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RESEARCH ARTICLE

Analytical Method Development and Method Validation for Escitalopram Oxalate in Pharmaceutical Dosage Forms by HPLC Method

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ABSTRACT

A simple, specific and accurate high performance liquid chromatographic method was developed for the Escitalopram Oxalate in pharmaceutical dosage form. The column used was Inertsil ODS-2 (250 x 4.6 mm, 5 μ m) with mobile phase containing buffer, Acetonitrile and Methanol (670:280:50 v/v/v). The buffer is prepared by adding 3.4gm of potassium dihyrogen phosphate in a 1000ml of volumetric flask with water. Add 1ml of triethylamine. Adjust the pH to 3.8 with diluted Orthophosphoric acid solution. Filter with nylon 0.45 μ m it. The flow rate was 1.0 ml/ min and effluents were monitored at 238 nm. The retention times of Escitalopram Oxalate were found to be 14 min. The proposed method was validated and successfully applied to the estimation of Escitalopram Oxalate in pharmaceutical dosage forms.

KEYWORDS

Escitalopram Oxalate, Buffer, Acetonitrile, Methanol, Validation

INTRODUCTION

Modified release dosage form is a general term used to describe the dosage forms having drug release features based on time, course and/or location and which are designed to accomplish therapeutic or convenience objectives not offered by conventional or immediate release forms. There are several terms which are used interchangeably with respect to modified release dosage for Escitalopram Oxalate is an orally selective administered serotonin reuptake inhibitor (SSRI). Escitalopram is the pure Senantiomer of the racemic bicyclic phthalane derivative citalopram. It is chemically designated (IUPAC) S-(+)-1-[3-(dimethyl-amino) propyl]-1-(p-fluorophenyl)-5-phthalancarbonitrile oxalate.

*Address for Correspondence: Ms. Vanita M. Lasan Assistant Professor, Department of Quality Assurance, L. M. College of Pharmacy, Ahmedabad-380009, Gujarat. India. E-Mail Id: vanita lasan@yahoo.co.in Its empirical molecular formula is $C_{20}H_{21}FN_2O \cdot C_2H_2O_4$. Chemical structure formula of Escitalopram Oxalate was Figure 1.



Figure 1: Structure of Escitalopram Oxalate

Escitalopram Oxalate is a white to slightlyyellow powder with a molecular weight of 414.40 g/mol. Escitalopram Oxalate is available as tablets or as an oral solution. Lexapro tablets are film-coated, round tablets containing escitalopram oxalate in strengths equivalent to 5 mg, 10 mg and 20 mg escitalopram base. The 10 and 20 mg tablets are scored¹.

Different analytical methods have been reported in the literature for by HPLC method with PDA and ESI-MS and UV as detector for estimation of Escitalopram Oxalate in plasma. Determination of Escitalopram Oxalate in tablet by HPLC, spectrophotometer method Potentiometers using Escitalopram Oxalate -selective membrane electrode was reported. No spectrophotometer first derivative and colorimetric method with gallic acid reagent is reported for the estimation of Escitalopram Oxalate as a bulk drug and in its dosage form²⁻⁹. The present study was to establish a simple, reproducible and reliable HPLC method for estimation of Escitalopram Oxalate in bulk drug and in its formulation. To estimate the amount of drug in formulation by HPLC method. To develop methods and do validation parameter.

MATERIAL AND METHODS

Materials, Reagents and Chemicals

Escitalopram Oxalate tablet was kindly give a Torrent Research Center, Ahmedabad, Gujarat, India. Methanol and Acetonitrile (HPLC grade) all the reagents of AR grade were purchased from Rankem Company, RFCL Ltd., New Delhi. Hydrochloric Potassium acid. dihydrogen orthophosphate Orthophosphoric and acid reagents of GR grade were purchased from Rankem Company, RFCL Ltd., New Delhi and Water (Quntum TM VX) purchased from Milli-Q.A tablet Escitalopram Oxalate (Lexapro) containing 20mg and 15mg were used.

Instrumentation

The High performance liquid Chromatography system was Shimadzu Company consisted of a Model no: LC-2010CHT with UV detector with 20 μ L injection volume. The output signals were monitored and integrated using class-vp software.

Experimental

Chromatographic Conditions

For determination Inertsil ODS-2 (250 x 4.6 mm, 5μ m) column was used. The elution of mobile phase consisted of a mixture of Buffer (Dissolve

3.4 gm KH₂PO ₄ to 1000 ml of volumetric flask with water. Add 1 ml triethylamine. Adjust the pH to 3.8 with diluted orthophosphoric acid solution. Filter it with Nylon 0.45 μ m), Acetonitrile and Methanol (670:280:50 v/v/v).The flow rate was 1.0 ml/min and the column was operated at temperature (37 ± 5 ° C). The volume of sample injected was 20 μ L. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 238nm.

