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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF VENLAFAXINE HYDROCHLORIDE IN BULK AND DOSAGE FORM

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ABSTRACT

The presented experiment was intended to develop a sensitive, precise, economic and accurate RP-HPLC method for the analysis of Venlafaxine (VNF) in bulk and pharmaceutical dosage forms. Mobile phase composition of Acetonitrile: Water dissimilar amalgamations were studied as mobile phase on a C18 stationary phase. A mobile phase composition in the ratio of 55:45 at pH 4.3 adjusted with ortho phosphoric acid was found to be the most appropriate of all amalgamations studied. Seeing as, the chromatographic peaks were better definite and determined and approximately complimentary from tailing. The retention time estimated for VNF was 4.61min (Mean RT of SST). System suitability testing was carried out by means of six repeated injections of standard solution of the VNF (10µg/ml) and the calculated parameters were obtained within the acceptance criteria as per ICH Q2R1 guideline. The peak areas of Venlafaxine were reproducible as indicated by % RSD within acceptance criteria. A good linear relationship (r2 = 0.9987) was observed between the standard concentration of VNF and the respective mean peak areas in the range of 5-30µg/ml. The regression curve was constructed by linear regression fitting and its regression equation was y = 15121x – 1994.3. Where, Y gives mean peak area and X is the concentration of the drug). Validation of the developed RP-HPLC method of VNF was performed by ICH guideline Q2R1.

KEYWORDS: RP-HPLC, Venlafaxine, Validation, ICH guidelines.

INTRODUCTION

Venlafaxine (Effexor) is an antidepressant of the serotonin-norepinephrine reuptake inhibitor (SNRI) class first introduced by Wyeth in 1993. It is prescribed for the treatment of clinical depression and anxiety disorders. Due to the pronounced side effects and suspicions that venlafaxine may significantly increase the risk of suicide it is not recommended as a first line treatment of depression. However, it is often effective for depression not responding to SSRIs. Venlafaxine was the sixth most widely-used antidepressant based on the amount of retail prescriptions in the US (17.1 million) in 2006. For the management of major depressive disorder (MDD), generalized anxiety disorder (GAD), social anxiety disorder (social phobia), panic disorder with or without agoraphobia, vasomotor symptoms in women with breast cancer and in postmenopausal women, and neuropathic pain. Venlafaxine is usually categorized as a Serotonin-Norepinephrine reuptake inhibitor (SNRI), but it has been referred to as a serotonin-Norepinephrine-dopamine reuptake inhibitor (SNDRI). It works by blocking the transporter "reuptake" proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse. The neurotransmitters affected are serotonin and Norepinephrine. Additionally, in high doses it weakly inhibits the reuptake of Dopamine, with recent evidence showing that the Norepinephrine transporter also transports some Dopamine as well, since dopamine is inactivated by norepinephrine reuptake in the frontal cortex.

MATERIALS AND METHODS

An analytically pure sample of Venlafaxine standard was procured as gift sample Intas Pharmaceutical gujrat, India. All the chemicals were analytical grade, water and methanol also HPLC grade. All reagents and chemicals used were as listed in **Table No 1.** and of HPLC grade hence no further characterization was done.

Table 1: Characterization of reagents and chemicals.

Sr. No.	Chemical	Grade	Make	Characterization
1	Water	HPLC	--	As HPLC grade no further characterization performed
2	Acetonitrile	HPLC	Fisher Scientifics	As HPLC grade no further characterization performed
3	Triethyl amine	HPLC	Merck	As HPLC grade no further characterization performed
4	Ortho phosphoric acid	HPLC	Finar	As HPLC grade no further characterization performed

Preparation of standard stock solution of VNF for trial runs

Accurately weighed 10mg of VNF and transferred to100 ml volumetric flask holding a small amount of mixture of solvent (ACN 50: Water 50). The volume was made up to the mark using same mixture of solvent slowly and with little quivering to make the resultant solution of 100µg/ml. This solution was ultrasonicated for 30 min in three cycles each of 10 min. In addition, it was the filtered through 0.45µ membrane filter in order to eliminate particulate matter if any.

