Quantitative Estimation of Felodipine by Zero and First Order Derivative Area under Curve Spectrophotometric Methods in Bulk and in-house Tablets

Jain PS*, Ansari NA and Surana SJ

RC Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, India

*Corresponding author: Pritam S Jain, R C Patel Institute of Pharmaceutical Education and Research, Karwand Naka, Shirpur, Dist. Dhule 425 405 (MS), India, E-Mail: pritash79@yahoo.com

Received: February 24, 2018; Accepted: April 08, 2018; Published: April 28, 2018

Abstract

The main objective of the proposed methods is to disclose simple, accurate, precise, reproducible and sensitive UV-spectrophotometry methods for the estimation of Felodipine in Bulk and in-house formulation tablets. Further, this study is designed to validate the developed methods as per ICH guidelines. Felodipine is a long acting calcium channel blocker anti-hypertensive agent. Four simple UV-Spectrophotometric methods were established by using Double beam UV-Spectrophotometer (UV-2450, Shimadzu), Felodipine showed maximum absorbance at 237.60 nm (λ max). The calibration curves were plotted in the concentration range of 03-18 μg/mL. The % recovery was found to be in the range of 99-101%. The precision of the proposed method was calculated in terms of %RSD <2, methods are rugged and precise. Proposed methods can be used for routine analysis of Felodipine in bulk and in-house formulations.

Keywords: Curve spectrophotometric Methods; Felodipine.

Introduction

Felodipine (FDP), (Molecular formula- C18H19Cl2NO4 M. W. - 384.3 gm.), chemically is 4-(2, 3-Dichlorophenyl)-1, 4-dihydro-2, 6-dimethyl-3, 5 pyridinedicarboxylic acid ethyl methyl ester (FIG. 1) [1,2].

![Chemical structure of Felodipine](image-url)
Felodipine is a long-acting calcium channel blocker (dihydropyridines class) used as an anti-hypertensive and in the treatment of angina [3]. The literature review shows the various methods for the determination of Felodipine by High Performance Liquid Chromatography (HPLC) [4], LC-MS Method for estimation of Felodipine in Human Plasma [5], Estimation of Felodipine in Rabbit Plasma by HPLC, regioisomeric impurity by UV Spectrophotometric method [6]. The objective of this work is to establish zero and first derivative UV Spectroscopy and its AUC technique. The current works emphasize simple, precise, sensitive and effective UV Spectroscopy method for estimation of Felodipine in bulk and in-house tablets. The method was validated as per ICH guidelines.

**Experimental Works**

**Materials and method**

Felodipine (Pure) were obtained as a gift sample from Glenmark Pharmaceutical Ltd, Mumbai. As the tablet formulation was not available in Indian market, tablet containing 10 mg Felodipine were prepared in-house using Dry Granulation technique. Prepared tablets were used as pharmaceutical formulation for further analysis.

**Instrument**

A double beam UV-VIS spectrophotometer (UV-2450, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 1 cm quartz cells was used. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

**Preparation of standard stock solution and determination of λ max**

The standard stock solution of Felodipine was prepared by dissolving 10 mg of drug into a 100 mL volumetric flask and diluted up to the mark with methanol to make the concentration of 100 μg/mL. From the standard stock solution, 1 mL of solution was transferred into 10 mL of volumetric flask and diluted up to the mark obtaining 10 μg/mL and scanned in UV-visible range 400-200 nm, Felodipine shows a maximum absorbance at 237.40 nm (λ max).

**Method I (Zero Order Spectrometry) and method II (AUC-Zero Order Spectrometry)**

From the standard stock solution (100 μg/mL of Felodipine), different dilutions were prepared in the range of 3-18 μg/mL. In Method I, the maximum absorbance was obtained at 237.40 nm, Shown in FIG. 2.
And in the Method II, Area under Curve was chosen between the range of 233.00-240.20 nm, Shown in FIG. 3. The calibration curves were plotted as concentration versus absorbance for Zero Order and concentration versus AUC for Zero Order AUC in Method I and Method II, respectively Shown in FIG. 4 and 5.

