*REDVET - Revista electrónica de Veterinaria* - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: 3 July 2024 Revised: 22 August 2024 Accepted: 15 September 2024

# A Novel and Sensitive Spectrophotometric Approach for Quantifying Vardenafil Hydrochloride Trihydrate in Tablets: Application to Dissolution Testing

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**Abstract:**
**Aims:** To develop and validate a novel Zero-order Absorbance UV-Spectrophotometric method for the quantification of Vardenafil Hydrochloride Trihydrate in commercial tablets and its application for dissolution testing.
**Background:** Erectile Dysfunction (ED) is a significant disorder affecting men's sexual health, with Vardenafil being a potent and selective inhibitor of phosphodiesterase type 5 (PDE5). Vardenafil is commonly used as an oral therapy for treating ED. Accurate and reliable methods for its quantification in pharmaceutical formulations and during dissolution tests are essential for quality control.
**Objective:** To establish and validate a simple, accurate, precise, economical, and sensitive method for determining the concentration of Vardenafil hydrochloride trihydrate in commercial tablets and during dissolution tests using UV-Spectrophotometry.
**Method:** A Zero-order Absorbance UV-Spectrophotometric approach was employed, utilizing 0.1N HCl as the solvent. The method was optimized to determine Vardenafil hydrochloride trihydrate within a linear range of 2-12 µg/mL, with a coefficient of correlation value greater than 0.99. The method was validated for accuracy, precision, sensitivity, and ruggedness according to ICH guidelines. Additionally, the method was applied for the quantification of Vardenafil hydrochloride trihydrate in dissolution tests.
**Result:** The method successfully determined Vardenafil hydrochloride trihydrate concentration in commercial tablets, showing a % recovery between 99.85% and 100.07%. The % amount of drug estimated was in good agreement with the label claims. The method also allowed direct measurement of samples from the dissolution vessel without pH correction.
**Conclusion:** The developed UV-Spectrophotometric method is a reliable, accurate, and cost-effective approach for quantifying Vardenafil hydrochloride trihydrate in commercial tablets and during dissolution tests. The method's validation as per ICH guidelines ensures its suitability for routine quality control.
**Other:** This analytical approach provides the simple and economical technique for the analysis of Vardenafil hydrochloride in pharmaceutical formulation.

**Keywords:** Vardenafil hydrochloride trihydrate; phosphodiesterase 5 inhibitor; UV-Spectrophotometry; Dissolution etc.

## Introduction

Erectile dysfunction (ED) is the most frequent sexual condition. The inability to produce and maintain an adequate erection for a satisfactory sexual act is classified as ED[1]. ED is a serious medical disorder that can reduce one's quality of life and cause anxiety, low self-esteem, and dissatisfaction. The mental stress brought on by ED can have a range of effects on how patients interact with others. Diabetes, hypertension, cardiovascular disease, hyperlipidemia, injuries, obesity, anxiety, aging, stress, smoking, drug abuse, and alcohol abuse are all significant causes of ED. Phosphodiesterase type 5 (PDE-5) inhibitors are largely regarded as the therapy of choice for those with ED who do not have any contraindications to using them[2-3]. Because PDE-5 is responsible for the breakdown of cGMP in the penis, blocking the enzyme causes vasodilation and the maintenance of penile blood flow. Due to side effects such headaches, face flushing, nasal congestion, back discomfort, and visual disturbances, as well as interactions with nitrates or α-blockers that may cause hypotension or syncope.[5-8]

Vardenafil hydrochloride trihydrate (VAR) (Fig. 1) is a benzenesulfonamide derivative and selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). It is used an oral therapy for the treatment of ED. It has shown some efficacy in the treatment of pulmonary arterial hypertension (PAH). Vardenafil hydrochloride

