WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 11, 2568-2579. Research Article ISSN 2277–7105

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF A NEW RP-HPLC METHOD FOR THE ESTIMATION OF VENLAFAXINE HYDROCHLORIDE AND THE RELATED SUBSTANCES IN CAPSULE DOSAGE FORM

Dr. Ch. Saibabu*[1], G. Naga Jyothirmai[2], Dr. M. Bhaskar[3]

[1]*Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhra Pradesh, India.

[2]Department of Pharmacy, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhra Pradesh, India.

[3]Professor, Department of Pharmaceutical Analysis, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhra Pradesh, India.

Article Received on 05 May 2026,
Article Revised on 25 May 2026,
Article Published on 03 June 2026.

https://doi.org/10.5281/zenodo.20537138

***Corresponding Author**

Dr. Ch. Saibabu

Professor, Department of Pharmaceutics, Malineni Lakshmaiah College of Pharmacy, Singarayakonda, Prakasam-523101, Andhra Pradesh, India.

How to cite this Article: Dr. Ch. Saibabu*[1], G. Naga Jyothirmai[2], Dr. M. Bhaskar[3] (2026). Analytical Method Development and Validation of A New Rp-Hplc Method For The Estimation of Venlafaxine Hydrochloride And The Related Substances In Capsule Dosage Form. World Journal of Pharmaceutical Research, 15(11), 2568-2579.

ABSTRACT

A simple, accurate, precise and validated new RP-HPLC method for determination of Venlafaxine Hydrochloride has been developed. Analysis was carried out on Shimadzu HPLC system with Inertsil ODS- 3V C_{18} column, (150mmx4.6mm, 5 µm) using filtered and degassed solutions containing a mixture of buffer and methanol (55:45) in isocratic mode as mobile phase with flow rate of 1.0mL/ minute. The detection was carried out using UV detector set at 225nm. The method, obeyed Beer's law in the concentration range of linear from 80% - 120% of Venlafaxine Hydrochloride. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantification. The procedure is simple, reproducible, cheap and less time consuming. To Validate the RP-HPLC method used for the analysis of related substances present in Venlafaxine Hydrochloride and to demonstrate that the method is appropriate. The HPLC method used for determination of related substances in Venlafaxine Hydrochloride has been developed in house at Ra Chem Pharma ltd. The method needs to be

validated in order to give evidence of its reliability and suitability. The validation will be performed according to the current requirements as laid down in the ICH Guidelines Q2 (R1). Analysis was carried out on Shimadzu HPLC system with Column: Altima C8, 250 mm x 4.6 mm, 5µm using filtered and degassed solutions containing a mixture of buffer and ACN (1490:510) in isocratic mode as mobile phase with flow rate of 1.2mL/ minute. The detection was carried out using UV detector set at 225nm. The method was validated for impurity -F in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantification. The percentage recovery 100.76% from the capsule formulation. The results obtained in this study demonstrate that the Venlafaxine Hydrochloride HPLC method described in the protocol is selective, linear, precise, rugged and robust for the determination of Related Substances in Venlafaxine Hydrochloride drug.

KEYWORDS: Venlafaxine hydrochloride, antidepressant, RP-HPLC, validation.

INTRODUCTION

Venlafaxine is a bicyclic antidepressant, and is usually categorized as a serotonin-norepinephrine reuptake inhibitor (SNRI), but it has been referred to as a serotonin-norepinephrine-dopamine reuptake inhibitor. Venlafaxine hydrochloride is designated (R/S)-1-[2-(dimethylamino)-1-(4 methoxyphenyl) ethyl] cyclohexanol hydrochloride or (±)-1-[a [α-(dimethylamino) methyl] p-methoxybenzyl] cyclohexanol hydrochloride salt and has the empirical formula of C17H27NO2. HCl.

