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VALIDATED RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF TADALAFIL AND DAPOXETINE HYDROCHLORIDE IN COMBINED PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, efficient and reproducible Reverse Phase-High Performance Liquid Chromatography (RP-HPLC) method for simultaneous determination of Tadalafil and Dapoxetine hydrochloride in combined pharmaceutical solid dosage form has been developed. The separation was carried out on Hypersil BDS C8, 250 × 4.6mm column using buffer (6.8g of potassium dihydrogen orthophosphate and 0.94g of sodium hydroxide in 900ml water, adjusted to pH 6.8 with ortho phosphoric acid and make up the volume to 1000ml with water): acetonitrile in the ratio 55:45 v/v as eluent. The flow rate was 1.0 ml/min and effluent was detected at 254 nm. The retention times of tadalafil and dapoxetine hydrochloride were 4.473 min and 5.836 min respectively. The percentage recovery was within the range between 99.88% to 100.37% for tadalafil, 99.87% to 100.08% for dapoxetine hydrochloride. The linear ranges were found to be 50-150µg/ml (r^2 = 0.9984) for tadalafil, and 50-150µg/ml (r^2 = 0.9962) for dapoxetine hydrochloride. The percentage relative standard deviation for accuracy and precision was found to be less than 2%. Hence, the method could be successfully applied for routine analysis of tadalafil and dapoxetine hydrochloride in the combined solid dosage form.

KEY WORDS

Tadalafil, Dapoxetine hydrochloride, Solid dosage form, RP-HPLC

INTRODUCTION

Tadalafil (**Fig.1**) Chemically, 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) is classified as potent and highly selective phosphodiesterase type 5(PDE5) inhibitor. When the enzyme is inhibited, relaxation of the smooth muscle in the corpus cavernosum occurs and leads to inflow of blood, potentiating erection [1].

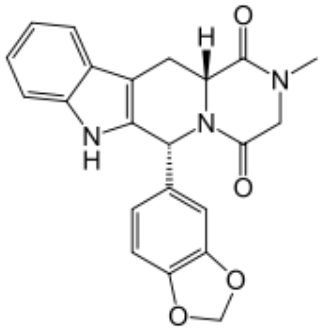
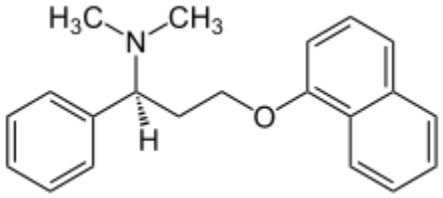


Figure 1: Structure of Tadalafil

Dapoxetine hydrochloride (Fig.2) chemically, N, N-dimethyl - 3 –naphthalen - 1 - yloxy- 1 – phenylpropan -1-amine hydrochloride, is used for treatment of premature ejaculation in men. It is a short-acting

selective serotonin reuptake inhibitor (SSRI). It can also sometimes be effective and used in treating premature ejaculation problems, impotence and some cases of insomnia.



. HCL

Figure 2: Structure of Dapoxetine hydrochloride

Literature review reveals that methods have been reported for analysis of Tadalafil and Dapoxetine hydrochloride individually such as, validation and stability indicating RP-HPLC method for the determination of Tadalafil API in pharmaceutical [2], a sensitive and simple high performance liquid chromatographic method for quantification of Tadalafil in human serum [3], stress degradation studies on Tadalafil and development of a validated stability-indicating LC assay for bulk drug and pharmaceutical dosage form and Tadalafil pharmacokinetics in healthy subjects [4], development and validation of a RP-HPLC method for the determination of Dapoxetine hydrochloride in pharmaceutical formulation using an experimental design [5], determination of Dapoxetine hydrochloride, 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 chromatography [6] and pharmacokinetics of Dapoxetine hydrochloride is also reported [7]. However, no method has been reported for the simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in combined solid dosage form. Hence, a sample, rapid, precise, accurate RP-HPLC method for the simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in combined solid dosage form is developed and validated.

MATERIALS AND METHODS

Experimental

Chemicals and reagents

Acetonitrile of HPLC grade and Methanol were purchased from E.Merck (India) Ltd., Mumbai. Orthophosphoric acid of AR grade was obtained from Qualigens Fine Chemicals Ltd., Mumbai. Tadalafil and Dapoxetine hydrochloride were a gift sample by Sai Mirra Innopharm Pvt. Ltd., Chennai – 600 098, Tamil Nadu, India. The commercially available tablets containing Tadalafil and Dapoxetine hydrochloride were procured from the local market.

