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Original article

APPLICATION OF UV-SPECTROPHOTOMETRY AND HPLC FOR DETERMINATION OF VENLAFAXINE AND ITS FOUR RELATED SUBSTANCES IN PHARMACEUTICAL DOSAGE FORMS

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Abstract

Venlafaxine belongs to a group of Antidepressant with a novel chemical structure. A rapid, specific reversed – phase HPLC method has been developed for assaying Venlafaxine in pharmaceutical dosage forms involving an isocratic elution in C_{18} column using a mobile phase composition of phosphate buffer (pH: 3.6)-acetonitril (60: 40, v/v). The flow rate was 1 mL/min at 225 nm. For separation of impurities composition of mobile phase was phosphate buffer pH:5 - acetonitril - methanol (80:13:7, v/v/v). Also, simple, rapid and precise an UV spectrophotometric method was developed for dissolution studies. All the validation parameters were within the acceptance range.

The proposed methods are sensitive, accurate, reproducible and useful for the routine determination of venlafaxine in tablets.

Key words: *Liquid chromatography, Venlafaxine, Related substances, Spectrophotometry, Pharmaceutics.*

Venlafaksin ve İlgili Dört Maddenin Farmasötik Dozaj Formlarında Miktar Tayini için UV Spektrofotometri ve YPSK'nın Uygulanması

Venlafaksin, yeni bir kimyasal yapıya sahip antidepresan grubundan bir ilaçtır. Venlafaksin'in farmasötik dozaj formlarından tayini amacıyla, C_{18} kolonda, fosfat tamponu (pH: 3.6)- asetonitril (60:40, h/h) bileşimi bir hareketli faz kullanılarak izokratik elüsyonla, hızlı, spesifik karşıt-faz YPSK yöntemi geliştirilmiştir. Akış hızı 225 nm'de 1 mL/dk olarak ayarlanmıştır. Safsızlıkların ayırımı amacıyla hareketli faz olarak, fosfat tamponu (pH:5)- asetonitril - metanol (80:13:7, h/h/h) karışımı kullanılmıştır. Ayrıca disolüsyon çalışmaları için basit, hızlı ve kesinliği yüksek bir UV spektrofotometrik yöntem geliştirilmiştir. Tüm validasyon parametreleri kabul sınırları içinde bulunmuştur.

Önerilen yöntemler tabletlerde venlafaksin'in rutin miktar tayini için duyarlı, doğru, tekrarlanabilir ve uygun bulunmuştur.

Anahtar kelimeler: *Sıvı kromatografi, Venlafaksin, İlgili maddeler, Spektrofotometri, Farmasötikler.*

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INTRODUCTION

Venlafaxine (Fig.1) is designated (R/S) - 1- [2- (dimethylamino) - 1- (4 - methoxyphenyl) ethyl] cyclohexanol hydrochloride or (RMG) - 1 - [α - [(dimethylamino) methyl] - p - metoxy benzyl] cyclohexanol hydrochloride and has the empirical formula of $C_{17}H_{27}NO_2HCl$. Its molecular weight is 313.87 (1). It is a particularly effective second generation antidepressant chiral drug, administered as a racemic mixture, exerting a dual mechanism of action on the monoaminergic system (2).

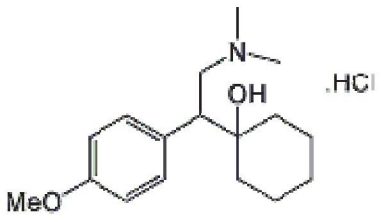


Figure 1. Structure of venlafaxine (R/S) -1-[2-(dimethylamino)-1-(4-methoxy phenyl) ethyl] cyclohexanol hydrochloride

Venlafaxine is rapidly absorbed and metabolized in the liver to its active metabolite des-methyl venlafaxine. It is a highly soluble drug with a solubility of greater than 500 mg/mL in water and an octanol - water partition coefficient of 0.43.

Approximately 92% of an oral dose is absorbed in the gastrointestinal tract. However, due to extensive first pass metabolism, only 12.6% is available in systemic circulation. The bioavailability of Venlafaxine does not differ significantly between a tablet and oral solution based upon maximum plasma concentration and area under the curve (3).