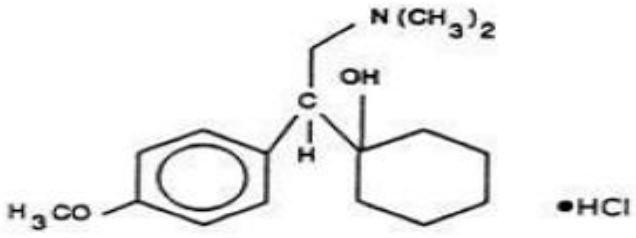

Figure-1. Chemical structure of Venlafaxine HCl.

Various methods have been reported for estimation of venlafaxine hydrochloride in biological matrices such as plasma, which includes the use of LC with UV detection1, LC with electrospray ionization mass spectrometry2, LC with coulometric detection3, LC with fluorimetric detection 4,5, LC with diode array detection6,7, GC-MS8, LC-MS-MS9 and for estimation of it in serum by use of LC10. Stability indicating methods have also been reported for its invitro determination in gastric and intestinal fluids11 and pharmaceutical

formulations12. Both the reported stability indicating methods uses acetonitrile and buffer in various proportions for quantification of venlafaxine hydrochloride. Present study involves development of RP-HPLC method using simple mobile phase containing methanol and buffer for quantitative estimation of venlafaxine hydrochloride in tablet dosage forms which is sensitive and requires shorter analysis time. The developed method was validated as per ICH guidelines13,14.

MATERIALS AND METHODS

The Liquid chromatographic system consisted of following components: Shimadzu HPLC model (VP series) containing LC-10AT (VP series) pump, Variable wavelength programmable UV/VIS detector SPD-10AVP and Rheodyne injector (7725i) with 20 µL fixed loop. Chromatographic analysis was performed using Spinchrom software on a Phenomenex Gemini C18 column with 250 × 4.6 mm i.d. and 5 µm particle size. The Shimadzu electronic balance (AX 200) was used for weighing purpose.

Table No: 1: Chemicals/ reagents used during the assay method development.

S. No	Name	Grade	Supplier	Lot No/ B.No
1.0	Ortho -phosphoric acid	HPLC	Merck	AE0A600448
2.0	Methanol	HPLC	Finar	9925P1028H11
3.0	Tri –ethylamine	MERCK	Merck	SJS610460

Table No: 2. Grade and Batch/lot no. of venlafaxine HCl and impurity-F.

S.No	Name	Grade	Lot No/B.No
1.0	Venlafaxine Hydrochloride WRS	IH*	VEN/WRS/001/11
2.0	Venlafaxine 150mg Capsules	IH*	VEOC/N/11/002
3.0	Placebo	IH*	VEXPP/N/11/001
4.0	Impurity-F	IH*	VNS/IMP-F/1651/05

Preparation of Buffer solution

Mix 1.6 ml of ortho phosphoric acid with 1000ml of distilled water and adjust the pH to 3.0 with Triethyl amine.

Preparation of Mobile Phase

Prepare filtered and degassed solutions containing a mixture of buffer and methanol (55:45)

Preparation of Standard Solution

Transfer about 25mg of Venlafaxine Hydrochloride WS, accurately weighed, to a 50 ml volumetric flask and dissolve in dilute with mobile phase to volume. Mix and filter. (This is standard stock solution).

Transfer 5.0ml of this solution to a 50 ml volumetric flask, dilute with mobile phase to volume and mix.

Preparation of Sample Solution

Transfer an accurately weighed quantity of the powdered pellets equivalent to about 31mg of Venlafaxine Hydrochloride to a 250 ml volumetric flask. Add 60 mL of mobile phase and sonicate to dissolve for 15 minutes with intermittent shaking and make up to the volume with mobile phase. Mix and filter through 0.45µm nylon membrane filter. Transfer 10.0 ml of this solution to a 25 ml volumetric flask, dilute with mobile phase to volume, and mix.

Impurity F preparation

Transfer 20mg of impurity F in 20ml volumetric flask add 10ml of acetonitrile, sonicate to dissolve and make up to the volume with Acetonitrile. Transfer 1ml of this solution into 100ml volumetric flask, make up to volume with diluents (10ppm).

