https://doi.org/10.48047/AFJBS.6.7.2024.3263-3276

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

METHOD DEVELOPMENT AND VALIDATION OF BCS CLASS-I DRUG BY RP-HPLC METHOD

Amol Shirode[1], Shubham Zarikar[2*], Bhaskar Aher[1], Vinod Bairagi[3], Ganesh Sonawane[4], Pravin Jadhav[1]

[1]Department of Pharmaceutical Chemistry, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

[2]Department of Pharmaceutical Quality Assurance, K.B.H.S.S Trust's Institute of Pharmacy, Malegaon, Nashik-423301, Maharashtra, India

[3]Department of Pharmacology, K.B.H.S.S. Trust's Institute of Pharmacy Malegaon, Nashik-423105, Maharashtra, India

[4]Department of Pharmaceutical Chemistry, Divine College of Pharmacy, Satana, Nashik-423301, Maharashtra, India

***Corresponding Author:** shubahamzarikar2000@gmail.com

Volume 6 issue 7 2024
Received:01June2024
Accepted:30June2024
doi:10.48047/AFJBS.6.7.2024.3263-3276

ABSTRACT

A stability indicating RP-HPLC method was developed to quantitatively measure Venlafaxine in both its pure form and pharmaceutical formulations, following ICH guidelines for validation. The method employed an Inertial ODS C18 column (150mm x 4.6mm, 5µ) at ambient temperature (30°C) with a flow rate of 0.8 ml/minute using isocratic elution. UV detection at 232 nm was performed following injection of 20 µl of the sample. The mobile phase, consisting of phosphate buffer and acetonitrile (30:70, v/v), also served as the diluent. The retention time for Venlafaxine standard was determined to be 5.011 minutes. The method demonstrated linearity over a concentration range of 12.5-75 µg/ml, with a correlation coefficient of 1. Precision and accuracy studies indicated % RSD values below 2. This validated method is suitable for routine assay determination of Venlafaxine in both its pure form and pharmaceutical formulations.

Keywords: Venlafaxine, RP-HPLC, Method Devlopment, Validation, etc

INTRODUCTION

Chromatography was pioneered by the Russian chemist and botanist Mikhail Tsvet (1872-1919). He coined the term "chromatography" from the Greek words "chroma" (color) and "graphein" (writing) to describe his research on separating colored plant pigments into bands. The principle of chromatographic separation is unique among separation methods because it relies on the interaction between two phases that do not mix: a stationary phase and a mobile phase. A column is employed to carry a sample through the mobile phase, which contains the dispersed stationary phase (1). HPLC is a crucial analytical tool used in every stage of drug discovery, development, and production. Today, High Performance Liquid Chromatography (HPLC) is a powerful asset in analytical chemistry (2). It has the ability to separate, detect, and quantify compounds in liquid samples. HPLC is well-known as one of the most accurate analytical methods, widely used for both quantitative and qualitative analysis of pharmaceutical products (3). The main advantage of HPLC compared to traditional column chromatography is its ability to achieve higher resolution of separated substances, faster separation times, and improved accuracy, precision, and sensitivity (4). Process validation involves verifying and documenting that processes operate within their specified design parameters, demonstrating their ability to consistently and reliably produce a finished product that meets required quality standards (5). The Biopharmaceutics Classification System (BCS), established in 1995, is the standard for assessing the bioequivalence of oral dosage forms. It classifies drugs based on their solubility in water and permeability in the intestines. BCS evaluates these factors along with the dissolution rate to determine the bioavailability of Immediate Release (IR) solid oral medications (6). Developing and validating a method for the BCS Class-I drug venlafaxine using RP-HPLC (Reverse Phase High-Performance Liquid Chromatography) is crucial for ensuring pharmaceutical quality and regulatory compliance. Regulatory agencies such as the FDA and EMA require validated analytical methods to confirm that drugs are safe, effective, and of high quality. Venlafaxine, a highly soluble and permeable antidepressant, necessitates a robust RP-HPLC method to accurately characterize its properties, ensure consistent quality control, and monitor stability. RP-HPLC is particularly suited for this purpose due to its precision, accuracy, and efficiency in analyzing complex mixtures (7). By establishing a validated RP-HPLC method for venlafaxine, manufacturers can guarantee that the drug meets required standards throughout its shelf life, thus safeguarding patient health and maintaining its therapeutic efficacy. The chemical structure of venlafaxine is shown in Figure 1.

