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A NEW VALIDATED SIMULTANEOUS METHOD DEVELOPMENT BY RP-UPLC FOR THE ESTIMATION OF TADALAFIL AND DAPOXETINE IN BULK AND PHARMACEUTICAL DOSAGES

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ABSTRACT

RP-UPLC method has been developed and described for simultaneous estimation of Tadalafil and Dapoxetine in formulation. The estimation was carried out on a UPLC (IWATERS)_SYMMETRY® C 18(4.6x75mm) 3.5µm column with a mixture of 45% methanol: 55% buffer (0.3 w/v of glacial acetic acid and 0.64 % w/v of sodium pentasulphonate) and pH adjusted to 5 with NaOH. UV detection was performed at 254 nm. The flow rate was 0.6ml/min. The retention time by proposed method were found to be 1.21 and 2.81 min for Tadalafil and Dapoxetine respectively. The drugs show linearity in the range of 10-30 µg/mL for Tadalafil and 30-90 µg/mL for Dapoxetine with correlation coefficients 0.999. The accuracy of the proposed method was determined by recovery studies and found to be 101.13% for Tadalafil and 99.78% for Dapoxetine. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantification (LOQ) was 0.0054 µg/mL and 0.0218µg/mL for Tadalafil and 0.0149 µg/mL and 0.0596 µg/mL for Dapoxetine, respectively. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. The proposed method was validated as per ICH guideline, obtained results were found to be within limits. Hence, the method could be successfully applied for routine analysis of Tadalafil and Dapoxetine in the combined solid dosage form.

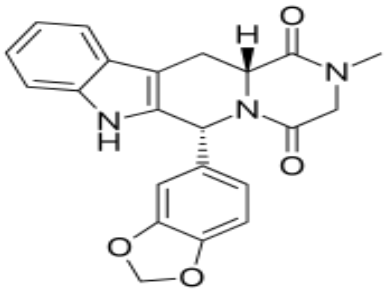
KEY WORDS

Dapoxetine, ICH guidelines, Simultaneous estimation, Tadalafil.

INTRODUCTION

Tadalafil [1-2] (TAD) is chemically named as hydro-2-methyl-6-[3,4-(methylenedioxy) phenyl] pyrazino-[1,2:1,6] pyrido [3,4-b] indole-1,4-dioneand phosphodiesterase type5 inhibitor1-4 (Fig.1). TAD is a potent and selective, reversible inhibitor of cyclic

guanosinemono phosphate (CGMP) specific phoshpodiesterase type 5(PDE) inhibitor used in the management of erectile dysfunction. The inhibition of phosphodiesterase type5 (PDE5) enhances erectile function by increasing the amount of cGMP. However, because sexual stimulation is required to initiate the local penile release of nitric oxide, tadalafil's inhibition of PDE5 will have no effect without direct sexual stimulation of the penis. The recommended tadalafil starting dose for most men is 10 mg.

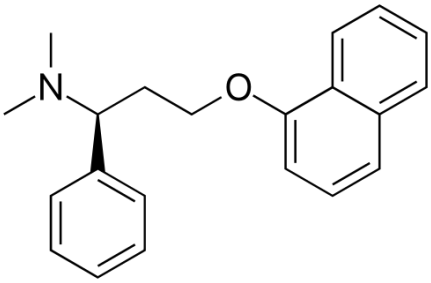


(Fig.1) Tadalafil

The purpose of this study was to develop a shorter run time and also to maintain pH for reducing the retention time. Thus, the peak tailing is minimized. So that it is a simple, rapid, precise and accurate RP-UHPLC method for the simultaneous estimation of both the drug in combined tablet dosage forms. Literature survey reveals that a few RP-HPLC [4-5], Spectrophotometric [6-7] methods are reported for the estimation of Tadalafil while RP-HPLC methods for simultaneous estimation of above mentioned drugs [8-9] (TAD&DAP).