Venlafaxine hydrochloride diluted standard preparation

Transfer 25mg of Venlafaxine hydrochloride WS in 25ml volumetric flask add 20ml Of mobile phase sonicate to dissolve and make up to volume With mobile phase. Transfer 1ml of this solution in to 100 ml volumetric flask, make up to volume diluents (10ppm).

METHOD DEVELOPEMNT

Selection of wavelength

Accurately weigh and quantitatively transfer about 48.0 mg of Venlafaxine Hydrochloride WRS into a 25-mL volumetric flask. Add 10 mL diluent and sonicate to dissolve and dilute to volume with diluent. Transfer 5 mL of above solution into a 50 mL volumetric flask and make up to the mark with dissolution medium. Filter through 0.45 µ nylon membrane filter paper. Adjust the baseline to zero using water as blank. Take the UV spectrum for venlafaxine hydrochloride as shown in the figure no.2.

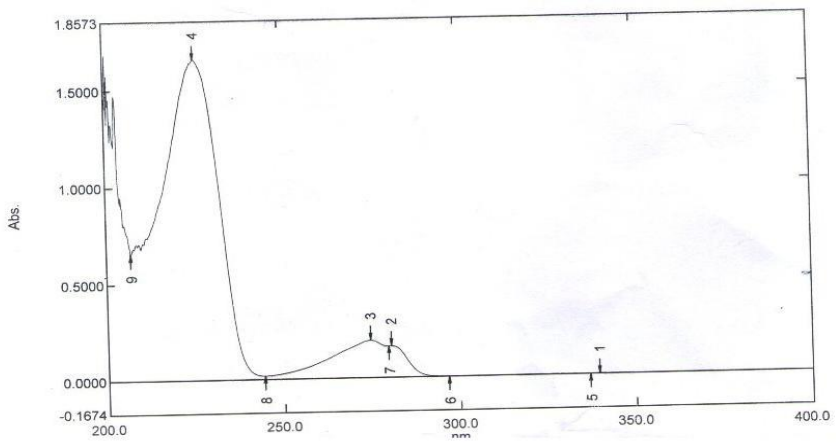

Fig. No. 2: UV spectrum of venlafaxine HCl.