Diluent: Water: Methanol (20:80)

Preparation of Standard Solutions

Weigh and transfer accurately 63.9 mg of Escitalopram Oxalate Working/reference standard in to a 50 ml clean and dried volumetric flask. Add 10 ml of methanol and sonicate to dissolve. Make up the volume to 50 ml with diluent, mix. Diluent 5 ml to 50 ml with diluent mix.

Preparation of Sample Solutions

Weigh and transfer accurately 10 tablets into a 250 ml clean and dried volumetric flask. Add 20 ml of 0.1 N HCL sonicate with intermittent shaking to disperse the tablets. Add about 150 ml Methanol and sonicate for 30 minute with intermittent shaking. Dilute Up to the mark with Methanol and mix. Filter through 0.45μ Nylon filter after discarding first 3 ml and dilute 5 ml of the filtrate to 20 ml with dilute, mix. Determination of Escitalopram Oxalate in tablet dosage form was shown in Table 1.

Preparation of Calibration Curve

Perform linearity at five levels over the range of 50μ g/ml to 200μ g/ml of test concentration. A standard stock solution was prepared and further diluted to attain concentration at about 50.1μ g/ml, 80.2μ g/ml, 100.3μ g/ml, 120.3μ g/ml, 160.4μ g/ml and 200.6μ g/ml of the test concentration. The solution of $(20\mu$ L) was injected into column. Inject all above standard preparations in duplicate. Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of

escitalopram oxalate at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance versus concentration of the drug.

RESULTS

Method Development and Optimization

The proposed validated method was successfully applied to Escitalopram Oxalate in bulk powder and in tablet dosage forms. The results obtained can be classified as being rapid, simple, and sensitive. No interference of the excipients with the peaks of interest appeared, hence the proposed method is applicable for the routine simultaneous estimation of Escitalopram Oxalate in pharmaceutical dosage forms. Media should be selected on the basis of physicochemical properties of drug substance and dosage form. Pre formulation information of mobile phase like Solubility, pH stability profile is needed before selection of mobile. Physicochemical properties of drug substance include solubility and solution state stability. In Mobile phase commonly used Purified water, Tri ethyl amine, Ortho-Phosphoric acid, buffered aqueous solution pH is 3.8 & 3.0, filter the buffer. Prepare the mobile phase and final p^{H} is 4.5 & 3.0. Well separation observed in the chromatogram. is The wavelength of detection selected was 238nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of Escitalopram Oxalate were about 14 minute. The Chromatogram of Escitalopram Oxalate was shown in Figure: 2.

Method Validation

Linearity

Perform linearity at five levels over the range of 50μ g/ml to 200μ g/ml of test concentration. A standard stock solution was prepared and further diluted to attain concentration at about 50.1μ g/ml, 80.2μ g/ml, 100.3μ g/ml, 120.3μ g/ml, 160.4μ g/ml and 200.6μ g/ml of the test concentration. The solution of $(20\mu$ L) was injected into column. Inject all above standard preparations in duplicate. Calculate mean area for each level. Plot a graph of mean area versus concentration. The calibration curve was plotted between absorbance versus concentration μ g/ml of Escitalopram Oxalate was shown in Figure 3 and linearity data was shown in Table 2.



Figure 2: Chromatogram of Escitalopram Oxalate

Name	Retention Time	Area	Theoretical plates	Asymmetry
ESCITALOPRAM	15.842	12078563	10090	1.47

Chromatogram of Escitalopram Oxalate

Drug		Label claim (mg)	Amount found (mg)	% Purity	% RSD
Escitalopram	Standard	15	63.7	99.37	0.2
Oxalate	Sample	15	1381.1	99.40	0.1

Table 1: Determination of Escitalopram Oxalate in Tablet dosage form

Sr No	Escitalopram Oxalate			
51.110	Conc (%)	Area		
1	50.10	5514836		
2	80.20	9352761		
3	100.30	11766941		
4	120.30	13583910		
5	160.40	18151433		
6	200.60	22094906		
Slope	10902.0306			
Intercept	550924.3714			
Correlation co-efficient	0.99747			

Table 2: Linearity data of Escitalopram Oxalate

Table 3: Recovery Studies of Escitalopram Oxalate

Drug	Amount taken (mg)	Amount added (%)	% Recovery	Average % recovery	SD	% RSD
Escitaloprom		50 %	99.70		12387.10	
Ovalate	64.00	100 %	99.60	99.43	20327.91	0.1
Oxalate		200 %	99.00		63377.98	

Table 4: Results of Intraday Precision, Interday Precision, LOD and LOQ of Escitalopram Oxalate

Drug	Intraday Precision %RSD	Interday Precision %RSD	LOD	LOQ
Escitalopram Oxalate	0.13	0.13	1.54	4.67

