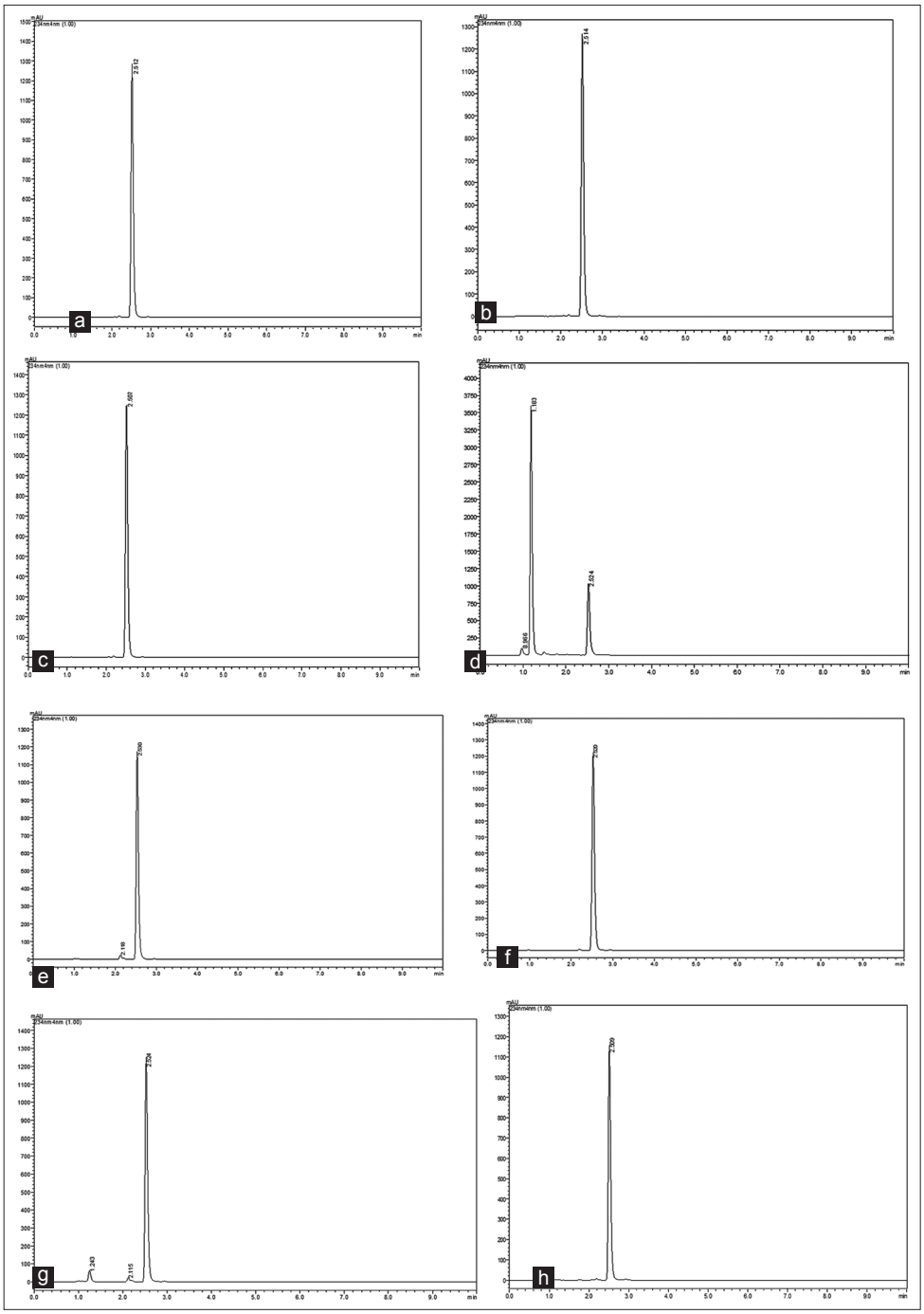

Figure 3: Representative chromatograms of Felodipine. (a) Felodipine, (b) Felogard tablet, (c) Plendil tablet, (d) oxidative degradation, (e) acidic degradation, (f) thermal degradation, (g) basic degradation, and (h) photolytic degradation

and 100 µg/ml of drug solution was used for all the degradation studies.

100 µg/ml FLD solution was exposed to acidic degradation with 0.1 M HCl for 20 min at 70°C the stressed sample was cooled, neutralized and diluted with mobile phase. Similarly, stress studies were conducted in alkaline conditions with 0.1 M NaOH at 70°C for 20 min and neutralized after cooling with proper dilution with mobile phase.

Oxidative stress studies were performed using 30% H2O2, and thermal stress studies were conducted in a thermostat at 70°C for 20 min. 20 µL solution of each of the solutions under forced degradation studies were injected into the HPLC system, and the chromatograms were recorded from

which the percentage recovery as well as the degradants were studied.

