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Research Article

Analytical Method Development and Validation for Venlafaxine Hydrochloride: UV-Visible Spectrophotometric Estimation in Bulk and Pharmaceutical Dosage Forms

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ABSTRACT

This abstract presents the development and validation of a UV-visible spectrophotometric method for the accurate estimation of Venlafaxine HCl, a commonly prescribed antidepressant, in bulk and pharmaceutical formulations. The method utilizes purified water as the solvent, with a maximum absorbance identified at 224.60 nm. It exhibited excellent linearity within a concentration range of 5-35 µg/ml, with a high correlation coefficient ($R^2 = 0.999$) and consistent mean recovery rates between 99.00% and 100.0%. The method's cost-effectiveness and simplicity make it an ideal candidate for routine quality control. Validation was performed following ICH guidelines, ensuring reliability across parameters such as linearity, repeatability, precision, accuracy, ruggedness, and determination of LOD & LOQ. Recovery studies further confirmed the method's applicability for the routine analysis of Venlafaxine HCl in both active pharmaceutical ingredients and commercial tablet formulations. In conclusion, this validated method provides a reliable, swift, and economical approach to the quality control of Venlafaxine HCl, making it a valuable addition to existing analytical techniques.

Keywords: Antidepressant; Venlafaxine HCL; Analytical Validation; Method development; ICH guidelines

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1. INTRODUCTION

The antidepressant Venlafaxine HCl is (Bicyclic antidepressant). It is known as SNRI (inhibitor of serotonin-norepinephrine reuptake), but it has also been referred to as SNDRI (serotonin-norepinephrine-dopamine reuptake inhibitor). It functions by blocking the reuptake of the transporter protein for the mood of the main neurotransmitter [1]. This leaves the neurotransmitter more involved in the synapse. The impaired neurotransmitters are serotonin and norepinephrine, although in large concentrations it often weakly stimulates dopamine reuptake, with new research suggesting that some dopamine is also transmitted through the norepinephrine transporter, as dopamine is inactivated in the frontal cortex through norepinephrine reuptake. Dopamine transporters are primarily used in the frontal cortex, so Venlafaxine HCl can improve dopamine neurotransmission in these areas of the brain [2]. Venlafaxine HCl has been shown to increase the pain threshold in mice, indirectly influencing opioid receptors as well as alpha 2-adrenergic receptors. The naloxone and opioid blockers reversed these pain benefits, thereby endorsing an opioid mechanism [3].

The literature survey on the estimation of venlafaxine also focuses on the estimation of venlafaxine for other HPLC and UV Spectrometric products. Venlafaxine HCl is a 2007 British Pharmacopoeia official. Via potentiometric titration, the assay of the drug according to BP is processed. It needs and performs simple, quick and cost-effective analytical methods for routine analysis. A UV spectroscopic approach for estimating venlafaxine biological fluids, such as serum plasma urine, was disclosed in a literature survey. The

chromatographic process is time-consuming, expensive and highly trained. The UV spectrometric technique is simple and precise. It can be incredibly beneficial for routine bulk analysis, sample composition and dissolution. The main objective of this study was to establish a simple, precise accurate and economical method for estimating Venlafaxine HCl in bulk pharmaceutical formulations with a better detection range [4].

2. UV- VISIBLE SPECTROPHOTOMETRIC METHOD

UV-visible spectroscopy examines a typical sample's wavelength. Broad use for test detection is beneficial for detailed estimates of UV-visible spectra with vast highlights [4]. The most important technique of the spectrophotometric method is to identify the substance factor on-premise of the material's transmitting or reflection properties as the wavelength power, adhering to the law of Beer-Lambert and a synthetic compound bearing a bunch of chromophores for light retention. When compared with other methods, it devotes less time and offers phenomenal precision in viability [5]. The writing description teaches the UV techniques and the guarantee of venlafaxine HCL is provided for by the RP-HPLC approach solely with various medications. The present review involves enhancement and acceptance of modern UV spectroscopy techniques for unadulterated assurance of venlafaxine HCL and economic conditions are indicated by its pharmaceutical plans. The investigation plan was approved by the criteria of ICH rules acceptance [6][7].

To develop a fair UV-visible spectrophotometric technique for the investigation of venlafaxine hydrochloride in-depth, distilled water was studied.