Several methods can be found in the literatures for the determination of venlafaxine in pharmaceutical dosage forms have been established by the use of HPLC method. Some of these methods were done with photodiode array UV detection by HPLC method but they are gradient and need column temperature (4,5), with using a gradient method (6), LC-MS method in human plasma and application to a pharmacokinetic (7,8,9) and bioequivalence studies (10). For dissolution method a flow-injection assay study was developed (11).

In the present study, a rapid, specific, precise and validated an HPLC method with an isocratic elution and spectrophotometric method for the quantitative estimation of venlafaxine and its four related substances in pharmaceutical dosage forms without using column temperature is reported. In addition a simple, rapid and specific a UV spectrophotometric method for dissolution of venlafaxine is developed and validated.

MATERIALS AND METHODS

Chemicals and reagents

Venlafaxine (100 % pure) was a sample form Aarti Health care APL, India, Acetonitril (HPLC grade) was obtained from (Caledon Labratories LTD. Canada). Potassium dihydrogen phosphate was purchased from Merck (Darmstadt, Germany).

The other entire chemicals used were of analytical grade.

Instrumentation

The HPLC system consisted of an Agilent Technologies 1200 series, USA) equipped with an Agilent Technologies G 1311A Quat pump in a quaternary gradient mode and an Agilent Technologies 1200 series VWD detector. Data acquisition was performed by Chemstation software operated on a Pentium IV microprocessor. Analytical was carried out at 225 nm, with a perfectsil Target C_{18} column of 250x4.6 mm i.d., 5µm dimensions. (M.Z – Analysentechnik GmbH. Germany) at ambient temperature. The mobile phase consisted of potassium dihydrogen phosphate (pH: 3.6±0.05, 25mM) - acetonitril (60: 40, v/v) that was set at a flow rate of 1 mL/min. The pH was determined by Metrohm pH meter (model:78) and UV spectrophotometer was Perkin Elmer (550-SE) .

Preparation of Solutions

Stock solution

Stock solution of venlafaxine was prepared by dissolving accurately weighted 20 mg of the drug in 100 ml of water (final concentration, 0.2 mg/mL). From this stock, 0.01 mg / mL solution standard was freshly prepared during the analysis day.

Sample preparation

For the preparation of linearity curve, the volume of calibration standards were made up with water to get the linearity range of 50 to 160 µg / mL. Concentration of 80,100,120 µg / mL were taken as quality control samples . An aliquot of 20 µL of this solution was injected for the HPLC analysis. For the estimation in dosage forms, 20 tablets from each batch were randomly selected and powdered.Amount equivalent to 20 mg of venlafaxine from powdered formulation was accurately weighted and taken in a volumetric flask, 50 mL of water was added, this mixture was subjected to vigorous shaking for 15 minutes for complete extraction of the drug, then made up the volume to 100 mL, and then centrifuged at 4500 RPM for 30 minutes (Sigma 101, Germany). 5 mL of the clear supernatant was taken and diluted with waters (5/100) and 20 µL of this solution was injected for HPLC analysis.

Method Validation

Linearity (Calibration curve)

The calibration curves were constructed with eight concentrations from 50 to 160 µg/mL of working concentration for venlafaxine (Fig. 2).

Each solutions was injected in three replicated and the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method (Table 1). According to this method, linearity was obtained in the concentration range of 50 -160 µg/mL, with regression coefficient of 0.999, intercept -0.00025 and slope 2.02553 10^{-5}.

