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# DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR QUANTITATIVE DETERMINATION OF VENLAFAXINE HCL IN BULK AND TABLET FORMULATION.

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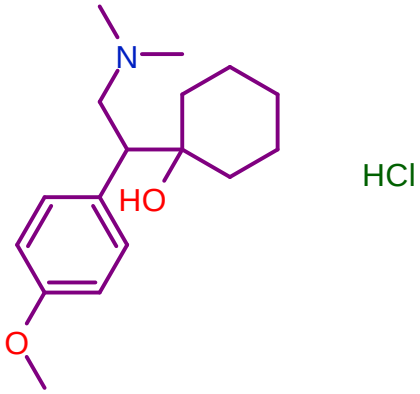
***Abstract:*** A simple, precise, accurate and rapid high performance thin layer chromatographic method has been developed and validated for the determination of Venlafaxine HCL in bulk drug and tablet dosage form. The stationary phase used was precoated silica gel 60 NP-18 $F_{254}$. The mobile phase used was a mixture of Carbon tetrachloride: n-hexane: Triethylamine (4:1:0.3v/v.) The detection of spot was carried out at 225 nm. The method was validated in terms of linearity, accuracy, ruggedness, Robustness, precision, Sensitivity and specificity. The calibration curve was found to be linear between 500 to 3000 ng/band for Venlafaxine HCL. The method was successfully employed for the estimation of Venlafaxine HCL as a bulk drug and in Tablet dosage form.

**Keywords:** HPTLC method of estimation, Venlafaxine HCL, Tablets

## I. INTRODUCTION

Venlafaxine Hydrochloride is a synthetic ethyl-cyclohexanol derivative used for the treatment of depression, Venlafaxine Hydrochloride is metabolized to O-desmethylvenlafaxine, which potentiates CNS activity. Both venlafaxine and its active metabolite inhibit neuronal reuptake of norepinephrine, dopamine and serotonin. It is usually sold as a mixture of the respective hydrochloride salts, (R/S)-1-[2-(dimethylamino)-1-(4 methoxyphenyl) ethyl] cyclohexanol hydrochloride, $C_{17}H_{28}ClNO_2$ , which is a white to off-white crystalline solid. [1-3]

Venlafaxine extended-release (long-acting) capsules are also used to treat generalized anxiety disorder (GAD; excessive worrying that is difficult to control), social anxiety disorder (extreme fear of interacting with others or performing in front of others that interferes with normal life), and panic disorder (sudden, unexpected attacks of extreme fear and worry about these attacks). Venlafaxine is in a class of medications called selective serotonin and norepinephrine reuptake inhibitors (SNRIs). [1,4] It works by increasing the amounts of serotonin and norepinephrine, natural substances in the brain that help maintain mental balance. The detailed literature survey revealed that there was no HPTLC method reported, Hence, the attempts was made to develop and validate the HPTLC method on Venlafaxine HCL.




**Venlafaxine HCL**

## II. MATERIAL AND METHOD:

The Venlafaxine HCL reference standard was obtained from the GLENMARK Pharmaceutical LTD, Mumbai, India. Tablets (Effexor XR 100 mg) were obtained from 1mg online medical store and healthcare. The methanol used for preparation of stock and sample solution. HPLC grade methanol purchase Mark Life Science Private Limited, Godrej One, 8th Floor, Eastern Express Highway Vikhroli (East), Mumbai 400079.

### HPTLC Instrumentation:

| | |
|---|---|
| HPTLC system | Camag TLC system (Muttenz, Switzerland) |
| Sample applicator: | Linomat 5 |
| Scanner: | TLC scanner 3 |
| Data processor: | winCATs (version 1.3.0) |
| Development chamber: | Camag twin trough chamber (20 x 10 cm) |
| Syringe for application: | Hamilton syringe (100 µL) |
| Ultrasonicator: | Enertech Electronics Pvt. Ltd., India |

### Selection of Chromatographic Mode

The Normal-phase HPTLC was selected for method development.