Preparation of working solution of VNF

1.0ml of above standard stock solution was pipetted out in 10 ml volumetric flask and diluted to 10ml with mixture of solvent (ACN 50: Water 50) to make the resultant solution of 10µg/ml. This initial standard solution was ultrasonicated for 30 min in three cycles each of 10 min. Also, it was then filtered through 0.45µ membrane syringe filter before injection to HPLC.

Preparation of standard stock solution of VNF in mobile phase

Accurately weighed 10mg of VNF and transferred to 100ml volumetric flasks already containing some amount of mobile phase (ACN: Water, 55:45 at pH 4.3). The volume was made up to the mark slowly with occasional shaking using mobile phase. The resultant solution of VNF was attained as 100µg/ml. This solution was further filtered through 0.45µ membrane filter paper. Also, it was ultrasonicated for 30 min in three cycles each of 10min for degassing purpose.

Preparation of standard working solution of VNF in mobile phase

1.0ml of above standard stock solution (100µg/ml) was pipette out using micropipette and kept in 10 ml volumetric flask. The volume was then made up to the mark with mobile phase (ACN: Water, 55:45 at pH 4.3) to form the resulting solution of 10µg/ml. The consequential solution was then filtered through 0.45µ syringe filter paper and ultrasonicated for 10min for degassing. The working solution was then filled in HPLC vials for further analysis.

Selection of mobile phase for quantification of venlafaxine

The mobile phase selection was achieved by performing trial runs. The details of conditions for trial runs of Venlafaxine were as listed in **Table** no 2.below.

Determination of absorption maxima of VNF

At the beginning of the study, the quantification of Venlafaxine was planned in reverse phase mode. The detector was set at 225nm (as per compendial monograph) and the mobile phase composition was Acetonitrile and Water in the proportion of 50: 50. First injection was performed as a blank run to observe baseline characteristics. The Baseline was found to be stable. The trial run was performed and first chromatogram was recorded as shown in **Figure 1** and observed for the results with respect to retention time, peak shape, peak area, number of theoretical plates etc. The peak was observed for VNF. So the chromatographic conditions of trial 1 were rejected and another modification as shown in. The trial 2 chromatogram was recorded as shown in **Figure 2**. The chromatogram showed peak but its shape was not appropriate. So, it was also rejected. Also, it was assumed from the peak shape that, aqueous phase should consists of some ion pairing reagent in order to get suitable peak shape and chromatographic results.

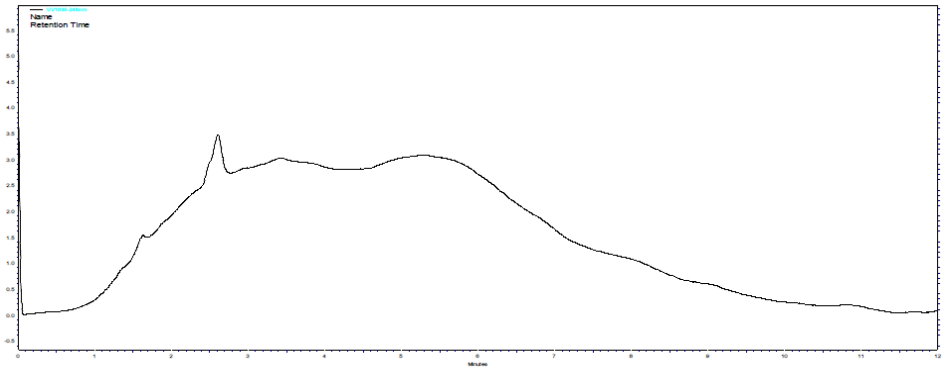

Figure 1: Trial 1 with 50% v/v ACN at 1.0mL/min flow rate.

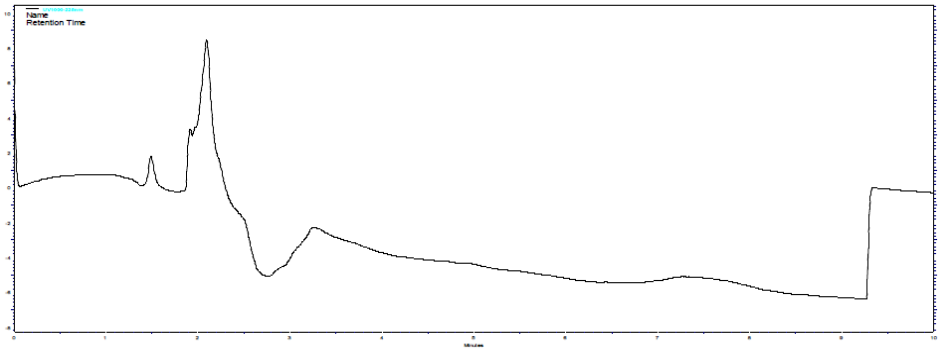

Figure 2: Trial 2 with 70% v/v ACN at 1.0mL/min flow rate.