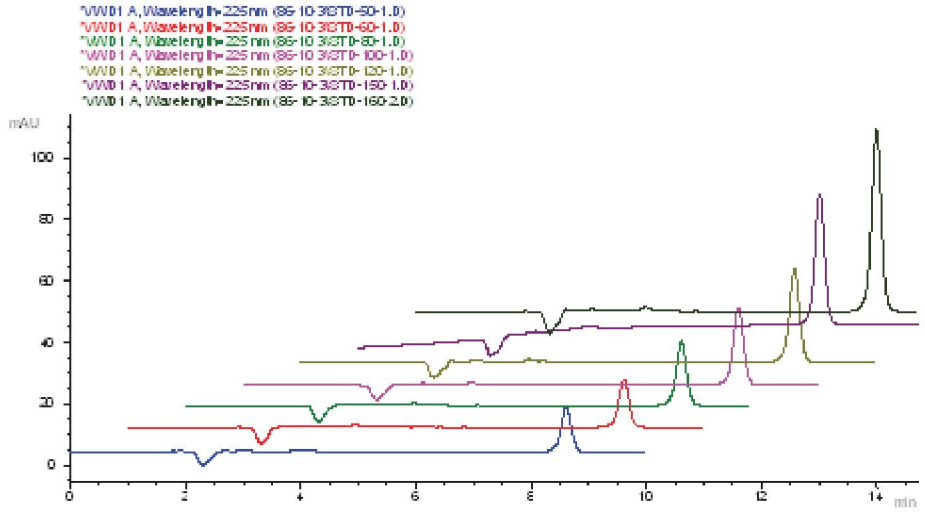


Figure 2. Chromatograms of venlafaxine (from top to bottom), standard solution (10 µg/mL) venlafaxine elutes approximately at 4.4 min.

Accuracy and precision

Accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicate analysis of the QC samples at three concentration levels (80%, 100% and 120%) of working concentrations for venlafaxine. Precision of the assay was determined by repeatability (intra-day) and intermediate precision (inter-day). Repeatability refers to the use of the analytical procedure within a laboratory over short period of time that was evaluated by assaying the quality control samples during the same day and on different days using new solutions and different chromatographic systems (Figure 3). Intermediate precision was assessed by comparing the assay on different days (3 days), (Table 1).

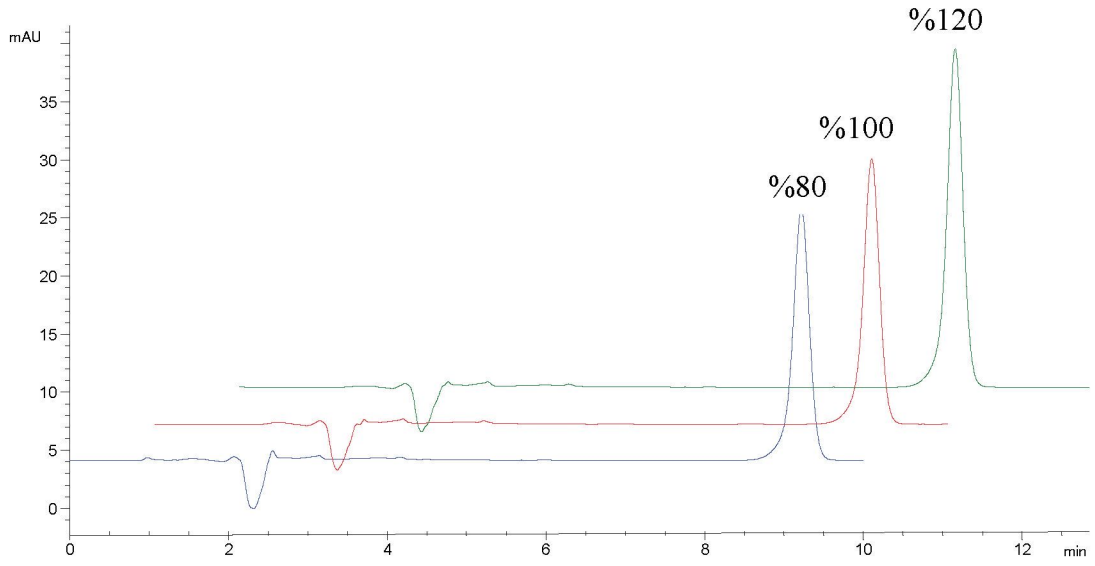


Figure 3. Chromatograms of sample solutions in different concentrations of venlafaxine.

Table 1. Intra-day and inter-day precision and accuracy for the determination of venlafaxine 50 mg by HPLC method.