The parameters used for the selection of the media were affectability of the procedure, ease of research preparation, dissolvability of the drug, solvent costs and suitability of the approach.

3. MATERIAL AND METHOD

3.1 Apparatus

A Shimadzu UV visible double beam spectrophotometer (UV mini-1900, Shimadzu Corporation, Kyoto, Japan) was used for all absorbance measurements with matched quartz cells.

3.2 Materials

All chemicals and reagents were of analytical or HPLC grade. Venlafaxine HCl was supplied from Mylan Laboratories Hyderabad as a gift sample and distilled water, and methanol should be used for the method utilization. Pharmaceutical grade excipients were obtained from the lab of R.C Patel Institute of Pharmaceutical Education and Research, Shirpur Dhule.

3.3 Determination of Wavelength of Maximum Absorption

The bulk solution was prepared by using purified water as a solvent. By dissolving 10 mg of venlafaxine bulk drug into 100 ml, the normal stock solution of venlafaxine HCl was prepared. Then 1 ml of the sample was withdrawn from 100 ml of stock solution to assess the lambda max of the substance and diluted up to 10 ml of distilled water, so the concentration is 10 µg/ml. The sample concentration is filled in the UV cuvette and the UV visible spectrometer tracks the lambda max in spectrum mode. 10 µg/ml of the sample was scanned in the 400-200nm UV visible spectrometer range.

The lambda max was determined to be 224.60 nm of Venlafaxine HCl [8].

3.4 Assay Determination

In a 100 ml volumetric flask, a quantity of venlafaxine HCl equal to 100 mg of venlafaxine hydrochloride was taken and dissolved in 10 ml of water and mixed with water up to the level. For 5 minutes, the resulting solution was ultrasonicated. Using Whatman filter paper No.40, the solution was then filtered. Relevant dilutions of water are made from the filtrate. Preferred concentration (10µg/ml) to be obtained. This solution was then tested in UV and the outcome was calculated by the percentage amount found (Table 1) [9].

4. PARAMETERS

4.1 Linearity

The calibration curve was reported by the UV visible spectrometer photometric process. The linearity spectrum should be based on a 10 µg/ml concentration assay of lambda peak absorbance. The linearity spectrum was known to be 5µg/ml, 10 µg/ml, 15 µg/ml, 20µg/ml, 25 µg/ml, 30 µg/ml, and 35 µg/ml, based on absorbance so the absorbance vs concentration calibration curve is plotted and it is necessary to record the graph.

4.2 Repeatability

The repeatability of the procedure was calculated by the preparation of six 15 µg/ml dilutions of the same mean concentration concentrations as those of the Lambert beer range.

4.3 Accuracy

The accuracy of Venlafaxine HCl was calculated by the preparation of two stock solutions as a bulk drug

and the dosage type being marketed. Dilutions of 80 %, 100 %, 120 % are prepared for precision determination, in which the quantity is maintained constant if 15 µg/ml is marketed. The quantity of pure drugs ranged from 12 µg/ml, 15 µg/ml, and 18 µg/ml to 80%, 100%, and 120%, respectively. The dilutions were prepared in triplicate and the percentage recovery indicated precision [12].

4.4 Precision

By preparing three middle levels of concentration dilution to become 10 µg/ml, 15 µg/ml, and 20µg/ml, respectively, the precision form of Venlafaxine HCl was shown. The methodology was revealed by Intraday and Inter-day variance research. In the intraday analysis, the solution is prepared for three separate ranges and analysed three times a day, morning, midday, and evening. In an inter-day analysis, three solution concentration ranges were prepared and analysed three times a day, i.e. day 1, day 2, and day 3. The outcome was reported by Percentage RSD [13].

4.5 Ruggedness

It was calculated by the preparation of 6 medium concentration dilutions of 15µg/ml. The findings evaluated by two separate observers were observed, as well as on two different instruments and the

respective absorption. The outcome was reported by Percent RSD [14].

4.6 Limit of detection (LOD)

The detection limit for venlafaxine HCl was determined by the preparation of dilutions ranging from 5-10 µg/ml at various concentrations. The detection limit of an individual analytical technique is the smallest quantity of analyte in a sample that can be detected as an exact value but not necessarily quantitated.

