INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

A Path for Horizing Your Innovative Work

DEVELOPMENT AND VALIDATION OF DUAL WAVELENGTH UV SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF TADALAFIL AND DAPOXETINE HYDROCHLORIDE IN THEIR COMBINED TABLET DOSAGE FORM

*Gunjan Amin, Bhavin Chapla, Ashutosh Pandya, Dr. Jagdish Kakadiya, Dharmendrasinh Baria

Department of Pharmacy, Indubhai Patel College of Pharmacy and Research Centre, Dharmaj, Petlad-Khambhat road, Anand, Gujarat, India.

Corresponding Author Email: **amin_gunjan@yahoo.com**

Abstract: The present manuscript describes simple, sensitive, rapid, accurate, precise and economical dual wavelength spectrophotometric method for the simultaneous determination of Tadalafil and Dapoxetine Hydrochloride in combined tablet dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of Dapoxetine Hydrochloride at the absorbance difference between 280.0 nm and 295.4 nm and Tadalafil at the absorbance difference between 255.0 nm and 298.2 nm. The linearity was obtained in the concentration range of 4-24 μg/ml and 10-60 μg/ml for Tadalafil and Dapoxetine Hydrochloride respectively. The method was successfully applied to pharmaceutical dosage form because no interference from the tablet excipients was found. The suitability of these methods for the quantitative determination of Tadalafil and Dapoxetine Hydrochloride was proved by validation and recovery study. The proposed methods were found to be simple and sensitive for the routine quality control application ofTadalafil and Dapoxetine Hydrochloride in pharmaceutical tablet dosage form.

Keywords: Tadalafil, Dapoxetine Hydrochloride, dual wavelength UV spectrophotometric method

INTRODUCTION

Tadalifil (TAD) is a potent and selective, reversible inhibitor of cyclic guanosine mono phosphate (CGMP) specific phoshpo diesterase type 5(PDE) inhibitor used in the management of erectile dysfunction. Tadalafil is chemically named hydro-2-methyl-6-[3,4-(methylenedioxy)phenyl] pyrazino-[1,2:1,6] pyrido [3,4-b] indole-1,4-dione and phosphodiesterase type 5 inhibitor[1-4] used in the management of erectile dysfunction. It is not official in any Pharmacopoeia. Literature survey also reveals Spectrophotometric[5-6] and HPLC[7-12] methods for determination of TAD with other drugs. Dapoxetine HCl is designated chemically as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine[13]. It is used for erectile dysfunction. Cefpodoxime is not official in any Pharmacopoeia. Literature survey reveals RP-HPLC[14-15] methods for determination of DAP with other drugs. The combined dosage forms of TAD 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 TAD and DAP in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric or other method for simultaneous estimation of TAD and DAP in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate and economical spectrophotometric method based on Dual Wavelength UV spectrophotometric method for simultaneous estimation of both drugs in their combined tablet dosage forms.

MATERIALS AND METHODS

Apparatus

A double beam UV/Visible spectrophotometer (shimadzu model 1800, Japan) 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. An analytical balance (K.ROY instruments Pvt. Ltd., Varanasi, India), an ultrasonic bath (Janki Impex Pvt. Ltd., Ahmedabad, Gujarat, India) was used in the study.

Reagents and Materials

TAD and DAP bulk powder was kindly purchased by OHM Pharmaceuticals Ltd.,

Ahmedabad, Gujarat, India and Jai Radhe Sales, Ahmedabad, Gujarat, India respectively. The commercial fixed dose combination product TADAPOX (TAD – 20 mg, DAP – 60 mg) was procured from the local market which is manufactured by RSM Enterprises. methanol solution is used as solvent for the preparation of different concentration of both drugs TAD and DAP.

Preparation of standard stock solutions

An accurately weighed quantity of TAD (100 mg) and DAP (100 mg) were transferred to a separate 100 ml volumetric flask and 50 ml methanol is added to both volumetric flask and sonicated for 5 minutes. Volume was adjusted up to the mark with methanol to obtain standard solution having concentration of TAD (1000 μg/ml) and DAP (1000 μg/ml). 10 ml solutions of TAD (1000 μg/ml) and DAP (1000 μg/ml) were transferred to a separate 100 ml volumetric flask and diluted up to concentration of TAD (100 μg/ml) and DAP (100 μg/ml) with methanol.