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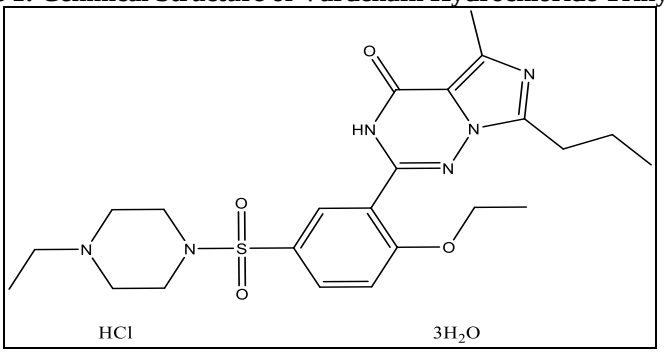
trihydrate is chemically, 2-[2-ethoxy-5-(4-ethylpiperazin-1-yl)sulfonylphenyl]-5-methyl-7-propyl-3H-imidazo[5,1-f][1,2,4]triazin-4-one;trihydrate; hydrochloride[9].

**Figure 1: Cehmical Structure of Vardenafil Hydrochloride Trihydrate**




Several analytical techniques for the determination of VAR have been published in the literature, including HPLC[10-16], UPLC[17,18], HPLC/MS[19-22], LC-ESI-MS[23, 24], LC-ESI-MS/MS[25-27], LC–UV–ESI-MS[28], Imunoassay[29], and UPLC-MS/MS[30]. Furthermore, HPTLC[31-33], UV spectrophotometric[34-39], Capillary Chromatography[40], Capillary Electrophoresis[41], GC-MS[42], and Human Plasma Determining LC technique[43], stability-indicating LC method[44] were previously conducted. The present work undertakes a UV-Spectrophotometry approach and its application for dissolution study, which has before been unexplored. Furthermore, validation was achieved by assessing the analytical characteristics of linearity, accuracy, precision, assay, and robustness according to the ICH procedures[45].

## Materials And Methods

### Instrumentation

All absorption spectra were obtained using a Shimadzu UV–Vis double beam spectrophotometer, Model UV-2450, with a 10 mm matched quartz cell for absorbance measurements. In the wavelength range of 200–800 nm, this spectrophotometer has a wavelength accuracy of 0.2 nm, a scanning speed of 200 nm/min, and a bandwidth of 2.0 nm. On an analytical balance, all the weights were taken (Shimadzu AUX 120). The dissolution tests were carried out using an Electro Lab Dissolution Tester TDL-08L dissolution test system USP Type II with one vessel with a capacity of 1 L and a rotating paddle device with temperature control.

### Reagents and Standards

Reference standard of pharmaceutical grade Vardenafil Hydrochloride Trihydrate (99.5%) was received as a gift sample from Macleods Pharmaceuticals Pvt Ltd, Mumbai. The tablets Varimax10® were obtained from a local pharmacy.

### Experimental

### Preparation of standard stock solution

Accurately weighed quantity of 10 mg of Vardenafil HCl Trihydrate transferred to 100 ml volumetric flasks. It was dissolved in 0.1N HCl solution and was shaken manually for 10 min. To get a final strength of 100 µg/ml, the volume was filled to the mark using the same solvent.

### Selection of wavelength for analysis

An appropriate volume of 1 ml of standard stock solution of Vardenafil HCl Trihydrate was transferred into a 10 ml volumetric flask, diluted to mark with 0.1N HCl solution to give a concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200 nm – 400 nm). In spectrum, Vardenafil HCl Trihydrate indicated maximum absorbance at 226 nm.

### Linearity Studies

Into a series of 10.0 mL volumetric flasks, different aliquots of a VAR stock standard solution, equivalent to 0.2–1.2 mL, were transferred to separate flasks. The volume in each flask was adjusted to the mark with 0.1N HCl, resulting in concentrations ranging from 2–12 µg/mL. The solutions were scanned in the UV region from 200–400 nm. Absorbances of these solutions were recorded at 226.00 nm, and a calibration curve of absorbance versus concentration was plotted.

### Validation of the method

The method was validated in terms of accuracy, precision, ruggedness, and sensitivity in accordance with ICH Q2 (R1) criteria.

