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Original Research Article

A NOVEL VALIDATED RP-HPLC METHOD FOR THE DETERMINATION OF ESCITALOPRAM OXALATE IN BULK AND PHARMACEUTICAL TABLET DOSAGE FORMS

[a,b]P.RAVISANKAR*, [a]B. KRISHNA SWAPNA, [a]K.V.S. SANTOSH KUMAR, [a]CH.DEVADASU, [a]P.SRINIVASA BABU, [c]G.DEVALA RAO

[a] Department of Pharmaceutical Analysis and Quality Assurance, Vignan Pharmacy College, Vadlamudi, Guntur - 522213, A.P., India.

[b] Faculty of Science, Sri Chandrasekharendra Saraswathi Viswa Maha Vidyalaya (SCSVMV University), Enathur, Kanchipuram – 631561, T.N., India.

[c] Department of Pharmaceutical Analysis, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada - 521108, A.P., India.

Author for Correspondence: banuman35@gmail.com

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ABSTRACT

An accurate, novel, highly sensitive, precise, simple, efficient and reproducible, isocratic Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was developed and validated for the quantitative determination of Escitalopram oxalate in pharmaceutical tablet dosage forms. RP-HPLC method was developed by using Welchrom C_{18} Column (4.6 X 250mm, 5µm), Shimadzu LC-20AT Prominence Liquid Chromatograph. The mobile phase composed of Phosphate buffer (pH-7.48, adjusted with triethylamine): acetonitrile (50:50 v/v). The flow rate was set to 1.0 mL/min with the responses measured at 240 nm using Shimadzu SPD-20A Prominence UV-Vis detector. The retention time of Escitalopram oxalate was found to be 5.43 min. Linearity was established for Escitalopram oxalate in the range of 2-10 µg/mL with correlation coefficient 0.999. The percentage recovery was found to be 99.14% to 101.3%. Validation parameters such as specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ) were evaluated for the method according to the International Conference on Harmonization (ICH) Q_2 R_1 guidelines. The developed method was successfully applied for the quantitative analysis of commercially available dosage form.

Key words: Escitalopram oxalate, Isocratic RP-HPLC, UV-Vis detector, Method Validation.

INTRODUCTION

Escitalopram oxalate is chemically S-(+)-1-[3-(dimethyl-amino)propyl]-1-(pfluorophenyl)-5- phthalancarbonitrile oxalate (Fig.1) is a class of antidepressants[1] known as selective serotonin reuptake inhibitors (SSRIs). The antidepressant, antiobsessive-compulsive, and antibulimic actions of escitalopram are presumed to be linked to its inhibition of CNS neuronal uptake of serotonin. Escitalopram blocks the reuptake of serotonin at the serotonin reuptake pump of the neuronal membrane, enhancing the actions of serotonin on $5HT_{1A}$ autoreceptors. SSRIs bind with significantly less affinity to histamine, acetylcholine, and norepinephrine receptors than tricyclic antidepressant drugs. As per the literature survey revealed that very few analytical methods for the separation and estimation of Escitalopram oxalate have been reported such as Colorimetric method[2] HPTLC[3-5], HPLC[6-8] , Electrospray ionization tandem mass spectrometric analysis, LC-MS/MS[9], Capillary electrphoresis[10], Isolation and characterization of process related impurities in Escitalopram oxalate active pharmaceutical ingredients have been determined . Very few analytical HPLC methods were reported in literature for the determination of Escitalopram oxalate in bulk and pharmaceutical dosage forms. The reported HPLC methods so far in the literature are considered to be uneconomical, time consuming and have poor symmetry. In fact there is a need for the development of a novel, simple, rapid, efficient RP-HPLC analytical method with reproducibility for determination of Escitalopram oxalate in bulk and pharmaceutical dosage forms. The aim of the work was to develop a novel, simple, rapid, economic, precise, efficient RP-HPLC method for quantitative analysis of Escitalopram oxalate, and to validate the method according with ICH guidelines[11]. This method showed advantage of shorter retention time, runtime, simple mobile phase preparation.

MATERIALS AND METHODS

Chemicals and Reagents

The reference sample of Escitalopram oxalate standard was kindly supplied as gift sample by Hetero Drugs Ltd., Hyderabad, Andhra Pradesh, India. All the chemicals were analytical grade. Potassium dihydrogen orthophosphate and phosphoric acid from Rankem Ltd., Mumbai, India, while acetonitrile (HPLC grade) and triethylamine (HPLC grade) from Merck Pharmaceuticals Private Ltd., Mumbai, India. Ortho phosphoric acid used was of HPLC grade and purchased from Merck Specialties Private Ltd., Mumbai, India. Commercial tablets of Escitalopram oxalate

formulation was procured from local market. Lexapro 5mg tablets are manufactured by Cipla Ltd., Mumbai, India.

Instruments

Quantitative HPLC was performed on a isocratic high performance liquid chromatograph (Shimadzu LC-20AT Prominence Liquid Chromatograph) with a LC-20AT VP pump, manual injector with loop volume of 20 µL (Rheodyne), programmable variable wavelength Shimadzu SPD-20A Prominence UV-Vis detector and Welchrom C_{18} Column (4.6 X 250mm, 5µm particle size). The HPLC system was equipped with "Spinchrome" software. In addition an electronic balance (Shimadzu TX223L), digital pH meter (Systronics model 802), a sonicator (spectra lab, model UCB 40), UV-Visible Spectrophotometer (Systronics model-2203) were used in this study.

