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Original Article

DEVELOPMENT AND EVALUATION OF NOVEL ESTIMATION TECHNIQUES FOR *IN VITRO* DISSOLUTION STUDY AND VALIDATION PROTOCOL FOR ESCITALOPRAM AS ANTIDEPRESSANT DRUG AND THEIR FORMULATION

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ABSTRACT

Objective: To develop and validate the RP-HPLC method and *in vitro* dissolution study for escitalopram as antidepressant drug and their formulation.

Methods: The chromatographic separation was done by using a C-18, 150 mm column and a mobile phase consisting of phosphate buffer (40%) and acetonitrile HPLC grade (60%). Detection was carried out at 211 nm with a flow rate of 1 ml/min with an injection of 20 µl. The method was validated with different parameters such as linearity, precision, accuracy, robustness, and limit of detection (LOD), the limit of quantification (LOQ) according to ICH guidelines.

Results: The linear calibration curve was obtained in the concentration range of 0-50 µg/ml and gave an average correlation factor 0.992. The retention time was observed at 2.96 min. The Minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.03 and 0.09 µg/ml, respectively. The relative standard deviation of intra and the inter-day assay was found to be less than 2. The dissolution studies show moderate dissolution (23.4%) after 45 min, but it reaches a plateau after approximately 25 min.

Conclusion: This method was found to be simple, rapid and economic with less run time. The validated parameters manifest the method is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters. Therefore, the developed method can be applied for both routine analysis and quality control assay and it could be a very powerful tool to investigate the stability of escitalopram.

Keywords: Escitalopram, RP-HPLC, Dissolution studies, Anti-depressant activity

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INTRODUCTION

Escitalopram is chemically (1S)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile. The typical chemical structure is shown in fig. 1. The drug having the molecular formula: $C_{20}H_{21}FN_2O•C_2H_2O_4$. Escitalopram is a selective serotonin reuptake inhibitor (SSRI). The S-enantiomer of racemic citalopram [1]. In the treatment of depression and anxiety, it is used to restore serotonergic functions. Escitalopram is approximately 150 times more potent than citalopram's R-enantiomer. Amongst SSRIs, escitalopram exerts the highest degree of selectivity for the serotonin transporter (SERT) relative to others [2]. Escitalopram also differentiates itself from other SSRIs via allosteric action on its target; this may be the mechanism responsible for its observed superior efficacy and faster onset compared to other SSRIs.

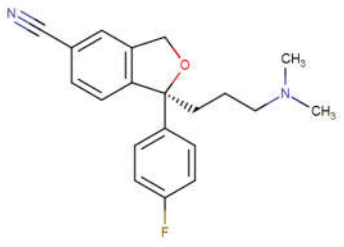

Fig. 1: Structure of escitalopram

Literature survey reveals spectrophotometric, RP-HPLC [3-13], and HPTLC [14] methods for these compounds either individually or in combination with other dosage forms. The literature review does not show any stability indicating RP-HPLC method for quantification of escitalopram. Hence, it was felt that there was a need for a new analytical method by RP-HPLC. The present research work was aimed to develop a single, simple, fast, rapid suitable stability-indicating RP-HPLC method for the determination of escitalopram. The developed method was validated concerning specificity, the limit of detection (LOD), the limit of quantitation (LOQ), linearity, precision, accuracy and robustness. The method is in the accordance with ICH guidelines [15-17].

MATERIALS AND METHODS

Chemicals and reagents

Samples of escitalopram pure drugs were received from, Cipla Limited (Mumbai, India). HPLC-grade acetonitrile was purchased from Merck (Mumbai, India). Ortho-phosphoric acid was purchased from Qualigens Fine Chemicals (Mumbai, India). HPLC grade water was prepared by using a Millipore Milli-Q plus purification system.

HPLC instrumentation and conditions

The HPLC system employed was Hitachi L2130 with D elite 2000 Software with isocratic with UV-visible detector (L-2400).