Chromatographic conditions

Column : Altima C8, 150 mm x 4.6 mm, 5µm or it's equivalent

Flow rate : 1.0 ml/min

Wave length : 225 nm

Injection Volume : 20 µL

Run Time : 90 minutes

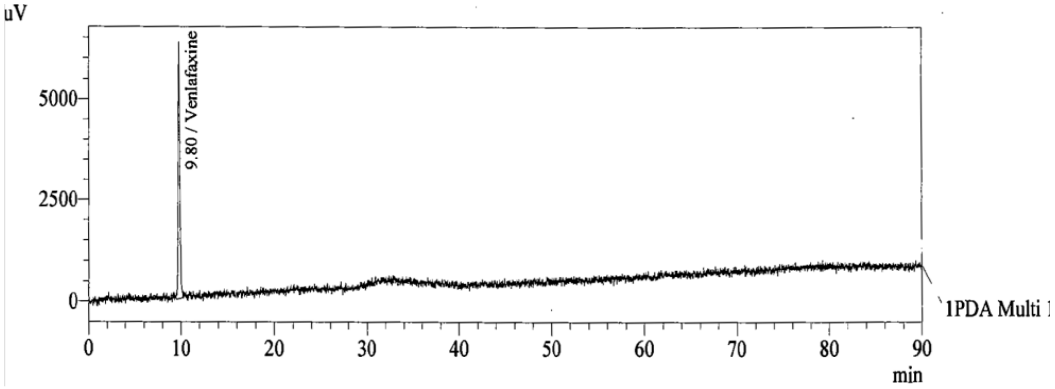

Fig. no.3. **Chromatogram for Working Standard Solution of drug.**

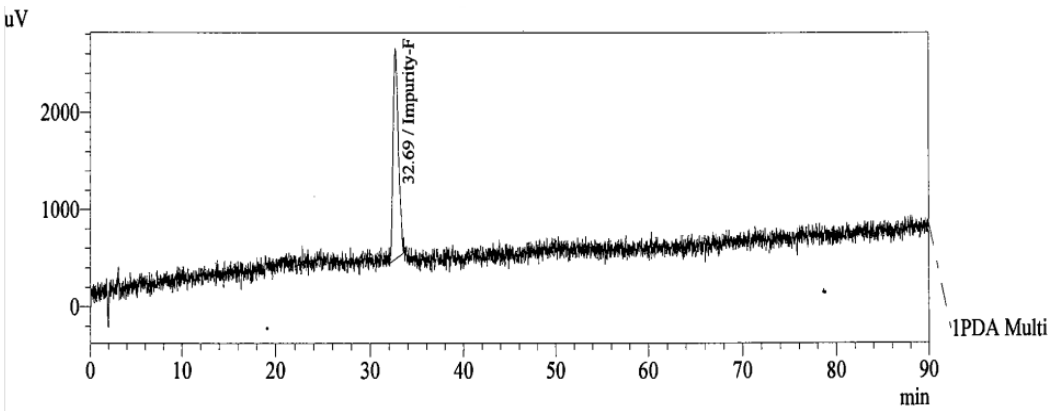

Fig. no. 4: **Chromatogram for Impurity-F Solution**

METHOD VALIDATION

LINEARITY

Demonstrate the linearity of Venlafaxine Hydrochloride over the range of 80% to 120% of specification limit as mentioned below. Preparation of standard stock solution as per methodology. The linearity of the HPLC method was demonstrated for Venlafaxine HCl assay solutions ranging from 80% to 120% of the standard concentrations. Results obtained are shown in Table 3 and Figure 1 show the line of best fit for concentration versus peak area of Venlafaxine HCl. The corresponding typical chromatograms are enclosed as Appendix-III.

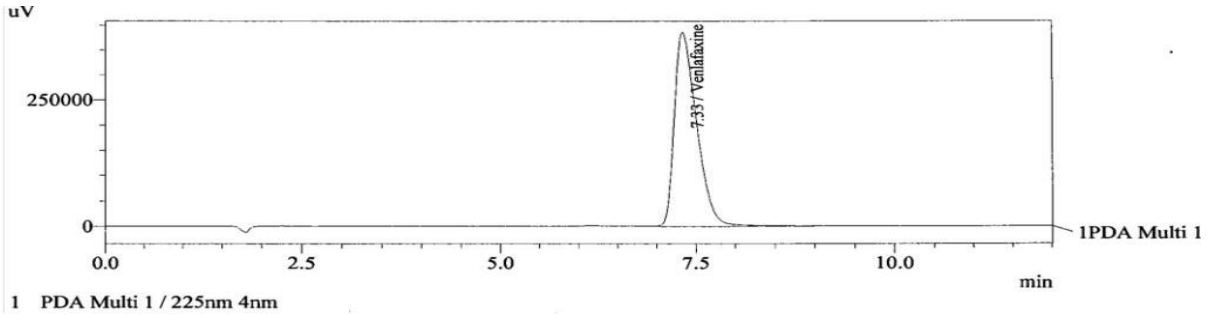

Fig no.: 5. **Chromatograms for linearity 120% solution.**

Table no: 3: Linearity for Venlafaxine HCl.