Table 5: Results robustness testing of the Escitalopram Oxalate

Parameter	%Recovery
Flow rate (0.9 ml/min)	0.12
Flow rate (1.1 ml/min)	0.14
Column temperature (30°C)	0.07



Figure 3: The calibration curve was plotted between absorbance vs % concentration of Escitalopram Oxalate

 Table 6: Summary of the accepted system suitability requirements

Validation	Escitalopram	Acceptance
parameter	Oxalate	Criteria
Retention time	15.842	
Asymmetry (TF)	1.47	
Resolution (Rs)	5.38	
Number of theoretical plates (N)	10090	NLT 3000
Calibration range	50% - 200%	
Slope (m)	109027	Ma-Jac
Intercept (c)	550924	
Standard deviation	50995.8	
Coefficient of variance	1	
Correlation Coefficient(r ²)	0.997	NLT 0.999
Accuracy	99.00 % to 99.70 % 0.23 ,0.19,0.29	98.0 % to 102.0 %. % RSD NMT 2.0%.
Precision (%RSD)	0.13	NMT 2.0
Inter mediate Precision (% RSD)	0.13	NMT 2.0
Flow rate:(0.9ml/min & 1.1ml/min)	0.12 & 0.14	NMT 2.0

Perform accuracy at 3 level 50%, 100% & 200% of the test concentration. Transfer the placebo blend equivalent to 10 Average weights of the escitalopram oxalate 20mg tablets and add to it 50%,100% and 200% of the drug with respect to the test concentration. Prepare the sample in triplicate at each level and inject each preparation in duplicate and average recovery (%), SD and % RSD was calculated. The accuracy results data was shown in Table 3.

Precision

The precision was studied both intra-day and inter-day. Performing assay of six sample preparations under sample conditions as per method. Prepare six replicates of sample at the test concentration by one analyst and inject on the same equipment and on the same day for intra-day. For inter-day repeat the procedure followed for method precision on a different day by different analyst using a different column and HPLC system and using same lot of sample. Results are shown in table: 4 and 5. The RSD of six replicate determinations should not be more than 2.0%. From the data obtained, the developed HPLC method was found to be precise and accurate. The precision results data was shown in Table 4.

Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantitation (LOQ) for the procedure were performed on samples containing very low concentrations of analytes. Based on the Standard Deviation of the Response and the Slope the LOD and LOQ were determined. LOD and LOQ were calculated by use of the equations $LOD = 3.3\sigma/S$ and $LOQ = 10\sigma/S$, where σ is the standard deviation of the blank and S is the slope of the calibration plot. The limit of detection and limit of quantitation results data was shown in Table 4.

Specificity and Selectivity

Inject blank, standard preparation five times, placebo preparation once and sample preparation as such in duplicate. The % assay for such sample and the peak purity index for the main peak in standard preparation, sample preparation and sample preparation.

The difference in the assay of as such sample and sample spiked with known impurities should not be more than 2.0% absolute. Peak purity for the main peak in standard preparation, sample preparation and sample preparation spiked with knows impurity should be equal to or above 0.990. The method was also specific and selective because there was no interference from excipients in the tablets.

Robustness

Established the robustness of the method by making deliberate minor variations in the following method parameters. Change the flow rate 0.9 & 1.1 ml/min, Change column temperature 30 °C and Change the column lot. Check the effect of change in parameter on system suitability and assay. The robustness results data was shown in Table 5.

System suitability

Perform system suitability test as per method before performing any parameter. e.g. % RSD, theoretical plates and tailing factor. Summary of the accepted system suitability requirements in Table 6.

DISCUSSION

The observation and results obtained shall be compared with acceptance criteria and discussed in validation report. % RSD for Escitalopram peak in chromatogram of standard Preparation. Not more than 2.0. There should not be any interference from peaks due to blank, placebo and impurities with the main peak. Peak purity for the main peak in standard preparation, sample preparation and sample. Preparation spiked with knows impurity should be equal to or above 0.990.The correlation coefficient value should not be less than 0.99 over the working range. The recovery for individual and mean value at each level should be between 98 % to 102% with RSD not more than 2.0 %. The system suitability criteria should meet the requirement as per method e.g. % RSD, theoretical plates and tailing factor.

CONCLUSION

Based on observations and results obtained in validation study by applying the suggested procedures, for suitability of method for routine and stability analysis shall be drawn. The observation and results obtained shall be compared with acceptance criteria and discussed in it. It is obvious that they are applicable for the determination of Escitalopram Oxalate should not be any interference and with good sensitivity. The proposed validated method was successfully applied to Escitalopram Oxalate in bulk powder and in tablet dosage forms. The results obtained can be classified as being rapid, simple and sensitive.

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