Standard and sample preparation for UV-spectrophotometer analysis

The standard and sample stock solutions were prepared separately by dissolving standard and sample in a solvent in the mobile phase diluting with the same solvent. After the optimization of all conditions for UV analysis, it scanned in the UV spectrum in the range of 200 to 400 nm. This has been performed to know the maxima of escitalopram so that the same wavenumber can be utilized in HPLC UV detector for estimating the escitalopram. While scanning the escitalopram solution, we observed the maxima at 211 nm. The UV spectrum has been recorded on ELICO SL-159 make UV-Visible spectrophotometer model UV-2450.

Standard Solution preparation

25 mg of escitalopram standard was transferred into 25 ml volumetric flask, dissolved and makeup to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10 ml volumetric flask and makeup to volume with mobile phase.

Sample solution preparation

20 tablets of the marketed drug were weighed and the average weight was calculated. The sample equivalent to 25 mg of escitalopram was accurately weighed and transferred into a 25 ml volumetric flask. About 20 ml of diluent was added and sonicated to dissolve drug completely and the volume was made up to the mark with diluent, which gave the stock solution of 1000 ppm. The solution was mixed well and filtered through a 0.45 µm filter. 1 ml of the above stock solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent to prepare 100 ppm solution. Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and diluted up to the mark with diluent, which gave 10 ppm escitalopram working standard solution. It was mixed well and filtered through a 0.45 µm filter.

Preparation of phosphate buffer

About 6.8 gm of potassium dihydrogen orthophosphate was weighed and transferred into a 1000 ml beaker, dissolved and diluted to 1000 ml with HPLC water. The pH was adjusted to 3.0 with orthophosphoric acid.

Preparation of the mobile phase

400 ml (40%) of the above buffer and 600 ml of acetonitrile HPLC (60%) were mixed well and degassed in an ultrasonic water bath for 15 min. The solution was filtered through a 0.45 µm filter under vacuum filtration [18].

Diluent preparation: Mobile phase as diluent.

RESULTS

Initialization of the instrument

The HPLC instrument was switched on. The column was washed with HPLC water for 45 min. The column was then saturated with the mobile phase for 45 min. The mobile phase was run to find the peaks. After 20 min the standard drug solution was injected in HPLC.

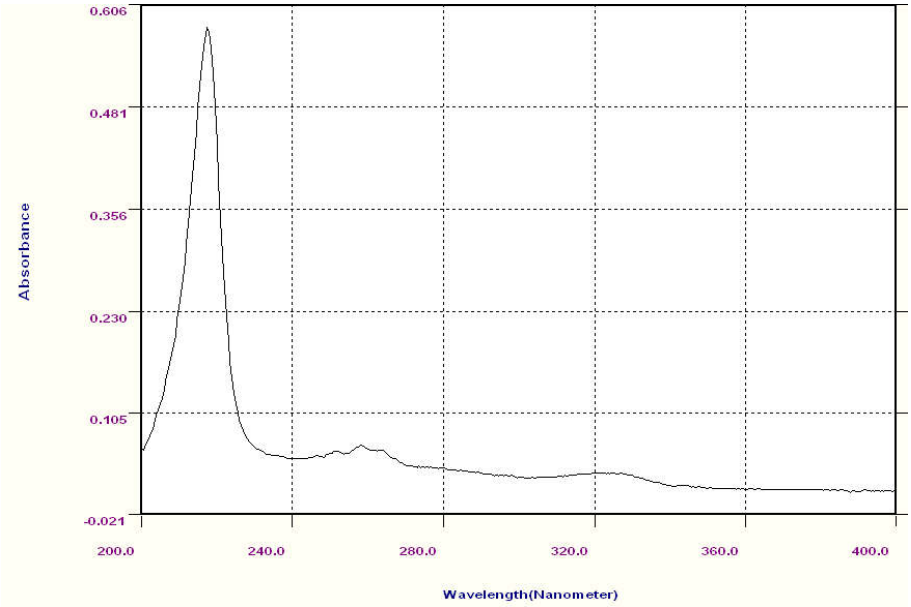

Fig. 2: Chromatogram escitalopram

Table 1: Optimized chromatographic conditions

Column:	C18 Develosil ODS HG-5 RP 150 mm x4.6 mm 5 µm particle size
Mobile Phase:	Buffer: Acetonitrile (60;40) (pH 2.9)
Flow Rate:	1.0 ml/min
Wavelength:	211 nm
Injection volume:	20 µl
Run time:	10 min
Column temperature:	Ambient