To date, there have been no published reports about the simultaneous quantification of Tadalafil and Dapoxetine by RP-UPLC in bulk drug and in tablet dosage form. This present study reports for the first-time simultaneous quantification of Tadalafil and Dapoxetine by RP-UPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines [10-12]

Dapoxetine[3](DAP)(1S)-N, N-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine (Fig.2) is used for treatment of premature ejaculation in men, impotence and some cases of insomnia. Dapoxetine is a short acting selective serotonin reuptake inhibitor (SSRI). Increasing serotonin's action at the post synaptic cleft, and consequently promoting ejaculatory delay SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders.



(Fig.2) Dapoxetine

MATERIALS AND METHODS

Instrumentation:

Chromatography was performed with uplc_agilent_1220 infinity LC with high speed auto sampler with open lab_ chem station software using a UV detector at 254nm.

Reagents and Chemicals:

The sample reference of TAD and DAP were provided as gift samples from Reddys Laboratories, Hyderabad**.** water, methanol, glacial acetic acid were obtained from Merck, Mumbai. and pentasulphonate buffer, obtained from RANKEM Mumbai. All solvents used in this work are HPLC grade. Marketed formulation (TD Pill, Sunglow Pharmaceutical Ltd. containing Dapoxetine 30 mg & Tadalafil 10 mg) was purchased from local market.

Chromatographic conditions:

Column: UPLC (IWATERS)_SYMMETRY® C 18(4.6x75mm) 3.5µm

Mobile phase: MeOH (45): Buffer buffer (55) (0.3 w/v of glacial acetic acid and 0.64 % w/v of sodium pentasulphonate)

Flow rate: 0.6mL/min
Detector wavelength: 254 nm
Injection volume: 2 µl
Temperature: 25^{o} C Ambient temperature

Solutions and sample preparation

Preparation of Phosphate buffer

Buffer was prepared by dissolving 0.3 w/v of glacial acetic acid and 0.64 % w/v of sodium pentasulphonate in 100ml of HPLC grade water and pH was adjusted to 5.0 with Sodium hydroxide. The buffer was filtered through 0.45ìm nylon membrane filter to remove all fine particles and gases.

Preparation of mobile phase (Used as Diluent)

The above prepared buffer and Methanol HPLC grade were mixed in the proportion of 55:45 v/v and was filtered through0.45µm nylon membrane filter and degassed by sonication.

Standard stock solution Preparation

Accurately weighed and transferred 10.07mg and 30.38mg of TAD and DAP working standards in to two 50ml clean and dry volumetric flask separately, add ¾ volume of diluent, sonicated for 30 minutes and make up to the volume with diluents. From the above stock solution 5.0ml was pippeted out in to a 50ml volumetric flask and then make up to the final volume with diluent.

Working Standard Solutions Preparation

Aliquot of 2.5, 3.5, 4.5, 5.5, 6.5, 7.5mL were pipette out from stock solution into 50 ml volumetric flask separately for both TAD and DAP and volume was made up to 50 ml with diluent. This gives the solutions of 10,14,18,22,26,30µg/ml for TAD and 30,42,55,65,77,90µg/ml for DAP respectively.

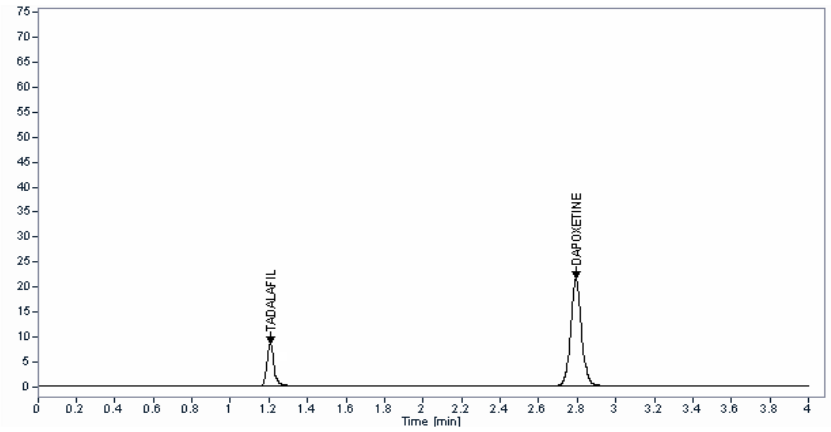
Sample preparation

Twenty tablets were weighed and crushed into fine powder. The average weight of tablet was weighed and dissolved in 50 ml diluent, sonicated for 20 min and filtered through PVDF 0.45µ filter. From the filtrate, 5 ml was pipette out and transferred into a 50 ml volumetric flask and the solution was made up to the volume with diluent.