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**Accuracy**
To assess the accuracy of the proposed method, recovery studies were carried out at three different levels i.e., 80%, 100%, and 120%. To the pre-analyzed sample solution, a known amount of standard drug solution was added at three different levels, and absorbance was recorded.

**Precision**
The precision of the method was assessed through investigation of intra-day precision, inter-day precision, and repeatability. Intra-day precision was determined by analyzing VAR solutions at concentrations of 4, 6, and 8 µg/mL at different times on the same day. Inter-day precision was confirmed by assessing the determinations of VAR concentrations at 6, 9, and 12 µg/mL over three consecutive days. To determine the degree of repeatability of the developed method, statistical evaluation was carried out. Repeatability was determined by analyzing VAR solution of 6 µg/mL for six times.

**Sensitivity**
Sensitivity of the method was investigated using the standard deviation of the response and the slope. Detection Limit (DL) and Quantitation Limit (QL) were calculated using the formulae of DL = 3.3 × SD/S and QL = 10 × SD/S. The six estimations of calibration plots were selected to determined lower detection and Quantitation limits of VAR.

**Ruggedness**
The ruggedness of the proposed method was determined by analysis of aliquots from the homogenous slot by two analysts using the same operational and environmental conditions.

**Assay of Vardenafil Tablets**
The average weight was determined by weighing twenty tablets containing 10 mg of Vardenafil Trihydrate HCl. In a 100 ml volumetric flask containing 50 ml 0.1 N HCl solution, tablet powder corresponding to 10 mg of Vardenafil Trihydrate HCl was transferred and the volume was built up to volume. After that, the mixture was sonicated for 15 minutes. The solution was filtered using Whatman filter paper no.41. From the filtrate, further dilution was made to bring a final concentration of 6 µg/ml with same solvent and used for the analysis. The absorbance was measured at 226.00 nm against the blank.

**In-vitro Dissolution Study**
A dissolution testing apparatus (ELECTROLAB DISSOLUTION TESTER TDL-08L) was used to determine the in-vitro release rate of VAR from VAR tablets. The dissolution test was performed using 900 mL of 0.1 N HCl at 37 ± 0.50°C and 100 rpm according to the Indian Pharmacopeia (IP) Dissolution testing apparatus I (paddle) method. The tablet was placed inside the dissolution vessel. 5 ml of sample were withdrawn at time intervals of 10, 20, 30, 40, 50, and 60 minutes. The withdrawn volume was replaced with 5 ml of fresh dissolution medium. The solutions' absorbance was then measured using a UV-Visible double beam spectrophotometer (SHIMADZU), model UV-2450 at 226 nm. Cumulative percentage drug release was calculated based on the standard curve.
To determine the mechanism of drug release from the tablet, the findings of the in-vitro dissolution study were fitted into the following kinetic equations:
a. Zero-order drug release: Cumulative % drug release Vs Time.
b. First-order drug release: Log cumulative % drug retained Vs Time.
c. Higuchi's classical diffusion equation: Cumulative % drug release Vs Square root of time.
d. Hixson-Crowell cube root law: Cube root of cumulative % drug retained Vs time
e. Peppas Korsemeyer Model: Cumulative % drug release Vs Log time.

**RESULTS AND DISCUSSION**
Vardenafil hydrochloride trihydrate is used an oral therapy for the treatment of ED. It shows its effect by selectively inhibiting cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). In pharmaceutical quality control, the most challenging task is creating a consistent and reproducible spectrophotometric method for determining the concentration of the target analyte in pharmaceutical media. The goal of the research reported here was to establish a simple, quick, and unique spectrophotometric method for determining VAR in tablets and extended its application for conducting dissolution studies. The conventional approaches have used more comprehensive ranges of the calibration curve and wavelength of analysis; the developed novel zero order absorbance approach was found to be more sensitive and precise to estimate the VAR in the range of 2 – 12 µg/mL. Moreover, the proposed work explored 0.1 N HCl as a substitute to organic solvents, which is more economical than methanol and ethanol, thus considered to ecofriendly solvent. Wherefore, the developed spectrophotometric approach has become more environmentally sustainable than the previously documented reports. The applicability and reproducibility of proposed approach for routine quality control analysis of VAR was addressed by determining the stability of VAR in a 0.1N HCl solvent. The same was verified with keeping the 6 µg/mL working solution of VAR for 72 hours; neither formation of precipitate nor change in color of 6

