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**Q-ABSORBANCE RATIO SPECTROPHOTOMETRIC METHOD FOR THE
SIMULTANEOUS ESTIMATION OF VARDENAFIL AND DAPOXETINE
HYDROCHLORIDE IN THEIR COMBINED DOSAGE FORM**

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ABSTRACT

The present manuscript describes simple, sensitive, rapid, accurate, precise and economical Q-absorbance ratio method for the simultaneous determination of Vardenafil and Dapoxetine Hcl in combined dosage form. Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an is absorptive point and other being the λ -max of one of the two components. Vardenafil and Dapoxetine Hcl show an is absorptive point at 270 nm in water. The second wavelength used is 292 nm, which is the λ -max of Dapoxetine Hcl in water. The linearity was obtained in the concentration range of 1-5 μ g/ml for Vardenafil and 10-30 μ g/ml for Dapoxetine Hcl. The concentrations of the drugs were determined by using ratio of absorbances at is absorptive point and at the λ -max of Dapoxetine Hcl. The method was successfully applied to pharmaceutical dosage form because no interference. The results of analysis have been validated statistically and by recovery studies.

KEY WORDS

Dapoxetine Hcl, Vardenafil, Absorbance ratio method, Is absorptive point and Validation.

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INTRODUCTION

Vardenafil¹ (VAR) is chemically (1-[[3-(1,4-dihydro-5-methyl-4-oxo-7-propylyl-midazo [5,1f] [1,2,4] triazin-2-yl)-4-ethoxyphenyl]sulfonyl] -4-ethylpiperazine. Vardenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP). Vardenafil is used to treat male erectile dysfunction (impotence) and pulmonary arterial hypertension (PAH) (Figure No.1).

Dapoxetine hydrochloride² (DAP Hcl) is chemically (S)-N,N dimethyl- 3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine. Dapoxetine hydrochloride is indicated for the treatment of premature ejaculation and erectile dysfunction in men aged 18-64 years (Figure No.2).

The combined dosage forms of VAR and DAP Hcl 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 Hcl in their combined dosage forms. Literature survey does not reveal any spectrophotometric method for simultaneous estimation of VAR and DAP Hcl in combined dosage forms. The present communication describes simple, specific, rapid, accurate and precise chromatographic method based on Q-absorption spectrophotometric method³ for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIAL AND METHODS

Instrument

A Shimadzu UV-1800 double beam UV/Visible spectrophotometer with spectral width of 2 nm, wavelength accuracy of 0.5 nm and a pair of 10 mm matched quartz cell was used to measure absorbance of all the solutions. Spectra were automatically obtained by UV-Probe system software. A Scale- tecTM analytical balance and ultrasonic bath was used in the study.

Reagents and Materials

VAR bulk powder was kindly purchased by Centurioan Laboratories Private Limited, Baroda, Gujarat, India and DAP Hcl bulk powder kindly supplied as a gift sample by Ami Life science pvt. Ltd. Akota, Baroda, Gujarat, India. The commercial fixed dose combination product Super Vilitra was kindly purchased by Centurioan Laboratories, Baroda, India. Were used in study.

Preparation of Standard Solutions

A 10 mg of standard VAR and DAP Hcl were weighed and transferred to 100 ml separate

volumetric flasks and dissolved in distil water. The flasks were shaken and volumes were made up to mark with distil water to give a solution containing 100µg/ml each of VAR and DAP Hcl.

Methodology

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ -max of one of the two components. From the overlay spectra of two drugs, it is evident that VAR and DAP Hcl show an isoabsorptive point at 270 nm. The second wavelength used is 292 nm, which is the λ -max of DAP Hcl. Working standard solutions having concentration 1, 2, 3, 4, and 5 µg/ml for VAR and 10, 15, 20, 25, and 30 µg/ml for DAP Hcl were prepared in distil water and the absorbances at 270 nm (isoabsorptive point) and 292 nm (λ -max of DAP Hcl) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM - QY) / (QX - QY)] \times A_1/ax_1 \dots\dots\dots(1)$$

$$CY = [(QM - QX) / (QY - QX)] \times A_1/ay_1 \dots\dots\dots(2)$$

Where, A₁ and A₂ are absorbances of mixture at 270 nm and 292 nm; ax₁ and ay₁ are absorptivities of VAR and DAP Hcl at 270 nm; ax₂ and ay₂ are absorptivities of VAR and DAP Hcl respectively at 292 nm; QM = A₂ / A₁, QX = ax₂ / ax₁ and QY = ay₂ / ay₁.

Validation of the proposed method

The proposed method was validated according to the International Conference on Harmonization (ICH) guideline.

