Development and Validation of a Stability-Indicating LC Method for the Determination of Venlafaxine in Extended-Release Capsules and Dissolution Kinetic Studies

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Abstract

A stability-indicating reversed-phase high-performance liquid chromatography method is developed and validated for the determination of venlafaxine hydrochloride (VEN) in extended-release capsules containing spherical beads and for dissolution studies. The method is carried out on a Luna C_{18} column (250 mm × 4.6 mm) maintained at 35°C. The mobile phase is composed of ammonium–acetate buffer 32 mM, adjusted to pH 6.8 with phosphoric acid–acetonitrile–methanol (62:30:8, v/v/v), run at a flow rate of 1.0 mL/min, and detection at 226 nm. Validation parameters such as the specificity, linearity, precision, accuracy, and robustness are evaluated, giving results within the acceptable range. In order to evaluate the best dissolution condition, the dissolution profiles are performed under different conditions, such as media (HCl, water, phosphate buffer), apparatus (I and II), and dissolution rates (50, 75, and 100 rpm). The kinetics release mechanism is evaluated by fitting different models, such as the zero order rate, first order, and Higuchi. Moreover, the proposed method is successfully applied for the assay of VEN in extended-release capsules.

Introduction

Venlafaxine hydrochloride (VEN) is a phenethylamine bicyclic derivative, chemically known as 1-[2-(dimethylamino)-1-(4-methoxy-phenyl)ethyl]cyclohexanol hydrochloride. It is a novel, non-tricyclic antidepressant, and the mechanism of action in humans is believed to be associated with potential of neurotransmitter activity in the central nervous system. Preclinical studies have shown that VEN is a potent inhibitor of neuronal serotonin and norepinephrine reuptake and a weak inhibitor of dopamine reuptake (1–3).

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VEN extended-release capsules, which contains spherical beads, are formulated for once-a-day oral administration. In this formulation, drug release is controlled by diffusion through the coating membrane on the beads and is not pH-dependent (4). Spherical beads are good-quality particulate solid dosage forms due to their technological characteristics, which include high flowability and large surface area (5). Beads are frequently used in controlled release systems because they offer flexibility for further modifications, such as coating. Moreover, they are freely dispersed in the gastrointestinal tract with low risk of side effects and dose dumping, and consequently, low inter- and intra-patient variability (6,7).

A stability-indicating method is defined as an analytical method that accurately quantitates the active ingredients without interference from degradation products, process impurities, excipients, or other potential impurities. These interfering compounds can be originated from various sources of the synthetic route and from the process employed in the manufacturing of pharmaceutical dosage forms. The presence of degradants and impurities in pharmaceutical formulations can result in changes in their chemical, pharmacological, and toxicological properties, which affect their efficacy and safety. Therefore, the adoption of stability-indicating methods is always required to control the quality of pharmaceuticals during and after their production (8–10).

The majority of VEN analytical methods documented in the scientific literature, such as capillary electrophoresis (11,12), high-performance liquid chromatography (HPLC) (13), and liquid chromatography (LC) tandem mass spectrometry (14–16), are bioanalytical in nature and utilized for VEN drug monitoring, metabolism, and pharmacokinetics studies. For determining VEN in bulk material and pharmaceutical formulations, two stability-indicating methods using HPLC have been described (17,18). Additionally, the in vitro determination of gastric and intestinal stability of VEN was studied (19). However, a validated

method for the quantitation of VEN in extended-release capsules containing spherical beads has not been described.

The in vitro dissolution studies are relevant to the prediction of in vivo performance of the products as an attempt to identify potential problems of drug release and absorption. This is to ensure the physiological availability of the drug. Dissolution studies are a very important tool for quality control of finished products to assess batch-to-batch consistency of drug release from solid dosage forms (20–23). Studies on the bioavailability of drugs from a given dosage form revealed that, in many cases, several dosage forms with the same content of the active compound did not give the same therapeutic effect (24).

