

DEVELOPMENT AND VALIDATION OF KINETIC SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF VENLAFAXINE HYDROCHLORIDE

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A kinetic spectrophotometric method that is sensitive and simple has been developed for the quantification of the venlafaxine hydrochloride in bulk as well as in pharmaceutical preparations. In this technique, venlafaxine is oxidized in the presence of alkaline potassium permanganate as an oxidizing agent and sodium hydroxide as a basic medium, and the reaction is carried out at room temperature. Due to the production of a manganate ion, the spectrophotometric measurement was performed at 610 nm. A fixed-time-based approach (15 min) was used to plot calibration graphs. The proposed method was optimized for all the conditions of reaction, whereby the linearity range was found to be 5–30 µg/mL with a limit of detection of 0.07 µg/mL. For the quantification of venlafaxine hydrochloride in a dosage form, the developed method was successfully applied and the percentage recovery was 98.87–100.22%. Statistical comparison was done for the obtained results with those of an official method and no significant differences regarding precision and accuracy were found.

Keywords: kinetic spectrophotometric method, venlafaxine hydrochloride, potassium permanganate, pharmaceutical preparations.

РАЗРАБОТКА И ВАЛИДАЦИЯ КИНЕТИЧЕСКОГО СПЕКТРОФОТОМЕТРИЧЕСКОГО МЕТОДА ОПРЕДЕЛЕНИЯ ВЕНЛАФАКСИНА ГИДРОХЛОРИДА

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Для количественного определения венлафаксина гидрохлорида в нерасфасованном виде, а также в фармацевтических препаратах разработан чувствительный и простой кинетический спектрофотометрический метод. Реакция окисления венлафаксина проведена в присутствии щелочного перманганата калия в качестве окислителя и гидроксида натрия в качестве основной среды при комнатной температуре. Из-за образования манганинового иона спектрофотометрическое измерение проводили на длине волны 610 нм. Для построения калибровочных кривых использован метод фиксированного времени (15 мин). Метод оптимизирован для всех условий проведения реакции, диапазон линейности 5–30 мкг/мл, предел обнаружения 0.07 мкг/мл. Разработанный метод успешно применен для количественного определения венлафаксина гидрохлорида в лекарственной форме, процент извлечения 98.87–100.22%. Статистическое сравнение полученных результатов с данными традиционного метода не выявило существенных различий в точности.

Ключевые слова: кинетический спектрофотометрический метод, венлафаксина гидрохлорид, перманганат калия, фармацевтические препараты.

Introduction. Chemically Venlafaxine (VEN) is 1-[2-(dimethylamino)-1-(4 methoxyphenyl)ethyl] cyclohexanol, which is used as an antidepressant and is of the serotonin-norepinephrine reuptake inhibitor (SNRI) class [1]. In clinical depression as well as anxiety disorders, it is given as a medication [2]. The molecular weight of VEN is 313.9 g/mol and it is freely soluble in water and eliminates a half-life of 4–5 h.

Venlafaxine hydrochloride is a third-generation antidepressant, and as per the biopharmaceutical classification system, it belongs to the class 1 drug. The drug in its hydrochloride salt form was administered to adults in the range of 75 to 350 mg/day.

According to the findings of the literature review, several analytical techniques have been described for the determination of the active pharmaceutical ingredient, including venlafaxine hydrochloride, as well as formulations including spectrophotometric [3–10], HPLC [11–18], and LC MS [19–21]. There have been no documented kinetic spectrophotometric techniques [22–24] for the determination of venlafaxine hydrochloride in pharmaceutical formulations. In pharmaceutical analysis, kinetic methods have certain advantages with respect to selectivity and are free from the effect of additive interference, when compared to direct spectrophotometric methods. As there is no involvement of tedious processes such as filtration and extraction, the kinetic method is considered to be superior to others as it is rapid as well as simple. This method has high selectivity as the absorbance measurement is made as a function of the reaction time, and the other components do not respond at the given optimized reaction conditions.

