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BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ESCITALOPRAM IN HUMAN PLASMA USING RP-HPLC METHOD

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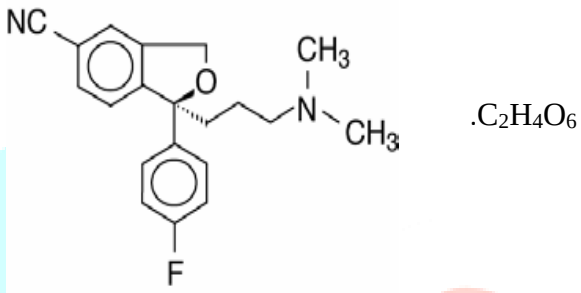
Abstract

A solid phase extraction method was developed for the simultaneous quantification escitalopram in human plasma by reverse –phase high performance liquid chromatography (RP-HPLC).Tinidazole was used as an internal standard. Samples were separated on Enable C_{18} column using 0.2 % ortho phosphoric acid and acetonitrile in the ratio of 65:35 % v/v as mobile phase at a flow rate of 1.0 ml/min at ambient temperature. Detection was carried out at 240nm. Calibration plots were linear (R2>0.99) over the concentration range of 100-800 ng/ ml for escitalopram. All the analytical validation parameters were determined as per ICH guidelines. The bioanalytical method developed was selective, robust and reliable as accuracy, precision, recovery and other validation parameters were within the limits as specified by the guidelines. The overall recovery of escitalopram was 99.75%, respectively. Precision was 5.34% (intra-day) and 6.31% (inter-day). The validated method was successfully applied to a pharmacokinetic study of human plasma. The method can be very useful for the therapeutic drug monitoring (TDM), in bioequivalence studies, and biomedical investigations.

Key words: RP-HPLC, Escitalopram oxalate, Solid phase extraction, Human plasma

INTRODUCTION

Escitalopram (ESC) is the pure S-enantiomer of the racemic bicyclic phthalane derivative citalopram. It is chemically designated S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluorophenyl)- 5 phthalancarbonitrile oxalate. ESC is a drug under the selective serotonin reuptake inhibitor (SSRI). ESC acts as anti depressant drug by blocking the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on $5HT_{1A}$ auto receptors. According to the Literature survey fewer chromatographic methods were reported for individual determination of escitalopram [ ] and in mixtures with their metabolites and other antipsychotic drugs [ ] in human plasma/serum. The drug dosage is individualized by maintaining the plasma or blood drug concentration within the targeted therapeutic window thus improper drug dosing leads to the risk of adverse effects due to the elevated levels of drug in the blood. Hence, monitoring of escitalopram is of great importance for diagnostic and therapeutic purposes. The main objective of the present study is to develop a precise, accurate, rapid and sensitive analytical method for the estimation of escitalopram oxalate in human plasma.[1-6]



$.C_2H_4O_6$

Fig 1: Structure of escitalopram oxalate

1. MATERIALS AND METHODS:

1.1.Chemicals and Solutions

Escitalopram and Tinidazole were obtained as gift sample from Aurobindo Pharmaceuticals, Hyderabad, India, HPLC grade acetonitrile (Hplc grade), water, Ortho phosphoric acid(OPA) (analytical grade), High purity water was prepared with Milli-Q water purification system were used for the analysis All solutions were filtered through 0.45 µm nylon membrane filter using vacuum pump Blank (drug free) human plasma was collected from Hi-tech laboratory, Coimbatore, and refridgerated until use.

1.2.Instrumentation

Quantitative HPLC was performed on Shimadzu LC-20, SPD-M20A Prominence diode array detector and Shimadzu 1700 LC-UV Spectrophotometer. A reverse phase Enable C18 analytical column was used. Weighing was done on Shimadzu electronic balance AY 220.The SPE catridge employed was strata ^{TM}-X (Phenomenex)

1.3.Chromatographic conditions: [7-10]

Preliminary studies were conducted and trails are made for the bioanalytical method development. Separation and analysis was carried out on Enable C_{18} column. The optimized mobile phase consists of 0.2 % OPA in water and acetonitrile in the ratio of 65:35 % v/v. Flow rate was maintained at 1 ml/min and prior to sample injection, column was saturated with mobile phase for 30 min. The detection response was measured at 240 nm and maintained at ambient temperature.

