SIMULTANEOUS ESTIMATION OF ESCITALOPRAM OXALATE AND CLONAZEPAM IN BULK AND COMBINED PHARMACEUTICAL DOSAGE FORM BY UV SPECTROSCOPY

1Veena Sri. DS*, 2D. Asha Madhuri, 3V.V.L.N.Prasad, 4Sanayaima Huidrom, 5Vasudha Bakshi

Department of Pharmaceutical Analysis and Quality Assurance, School of Pharmacy, Anurag group of institutions, Venkatapur, Rangareddy district, AP, INDIA

Abstract

A simple, accurate, precise and economical procedure for simultaneous estimation of Escitalopram oxalate and Clonazepam in combined tablet dosage form has been developed utilizing concept of standard addition. The method is based upon simultaneous determination of Escitalopram oxalate and Clonazepam at 238nm and 222nm in methanol. Different analytical parameters such as linearity, accuracy and precision were determined according to ICH guidelines. Escitalopram oxalate and Clonazepam at their respective wavelength shows linearity in the concentration range of 10-24 and 2-14 µg/ml. The method was validated statistically. The results of analysis formulation given as percentage of label claim were found to be 97.5% and 95.8% for Escitalopram oxalate and Clonazepam respectively. The value of limit of detection and limit of quantification was found to be 0.44 µg/ml and 1.33 µg/ml for Escitalopram oxalate and 0.53 µg/ml and 1.61 µg/ml .Therefore, the proposed method can be used for the routine analysis of both drugs in quality control laboratories.

Keywords: Escitalopram oxalate, Clonazepam, Simultaneous estimation, Methanol.

Corresponding Author:

Veena Sri. DS
M.Pharm (Pharm. Analysis and quality assurance)
H.no:3-1-105/1/2,Shathavahana nagar,
Lb nagar, Hyderabad
Telangana, INDIA
Email: veenasripharma9@gmail.com
Mobile: +91 9550183752
Available online: www.ijipsr.com October Issue 2457
INTRODUCTION

Escitalopram Oxalate (ESC) is chemically 1-[3-(Dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile ethanedioate is a pure s-enantiomer of the racemic bicyclic phthalane derivative of citalopram (Fig.1). It is selective serotonin reuptake inhibitors (SSRI), clinically effective drug in the treatment of major depressive disorder and generalised anxiety disorder [1]. It is soluble in methanol, chloroform and 0.1N HCl and sparingly soluble in water. Escitalopram oxalate is not official in any pharmacopoeia; hence no official method is available for estimation of Escitalopram oxalate in tablet formulation. Chiral liquid chromatography, HPTLC and zero order spectrophotometric methods have been developed for determination of Escitalopram Oxalate individually. [5,6,7]. Clonazepam (CLZ), 1,3-dihydro- 7-nitro- 5- (2-chlorophenyl) -2H -1, 4- benzodiazepine -2-one [2] is a benzodiazepine derivative (Fig.2). It is an anticonvulsant, muscle relaxant and anxiolytic agent soluble in methanol, acetone, chloroform and practically insoluble in water, hexane, 0.1N HCl. It is official in USP [3] and BP [4]. For this combination HPLC [8] [9], HPTLC [10] are reported and HPLC methods for ESC in combination with etizolam [11] and CLZ in combination desvenlafaxine [12] with are reported. There is however no work reported on combination of these drugs by simultaneous equation method. The significant feature of these combinations lies in the fact that Clonazepam is present in minute amount compared to Escitalopram Oxalate which makes its analysis more complicated and tedious. Hence in the present communication we propose fast, simple, and accurate spectrophotometric method, without tedious extraction procedure, was developed by applying standard addition method, for the simultaneous estimation of both the drugs in tablet dosage form by UV spectrophotometry.

**Fig.1. Structure of Escitalopram Oxalate**

**Fig.2. Structure of Clonazepam**
MATERIALS AND INSTRUMENTS

INSTRUMENT SPECIFICATIONS: UV Spectrophotometer, Shimadzu, model 1800

CHEMICALS AND REAGENTS: Methanol obtained from local market, Pure Escitalopram Oxalate and Clonazepam were obtained as gift sample from Suraksha Pharmaceuticals Pvt. Ltd., Roorkee. The tablet dosage form STALOPAM PLUS (claim: 10mg Escitalopram Oxalate and 0.5mg Clonazepam) was manufactured by Lupin Pharmaceuticals procured from local market.

SELECTION OF SOLVENT AND WAVELENGTH:
The absorbance of both drugs was found to be maximum in methanol. So, Methanol is used as solvent (Fig.3). Different concentrations of Escitalopram oxalate, Clonazepam and a mixture of Escitalopram oxalate and Clonazepam were prepared, scanned in the UV region, from which the wavelengths of 238 and 222 nm were selected for Escitalopram oxalate and Clonazepam respectively for further studies.

