Absorption correction spectrophotometric method for simultaneous estimation of desvenlafaxine and clonazepam in their combined dosage form

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ABSTRACT

A new sensitive, simple, rapid and precise spectrophotometric method has been developed for simultaneous estimation of Desvenlafaxine and Clonazepam in pharmaceutical dosage form. This method was based on UV spectrophotometric determination of two drugs, using absorbance correction method. It involves measurement of absorbances at two wavelengths 226 nm (λmax of Desvenlafaxine (DESV)) and 309 nm (λmax of Clonazepam (CLO)) in methanol for the simultaneous quantitative determination of Desvenlafaxine and Clonazepam in the binary mixture without previous separation. The linearity was observed in the concentration range of 5 - 25 µg/ml for Desvenlafaxine and 5-25 µg/ml for Clonazepam. The accuracy and precision of the method was determined and validated statically. The method showed good reproducibility and recovery with % RSD less than 2. Method was found to be rapid, specific, precise and accurate, can be successfully applied for the routine analysis of Desvenlafaxine and Clonazepam in combined dosage form without any interference by the excipients. The method was validated according to ICH guidelines.

Keywords: Clonazepam, Desvenlafaxine, Simultaneous estimation, Absorption correction method.

INTRODUCTION

Desvenlafaxine (O-desmethylvenlafaxine) the major active metabolite of venlafaxine, is an antidepressant from the serotonin-norepinephrine reuptake inhibitor (SNRI class). Desvenlafaxine may be used to treat major depressive disorder and is being studied for use in the management of vasomotor symptoms in postmenopausal women. It is formulated as an extended release tablet. FDA approved in 2008. The structure of Desvenlafaxeine is shown in Fig 1.

![Desvenlafaxeine Structure](http://scholarsresearchlibrary.com/archive.html)

Fig 1. Structure of Desvenlafaxine

An anticonvulsant used for several types of seizures, including myotonic or atonic seizures, photosensitive epilepsy, and absence seizures, although tolerance may develop. It is seldom effective in generalized tonic-clonic or partial seizures. The mechanism of action appears to involve the enhancement of gamma-aminobutyric acid receptor responses. The structure of Clonazepam is shown in Fig 2.
Literature survey revealed that, Clonazepam is official in Indian Pharmacopoeia\(^{(5)}\), Desvenlafaxine is not official in any Pharmacopoeia. However, the combination is not official in any pharmacopoeia. On detailed literature survey, it was found that through individually these drugs have been analyzed by many methods, but there is no method available for the estimation of Desvenlafaxine and Clonazepam drugs in the combine dosage form. Therefore, it was thought worthwhile to develop simple, precise, accurate UV spectrophotometric method for simultaneous determination of Desvenlafaxine and Clonazepam in tablets. The proposed method was applied to the determination of analyts in pharmaceutical preparations, with satisfactory result. Validation was done with respect to various parameters, as per ICH guideline.

### MATERIALS AND METHODS

**Instrumentation**

A UV-visible spectrophotometer, model UV 1800 (Shimadzu) was used to measure absorbance of the resulting solutions using UV-Probe software version 2.31. A Digital analytical balance (Wensar DA13-220) and ultrasonic sonicator (Equitron) were used in the study.

**Reagents and materials**

Pure Desvenlafaxine (DESV) & Clonazepam (CLO) kindly gifted as a gift sample by Intas (astron) pharma, Ahemdabad, India and ZydusCadila, Ahemdabad, India. ZYVEN OD PLUS 50 Tablet formulation procured from local market. All analytical grade chemicals and solvents were obtained from Merck (India). Methanol used as solvent in the study. Borosil volumetric flasks of 10, 50, 100 ml capacity and pipettes – 1ml, 5ml, 10ml, beakers, measuring cylinders etc.

**PREPARATION OF SOLUTIONS**

**Preparation of Standard Stock Solutions**

**Desvenlafaxine (1000 µg/ml)**

Accurately weighed DESV (100 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

**Clonazepam (1000 µg/ml)**

Accurately weighed CLO (100 mg) was transferred to a 100 ml volumetric flask, dissolved in methanol and diluted to the mark with same solvent to obtain a standard stock solution (1000 µg/ml).

**Preparation of Working Standard Solutions**

**Desvenlafaxine (100 µg/ml)**

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

**Clonazepam (100 µg/ml)**

Standard Stock solution (5 ml) was transferred to a 50 ml volumetric flask and diluted up to the mark with methanol.

**Preparation of calibration curve**

**Calibration curve for Desvenlafaxine**

Aliquots of working standard solution of DESV (100 µg/ml) 0.5, 1, 1.5, 2 and 2.5 ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 5, 10, 15, 20 and 25 µg/ml. Absorbance of each solution was measured at 226 nm using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.
Calibration curve for Clonazepam
Aliquots of working standard solution of CLO (100 µg/ml) 0.5, 1, 1.5, 2 and 2.5ml were transferred into a series of 10 ml volumetric flasks and volume was adjusted to the mark with methanol to get concentrations 5, 10, 15 20 and 25 µg/ml. Absorbance of each solution was measured at 226 nm and 309 nm using methanol as a blank. Calibration curve was obtained by plotting respective absorbance against concentration in µg/ml and the regression equation was computed.