Level	Peak Area
80%	1570991
90%	1767996
100%	1959098
110%	2162998
120%	2396471
Slope	**38704994.7**
Intercept	**12993.0**
Correlation Coefficient	**0.9999**
R^2	**0.9999**

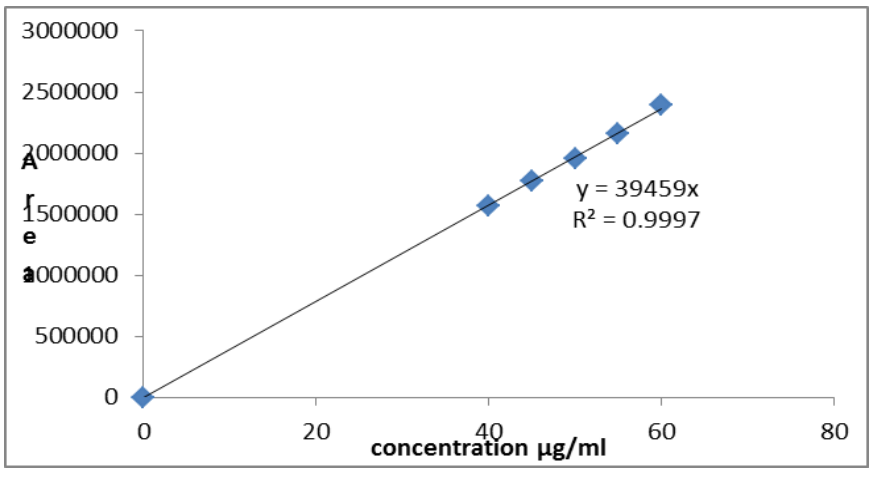

Fig. no. 6: Linearity data of venlafaxine HCl.

Precision

Prepared a standard solution (This solution contains 0.05mg/mL of Venlafaxine Hydrochloride in diluent. The solution was analyzed as per the HPLC method described in the protocol. Table 1 summarizes the retention time, peak, area, Theoretical plates, Tailing factor, and %RSD of Venlafaxine Hydrochloride. Figure-1 represents the working standard solution.

SPECIFICITY/SELECTIVITY

Prepared a blank solution, placebo solution, Impurity-F solution and standard solution. As described in methodology, all these solutions were analyzed using the PDA detector.

Table-15 summarizes the retention time**,** the peak area and peak purity values of blank, placebo, Impurity-F and Venlafaxine Hydrochloride standard solution. Figure-2 represents the blank solution, Figure-3 represents the placebo solution, Figure-4 represents the Impurity-F solution and Figure-5 represents the working sample solution.

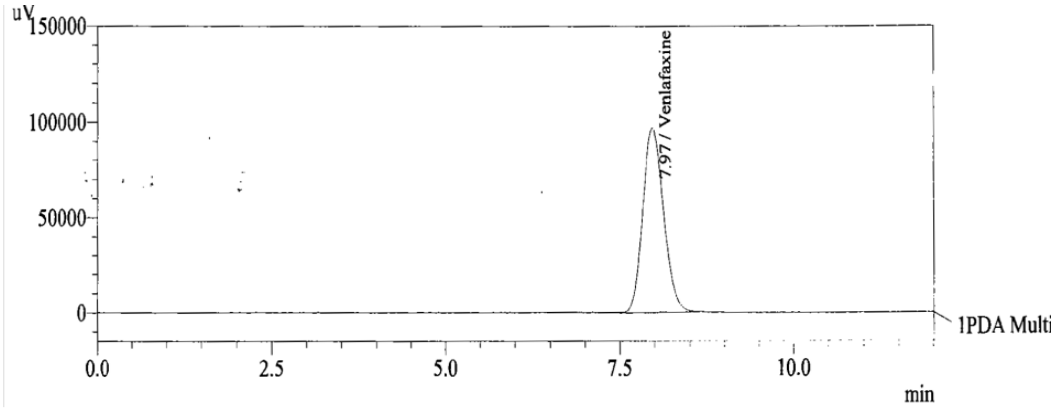

Fig. no. 7: Chromatogram for Working Standard Solution(API).