Methodology

The working standard solutions of TAD and DAP were prepared separately in 25 ml volumetric flask using methanol as a solvent. They were scanned in the UV range of 200-400 nm. From the overlain spectra, four wavelengths 280.0 nm (λ_1), 295.4 nm (λ_2), 255.0 nm (λ_3) and 298.2 nm (λ_4) were selected for quantitation of both the drugs by proposed dual wavelength spectrophotometric method. The quantitative determination of DAP is carried out by measuring the absorbance difference value at between 280.0 nm and 295.4 nm where TAD have same absorbance at both the wavelengths. The absorbance difference between 280.0 nm and 295.4 nm is directly proportional to concentration of DAP. The quantitative determination of TAD is carried out by measuring the absorbance difference value at 255.0 nm and 298.2 nm where DAP has same absorbance at both the wavelengths. The absorbance difference between 255.0 nm and 298.2 nm is directly proportional to concentration of TAD.
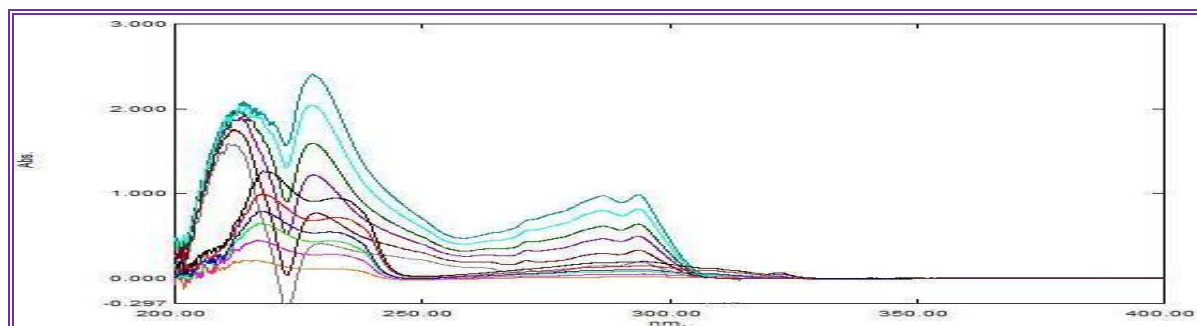

Figure 1 Overlain zero-order absorption spectra of TAD and DAP in methanol
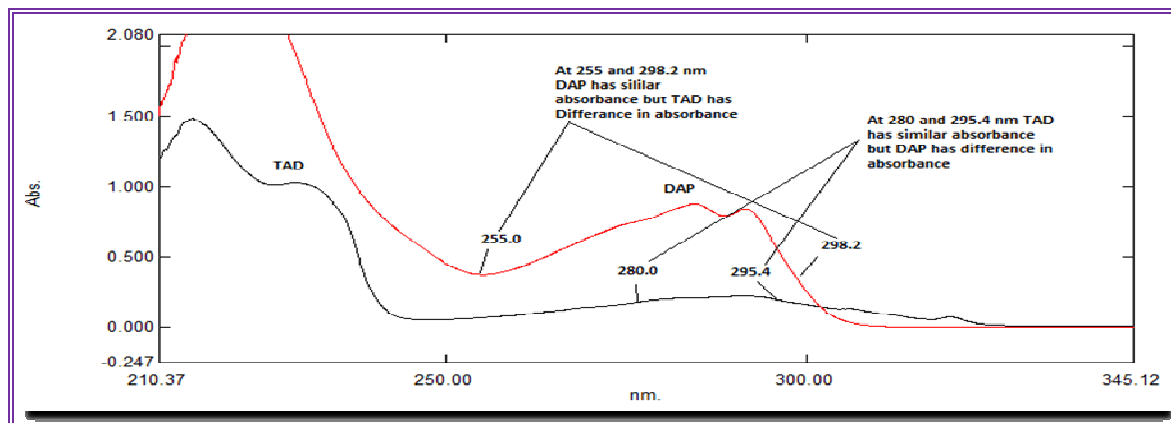
Figure 2 Overlain Spectra at 280 nm and 295.4 nm were selected for the estimation of TAD while 255 nm & 298.2 nm were selected for the estimation of DAP

VALIDATION OF THE PROPOSED METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[18].

