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A VALIDATED, STABILITY-INDICATING HPLC METHOD FOR THE DETERMINATION OF FELODIPINE AND ITS RELATED SUBSTANCES

Liandong Hu [*1, 2], Qiaofeng Hu [1, 2] and Na Gao [1, 2]

School of Pharmaceutical Sciences, Hebei University [1], PR China
Key Laboratory of Pharmaceutical Quality Control of Hebei Province [2], Baoding, PR China

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Correspondence to Author:

Liandong Hu

School of Pharmaceutical Sciences & Key Laboratory of Pharmaceutical Quality Control of Hebei Province, Hebei University, No.180, WuSi Road, Baoding, 071002, PR China

Email: hbupharm@126.com

ABSTRACT: An HPLC method was developed and validated to determine felodipine and related substances. A C18 column (5µm, 250 ×4.6 mm) was used for the separation at room temperature, with methanol: acetonitrile: water (50:15:35%, v/v/v) as the mobile phase at the flow rate of 1.0mLmin^{-1}. The detection wavelength was 238 nm. The validation characteristics included accuracy, precision, specificity, linearity, and robust, and stability-indicating. The method showed good linearity for felodipine and its related substances with correlation coefficients in the range of 5.05-40.4µg/ml and 0.31-15.50µg/ml. Method accuracy was assessed for felodipine and its related substances at three levels, the recovery ranged from 98.86 to 101.03%. The intermediate precision were 0.42% for felodipine and was 1.01% for the related substances (n = 9). Limit of detection and quantification for felodipine and its related substances were 1 and 4ng, respectively. The solution remains stable within eight hours at room temperature. Finally, the method was demonstrated to be robust, resistant to small variations of chromatographic variables. This method is fast, simple, can be used for direct determination of felodipine and its related substances in the pharmaceutical preparation.

INTRODUCTION: Hypertension is a major risk factor for arteriosclerosis and the beneficial effects of lowering blood pressure on the vascular morbidity and mortality are well documented and demonstrated. Felodipine, a new generation calcium channel antagonist, belonging to the class of dihydropyridines, is a practical advance in the treatment of hypertension [1].

Felodipine is a highly vasoselective calcium antagonist that effectively reduces arterial blood pressure [2]. It does not have any negative inotropic effects at doses used for antihypertensive therapy [3, 4]. It has a direct relaxing effect on smooth muscle cells, especially those of resistance vessels.

Clinical studies have shown that felodipine is an effective drug for the treatment of essential hypertension [5-7].

There are impurities/related substances associated with the manufacture of felodipine drug substance. The process related impurities are observed with synthetic routes and/or manufacturing processes.

Structures of felodipine and its related substances and their chemical names are provided in **Table 1**. These related substances are monitored during the release of drug substance raw material and finished drug products.

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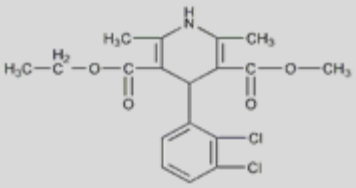
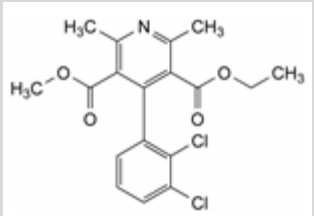
The primary goal of this study was to develop and validate an HPLC method that could separate felodipine from its potential related substances and has sufficient sensitivity for quantitation of these impurities at very low concentrations. The present analytical method discussed in this article is a simple isocratic high performance liquid chromatography with ultraviolet detection for the determination of felodipine and its related substances.

TABLE 1: CHEMICAL NAMES AND STRUCTURES FOR FELODIPINE AND ITS RELATED SUBSTANCES IMPURITY I

Chemical name	structure
Felodipine: 4-(2,3-dichlorophenyl)-1,4-dihyro-2,6-dimethyl-3,5-pyridinedicarboxylic acid ethyl methyl ester	
Impurity I: Ethyl methyl4-(2,3-dichlorophenyl)-2,6-dimethylpyridine-3,5-dicarboxylate	

EXPERIMENTAL:

Materials and reagents: Methanol, acetonitrile (HPLC grade) and phosphate buffer was obtained from Tianjin Kermel Chemical Reagent Co. Ltd (Tianjin, China).Standard Felodipine (99.9% of purity) and Impurity I (ethyl methyl4-(2,3-dichlorophenyl)-2,6- dimethylpyridine-3,5-dicarbo xylate) (98.6% of purity)were obtained from The National Institute for Control of Pharmaceutical and Biological Products. All other chemicals were of analytical grade. Water was purified by redistillation and passed through a 0.22μm membrane filter before use.

