Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

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Analytical Method Development and Validation of RP-HPLC Method for Estimation of Escitalopram in Bulk and Pharmaceutical dosage form

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Cite this paper as: Shital Khedkar, Vijaya Barge, (2025) Analytical Method Development and Validation of RP-HPLC Method for Estimation of Escitalopram in Bulk and Pharmaceutical dosage form. *Journal of Neonatal Surgery*, 14 (32s), 5234-5243.

ABSTRACT

Objective: To develop and validate the RP-HPLC method for the estimation of an antidepressant drug Escitalopram

Methods: A Phenomenex C-18, 250 mm x 4.6 mm, 5 µm, and a mobile phase made of 0.05% OPA in water: methanol (40:60 v/v) were used for the chromatographic separation. The detection was performed at 238 nm using a 20 µl injection and a flow rate of 1 ml/min. According to ICH Q2 (R1) requirements, the method was validated using a variety of factors, including linearity, precision, accuracy, and robustness.

Results: The retention time was observed at 2.70 min. The method was found to be linear with a correlation coefficient (r^2) of 0.9997.

Conclusion: This approach was proven to be quick, easy, cost-effective, and run time-efficient.The validated parameters manifest that the method is reliable, linear, accurate, and precise, as well as robust with minor variations in chromatographic parameters.

Keywords: *Escitalopram, RP-HPLC, Anti-depressant activity, Acetonitrile and Methanol, OPA*

1. INTRODUCTION

Escitalopram is chemically (1S)-1- [3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrile[1].

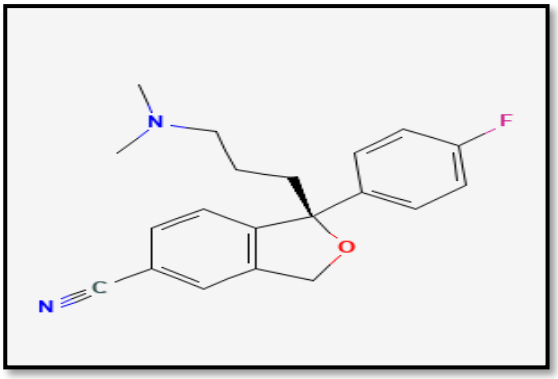

Fig. 1: Structure of Escitalopram

Escitalopram is the (S)-enantiomer of the racemic selective serotonin reuptake inhibitor (SSRI)[2] antidepressant, and it is available in marketed formulations under the brand names Sipralexa, Lexapro, Cipralex, Nexito, and Seroplex. People use escitalopram as an antidepressant3. Additionally, it works well for treating anxiety problems. SSRIs and escitalopram have a reduced toxicity profile compared to previous antidepressants[4,5]. Escitalopram is a white to off-white powder. It is sparingly soluble in water and freely soluble in methanol and dimethyl sulfoxide (DMSO). The elimination half-life of escitalopram is 27-32 hours. Escitalopram has a pKa value of 9.78. It restores serotonergic functioning and is used to treat anxiety and depression. The potency of escitalopram is roughly 150 times that of its R-enantiomer. In comparison to other SSRIs, escitalopram exhibits the highest level of selectivity for the serotonin transporter (SERT)[11].

Mechanism of action[8]:

Escitalopram blocks serotonin's absorption into the presynaptic neuron, increasing serotonergic activity by binding to the orthosteric (or primary) binding site on the serotonin transporter (SERT), which is also where endogenous 5-HT (serotonin) interacts[2,6,8].

In addition to their orthosteric action, escitalopram and paroxetine are believed to be allosteric serotonin reuptake inhibitors because they bind to a secondary allosteric location on the SERT molecule, which increases the inhibition of 5-HT reuptake[8].

In comparison to other SSRIs, this combination of orthosteric and allosteric activation raises extracellular 5-HT levels, which causes a quicker onset of effect and increased efficacy[7].

