Journal of Pharmaceutical Research Vol. 13, No. 3, July - September 2014 : 71-73

DEVELOPMENT AND VALIDATION OF HPTLC/DENSITOMETRY METHOD FOR THE ESTIMATION OF VILAZODONE HCl IN BULK AND IN TABLET FORMULATION

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Received on: 26.06.2014 Revised: 26.08.2014 Accepted: 03.09.2014

ABSTRACT
A simple, economic, precise, sensitive and accurate HPTLC/densitometry method of analysis for determination of vilazodone HCl in both bulk drug and in tablet formulation was developed and validated. The method employed TLC aluminum plates precoated with silica gel 60 F_{254} S as the stationary phase. The solvent system consists of toluene:chloroform: methanol (2:2:1). This system was found to give compact spots for vilazodone HCl (R_f value=0.45). Densitometric analysis of vilazodone HCl was carried out in the absorbance mode at 242 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r^2=0.997 with respect to peak height and peak area, in the concentration range of 200-1200 ng/band. The method was validated as per the ICH guidelines for various parameters like recovery, precision, repeatability and reproducibility. The results obtained proved that the method can be employed for routine analysis of Vilazodone HCl in bulk as well as in tablet formulation. The proposed method also indicates no interference of excipients from tablet formulation.

Keywords: *Vilazodone HCl; HPTLC; ICH guidelines; validation.*

INTRODUCTION
General Background
Vilazodone HCl (VLN, Fig. 1) is chemically 5-(4-[4-(5-cyano-1*H*-indol-3-yl)butyl] piperazin-1-yl)benzofuran-3-carboxamide hydrochloride[1]. It contains an indole-piperazine that utilizes its function as an SSRI and 5-HT_{1A} receptor partial agonist[2]. It belongs to the category of serotonergic antidepressant approved by FDA (Food and Drug Administration) for treatment of depressive disorder [3,4]. It is a novel serotonin reuptake inhibitor and serotonin 1A receptor partial agonist[5] having strong affinity for D2 dopaminergic receptors[6]. Most of literature available highlights pharmacological profile of drug. Recently we published the spectroscopic method for vilazodone HCl estimation in bulk and in tablet formulation[7]. After the success of primary results, we are extending the work by more sophisticated technique i.e. HPTLC/densitometry.

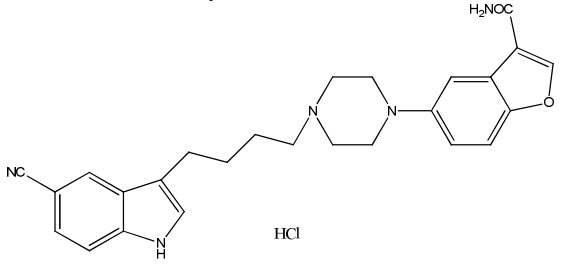

Fig. 1 : *Chemical structure of vilazodone HCl*

Thus, the present study illustrates the development and validation of a rapid, simple, specific, sensitive, accurate and precise HPTLC method for the determination of VLN in bulk and in tablet dosage form. The proposed method is optimized and validated according to ICH guidelines[8].

EXPERIMENTAL
Reagents and chemicals
Vilazodone HCl was supplied as gift sample by Glenmark Pharmaceuticals Ltd., Mumbai (India). All chemicals and reagents used were of analytical grade by Merck Chem. Ltd., Mumbai (India). Methanol was selected as the solvent for sample preparation. Tablet formulation Viibryd from Forest Laboratories was used as pharmaceutical preparation for analysis.

Instrumentation
Chromatography was performed on 10 cm × 10 cm aluminum backed TLC plates coated with 200 µm layers of silica gel 60F_{254} S (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 100-110°C for 10 min prior to chromatography. The samples were applied on the plates as 6 mm wide bands, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with 100 µl sample syringe (Hamilton, Bonaduz, Switzerland). Mobile phase was kept in Camag twin-trough glass chamber. Densitometric scanning was performed using Camag TLC Scanner 3 equipped with WinCATS software version 1.3.0.

Preparation of standard stock solution
An accurately weighed 10 mg of VLN was transferred to 10 ml volumetric flask; dissolved in methanol and the volume was made up to mark with the same solvent to give 1000 ng/µl solution.

