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RESEARCH ARTICLE

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A NOVEL RP-HPLC METHOD FOR THE DETERMINATION OF VENLIFAXINE IN PHARMACEUTICAL DRUG PRODUCTS

ABSTRACT

Sensitive, simple and accurate high performance liquid chromatographic (HPLC) methods for the determination of Venlafaxine in bulk drug and pharmaceutical dosage form. The quantification was carried out using C 18 (chromosil, 250mm, 4.6mm) column and mobile phase comprised of Water, Tri ethyleamine, 1% Ortho Phosphoric acid in proportion of 50:30:20 (v/v/v) at pH 4.2. The flow rate was 1.5 ml/min and the effluwas monitored at 245 nm. The retention time of Venlafaxine is 4.0min. The method was validated in terms of linearity, precision, accuracy, and specificity, limit of detection and limit of quantitation. Linearity of Venlafaxine is 3-21ppm. The percentage recoverie is 99.85% from the tablet formulation. The proposed method isuitable for simultaneous determination of pioglitazone and glimepiride in pharmaceutical dosage form and bulk drug.

KEYWORDS: Venlafaxine, HPLC, Development, Method validation.

INTRODUCTION

Venlafaxine (1-[2-dimethylamino)-1-(4-methoxy phenyl) ethyl] Cyclohexanol) (brand name: Effexor or Efexor) is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class. introduced by Wyeth in 1993. Venlafaxine used to treat the major depressive disorder (MDD), as a treatment for generalized anxiety disorder with depression social phobia, panic disorder, and vasomotor symptoms.[4] Neurotransmitters are the chemical messengers which produced and release by nerve cells to cause the cells to become more or less active. Imbalance in these neurotransmitters is the cause of depression and also may play a role in anxiety. Venlafaxine affects on chemical messengers like neurotransmitters, serotonin, dopamine, and norepinephrine within the brain. Venlafaxine is work by inhibiting the release or affecting the action of these neurotransmitters.

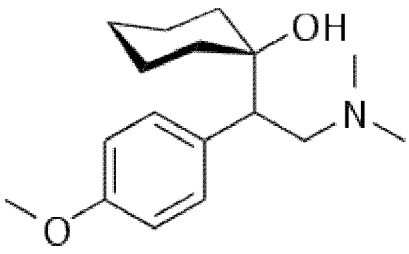

Fig: 1 Structure of Venlafaxine

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Literature survey reveals most of the methods are reported for analysis Cenlafaxine in Serum and biological fluids. There was very few HPLC methods have been reported for simultaneous estimation of Venlafaxine in pharmaceutical dosage form, which prompted to pursue the present work. The objective of the present work is to develop and validate new analytical methods for simultaneous determination of Venlafaxine.

MATERIALS AND METHOD

Apparatus and Chromatographic conditions:

Chromatographic separation was achieved on a A Series 200 HPLC system PEAK LC7000 equipped with isocratic HPLC with PEAK 7000 delivery system. PEAK LC7000 UV/Visible detector with soft ware, Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, Electronic balance-DENVER (SI234) was used for separation. Injection volume is 20µL and UV absorbance measured at 245nm. Mobile phase consisting of Water, Tri ethyleamine, 1% Ortho Phosphoric acid in proportion of 50:30:20 (v/v/v) at pH 4.2.. The mobile phase was filtered through a 0.45µ membrane filter and sonicated for 15min.

Reagents and Solutions:

Pure (not less than 98.5%) standards HPLC grade Water is used for this study. All other reagents (Ortho phosphoric acid, Tri ethyleamine) used in this study were of AR grade. HPLC grade Water was purchased from E. Merck (Mumbai, India).

Determination of wavelength of maximum absorbance

The standard solutions of Venlafaxine was scanned in the range of 200 -400 nm against mobile phase as a blank. Venlafaxine sh Venlafaxine owed maximum absorbance at 245nm. So the wavelength selected for the determination of Venlafaxine was 245 nm.

Standard solution:

Weighed accurately 50mg of pure standard and transferred into 100 ml of volumetric flask, dissolved the contents with 50ml of diluent, sonicated for 15min and diluted to 100ml volume with diluents. The above resulting solution diluted in to a suitable volumetric flask (50ppm for each active ingredient).

Sample solution:

Market available dosage form was analyzed with a concentration of 50ppm for each ingredient.