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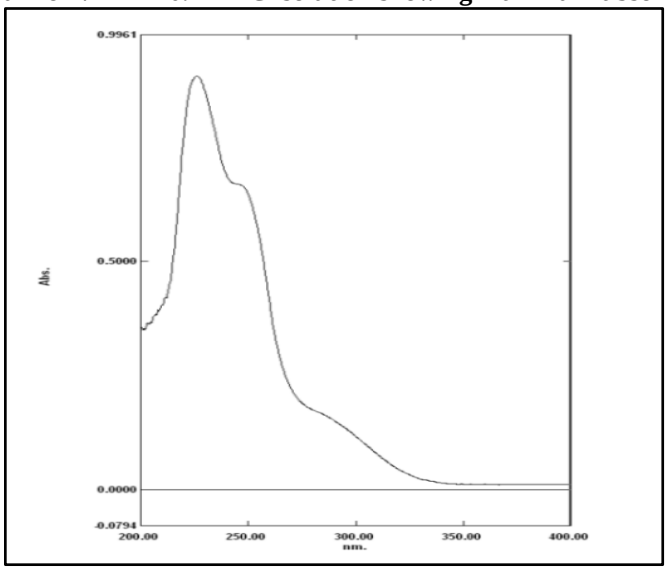
µg/mL solution of VAR was noticed; further, there was no significant decrease of absorbance recorded at specified wavelength. The zero-order spectrum of VAR was recorded as shown in Figure 2, demonstrated maximum absorbance at 226.00 nm.

**Fig 2: UV Spectrum of VAR in 0.1N HCl solution showing maximum absorbance at 226 nm.**

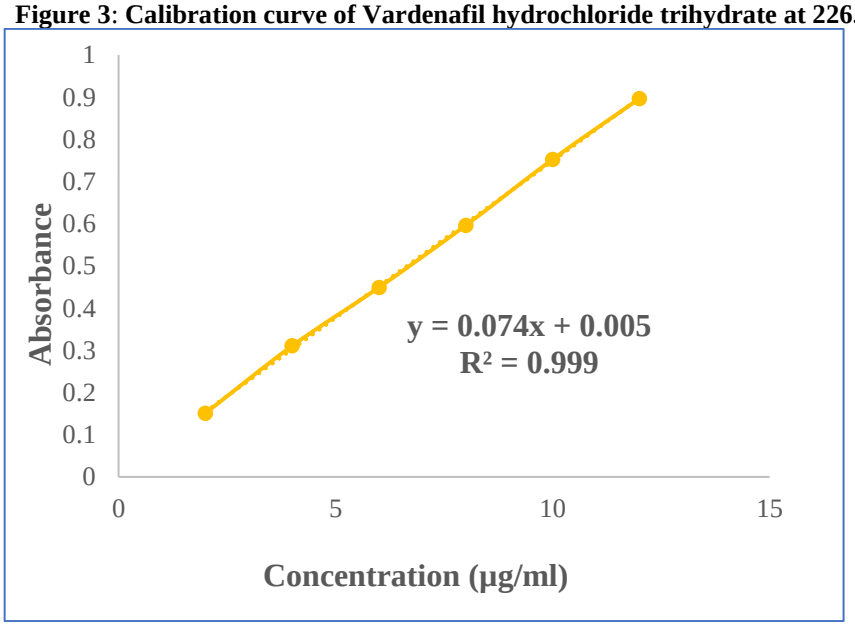



The developed approach showed a good linear correlation between VAR concentration and response over the range of 2-12 µg/ml, with a coefficient of correlation greater than 0.99 as depicted in Figure 3. The analytical findings of the calibration plots, including correlation coefficient, slope, intercept, DL and QL are summarized in Table 1.

