

VALIDATED HPLC METHOD FOR SIMULTANEOUS QUANTITATION OF TADALAFIL AND DAPOXETINE HYDROCHLORIDE IN BULK DRUG AND FORMULATION

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ABSTRACT

HPLC method has been described for simultaneous determination of Tadalafil and Dapoxetine Hydrochloride in formulation. This method is based on HPLC separation of the two drugs on the Thermo Hypersil BDS-C₁₈ (250 mm × 4.6 mm, 5.0 μ) from Germany with isocratic conditions and simple mobile phase containing acetonitrile: 0.1 % triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid (80: 20) at flow rate of 1 mL/min using UV detection at 229 nm. This method has been applied to formulation without interference of excipients of formulation. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 0.25-4 μg/mL for Tadalafil and 0.75-12 μg/mL for Dapoxetine Hydrochloride respectively. The mean values of the correlation coefficient, slope and intercept were 0.9995 ± 1.27, 133612 ± 0.72 and 49109 ± 1.21 for Tadalafil and 0.9992 ± 0.78, 155825 ± 0.87 and 132842 ± 0.54 for Dapoxetine Hydrochloride, respectively. The method was validated for precision, robustness and recovery. The limit of detection (LOD) and limit of quantitation (LOQ) was 0.010 μg/mL and 0.025 μg/mL for Tadalafil and 0.005 μg/mL and 0.020 μg/mL for Dapoxetine Hydrochloride, respectively. Statistical analysis showed that the method is repeatable and selective for the estimation of Tadalafil and Dapoxetine Hydrochloride.

Keywords: Tadalafil; Dapoxetine Hydrochloride; HPLC; Validation.

INTRODUCTION

Chemically Tadalafil is pyrazino-[1',2':1,6]pyrido[3,4-b]indole-1,4-dione,6-(1,3-benzodioxol-5-yl)-2,3,6,7,12,12a-hexahydro-2-methyl-,(6R,12aR) (Figure 1)¹. Tadalafil is classified as potent and highly selective phosphodiesterase type 5 (PDE5) inhibitor. Inhibition of PDE5 prevents cyclic guanosine monophosphate (cGMP) from being degraded in penile tissue. When the enzyme is inhibited, relaxation of the smooth muscle in the corpus cavernosum occurs and leads to inflow of blood, potentiating erection².

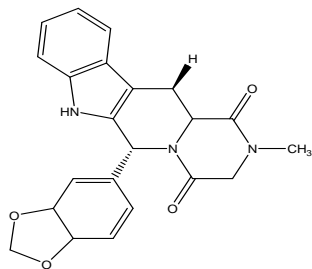


Fig. 1: Structure of Tadalafil

Dapoxetine Hydrochloride, (1S)-N,N-dimethyl-3-naphthalen-1-yloxy-1-phenylpropan-1-amine hydrochloride (Figure 2) is used for treatment of premature ejaculation in men³. Dapoxetine is a short-acting selective serotonin reuptake inhibitor (SSRI). SSRIs are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders. They can also sometimes be effective and used in treating premature ejaculation problems, impotence and some cases of insomnia⁴.

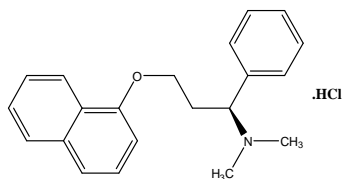


Fig. 2: Structure of Dapoxetine Hydrochloride

Literature review reveals that methods have been reported for analysis of Tadalafil and Dapoxetine individually such as, validation and stability indicating RP-HPLC method for the determination of Tadalafil API in pharmaceutical⁵, a sensitive and simple high performance liquid chromatographic method for quantification of Tadalafil in human serum⁶, stress degradation studies on Tadalafil and development of a validated stability-indicating LC assay for bulk drug and pharmaceutical dosage form⁷ and Tadalafil pharmacokinetics in healthy subjects⁸, development and validation of a RP-HPLC method for the determination of Dapoxetine Hydrochloride in pharmaceutical formulation using an experimental design⁹, determination of Dapoxetine, an investigational agent with the potential for treating depression, and its mono- and di-desmethyl metabolites in human plasma using column-switching high-performance liquid chromatography¹⁰, and pharmacokinetics of Dapoxetine Hydrochloride is also reported¹¹.

To date, there have been no published reports about the simultaneous quantitation of Tadalafil and Dapoxetine Hydrochloride by HPLC in bulk drug and in tablet dosage form. This present study reports for the first time simultaneous quantitation of Tadalafil and Dapoxetine by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines^{12,13,14}.