RESULTS AND DISCUSSIONS

Method development:

Method development trials were performed with different buffer salts, organic modifiers and columns. Finally the separation was achieved with Water, Tri ethyleamine, 1% Ortho Phosphoric acid in proportion of 50:30:20 (v/v/v) at pH 4.2 at 245nm. Report of Standard solution represented in figure-2. The active ingredient was well separated and the peak shape, resolution (not less than 5.0) and tailing factor (not less than 2) were also within the limit.

Method validation:

Validated the finalized method as per ICH and FDA with parameters like specificity, precision, accuracy, linearity and range, ruggedness, robustness etc.

Specificity

Different forced degradation studies were performed with acid, alkali, peroxide, UV and photo degradation conditions. The sample was passed the purity test. The purity angles for drug components in all stress conditions were found to be less than the threshold angle and no interference was observed with diluent and placebo.

Linearity:

The linearity of method was evaluated by analyzing different concentrations (0.3ppm to 21ppm for each ingredient) of the standard solution. Calibration graph was plotted against peak area and concentration of solution. The Intercept value (0.999) found to be within the limit 0.999. The linearity results tabulated in table-3 and linearity plots were represented in graph-1.

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Linearity test

S.NO	CONC mg/ml	AREA
1	3 ppm	51043.8
2	6 ppm	103463.4
3	9 ppm	149327.2
4	12 ppm	204588.9
5	15 ppm	256138.2
6	18 ppm	316965.6
7	21 ppm	364387.4

Table 1: Linearity of Venlafaxine

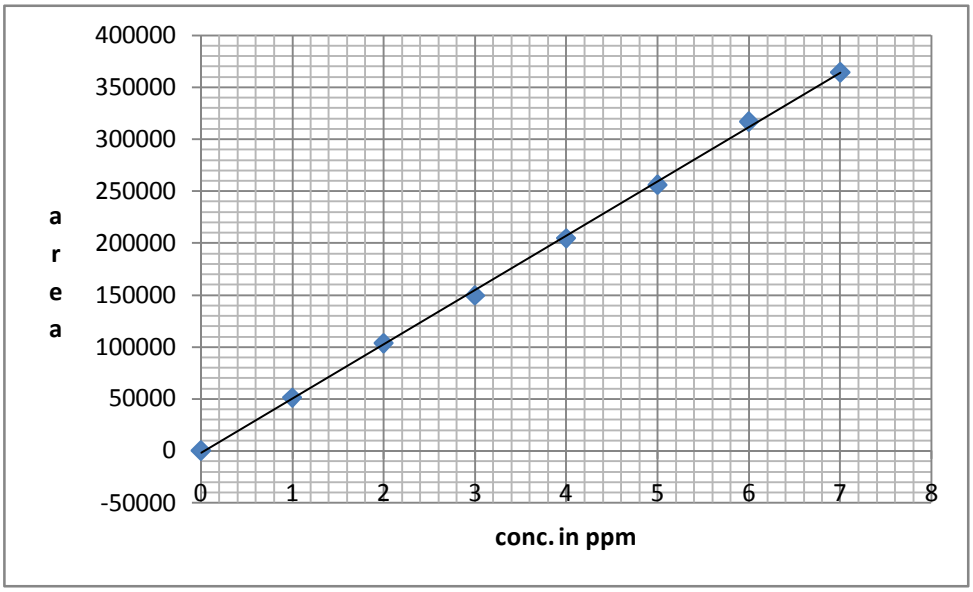

Figure2 Calibration curve of Venlafaxine

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Drug	Carvedilol
Concentration range	3-21ppm
Slope (m)	104609.64
Intercept (b)	-2328
Correlation coefficient	0.999
% RSD	

Table.2 Linear Regression Data for Calibration curve

Precision:

Precision was evaluated by repeatedly injecting (n = 6) solution(12ppm) of Venlafaxine at proposed method conditions. Percentage relative standard deviation (% RSD) was found (RSD-0.198) to be less than 1% for within a day, which proves that the developed method is precise. Results were tabulated in Table-1.

INJECTION	CONCENTRATION	PEAK AREA
1	12ppm	206748.7
2	12ppm	206073.6
3	12ppm	206439.9
4	12ppm	206892.5
5	12ppm	206038.7
6	12ppm	206985.3

Table 3: Precision parameters of Venlafaxine

Accuracy:

Accuracy of the method was carried out with a known quantity of the pure drug was added to the placebo sample at the levels of 50%, 100% and 150% of the test concentration. The contents were determined from the respective chromatograms. The concentration of the drug product in the solution was determined using assay method. The mean recoveries were (101.33, 99.3, 99.68**)** in range of 99.66-100.611 % which shows that there is no interference from excipients. Table-4 represents the recovery results.

