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Validated RP - HPLC Method for Simultaneous Estimation of Tadalafil and Dapoxetine in Combined Pharmaceutical Dosage Forms

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ABSTRACT

Aim: To develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the simultaneous estimation of Tadalafil and Dapoxetine.

Background: Estimation of Tadalafil and Dapoxetine is a combination drug of choice used to treat preamature ejaculation in men.

Objective: The main objective of the Simultaneous estimation of combined drug is to establish identity, physical characteristics and potency of the drugs and to demonstratethe suitability of the assay method to provide useful data to ensure the technique gives satisfactory and consistent results.

Materials and methods: A HPLC (Inertsil, Water2695) with UV/VIS Detector/PDA detector, UV (lab India, UV 3000$^+$ series) and Inertsil C18 250mm × 4.6mm × 5µm column was used. A new method was established for simultaneous estimation of Dapoxetine and Tadalafil by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Dapoxetine and Tadalafil by using inertsil C18 5µm (4.6*250mm) column, flow rate was 1ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 3: MEOH (30:70%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 260nm.

Results: The results were in good agreement with those obtained with official HPLC with absorption maximum of 260 nm by preparing mobile phase 70:30 methanol: phosphate bufferwith flow rate 1 ml/min and it run for 30 minutes by selecting column Inertsil C18 4.6mm×250 mm. All the results obtained with good precise, accurate and robustness as per international conference on Harmonization (ICH) guidelines.

Conclusion: It can be concluded that the proposed RP-HPLC method is accurate, precise, sensitive, robust and reproducible for the simultaneous analysis of Tadalafil and Dapoxetine with less tailing factor and is also economical.Inertsil C18 column (4.6×250mm)5µ, flow rate was 1ml/min. Both samples scan in the range of 200 to 400 nm and maximum wavelength was identified at 260 nm.

KEYWORDS: Dapoxetine and Tadalafil, Inertsil C18, RP-HPLC.

INTRODUCTION

Tadalafil is a phosphodiesterase type 5 (PDE 5) inhibitor which works by increasing blood flow to the penis during sexual stimulation and enables its erection following sexual stimulation. Dapoxetine is a selective serotonin reuptake inhibitor (SSRI) which increases the level of serotonin in nerves to improve control over ejaculation and increase the time taken to ejaculate.[1-3]

Chemicals and Reagents:

Tadalafil and Dapoxetine HCL drug was obtained from MSN Laboratories, Hydrabad. We used HPLC grade acetonitrile, water and GR grade KH_2PO_4 and ortho phosphoric acid .[4-5]

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Instrumentation:
A HPLC (Inertsil, Water2695) with UV/VIS Detector/PDA detector, UV (lab India, UV 3000+series) and Inertsil C18 250mm × 4.6mm × 5µm column was used. The HPLC system was equipped with Empower software for data processing.[6]

Chromatographic Condition:
The mobile phase includes mobile phase 70:30 methanol: phosphate buffer was found to resolve Tadalafil and Dapoxetine. Ortho phosphoric acid was pre-owned for pH adjustment of buffer to 6.8. The mobile phase was strained through 0.45 nylon filter and then ultrasonicated for 30 min. The flow rate was set to 1.0ml/min. The drug shows good absorbanceat 260nm, which was selected as wavelength for further analysis.[7-8]

Preparation of Mobile Phase: Mix a mixture of above methanol 700 ml (70%) and 300 ml ofbuffer (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.45 µ filter under vacuum filtration.[9]

Preparation of Sample solution: Accurately weigh and transfer 10 mg of Dapoxetine and 10mg Tadalafil Tablet powder into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the standard with the same solvent. Further pipette 0.6ml of Dapoxetine and Tadalafil from the above stock solution into a 10ml volumetric flask and dilute up to the norm with diluent.[10-12]