	Within day precision			Between day precision		
Amount	Mean area	SD	RSD %	Mean area	SD	RSD%
80	389.4	3.25	0.822	387.033	2.74	0.709
100	473.88	4.2	0.88	468.62	5.8	1.23
120	575.03	0.001	0.54	576.92	1.77	0.297
	Acceptance criteria RSD<2			Acceptance criteria RSD<3		

Selectivity / Specificity

During specificity study number of different solutions was prepared. Venlafaxine, individual impurities, standard solution, sample solution and placebo solution were injected. The spectra and purity plots solutions were traced through a diode array detector for each ingredient in the standard. Furthermore, forced degradation studies were conducted in order to prove selectivity of the method.

Robustness

Several parameters of the method were purposely altered in order to determine the robustness of the method. The system suitability parameters as well as the recovery for the main ingredients in the sample solution were examined. The method parameters altered were the columns temperature, the flow rate and the buffers' pH (Table 2).

Table 2. Venlafaxine and impurities in sample solution and system suitability parameters through robustness study.

impurity E	impurity C	impurity B	impurity A	Venlafaxine	Method parameters
101.5	98.9	99.5	101.2	99.80	working conditions
100.2	97.4	98	99.5	100.02	column temperature: 30
96.8	98.4	97.5	100.4	100.30	column temperature: 35
95.8	97.8	101.6	96.8	100.23	Flow rate:0.8 ml/min
99.20	100.80	102.60	97.30	99.98	Flow rate:1.4 ml/min
No Resolution					pH: 2.8
100.5	103.8	97.7	101	98.87	pH: 3.2
95-105%	95 -105%	95-105%	95-105%	98-102%	Acceptance criteria

Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard method. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 3.

Table 3. Recovery studies of venlafaxine in different sample solutions.

Mean	SD	RSD
484.2	4.9	1.01%

Detection and quantitation limits (Sensitivity)

Limits of detection (LOD) and quantitation (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak area of three times the baseline noise. The quantitation limit was defined as the lowest concentration level that provided a pick area with a signal-to-noise higher than 10, with precision (% RSD) and accuracy (% bias) within ±10 %.

Application of method

In order to evaluate the application of method, commercial preparations and the new formulation were analyzed. The samples were prepared as described above and the content of venlafaxine was calculated (Table 4).

Table 4. Comparison of commercial preparations (Reference) and new formula (Test)

	Test samples	Reference samples (Effexor)
Labeled (mg/mL)	50	50
[a]Found (mg/mL) ± SD	49.6±0.56	50.4±0.44
% Recovery	99	101
% RSD	1.41	1.08

System suitability

Sample solution was injected three times in order to obtain the retention times of the components and all the important parameters of system suitability testing were calculated (RSD of area of venlafaxine peak and h /v ratio of impurity B, where h is the peak height of impurity B and v is the distance between the top of the peak of impurity B and the lowest point of the valley defined between the peak due to Impurity B and venlafaxine). All the other impurities were well separated as indicated in Fig. 4.