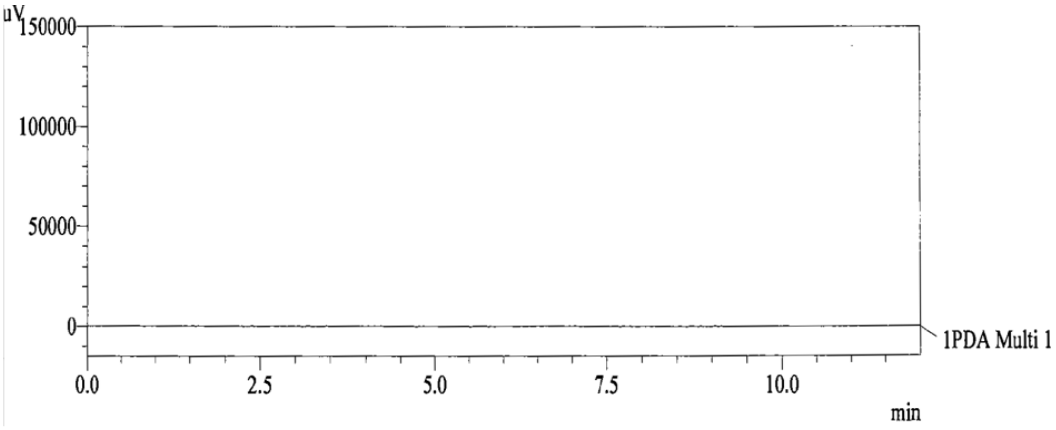

Fig. no.8: Chromatogram for Blank Solution.

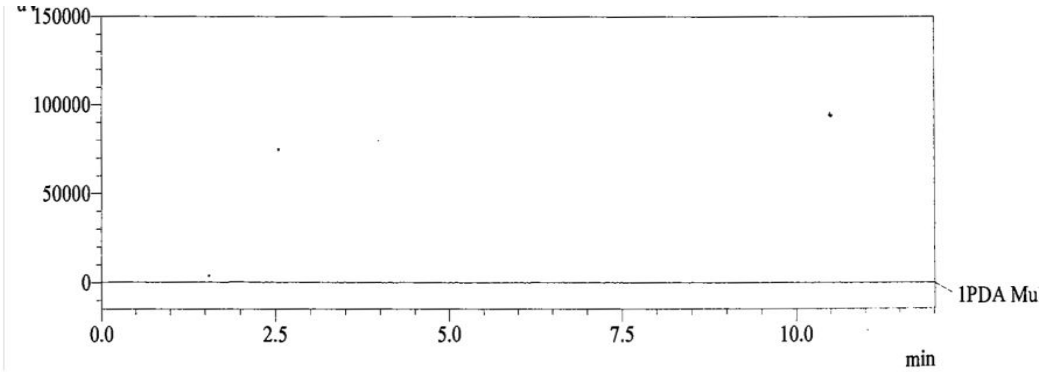

Fig. no. 9: Chromatogram for Placebo Solution.

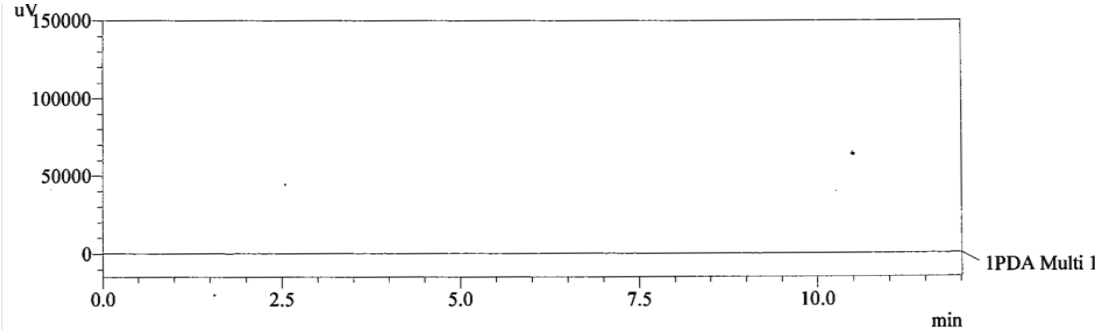

Fig. no. 10: Chromatogram for Impurity-F Solution.