Linearity (Calibration curve)

The calibration curves were plotted over a concentration range of 4-24 µg/ml for TAD and 10-60 µg/ml for DAP. Appropriate volume of aliquot from standard stock solution TAD (100 µg/ml) and DAP (100 µg/ml) was transferred to different volumetric flasks of 25 ml capacity. The volume was adjusted to the mark with the methanol to obtain concentration of 4, 8, 12, 16, 20 and 24 µg/ml TAD and 10, 20, 30, 40, 50 and 60 µg/ml DAP. These solutions scanned separately in the UV range of 200-400 nm. The absorbances of the solutions were measured at 280.0 nm (λ_1), 295.4 nm (λ_2), 255.0 nm (λ_3) and 298.2 nm (λ_4). The difference in absorbance between 280.0 nm (λ_1) and 295.4 nm (λ_2) is due to the DAP and was plotted against DAP concentration (µg/ml). The difference in absorbance between 255.0 nm (λ_3) and 298.2 nm (λ_4) is due to the TAD and was plotted against TAD concentration (µg/ml) and two different regression equations were obtained.

Method precision (repeatability)

The precision of this method was checked by repeated scanning and measurement of absorbance of solution (n = 6) for TAD (4, 8, 12, 16, 20 and 24 µg/ml) and DAP (10, 20, 30, 40, 50 and 60 µg/ml) 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 TAD and DAP (12,16,20 μg/ml for TAD and 30,40,50 μg/ml for DAP). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating recovery of TAD and DAP by the standard addition method. Known amounts of standard solutions of TAD and DAP were added at 80, 100 and 120 % level to prequantified sample solutions of TAD and DAP (15μg/ml and 45μg/ml for TAD and DAP, respectively). The amounts of TAD and DAP were estimated by applying obtained values to the respective regression line equations. The experiment was repeated for three times.

ANALYSIS OF TAD AND DAP IN COMBINED TABLET DOSAGE FORM

Twenty Tablets were weighed and powdered. The powder equivalent to 20 mg of TAD and 60 mg of DAP was transferred to a 100 ml volumetric flask. Mehanol (50 ml) was added to it and sonicated for 20 min. The solution was filtered through Whatman filter paper No. 41 and the volume was adjusted up to the

mark with methanol. This solution is expected to contain 200 μg/ml of TAD and 600 μg/ml of DAP. This solution (10 ml) was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of TAD (20 μg/ml) and DAP (60 μg/ml). From this solution 2.5 ml was taken in to a 100 ml volumetric flask and the volume was adjusted up to mark with methanol to get a concentration of TAD (20 μg/ml) and DAP (60 μg/ml). The responses of the sample solution were measured at 280.0 nm (λ_1), 295.4 nm (λ_2), 255.0 nm (λ_3) and 298.4 nm (λ_4) for quantification of TAD and DAP. The amounts of the TAD and DAP present in the sample solution were calculated by fitting the responses into the regression equation for TAD and DAP in the proposed method.

RESULTS AND DISCUSSION

The standard solutions of TAD and DAP were scanned separately in the UV range 200 – 400 nm. From the overlain spectra of both drugs, four specific wavelengths are selected. The absorbance at 280.0 nm (λ_1) and 295.4 nm (λ_2) wavelengths was found to be with same absorbance for TAD. The difference in absorbance at these two wavelengths ($A_{295.4} - A_{280.0}$) cancels out the contribution of absorbance

of TAD. These two selected wavelengths were employed to determine the concentration of DAP. Similarly, the absorbance at 255.0 nm (λ_3) and 298.2 nm (λ_4) wavelengths was found to be with same absorbance for DAP. The difference in absorbance at these two wavelengths ($A_{255.0} - A_{298.2}$) cancels out the contribution of absorbance of DAP. These two selected wavelengths were employed to determine the concentration of TAD.

The proposed method was found to be simple, sensitive, rapid, accurate, precise and economic for the routine simultaneous estimation of two drugs. The linearity range for TAD and DAP were found to be 4-24 μg/ml and 10-60 μg/ml 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 0.023 and 1.803μg/ml respectively for TAD and 0.020 and 1.045μg/ml respectively for DAP indicates sensitivity of the proposed method. The method was successfully used to determine the amounts of TAD 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 sensitive, accurate and precise. Hence the method can be employed for the routine analysis of these drugs in combinations.

CONCLUSION

Based on the results, obtained from the analysis of described method, it can be concluded that the method has linear response in the range of 4-24 μg/ml and 10-60 μg/ml for TAD and DAP, respectively with co-efficient of correlation, (R^2)=0.9992 and (R^2) = 0.9987 for TAD and DAP, respectively. The proposed spectrophotometric method was found to be simple, sensitive, accurate and

precise for determination of TAD and DAP in tablet dosage form. The method utilizes easily available and cheap solvent for analysis of TAD and DAP hence the method was also economic for estimation of TAD and DAP from tablet dosage form. The common excipients and other additives are usually present in the tablet dosage form do not interfere in the analysis of TAD and DAP in method, hence it can be conveniently adopted for routine quality control.