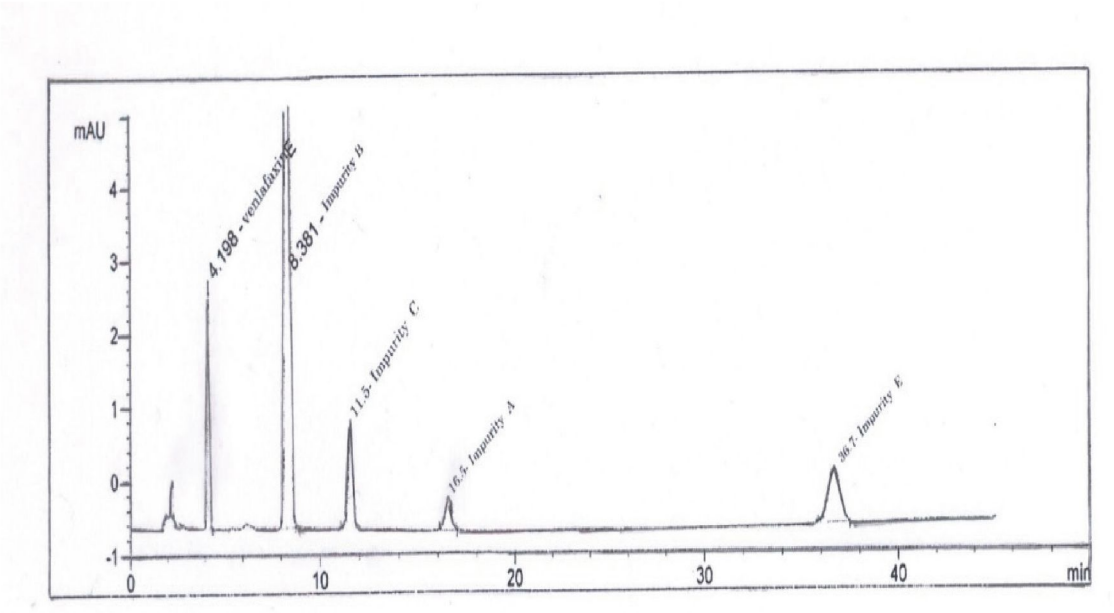


Figure 4. Chromatograms of venlafaxine and its four impurities in one injection: venlafaxine (t_R= 4.1), impurity B (t_R= 8.3), impurity C (t_R=11.5), impurity A (t_R= 16.5), impurity E (t_R=36.7).

Stability

The stability of the drug solution was determined using the QC samples for short-term stability by keeping at room temperature for 36 hours, and then analyzing. The long term stability was determined by storing at 45 ºC and 75 % relative humidity (R.H.) for 90 days.

Stability of tablets

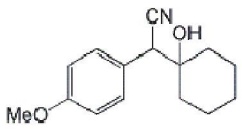
The stability of the tablets was determined using the QC samples for short- term stability by keeping at room temperature for 36 hours, and then analyzing. The samples (n = 3) were taken out 30, 60, 90 days and evaluated for the drug content and physical parameters like color change, friability and hardness, and dissolution.

Stability of solutions

Both standard and sample solutions were prepared and analyzed for recovery of venlafaxine and four impurities at Oh,5h,12h and 24 h at room temperature.

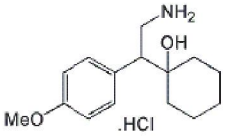
Impurities

Chemical structure of venlafaxine impurities are shown in (Fig. 5). Drug stock solution of the impurities was prepared by dissolving accurately weighted 5 mg of their working standards in 20 mL of mobile phase (Final Concentration 0.25 mg/mL). From these stock, 0.01 mg/mL solutions standard were prepared. The optimal composition of mobile phase was determined to be phosphate buffer pH:5 acetonitril-methanol (80:13:7, v/v/v). Flow rate was set at 0.8 mL/min. As can be seen in Figure 6, they have good resolutions and are well separated.



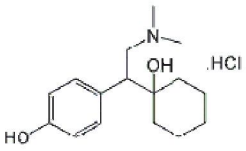
(impurity E)

1-[Cyano-(4-methoxyphenyl) methyl]cyclohexanol



(impurity C)

1-[2-Amino-1-(4-methoxyphenyl)ethyl] cyclohexanol hydrochloride



(impurityy A)

O-Desmethyl Venlafaxine Hydrochloride

(impurity B)

N-Desmethyl Venlafaxine Hydrochloride

Figure 5. The structure of some venlafaxine related substances.

Method validation

Calibration curves were linear over the range of concentration used (50-120 µg/mL), which Table 5 summarizes the results of the method. Method precision, as indicated in Table 7, was <2.5. The accuracy, as measured by the relative errors, ranged from 98.5 to 100.03% for all impurities. To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard method. The results are reported in Table 6.

Table 5. Linearity results (n=3) for impurities of venlafaxine.

