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Spectrophotometric Analysis of Vardenafil in Tablet Dosage Forms by Using Electrophilic Coupling Reagents

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Two new spectrophotometric methods (M1 and M2) are developed for the determination of vardenafil in bulk and in tablet dosage forms. The method M1 involves oxidative coupling of vardenafil with 3-methyl-2-benzothiazolinone hydrazone hydrochloride in presence of ferric chloride in acidic medium yielding green colored chromogen with absorption maxima at 625 nm. The method M2 is based on the oxidation of 4-aminoantipyrine by potassium periodate, which subsequently couples with vardenafil in an alkaline medium to form a red colored product having absorption maxima at 530 nm. The absorbance concentration graphs were rectilinear over the range of 4-40 μg ml^{-1} for the method M1 and 4-60 μg ml^{-1} for the method M2. The limit of detection values was found to be 0.044 μg ml^{-1} and 0.035 μg ml^{-1} for the methods M1 and M2, respectively. The analytical performance of the developed methods was fully validated as per the guidelines prescribed by International Conference on Harmonization. The results were found to be acceptable. The application of the proposed methods in the determination of vardenafil in their commercial tablet dosage forms was successful showing good percentage recoveries.

Keywords: Vardenafil, Electrophilic coupling agent, 3-Methyl-2-benzothiazolinone hydrazone hydrochloride, Aminoantipyrine, Analysis

INTRODUCTION

Vardenafil (VDL) [1-4], chemically known as 2-[2-ethoxy-5-(4-ethylpiperazine-1-sulfonyl)phenyl]-5-methyl-7-propyl-1H,4H-imidazo[4,3-f][1,2,4]triazin-4-one (Fig. 1), belongs to a class of medications called phosphodiesterase inhibitors. Cyclic guanosine monophosphate (GMP) causes relaxation of smooth muscle and increases blood flow into the corpus cavernosum located around the penis. This results in penile erection during sexual stimulation. Degradation of cyclic GMP in the corpus cavernosum by cyclic GMP specific phosphodiesterase type 5 leads to erectile dysfunction. VDL does not heal erectile dysfunction or increase sexual desire but it works selectively by inhibiting the action of a cyclic GMP specific phosphor-

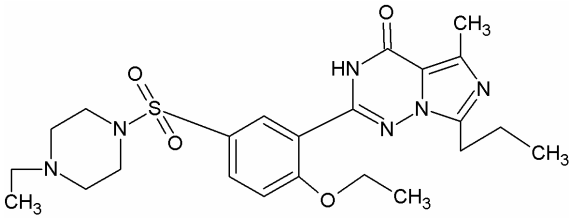

Fig. 1. Structure of verdenafil.

diesterase type 5 which helps to widen blood vessels and improves the blood flow. Following sexual stimulation, in erectile dysfunction, VDL improves the blood flow to the penis and helps to maintain an erection.

The efficacious and safe use of VDL primarily depend on the quality of its tablet preparation and determining its concentrations in tablets for the purpose of quality control. As a result, there is an increasing demand for a proper

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analytical method for determination of VDL in bulk drug and tablet dosage forms.

Literature review revealed that few analytical methods have been published for the quantification of VDL in biological fluids. These include HPLC with amperometry detection [5], HPLC with UV detection [6], HPLC with fluorescence detection [7], LC-MS [8,9], GC-MS [10,11] and square-wave adsorption anodic stripping voltammetry [12] methods. A few methods are found in the literature for the analysis of VDL in pharmaceutical dosage forms including HPLC with UV detection [13], stability indicating liquid chromatography with UV detection [14], stability indicating UPLC [15], capillary electrophoresis with diode-array detection [16] and micellar electrokinetic capillary chromatography [17] methods. LC-ESI-MS [18] and HPLC with chemiluminescence detection [19] methods have been reported in the literature for VDL analysis in dietary supplements. Though most of the reported methods are sensitive, they are cumbersome, need complex and expensive instrumentation and require expert operational personnel. On this basis, development of an alternate simpler method for the analysis of VDL seems quite necessary.

Spectrophotometry has a significant importance in drug analysis and the spectrophotometric methods are widely applied in pharmaceutical analysis. In pharmaceutical analysis field the importance of spectrophotometric method has greatly increased because of their simplicity, reproducibility, speed and less analysis time.