### Selection of Detection Wavelength

From the UV-Spectrum of Venlafaxine HCL, wavelength **225 nm** was selected for the estimation of drug; showing

**Fig.1**

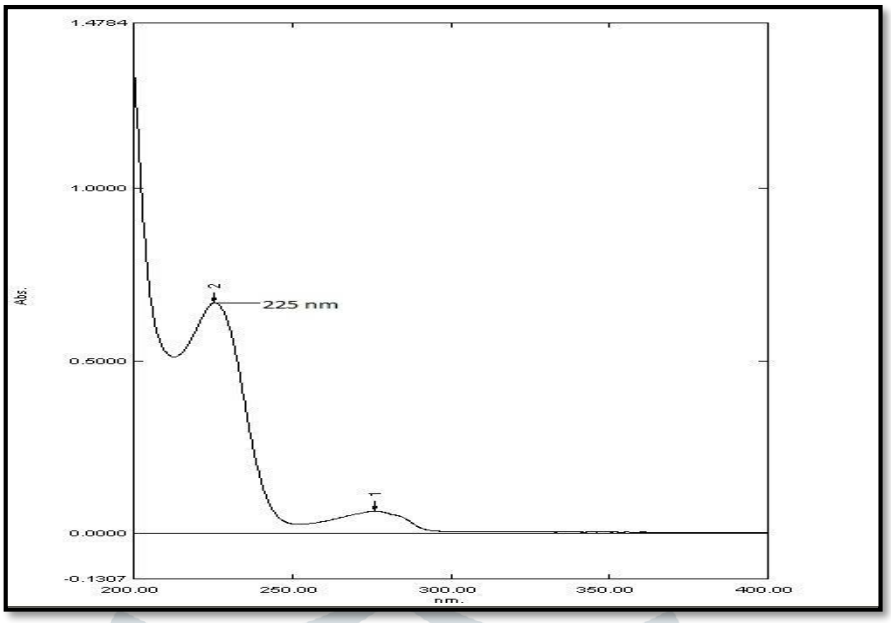



**Fig.1**: **Wavelength of Venlafaxine HCL**

**Preparation of Stock Standard Solution**

Standard stock solution was prepared by accurately weigh 10 mg of Venlafaxine HCL powder was transfer to 10 mL volumetric flask volume make up by methanol up to the mark to get final concentration (1000 µg/mL).

**Selection of Chromatographic Layer**

Identification and determination were performed on (10 cm x 10 cm, layer thickness 0.2 mm, E-Merck, Darmstadt, Germany) aluminum backed silica gel 60 NP-18 $F_{254}$ TLC plates, prewashed with methanol.

**Development of optimum mobile phase**

To obtain high resolution and reproducible peaks, various mobile phase compositions were experimented. The details about the mobile phase composition and its effects are given below. The essential parameters were found optimum with use of **Carbon tetrachloride: n-hexane: Triethylamine (4:1:0.3*v/v/v*)** as mobile phase. The wavelength of 225 nm was selected to be optimal for the highest sensitivity. A sharp and well resolved peak was obtained for Venlafaxine HCL at $R_{f\,of}$ **0.6± 0.02**. The TLC chamber was saturated with mobile phase for **25 min.** at room temperature.

**Optimization of Mobile Phase**

| Solvent System | Composition of Mobile Phase | R*f* | Comment |
|---|---|---|---|
| Ethyl acetate | 10 | 0.0 | No spot |
| $CCl_4$: MeOH | 9.5:0.5*v/v* | 0.93 | tailing |
| $CCl_4$: ACN: Triethylamine | 4.5:0.5:0.2 *v/v/v* | 0.71 | $R_f$ but tailing |
| $CCl_4$: *n*-hexane: Triethylamine | 4 : 1:0.3*v/v/v* | 0.62 | Resolved spot |

**Chromatographic Conditions**

The samples were spotted in the form of bands of width 6 mm with Camag microliter syringe on aluminum backed plates pre-coated with silica gel plate 60 NP-18 $F_{254}$ (0.2 mm thickness E. Merck, Germany) using Linomat 5 (Camag, Muttenz, Switzerland) sample applicator. Before chromatography, the plates were pre-washed with methanol and activated at $105^0$C for 5 min. linear ascending development of TLC plates was performed. The twin trough glass chamber previously saturated with mobile phase for 25 min. The development distance was 80 mm. Subsequent to the development; TLC plates were dried in air with the help of an air dryer. Using the absorbance mode at 225 nm on the

Camag TLC scanner 3, densitometric scanning was carried out. The radiation source that was used was a deuterium lamp that continuously emitted UV light between 200 and 400 nm in wavelength.