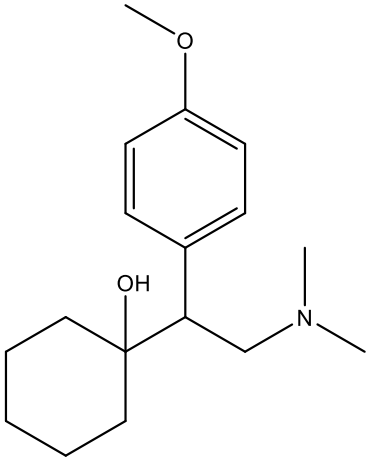

Figure 1: Chemical structure of venlafaxine

Venlafaxine hydrochloride is a selective serotonin and norepinephrine reuptake inhibitor (SNRI) prescribed for anxiety and various types of depression. Introduced by Wyeth in 1993, venlafaxine hydrochloride and its metabolite O-dimethylvenlafaxine reach steady-state plasma concentrations within three days of oral multiple-dose therapy. These concentrations follow linear kinetics across a dosage range of 75–450 mg/day. SNRIs, including venlafaxine, are effective in managing chronic pain (8). As a novel type of antidepressant distinct from tricyclic antidepressants, venlafaxine is believed to modulate neurotransmitter activity in the central nervous system to exert its therapeutic effects (9). Its pharmacological mechanisms involve the inhibition of serotonin and norepinephrine neurons. Since its introduction in 1994, venlafaxine has gained popularity, particularly among patients who do not respond adequately to selective serotonin reuptake inhibitors (10).

MATERIAL AND METHOD

Material

The chemicals and equipment's used are mentioned in Table 1 and 2.

Table 1: Chemicals used

Chemicals	Reagents
Potassium Di-hydrogen orthophosphate Di-potassium hydrogen orthophosphate	Ranke/ARGrade
Acetonitrile	Standard reagents
Water	Standard reagents
Methanol	Standard reagents
O-Phosphoric acid	Standard reagents

Table 2: Equipment used

Chromatographic mode	Chromatographic condition
HPLC	Water2695
Software for HPLC	Empower software
UV-spectrometry	Nicoletevolution100
Software for UV spectrometry	Vision pro
Column	SymmetryC18 column
pKa	9.37
Mobile phase	Methanol: Water
Detection wavelength	227nm
Radical sonicator	Citizen,Digitalultrasonic cleaner
PH measure	Thermo scientific

Method

Development and Validation of HPLC Methods

The method development for simultaneous estimation of venlafaxine in pharmaceutical dosage forms involves the following steps:

1. Selection of Detection Wavelength:

Venlafaxine (10 mg) was dissolved in the mobile phase, and a spectrum was generated by scanning the solution from 200 to 400 nm. The wavelength for venlafaxine was chosen based on the overlay spectrum.

2. Selection of Column:

The choice of column was based on the chemical properties, polarity, and solubility differences in the analyses.

3. Selection of Mobile Phase:

Acetonitrile and water (HPLC grade) were mixed in an 80:20 volume ratio to prepare the mobile phase. The mobile phase was degassed by sonicating it for ten minutes to remove any trapped gases.

4. Selection of Flow Rate:

A flow rate of 1 ml/min was selected based on criteria such as retention time, column backpressure, achieving peak symmetry, and effective separation of impurities.

5. Preparations and Procedures:

Preparation of Buffer:

Potassium dihydrogen orthophosphate (2.95 g) and potassium hydrogen orthophosphate (0.58 g) were dissolved in 1000 ml of distilled water and sonicated to prepare the buffer solution.