Instrumentation and chromatographic conditions

The chromatographic separation was carried out on HPLC system (Shimadzu 1100 Series, Germany) with UV- Visible dual absorbance detector (PDA), Hypersil BDS C_8 column (250 x 4.6mm). The mobile phase consisting of buffer (6.8g of potassium dihydrogen orthophosphate and 0.94g of sodium hydroxide in 900ml water, adjusted to pH 6.8 with ortho phosphoric acid and make up the volume to 1000ml with water) : acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 55:45 v/v was pumped into the column at a flow rate of 1.0 ml/min. The detection was monitored at 254 nm. The volume of injection loop was 10 µl prior to the injection of the drug solution; the column was equilibrated for at least 30 min. with the mobile phase following through the system.

Preparation of Standard solutions

Accurately weighed about 20mg of Tadalafil working standard and 67.2mg of Dapoxetine hydrochloride working standard into 200ml volumetric flask. Dissolved and diluted to the required volume with mobile phase and mixed well.

Analysis of Sample Preparation

Accurately weighed about 275 mg of the sample into a 100 ml volumetirc flask. 50 ml of mobile phase was added and sonicated for 30 minutes. Cooled and diluted to the required volume with mobile phase and mixed well. Filtered through 0.45µ membrane filter.

Calculation: Calculate the amount of Tadalafil and Dapoxetine hydrochloride present in mg per average weight of tablet using the formula: Tadalafil

Test area Std. wt 100 P
---------- x -------- x ------- x --------- x Avg.wt.
Std. area 200 Test wt. 200
Dapoxetine hydrochloride equivalent to Dapoxetine:

Test area Std. wt 100 305.41 P
---------- x -------- x -------- x---------- x --------- x Avg.wt.
Std. area 200 Test wt. 341.87 100

RESULTS AND DISCUSSION

All of the analytical validation parameters for the proposed method were determined according to International Conference on Harmonization (ICH) guidelines [8].

System Suitability

It is essential for the assurance of the quality performance of chromatographic system. Five injections of standard drug solutions, tadalafil and dapoxetine hydrochloride were given separately to the system. The system suitability parameters such as retention time, peak area response and Number of theoretical plates and also their Mean, Standard deviation & %RSD were also be calculated for the standard drug solutions and mentioned in Table 1 and 2. It was observed that all the values are within the limits.

Table 1: System suitability for Tadalafil

S.No.	Standard	System suitability parameters		
		Retention time (min)	Peak area response	Number of theoretical plates
1.	Standard -1	4.478	1304610	6164
2.	Standard -2	4.474	1305629	6143
3.	Standard -3	4.472	1304074	6187
4.	Standard -4	4.471	1304677	6182
5.	Standard -5	4.470	1305888	6168
Mean		4.473	1304976	6169
Standard deviation		0.0031	757.48	17.28
RSD in %		0.07	0.06	0.28

Table 2: System suitability for Dapoxetine hydrochloride

S.No.	Standard	System suitability parameters		
		Retention time (min)	Peak area response	Number of theoretical plates
1.	Standard -1	5.844	1658051	2354
2.	Standard -2	5.839	1658091	2357
3.	Standard -3	5.835	1656954	2366
4.	Standard -4	5.832	1656463	2368
5.	Standard -5	5.832	1656398	2372
Mean		5.8364	1657191	2363
Standard deviation		0.0051	831.35	7.60
RSD in %		0.09	0.05	0.32

Specificity

The specificity of the HPLC method is illustrated in Fig. 3, where complete separations of tadalafil and dapoxetine hydrochloride were noticed in presence of other inactive excipients used in tablets. In addition, there was no any interference at the retention time of in the chromatogram of placebo solution. In peak purity analysis with PDA, purity angle was always less than purity threshold for the analyte. This shows that the peaks of analyte were pure and excipients in the formulation does not interfere the analyte. The data were presented in the Table 3 and 4.