RESULTS AND DISCUSSION

Method Development

The identification wavelength was adopted by dissolving the drug in mobile phase to get a concentration of 10µg/ml for individual and mixed standards. The resulting solution was scanned in Ultraviolet (U.V) range from 200-400nm. The overlay spectrum of Tadalafil and Dapoxetine was obtained and the isobestic point of Tadalafil and Dapoxetine showed absorbance's maxima at 260 nm. Chromatographic method development was optimized by various parameters both in Active Pharmaceutical Ingredient (API) and pharmaceutical dosage form in Figure 1.
The Optimized Chromatographic conditions by preparing mobile phase 70:30 methanol:phosphate buffer with flow rate 1 ml/min and it run for 10 minutes by selecting column Inertsil C18 4.5×250 mm 5.0 µm of ambient temperature.
The retention time of Tadalafil and Dapoxetine was found to be 2.5 mins and 3.9mins respectively was shown in figure no 2.

VALIDATION REPORT

Linearity

The linearity study was performed for the concentration of 100 ppm to 500 ppm for Tadalafil and 1ppm to 5ppm for Dapoxetine was shown in Table no 1**Accuracy**
The accuracy study was performed for 50%, 100% and 150 % for Tadalafil and Dapoxetine.The percentage % retrieval was found to be 99.84% and 100.51% was shown in Table no 2 and 3.

Precision (Repeatability)

The precision evaluation was performed for five injections of Tadalafil and Dapoxetine. Each standard injection was injected into chromatographic system. Theintermediate precision study was performed for five injections was shown in Table no 4.

Intermediate Precision (Ruggedness)

The intermediate precision evaluation was performed for five injections of Tadalafil and Dapoxetine. Each standard injection was injected into chromatographic system. Theintermediate precision study was performed for five injections was shown in Table no 5.

LOD and LOQ

The LOD was performed for Tadalafil and Dapoxetine was estimated to be 2.9 and 3 respectively.The LOQ was performed for Tadalafil and Dapoxetine was estimated to be 10.03 and 10.1 respectively was shown in Fig No 3.

Robustness

The robustness was performed for the flow rate variations from 0.6ml/min to 1.2ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Tadalafil and Dapoxetine which can be resulted that the variation in flow rate affected

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the method significantly was shown in Table no 6.