Table 2: Different trials for chromatographic conditions

Column used	Mobile phase	Flow rate	Wavelength	Observation	Result
Waters C18, 5µm, 25 cmx4.6 mm i.d.	Methanol: Water = 80: 20	0.5 ml/min	211 nm	Low response	Method rejected
Waters C18, 5µm, 25 cmx4.6 mm i.d.	ACN only	0.5 ml/min	211 nm	Very low response	Method rejected
Waters C18, 5µm, 25 cmx4.6 mm i.d.	ACN: water = 50: 50	1.0 ml/min	211 nm	Tailing peak	Method rejected
Waters C18, 5µm, 25 cmx4.6 mm i.d.	ACN: acetate buffer = 50:50	1.0 ml/min	211 nm	Broad Peak	Method rejected
Waters C18, 5µm, 25 cmx4.6 mm i.d.	ACN: phosphate buffer = 60:40 (pH 2.9)	1.0 ml/min	211 nm	Good response	Method accepted

Method validation

Accuracy and recovery study

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of escitalopram were taken and added to the pre-analyzed formulation of concentration 10 µg/ml. From that percentage, recovery values were calculated. The results were shown in table 3.

Precision

As per USP, it is the degree of agreement among individual test results obtained upon repeated application of analytical methods to multiple samplings of a homogenous sample or measure of the extent to which the data values are close to each other for many measurements (under similar conditions).

Preparation of working standard of 10 ppm of escitalopram

25 mg of escitalopram working standard was accurately weighed and transferred into a 25 ml volumetric flask, and about 20 ml of diluent was added to it and sonicated to dissolve drug completely and volume was made up to the mark with the same solvent which gave a Stock solution of 1000 ppm. 1 ml of the above stock solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents to prepare 100 ppm solution. Further 1 ml of prepared 100 ppm solution was pipetted into a 10 ml volumetric flask and was diluted up to the mark with diluents, which gave 10 ppm escitalopram working standard solution. The solution was mixed well and filtered through a 0.45 µm filter.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2%.

Table 3: Accuracy study of escitalopram

Sample ID	Concentration (µg/ml)		% recovery of pure drug	Statistical analysis
	Pure drug	Formulation		
S_1: 80 %	8	10	99.13	Mean*= 98.94667%
S_2: 80 %	8	10	98.79	SD = 0.171561
S_3: 80 %	8	10	98.92	% R. SD= 0.1733
S_4: 100 %	10	10	99.72	Mean*= 99.76%
S_5: 100 %	10	10	99.81	SD = 0.045826
S_6: 100 %	10	10	99.75	% R. SD= 0.0459
S_7: 120 %	12	10	99.36	Mean*= 99.37667%
S_8: 120 %	12	10	99.28	SD = 0.105987
S_9: 120 %	12	10	99.49	% R. SD = 0.1066

*mean assay values of 3 replicates (n=3).