Recently the authors of this manuscript have proposed three spectrophotometric methods (1, 2 and 3) for the determination of VDL [20]. The methods are based on the oxidation of VDL with $KMnO_4$ in 0.6 M NaOH at room temperature (method 1), formation of yellow colored chloroform extractable ion-pair complex of VDL with bromocresol green under acidic condition (method 2) and inner molecular complex formation of the VDL with acetaldehyde and sodium nitroprusside in alkaline medium (method 3).

In continuation of our work with VDL, present study is devoted to the development of two spectrophotometric methods for the determination of VDL in bulk and in its tablet dosage forms using 3-methyl-2-menzothiazolinone hydrazone hydrochloride (MBTH) and 4-aminoantipyrine (AP) as analytical reagents.

METHODOLOGY

Apparatus

ELICO double beam model SL 159 digital ultraviolet-visible spectrophotometer with matched 1-cm quartz cells was used for all the spectrophotometric measurements. A Shimazdu (Tokyo, Japan) electronic weighing balance model BL 220H was used for weighing the samples.

Chemicals and Reagents

Analytical reagent grade chemicals and doubly distilled water were used throughout the investigation. Aqueous solutions of 0.2% MBTH (Merck, Mumbai), 0.7% $FeCl_3$ (Sdfine-Chem limited, Mumbai), and 0.5% HCl (Fisher Scientific, Mumbai) were prepared for the assay of VDL by the MBTH method. For the analysis of VDL by the AP method, aqueous solutions of 0.2% AP (Merck, Mumbai), 0.23% KIO_4 (Sdfine-Chem limited, Mumbai) and 2 N NaOH (Sdfine-Chem limited, Mumbai) were prepared.

Standard Solutions

Vardenafil standard was obtained from Rainbow Pharma Training Labs, Hyderabad and was used as received. An accurately weighed amount (100 mg) of VDL was transferred into a 100 ml calibrated flask and dissolved in 50 ml water. The solution was completed to volume to obtain a stock standard solution of 1 mg ml^{-1} with the same solvent. The stock solution was further diluted appropriately with water to obtain working standard solutions equivalent to 200 μg ml^{-1} (for the method M1) and 400 μg ml^{-1} (for the method M2) of VDL. Levitra tablets (Bayer healthcare pharmaceuticals Inc, Wayne), containing 10 mg, 20 mg and 25 mg of VDL/tablet were purchased from the local market.

GENERAL ANALYTICAL PROCEDURE

Method M1

Varying aliquots (0.2-2.0 ml) of standard VDL solution (200 μg ml^{-1}) equivalent to 4-40 μg ml^{-1} were accurately transferred into a series of calibrated flasks with capacity 10 ml. By adding water, the total volume in each flask was brought to 2 ml. Then, 1.5 ml of 0.2% MBTH solution, 1 ml

of 0.7% $FeCl_3$ and 1 ml of 0.5% HCl were transferred to each flask. The contents of the flask were mixed well and kept aside at room temperature for 10 min. The mixture in each flask was diluted to 10 ml with water and mixed well. The absorbance of the green colored complex was measured at 625 nm against the reagent blank.

Method M1

Varying aliquots (0.1-1.5 ml) of standard VDL solution (400 µg ml^{-1}) equivalent to 4-60 µg ml^{-1} were accurately transferred into a series of calibrated flasks with capacity 10 ml. By adding water, the total volume in each flask was brought to 1.5 ml. Then, 1.5 ml of 0.2% AP solution, 1 ml of 0.23% KIO_4 and 1 ml of 2 N NaOH were transferred to each flask. The contents of the flask were mixed well and kept aside at room temperature for 5 min. The mixture in each flask was diluted to 10 ml with water and mixed well. The absorbance of the reddish colored complex was measured at 530 nm against the reagent blank.

For both methods (M1 and M2), calibration curves were constructed by plotting absorbance values against the corresponding concentrations of VDL. The concentration of the unknown sample was determined from the calibration curve or computed using the regression equation.