4.7 Limit of quantitation:

For a defined degree of precision and accuracy, the LOQ is the concentration that can be quantified accurately. Using a formula involving standard deviation response and calibration curve slope, the LOQ was calculated.

5. RESULTS AND DISCUSSION

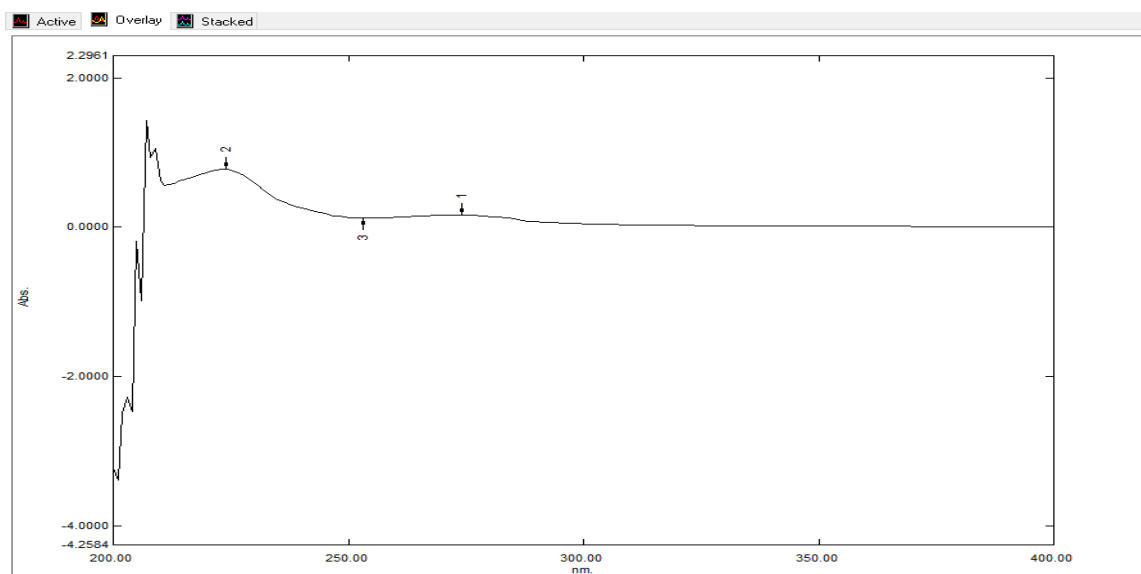
Venlafaxine is a drug used as an antidepressant. The analysis of validity was processed by the UV spectroscopic system. For the routine estimate of venlafaxine HCL in bulk drug and pharmaceutical dosage forms, the proposed method was found to be quick, sensitive, reliable, accurate, economical, and rapid. The validation parameter summary of the spectrometric method proposed is shown in Table 1

Table 1: Summary of validation

| Parameter | Results |
|--|--|
| Lambda max | 224.60 nm |
| Linearity range | 5-35µg/ml |
| Linearity indicated by the correlation coefficient | 0.9955 |
| Linear regression equation | $y = 0.0253x + 0.2405$ |
| Repeatability indicated as % RSD | 1.7081% |
| Accuracy indicated by % recovery | 99-100 % |
| Precision indicated by %RSD | Intraday- 1.6392% Interday- 1.7612% |
| Ruggedness indicated by %RSD | 1.8285% |
| LOD | 0.308µg/ml |
| LOQ | 3µg/ml |
| Assay % | 98% |

5.1 Selection of wavelength

Scan the standard UV spectrophotometer solution in spectrum mode from 200 nm and 400 nm, using diluents as blanks. Venlafaxine HCL shows the 224 nm λ_{max} seen in Figure 1.

**Figure 1: Lambda max of venlafaxine HCL**

5.2 Linearity and range

The obtained calibration curve was assessed by its coefficient of correlation. The absorption of 5-30 $\mu\text{g/ml}$ samples was linear with a correlation coefficient (R^2) of 0.9955, as shown in Figure 2 and Table 2.

