# SIMULTANEOUS ESTIMATION AND VALIDATION OF VARDENAFIL AND DAPOXETINE HYDROCHLORIDE IN PHARMACEUTICAL FORMULATION BY THIN LAYER CHROMATOGRAPHIC DENSITOMETRIC METHOD

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**ABSTRACT**
The present manuscript describes new, simple, accurate, and precise high performance thin layer chromatography method for the simultaneous determination of Vardenafil and Dapoxetine in combined tablet dosage form. Chromatographic separation of the drugs was performed on aluminium plates pre coated with silica gel 60 $F_{254}$ as the stationary phase and the solvent system consisted of Chloroform: Methanol: Acetonitrile: Formic acid (4: 0.8: 4: 1 v/v/v/v). Densitometric evaluation of the separated zones was performed at 232 nm. The two drugs were satisfactorily resolved with $R_f$ values 0.47 and 0.79 for Vardenafil and Dapoxetine Hydrochloride, respectively. The linear regression data for the calibration plots showed good relationship with $r^2 = 0.9995$ from 150-750 ng/spot for Vardenafil and $r^2 = 0.9980$ from 450-2250 ng/spot for Dapoxetine Hydrochloride. The methods were validated for precision, accuracy, and recovery. The percentage recovery for Vardenafil was found to be 99.58 – 100.72 % and 99.97 – 100.21% for Dapoxetine Hydrochloride. The limits of detection and quantification were 21.86 and 66.25 ng/spot per spot for Vardenafil and 128.58. and 389.64 ng/spot per spot for Dapoxetine Hydrochloride, respectively.
**Keywords:** Vardenafil, Dapoxetine Hydrochloride, High Performance Thin Layer Chromatography Method.

## INTRODUCTION
Vardenafil (VAR) is chemically (1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propyli-midazo[5,1f][1,2,4]triazin-2-yl)-4-ethoxyphenyl]sulfonyl]-4-ethylpipera-zine Vardenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)[1-6]. Vardenafil is used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH)[4]. Literature survey also reveals RP-HPLC[7-9] method for determination of Vardenafil.
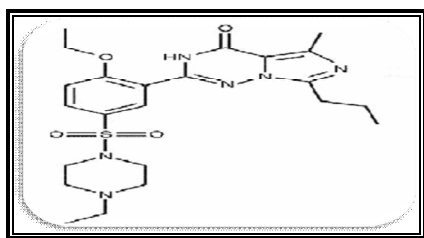


**Figure 1: Vardenafil**

Dapoxetine Hydrochloride (DAP) is chemically (S)-N, Ndimethyl- 3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine[10-13]. Dapoxetine Hydrochloride is indicated for the treatment of premature ejaculation and erectile dysfunction in men aged 18-64 years [13]. Literature survey reveals RP-HPLC methods for determination of DAP with other drugs [12,14].
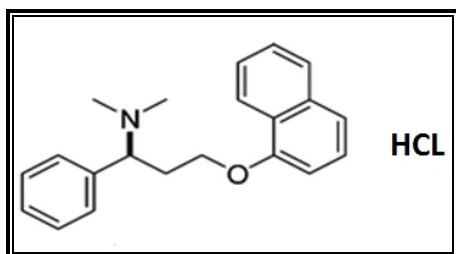


**Figure 2: Structure of Dapoxetine Hydrochloride**

The combined dosage forms of VAR and DAP are available in the market for the treatment of erectile dysfunction and premature ejaculation. The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of VAR and DAP in their combined dosage forms. Literature survey does not reveal any simple HPTLC method for simultaneous estimation of VAR and DAP in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on High Performance Thin Layer Chromatographic method for simultaneous estimation of both drugs in their combined tablet dosage forms.

## MATERIALS AND METHODS
### Reagents and Materials
VAR and DAP bulk powder was kindly purchased by Sunrise Laboratory Ahmedabad and Jai Radhe Sales Ahmedabad, Gujarat, India respectively. The commercial fixed dose combination product Levitra Super Force (VAR – 20 mg, DAP – 60 mg) was procured from the local market which is manufactured by Centurion Laboratories Private Limited, Vadodara.

### Instrumentation
CAMAG HPTLC instrument (Camag Muttenz, Switzerland) was used in this method. CAMAG HPTLC is equipped with CAMAG TLC scanner-3, Linnomate V Automatic sample applicator controlled by WIN CATS software (1.4.3 version). Aluminium packed silica Gel 60 $F_{254}$ HPTLC plates (100 X 100 mm, layer thickness 0.2mm, E.MERCK). Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag Muttenz, Switzerland). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 to 400 nm.