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Recovey	Conc. of sample ppm	Recovery	% of recovery
50%	6	5.98	99.66
100%	12	11.975	99.792
150 %	18	18.11	100.611

Table 3: Accuracy results of Venlafaxine

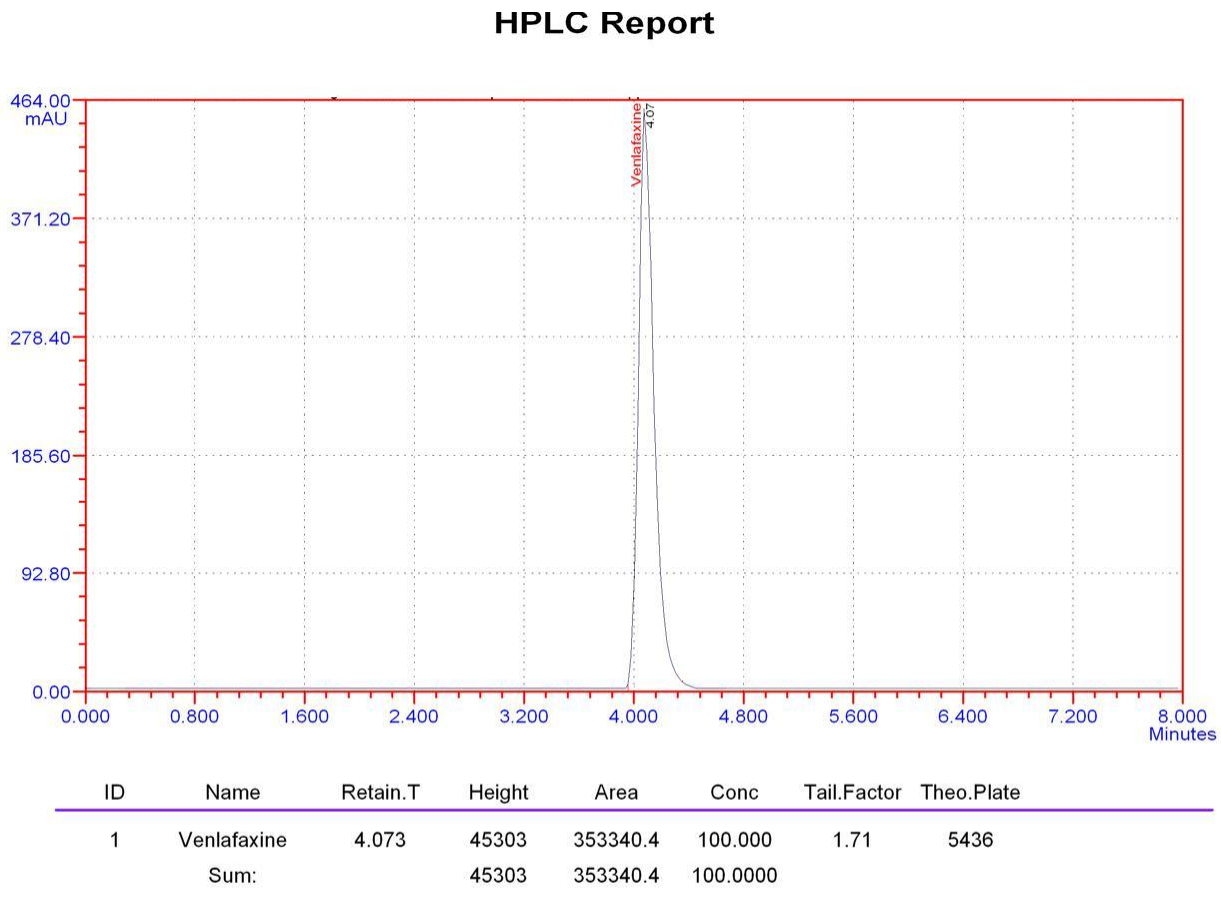

Figure 3: Typical chromatogram of Venlafaxine Standard.

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Ruggedness and Robustness:

The ruggedness of the method was determined by carrying out the experiment on different analysts using different columns of similar types. The percentage of assay of different preparations assay values with two different analysts and columns were 99.5%, 98.7% respectively.