Procedure for Tablet Formulation

Twenty tablets were exactly weighed and finely powdered. A powdered amount equivalent to 100 mg of VDL was dissolved in 50 ml of water by shaking continuously for 10 min. Whatmann no. 1 filter paper was used for filtering the solution. The filtrate was transferred into a 100 ml volumetric flask and made up to the volume with water. This solution was further diluted properly to get 200 µg ml^{-1} and 400 µg ml^{-1} concentrations for the analyses by the methods M1 and M2, respectively. The VDL content in the tablets was quantified using the corresponding regression equation or calibration curve.

RESULTS AND DISCUSSION

The electrophilic coupling reagents, 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) and 4 amino antipyrine (AP) are the most important and widely used chromogenic reagents for the spectrophotometric analysis of many pharmaceutical compounds [21-29]. The literature survey revealed that the reaction between VDL and MBTH and AP has not been investigated yet. Therefore, in the present study the use of MBTH and AP as electrophilic coupling reagent is proposed for the spectrophotometric determination of VDL by MBTH and AP methods, respectively.

The chemical reaction in the present spectrophotometric analysis of VDL, involves the oxidation of coupling reagent [MBTH by $FeCl_3$ in acidic medium-method M1; AP by KIO_4 in alkaline medium- method M2] to form an active coupling species. The active coupling species undergo electrophilic substitution with VDL to form a green colored product having maximum absorption at a wavelength of 625 nm in method M1 (Fig. 2) and a red colored product having absorption maxima at 530 nm in method M2 (Fig. 3). The proposed mechanism of the oxidative coupling reaction in methods M1 and M2 could be presented as shown in schemes 1 and 2, respectively.

Association Constant and Standard Free Energy Change of the Complexes

The association constants for VDL-MBTH and VDL-AP complexes were evaluated at 625 nm and 530 nm, respectively using the Benesi-Hildebrand equation [30]:

$$[A_o]/A_\lambda = 1/\varepsilon + (1/K_c\,\varepsilon)\,1/[D_o]$$

where $[D_o]$ = Concentration of the VDL
$[A_o]$ = Concentration of the electrophilic coupling reagent
A_λ =Absorbance of the VDL-MBTH and VDL-AP complexes at 625 nm and 530 nm, respectively
E = Molar absorptivity of the VDL-MBTH and VDL-AP complexes at 625 nm and 530 nm, respectively
K_c = Association constant of the complex.
The standard free energy ($\Delta G°$) of complexation is related to the association constant Kc according to the following equation [31]:

$$\Delta G° = -2.303RT\log Kc$$

where $\Delta G°$ = Free energy change of the complex
R = Gas constant (1.987 cal mol^{-1} $degree^{-1}$)
T = Temperature in Kelvin

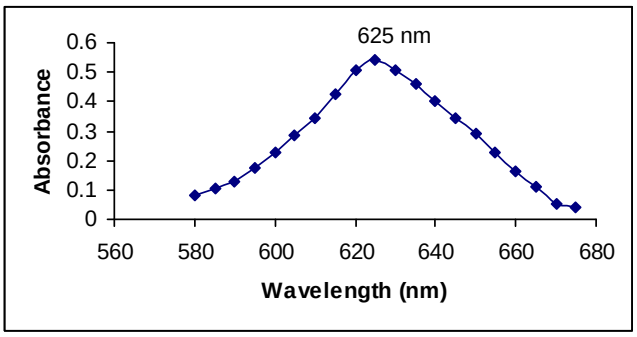


Fig. 2. Absorption spectra of VDL-MBTH complex.

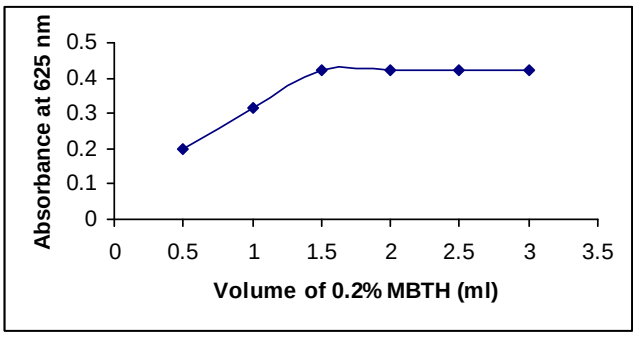


Fig. 4. Effect of MBTH concentration.