**Method III (1st Order derivative spectrometry) and method IV (AUC-1st Order Derivative Spectrometry)**

In Method III and Method IV, For Method III, Spectra of the above dilutions were derivative into a 1st order derivative by using UV-Probe 2.21 software, in this the delta lambda and scaling factor were selected 4 and 10, respectively. In this method the amplitude was recorded at 245.00 nm shown in FIG. 6. While Method IV, the AUC range for 1st derivative 242.20-248.80 nm selected, shown in FIG. 7. The calibration curves were plotted as concentration versus amplitude and concentration versus AUC for Method III and Method IV, respectively shown in FIG. 7 and 8.
FIG. 3. Zero order AUC spectra of Felodipine.

FIG. 4. Linearity curve of Felodipine by zero order Spectrometry.
FIG. 5. Linearity curve of Felodipine by zero order AUC spectrometry.

FIG. 6. 1st Order spectra of Felodipine.
FIG. 7. 1st Order AUC spectra of Felodipine.

FIG. 8. Linearity curve of Felodipine by 1st order spectrometry.
Validation of the Method

Study of linearity curves

From the standard stock solution, aliquots portion in the range of 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mL were transferred into a 10mL volumetric flask and diluted up to the mark using methanol to prepare 3, 6, 9, 12, 15 and 18 µg/mL. The dilutions were scanned on a spectrophotometer in the range of 400-200 nm. The graph was plotted between concentration versus absorbance, amplitude and AUC.

Accuracy/Recovery studies

The accuracy of the method was determined by calculating recoveries of Felodipine by the standard addition method. The study was carried out by adding known amount of standard drug to the developed in-house tablet formulation at three 80, 100 and 120% level. The solutions were reanalyzed by proposed method. The accuracy of the method noted.

Precision
Precision of the method was performed as intra-day and inter-day. Intra-day precision was determined by examining the 6, 9 and 12 μg/mL of Felodipine for three times in the same day. Inter-day precision was determined by analyzing the 6, 9 and 12 μg/mL of Felodipine for three days.

**Sensitivity (LOD and LOQ)**

The Sensitivity measurements of Felodipine by proposed method were determined in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ) by using the equation designed by International Conference on Harmonization (ICH) guidelines.

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S} \\
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where \(\sigma\) = Standard Deviation

\(S\) = slope

**Repeatability**

Repeatability of the proposed method was determined for 9 μg/mL concentration of Felodipine by analyzing for six times for all the methods.

**Ruggedness**

Ruggedness of the proposed method was determined for 9 μg/mL concentration of Felodipine by analyzing of aliquots from a homogenous slot by two analysts using same environmental and operational conditions. The results are in acceptable range that is %RSD values <2 for all the methods.

**Results and Discussion**

**Method validation**

The proposed method was validated as per ICH guidelines. The solutions of the drug were prepared as per the earlier adopted procedure given in the experiment.

**Linearity studies**

Felodipine showed a good linear relationship for all the methods are shown in TABLE 1. The given concentration ranges 03-18 μg/mL.
### Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Method I</th>
<th>Method II</th>
<th>Method III</th>
<th>Method IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer-Lambert’s Range (µg/mL)</td>
<td>3-18</td>
<td>3-18</td>
<td>3-18</td>
<td>3-18</td>
</tr>
<tr>
<td>λ max (nm)</td>
<td>237.60</td>
<td>233.00-240.20</td>
<td>245.00</td>
<td>242.20-248.80</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0569</td>
<td>0.0096</td>
<td>0.0297</td>
<td>0.0222</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0185</td>
<td>0.0023</td>
<td>0.0071</td>
<td>0.0004</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>Limit of detection (µg)</td>
<td>0.1514</td>
<td>0.7080</td>
<td>0.1260</td>
<td>0.2764</td>
</tr>
<tr>
<td>Limit of quantitation (µg)</td>
<td>0.4590</td>
<td>2.1454</td>
<td>0.3819</td>
<td>0.8378</td>
</tr>
</tbody>
</table>

**TABLE 1. System suitability test results (n=5).**

### Accuracy

The % recovery of Felodipine at three concentration level 80, 100 and 120% were calculated for all the methods and the results shown in **TABLE 2.**

<table>
<thead>
<tr>
<th>% Value</th>
<th>Initial amount (µg/mL)</th>
<th>Amount added (µg/mL)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Method I</td>
</tr>
<tr>
<td>80</td>
<td>6</td>
<td>4.2</td>
<td>100.0659</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>6</td>
<td>99.9853</td>
</tr>
<tr>
<td>120</td>
<td>6</td>
<td>7.2</td>
<td>100.0049</td>
</tr>
</tbody>
</table>

