Original Article

DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMERIC METHOD FOR THE DETERMINATION OF VENLAFAXINE HYDROCHOLORIDE IN BULK AND SOLID DOSAGE FORMS

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Abstract

Determination of Venlafaxine Hydrochloride in a fixed dosage form was carried out by UV Spectrophotometric method. The absorbance values were observed for different dilutions of drug at 224nm and which are used for the estimation of drug without mutual interference of excipients. The solvent used for the dilution is Distilled water. This method obeys Beer's Lambert's law in the concentration range of 2-14 µg/ml. The results of analysis have been validated statistically and the recovery studies confirmed the accuracy of this proposed method.

Key words: Venlafaxine Hydrochloride, UV Spectrophotometer, Recovery studies.

Introduction

Venlafaxine hydrochloride is an anti-depressant agent and chemically it is a cyclohexanol hydrochloride derivative. Its IUPAC name is [R/S]-1-[2-dimethylamine)-1-[4-Methoxy phenyl)ethyl] cyclohexanol hydrochloride or (+)-1-[2-[(dimethylamino)methyl]p-Methoxybenzyl] cyclohexanol hydrochloride. Venlafaxine is official drug in British Pharmacopoeia [1-3]. HPLC measurements of Venlafaxine and o-desmethyl venlafaxine in human plasma for its determination in pharmaceutical preparations when present alone. HPLC methods were reported for the estimation of Venlafaxine dosage forms [4]. The review of literature revealed that no method is reported for the Venlafaxine hydrochloride in fixed dosage products by UV-spectroscopy. The present paper describes a simple, rapid, accurate and reproducible method for the estimation of Venlafaxine hydrochloride in tablet formulation by first order derivative spectrophotometry.

Materials and Methods:

Materials

Venlafaxine hydrochloride was a gift sample from Lee Pharma Pvt. Ltd, Hyderabad. The commercial fixed dose product VENLOR 37.5mg and DALIUM 37.5mg was procured from the local market.

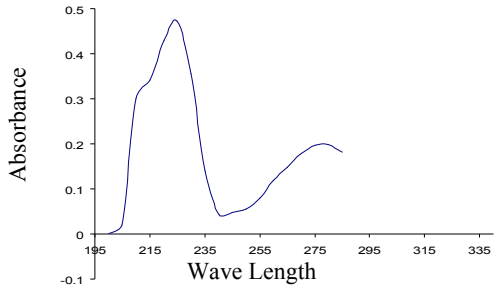
Equipments

PERKIN ELMER UV-Visible spectrophotometer Lamda 25 with 1cm matched quartz cell was used for the measurement of absorbance. SHIMADZU-AX-200 electronic balance was used for weighing the samples. Class-A volumetric glass wares were used

Development of the method

The solution of Venlafaxine was prepared in distilled water at the concentration of 10µg/ml. This was scanned in the wavelength range of 200-400 nm. Data were recorded at an interval of 5nm. The wavelength selected for Venlafaxine hydrochloride analysis was 224 nm. The spectral data of the drug shown in Fig 1

Fig 1: Absorption Spectral data (at 224nm)



Analysis of Marketed Formulation

20 tablets were weighed accurately and powdered. Each containing of 37.5 mg quantity equivalent to 10 mg of Venlafaxine hydrochloride was weighed and dissolved in 100ml distilled water. The solution was filtered through whatmann filter paper No: 41. Further dilution was made up to 100ml with distilled water in volumetric flask. To set the theoretical concentration of the drug was 10µg/ml and the concentration of the drug was determined. The data was shown in Table 1.

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Table 1: Analysis of Formulation

S. No.	Brand Name	Amount of drug (in mg)		% Labelled claim
		Labelled	Estimated	
1	VENLOR [Cipla]	37.5	36	96.26
2	DALIUM [Npil]	37.5	36	96.26