**Table 1. Optical and regression parameters for zero order UV-Spectrophotometric estimation of VAR**

| Sr. No. | Parameters | Zero-Order Method |
|---|---|---|
| 1. | Wavelength for maximum absorbance (λ max) | 226 nm |
| 2. | Beer's Law Limit (µg/mL) | 2 -12 |
| 3. | Slope (M) | 0.074 |
| 4. | Intercept (C) | 0.005 |
| 5. | correlation coefficient ($r^2$) | 0.999 |
| 6. | DL (µg/mL) | 0.10 |
| 7. | QL (µg/mL) | 0.33 |

**Figure 3**: **Calibration curve of Vardenafil hydrochloride trihydrate at 226.00 nm**



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The findings obtained in Table 2 demonstrated exceptional accuracy of approach that was evaluated by carrying out the standard addition technique on Varimax10® tablets at 80, 100, and 120 % levels, presented a good recovery rate at the levels studied, and it was found that % RSD is less than 2 for optimized approach that meet the requirements for acceptance of an accuracy study.

**Table 2: Results of accuracy studies**

| Drug | Initial Amount [µg/ml] | Amount added [µg/ml] | Amount recovered [µg/ml, n = 3] | % Recovery | % RSD |
|---|---|---|---|---|---|
| VAR | 4 | 3.2 | 7.20 | 100.07 | 0.24 |
| | 4 | 4.0 | 7.99 | 99.85 | 0.83 |
| | 4 | 4.8 | 8.79 | 99.93 | 0.45 |

**n = number of determinations**

Precision was assessed as intra-day precision, inter-day precision, and repeatability assay. It was examined using concentrations of 4, 6, and 8 µg/ml with a repeatability assay at 6 µg/mL. The % RSD was found to be less than 2%, representing that the proposed approach is precise and reproducible for the analysis of VAR. The results are shown in Table 3.

**Table 3: Results for precision (Intra-day, Inter-day, and repeatability ) studies**

| Conc. (µg/ml) | Intra-day | | Inter-day | |
|---|---|---|---|---|
| | % Amount found | % RSD [n = 3] | % Amount found | % RSD [n = 3] |
| 4 | 99.26 | 0.11 | 100.07 | 0.12 |
| 6 | 99.71 | 0.59 | 99.78 | 0.34 |
| 8 | 100.94 | 0.06 | 100.88 | 0.08 |

| Repeatability studies | | |
|---|---|---|
| Conc. (µg/ml) | % Amount found | % RSD [n = 6] |
| 6 | 99.30% | 0.76% |

**n = number of determinations**

To establish the ruggedness of the proposed approach, the VAR solution (6 µg/ml) was analyzed by two different analysts, with each analyst performing six repetitions. The results, expressed in terms of %RSD, were found to be less than 2%, confirming that the approach is validated for ruggedness. The results are shown in Table 4.

**Table 4: Results for ruggedness studies**

| Drug | Analyst I | | Analyst II | |
|---|---|---|---|---|
| | Amount found % ± SD | % RSD [n = 6] | Amount found % ± SD | % RSD [n = 6] |
| VAR | 99.97 ± 0.15 | 0.16 | 99.94 ± 0.23 | 0.24 |

**n = number of determinations**

VAR tablets, Varimax10® with Label claim 10 mg/tablet, when analysed with the proposed method, proved to be excellent. The percent amount of VAR in the tablet matrix was found to be 99.56 using proposed approach. As a result, no components of the matrix were found to interfere with VAR. Thus, the proposed approach can be used to study VAR in a pharmaceutical quality control of VAR in pharmaceutical formulations. The selectivity and specificity were also verified with RSD values of 0.46 for six replicates, indicating that no interferences of the excipients were observed under these conditions with the determination of VAR in Varimax10®.