HPLC conditions [8]: The HPLC analysis system consisted of a LC-20AT liquid chromatogram and SPD-20A UV/VIS detector (Shimadzu, Kyoto, Japan) and the chromatographic column was a Kromasil C-18 (5μm, 250 ×4.6 mm).Mixture of methanol: acetonitrile: water (50:15:35%, v/v/v) was used as the mobile phase. The flow rate was 1.0 ml/min; UV-detection was at a wavelength of 238 nm.

Sample preparation: 10 tablets were weighed and finely powdered. A portion of powder equivalent to felodipine 5 mg was accurately weighed into 50 ml volumetric flasks and 40ml ethanol was added. The volumetric flasks were sonicated for 5min, then the solutions were then made up to volume with ethanol, shaken, centrifugal, take supernatant fluid, the solution was filtered through 0.45μm filter.

Accurately measuring continued filtrate 5m1, put it into 25 ml volumetric flasks, the solutions were then made up to volume with mobile phase, shaken, 20μl of the test solution was injected and chromatogram was recorded; then accurately weighed felodipine reference substance drying to constant at 105^{o}C, the solutions were then dissolved and made up to per 1ml contains 20μg samples with mobile phase, and determined with the same method, calculated peak area with external standard.

Related substances preparation: Impurity I reference substance were weighed, methanol was added to dissolve and quantitative dilution made 1ml solution contains 0.3mg, then 1ml was accurately measured and transferred to a 100ml volumetric flask, 1 ml test solution was added and then diluted to volume with the mobile phase, and mixed.

RESULTS AND DISCUSSION:

Method validation: The method was validated according to the high performance liquid chromatography (the Chinese pharmacopoeia 2010 edition appendix V D).

The following validation parameters were addressed: linearity, range, accuracy, precision, specificity and limit of detection, limit of quantitation, solution stability destructive and robustness.

1. **System suitability:** System suitability tests were performed to ensure that the HPLC system and procedure are capable of providing quality data. All those parameters reported in **Table 2**.

TABLE 2: VALIDATION OF THE METHOD REGARDING SYSTEM SUITABILITY, LINEARITY, LIMITS OF DETECTION AND QUANTITATION.

Analytical parameter	Criteria	Felodipine	Impurity I
Retention time Fel RT		6.5	5.5
Resolution (R)	≥1.5	2.9	3.5
Tailing factor (T)	0.95-1.05	1.045	1.033
Number of theoretical plates (N)	≥3500	7440	7250
Linear equation		y = 64193 x – 2218	y = 34701x+3825.2

2. **Linearity and range**: The linearity of felodipine and Impurity I were evaluated at five concentrations in the range of 5.05-40.4µg/ml and 0.31-15.50µg/ml, respectively. The linearity curves were defined by the following equations

$y = 64193 x - 2218$, $R^2 = 0.999$ for felodipine

$y = 34701x+3825.2$, $R^2 = 0.9994$ for Impurity I

Where y is the peak area of analyte and x is the analyte concentration. The results show excellent correlations between peak area and concentration over the desired concentration range.

3. **Accuracy and Precision**: Accuracy and precision were established across the analytical range for felodipine. Accuracy of the method was calculated by recovery studies by standard addition method. The method was determined by preparing the samples of the same batch in nine determinations with three concentrations and three replicate each. The accuracy of the method is shown in **Table 3.** The precision is shown in **Table 4**.

