SIMULTANEOUS ESTIMATION OF ESCITALOPRAM AND CLONAZEPAM BY RP-HPLC IN PHARMACEUTICAL FORMULATION

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Abstract

This paper presents a RP-HPLC method for simultaneous estimation of Escitalopram and clonazepam in pharmaceutical formulations. The process was carried out on a 250 × 4.6 mm, 5µ, C18 column. The flow rate was 1ml/min and eluent was monitored by absorbance at 248 nm using a mixture of Methanol and Buffer (pH 4.0) in the ratio of 90:10 (v/v). The retention times of Escitalopram and Clonazepam was found to be 3.22 and 4.29 min respectively. Calibration plots were linear in the concentration range of 2.5-80 µg mL⁻¹ and 0.125-4 µg mL⁻¹ for Escitalopram and clonazepam respectively. The total run time is 10 min. The proposed method was validated by testing its linearity, recovery, specificity, system suitability, precision (Interday and intraday), robustness and LOD/LOQ values and it was successfully employed for the simultaneous estimation of Escitalopram and clonazepam in pharmaceutical tablet formulations.

Keywords: Escitalopram, Clonazepam, RP-HPLC Estimation

1. Introduction

Escitalopram oxalate (ESC) is the (Figure 1) Selective serotonin reuptake inhibitor, Antidepressant agent, chemically it is S-(+)-5-Isobenzofurancarbonitrile, 1-[3-(dimethyl amino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-oxalate[1]. Clonazepam (CLZ) is Benzo diazepine, Anticonvulsant drug, chemically it is 5-(o-chlorophenyl)-1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one. Literature survey reveals several spectroscopic [2-4], HPLC [5,6] and HPTLC [7-15] methods for estimation of Escitalopram and clonazepam individually as well as combination with other drugs. All the reported HPLC methods used buffer in the mobile phase in longer retention time. The present study was aimed to develop a simple, rapid, precise, accurate, and selective chromatographic method for simultaneous estimation of Escitalopram and clonazepam in bulk and dosage forms with the use of buffer in the mobile phase in short duration.

2. Experimental

a. Chemicals: Bulk samples of ESC and CLZ were obtained from Plethico Pharmaceuticals Ltd., Indore, India. The commercial samples of combination tablets containing 200 mg and 10 mg of ESC and CLZ respectively were purchased from local market. Methanol (HPLC Grade), Water (HPLC Grade), and Buffer (pH 4.0) were used for the study.
Grade), Potassium di hydrogen phosphate (AR Grade), ortho-phosphoric acid (AR Grade) were purchased from RANCHEM (India). Mili-Q water was used throughout the experiment.

**b. Equipments:** Quantitative HPLC was performed on an isocratic HPLC of SHIMADZU prominence consisting of LC-20AT liquid pump, manual with 20µL sample injection loop and SPD 20A UV-Visible absorbance detector. The output signal was monitored and integrated by Shimadzu spin chrome software.

**c. Liquid chromatographic conditions:** Chromatographic conditions were obtained using a stainless steel column (C₁₈ 250mm x 4.6mm 5µm), which was maintained at 40°C. The analytical wavelength was set at 248 nm and samples of 20µL were injected to HPLC system. The mobile phase was methanol and Phosphate Buffer in ratio of 90:10 (pH=4) at a flow rate of 1ml/min. The mobile phase was filtered through 0.22µm filter and degassed for 10 minutes by sonication.

**d. Preparation of standard stock solutions:** Accurately weighed 10.0 mg of ESC and CLZ was transferred separately to a 10.0 mL volumetric flask, sufficient amount of methanol was added to dissolve it and then volume was made up to the mark (1000 µg/mL) with mobile phase. 1.0 mL of stock A was taken into 10.0 mL volumetric flask and further diluted up to 10.0 mL with methanol (stock, 100 µg/mL). Aliquots of stock were further diluted up to 10.0 mL to get concentration of 2.5, 5, 10, 20, 40, 80, 100 µg/mL and 0.125, 0.25, 0.5, 1, 2, 4 and 8 µg/mL for ESC and CLZ respectively.

