Development and Validation of Venlafaxine Hydrochloride in Bulk and in Capsule Formulation by HPTLC

Abstract

A simple, sensitive and rapid high performance thin layer chromatographic (HPTLC) method has been developed and validated for quantitative estimation of Venlafaxine hydrochloride in bulk and in capsule formulation. The drug was chromatographed on silica gel 60F_{254}TLC plate using methanol: ammonia (4.5:0.5*v/v*) as mobile phase. Densitometric scanning of was carried out at 224 nm and resolution was found with R_f value 0.65±0.02. The linear regression analysis for the calibration plots showed good linear relationship with r^2=0.998 in concentration range 500-3000ng/spot. The minimum detectable amount was found to be 7.7ng/spot, whereas the limit of quantitation was found to be 23.3ng/spot. The method was validated for precision, recovery, repeatability and robustness as per the ICH guidelines. Statistical analysis of the data showed that the method is precise, accurate, reliable, reproducible, and selective for the analysis of Venlafaxine hydrochloride. The proposed method also indicates no interference of excipients from capsule formulation.

Keywords: High performance thin layer chromatography; Venlafaxine HCl; Validation; ICH guidelines; Precision; Recovery; Repeatability; Robustness; Concentration; Statistical analysis; Precise; Accurate; Reliable; Reproducible; Quantitative

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Introduction

Venlafaxine hydrochloride (VEN; Figure 1) is serotonin or epinephrine reuptake inhibitor (SNRI) class to be used clinically as antidepressant [1,2]. Chemically it is (R/S)-1-[2-(dimethylamino) -1-(4-methoxyphenyl) ethyl] cyclohexanol hydrochloride [2-4]. It works by blocking the transporter "reuptake" proteins for key neurotransmitters affecting mood, thereby leaving more active neurotransmitters in the synapse [5,6]. It has a simultaneous effect on noradrenaline reuptake and some weak effects on dopamine reuptake. The combination of the effects on the reuptake mechanisms appears to be responsible for the antidepressant action of the drug [7,8].

Chromatographic estimation of Venlafaxine HCl was reported by using few HPLC methods either in combination or individual in pharmaceutical dosage form [9-12]. In the same context a method for estimation by HPTLC was also available as reported to study the degradation kinetics [13] and also HPTLC analysis of Venlafaxine hydrochloride in the bulk drug and tablets [14].

Based on the available literature sources, it was thought that there is scope for development of simplified HPTLC method for VEN. Even though some methods of analysis are available but it was thought to perform the estimation by more sophisticated technique. Therefore; the present study illustrates the development and validation of simple, sensitive and rapid HPTLC method for estimation of VEN in bulk and in capsule dosage form. The proposed method is optimized and validated according to ICH guidelines.

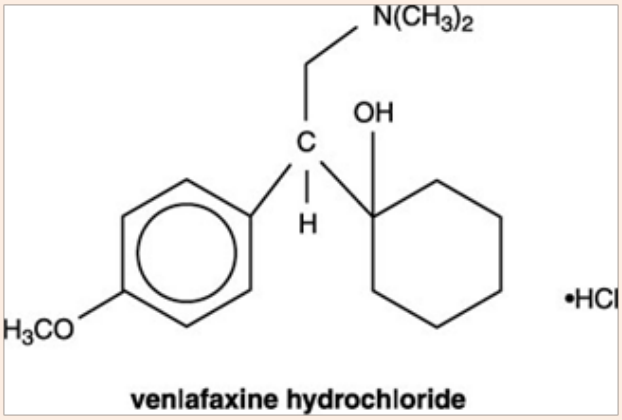

Figure 1: Chemical structure of Venlafaxine hydrochloride.

Materials and Methods

Instrumentation and chemicals

The HPTLC system (Camag, Muttenz, Switzerland) equipped with a sample applicator Linomat V connected to a nitrogen cylinder, twin trough plate development chamber (10×10cm), TLC Camag Scanner III and Wincats 4.02. Pre-coated silica gel 60 F_{254} TLC aluminium plates (0.2mm thick) were obtained from E. Merck Ltd., Mumbai (India); Densitometric analysis was carried out using TLC scanner III winCATS software.

Venlafaxine hydrochloride was kindly supplied as a gift sample by Zydus Pharmaceuticals Ltd., India. Methanol and ammonia employed were of analytical grade and used as solvents to prepare the mobile phase. Capsule formulation Veniz XR (Sun Pharmaceuticals Ltd., India) was used as pharmaceutical preparation for analysis.

Method

Standard stock solution preparation

An accurately weighed 10 mg of drug was transferred to 10 mL volumetric flask; dissolved in methanol and the volume was made upto the mark with same solvent to obtain working standard of 1000 ng/µl.

Optimization of mobile phase and chromatographic conditions

On the basis of polarity, ethyl acetate was selected as trail solvent for mobile phase. It was followed by further trials by combining with methanol in varying ratios. The developed spot was diffused and tailing was observed. To the above mobile phase, 1mL methanol was added. The peak was found to be symmetrical in nature with tailing hence; ammonia was added to remove tailing effect. Finally, the mobile phase methanol: ammonia (4.5:0.5*v/v*) gave good, sharp and symmetrical peak with R_f value of 0.65 for VEN. Plates were developed to a distance of 8cm in Camag twin-trough glass chamber previously saturated with mobile phase vapors for 25min at ambient temperature. Densitometric scanning was performed at 22 nm. A typical chromatogram of VEN in bulk showing R_f value 0.65 is shown in Figure 2.

