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ABSTRACT
A simple, sensitive, accurate, linear, reproducible, ecofriendly, economic and fast HPLC method has been developed. The proposed method was validated for according to ICH guidelines. Reverse phase HPLC method has been developed for simultaneous estimation of anti-psychotic drug Olanzapine and antidepressant drug Escitalopram Oxalate in bulk drug and dosage forms. The mobile phase used for to developed the method was Methanol: Water (80:20 v/v). The flow rate of mobile phase delivery is 1.0 ml/min. at a wavelength detection at 217nm. The retention time of Olanzapine and Escitalopram Oxalate were 2.21 min 3.09 min. respectively. The developed method was accurately validated follows all the guidelines prescribed by the ICH. The proposed method used for determination of concentration of these drugs in bulk and combined dosage forms.

Key words: RP-HPLC, Olanzapine, Escitalopram oxalate, Validation and Pharmaceuticals.

1. INTRODUCTION
Olanzapine (OLZ) is chemically 2 methyl – 4 – (4- methyl) 1-piperazinyl- thieno (2,3b) (1,5) benzodiazepine is an atypical antipsychotic drug approved for the treatment of Schizophrenia and bipolar disorders and other psychotic disorders such as delusion, hallucination, emotional and social withdrawal and poverty of speech by the U.S. food and drug administration (FDA). Pharmacological research has been shown that Olanzapine has receptor affinity for Dopamine D1-D5, serotonin 5HT, 2A/2B/2C, 5HT3 and 5HT6 receptor. (2,3,4,5) Olanzapine lower the risk of extra pyramidal side effects and minimal increase in prolactin level. (6)

Structure of Olanzapine

![Fig. 1: Olanzapine](https://example.com/olanzapine.png)

Escitalopram Oxalate (ESC) chemically it is (S) 1-3 [3-( Dimethyl amino) propyl ]-1-(4- Fluoro phenyl)-1-3 dihydro 5-isobenzofuran 5 carbonitril oxalate. Escitalopram Oxalate is one of the most prescribed drug being a selective and potent reuptake inhibitor. This antidepressant is widely use because it is safer, less toxic, more tolerable than the tricyclic antidepressant and monoamine oxidase inhibitor. It is used for the treatment of in the adults with major depressive disorder, generalized anxiety disorder and social anxiety disorder or panic disorder. It is pure S-enantiomer (single isomer) of the racemic bicyclic phthalate derivatives of citalopram. (7)

Structure of Escitalopram Oxalate

![Fig. 2: Escitalopram Oxalate](https://example.com/escitalopram.png)
The combination drug therapy is chosen because of many patients suffering from two diseases simultaneously then two different categories drug are given to the patient but it has many side effects, so we consider that to formulate the different categories of drug in to the combination by considering its compatibility so that it will becomes more beneficial to the patient in future.

In literature, a few methods were reported for the determination of Olanzapine by using HPLC method with UV detection. Likewise, there are number of methods for the determination of Escitalopram oxalate by using HPLC method with UV detection. In addition, several LC-MS methods developed for determination of several antipsychotic and antidepressant including Olanzapine and Escitalopram Oxalate was reported. According to our knowledge very few methods for the co-analysis for these two active substances. The aim of these study is to develop an effective, simple, rapid HPLC method for simultaneously quantification of Olanzapine and Escitalopram Oxalate. (6)

2.EXPERIMENTAL
2.1 Chemicals
The antipsychotic drug Olanzapine and antidepressant drug Escitalopram Oxalate are obtained from Cipla Pharmaceutical Ltd. In bulk quantity. All solvents and reagents used were in bulk quantity. All solvent, chemicals, and reagents used were of high performance liquid chromatography grade respectively. HPLC grade Methanol, Water obtained from S.D. fine chem. Ltd. Mumbai. All the solutions for analysis were prepared freshly and analyzed.

3. INSTRUMENTATION AND ANALYTICAL CONDITION
Chromatography was performed by using JASCO HPLC model number LCC-NET2/ADC pump, C361860860 and sample injection port 772i (20µl). The mobile phase were prepared freshly, filter through 0.45µm membrane filter and sonicator (ultrasonic USB 40D) for 30 min. before used.

Table 1: The various chromatographic conditions optimized for analysis of Olanzapine and Escitalopram oxalate by HPLC.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mobile phase</th>
<th>Flow rate ml/min</th>
<th>Detection wavelength (nm)</th>
<th>Injection volume ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olanzapine</td>
<td>Methanol: water (80:20)</td>
<td>1</td>
<td>226</td>
<td>2</td>
</tr>
<tr>
<td>Escitalopram oxalate</td>
<td>Methanol: water (80:20)</td>
<td>1</td>
<td>239</td>
<td>2</td>
</tr>
</tbody>
</table>

3.1 Preparation of mobile phase:
Mobile phase was prepared by using HPLC grade methanol and Water had ratio Methanol: Water (80:20) was found to resolve Olanzapine and Escitalopram Oxalate. The degassing of mobile phase was done by sonication for 30 min. the flow rate was set to 1ml/min. both drug shows good absorbance at 231nm which was selected as wavelength for further analysis. The column temperature was maintained at room temperature.

3.2 Preparation of stock solution:
Standard stock solution of Olanzapine and Escitalopram Oxalate were prepared individually by dissolving 1mg drug in 100 ml volumetric flask by using mobile phase and make the volume up to the mark. This will get 100µg/ml of solution from this 5ml of solution was pipette out and add in 10ml volumetric flask and dilute up to the mark with mobile phase which will get 50µg/ml solution respectively for both the drug and used for sample injection.