Preparation of Mobile Phase:

The same phosphate salts were dissolved in 1000 ml of distilled water and sonicated to prepare the mobile phase. The final solution was filtered using a 0.45 micron filter under vacuum.

Diluents Preparation:

The mobile phase was used as the diluents.

Preparation of Individual Standard Preparation:

A working standard (50 mg) was dissolved in 70 ml of diluent in a 100 ml volumetric flask. After sonication and adjustment to volume, 1.5 ml was pipetted into a 10 ml volumetric flask and diluted with diluent.

Preparation of Sample Solution:

Venlafaxine (API) powder (100 mg) was dissolved in 50 ml of diluent in a 100 ml volumetric flask, sonicated, and adjusted to volume with diluent. Approximately ten venlafaxine tablets were crushed into a powder using a mortar and pestle.

Procedure:

After injecting 20 ml of the standard and sample into the chromatographic system, peak areas were measured. The assay percentage was calculated according to established procedures.

System Suitability:

The peak tailing factor for venlafaxine in the standard solution should not exceed 2.0. Additionally, the venlafaxine peak in the standard solution should exhibit a minimum of 2000 theoretical plates.

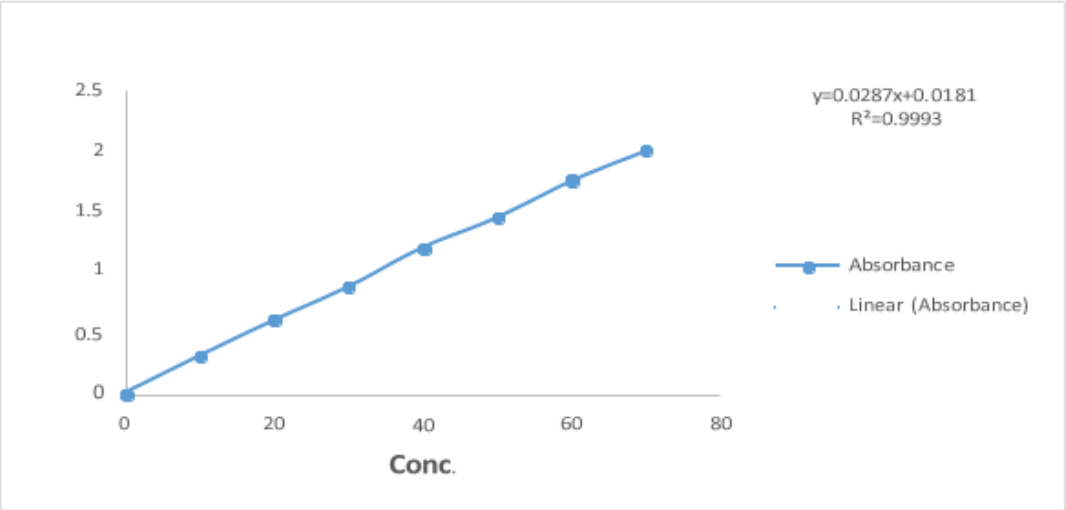

Figure 2: Calibration curve of venlafaxine

Preparation of Sample Solutions: For the preparation of an 80% solution: Precisely 8 mg of venlafaxine working standard was weighed and added to clean, dry 10 ml and 100 ml volumetric flasks. Approximately 7 ml of diluent was added, and the mixture was sonicated until the standard completely dissolved. The volume was adjusted with the same solvent to create the stock solution. From this stock solution, 3 ml and 0.3 ml were pipetted into separate 10 ml volumetric flasks and diluted with diluent to the appropriate volume.

For the preparation of a 100% solution: Exactly 10 mg of venlafaxine working standard was measured and placed into two separate clean, dry volumetric flasks (one 10 ml and one 100 ml). Approximately 7 ml of diluent was added to each flask, and the mixture was sonicated until the sample completely dissolved. The volume was adjusted with the same solvent to create the stock solution. From this stock solution, 3 ml and 0.3 ml were pipetted into a 10 ml volumetric flask and diluted with diluent to the appropriate volume.