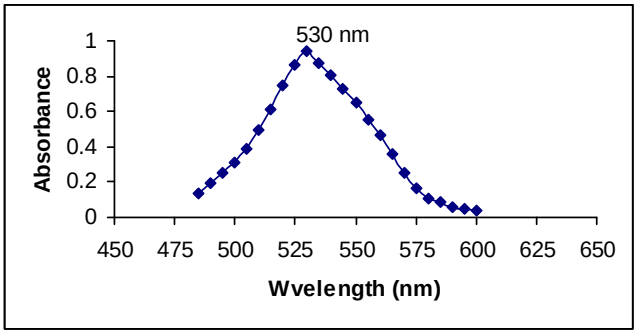


Fig. 3. Absorption spectra of VDL-AP complex.

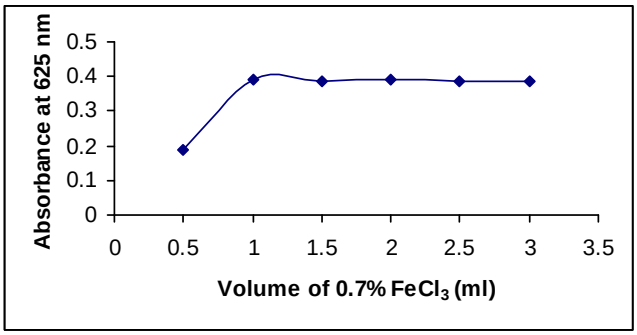


Fig. 5. Effect of $FeCl_3$ concentration.

K_c = Association constant (M^{-1}) of the drug-reagent complex.

The association constants and free energy change values were found to be 4.108×10^4 M^{-1} and -5.762×10^3 kJ mol^{-1} for VDL-MBTH complex (method M1), 8.190×10^4 M^{-1} and -6.137×10^3 kJ mol^{-1} for VDL-MBTH complex (method M2), respectively.

Optimization of Experimental Conditions

The experimental parameters (concentration of MBTH, $FeCl_3$, HCl and reaction time in method M1; concentration of AP, KIO_4, NaOH and reaction time in method M2) affecting the development of colored complexes and their stability were carefully studied and optimized.

Effect of Concentration of 3-Methyl-2-benzo-thiazolinone Hydrazone Hydrochloride (Method M1)

The effect of the MBTH concentration on the color development was studied by adding different volumes (0.5-3.0 ml) of 0.2% MBTH to 1 ml of VDL (20 µg ml^{-1}). The maximum absorbance of the color was reached at 1.5 ml of the MBTH and remained constant with higher volumes (Fig. 4) Therefore, the optimum value was chosen as 1.5 ml of 0.2% MBTH.

Effect of Concentration of $FeCl_3$ (Method M1)

The influence of the volume of 0.7% $FeCl_3$ on the formation of color was studied. This was performed by adding different volumes (0.5-3.0 ml) of 0.7% $FeCl_3$ to 1 ml of VDL (20 µg). The maximum absorbance was obtained with 1 ml of 0.7% $FeCl_3$. Above this volume, the absorbance remained constant (Fig. 5). As a result, the above said volume was used for all the measurements.

Effect of HCl (Method M1)

The influence of acidity on the reaction of MBTH with VDL in the presence of $FeCl_3$ was studied by adding different volumes (0.5-3.0 ml) of 0.5% HCl to a fixed concentration of VDL (20 µg ml^{-1}). The maximum

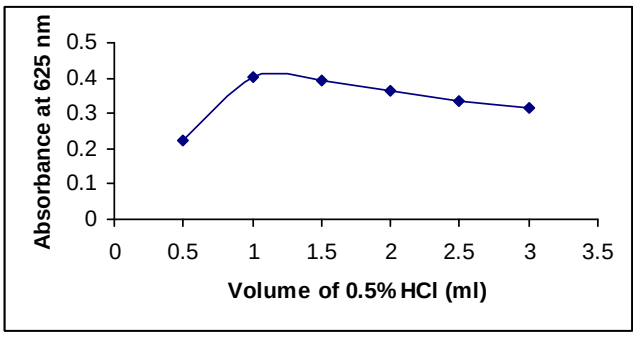


Fig. 6. Effect of acidity.