LOD(mg/mL)	LOQ(mg/mL)	R^2	Regression equation	Component
0.13	0.38	0.9999	y=25320208x-1291	impurity A
0.12	0.35	0.9989	y=14899753x-1111	impurity B
0.08	0.18	0.9995	y=17566832x-30051	impurity C
0.16	0.37	0.9997	y=22032123x-1128	impurity E

Table 6. Accuracy, precision and recovery results for impurities of venlafaxin.

impurity A	impurity B	Impurity C	impurity E		Acceptance Criteria
99.80 %	98.50 %	100.03 %	99.50 %	Accuracy	95-105.0 %
2.50 %	0.85 %	1.80 %	0.56 %	precision	< 5%
impurity A	impurity B	impurity C	impurity E	Venlafaxine	Recovery
100.02	100.02	100.40	99.20	98.90	Recovery of standard
98.7	100.5	99.8	100.8	99.6	Recovery of sample
95-105 %	95-105 %	95-105 %	95-105 %	98-102 %	Acceptance criteria

Dissolution studies

The method is based on a direct measurement of the absorbance of the analyte in water medium, at 225 nm by UV-Spectrophotometry Fig. 5.

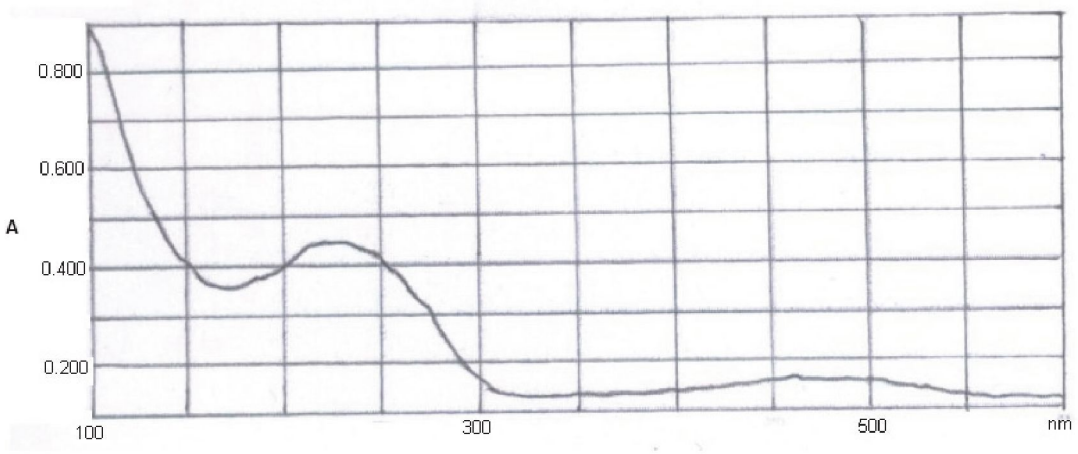


Fig 5. UVspectra of venlafaxine showing the purity in tablets.

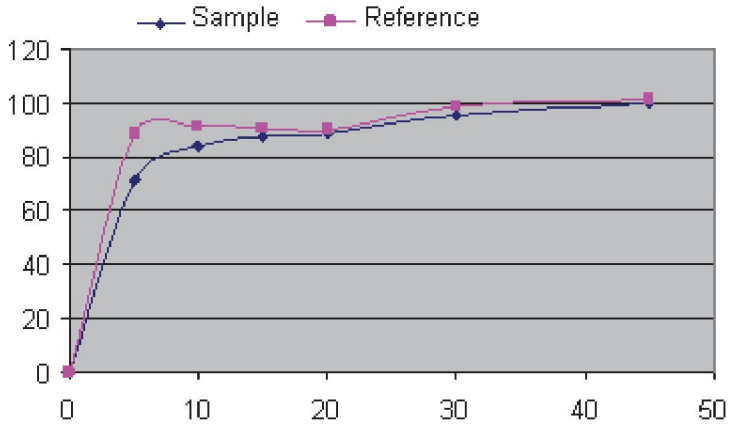


Fig 6. Compression between sample and reference for dissolution study of venlafaxine.

Dissolution study was carried out using USP Type 2 dissolution apparatus. The study was carried out in 900 ml of water .The dissolution medium was kept in thermostatically controlled water bath , maintained at 37 ± 0.5 ºC . The paddle was lowered so that the lower end of the stirrer was 25 mm above from the base of the beaker the pre-weighed tablet was then introduced in to the dissolution jar and the paddle was rotated at 50 rpm. At different time intervals , 15 ml sample was withdrawn, filtered the solution through watman filter paper no 42 , then 5 ml of the resulting solution was transferred to 50 ml volumetric flask and volume was make up with distilled water. The proposed method was validated in terms of linearity, repeatability, accuracy and selectivity. Release of venlafaxine in reference and new formulation was same as each other and the values were calculated as follows (Fig 6) (Table 7).

