SIMULTANEOUS ESTIMATION OF VARDENAFIL HYDROCHLORIDE AND DAPoxetine HYDROCHLORIDE IN COMBINED PHARMACEUTICAL DOSAGE FORM BY SPECTROPHOTOMETRY AND RP-HPLC

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Abstract

Literature review revealed that no method has been reported for simultaneous estimation of Vardenafil and Dapoxetine by spectrophotometry or RP-HPLC. In present research work attempt was made to develop the methods for simultaneous estimation of Vardenafil hydrochloride (VRDC) and Dapoxetine hydrochloride (VRDC) in combined pharmaceutical dosage form by spectrophotometry and RP-HPLC. Two simple, rapid, accurate, precise, reliable and economical methods have been proposed. They are absorbance ratio (Q-ratio) method and RP-HPLC. For absorbance ratio method wavelength selected were 280 nm, isoabsorptive point and 292 nm (λ max of DPH). The linearity range for VRDC and DPH were found to be 6-18 µg/ml and 18-54 µg/ml for absorbance ratio method. A RP-HPLC method was developed on Lichrospher® 100, RP-18 C (5 µm), Merck Ltd., India, 250 mm L x 4.6 mm Ø in size using mobile phase Methanol: Acetonitrile (95: 5 v/v) with 0.5 % triethyl amine. The flow rate was 1.2 ml/min and column effluents were monitored at 280 nm. Retention time were 2.25 min and 3.45 min for VRDC and DPH respectively. For RP-HPLC method linearity range were found to be 10-30 µg/ml and 30-90 µg/ml for VRDC and DPH respectively. Both the developed methods were validated in terms of linearity, limit of detection, limit of quantification, accuracy, precision, and robustness according to ICH guideline. The value of %RSD for intraday and interday precision was found to be less than 2 for both the methods. This value confirms that methods are precise. The value of % recovery between 98-102 shows that the methods are accurate and free from the interference of excipients used in formulation. The developed methods were successfully applied to perform assay of marketed tablet dosage form and the results were found in good agreement with % labelled claim.

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INTRODUCTION

Day to day number of newer drugs and their formulations either in single or in combined dosage forms are marketed. The combination of Vardenafil hydrochloride and Dapoxetine hydrochloride is used in the treatment of erectile dysfunction and premature ejaculation. It is well known that variation in quality is observed for different brands of dosage form as well as in the same brands between different batches. Therefore it was thought of interest to study the quality of marketed formulations of Vardenafil hydrochloride and Dapoxetine hydrochloride. The present research work was aimed to develop and validate UV-absorbance ratio method and RP-HPLC method for simultaneous estimation of Vardenafil hydrochloride and Dapoxetine hydrochloride in combined pharmaceutical dosage form. Analysis of pharmaceutical product is very important as it is concerned with quality of life. Vardenafil hydrochloride is a potent inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase (PDE 5). It is used in the treatment of erectile dysfunction. Dapoxetine hydrochloride is selective serotonin reuptake inhibitor used in treatment of anxiety as well as premature ejaculation.1-3

The chemical structures of Vardenafil hydrochloride and Dapoxetine hydrochloride are shown in Figure 1(A),(B).1,3

![Chemical structures](image)

**Figure 1:** Chemical structure of [A] Vardenafil hydrochloride and [B] Dapoxetine hydrochloride

Vardenafil hydrochloride and dapoxetine hydrochloride are not official in any Pharmacopoeia. So, not any official method described in for estimation of both drugs. But various other methods have been reported for estimation of Vardenafil hydrochloride. They are validated rapid stability-indicating method for the determination of related substances in Vardenafil hydrochloride by ultra-performance liquid chromatography, high performance liquid chromatography: chemiluminescence method for potential determination of Vardenafil in dietary supplement, determination of vardenafil in human plasma by high performance liquid chromatography with UV detection, liquid chromatography/tandem mass spectrometry method for the simultaneous determination of vardenafil and its major metabolite, N-desethylvardenafil, in human plasma: application to a pharmacokinetic study, multi-response optimization of a capillary electrophoretic method for determination of vardenafil in the bulk drug and in a tablet formulation.4-8 Methods reported for Dapoxetine hydrochloride are: UV Spectrophotometric method, RP-HPLC method, HPTLC, Thin layer chromatography densitometric method.9-14
MATERIALS AND METHOD

Standard of Vardenafil hydrochloride and Dapoxetine hydrochloride as well as tablet formulation (SuperZhewitra) were received as gift sample from Sunrise Remedies Pvt. Ltd., Santej, Ahmedabad.