Table No. 4: **Summary of selectivity data.**

Name of the Peak	RT	Area	Peak Purity
Blank	---	---	---
Placebo	---	---	---
Impurity-F	ND	ND	ND
Venlafaxine Hydrochloride	7.97	2072834	1.0000

ACCURACY

Demonstrate the accuracy of the test method by preparing recovery sample (i.e. Venlafaxine Hydrochloride API spiked to the Placebo) at the levels 80%, 100% and 120% of test concentrations. Prepare the recovery sample in triplicate in each level. Preparation of standard stock solution as per methodology.

Table No.: 5 Accuracy results of venlafaxine HCl.

Level	% Venlafaxine HCl Working strength	Theoretical Conc. (mg/mL)	Measured Conc. (mg/mL)	% Recovery	%RSD
80%	40.11	0.04011	0.03996	99.6	**0.06**
	40.09	0.04009	0.03991	99.6	
	40.12	0.04012	0.03992	99.5	

100%	49.99	0.04999	0.05008	100.2	**0.06**
	50.01	0.05001	0.05008	100.1	
	49.98	0.04998	0.05009	100.2	
120%	60.21	0.06021	0.05996	99.6	**0.00**
	60.19	0.06019	0.05993	99.6	
	60.22	0.06022	0.05998	99.6	

RESULTS AND DISCUSSION

This study showed that there was no interference observed due to impurity-F, blank and placebo with Venlafaxine Hydrochloride peak. Therefore the method is selective for the determination of Assay of Venlafaxine Extended Release Capsules 150mg. The study proves that the response for Venlafaxine HCl peak is linear over the range of 80% to 120% of standard concentration. This proves that as the concentration of the drug was increased the peak area is also increased which says that the drug obeys the beers law over the particular range. The result obtained meets the system suitability requirement, which indicates that the system is suitable for analysis. The drug showed acceptable precision values and obeyed athe specifications in ICH indicating that the method was suitable for the analysis of this particular drug. As the recovery results are between the ranges of 98.0% - 102.0%, with a precision at each level of less than 2.0% RSD, the study proves that the method is accurate for determination of Venlafaxine HCl in over the range of 80% to 120% of target concentration. The method was validated for specificity, linearity, accuracy, precision, limit of detection, limit of quantification, robustness and solution stability. Optimization of mobile phase was performed based on resolution, asymmetric factor and peak area obtained. Different mobile phases were tried but satisfactory separation, well resolved and good symmetrical peaks were obtained with the mobile phase buffer and methanol (55:45).

Calibration curve was prepared using concentrations in the range of (expected detection limit range). The standard deviation of y-intercepts of regression line was determined and kept in following equation for the determination of detection limit and quantification limit. Detection limit = 3.3σ/s; Quantification limit = 10σ/s, where σ is the standard deviation of y-intercepts of regression lines and s is the slope of the calibration curve. Calibration curve for venlafaxine hydrochloride and its impurity was obtained by plotting the peak area ratio versus the concentration of venlafaxine hydrochloride over the range of 0.08-0.12 µg/ml, slope and intercept value for calibration curve was y = 12993.0, and it was found to be linear over entire calibration range studied with r^2 value of 0.999. The recovery studies were carried out three times over a specified concentration range and the amount of venlafaxine

hydrochloride and its impurity was estimated by measuring the peak area ratios by fitting these values to the straight-line equation of calibration curve. From above determination, percentage recovery and standard deviation of percentage recovery were calculated.

CONCLUSION

Proposed study describes new RP-HPLC method using simple mobile phase for the estimation of venlafaxine hydrochloride in tablet formulations. The method was validated and found to be simple, sensitive, accurate and precise. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore the proposed method can be used for routine analysis for estimation of venlafaxine hydrochloride in its capsule formulations. The results obtained in this study demonstrate that the Venlafaxine Hydrochloride HPLC method described in the protocol is selective, linear, precise, rugged and robust for the determination of Related Substances in Venlafaxine Hydrochloride drug.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Azidus Laboratories, Chennai, India, for their valuable support in providing a literature survey and facilitating this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this research.