KMnO₄ and VEN react with each other in alkaline conditions in the present study, produce a greenish manganate with a λ_{max} at 610 nm, and form a basis for the direct spectrophotometric determination of VEN. Optimization of reaction conditions was done and the technique was validated in accordance with the International Conference on Harmonization (ICH) guidelines [25] for accuracy, sensitivity, linearity as well as precision. When applied to formulations, the validated technique results were consistent with the label claim for VEN in the formulation.

Experimental. We used a LAB India double beam UV-Vis spectrophotometer with the UV WIN5 software (version 5.2.0.1104) and a pair of identical quartz cells (1 cm thickness) to conduct our experiments. The sample solution was sonicated using an ultra sonicator (Citizen Inia) before being analyzed, and weighing was done using a digital weighing balance (Shimadzu AUX 220). The chemicals, as well as reagents that were utilized, were of the highest analytical quality; throughout the research, high-purity double-distilled water was utilized. The standard 1000 $\mu\text{g/mL}$ VEN stock solution was prepared in double-distilled water. VEN (purity % is 99.82) was a gift sample from the R.A. Chem, Hyderabad. The solutions for both the stock and working standard solutions were freshly prepared. The commercial dosage form of VENLOR-XR is 37.5 mg/capsule (product of CIPLA Ltd., India). Potassium permanganate (Merck, India), 0.05 M solution was freshly prepared (7.9 g potassium permanganate in 100 mL double-distilled water) and standardization was done by the titrimetric method. A 0.4 M solution of NaOH (SD Fine Chemicals, Mumbai, India) was produced by dissolving 1.6 gm of NaOH in 100 mL double-distilled water.

General recommended procedure. Accurate volumes of VEN working solution over the concentration range of 5–30 $\mu\text{g/mL}$ were transferred into a series of 10 mL standard flasks. Firstly, 1.0 mL of 0.05 M potassium permanganate was added to each flask, followed by 1.0 mL of 0.4 M sodium hydroxide. The volume of flasks was made up to the mark with double distilled water. After 15 mins, the absorbance of the prepared solutions was observed at 610 nm and compared with a similar treatment using the given reagent blank. The calibration graph was developed by plotting the final concentration of the drug on the *x*-axis against the absorbance values in the *y*-axis. The least-squares technique within the Microsoft Excel software was used to calculate the appropriate regression of *y* on *x*.

Procedure for pharmaceutical formulation. Twenty capsules each containing 37.5 mg of VEN were weighed out and the average weight of the capsule was computed. Next, the powder present in 20 capsules was completely evicted from the shells. The powder weight that was 10 mg of VEN was weighed and was dissolved in 10 mL of double distilled water, sonicated for a time period of 10 min and filtered through Whatman filter paper No. 42. The filtrate was transferred to a calibrated 100 mL flask and diluted to the volume with double distilled water. The method mentioned in the general recommended procedure was applied to the prepared solution.

Results and discussion. VEN's absorption spectra in aqueous potassium permanganate solution in basic media show an absorption band at 610 nm (Fig. 1). This band is formed as a result of the production of a manganate ion, which occurs as a result of the oxidation of VEN with potassium permanganate in an alkaline environment. Because the product's color density rises with time, a kinetically based technique approach was used to estimate VEN in the pharmaceutical dosage form under study. The experimental factors that influence the formation and stability of the reaction product were identified and optimized. The variables investigated were the concentration of the additional reagents (potassium permanganate and sodium hydroxide), the sequence in which they were added, the temperature, and the length of time the mixture was heated.

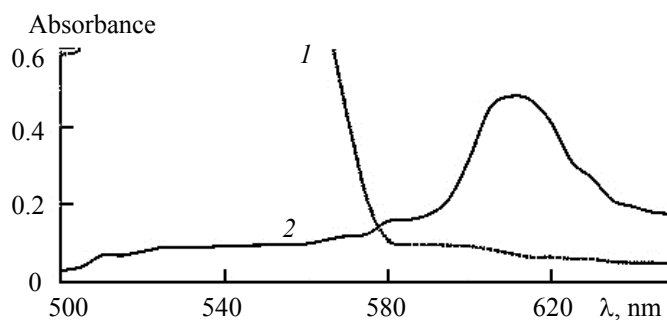


Fig. 1. Overlay spectra of (1) reagent blank (0.05 M KMnO_4) and (2) VEN (20 $\mu\text{g/mL}$).