Method – A: Absorbance ratio (Q-ratio) method

Instrumentation

For the Spectrophotometric method, UV-Visible spectrophotometer (Shimadzu 1800 with UV Probe 2.21 software) and a pair of 1 cm matched quartz cells were used. Shimadzu AUX 220 weighing balance.

Selection of Solvent

Vardenafil hydrochloride and Dapoxetine hydrochloride are freely soluble in water and methanol. Distilled water was selected as common solvent due to cost effectiveness.

Preparation of Standard Solutions

Accurately weighed quantity of 100 mg of VRDC and 100 mg of DPH were transferred into 100 ml volumetric flask, dissolved and diluted up to mark with distilled water. This will give a stock solution having strength of 1000 μg/ml VRDC and 1000 μg/ml DPH.

Selection of Analytical Wavelength

6-18 μg/ml solutions of VRDC and 18-54 μg/ml solutions of DPH were prepared in distilled water by appropriate dilution of standard solutions of VRDC and DPH respectively and spectrum was recorded between 200-400 nm. From overlain spectra of VRDC and DPH is absorptive point selected was 280 nm. Another wavelength selected was 292 nm.

Preparation of Calibration Curves

Series of standard solutions were prepared by dilution of the working standard solutions with distilled water to reach concentration range of 6, 9, 12, 15, and 18 μg/ml for VRDC and 18, 27, 36, 45, and 54 μg/ml for DPH.
The diagrams depict overlaid spectra of VRDC and DPH at 280 nm and 292 nm, with the following annotations:

- **Fig 3** Overlaid Spectra of VRDC at 280 nm and 292 nm
  - $\lambda_1 = 280$ nm (isoabsorptive point)
  - $\lambda_2 = 292$ nm

- **Fig 4** Overlaid Spectra of DPH at 280 nm and 292 nm for Q-Ratio Method
  - $\lambda_1 = 280$ nm (isoabsorptive point)
  - $\lambda_2 = 292$ nm

- **Fig 5** Calibration curve of VRDC at 280 nm by Q-Ratio Method
  - $y = 0.016x - 0.001$
  - $R^2 = 0.999$
Fig 6 Calibration curve of VRDC at 292 nm by Q - Ratio Method

Fig. 7 Calibration curve of DPH at 280 nm by Q - Ratio Method

Fig 8 Calibration curve of DPH at 292 nm by Q - Ratio Method
Analysis of Pharmaceutical Dosage Form

Twenty tablets of brand Super Zhewitra (Sunrise Remedies Pvt. Ltd) were weighed and average weight was determined (510mg). The quantity of the powder equivalent to 10 mg of VRDC and 30mg of DPH (255mg) was transferred to a 100 ml volumetric flask. The content was mixed with distilled water (70 mL) and sonicated for 5 min to dissolve the drug as completely as possible. The solution was then filtered through 0.45 μm membrane filter paper. The volume was made up with distilled water. Filtrate contained mixture of 100 μg/ml VRDC and 300 μg/ml DPH. The filtrate solution was suitably diluted with distilled water to get a final concentration of 12 μg/ml of VRDC and 36μg/ml of DPH. The absorbance of prepared sample solution i.e. A1 and A2 were recorded at 280 nm and 292 nm respectively and ratio of absorbance (Q_M) was calculated, i.e. 

\[ Q_X = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{aX_1} \]  

\[ Q_Y = \frac{Q_M - Q_X}{Q_Y - Q_X} \times \frac{A_1}{aY_1} \]  

Where, \( Q_X = \frac{aX_2}{aX_1} \); \( Q_Y = \frac{aY_2}{aY_1} \); \( Q_M = \frac{A_2}{A_1} \) 
\( \lambda_1 = 280 \text{ nm} \) (isosorptive point); \( \lambda_2 = 292 \text{ nm} \) (\( \lambda_{\text{max}} \) of DPH), 
\( A_1 = \text{absorbance of mixture at 280 nm} \), 
\( A_2 = \text{absorbance of mixture at 292 nm} \), 
\( aX_1 = \text{absorptivity of VRDC at 280 nm} \), 
\( aY_1 = \text{absorptivity of DPH at 280 nm} \), 
\( aX_2 = \text{absorptivity of VRDC at 292 nm} \), 
\( aY_2 = \text{absorptivity of DPH at 292 nm} \).