Therefore, another trial 3 was executed with two major modifications in the mobile phase. The ACN concentration was reduced to 55% v/v and Triethylamine was used as an ion paring reagent. Also, the pH of the aqueous phase was adjusted to 4.3 with the help of phosphoric

acid (HPLC grade). The trial 3 chromatogram was recorded as shown in **Figure 3.** The chromatogram showed acceptable chromatographic characteristics as retention time, peak area, number of theoretical plates and tailing factor as 4.10min, 146382, 4374 and 1.14 respectively.

Therefore, chromatographic conditions of trial 3 were further maintained throughout RP-HPLC method development of VNF in the later sections.

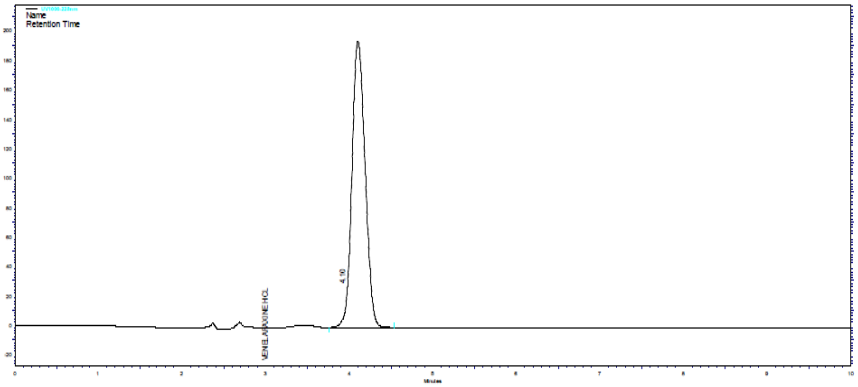

Figure 3: Trial 3 with 55% v/v ACN at 1.0mL/min flow rate, pH of aqueous phase 4.3.

Table 2: Characterization of Reagents and Chemicals.

S. no.	Organic concentration (% v/v)	pH of aqueous phase	Wavelength (nm)	Flow rate In mL/min
1	50 % Acetonitrile	--	225	1.0
2	70 % Acetonitrile	--	225	1.0
3	55 % Acetonitrile	4.3 of aqueous phase	225	1.0

This study was performed in order to set the position of HPLC detector for analysis of VNF during HPLC method development. Also, it was carried out to confirm the sample purity of VNF obtained as a gift sample. UV spectroscopy is a powerful technique as per as the identification of drug substances are concerned. The drug substances shows different wavelength maximum in various solvents and solvent compositions. Almost all drug substances consist of chromophore in their structure which can be identified in UV spectroscopy.

The wavelength maximum of VNF was determined in solvent mixture of Methanol and water in the proportion of 50:50. The 10ppm solution of VNF was used for this study. The spectrum observed was shown in **Figure 4**. The absorbance values obtained by peak to peak calculations were as tabulated in **Table 3.** As shown in observation table, the maximum

absorbance was seen at 225nm. Therefore, this wavelength was used for further analysis using RP-HPLC. The RP-HPLC detector was tuned to this specific wavelength to detect VNF.

Table 3: Observation table for absorbance values recorded during wavelength determination using UV spectroscopy.