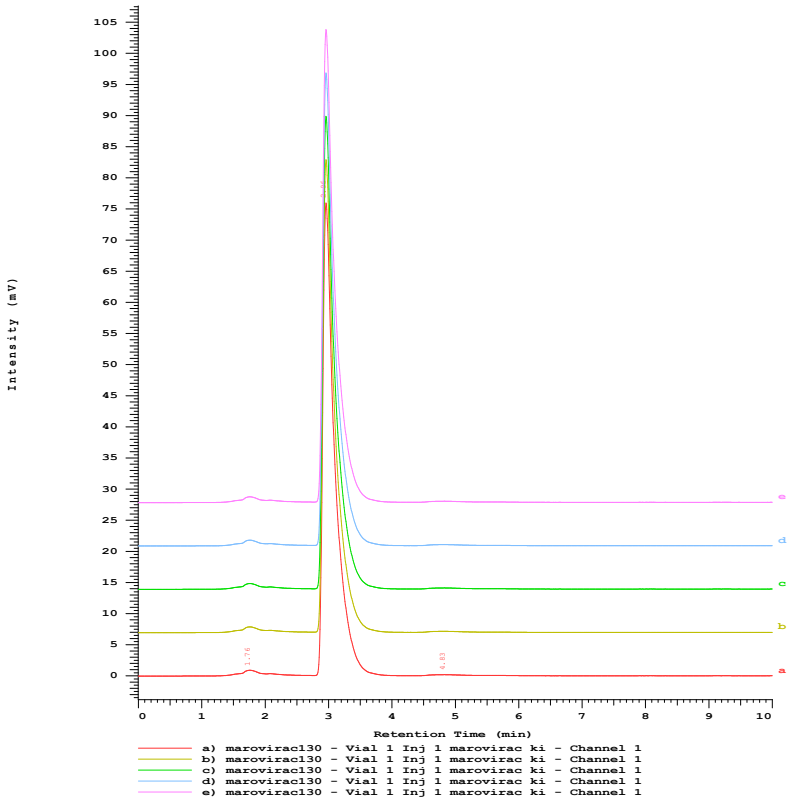

Fig. 3: Repeatability for escitalopram

Table 4: Precision results

HPLC injection replicates of escitalopram	Retention time	Area
Replicate–1	2.96	1025457
Replicate–2	2.94	1003224
Replicate–3	2.97	995798
Replicate–4	2.96	992259
Replicate–5	2.97	998740
Average	2.958	1003096
Standard Deviation*	0.013038	13131.13
% RSD	0.440784	1.309061

*Standard deviation (n=5) calculated in five replicates.

Table 5: Results of intra-assay and inter-assay

Conc. of escitalopram (API) (µg/ml)	Observed Conc. of escitalopram (µg/ml) by the proposed method			
	Intra-day assay		Inter-day assay	
	Mean* (n=6)	% RSD	Mean* (n=6)	% RSD
10	10.01	0.86	10.03	0.87
30	30.02	0.30	30.03	0.32
100	99.97	0.13	99.95	0.11

*Mean (n=6)

Intra-assay and inter-assay

The intra and inter-day variation of the method was carried out and the high values of mean assay and low values of standard deviation and % RSD (% RSD<2%) within a day and day to day variations for escitalopram revealed that the proposed method is precise.

Linearity and range

Linearity indicates the ability of analytical procedures to produce results that are directly proportional to the concentration of analyte in the given sample.

Preparation of stock solution (100 ppm)

25 mg of escitalopram was dissolved in 25 ml of the mobile phase, which gave a solution of the strength of 1000 ppm. 1 ml of this solution was pipetted into a 10 ml volumetric flask and the volume was made up to mark with diluents (mobile phase), which finally gave the stock solution of the strength of 100 ppm. The stock solution was degassed in an ultrasonic water bath for 5 min and filtered through a 0.45 µm filter under vacuum filtration.

Procedure

Each level was injected into the chromatographic system and the peak area was measured. A graph of peak area versus concentration (on X-axis concentration and Y-axis peak area) was plotted and the correlation coefficient was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.999.

Calibration curve

Linearity plot information

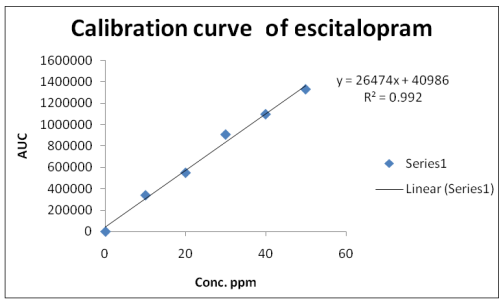

Fig. 4: Calibration curve of escitalopram (API)

The calibration curve showed good linearity in the range of 0-50 µg/ml, for escitalopram (API) with a correlation coefficient (r^2) of 0.992 (fig. 4). A typical calibration curve has the regression equation of y = 26474x+40986 for escitalopram.

Table 6: Linearity results

Conc.	AUC (n=6)
0	0
10	339085
20	548749
30	905740
40	1095457
50	1327962

Method robustness

Influence of small changes in chromatographic conditions such as a change in flow rate (± 0.1 ml/min), temperature (±2 °C), the wavelength of detection (±2 nm) and acetonitrile content in the mobile phase (±2%) studied to determine the robustness of the method are also in favour of (table 7, % RSD<2%) the developed RP-HPLC method for the analysis of escitalopram (API).

