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A stability indicating LC method for felodipine

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Abstract

A stability indicating reversed-phase liquid chromatographic (RP-LC) method was developed for the assay of felodipine as a bulk drug and in pharmaceuticals. The chromatography was performed on a C18 column. Eluents were monitored by UV detection at 238 nm using the mobile phase methanol–potassium dihydrogen orthophosphate (pH 3.5; 0.01M) (75:25, v/v). The method was statistically validated for linearity, accuracy, precision and specificity. The linearity of felodipine peak area responses was demonstrated within the concentration range of 1–7 μg/ml. The limits of detection and quantitation were 150 and 500 ng/ml, respectively. The method was demonstrated to be precise, accurate and specific with no interference from the tablet excipients and separation of the drug peak from the peaks of the degradation products (oxidative degradation, photodegradation, acid and base degradation). The results indicated that the proposed method could be used in a stability assay.

Keywords: Felodipine; RP-LC; Stability indicating; Tablets

1. Introduction

Felodipine is a vasoselective calcium channel antagonist of the dihydropyridine class used in the management of hypertension [1,2]. It is chemically described as ethyl methyl (RS) 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate. It is official in the European Pharmacopoeia [3]. Estimation of felodipine in biological samples has been reported by gas chromatographic (GC) [4–6] and liquid chromatographic (LC) [4] methods. GC [7–10] methods have been used for the determination of felodipine and its metabolites in plasma. Separation and estimation of the metabolites of felodipine in biological samples have been achieved by LC methods as reported by Gabrielsson et al. [11] and Weidolf [12].

Spectrophotometric [13–16] and reversed-phase liquid chromatographic (RP-LC) [17,18] methods are cited in literature for the determination of felodipine in pharmaceuticals. However, none of these methods is stability indicating. A RP-LC method developed and validated by Qin et al. [19] allows for the simultaneous determination of enalapril, felodipine and their degradation prod-

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ucts in the combined enalapril/felodipine formulation. However, the long chromatogram run time (35 min) precludes the use of this method for routine stability estimation of the drug. Furthermore, the method detects and separates only the major degradation product of felodipine (dehydrofelodipine) from the undegraded drug. A simple analytical method is required that can quantitatively estimate felodipine in the presence of its potential degradation products and formulation excipients.

This paper describes the development and validation of a stability indicating RP-LC method for the assay of felodipine as a bulk drug and in its pharmaceutical dosage forms.

2. Experimental

2.1. Materials

Felodipine was a gift from Cipla Ltd. (Mumbai, India) and Astra IDL Ltd. (Bangalore, India). A reference sample of dehydrofelodipine (H152/37) was obtained from Astrazeneca (Sweden). The structures of these components are shown in Fig. 1. Methanol (Ranbaxy Chemicals, India) was of HPLC grade. Double distilled water was used. All other chemicals and solvents were of analytical–reagent grade and were purchased from Ranbaxy Chemicals, India.

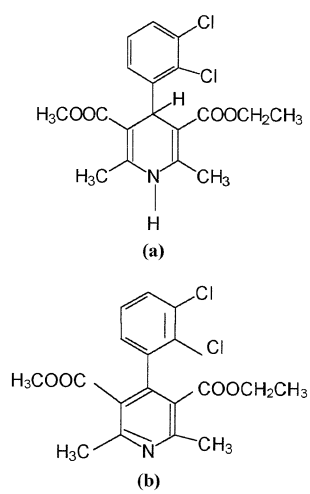

Fig. 1. Structures of (a) felodipine and (b) dehydrofelodipine (H152/37).

Local commercial tablet formulations of felodipine of two dose strengths (5 and 10 mg of felodipine per tablet) were used. Extended release tablet formulation developed in our laboratory and containing 5 or 10 mg of felodipine per tablet in a matrix of cellulose acetate, lactose, talc and magnesium stearate was also analyzed using the proposed method. The commercial formulations were coded as A5 and A10 whilst the tablets developed in-house were coded as B5 and B10 during this study.

2.2. LC instrumentation

The liquid chromatographic system consisted of a Jasco PU-980 Intelligent pump (Jasco, Japan) coupled with a Jasco UV-975 Intelligent UV/VIS detector and a Rheodyne injector (model 7725) fitted with a 20 μl sample loop. Data integration was done using Borwin chromatography software version 1.21.