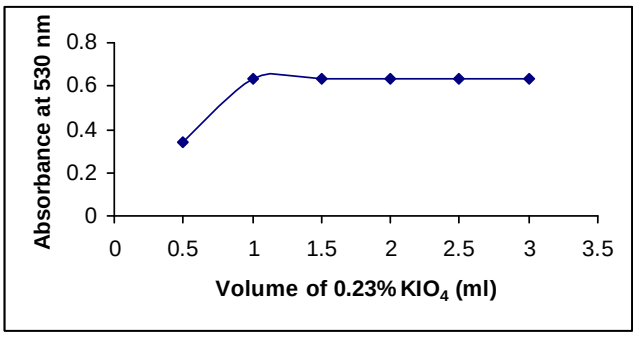


Fig. 8. Effect of KIO_4 concentration.

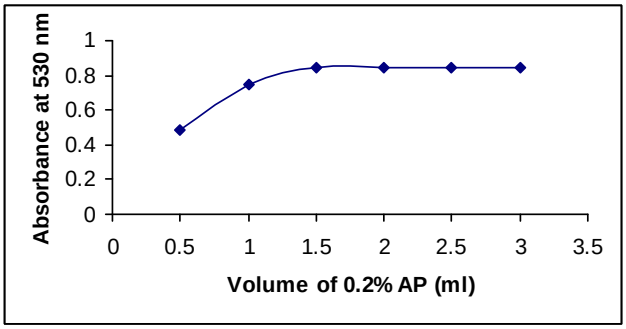


Fig. 7. Effect of AP concentration.

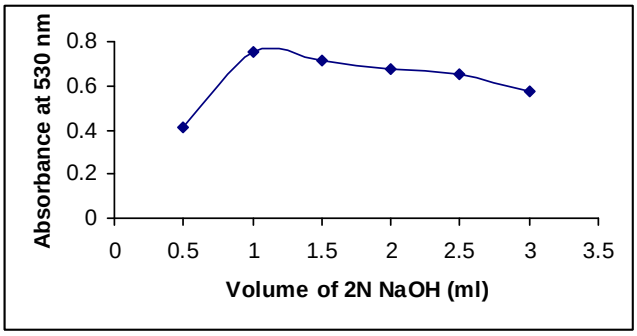


Fig. 9. Effect of alkalinity.

absorbance was found with 1 ml of 0.5% HCl, beyond which the absorbance decreased (Fig. 6). Thus, 1 ml of 0.5% HCl was chosen for the determination.

Effect of Concentration of 4-Aminoantipyrine (Method M2)

To optimize the concentration of AP, different volumes (0.5-3.0 ml) of 0.2% AP were added to the mixture under study (40 µg ml^{-1} VDL). It was found that 1.5 ml of 0.2% AP is sufficient for the formation of maximum and stable colored complex. At lower concentration of 0.2% AP, there was a decrease in the absorbance. Whereas, no change in the absorbance was observed at higher concentration (Fig. 7).

Effect of Concentration of KIO_4 (Method M2)

To study the effect of the volume of 0.23% KIO_4 on the absorbance of the colored complex, varying volumes of (0.5-3.0 ml) 0.23% KIO_4 were mixed with 1 ml of VDL (40 µg). The results showed that the highest absorbance is obtained with 1 ml, which remains unaffected by the further addition of 0.23% KIO_4 (Fig. 8). Hence, 1 ml of 0.23% KIO_4 was used for the determination.

Effect of NaOH (Method M2)

The influence of alkalinity on the development and stability of the color in method M2 using different volumes (0.5-3.0 ml) of 2 N NaOH were tested in the current study. The highest color intensity was observed with 1 ml of 2 N NaOH (Fig. 9) and therefore 1 ml of 2 N NaOH was used throughout the experiment.

Effect of Reaction Time and Stability of Colored Complex (Methods M1 and M2)

The optimum reaction time for the development of colored complexes at room temperature was studied. It was found that 10 min and 5 min of standing time is required for the formation of complete color in methods M1 and M2,

respectively. The colored complex formed was stable for 6 h in method M1 whereas it was 30 min in method M2.

Method Validation

According to International Conference on Harmonization (ICH) guidelines, validation of the methods was performed [32].