**Linearity Studies**

A fixed volume in the range of 0.5–3.0 mL was transferred from stock solution into series of 10 mL volumetric flasks and volumes were adjusted up to mark with methanol. From each volumetric flask, 10 µL of solution was applied on HPTLC plate to get concentration in the range of 500 - 3000 ng/band. After evaporation of solvents at room temperature for 20 min, chromatography was performed as described above. The calibration curve was developed by plotting peak-area against concentration of drug per band. Calibration equations were determined by use of linear regression analysis and correlation coefficients ($r^2$) were calculated. The results are reported in **Table 1** and calibration curve 3-D graph and the typical chromatogram of Venlafaxine HCL shown in **Fig. 2, 3** and **4**

**Table 1: Linearity Studies of Venlafaxine HCL**

| Sr. No | Concentration of Venlafaxine HCL [ng/band] | Peak Area ± SD [n = 6] | % RSD |
|---|---|---|---|
| **1** | 500 | 6652.70±63.52 | 0.95 |
| **2** | 1000 | 8668.06 ± 61.70 | 0.71 |
| **3** | 1500 | 10695.56 ± 87.67 | 0.81 |
| **4** | 2000 | 12919.52 ± 53.63 | 0.41 |
| **5** | 2500 | 14815.20 ± 99.45 | 0.67 |
| **6** | 3000 | 16843.76 ± 99.70 | 0.59 |

