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Stability Indicating UV Spectrophotometric Method For The Estimation Of Venlafaxine Hydrochloride In Commercial Dosage Formulations

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Abstract: A simple, selective and stability indicating UV spectrophotometric method was developed and validated for the estimation of venlafaxine hydrochloride in pharmaceutical dosage forms. In this method venlafaxine hydrochloride exhibits maximum absorbance at 225nm with the solvent system water. The method was validated as per the International Conference on Harmonization (ICH) guidelines. Drug obeys Beer-Lambert's law in the concentration range of 1-32 µg/ml. Results of percentage recovery study shows that the method was not affected by the presence of common excipients in tablet and capsules. Therefore, the proposed method could be applied for the routine analysis of pharmaceutical dosage forms containing venlafaxine hydrochloride.

Key words: *venlafaxine hydrochloride, anti- depressant, UV-Spectrophometer, Validation, stress studies.*

1. **Introduction:**

Venlafaxine 1-[(1RS)-2-(Dimethylamino)-1-(4-methoxyphenyl)ethyl] cyclohexanol hydrochloride[1] is official in European Pharmacopoeia[2] and British Pharmacopoeia[3]. It is white powder freely soluble in water and methanol. Venlafaxine is a bicyclic phenylethylamine derivative, which is a unique anti-depressant, structurally differs from other currently available anti-depressants[1]. Venlafaxine and its active metabolite, O-desmethylvenlafaxine, inhibit the neuronal uptake of norepinephrine, serotonin, and, to a lesser degree, dopamine, but have no monoamine oxidase inhibitory activity and a low affinity for brain muscarinic, cholinergic, histaminergic, or alpha-adrenergic receptors[2]. Hence, it lacks the adverse anti-cholinergic, sedative, and cardiovascular effects of tricyclic anti-depressants[3]. Venlafaxine has an established tolerability and efficacy profile for the treatment of depressive disorders[2].

It has been proposed that anti-depressants with a dual action of inhibiting the re-uptake of both noradrenalin and serotonin (5-hydroxytryptamine, 5-HT) may be more effective than drugs acting on a single monoamine (e.g., selective serotonin re-uptake inhibitors, SSRIs). Venlafaxine is the first drug to be marketed that inhibits both noradrenalin and 5-HT re-uptake without actions in other receptors[4].

Venlafaxine is available in immediate-release (IR) formulation: ODV half-life is about 10 h (and venlafaxine half-life = 4 h), so this drug can be given in two divided doses. An extended-release (XR) preparation is also available in 37.5, 75, and 150 mg doses: Once-a-day XR dosing achieves bioavailability equivalent to that of twice-a-day dosing with IR formulation[5]. The XR preparation is also associated to a better compliance and demonstrates both anti-depressant and, after 2–3 weeks of treatment, also a good anxiolytic effect[6]. Metabolism of venlafaxine occurs primarily via O-demethylation (mediated by cytochrome P450 [CYP] 2D6) and, to a lesser extent, by N-demethylation (mediated by CYP3A4)[7]. This drug is a racemic mixture of the (−)-R-enantiomer and (+)-S-enantiomer. The R-enantiomer inhibits both serotonin and noradrenaline reuptake in vitro, while the S-enantiomer inhibits only serotonin re-uptake. Venlafaxine undergoes extensive first-pass metabolism to the major O-dimethyl metabolite, and 2 minor metabolites. O-demethylvenlafaxine (Referred here O-de-MVX) inhibits noradrenaline and serotonin re-uptake with similar potencies to those of the parent compound and exhibit linear kinetics with an elimination half-life of 5 and 11 hours. Two minor metabolites are N-desmethylvenlafaxine and N,O-didesmethylvenlafaxine (Referred here as N-des-MVX and N,O-dides-MVX). These two metabolites may also be considered as pharmacologically active, but they are claimed to be less potent than the parent drug.

