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# STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF RELATED SUBSTANCE IN VENLAFAXINE HYDROCHLORIDE TABLETS

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**Keywords:**

RP- HPLC, Impurity, Related substances, Venlafaxine tablets, ICH Guidelines

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**ABSTRACT:** Recently, several methods have been developed for the determination of drugs and their impurities products by Reverse Phase-High Performance Liquid chromatography (RP-HPLC). The present paper describes highly specific, linear, precise, rugged, accurate, robust, and stability, indicating the RP-HPLC method for the determination of related substances present in Venlafaxine tablets as per ICH guidelines. Chromatographic separation of impurities at satisfactory level was achieved at the detection wavelength of 235 nm using column Agilent C18 column (4.6 × 150 mm, 5 µ) and a mobile phase of Methanol: Phosphate buffer of pH 6.8 (60:40% v/v), pumped at a flow rate of 1 ml/min. The % recovery of Venlafaxine and Impurity-A was found to be 98.19% and 98.97%, respectively. The % RSD of Venlafaxine and Impurity-A was found to be 0.52 and 0.83, respectively. The proposed method was found to be specific, linear, precise, accurate, robust, stable, and can be successfully used for the determination of related substances.

**INTRODUCTION:** The impurities in pharmaceuticals are undesirable chemicals that stay with the active pharmaceutical ingredient (APIs) or develop during formulation or after aging of both API and formulation. The presence of these undesirable synthetic compounds, even in small amounts, may impact the efficacy and safety of the pharmaceutical item. Venlafaxine is a cyclohexanol and phenylethylamine derivative that functions as a Serotonin and Noradrenaline Reuptake Inhibitor (SNRI) and is used as an anti-depressive agent with unique pharmacological properties that may enhance its efficacy as well as its safety profile.

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Venlafaxine chemically it is 1-[2-(dimethylamino)-1- (4-methoxyphenyl) ethyl]cyclohexan -1-ol hydr-ochloride. It is white to off-white crystalline solid, soluble in water, with Molecular formula $C_{17}H_{28}ClNO_2$, and is of 313.866 g/mol weight [1]. The chemical structure of Venlafaxine hydrochloride is shown in **Fig. 1**.

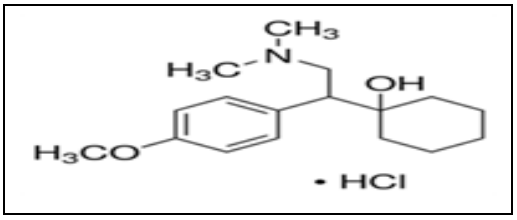


**FIG. 1: STRUCTURE OF VENLAFAXINE HYDRO-CHLORIDE**

**Impurity- A (Descyclohexanol):** The Chemical name is 2-(4- methoxyphenyl)-n, n-dimethyl-ethanamine with molecular weight 179.25 g/mol and Molecular formula $C_{11}H_{17}NO$. The chemical structure of Impurity-A is shown in **Fig. 2**.

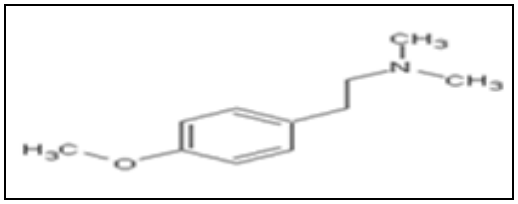

**FIG. 2: STRUCTURE OF IMPURITY-A (DESCYCLO-HEXANOL)**

Literature Review reveals that few methods [2-6] were reported for the determination of impurities in Venlafaxine API. No methods are available for the determination of impurities present in Venlafaxine tablets. Impurities present in drug products depend on synthetic route of active pharmaceutical ingredients and different processes followed for manufacturing. Since HPLC is the most available instrument in quality control laboratories with better selectivity and sensitivity, it was proposed to develop a method based on RP-HPLC mode as per ICH guidelines [7-9].