Linearity (calibration curve)

The calibration curves were plotted over a concentration range of 1-5 µg/ml for VAR and 10-30 µg/ml for DAP Hcl. Appropriate aliquots from the standard stock solutions of VAR and DAP Hcl were used to prepare two different sets of dilutions: Series A, and B as follows. Series A consisted of different concentration of VAR (1-5 µg/ml). Aliquot from the stock solution of VAR (100 µg/ml) was pipette out in to a series of 10 ml volumetric flask and diluted with distilled water to

get final concentration in range of 1-5 µg/ml (0.1, 0.2, 0.3, 0.4, and 0.5ml). Series B consisted of varying concentrations of DAP Hcl (10-30 µg/ml). Appropriate volume of the stock solution of DAP Hcl (100 µg/ml) was transferred into a series of 10 ml volumetric flask and the volume was adjusted to the mark with distil water to get final concentration in range of 10-30 µg/ml (1, 1.5, 2.0, 2.5, and 3.0 ml). The absorbances of solution were then measured at 270 nm and 292 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated (Figure No.3).

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions ($n = 6$) for VAR and DAP Hcl 3 µg/ml and 15 µg/ml respectively without changing the parameter of the proposed Spectrophotometry method.

Intermediate precision (reproducibility)

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 (2, 3, and 4 µg/ml) and for DAP Hcl (15, 20 and 25 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of VAR and DAP Hcl by the standard addition method. Known amounts of standard solutions of VAR and DAP Hcl were at added at 80, 100 and 120 % level to prequantified sample solutions of VAR and DAP Hcl (6µg/ml for VAR and 15µg/ml for DAP Hcl). The amounts of VAR and DAP Hcl were estimated by applying obtained values to the respective regression line equations.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by Calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following

equations designated by International Conference on Harmonization (ICH) guidelines.

$$\text{LOD} = 3.3 \times \sigma/S \dots\dots\dots (3)$$

$$\text{LOQ} = 10 \times \sigma/S \dots\dots\dots (4)$$

Where, σ = the standard deviation of the response and S = slope of the calibration curve.

Analysis of drugs in sample

The absorbances of the sample solution i.e. A1 and A2 were recorded at 270 nm and 292nm (λ -max of DAP) respectively, and ratios of absorbance were calculated, i.e. A2/A1. Relative concentration of two drugs in the sample was calculated using above equation (1) and (2). The analysis procedure was repeated six times with tablet mixture.

RESULTS AND DISCUSSION

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 270 nm (isoabsorptive point) and 292 nm (λ -max of DAP Hcl) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of VAR (214 nm) and DAP (292 nm) showing isoabsorptive point (270 nm) in distil water is shown in Figure No.4. The validation parameters were studied at all the wavelengths for the proposed method. Accuracy was determined by calculating the recovery and the mean was determined (Table No.2). The method was successfully used to determine the amounts of VAR and DAP Hcl present in the tablet mixture. The results obtained were in good agreement with the corresponding labeled amount (Table No.3). Precision was calculated as repeatability and intra and inter day variations (% RSD) for both the drugs. Optical characteristics and summary of validation parameters for method is given in Table No.1. By observing the validation parameters, the method was found to be simple, sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these two drugs in combined dosage form.

Table No.1: Regression analysis data and Summary of validation parameters for VAR and DAP Hcl by Q-Absorbance Spectrophotometric method

S.No	Parameters	Q-Absorbance ratio method	
		Isoabsorptive point of VAR and DAP Hcl at 270 nm	λ -max of DAP Hcl at 292 nm
1	Concentration range ($\mu\text{g/mL}$)	1-5	10-30
2	Slope	0.038	0.021
3	Intercept	0.015	0.007
4	Correlation coefficient	0.997	0.999
5	Accuracy \pm SD ^a	99.78 \pm 0.64 - 100.32 \pm 0.32	99.78 \pm 0.24 - 100.87 \pm 0.18
6	LOD ^b ($\mu\text{g/mL}$)	0.1193	0.314
7	LOQ ^c ($\mu\text{g/mL}$)	0.363	0.952
8	Repeatability (% RSD ^d , n = 6)	1.07	0.451
9	Precision (% RSD) Interday (n = 3)	0.97-1.4	0.28-0.61
	Intraday (n = 3)	0.97-1.53	0.28-0.73

^aSD= Standard deviation, ^bLOD = Limit of detection, ^cLOQ = Limit of quantification, ^dRSD = Relative standard deviation.