1.4.Preparation of stock solutions of analyte and IS

Ten milligrams of standard escitalopram oxalate was dissolved in the few ml of methanol and made up the volume with methanol. Stock solution of tinidazole (IS) was prepared separately.

1.5.Preparation of working standard solution:

From the stock solution, dilutions were made to get a final concentration of 1 mg/ml. Serial dilutions were made with the working standard to get a final concentration of 100, 200, 400,600, 800 ng/ml solutions. The working standard was prepared for internal standard also.

1.6.Plasma spiking procedure:[11-12]

Hundred micro litre of drug and internal standard were added to 0.5 ml plasma and vortexed for 2 mins. To this 1 ml ACN was added and again vortexed for 2-3 mins. It was then centrifuged at 3000 rpm for 5 mins and the supernatant was collected and refrigerated until analysis.

1.7.Sample preparation by SPE:

Before sample extraction the solid phase extraction cartridge were conditioned with 1.0ml of methanol followed by 1.00ml of purified water and 1.00ml of 2% ortho phosphoric acid buffer solution. Prepared plasma samples were then loaded on to the SPE cartridges and eluted completely under slow vacuum and the cartridges were then washed with 1.00 ml 2% ortho phosphoric acid buffer solution and 1.00 ml of methanol and allowed to dry. The analyte and the internal standard were then eluted with 1.00ml acetonitrile. The eluted samples were then injected into the HPLC system.

1.8.Validation of the method [13-18]

After developing a method its validation is necessary to prove the suitability of the method for the intended purpose. Here the procedure followed for the validation of the developed method is described.

a) Precision*:*

Intraday and interday precision studies were conducted. In intraday precision of plasma sample containing drug at three different concentrations with internal standard were injected and chromatogram was recorded. Similarly interday precision over a two week period time was evaluated.

Acceptance criteria: RSD of the mean concentration of five readings should be less than 15% for bioanalytical method

b) Accuracy:

It is the closeness of mean tests results obtained by the method to true concentration of Analyte. In this study the selected concentration of the plasma were injected six times and mean peak area for each concentration was calculated. Concentration of the each injection was calculated and the standard deviation between the readings is calculated.

c) Recovery studies:

The relative recovery of drug from plasma was calculated by comparing the readings of concentration obtained from the drug spiked plasma to that of equal concentration from standard sample. Recovery studies were carried out six times for sample concentration at three levels within the calibration curve.

Acceptance Criteria: For an assay method, mean recovery should be 85-105% ± 2%.

d) Linearity and Range:

Linearity and range were estimated by using calibration curve. By using standards prepared by spiking plasma and internal standard at different concentrations like 50ng/ml to 250ng/ml the calibration graph was plotted with concentration of spiked plasma on x-axis and peak area on y-axis . The linearity is determined from 50% to 250% of the proposed concentration.

Acceptance Criteria: Coefficient of correlation of the calibration should be not less than 0.99

e) Lower Limit of Quantification (LLOQ):

The LLOQ is determined by using the calibration curve. Limit of quantitation is the concentration of substance in the sample that will give a signal-to-noise ratio of 10:1.Detection limit corresponds to the concentration that will give a signal-to-noise ratio of 3:1. The signal to noise ratio were performed by comparing measured signal of blank plasma sample with those of known low concentration of drug.

f) Specificity:

Specificity of the method was demonstrated by using diode array detector peak purity test. The diode array spectrum of both standard and sample peak were recorded and compared. The other way for doing specificity based in measurement of absorbance ratio of drug peaks at two different levels. The retention time (Rt), resolution factor (Rs) and tailing (T) were noted for the peaks of escitalopram oxalate. Peak purity study is done to prove that a developed method is specific for the drug of interest.

Acceptance criteria: Purity angle should be less than purity threshold i.e.0.99-1.00

g) Selectivity:

Selectivity is the analytical method ability to differentiate and quantify the analyte in the presence of other components in the sample. The selectivity was established by two different methods.

Method I: Chromatograms of six blank plasma samples were compared with chromatogram obtained from standard solutions. Each chromatogram was tested for interferences due to endogenous plasma component on the retention times of the selected drugs.

