

DETERMINATION OF ESCITALOPRAM OXALATE IN PHARMACEUTICAL FORMULATION BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

M. S. Charde

Government College of Pharmacy, Amravati

Corresponding author*: manojudps@rediffmail.com

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ABSTRACT:

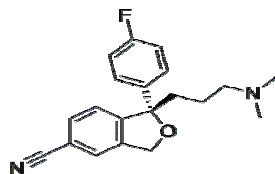
A simple, precise and sensitive reverse-phase high performance liquid chromatographic (RP-HPLC) method has been developed for the quantitation of Escitalopram oxalate in pharmaceutical formulations. Chromatographic separation was achieved on a 250×4.6 mm, 5μ , C_{18} column. The flow rate was 1ml/min and eluent was monitored by absorbance at 240 nm using a mixture of Acetonitrile and Buffer (pH 4.0) in the ratio of 25:75 (v/v). The retention times of Escitalopram oxalate was found to be 3.2 min. Calibration plots were linear in the concentration range of 2.5 - $80\mu\text{g mL}^{-1}$ for Escitalopram oxalate. 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 determination of Escitalopram oxalate in pharmaceutical tablet formulations.

Keyword: HPLC, Acetonitrile, isocratic, Escitalopram oxalate

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]. Literature survey reveals several spectroscopic [2-4], HPLC [5,6] and HPTLC [7-15] methods for estimation of Escitalopram oxalate individually as well as combination with other drugs. All the reported HPLC methods used buffer in the mobile phase and long retention time. The present study was aimed to develop a simple, rapid, precise, accurate, and selective chromatographic method for estimation of Escitalopram oxalate in bulk and dosage forms with the use of buffer in the mobile phase in short duration.

Figure 1: Structure of Escitalopram



2. EXPERIMENTAL

a. Chemicals: Bulk sample of ESC was obtained from Plethico Pharmaceuticals Ltd., Indore, India. The commercial samples of

tablets containing 100 mg and 200 mg of ESC were purchased from local market. Acetonitrile (HPLC Grade), Water (HPLC 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 a isocratic HPLC of SHIMADZU prominence consisting of LC-20AT liquid pump, manual with $20\mu\text{L}$ 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 (C_{18} $250\text{mm} \times 4.6\text{mm}$ $5\mu\text{m}$), which was maintained at 40°C . The analytical wavelength was set at 240 nm and samples of $20\mu\text{L}$ were injected to HPLC system. The mobile phase was Acetonitrile and Phosphate Buffer in ratio of 90:10 (pH=4) at a flow rate of 1ml/min. The mobile phase was filtered through $0.22\mu\text{m}$ filter and degassed for 10 minutes by sonication.

d. Preparation of standard stock solutions: Accurately weighed 10.0 mg of ESC was transferred to a 10.0 mL volumetric flask, sufficient amount of methanol was added to dissolve it and then volume was made up to

mark (Stock A, 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 B, 100 µg/mL). Aliquots of stock B were further diluted up to 10.0 mL to get concentration of 2.5, 5, 10, 20, 40, 80, and 100 µg/mL.

e. Sample preparation: Twenty tablets were weighed (each tablet contain 200 mg of ESC). The average weight was determined. It was finely powdered and mixed thoroughly. The powder equivalent to 10 mg was taken and dissolved in 10.0 mL mobile phase, it was sonicated for 15 min and solution was filtered through 0.22µ membrane filter (Stock, 1000 µg/mL). 1.0 mL of stock was taken into 10.0 mL volumetric flask and further diluted up to 10.0 mL with methanol to get a concentration 100 µg/mL.

f. Determination of Assay: Five replicates of sample (100 µg) 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 amount of drug present per tablet was calculated by comparing a sample peak with that of standard solution. The % label claim reported is 99.8%±0.174.

g. Recommended procedure for standard graph: In order to established the linearity of analytical method, a series of dilutions were made for ESC ranging from 2.5-80 µg/mL. All the solutions were filtered through 0.22µ 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 to obtained a regression equations.

h. Recovery Study: Accuracy was determined by recovery studies of ESC, 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: The standard curve was prepared in the concentration range of 2.5 to 80 µg/mL for ESC. 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.

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 repeatability studies. Standard solutions were prepared and were injected in triplicate. The response of each injection was measured and the precision was calculated using \pm S.D and % RSD equations. The % RSD values for repeatability precision study should be $\leq 1\%$, 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.

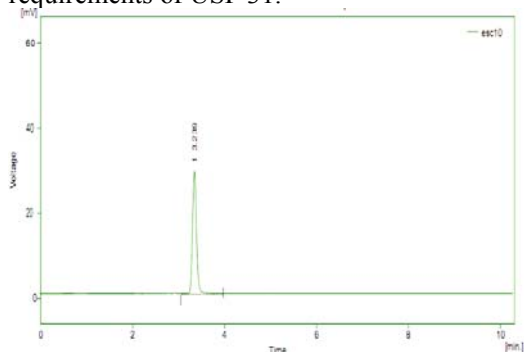
3. RESULTS AND DISCUSSION

To develop a suitable and robust LC method for the determination of ESC 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 (less than 4 min) with the use of buffer mobile phase. The retention time is 3.22 min. The chromatogram is shown in Figure 2.

Figure 2. Chromatogram of Escitalopram

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 of ESC 100 µg/mL. This was repeated five times. The RSD values

of ESC were ± 0.46 . 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 1: Summary of regression analysis and validation parameters

Parameters	Values
Regression analysis	
Slope	3.601
Intercept	0.0000
Correlation coefficient	0.9994
Validation parameters	
LOD	239.0 ng/mL
LOQ	724 ng/mL
Accuracy (%RSD)	0.28
Precision (%RSD)	0.03
Repeatability	
Robustness (%RSD)	0.16
System Suitability test parameters	
Retention time (min) \pm SD	3.208 ± 0.004
Tailing factor \pm %RSD	1.0183 ± 0.007
Theoretical plates \pm SD	2893 ± 63.996

b. Recovery study

Accuracy was determined by recovery studies of ESC, 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 are shown in Table 2.

Table 2. Recovery of ESC standard solution added to sample

Concentration $\mu\text{g/mL}$	Recovery %
80	99.51 ± 0.239
100	100.1 ± 0.239
120	99.9 ± 0.239

c. Specificity: Subjecting the drug solution in different stress conditions such as acid, base, and peroxide, and the degradation was noted. The specificity data is shown in Table 3.

Table 3 Specificity data for ESC

Specificity	RT of ESC	RT of degraded product
Acid stress (0.5N HCl)	3.212	4.439
Base stress (5N NaOH)	3.215	2.195
Peroxide stress (3% H_2O_2)	3.215	3.367

d. Linearity: Linearity was evaluated by analysis of working standard solutions of ESC of six different concentrations. The range of linearity was from 2.5 to 80 $\mu\text{g/mL}$. 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.

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

f. 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 respectively.

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

h. 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 quantitative determination of ESC 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 of ESC in commercial samples.

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