Sr. No.	Wavelength (nm)	Absorbance
1.	248	0.944
2.	203	1.954

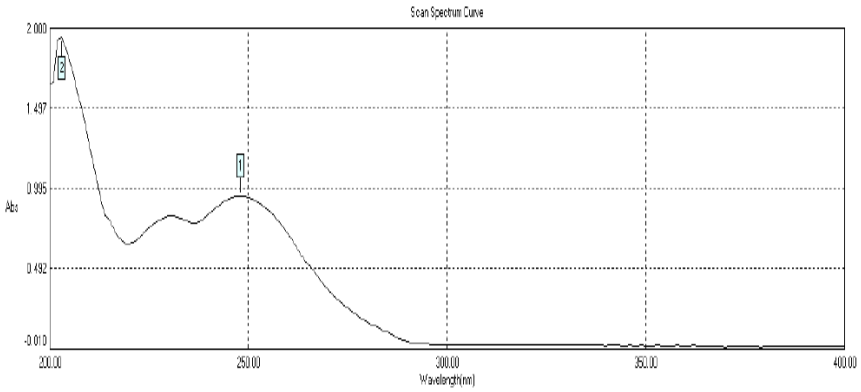

Figure 4: UV spectrum of venlafaxine in methanol water 50:50.

System suitability testing (SST)

System suitability test was performed with six repeated measurements of 10µg/ml standard working solution of VNF. The chromatograms recorded were integrated for determination of retention, peak area, number of theoretical plates and asymmetry factors. The results obtained are as tabulated in Table 5. The results are compared with standard prescribed in ICH Q2R1 guideline. As shown in Table 5 the results obtained for percent RSD of retention time and mean peak area were found to be 0.32 and 1.80 respectively. Also, the number of theoretical plates (NOP) and asymmetry factor for representative chromatogram as shown in **Figure 5** were 5627 and 1.58 respectively. From the results obtained, it was accomplished that the current method successfully passed for SST as the results obtained for all parameters were in agreement with ICH guideline.

System suitability testing

A laboratory would run the SST before submitting a sample batch to ensure that each component of the system (mobile phases, column, pumps, auto-sampler, mass spectrometer,

acquisition method, etc.) meet the in-house performance criteria for that method. Ideally, a lab would make the SST material in bulk, then aliquot and store it for quick and easy use.

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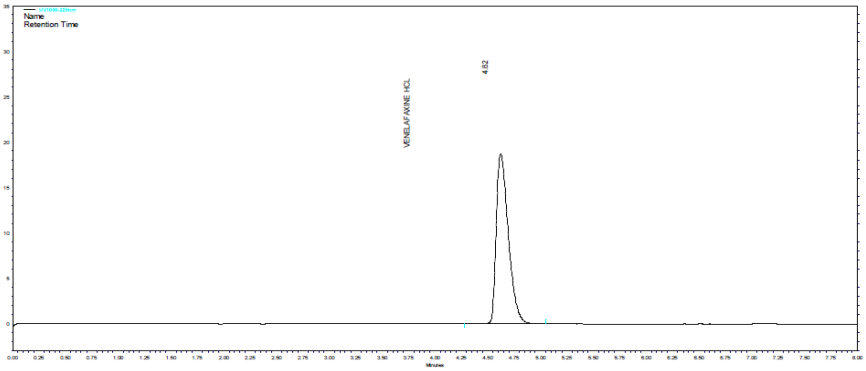

Figure 5: Chromatogram obtained in SST of Venlafaxine.

RP-HPLC method validation

Linearity and Range

Mathematically speaking, linearity is a function of values that can be graphically represented as a straight line. Similarly, as per the ICH Q2 (R1) guideline, the linearity of an analytical method can be explained as its capability to show *"results that are directly proportional to the concentration of the analyte in the sample"*. Linearity is often measured within a given range.

Table 4: Observations recorded for 10µg/ml conc. of VNF in SST.

Sr. No.	Parameter	Mean observations	SD	% RSD	Acceptance criteria	Inference
1	Peak Area	143589.50	2589.10	1.80	< 2	Pass
2	Retention	4.62	0.0149	0.32	< 0.5	Pass

	time					
3	Number of Theoretical plates*	5627	--	--	> 2000	Pass
4	Tailing factor*	1.58	--	--	< 2	Pass

The results were represented in terms of the correlation coefficient r2 = 0.9987, Y-intercept = -1994.3, slope of the regression line as 15121. The regression equation was therefore:
Y = 15121x – 1994.3.

The results recorded for all six standard solutions of VNF in the range of 5-30µg/ml were as tabulated in **Table 5**. Also, the calibration curve plotted between standard concentrations of VNF corresponding to mean peak area was as shown in **Figure 6**. Finally, it was concluded that the method was found to be linear in the concentration range of 5-30µg/ml of VNF. Also linearity showed that the method was sensitive to determine equivalent concentration of VNF in the given range of 5-30µg/ml.