**TABLE 2. System suitability test results (n=5).**

### Precision

The precision of the developed method was expressed in terms of intra-day and inter-day. The % relative standard deviation’s (RSD) values for all the methods were found to be less than 2 and results shown in **TABLE 3.**

### Sensitivity

The LOD and LOQ for all the proposed methods were shown in **TABLE 1.**

### Repeatability

Repeatability for Felodipine was determined for all the methods by analyzing 9 µg/mL concentration for six times and the % amount found was determined with % RSD <2. The results of repeatability are summarized in **TABLE 4.**
Conc. (µg/mL) & Intra-day\(^a\) & Inter-day\(^a\) \\
\hline
Method I & Method II & Method III & Method IV & Method I & Method II & Method III & Method IV \\
\hline
6 & 0.0610 & 0.5016 & 0.182781 & 0.504148 & 0.5440 & 0.1002 & 0.064877 & 0.380348 \\
9 & 0.5431 & 0.0442 & 0.173815 & 0.15015 & 0.02953 & 0.4938 & 0.037418 & 0.195403 \\
12 & 0.3407 & 0.4108 & 0.14044 & 0.357009 & 0.2631 & 0.1892 & 0.049225 & 0.434371 \\
\hline
\(^a\)Average of three estimates

<table>
<thead>
<tr>
<th>Method</th>
<th>Amount taken (µg/mL) (n=6)</th>
<th>Amount found(^a) (%)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method I</td>
<td>9</td>
<td>99.9934</td>
<td>0.0806</td>
</tr>
<tr>
<td>Method II</td>
<td>9</td>
<td>99.9807</td>
<td>0.1703</td>
</tr>
<tr>
<td>Method III</td>
<td>9</td>
<td>99.9688</td>
<td>0.1305</td>
</tr>
<tr>
<td>Method IV</td>
<td>9</td>
<td>99.9499</td>
<td>0.1952</td>
</tr>
</tbody>
</table>

\(^a\)Average of six estimations

### TABLE 3. Precision.

**Ruggedness**

The method was performed by changing analyst and the method was found to rugged with % Relative standard deviation (RSD) <2.

<table>
<thead>
<tr>
<th>Method</th>
<th>Amount taken (µg/mL) (n=3)</th>
<th>Amount found(^a) (%)</th>
<th>Analyst I</th>
<th>Analyst II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method I</td>
<td>9</td>
<td>99.9824</td>
<td>99.9479</td>
<td></td>
</tr>
<tr>
<td>Method II</td>
<td>9</td>
<td>99.9228</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Method III</td>
<td>9</td>
<td>99.9812</td>
<td>100.0062</td>
<td></td>
</tr>
<tr>
<td>Method IV</td>
<td>9</td>
<td>99.9916</td>
<td>100.0083</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Average of six estimations

### TABLE 4. Precision.

**Determination of Felodipine in in-house tablet**

The % amounts reveal from in-house tablet, that there is no interruption from excipients present in it. The % amount for all the method was determined results shown in TABLE 6.

### Conclusion

Method (I, II, III, IV) has been developed for quantitative analysis of Felodipine in Bulk and i-house formulation. The results show that the developed UV Spectrophotometric methods are simple, accurate, precise, reproducible and sensitive.
Consequently, these methods can be used routine analysis and quality control for estimation of Felodipine in Bulk and Pharmaceutical formulations.

The authors are thankful to the Principal, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur - 425405 (MS), India, for providing the laboratory facility.

<table>
<thead>
<tr>
<th>Method</th>
<th>Concentration (µg/mL) (n=6)</th>
<th>Amount found (µg/mL) (n=6)</th>
<th>Amount founda (%)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method I</td>
<td>9</td>
<td>8.9988</td>
<td>99.9869</td>
<td>0.0834</td>
</tr>
<tr>
<td>Method II</td>
<td>9</td>
<td>8.9930</td>
<td>99.9228</td>
<td>0.2028</td>
</tr>
<tr>
<td>Method III</td>
<td>9</td>
<td>8.9960</td>
<td>99.9563</td>
<td>0.1595</td>
</tr>
<tr>
<td>Method IV</td>
<td>9</td>
<td>8.9992</td>
<td>99.9916</td>
<td>0.2085</td>
</tr>
</tbody>
</table>

aAverage of six estimations

TAB. 6. Analysis of in-house Tablet.

REFERENCES


