HPTLC AND FIRST DERIVATIVE UV-SPECTROPHOTOMETRIC METHODS FOR ESTIMATION OF VILAZODONE IN PHARMACEUTICALS

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ABSTRACT

A new HPTLC method and a sensitive UV spectrophotometric method were established for estimating vilazodone hydrochloride in its pharmaceutical formulations. An environment-friendly chromatographic mobile phase consisting of ethyl acetate: toluene: formic acid (5:4:1, V/V/V) along with ultraviolet densitometric detection at 240 nm using pre-coated silica gel plates was used. First derivative UV spectrophotometric determinations were performed using methanol as solvent, and amplitudes were measured for quantification purposes. Validation studies were compliant with ICH guidance. Vilazodone shows an optimum Rf value of 0.73 ± 0.02 by HPTLC method. First derivative spectrophotometric measurements were done at 230 nm (peak) and 247 nm (valley), respectively. Best method linearity was observed in the range of 100 - 600 ng band^{-1} and 100 - 600 ng mL^{-1} for the chromatographic and spectrophotometric methods, respectively. Method detection limits were 61.4 ng band^{-1} and 36.03 ng mL^{-1}, for chromatography and spectrophotometry, respectively. Higher % recovery (> 99 %) of vilazodone from the in-house formulation satisfied study intent as well as instituted selectivity of both the methods. Both the newly developed methods have the potential of application in routine quantification of vilazodone in pharmaceuticals.

Keywords: High-Performance Thin layer chromatography, densitometry, derivative spectroscopy, vilazodone

INTRODUCTION

Vilazodone hydrochloride (VLN) is a novel antidepressant agent and chemically known as 5-[4- [4-(5-cyano-1*H*-indol-3-yl) butyl]-1-piperazinyl]-2-benzofuran carboxamide hydrochloride (1:1) (Fig. 1)[1]. Serotonin partial agonism and reuptake inhibition are pharmacological activities of VLN[2]. The literature review revealed analytical procedures such as spectrophotometric, high-performance liquid chromatography (HPLC), high-performance thin-layer chromatography (HPTLC), and liquid chromatography-mass spectrometry (LC-MS) for VLN in diverse sample matrixes[3-15]. It was noticed during the review that only two HPTLC methods are reported to estimate VLN, and both of them have one or the other drawbacks like the use of non-environment friendly organic solvent systems and co-elution of unknown peaks with the analyte, which suggests poor elution as well as decreased resolution. Co-elution in HPTLC is a common issue when there is interaction between the ionized as well as unionized forms of an analyte with the stationary phase material. With the intent to provide an error-free

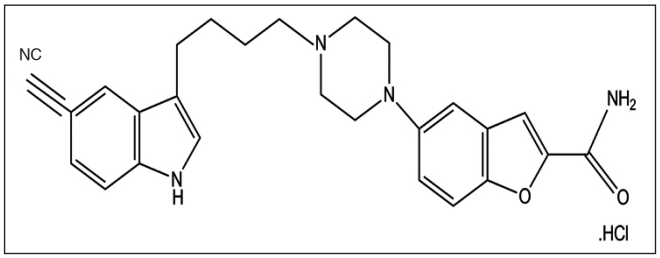

Fig. 1: Chemical structure of VLN

simple alternative analytical procedure for VLN, a UV spectrophotometric method was found befitting. The reported spectrophotometric methods have issues such as lower sensitivity, which rendered a challenge to compete with other sensitive analytical techniques in everyday practice. Hence, the authors attempted to utilize derivative spectroscopy, which efficiently eliminates possible variation in λ_{max} values and less deviation in absorbance measurement. The transformed zero-order spectra are generated considering a change in absorbance values with wavelengths *vs.* wavelength ($dA/d\lambda$)[16]. Moreover, derivatization of zero-order spectra leads to the correction of a systematic error by suppression of background matrix interference, and it also improves finer spectral features[17]. Because of the above facts, systematized

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Table I: Comparison of method performance of current methods with reported chromatographic and spectrophotometric methods