Table 5: Observations for linearity experiment of venlafaxine.

Conc. of VNF std solution (µg/ml)	Mean peak Area of VNF*
5	72547
10	145539
15	226123
20	310173
25	372579
30	448733

Mean peak area is of three repeated measurements

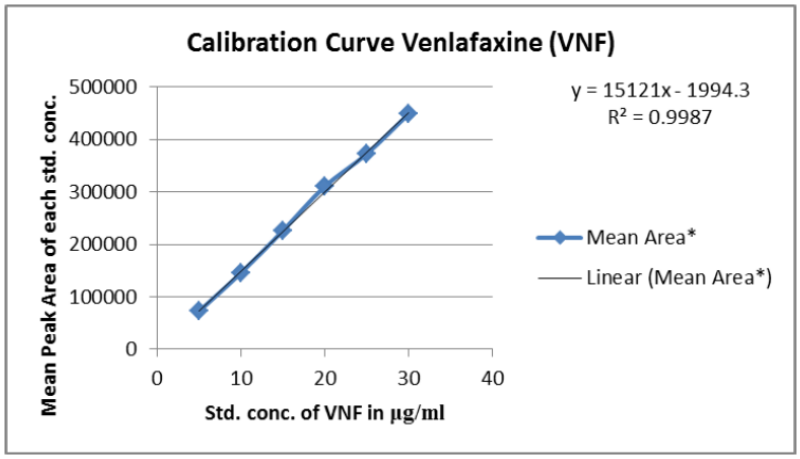

Figure 6: Calibration curve of Venlafaxine showing linearity in the range of 5-30µg/ml.

Precision

Precision may be considered at three levels: repeatability, intermediate precision, and reproducibility. Repeatability reflects precision under the same operating conditions over a short interval of time, while intermediate precision expresses within-laboratory variation, such as different days, different analyst, different equipment, and reproducibility is an indication of precision between laboratories.

Repeatability (inta-day precision) should be assessed using a minimum of 9 determinations covering the specified range for the procedure by 3 replicates or 6 determinations at 100% of the test concentration.

Intermediate precision (inter-day precision) depends upon the circumstances under which the procedure is intended to be used. The specific day, analyst performing, equipment is the random events that cast effect on the precision of the analytical procedure. It is not considered necessary to study these effects individually.

Three quality control (QC) standards were defined in the given range of linearity viz. 7, 18 and 28µg/ml. The standards were used to study repeatability as well as intermediate precision.

Repeatability of the method was studied by planning an experiment in a day. Three repeated measurements of QC standards were carried by injecting its solution to the predetermined chromatographic conditions as per chapter 6, section 6.2.2. The same measurements are repeated three times in a day at an interval of three hrs for all QC standards as above.

The intermediate precision was estimated by planning an experiment on three different days with identical samples of three QC standards. For inter day precision (intermediate precision) three QC standards measured on three days in triplicate. The results obtained were as shown in **Table 6**. The results obtained in terms of peak area were then subjected for statistical evaluation by calculating SD and % RSD in both case i.e. repeatability and intermediate precision. The percent RSD found for repeatability was in the range of 0.98 to 1.84. However, it was for intermediate precision in the range of 0.41 to 1.09 as shown in **Table 6**. The results were compared for acceptance criteria as per ICH guideline and were found in agreement with the limits prescribed.

Therefore, it was concluded that the present method passes for test for precision.

Table 6: Observation table for results of Repeatability and Intermediate precision.

Conc. (µg/ml)	Intra-day precision			Inter-day precision		
	Mean area ± SD	% RSD	Inference	Mean area ± SD	% RSD	Inference
15	102482.33 ± 1004.47	0.98	Pass	102886.33 ± 976.81	0.95	Pass
35	274419.00 ± 4641.83	1.69	Pass	271364.56 ± 2958.07	1.09	Pass
55	415721.33 ± 7653.34	1.84	Pass	415429.78 ± 1720.30	0.41	Pass

% Accuracy

Accuracy is a measure of the closeness of the experimental value to the actual amount of the substance in the matrix. Precision measures of how close individual measurements are to each other.