Robustness of the method was determined by making slight changes in the chromatographic conditions, such as flow rate and column pH, Mobile phase ratio. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed is rugged and robust. The robustness limit for mobile phase variation, flow rate variation and pH variation were well within the limit, which shows that the method is having good repeatability under given set of conditions and results were within the limit. Robustness results were tabulated in table-5

Condition	**Mean area**	**% assay**	**% difference**
Unaltered	353340.4	100.0	0.0
Flow rate at 1.4 mL/min	348561.5	98.64	1.36
		1.043	1.43
Flow rate at 1.6mL/min	358412.4		
Mobile phase: Water : TEA : OP			
50% 28% 18%	351462.8	99.46	0.54
52% 26% 22%	353655.2	100.08	0.08
pH of mobile phase at 6.0	355742.9	100.67	0.67
pH of mobile phase at 5.6	348965.2	98.76	1.24

Table-5 Result of Ruggedness and Robustness

System suitability

System suitability parameters were established by injecting the freshly prepared standard solution (each active 21ppm/five replicate injections) in to the chromatographic system. Calculated the percent relative standard deviation for peak area and retention time and results found to be satisfactory. The system suitability results are within their parameter range like Tailing Factor ≤2, Theoretical Plates ≥2000. System suitability results tabulated in table-1.Standerd peak was shown in Fig-2

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Parameters	Values
λ max (nm)	250
Beer's law limit (µg/ml)	3-21mg/ml
Correlation coefficient	0.999
Retention time	4.0 min
Theoretical plates	364387
Tailing factor	1.71
Limit of detection	15ng/mll
Limit of quantification	50ng/ml

Table 5: System suitability parameters of Venlafaxine

Limit of detection and Limit of quantification

Limit of detection (LOD) is defined as the lowest concentration of analyte that gives a detectable response. Limit of quantification (LOQ) is defined as the lowest Concentration that can be quantified reliably with a specified level of accuracy and Precision. For this sample was dissolved by using Mobile Phase and injected until peak was diapered. After 15ng/ml dilution, Peak was not clearly observed. So it confirms that 15ng/ml is limit of Detection. For this study six replicates of the analyte at lowest concentration were Measured and quantified. The LOD and LOQ of Venlafaxine are given in Table-6.

Parameter	Measured
LOD	15ng/ml
LOQ	50ng/ml

Table 6: LOD and LOQ values of Venlafaxine.

RESULT AND DISCUSSION

The nature of the sample, its molecular weight and solubility decides the proper selection of the stationary phase. The drug Venlafaxine being non-polar is preferably analyzed by reverse phase columns and accordingly C18 column was selected. So the elution of the compound from the column was influenced by polar mobile phase. The concentration of the methanol and Acetonitrile were optimized to give symmetric peak with short run time based on 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 Water, Tri ethyleamine, 1% Ortho Phosphoric acid in proportion of 50:30:20 (v/v/v).The retention time of Venlafaxine was found to be 4.0

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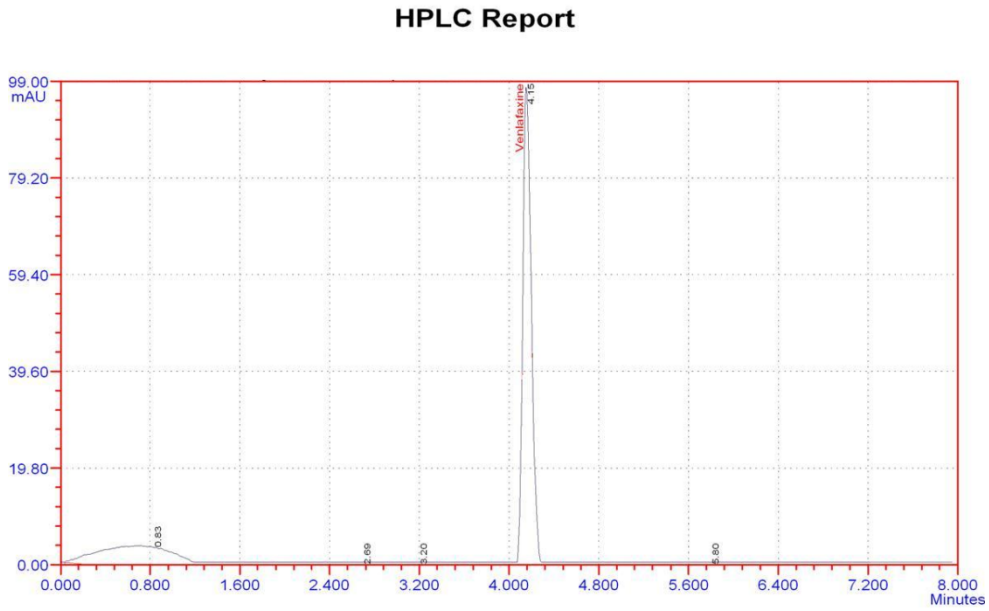
min, which indicates a good base line. The RSD values for accuracy and precision studies obtained were less than 2% which revealed that developed method was accurate and preciseThe high percentage of recovery of Venlafaxine was found to be 99.65 indicating that the proposed method is highly accurate. Proposed liquid chromatographic method was applied for the determination of Venlafaxine in tablet formulation. The result for Venlafaxine was comparable with a corresponding labelled amount (Table 7). The absence of additional peaks indicates no interference of the excipients used in the tablets.