Method II**:** This method involves the peak purity test method using diode array detector. The PDA spectrum, UV spectrum, absorbance ratio curve and first derivative spectrum of the standard and sample peaks was recorded using PDA detector and compared for the peak purity of drug.

2. RESULTS

2.1.Method development

3.1.1. Chromatographic separation

A number of HPLC chromatographic systems were investigated to optimize the separation of escitalopram oxalate. Retention time for escitalopram oxalate function of stationary phase (PhenomenexC18 column), the mobile phase and the other optimized chromatographic conditions are shown in Table

3.1.2. Calibration curve.

The coefficient of determination (R^2), slope and intercept for escitalopram oxalate were 0.999, 23.427 and 37.732, respectively .The retention time for escitalopram oxalate was 8.323 min. HPLC overlay chromatogram of escitalopram oxalate at 249nm and calibration curve are shown in Fig. .

3.2. Method validation

3.2.1. Linearity, accuracy and precision

The coefficient of determination (R^2) for escitalopram oxalate was 0.995 as shown in Fig. 2.

The accuracy of the method was determined and indicated by the recovery. The results are shown in Table2

Intra-and inter-day precision data of the RP–HPLC method for escitalopram oxalate are presented in Table3..

3.2.2. Ruggedness

Ruggedness of the method was carried out by different analysts in two different days. The results are displayed in Table.

3.2.3. LOD and LOQ

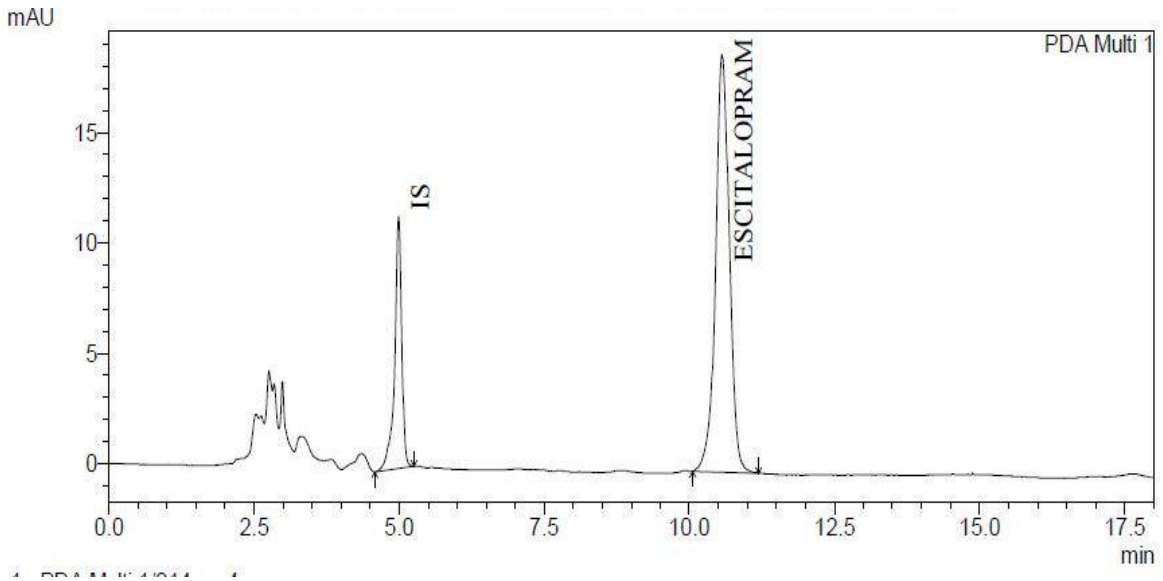
The LOD and LOQ of escitalopram oxalate were determined to be 0.09 mg/mL and 0.23 mg/mL, respectively