## MATERIALS AND METHODS:

**Reagents and Chemicals:** The drug sample of Venlafaxine and impurity A (Descyclohexanol) obtained from Dyalabs Mumbai. Acetonitrile (HPLC Grade), water (HPLC Grade), Potassium dihydrogen phosphate (AR Grade), Methanol (HPLC Grade), Sodium dihydrogen phosphate (AR Grade) was obtained from Merck, Hyderabad.

**Preparation of Mobile Phase:** Mix 60 ml Methanol (HPLC grade) and 40 ml pH 6.8 Phosphate buffer degassed in ultrasonicator. It is utilized as a diluent.

**Chromatographic Conditions:** HPLC autosampler – UV detector Separation module 2695, Empower software version-2 manufactured by Waters have been used to achieve study, equipped with pump and degasser, UV detector 2487, injector with 10 µl loop and Agilent C18 column (4.6 mm × 150 mm) with 5 µ internal diameter. In addition, UV double beam spectrometers UV 3000+, UV win software of Lab India, pH meter, Digital weighing balance, Sonicator.

**The Mobile Phase Composed of Methanol:** pH 6.8 Phosphate buffer (60:40% v/v) pumped at a flow rate of 1 ml/min using a detection wavelength of 235 nm and an injection volume of 10 µl. The mobile phase was filtered through 0.45 µ pore size filter and degassed ultrasonically after mixing. The run time was set at 10 min with the HPLC system operating at ambient temperature. The obtained chromatogram as shown in **Fig. 3**.

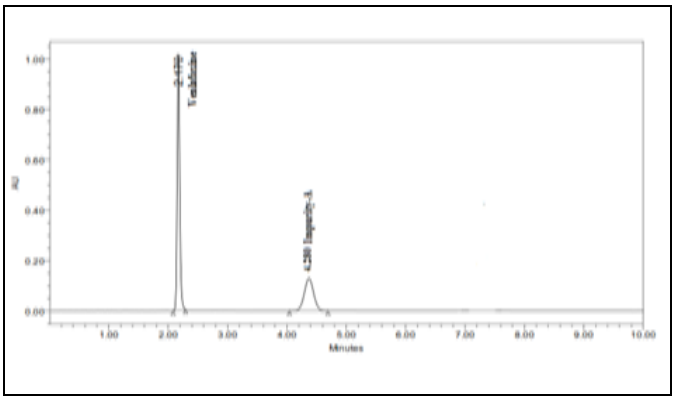

**FIG. 3: OPTIMIZED CHROMATOGRAM**

**Preparation of Solutions:**

**Preparation of Standard Venlafaxine Solution:** 10 mg of Venlafaxine working standard was accurately weighed transferred into a 10 ml volumetric flask sonicate to dissolve it completely and make volume up to the mark with the same solvent. Pipette out 0.5 mL from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent (50 µg/mL).

**Preparation of Standard Impurity-A solution:** 1 mg of Impurity-A weighed and transferred into a 10 ml volumetric flask and made volume up to the mark. Pipette out 1 ml from the above stock solution diluted up to 10 mL with diluents (1 µg/mL).

**Sample Solution Preparation:** Take 10 venlafaxine tablets, weigh and crush. Accurately weigh the powder, quantity equivalent to 10 mg of venlafaxine.

To that add 1 mg of impurity A. Transfer the contents into 10 ml volumetric flask, dissolve it completely and make the volume up to the mark. (Final Concentration is 10 µg/ml and 1 µg/ml)

**Methodology:** Equilibrate the column with the mobile phase for 45 min before analysis. The chromatographic system should satisfy the system suitability limits before analysing sample. Tailing factor (T), theoretical plate number (N) and resolution (Rs) for standard Venlafaxine and standard impurity A were tested.

**RESULTS AND DISCUSSION:** System Suitability (SST) Solution Preparation: Accurately weigh 10 mg Venlafaxine, and 1 mg Impurity-A working standard into a 10 mL volumetric flask, dissolve it and make volume up to the mark. Pipette out 0.1 ml of the above stock solution dilute up to the 10 mL with diluent. (Final Concentration is 10 µg/mL and 1 µg/mL respectively). The SST results were reported in **Table 1**.

