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QUANTITATIVE DETERMINATION OF FELODIPINE IN PHARMACEUTICALS BY HIGH PRESSURE LIQUID CHROMATOGRAPHY AND UV SPECTROSCOPY

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Abstract

In this study, HPLC and UV Spectroscopic methods were developed for the quantitative determination of pharmaceutical samples containing of felodipine

In HPLC method, felodipine was determined by isocratic system using methanol-0.055M phosphate buffer (83:17 v/v- pH=3±0.1) as mobile phase. Disulfiram was chosen as an internal standard. Detection was carried out with UV detection at 275 nm. UV spectroscopic method was also used in the quantitative determination of felodipine and 234 and 360 nm (λ_{max}) were chosen for the calculation.

Key Words: *Felodipine, HPLC, UV Spectroscopy, Quantitative determination*

Farmasötik Preperatlarda Felodipinin Yüksek Basınçlı Sıvı Kromatografisi ve UV Spektroskopisi ile Miktar Tayini

Bu çalışmada felodipin içeren farmasötik ürünlerde yüksek basınçlı sıvı kromatografisi ve UV Spektroskopisi yöntemi uygulanarak nicel tayinler yapılmıştır. Yüksek basınçlı sıvı kromatografisi yönteminde isokratik sistemde çalışılmış ve hareketli faz sistemi olarak metanol-0.055M fosfat tamponu (83:17 v/v- pH=3±0.1) kullanılmıştır. Akış hızı 0,7 mL.dak^{-1} olarak seçilmiştir. Sabit faz olarak Luna C_{18} 5µ (250x4.6 mm) kolon sistemi kullanılmış ve ölçümler UV dedektörde 275 nm'de gerçekleştirilmiştir. Çalışmalarda disulfiram internal standart olarak kullanılmıştır. Felodipin tayini için diğer bir yöntem olarak UV spektroskopisi kullanılmış ve hesaplamalar için λ_{max} 234 ve 360 nm dalga boyları seçilmiştir.

Anahtar Kelimeler: *Felodipin, YBSK, UV Spektroskopisi, Miktar tayini*

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Introduction

Felodipine is a substituted 1,4 dihydropyridin derivative and chemically designed as ethylmethyl-1,4-dihydro-2,6-dimethyl-4-(2,3 dichlorophenyl)-3,5-pyridindicarboxylate (Figure 1). It is mainly used for the management of hypertension and angina pectoris like the other calcium channel blockers (1).

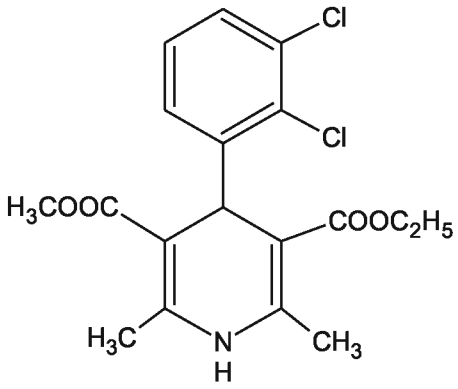

FIGURE 1. Felodipine.

Felodipine is a peripheral and coronary vasodilator but unlike the calcium- channel blockers verapamil or diltiazem, has little or no effect on cardiac conduction and negative inotropic activity is rarely seen at therapeutic doses (2,3). In the quantitative determination of felodipine in body fluids and pharmaceutical dosage forms, the previous studies were realized by titrimetry (4), spectrophotometry (5), HPLC (6-11) and gas-liquid chromatography (12).

In this study, we advised two assay procedures which would serve as a rapid and reliable methods for the quality control of felodipine in commercial samples.

Experimental

Chemicals

Felodipine and disulfiram (internal standard) were kindly donated by Astra-Zeneca and Nobel İlaç Sanayi A.Ş. in İstanbul-Turkey respectively.

All the solvents and chemicals were of analytical grade. HPLC grade methanol and water were purchased from Merck Company (Germany). Plendil® tablets (batch no: 202171), Astra-Zeneca İlaç Sanayi, İstanbul, Turkey. Containing 5 mg felodipine were purchased from local pharmacies in Ankara-Turkey.

Apparatus

HPLC system consists of Hewlett-Packard Co.Ltd.1050 series delivery pump system equipped with a 1050 UV-Vis detector. Peak areas were integrated automatically by a 3396 A multimode integrator.

UV-Vis spectrophotometer: A Beckman DU 650 series, double beam with a fixed slit width (2 nm). 1 cm quartz cell was employed over the range 200-400 nm.