The sustained elevation of synaptic 5-HT eventually leads to desensitization of 5-HT$_1$A auto receptor, which normally inhibit endogenous 5-HT release in response to elevated serotonin levels[9]. This desensitization is believed to be necessary for the full clinical effect of SSRIs and may explain their typically delayed onset of therapeutic action.

2. MATERIALS AND METHODS

We performed High-performance liquid chromatography (HPLC) using anAgilentinstrument equipped with anAuto sampler, a UV detector, and Openlab EZ Chrome workstationSoftware (1260 Infinity II). A Phenomenex C18 column (5 µm, 250 mm × 4.6 mm) was used for the separation.

Standard drugs were procured from Aarti Pharma, Bhandup West, Mumbai. Tablets containing 20 mg escitalopram (Nexito 20) were procured from the local market.

The reagents used in this work—methanol, water, acetonitrile, and ortho-phosphoric acid—were all of HPLC grade.

- **Chromatographic condition**

After several trials with the different combination and ratio of solvents, the mobile phase consisting of 0.05% OPA in water: methanol (40:60 v/v) was pumped at a flow rate of 1 mL/min. The elution was observed at 238 nm, and the injection volume was 20 µL. The validation of the method was followed in the ICH guidelines.

3. CHARACTERISATION OF DRUG SUBSTANCE.

- **Selection of solvent**

Table 1: Drug Solubility Summary

Sr. No.	Name of Solvent	Observation	Conclusion
1	Water	Drug Particles seen after sonication	Drug was not found soluble in water.
2	Methanol	No Drug Particles seen after sonication	Drug was found soluble in methanol.
3	Water: Methanol (10:90)	No Drug Particles seen after sonication	Drug was found soluble in methanol.

Final Conclusion: Water:Methanol (10:90% v/v) will be used as a diluent for preparing stock solution.

- **Preparation of mobile phase**

✓ **Preparation of 0.05 % Orthophosphoric Acid:** Measure and transfer accurately about 0.5 ml of orthophosphoric acid in 1000 ml of water. Mix well. Filter through a 0.45 µ nylon membrane disc filter and degas.

- ✓ **Preparation of mobile phase:** Prepare a mixture of 0.05 % OPA and methanol in the ratio of 40:60 v/v. Mixed well.
- ✓ **Preparation of Diluent:** Water: Methanol 10:90 % v/v. which is used as blank.

- **Preparation of Standard solution:**

- ✓ **Preparation of Escitalopram Standard stock solution:** Weigh accurately about 63.8 mg of escitalopram oxalate (equivalent to 50 mg of escitalopram) working standard and transfer it into a 100 mL volumetric flask. Add about 70 mL of diluent, sonicate to dissolve, and make up to the mark with diluent.

Further dilute 4 mL each of escitalopram stock solution to 50 mL with diluent (40 ppm escitalopram).

- **Preparation of Sample Solution:**Take the 20 tablets and measure the average weight of the tablets. Weighed 20 tablets and transferred them to a mortar and pestle and crushed them to a fine powder. Mixed the contents uniformly. Weighed and transferred Nexito 20 (Escitalopram 20 mg) tablet powder Equivalent of 100 mg of escitalopram in a 250 mL clean and dry volumetric flask. Add about 200 mL of diluent, sonicate for 60 minutes with intermittent shaking at control room temperature, and make the volume up to the mark with diluent and mix. Filter the sample solution through a 0.45µ membrane PVDF filter. Discard the first 4.0 mL of filtrate and then collect the sample.

Further dilute 5 mL of sample stock solution to a 50 mL volumetric flask, make up with diluent up to the mark, and mix well. (Concentration of Sample Solution: 40 ppm)

4. ANALYTICAL METHOD VALIDATION

A simple, precise and economic RP-HPLC method was developed and validated for estimation of Escitalopramin bulk and tablet. The method was validated as per ICH guidelines by using various validation parameters such as Linearity, accuracy, precision, specificity and robustness[11].