Table No.2: Recovery data of VAR and DAP Hcl by Spectrophotometric method

S.No	Drug	Level	Amount of sample taken ($\mu\text{g/mL}$)	Amount of standard spiked (%)	% Recovery \pm SD (n=5)
1	VAR	I	6	80 %	100.32 \pm 0.32
		II	6	100 %	100 \pm 0.48
		III	6	120 %	99.78 \pm 0.64
2	DAP Hcl	I	15	80 %	100.87 \pm 0.18
		II	15	100 %	99.75 \pm 0.24
		III	15	120 %	100.37 \pm 0.06

Table No.3: Analysis of VAR and DAP Hcl by Spectrophotometric Method

S.No	Mixture	Label claim		Amount found		% Label claim (%RSD, n=6)	
		VAR/TAB	DAP.Hcl/TAB	VAR/TAB	DAP.Hcl/TAB	VAR	DAP.Hcl
1	VAR 20 + DAP 60	VAR/TAB 20 mg	DAP.Hcl/TAB 60 mg	VAR/TAB 20.08 mg	DAP.Hcl/TAB 59.84 mg	VAR 100.46 \pm 0.86 %	DAP.Hcl 99.73 \pm 2.09 %

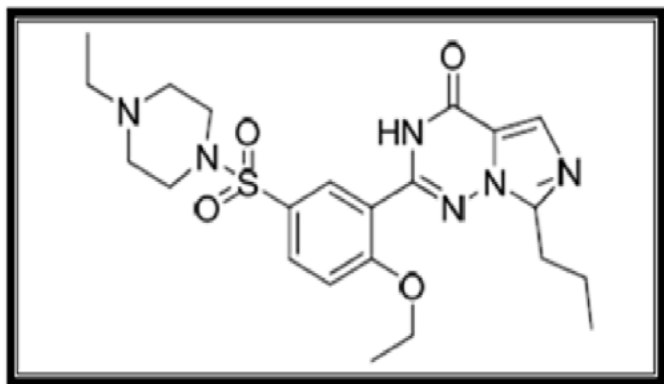


Figure No.1: Structure of Vardenafil

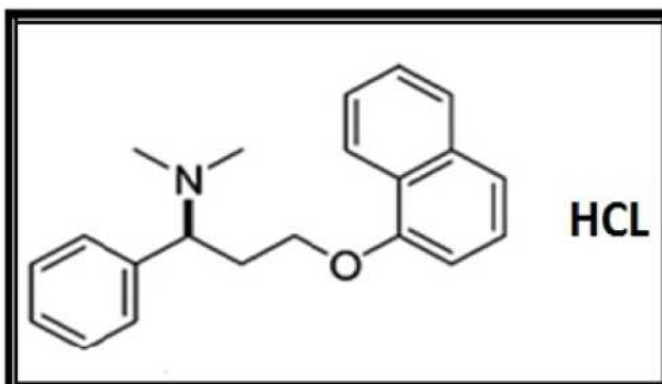


Figure No.2: Structure of Dapoxetine Hydrochloride

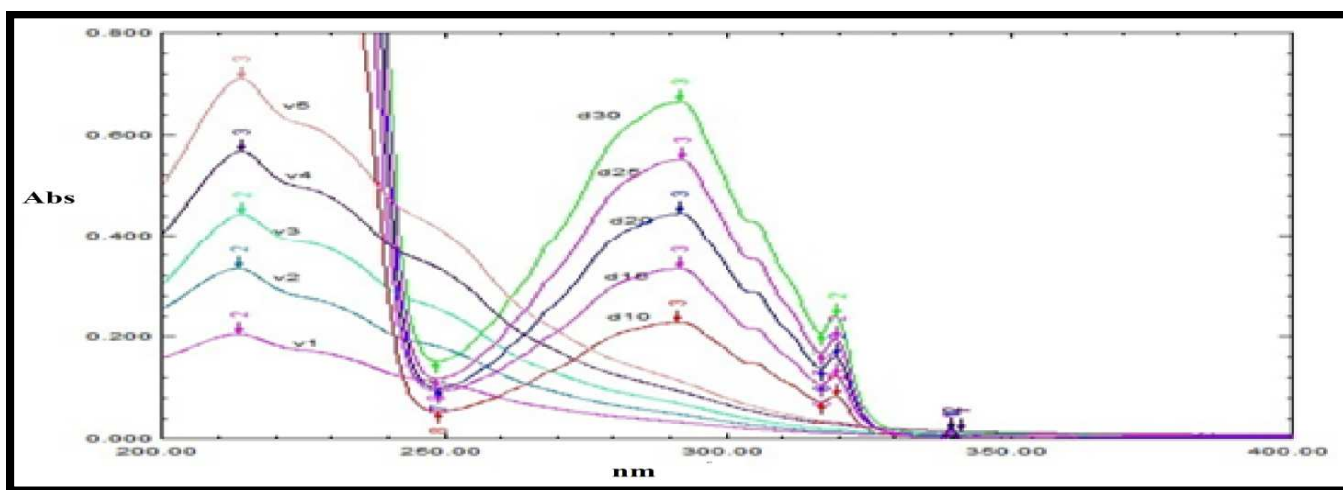


Figure No.3: Linearity of VAR and DAP Hcl in Distilled water

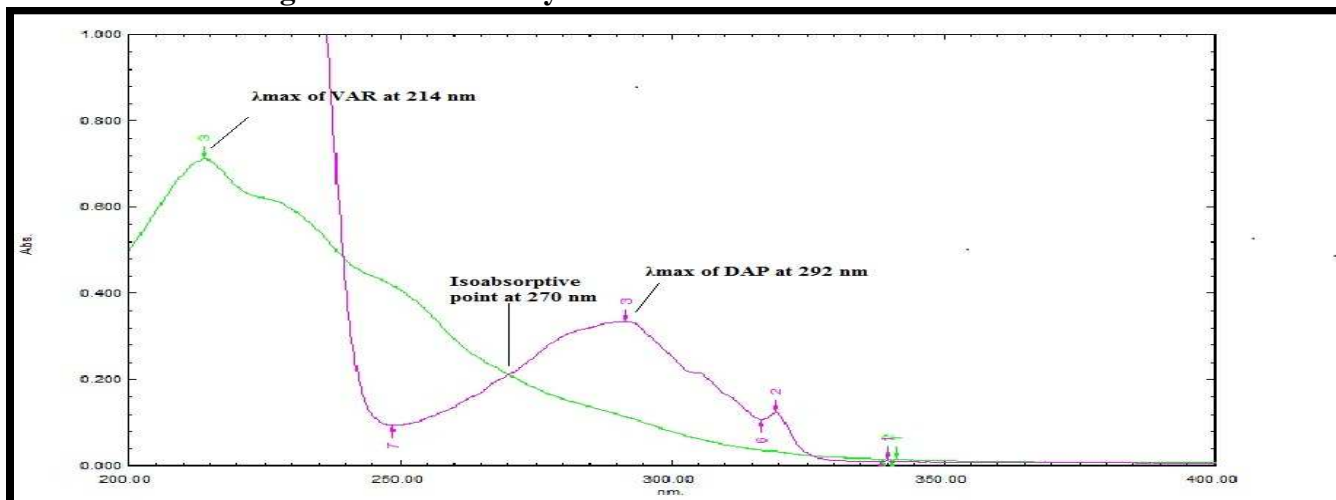


Figure No.4: Overlain absorption spectra of Vardenafil (214 nm) and Dapoxetine HCL (292 nm) showing isoabsorptive point (270 nm) in distil water

CONCLUSION

The proposed spectrophotometric method was found to be simple, sensitive, accurate and precise for determination of VAR and DAP Hcl in Tablet mixture. The method utilizes easily available and cheap solvent for