Optimization of reaction conditions. The spectrophotometric characteristics of the produced colored product, as well as different experimental factors affecting color development and stability, were investigated and improved using a one-variable-at-a-time approach. The effects of potassium permanganate on the reaction were investigated at concentrations ranging from 0.01 to 0.06 M. The high absorbance was obtained when 1 mL of 0.05 M KMnO_4 was added. The green color development occurs in basic media, so NaOH was chosen. The influence of the concentration of sodium hydroxide on the reaction was studied over the range of 0.1–0.5 M. The concentration of sodium hydroxide was optimized at 0.4 M to produce good absorbance. It was also found that no change in absorbance values occurs if the volume of sodium hydroxide was above 1.0 mL. The decrease in the volume of NaOH results in the lowering of the values of absorbance; therefore, 1.0 mL of 0.4 M NaOH was optimized.

To study the influence of time, a fixed concentration of VEN (20 $\mu\text{g/mL}$) was added to 1.0 mL of 0.05 M KMnO_4 solution, and readings of absorbance were recorded in times ranging from 5 to 40 min. The entire color development takes place in 35 min, and the color remains stable for up to 60 min while the reaction product is present. Via a process of heating the resultant solution in a water bath that was thermostatically controlled, it was determined that a full-color formation had occurred. From the preliminary test, it was confirmed that a complete color formation took place by heating the resultant solution in a thermostatically controlled water bath. Incrementing the temperature of the water bath resulted in a proportional increase in absorbance. Thus, trials were carried out at higher temperatures, although there was a chance of the occurrence of the precipitation of manganese(II) dioxide. For this purpose, the optimal temperature of $(50 \pm 2)^\circ\text{C}$ was chosen to determine VEN. The heating time is an important part of the experiment. To determine the time at which the solution attains its peak absorbance, different time intervals were tried and the heating time of 15 min was found to produce the highest absorbance readings.

Quantitation methods. Because of the intensity of the generated color changes with time at 610 nm, a kinetic approach for quantification of VEN measurement is desirable. The fixed time, rate constant, as well as initial rate methods were utilized, while the selection of suitable analytic methods was based on the value of intercept, applicability, coefficient correlation as well as sensitivity.

Initial rate method. The quantification of VEN from the optimized experimental conditions described previously results in a pseudo-first order reaction in which the KMnO_4 concentration was 30 times smaller and the NaOH concentration was 600 times smaller than the initial concentration of VEN. According to the pseudo-first-order-rate equation using the following formula, the rate of the reaction will be exactly proportional to the concentration of the drug:

$$v = K'[C]^n. \quad (1)$$

Equation (1) was the substructure for several experiments, which were disbursed to get drug concentration. Taking rates and concentrations of logarithms (Table 1), the preceding equation becomes:

$$\log v = \log(\Delta A/\Delta t) = \log K' + n \log C, \quad (2)$$

where A is the absorbance, t is the time in seconds, and K is the pseudo-first order rate constant. Regression of $\log K$ versus $\log C$ gave the regression equations $\log v = \log(\Delta A/\Delta t) = 0.6448 + 0.9342 \log C$; $r = 0.9922$. A straight line with slope values ($n \approx 1$) was obtained confirming that the reaction was first order.

TABLE 1. Relation between Reaction Rates and Concentrations for Drug VEN

$\log(\Delta A/\Delta t)$	-3.87	-3.59	-3.37	-3.32	-3.20	-3.13
$\log[\text{drug}]$	-4.824	-4.524	-4.346	-4.221	-4.125	-4.025

Fixed-time method. For the varied concentrations of VEN, reaction rates were determined. The absorbance measurements were carried out at preselected fixed time. At a fixed duration of 5, 10, 15, 20, 25, 30, and 40 min (Fig. 2) the calibration graph of initial concentration versus absorbance was established with the regression equation assembled in Table 2. The most acceptable values of the correlation coefficient, as well as intercept, were achieved at a fixed period of 15 min, a rate which was therefore chosen due to its having the best suited time for measurement. The analytical parameters for the quantification of VEN by the fixed time method were presented in Table 3. The fixed time technique was used to determine the concentration of VEN in the concentration range of 5–30 $\mu\text{g/mL}$ based on the optimal reaction conditions developed.