2.3. Chromatography

Chromatography was performed on a LiChroCART® 250*4 mm cartridge prepacked with LiChrospher® 100 RP-18 (5 μm) (Merck, Germany). The mobile phase was prepared by first preparing a solution of 0.01 M KH_2PO_4, then adjusting its pH to 3.5 with phosphoric acid, and finally mixing the solution with methanol in a ratio of 25:75 (v/v), respectively. The mobile phase was filtered through a nylon membrane (0.45 μm) and degassed by sonication prior to use. Chromatography was performed at room temperature under isocratic conditions at a flow rate of 1.5 ml/min. Detection was done at 238 nm and the detector sensitivity was set at 0.16 AUFS.

2.4. Preparation of standard solutions

A stock solution of felodipine (100 μg/ml) was prepared in methanol and diluted further with mobile phase to obtain a standard solution of 4 μg/ml. A standard solution containing both felodipine and H152/37 (4 μg/ml each) in the mobile phase was also prepared.

2.5. Preparation of sample solutions

Each tablet of felodipine was crushed to a fine powder. The powder was quantitatively transferred to a volumetric flask (capacity 25 ml for lots A5 and B5; 50 ml for lots A10 and B10) and stirred with methanol for 30 min. The volume was made up with methanol and the resulting extract was centrifuged. The clear centrifugate was used for the analysis after suitable dilution with the mobile phase.

2.6. Forced degradation of felodipine

Forced degradation studies were performed to provide an indication of the stability indicating property and specificity of the proposed method. Intentional degradation was attempted using heat, oxidation, light, acid and base. For thermal degradation, the drug in the solid state as well as in solution (1 mg/ml in methanol) was stored in the dark at 60 ± 2 °C for a period of 7 days. Photodegradation was attempted by exposing a 1 mg/ml methanolic solution of felodipine to direct sunlight for a period of 48 h. Oxidative degradation was induced by adding 3 ml of 0.1 M ceric ammonium sulfate solution to 25 mg of felodipine, vortex-mixing and allowing to stand at room temperature for 30 min. The resultant solution was extracted with chloroform, which was then evaporated. The residue obtained was dissolved in methanol and diluted further with mobile phase. Acid degradation was attempted by refluxing felodipine with 1M methanolic HCl (2 mg/ml) for 3 h. Base degradation was performed by refluxing felodipine with 1 M methanolic NaOH (2 mg/ml) for 1 h. To exclude the possible degradative effect of refluxing, the drug was also refluxed with methanol (2 mg/ml) for 3 h. The oxidative, acid and base degradation studies were performed in the dark to exclude the possible degradative effect of light on felodipine.

After completion of the degradation treatments, the samples were allowed to cool to room temperature, neutralized (when required) and injected into the chromatographic system after appropriate dilution with the mobile phase. The degraded samples were analyzed against a control sample (lacking degradation treatment).

2.7. Validation of the assay method

2.7.1. Stability of analyte in solution

The stability of felodipine in the mobile phase was assessed by injecting the standard solution (4 μg/ml) at 0, 6, 24 and 48 h post preparation.

2.7.2. Precision and accuracy

System precision (repeatability) was evaluated by performing six consecutive injections of a 4 μg/ml felodipine standard solution. Method precision was determined by six repeated assays of the same lot of the tablet formulations. The accuracy of the proposed method was determined by recovery experiments using tablets from the same lot of the commercial and developed formulations. The recovery was assessed at three levels (50, 100 and 150%).

2.7.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ concentrations were determined at signal-to-noise ratios of 3:1 and 10:1, respectively.

2.7.4. Linearity

Seven solutions were prepared in the mobile phase containing 1–7 μg/ml of felodipine. The peak area versus concentration data was treated by least-squares linear regression analysis.

2.7.5. Specificity

To demonstrate the specificity of the method, placebo formulations, containing the tablet ingredients other than the drug, were subjected to the sample preparation method as outlined in Section 2.5 and the samples thus prepared were injected into the chromatographic system.

2.7.6. Robustness

The influence of intentional variations in chromatographic conditions (pH of 0.01M KH_2PO_4, ionic strength of KH_2PO_4 and lots of bulk drug from two sources) on the assay results was evaluated.