1) **System Suitability Test**: System suitability test is a pharmacopoeias requirement and is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done.The tests were performed by collecting data from Single injection of blank (Diluent) and six replicate injections of Standard solution were injected into the chromatograph.

2) **Specificity: (Identification, Interference & Peak Purity)**

Inject Blank (Diluent), standard solution, placebo solution and sample solution.

3) **Linearity:**

Linearity was evaluated in the range of 50 % to 150 % of Escitalopram for working concentration. The working concentration of Escitalopram in solution is 40 µg/mL

4) **Accuracy (Recovery):**

Evaluated accuracy from 50% to 150% of Escitalopram tablet, working concentration level. Each level prepared in triplicates.

5) **Precision:**

i. **Method Precision:**

Single injection of blank (Diluent), Standard solution (Five replicates) and sample solution (six preparations) was injected on the system.

ii. **Intermediate Precision:**

Six independent sample preparations were prepared on different day and by different analyst and injected on the HPLC.

6)Robustness:

This parameter was studied by making small, deliberate changes in the chromatographic conditions and Assay parameters, observing the effect of these changes on the system suitability and results obtained by injecting the standard and sample solutions.

5. RESULTS AND DISCUSSION

- **Reverse Phase High Performance Liquid Chromatography Method Development and Optimization**

- ➢ **Chromatographic Condition:**

After several trials with the different combination and ratio of solvents and sharp peak

Column	:	Phenomenex C18, 250 mm X 4.6 mm, 5 µm
Flow Rate	:	1.0 mL/min
Injection Volume	:	20 µL
Wavelength	:	238 nm
Column oven Temp	:	40ºC
Auto Sampler Temp	:	25ºC
Run time	:	7 minutes
Retention time	:	About 2.7 minutes for Escitalopram
Seal wash	:	Water: Acetonitrile, 90:10 v/v
Needle wash	:	Water: Acetonitrile, 10:90 v/v

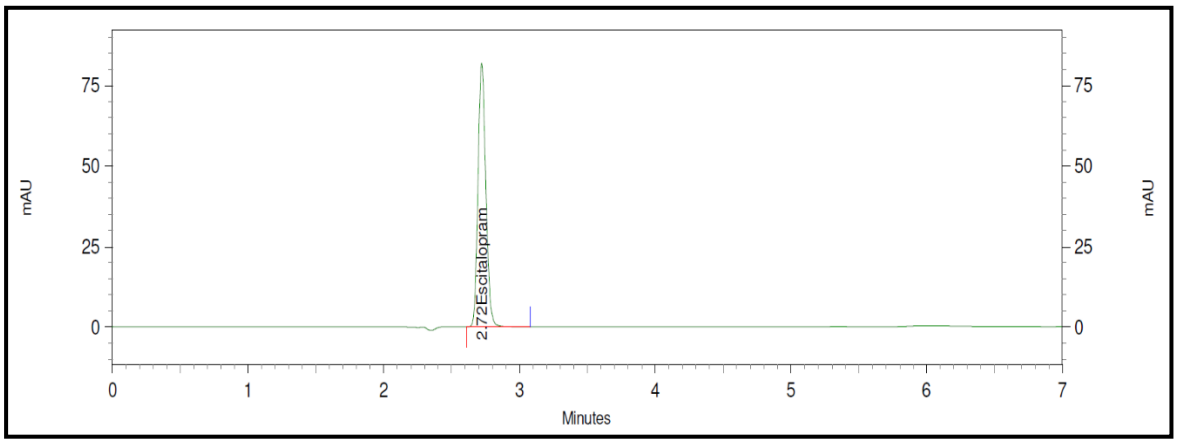

Fig. 2: Typical chromatogram for Escitalopram

Observation: Escitalopram eluted at 2.70 minutes with acceptable chromatography. (Asymmetry: 1.13 and Theoretical plates 12865)

Conclusion: Method can be used for further analysis.