Currently, there is no published method validated for the dissolution profile studies and for the quantitative analysis of VEN extended-release capsules. As LC with UV detection has been used for quality control of most of pharmaceuticals, this study aims at developing and validating a sensitive stability-indicating HPLC method to be applied to the analysis of VEN in extended-release pharmaceutical products and dissolution profiles studies.

Experimental

Chemicals and reagents

VEN was purchased from DEG (lot: VH06/0106/07, São Paulo, Brazil). A total of six batches of capsules containing 150 mg and 75 mg of VEN extended-release pellets (reference product Effexor and two generic formulations from distinct laboratories) were obtained from commercial sources within their shelf life period. HPLC-grade acetonitrile and methanol were obtained from Tedia (Fairfield, OH). All chemicals used were pharmaceutical- or special analytical-grade. During all the analyses, ultrapure water obtained using a Milli-Q Gradient System (Millipore, Bedford, MA).

Apparatus and chromatographic conditions

The HPLC analysis was performed on a Shimadzu LC-10A system (Shimadzu, Kyoto, Japan) equipped with a LC-10AD pump, DGU-14A degasser, SPD-10AV variable-wavelength detector (set at 226 nm), SPD-M10A$_{VP}$ photodiode array (PDA) detector, and a SCL-10Avp system controller unit. The experiments were carried out on a reversed-phase Phenomenex (Torrance, CA), Luna C_{18} column (250 mm × 4.6 mm i.d., 5 µm). A security guard holder (4.0 mm × 3.0 mm i.d.) has been employed to protect the analytical column. The LC system was operated isocratically at 35ºC using a mobile phase of ammonium–acetate buffer 32 mM, adjusted to pH 6.8 with phosphoric acid–acetonitrile–methanol (62:30:8, v/v/v). It was filtered through a 0.45-µm membrane filter and run at a flow rate of 1.0 mL/min. The injection volume was 20 µL for both standards and samples. Data acquisition was performed using CLASS-VP software to measure the detected peak areas. The dissolution test was performed using rotating basket and paddle apparatus in a Vankel 7000 dissolution tester (VanKel Technology Group, Cary, NC), in accordance with the United States Pharmacopoeia, USP 32 (22).

Procedure

Preparation of reference solutions

The stock solutions of VEN were prepared by weighing out 10 mg of the solution, transferring them to individual 10-mL volumetric flasks, and diluting to volume with water–methanol (50:50, v/v) to obtain a concentration of 1 mg/mL. The stock solutions were stored at 4–8°C and protected from light. Working standard solutions were prepared daily by diluting the stock solutions to an appropriate concentration with the mobile phase.

Preparation of sample solutions

To prepare the sample stock solution, the pellets from the capsules containing 150 and 75 mg of VEN were accurately weighed and crushed to a fine powder. An appropriate amount was transferred into a 50-mL volumetric flask, diluted to volume with water–methanol (50:50, v/v), and sonicated for 30 min, obtaining a final concentration of 1 mg/mL of the active pharmaceutical ingredient. This solution was stored at 4–8ºC and protected from light. Working sample solutions were prepared daily by diluting the stock solution to an appropriate concentration with the mobile phase.

Method Validation

The method was validated in samples of pharmaceutical formulations with the label claim of 150 mg of VEN by the determination of the following parameters: specificity, linearity, range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), and robustness, following the International Conference on Harmonization (ICH) guidelines (25).

Specificity

The specificity of the method was determined by subjecting a sample solution (1 mg/mL) to accelerated degradation by acidic, basic, oxidative, and photolytic conditions to evaluate the interference in the quantitation of VEN. After the procedures, the samples were diluted in mobile phase to a final concentration of 40 µg/mL. A sample solution in 5 M hydrochloric acid, refluxed at 70ºC for 5 h, was used for the acidic hydrolysis. For the basic hydrolysis evaluation, a sample solution in 5 M sodium hydroxide was refluxed at 100ºC for 7 h. The oxidative degradation was induced by storing the sample solutions in 30% hydrogen peroxide, at ambient temperature for 6 h, and protected from light. Photodegradation was induced by exposing the sample to 200 Wh/m^2 near UV light for 15 days. Then, the specificity of the method was established by determining the peak purity of VEN in degradated samples using a PDA detector.

