UV SPECTROPHOTOMETRIC ESTIMATION OF DILTIAZEM HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM

Rohan D. Kuntawar*, Sugandha V. Mulgund

Department of Quality Assurance Techniques, STES’s Sinhgad College of Pharmacy, Vadgaon(Bk), Pune 411041, India.

ABSTRACT
A simple, precise, accurate and economical spectrophotometric method has been developed for estimation of Diltiazem hydrochloride. The standard and sample solutions were prepared by using distilled water as a solvent. Quantitative determination of the drug was performed at 236 nm. The linearity was established over the concentration range of 6-16 µg/ml for Diltiazem hydrochloride with correlation coefficient value of 0.999. Precision studies showed that % relative standard deviation (%RSD) was within range of acceptable limits (<2). The mean percentage recovery was found to be 104.18%. The proposed method has been validated as per ICH Q2 (R1) guidelines. This method can be used for routine quality control analysis of Diltiazem Hydrochloride in bulk and pharmaceutical formulations.

KEYWORDS: Diltiazem hydrochloride, UV spectrophotometry, Validation.

INTRODUCTION
Diltiazem (Figure 1) is a calcium channel antagonist used in the treatment of angina, hypertension, and arrhythmias in humans as well as hypertrophic cardiomyopathy in cat [1]. Diltiazem is widely used as antihypertensive agent, vasodilator agents, calcium channel blockers and cardiovascular agents [1,2]. Chemically, Diltiazem is (2S,3S)-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-4-oxo-2,3,4,5-tetrahydro-1,5-benzothiazepin-3-yl acetate [1,6]. Diltiazem is official in IP 2010 [4], BP 2009 [5] and USP 2005 [6]. HPLC [7] and HPTLC [8] methods are reported for the estimation of Diltiazem from its formulation or biological fluids. Only few papers have been available in the literature which employed
spectrophotometric method for estimation of Diltiazem hydrochloride as single drug and combined dosage forms \cite{7,10,11}. In this context, we wish to further explore spectrophotometric technique for estimation of Diltiazem hydrochloride in bulk and tablet dosage form.

![Chemical Structure of Diltiazem hydrochloride](image)

**Fig. 1: Chemical Structure of Diltiazem hydrochloride.**

**MATERIALS AND METHODS**

**Apparatus and instrumentation**
Shimadzu UV 1800 (Japan) double beam spectrophotometer with matched quartz cells, connected to computer loaded with UV Prob Software, was employed for this work. Single pan electronic balance (Shimadzu, AX 200, Japan) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India). Calibrated volumetric glass wares (Borosil®) were used in this study.

**Materials**
Reference standard of Diltiazem Hydrochloride API was supplied as gift sample by Micro Labs Ltd., Bangalore, India. Commercially available tablets (Dilzem® label claim 30 mg, Torrent, Batch. No. – 20013011) were obtained from local pharmacy. Distilled water was used as solvent and was obtained from Elga (Bucks, England) water purification unit.

**Method development**

**Preparation of standard solution**
The standard stock solution of Diltiazem Hydrochloride was prepared by transferring, accurately weighed, 10 mg of API to 100 ml of volumetric flask. The drug was dissolved with sonication in 50 ml of distilled water and volume was made up to the mark by using distilled water. The standard stock solution (100 µg/ml) was further diluted with distilled water to obtain the solution of 10 µg/ml Diltiazem Hydrochloride.
Determination of $\lambda$ max
The standard solution of Diltiazem Hydrochloride (10 $\mu$g/ml) was scanned in the range of 200-400 nm against distilled water as blank. $\lambda$ max of this solution was found to be 236 nm (Figure 2).

Calibration curve for Diltiazem Hydrochloride
Adequate dilutions were made from standard stock solution to obtain concentrations of 6, 8, 10, 12, 14, 16 $\mu$g/ml respectively. The absorbances were recorded at 236 nm. The relationship between absorbance and concentrations were established by simple regression equation method. The regression equation was obtained and this relationship is presented in the calibration curve (Figure 3).

Assay of tablet formulation
Twenty tablets each containing 30 mg of Diltiazem hydrochloride were weighed, powdered and average weight was calculated. Powder equivalent to 10 mg of Diltiazem hydrochloride was dissolved in 30 ml of distilled water with help of sonication. This was further diluted upto the mark with distilled water. The solution was filtered using whatmann filter paper no. 41 and first 5 ml of filtrate was discarded. This solution was further diluted to obtain 10 $\mu$g/mL solution with same solvent and subjected for UV analysis. This procedure was repeated in triplicate (Table 1).

Method validation
The objective of validation of an analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) and Robustness according to ICH Q2 (R1) guideline.

Linearity and Range
The linearity was determined by using working standard solutions between 6-16 $\mu$g/ml. The spectras of these solutions were recorded. Calibration curve of absorbances vs. concentrations was plotted and linear regression was performed. Regression equation and correlation coefficient were obtained (Figure 3).
Method Precision

Repeatability: The precision of the method was checked by repeatedly analysis of (n = 6) standard solutions of Diltiazem Hydrochloride (10 μg/mL). Absorbances of each of these solutions were measured at 236 nm. Percentage relative standard deviation (% RSD) was calculated (Table 2).