**TABLE 1: SYSTEM SUITABILITY RESULTS (SST)**

| S. no. | Name | Retention time(min) | Area (µV sec) | USP resolution | USP tailing | USP plate count |
|---|---|---|---|---|---|---|
| 1 | Venlafaxine | 2.170 | 11235 | | 1.6 | 5038 |
| 2 | Impurity-A | 4.257 | 3245 | 2.4 | 1.3 | 3428.2 |
| | | Acceptance criteria | | NLT 2.0 | NMT 2.0 | NLT 2000 |

**Specificity:** The system suitability for specificity was carried out to determine whether there is any interference of any impurities in the retention time of an analytical peak. The specificity was performed by injecting blank and was found that there were no interferences. The chromatogram as shown in **Fig. 4**.

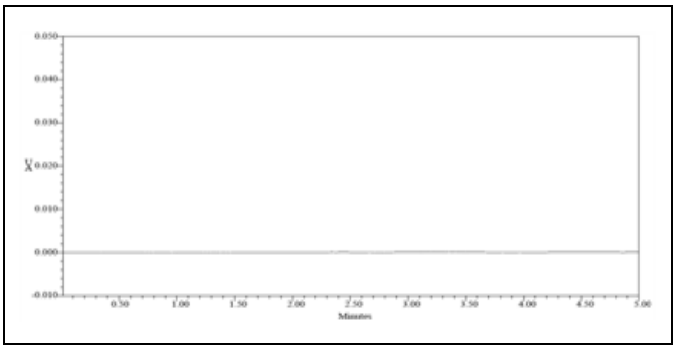

**FIG. 4: CHROMATOGRAM OF BLANK**

**Linearity:** 10 mg of Venlafaxine and 1mg of Impurity-A working standard was accurately weighed and were transferred into a 10 ml clean dry volumetric flask, add about 2 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent

**Preparation of Level – I (50 ppm of Venlafaxine and 5 ppm of Impurity-A):** 0.05 ml of stock solution was taken into 10ml of volumetric flask and diluted up to the mark with diluent.

**Preparation of Level – II (100 ppm of Venlafaxine and 10 ppm of Impurity-A):** 0.10 ml of stock solution was taken into 10ml of volumetric flask and diluted up to the mark with diluent.

**Preparation of Level – III (150 ppm of Venlafaxine and 15 ppm of Impurity-A):** 0.15 ml of stock solution was taken into 10ml of volumetric flask and diluted up to the mark with diluent.

**Preparation of Level – IV (200 ppm of Venlafaxine and 20 ppm of Impurity-A):** 0.20 ml of stock solution was taken into 10 ml of volumetric flask and diluted up to the mark with diluent.

**Preparation of Level – V (250 ppm of Venlafaxine and 25ppm of Impurity-A):** 0.25 ml of stock solution was taken into 10 ml of volumetric flask and diluted up to the mark with diluent.

**Procedure:** Each level was injected, and the peak area was measured. Plot a graph of a peak on X-axis concentration and on the Y-axis Peak area, and the $r^2$ was calculated.

The method for the determination of impurity in venlafaxine was found to be linear, and the correlation coefficient was found to be 0.993. These results were reported in **Tables 2** and **3** and **Fig. 5** and **6**.

**TABLE 2: LINEARITY RESULTS OF VENLAFAXINE**

| S. no. | Linearity Level | Concentration (µg/ml) | Area |
|---|---|---|---|
| 1 | I | 50 | 5461 |
| 2 | II | 100 | 11016 |
| 3 | III | 150 | 15930 |
| 4 | IV | 200 | 21336 |
| 5 | V | 250 | 26580 |
| | Correlation Coefficient | | 0.999 |

**Accuracy:** Preparations of 50% 100% 150% solutions were prepared by using the stock solution in 10 ml volumetric flask, and dilutions were done as follows.