Table 1
Optimization of chromatographic conditions

Methanol: KH_2PO_4 (pH 3.5; 0.01 M) (v/v)	Flow rate (ml/min)	Retention time (RT) (min)		Resolution
		H152/37	Felodipine	
70:30	1.0	11.11	16.41	7.22
70:30	1.5	7.43	10.68	5.45
75:25	1.0	7.10	9.82	4.93
75:25	1.5	4.44	5.96	3.58
80:20	1.0	5.17	6.65	2.92

3. Results and discussion

3.1. Optimization of the chromatographic procedure

Felodipine is metabolized in vivo by oxidation of the 1,4-dihydropyridine moiety to its pyridine analog (dehydrofelodipine), followed by ester hydrolysis, hydroxylation of the alkyl groups and conjugation [20]. Oxidative and hydrolytic degradation of the bulk drug were anticipated to yield the pyridine derivative of the drug (dehydrofelodipine) and the carboxylic acids, respectively. The chromatographic conditions were optimized so as to obtain a good separation between the drug and its degradation products. Detection was performed at 238 nm, the λ_{max} of felodipine. The anticipated degradation products were expected to absorb at this wavelength and therefore be detected.

A gradient LC method reported by Gabrielsson et al. [11] employed a mobile phase comprising methanol and phosphate buffer (0.05M; pH 3.5) for the separation of felodipine from its carboxylic acid metabolites. Hydrolysis of the ester functionality of felodipine during acid and base degradation was anticipated to produce products containing the carboxylic acid group. The European pharmacopoeia [3] also recommends a pH of 3 for the aqueous component of the mobile phase. Published methods vary with respect to the molarity of the phosphate buffer used; KH_2PO_4 solutions of molarity 0.05 and 0.001 have been used [11,19]. The retentions of felodipine and its major degradation product, dehydrofelodipine, are not significantly affected by the salt concentration in the mobile phase; the controlling factor being the percentage of organic solvent in the mobile phase. Based on these considerations, the aqueous component of the mobile phase was selected to be KH_2PO_4 (pH 3.5; 0.01 M).

As a preliminary guide to the selection of the mobile phase, the standard solution of felodipine and reference degradation product (dehydrofelodipine; H152/37) was injected into the chromatographic system and the elution was studied using mobile phases comprising binary mixtures of methanol and KH_2PO_4 (pH 3.5; 0.01M) in varying ratios. The results are shown in Table 1. The chromatographic conditions were optimized after taking into consideration the resolution between the drug and its major degradation product (dehydrofelodipine) and the run time for the chromatogram. A mobile phase of methanol–KH_2PO_4 (pH 3.5; 0.01 M) (75:25, v/v) at a flow rate of 1.5 ml/min was found to well separate felodipine from dehydrofelodipine with a reasonably short run time. These optimized conditions also afforded separation of the drug from its degradation products formed by pathways other than oxidation (Fig. 2). The peaks of all the degradation products were well resolved from the drug peak (resolution > 3). The percentage of intact felodipine, after forced degradation through various routes, is shown in Table 2.

Felodipine was quantitatively oxidized to dehydrofelodipine by ceric ammonium sulfate under the conditions employed as evidenced from the absence of the drug peak in the chromatogram. This was in agreement with the quantitative yield of the pyridine derivative reported on oxidation of the drug with Ce^{4+} ions [21]. The drug was found to be stable to heat under the conditions of the study, both in the dry state as well as in solution.

Exposure to direct sunlight resulted in a degradation product with a retention time (RT) similar to that of H152/37, suggesting that photodegradation resulted in formation of dehydrofelodipine. Felodipine was more susceptible to degradation under basic conditions than under acidic conditions, as evidenced from the percentage of intact drug and difference in hydrolysis times. No degradation was observed upon refluxing the drug with methanol, suggesting that the degradation under acidic and basic conditions was the result of hydrolysis with no influence of heat on the degradation. The degradation product formed on acid and base hydrolysis had RT values similar to that of H152/37. TLC analysis of the acid- and base-degraded samples [mobile phase: ethyl acetate: toluene (4:6, v/v)] yielded R_f values for the degradation products, which matched the R_f value of H152/37. Degradation under acidic and basic conditions was thus inferred to produce dehydro-