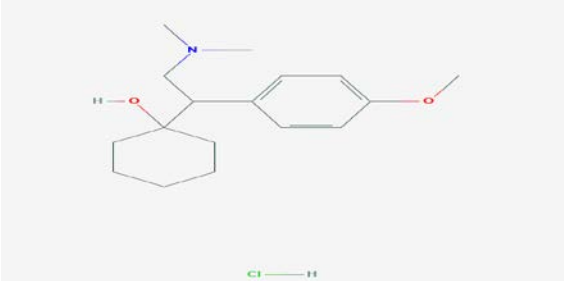


Fig: 1 structure of venlafaxine hydrochloride

2. Materials and Methods:

2.1 **Chemicals and reagents**: Venlafaxine hydrochloride was gifted by pharma company, Hyderabad, Telangana, India. HPLC grade methanol was procured from Rankem chemicals limited, New Delhi, India, distilled water, double distilled water, Acetonitrile. The marketed formulation, VENTAB XL 75mg and Venlor – XR 150mg was procured from the local market.

2.2 Equipments: Double beam UV spectrophotometer; Model: SL 210; Make: ELICO. The output signal was checked and the acquisition and integration of data was performed using spectral treats Software on a computer. Contech electronic balance was used for weighing.

3. Method development:

3.1 Selection of wavelength:10mg of Venlafaxine hydrochloride drug was accurately weighed and transferred into 10 ml of volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 1000µg/ml. From the above solution 1 ml was pipetted out and transferred into another 10 ml volumetric flask and the volume was made up to the mark with distilled water to obtain the concentration of 100µg/ml. From the above 1ml was pipetted out and transferred into a 10ml volumetric flask and the volume was made up to the mark with the distilled water to obtain the concentration of 10µg/ml and was scanned between 200-400nm against water as blank.

Fig 2: UV spectrum of venlafaxine hydrochloride

4. Assay:

4.1 **Preparation of standard stock solution**:10mg of Venlafaxine hydrochloride pure was accurately weighed and transferred into 10ml of volumetric flask and the volume was made up to the mark with distilled water to get concentration of 1000µg/ml. From this 0.1 ml was pipetted out and transferred into 10ml of volumetric flask and the volume was made up to the mark to get 100µg/ml solution and its absorbance was measured at 225nm.

4.2 Preparation of sample solution-1: 10 tablets were weighed and powdered. Powdered tablet equivalent to 10 mg of venlafaxine hydrochloride was weighed accurately and taken into 10ml volumetric flask, dissolve in distilled water and sonicated for 15min. Then the volume was made up to the mark with distilled water. The solution was filtered through whatmann filter paper no. 41. From the above solution 0.1 ml of solution was pipetted out and taken in 10ml volumetric flask. The volume was made up to 10ml to get 10ppm solution and its absorbance was measured at 225nm.

The % Assay was calculated using the following formula:

% Assay= (absorbance of the sample/absorbance of the standard) *(concentration of the standard/concentration of the sample) *100

Absorbance of sample= 0.5777

Absorbance of standard= 0.5906

Concentration of standard= 15

Concentration of sample= (absorbance of sample/absorbance of standard) x concentration of standard.

=14.6

%Assay= 100.4%

4.3 Preparation of sample solution-2: contents of 5 capsules were weighed and grinder with the help of pestle and mortar. venlafaxine equivalent to 10mg was taken and transferred to a 10ml volumetric flask, dissolved in distilled water and sonicated for 15 min. after sonication volume was made up to the mark to obtain 1000µg/ml. the resulting solution was filtered through whatmann filter paper no. 41. From the above solution 0.1ml was pipetted out and was transferred to 10ml volumetric flask and made up to the mark with distilled water to obtain the concentration of 10µg/ml and the absorbance was measured at 225nm.

The % Assay was calculated using the following formula:

% Assay= (absorbance of the sample/absorbance of the standard) *(concentration of the standard/concentration of the sample) *100

Absorbance of sample = 0.5619

Absorbance of standard = 0.5906

Concentration of standard= 15ppm

Concentration of sample = absorbance of sample/absorbance of standard* concentration of standard

= 14.2

% Assay = (absorbance of sample/ absorbance of standard)*(concentration of standard/concentration of sample)*100

= (0.5619/ 0.5906)* (15/113.7)*100

=100.5%

5. Method validation:

validation is the process of “establishing documented evidence” which provides high degree of assurance that a specific activity will consistently produce desired results or product meeting it’s predetermined specifications and quality specifications.

5.1 Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.