**e. Sample preparation:** Twenty tablets were weighed (each tablet contain 200 mg of ESC and 10 mg of CLZ). The average weight was determined. It was finely powdered and mixed thoroughly. Powder equivalent to 200 mg of ESC and 10 mg of CLZ was taken and dissolved in 100 mL mobile phase, it was sonicated for 15 minutes and solution was filtered through 0.22µm membrane filter. Further dilutions were done with mobile phase to get concentration of 20:1 (ESC: CLZ).

**f. Preparation of laboratory mixture:** Methanolic solutions of each of ESC and CLZ was prepared by transferring 200 mg and 10 mg into two separate 10.0 mL volumetric flask. Dissolving and raising the volume up to the mark with mobile phase to produce solutions of 20 mg/mL and 1.0 mg/mL of ESC and CLZ respectively. Into 10.0 mL volumetric flask aliquots of 5.0 mL each of ESC and CLZ were transferred respectively. The final concentration of the prepared mixture is similar to the concentration ratio of the mentioned drug in tablets.

**g. Determination of Assay:** Five replicates of sample in equal volume (20µL) were injected separately into the stationary phase. The chromatograms were recorded and the response i.e. peak area of major peaks were measured. The amount of drug present per tablet was calculated by comparing a sample peak with that of standard solution. The % label claim reported is 99.6% ±0.02%.

**h. Recommended procedure for standard graph:** In order to established the linearity of analytical method, a series of dilutions were made for ESC and CLZ ranging from 2.5-80 µg/mL and 0.125-4 µg/mL respectively were prepared. All the solutions were filtered through 0.22µm membrane filter. The solutions were injected in triplicate into the HPLC column, keeping the injection volume constant (20µL). The chromatograms were recorded and calibration curve was plotted between the mean peak area vs. respective concentration and regression equations were obtained.

**i. Recovery Study:** Accuracy was determined by recovery studies of ESC and CLZ, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis.

3. **Method Validation**

**a. Specificity:** Subjecting the drug solution in different stress conditions such as acid, base, and peroxide, and the degradation was noted.

**b. Linearity:** To get the working concentration range, linearity was observed in the range of 2.5-80 µg/mL and 0.125-4 µg/mL for ESC and CLZ respectively. The linearity of these methods was evaluated by linear regression analysis, using least square analysis method.

**c. Accuracy:** The accuracy of the developed method was determined by recovery studies. The recovery studies are usually made by spiking the known amount of pure drug with the formulation. It is usually done by adding 80, 100, and 120 % of the pure drug with the formulation taken for analysis. The % recovery for ESC and CLZ reported respectively, which showed excellent recoveries were made with each added concentration.
d. Limit of Detection and Limit of Quantitation: The LOD and LOQ were determined for HPLC method. The limits were determined based on the standard deviation amongst response and slope of the curve at lowest concentrations (International conference of Harmonization, 1997) [16,17].

e. Precision: The precision studies were performed by interday and intraday studies. Standard solutions were prepared and were injected in triplicate. The response of each injection was measured and the precision was calculated using ± S.D and % RSD equations. The % RSD values for repeatability precision study should be ≤ 1%, which confirm that method is sufficiently precise.

f. Robustness: The robustness was performed by adding 80, 100, and 120 % of the pure drug with the formulation taken for analysis in two different mobile phase compositions of 70/30 and 80/20. The % recovery was calculated for each added concentration in both mobile phases.

4. Results and Discussion
To develop a suitable and robust LC method for the simultaneous estimation of ESC and CLZ different mobile phases and columns were employed to achieve the efficient separation and resolution. The criteria employed for selecting the mobile phase for the analysis of the drugs were cost involved and time required for the analysis. Attempts with traditional reverse phase columns presented poor peak symmetry and tailing problem. Most of the separation methods in literature overcame these problems by use of buffers in mobile phase [6]. The proposed method was able to selectively separate ESC in a short chromatographic run with the use of buffer mobile phase. The retention time for ESC and CLZ are 3.22 and 4.29 min respectively. The chromatogram is shown in Figure 2.