For the preparation of a 120% solution: A clean, dry 10 ml and 100 ml volumetric flask were filled precisely with 12 mg of venlafaxine working standard. Approximately 7 ml of diluent was added, and the flask was sonicated until the drug completely dissolved. The volume was then adjusted with the same solvent to create the stock solution. From this stock solution, 3 ml and 0.3 ml were pipetted into a 10 ml volumetric flask and further diluted with diluent to the appropriate volume.

Procedure: Injections of the standard solution at accuracies of -80%, -100%, and -120%

were conducted. The amounts of venlafaxine added and found were recorded, along with their mean and individual recovery values.

RESULTS AND DISCUSSION

Selection of solvent:

Methanol was selected as the solvent for dissolve in given Venlafaxine.

Selection of analytical wavelength:

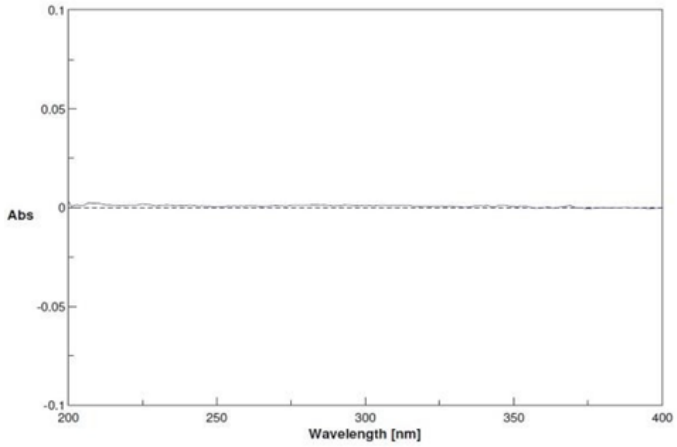

Figure 3: UV spectrum of Methanol as a blank

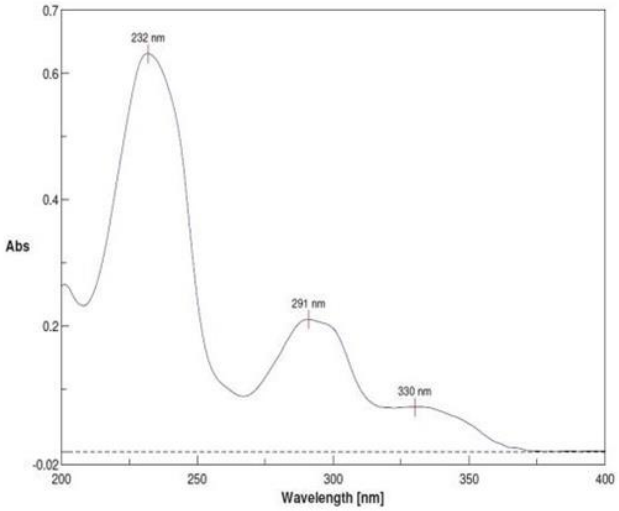

Figure 4: UV spectrum of Venlafaxine

Observation:

The standard solution (5 parts per million) underwent a scan from 200 nm to 400 nm to identify the wavelength at which the drug showed maximum absorption. Venlafaxine exhibited its highest absorbance at 232 nm. Therefore, 232 nm was chosen as the analytical wavelength for subsequent determinations. On average, the venlafaxine assay percentages ranged around 99.71%.

Assay calculations for venlafaxine:

The investigation into venlafaxine testing was conducted. The chromatographic apparatus was injected with three injections each of the sample, standard, and blank.

Assay of Pharmaceutical Formulation:

A 20 mL solution of Effexor tablets of various dosages was individually injected into the HPLC system, and chromatograms were recorded for each.

Table 3: Result of assay pharmaceutical

Sr. No.	Dosage in mg	Found	%Assay
1	25	24.915	99.65
2	37.5	37.421	99.74
3	50	50.06	100.11
4	75	75.54	100.73
5	100	100.31	100.31

Acceptance criteria: The % of Assay was found to be within the limits of 99.