Reported method	Linearity (μg mL^{-1})	Accuracy (%)	Precision (%RSD)	Assay (%)	Remarks
Spectrophotometry[3]	1 - 3	98.91 - 101.16	Method:0.12	99.78	Use of multiple solvents.
			System:0.08		
Spectrophotometry[4]	5 - 60	98.93 - 100.1	Intraday:0.16	100.58	Method sensitivity needs revival.
			Interday:0.09		
RP-HPLC[5]	1 - 5	99.8 - 99.92	Intraday: 0.14	99.902	Triethylamine used as peak modifier demand more time for column equilibration as well as clean up.
			Interday:0.14		
RP-HPLC[6]	2 - 12	80.66 - 118.14	Intraday:1.16	99.93	Data inconsistency, poor recovery.
			Interday:1.19	100.66	
RP-HPLC[7]	50 - 180	98.6 - 102.4	0.2	99.16	Low sensitivity, longer runtime, gradient programming.
RP-HPLC[8]	0.4 - 1.2	98.87 - 99.59	Intraday:0.03 -1.24	101.61	Uses 100% methanol as mobile phase and uses column oven at 30°C.
			Interday:0.57 -1.23		
RP-HPLC[9]	25 - 75	98.21 - 99.07	Intraday:0.72 - 1.41	98.09	Lower method sensitivity.
			Interday:0.47 - 0.8		
RP-HPLC[10]	1.1 - 120	98.2 - 101.21	0.8 - 1.12	99.4	Fails to establish chromatograms of study.
RP-HPLC[11]	4 - 20	100.34 - 100.48	Intraday:0.09 - 0.13	99.75	Fails to establish method selectivity.
			Interday:0.11 - 0.17		
HPTLC[12]	200 - 1200 ng $band^{-1}$	99.88 - 100.01	Intraday:0.38 -1.42	99.41	Lower sensitivity.
			Interday:0.12 - 0.52 System:0.4		
HPTLC[13]	100 -500 ng $spot^{-1}$	99.36 - 101.98	Intraday:0.94	100.2	Co-elution observed.
			Interday:0.86		
Current Method					
HPTLC	100 - 600 ng $band^{-1}$	98.67 - 102.15	Intraday:0.28 -1.4	100.33	
			Interday:0.39 - 0.86		
dA/dλ	100 - 600 ng mL^{-1}	100.08 - 102.66	Intraday:0.2 - 0.78		
			Interday:0.45 - 0.78	99.83	
Remarks for Current Methods	The current methods are found superior than the above enlisted methods in terms of being highly sensitive with a wider linear range, lack of co-elution, superior recovery than the reported HPTLC and spectrophotometric methods.				

and simple analytical methods for estimating this novel antidepressant agent present in bulk and in-house tablet dosage form was developed.

MATERIALS AND METHODS

Chemicals

The standard VLN (purity > 99.5 %) was kindly supplied by Glenmark Pharmaceuticals Ltd., India. Ethyl acetate, toluene, formic acid, and methanol were of analytical reagent grade and were procured from Merck, Mumbai. In-house tablets of VLN containing 40 mg of analyte were studied for assay purposes.

Instrumentation

An HPTLC system (CAMAG, Muttenz, Switzerland) equipped with a TLC Scanner III controlled by winCATS software version 1.4.4 was used for the purpose. The stationary phase comprised of pre-coated silica gel aluminum plates 60F254 (10×10 cm of layer thickness 0.2 µm) from E.Merck, Darmstadt, Germany. The samples were applied at an application rate of 150 nL s^{-1} constantly on TLC plates by using a Linomat-V autosampler connected to a nitrogen cylinder having a 100 µL syringe (Hamilton, Switzerland). The slit dimensions of the TLC scanner were set at 5 mm × 0.45 mm, and 20 mm s^{-1} scanning. A twin trough chamber of dimensions 10 × 10 cm served the chromatographic development purpose. A Shimadzu 1800 UV spectrophotometer with 10 mm matched quartz cuvettes was used for the spectrophotometric studies. The instrument was controlled using UV Prove 2.32 software.

Preparation of standard and sample solution

About 5 mg of standard VLN was placed in a 5 mL volumetric flask containing 2 mL of methanol and vortexed for the 30 s. A stock solution of 1000 µg mL^{-1} was obtained by making up the volume with methanol. Further linearity solutions from 100 to 600 ng $band^{-1}$ and 100 to 600 ng mL^{-1} were obtained by diluting suitable aliquots of the above VLN solution using methanol.

Tablet powder equivalent to 5 mg of standard VLN was transferred to a 5 mL volumetric flask containing 2 mL of methanol and sonicated for about 20 min. Post volume make up using methanol, centrifugation (10 min at 10,000 × g) ensured maximal extraction of the analyte while settling down of the tablet components. Further, the solution was membrane filtered (0.2 µm). The filtered solution was utilized to obtain sample concentrations within the linearity range. Around 2 µL of the sample, solution was used for the chromatographic estimation of VLN.