Chromatographic conditions

Escitalopram oxalate separation was performed on C_{18} (250mmX4.6mm, 5µm) column. The mixture of Phosphate buffer (pH adjusted to 7.48 using triethylamine) and Acetonitrile in ratio of 50:50 v/v was selected as mobile phase and UV detection wavelength was 240nm with a flow rate of 1mL/min. Injection volume was 20µL, with ambient temperature, run time was 10 min. and retention time was 5.43 min.

Preparation of mobile phase

Phosphate buffer was prepared by dissolving 1.488 g of potassium dihydrogen orthophosphate and 0.288 g dipotassium hydrogen phosphate in 500 mL of HPLC grade water. pH was adjusted to 7.48 with triethylamine. The above prepared buffer and acetonitrile were mixed in the proportion of 50:50 v/v and was filtered through 0.45 µm nylon membrane filter and degassed by sonication.

Preparation of Standard solution

About 10 mg of pure Escitalopram Oxalate was accurately weighed and dissolved in 10 mL of mobile phase at to get 1 mg/mL stock solution. Working standard solution of Escitalopram Oxalate was prepared with mobile phase. The final volume was made with the mobile phase. The standard solution was filtered through 0.45 µm nylon membrane filter and degassed by sonicator.

Selection of detection wavelength

The UV spectra of various diluted solutions of Escitalopram Oxalate in mobile phase were recorded using UV spectrophotometer. The peak of maximum absorbance was observed at 240 nm. This wavelength was used for detection of Escitalopram Oxalate.

VALIDATION OF THE DEVELOPED METHOD:

The developed method of analysis was validated as per the ICH for the parameters like system suitability, specificity, linearity, precision, accuracy, robustness and system suitability, limit of detection (LOD) and limit of quantitation (LOQ).

System suitability

System suitability tests are an integral part of chromatographic method which was used to verify reproducibility of the

chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 10 µg/mL for Escitalopram Oxalate to check the reproducibility of the system.At first the HPLC system was stabilized for 40 min. One blank followed by six replicates of a single calibration standard solution of Escitalopram Oxalate was injected to check the system suitability.

Specificity

The effect of wide range of excipients and other additives usually present in the formulations of Escitalopram Oxalate in the determinations under optimum conditions was investigated. The specificity of the RP-HPLC method was established by injecting excipients such as lactose anhydrous, microcrystalline cellulose and magnesium stearate have been added to the placebo solution and injected and tested.

Linearity

The linearity graphs for the proposed assay methods were obtained over the concentration range of 2-10 µg/mL of Escitalopram Oxalate. Method of least square analysis was carried out for getting the slope, intercept and correlation coefficient.

Precision

Intraday and Interday precision study of Escitalopram Oxalate was carried out by estimating corresponding responses 3 times on the same day and on 3 different days for the concentration of 10µg/mL. Every sample was injected in triplicate.

Accuracy (Recovery studies)

The accuracy of the method was determined by calculating recovery of Escitalopram Oxalate by the method of addition. Known amount of Escitalopram Oxalate at 80%, 100% and 120% was added to a pre quantified sample solution. The recovery studies were carried out in the tablet in triplicate each in the presence of placebo.

Robustness

The Robustness was evaluated by the analysis of Escitalopram Oxalate under different experimental conditions such as making small changes in flow rate (± 0.2 mL/min), detection wavelength (±5nm) and Mobile phase composition (±5%).

Limit of Detection and Limit of Quantitation

Limit of Detection is the lowest concentration in a sample that can be detected, but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. Limit of Detection and Limit of Quantitation were calculated using following formula LOD = 3.3(SD)/S and LOQ= 10 (SD)/S, where SD = standard deviation of response (peak area) and S = slope of the calibration curve.

Assay

The content of twenty tablets were accurately weighed and transferred into a

mortar and ground to a fine powder. From this, tablet powder which is equivalent to 10 mg of Escitalopram Oxalate was taken and the drug was extracted in 10 mL of mobile phase. The resulting solution was filtered using Whatman Grade No. 1 filter paper and degassed by sonication. This solution was further suitably diluted for chromatography. The test solutions were injected into the system by filling a 20 µL fixed volume loop manual injector. The chromatographic run time of 10 min. was maintained for the elution of the drug from the column. The elutes were monitored with UV detector at 240 nm. A 20 µL volume of standard and sample solutions were separately injected on HPLC system. From the peak area of Escitalopram Oxalate the amount of drug in the sample were computed. The content was calculated as an average of six determinations.

RESULTS

Table.1.Optimized Chromatographic Conditions and System Suitability Parameters

Parameter	Chromatographic conditions
Instrument	SHIMADZU LC-20AT prominence liquid chromatograph
Column	THERMO ODS C_{18} Column, (4.6 X 250mm, 5µm)
Detector	SHIMADZU SPD-20A prominence UV-Vis detector
Diluents	BUFFER (pH-7.48) : ACETONITRILE (50:50v/v)
Mobile phase	BUFFER (pH-7.48) : ACETONITRILE (50:50v/v)
Flow rate	1mL/min
Detection wave length	By UV at 240 nm.
Run time	10 minutes
Column back pressure	134 (kg/cm^2)
Temperature	Ambient temperature (25°C)
Volume of injection loop	20 (µL)
Retention time	5.43 minutes
Theoretical plates [th.pl] (Efficiency)	10526
Theoretical plates per meter [t.p/m]	210313
Peak asymmetry	1.05

Table.2.Specificity Study

Name of the solution	Retention time (R_t) min.
Mobile phase	No peaks
Placebo	No peaks
Escitalopram oxalate (10 µg/ml)	5.43 min.