Linearity

The linearity of the proposed methods was determined by constructing a calibration curve with seven calibration points for VDL, in the concentration range of 4-40 μg ml^{-1} for method M1 and 4-60 μg ml^{-1} for method M2. The absorbance values were plotted against the respective concentrations of VDL to obtain the calibration curves. The results were subjected to the regression analysis by the least squares method to calculate the intercept, slope and regression coefficient. The results are presented in Table 1.

Sensitivity

Sensitivity parameters such as Sandell's sensitivity, molar absorptivity, limit of detection (LOD) and limit of

Table 1. Linearity and Sensitivity Data

Parameters	Method M1	Method M2
Beer's Limit (μg ml^{-1})	4-40	4-60
Molar Absorbtivity (M^{-1} cm^{-1})	3.774×10^4	4.605×10^4
Sandell's sensitivity (μg cm^{-2}/0.001 Absorbance unit)	3.898×10^{-3}	3.268×10^{-3}
Regression equation (Y = mx + c)[$]	-	-
Slope (m)	0.0261	0.0237
Intercept (c)	-0.0061	-0.0112
Correlation coefficient (r)	0.9993	0.9994
LOD (μg ml^{-1})	0.044	0.035
LOQ (μg ml^{-1})	0.133	0.106

[$]Y = mx + c, where Y is the absorbance and x is the concentration of VDL in μg ml^{-1}.

Table 2. Intra- and Inter-Day Precision and Accuracy Data

Method M1				Method M2			
Intra-day precision and accuracy							
Concentration of VDL (μg ml^{-1})				Concentration of VDL (μg ml^{-1})			
Taken	Found	RSD (%)	Recovery (%)	Taken	Found	RSD (%)	Recovery (%)
4	3.98	0.376	99.50	4	3.90	0.355	97.aA50
20	19.89	0.14	99.45	30	29.91	0.207	99.70
40	40.02	0.187	100.05	60	59.93	0.123	99.88
Inter-day precision and accuracy							
4	4.05	0.518	101.25	4	3.86	0.829	96.50
20	19.94	0.18	99.7	30	29.98	0.236	99.93
40	39.82	0.213	99.67	60	59.87	0.131	99.78

Table 3. Recovery Study Data

Method	Brand name of tablet	Labeled claim (mg)	Pure drug added (mg)	Found[$]	Recovery (%)	RSD (%)
		10	10	19.96	99.80	0.222
M1	Levitra	20	10	29.96	99.86	0.122
		25	10	34.98	99.94	0.165
		10	10	19.95	99.75	0.202
M2	Levitra	20	10	29.99	99.96	0.143
		25	10	34.93	99.80	0.105

[$]Average of five determinations.