analysis of VAR and DAP Hcl hence the method was also economic for estimation of VAR and DAP Hcl from Tablet mixture. The common excipients and other additives are usually present in the Tablet mixture do not interfere in the analysis of VAR and DAP Hcl in distil water, hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

1. Vardenafil-Wikipedia, (<http://www.urology-textbook.com/vardenafil.html>) Accessed 10 February 2013.
2. Dapoxetine-Wikipedia, (<http://www.urology-textbook.com/vardenafil.html>) Accessed 10 February 2013.
3. Beckett A H and Stenlake J B. Practical pharmaceutical chemistry, *CBS publishers and distributors, New Delhi*, 3rd edition, 2, 1998, 275.
4. Nataraj K S, Kesinath R, Kiran D, Suresh S. Simple validated UV-Spectrophotometric method for estimation of Dapoxetine, *JCPS*, 4(3), 2011, 105-107.

5. Bhavin Chapla, Gunjan Amin Ashutosh Pandya, Jagdish Kakadiya, Nehal Shah. Simultaneous estimation and validation of Vardenafil and Dapoxetine hydrochloride in pharmaceutical formulation by thin layer chromatographic densitometric method, *IRJP*, 3(5), 2012, 480-483.
6. Abirami G, Anandakumar K, Velmurugan R. Development and Validation of UV-Spectroscopy method for the determination of Dapoxetine Hydrochloride in pharmaceutical formulation, *Journal of Pharmacy Research*, 5(4), 2012, 1949-1951.

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