Method Validation\(^{15}\)

Validation of developed method was carried out according to ICH guideline for Validation of Analytical Procedures Q2 (R1).

Linearity and Range

Solutions having concentration 6, 9, 12, 15, and 18 μg/ml for VRDC and 18, 27, 36, 45, and 54 μg/ml for DPH were prepared from working standard solution. Prepared solutions were analyzed as per the proposed method. Six replicate analyses were carried out. The mean absorbance with its standard deviation and % relative standard deviation were calculated for both the drugs. Mean absorbance against concentration were plotted to obtain the calibration curves. Regression equations, co-relation coefficients were computed from calibration curves.

Precision

a) Repeatability

Six replicate solutions containing mixture of 12 μg/ml VRDC and 36 μg/ml DPH were prepared from their respective stock - working standard solution. Prepared solutions were analyzed as per the proposed
method. The mean % labelled claim with its standard deviation and % relative standard deviation were computed for both the drugs.

b) **Intraday Precision**
Solution containing mixture of 12 μg/ml VRDC and 36 μg/ml DPH was prepared from their respective stock - working standard solution. Prepared solution was analyzed as per the proposed method. Analysis was replicated for 6 different times within same day. The mean % labelled claim with its standard deviation and % relative standard deviation were computed for both the drugs.

c) **Interday Precision**
Solution containing mixture of 12 μg/ml VRDC and 36 μg/ml DPH was prepared from their respective stock - working standard solution. Prepared solution was analyzed as per the proposed method. Analysis was replicated for 6 different days. The mean % labelled claim with its standard deviation and % relative standard deviation were computed for both the drug.

**Accuracy**
Accuracy may be determined by application of the analytical method to the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i.e. “to spike”). In present studies, the later technique was adopted. Here, accuracy of the method was carried out at three levels (80%, 100%, and 120%). Known amount of VRDC (8, 10, 12 mg) and DPH (24, 30, 36 mg) were added to a pre quantified sample powder, and the amount of VRDC and DPH were estimated from calibration curve.

**Limit of Detection and Limit of Quantification**
The LOD and LOQ of developed method were calculated by using equations:
Limit of Detection (LOD) : \[3.3 \times \sigma/S\]
Limit of Quantification (LOQ) : \[10 \times \sigma/S\]
Where, \(\sigma\) = The Standard deviation of the response,
\(S\) = Slope of calibration curve.

**Method – B: RP-HPLC Method**
**Instrumentation**
**RP-HPLC System:** One of most advanced Chromatographic system available now-a-days
**Make and Model:** Young - Linn Clarity 9100 HPLC System
**Degasser:** Vacuum degasser YL - 9101
**Pump:** Quaternary pump YL - 9110
**Detector:** PDA detector YL - 9160
**Column:** Hibar® 250-4.6mm, Lichrospher® 100, RP-18 e (5μm), Merck ltd., India; 250 mm L × 4.6 mm Ø in size
**Temperature:** 20 ± 5 °C
**Pressure:** 1500 - 3000 psi
**Ultra-Sonicator:** Fast clean, Ultrasonic cleaner
Preparation of Standard Solutions

A 25 mg of both standard VRDC and DPH were accurately weighed and transferred to a 25 ml volumetric flask and dissolved in 15 ml optimized mobile phase as a diluent. The flask was sonicated for 5 min. The flask was shaken and volume was made up to the mark with diluent to give a solution containing 1000 μg/ml of VRDC and DPH.

Selection of Analytical Wavelength

Solutions containing appropriate concentration of VRDC and DPH in methanol were scanned using UV spectrophotometer in “Spectrum mode” in the range of 400 - 200 nm and their spectra were overlaid. From overlaid spectra of both the drugs 280nm was selected as analytical wavelength for detection.

![](image)

Fig 9 Overlaid Spectra of VRDC and DPH in methanol.

Selection of Mobile Phase

The pure drug mixture of VRDC and DPH were injected into HPLC system and run in different solvent systems. All the solvents were filtered through 0.45 μm membrane filter paper and sonicated for 20 min. Different mobile phases like, Methanol : Acetonitrile, Methanol : Buffer, and Methanol : Water were tried in order to find best condition for separation of VRDC and DPH. It was found that methanol : acetonitrile with 0.5% triethyl amine gives satisfactory results as compared to other mobile phases. This mobile phase system was tried with different proportions and different flow rates to optimize mobile phase for best possible separation of both the drugs.