3.3 Determination of appropriate UV wavelength:
Appropriate wavelength for the detection of the drug in mobile phase was determine by scanning standard solution of both drugs over the wavelength range 200-400nm and after scanning an appropriate wavelength was selected. From overlain UV. spectra of Olanzapine and Escitalopram oxalate 231nm was selected as wavelength for HPLC analysis of both drugs.

3.3 Selection of HPLC stationary phase
The best result was obtained by using hibarR 250-4.6 HPLC column purosphensR STAR RP-18 as compared to other different stationary phase.

4. METHOD OF VALIDATION
Linearity
The methods were validated according to International Conference of Harmonization (ICH) Q2B guideline for validation of analytical procedures in order to determine the linearity, sensitivity, precision and accuracy for each analyte. (8,9) six-point calibration curve were generated with appropriate volume of working standard solution for both HPLC and UV method. In case of UV the range was optimised at 5-35µg/ml for Olanzapine and 5-40 µg/ml for Escitalopram Oxalate respectively.

Precision and Accuracy
Both precision and accuracy were determined with standard quality control sample prepared in triplicates at different concentration levels covering the entire linearity range. The precision is (%) degree of repeatability of an analytical method under normal operational condition. The precision is the assay was determined by repeatability (Inter-day) and intermediate (Intra-day) precision and reported as % R.D.S. for a statistically significant number of replicate measurement. (9)
The method specificity was assessed by comparing the chromatogram (HPLC) and scan (UV) the drug and the most commonly used excipients mixture with those obtained from blank (excipients solution in water without drug).

**LOD and LOQ**

The (LOD) limit of detection is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from background levels. The (LOQ) limit of quantification is defined as the lowest concentration of the standard curve that can be measured with acceptable accuracy, precision and variability. (10) The LOD and LOQ were calculated as

\[
\text{LOD} = 3.5 \sigma / s \quad \text{and} \quad \text{LOQ} = 10 \sigma / s
\]

Where \( \sigma \) the standard deviation of the lowest standard concentration and \( S \) is the slope of the standard curve.

**5.RESULT AND DISCUSSION**

The UV spectroscopy method developed a simple, sensitive, accurate and rapid analytical method for quantitative determination of drug compounds which will reduce the unnecessary sample preparation and cost of materials. Olanzapine and Escitalopram Oxalate are UV absorbing drug compounds which absorb a light of a particular wavelength and this property has been successfully employed for their quantitative determination with spectrophotometric method.

**Table 1: Validation parameters for UV method of analysis of Olanzapine and Escitalopram Oxalate.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Olanzapine At 226nm</th>
<th>Escitalopram oxalate At 239nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer’s Law Range</td>
<td>5-35 (μ/ml)</td>
<td>5-40 (μ/ml)</td>
</tr>
<tr>
<td>Regression Equation ( (y = mx + c) )</td>
<td>( y = 0.0342x + 0.0135 )</td>
<td>( y = 0.0316x + 0.0721 )</td>
</tr>
<tr>
<td>Slope (m)</td>
<td>0.042</td>
<td>0.039</td>
</tr>
<tr>
<td>Intercept (c)</td>
<td>-0.4</td>
<td>0.035</td>
</tr>
<tr>
<td>Correlation Coefficient ( R^2 )</td>
<td>( R^2 = 0.9866 )</td>
<td>( R^2 = 0.9945 )</td>
</tr>
<tr>
<td>LOD</td>
<td>0.490</td>
<td>0.139</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.149</td>
<td>0.301</td>
</tr>
<tr>
<td>% Recovery</td>
<td>93.2 %</td>
<td>96.8%</td>
</tr>
</tbody>
</table>

**Precision**

Precision was determined by using Olanzapine and Escitalopram oxalate three times.

**Table 2: Result of Precision study.**

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration of Drug (μ/ml)</th>
<th>Absorbance</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Olanzapine</td>
<td>Escitalopram oxalate</td>
<td>Olanzapine</td>
</tr>
<tr>
<td>1.</td>
<td>10 (n=3)</td>
<td>0.2859</td>
<td>0.3551</td>
<td>0.0065</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2864</td>
<td>0.3658</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2739</td>
<td>0.3740</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>15 (n=3)</td>
<td>0.64770</td>
<td>0.5450</td>
<td>0.0050</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6539</td>
<td>0.5639</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6570</td>
<td>0.5648</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>20 (n=3)</td>
<td>0.8695</td>
<td>0.7220</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8716</td>
<td>0.7868</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8678</td>
<td>0.7430</td>
<td></td>
</tr>
</tbody>
</table>

**Linearity**

The linearity studies were determined at different concentration ranging from 5 to 35μg/ml for Olanzapine and 5 to 40μg/ml for Escitalopram Oxalate.
Fig. 3: Calibration curve for Olanzapine

\[ y = 0.0342x + 0.0135 \]
\[ R^2 = 0.9866 \]

- Absorbance
- Linear (Absorbance)

Fig. 4: Calibration curve for Escitalopram Oxalate

\[ y = 0.0316x + 0.0721 \]
\[ R^2 = 0.9945 \]

- Absorbance
- Linear (Absorbance)

Fig. 5: Spectra of Olanzapine

Overlain spectra of Escitalopram Oxalate
6. CONCLUSION

The UV spectrophotometry method is the method of analysis and it is simple as well as reliable more useful and selective providing acceptable accuracy and precision with lower limit of detection (LOD) and quantification (LOQ). These method has shorter duration of analysis for both Olanzapine and Escitalopram oxalate. These method is suitable for routine analysis for both laboratory purpose and Pharmaceutical dosage forms. The recovery achieved by UV spectrophotometry are good.

REFERENCES