MATERIALS AND METHODS

Materials

Om Laboratories Gandhinagar, India, kindly supplied pure drug sample of Tadalafil as a gift sample of Batch No.: TDF/11-12/04 and Dapoxetine Hydrochloride of Batch No.: DP/01/11-12. It was used without further purification and certified to contain 99.80 % (w/w) for Tadalafil and 99.00 % (w/w) for Dapoxetine Hydrochloride on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Instrumentation

The HPLC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 μL capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 229 nm. Data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was Thermo Hypersil BDS-C₁₈ (250 mm × 4.6 mm, 5.0 μ) from Germany.

Preparation of Standard Stock Solutions

Standard stock solution of concentration 1000 µg/mL of Tadalafil and 1000 µg/mL of Dapoxetine hydrochloride was prepared using methanol. From the standard stock solution, the mixed standard solutions were prepared using mobile phase to contain 1 µg/mL of Tadalafil and 3 µg/mL of Dapoxetine Hydrochloride.

Optimization of HPLC Method

The HPLC procedure was optimized with a view to develop a simultaneous assay method for Tadalafil and Dapoxetine Hydrochloride, respectively. The mixed standard stock solution (1 µg/mL of Tadalafil and 3 µg/mL of Dapoxetine Hydrochloride) was injected in HPLC. For HPLC method optimization methanol and water was tried in the ratio of 80: 20 but it was found that the

peak shape was not good for both the drugs. Hence water was replaced with buffer. Methanol: phosphate buffer pH 4.0 adjusted with ortho phosphoric acid in the ratio of 80: 20 was taken, it was found that sharp peak was obtained for Tadalafil but hump shape peak was obtained for Dapoxetine Hydrochloride. Then buffer was replaced with triethyl amine. Methanol: 0.1 % triethyl amine in water pH adjusted to 4 with ortho phosphoric acid in the ratio of 80: 20 was tried and it was found that sharp peak was obtained for Tadalafil but slight tailing was observed for Dapoxetine Hydrochloride. Finally, methanol was replaced with acetonitrile and the mobile phase used was acetonitrile: 0.1 % triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid in the ratio 80: 20 v/v, at flow rate 1 mL/min gives acceptable retention time (t_R), plates and good resolution for Tadalafil and Dapoxetine Hydrochloride (Figure 3).

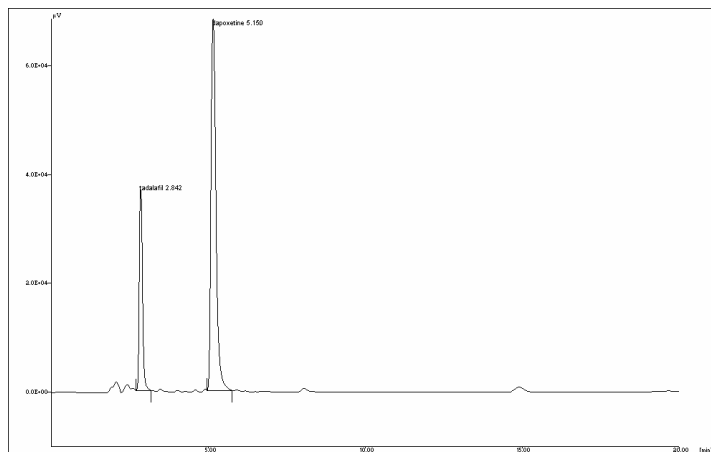


Fig. 3: HPLC chromatogram of standard Tadalafil and Dapoxetine Hydrochloride (1 µg/mL and 3 µg/mL)

Validation of the method

Validation of the optimized HPLC method was carried out with respect to the following parameters:

Linearity and range

The mixed standard stock solution (1000 µg/mL of Tadalafil and 1000 µg/mL of Dapoxetine Hydrochloride) was further diluted to get Tadalafil and Dapoxetine Hydrochloride concentration in the range of 0.25-4 µg/mL and 0.75-12 µg/mL, respectively. Linearity of the method was studied by injecting six concentrations of the drug prepared in the mobile phase in triplicate into the LC system keeping the injection volume constant. The peak areas were plotted against the corresponding concentrations to obtain the calibration graphs.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations 0.25, 1.0, 3.0 µg/mL for Tadalafil and 0.75, 3.0, 9.0 µg/mL for Dapoxetine Hydrochloride six times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitation

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. To determine the LOD and LOQ, serial dilutions of mixed standard solution of Tadalafil and Dapoxetine Hydrochloride was made from the standard stock solution. The samples were injected in LC system and measured signal from the samples was compared with those of blank samples.