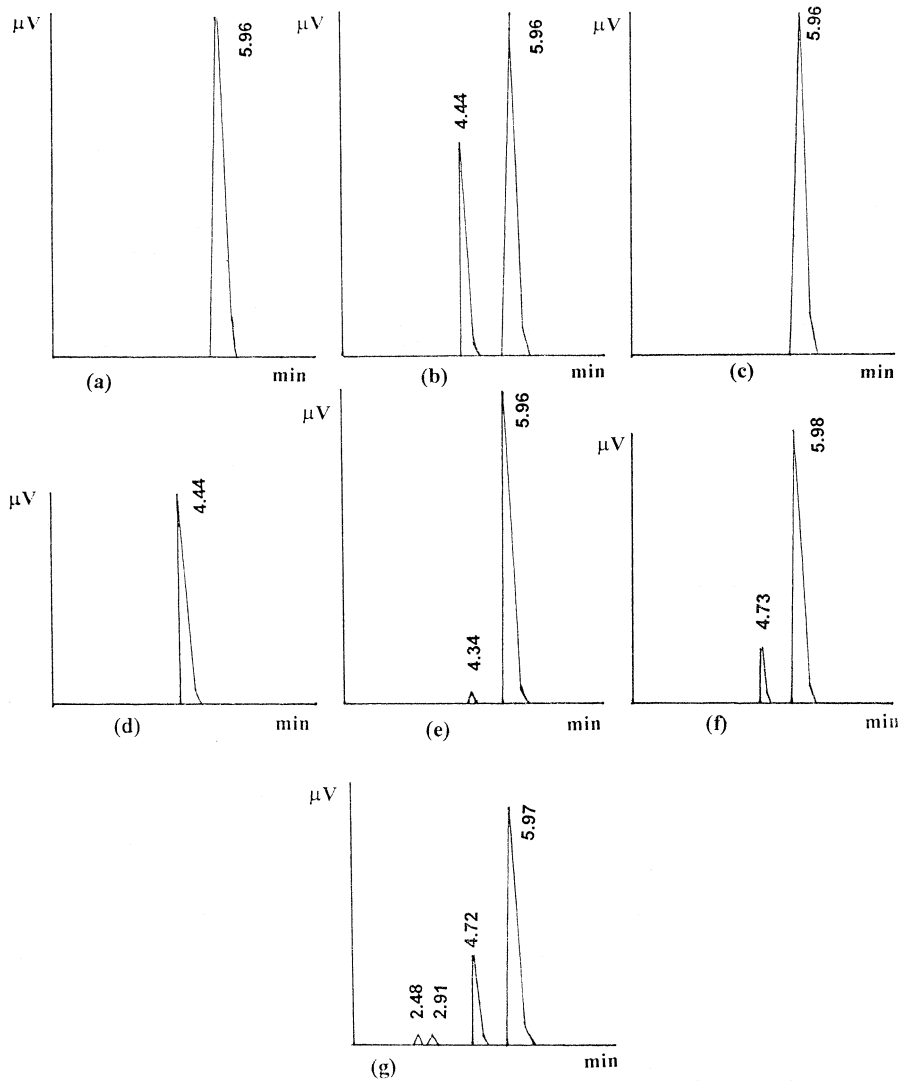

Fig. 2. Chromatograms of felodipine and its degraded products: (a) undegraded felodipine, (b) felodipine and H152/37, (c) thermal degradation, (d) oxidative degradation, (e) photodegradation, (f) acid degradation, (g) base degradation.

Table 2
Forced degradation of felodipine

Degradative condition	Time (h)	Intact drug (%)	RT of degradation products (min)
Thermal (solid drug)	168	99.65	None detected
Thermal (solution)	168	98.92	None detected
Sunlight	48	95.57	4.34
Oxidation	0.5	0	4.44
Acid hydrolysis	3	79.81	4.73
Base hydrolysis	1	70.34	2.48, 2.91, 4.72

felodipine. The two additional products formed upon base degradation were postulated to be the methyl monoacid (RT 2.48 min) and ethyl monoacid (RT 2.91 min) analogs of felodipine.

3.2. Validation of assay method

3.2.1. Stability of analyte in solution

Felodipine was found to be stable in solution in the mobile phase, when the standard solution was analyzed at 0, 6, 24 and 48 h post preparation. No peaks corresponding to the degradation products were observed and there was no significant change in the drug peak area.