Preparation of mobile phase:

The HPLC grade methanol, acetonitrile and triethyl amine were filtered through 0.45 μm membrane filter paper separately. Filtered solutions were ultrasonicated for 20 min. Solutions were allowed to come at room temperature if they were warmed due to sonication.

Chromatographic Conditions:

Stationary Phase: Hibar ® 250-4.6mm, Lichrospher ® 100, RP-18 e (5μm), Merck ltd., India; 250 mm L × 4.6 mm Ø in size
Mobile Phase: Methanol: Acetonitrile 95:5 with 0.5% TEA.
Flow Rate: 1.2 ml/min
Detection: 280 nm
Injection Volume: 20 μl
Temperature: 20 ± 5 °C
Pressure: 1500 - 3000 psi

Preparation of Standard stock solution
A 25 mg of both standard VRDC and DPH were accurately weighed and transferred to a 25 ml volumetric flask and dissolved in 15 ml optimized mobile phase as a diluent. The flask was sonicated for 5 min. The flask was shaken and volume was made up to the mark with diluent to give a solution containing 1000 μg/ml of VRDC and DPH.

Preparation of working standard solution of VRDC and DPH
100 μg/ml of VRDC and 100 μg/ml of DPH solutions were prepared by diluting 1ml of respective stock solutions to 10ml with methanol.

Preparation of binary mixture of VRDC and DPH
By appropriate dilution using mobile phase, from the working standard solutions of VRDC and DPH, binary mixture containing 10+30, 15 + 45, 20 + 60, 25 + 75, 30 +90 μg/ml VRDC + DPH were prepared and sonicated for 5 min. Six replicate series of binary mixture were prepared.

Preparation of Calibration Curves
Prepared standard solutions were injected to system with stated chromatographic conditions as described in section above. The chromatogram was stopped after separation achieved completely. Peak areas were recorded for all the peaks using YL -Clarity software (Ver.3.0.04.444). Standard calibration curve of AUC against concentration were plotted.

![Overlay chromatogram](image)

**Fig. 10** Overlaid chromatogram in 3D view (10-30μg/ml VRDC) and (30-90μg/ml DPH)
Analysis of Pharmaceutical Formulation

Twenty tablets were weighed accurately and their average weight was determined (510mg). The tablets were crushed to fine powder and from the triturate, tablet powder equivalent to 25 mg of VRDC and 75 mg of DPH were weighed and transferred to 25 ml volumetric flask. To this flask, 15 ml methanol was added and the flask was sonicated for 5 min. The volume was adjusted up to the mark with methanol. The solution was then filtered through 0.45 μm membrane filter paper. Filtrate contained mixture of 1000 μg/ml VRDC and 3000 μg/ml DPH. The filtrate solution was suitably diluted with mobile phase to get a final concentration of 20μg/ml of VRDC and 60μg/ml of DPH and sonicated for 5 min. Prepared solution was injected to system with stated chromatographic conditions as described above. The chromatogram was stopped after separation achieved completely. Peak areas were recorded using YL - Clarity software (Ver.3.0.04.444). The analysis procedure was repeated six times with tablet formulation Concentration of VRDC and DPH were computed by putting value of their peak areas in respective standard regression equation obtained from calibration curve.

Validation of Method\textsuperscript{15}

Validation of developed method was carried out according to ICH guideline for Validation of Analytical Procedures Q2(R1).
Linearity:

Binary mixture containing 10 + 30, 15 + 45, 20 + 60, 25 + 75, 30 + 90 μg/ml VRDC + DPH were prepared from working standard. Prepared solutions were analyzed as per the proposed method. Six replicate analyses were carried out. The mean area with its standard deviation and % relative standard deviation of peak areas were calculated. Mean AUC against concentration were plotted to obtain the calibration curve. Regression equations, co-relation coefficients were computed from calibration curves.

Precision
Repeatability

Six replicate solutions containing mixture of 20 μg/ml VRDC and 60 μg/ml DPH were prepared from their respective stock - working standard solution. Prepared solutions were analyzed as per the proposed method. The mean peak area with its standard deviation and % relative standard deviation was computed for both the drugs.