Table.3.Linear Regression Data

Parameter	Method
Detection wavelength(λ_{max})	UV at 235nm
Linearity range (µg/ml)	2-10µg/ml
Regression equation (Y = a + bX)	Y= 6.7286 + 103.3X
Slope(b)	103.3
Intercept(a)	6.7286
Standard error of slope (S_b)	1.271132
Standard error of intercept (S_a)	7.697088
Standard error of estimation (S_e)	10.63565
Regression coefficient (R^2)	0.9994
% Relative standard deviation* i.e., Coefficient of variation(CV)	1.191619
Percentage range of errors* (Confidence limits) 0.005significance level 0.001 significance level	 2.02357 3.74269

***Average of 6 determinations; acceptance criteria <2.0**

Table.4.Calibration Data

S.No	Linearity level (µg/mL)	Peak area	Slope	Y-intercept	Correlation Coefficient(r^2)
1	2 µg/mL	213.101	**103.8**	**5.979**	**0.999**
2	4µg/mL	426.205			
3	6µg/mL	630.309			
4	8µg/mL	850.407			
5	10µg/mL	1030.12			

Table.5.Results of Precision Study (Intraday)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD (acceptance criteria < 2.0)
Escitalopram oxalate	10	1	1019.213	0.954
		2	1022.317	
		3	1023.211	
		4	1010.397	
		5	1017.235	
		6	1039.427	

Table.6.Results of Precision Study(Interday)

Sample	Concentration (µg/mL)	Injection no.	Peak area (mV.s)	%RSD(acceptance criteria < 2.0)
Escitalopram oxalate	10	1	1039.144	1.455
		2	1052.167	
		3	1031.216	
		4	1011.129	
		5	1023.225	
		6	1044.756	

Table.7.Recovery Data

S. No	Concentration level	Amount added (µg/mL)	Amount found (µg/mL)	Mean % Recovery ± SD*	% RSD #
	80%	8	8.186743088	101.364±0.734	0.718
1		8	8.131652057		
		8	8.131652057		
	100%	10	9.955146903	99.544±0.6194	0.622
2		10	10.01605626		
		10	9.892166458		
	120%	12	12.03910799	100.3181±0.334	0.333
3		12	11.99762836		
		12	12.07779405		

***SD is standard deviation**
 # % RSD is percentage of relative standardation

Table.8.Robustness Data

S. no	Parameter	Optimized	Used	Peak area	Retention time (R_t), min	Plate count	Peak asymmetry
1.	Flow rate (±0.2mL/min)	1.0 mL/min	0.8mL/min	1033.451	5.83	10876	1.066
			1.0mL/min	1030.12	5.43	10526	1.05
			1.2mL/min	1021.98	5.39	10268	1.058
2.	Detection wavelength (±5nm)	240 nm	235nm	1039.12	5.39	10792	1.146
			240nm	1030.12	5.43	10526	1.05
			245nm	1036.566	5.37	10206	1.128
3.	Mobile phase composition (±5%)	50:50v/v	55:45v/v	1027.99	5.46	10564	1.102
			50:50v/v	1030.12	5.43	10526	1.05
			45:55v/v	1029.23	5.69	10518	1.107

Table.9.Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection(LOD)	0.2447 µg/mL
Limit of Quantitation(LOQ)	0.741 µg/mL