Linearity and range

Linearity was determined by constructing three calibration curves. For the construction of each calibration curve, appropriate amounts of the stock solution were diluted with mobile phase, yielding concentrations of 10, 20, 30, 40, 50, 60, and 70 µg/mL. Before injection of the solutions, the column was equili-

brated for at least 20 min with mobile phase flowing through the system. Three replicate 20 µL injections of standard solution were made to verify the repeatability of the detector response at each concentration. The peak areas of the chromatograms were plotted against the concentrations of VEN to obtain the respective calibration curves. The seven concentrations of the standard solutions were subjected to regression analysis by the least-squares method to calculate the calibration equation and correlation coefficient (r). Linearity was expressed as correlation coefficient; the value must be > 0.9990.

Precision

The precision of the method was determined by repeatability and intermediate precision studies. Repeatability was examined by six evaluations of the same concentration sample of VEN on the same day and under the same experimental conditions. The intermediate precision of the method was assessed by performing the analysis on three different days (inter-days) and also by other analysts performing the analysis in the same laboratory (between-analysts). Precision was expressed as relative standard deviation (RSD); the results must be lower than 2%.

Accuracy

The accuracy was evaluated by the recovery of known amounts of the reference substance added to a sample solution (containing 20 µg/mL of VEN and excipients) to obtain solutions with final concentrations of 32, 40, and 48 µg/mL, equivalent to 80, 100, and 120% of the nominal analytical values, respectively. The accuracy was calculated as the percentage of the drug recovered from the formulation matrix.

Limits of quantitation and detection

The LOQ was taken as the lowest concentration of analyte in a sample that could be determined with acceptable precision and accuracy. The LOD was taken as the lowest absolute concentration of analyte in a sample that could be detected but not necessarily quantified. The LOD and LOQ were calculated from the slope and the standard deviation (SD) of the intercept of the mean of three calibration graphs, which were determined by a linear regression model as defined by the ICH (25). The values were also evaluated in an experimental assay.

Robustness

The robustness of an analytical method 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 (25). According to this definition, two approaches are possible to evaluate robustness: either a one-variable-at-a-time (OVAT) procedure or an experimental design procedure. The OVAT procedure varies the levels of one factor while keeping the other factors at nominal levels. The experimental design is an experimental set-up that allows simultaneously sutdying a number of factors in a low number of experiments. In robustness testing, usually two-level screening designs, such as factorial, fractional factorial or Plackett-Burman designs are applied (26–28). The robustness of the proposed method was determined by analyzing the same samples under a variety of conditions of the method parameters, such as: flow rate, column temperature, changing the mobile phase composition, and pH. The factorial 2^4 was applied to evaluate the relationships between one or more measured responses.

System suitability

To ensure the validity of the analytical procedure, a system suitability test was carried out. Data from six injections of 20 µL of the working standard solution containing 40 µg/mL were used for evaluating the system suitability parameters, such as asymmetry, theoretical plates, retention time, and peak area.

Method Application

Analysis of pharmaceutical extended-release capsules

For the quantitation of VEN in the dosage forms, 20 capsules of each pharmaceutical sample containing 150 and 75 mg of VEN extended-release beads were separated, accurately weighed, and crushed to a fine powder. An appropriate amount of each sample was transferred into a 100-mL volumetric flask, diluted to volume with water–methanol (50:50 v/v), and sonicated for 30 min, obtaining final concentrations of 1 mg/mL (stock solutions). For the analysis, the stock solutions were diluted daily to appropriate concentrations with mobile phase. An aliquot of 20 µL was injected for the analysis, and the amount of each drug per capsules was calculated against the respective reference standard.