TABLE 3: RESULTS OF ACCURACY

Analyte	Level	Added (mg)	Recovered (mg)	Recovery (%)	Average recovery (%)	R.S.D. (n= 3 (%))
Felodipine	0.8	0.1616	0.160	99.21	99.25	0.18
	0.8	0.1616	0.160	99.11		
	0.8	0.1616	0.161	99.45		
	1.0	0.202	0.204	101.03	100.09	0.83
	1.0	0.202	0.202	99.77		
	1.0	0.202	0.201	99.47		
	1.2	0.2424	0.242	99.76	99.77	0.35
	1.2	0.2424	0.243	100.12		
	1.2	0.2424	0.241	99.42		
Impurity I	0.8	0.0252	0.025	99.22	99.59	0.43
	0.8	0.0252	0.0251	99.50		
	0.8	0.0252	0.0252	100.06		
	1.0	0.0315	0.0317	100.59	100.22	0.6
	1.0	0.0315	0.0317	100.54		
	1.0	0.0315	0.0314	99.52		
	1.2	0.0378	0.0376	99.35	99.03	0.28
	1.2	0.0378	0.0374	98.87		
	1.2	0.0378	0.0374	98.86		

TABLE 4: INTERMEDIATE PRECISION OF FELODIPINE AND IMPURITY I

Sample	Peak area	Average peak area	R.S.D. (n=6)
Felodipine	1302389	1298173	0.42
	1294174		
	1292493		
	1297613		
	1306840		
	1295529		
Impurity I	51463	51286	1.01
	51662		
	50273		
	51664		
	51331		
	51325		

4. Specificity: The specificity of the HPLC method is illustrated in **Fig. 1**, Impurities I were spiked and were found to be well separated from felodipine.

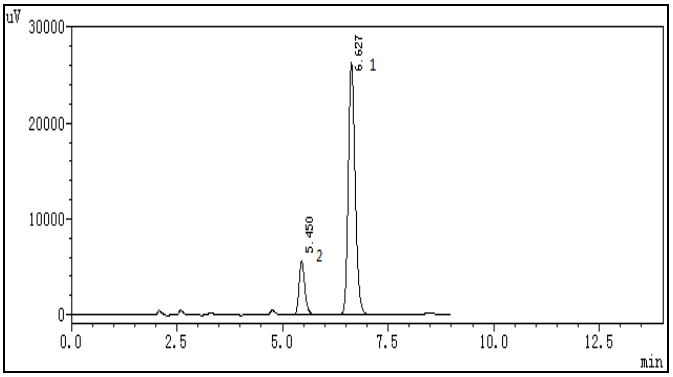


FIG. 1: CHROMATOGRAPHY OF FELODIPINE (1) AND IMPURITIES I (2)

5. **Stability:** Sample solution was performed after they were stored at room temperature and under refrigerated conditions over time. All solutions are stable for at least 8h at room temperature, the data was shown in **Table 5**, and the result indicated that the solution was stable in 8 hours at room temperature.

6. **Destruction test:** In order to determine the specificity of felodipine, a raw materials destruction test was carried out. The test involved exposure to acid, base, light, H_2O_2 (oxidative medium) and heat. **Fig. 2** shows the chromatograms of felodipine raw materials after treatment with acid, base, light, heating and oxidation by H_2O_2. It can be seen that the related substances can be detected and separated effectively.

7. **Robustness**: The robustness of the method was examined by small variations of critical parameters, such as changes of pH of the mobile phase, flow rate, percentage of acetonitrile in the mobile phase. The degree of reproducibility of the results obtained as a result of small deliberate variations in the method parameters has proven that the method is robust (**Table 6**).

TABLE 5 STABILITY OF FELODIPINE SAMPLES AND IMPURITY I UNDER INDICATED CONDITIONS

Components	**Hour**	**Peak area**	**R.S.D.**
Felodipine	0	1309155	0.18
	1	1304339	
	2	1307175	
	4	1304678	
	8	1309106	
Impurity I	0	51463	1.13
	1	51662	
	2	50273	
	4	51664	
	8	51325	