The commercial brand Varimax10® was subjected to dissolution test and concentration of VAR was calculated by the optimized UV-Spectrophotometry approach. The dissolution behavior of VAR tablets was determined by finding the $R^2$ value for each release kinetic model following the Zero order, First order, Hixson-Crowell, Peppas Korsemeyer, and Higuchi models. The highest correlation coefficient ($R^2$) value, which was derived according to its respective kinetic model, indicated its pattern of release. Regression coefficient ($R^2$) values for different kinetic models and Dissolution Profile of VRA tablets depicted in Table 5 and Figure 4 respectively. The dissolution study for VAR tablets presented here shows $R^2$ value of first order model is very near to 1 than the $R^2$ values of other kinetic models. Thus, the drug release is considered to follow first-order kinetics.

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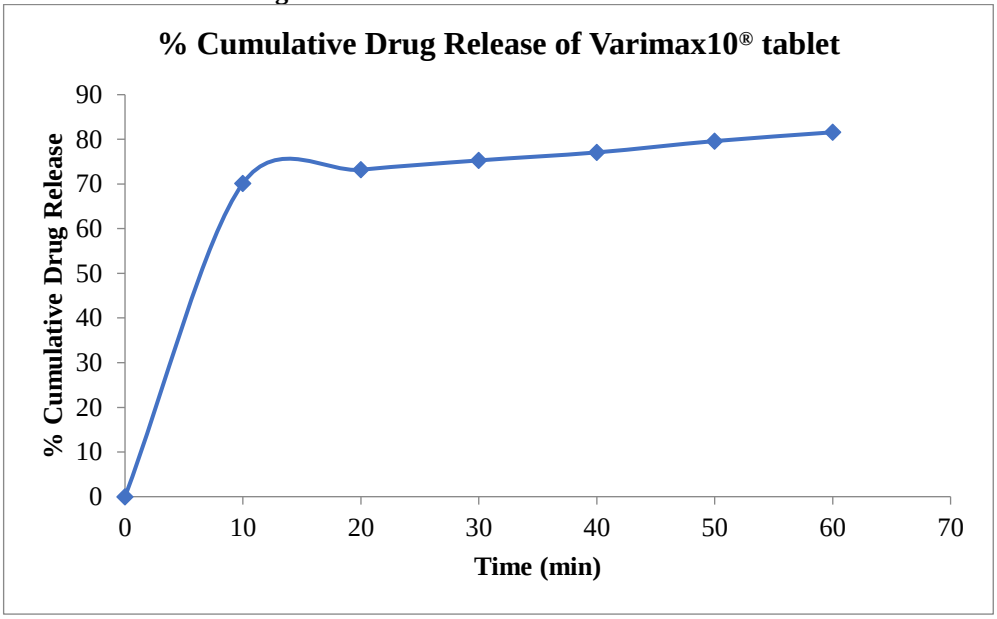
**Table 5: Regression coefficient (R2) values for different kinetic models**

| Kinetic Model | Regression coefficient ($R^2$) |
|---|---|
| Zero-order drug release | 0.482 |
| First-order order drug release | 0.995 |
| Higuchi's classical diffusion equation | 0.776 |
| Hixson-Crowell cube root law | 0.966 |
| Korsmeyer-Peppas model | 0.966 |

**Figure 4**: **Dissolution Profile of VAR tablets**




**Conclusion:**

The proposed UV spectrophotometry method explores the development and validation of new, rapid and eco-friendly approach using the zero-order absorbance technique for a specific estimation of Vardenafil hydrochloride trihydrate in bulk, pharmaceutical formulation (tablets) and extended to the dissolution medium. Hydrochloric acid (0.1 N) solution as an ecological solvent agent was explored to develop approach for usage as a potential alternative candidate for the common polar organic solvent used in spectrophotometric methods. The established approaches have many benefits over the addressed analytical reports of being green, economical, rapid, and sensitive; moreover, this method does not require any tedious, sophisticated, and expensive techniques like HPLC and LC-MS/MS. The findings and statistical limits revealed that the proposed approaches are simple, accurate, precise, and specific. Therefore, it can be considered a viable solution for quality control laboratories to monitor VAR in pharmaceutical specimens and dissolution tests.

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