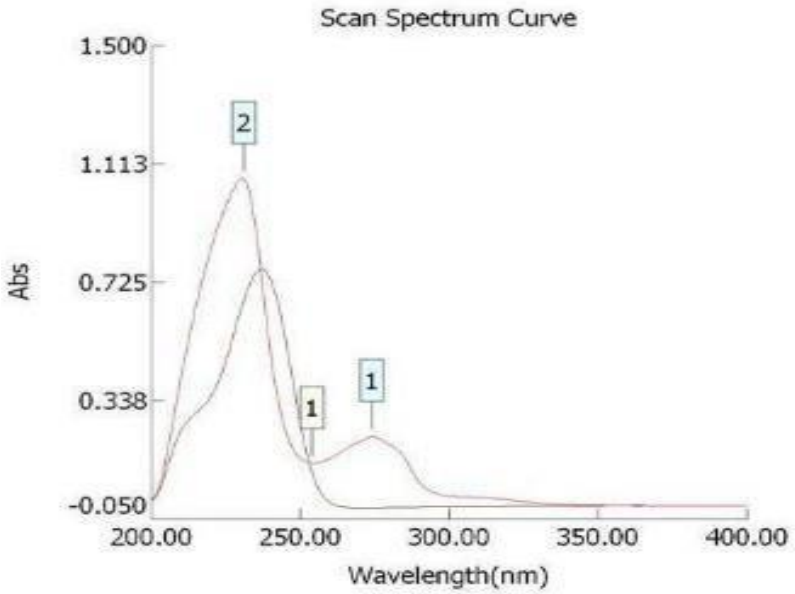

Fig No 1. Spectrum showing overlapping spectrum of Tadalafil and Dapoxetine

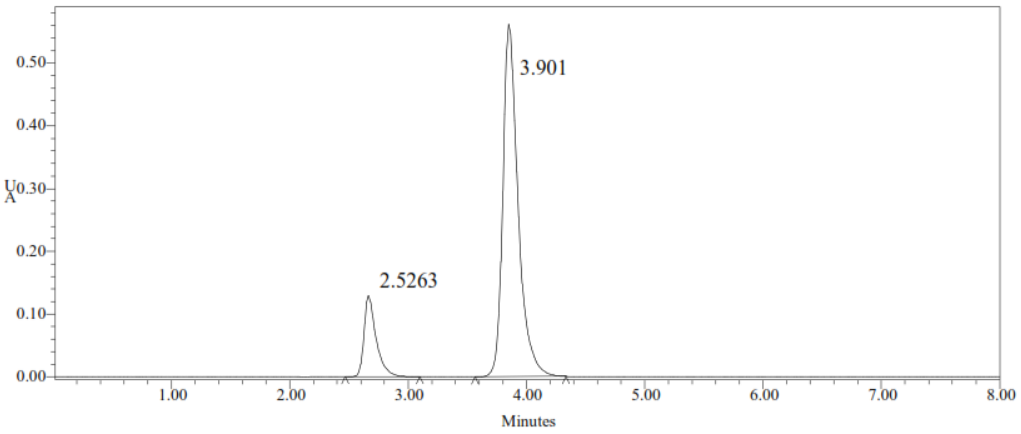

Fig No 2. The retention time of Tadalafil and Dapoxetine was found to be 2.5 mins and 3.9 mins respectively**.**

Table no 1. Linearity Results for Tadalafil and Dapoxetine

S. No	Linearity Level	Concentration Tadalafil	Concentration Dapoxetine	Area (Tadalafil)	Area (Dapoxetine)
1	I	100ppm	1ppm	668934	66510
2	II	200ppm	2ppm	956781	94701
3	III	300ppm	3ppm	1313873	124802
4	IV	400ppm	4ppm	1563458	152731
5	V	500ppm	5ppm	1867084	179732
Correlation Coefficient				**0.999**	

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Table no 2. Accuracy Results for Tadalafil

%Concentration (at specification Level) Tadalafil	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table no 3. Accuracy results of Dapoxetine

%Concentration (at specification Level) Dapoxetine	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table no 4.Results of Precision for Tadalafil and Dapoxetine

injection	Area Tadalafil	Area Dapoxetine
Injection-1	1302729	123149
Injection-2	1302947	123766
Injection-3	1303236	124271
Injection-4	1303977	124691
Injection-5	1309759	124956
Average	1304529.8	12412.7
StandardDeviation	2961.1	725.6
%RSD	0.2	0.6

Table no 5. Results of Intermediate precision for Tadalafil and Dapoxetine

injection	Area Tadalafil	Area Dapoxetine
Injection-1	1300148	122487
Injection-2	1304520	122626
Injection-3	1305937	122632
Injection-4	1306476	122702
Injection-5	130871	122962
Average	1305070.2	122681.8
StandardDeviation	3061.8	174.8
%RSD	0.2	0.1