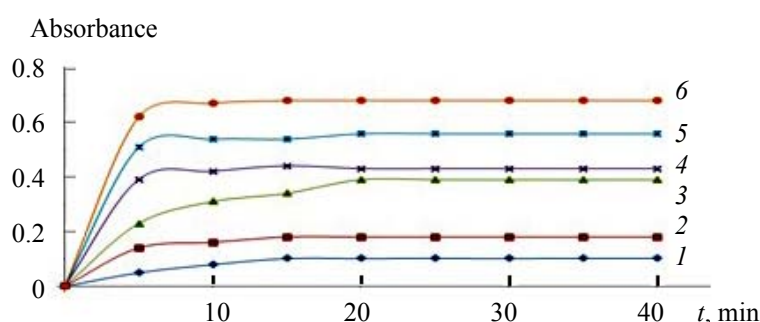


Fig. 2. Absorbance-time curves for the reaction between VEN and KMnO_4 in aqueous medium: 1.0 mL of 5×10^{-2} M KMnO_4 (1) 1.59×10^{-5} M, (2) 3.18×10^{-5} M, (3) 4.77×10^{-5} M, (4) 6.36×10^{-5} M, (5) 7.95×10^{-5} M, and (6) 9.54×10^{-5} M.

TABLE 2. Regression Equations $A = a + bC$ and Correlation coefficient (r) for the Studied Drugs of Different Concentrations at Different Time Intervals Using the Fixed Time Method

Time, min	Regression equation*	r	Time, min	Regression equation*	r
5	$-0.1047 + 0.0237C$	0.9941	25	$-0.0276 + 0.0231C$	0.9964
10	$-0.0633 + 0.0237C$	0.9937	30	$-0.0267 + 0.0207C$	0.9965
15	$-0.0237 + 0.066C$	0.9989	35	$-0.0269 + 0.0232C$	0.9965
20	$-0.0273 + 0.0235C$	0.9965	40	$-0.0302 + 0.0276C$	0.9965

Note. A is the absorbance at 610 nm and C is the concentration in $\mu\text{g/mL}$.

TABLE 3. Analytical Parameters for the Determination of VEN in Pure form Using the Fixed Time Method

λ_{max} , nm	610
Beer's law limit, $\mu\text{g/mL}$	5–30
Slope	0.0666
Correlation coefficient (r)	0.9989
Regression equation $A = a + bC$, where C is the concentration of drug ($\mu\text{g/mL}$)	$-0.0237 + 0.066C$
Sandell's sensitivity ($\mu\text{g/cm}^2/0.001$ absorbance unit)	50.54×10^{-3}
LOD ($\mu\text{g/mL}$)	0.07
LOQ ($\mu\text{g/mL}$)	0.22
Molar absorptivity (L/mol/cm)	6.21×10^3
Stoichiometric relationship, VEN: KMnO_4	1:1

Rate constant method. Graphs of $\log A$ versus time for the VEN concentrations within the range of 1.5×10^{-5} to 9.0×10^{-5} M was plotted and the pseudo-first-order rate constants (K) corresponded to different concentrations of the investigated drug $[C]$, which were calculated from the slopes multiplied by 2.303. Regression of K values versus $[C]$ gave the equations (Table 4):

$$K = -0.1115 + 0.0223C, r = 0.6777, \quad (3)$$

where C represents the molar concentration and A represents the absorbance at 610 nm. Since the method suffered from poor linearity as designated by the r value, this method was therefore omitted.

TABLE 4. Values of K Calculated from Slopes of $\log A$ Versus t Graphs Multiplied by -2.303 for Different Concentrations of VEN

[Drug]	K	[Drug]	K
1.59×10^{-5}	-2.487×10^{-3}	6.36×10^{-5}	-9.948×10^{-3}
3.18×10^{-5}	-4.974×10^{-3}	7.95×10^{-5}	-12.436×10^{-3}
4.77×10^{-5}	-7.461×10^{-3}	9.54×10^{-5}	-14.925×10^{-3}

Of all the methods described previously, the most acceptable value of r was achieved for a fixed time of 15 min, making 15 min the best choice for the VEN quantification. And as the most acceptable values of the correlation of coefficient and linear range were obtained from the fixed time method, it was the fixed time method that was applied in the quantification of VEN in both the pure form and pharmaceutical formulations.