✓ **Analytical Method Validation of RP-HPLC**

1. **System Suitability:**

Table 2: System Suitability Test of Escitalopram

Tailing Factor	**1.12**
Theoretical plates	**12453**
Injection No.	**Area**
1	5345268
2	5339547
3	5340259
4	5351429
5	5345209

Mean	5344342
%RSD	**0.1**

Conclusion:

- The data demonstrates that the system suitability is within the acceptance criteria, thus the system is suitable.

2. Specificity: (Identification, Interference & Peak Purity)

Table 3: Specificity (Identification and Interference)

Solution	**Specificity data**		
	Retention time (min)	**Purity Match**	
Blank solution	NA	NA	
Placebo solution	NA	NA	
Standard solution	2.72	Purity angle	Purity threshold
		3.87	5.87
Sample solution	2.71	3.21	5.36

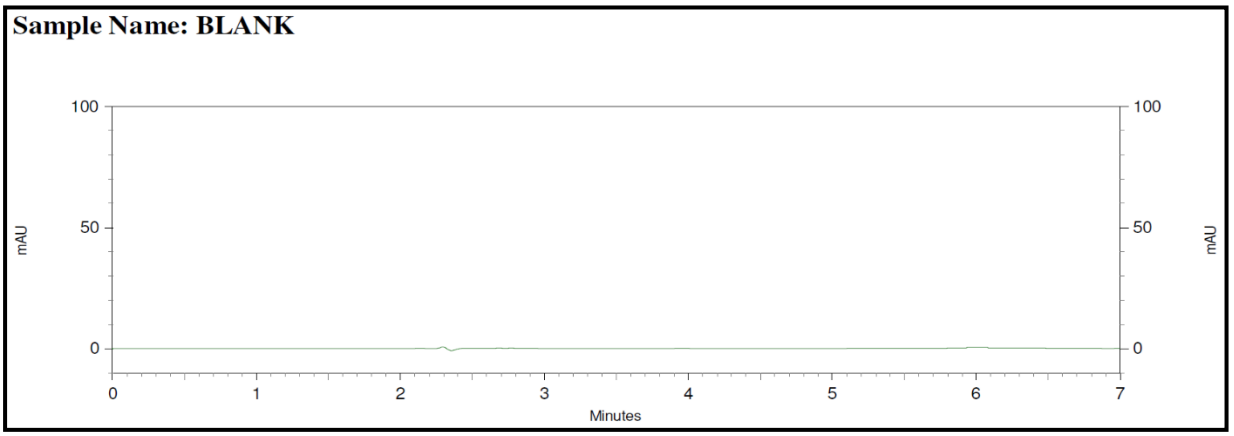

Fig. 3: Chromatogram of Blank

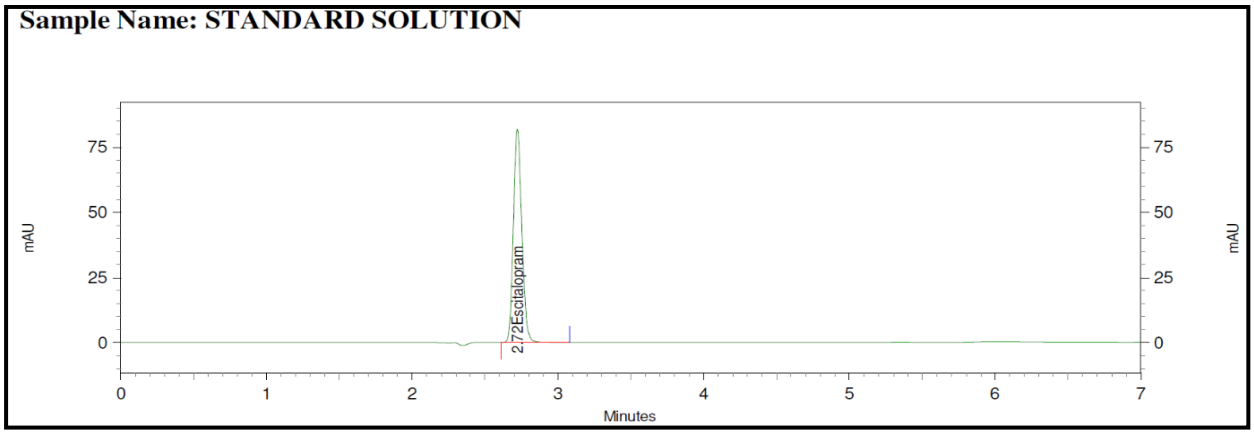

Fig. 4: Chromatogram of Standard

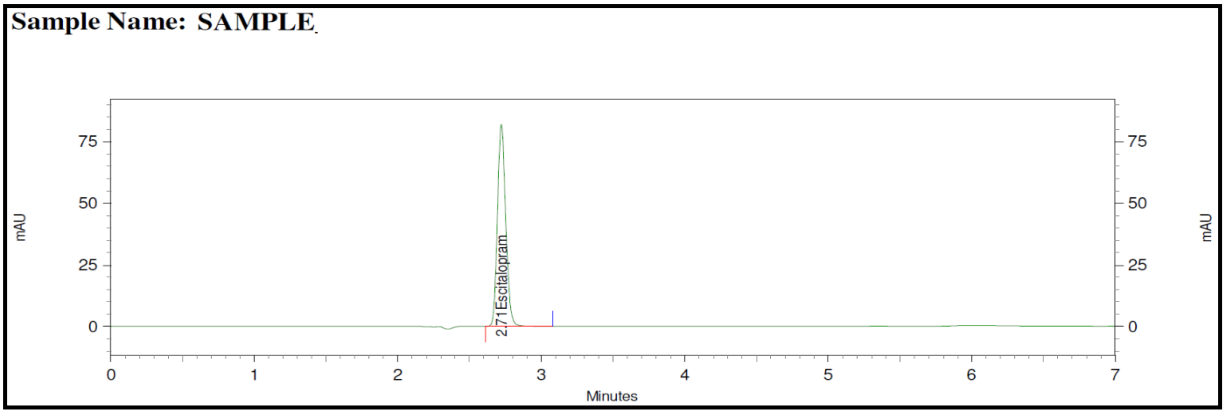

Fig. 5: Chromatogram of Sample

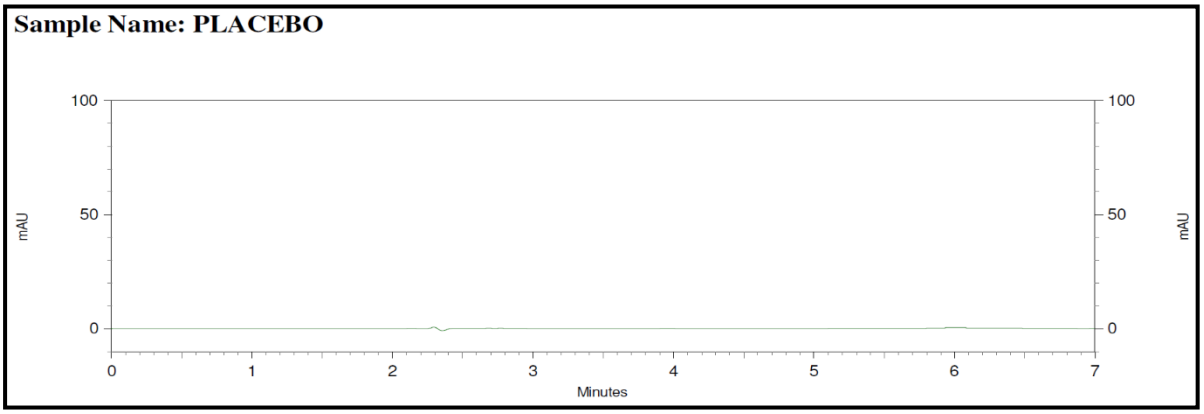

Fig. 6: Chromatogram of Placebo

Conclusion:

- The data demonstrates that retention time in standard and sample is same for Escitalopram peak.
- The data demonstrates that there is no interference in blank and placebo at the retention time of Escitalopram peak. Peak Purity match in both chromatograms obtained from Standard and Sample solution.