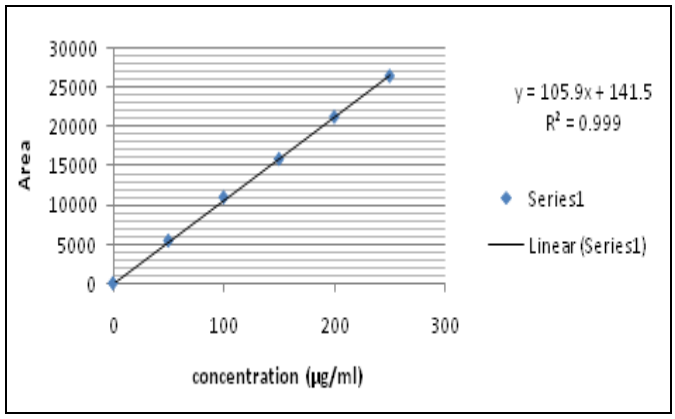


**FIG. 5: LINEARITY GRAPH OF VENLAFAXINE**

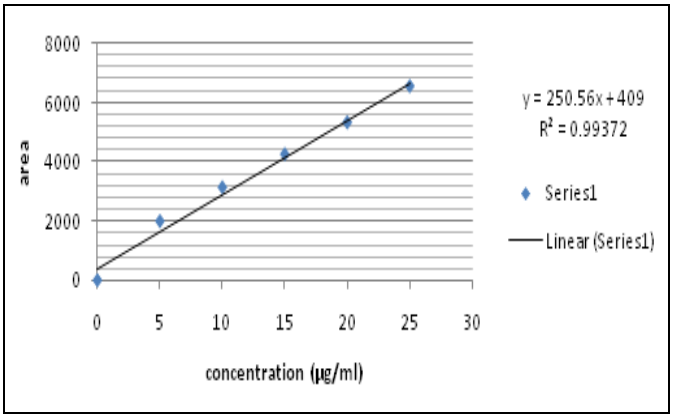


**FIG. 6: LINEARITY GRAPH OF IMPURITY**

**TABLE 3: LINEARITY RESULTS OF IMPURITY -A**

| S. no. | Linearity Level | Concentration (µg/ml) | Area |
|---|---|---|---|
| 1 | I | 5 | 1989 |
| 2 | II | 10 | 3135 |
| 3 | III | 15 | 4253 |
| 4 | IV | 20 | 5324 |
| 5 | V | 25 | 6545 |
| Correlation Coefficient | | | 0.993 |

**Preparation of 50% solution (With Respect to Target Assay Concentration):** To 5 mg of Venlafaxine, 0.5 mg of Impurity-A was added and diluted with diluent. Pipette out 1ml into a 10 mL volumetric flask and dilute up till the mark on the flask.

**Preparation of 100% solution (with Respect to Target Assay Concentration):** To 10 mg of Venlafaxine, 1 mg of Impurity-A was added and diluted with diluent. Pipette out 1ml into a 10 mL volumetric flask and dilute up till the mark on the flask.

**Preparation of 150% solution (With Respect to Target Assay Concentration):** To 15 mg of Venlafaxine, 2 mg of Impurity-A was added and diluted with diluent. Pipette out 1ml into a 10 mL volumetric flask and dilute up till the mark on the flask.

**Procedure:** The standard solutions of accuracy 50%, 100%, and 150% were injected into the chromato-graphic system.

The % recovery was found to be 98.19% and 98.97%, respectively (NLT 98% and NMT 102%). Accuracy values of the drug and impurity were tabulated in **Table 4**.