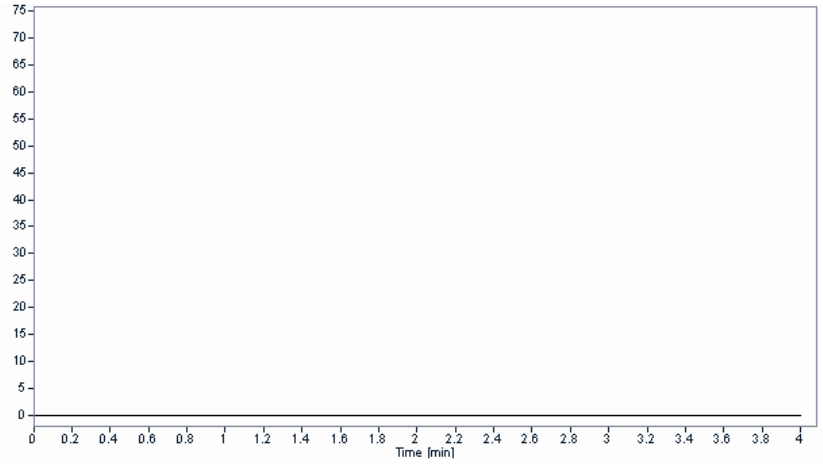
RESULTS AND DISCUSSION

Method development

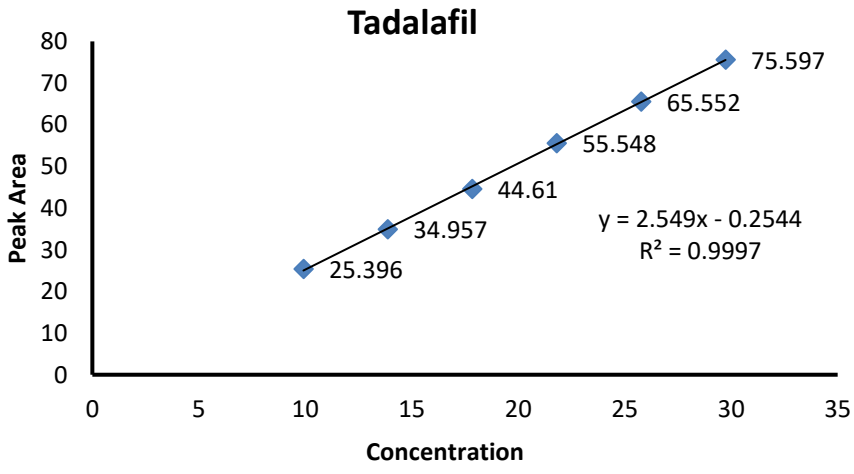
Reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol and Water, Acetonitrile and Water as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. The organic content of mobile phase was also investigated to optimize the separation of both drugs. To improve the tailing factor, the pH of mobile phase becomes important factor. At pH: 5.0 (NaoH) both drugs eluted with better separation. Thereafter, buffer: methanol were taken in isocratic ratio, %buffer / %methanol: 55/45, with flow rate of 0.6mL/min was employed ODS (4.6x75mm) 3.5µm particle size was used as the stationary phase to improve resolution and the tailing of both peaks were reduced considerably and brought close to1. To analyze both drugs detection was tried at various wavelengths from 200nm to 280nm. Both TAD and DAP showed maximum absorption at 254nm with UV detector. The chromatogram obtained was shown in the Fig.3.