Various aliquots were prepared from the stock solution(100µg/ml) ranging from 1-32µg/ml and the absorbance was measured at 225nm using water as blank. It was found that venlafaxine hydrochloride obeys beer -lambert’s law.

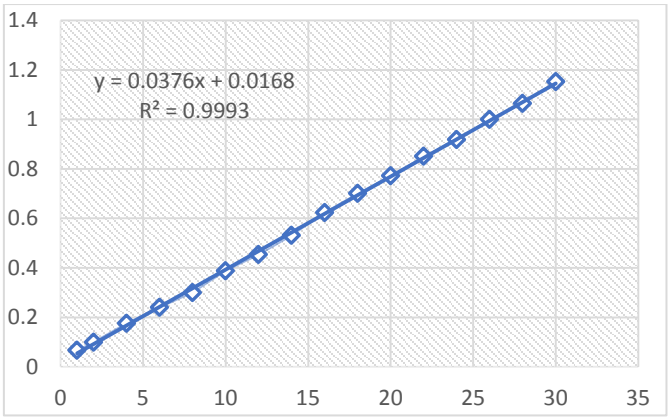


Fig: 3 linearity curve of venlafaxine hydrochloride

5.2 Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

15ppm standard solution of Venlafaxine HCl pure drug is selected for Precision study. From the standard stock solution(100µg/ml) 1.5ml was pipetted out and transferred into 10ml volumetric flask and the volume was made up to 10ml using distilled water to give 15ppm solution. This procedure is repeated 6 time and observances of all were measured at 225nm using distilled water as blank and its %RSD was calculated by using the formula %RSD = (standard deviation of the measurement / mean value of measurement) *100

Table 1: precision

S. No.	Concentration (µg/ml)	absorbance
1	15	0.5518
2	15	0.5516
3	15	0.5501
4	15	0.5502
5	15	0.5514
6	15	0.55084
Average		0.55084
SD		0.00068
%RSD		0.123528

5.3 Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

The accuracy of the method was determined by preparing different concentrations that is 50%, 100%, 150% in which the amount of marketed formulation was kept constant and the amount of pure drug was varied that is 5 µg/ml, 10 µg/ml, 15 µg/ml for 50%, 100%and 150%.

The accuracy was indicated by % recovery and calculated using the formula:

% Recovery = (amount found / amount added) *100

Table 2: accuracy

S.No.	Fixed conc. (µg/ml)	Conc. Added (µg/ml)	% spiked	% recovery
1	15	5	50%	99.7%
2	15	10	100%	99.8%
3	15	15	150%	99.83%

5.4 Robustness:

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 determined by carrying out the analysis at two different wavelengths i.e., at ±1nm

Table 3: robustness

S.No.	Concentration (µg/ml)	Wavelength (nm)	Absorbance	Calculations
1	15	224	0.54994 0.5504 0.5493	Mean- 0.54988 S.D-0.000552 %RSD-0.100467
2	15	226	0.5434 0.5459 0.5453	Mean-0.544867 S.D- 0.001305 %RSD-0.23953

5.5 Ruggedness:

Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment etc. Ruggedness of the method was determined by carrying out the analysis using two different instruments and the respective absorbance was noted and the result was indicated by %RSD

Table 4: ruggedness (instrument-1)

S.No	Concentration (µg/ml)	Absorbance	Statistical analysis
1	15	0.581	Mean-0.581667 S.D-0.000577 %RSD-0.099258
2	15	0.582	
3	15	0.582	

Table 4: ruggedness (instrument-2)

S.No	Concentration (µg/ml)	Absorbance	Statistical analysis
1	15	0.5846	Mean- 0.581033 S.D- 0.003967 %RSD-0.682716
2	15	0.5755	
3	15	0.5830	

5.6 Limit of detection:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit (L.O.D) may be expressed as:

L.O.D=3.3 σ/S

Where σ = the standard deviation of the response

S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

L.O.D = 3.3*0.00068/0.0376

= 0.05425µg/ml

5.7 Limit of quantification:

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The Quantitation limit (QL) may be expressed as:

L.O.Q=10 σ /S

Where σ = the standard deviation of the response

S = the slope of the calibration curves the slope S may be estimated from the calibration curve of the analyte.