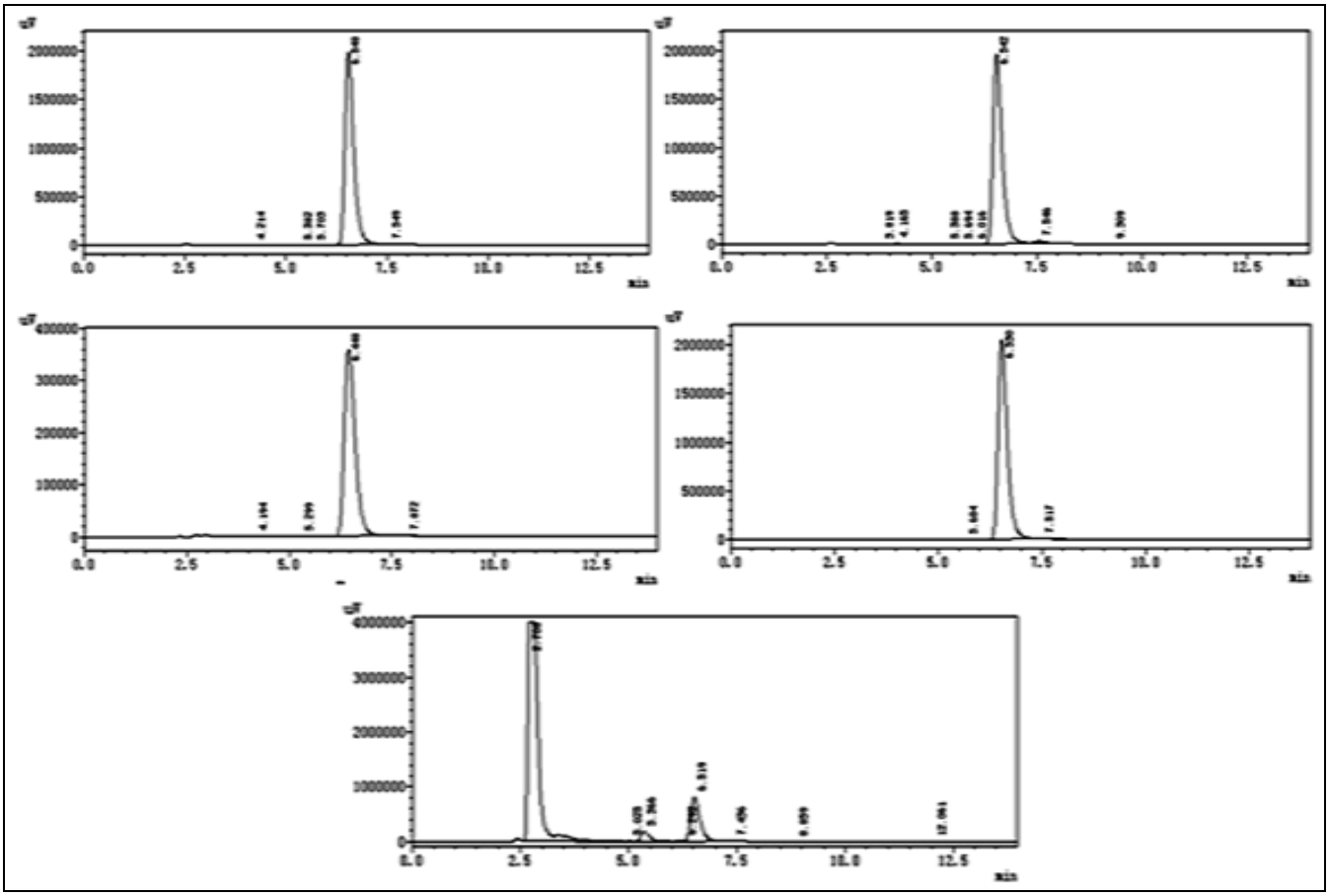


FIG. 2: REPRESENTATIVE CHROMATOGRAMS OF FELODIPINE RAW MATERIAL AFTER TREATMENT WITH 0.1MOL/L HYDROCHLORIDE (A); 0.1MOL/L SODIUM HYDROXIDE (B); LIGHT(C);HEAT (D); H_2O_2 (E).

TABLE 6: RESULTS OF ROBUSTNESS STUDY

Factor	Level	Mean % assay	Impurities I	%R.S.D. of results	Impurities I
		Felodipine		Felodipine	
Flow rate (ml/min)	0.9	99.6	101.3	0.6	0.8
	1.1	100.1	100.7		
% of methanol	50	100.3	99.6	0.3	0.4
	55	100.4	99.8		
% of acetonitrile	15	100.1	100.2	0.4	0.5
	10	100.3	99.8		

CONCLUSIONS: An HPLC method has been developed and validated for the determination of felodipine and related substances. The developed method is accurate, precise and linear across the analytical range. The method is stability-indicating and specific for the determination and quantitation of trace levels of felodipine and its related substances. This method has especially low limits of detection and quantitation, and was proven to be robust, accurate and precise.

The method is therefore suitable for felodipine and its related substances and applied to other pharmaceutical dosage forms.

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