Table 2: Preparation of Standard Calibration Curve

| Conc.($\mu\text{g/ml}$) | Absorbance |
|---------------------------|------------|
| 5 | 0.380 |
| 10 | 0.491 |
| 15 | 0.595 |
| 20 | 0.749 |
| 25 | 0.894 |
| 30 | 0.994 |

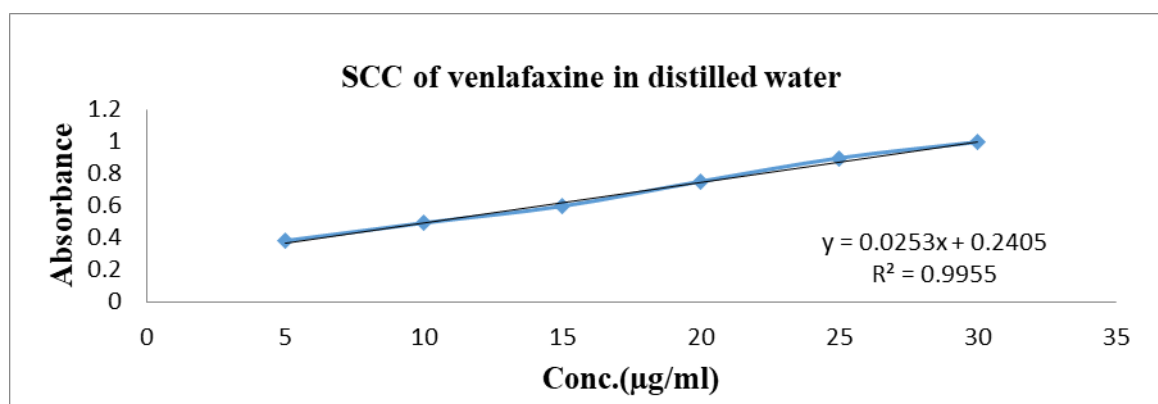


Figure 2: Calibration Curve of Venlafaxine HCl

5.3 Repeatability

Repeatability (% R.S.D.) at all concentration levels ranged from 1.70 % (Table 3). Repeatability results indicate precision over a limited interval of time and inter-assay accuracy under the same operating conditions.

Table 3: Repeatability of venlafaxine HCl

| Sr. no | Conc.(µg/ml) | Absorbance | Amount | % Amount |
|--------|--------------|-----------------|-----------------|-----------------|
| 1 | 15 | 0.525 | 12.25 | 81.66667 |
| 2 | 15 | 0.531 | 12.4 | 82.66667 |
| 3 | 15 | 0.535 | 12.5 | 83.33333 |
| 4 | 15 | 0.511 | 11.9 | 79.33333 |
| 5 | 15 | 0.523 | 12.2 | 81.33333 |
| 6 | 15 | 0.521 | 12.15 | 81 |
| | Average | 0.524333 | 12.23333 | 81.55556 |
| | SD | 0.008359 | 0.208966 | 1.393105 |
| | %RSD | 1.594144 | 1.708167 | 1.708167 |

5.4 Accuracy

The similarity of the test results to the true value is the accuracy of an analytical method. The application of the analytical procedure to recovery studies where a sufficient concentration of standard is spiked in pre-analysed sample solutions has been determined. The % recovery for the standard

analysis and reference analysis method for all the three concentration levels 80%, 100%, and 120% ranged from 99.6770 %, 91.0666%, and 100.3958% with % RSD, 0.01608%, 0.01580%, 0.2214% respectively become showing that any small change in the drug concentration can be accurately determined with high accuracy. The results were obtained from the standard method.

Table 4: Accuracy Readings of Venlafaxine hydrochloride

| Observation/ Results | | | | | | |
|----------------------|---------------|-----------|------------|---------------------|--------|---------|
| No. of preparation | Conc. (µg/ml) | | % Recovery | Statistical Results | | |
| | Formulation | Pure Drug | | Mean | SD | %RSD |
| 80% | 15 | 8 | 97.9065 | 99.6770 | 1.6036 | 0.0160 |
| 80% | 15 | 8 | 101.0313 | | | |
| 80% | 15 | 8 | 100.0938 | | | |
| 100% | 15 | 10 | 91.1500 | 91.0666 | 0.1443 | 0.01585 |
| 100% | 15 | 10 | 90.9000 | | | |
| 100% | 15 | 10 | 91.1500 | | | |

| | | | | | | |
|------|----|----|----------|-----------------|--------|----------|
| 120% | 15 | 12 | 102.9583 | 100.3958 | 2.2233 | 0.022174 |
| 120% | 15 | 12 | 98.9791 | | | |
| 120% | 15 | 12 | 99.2500 | | | |