Chromatogram of the Venlafaxine sample:

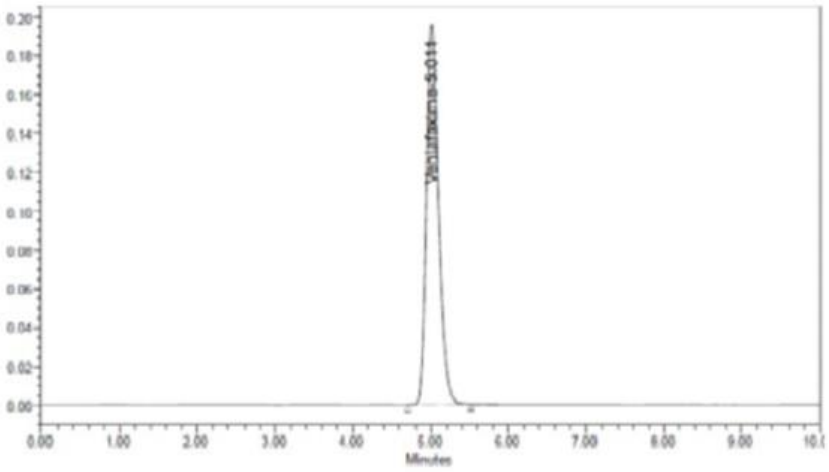

Figure 5: Chromatogram of the Venlafaxine standard

Table 4: Venlafaxine standard table

Sr. No.	Peak Name	RT	Area	% Area	Height	USP Plate count	USP Tailing
1	Venlafaxine	5.011	2118148	100	196104	4957	1.18

Chromatogram of venlafaxine sample graph:

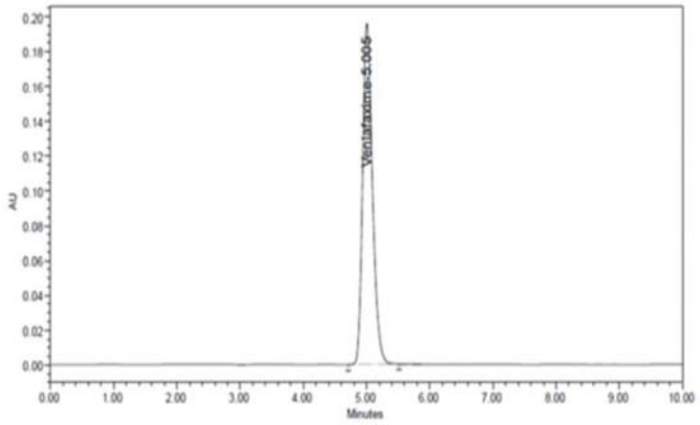

Figure 6: Chromatogram of the Sample graph

Table 5: Chromatogram of venlafaxine sample table

Sr. No.	Peak Name	RT	Area	% Area	Height	USP Plate count	USP Tailing
1	Venlafaxine	5.005	211554	100	196125	4952	1.15

Validation results:

Accuracy: A study on accuracy was conducted for Venlafaxine at levels of 80%, 100%, and 120%. Each level was injected into the chromatographic system three times. The area measured for each level was utilized to determine the percentage recovery.

Table 6: Accuracy results of Venlafaxine

Sr. No.	Level %	Amount added (mg)	Amount Recovered (mg)	% Recovery
1	80	40	39.69	99.20
2	100	50	49.83	99.61
3	120	60	59.77	99.63

Acceptance Criteria: The percentage recovery for each level should fall within the range of 98.0 to 102.0%.

System Suitability:

The system suitability was evaluated by performing six replicate analyses of the drug at a concentration of 20.0µg/ml. The acceptance criterion is ± 1% for the percent coefficient of variation of peak area and retention times of the drug. The relative standard deviation (RSD) of peak area and retention time for both the drug and internal standard are within acceptable limits. The column efficiency, expressed as the number of theoretical plates for the six replicate injections, was approximately 5774 ± 11, and the USP tailing factor was 1.14 ± 0.0005.