PREPARATION OF STANDARD STOCK SOLUTIONS:
The 10 mg of standard Escitalopram Oxalate and Clonazepam were weighed accurately and transferred into two different 10 ml volumetric flasks. Both the drugs were dissolved in methanol and diluted up to the mark by using the solvent methanol to obtain a final concentration of 1000$\mu$g/ml. 100 $\mu$g/ml of ESC and CLZ solution were prepared by diluting 1 ml of stock solution to 10ml with methanol in separate 10ml volumetric flask The resulting solution was used as a working standard solution. The aliquot portion of stock solutions of Escitalopram Oxalate and Clonazepam were diluted approximately with methanol to obtain concentration of 10$\mu$g/ml of each drugs. These solutions were scanned in the range of 200-400 nm in 1cm cell against blank .From the overlain spectra the wavelength selected for the estimation are 238 nm and 222 nm for Escitalopram Oxalate and Clonazepam respectively.

SIMULTANEOUS EQUATION METHOD
In Simultaneous equation method two wavelengths i.e, 222nm of CLZ and 238nm of ESC were selected as their respective $\lambda_{max}$ from the overlain spectrum, at which both drugs have maximum absorbance. Two simultaneous equations were formed using absorptivity coefficients at selected wavelengths. The concentrations of two drugs in the mixture were calculated using the following two simultaneous equations.

$C_x = A_2 ay_1 - A_1 ay_2 / ax_2 ay_1 - ax_1 ay_2$ \hspace{1cm} (1)

$C_y = A_1 ax_2 - A_2 ax_1 / ax_2 ay_1 - ax_1 ay_2$ \hspace{1cm} (2)
Where, Cx and Cy are the concentrations of x and y. A_1 is the absorbance of mixture at λ_1, A_2 is the absorbance of mixture at λ_2, ax_1 is the absorptive value of x at λ_1, ax_2 is the absorptive value of x at λ_2, ay_1 is the absorptive value of y at λ_1, ay_2 is the absorptive value of y at λ_2.

VALIDATION OF THE METHOD

1) LINEARITY:
Escitalopram oxalate and Clonazepam was found to be linear in a concentration range of 10-24µg/ml and 2-14µg/ml respectively. The absorbances of these solutions were noted at wavelength 238nm and 222 nm for ESC and CLZ respectively. Calibration curves were plotted using concentration Vs absorbance and the slope, intercept and correlation coefficient values were found to be 0.0311, 0.1098 and 0.9992, respectively for ESC 0.0969, 0.012 and 0.9992, respectively, for CLZ. The linearity values were shown in Table 1, and Fig.4 (ESC) and Fig.5 (CLZ)

2) PRECISION:
The precision studies were carried in terms of intraday and interday, the % relative standard deviation (%RSD) values were found to be less than 2, which indicate that the method is precise [14]. The precision values were shown in Table 2(a,b).

3) ACCURACY:
The accuracy of the method was ascertained by carrying out recovery studies using standard addition method. The recovery studies are performed to determine if there was any positive or negative interference from excipients present in the formulation. The percentage recovery results revealed that the values were near to 100%, which indicates that the proposed method is accurate as the results are within the official limits. It also reveals that the commonly used excipients and additives in the formulation were not interfering with the proposed method.(Table no:3)

4) LIMIT OF DETECTION AND LIMIT OF QUANTITATION:
The LOD and LOQ were separately determined based on calibration curve. The residual standard deviation of a regression line or the standard deviation of y- intercepts of regression lines were used to calculate the LOD and LOQ [13]
I. Formula for LOD (µg/ml);
\[ \text{LOD} = 3.3 \times \frac{\text{SD}}{\text{S}} \]

Where,
SD = the standard deviation of the response
S = the slope of the calibration curve (mean)
II. Formula for LOQ (µg/ml);

LOQ = 10 x SD / S

Where,

SD = the standard deviation of the response

S = the slope of the calibration curve (mean)

The value of limit of detection and limit of quantification was found to be 0.44 µg/ml and 1.33 µg/ml for Escitalopram oxalate and 0.53 µg/ml and 1.61 µg/ml.

5) ANALYSIS OF FORMULATION

For the preparation of sample solution, 5 tablets were taken and weighed, powdered and weight equivalent to 20mg was taken. The tablet powder was weighed, it contains 20mg of Escitalopram Oxalate and 1 mg of Clonazepam. So 11mg of API of Clonazepam was added to make 12 mg of clonazepam (standard addition method). This powder was transferred into 10 ml volumetric flask and dissolved in methanol. This solution was sonicated for 3 mins and filtered through whatman filter paper. From the filtrate 0.1 ml was taken and made up to 10 ml with methanol to give 20 µg/ml of ESC and 12 µg/ml of CLZ drugs. This solution was scanned over the range of 200-400 nm, using two sampling wavelengths 222nm and 238nm determined the concentration of these drugs in tablet formulation and the results are shown in Table 4., and Fig.6.