The accuracy of the present method was determined from the results obtained in intermediate precision experiment for three QC standards (as they are defined across range of linearity and measured in triplicate). The mean peak area obtained in each of this case was subjected to determine corresponding mean measured concentration using regression equation. The percent accuracy was then finally determined from mean measured concentration using following formula.

$$\% \text{ Accuracy} = \frac{\text{Mean measured concentration}}{\text{Nominal Concentration}} \text{ x } 100$$

The results obtained for this study were as tabulated in **Table 7**. The percent accuracy from the results of intermediate precision was found in the range of 98.59 to 100.43 % w/w of VNF (**Table 7**). From the results obtained it was concluded that the present method passes for test of accuracy as per ICH Q2R1 guideline.

Table 7: Observations for percent accuracy determined for three QC standards of intermediate precision.

Sr. No.	Conc. (µg/ml)	Mean Peak Area*	Mean Measured Conc. (µg/ml)	% Accuracy (w/w)	Inference (Std. for VNF 95-105% w/w)
1	07	102886.33	6.94	99.09	Passed
2	18	271364.56	18.08	100.43	Passed
3	28	415429.78	27.61	98.59	Passed

Robustness

Robustness is typically evaluated during method development/optimization, but can have a pronounced effect on the validation of a method. Robustness experiments measure a method's ability to remain unaffected by small but deliberate variations in method parameters. Examples of potentially sensitive processes include. Column oven temperature, the percent organic phase, pH, or buffer concentration of mobile phase, wavelength may also be an important for chromatographic separations.

The robustness of the present method was studied by variation of two method parameters viz. concentration of Acetonitrile (±5%) and wavelength (±1nm), initially, the organic concentration was varied and the chromatograms recorded with 10µg/ml standard solution of VNF. This concentration was kept constant throughout the experiment. The results were recorder for peak area. The mean area was then utilized to calculate mean measured concentration of standard VNF using regression equation. The later was then used to calculate percent assay of the VNF corresponding to each concentration at level of change.

Table 8: Observations for robustness study with variation in ACN concentration.

Methanol Concentration (%)	Standard Conc. (µg/ml)	Mean peak area*	Mean measured conc. (µg/ml)	% Assay (%w/w)	Inference (Std for VNF is 95-105 % w/w)
60	10	145539	9.76	97.57	Passed
64	10	148829	9.97	99.74	Passed
56	10	142035.00	9.53	95.25	Passed

*Mean area of three repeated measurements of VNF 10µg/ml to predetermined chromatographic conditions.

Table 9: Observations for robustness study with variation in wavelength of the detector.

Flow Rate (ml/min)	Standard Conc. (µg/ml)	Mean peak area*	Mean measured conc. (µg/ml)	% Assay (%w/w)	Inference (Std for VNF is 95-105 % w/w)
1.0	10	145539	9.76	97.57	Passed
1.1	10	147835	9.91	99.09	Passed
0.9	10	150490	10.08	100.84	Passed

*Mean area of three repeated measurements of VNF 10µg/ml to predetermined chromatographic conditions.

Identical procedure was employed to study the robustness with another method parameter i.e. wavelength and percent assay values of VNF corresponding to mean measured concentration were calculated. The results for robustness experiment for ACN concentration variation in terms of percent assay were found in the range of 95.25 to 99.74% w/w of VNF .Similarly percent accuracy noted for variation in wavelength was found in the range of 97.57 to 100.84 % w/w of VNF .The results obtained were evaluated for their acceptance criteria for VNF standards and were found within limits (95-105 % w/w of VNF).

% Recovery

The purpose of analysis of drug substances and other natural products is quantitation of target compounds in the matrix (drug product) in which the compounds occur. The most common technique for determining accuracy in drug product studies is the spike recovery method, in which the amount of a target compound is determined as a percentage of the theoretical amount present in the matrix. In a spike recovery experiment, a measured amount of the constituent of interest is added to a matrix (spiked) and then the analysis is performed on the spiked material, from the sample preparation through chromatographic determination. A comparison of the amount found versus the amount added provides the recovery of the method, which is an estimate of the accuracy of the method. In an ideal situation, such as the determination of a synthetic pesticide in food, the matrix will be devoid of the target analyte(s).