ID	Name	Retain.T	Height	Area	Conc	Tail.Factor	Theo.Plate
1		0.825	505	27579.5	40.282	0.84	5
2		2.690	549	16738.1	24.448	0.92	155
3		3.197	377	2184.4	3.191	1.84	6066
4	Venlafaxine	4.152	5747	19508.0	28.493	1.29	29813
5		5.800	327	2455.2	3.586	1.32	11893
	Sum:		7505	68465.4	100.0000		

Figure 4: Typical chromatogram of Venlafaxine Formulation

Formulation	Tablet dosage	Sample concentration	Amount of drug estimated	% OF DRUG ESTIMATED
VENAXIN	25 mg	12.5 PPM	12.483 PPM	99.864

Table 7: Chromatographic conditions of Venlafaxine

CONCLUSION

The complete study results reveals that the developed and validated method has applicable for the determination of Venlafaxine in pharmaceutical drug products. The developed method has potential application for all ingredients and applicable for routine quality control analysis.

REFERENCES

1. Muth EA, Haskins JT, Moyer JA, Husbands GE, Nielsen ST, Sigg EB (December 1986). "Antidepressant biochemical profile of the novel bicyclic compound Wy-45,030, an ethyl cyclohexanol derivative". *Biochemical Pharmacology* **35** (24): 4493–7.
2. Yardley JP, Husbands GE, Stack G, *et al.* (October 1990). "2-Phenyl-2-(1-hydroxycycloalkyl)ethylamine derivatives: synthesis and antidepressant activity". *Journal of Medicinal Chemistry* **33** (10): 2899–905.
3. Bymaster FP, Dreshfield-Ahmad LJ, Threlkeld PG, *et al.* (December 2001). "Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors". *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* **25** (6): 871–80.
4. "venlafaxine-hydrochloride". *The American Society of Health-System Pharmacists*. Retrieved 3 April 2011.
5. Mandrioli R, Mercolini L, Cesta R, Fanali S, Amore M and Raggi Chromatogr B., 2007, 856(1-2), 88-94.
6. Liu W, Wang F and Li H, J Chromatogr B., 2007, 850(1-2), 183-189.
7. Ebenezer B, Asafu-Adjaye, Faustino P J, Tawakkul M A, Anderson L W, Kwon H and Volpe D A, J Pharm Biomed Analysis, 2007, 43(5), 1854-185910.
8. Kirchherr H and Kuhn-velten W N, J Chromatogr B., 2006, 843(1), 100-113.11.
9. Juan H, Zhiling Z and Huande L, J Chromatogr B., 2005, 820(1), 33-39.
10. . Waschgler R, Moll W, Konig P and Conca A, Int J Clin Pharmacol Ther., 2004, 42, 713.

11. Raut B B, Kolte B L, Deo A A, Bagool M A and Shinde D B, J Liq ChromaTechnol., 2003, 26(8), 1297-1313.
12. Frahnert C, Rao M L and Grasmader K, J Chromatogr B., 2003, 794(1), 354.
13. International Conference on Harmonization, Q2B: Validation of Analytical Procedures: Methodology and Availability, Federal Register, 1997, 62 (96), 27463–27467.
14. FDA, Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation, Availability, Federal Register (Notices), 2000, 65(169), 52776–52777.
15. www.fda.gov/cder/guidance/cmc3.pdf
16. USP 25–NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopeal Convention, Rockville, Maryland, USA, 2002), 2256.

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