Table.10.Assay Results

S. No	Formulations	Labeled amount	Amount found	% Assay ±SD*
1	LEXAPRO TABLETS	5mg	4.99mg	99.81±1.195

*** Average of 6 determination**

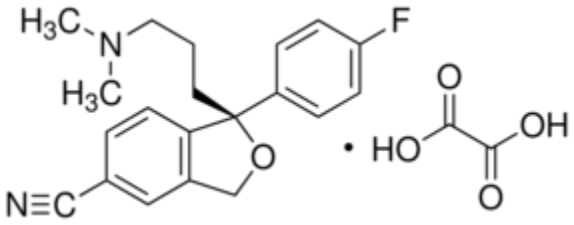

Fig.1. Structure of Escitalopram oxalate

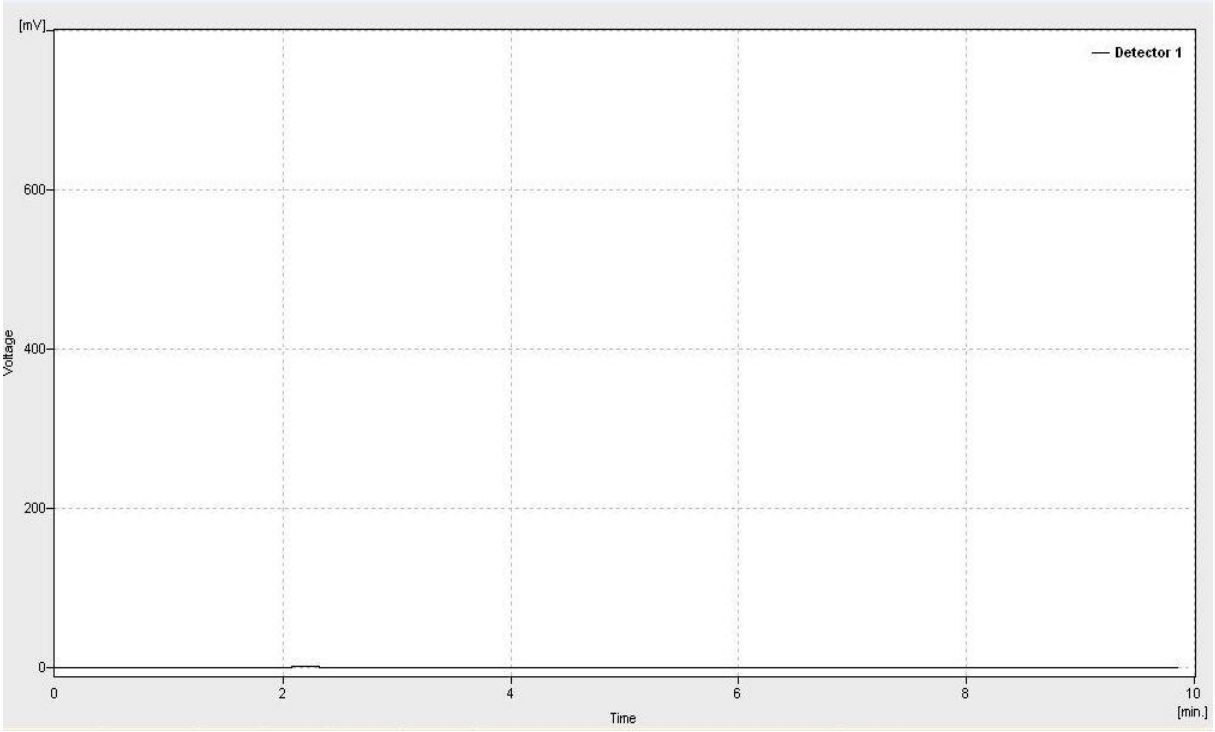

Fig.2.Chromatogram of placebo

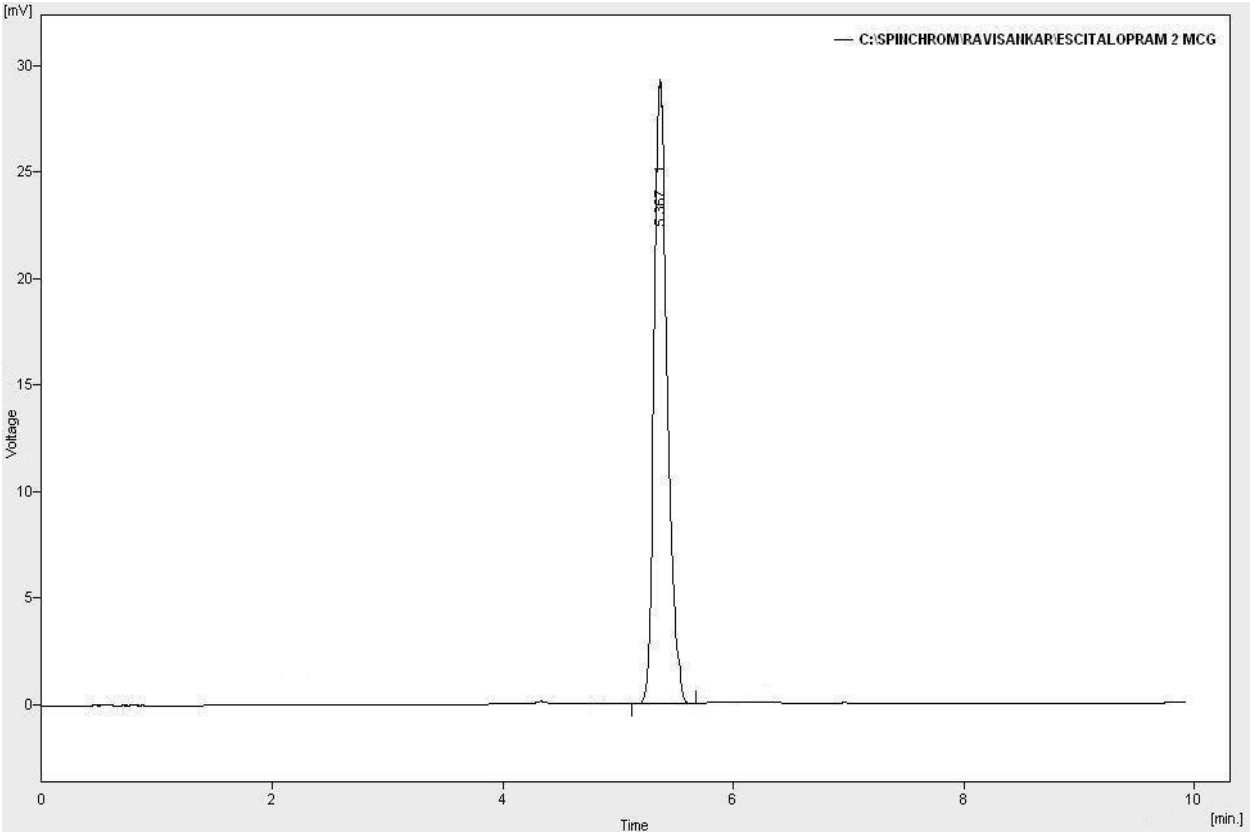

Fig.3. Standard chromatogram of Escitalopram oxalate (2 µg/mL)