Table 7. Dissolution results based on F1 and F2 calculation.

Final result	Standard
57.91	≥ 50
5.99	≤ 15

$F1 = \sum (|R t - T t|) / (\sum R t) * 100$

$F2 = 50 * \log \{ [1 + 1/n \sum(R t - T t)^2]^{-½} * 100$

Validation of UV-spectrophotometric method

The calibration curves were constructed with eight concentrations from 50 to 160 µg/mL of working concentration for venlafaxine. Each solutions was measured in three replicated and the linearity was evaluated by linear regression analysis, which was calculated by the least square regression method (Table 8), accuracy of the assay method was determined for both intra-day and inter-day variations using the triplicated analysis of the QC samples and inter-day precision for the analyte was shown in Table 9. The intra and inter-day RSD were less than 2 %. Also, during specificity study number of different solutions were prepared. Venlafaxine, standard solution, sample solution and placebo solution were measured.

Table 8. Results of regression of the linearity of venlafaxine by UV method.

Parameter	
Slope	0.0297
Intercept	7.37×10^{-5}
Correlation Coefficient (r^2)	0.99952
Recovery %	99.5
LOQ (µg/mL)	1.01
LOD (µg/mL)	0.29

Table 9. Intra-day and inter-day precision and accuracy for the determination of venlafaxine 50 mg by UV-Spectrophotometric method

	Within day precision			Between day precision		
Amount	Mean area	SD	RSD%	Mean area	SD	RSD%
80(ppm)	0.274	0.001	0.573	0.273	0.003	1.283
100(ppm)	0.33	0.002	0.651	0.33	0.003	0.88
120(ppm)	0.38	0.001	0.358	0.38	0.004	10158
	Acceptance criteria RSD<2			Acceptance criteria RSD<3		

RESULTS

A rapid, specific isocratic HPLC method has been developed for the determination of Venlafaxine using a UV detector. The method was validated for accuracy, precision, linearity and stability. The method uses a simple mobile phase composition, easy to prepare with little or no variation. The rapid run time of 4 min allows the analysis of a large number of samples with less mobile phase, that proves to cost- effective. It can be achieved without column temperature.

For assay method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate Venlafaxine and all the impurities. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with phosphate buffer pH:5-acetonitril-methanol (80:13:7, v/v/v) and 1 min/mL flow rate is quite robust. In separation of impurities compounds with similar structure elute close to each other. Parameters that effect retention and separation are percentage of organic solvent and type of organic solvent, because they defined the polarity of the mobile phase and kind of interaction between molecules and stationary phase. Different percentages of mobile phases were tried in order to achieve the best separation. The peaks elute from the C18 column in order of decreasing polarity, that is more polar compounds elute first and less polar and bigger compounds retained strongly and elute later. The optimum wavelength for detection was at 225 nm which better detector response for venlafaxine and its four impurities was obtained. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. Calibration curve was linear.

Sample- to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day-to-day variability was assessed using three concentrations analyzed on three different days, over a period of two weeks. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values reported in Table 1 and 9, shows that proposed methods provides acceptable intra – day and inter – day variation. Furthermore, it is necessary, to develop a simple, specific, rapid, analytical method for the quantitation of the venlafaxine. Therefore, spectrophotometric method for dissolution was found so fast. For UV spectrophotometric method, in dissolution study, linearity was obtained in concentration range of 50 -160 μg/ml, with regression 0.999, intercept 7.37×10^{-5} and slope 0.0297. The value of standard deviation and RSD in recovery was found to be less than 2%; shows the high precision of the method.

DISCUSSION

The proposed methods are accurate, simple, rapid and selective for determination of venlafaxine and especially for its impurities with an isocratic solution, in pharmaceutical dosage forms.

Hence, it can be conveniently adopted for the routine quality control analysis.

The retention time of 4.1 min allows the analysis of a large number of samples with less mobile phase, and that proves to cost – effective. Furthermore, UV spectrophotometric method for dissolution tests is easy and so time consuming.

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