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STUDIES ON ZERO ORDER UV-SPECTROPHOTOMETRIC METHOD USING ABSORBANCE AND AREA UNDER CURVE TECHNIQUE FOR DETERMINATION OF VENLAFAXINE HCL IN BULK AND TABLET FORMULATION

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Abstract: A new, simple, fast and reliable zero order spectrophotometric method has been developed for determination of Venlafaxine HCL in Bulk and Tablet formulation. The quantitative determination of drug was carried out using the zero order values (absorbance) measured at 225 nm. Calibration graph constructed at 225 nm was linear in concentration range of 5-30 µg/ml with correlation coefficient 0.999. The method was found to be precise, accurate, specific, and validated as per ICH guidelines and can be used for determination of Venlafaxine HCL in Tablet formulation

Index Terms - Venlafaxine HCL, Zero Order, AUC, Validation.

I. INTRODUCTION

1-[2-(dimethylamino)-1-(4-methoxyphenyl) ethyl] cyclohexan-1-ol, also known as venlafaxine, is an antidepressant that neither serotonin nor adrenaline can produce. It belongs to SNRI class of reuptake inhibitors. It is recommended for the management of anxiety disorders and clinical depression [1-4]. Venlafaxine's (VNL) molecular basis shows that, according to the UV method of assessment, it is a complex molecule whose therapeutic action and quality control parameters are attributed to alcoholic, tertiary amine, and OCH_3 groups [2, 3]. A review of the literature reveals that no clear, simple data on the UV technique for Venlafaxine estimate has been published. After determining that the absorbance was 225 nm, the medication dissolved in methanol was searched for in the spectrum. The procedure has been verified in compliance with ICH recommendations [4].

EXPERIMENTAL

Selection of Solvent

Methanol was selected as the solvent for dissolving Venlafaxine HCL.

Preparation of Stock Standard Solution of Venlafaxine HCL

Stock standard solution was prepared by dissolving 10 mg of Venlafaxine HCL in 100 mL of Methanol to achieve concentration100 µg/mL.

Determination of λ max and Selection of Area under Curve (AUC)

From the stock solutions, 1mL of Venlafaxine HCL was transferred into 10 mL volumetric flask containing 70 ml of methanol and the volume was adjusted to the mark with same solvent to get concentration 10µg/mL. The solution was scanned in the UV range 400 – 200nm. In **Method A**, absorbance was recorded at **225 nm** while in **Method B** AUC was recorded in the wavelength range of **216.80 - 232 nm**. The calibration curves were constructed by plotting concentration *versus* absorbance/ AUC of zero order spectrum in **Method A** and **B**, respectively. **Shown in Figure 1 and 2.**