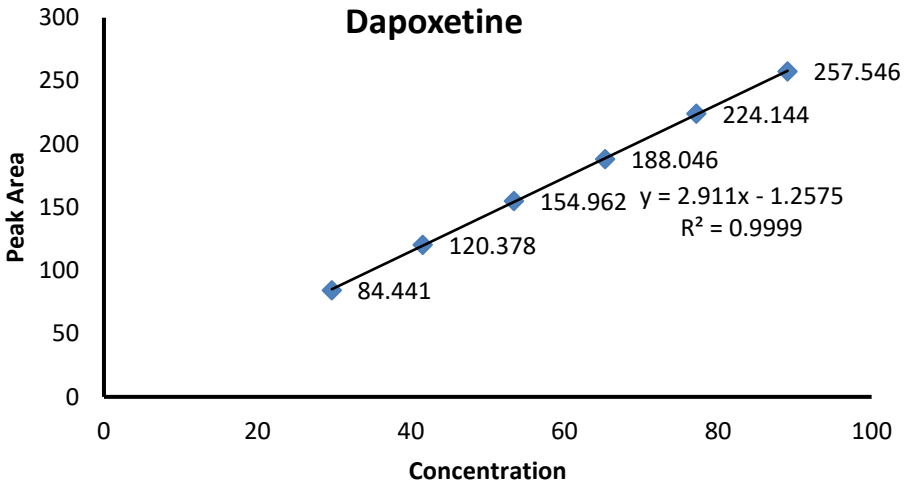
(Fig.3) Developed chromatogram



(Fig.4) Blank



(Fig.5) Linearity of Tadalafil



(Fig.6) Linearity of Dapoxetine

Method Validation:

The validation of the method was carried out as per ICH guidelines the parameter assessed were System suitability, specificity, linearity, accuracy & precision, Robustness, LOD and LOQ.

System suitability

The UPLC system was optimized as per the chromatographic conditions. One blank followed by five replicates of a single calibration standard solution of 20µg/ml of TAD and 60µg/ml of DAP was injected to check the system suitability. To ascertain the system suitability for the proposed method, the parameters such as retention time, theoretical plates, peak asymmetry and resolution were taken, and results were presented in (Table 1).

(Table 1). System suitability

S.No	Parameter	Tadalafil*	Dapoxetine*
1.	RT	1.21	2.81
2.	Theoritical plates	2213.768	3400.126
3.	Tailing factor	1.234	1.048
4.	Resolution	9.7296	9.7296
5.	Area%	22.308	77.692
6.	SD	0.09	0.51
7.	%RSD	0.184	0.301

*mean average of five determinations

(Table 2). Specificity

SNo.	Injection	Tadalafil RT	Area	Dapoxetine RT	Area	SD	%RSD
1.	Tadalafil (5)	1.2	48.833	NIL	NIL	0.14	0.28
2.	Dapoxetine (5)	NIL	NIL	2.81	169.26006	0.80	0.48
3.	Blank	About1.2	NIL	About2.81	NIL	NIL	NIL
4.	Placebo	About1.2	NIL	About2.81	NIL	NIL	NIL

Specificity

The effect of excipients and other additives usually present in the combined tablet dosage form of TAD and DAP in the determination under optimum conditions was investigated. The specificity of the RP-UPLC method was established by injecting the blank and placebo solution into the UHPLC system. The peak purity of TAD and DAP was determined by comparing the spectrum at three different regions of the spot i.e. peak start, peak apex and peak end. Effect of excipients of formulation was studied for whether it interfered with the assay. The representative chromatogram of blank was shown in (Fig .4) and the readings are shown (Table 2).

Linearity

TAD showed a linearity of response between 10-30µg/mL and DAP showed a linearity of response between 30-90µg/ml. These were represented by a linear regression equation as follows: y (TAD) = 2.549x-0.254 (r2=0.999), y (DAP) = 2.911x-1.257 (r^2=0.999) and regression line was established by least squares method and correlation coefficient (r^2) for TAD and DAP is found to be greater than 0.98. Hence the curves established were linear, shown in Fig.5 & 6 and in Table 3.