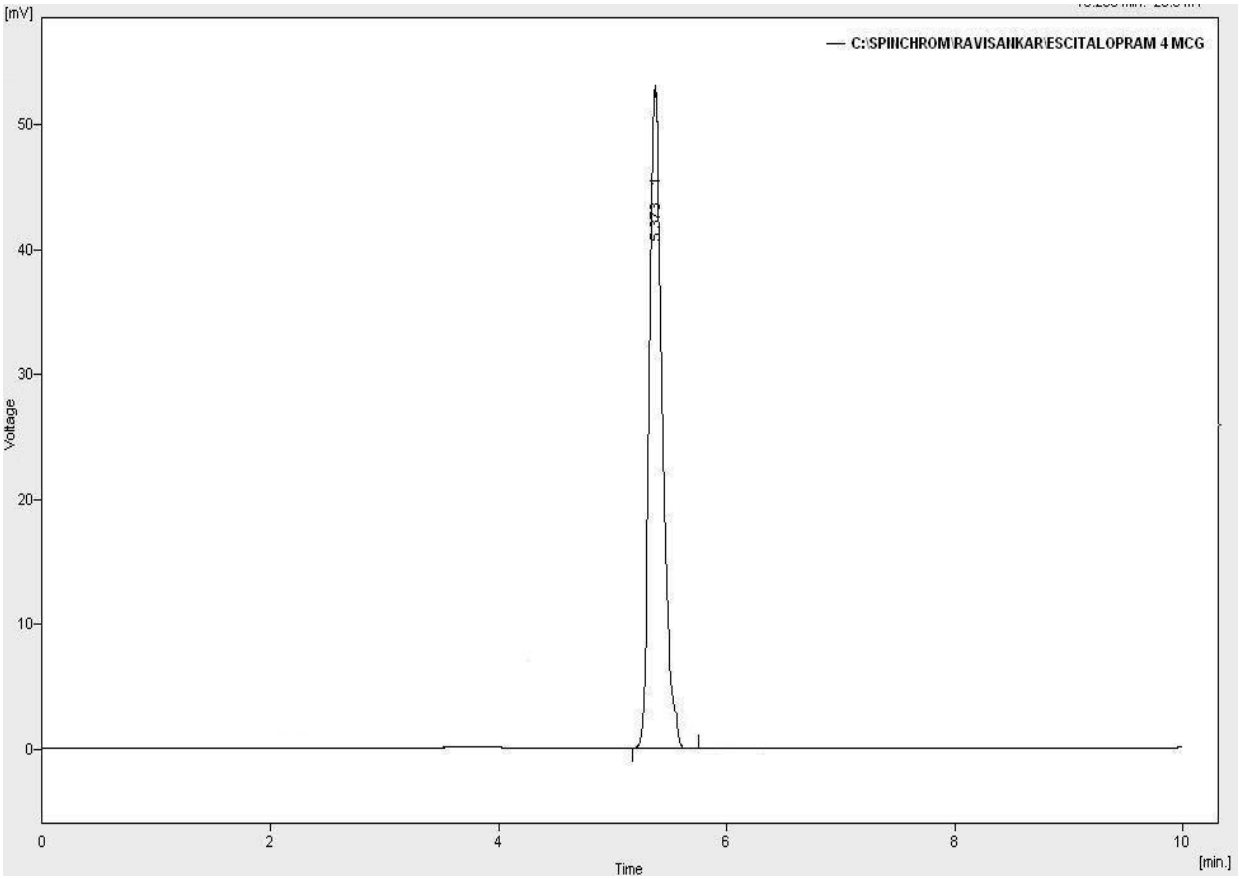

Fig.4. Standard chromatogram of Escitalopram oxalate (4 µg/mL)