RESULTS AND DISCUSSION

The present work was compared with the previously published methods in Table 1. Mobile phase mixture consisting of phosphate buffer (pH 7.0) and acetonitrile 20:80, v/v with flow rate 1.2 ml/min was found to be the suitable chromatographic condition to get a sharp peak (ultraviolet detection at 234 nm). FLD was eluted at 2.512 min. FLD shows linearity over a concentration range of 0.1–150 µg/mL [Table 2], and the calibration curve was shown in Figure 2. The typical chromatograms of FLD in its pure form were shown in Figure 3. The LOQ was found to be 0.0852 µg/mL and the LOD was found to be 0.0279 µg/mL. The percentage RSD in precision (intraday and interday), and accuracy studies [Table 3], and robustness study [Table 4] was found to be <2.0 indicating that the proposed method is precise, accurate, and robust.

The proposed validated method was applied to the tablet formulations, and the percentage recovery was 98.96–99.18 [Table 5] without the interference from the excipients. The typical chromatograms of FLD in its marketed formulations were shown in Figure 3. FLD has shown <15% degradation in all the degradations, and in oxidation, it is 20.34%. FLD is found to be more resistant in all the stressed conditions [Table 6]. The present stability-indicating method for the determination of FLD in pharmaceutical formulations is specific because the drug peak was well separated and the overall analytical data demonstrated that the excipients did not interfere with the drug peak and the system suitability parameters are in acceptance criteria, i.e. theoretical plates were more than 2000 and the tailing factor was <2 (or <1.5–2.0) in the entire chromatographic study.

Table 2: Linearity of FLD

Conc. (µg/mL)	*Mean peak area±SD	RSD (%)
0.1	4865±21.89	0.45
1	48263±111	0.23
5	241315±1399.62	0.58
10	480431±1681.50	0.35
20	980927±6081.74	0.62
50	2538824±12440.23	0.49
100	5353490±32656.28	0.61
150	8030235±29711.86	0.37

*Mean of three replicates. FLD: Felodipine, RSD: Relative standard deviation

Table 3: Precision and accuracy study of FLD

Conc. (µg/mL)	Intraday precision	Interday precision	
	*Mean peak area±SD (%RSD)	*Mean peak area±SD (%RSD)	
10	480431±1681.50 (0.35)	481652±3227.06 (0.67)	
20	980927±6081.74 (0.62)	983580±9245.65 (0.94)	
50	2538824±12440.23 (0.49)	2569213±21324.46 (0.83)	
Accuracy	***Mean peak area±SD (% RSD)**	**Drug found (µg/mL)**	***Recovery (%)**
18	883839±4065.65 (0.46)	17.49	97.16
20	980927±6081.74 (0.62)	19.76	98.80
22	1109018±5988.69 (0.54)	21.53	97.86

*Mean of three replicates. FLD: Felodipine, RSD: Relative standard deviation

Table 4: Robustness study of FLD

Parameter	Condition	*Mean peak area	Statistical analysis	*Retention time
Flow rate (mL/min)	1.1	2531653	Mean=2552205	2.912
	1.2	2538824	SD=29605.34	2.518
	1.3	2586139	% RSD=1.159	2.069
Detection wavelength (±2 nm)	232	2537652	Mean=2537029	2.519
	234	2538824	SD=2173.941	2.518
	236	2534612	% RSD=0.856	2.516
Mobile phase (v/v) Phosphate buffer (pH 7): Acetonitrile	18:82	2536512	Mean=2538330	2.693
	20:80	2538824	SD=1628.21	2.518
	22:78	2539654	% RSD=0.064	2.320
pH (±0.1 unit)	6.9	2510642	Mean=2539399	2.620
	7	2538824	SD=29048.26	2.518
	7.1	2568730	%RSD=1.143	2.415

*Mean of three replicates. FLD: Felodipine, RSD: Relative standard deviation

Table 5: Assay of FLD tablets

Sample No.	Formulation	Label claim (mg)	*Amount found (mg)	*Recovery (%)
1	Brand I	10	98.96	98.96
2	Brand II	10	99.18	99.18

*Mean of three replicates. FLD: Felodipine

Table 6: Stress degradation studies of FLD

Stress Conditions	*Mean peak area	*Drug recovered (%)	*Drug decomposed (%)	Theoretical plates	Tailing factor
Standard drug	5353490	100	-	6492.887	1.411
Acidic degradation	4748581	88.70	11.3	7007.350	1.429
Alkaline degradation	4987204	93.15	6.85	7136.644	2.115
Oxidative degradation	4264839	79.66	20.34	6941.404	2.524
Thermal degradation	5081474	94.91	5.09	6492.344	1.401

*Mean of three replicates. FLD: Felodipine

CONCLUSION

The proposed stability-indicating HPLC method was validated as per the ICH guidelines and applied for the determination of Felodipine in pharmaceutical dosage forms and can be successfully applied to perform long-term and accelerated stability studies of Felodipine formulations.

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