Robustness of the method

To evaluate robustness of a HPLC method, few parameters were deliberately varied. The parameters included variation of flow rate, percentage of acetonitrile in the mobile phase and solvents from different lot were taken. Robustness of the method was done at three different concentration levels 0.25, 1.0, 3.0 µg/mL and 0.75, 3.0, 9.0 µg/mL for Tadalafil and Dapoxetine Hydrochloride, respectively.

Specificity

The specificity of the method towards the drug was established through study of resolution factor of the drug peak from the nearest resolving peak. The peak purity of Tadalafil and Dapoxetine Hydrochloride 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). Effect of excipients of formulation was studied for whether it interfered with the assay.

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Tadalafil and Dapoxetine Hydrochloride combination tablet) to which known amount of Tadalafil and Dapoxetine Hydrochloride 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 a marketed formulation

To determine the content of Tadalafil and Dapoxetine Hydrochloride in conventional tablet (Brand name: Super Vidalista, Label claim: 20 mg Tadalafil and 60 mg Dapoxetine Hydrochloride per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 20 mg of Tadalafil and 60 mg Dapoxetine Hydrochloride was

transferred into a 50 mL volumetric flask containing 30-35 mL methanol, sonicated for 30 min and diluted upto 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (400 and 1200 µg/mL for Tadalafil and Dapoxetine Hydrochloride, respectively). Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 micron filter (Millipore, Milford, MA). The above stock solution was further diluted to get sample solution of 2 and 6 µg/mL for Tadalafil and Dapoxetine Hydrochloride, respectively. A 20 µL volume of sample solution was injected into HPLC, six times, under the conditions described above. The peak areas were measured at 229 nm and concentrations in the samples were determined using multilevel calibration developed on the same HPLC system under the same conditions using linear regression equation.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Tadalafil and Dapoxetine Hydrochloride in the current

study involving acetonitrile: 0.1 % triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid (80: 20) are given below.

Linearity

Tadalafil and Dapoxetine Hydrochloride showed good correlation coefficient ($r^2 = 0.9995$ for Tadalafil and 0.9992 for Dapoxetine Hydrochloride) in given concentration range (0.25-4 µg/mL for Tadalafil and 0.75-12 µg/mL for Dapoxetine Hydrochloride). The mean values of the slope and intercept were 133612 ± 0.72 and 49109 ± 1.21 for Tadalafil and 155825 ± 0.87 and 132842 ± 0.54 for Dapoxetine Hydrochloride, respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were $< 2\%$, respectively as recommended by ICH guidelines.

Table 1: Precision studies

Concentration (µg/mL)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
Tadalafil						
0.25	0.248 ± 0.98	1.16	99.20	0.245 ± 1.32	0.91	98.00
1	0.988 ± 2.52	0.92	98.80	0.991 ± 2.24	0.72	99.10
3	2.950 ± 5.32	0.95	98.33	2.980 ± 2.57	1.56	99.33
Dapoxetine Hydrochloride						
0.75	0.739 ± 1.25	0.52	98.53	0.758 ± 3.72	1.51	101.07
3	2.980 ± 3.39	1.04	99.33	2.950 ± 5.81	1.72	98.33
9	8.950 ± 5.21	1.24	99.44	8.820 ± 2.25	1.91	98.00

Table 2: Robustness testing^a (n = 3)

Factor ^a	Level	Retention time	Retention factor	Asymmetry
Tadalafil				
A: Flow rate (mL/min)				
0.9	-1	2.89	0.15	1.40
1.0	0	2.85	0.14	1.38
1.1	+1	2.84	0.13	1.34
Mean ± SD (n = 3)		2.85 ± 0.07	0.25 ± 0.03	1.38 ± 0.04
B: % of acetonitrile in the mobile phase (v/v)				
79	-1	2.88	0.15	1.41
80	0	2.85	0.14	1.38
81	+1	2.82	0.12	1.35
Mean ± SD (n = 3)		1.85 ± 0.05	0.14 ± 0.02	1.15 ± 0.03
C: Solvents of different lots				
First lot		2.85	0.14	1.38
Second lot		2.87	0.15	1.39
Mean ± SD (n = 3)		2.86 ± 0.01	0.14 ± 0.01	1.38 ± 0.01