3.2.2. Precision and accuracy

System precision (repeatability) was evaluated by performing six consecutive injections of the 4 μg/ml standard solution, giving a low RSD value of 1.78% and no change in retention time of the drug. The felodipine contents found in the tablet formulations using the proposed method, are listed in Table 3. The low RSD values indicate that the proposed method is precise. Recovery data (Table 4) obtained from the study of the tablet formulations ranged from 97–102% with low RSD values ($< 3\%$). The experimental average felodipine content for each lot of tablets was not significantly different from the true value (two-tailed Student's t-test, $\alpha = 0.05$). The accuracy of the method was demonstrated by plotting the amount of felodipine found (expressed in mg) against the amount present. Linear regression analysis rendered slopes not significantly different from one (t-test; $\alpha = 0.05$), intercepts not significantly different from zero (t-test; $\alpha = 0.05$) and correlation coefficients close to unity. The quantitative recovery of felodipine achieved indicates that there was no interference from excipients present in the tablet formulations.

3.2.3. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ concentrations were found to be 150 and 500 ng/ml, respectively.

3.2.4. Linearity

The plot of the drug peak area versus concentration was linear over the concentration range of 1–7 μg/ml (Table 5). The regression line equation calculated by the least-squares method was $y = 33424.21x + 71.43$ with a coefficient of correlation of 0.9994. The intercept value was not significantly different from zero (95% confidence).

3.2.5. Specificity

Placebo formulation samples yielded clean chromatograms with no interference from the tablet excipients. The ability of the method to separate the drug from its degradation products

Table 3
Precision of the assay method

Sample No.	Felodipine content (% label claim)			
	A5	A10	B5	B10
1	97.17	101.05	98.98	98.60
2	99.14	100.45	99.36	99.03
3	101.02	102.78	97.86	97.34
4	99.56	100.97	99.03	98.66
5	97.24	100.11	99.58	99.28
6	101.08	102.86	96.97	95.98
Mean	99.20	101.37	98.63	98.15
RSD (%)	1.74	1.16	1.02	1.28

Table 4
Recovery studies

% w/w	Amount present (mg)	Amount found (mg)	Average recovery (%)	RSD (%)
Lot A5				
50	7.5	7.310, 7.420, 7.151	97.25	1.85
100	10.0	10.301, 10.124, 10.080	101.68	1.15
150	12.5	12.528, 12.046, 12.335	98.42	1.97
Mean ($n = 9$)			99.12	2.48
Lot B5				
50	7.5	7.385, 7.710, 7.602	100.88	2.19
100	10.0	9.756, 9.946, 9.825	98.42	0.98
150	12.5	12.736, 12.241, 12.350	99.54	2.09
Mean ($n = 9$)			99.61	1.92
Lot A10				
50	15.0	15.327, 15.023, 15.280	101.40	1.08
100	20.0	20.142, 20.452, 19.725	100.53	1.81
150	25.0	25.682, 24.538, 25.714	101.25	2.65
Mean ($n = 9$)			101.06	1.74
Lot B10				
50	15.0	15.260, 14.720, 15.236	100.48	2.02
10	20.0	19.452, 19.665, 20.171	98.81	1.87
150	25.0	25.658, 25.145, 25.701	102.01	1.21
Mean ($n = 9$)			100.43	2.04

and the non-interference from the tablet excipients indicates the specificity of the method.

3.2.6. Robustness

There was no significant change in retention time, peak shape and assay results upon introduction of intentional variations in parameters such as pH of 0.01 M KH_2PO_4, (from 3.3 to 3.7), ionic strength of KH_2PO_4 (from 0.009 to 0.011 M) and lots of bulk drug from two sources.

Table 5
Linearity data

Concentration (μg/ml)	Mean peak area ($n = 6$)	RSD (%)
1	34086	2.20
2	67888	2.05
3	100352	1.18
4	129742	1.86
5	166905	0.98
6	204856	1.53
7	232549	1.60
Slope[a]	33424.21 ± 1335.30	
Intercept[b]	71.43 ± 5971.66	

[a] 95% confidence limits of the slope.
[b] 95% confidence limits of the intercept.

4. Conclusion

The RP-LC assay method developed for felodipine is rapid, precise, accurate, specific and stability indicating. The method may be used for assessing the stability of felodipine as a bulk drug and in its pharmaceutical formulations. The method may be extended to study the degradation kinetics of felodipine and also for the estimation of felodipine and its pyridine metabolite (dehydrofelodipine) in biological fluids.

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