![Chromatogram for ESC (3.280) and CLZ (4.293)](image)

a. System Suitability: System suitability tests were performed as per the USP 31 to confirm the suitability and reproducibility of the system. The test was carried out by injecting 20μL standard solutions containing ESC of 100μg/mL and CLZ of 5μg/mL. This was repeated five times. The RSD values of ESC and CLZ were ±0.46 and ±0.84. The RSD values were found to be satisfactory and meeting the requirements of USP 31. Theoretical plates, tailing factor were determined and are presented in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
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<tbody>
<tr>
<td>ESC</td>
<td>CLZ</td>
</tr>
<tr>
<td>Regression analysis</td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>3.597</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0000</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9997</td>
</tr>
<tr>
<td>Validation parameters</td>
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<tr>
<td>LOD</td>
<td>3.03 μg</td>
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<tr>
<td>LOQ</td>
<td>9.25 μg</td>
</tr>
<tr>
<td>Accuracy (%RSD)</td>
<td>0.076</td>
</tr>
<tr>
<td>Precision (%RSD) Interday</td>
<td>0.06</td>
</tr>
<tr>
<td>Precision (%RSD) Intraday</td>
<td>0.09</td>
</tr>
<tr>
<td>Robustness (%RSD)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Table 1: Summary of regression analysis and validation parameters

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Retention time (min) ± SD 3.208±0.057 4.614±0.004
Tailing factor ± %SD 1.0183±0.007 1.0192±0.005
Theoretical plates ± SD 20.83±0.752 32.35±0.552

b. Recovery study: Accuracy was determined by recovery studies of ESC and CLZ, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Good recoveries were obtained when a mixtures of sample was spiked with the drug. The recovery study is shown in Table 2.

Table 2. Recovery of ESC standard solution added to sample

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ESC</td>
</tr>
<tr>
<td>80%</td>
<td>99.7±0.15</td>
</tr>
<tr>
<td>100%</td>
<td>99.83±0.15</td>
</tr>
<tr>
<td>120%</td>
<td>100.2±0.15</td>
</tr>
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</table>

c. Linearity: Linearity was evaluated by analysis of working standard solutions of ESC and CLZ of six different concentrations. The response for the drug was linear in the concentration range between 2.5-80 µg/mL and 0.125-4 µg/mL for ESC and CLZ respectively. The peak area and concentration of each drug was subjected to regression analysis to calculate the calibration equations and correlation coefficients. The regression data obtained are represented in Table 1. The results shows that with-in the concentration range mentioned above, there was an excellent correlation between peak area and concentration of drug.

d. Accuracy: Good recoveries were obtained when a mixtures of sample was spiked with the drug. The accuracy data are shown in Table 1.

e. Limit of detection and limits of quantitation: The limit of detection (LOD) and limit of quantitation (LOQ) were established as per the ICH guidelines. Limit of detection and limit of quantitation were found to be 239 ng/mL and 724 ng/mL of ESC and 442.95 µg and 134.5 µg respectively.

f. Precision: The method precision was evaluated by interday and intraday studies. Standard solutions were prepared and were injected in six replicates. The response of each injection was measured and the precision was calculated using ± S.D and % RSD equations. The % RSD values for repeatability precision study should be ≤ 1%, which confirm that method is sufficiently precise.

g. Robustness: The recovery studies for both mobile phases showed good recovery which indicate that the method is robust enough to withstand the variations in the mobile phase composition.

Conclusion
The proposed method for simultaneous determination of ESC and CLZ in pharmaceutical formulation is efficient and sensitive. The excipients of the commercial sample analyzed did not interfere in the analysis, which proved the specificity of the method for these formulations. The HPLC method was found to be simple, rapid, precise, accurate, and sensitive. Its advantages over other existing methods are its low-cost and less time consuming. This method can be used for routine quality control in commercial samples.

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References
2. S.V. Gandhi, N.D. Dhavale, V.Y. Jadhav, S.S. Sabnis, spectrophotometric and reversed-phase high-performance liquid chromatographic methods for simultaneous determination of