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Optimization of mobile phase and chromatographic conditions

On the basis of polarity, toluene was selected as trail solvent for mobile phase. It was followed by further trials combining toluene and chloroform in varying ratios. The developed spot was diffused and tailing was observed. To the above mobile phase, 1 ml methanol was added. The peak was found to be symmetrical in nature. Finally, the mobile phase toluene: chloroform: methanol (2:2:1) gave good, sharp and symmetrical peak with R_f value of 0.45 for VLN. Also the spot for VLN was compact and not diffused. Plate was developed to a distance of 8 cm in Camag twin-trough glass chamber previously saturated with mobile phase vapors for 25 min at ambient temperature. Densitometric scanning was performed at 242 nm. A typical chromatogram of VLN in bulk is shown in Fig. 2.

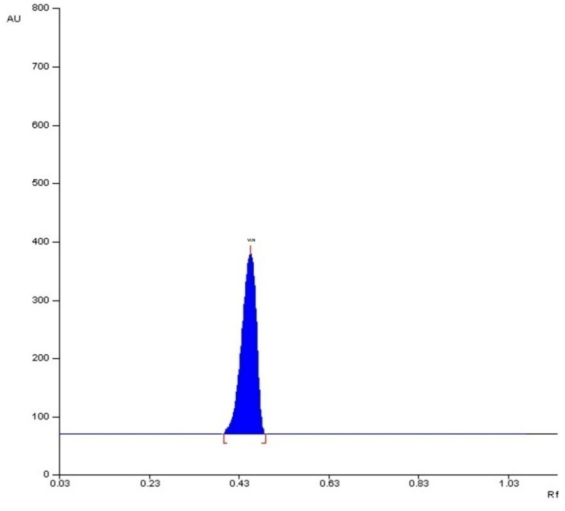

Fig.2: *A typical chromatogram of vilazodone HCl in bulk showing R_f value 0.45*

Validation of proposed method

Linearity study

Linearity was performed using working standard of VLN. Calibration was done by applying standard stock solution ranging from 0.2 - 1.2 µl on TLC Plate; which gives concentration of 200-1200 ng/band. The plate was developed and scanned as described under above chromatographic conditions. Calibration curve was constructed by plotting the peak area *vs*. corresponding drug concentration. The 3D linearity chromatogram is shown in Fig. 3.

Analysis of tablets

To determine the content of VLN in tablets, twenty tablets, each containing 10 mg VLN, were accurately weighed and finely powdered. An amount equivalent to 10 mg VLN was transferred to 100 ml volumetric flask and extracted with methanol for 20 min by shaking mechanically. The solution was diluted to volume with the same solvent and filtered; from it, the sample solution (6 µL, containing 600 ng of VLN) was applied on TLC plate, developed and scanned. Absorption spectra of VLN in standard and tablet solution are shown in Fig. 4.

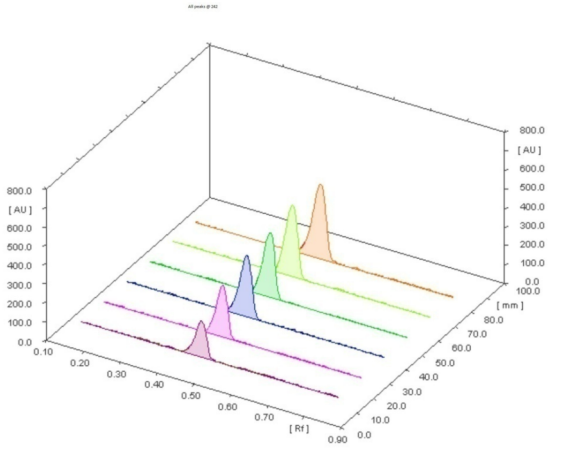

Fig. 3 : *The 3D linearity chromatogram of vilazodone HCl*

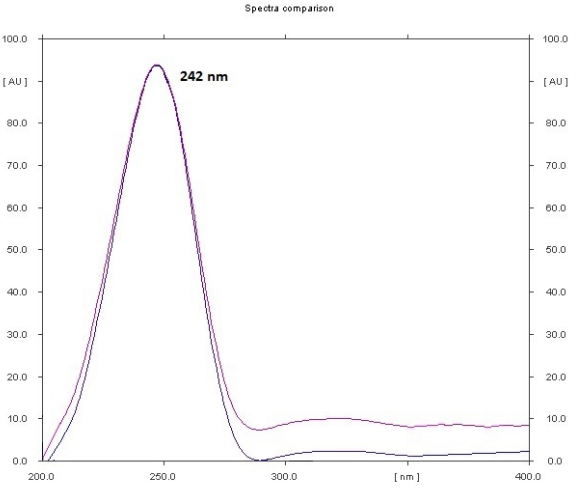

Fig. 4 : *Absorption spectra of VLN from standard and tablet solution are showing λ max at 242*

Accuracy

Recovery study was carried out by over spotting at 80, 100 and 120 % level where known amount of standard VLN was added to pre analyzed sample (400 ng of VLN) and subjected them to the proposed method for analysis. High recovery and low standard deviation confirmed that proposed method is accurate for determination of VLN in pharmaceutical formulation.