Chromatographic workflow

This HPTLC procedure utilized silica gel 60F254 plates cut into dimensions of 10 × 10 cm before the application of the analyte. Around 2 µL of the solutions containing VLN (standard and sample) were applied on to the plate as bands with a width of 4 mm. The mobile phase containing ethyl acetate: toluene: formic acid (5:4:1 V/V/V) was poured into the twin trough chamber, and the chamber was allowed to saturate for 20 min at ambient conditions for ascending mode of separation. Post-development drying of plates was carried out for 5 min in ambient conditions. Further, the plates were scanned at 240 nm, and the obtained data were processed using winCATS software.

Spectrophotometric method

The drug solutions of VLN with final concentrations from 100 to 600 ng mL^{-1} were scanned in zero-order spectrum mode under 200 - 400 nm. The generated spectra were transformed using UV Probe software, and first derivative spectrums were obtained. Absorbance was measured (triplicate) for every determinant.

Method validation

Validation studies were International Conference on Harmonization (ICH) compliant and were performed according to the following procedure[18].

Linearity

To establish the linear response range of VLN over the concentration range of 100 - 600 ng $band^{-1}$ (six points) and 100 - 600 ng mL^{-1} (six points), densitograms and first derivative spectra were studied, respectively. Calibration curves were prepared, placing peak area and absorbance on Y-axis and concentration (ng $band^{-1}$ and ng mL^{-1}) on X-axis with subsequent statistical analysis.

Accuracy and precision

Fixed concentration solutions of VLN present in tablet solution, were chosen and were spiked with 50 %, 100 % and, 150 % of standard VLN. The recovery of VLN was indicative of method trueness.

Method precision was assessed as intraday and interday precision at three quality control (QC) levels viz. low QC(LQC), mid-QC (MQC), and high QC(HQC) of VLN. Further, the % relative standard deviation (% RSD) was calculated.

Sensitivity

Method sensitivity was affirmed by instituting a limit

of detection (LOD) and limit of quantitation (LOQ). Both the limits were calculated using the following equation:

$$LOD\ (or) LOQ = A \times Sbc \ldots \text{(Eqn.1)}$$

where A= 3.3 for LOD and 10 for LOQ, 'Sb,' and 'c' are the standard deviation of the intercept and slope of the linearity curve, respectively.

Selectivity and specificity

The selectivity of both methods can be effectively assessed by estimating the % recoveries of VLN from the dosage form containing commonly used formulation additives and excipients. Visual evaluation of spectra and peak areas with R*f* values of analyte in standard, as well as the sample, serve as a useful tool for affirming the specific nature of the TLC process. Further, peak purity in terms of spectral comparisons of VLN band at start, apex, and end of the peak was performed[19].

RESULTS AND DISCUSSION

Optimization of the chromatographic method

Method optimization was performed for the development of a new mobile phase and the best spectral measurements. The various physicochemical properties of VLN were studied before early method development experiments. Further, an in-depth analysis of the reported methods (Table I) helped the authors to set up the method intent. In the present study, the authors have opted for greener alternatives of solvents like ethyl acetate and toluene and avoided the use of solvents like methanol and chloroform, etc. In one of the reported methods, co-eluting peaks with a low resolution to that of VLN was observed. Formic acid (98 %) was used to improve the compactness as well as a resolution between the analyte and extra peaks present, if any. The formic acid helps to convert the analyte into its protonated form and minimizes the peak tailing due to interaction with the stationary phase.

Further, using formic acid as a mobile phase component helped in increasing the polarity of the mobile phase, resulting in better elution of VLN. With the above study intent, a typical mobile phase comprising of ethyl acetate: toluene: formic acid (5:4:1 V/V/V) was found suitable. The detection conditions should be optimized to obtain an accurate estimation of the analyte. So, the drug in methanol was scanned within the UV light range, i.e., 200 - 400 nm. VLN portrayed an absorption maximum (λ_{max}) at 240 nm. Further, the detections were carried out at 240 nm. VLN was found to show an R*f* value of 0.73 ± 0.2 under the above chromatographic condition (Fig. 2(A,B)).