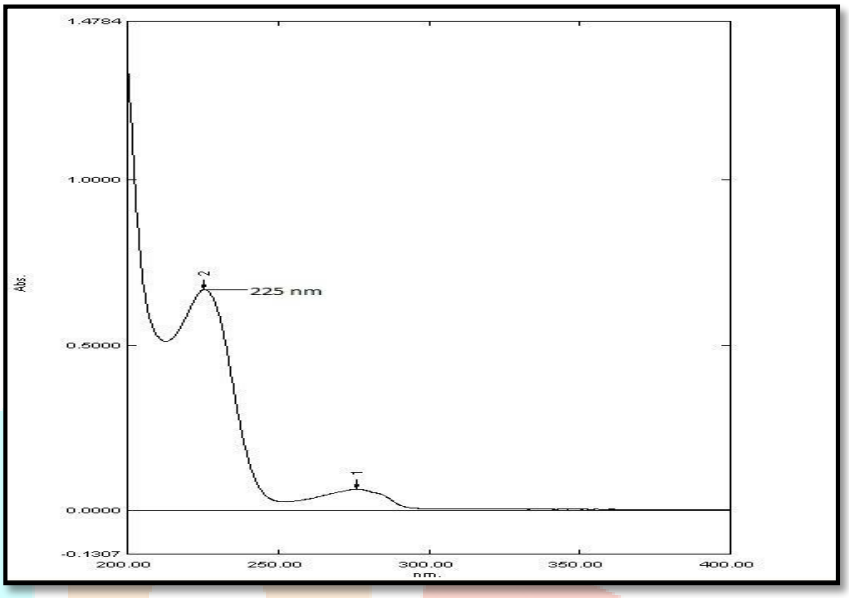

Fig. 1 UV-Spectrum of venlafaxine HCL in Methanol

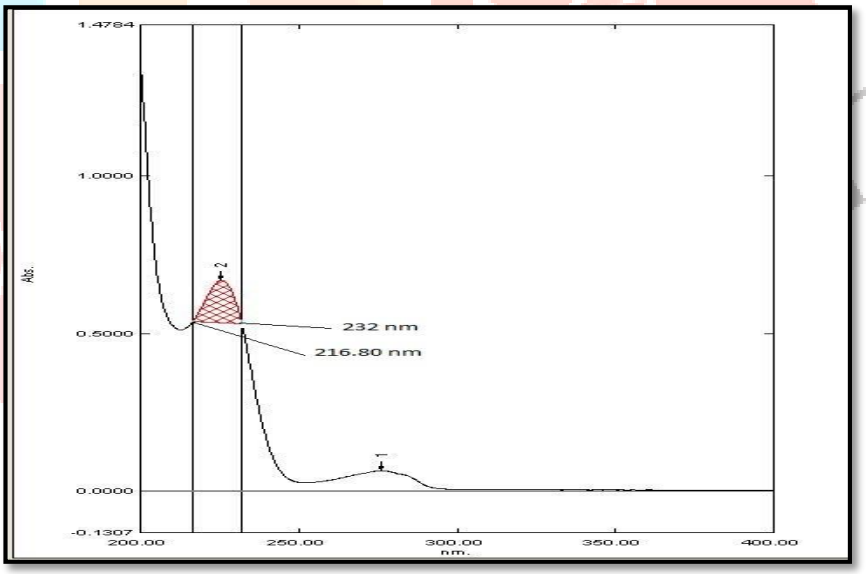

Fig.2 AUC of zero order derivative spectrum of venlafaxine HCL in wavelength range 216.80nm - 232 nm

Linearity Study:

An appropriate volume (0.5 – 3 mL) of Venlafaxine HCL from stock solution was transferred into six separate 10 mL volumetric flasks. The volume was adjusted to the mark with methanol to get concentrations of 5 - 30µg/mL.

In **Method A** absorbance of these solutions were recorded at **225nm** and in **Method B** AUC of zero order spectrum selected in **216.80 - 232 nm,** Results are showed in **Table 1**,the calibration curves were constructed by plotting concentration *versus* absorbance and AUC in **Method A** and **B**, respectively; shown in **Figure3 and Figure 4.**

Table 1: Linearity studies

Sr. No.	Concentration of Venlafaxine HCl [µg/mL]	Method A Absorbance Mean ± SD [n = 6]	% RSD	Method B Mean AUC ± SD [n = 6]	% RSD
1	5	0.1638 ± 0.00025	0.15	0.2907± 0.0029	1.00
2	10	0.3049 ± 0.00060	0.19	0.577 ± 0.0033	0.57
3	15	0.4567 ± 0.0017	0.38	0.885 ± 0.0016	0.18
4	20	0.6117 ± 0.0010	0.17	1.156 ± 0.0025	0.21
5	25	0.7565 ± 0.0048	0.63	1.444 ± 0.0026	0.18
6	30	0.9157 ± 0.0011	0.12	1.773 ± 0.0024	0.13

n- Number of determinations

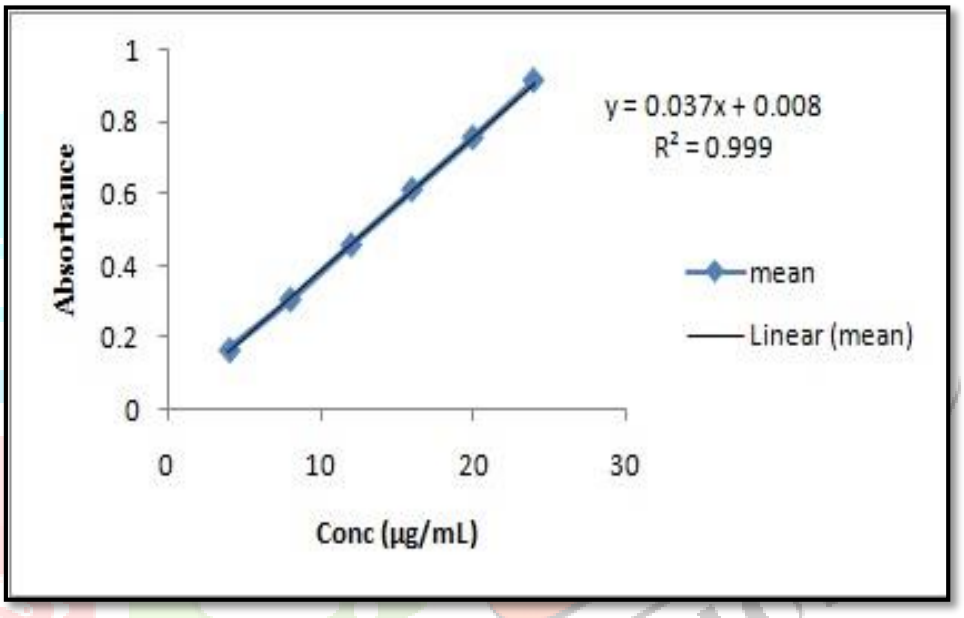

Fig.3: Calibration Curve of Venlafaxine HCl Y = 0.037 x + 0.008 where, Correlation coefficient = 0.999, Slope = 0.037 Intercept = 0.008