Application of absorption correction method
Two wavelengths from spectra, 226nm (DESV) and 309nm(CLO) were selected. Theabsorptivity values of DESV and CLO were determined at selected wavelengths. At 309 nm,estimation of CLO was done where no interference of DESV. The absorbance of working standard solution of CLO at 361 nm was calculated using equation (1) and then from the total absorbance of sample mixture at wavelength 226 nm, the contribution due to CLO was subtracted. The calculated absorbance was called as corrected absorbance for DESV. The concentrations of DESV (Cy) at 226 nm using the corrected absorbance was determine using absorptivity value. The concentration of two drugs in the mixture can be calculate using following equations,

\[ CX = \frac{A(309 \text{ nm})}{a(1\%, 1\text{cm})309 \text{ nm}} \]  

(1)

\[ AY(226 \text{ nm}) = CX \times a(1\%, 1\text{cm})226 \text{ nm} \]  

(2)

\[ CAx = A(226 \text{ nm}) - AY \]  

(3)

\[ CY = \frac{CAx}{a(1\%, 1\text{cm})226 \text{ nm}} \]  

(4)

Where,

Cx = Concentration of Clonazepam  
Cy = Concentration of Desvenlafaxine  
A226 nm = Absorbance of mixture at 226 nm  
A309 nm = Absorbance of mixture at 309 nm  
CAx226 nm = Corrected absorbance of Desvenlafaxine at 226 nm  
Ay226 nm = Absorbance of Clonazepam at 228nm.

Preparation of sample solution
For the estimation of the drug in tablet formulation twenty tablets were weighed and their average weight was determined. The tablets were then finely powdered. Appropriate quantity equivalent to 10 mg DESV and 0.1 mg CLO was accurately weighed. The powder was transferred to 100 ml volumetric flask and add standard of CLO 9.9 mg and shaken vigorously with methanol for 15 min and the solution was sonicated for 15 minutes and filtered through Whatman filter paper No. 41. Necessary dilutions are made with methanol to give final concentration 20 µg/ml of DESV and 20 µg/ml CLO respectively. The absorbance’s values were read at 226 and 309 nm and concentration was obtained by solving the absorption correction equations.

Method Validation
The developed method was validated with respect to linearity, accuracy, intraday and interday precision, limit of detection (LOD) and limit of quantitation (LOQ) and robustness in accordance with the ICH guideline.

Linearity and Range
Linearity was studied by preparing standard solutions at 5 different concentrations. The linearity range for Desvenlafaxine and Clonazepam were found to be 5-25 µg/ml and 5-25 µg/ml respectively. Linearity was assessed in the terms of slope, intercept and correlation coefficient for both the drugs.

Precision
The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate (intraday) precision and reproducibility (interday precision).

1) Intraday Precision
Solutions containing 10, 15, 20 µg/ml of DESV and 10, 15, 20 µg/ml of CLO were analysed three times on the same day and %R.S.D was calculated.
2) Interday Precision
Solutions containing 10, 15, 20 µg/ml of DESV and 10, 15, 20 µg/ml of CLO were analyzed on three different successive days and %R.S.D was calculated.

3) Repeatability
Method precision of experiment was performed by preparing the standard solution of DESV (15 µg/ml) and CLO (15 µg/ml) for six times and analysed as per the proposed method. Coefficient of variation (%CV) was not more than 2%.

Accuracy
Accuracy expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at 3 different concentration levels (80%, 100%, 120%) taking into consideration percentage recovery of added bulk drug samples. The experiment was repeated three times by spiking previously analysed samples of tablet with three different concentrations of standards.

Limit of Detection (LOD)
Limit of detection can be calculated using following equation as per ICH guidelines.

\[
LOD = 3.3 \times (N / S)
\]

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

Limit of Quantification (LOQ)
Limit of quantification can be calculated using following equation as per ICH guidelines.

\[
LOQ = 10 \times (N / S)
\]

Where, N is the standard deviation of the peak areas of the drug and S is the slope of the corresponding calibration curve.

RESULTS AND DISCUSSION

Selection of wavelength for simultaneous estimation of DESV and CLO
To determine the wavelength for measurement, DESV (20 µg/ml) and CLO (15 µg/ml) solutions were scanned between 400-200 nm. Absorbance maxima were obtained at their \( \lambda_{\text{max}} \) 226 nm and 309 nm for DESV and CLO respectively.

METHOD VALIDATION

Linearity & Range
The linearity of DESV and CLO was found to be in the range of 5-25 µg/ml and 5-25 µg/ml respectively. Linearity data for DESV and CLO are depicted in Table 1. Calibration curve of DESV and CLO are shown in Figure 7, 8 and 9 respectively. Linear regression equations and correlation-coefficient values for DESV and CLO are shown in Table 2.