Intermediate Precision

The intra-day and inter-day precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for standard solutions of 10 μg/ml Diltiazem Hydrochloride. The results were reported in terms of relative standard deviation (RSD) (Table 2).

Accuracy

The accuracy for the analytical procedure was determined at 75 %, 100 % and 125 % levels of 10 μg/ml standard solution. Absorbance was measured at the 236 nm and results were expressed in terms of % recoveries. Three determinations were performed at each level and % RSD was calculated at each level (Table 3).

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In this study, LOD and LOQ were based on the standard deviation of the response and the slope of the corresponding curve using the following equations-

LOD = 3.3 σ/S and LOQ = 10 σ/S

Where, σ is the standard deviation of the signal to noise ratio of the sample and S is the slope of the related calibrations graphs.

The limit of quantification (LOQ) is defined as the lowest concentration of the standard curve that can be measured with an acceptable accuracy, precision and variability. Six sets of known concentrations (6-16 μg/ml) were prepared and scanned. By using these spectras, regression equations were obtained. By taking average of slopes and standard deviation of y-intercept, LOD and LOQ were calculated. The values of LOD and LOQ are given in table 4.
RESULTS AND DISCUSSION

Fig. 2: UV spectrum of Diltiazem hydrochloride (10 μg/ml)

Fig. 3: Calibration Curve of Diltiazem (6-16 μg/ml)

Developed UV-spectrophotometric method for the estimation of Diltiazem Hydrochloride in tablet was found to be simple, accurate, economical and reproducible. The correlation coefficient value was 0.999. Drug concentrations were found to be linear in the range of 6-16 μg/ml. For repeatability, the % relative standard deviation (% RSD) was found to be 0.6134 while, intra-day and inter-day precision results in terms of percent relative standard deviation values were found to be 0.2527 and 0.4579, respectively. The results were satisfactory and method was precise. The accuracy of the method was assessed by recovery studies at three different levels i.e. 75%, 100%, 125%. The values of standard deviation were satisfactory and the mean % recovery was 104.18%. The % RSD value is ≤ 2 indicates the
accuracy of the method. The Limit of Detection and Limit of Quantitation values were found to be 0.2756 μg/ml and 0.8351 μg/ml respectively. The result of the analysis of pharmaceutical formulation by the developed method was consistent with the label claim, highly reproducible and reliable. The validation parameters are summarized in Table 4.

Table 1. Assay of Tablet Dosage Form (Dilzem®)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample solution concentration (μg/ml)</th>
<th>Amount found (%)</th>
<th>Mean ± SD</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>100.48</td>
<td>100.91±0.3798</td>
<td>0.307</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>101.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>101.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n=3, SD=Standard Deviation, % RSD = % Relative Standard Deviation

Table 2. Precision Results for Diltiazem hydrochloride

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration of drug (μg/ml)</th>
<th>Absorbance (Mean ± S.D)*</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diltiazem (n=6)</td>
<td>10</td>
<td>0.52112±0.0018</td>
<td>0.3541</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>10</td>
<td>0.52439±0.0013</td>
<td>0.2527</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>10</td>
<td>0.52321±0.0023</td>
<td>0.4579</td>
</tr>
</tbody>
</table>

*n=3

Table 3. Accuracy Results for Diltiazem hydrochloride

<table>
<thead>
<tr>
<th>Accuracy Level</th>
<th>Amount added (μg/ml)</th>
<th>Amount recovered (μg/ml)</th>
<th>% Recovery ± SD *</th>
<th>Mean Recovery (%)</th>
<th>% RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (75%)</td>
<td>17.5</td>
<td>18.5</td>
<td>105.74 ± 0.40</td>
<td>104.18</td>
<td>0.6079</td>
</tr>
<tr>
<td>II (100%)</td>
<td>20</td>
<td>20.3</td>
<td>101.56 ± 0.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III (125%)</td>
<td>22.5</td>
<td>23.6</td>
<td>105.25 ± 1.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*n=3

Table 4. Summary of Validation Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ max</td>
<td>236</td>
</tr>
<tr>
<td>Linearity range (μg/ml)</td>
<td>6-16</td>
</tr>
<tr>
<td>Regression Equation (y=mx+c)</td>
<td>y = 0.051x + 0.009</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.999</td>
</tr>
<tr>
<td>Precision (% R.S.D)</td>
<td></td>
</tr>
<tr>
<td>Repeatability (n=6)</td>
<td>0.6134</td>
</tr>
<tr>
<td>Intraday (n=3)</td>
<td>0.2527</td>
</tr>
<tr>
<td>Interday (n=3)</td>
<td>0.4579</td>
</tr>
<tr>
<td>Accuracy (Mean % Recovery)</td>
<td>104.18</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Limit of Detection (LOD) μg/ml</td>
<td>0.2756</td>
</tr>
<tr>
<td>Limit of Quantitation (LOQ) μg/ml</td>
<td>0.8351</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The proposed UV-spectrophotometric method was simple, precise, accurate and economical for determination of Diltiazem Hydrochloride in tablet dosage form. The method was validated as per ICH guidelines in terms of linearity, accuracy, precision, limits of detection (LOD), limit of quantification (LOQ) and reproducibility. This method can be used for routine quality control analysis of Diltiazem Hydrochloride in bulk and pharmaceutical formulations.

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**REFERENCES**