In vitro dissolution studies

The in vitro dissolution studies of VEN capsules containing extended-release beads were performed using 900 mL of the medium and evaluated using the following parameters: dissolution rates, dissolution media, and apparatus. The dissolution media tested were ultrapure water (50, 75, and 100 rpm), HCl 0.1 M (75 rpm), and phosphate buffer pH 6.8 (75 rpm). The media were degassed by heating followed by vacuum filtration. Apparatus I (basket) and apparatus II (paddle) with sinkers (to maintain the capsule in the vessel bottom) were used. The temperature of the cell was maintained at 37 ± 0.5°C by using a thermostatic bath. At each sample time interval, an exact volume (10 mL) of the sample was withdrawn from flask, filtered, and immediately replaced with an identical volume of fresh medium to maintain the dissolution sink condition. At predetermined time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h), the concentration of VEN in the dissolution medium was determined by the proposed HPLC method. The cumulative percentage of drug release was plotted against time in order to obtain the release profile and to calculate the in vitro dissolution data (n = 6).

The dissolution profiles of VEN extended release beads were evaluated using the concept of dissolution efficiency (DE). The DE was proposed by Khan in 1975 (29) and is calculated from area under curve (AUC). The calculations were performed for each individual vessel of the dissolution profiles. The resulting DE values were submitted to statistical analysis using one-way analysis variance (ANOVA) in order to detect the existence of significant differences between the respective profiles. Tukey-Kramer HSD (Honestly Significant Difference) test was used for evaluation of quantitative effects of the differences using 5% significance level.

The release kinetics of VEN was assessed by first order, zero order, and Higuchi equations (30,31). The mathematical evaluation was performed considering the points up to 12 h of drug release because those precede the beginning of the profile plateau.

Results and Discussion

A reversed-phase HPLC method was proposed as a suitable method for determining VEN in drug dissolution studies and pharmaceutical dosage form. To obtain the best chromatographic conditions, the wavelength for detection, the column, and the mobile phase composition were adequately selected. The use of ammonium acetate 32 mM (pH 6.8) in combination with acetonitrile and methanol (62:30:8, v/v/v) at 35ºC resulted in a better peak symmetry (1.3) and in a relatively short retention time (6.8 min), which allowed a rapid determination of the drug. This is important for routine analysis. The optimized conditions of the method were validated and a typical chromatogram is shown in Figure 1A.

The specificity of the analytical method for VEN is indicated in Figure 1, where the acidic condition generated one additional peak. Under basic, oxidative, and photolytic conditions, there was neither change in the area nor additional peak detected. In a previously published paper, Makhija and Vavia (17) obtained similar results under acid, basic, and oxidative conditions using different chromatographic conditions. However, the photo-stability of the drug was not evaluated by these authors. According to them, the acid-degraded product would result by the *o*-demethylation of the 4-methoxy group attached to the phenyl ring. The studies with the PDA detector has shown that the VEN peak was free from any coeluting peak with values of peak purity index > 0.9999, thus demonstrating that the proposed method is specific.

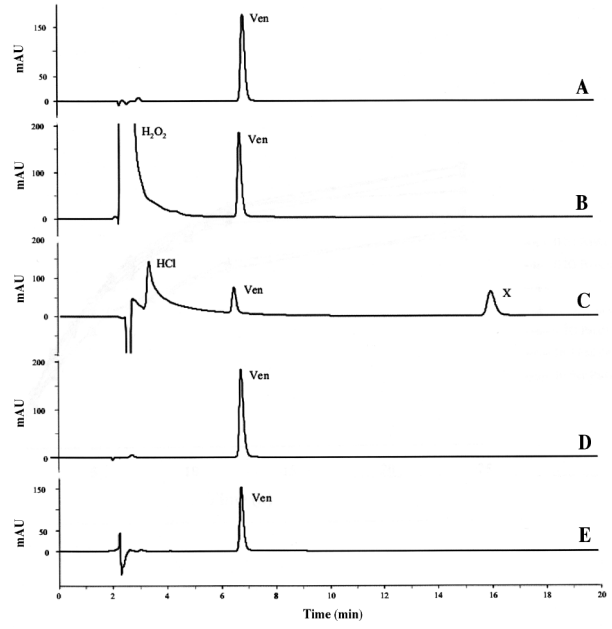

Figure 1. HPLC chromatograms of VEN (A) extended-release capsules (40 µg/mL), after oxidative condition, (B); after acidic condition, (C); after photolytic condition, (D); after basic condition, (E).