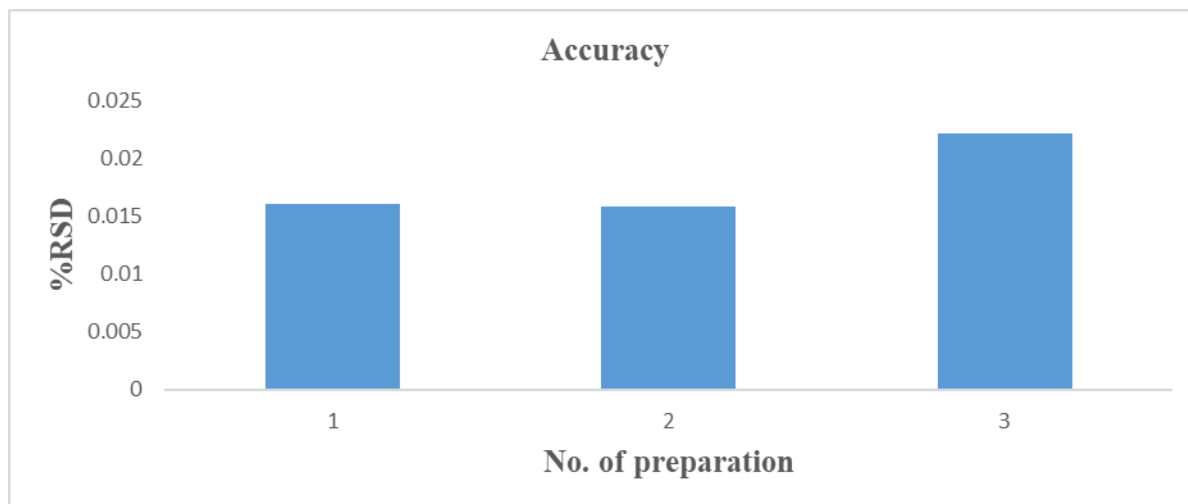


Figure 3: Accuracy Readings of Venlafaxine hydrochloride

5.5 Precision

The precision of the analytical method specifies, under recommended conditions, the degree of dispersion between a set of measurements obtained from multiple samples of the same homogeneous sample. Intraday precision refers to the short use of the analytical technique within a laboratory using

the same operator and the same equipment, while Interday precision involves the estimation of analysis differences when different analysts use a method inside a laboratory on different days. The developed method's intra-day and inter-day precision study confirmed adequate sample stability and method reliability where all the % RSDs were less than 2% (Table 5, 6) (Figure 4, 5).

Table 5: Precision (Intraday) readings of Venlafaxine hydrochloride

| Conc.(µg/ml) | Morning | | | Afternoon | | | Evening | | |
|------------------|---------|-------|--------|-----------|-------|---------|---------|--------|---------|
| | Abs. | Amt. | % Amt. | Abs. | Amt. | % Amt. | Abs. | Amt. | % Amt. |
| 10 | 0.3930 | 8.94 | 89.4 | 0.3880 | 8.825 | 88.25 | 0.399 | 9.1 | 91.00 |
| | 0.5760 | 13.53 | 90.2 | 0.571 | 13.4 | 89.3333 | 0.598 | 14.075 | 93.8333 |

| | | | | | | | | | |
|-------------|--------|--------|---------|--------|---------|--------|--------|--------|---------|
| 20 | 0.7300 | 17.377 | 86.88 | 0.734 | 17.475 | 87.375 | 0.788 | 18.825 | 94.125 |
| AVG | | 13.282 | 88.8291 | | 13.2333 | 88.319 | | 14 | 92.9861 |
| SD | | 4.2241 | 1.7284 | | 4.3274 | 0.9810 | | 4.8629 | 1.7261 |
| %RSD | 1.9458 | | | 1.1107 | | | 1.8564 | | |