Table 1: Linearity values of ESC and CLZ

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>ESC</th>
<th>CLZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>10-24µg/ml</td>
<td>2-14µg/ml</td>
</tr>
<tr>
<td>Slope</td>
<td>0.0311</td>
<td>0.0969</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.1098</td>
<td>-0.012</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9992</td>
<td>0.9992</td>
</tr>
</tbody>
</table>

Table 2a: Intraday precision studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>%* RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escitalopram oxalate</td>
<td>10</td>
<td>0.41641</td>
<td>0.41512</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.4852</td>
<td>0.4942</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>10</td>
<td>0.95186</td>
<td>0.94110</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.153</td>
<td>1.2011</td>
</tr>
</tbody>
</table>

2b: Interday precision studies

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>%* RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escitalopram oxalate</td>
<td>10</td>
<td>0.4216</td>
<td>0.4251</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.4928</td>
<td>0.5031</td>
</tr>
<tr>
<td>Clonazepam</td>
<td>10</td>
<td>0.9671</td>
<td>0.9549</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.1621</td>
<td>1.1892</td>
</tr>
</tbody>
</table>

* mean of three observations, %* RSD-Relative Standard Deviation

Available online: www.ijipsr.com  October Issue 2461
**Table 3: Results of recovery studies**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount added (mcg/ml) (%)</th>
<th>Amount recovered (mcg/ml)</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESC</td>
<td>20 (100%)</td>
<td>20.22-20.32</td>
<td>101.1%-101.6%</td>
<td>0.348</td>
</tr>
<tr>
<td></td>
<td>10 (50%)</td>
<td>10.02-10.12</td>
<td>100.2%-101.2%</td>
<td>0.702</td>
</tr>
<tr>
<td>CLZ</td>
<td>12(100%)</td>
<td>12.1-12.2</td>
<td>100.83%-101.66%</td>
<td>0.579</td>
</tr>
<tr>
<td></td>
<td>6 (50%)</td>
<td>5.96-5.89</td>
<td>99.3%-99.66%</td>
<td>0.255</td>
</tr>
</tbody>
</table>

* * mean of three observations, %* RSD - Relative Standard Deviation

**Table 4: Results of analysis of tablet formulation**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESC</td>
<td>10</td>
<td>9.75</td>
<td>97.5</td>
<td>0.7214</td>
</tr>
<tr>
<td>CLZ</td>
<td>0.5</td>
<td>0.479</td>
<td>95.8</td>
<td>0.6611</td>
</tr>
</tbody>
</table>

Fig. 3: Overlaid spectra of Escitalopram oxalate and Clonazepam in Methanol (Selection of Wavelength)

Fig. 4: Overlaid normal spectra of Escitalopram Oxalate in different concentrations (10-24 µg/ml)

Fig. 5: Overlaid normal spectra of Clonazepam in different concentrations (2-14 µg/ml)

Fig. 6: UV spectrum of Formulation Sample
RESULTS & DISCUSSION

Escitalopram Oxalate and Clonazepam showed maximum absorbance in Methanol at 238 and 222 nm. The proposed method for simultaneous estimation of both the drugs was validated as per the ICH guidelines. The linearity was observed in the concentration range of 10-24 mcg/ml for Escitalopram Oxalate and 2-14mcg/ml for Clonazepam with regression co-efficient of 0.9992 and 0.9992. Amount of drugs estimated by the proposed method was in good agreement with the label claim. The accuracy of the method was assessed by recovery experiments. The precision of the method was studied as repeatability, intra-day and inter day variations; the %RSD less than 2, indicates proposed method is precise. Recovery was close to 100% for both the drugs.

CONCLUSION

The present study comprises a UV spectroscopic method of analysis for the simultaneous estimation of Escitalopram Oxalate and Clonazepam in tablet dosage form. From the study of validation parameters, it was observed that the method is specific, accurate, precise, reproducible. The proposed method could be applied to routine analysis in quality control laboratories.

ACKNOWLEDGEMENT

I am very much thankful to School of Pharmacy, Anurag Group of Institutions, Hyderabad, for giving permission to carry out my work.

REFERENCES

1. www.drugbank.com for Escitalopram oxalate drug profile(DB01175)
2. www.drugbank.com for Clonazepam drug profile(DB01068)

Available online: www.ijipsr.com October Issue 2463