LOD and LOQ

The minimum concentration level at which the analyte can be reliably detected (LOD) and quantified (LOQ) were found to be 0.03 and 0.09 µg/ml, respectively.

Assay of escitalopram in dosage form

Twenty tablets containing drug escitalopram having brand name Cipralex-10 mg were taken and the I. P. method was followed to determine the average weight. Above weighed tablets were finely powdered and triturated well. A quantity of powder equivalent to 100 mg of drugs was transferred to 100 ml volumetric flask, and 70 ml of diluents was added and the solution was sonicated for 15 min, thereafter volume was made up to 100 ml with the same solvent. Then 10 ml of the above solution was diluted to 100 ml with diluents. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (1 ml) was transferred to five different 10 ml volumetric flasks and volume were made up to 10 ml with the same solvent system. The solution

prepared was injected in five replicates into the HPLC system and the observations were recorded. A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in table 8.

Table 7: Result of the method robustness test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.06
Flow (0.9 ml/min)	0.04
Temperature (27 °C)	0.08
Temperature (23 °C)	0.11
Wavelength of detection (213 nm)	0.03
Wavelength of detection (209 nm)	0.02

*% RSD<2%

Table 8: Assay of escitalopram tablets

Brand name of the tablet	Labelled amount of drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (±SD) assay (n = 6)
Cipralex-10 mg	10	09.34 (±0.06)	99.56 (±0.48)

*±SD (n=6) for the assay of tablets containing escitalopram was found to be 99.56 %.

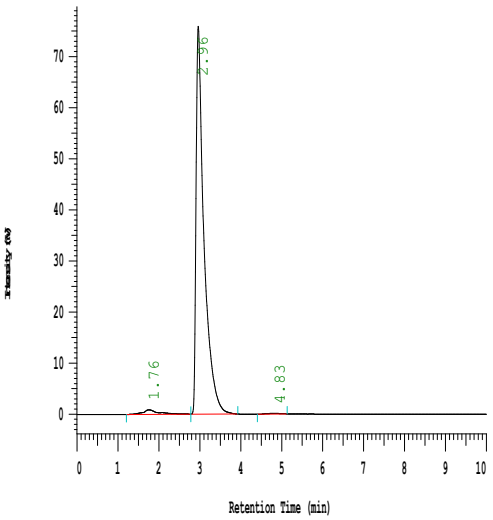

Fig. 5: Chromatogram showing peak of escitalopram in tablet formulation

Dissolution profile

Dissolution was done according to USP using the paddle dissolution apparatus. The dissolution test was performed in three different pH media of 1.2 (0.1N HCl), 4.5 and 6.8 phosphate buffer. The dissolution profiles were performed for the two prepared formulae to select the best

f2= 50 log {[1+(1\n)Sn (R-T)2]-0.5 ×100}. (1)

f1= {[Sn |R-T |]/[Sn R]}x100. (2)

Table 9: Dissolution data of escitalopram

Time (min)	% dissolved formula-1
0	0±.00
5	7±0.55
10	11.6±0.68
15	15.2±0.14
20	17.9.7±0.36
25	19.8±0.27
30	20.8±0.61
35	21.9±0.62
40	22.7±0.49
45	23.4±0.50

*% dissolution studies done with (n=10) with respect to time in min, the result shows a moderate dissolution (23.4%) after 45 min, but it reaches a plateau after approximately 25 min.