L.O.Q= 10 *0.00068 /0.0376

= 0.1808 µg/ml

6. Forced degradation studies:

Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates degradation products that can be studied to determine the stability of the molecule. The ICH drug stability testing guideline *Q1A (R2)* emphasizes that the analysis of samples of active pharmaceutical ingredients, which are subjected to stress conditions, should be carried out, to establish their inherent stability characteristics, thereby leading to identification of the degradation products through the use of validated stability-indicating analytical methods[11]. Stability-indicating-assay-methods (SIAMs) are specific ones, which evaluate the drug in the presence of its degradation products, excipients, and additives.

Acid hydrolysis:

A 1ml aliquot of the standard 15µg/ml venlafaxine hydrochloride was taken in a 10 ml volumetric flask and mixed with 1 ml of 1 N HCl. The absorbance was measured at 227nm after 24hrs after neutralizing the acid by base of same strength to terminate the reaction.

Alkaline hydrolysis:

1ml aliquot of the standard 15µg/ml venlafaxine hydrochloride was taken in a 10 ml volumetric flask and mixed with 1 ml of 1 N NaOH. The absorbance was measured after 24hrs after neutralizing the acid by base of same strength to terminate the reaction.

Oxidative degradation:

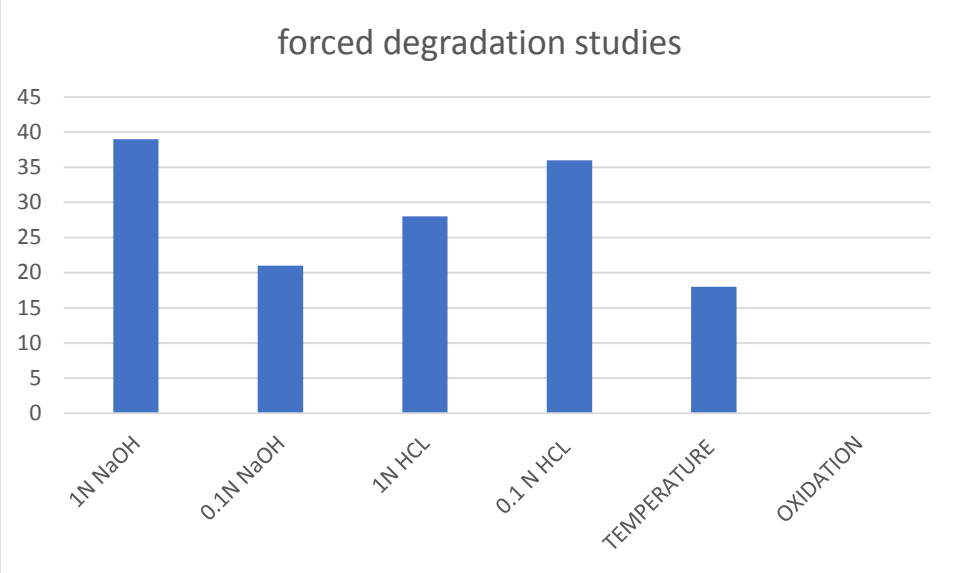
A 1ml aliquot of the standard 15µg/ml venlafaxine hydrochloride was taken in a 10 ml volumetric flask and mixed with 1 ml of 3% hydrogen peroxide. The absorbance was measured at 227nm after 24 hours.

Thermal degradation:

15µg/ml standard drug solution was exposed to dry heat 40 [illegible] in the hot air oven for 3hrs. Then the absorbance was measured at 225nm and the percentage of Degradation was calculated.

Photolytic degradation:

15µg/ml standard drug solution was exposed to near ultraviolet lamp in the UV chamber for 12 hours. The absorbance was measured at 225nm.



7. Conclusion:

The proposed method is specific in estimating the commercial formulation without interference of excipients and the other additives. Hence, this method can be used for routine determination of venlafaxine hydrochloride in the bulk sample and pharmaceutical formulation. The proposed method for stability study shows that there is appreciable degradation of venlafaxine found in stress conditions. A new simple analytical method has been developed to apply for the evaluation of the stability of venlafaxine to quantify and its degradation products in a solid premix dosage form.

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