HPLC Method

Chromatographic conditions

Chromatographic separation was carried out on Luna C_{18} 5μ (250x4.6 mm) column. Felodipine was determined by isocratic system with mobile phase consisting of methanol-0.055 M phosphate buffer (83:17 v/v). The phosphate buffer is adjusted pH=3±0.1 with o-phosphoric acid. The mobile phase was prepared daily and filtered through a millipore, 0.45 μm membrane and degassed for 15 minute in an ultrasonic bath before use. Flow-rate was 0.7 mL.min^{-1} and the detector was set at 275 nm. Disulfiram was used as internal standard. Injection volumes were 20 μL and all assays were performed at ambient temperature.

Stock Solutions

The stock solution of felodipine (100 mcg mL^{-1}) and disulfiram (int. st. 64 mcg mL^{-1}) were prepared in methanol. These solutions were stable for a week if stored at 4°C. Calibration graphs were prepared in synthetic mixtures of felodipine and disulfiram. Standard solutions of felodipine were prepared in the concentration range of (2-20 mcg mL^{-1}) and internal standard disulfiram concentration was fixed in 2.56 mcg mL^{-1} for all standard mixtures. All appropriate dilutions were prepared with methanol. 20 μL volume of each synthetic mixture was injected and all applications were repeated three times. The peak height ratios of active substances to internal standard were plotted against corresponding concentration of felodipine.

Sample Preperation

Twenty tablets were weighed and powdered. A portion of the powder equivalent to about 5 mg felodipine was weighed accurately, transferred to a 50 mL volumetric flask and stirred with 40 mL methanol on a magnetic stirrer for 15 minutes. The solution was filtered and diluted up to 50 mL with methanol. 2.5 mL of this solution and 1 mL internal standard solution were pipeted into 25 mL volumetric flask and completed with methanol to the mark (sample solutions contain 10 mcg mL^{-1} felodipine and 2.56 mcg mL^{-1} disulfiram). 20 μL volume of sample solution was injected into the column.

UV Spectroscopic Method

Calibration Procedure

Two different concentrations of standard felodipine solutions were prepared in methanol (Standard A solution: 150 mcg mL^{-1}. Standard B solution: 250 mcg mL^{-1}). 1, 1.5, 2, 3, 4 mL of these solutions were transferred into 25 mL volumetric flasks and diluted with methanol separately.

The absorption of solutions of A and B were measured at 234 and 360 nm respectively. Molar absorptivity and A^{1}_{1} values were calculated and regression equations were established separately.

Sample Preparation

An accurately weighed portion of the powder equivalent 10 mg felodipine was extracted with 40 mL methanol, filtered and diluted up to 50 mL with methanol. 4 mL of the solution was pipeted into 50 mL volumetric flask and completed with methanol. Absorbance of solution was measured at 234 and 360 nm.

Results and Discussion

In this study, high performance liquid chromatography and UV spectroscopic methods were developed for the determinations of felodipine. To find the appropriate HPLC conditions for determination of felodipine, various mobile phase systems were tested. The optimum wavelength for detection was 275 nm at which much better detector responses for active substance and internal standard were obtained. The mobile phase was

chosen as methanol-0.055 M phosphate buffer (83:17 v/v- pH=3±0.1). The mobile phase was found to be essential to improve the sharpness and thinness of the felodipine and disulfiram (int. st.). The elution order were t_r= 12.52 min for felodipine and t_r= 9.64 min for internal standard at a flow rate of 0.7 mL min^{-1}. (Figure 2). Linear relationship in the concentration range of 2-20 mcg mL^{-1} and the regression equation was obtained as y=0.04365x+0.1356 (r=0.9986). (Table1).

