Validated First Order Derivative UV Spectrophotometric Method for the Determination of Clonazepam

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ABSTRACT
A simple, precise and economical procedure has been developed for estimation of Clonazepam in bulk drug and pharmaceutical dosage form, Using UV- spectrophotometer. The first order derivative spectrophotometric method was employed for estimation of Clonazepam using analytical grade methanol as solvent. Clonazepam has maximum absorbance at 243nm and Clonazepam obeys Beer’s law in concentration range 8-24µg/ml. The recovery studies verified accuracy of the purposed method; result validated as per ICH guideline and results were found satisfactory and reproducible. The method was successfully performed for the estimation of Clonazepam in tablet dosage form without the interference of common excipients.

Keywords: Clonazepam, UV Spectrophotometric, First derivative, Estimation.

INTRODUCTION
Clonazepam belongs to category of benzodiazepine drug. Chemically it is 5-(2-Chlorophenyl)-7-nitro-2, 3-dihydro-1, 4-benzodiazepin-2-one used for epilepsy or anxiety disorders5. Only very few analytical methods are reported so far for the determination of Clonazepam in pharmaceutical formulations and bulk drugs. Four HPLC methods2-5 and only one UV Spectrophotometric method6 have been reported which are useless for routine analysis. There for; the aim of the present study was to develop new method which is simple, rapid, economical and suitable for the routine determination of Clonazepam in its bulk drug and dosage forms.

Materials and Methods
Materials
Shimadzu 1800 spectronic model UV Spectrophotometer with 1cm matched quartz cells was used as the instrument for data collection and analysis. Methanol 95% was used as the solvent obtained from Qualigen. Tablet brands were obtained from the local market for assay and recovery studies.

Methodology
Preparation of Standard Stock Solution
Standard stock solution of Clonazepam was prepared by dissolving accurately weighed amount of clonazepam (10mg) in 100 ml of methanol with sonication and transferred it to 100 ml of volumetric flask.

Volume was made up to the mark with methanol for obtaining standard stock solution of 100 µg/ml concentration.

Selection of Analytical Wavelength Range
Standard stock solution having 100µg/ml of clonazepam was prepared by dissolving 10mg of drug in 100 ml. the subsequent dilutions of standard stock solution was made with methanol to get final concentration (20µg/ml) of standard solution.

These were scanned in the spectrum mode of an instrument from 280 nm to 230 nm. The first order derivative of the spectrum with N=5 were proposed to proceed for selection of analytical wavelength. The maximum absorbance was found to be at 243nm, selected as wavelength for the determination of Clonazepam.

Determination of Amplitude
The standard solution of clonazepam (20µg/ml) was scanned in the range of 280-230 nm and the amplitude was found to be 0.004 at 243nm.

Stability of Drug in Selected Solvent
The stability of drug in selected solvent was determined by measuring the absorbance of the drug solution...
(20µg/ml) at different time intervals. After every 5 min. absorbance was measured and solution was found to be stable.

**Linearity**

From the standard stock solution of Clonazepam, appropriate aliquots were pipette out into 25 ml volumetric flask and dilutions were made up with methanol to obtain working standard solution of Clonazepam 8, 12, 16, 20, 24, µg/ml.

The difference in amplitude of Clonazepam were measured in the first derivative mode with n=5 of instrument at 243 nm. The calibration plot of the drug Clonazepam was plotted.

The concentration range over which the drug followed linearity was chosen as an analytical concentration range i.e. 8-24 µg/ml for Clonazepam. (Table 1, Figures 1 to 4).

**Table 1:** Standard Calibration Data for Clonazepam

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Conc.(µg/ml)</th>
<th>Amplitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>08</td>
<td>0.001</td>
</tr>
<tr>
<td>2.</td>
<td>12</td>
<td>0.002</td>
</tr>
<tr>
<td>3.</td>
<td>16</td>
<td>0.003</td>
</tr>
<tr>
<td>4.</td>
<td>20</td>
<td>0.004</td>
</tr>
<tr>
<td>5.</td>
<td>24</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Figure 1: Calibration plot of Clonazepam at 243 nm.

Figure 2: First derivative spectrum of clonazepam at 8µg/ml

**Figure 3:** First derivative spectrum of clonazepam 16µg/ml

**Figure 4:** First derivative spectrum of clonazepam 24µg/ml

**Validation of Proposed Method**

**Estimation of Drug from Dosage Form: (Tablet Assay Study)**

Brand name- Lonazep

**Standard**

From the standard stock solution of Clonazepam, appropriate aliquots were pipette out into 25 ml of volumetric flask and dilutions were made by using methanol to get working standard solution of Clonazepam 20µg/ml.

This concentration was scanned at wavelength of 243nm with derivative mode N=5.

**Sample**

Twenty tablets of brand Lonazep and containing 0.5 mg of clonazepam weighed, and finally powered.