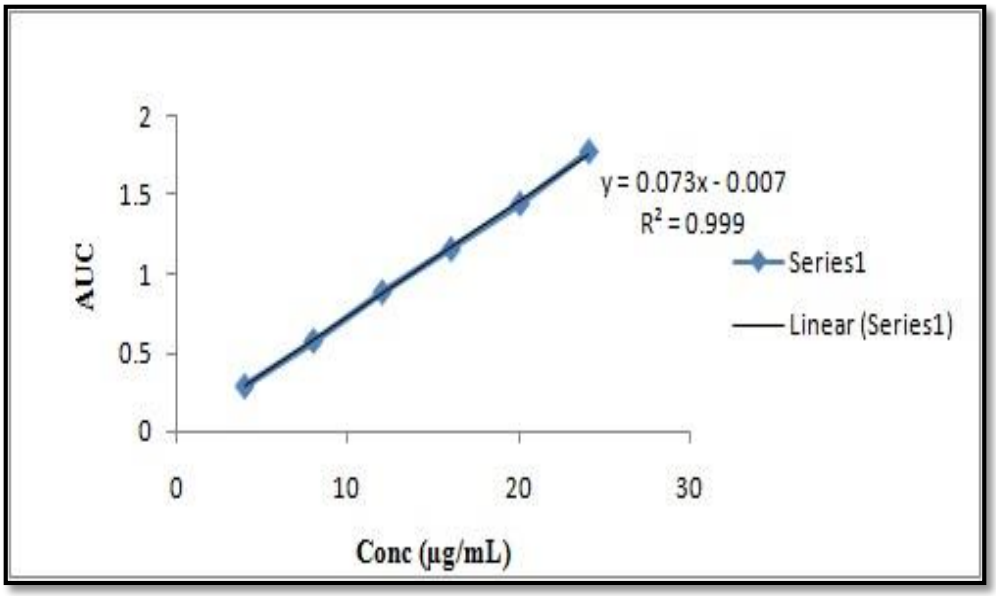

Fig.4: Calibration Curve of Venlafaxine HCl Y = 0.073 x – 0.007 where, Correlation coefficient = 0.999, Slope = 0.073 Intercept = 0.007

Analysis of Bulk Material

An accurately weighed 10 mg of Venlafaxine HCL was transferred into 100 mL volumetric flask; dissolved in Methanol and the volume was made up to mark with the same solvent. An appropriate volume 1.5 mL was transferred into 10 mL volumetric flask and volume made up to the mark with Methanol.

The concentration was determined by regression equation. The resulting solutions were scanned using UV-Spectrophotometer in the range of 400 - 200 nm and against water as blank; results are reported in **Table 2.**

Table 2: Analysis of Bulk Material

Drug	Amount Taken [µg/mL]	Method A Amount found [µg/mL]	% Amount found [n=6]	Method B Amt. found [µg/mL]	% Amount Found
VH	15	11.93	99.41	12.22	101.83
	15	11.96	99.68	12.41	103.43
	15	12.05	100.47	12.17	101.49
	15	12.07	100.58	12.19	101.62
	15	12.15	101.28	12.02	100.23
	15	12.18	101.53	12.11	101.60
	Mean± SD	12.05 ±0.100	99.64 ± 0.14	12.19 ± 0.12	101.60±1.06
	% RSD	0.83	1.01	1.04	1.04

n- Number of determinations

Analysis of *in-house* Tablets

Since the marketed formulation of Venlafaxine HCL was not available during the study; therefore, *in house* tablets were prepared**.** Ten Venlafaxine HCL tablets were accurately weighed, average weight determined and ground into fine powder. A quantity of powder drug equivalent to 10 mg of Venlafaxine HCL was transferred into 100 mL volumetric flask containing 70 ml water shaken manually for 20 min and volume was adjusted to mark using same solvent. From it, 1.5 mL was transferred into 10 mL of volumetric flask and diluted to mark using water to get concentration 15µg/mL. The resulting solutions were scanned using UV-Spectrophotometer in the range of 400 - 200 nm. Results are shown in **Table 3.**

Table 3: Analysis of *in-house* Tablets

Drug	Amt. Taken [µg/mL]	Method A Amt. found [µg/mL]	% Amount found	Method B Amount found [µg/mL]	% Amount Found
VH	15	12.19	101.59	12.2	101.66
	15	11.93	99.41	12.10	100.86
	15	11.89	99.12	12.20	101.72
	15	11.84	98.73	12.05	100.47
	15	12.24	102.00	12.14	101.21
	15	12.22	101.86	12.09	100.75
	Mean ± SD	12.05 ± 0.18	100.4 ± 1.51	12.13 ± 0.06	101.11 ± 0.5
	% RSD	1.51	1.51	0.5	0.5

Validation

The Proposed method was validated as per the ICH guidelines for linearity and Range, accuracy, precision, ruggedness.