Optimization of the spectrophotometric method

VLN in methanol shows a λ_{max} at 240 nm (Fig. 3(A)) when scanned under UV light within 200- 400 nm. However, when plotted in the linearity curve, an R^2 value less than 0.9 was obtained for the obtained absorbances. This presented a need for transformation of the zero-order spectra to obtain suitable derivative

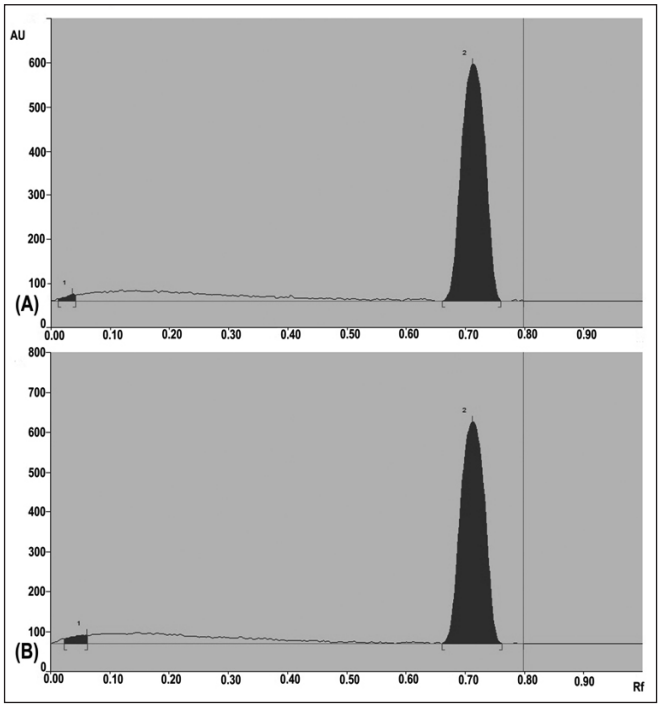

Fig. 2: Densitograms comparing R_f values of (A) standard VLN and (B) VLN in tablet solution

spectra for getting optimum measurements. A first-order (dA/dλ) smoothing provided amplitudes at 230 nm (peak) and 247 nm (valley). However, to obtain the best signal-to-noise response as well as reproducible results, the total amplitudes at 230 nm and 247 nm (Fig. 3(B)) were used. The molar extinction coefficient ('ε') was found to be 1.519×10^5 (L mol cm^{-1}). Further, the measurements were carried out considering total amplitudes at both the wavelengths.

Validation

Linearity

The methods were found linear over the concentration range of 100 - 600 ng $band^{-1}$ and 100 - 600 ng mL^{-1} (R^2 > 0.99) for HPTLC and spectrophotometric methods, respectively. Further, satisfactory results obtained through regression statistics of linearity data indicated goodness of fit (Table II).

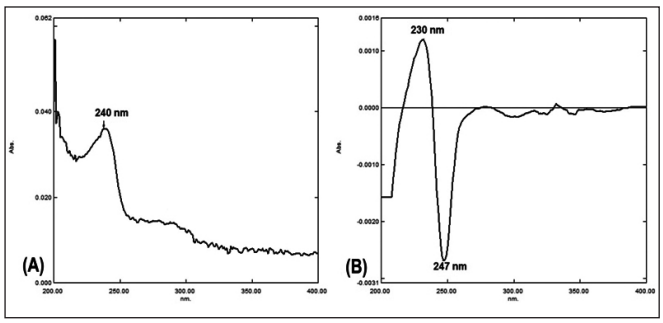

Fig. 3: UV absorption spectrum of VLN at LQC: 100 ng mL^{-1} (A) zero order, (B) first derivative (dA/dλ)

Table II: Summary of linearity data

Linearity	Correlation coefficient (R^2)	Multiple R	Adjusted R^2	Slope	Inter-cept
100 - 600 ng band^{-1}	0.9941	0.9970	0.9926	20.12	964.6
100 - 600 ng mL^{-1}	0.9992	0.9996	0.999	0.00002	0.0

Accuracy and precision

Satisfactory recoveries of VLN between 98.67 - 102.15 % and 99.18 - 99.49 % advocated the accuracy and reliability of the developed HPTLC and spectrophotometric methods, respectively.

The precision study revealed acceptable values of % RSD (< 2%). The values were indicative of method preciseness during the study period (Table III).

Table III: Results of intraday and interday precision study

Type	Concentration Taken		%RSD	
	HPTLC (ng band^{-1})	dA/dλ (ng mL^{-1})	HPTLC	dA/dλ
Intraday	100	100	0.97	0.78
	300	300	1.08	0.34
	600	600	0.3	0.2
Interday	100	100	0.55	0.78
	300	300	0.98	0.75
	600	600	0.43	0.045

% RSD = Relative standard deviation estimated for three determinations

Sensitivity

The method sensitivities, i.e., LOD were 61.4 ng band^{-1}, and 36.03 ng mL^{-1} and LOQ were 186.2 ng band^{-1} and 120.11 ng mL^{-1} obtained for HPTLC and spectrophotometric methods, respectively. Further, Sandell's sensitivity value (0.0029 µg cm^2 0.001 absorbance unit^{-1}) for the spectrophotometric method was also determined.