Table 4. Robustness Data

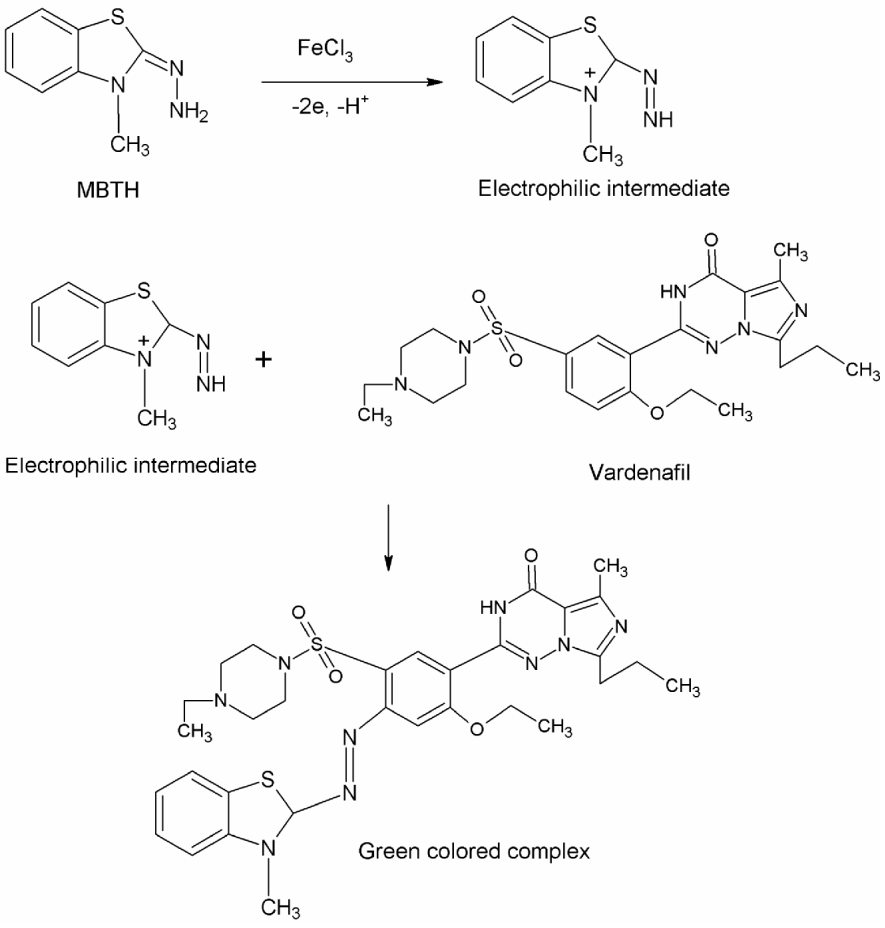
Experimental parameter	Concentration of VDL ($\mu g\ ml^{-1}$)			
	Taken	Found[$]	RSD (%)	Recovery (%)
Method M1				
Volume of 0.2% MBTH	4	3.92	0.918	98.00
(1.5 ± 0.1 ml)	40	39.89	0.230	99.80
Volume of 0.7% $FeCl_3$	4	4.05	0.716	101.25
(1.0 ± 0.1 ml)	40	39.84	0.245	99.60
Volume of 0.5% HCl	4	3.88	0.824	98.00
(1.0 ± 0.1 ml)	40	39.95	0.222	99.87
Reaction time	4	4.01	0.448	100.25
(10 ± 1 min)	40	39.91	0.23	99.77
Method M2				
Volume of 0.2% AP	4	3.94	0.964	98.50
(1.5 ± 0.1 ml)	60	59.97	0.140	99.95
Volume of 0.23% KIO_4	4	3.98	0.477	99.50
(1.0 ± 0.1 ml)	60	60.04	0.113	100.06
Volume of 2N NaOH	4	3.93	1.043	98.25
(1.0 ± 0.1 ml)	60	59.95	0.121	99.91
Reaction time	4	3.96	0.732	99.00
(5 ± 1 min)	60	59.9	0.156	99.83

[$]Average of three determinations.

Table 5. Assay Results for the Determination of VDL in Tablet Dosage Forms

Method	Labeled claim (mg)	Found[$]	RSD (%)	Recovery (%)
	10	9.94	0.408	99.40
M1	20	19.96	0.260	99.80
	25	24.93	0.166	99.72
	10	9.96	0.561	99.60
M2	20	19.95	0.209	99.75
	25	24.97	0.166	99.88

[$]Average of five determinations.



Scheme 1. Reaction of VDL with MBTH (method M1)

quantification (LOQ) were calculated for the proposed methods. The results are compiled in Table 1. The values of molar absorbtivity, Sandell's sensitivity, LOD & LOQ confirmed the sensitivity of the proposed methods.

Precision and Accuracy

The intra and inter-day precision and accuracy of the proposed methods were examined by the analysis of VDL at three different levels of concentration (4, 20 and 40 μg ml^{-1} - method M1; 4, 30 and 60 μg ml^{-1} -method M2). The intra- and inter-day precision and accuracy was assessed by the analysis of each concentration in one day and on three different days, respectively. The results are expressed as percentage recovery and percentage relative standard deviation. The precision and accuracy of the proposed methods were fairly good, as indicated by the low values of relative standard deviation and high values of recovery (Table 2). This level of precision and accuracy was satisfactory for the analysis of VDL.