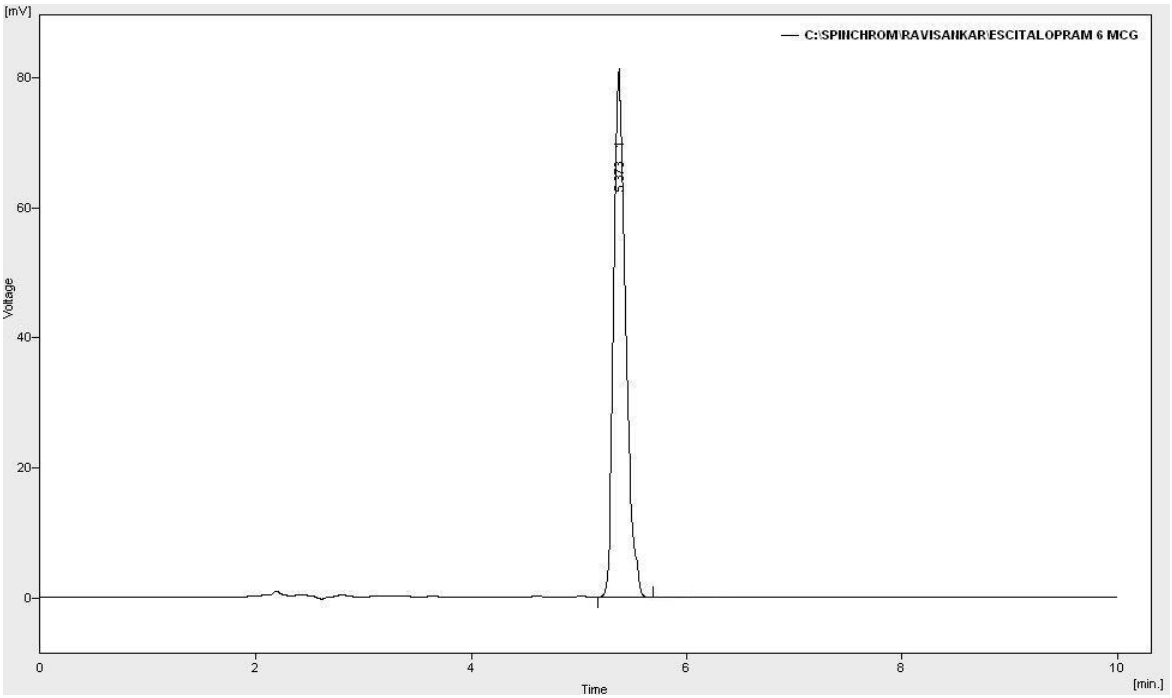

Fig.5. Standard chromatogram of Escitalopram oxalate (6 µg/mL)

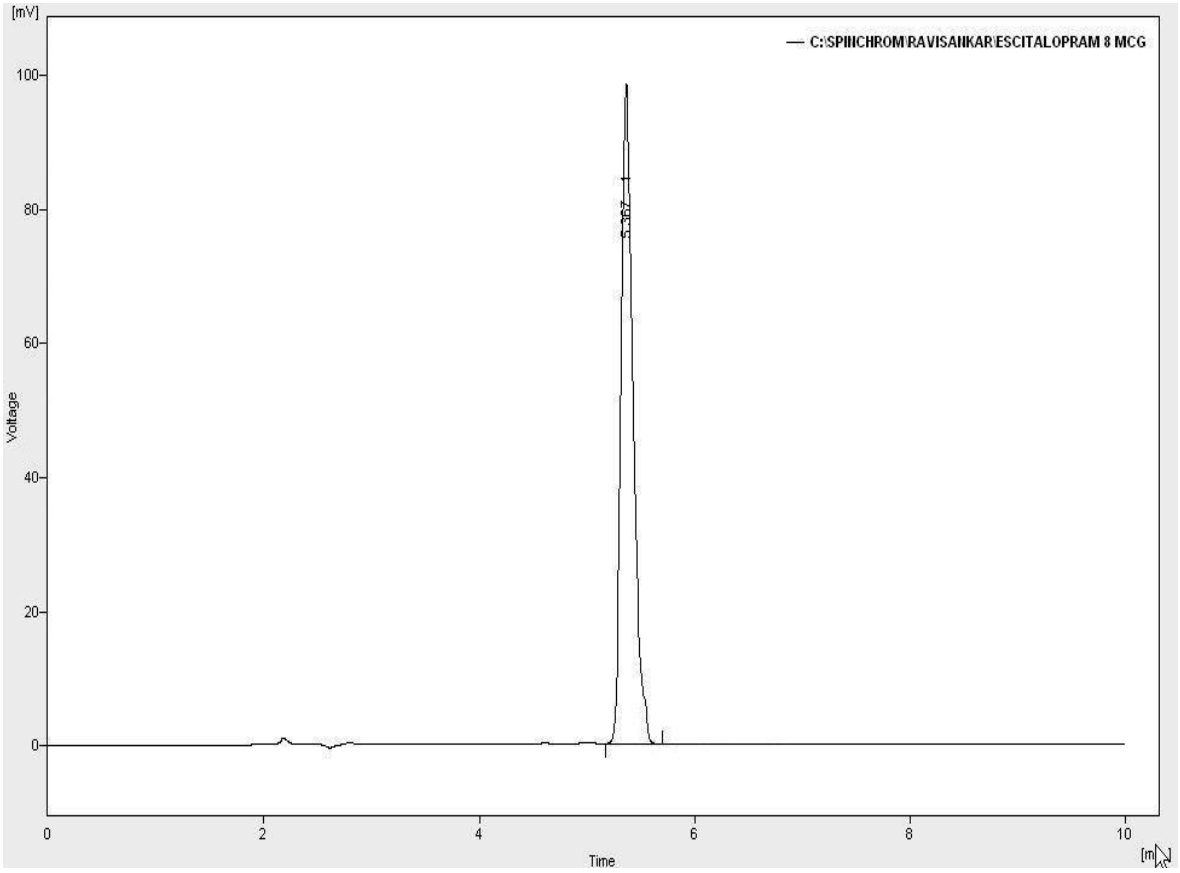

Fig.6. Standard chromatogram of Escitalopram oxalate (8 µg/mL)