Precision

Precision of the method was studied as intra-day and inter-day variations. Intra-day precision was determined by analyzing three different concentrations 400 ng, 600 ng and 800 ng of VLN, for three times within the day. Day to day variability was assessed using above mentioned three concentrations and analyzing it for three consecutive days, which shows reproducibility of the method.

Repeatability

Repeatability of sample application was assessed by applying 0.6 µl (600 ng) of drug solution six times on a

TLC plate followed by development of plate and recording the peak height and area for six bands.

Sensitivity

Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantitation (LOQ). LOD and LOQ were calculated by the method which was based on the SD of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ, LOD = 3.3 (SD/S) and LOQ = 10 (SD/S). Stock solution of VLN was prepared and different volume of stock solution in the range 200–400 ng/band were applied in triplicate.

Ruggedness

Ruggedness of the method was checked by analyzing 600 ng (n = 6) of VLN, with the help of two analysts and the variations in the results were checked.

Robustness

Robustness was studied at the concentration level of 600ng/band. In this study, few parameters like mobile phase composition (± 0.5 ml), development distance (± 0.5 cm) and duration of saturation (± 5 min.) were changed deliberately and the effects on the results were examined.

RESULTS AND DISCUSSION

An HPTLC / densitometric method has been developed successfully for the determination of vilazodone HCl in bulk and in tablet formulation. The estimation of drug was performed on HPTLC aluminum plates precoated with silica gel 60F254 S using toluene:chloroform: methanol (2:2:1) as mobile phase. The densitometric quantification for the drug was carried out at 242 nm. VLN obeyed linearity over the investigated concentration range of 200-1200 ng/band with coefficient of correlation (r^2) 0.997. The R_f for VLN was found to be 0.45. The limits of detection (LOD) and quantitation (LOQ) was found to be 8 and 24 ng/band respectively.

The proposed method was applied for pharmaceutical tablet formulation and % label claim for VLN was found to be 99.41. This indicates no interference of the excipients present in the tablet. The method was validated for accuracy, precision, repeatability, sensitivity, ruggedness and robustness. Accuracy of the method was checked by recovery studies at three different levels i.e. 80 %, 100 % and 120 %. The recovery studies indicate the drug has almost recovered. The mean % recovery of VLN was found to be in the range of 99.88–100.01 %; the % RSD value of less than 2 is an indicative of accuracy of the proposed method. The method was found to be precise as indicated by the inter-day, intra-day and repeatability analysis; showing % RSD less than 2. Repeatability indicates performance of the HPTLC instrument under optimized chromatographic conditions. The results did not show any statistical difference between operators showing that developed method was rugged. The robustness studies were done by making small deliberate change in amount of toluene by 0.5 ml, development distance by 0.5 cm and duration of saturation time by 5 min. The obtained results and % RSD values below 2 showed that method is highly robust. The results showing summary of all parameters for the developed method are highlighted in Table 1. This is an indicative that the developed method can be applied successfully for the determination of Vilazodone HCl in tablet formulation.

Table 1: *Summary of validation parameters of proposed HPTLC method*

Parameters	Results
Linearity range	200-1200 (ng/band)
Correlation coefficient	0.997
Limit of detection	8 ng/band
Limit of quantitation	24 ng/band
% Recovery (n = 3)	99.88–100.01 %
% RSD	0.54 – 0.66
Precision (%RSD)	
Intra-Day (n = 3)	0.38 – 1.42
Inter-Day (n = 3)	0.12 – 0.52
Repeatability (n = 6)	0.40
Ruggedness (%RSD)	
Analyst I (n = 6)	1.01
Analyst II (n = 6)	1.09
Tablet assay (% amount found)	99.41 %

ACKNOWLEDGMENT

The authors are thankful to Glenmark Pharmaceuticals Ltd., Mumbai (India) for providing gift sample of vilazodone HCl.

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