(Table 3). Linearity

S. No	Linearity of Tadalafil		Linearity of Dapoxetine	
	Conc (µg/ml)	Peak area	Conc (µg/ml)	Peak area
1	9.912	25.396	29.688	84.441
2	13.876	34.957	41.563	120.378
3	17.841	44.610	53.438	154.962
4	21.806	55.548	65.313	188.046
5	25.771	65.552	77.188	224.144
6	29.736	75.597	89.064	257.546
Slope	2.549		2.911	
Y-Intercept	0.254		1.257	
Co-Relation Co-Efficient	0.999		0.999	

Recovery

To pre-analyzed sample solution, a definite concentration of standard drug (10%, 20% & 30 % level) was added and recovery was studied. The % Mean recovery for TAD and DAP are 101.13 and 99.78 respectively and these results are within acceptable limit of 98-102. The % RSD for TAD and DAP are 0.13 and 0.27 respectively and %RSD is within limit of ≤ 2. Hence the proposed method is accurate, and the results were summarized in Table 4& 5.

Precision: (Repeatability)

Six replicates injections in same concentration were analyzed in the same day for repeatability and the % RSD for TAD and DAP found to be 1.48 and 0.84 respectively and % RSD for TAD and DAP found to be within acceptable limit of ≤ 2 and hence method is reproducible, and the results are shown in Table 6.

Robustness:

The robustness was established by changing the flow rate, composition of the mobile phase and change in wavelength within allowable limits from actual chromatographic conditions. It was observed that there were no marked change in mean Rt and RSD is within limit of ≤ 2. The tailing factor, resolution factor and no. of theoretical plates are found to be acceptable limits for both TAD and DAP. Hence the method is reliable with variations in the analytical conditions and the results of TAD are shown in Table 7 and results of DAP shown in Table 8.

(Table 4). Recovery of Tadalafil

S No.	Sample ID	Standard Area	Sample Area	Calculated content (mg of drug)	Actual Assay	Recovered Amount	Actual amount added	Recovery Percentage (%)
					Mg of drug/ avg.Wt			
1	Spiked with 10%	50.496	56.269	11.0498	10.0323	1.0174	1.0055	101.19
2	Spiked with 20%	50.496	61.661	12.0535	10.0323	2.0212	2.0018	100.97
3	Spiked with 30%	50.496	66.770	13.0775	10.0323	3.0452	3.0085	101.22

(Table 5). Recovery of Dapoxetine

S No.	Sample ID	Standard Area	Sample Area	Calculated content (mg of drug)	Actual Assay	Recovered Amount	Actual amount added	Recovery Percentage (%)
					Mg of drug/ avg.Wt			
1	Spiked with 10%	167.667	185.708	32.8852	29.9450	2.9402	2.9542	99.53
2	Spiked with 20%	167.667	203.163	35.8120	29.9450	5.8670	5.8814	99.75
3	Spiked with 30%	167.667	219.635	38.7906	29.9450	8.8456	8.8393	100.07

(Table 6). Accuracy & precession

S. No	Drug	Sample ID	Standard Area	Sample area (Avg)	mg/tab (Avg)	Average at Individual Conc Levels & Assay %	SD at Individual Conc Levels	% CV at Individual Conc Levels	% RSD
		Low level		39.55	9.952	99.53	1.29	1.30	
1.	Tadalafil	Middle level	49.66	50.44	10.052	100.52	0.41	0.41	1.48
		High level		60.75	10.092	100.92	0.59	0.58	
		Low level		133.543	29.964	99.88	0.59	0.59	
2	Dapoxetine	Middle level	168.08	167.912	29.839	99.47	0.80	0.80	0.84
		High level		203.166	30.030	100.10	0.78	0.78	

(Table7). Robustness of Tadalafil

S.No.	Parameter	Drug (Tadalafil)	Avg Peak Area	SD	% RSD
1.	Flow rate	0.4ml/min	63.49	0.32	0.51
		0.8ml/min	38.07	0.18	0.47
2.	Mobile phase Change	10% increase in Aq phase	65.18	0.18	0.28
		10% increase in Org phase	34.07	0.07	0.21
3.	Change in Wave length	256nm	178.47	1.22	0.68
		252nm	60.78	1.13	1.86