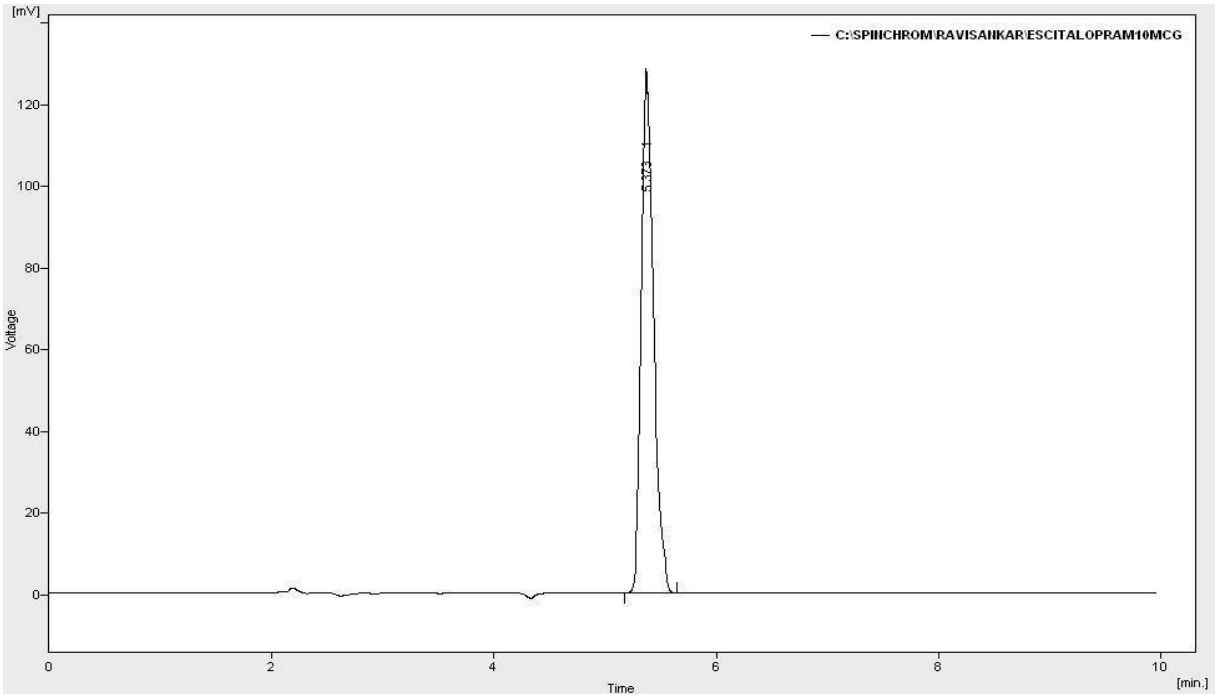

Fig.7. Standard chromatogram of Escitalopram oxalate (10µg/mL)

