

THE ASSAY OF FELODIPINE BY SECOND DERIVATIVE SPECTROPHOTOMETRY

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Abstract

The present paper aimed the development of a new felodipine assay method, a calcium channel antagonists, used as an antihypertensive drug. The second derivative of the felodipine absorption spectra was used at wavelengths between 239 - 230 nm, in methanolic solution. It was established the concentration range for Beer's law. It was assessed that there is a linear relationship between the second derivative and the concentration of the felodipine solutions, in the range between $1.946 \cdot 10^{-6}$ and $19.46 \cdot 10^{-6}$ g/mL. The correlation coefficient and the regression equation were found to be $r = 0.99975$ and $y = 0.00883x + 0.00487$. This method proved to be sensitive, reproducible and accurate.

Rezumat

Lucrarea de față prezintă dezvoltarea unei noi metode de dozare a felodipinei, blocant al canalelor de calciu, care acționează ca medicament antihipertensiv. S-a utilizat derivata de ordinul al doilea a spectrului de absorbție al felodipinei, determinat în metanol, la lungimi de undă cuprinse între 239 și 230 nm. S-a stabilit domeniul de concentrații pentru care se respectă legea Beer. Pe domeniul de concentrații cuprins între $1,946 \cdot 10^{-6}$ și $19,46 \cdot 10^{-6}$ g/mL există o relație liniară între derivata de ordinul al doilea și concentrația soluției de felodipină. S-au calculat coeficientul de corelație $r = 0,99975$ și ecuația dreptei de regresie $y = 0,00883x + 0,00487$. Metoda este sensibilă, reproductibilă și exactă.

Keywords: felodipine, second derivative spectrophotometry

Introduction

Among the calcium antagonists, considered to be the only drugs which modify the calcium influx into cells by blocking, thereby causing vasodilation, felodipine: ethyl methyl ester of (RS)-4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridine-dicarboxylic acid (Figure 1), is characterized by a certain therapeutic effectiveness being used in the treatment of various cardiovascular disorders [2].

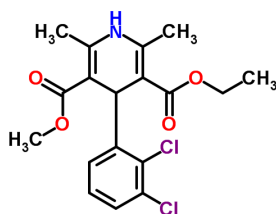


Figure 1.

The chemical structure of felodipine

The literature includes many methods for the assay of felodipine based on high performance liquid chromatography [3-13]. There are very little references for the spectrophotometric method of felodipine assay [14-16]. The spectrophotometric

methods are simpler and have lower costs compared with chromatographic methods and therefore are used to determine numerous of drugs [17-19]. Therefore, we proposed to establish a new method by derivative spectrophotometry for the determination of felodipine in bulk.

Materials and Methods

Instrument: Perkin Elmer UV-VIS spectrometer Lambda 2 (Perkin Elmer) (10 mm quartz cells); analytical balance (Mettler Toledo AT261 Delta Range); ultrasonic bath Elma 9331-1 (Barnstead, Lab-Line, USA).

Chemicals and reagents: felodipine (Labormed Pharma); spectrophotometric grade methanol (Merck KGaA, Germany). All reagents were of analytical grade.

Method

Procedure for calibration curve

A $2.435 \cdot 10^{-4}$ g/mL stock standard solution of felodipine (solution A) was obtained by dissolving an appropriate amount of drug in methanol. A $9.74 \cdot 10^{-6}$ g/mL standard solution of felodipine (solution B) was prepared by dilution of A solution. Ten standard solutions of felodipine were prepared

individually by dilution of the B solution with methanol for spectrophotometric determination to obtain a concentration range of $1.946 \cdot 10^{-6}$ and $19.46 \cdot 10^{-6}$ g/mL. The variation in the amplitude of the second derivative was registered.

Results and Discussion

The spectrophotometric analysis was performed by recording the UV absorption spectrum, first derivative (D1) and second (D2) order derivatives of UV absorption spectrum of felodipine methanolic solutions (Figures 2, 3 and 4).

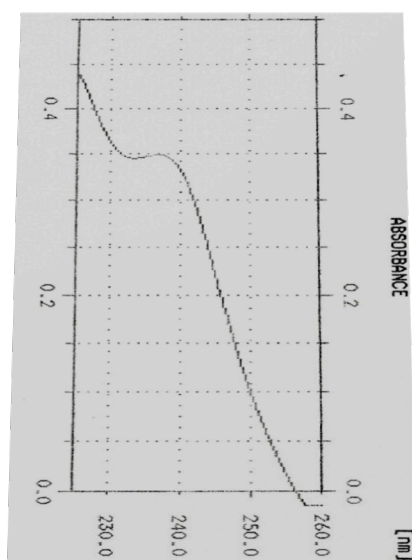


Figure 2.