Selectivity and specificity

The excellent results obtained (Table IV) during the recovery study by standard addition method depicted noninterference from the excipients and ensured method selectivity to estimate VLN. Visual comparison of chromatograms of the standard VLN and VLN in in-house tablet formulation (Fig. 2(A,B)) depicted identical responses for VLN in both standard and sample bands. Further, a three-dimensional view (Fig. 4) of the three QC levels studied during accuracy study, and the sample served the purpose. The results of the above research advocated method specificity.

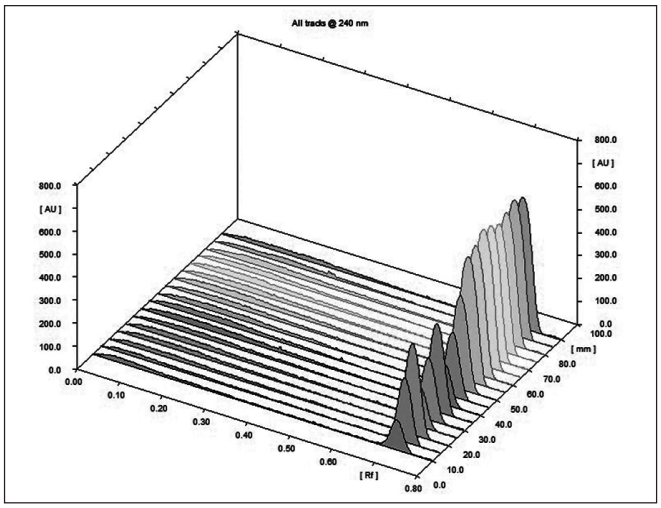

Fig. 4: Three dimensional chromatograms of VLN displaying peaks for the three QC levels studied during accuracy study and samples investigated

Table IV: Accuracy data of the method

Level (%)	Recovery (%) ± S.D.		% RSD	
	HPTLC	dA/dλ	HPTLC	dA/dλ
50	102.15 ± 0.51	100.27 ± 0.34	0.50	0.34
100	100.31 ± 0.34	100.08 ± 0.31	0.34	0.31
150	98.67 ± 0.47	102.66 ± 0.4	0.47	0.39

100 % = 200 ng band^{-1} and 200 ng mL^{-1} for HPTLC and dA/dλ method, respectively, Recovery = Mean of three determinations at each level, S.D. = Standard deviation

Analysis of tablet solution

The visual evaluation of chromatograms obtained for tablets indicated method selectivity because of the noninterference of any of the formulation components. The mean content of VLN was found to be > 99% for both the methods (Table V). Further, a student's t-test

result revealed no significant difference in the results of the assay by the two methods as the calculated values were well below the theoretical values (Table V).

Table V: Results of tablet analysis by both the methods

Mean Recovery (%) ± S.D.		% RSD		t-Value	
HPTLC	dA/dλ	HPTLC	dA/dλ	Tabulated	Obtained
100.33 ± 0.87	99.83 ± 1.01	0.50	0.34	4.3	0.96

Mean Recovery = Mean of three determinations, Tabulated= t-value at P = 0.05, degree of freedom=2

CONCLUSION

A new HPTLC and a simple first derivative spectrophotometric method were established for estimating VLN. The current study possesses advantages such as the use of environment-friendly solvents and compact elution of the analyte with no co-eluting peaks. The R*f* values obtained for VLN are suitable and optimum for HPTLC analysis purpose. Collaterally, a simple derivative spectrophotometric method was instituted, with improved sensitivity. The transformation of zero-order spectra to first derivative spectra produced reproducible results with reliability. An overall critical comparison of the current methods with that of reported ones indicated the superiority of the newly developed techniques. Overall, these analytical methods are apt, trustworthy for estimating VLN in pharmaceutical dosage forms and lack reported drawbacks. Results of the validation study were found compliant with ICH guidelines. Besides, these methods can be effectively used as an alternative analytical tool for analyzing VLN in bulk and tablets for everyday purposes. Further, these methods may serve as a basis for estimating VLN in biological fluids.

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