The difference between the theoretical amount and the amount analytically determined in the spiked matrix provides an estimate of accuracy. Other approaches to spike recovery studies include adding the target analyte to a similar matrix that does not contain the target and spiking the target analyte into natural matrix from which the target has been exhaustively extracted and then dried. Recovery is frequently concentration dependent; the FDA guidance for drugs suggests that matrices be spiked at 80, 100, and 120% of the expected value, and that the experiment be performed in triplicatei.

The percent recovery in this method was planned by spike method. A known amount of standard (10µg/ml) was spiked in sample solution prepared from tablet dosage form VNF and final test solution was prepared at three levels viz. 80%, 100% and 120%. The test solutions at these three levels were then injected given of chromatographic conditions in triplicate and chromatograms are recorded. The total peak area (peak area corresponding to sample solution and standard solution spiked of VNF together) was then determined in each case. The peak

area of the spiked standard concentration of VNF was then subtracted from total peak are to get peak area corresponding to sample solution of VNF prepared from powder of 20 tablets (dosage form). The percent recovery at all three levels viz. 80, 100 and 120% was then determined using sample, standard concentrations and corresponding peak area of VNF by using formula given in section 6.2.3.5 of chapter 6.

The results obtained for percent recovery study were as tabulated in **Table 10**. Also, the representative chromatogram of this study obtained at 120% level was as shown in **Figure 7**. From the results obtained, the percent accuracy for VNF from tablet dosage form was found in the range of 95.87 to 101.90 % w/w of the labeled claim of VNF. Also, mean measured concentration corresponding to each sample concentration was determined using regression equation and was found as 7.70, 9.90 and 12.19 µg/ml for 8, 10 and 12µg/ml of VNF.

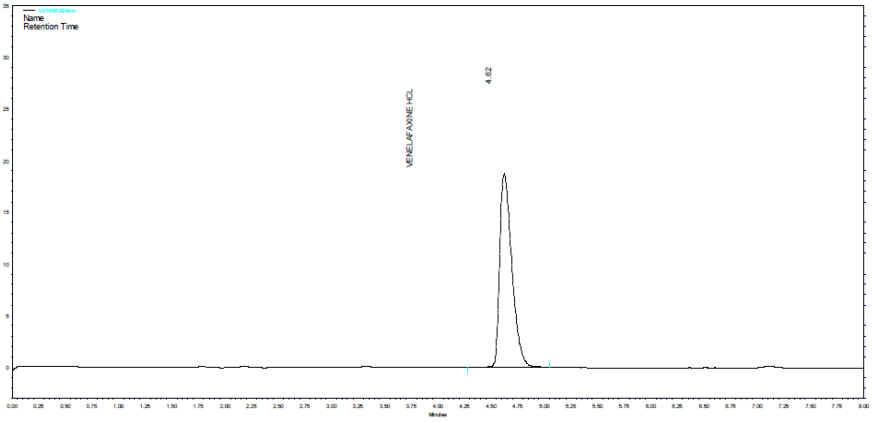

Figure 7: Chromatogram obtained for percent recovery experiment at 80% level.

Results obtained were compared with the standards prescribed for VNF in compendial monograph for Venlafaxine tablets (95-105% w/w of VNF). The results observed for this experiment were found in conformity with that of standards prescribed for Venlafaxine in compendial monographs.

Table 10: Observations for percent recovery experiment of VNF at 80, 100 and 120% levels.

% Recovery Level	Conc. of stad. spiked (µg/ml)	Conc. of sample (µg/ml)	Mean Total peak area*	Mean peak Area of sample conc.*	Amount recovered (µg/ml)	% Recovery	Inference (Std. For PRP 90-110 %w/w)
80	10	08	259939	114400	7.70	95.87	Pass
100	10	10	293243	147704	9.90	99.02	Pass
120	10	12	327937	182398	12.19	101.90	Pass

Therefore, from this experiment of percent recovery, it was concluded that the presented method passed for test for accuracy by percent recovery method. In addition, absence of any additional peaks in the chromatogram test solution of VNF suggested that the present is method is specific for determination of Venlafaxine in sample matrix. Also, with this author have attained third objectives of the research work i.e. exploration of applicability of the method for determination of Venlafaxine in tablet dosage available in the market.