Intraday precision:
Replication within same day at different time:

Solution containing mixture of 20 μg/ml VRDC and 60 μg/ml DPH was prepared from their respective stock - working standard solution. Prepared solution was analyzed as per the proposed method. Analysis was replicated at 6 different times within same day. The mean peak area with its standard deviation and % relative standard deviation was computed for both the drugs.

Interday Precision:
Replication in different days:

Solution containing mixture of 20 μg/ml VRDC and 60 μg/ml DPH was prepared from their respective stock - working standard solution. Prepared solution was analyzed as per the proposed method. Analysis was replicated for 6 different days. The mean peak area with its standard deviation and % relative standard deviation was computed for both the drugs.

Accuracy (Recovery Studies)

In case of the assay of a drug in a formulated product, Accuracy is determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i.e. to spike). In present studies, the later technique was adopted. Here, accuracy of the method was carried out at three levels (80%, 100%, and 120%). Known amount of VRDC (8, 10, 12 mg) and DPH (24, 30, 36 mg) were added to a pre quantified sample powder, and the amount of VRDC and DPH were estimated by mean peak area and fitting these value to the straight-line equation of calibration curve.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ were calculated from the data obtained from the linearity studies. For each of the six replicate determinations, slope and y-intercept of the linearity plot was determined. Average of slope (S) and standard deviation of the y intercept (σ)were computed. From these values, the parameters LOD and LOQ were determined using following equations (On the basis of response and slope of the regression equation):

\[ \text{LOD} = 3.3 \sigma \sqrt{S} \]
Where; $\sigma$ = Standard deviation of Response, $S$ = Slope of calibration curve

### Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present. Typically, these might include impurities, degrades etc. A solution of blank in methanol was prepared and spectrum was taken. The spectrum of blank was compared with those acquired from VRDC (20 μg/ml) and DPH (60 μg/ml) standards. Peak purities of main peaks were computed using PDA detector YL - 9160 and YL - Clarity software (Ver.3.0.04.444).

### Robustness

Solution containing mixture of 20 μg/ml VRDC and 60 μg/ml DPH was prepared from their respective stock - working standard solution. Prepared solution was analyzed six times as per proposed method with small but deliberate change in chromatographic conditions as listed below:
1) Change in flow rate: 1.6 ml/min; 1.7 ml/min; 1.8 ml/min
2) Change in mobile phase composition:
Methanol: ACN: 95:5 v/v; 96:4 v/v; 94:6 v/v

The mean peak area with its standard deviation and % relative standard deviation was computed at each level.

### System Suitability Parameters

Solution containing mixture of 20 μg/ml VRDC and 60 μg/ml DPH was prepared from their respective stock - working standard solution. Data related to peak like area, height, width, retention time, resolution, tailing (symmetry) factor, column efficiency (theoretical plates) etc. was recorded using YL - Clarity software (Ver.3.0.04.444). All system suitability parameters were computed using these recorded data.

### RESULTS AND DISCUSSION

#### Method-A: Absorbance Ratio (Q-ratio) Method

Absorbance ratio (Q-ratio) method was developed for simultaneous determination of VRDC and DPH. The proposed method has been extensively validated as per ICH guidelines.

#### Analysis of Marketed Formulations

The proposed method was successfully applied to determine VRDC and DPH in pharmaceutical formulation (Table 1). The results obtained for VRDC and DPH were comparable with the corresponding label claims.

#### Linearity

The calibration curves were found to be linear over the ranges 6 – 18 μg/ml and 18 – 54 μg/ml for VRDC and DPH respectively. Characteristic parameters for the regression equation and correlation coefficients are given in Table 3. The linearity of the calibration curves was validated by the high value of correlation coefficients of the regression.

#### Accuracy

The recovery experiments were carried out by the standard addition method. The percent recoveries obtained were 99.10 and 98.83 for VRDC and DPH respectively (Table 2).

#### Precision

The results of the repeatability, intraday and interday precision are shown in Table 3. The low values of relative standard deviation (RSD) of the repeatability, intraday and interday determinations show that the proposed method is precise.
Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of the drugs are given in Table 3. These data shows that this method is sensitive for the determination of VRDC and DPH.

Method –B: RP-HPLC METHOD

RP-HPLC method was developed for simultaneous determination of VRDC and DPH. The proposed method has been extensively validated as per ICH guidelines.