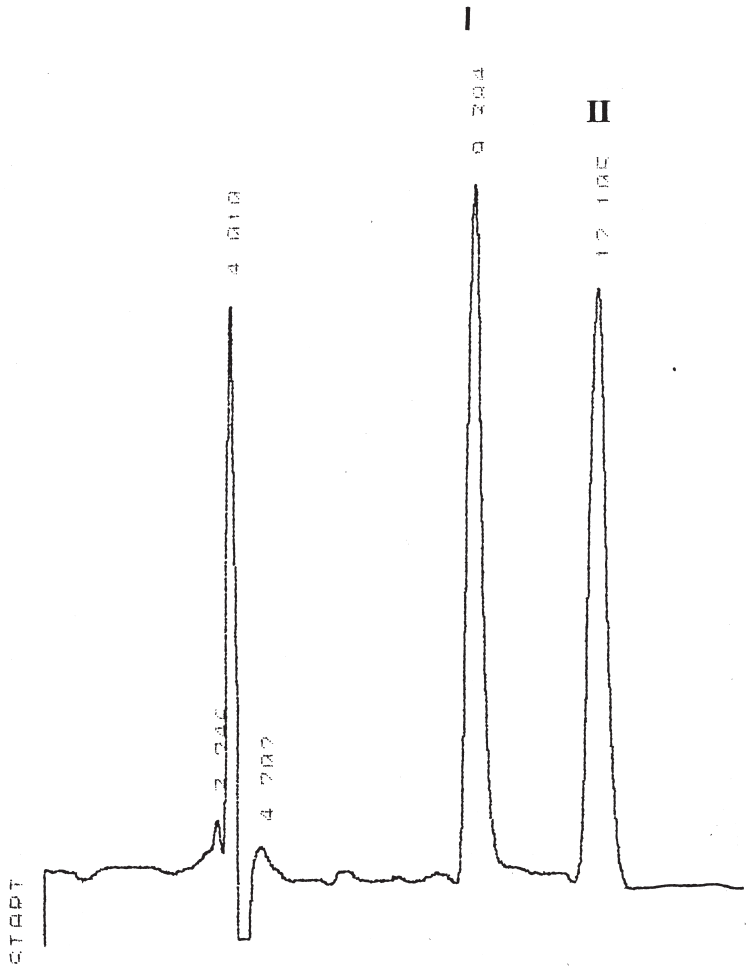

FIGURE 2. Chromatogram of a commercial tablet containing felodipine
Disulfiram (I) t_r = 9.64 min 2.56 mcg mL^{-1} in methanol
Felodipine (II) t_r = 12.52 min 10 mcg mL^{-1} in methanol.

TABLE 1. Statistical analysis of the results in the determination of felodipine by using HPLC and UV spectroscopy.

Parameters	HPLC	UV	
		234 nm	360 nm
Range mcg mL^{-1}	2-20	4-24	8-60
Detection limit mcg mL^{-1}	0.4	2	2.5
Quantitation limit mcg mL^{-1}	1	1	5
Regression equation			
Slope (a)	0.04365	0.4783	0.1927
$Slope_{SD}$	$8.6x10^{-4}$	$9.3x10^{-3}$	$2.96x10^{-3}$
Intercept (b)	0.1356	0.007	-0.02
$Intercept_{SD}$	$2.36x10^{-3}$	$4.5x10^{-4}$	$8.75x10^{-4}$
Corr. coeff. (r)	0.9986	0.9990	0.9987
RSD %	0.79	0.73	1.01
ε		18630	6763
A^{1} % 1,1 cm		484	176

The mean percentage of recoveries (± conf. lim.) and relative standard deviation were found to be 99.9±0.75 and 0.79 % respectively (Table 2).

TABLE 2. Assay results of laboratory prepared sample by HPLC.

Standard Synthetic Mixture	X		$Y=H_F/H_D$	Recovery* %
	Added	Found		
St_1	8	7.94	0.482	99.2
St_2	10	10.07	0.575	100.7
St_3	12	12.01	0.659	100.1
St_4	14	13.85	0.745	98.9
St_5	16	16.08	0.837	100.5
$\overline{X}$				99.9
RSD %				0.79
Conf. Lim. (p=0.05)				0.75

*Results obtained are the mean of five determinations for laboratory prepared samples
x= Concentration of felodipine mcg mL^{-1}.
y= Peak height ratio of felodipine to disulfiram.

The other method, UV spectroscopy, 234 and 360 nm were chosen λ max absorption points. A linear concentration was obtained as 4-24 mcg mL^{-1}, 8-60 mcg mL^{-1} at 234 and 360 nm respectively.

The mean percentage recoveries (± conf. lim.) were found to be 100.2±0.71 and 99.6±0.98 and relative standard deviations were found 0.73 and 1.01 % in laboratory samples at 234 and 360 nm respectively. (Table 3).

TABLE 3. Assay results of laboratory prepared samples by UV spectroscopic method.

Standard Synthetic Mixture	234 nm			360 nm		
	mcg mL^{-1}			mcg mL^{-1}		
	Added	Found	Recovery*	Added	Found	Recovery*
St_1	4	4.05	101.2	8	8.04	100.5
St_2	8	8.04	100.5	12	11.98	99.8
St_3	12	11.95	99.7	16	15.72	98.3
St_4	16	15.89	99.3	24	24.14	100.6
St_5	20	20.06	100.3	40	39.6	98.9
$\bar{X}$	100.2			99.6		
RSD %	0.73			1.01		
Conf. Lim. (p=0.05)	0.71			0.98		

*Results obtained are the mean of five determinations for laboratory samples

The regression equations were found as y=0.4783x+0.007 (r= 0.9990) and y=0.1927x-0.02 (r=0.9987) at 234 and 360 nm respectively. Molar absorptivity (ε) and A_1^1 (1 %,1 cm) values were calculated as 18630, 484 at 234 nm and 6763, 176 at 360 nm (Table1).