REFERENCES

1. Raut BB, Kolte BL, Deo AA, Bagool MA, Shinde DB. A rapid and sensitive high performance liquid chromatographic method for determination of venlafaxine and o-desmethyl venlafaxine in human plasma with UV detection. J. Liq. Chromatogr. Technol., 2003; 26: 1297–313. [Google Scholar]
2. Juan H, Zhiling Z, Huande L. Simultaneous determination of fluoxetine, citalopram, paroxetine, venlafaxine in plasma by high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) J Chromatogra. Bio. Sci. Appl., 2005; 820: 33–9. [PubMed] [Google Scholar]
3. Clement EM, Odontiadis J, Franklin M. Simultaneous measurement of venlafaxine and its major metabolite, O-desmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction. J. Chromatogra. Bio. Sci. Appl., 1998; 705: 303–8. [PubMed] [Google Scholar]

4. Vu RL, Helmeste D, Albers L, Reist C. Rapid determination of venlafaxine and O-desmethylvenlafaxine in human plasma by high-performance liquid chromatography with fluorimetric detection. J. Chromatogra. Bio. Sci. Appl., 1997; 703: 195–201. [PubMed] [Google Scholar]
5. Li HD, Ding DR, Zhang BK, Yuan HY. Studies on determination of new drug venlafaxine in human plasma by high performance liquid chromatography with fluorimetric detection. Yaowu. Fenxi. Zazhi., 2001; 21: 240–2. [Google Scholar]
6. Titier K, Castaing N, Scotto-Gomez E, Molimard M. High-performance liquid chromatographic method with diode array detection for identification and quantification of the eight new antidepressants and five of their active metabolites in plasma after overdose. Ther. Drug Monit., 2003; 25: 581–7. [PubMed] [Google Scholar]
7. Duverneuil C, de la Grandmaison GL, de Mazancourt P, Alvarez JC. A high-performance liquid chromatography method with photodiode-array UV detection for therapeutic drug monitoring of the nontricyclic antidepressant drugs. Ther. Drug Monit., 2003; 25: 567–73. [PubMed] [Google Scholar]
8. Sarah MR, Lambert EE. Development of a solid phase extraction for 13 ‘new’ generation antidepressants and their active metabolites for gas chromatographic-mass spectrometric analysis. J. Chromatogra. A., 2005; 1098: 19–29. [PubMed] [Google Scholar]
9. Bhatt J, Jangid A, Venkatesh G, Subbaiah G, Singh S. Liquid chromatography-tandem mass spectrometry (LC-MS-MS) method for simultaneous determination of venlafaxine and its active metabolite *O*-desmethyl venlafaxine in human plasma. J. Chromatogra. Bio. Sci. Appl., 2005; 829: 75–81. [PubMed] [Google Scholar]
10. Frahnert C, Rao ML, Grasmader K. Analysis of eighteen antidepressants, four atypical antipsychotics and active metabolites in serum by liquid chromatography: A simple tool for therapeutic drug monitoring. J. Chromatogra. Bio. Sci. Appl., 2003; 794: 35–47. [PubMed] [Google Scholar]
11. Asafu-Adjaye EB, Faustino PJ, Tawakkul MA, Anderson LW, Yu LX, Kwon H, et al. Validation and application of a stability-indicating HPLC method for the invitro determination of gastric and intestinal stability of venlafaxine. J. Pharm. Biomed. Anal., 2007; 43: 1854–9. [PubMed] [Google Scholar]
12. Makhija SN, Vavia PR. Stability indicating LC method for the estimation of venlafaxine in pharmaceutical formulations. J. Pharm. Biomed. Anal., 2002; 28: 1055–9. [PubMed] [Google Scholar]

13. ICH, Q2A Text on validation of analytical procedures, international conference on harmonization. 1994 Oct. [Google Scholar]
14. ICH, Q3B validation of analytical procedures: methodology, international conference on harmonization. 1996 Nov. [Google Scholar]