**TABLE 4: SUMMARY OF ACCURACY RESULTS**

| %Concentration (at specification Level) | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|
| **Accuracy results of Venlafaxine** | | | | | |
| 50% | 5624 | 4.30 | 4.25 | 98.83% | 98.19 % |
| 100% | 11657 | 8.48 | 8.25 | 97.28% | |
| 150% | 18043 | 12.39 | 12.2 | 98.46% | |
| **Accuracy results for Impurity-A** | | | | | |
| 50% | 2242 | 7.1 | 7.05 | 99.29% | |
| 100% | 3170 | 13.2 | 13.1 | 99.24% | 98.97% |
| 150% | 3881 | 18.8 | 18.5 | 98.40% | |

**Precision:**

**Preparation of Stock Solution:** The 100% concentration Level of Venlafaxine and Impurity -A solution was prepared.

**Procedure:** The stock solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicates of Venlafaxine and Impurity-A was found to be 0.52 and 0.83, respectively. These results were reported in **Table 5**.

**Range:** Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 50 µg/ml - 250 µg/ml and 5 µg/ml – 25 µg/ml of Venlafaxine and Impurity-A respectively.

**TABLE 5: PRECISION RESULTS OF VENLAFAXINE AND IMPURITY A**

| S. no, | % RSD results for Venlafaxine | | % RSD results for Impurity A | |
|---|---|---|---|---|
| | Area | $R_t$ (min) | Area | $R_t$ (min) |
| 1 | 11756 | 2.170 | 3576 | 4.445 |
| 2 | 11615 | 2.166 | 3085 | 4.657 |
| 3 | 11817 | 2.175 | 3351 | 4.937 |
| 4 | 11674 | 2.202 | 3276 | 4.626 |
| 5 | 11768 | 2.191 | 3901 | 4.515 |
| Average | 11716 | | 3684 | 4.445 |
| Standard Deviation | 7664.08 | | 235.28 | |
| %RSD | 0.52 | | 0.83 | |

**Limit of Detection (LOD) and Limit of Quantitation (LOQ):** LOD and LOQ are calculated with respect to the standard deviation (SD) and the slope of the calibration curve (S) by the given formulae.

The LOD was found to be 0.0010 µg/mL and 0.0058 µg/mL for venlafaxine and impurity A respectively.

The LOQ was found to be 0.0029 µg/mL and for 0.013 µg/mL venlafaxine and impurity A, respectively. The results were reported in the **Table 6** and **7**.

**Formulae:**

$$LOD=3.3 \times \sigma/S$$

$$LOQ=10 \times \sigma/S$$

Where, σ - Standard deviation, S - Slope

**TABLE 6: LOD RESULTS**

| Drug name | LOD(µg/mL) | S/N ratio |
|---|---|---|
| Venlafaxine | 0.0010 | 2:0.8 |
| Impurity-A | 0.0058 | 3:1 |

**TABLE: 7 LOQ RESULTS**

| Drug name | LOQ(µg/mL) | S/N ratio |
|---|---|---|
| Venlafaxine | 0.0029 | 10:09 |
| Impurity-A | 0.013 | 10 |

**Robustness:** Standard solution 150 µg/ml of Venlafaxine and 15 µg/ml of Impurity-A were analyzed. Deliberate changes in the flow rate and mobile phase (MP) composition were made to evaluate the impact on the method.

**A.** The flow rate was varied at 0.8 ml/min to 1.1 ml/min compared with the actual flow rate. These results were reported in **Table 8**.

**B.** The organic composition in the mobile phase was varied and compared with that of the actual MP composition. These results were reported in **Table 9**.

**TABLE 8: ROBUSTNESS RESULTS VARIATION IN FLOW RATE**

| S. no. | Flow rate (ml/min) | System suitability results of venlafaxine | | System suitability results of impurity A | |
|---|---|---|---|---|---|
| | | USP plate count | USP tailing | USP plate count | USP tailing |
| 1 | 0.8 | 7515.5 | 0.9 | 8573.5 | 1.0 |
| 2 | 1.1 | 10026.7 | 1.0 | 12458.5 | 1.2 |

**TABLE 9: ROBUSTNESS RESULTS VARIATION IN MOBILE PHASE COMPOSITION**

| S. no. | Change in Organic Composition in the Mobile Phase | System suitability results of venlafaxine | | System suitability results of impurity A | |
|---|---|---|---|---|---|
| | | USP plate count | USP tailing | USP plate count | USP tailing |
| 1 | 50:50 | 6953.5 | 1.0 | 7079.0 | 1.0 |
| 2 | *Actual | 5038 | 1.6 | 3428.2 | 1.3 |
| 3 | 70:30 | 6048.5 | 1.0 | 6228.5 | 1.1 |

*Actual – Methanol: pH 6.8 Phosphate Buffer in 60:40 v/v ratio

**Stability Studies:**

**Forced Degradation Studies:** Forced degradation of the Test sample was performed under acidic, alkaline, heat, photolytic, and oxidative stress conditions.