A quantity of powder sample of Lonazep was taken into volumetric flask and dilutions were made by using methanol to obtain conc. of 20µg/ml respectively. These concentrations were scanned at wavelength of 243nm with derivative mode N=5. (Table 2)
Table 2: Assay of Clonazepam (Lonazep Brand) in Tablet Form

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>Label Claim (mg/tablet)</th>
<th>Amount Found (mg/tablet)</th>
<th>% of Label Claim</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lonazep</td>
<td>0.5</td>
<td>0.49</td>
<td>98</td>
<td>99.64</td>
<td>1.5773</td>
<td>2.4880</td>
</tr>
</tbody>
</table>

Table 3: Accuracy parameters of Clonazepam

<table>
<thead>
<tr>
<th>Label % recovery</th>
<th>* Amount present (mg/tablet)</th>
<th>Amount of Standard Added (mg/tablet)</th>
<th>Total Amount Recovered (mg/tablet)</th>
<th>% recovery</th>
<th>% mean recovery</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.5</td>
<td>0.4</td>
<td>0.39</td>
<td>99.93</td>
<td></td>
<td>0.0602</td>
<td>0.0036</td>
</tr>
<tr>
<td>100</td>
<td>0.5</td>
<td>0.4</td>
<td>0.399</td>
<td>99.98</td>
<td></td>
<td>0.2437</td>
<td>0.0594</td>
</tr>
<tr>
<td>120</td>
<td>0.5</td>
<td>0.6</td>
<td>0.605</td>
<td>100.80</td>
<td></td>
<td>0.521</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Table 4: Determination of Precision

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Assay of Clonazepam as % of Labelled amount (inter – day precision)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analyst 1</td>
</tr>
<tr>
<td>1</td>
<td>100.15</td>
</tr>
<tr>
<td>2</td>
<td>100.13</td>
</tr>
<tr>
<td>3</td>
<td>100.20</td>
</tr>
<tr>
<td>4</td>
<td>100.01</td>
</tr>
<tr>
<td>Mean</td>
<td>100.12</td>
</tr>
<tr>
<td>SD</td>
<td>0.08057</td>
</tr>
<tr>
<td>CV</td>
<td>0.00649</td>
</tr>
</tbody>
</table>

Accuracy (Recovery Study)

A recovery experiment is used for study of accuracy of method. The recovery study was performed by adding known amount bulk drug to tablet powder. The recovery experience was performed at 3 levels, 80, 100 and 120% of clonazepam standard concentration.

Recovery samples were prepared in aforementioned procedure. 3 samples were prepared for each recovery level. The solutions were then analyzed. % recoveries were calculated by using following formula.

\[
\text{% Recovery} = \frac{\text{observed amount of compound in sample}}{\text{Amount of all compound present in sample}} \times 100
\]

The recovery values are summarized in following Table 3.

Precision

The precision (inter–day) was evaluated by using 4 independent sample of celecoxib. The intermediate precision (inter-day precision) of the process was also determined by using four different analysts in the same laboratory. The values obtained by four analysts were summarized in Table 4.

RESULTS AND DISCUSSION

The standard solutions of Clonazepam in Methanol (20µg/ml each) subjected to a scan at the series of wavelengths of 280nm to 230nm at First order and the derivative spectra were taken at N=5 using Shimadzu 1800 spectronic UV-Visible spectrophotometer. And amplitude found to be 0.004. The calibration plot of Clonazepam was found to be linear at conc. Range 8 to 24
μg/ml at 243nm. There for, it was clear that Clonazepam can be determined in presence of methanol with no intervention of any irrelevant substance in pharmaceutical products.

With the intention of determining the practicability of the developed technique for the assessment of commercially available brands (Lonazep) of medicinal formulations, the technique was initially attempted on bulk drugs in their synthetic mixture sample as well as concentrations were estimated. Then the technique was subjected to the assay of in marketed dosage forms and satisfactory results were attained within the appropriate limits as per the content of the label claim for Clonazepam.

The newly developed method was validated as per the international guidelines and parameters. The novel method for the quantitative investigation of Clonazepam was subjected to different validation parameters like specificity and selectivity in presence of formulation additives and excipients, studied for Linearity and range at different levels of concentrations and calibration standards where the determination range was optimized, accuracy was proved by recovery studies at different concentration levels, precision was established through inter day precision studies, where the samples were subjected to changed conditions other than optimized parameters.

CONCLUSION

From the above experimental studies it can be concluded that First Order Derivative method by UV spectrophotometry instrument developed for estimation of Clonazepam. The proposed methods for the selected drugs were found to be precise and accurate. The most important features of spectrophotometric methods are their rapidity and simplicity. Results of validation parameters demonstrate that these performed analytical procedures are suitable for its intended purpose and meet the criteria defined in ICHQ2A/B guidelines. The method is an excellent alternative to HPLC methods for routine analysis and accurate and rapid than the zero order UV spectrophotometric method.

REFERENCES

1. URL: https://en.wikipedia.org/wiki/Clonazepam

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