Accuracy

Recovery studies were conducted at three different percentage drug recovery levels (i.e., 80, 100, and 120%), where a known quantity of standard drug was added to the pre-analyzed sample and the sample was then exposed to the proposed zero order UV-Spectrophotometric method. This was done to evaluate the accuracy of the proposed method. **Tables 4 and 5** present the findings.

Table.4: % Recovery Studies (Method A)

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount Recovered [µg/mL, n=3]	% Recovered	% RSD
VH	15	12	7.93	99.49	0.83
	15	15	8.12	100.77	1.10
	15	18	7.76	98.64	0.63

Table 5: % Recovery Studies (Method B)

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount Recovered µg/mL[n=3]	% Recovered	% RSD
VH	15	12	8.18	101.29	1.59
	15	15	8.15	100.96	1.17
	15	18	8.11	100.67	0.92

Precision

The repeatability, intra-day, and inter-day precision of the procedure were examined. Venlafaxine HCL (15µg/mL) was analyzed six times to assess repeatability. The findings are shown in **Table 6.**

Three separate analyses of Venlafaxine HCL at 5, 10, and 15 µg/mL were performed to establish the intra-day precision. The same concentration of the solutions was analyzed every day for three days in order to evaluate the inter-day precision; the findings are shown in **Table 7.**

Table 6: Repeatability Studies

Drug	Amount Taken [µg/mL]	Method A Amount found [µg/mL]	Method A % Amount found [n=6]	Method B Amount found [µg/mL]	Method B % Amount Found
RE	15	12.04	100.40	12.20	101.72
	15	11.70	97.5	12.10	100.90
	15	11.98	99.84	12.16	101.39
	15	11.96	99.70	12.02	100.17
	15	11.85	98.78	12.21	101.18
	15	11.97	99.75	12.14	101.18
	Mean ± SD	11.92 ± 0.12	99.33± 1.03	12.14± 0.07	101.19 ± 0.60
	% RSD	1.04	1.04	0.59	0.59

n- Number of determinations

Table 7: Precision Studies [Intra-day and Inter-day]

Method A	Method B					
Standard Concentration (µg/mL)	**Amt. Found [µg/mL]**	**% Amount found**	**% RSD**	**Amount found [µg/mL]**	**% Amount found**	**% RSD**
Intra-day Precision						
10	7.92	99.12	0.38	8.03	100.48	0.09
15	12.03	100.29	0.41	12.19	101.65	0.61
20	16.02	100.13	1.50	15.97	99.85	0.35
Inter-day Precision						
10	7.86	98.27	1.57	7.86	98.30	1.95
15	11.87	98.97	0.48	11.76	98.0	0.47
20	16.02	100.16	0.49	16.32	102.0	2.0

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantification (LOQ). The DL and QL were calculated by the use of the equation DL = 3.3 × ASD/S and QL = 10 × ASD/S; Where, 'ASD' is Average standard deviation of the peak height and areas of the drug (n = 3), taken as a measure of noise, and 'S' is the slope of the corresponding calibration curve.
Different volume of stock solution in the range 0.5 - 1µg/mL was prepared. The procedure was repeated in triplicate. LOD and LOQ were found to be **0.266 µg** and **0.806µg** for **Method A** and **0.177µg** and **0.538µg** for **Method B**.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two different analysts using same operational and environmental conditions and the results are reported in **Table 8.**

Table 8: Ruggedness Studies

Method A			**Method B**	
Analysts	**[%] Amount found ± SD [n= 6]**	**% RSD**	**[%] Amount found ± SD [= 6]**	**% RSD**
I	100.93 ± 0.80	0.79	101.80 ± 0.68	0.68
II	100.23 ± 0.37	0.37	101.10 ± 0.75	0.74

n- Number of determinations

Conclusion:

It was discovered that the suggested technique for estimating venlafaxine hydrochloride was simple, sensitive, dependable, and had good precision and accuracy. When calculating commercial formulations, the procedure is precise and does not take into account the effects of excipients or other additives. Therefore, the routine analysis of Venlafaxine HCL in pharmaceutical and pure formulations can be performed using this method.

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