**Stock Solution Preparation:** Twenty Tablets were weighed and powdered. Tablet powder having weight equivalent to 20 mg was weighed accurately and taken in a 10 mL volumetric flask. To it 5 mL of the mobile phase was added and sonicated for 15 min to dissolve the drugs. The volume was made up to 10 mL with the mobile phase. The resulting solution was then filtered through a 0.45 µm membrane filter to prepare a stock solution of the tablet sample. Further dilution was done by diluting 0.1 mL of stock solution to 10 ml mobile phase.

The concentration of Venlafaxine and venlafaxine in the solution was 10 µg/mL, 25 µg/mL, respectively.

**Acid Hydrolysis:** Forced degradation in acidic media was performed by adding 2 mL 0.1 M HCl to 10 mL of stock solution, and the mixture is heated at 60 °C for approximately 26 h, and the solution is neutralized by addition of 0.1 M NaOH. The prepared solution is injected, and chromatograms were recorded.

**Alkaline Hydrolysis:** Forced degradation in basic media was performed by adding 2 mL 0.1M NaOH to 10 mL of stock solution, and the mixture is heated at 60 °C for approximately 26 h, and the solution is neutralized by addition of 0.1M HCl. The prepared solution is injected, and chromatograms were recorded.

**Oxidative Degradation:** To study the effect of oxidizing conditions, an aliquot of stock solution was added to 1 ml 30% $H_2O_2$ solution. The prepared solution 10 ml is injected, and chromatograms were recorded.

**Thermal Degradation:** To study the effect of temperature, an aliquot of stock solution was kept at 70 ºC for 26 h. 10 ml of the resulting solution was injected into HPLC, and chromatograms were recorded.

**Photolysis:** To study the effect of photolysis, an aliquot of stock solution was exposed to UV light for 4 h. 10 ml of the resulting solution was injected into HPLC, and chromatograms were recorded.

From the stability studies, it was found that both Venlafaxine and Impurity-A were degraded when subjected to acid hydrolysis, alkaline hydrolysis, and thermal degradation and were found to be stable when subjected to Photolysis, Oxidation, *i.e.*, no significant peaks were found. The results of Forced degradation studies were reported in **Table 10**.

**TABLE 10: RESULTS OF STRESS DEGRADATION STUDIES**

| Stress Condition | Sample-1 (Venlafaxine) | | | Sample-2 (Impurity-A ) | | |
|---|---|---|---|---|---|---|
| | Area | %Assay | %Degradation | Area | %Assay | %Degradation |
| Acidic | 110476 | 91.7 | 8.7 | 3575 | 92.3 | 8.2 |
| Alkaline | 128364 | 92.0 | 12.7 | 3678 | 81.7 | 12.8 |
| Photolytic | 153267 | 87.5 | 13.8 | 3396 | 86.4 | 12.4 |
| Thermal | 101678 | 96.3 | 13.5 | 3424 | 85.5 | 12.3 |
| Oxidative | 105735 | 94.2 | 11.3 | 3424 | 95.2 | 11.2 |

**CONCLUSION:** The results obtained in this study demonstrate that the RP- HPLC method described in the method of analysis is selective, accurate, precise, linear, rugged, and robust for the determination of related substances in venlafaxine hydrochloride drug substance. This stability-indicating RP-HPLC method has the practical advantage of shorter retention time, and satisfactory results were obtained from method validation.

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**CONFLICTS OF INTEREST:** The authors declare no conflicts of interest

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