Recovery Studies

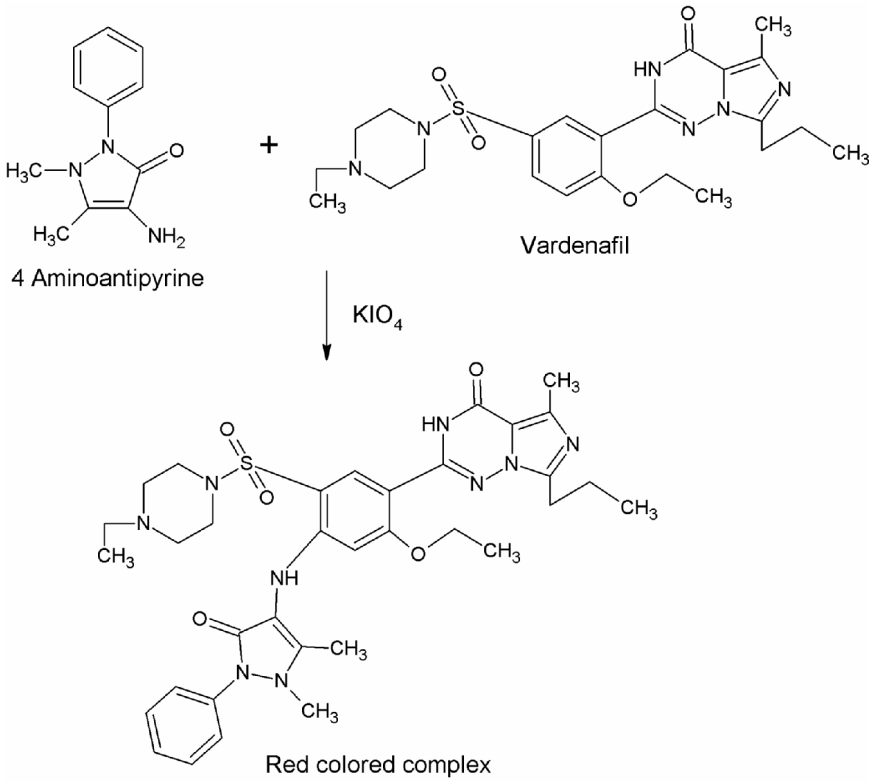
Accuracy of the proposed methods was further evaluated by spiking the preanalyzed tablet solution with pure drug. The total concentration of VDL was once again analyzed by the proposed methods and the percentage recovery was then calculated. The results of recovery studies are shown in Table 3. Good recoveries were obtained, which is an indication of high accuracy of the proposed methods. The proposed methods suffered no interference from common excipients indicating the high selectivity of the methods and its applicability for routine determination of VDL in tablet dosage forms.

Robustness

Method robustness was examined by making small deliberate changes in the experimental parameters. The investigated parameters are:

Method M1

- Volume of 0.2% MBTH (1.5 ± 0.1 ml)



Scheme 2. Reaction of VDL with AP (method M2)

- Volume of 0.7% $FeCl_3$ (1.0 ± 0.1 ml)
- Volume of 0.5% HCl (1.0 ± 0.1 ml)
- Reaction time (10 ± 1 min)

Method M2

- Volume of 0.2% AP (1.5 ± 0.1 ml)
- Volume of 0.23% KIO_4 (1.0 ± 0.1 ml)
- Volume of 2N NaOH (1.0 ± 0.1 ml)
- Reaction time (5 ± 1 min)

The robustness was checked at two different VDL concentration levels (4 & 40 μg ml^{-1} -method M1; 4 & 60 μg ml^{-1} -method M2). The results (Table 4) indicated that the changes have insignificant influence on the results as revealed by small values of relative standard deviation (≤1.043).

Application of the Proposed Methods for the Analysis of Dosage Forms

The satisfactory validation results made the proposed methods suitable for the analysis of VDL in tablet dosage forms (Levitra tablets, labeled to contain 10 mg, 20 mg and 25 mg of VDL per tablet). The results obtained were recorded in Table 5. The high percentage recoveries and the small values of the relative standard deviations indicate the high accuracy and precision of the proposed methods, respectively.

CONCLUSIONS

Two simple, precise, accurate and cost effective spectrophotometric methods (M1 and M2) were developed and validated for the determination of VDL in bulk and in its tablet dosage forms. From the sensitivity data, the method M2 is more sensitive than the method M1. In comparison with the earlier reported chromatographic and voltametric methods, the proposed methods employ simple procedures, do not require expensive instrumentation and expert operational personnel.

RECOMMENDATION

The proposed methods can be used as alternative methods to the reported chromatographic and voltametric methods for the routine determination of VDL in pure and tablet dosage forms.

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