Validation Methods

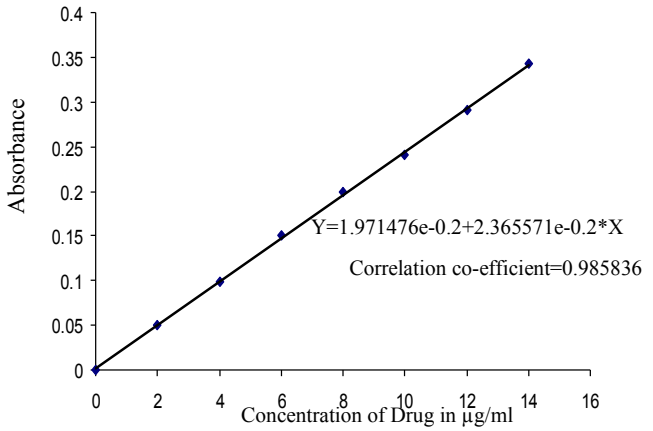
Linearity

Standard stock solution was prepared by dissolving 100 mg of each standard drug sample in 100 ml volumetric flask separately and the volume was made up with distilled water [5]. To get a concentration of 1mg/ml, from this suitable dilution was made in distilled water to get the working standard solution of 2-14 µg/ml for Venlafaxine. The absorbance was measured at 224 nm [6]. For Venlafaxine hydrochloride seven replicated analysis were carried out. Absorbance Vs concentration were plotted to obtain the calibration graph. The drug obeyed to the Beer's law to the above concentration range with R^2 value 0.995836 (Table 2 & Fig 2).

Table 2: Linearity

S.No.	Concentration	Absorbance at 224 nm
1	2	0.0499
2	4	0.0990
3	6	0.1498
4	8	0.1986
5	10	0.2409
6	12	0.2901
7	14	0.3422

Fig 2: Beer's Law Plot



Precision

Precision is the degree of aggregate among individual test results when a method is applied repeat ably to multiple sampling of a homogenous sample is known as precision of the analytical method.[7]

Repeatability

Repeatability studies were done by repeatedly observing the standard solution containing 10 µg/ml of Venlafaxine hydrochloride % RSD (percentage relative standard deviation) was calculated and the data was shown in Table 3.

Table 3: Repeatability

Venlafaxine hydrochloride			
S.No.	Concentration (µg/ml)	Absorbance	% RSD
1		1.128	
2		1.138	
3	10 µg/ml	1.141	0.005
4		1.149	
5		1.154	
6		1.159	

Average of Six Determinations

Inter day

Inter day precision was found out by preparing 10 µg/ml equivalent concentration of formulation for three days and standard deviation was calculated [8]. The peak area and % RSD (percentage relative standard deviation) are shown in Table 4.

Table 4: Interday

Day/Date	Venlafaxine hydrochloride (10 µg/ml)	
	Absorbance	% RSD
1st day (16.03.2009)	1.122 1.235 1.247	0.39
2nd day (17.03.2009)	1.235 1.121 1.246	0.39
3rd day (18.03.2009)	1.125 1.237 1.249	0.38

Average of three determinations

Intra day precision

Intra day precision was found out by preparing 10 µg/ml equivalent concentration of Venlafaxine hydrochloride in the formulation for six times on the same day. The absorbance of solution was noted and the concentration corresponding was calculated [9]. The data was presented in Table 5.

Table 5: Intraday

S.No.	Sample concentration (µg/ml)	Drug	Absorbance	% RSD
1	10 µg/ml	Venlafaxine Hydrochloride	1.118	0.62
2			1.232	
3			1.359	
4			1.246	
5			1.389	
6			1.451	

Average of six determinations

Accuracy and Recovery studies

The accuracy of the experiment was established by using recovery studies, ie, by external dilution method. The known amount of standard was added at 3 different levels of 80 %, 100 % and 120 % of samples [10]. The result of recovery analysis was presented in Table 6.

Table 6: Accuracy and Recovery studies

S.No.	Brand Name	Wt. of Std Drug added (mg)	Wt. of Powdered Tablet (mg)	Test Absorbance	Content of Drug in percentage Powdered Tablet (mg)	% Average Content (mg)
1			10	1.923	95.48	
2	VENLOR	2	11	1.946	96.61	96.09
3	[Cipla]		12	1.914	95.02	
4			13	1.959	97.28	
1			10	1.948	96.8	
2	DALIUM	2	11	1.961	97.4	95.83
3	[Npil]		12	1.926	95.6	
4			13	1.876	93.5	

Average of four determinations

Ruggedness

The ruggedness of the UV spectroscopy method was evaluated by difference in the absorbance and consistent using a standard working solution. Small differences in the absorbance and good constancy were observed after three days. An RSD of less than 0.06% were obtained.