LOD and LOQ

The Limit of Detection (LOD) is defined as the smallest amount or concentration of an analyte that can be reliably detected in a given type of sample or medium by a specific measurement processii. The United States Pharmacopeia defines the LOD as 2 or 3 times the baseline noiseiii. This is derived from the assumption that 3 times the noise will contain approximately 100% of the data from a normal distribution.

Detection limit and quantitation limit were calculated from the standard error of response, 'y' (STEYX) for corresponding standard concentrations, 'x' (5-30µg/ml) of VNF in linearity experiment. The STEYX was found to be 5737.92. The slope of regression equation was 15121. Therefore, LOD and LOQ were calculated by putting the value of STEYX and slope consequent formulae.

$$LOD = \frac{3.3 * 5737.92}{15121}$$

$$LOQ = \frac{10 * 5737.92}{15121}$$

The LOD and LOQ obtained for VNF were as tabulated in **Table 11** and found to be 1.25 and 3.79 respectively.

Table 11: Observations for LOD and LOQ.

Standard Drug Solution	LOD (µg/ml)	LOQ (µg/ml)
Venlafaxine (VNF)	1.25	3.79

From the results as tabulated in **Table 11**, it is restated that the quantity equivalent to 1.25µg/ml and 3.79µg/ml of Venlafaxine (VNF) can be productively sensed and measured

from the planned method correspondingly. Consequently, it was perceived that method passed for test for LOD and LOQ as per ICH guidelines Q2R1.

SUMMARY AND CONCLUSION

The presented experiment was intended to develop a sensitive, precise, economic and accurate RP-HPLC method for the analysis of Venlafaxine (VNF) in bulk and pharmaceutical dosage forms. Mobile phase composition of Acetonitrile:Water dissimilar amalgamations were studied as mobile phase on a C18 stationary phase. A mobile phase composition in the ratio of 55:45 at pH 4.3 adjusted with ortho phosphoric acid was found to be the most appropriate of all amalgamations studied. Seeing as, the chromatographic peaks were better definite and determined and approximately complimentary from tailing. The retention time estimated for VNF was 4.61min (Mean RT of SST).

System suitability testing was carried out by means of six repeated injections of standard solution of the VNF (10µg/ml) and the calculated parameters were obtained within the acceptance criteria as per ICH Q2R1 guideline. The peak areas of Venlafaxine were reproducible as indicated by % RSD within acceptance criteria. A good linear relationship (r2 = 0.9987) was observed between the standard concentration of VNF and the respective mean peak areas in the range of 5-30µg/ml. The regression curve was constructed by linear regression fitting and its regression equation was y = 15121x – 1994.3. Where, Y gives mean peak area and X is the concentration of the drug). Validation of the developed RP-HPLC method of VNF was performed by ICH guideline Q2R1 for parameters like,

System suitability

- Linearity and Range
- Precision
- Accuracy
- Robustness
- Limit of Detection
- Limit of Quantitation

Precision of the method was studies across given range of linearity i.e. 5-30µg/ml. Three QC standards were defined for precision study viz. 7, 18 and 28 µg/ml. When VNF standards solutions containing 7, 18 and 28µg/ml of VNF were analyzed by the proposed method to

establish intra and inter-day precision, low % RSD in acceptance criteria was found in all cases tested (0.98 to 1.84 and 0.41 to 1.09 for intra-day and inter-day precision study).

Robustness of the method was studied to assess an influence of method parameters on method performance in terms of percent assay of VNF. The results obtained for deliberate variations in method parameters viz. ACN concentration and wavelength, were in conformity with an acceptance criteria for VNF in terms of percent assay i.e. within 95-105% w/w.

Applicability of the method for estimation of VNF in marketed formulations was also tested in tablet dosage forms. Percent recovery values were determined at three levels of test concentrations. The results obtained from the tablet dosage form by the proposed method successfully recovered amount of VNF in presence of matrix. The amount recovered for VNF was found in the range of 95.87 to 101.90% w/w. The absence of additional peaks indicates non-interference of common excipients used in the tablets formulation while manufacturing. In addition, it proved that the developed method was specific for determination of VNF in tablet dosage form.

For this reason, ultimately it was concluded that the proposed HPLC method was sensitive and reproducible for the analysis of VNF in pharmaceutical dosage form (tablet) with small analysis time of 8min. Hence, author concluded that, we have attained all predetermined objectives of the proposed research work.

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