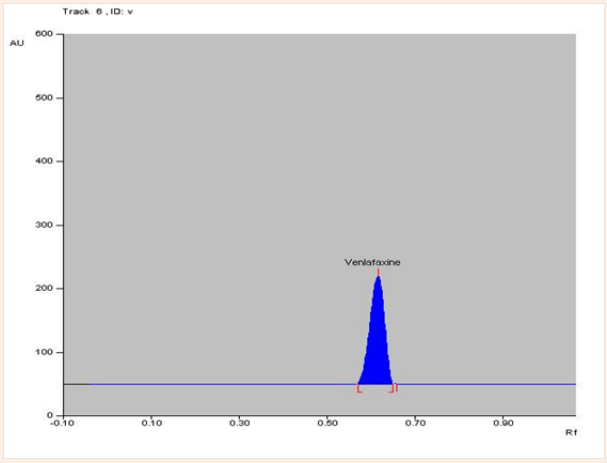

Figure 2: Chromatogram of standard Venlafaxine HCl showing Rf value 0.65.

Validation of proposed method

The proposed method was validated across the various parameters like linearity, accuracy, precision, sensitivity, reproducibility, repeatability and robustness studies as per the ICH guidelines [15]. System suitability test of the chromatography system was performed before each validation run.

Linearity

Linearity was performed using working standard of VEN. Calibration was done by applying standard stock solution ranging from 0.5-3.0µL on TLC Plate; which gives concentration of 500-3000ng/band. The plate was developed and scanned as described under chromatographic conditions.

Bulk assay

Bulk assay was assessed by six replicates determinations covering the specified range for the procedure *viz*; 1500ng/band of VEN on a TLC plate followed by development and scanning as described above.

Accuracy

Recovery experiment was assessed at three different concentrations (80%, 100% and 120% concentration). To the pre-analyzed sample solutions (1500ng/band), a known amount of drug standard solution of VEN was over applied in triplicate and analyzed. This procedure was repeated for three consecutive days. Calibration curves to estimate the concentration of drug per spot were measured daily on the same plates as the samples. The accuracy was determined and expressed as percentage recovery.

Precision

Precision of the method was studied as repeatability and intra-day and inter-day variations. Intra-day and Inter-day variation was assessed at three different concentrations 500, 1000 and 1500ng of drug solutions. Intra-day assay precision was found by analysis of standard drug at three times on the same day. Inter-day assay precision was carried out using at three different days and percentage relative standard deviation (%RSD) was calculated.

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOD). The LOD and LOQ were calculated by the use of the equation LOD =3.3×N/B and LOQ = 10×N/B; where, 'N' is standard deviation of the peak areas of the drugs (n=3), taken as a measure of noise, and 'B' is the slope of the corresponding calibration curve. Different volumes of stock solution in the range 500–1000ng/band were applied on HPTLC plate in triplicate.

Repeatability

It is measured by multiple injections of a homogenous sample of 1500ng/band of VEN that indicates the performance of the HPTLC instrument under chromatographic conditions followed by development of plate and recording the peak height and area for six bands.

Robustness

By introducing small but deliberate changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation, the effects on the results were examined.

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Formulation assay

To determine the content of VEN in pharmaceutical dosage form, Contents of twenty capsules were crushed to make a uniform powder having label claim of 37.5mg. A quantity of powder which is equivalent to 37.5mg of VLN was weighed accurately and transferred into a 10ml calibrated volumetric flask; finally the volume was adjusted to the mark. The resulting solution obtained was then filtered, through 0.45µm filter for complete removal of particulate matter. 5ml of the filtrate was diluted to 25ml in the volumetric flask with the diluent for analysis. A single spot at R_f 0.61 was observed in the chromatogram of the drug samples extracted from conventional capsules. There was no interference from the excipients commonly present in the conventional capsules. The drug content was found to be 100.9% with a % R.S.D. of less than 2 *viz*; 1.01. The low % R.S.D. value indicated the suitability of this method for routine analysis of VEN in pharmaceutical dosage forms.

Results and Discussion

An HPTLC/densitometric method has been developed successfully for the determination of Venlafaxine hydrochloride in bulk and in capsule formulation. The estimation of drug was performed on pre-coated silica gel 60 F_{254} TLC aluminium plates (0.2mm thick) using methanol: ammonia (4.5:0.5*v/v*) as mobile phase. The densitometric quantification for the drug was carried out at 224 nm. The R_f value for VEN was found to be 0.65.