Dapoxetine Hydrochloride

A: Flow rate (mL/min)				
0.9	-1	5.30	0.40	1.40
1.0	0	5.25	0.39	1.36
1.1	+1	5.20	0.37	1.32
Mean ± SD (n = 3)		5.25 ± 0.05	0.39 ± 0.01	1.36 ± 0.04
B: % of acetonitrile in the mobile phase (v/v)				
79	-1	5.27	1.10	1.38
80	0	5.25	1.10	1.36
81	+1	5.22	1.08	1.33
Mean ± SD (n = 3)		5.25 ± 0.05	1.10 ± 0.02	1.15 ± 0.03
C: Solvents of different lots				
First lot		5.25	1.10	1.36
Second lot		5.27	1.11	1.37
Mean ± SD (n = 3)		5.26 ± 0.01	1.1 ± 0.01	1.36 ± 0.01

^a Three factors were slightly changed at three levels (-1, 0, 1)

LOD and LOQ

Signal-to-noise ratios of 3:1 and 10:1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 0.010 µg/mL and 0.025 µg/mL for Tadalafil and 0.005 µg/mL and 0.020 µg/mL Dapoxetine Hydrochloride, respectively.

Robustness of the method

Each factor selected (except columns from different manufacturers) was changed at three levels (-1, 0 and 1). One factor at the time was changed to estimate the effect. Thus, replicate injections (n = 6) of mixed standard solution at three concentration levels were performed under small changes of three chromatographic parameters (factors). Insignificant differences in peak areas and less variability in retention time were observed (Table 2).

Specificity

The peak purity of Tadalafil and Dapoxetine Hydrochloride was assessed by comparing their respective spectra at the peak start, apex and peak end positions i.e., r (S, M) = 0.9992 and r (M, E) =

0.9990. A good correlation (r = 0.9989) was also obtained between the standard and sample spectra of Tadalafil and Dapoxetine Hydrochloride, respectively. Also, excipients from formulation were not interfering with the assay.

Recovery Studies

As shown from the data in Table 3 good recoveries of the Tadalafil and Dapoxetine Hydrochloride in the range from 99 to 101 % were obtained at various added concentrations.

Analysis of a formulation

Experimental results of the amount of Tadalafil and Dapoxetine Hydrochloride in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present. The drug content was found to be 99.83 % for Tadalafil and 100.93 % for Dapoxetine Hydrochloride. Two different lots of Tadalafil and Dapoxetine Hydrochloride combination tablets were analyzed using the proposed procedures as shown in Table 4.

Table 3: Recovery studies (n = 6)

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Tadalafil				
20	16 (80%)	36	36.70 ± 0.96	101.94
20	20 (100%)	40	39.75 ± 1.01	99.37
20	24 (120%)	44	43.80 ± 0.78	99.54
Dapoxetine Hydrochloride				
60	48 (80%)	108	106.5 ± 1.16	98.61
60	60 (100%)	120	120.5 ± 1.40	100.41
60	72 (120%)	132	130.0 ± 0.98	98.48

Table 4: Analysis of commercial formulation

	Tadalafil found (mg per tablet)	Recovery (%)
Tadalafil (20 mg)	Mean ± SD (n= 6)	
1 st Lot	19.85 ± 1.06	99.25
2 nd Lot	20.08 ± 0.94	100.40
Dapoxetine Hydrochloride (60 mg)	Dapoxetine Hydrochloride found (mg per tablet)	Recovery (%)
1 st Lot	Mean ± SD (n= 6)	
1 st Lot	60.40 ± 1.16	100.66
2 nd Lot	60.72 ± 1.04	101.20

CONCLUSION

HPLC method was developed and validated as per ICH guidelines. UV detection allowed an accurate quantitation of chromophoric compounds.

The drug was analysed by HPLC method using Thermo Hypersil BDS-C₁₈ (250 mm × 4.6 mm, 5.0 µ) from Germany with isocratic conditions and simple mobile phase containing acetonitrile: 0.1 % triethyl amine in water pH adjusted to 4.0 with ortho phosphoric acid (80: 20) at flow rate of 1 mL/min using UV detection at 229 nm. The procedure has been evaluated for the linearity, accuracy, precision and robustness in order to ascertain the suitability of the analytical method. The method was also applied to marketed samples. It has been proved that the method is selective and linear between concentration range 0.25-4 µg/mL for Tadalafil and 0.75-12 µg/mL for Dapoxetine Hydrochloride. LOD was found to be 0.010 µg/mL and LOQ was found to be 0.025 µg/mL for Tadalafil and LOD was found to be 0.005 µg/mL and LOQ was found to be 0.020 µg/mL for Dapoxetine Hydrochloride.

Statistical analysis proves that the method is suitable for the analysis of Tadalafil and Dapoxetine Hydrochloride as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Tadalafil and Dapoxetine Hydrochloride and also for its estimation in plasma and other biological fluids.

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