**n - Number of determinations**

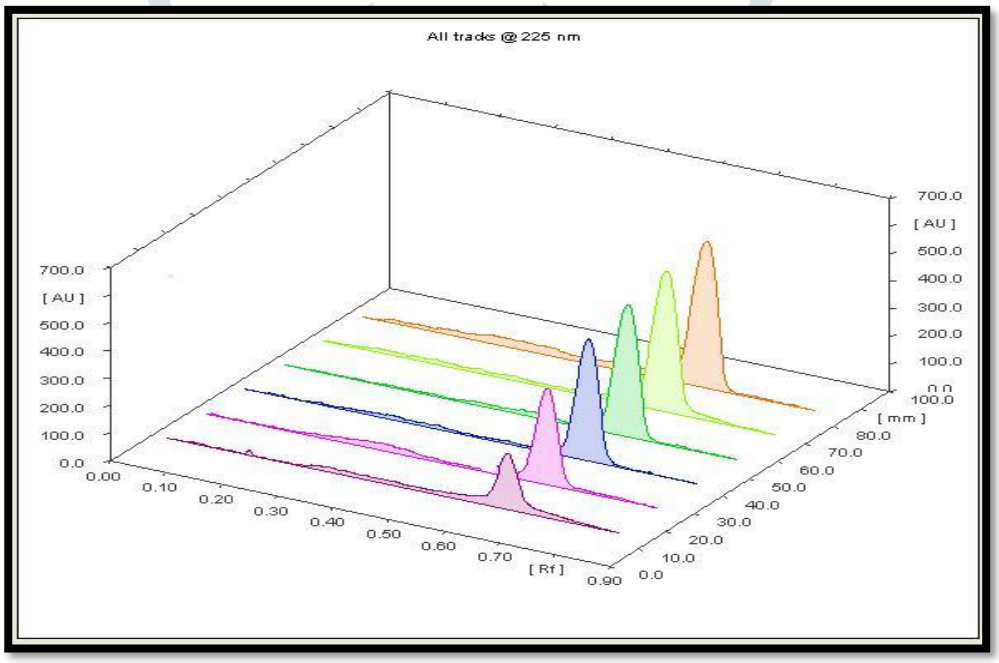



**Fig. 2: 3D linearity chromatogram of Venlafaxine HCL**

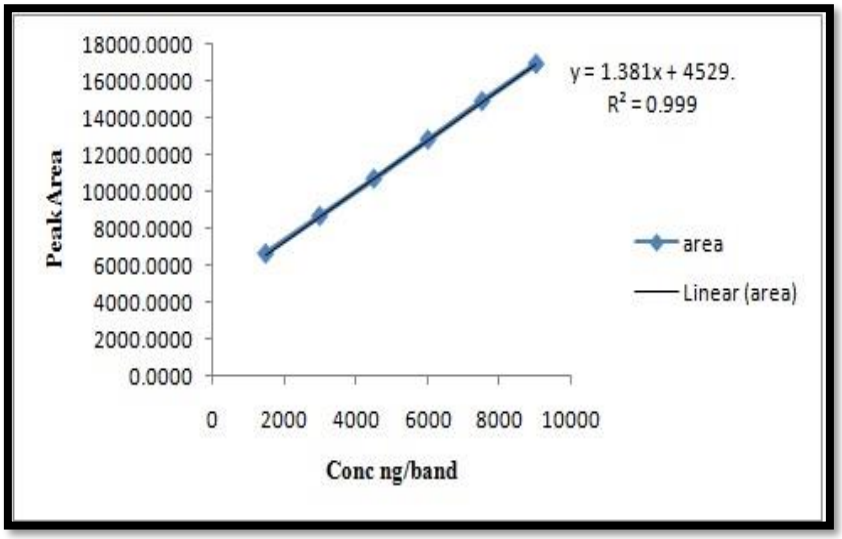



**Fig. 3: Calibration curve of Venlafaxine HCL**

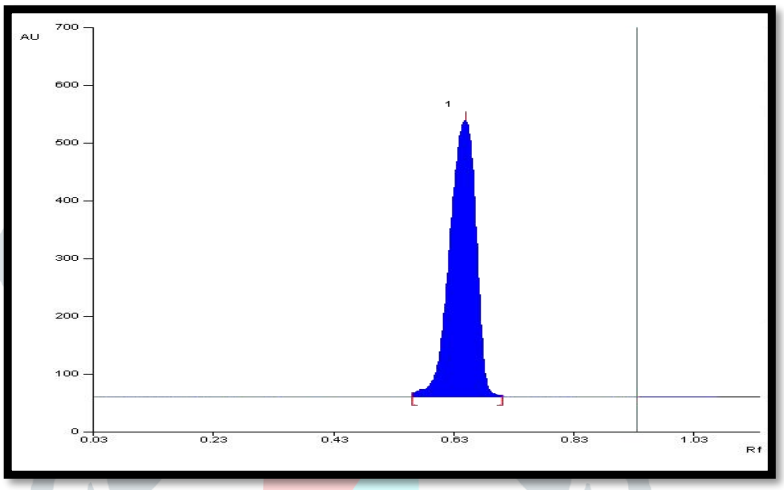



**Fig. 4: Chromatogram of Venlafaxine HCL**

**Analysis of Bulk Material**

An accurately weighed 100 mg of Venlafaxine HCL was transferred into 100 mL volumetric flask; dissolved in methanol and the volume was made up to mark with the same solvent. An appropriate volume 1.5 mL was transferred into 10 mL volumetric flask and volume made up to the mark with methanol. A fixed volume of 10 µL (containing 1500 ng/band) was spotted. The concentration was determined by regression equation. Results are shown in **Table 2**.

**Table 2: Analysis of Bulk Material**

| Drug | Amount taken [ng/band] | Amount found [ng/band] | % Amount found |
|---|---|---|---|
| VEN HCL | 1500 | 4465.04 | 99.22 |
| | 1500 | 4508.32 | 100.18 |
| | 1500 | 4417.23 | 98.16 |
| | 1500 | 4580.81 | 101.79 |
| | 1500 | 4516.22 | 100.36 |
| | 1500 | 4440.98 | 98.68 |
| | **Mean ± SD** | 4488.104± 59.27 | 99.73± 1.31 |
| | **% RSD** | 1.32 | 1.32 |

**Analysis of *in-house* Tablets**

Since the marketed formulation of Venlafaxine HCL was not available during the study; therefore, in house tablets were prepared**.** Ten Venlafaxine HCL tablets were accurately weighed, average weight determined and ground into fine powder. A quantity of powder drug equivalent to 100 mg of Venlafaxine HCL was transferred into 100 mL volumetric flask containing 70 ml methanol shaken manually for 20 min and volume was adjusted to mark using same solvent. From it; 1.5 mL was transferred into 10 mL of volumetric flask and diluted to mark using methanol. An appropriate volume of 10 µL (containing 1500 ng/band) was applied on NP-HPTLC plate, developed and scanned as described in chromatographic conditions. The experiment was repeated for five times and amount of Venlafaxine HCL tablet formulation was established by treating it with linearity equation. Results are shown in **Table 3**.