Analysis of Marketed Formulations

The proposed method was successfully applied to determine VRDC and DPH in pharmaceutical formulations (Table 4). The results obtained for VRDC and DPH were comparable with the corresponding label claims.

Linearity

The calibration curves were found to be linear over the ranges 10 – 30 µg/ml and 30 – 90 µg/ml for VRDC and DPH respectively. Characteristic parameters for the regression equation and correlation coefficients are given in Table 6. The linearity of the calibration curves was validated by the high value of correlation coefficients of the regression.

Accuracy

The recovery experiments were carried out by the standard addition method. The percent recoveries obtained were 99.23 and 99.96 for VRDC and DPH respectively (Table 5).

Precision

The results of the repeatability, intraday and interday precision are shown in Table 6. The low values of relative standard deviation (RSD) of the repeatability, intraday and interday determinations show that the proposed method is precise.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification of the drugs are given in Table 6. These data shows that this method is sensitive for the determination of VRDC and DPH.

Robustness

Results of robustness study is depicted in Table 6.

System Suitability Parameters

Result for all system suitability parameters is depicted in table 7

Table 1-Assay results of simultaneous estimation VRDC and DPH in marketed formulation by absorbance ratio (Q-ratio method)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Amount taken mg</th>
<th>Amount Obtained (mg) (Mean, n=6)</th>
<th>% Assay (Mean, n = 6) ± RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRDC</td>
<td>DPH</td>
<td>VRDC</td>
</tr>
<tr>
<td>Super zhewitra (20mg VRDC and 60mg DPH)</td>
<td>12</td>
<td>36</td>
<td>12</td>
</tr>
</tbody>
</table>
### Table 2- Summary of Recovery studies by absorbance ratio (Q-ratio method)

<table>
<thead>
<tr>
<th>Level</th>
<th>Amount taken (mg)</th>
<th>Standard added (mg)</th>
<th>Final amount (mg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRDC</td>
<td>DPH</td>
<td>VRDC</td>
<td>DPH</td>
</tr>
<tr>
<td>80%</td>
<td>10</td>
<td>30</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>30</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>120%</td>
<td>10</td>
<td>30</td>
<td>12</td>
<td>36</td>
</tr>
</tbody>
</table>

### Table 3- Summary of Validation Parameters of absorbance ratio (Q-ratio method)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>VRDC</th>
<th>DPH</th>
<th>Standard value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>6-18μg/ml</td>
<td>18-54 μg/ml</td>
<td></td>
</tr>
<tr>
<td>Equation</td>
<td>Y = 0.0165x-0.0015 (280 nm)</td>
<td>Y=0.0159x+0.0141 (280nm)</td>
<td></td>
</tr>
<tr>
<td>Linearity</td>
<td>Y = 0.0108x + 0.0034 (292 nm)</td>
<td>Y = 0.017x +0.0153 (292 nm)</td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.9995(280nm)</td>
<td>0.9995(280nm)</td>
<td>≥ 0.998</td>
</tr>
<tr>
<td>0.9994(292nm)</td>
<td>0.9995(292nm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%RSD</td>
<td>0.1961-1.0101</td>
<td>0.4973-0.7732</td>
<td></td>
</tr>
<tr>
<td>0.5076-1.5074</td>
<td>0.5446-0.7902</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability (% RSD n=6)</td>
<td>0.4235</td>
<td>0.3542</td>
<td>≤ 2.0 % RSD</td>
</tr>
<tr>
<td>Intraday precision</td>
<td>0.4770</td>
<td>0.5664</td>
<td></td>
</tr>
<tr>
<td>(% RSD)</td>
<td>0.6331</td>
<td>0.6524</td>
<td>≤ 2.0 % RSD</td>
</tr>
<tr>
<td>Interday Precision</td>
<td>0.3061</td>
<td>0.1907</td>
<td></td>
</tr>
<tr>
<td>LOD (μg/ml)</td>
<td>0.9276</td>
<td>0.5780</td>
<td></td>
</tr>
<tr>
<td>LOQ (μg/ml)</td>
<td>99.10</td>
<td>98.83</td>
<td>98-102 %</td>
</tr>
<tr>
<td>% Recovery</td>
<td>98.83</td>
<td>99.10</td>
<td></td>
</tr>
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</table>
**Table 4**- Assay Results of simultaneous estimation VRDC and DPH in marketed formulation by RP-HPLC method