The calibration curves constructed for VEN were found to be linear in the 10–70 µg/mL range. The values of the correlation coefficients and calibration equations calculated were: r = 0.9999, $y = 44450.4 \pm 120.1x + 2826.6 \pm 3515.8$; where x is concentration and y is the peak absolute area. This r-value indicated the linearity of the calibration curves for the method. Furthermore, ANOVA demonstrated significant linear regression and non-significant linearity deviation ($P < 0.01$).

The precision evaluated as the repeatability of the method was studied by calculating the RSD for six determinations of VEN in a 40 µg/mL solution performed on the same day and under the same experimental conditions. The RSD value obtained was 0.2%.

The intermediate precision was assessed by analyzing one sample of the pharmaceutical formulation on three different days (inter-days); the RSD value obtained was 0.9%. Between-analysts precision was determined by calculating the RSD for the analysis of one samples of the pharmaceutical formulation by three analysts; the value was found to be 0.5% (Table I).

The accuracy was assessed from three replicate determinations of three different added standard solutions containing 12, 20, and 28 µg/mL of VEN. The absolute means obtained were 98.8, 100.6, and 101.2% with a mean value of 100.2% and RSD of 1.2% (Table II). These results show that the method was accurate within the desired range.

For calculating the LOD and LOQ, the calibration equation for VEN was generated by using the mean values of the three independent calibration graphs. The mean of the slope and the SD of the intercept of the independent curves were 444,450.4, and 3515.8, respectively. The values calculated for the LOD and LOQ were 0.24 and 0.80 µg/mL, respectively. These values were also confirmed experimentally.

Table I. Inter-day and Between-analysts Precision Data of the Method

Inter-day			Between-analysts		
Day	Recovery* (%)	RSD† (%)	Analysts	Recovery* (%)	RSD† (%)
1	99.0		A	100.4	0.5
2	100.0	0.9	B	100.7	
3	100.7		C	99.4	

* Mean of three replicates
† RSD = Relative standard deviation

Table II. Accuracy Data of the Method

Added Concentration (µg/mL)	Mean concentration found* (µg/mL)	RSD† (%)	Accuracy (%)
12	11.7	0.1	98.8
20	20.0	0.2	100.6
28	28.2	0.4	101.2

* Mean of three replicates
† RSD = Relative standard deviation

For robustness testing, the ½ fractionated factorial 2^4 design was applied because it considers possible combinations between the factors at low (–) and high (+) levels with a low number of experiments. The experimental ranges of the selected variables evaluated are given in Table III, and the results demonstrated that the method was robust. Moreover, the analysis of variance ANOVA was performed, and the model terms (variables) were not significant ($P < 0.05$). The stability of the analytical solution was also analyzed, and it was found to be stable up to 72 h (100.2%, assay).

The system suitability test was also performed to evaluate the resolution and reproducibility of the system for the analysis to be performed using six replicate injections of a standard solution containing 40 µg/mL of VEN. The RSD values calculated for peak area and retention time were 0.2 and 0.5%, respectively. The mean asymmetry and theoretical plates ± RSD were 1.3 ± 0.8% and 9814 ± 0.6%, respectively. The results showed that the parameters are within the suitable range.

The LC method validated in this paper was applied for the determination of VEN in extended release capsules without prior separation of the excipients of the formulation. The results are shown in Table IV.

In vitro dissolution studies is considered a fundamental requirement in the pharmaceutical industry in order to ensure the quality of solid pharmaceutical forms, guarantee the quality from lot to lot, orientate the development of new formulations, and secure the uniformity quality and performance of the drug even after modifications (23,32).