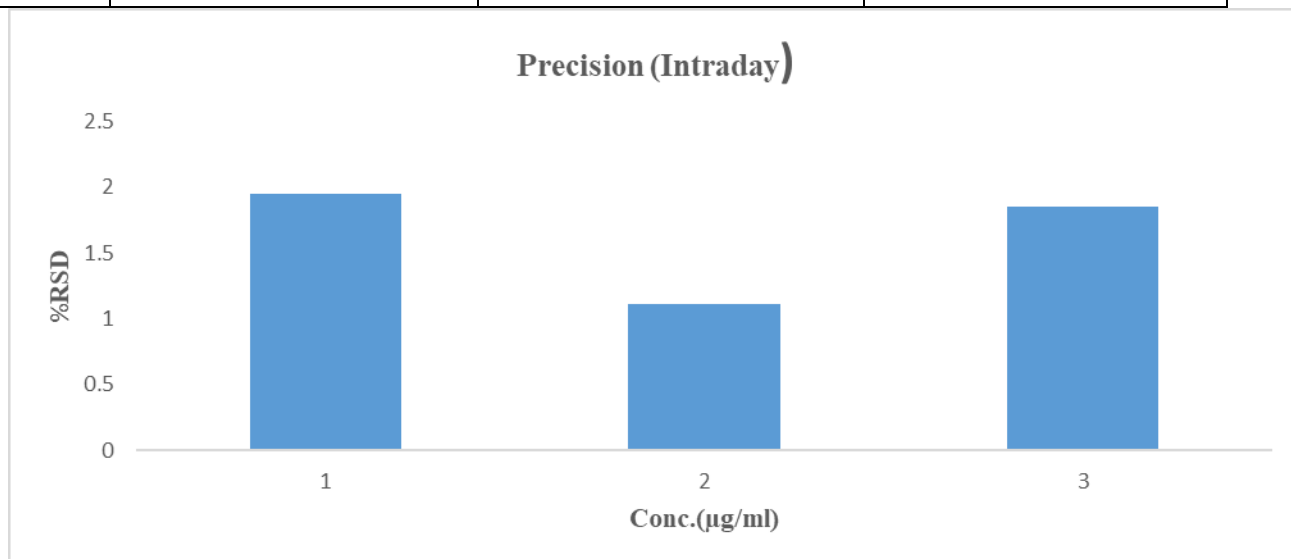


Figure 4: Precision (Intraday) readings of Venlafaxine hydrochloride

Table 6: Precision (Interday) analyses of Venlafaxine hydrochloride

| Conc.(µg/ml) | Day 1 | | | Day 2 | | | Day 3 | | |
|--------------|--------|--------|---------|--------|--------|---------|--------|---------|---------|
| | Abs. | Amt. | % Amt. | Abs. | Amt. | % Amt. | Abs. | Amt. | % Amt. |
| 10 | 0.3930 | 8.94 | 89.4 | 0.394 | 8.98 | 89.8 | 0.399 | 9.1 | 91.00 |
| 15 | 0.5760 | 13.53 | 90.2 | 0.555 | 12.99 | 86.6 | 0.555 | 12.99 | 86.6 |
| 20 | 0.7300 | 17.377 | 86.88 | 0.746 | 17.76 | 88.82 | 0.744 | 17.725 | 88.62 |
| AVG | | 13.282 | 88.8291 | | 13.245 | 88.4083 | | 13.2716 | 88.7416 |
| SD | | 4.2241 | 1.7284 | | 4.3980 | 1.6402 | | 4.3193 | 2.2023 |
| %RSD | 1.9458 | | | 1.8552 | | | 1.4817 | | |