3. Linearity:

Table 4: Linearity of Escitalopram

Level	Conc. (µg/mL)	Area	Mean
50%	20	2685419	2688432
		2689551	
		2690327	
75%	30	4059627	4058970
		4061429	
		4055854	
100%	40	5340254	5346727
		5349587	
		5350339	

125%	50	6632854	6635832
		6641893	
		6632749	
150%	60	8065749	8065241
		8061524	
		8068449	
Correlation Coefficient			0.9997
Intercept			26848
Slope			1333305
% Y-intercept			0.50

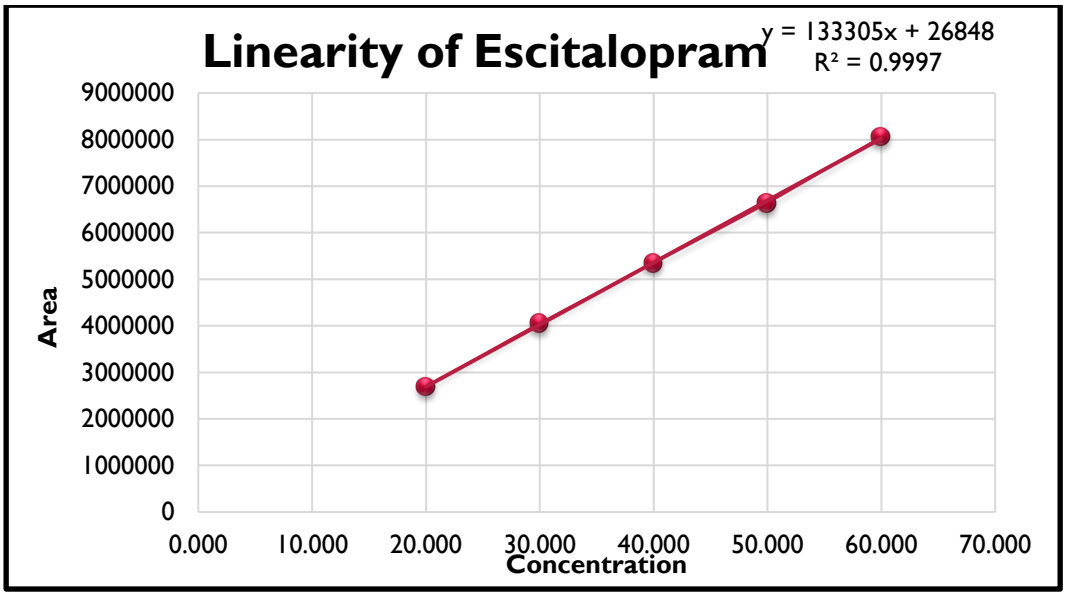

Fig. 7: Linearity plot of Escitalopram

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that the response is found to be linear.
- Co-relation coefficient (r^2 was found 0.9997).

4. Accuracy (Recovery):

Table 5: % Recovery for Escitalopram

Level (%)	Area	Escitalopram Added Conc. (µg/mL)	Escitalopram Added Conc. (µg/mL)	% Recovery	Mean % Recovery
50	2638451	19.89	19.9	100.07	99.57
	2705418	20.02	20.08	99.453	

	2532008	19.69	19.74	99.182	
100	5349521	40.05	40.01	99.862	99.89
	5339843	39.85	39.98	101.264	
	5320567	40.1	39.91	98.552	
150	8065419	60.19	60.1	99.854	100.12
	8059413	60.23	60.08	99.266	
	8135974	60.01	60.23	101.252	

Conclusion: The data shows that the Mean recovery for 50% to 150% is in the range of 98.0%-102.0% and individual recovery for 50% to 150% is in the range of 98.0% - 102.0%.