**Table 3: Analysis of *in-house* Tablets**

| Drug | Amount taken [ng/band] | Amount found [ng/band] | % Amount found |
|---|---|---|---|
| | 1500 | 4491.74 | 99.81 |
| | 1500 | 4556.11 | 101.24 |
| | 1500 | 4450.90 | 98.90 |
| **VEN** | 1500 | 4533.02 | 100.73 |
| **HCl** | 1500 | 4467.99 | 99.28 |
| | 1500 | 4591.89 | 102.0 |
| | **Mean ± SD** | 4515.27 ± 54.37 | 100.34± 1.20 |
| | **% RSD** | 1.20 | 1.20 |

## III. VALIDATION OF METHOD

**Precision studies**

The method's repeatability, intra- and inter-day fluctuations, and precision were all examined. The precision of the developed HPLC method was expressed in terms of % relative standard deviation (% RSD). Intra-day and Inter-day variation were checked by analyzing 1000, 1500 µg and 2000 ng/band of Venlafaxine HCL. The repeatability studies were performed by analyzing 1500 ng/band.

The results are shown in **Table 4 and 5.**

**Table 4: Precision Studies [Intra and Inter-day]**

| Standard Concentration (ng/band) | Amount Found [ng/band] | % Amount found [n=3] | % RSD |
|---|---|---|---|
| **Intra-day Precision** | | | |
| 1000 | 4518.32 | 100.40 | 0.52 |
| 1500 | 6073.57 | 101.22 | 0.47 |
| 2000 | 7417.74 | 98.90 | 0.24 |
| **Inter-day Precision** | | | |
| 1000 | 4552.71 | 101.17 | 0.53 |
| 1500 | 5954.15 | 99.23 | 0.50 |
| 2000 | 7417.37 | 98.89 | 0.50 |

**n - Number of determinations**

**Table 5: Repeatability Studies**

| Drug | Amount taken [ng/band] | Amount found [ng/band] | % Amount found |
|---|---|---|---|
| VEN HCL | 1500 | 4590.22 | 102.00 |
| | 1500 | 4565.60 | 99.23 |
| | 1500 | 4533.16 | 100.73 |
| | 1500 | 4428.67 | 98.41 |
| | 1500 | 4589.50 | 101.98 |
| | 1500 | 4448.22 | 98.84 |
| | **Mean ± SD** | 4509.23 ± 71.68 | 100.20 ± 1.59 |
| | **% RSD** | 1.58 | 1.58 |

**Accuracy**

Accuracy study was executed by standard addition method using three different levels. Recovery experiment was evaluated by over spotting the drug standard at 80%, 100% and 120% percentage of drug recovered, when known amount of standard drug was added to the pre-analyzed sample and subjected to proposed HPTLC method.

**Table 6: Recovery Studies results**

| Drug | Initial Amount [ng/band] | Excess drug added to the analyte [%] | Recovery [%] [n=3] | %RSD [n = 3] |
|---|---|---|---|---|
| VEN HCL | 1500 | 80 | 99.75 | 1.53 |
| | 1500 | 100 | 99.61 | 0.79 |
| | 1500 | 120 | 98.84 | 0.84 |

**Ruggedness**

The ruggedness of the method was studied by two different analysts using same operational and environmental conditions. The ruggedness of the proposed method was determined by 1500 ng/band concentration of Venlafaxine HCL. The results are shown in **Table 7**.

**Table 7: Ruggedness studies**

| Drug | Concentration [ng/band] | Amount Found (%) ± SD | |
|---|---|---|---|
| | | Analysts- I [n=6] | Analysts- II [n=6] |
| VEN HCL | 1500 | 100.53 ± 1.35 | 100.34 ± 1.57 |

**n - Number of determinations**

**Robustness**

Robustness was studied in six replicates at the concentration 1500 ng/band of Venlafaxine HCL. In this experiment, four parameters (mobile phase composition, mobile phase volume and development distance and saturation time) were studied. The results are shown in **Table 8.**

**Table 8: Robustness**

| Parameters | Venlafaxine HCL | |
|---|---|---|
| | ± SD of Peak area | % RSD |
| ▪ Mobile phase volume | 11.96 | 1.74 |
| ▪ Mobile phase composition | 11.07 | 1.54 |
| ▪ Development distance | 5.62 | 0.65 |
| ▪ Saturation time (min.) | 9.63 | 1.08 |