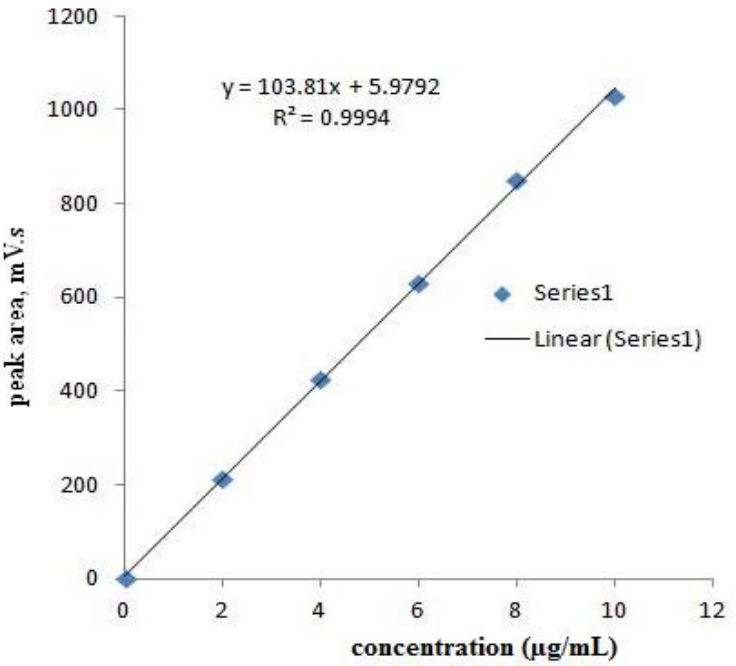

Fig.8.Calibration plot of Escitaloprm oxalate

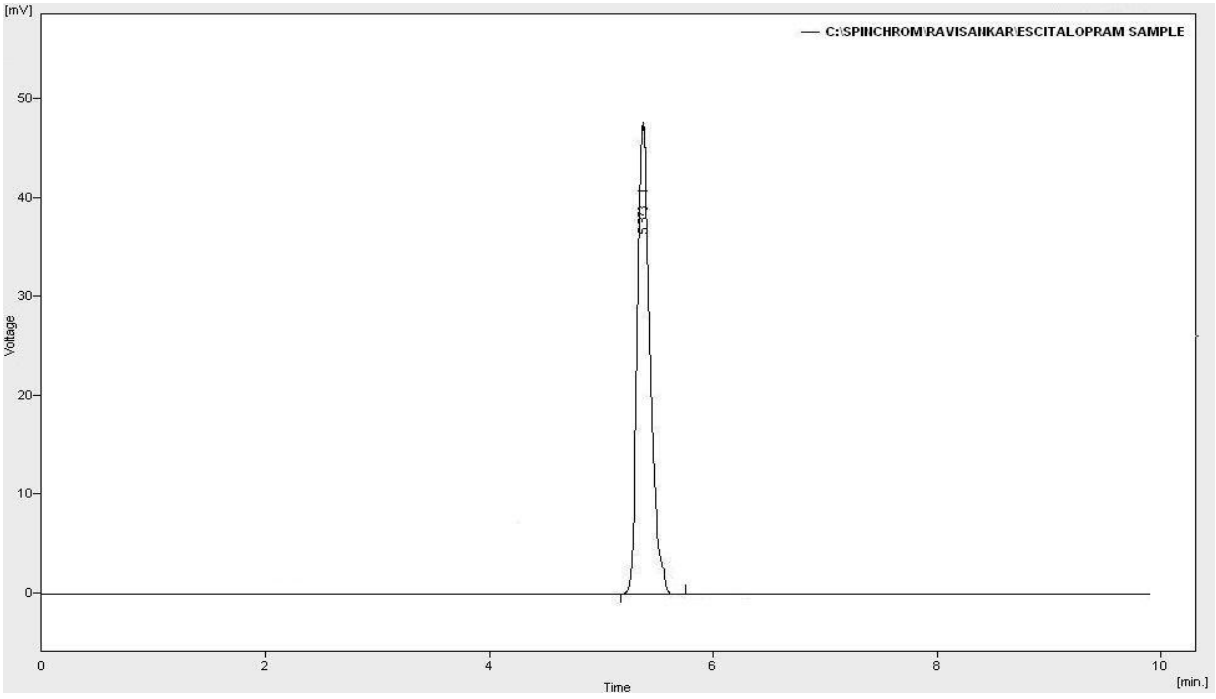

Fig.9. Chromatogram of market formulation (LEXAPRO tablets)

DISCUSSION

The mobile phase consisting of phosphate buffer (pH-7.48): acetonitrile (50:50 v/v) at 1 ml/min flow rate was optimized which gave sharp peak, minimum tailing factor with short run time for Escitalopram oxalate. The retention time for Escitalopram oxalate was 5.43 min. UV spectra of Escitalopram oxalate showed that the drug absorbed maximum at 240 nm, so this wavelength was selected as the detection wavelength. System suitability parameters and optimized chromatographic conditions are shown in Table 1. All the system suitability parameters were evaluated with the back ground of regulatory requirements which also suggests good chromatographic condition. The specificity was studied for the examination of the presence of interfering components, while the comparison of chromatograms there was no interference from placebo (Fig 2) with sample peak. They do not disturb the elution or quantification of Escitalopram oxalate furthermore the well-shaped peaks also indicate the specificity of the method. Therefore, it was concluded that the method is specific. The specificity results are summarized in Table 2. The calibration curve for Escitalopram oxalate was found to be linear over the range of 2-10 µg/mL. The data of regression analysis of the calibration curve is shown in Table 3 and Table 4. The regression equation was found to be Y=103.8x +5.979 with correlation coefficient is r^2=0.999 which indicates this method has good linearity. The representative chromatograms indicating the Escitalopram oxalate are shown in Fig. 3 to 7. The linearity of the graph is shown in Fig. 8. Precision was studied to find out intra and inter day variations in the test methods of Escitalopram oxalate for the three times on the same day and different day. The %RSD for intra-day and inter-day precision obtained was 0.954and 1.455 respectively. The values of % RSD (< 2.0) indicate that the proposed method is quite precise and reproducible and results are shown in Tables 5 and 6. Recovery studies of the drug were carried out for the accuracy parameter at three different concentrations levels i.e., multiple level recovery studies. A known amount of Escitalopram oxalate standard was added into pre-analyzed sample and subjected them to the proposed HPLC method. The % recovery was found to be within the limits as listed in Table 7. Generally the mean percentage recovery of Escitalopram oxalate at each level was not less than 98% and not more than 102%. In this case percentage recovery of Escitalopram oxalate was found to be in the range of 99.54 % to 101.3%. The method precision was done and the low % RSD (1.191) values indicates that the proposed method which was in good agreement with precision. Robustness was done by small changes in the chromatographic conditions like mobile phase flow rate, temperature, mobile phase composition etc., It was observed that there were no marked changes in the chromatograms. Infact the

parameters are within the limit which indicates that the method has robustness and suitable for routine use. The Robustness results are presented in Table 8. The limit of detection (LOD) and limit of quantitation (LOQ) was calculated based on the standard deviation (SD) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ. The limit of detection (LOD) was 0.2447 µg/mL and the limit of quantitation (LOQ) was 0.741 µg/mL which shows that this method is very sensitive. The results are presented in Table 9. The developed method was applied to the assay of Escitalopram oxalate tablets. The experimental results are given in Table 10. The results were very close to labeled value of commercial tablets. The sample chromatogram of Escitalopram oxalate are shown in Fig. 9

CONCLUSION

A New validated RP-HPLC method has been developed for the quantitative determination of Escitalopram oxalate in bulk and pharmaceutical tablet dosage forms. Statistical analysis of the results shows that the proposed procedure has good precision and accuracy. The method was completely validated shows satisfactory results for all the method validation parameters tested and method was free from interference of the other active ingredients and additives used in the formulation. As a matter of fact, results of the study indicates that the developed method was found to be simple, reliable, accurate, linear, sensitive, economical and reproducible and have short run time which makes the method rapid. Hence it can be concluded that this method may be used by the industries and employed for the routine quality control analysis of Escitalopram oxalate in active pharmaceutical ingredient (API) and pharmaceutical preparations.

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