5. Precision:

I. Method Precision:

Table 6: Method precision

Sample	**Area**	**% Assay**
Sample 1	5241059	98.01
Sample 2	5236854	97.88
Sample 3	5209718	97.61
Sample 4	5205416	96.96
Sample 5	5316258	99.27
Sample 6	5265843	97.99
Mean		**97.96**
Standard Deviation		**0.7534**
% RSD		**0.77**

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % RSD for % Assay is within the acceptance criteria and hence the method is precise.

II. Intermediate Precision:

Table 7:Intermediate Precision

Sample	**Area**	**% Assay**
Sample 1	5249524	98.26
Sample 2	5296511	99.05
Sample 3	5186502	96.94
Sample 4	5260143	98.66
Sample 5	5202689	97.01

Sample 6	5165871	96.70
Mean		**97.77**
Standard Deviation		**1.0074**
% RSD		**1.030**

Table 8: Intermediate Precision Pool Data

Parameter	**Method Precision (Analyst-I)**	**Intermediate Precision (Analyst-II)**
HPLC NO.	**AD/HPLC-02**	**AD/HPLC-04**
Column No.	**HPLC-20**	**HPLC-26**
Sample No.	**%Assay**	
1	98.01	98.26
2	97.88	99.05
3	97.61	96.94
4	96.96	98.66
5	99.27	97.01
6	97.99	96.70
Mean	**97.86**	**97.77**
Mean of Precision % Assay	**97.86**	
Absolute Mean difference % assay	**0.9**	

Conclusion:

- The data shows that system suitability is fulfilled.
- The data shows that % Assay is of six samples is not more than 2.0
- The data shows that % Assay is within the acceptance criteria and hence the method is rugged.

6)Robustness:

Table 9: Robustness for Escitalopram

Change in parameter	Condition	Area	Absolute difference of % Assay
Control	As per method	5241059	NA
Change in flow rate1.0 ml/min (±0.1 ml/min)	1.1 ml/min	5276524	-0.7
	0.9 ml/min	5175874	1.2
Change in wavelength (±2	240 nm	5306513	1.2

nm)	236 nm	5219482	-0.4

Conclusion:

- System suitability criteria were fulfilled.
- The difference of % assay value in each modified condition is within acceptance criteria.

6. CONCLUSION

HPLC has gained the valuable position in the field of analysis due to ease of performance, specificity, sensitivity, and the analysis of samples of a complex nature. This technique was employed in the present investigation for estimation of escitalopram tablet formulation. HPLC Agilent (1260 Infinity II) using Openlab EZ Chrome workstation software with Phenomenex C18 (250 mm X 4.6 mm), a 5µm column, and a UV/PDA detector was used for the study. The standard and sample solution of escitalopram were prepared in diluent. Different pure solvents of varying polarity in different proportions were tried as the mobile phase for the development of the chromatogram.

The mobile phase that was found to be most suitable was 0.05 % OPA and methanol. The wavelength of 238 nm was selected for the evaluation of the chromatogram of escitalopram, respectively. The selection of the wavelength was based on the λmax obtained by UV scanning of a standard laboratory mixture in water: methanol.

After establishing the chromatographic conditions, a standard laboratory mixture was prepared and analysed by the procedure described under Materials and Methods. It gave accurate, reliable results and was extended for estimation of escitalopram in tablet formulation.

The results from the table clearly indicate that the RP-HPLC technique can be successfully applied for the estimation of the above-mentioned drugs in their formulation.

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