The results obtained for felodipine determinations in commercial sample by using two methods were showed in Table 4. The purposed methods were compared with student's t and Fischer F test statistically. The results were showed that the differences between the results of the method were statistically insignificant (Table 5).

TABLE 4. Assay results of commercial samples.

Sample*	HPLC**		UV Spectroscopy**			
	mg	%	234 nm		360 nm	
			mg	%	mg	%
1	5.06	101.2	5.03	100.7	4.97	99.5
2	5.01	100.3	4.96	99.2	5.06	101.2
3	4.93	98.7	4.87	97.5	4.91	98.3
4	4.95	99.1	5.06	101.2	5.03	100.7
5	4.92	98.4	4.97	99.5	4.95	99.1
$\overline{X}$	4.97	99.5	4.98	99.6	4.98	99.8
RSD %		1.18		1.45		1.18
Conf.Lim. (p=0.05)		1.13		1.38		1.13

* Amount labeled 5 mg felodipine per tablet

** Results obtained are the mean of three determinations for each sample

TABLE 5. Statistically comparison of results with proposed methods.

	Student's t test	Fisher F test
HPLC-UV 360 nm	0.272	1.07
HPLC- UV 234 nm	0.250	1.48

For n=8 (p= 0.05)

$t_{theoretical} = 1.86$ $F_{theoretical} = 6.39$

Conclusion

As results it is observed that the proposed methods are practically applicable and give sensitive and dependable results. It is hoped that methods could be used routine analysis for commercial samples containing felodipine.

References

1. **Kayaalp, O. S.**, Rasyonel tedavi yönünden tıbbi farmakoloji, 9. baskı, I. Cilt, Konu: 38, pp. 421-493. Hacettepe-Taş, Ankara, **2000**.

2. **Parfitt, K. M**., The complete drug reference, 32. ed., Pharmaceutical Press, London, **1999**.

3. **Delego, J.N., Remers, W.A**., Wilson and Gisvold's textbook of organic medicinal and pharmaceutical chemistry, Tenth ed., Chapter: 19, pp. 588-593, Lippincott-Raven Publishers, Philadelphia, New York, **1998**.

4. European Pharmacopoeia 3rd ed. (Supplement), Strasbourg Codex 1, **1998**.

5. **Basavaiah, K., Chandrashekar U., Prameela H.C., "**Sensitive spectrophotometric determination of amlodipine a felodipine using iron (III) and ferricyanide" *Farmaco*, Feb. 58(2), 141-8, **2003**.

6. **Rapado, M.I., Garcia-Alvarez-Coque, M.C., Villanueva-Caman, R.M., "**Performance of micellar mobile phases in reversed-phase chromatography for the analysis of pharmaceuticals containing beta-blockers and other antihypertensive drugs" *Analyst***,** Nov. 121(11), 1677-82, **1996.**

7. **Weidolf, L., "**Bimodal column switching liquid chromatographic assay of metabolites of felodipine in rat urine" *J. Chromatogr*., Sep. 13,343(1), 85-97, **1985**.

8. **Cardoza, R.M., Amin, P.D., "**A stability indicating LC method for felodipine" *J. Pharm. Biomed. Anal*., Feb.1; 27(5), 711-8, **2002**.

9. **Zarapkar, S.S., Kolte, S.S., Rane, S.H., "**High performance liquid chromatographic determination of amlodipine and atenolol, simultaneously, from pharmaceutical preparations" *Indian Drugs*, 34(6), 350-353, **1997**.

10. **Lopez, J.A., Martinez, V., Alonso, R.M., Jimenez, R.M., "**High performance liquid chromatography with amperometric detection applied to the screening of 1,4-dihydropyridines in human plasma" J. Chromatogr.,A, 870(1+2), 105-114, **2000**.

11. Zhang, S., Zhen, Y., Zhang, L., Li, S., "Simultaneous determination of 6 different dihydropyridine calcium antagonist in human plasma by high performance liquid chromatography" *Sepu*, 13(2), 132-135, **1995**. (C.A. 122, 255372c, 1995).

12. Jiang, Y., Zhang, X., Ren,J., Liu, H., "Determination of felodipine in serum by capillary gas chromatography" *Zhongguo Yiyuan Yaoxue zazhi*, 18(3), 101-103, **1998**. (American Chem. Soc. 129, 130828, 1998).

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