(Table 8). Robustness of Dapoxetine

S.No.	Parameter	Drug Dapoxetine	Avg Peak Area	SD	%RSD
1.	Flow rate	0.4ml/min	196.52	0.58	0.30
		0.8ml/min	120.51	0.35	0.29
2.	Mobile phase Change	10% increase in Aq phase	200.93	0.50	0.25
		10% increase in Org phase	104.14	0.42	0.40
3.	Change in Wave length	256nm	142.91	1.28	0.90
		252nm	178.47	1.22	0.68

Limit of detection and limit of quantification:

The LOD can be define as the smallest level of analytes that gives a measurable response and LOQ was determined as the lowest amount of analytes that was reproducibly quantified. These two parameters were calculated using the formula based on the standard deviation of the response and the slope. Limit of detection (LOD) and limit of quantification (LOQ) were calculated by the use of the equation LOD = 3.3X N/B and LOQ = 10XN/B where N is the standard deviation of the peak areas of the corresponding drug sample, taken as the measure of the noise, and B is the slope of the corresponding calibration plot. LOD and LOQ for TAD were 0.0054&0.0218 µg/ml respectively and for DAP were 0.0149&0.0596µg/mL, respectively.

CONCLUSION

The proposed study describes new and simple RP-UPLC method for the simultaneous estimation of Tadalafil and Dapoxetine in combined solid dosage form with good retention time, economical mobile phase and quick run time. The method was validated as per ICH guidelines and found to be simple, sensitive, accurate and precise. Therefore, the proposed method can be successfully used for the routine analysis of simultaneous estimation of Tadalafil and Dapoxetine in combined solid dosage form without interference.

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REFERENCES:

[1] The United States Pharmacopoeia National Formulary 2014; USP-37/NF-32. *U.S.Pharmacopoeial Convention*; p. 4823-4825.(3)2014.

[2] The Martindale. The Complete Drug Reference. 34th ed. Sean C. Sweet man; p. 1751.

[3] Indian Pharmacopoeia 2014; Ministry of health and family welfare, Govt. of India. 7th ed. Ghaziabad: *Indian Pharmacopoeia Commission;* p. 1509-1510. (2)2014.

[4] Reddy.B, Reddy.A, Reddy.M, Validation and stability indicating RP-HPLC methods for the determination of Tadalafil API in pharmaceutical formulation, *Research in Pharmaceutical Biotechnology;*2:1: p. 001-006, 2010.

[5] Kumar.RS, Kumar.BR, Kumar.PA, A RP-HPLC method development and validation for the estimation of Tadalafil in bulk and pharmaceutical dosage forms, *Research Journal of Pharmacy and Technology*; 5:12: p. 1573-1576, 2012.

[6] Jain.V, Modi.A, Jain.A, Shrivastava.A, Telrandhe.U, Jain.S., Validated UV Spectrophotometric method for determination of Tadalafil in bulk and solid dosage form, *Journal of Advance in Drug Research*; p. 13-18, (2) 2012.

[7] Gunjan amin., Development and validation of dual wavelength UV Spectrophotometric method for simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in their combined tablet dosage form, *Int journal of pharmaceutical Research and BioSciences*; p. 247-255, (2)2012.

[8] Chenthilnathan.A, Rajeshwari.M, and Rama.K., Validated RP-HPLC method for simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in combined pharmaceutical dosage forms, *Int journal of pharmacy and Biological Sciences*; p.72-82.(4) 2014.

[9] Sunil.R.dhaneshwar, Abhad.giri, vidhyak.Bhusari., validated HPLC method for simultaneous quantitation of Tadalafil and Dapoxetine hydrochloride in bulk drug and formulation. *Int journal of pharmacy and pharmaceutical sci*;(2). p.654-658, (4)2012.

[10] ICH, Q2 (R1) Validation of Analytical Procedure, Test and Methodology, International Conference on Harmonization, Geneva, 2005.

[11] ICH, Q2A Validation of Analytical Procedures: Consensus Guidelines; ICH Harmonized Tripartite Guidelines, 1994.

[12] ICH, Q2B Validation of Analytical Procedures: Methodology, Consensus Guidelines; ICH Harmonized Tripartite Guidelines,1996.

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