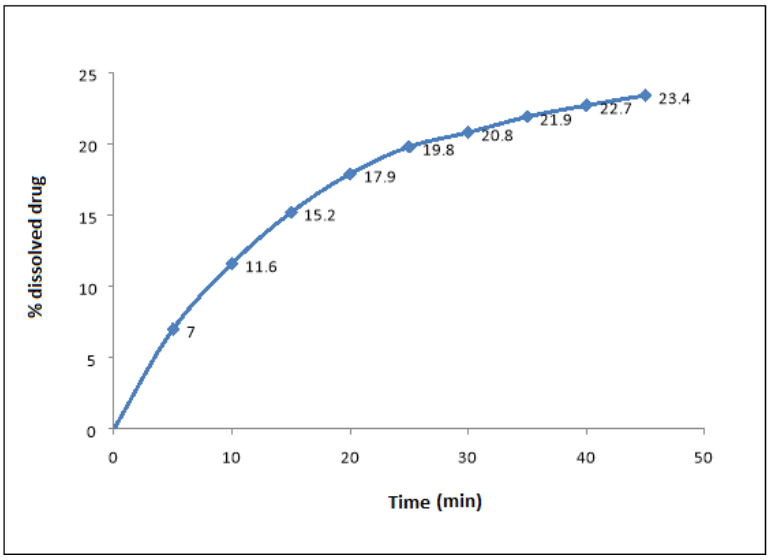

Fig. 6: Dissolution profile w. r. t. time

DISCUSSION

The various RP-HPLC methods have been studied for the analysis of escitalopram in bulk and various dosage forms [3-13]. However, the reported methods are less specific and also time-consuming. Therefore, we tried to develop a simple RP-HPLC method for the determination of escitalopram in bulk and its dosage form and also worked on the dissolution studies.

The sample preparation is a key step for accurate and reliable methods. The phosphate buffer is prepared by using potassium dihydrogen orthophosphate and the orthophosphoric acid is used to adjust the required pH. We have taken almost five trials with different types of mobile phases and out of that we have selected the ACN: phosphate buffer = 60:40 (pH 2.9) mobile phase, as it has given the good response as compare to the other methods, the indicative chromatogram is shown in the fig. 2. The separation was carried out by C-18, 150 mm column using mobile phase made up of phosphate buffer (40%) and acetonitrile HPLC grade (60%), the wavelength used is 211 nm with a flow rate of 1 ml/min and the injection volume 20 µl. The developed method was validated with different parameters such as linearity, precision, accuracy, robustness, the limit of detection (LOD), and limit of quantification (LOQ) according to ICH guidelines [15-17].

In the accuracy study, the SD found to be 0.171561, 0.045826, and 0.105987 the samples are analyzed in three replicates. The recovery study indicates that the average recovery is 99.36%. The precision results also hold the passing criteria, and which is taken in five replicates % RSD for the area of five standard injections results is not more than 2%. The result of intra-assay and inter-assay shown in table no. 5 also confirms that the proposed method is precise. The linearity plot indicates good linearity in the concentration range of 0-50 µg/ml, with a correlation coefficient (r2) of 0.992. A typical calibration curve has the regression equation of y = 26474x+40986 is shown in fig. 4.

The effect of small changes in chromatographic conditions such as the change in flow rate (±0.1 ml/min), temperature (±2 °C), the wavelength of detection (±2 nm) and acetonitrile content in the mobile phase (±2%) also studied to determine the robustness of the method, and are also in favour of developed method. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.03 and 0.09 µg/ml, respectively. As compared to the other developed methods the intra and inter-day assay was given a good result and it is found to be less than 2. The assay of tablets containing escitalopram was found to be 99.56 %. The fig. 5 shows the chromatogram of the tablet dosage form. The dissolution test was performed in three different pH media of 1.2 (0.1N HCl), 4.5 and 6.8 phosphate buffer respectively, and the study shows a moderate dissolution (23.4%) after 45 min, but it reaches a plateau after approximately 25 min. Fig. 6 indicates the graphic representation of the dissolution study with respect to time (min).

CONCLUSION

The RP-HPLC method was developed for the analysis of escitalopram in standard and their pharmaceutical formulation and is found to be simple, rapid and economic with less run time. The method has been validated and it has been shown that it is reliable, linear, accurate and precise as well as robust with minor variations in chromatographic parameters. The *in vitro* dissolution results show a moderate dissolution (23.4%) after 45 min, but it reaches a plateau after approximately 25 min. Therefore, it will be applied for both routine analysis and quality control assay and it could be a very powerful tool to investigate the stability of escitalopram.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally to this study.

CONFLICT OF INTERESTS

Declared none

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