The proposed HPLC method was successfully applied for determining VEN in dissolution studies. The selection of medium composition testing was related to physiological conditions. The media used were 0.1 M hydrochloric acid (pH 1.2), water (pH 5.0), and phosphate buffer (pH 6.8). Several types of dissolution apparatuses are described in USP 32 (22). Apparatus 1 and 2 are the most widely used for oral solid dosage forms. The basket (apparatus 1) is routinely used for capsules at dissolution rates of 75 and 100 rpm, whereas the paddle (apparatus 2) is mainly used for tablets at 50 and 75 rpm (21). In case of extended-release dosage forms, Jorgensen and Bhagwat suggested to use both of these apparatuses with water as the dissolution medium (33).

In order to investigate the release behavior of VEN, different conditions were tested, and the obtained profiles are presented in Figure 2.

Evaluating the dissolved percentage curves versus time, it can be observed that VEN presented quite a difference in the dissolution profiles. The results clearly indicate that in hydrochloric acid medium the drug content release was lower than in water and the buffer medium. The significant index of drug dissolution performance was verified using the concept of DE. The calculated DE are shown in Table V. The values were submitted to statistical analysis using ANOVA to detect the existence of significant differ-

Table III. Chromatographic Conditions and Range Investigated During Robustness Testing

	Factors				Responses*	
Experimental	Mobile phase pH	Temperature (ºC)	Buffer %	Flow rate (mL/min)	Assay (%)	RSD† (%)
1	7.1	40.0	60.0	0.9	100.0	0.6
2	6.5	30.0	60.0	0.9	99.9	0.4
3	7.1	30.0	64.0	0.9	100.1	0.2
4	6.5	40.0	60.0	1.1	100.1	0.6
5	6.5	30.0	64.0	1.1	99.9	0.2
6	7.1	30.0	60.0	1.1	100.0	0.3
7	7.1	40.0	64.0	1.1	100.2	0.1
8	6.5	40.0	64.0	0.9	99.9	0.5

* Mean of three replicates
† RSD = Relative standard deviation

Table IV. Determination of VEN in Pharmaceutical Dosage Forms

	Theoretical amount	Experimental amount*		
Sample	mg per capsule	mg	%	RSD† (%)
1	75	76.1	101.5	0.4
2	75	77.1	102.8	0.4
3	75	74.4	99.2	0.2
4	150	151.5	101.0	0.2
5	150	149.8	99.9	0.6
6	150	151.9	101.3	0.1

* Mean of three replicates
† RSD = Relative standard deviation

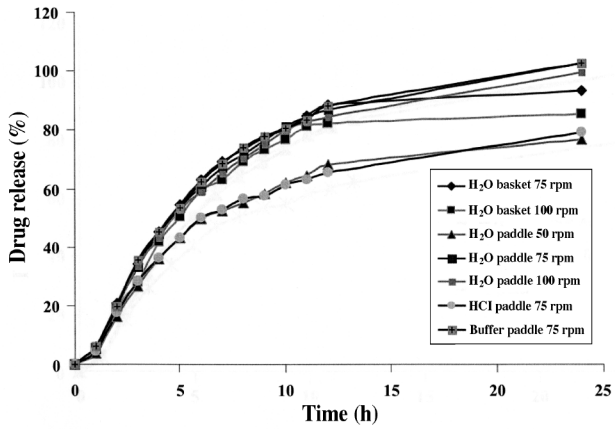

Figure 2. Release behavior of VEN in different conditions.

Table V. Dissolution Efficiency Values Obtained from Dissolution Tests Assessed with Different Dissolution Methods

Dissolution methods (medium and rotation)	ED (%) ± RSD	
	Basket	Paddle
H_2O – 50 rpm	–	57.0 ± 5.1
H_2O – 75 rpm	72.5 ± 0.9	73.9 ± 1.6
H_2O – 100 rpm	67.2 ± 1.9	72.0 ± 1.9
HCl – 75 rpm	–	57.1 ± 1.6
Buffer – 75 rpm	–	74.7 ± 2.0

ences. The ANOVA showed that all DE values were significantly different ($F_{calculated} = 82.22 > F_{critic} = 2.84; p < 0.05$).