Analytical method validation. The fixed-time technique was used to generate and analyze kinetic curves for various VEN concentrations under optimal circumstances. Calibration graphs of absorbance against starting VEN concentrations were produced at several fixed time intervals. The calibration graph, which was generated at a fixed time of 15 min, yielded the most suitable values of the correlation coefficient (r) as well as the intercept, and was thus chosen for quantitative analysis. The produced calibration curves demonstrated linearity across a concentration range of around 5–30 $\mu\text{g/mL}$. Regression analysis establishes linear correlations with insignificant intercepts. Table 3 shows the molar absorptivity, statistical analysis of the experimental data findings, calibration graph regression equations, and analytical parameters. Higher values of the coefficients of regression from the regression line indicate good linearity in accordance with Beer's law.

The limits of detection (LOD), as well as limits of quantitation (LOQ) of a technique, were established experimentally using a fixed time (15 min) approach. The LOD of VEN was attained as per ICH, $\text{LOD} (k = 3) = kSa/b$ (where b is the slope of the calibration curve and Sa is the standard deviation of the intercept), and was found to be 0.07 $\mu\text{g/mL}$. LOQ was also attained as per ICH, $\text{LOQ} (k = 10) = kSa/b$, and was found to be 0.22 $\mu\text{g/mL}$. Sandell's sensitivity coefficient value for VEN was found to be 0.056 $\mu\text{g/cm}^2$ per 0.001 absorbance units (Table 3). The high molar absorptivity value and low LOD, LOQ, and Sandell's sensitivity of the colored product indicate good sensitivity of the proposed technique.

The accuracy, as well as precision, were established for the suggested kinetic spectrophotometric technique. The precision of the method was determined in terms of the intermediate precision (intra-day and inter-day). For intra-day precision, three different VEN concentrations were tested in five repetitions on the same day, while for inter-day precision, three different concentrations were examined in 7 consecutive days. The percentage coefficient of variance (% RSD) for the results did not exceed 2%, demonstrating the high reproducibility of the results and the precision of the method. The precision findings demonstrate that the technique is appropriate for use in the analysis of VEN.

The stoichiometric ratio in which the permanganate of the potash and VEN were reacted was estimated by using the limiting logarithmic method which is carried out in two separate experiment sets. In the first set, the concentration of potassium permanganate was maintained in a constant manner, while the concentration of VEN was varied. While in contrast to this in the second set, the concentration of VEN remains constant and the concentration of the potassium permanganate was varied. The absorbance logarithm was compared with the logarithm of various concentrations of VEN or potassium permanganate. The slopes of the plotted straight line were recorded and found to be unity for each line. Hence the stoichiometric ratio for the reaction between VEN and KMnO_4 was found to be 1:1.

When it comes to the measurement of VEN in capsules, the fixed-time approach has been shown to be effective. In order to determine the concentration of VEN, the appropriate regression equations were used

in conjunction with a fixed period of 15 min. Application of the proposed kinetic spectrophotometric method to the determination of VEN in dosage form VENLOR-XR, labelled claim 37.5 mg, gave the following results: recovery $\pm$ SD 99.55 $\pm$ 0.677, *t*-test 1.21, *F*-test 2.36, reported method 99.99 $\pm$ 0.377. The theoretical values of *t* and *F* at *P* = 0.05 are 2.31 and 6.39, respectively. The statistical examination of data produced by the suggested technique as well as the published spectrophotometric method [10] showed that there was no major difference in precision as well as accuracy as revealed by *t*-test as well as *F*-test at 95% of the confidence level, respectively.

Conclusions. The proposed method can be easily applied for the quantification of Venlafaxine in pure and dosage forms and, moreover, the method which does not require treatment of the analyte and tedious extraction processes. The proposed fixed-time method is sensitive enough in the determination of a low concentration of Venlafaxine. These advantages encourage the application of the proposed method in quality control of Venlafaxine in pharma industries. No interference from excipients found in drug formulations was observed.

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