Table 7: Suitability of venlafaxine

Sr. No.	Area	T.P	T.F	RT
Inj.1	429411	5765	1.15	2.83
Inj.2	428559	5767	1.14	2.83
Inj.3	429159	5766	1.15	2.83
Inj.4	428066	5771	1.14	2.83
Inj.5	424032	5793	1.14	2.83
Inj.6	427085	5781	1.14	2.83
Mean	427718.7	5773.8	1.143	2.83
S.D	1987.88	11.07	0.005	0
RSD	0.46	0.19	0.456	0

Precision:

Repeatability:

In the precision study, five injections of Venlafaxine were performed. Each standard injection was administered once into the chromatographic system. The percentage relative standard deviation (RSD) was calculated based on the area of each standard injection.

Table 8: Repeatability results of Venlafaxine

Sr. No.	Venlafaxine	
	RT	Area
1	5.011	2118148
2	5.009	2117956
3	4.988	2124629

4	4.995	2130645
5	5.002	2125204
6	5.005	2122015
Avg	5.002	2123100
Std Dev	0.0088	4813.563
RSD	0.175	0.228

Acceptance Criteria: It is advised that the percentage RSD for the area of five standard injection results should not exceed 2%. The Venlafaxine % RSD determined in the Method precision study was found to be 0.2%.

Ruggedness:

For the intermediate accuracy evaluation, six injections of Venlafaxine were conducted. Each standard injection was administered once into the chromatographic system. The percentage relative standard deviation (RSD) was determined based on the area of each standard injection.

Table 9: Ruggedness results of Venlafaxine

Sr. No.	**Venlafaxine**	
	RT	**Area**
1	5.01	2128251
2	5.015	2130214
3	5.016	2125452
4	5.018	2124078
5	5.008	2126265
6	5.01	2118975
Avg	5.013	2125539
Std Dev	0.004	3872.101
RSD	0.08	0.181

LOD & LOQ:

The LOD and LOQ of the proposed method were determined using the slope (s) and standard deviation of the intercept of the calibration curve (σ). The LOD is 61.74 the LOQ is187.03.

Linearity:

Six distinct solutions (25%, 50%, 75%, 100%, 125%, and 150% of the standard stock solution) were prepared within the concentration range of 12.5–75 µg/mL. Each solution (20 mL) was injected, and chromatograms were collected to verify the method's linearity, resulting in a linear relationship. The slope, intercept, and correlation coefficient for the calibration curve of the current method are 52,481, 16,095, and the correlation coefficient, respectively.

Table 10: Linearity results for the drug Venlafaxine

Conc. (mcg)	Area
12.5	541656
25	1067131
37.5	1587197
50	2116076
62.5	2639479
75	3166192

Acceptance Criteria: Correlation coefficient should be not less than 0.99.

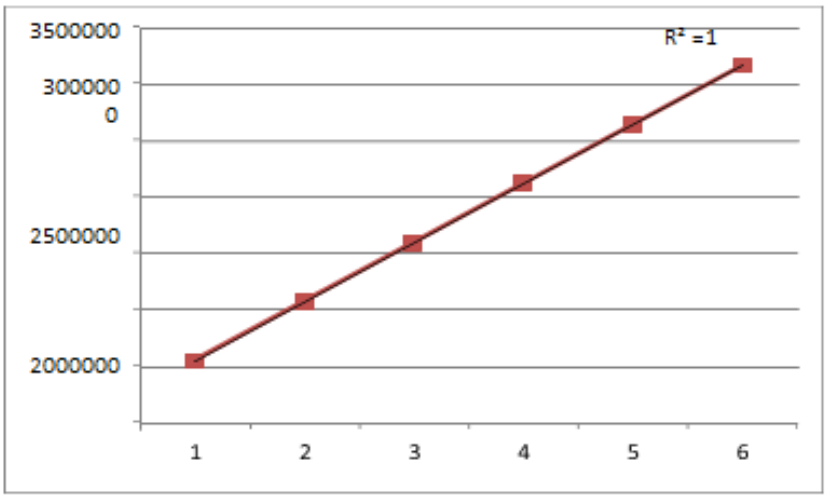

Figure 7: Linearity of venlafaxine

Range: After conducting a linearity analysis, the correlation coefficient for Venlafaxine across the concentration range of 10 µg to 50 µg was determined to be 0.9988.