However, it did not indicate which differences should be considered. In order to verify the significance of the differences, a pair wise comparison by Tukey-Kramer test using 5% significance level was performed. The results showed that the DE obtained in water-75 rpm-basket, water-75 rpm-paddle; water-100 rpm-paddle, and buffer-75 rpm-paddle do not have a significant difference ($p < 0.05$). The same was observed for water-50 rpm-paddle and HCl-75 rpm-paddle ($p < 0.05$). On the other hand, the most difference was observed in water-100rpm-basket; the results showed significant difference ($p > 0.05$).

Through the DE values, the best conditions to evaluate the mechanism of release were determined. In Figure 3, the superposition of the VEN drug release in different medium using 75 rpm dissolution rate and apparatus paddle is demonstrated.

The kinetics release mechanism of VEN extended-release beads was evaluated by fitting different models, such as the zero order rate equation ($Q_t = Q_0 - K_0t$) which describes the systems where the release rate is independent of the concentration of the dissolved species. The first-order equation ($ln\ Q_t = ln\ Q_0 - k_1t$), describes the release from systems where dissolution rate is dependent on the concentration of the dissolving species. The Higuchi square root equation ($Q_t = K_Ht^{1/2}$) describes the release from systems where the solid drug is dispersed in an insoluble matrix and the rate of drug release is related to the rate of drug diffusion. The mathematical evaluation was performed considering the points up to 12 h of drug release because those precede the beginning of the profile plateau.

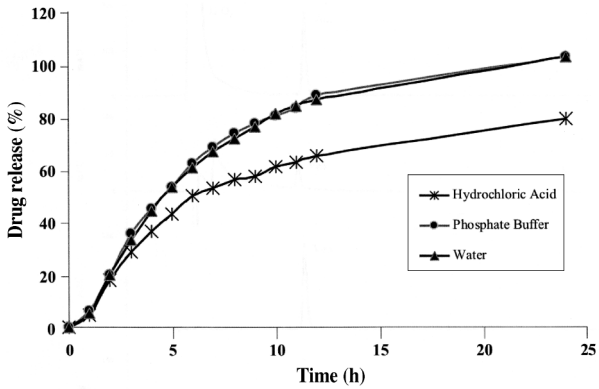

Figure 3. Release of VEN in different media using 75 rpm dissolution rate and apparatus II (paddle).

Table VI. Results of Fitting to Different Kinetic Release Equations

Medium	Zero Order	First Order	Higuchi
Hydrochloric acid	0.9569	0.9860	0.9806
Water	0.9729	0.9985	0.9807
Phosphate buffer	0.9598	0.9818	0.9807

Table VII. Kinetic Constants Calculated from First Order Kinetic Release: (k_1), ($t_{1/2}$), (Q_6), and (Q_{12})

Medium	K_1 (mg/h)	$t_{1/2}$ (h)	Q_{6h} (%)	Q_{12h} (%)
Hydrochloric acid	0.0912	7.1	44.7	68.0
Water	0.1761	4.4	62.0	86.8
Phosphate buffer	0.1735	4.6	34.5	86.3

The results of kinetics fitting are demonstrated in Table VI. It is evident that a linear relationship model, which best characterized these release profiles (*r*-value) is an apparent first order process. In this way, the first order kinetic release indicates that the amount of drug released is dependent on the matrix drug load (extended-release forms). In the pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in spherical beads, the release of the drug is proportional to the amount of drug remaining in its interior in such a way that the amount of drug released by unit of time diminishes (30).

The K_1 (kinetic constants), $t_{50\%}$ (first-order constant) of VEN dissolved at 6 h (Q_6) and 12 h (Q_{12}) were calculated and are demonstrated in Table VII.

According to the results, it is evident that the dissolution profile of VEN in acid medium exhibited lower dissolution constant rates than buffer and water medium. Using the HPLC developed method, the in vitro dissolution studies were successful performed.

Conclusion

The results of the validation studies show that the LC method is specific and accurate, and possesses significant linearity and precision characteristics without any interference from the excipients. The proposed method was successfully applied for the quantitation of VEN in extended-release pharmaceutical dosage forms and for dissolution kinetic studies. The new method contributes to improve quality control, ensuring the therapeutic efficacy of this medicine.

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