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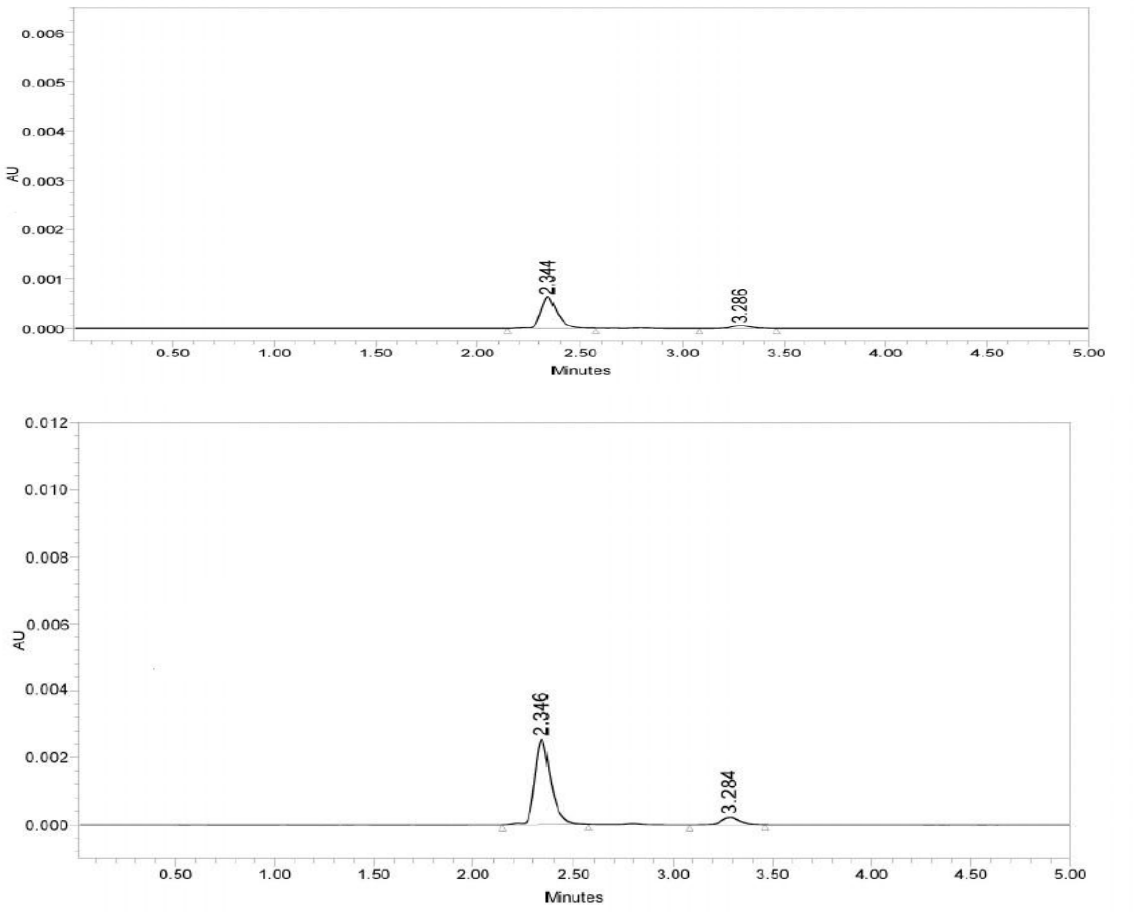

Fig No 3. The LOD was performed for Tadalafil and Dapoxetine was estimated to be 2.9 and 3 respectively and LOQ was found to be 10.03 and 10.1 respectively.

Table no 6. Robustness results for Tadalafil and Dapoxetine

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results			
		USP PlateCount Tadalafil	USP PlateCount Dapoxetine	USP Tailing Tadalafil	USP Tailing Dapoxetine
1	10% less	4508.4	6387.7	1.3	1.2
2	*Actual	4673.4	6090.3	1.4	1.2
3	10% more	4318.1	6232.5	1.3	1.2

CONCLUSION

The developed analytical method was validated as per ICH guidelines and it meets the acceptance criteria of each parameter. It is concluded that the developed method is simple, linear, precise, accurate, robust and sensitive to analyze the simultaneous analysis of Tadalafil and Dapoxetine with less tailing factor and is also economical. Inertsil C18 250mm × 4.6mm × 5µm, flow rate was 1ml/min. Both samples scan in the range of 200 to 400 nm and maximum wavelength was identified at 260 nm. The main advantage of developed UV method over HPLC method is that it is less time consuming and also economical.

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CONSENT AND ETHICAL APPROVAL
It is not applicable

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COMPETING INTEREST
Authors have declared that no completing interests exists

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