Precision
1. Intraday Precision
The data for Intraday precision for DESV and CLO is in range of % RSD was found to be 0.13-0.60% for DESV at 226 nm and 0.16-0.63% and 0.45 – 0.65 % for CLO at 226 nm and 309 nm respectively.

2. Interday Precision
The data for Interday precision for DESV and CLO is in range of % RSD was found to be 0.49-1.11% for DESV at 226 nm and 0.51-1.01 % and 0.42- 0.79 % for CLO at 226 nm and 309 nm respectively.

3. Repeatability
The data for repeatability for DESV and CLO was found to be 1.64 % for DESV at 226nm and 0.74%, 0.47% for CLO at 226 nm and 309 nm respectively.
Accuracy of the method was confirmed by recovery study from marketed formulation at three levels (80%, 100%, and 120%) of standard addition. Percentage recovery for DESV and CLO were found to be in the range of 99.15-99.83% and 98.33-100.95%. Data indicating recovery studies of Desvenlafaxime and Clonazepam shown in Table 3.

**LOD and LOQ**

LOD values for DESV and CLO were found to be 0.29µg/ml and 0.09µg/ml respectively. LOQ values for DESV and CLO were found to be 0.90µg/ml and 0.28g/ml respectively.

**Analysis of Marketed formulation**

Applicability of the proposed method was tested by analyzing the commercially available tablet formulation (ZYVEN OD PLUS 50). It is shown in Table:4

**Table: 1 Linearity data for DESV and CLO**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance mean ± S.D. (n = 5)</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DESVENLAFAXINE</td>
<td></td>
<td>226nm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.232 ±0.0004</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.467 ±0.0008</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0.730 ±0.0113</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.936 ±0.0012</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.188 ±0.0012</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>226nm</td>
<td>309nm</td>
<td></td>
</tr>
<tr>
<td>CLONAZEPAM</td>
<td>5</td>
<td>0.515±0.0094</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.957 ±0.0024</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>1.360 ±0.0038</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.835 ±0.0159</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.278±0.0012</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Table: 2 Regression data for DESV and CLO**

<table>
<thead>
<tr>
<th>Regression Parameters</th>
<th>DESV</th>
<th>CLO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.9995</td>
<td>0.9994</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.0476</td>
<td>0.0881</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>0.0057</td>
<td>0.0676</td>
</tr>
</tbody>
</table>

Figure 3: Overlain Spectra of DESV (20µg/ml) and CLO (15µg/ml)
Figure 4: Overlaid Spectra of DESV (5-25 µg/ml) at 226 nm

Figure 5: Overlaid Spectra of CLO (5-25 µg/ml) at 309 nm

Figure 6: Calibration curve of Desvenlafaxine at 226 nm

\[ y = 0.047x - 0.005 \]

\[ R^2 = 0.999 \]
<table>
<thead>
<tr>
<th>% Level of recovery</th>
<th>Amount of drug taken (µg/ml)</th>
<th>Amount of drug added (µg/ml)</th>
<th>Total amount found (µg/ml) ± S.D. (n=3)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>DESV 80%</td>
<td>10</td>
<td>8</td>
<td>17.97±0.0021</td>
<td>99.83</td>
</tr>
<tr>
<td>CLO 10</td>
<td></td>
<td></td>
<td>17.70±0.0010</td>
<td>98.33</td>
</tr>
<tr>
<td>DESV 100%</td>
<td>10</td>
<td>10</td>
<td>19.83±0.0015</td>
<td>99.15</td>
</tr>
<tr>
<td>CLO 10</td>
<td></td>
<td></td>
<td>20.19±0.0050</td>
<td>100.95</td>
</tr>
<tr>
<td>DESV 120%</td>
<td>10</td>
<td>12</td>
<td>21.90±0.0034</td>
<td>99.54</td>
</tr>
<tr>
<td>CLO 12</td>
<td></td>
<td></td>
<td>21.87±0.0055</td>
<td>99.44</td>
</tr>
</tbody>
</table>

Table: 4 Analysis of marketed formulation

<table>
<thead>
<tr>
<th>Tablet</th>
<th>Drug</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% label claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZYVEN OD PLUS 50</td>
<td>Desvenlafaxine</td>
<td>50</td>
<td>49.92±0.0037</td>
<td>99.89%</td>
</tr>
<tr>
<td></td>
<td>Clonazepam</td>
<td>0.5</td>
<td>0.507±0.0012</td>
<td>101.42%</td>
</tr>
</tbody>
</table>

CONCLUSION

A simple, rapid, accurate and precise spectrophotometric method has been developed and validated for the routine analysis of Desvenlafaxine and Clonazepam in API and tablet dosage forms. The spectrophotometric method is suitable for the simultaneous determination of Desvenlafaxine and Clonazepam in multi-component formulations without interference of each other. The absorbance correction method is rapid, simple and sensitive. The developed method is recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations.

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