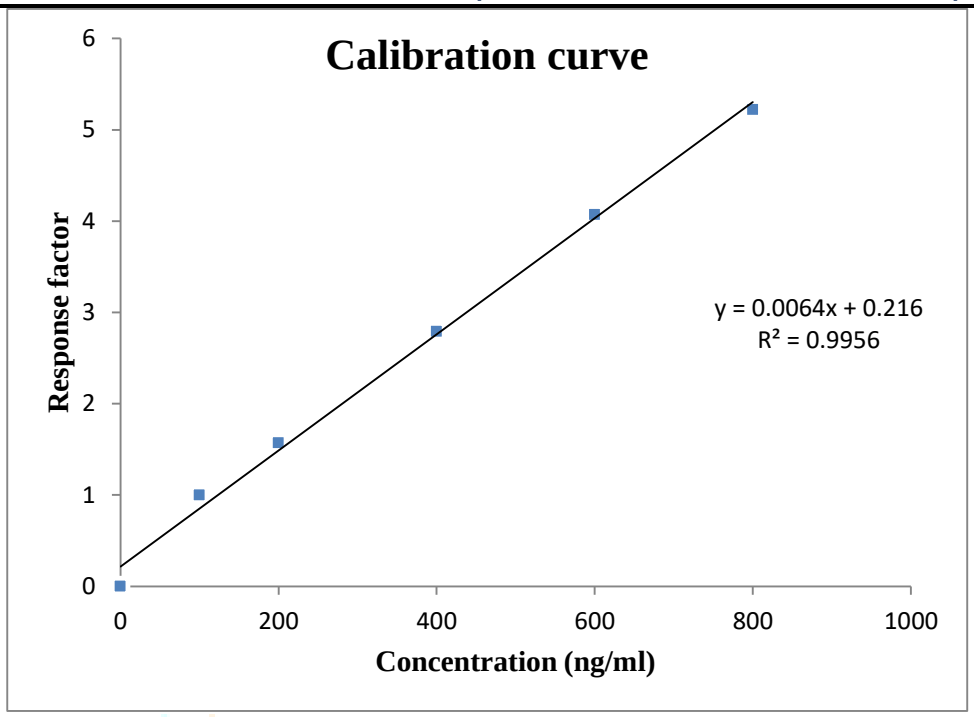
3. DISCUSSION

RP-HPLC method for the estimation of Escitalopram in human plasma was developed and the retention time for the selected internal standard tinidazole and the drug was at 4.8 and 10.5 min respectively. The extraction of drug from plasma was done by Solid Phase Extraction method (SPE), there by showing considerable reduction in interferences by plasma.

The method developed was validated as per ICH and FDA guidelines. The accuracy and the precision values obtained were within limits. Accuracy ranges from 99.2% to 100.3% with the precision 3.57% to 5.34% in intra-day method. In inter-day method the accuracy ranges from 99.5% to 100.08% with the precision 5.78% to 6.31%. The calibration curve plotted was linear at concentration range from (100ng/ml-800ng/ml) of the drug. The regression equation of Escitalopram oxalate shown was y= 0.006x+0.216 and correlation coefficient 0.995 which was within limits. The system suitability studies gave satisfactory results. So, by all these results present method developed produced symmetric peak shape, good resolution and less retention time.

3.RESULTS AND DISCUSSION:





Calibration curve for escitalopram oxalate

Table7:Accuracy and precision studies of escitalopram oxalate(Intraday)

Sl.no	Conc. of drug (ng/ml)	Mean peak Area*	Accuracy (%)	RSD (%)
1.	200	199526	99.2	4.98
2	400	312919	100.3	3.57
3	600	429303	99.9	5.32

*Average of six determinations.

Table 8: Accuracy and precision studies of escitalopram oxalate(Interday)

Sl.no	Conc. of drug (ng/ml)	Mean peak Area*	Accuracy (%)	RSD (%)
1	200	191206	99.6	5.78
2	400	336188	99.2	6.31
3	600	419568	100.08	6.11

*Average of three determinations.

Table11: Ruggedness studies for escitalopram oxalate

Drug	Concentration (ng/ml)	Mean peak area	%RSD
	Day I analyst – I		
Escitalopram oxalate	200	195392	2.76
	Day II analyst – II		
Escitalopram oxalate	200	199527	4.98

**Average of six determinations.

4. CONCLUSION

From the current work it was finally concluded that the developed RP-HPLC method was found to be very simple, reliable, precise, accurate, and sensitive method for the estimation of Escitalopram oxalate in human plasma. The peaks obtained for the drug of interest and internal standard were well resolved from each other without any plasma interferences by using solid phase extraction and the peaks were symmetrical in nature with acceptable tailing factor.

The method is suitable for the routine quantitative analysis in pharmaceutical dosage forms and can be used in therapeutic drug monitoring units, bioequivalence and bioavailability studies, pharmacokinetic and toxicology studies of Escitalopram oxalate.

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