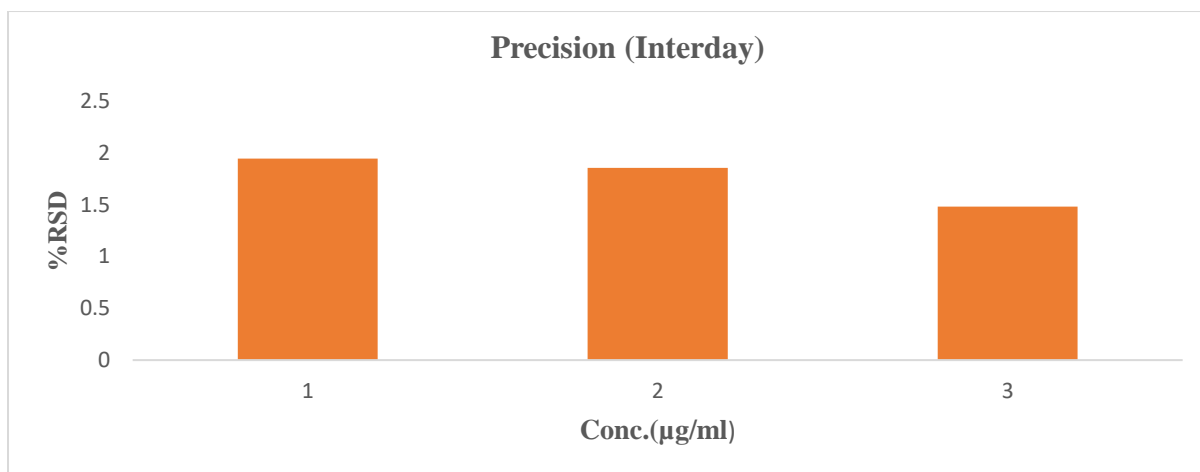


Figure 5: Precision (Interday) analyses of Venlafaxine hydrochloride

5.6 Ruggedness

The ruggedness of the analytical approach is the ability of a technique in unkindness of powerful environmental factors to resist the change in its results. Rugged analytical methods are chosen so because the effect of environmental factors is free from these methods. Two analysts 1 and 2,

respectively, analysed venlafaxine solution 15 ug/ml to determine the superiority of the proposed UV process. Test analysis and data processing resulted in RSD values of 1.7081 and 1.9489 percentage values. The results revealed that the proposed UV approach was robust as it showed less than 2 %RSD values, as seen in Table 7.

Table 7: Results Showing Ruggedness of Method for Venlafaxine hydrochloride

| Conc.(µg/ml) | Analyst-1 | | | Analyst-2 | | |
|--------------|------------|--------|---------|------------|--------|----------|
| | absorbance | amount | %amount | absorbance | amount | % amount |
| 15 | 0.525 | 12.25 | 81.66 | 0.510 | 11.87 | 79.16 |
| 15 | 0.531 | 12.40 | 82.66 | 0.531 | 12.40 | 82.66 |
| 15 | 0.535 | 12.50 | 83.33 | 0.532 | 12.42 | 82.83 |
| 15 | 0.511 | 11.90 | 79.33 | 0.511 | 11.90 | 79.33 |
| 15 | 0.523 | 12.20 | 81.33 | 0.521 | 12.15 | 81.00 |
| 15 | 0.521 | 12.15 | 81.00 | 0.524 | 12.22 | 81.50 |

| | | | | | | |
|-------------|--|--------|---------------|--|--------|---------------|
| AVG | | 15.23 | 81.55 | | 12.16 | 81.08 |
| SD | | 0.2089 | 1.390 | | 0.2370 | 1.580 |
| %RSD | | | 1.7081 | | | 1.9489 |

5.7 Limit of Quantitation (LOQ) and Limit of Detection (LOD)

LOQ represents the lowermost concentration that can be analysed with acceptable accuracy and precision. Generally, LOQ is the first calibration standard. LOD and LOQ of the proposed UV method were found to be 0.308 µg/ml and 3 µg/ml respectively.

6. CONCLUSION

The results and the statistical parameters validate the modest, rapid, specific, precise and accurate projected UV spectrophotometric method. Consequently, this method may even be used in bulk or dosage formulations for the determination of venlafaxine, without interference with normally used excipients and related substances.

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DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not Applicable

AUTHORSHIP CONTRIBUTION STATEMENT

Anil Pawar: Supervision, Validation, Methodology, Data Curation, Investigation, Writing – original draft, Tejas Pachpute: Conceptualization, Administration, Funding.

ABBREVIATIONS

Serotonin-norepinephrine reuptake inhibitor: SNRI

Serotonin norepinephrine-dopamine reuptake inhibitor: SNDRI

Reverse phase high-pressure liquid chromatography: RP-HPLC

International conference on harmonization: ICH

Standard Calibration Curve

Limit of Quantitation: LOQ

Limit of Detection: LOD

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