**Sensitivity**

The sensitivity of proposed methods was estimated in terms of Detection Limit (DL) and Quantification Limit (QL) determinations for both specified methods were based on the standard deviations of the responses and slopes of constructed calibration curves (n= 3) as described by International Conference for Harmonization guidelines Q2(R1). For the determination of DL and QL during HPTLC method validation; venlafaxine HCL solutions of 500, 600, 700, 800,900 and 1000 ng/band were applied on HPTLC plates. The DL and QL were calculated using equations DL = 3.3×N/B and QL = 10×N/B; where, 'N' is standard deviation of peak areas of the drug (n=3) taken as a measure of noise and 'B' is the slope of corresponding calibration curve.
The DL and QL values were found to be **0.553ng** and **1.675ng.**

**Specificity:**

The mobile phase designed for the method resolve the drug very efficiently. The $R_f$ value of venlafaxine HCL was found to be 0.5±0.02. The peak purity of venlafaxine HCL was assessed by correlation the spectra of venlafaxine HCL from tablet and venlafaxine HCL standard at peak start (S), peak-apex (M), and the peak end (E). Peak - purity spectra shown in **Fig. 5**

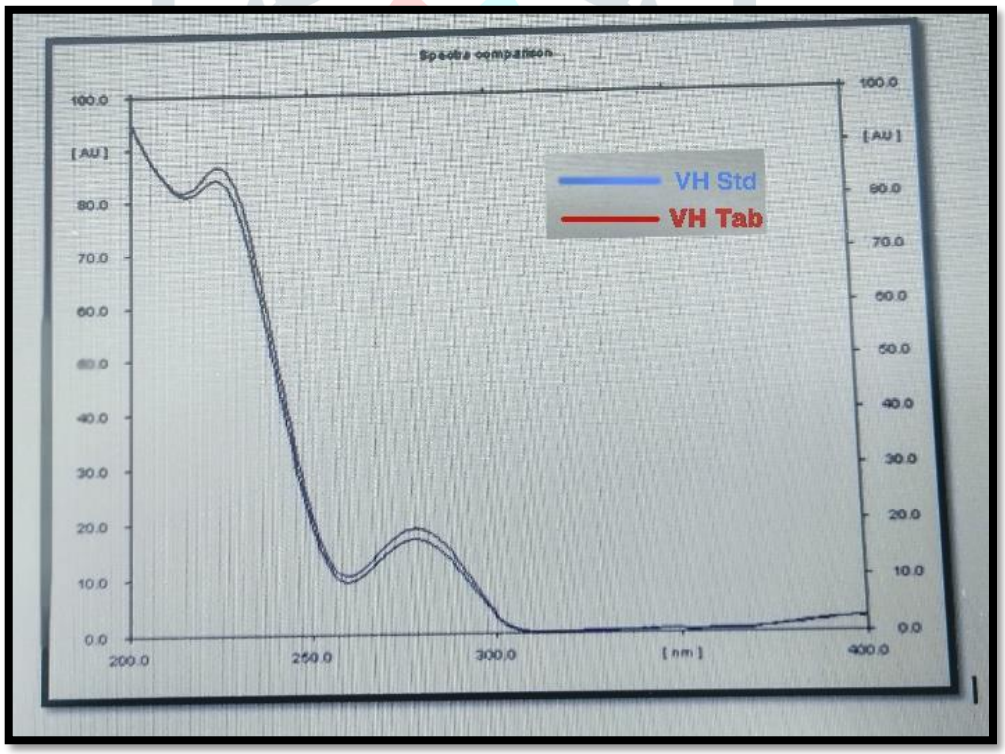



**Fig. 5 : An overlain UV-Spectrum of Venlafaxine HCL Standard and Venlafaxine HCL extracted from *in-house* Tablets with coefficient correlation value > 0.99.**

## IV. CONCLUSION

This HPTLC method is simple, precise, specific, accurate and rugged, and can be used for the usual study of venlafaxine HCL from its pharmaceutical formulations. The statistical analysis proved the method enables reproducible and selective analysis of venlafaxine hydrochloride in the bulk drug and in tablet formulations. The

methods are developed for quantification of Venlafaxine HCL tablets. It is also used in routine quality control of the formulations containing Venlafaxine HCL.

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