Table 1

Regression analysis data and summary of validation parameters for the proposed method

Parameters	Dual wavelength Spectroscopy method	
	TAD	**DAP**
Concentration Range (µg/ml)	4-24	10-60
Slope (m)	0.0326	0.0076
Intercept (c)	-0.0103	0.0623
Correlation Coefficient (R^2)	0.9992	0.9987
Accuracy (% recovery) (n = 3)	98.60 – 99.96 %	99.52 – 100.50%
Repeatability (%RSD) (n = 6)	0.15 %	0.22 %
Interday (n = 3) (%RSD)	0.09 – 0.14 %	0.11 – 0.15 %
Intraday(n = 3) (%RSD)	0.23 – 0.54 %	0.19 – 0.57 %
LOD (µg/ml)	0.023 µg/ml	0.020 µg/ml
LOQ (µg/ml)	1.803 µg/ml	1.045 µg/ml

Table 2

Recovery data of proposed method

Drug	Level	Amount taken (µg/ml)	Amount recovered (µg/ml) (n=3)	% Mean Recovery (n = 3)
TAD	80 %	12	11.96	99.96 ± 0.21
	100 %	15	15.2	98.6 ± 0.10
	120 %	18	17.85	99.16± 0.27
DAP	80%	36	35.83	99.52 ± 0.42
	100 %	45	45.65	101.44 ± 0.10
	120 %	54	54.27	100.5 ± 0.27

Table 3

Analysis of TAD and DAP by proposed method

	Label claim (mg)		Amount taken (µg/ml)		Amount Recovered (µg/ml) (n=3)		% Label claim	
Tablet	TAD	DAP	TAD	DAP	TAD	DAP	TAD	DAP
I	15	45	14.79	45.40	30.13	50.36	98.60	100.88

REFERENCES

1. Kiran Kumar D, Nataraj KS, Kesinath R and Suresh Kumar S: Method Development and Validation for Analysis of Dapoxetine in Bulk and Pharmaceutical Dosage Form by RP-HPLC"; International Journal of Chem Tech Research; 2010: 205-211.

2. SVV. Dhanu Radha. IJRRPAS. 2011 ; 1(3) : 172-178.

3. Drug Bank: Tadalafil November 2011. www.drugbank.ca/drugs/DB00820

4. Samuel L. Washington and Alan W Shindel. Drug Design, Development and Therapy. 2010; l (4):160-171.

5. Drug Bank: "Dapoxetine Hydrochloride" November 2011 www.drugbank.ca/drugs/DB00539

6. Mohammad Y, Gowrie Sankar, Pragati Kumar and Shahul H. E-journal of chemistry http://www.e-journals.net 2010; 7(3): 833-836.

7. Adlin Jino Nesalin, Jose Gnana Babu, Vijaya Kumar and Tamizh mani. E-journal of chemistry http://www.e-journals.net 2009; 6(3): 611-614.

8. B. Prasanna Reddy, K. Amarnadh Reddy and M. S. Reddy: Validation and stability indicating RP-HPLC method for the determination of tadalafil API in pharmaceutical formulations. Research in Pharmaceutical Biotechnology. 2010; 2(1): 1-6.

9. Suthar AS, Magdum CS, Patil SS and Naikawadi NS: Rp-hplc Estimation of Tadalafil in Tablet Dosage Form. Int. J. em. Sci. 2008; 6(2): 1223-1227.

10. Ashok K Shakya, Ahmed NA, Tewfik AA, Munther Melhim. Journal of Chromatography B Analytical Technologies In The Biomedical And Life Sciences", 2000; 852(1-2): 403-8.

11. Ramakrishna NVS' Vishwottam KN, Koteshwara M. Journal of Chromatography B, 2004; 809(2): 243–49.

12. Kannappan N, Yada D, Shashikanth and Mannavalan R: Method development and validation of stability indicating methods for assay of Tadalafil and sildenafil citrate by HPLC. International Journal of Chem Tech Research. 2010; 2(1): 329-333.

13. Mehta P, Sahoo U and Seth AK: Development and Validation of a RP-HPLC Method for the Determination of Dapoxetine Hydrochloride in Pharmaceutical Formulation Using an Experimental. International Journal of Pharma World Research, 2011; 6(2): 15.

14. Drug Bank: "Dapoxetine Hydrochloride" November 2011 www.drugbank.ca/drugs/DB00539 .

15. Martin Dale, Pharmaceutical press, 2005; 34th edition, 1751.