The proposed method has been validated for various parameters like linearity, accuracy, precision, sensitivity, reproducibility, repeatability and robustness as per ICH guidelines. The calibration curve obtained by plotting peak area against concentration showed linearity in the concentration range of 500-3000ng/µl per band and calibration curve constructed to be r^2= 0.998. The bulk assay for Venlafaxine hydrochloride was performed and the amount found is close to 100% when area was calculated for 1500ng/spot and six determinations. Calibration curve was constructed by plotting the peak area *vs.* corresponding drug concentration as shown in Figure 3 and 3-D linearity chromatogram is shown in Figure 4.

The proposed method was applied for pharmaceutical capsule formulation and % label claim for VEN was found to be 100.98%. As the retention time of VEN in bulk solution was same as marketed formulation solution and also there was no interference found of excipients. The mean recovery obtained for VEN was 99.64-100.68% and % RSD found was 1.7. The accuracy data tabulated in Table 1 show that the method is accurate within desired range.

The precision results expressed as %RSD and were found to be less than 2 for both intra-day and inter-day precision. There was no significant difference in the %RSD values, which indicates that the proposed method is precise. The detail results are tabulated in Table 2.

The LOD and LOQ for VEN were found to be 7.7ng and 23.3ng respectively. The values were low which indicates that the method is sensitive and no interference of the excipients with the peaks of interest appeared. It indicates the specificity of the method for quantitative estimation of drug in marketed formulation. The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters, and provides an indication of its reliability during normal usage. Robustness of the method was done in triplicate at a concentration level of 1000 ng/spot and the % RSD peak area was calculated and shown in Table 3. Hence, the proposed method is applicable for the routine estimation of VEN in pharmaceutical dosage form.

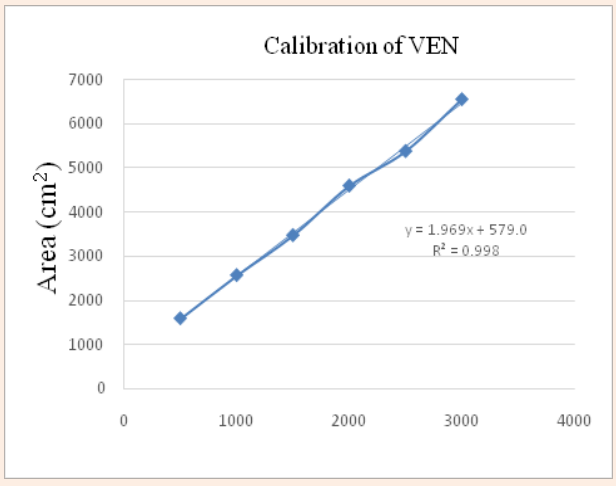

Figure 3: Calibration curve plot for Venlafaxine HCl.

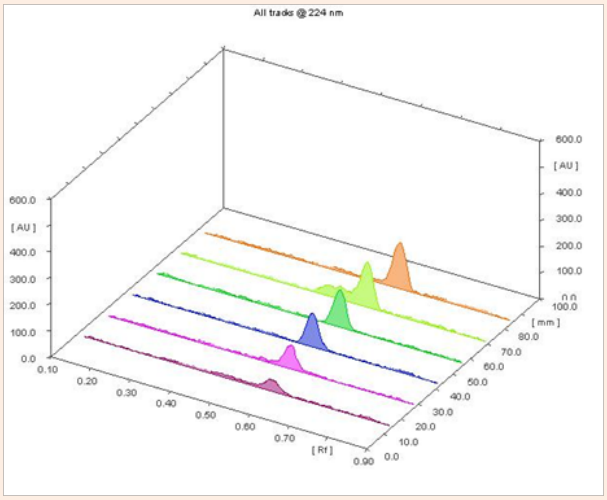

Figure 4: 3D Chromatogram of Venlafaxine HCl.

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Table 1: Recovery Study.

% level	Initial Amount (ng/band)	Amount Added (ng/band)	Amount found ± SD (ng/band) (n = 3)	% Recovery	% RSD
80	1500	1200	2690.4 ± 13.1	99.64	0.48
100	1500	1500	2997.8 ± 3.54	99.92	0.11
120	1500	1800	3322.5 ± 13.3	100.68	0.4

Table 2: Data of Precision Study.

Conc. (ng/band)	Intra Day		Inter Day	
	Amount Found (ng/band)		Amount Found (ng/band)	
	Mean ± SD	% RSD (n = 3)	Mean ± S.D.	% RSD (n = 3)
500	446.31± 2.41	0.16	443.98 ± 2.65	0.18
1000	959.99 ± 3.89	0.15	959.16 ± 4.26	0.17
1500	1519.95 ± 18.56	0.51	1510.97 ± 32.7	0.92

Table 3: Results of Robustness Study.

Parameters	± SD of peak area (n = 3)	% RSD
Mobile phase composition (Ammonia, ± 0.5mL)	8.09	0.32
Development distance (±0.5cm)	35.9	1.68
Duration of saturation (± 5min)	30.8	1.09

Conclusion

The reported HPTLC method was proved to be simple, rapid, and reproducible. The validation data indicate sensitivity, precision, accuracy, and reliability of the method for estimation of Venlafaxine HCl in bulk and capsule dosage form. The method was successfully validated as per ICH guidelines. The method is reproducible, sensitive, economical and simple for estimation of drug in bulk and marketed formulation without any excipients interference.

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