The UV spectrum of felodipine in methanol

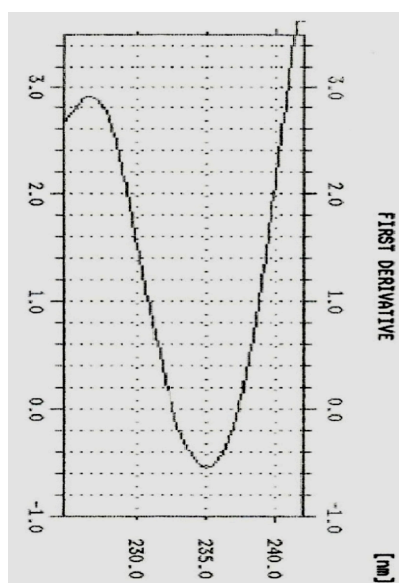


Figure 3.

D1 of UV spectrum

The D2 of the absorption spectrum recorded in the range 250 - 225 nm was used for the assay. The

calculation of the amplitude was carried out between wavelengths 239 and 230 nm. The wavelength scanning speed was 120 nm/min and the smoothing was set to 3 nm.

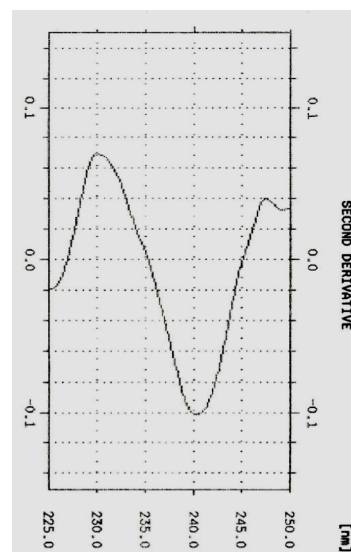


Figure 4.

D2 of UV spectrum

Method validation

The method validation followed the parameters: linearity, accuracy, precision (repeatability and intermediate precision), according to the International Conference on Harmonization (ICH) [20].

Linearity

The variation of amplitude of D2 of UV spectrum of felodipine *versus* concentration of drug was registered and the data were used in drawing the calibration curve. In the studied concentration range, a linear relationship between concentration and amplitude of D2 has been shown. The statistical parameters for calibration curve were given in the regression equation calculated from the calibration curve (Table I). The linearity of calibration curve in the studied concentration range was proved by the correlation coefficient which was found to be 0.99975.

Table I

Statistical data for the calibration curve

Parameter	Value
Beer's law limits (g/mL)	$1.946 \cdot 10^{-6}$ - $19.46 \cdot 10^{-6}$
Regression equation (y)	$0.00883x + 0.00487$
Intercept (a)	0.00487
Slope (b)	0.00883
Correlation coefficient (r)	0.99975
Regression coefficient (r ²)	0.9995

Accuracy was studied taking samples of felodipine so as to obtain concentrations between 80 - 120% of the amount of interest. The procedure described for the calibration curve plotting was used for

samples preparation. For each concentration level three solutions were prepared. The D2 of UV absorption spectra was registered. The results show

that in the range of the concentration from $7.2939 \cdot 10^{-6}$ to $14.9540 \cdot 10^{-6}$ g felodipine/mL, measurements were accurate (Table II).

Table II
Accuracy and precision results

Validation parameters		Parameters		
		Mean recovery	RSD%	Confidence range (95% confidence level)
Accuracy		100.04%	0.562	100.04 ± 0.43
Precision	Repeatability	99.48%	0.609	99.48 ± 0.46
	Intermediate precision	99.94%	0.736	99.94 ± 0.56

Method precision was evaluated by the repeatability and the intermediate precision, according to the ICH guidance.

Repeatability was determined using three samples of known concentration. For each of these samples, the average of the amplitude of the D2 of three successive determinations and the data of the statistical processing are shown in Table II. The relative standard deviation (RSD %) was 0.609 for the concentration range of $8.2915 \cdot 10^{-6}$ - $11.0380 \cdot 10^{-6}$ g felodipine/mL.

The *intermediate precision* was studied by determinations in another day with freshly prepared solutions using the same procedure for repeatability. Three solutions of felodipine were prepared and for each of them it was recorded the D2 of absorption spectrum for three times. Felodipine concentration was calculated using the regression equation. The data were processed statistically and are shown in Table II. The relative standard deviation was found to be $RSD\% = 0.736$.

Results of validation are summarized in Table III.

Table III
Summary of validation parameters

Value criteria		Results
Linearity	Regression equation	$y = 0.00883x + 0.00487$
	Correlation coefficient (r)	0.99975
Beer's law limits (g/mL)		$1.946 \cdot 10^{-6}$ - $19.46 \cdot 10^{-6}$
Accuracy		$RSD\% = 0.562$
Precision	Repeatability	$RSD\% = 0.609$
	Intermediate precision	$RSD\% = 0.736$

Conclusions

A new spectrophotometric method for the assay of felodipine, using second order derivative spectra, was established. The concentration range in which determinations can be performed is between 1.9468 mg/L and 19.468 mg/L. Under the set conditions, the method was precise, accurate and sensitive.

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