The comparable absorbance obtained on different days indicates that the method is capable of producing results with high precision on different days [11].

The high degrees of reproducibility of absorbance indicate that the method is fairly rugged and given in Table 7.

Table 7: Ruggedness

Venlafaxine Hydrochloride				
S.No.	Day	Wt. of Std Drug (in mg)	Std. Absorbance	% RSD
1	10th Mar 2009	10 µg/ml	0.2016 0.2182 0.2196 0.2294	0.06
2	14th Mar 2009	10 µg/ml	0.2206 0.2371 0.2393 0.2406	0.03

Average of four determinations

Stability

The sample was subjected for stability studies under room temperature. Stabilities were studied by performing experiment to check the changes the absorbance with the freshly prepared standarad solution [12]. The solution under room temperature was stable upto 5hrs. The data was shown in Table 8.

Table 8: Stability

S.No.`	Time (in hrs)	Venlafaxine Hydrochloride		% RSD
		Concentration (µg/ml)	Absorbance	
1	1hrs	10 µg/ml	1.118	0.0023
2	2 hrs		1.117	
3	3 hrs		1.115	
4	4 hrs		1.120	
5	5 hrs		1.112	

Average of five determinations

Results and Discussion

The UV spectrum of Venlafaxine hydrochloride was obtained by using distilled water as a solvent and then validated. The wave length was found to be 224 nm and linearity obtained at the concentration range 2-14 µg/ml for both bulk and solid dosage forms Fig. 1 shows Venlafaxine hydrochloride at a concentration of 10 µg/ml is evident that Venlafaxine hydrochloride shows absorbance at any of these wavelengths could be used for the estimation of Venlafaxine hydrochloride. The wavelength 224 nm was selected because it showed maximum absorbance by the solvent distilled water.

The absorption in first derivative mode of Venlafaxine hydrochloride at selected wavelength at 224nm was linear in concentration range of 2-14 µg/ml. the R^2 was found to be 0.995836.

The percentage recovery varied from 100-103 % w/w for Venlafaxine hydrochloride. The stability absorbance was measured in derivative mode at 224 nm for Venlafaxine hydrochloride at periodic intervals. There was no change in the absorbance at the wavelength up to 5 hours, indicating that the solution was stable for at least 5 hours. Commercial formulation containing Venlafaxine hydrochloride was analyzed by proposed method. Analysis of formulation was carried out. The content of the Venlafaxine was found to be 36 mg per tablet. The corresponding standard deviation was found to be 0.005 for Venlafaxine hydrochloride, indicating that the method has required precision. The accuracy of the method was determined by recovery studies. Venlafaxine hydrochloride was determined by recovery studies were carried out. The recovery was from 100-103 %, indicating that the method has required accuracy. To study the ruggedness of the prepared mixture was observed six times as a test sample. Small differences in the absorbance and good consistency were observed after three days. An RSD of less than 0.06 % were obtained. The comparable absorbance on different days indicates that the method is capable of producing results with high precision on different days.

Thus, the developed method is simple, accurate and precise and can be used for the routine analysis of Venlafaxine hydrochloride.

Conclusion

The method was validated with respect to linearity, precision, accuracy, selectivity and sensitivity. The calibration plots for each method were constructed. The regression equation and correlation coefficient of the mean of four consecutive calibrations were given

The method was established according to ICH guideline and definition. Accuracy was investigated by analyzing two different marketed formulations and percentage was found to be in the range of 100-103 % w/w.

The interday and intraday relative standard deviation (RSD) values with low percentage RSD values were obtained. This indicated that intermediate precision of the method was found to be good.

By applying the standard addition technique further assessed the validity of the suggested method. It was carried out by adding a known amount of sample to the marketed formulation at 50-150 % of concentration in the proposed method .The percentage recoveries of the concentration were found to be 100 %. Therefore it can be said that the method were highly selective.

The proposed method based on UV spectrophotometer is precise, accurate, simple to perform and economy in practice. It do not require expensive or sophisticated and chemicals in contrast with chromatographic method. Hence it can be used for routine analysis for bulk and solid dosage form.

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