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Labelled claim mg/tablet</th>
<th>Amount Obtained(mg)</th>
<th>% Assay (Mean, n = 6) ± RSD*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRDC</td>
<td>DPH</td>
<td>VRDC</td>
</tr>
<tr>
<td>Super zhewitra</td>
<td>20</td>
<td>60</td>
<td>20.01</td>
</tr>
<tr>
<td>(20mg VRDC and 60mg DPH)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 5**- Summary of Recovery studies by RP-HPLC method

<table>
<thead>
<tr>
<th>Level</th>
<th>Tablet powder (mg)</th>
<th>Standard added (mg)</th>
<th>Final amount (mg)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRDC</td>
<td>DPH</td>
<td>VRDC</td>
<td>DPH</td>
</tr>
<tr>
<td>80%</td>
<td>10</td>
<td>30</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>100%</td>
<td>10</td>
<td>30</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>120%</td>
<td>10</td>
<td>30</td>
<td>12</td>
<td>36</td>
</tr>
</tbody>
</table>

**Table 6**- Summary of Validation Parameters of RP-HPLC method

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Validation Parameters</th>
<th>Results</th>
<th>Standard Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Linearity Range</td>
<td>10-30μg/ml</td>
<td>30-90μg/ml</td>
</tr>
<tr>
<td>2</td>
<td>Straight line equation</td>
<td>y=8.2814x-6.8944</td>
<td>y=12.232x+58.741</td>
</tr>
<tr>
<td>3</td>
<td>Correlation Coefficient</td>
<td>0.9996</td>
<td>0.9994</td>
</tr>
<tr>
<td>4</td>
<td>Precision (% RSD)</td>
<td>0.1184</td>
<td>0.3846</td>
</tr>
<tr>
<td></td>
<td>Repeatability</td>
<td>0.7902</td>
<td>0.4517</td>
</tr>
<tr>
<td></td>
<td>Intraday</td>
<td>0.9218</td>
<td>0.5461</td>
</tr>
<tr>
<td></td>
<td>Interday</td>
<td>0.5275</td>
<td>0.0541</td>
</tr>
<tr>
<td></td>
<td>LOD (μg/ml)</td>
<td>1.5985</td>
<td>0.1640</td>
</tr>
<tr>
<td></td>
<td>Mean % Recovery</td>
<td>99.23</td>
<td>99.96</td>
</tr>
<tr>
<td>5</td>
<td>Specificity</td>
<td>Specific</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>LOQ (μg/ml)</td>
<td>0.5275</td>
<td>0.0541</td>
</tr>
<tr>
<td></td>
<td>Robustness:</td>
<td>1.5985</td>
<td>0.1640</td>
</tr>
<tr>
<td></td>
<td>(i)Changing flow rate</td>
<td>0.9063</td>
<td>0.4044</td>
</tr>
<tr>
<td></td>
<td>(ii)Changing Mobile</td>
<td>0.9753</td>
<td>0.5055</td>
</tr>
<tr>
<td></td>
<td>phase ratio</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table 6.32 System suitability parameter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value obtained</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VRDC</td>
<td>DPH</td>
</tr>
<tr>
<td>Retention time ($t_{R}$, min)</td>
<td>2.25</td>
<td>3.45</td>
</tr>
<tr>
<td>Theoretical plates (N)</td>
<td>3712</td>
<td>3688</td>
</tr>
<tr>
<td>Resolution ($R_s$)</td>
<td>9.17</td>
<td></td>
</tr>
<tr>
<td>Tailing factor ($A_s$)</td>
<td>1.02</td>
<td>0.93</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The proposed absorbance ratio method and RP-HPLC method provide simple, specific, precise, accurate and reproducible quantitative analysis for simultaneous determination of VRDC and DPH in combined tablet dosage form. The method was validated as per ICH guidelines in terms of specificity, linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ) and robustness. The proposed methods can be used for routine analysis and quality control assay of VRDC and DPH in combined dosage form.

**Recommend Future Research**: Stability indicating assay method for this combined dosage form.

**Conflict-of-Interest Notification Page**

We, the under mentioned author(s) of the manuscript entitled “Simultaneous estimation of Vardenafil hydrochloride and Dapoxetine hydrochloride in combined pharmaceutical dosage form by spectrophotometry and RP-HPLC” hereby declare that the above manuscript which is submitted for publication in the Indo American Journal of Pharmaceutical Research is NOT under consideration elsewhere.

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**REFERENCES**