Robustness:

To evaluate the robustness of the method, deliberate alterations were made to temperature variation, mobile phase composition, and flow rate. Testing the effects of minor, intentional modifications to the chromatographic conditions and associated peak regions enabled the determination of the method's robustness. Specifically, variations in flow rate and the percentage composition change in the mobile phase's phosphate buffer and acetonitrile were examined for this purpose. The approach was deemed sufficiently robust, as even slight variations in chromatographic conditions did not significantly alter the peak area.

Table 11: Results of Robustness

Sr. No.	Parameter	Venlafaxine	
		RT	Area
1	Standard	3.612	1450316
2	Robustness-Flow-1	3.173	1249667
3	Robustness-Flow-2	3.763	1595609
4	Robustness-OvenTemp-1	5.093	2128634
5	Robustness-OvenTemp-2	4.879	2106600

Specificity:

Specificity testing revealed that the established method effectively eliminates interference from tablet excipients, as evidenced by the absence of discernible differences between the chromatograms of the standard and the venlafaxine test sample.

Table 12: Specificity of Venlafaxine

Sample No.	Excipient Conc. (%)	Venlafaxine Input (mg)	Venlafaxine recovered (mg)	Venlafaxine recovered (%)	Mean Recovered (%)	S.D	% R.S.D
1	100%	10	9.95	99.8	100.03	1.06	1.06%
2	50%	10	9.12	101.2			
3	150%	10	9.91	99.1			

CONCLUSION

An HPLC method was developed and validated for venlafaxine, establishing a dependable analytical technique. This method proved effective in accurately measuring venlafaxine in various samples, including bulk drug substance and pharmaceutical formulations. The method's precision, accuracy, linearity, specificity, and robustness were verified, ensuring its suitability for routine analysis in pharmaceutical quality control labs. Furthermore, the method adhered to regulatory standards, including those outlined by the International Council for Harmonization (ICH), affirming its reliability and validity for pharmaceutical analysis.

REFERENCES

1) Chromatography K. Review Article: Chromatography Principle and Applications. 2018;(1).
2) Gupta M, Kapoor B, Gupta R. Paper Chromatography: A Review. 2018;5(10):462–8.
3) Article OR, Vidushi Y, Meenakshi B. A review on HPLC method development and validation. 2017;2(166):166–78.
4) Narendra D, Satyanarayana J, Phani KV, Reddy K, Ajay KVN, Aditya MB, et al. Simultaneous estimation of aceclofenac and paracetamol by using RP-HPLC. 2022;12(3):248–53.
5) Ahir KB, Singh KD, Yadav SP, Patel HS, Poyahari CB. Review Article: Overview of Validation and Basic Concepts of Process Validation. 2014;3(2):178–90.
6) Review on: Biopharmaceutics. 2021;9(5):738–43.
7) Article R, Singhal P, Singhal RV, Kumar VJ, Verma A, Kaushik RD, et al. The Biopharmaceutics Classification System (BCS). 7(1):269–83.
8) Mulay TA. A Review Article on Sustained Release Table of Venlafaxine Hydrochloride, an Antidepressant. 2023;11(1):632–41.
9) Swathi V, Reddy MS. International Journal of Research Publication and Reviews. Novel RP-HPLC Method Development and Validation for Estimation of Venlafaxine in Bulk Dosage Form. 2023;4(12):1176–82.
10) Howell C, Wilson AD, Waring WS. Cardiovascular toxicity due to venlafaxine poisoning in adults: a review of 235 consecutive cases. Br J Clin Pharmacol. 2007 Aug;64(2):192-7.