**Optimized chromatographic condition**

Stationary phase: Pre-coated silica gel 60 $F_{254}$ Aluminium Plates (10x10cm)

Mobile phase: Chloroform: Methanol: Acetonitrile: Formic acid (4:0.8:4:1 v/v/v/v)

Chamber saturation: 20 minutes

Development distance: 70mm

Development time: 15 minutes

Relative temperature: $25 \pm 2\degree C$

Scanning Speed: 20 mm/sec

Detection wavelength: 232 nm

VAR $R_f$: 0.47

DAP $R_f$: 0.79

**Preparation of standard stock solutions**

The powder equivalent to 60 mg VAR and 180 mg DAP was accurately weighed and transferred to volumetric flask of 100 ml capacity. 80 ml methanol was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper (0.45μ). 2.5 ml of this aliquot was added to 25 ml volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 60ng/μl VAR and 180ng/μl DAP. This solution was used for the estimation of VAR and DAP.



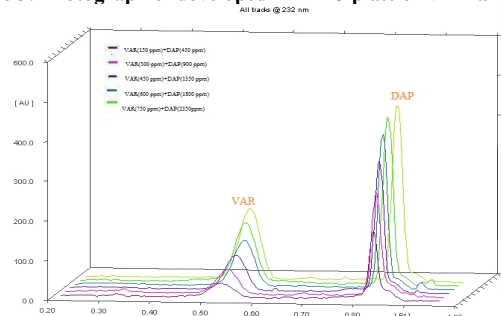**Figure 3: Photograph of developed HPTLC plate of VAR and DAP**



**Figure 4: Overlain view of all tracks of VAR and DAP at 232nm**
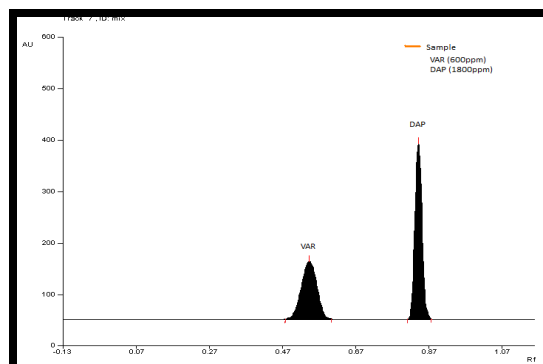


**Figure 5: Densitogram of marketed formulation containing 600 ng/spot VAR and 1800 ng/spot DAP**

**VALIDATION OF THE PROPOSED METHOD**

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[15].

**Linearity and range**

From the mixed standard stock solution 600 ng/μl of VAR and 1800 ng/μl of DAP, 1 to 5 μl solution spotted on HPTLC plate to obtain final concentration 150-750 ng/spot for AMB and 450-2250 ng/spot for DAP. Each concentration was applied six times to the HPTLC plate. The plate was then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

**Precision**

The precision of the method was verified by repeatability and intermediate precision studies.

**Repeatability**

Repeatability studies were performed by analysis of all concentrations (150, 300, 450, 600 and750 ng/spot for VAR and 450, 900, 1350, 1800 and 2250 ng/spot for (DAP) of the drug in six times on the same day.

**Intermediate precision**

The intermediate precision of the method was checked by intra day and inter day study. The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of VAR and DAP (150, 450, 750 ng/spot for VAR and 450, 1350, 2250 ng/spot for DAP). The result was reported in terms of relative standard deviation (% RSD).

**Specificity**

The specificity of the method was determined by analyzing standard drug and test samples. The spot for VAR and DAP in the samples was confirmed by comparing the $R_f$ and spectrum of the spot with that of a standard. The peak purity of VAR and DAP was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

**Accuracy**

Accuracy of the method was carried out by applying the method to drug sample (VAR and DAP combination tablet) to which know amount of VAR and DAP standard powder corresponding to 80, 100 and 120% of label claim had been added (standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

**ANALYSIS OF VAR AND DAP IN COMBINED TABLET DOSAGE FORM**

The powder equivalent to 60 mg VAR and 180 mg DAP was accurately weighed and transferred to volumetric flask of 100 ml capacity. 80 ml methanol was transferred to volumetric flask and sonicated for 10 minutes. The flask was shaken and volume was made up to the mark with methanol. The above solution was filtered through whatman filter paper (0.45μ). 2.5 ml of this aliquot was added to 25 ml volumetric flask and Volume was made up to the mark with Methanol to give a solution containing 60ng/μl VAR and 180ng/μl DAP. This solution was used for the estimation of VAR and DAP. 3.5 μl of the prepared sample was applied on pre-washed TLC plate, developed in the above mobile phase, dried in air and photo metrically analyzed by running chromatogram in optimized mobile phase. From the peak area obtained in the chromatogram, the amounts of both the drugs were calculated.

## RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for VAR and DAP in the current study involving Chloroform: Methanol: Acetonitrile: Formic acid (4: 0.8: 4: 1 v/v/v/v) as the mobile phase for HPTLC are given below.

The proposed method was found to be simple, specific, accurate, and precise for the routine simultaneous estimation of two drugs. The linearity range for VAR and DAP were found to be 150 – 750 ng/spot and 450-2250 ng/spot respectively. Regression analysis data and summary of all validation parameters is given in Table 1. Precision was calculated as repeatability (% RSD) and intra and inter day variation (% RSD) for both the drugs. Accuracy was determined by calculating the recovery and the mean was

determined. The LOD and LOQ were found to be 21.86 and 66.25 ng/spot respectively for VAR and 128.58 and 389.64 ng/spot respectively for DAP indicates sensitivity of the proposed method. The peak purity of VAR and DAP was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot. The peak purity was found to be 0.9996 and 0.9994 for VAR and DAP respectively. The method was successfully used to determine the amounts of VAR and DAP present in tablets. The results obtained are in good agreement with the corresponding labelled amount. By observing the validation parameters, the method was found to be specific, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

**Table 1: Regression analysis data and summary of validation parameters for the proposed method**

| Parameters | High Performance Thin Layer Chromatography method | |
|---|---|---|
| | **VAR** | **DAP** |
| **Concentration Range (ng/spot)** | 150 – 750 | 450 – 2250 |
| **Slope (m)** | 5.4242 | 2.3367 |
| **Intercept (c)** | 272.321 | 1183.510 |
| **Correlation Coefficient ($r^2$)** | 0.9995 | 0.9980 |
| **Accuracy (% recovery) (n = 3)** | 99.58 – 100.72 % | 99.97 – 100.21% |
| **Repeatability (%RSD) (n = 6)** | 0.38 % | 0.19 % |
| **Intraday (n = 3) (%RSD)** | 0.39 – 0.78 % | 0.37 – 0.47 % |
| **Interday(n = 3) (%RSD)** | 0.37 – 1.37 % | 0.35 – 0.37 % |
| **LOD (ng/spot)** | 21.86 | 128.58 |
| **LOQ (ng/spot)** | 66.25 | 389.64 |

**Table 2: Recovery data of proposed method**

| Drug | Level | Amount taken (ng/spot) | Amount Recovered (ng/spot) (n=3) | % Recovery (n=3) |
|---|---|---|---|---|
| **VAR** | 80 % | 480 | 481.4 | 100.29 ±0.045 |
| | 100 % | 600 | 604.3 | 100.72 ±0.017 |
| | 120 % | 720 | 716.9 | 99.58 ±0.035 |
| **DAP** | 80 % | 1440 | 1442.1 | 100.15 ±0.029 |
| | 100 % | 1800 | 1803.7 | 100.21 ±0.060 |
| | 120 % | 2160 | 2159.3 | 99.97 ± 0.080 |

**Table 3: Analysis of VAR and DAP by proposed method**

| Tablet | Label claim (mg) | | Amount taken (ng/spot) | | Amount Recovered (ng/spot) (n=3) | | % Label claim | |
|---|---|---|---|---|---|---|---|---|
| | VAR | DAP | VAR | DAP | VAR | DAP | VAR | DAP |
| **LEVITRA SUPER FORCE** | 20 | 60 | 600 | 1800 | 598.63 | 1797.33 | 99.77 % | 99.85 % |

## CONCLUSION

Introducing HPTLC into pharmaceutical analysis represents a major step in terms of quality assurance. Today HPTLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of HPTLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis. The developed HPTLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of VAR and DAP in pharmaceutical formulation without any interference from the excipients. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of VAR and DAP in method, hence it can be conveniently adopted for routine quality control.

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