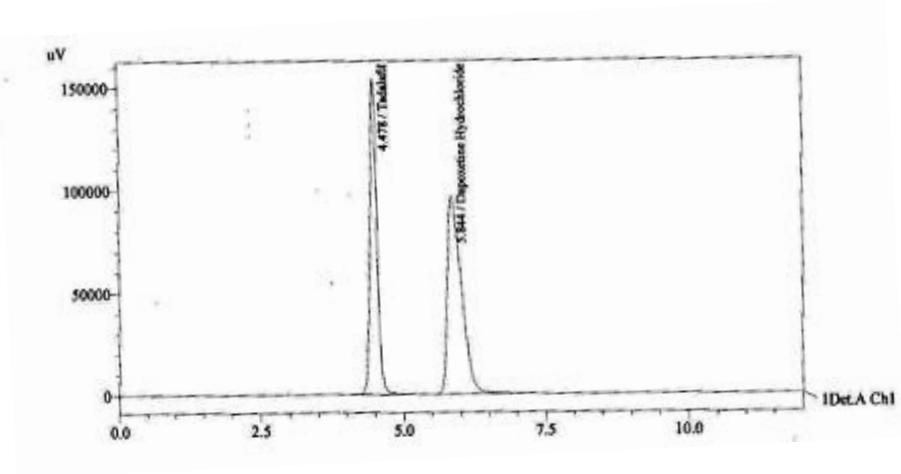
Table 3: Specificity for Tadalafil

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1304610
4.	Sample	1	1302381

Table 4: Specificity for Dapoxetine hydrochloride

S.No.	Name	No. of Injections	Area
1.	Blank	1	Nil
2.	Placebo	1	Nil
3.	Standard	1	1658051
4.	Sample	1	1655778

Figure 3 : Typical HPLC Chromatogram of Sample Tablets
(Tadalafil and Dapoxetine hydrochloride)



Linearity and Range

The Linearity of this method was determined at five levels from 50%– 150% of operating concentrations for tadalafil and dapoxetine hydrochloride and it was shown in Table 5 and 6. The plots of peak area of each sample against respective concentration of tadalafil and dapoxetine hydrochloride were found to be linear (Figure 5 and 6) in the range of 50%– 150% of operating concentrations. Beer's law was found to be obeyed over this concentration range. The linearity was evaluated by linear regression analysis using least square method. The regression equations were found to be Y= 12832x +2160.9 and Y= 5536.2x +1310.1 for tadalafil and dapoxetine hydrochloride respectively and correlation coefficient of the standard curves were found to be 0.9984 and 0.9962 for tadalafil and dapoxetine hydrochloride respectively. It observed

that correlation coefficient and regression analysis are within the limits.

Table 5: Linearity of response for Tadalafil

S.No	Linearity Level	Concentration (µg/ml)	Area
1.	Linearity -1	50	621922
2.	Linearity -2	80	1054945
3.	Linearity -3	100*	1298425
4.	Linearity -4	120	1531927
5.	Linearity -5	150	1919658

Table 6: Linearity of response for Dapoxetine hydrochloride

S.No	Linearity Level	Concentration (µg/ml)	Area
1.	Linearity -1	150	831010
2.	Linearity -2	240	1351602
3.	Linearity -3	300*	1660674
4.	Linearity -4	360	1927109
5.	Linearity -5	450	2527420

*** Operating concentration**

Figure 5: Linearity of response for Tadalafil

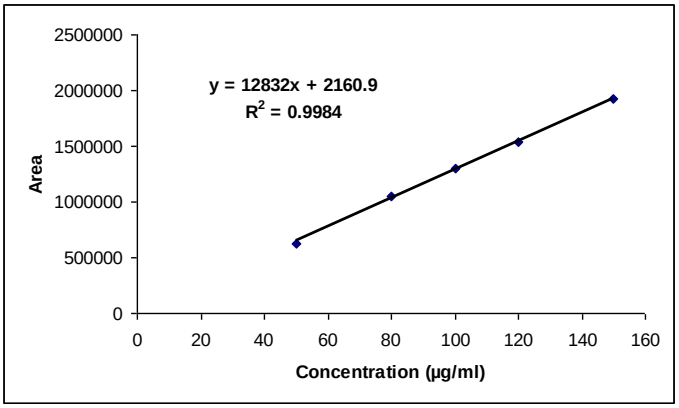
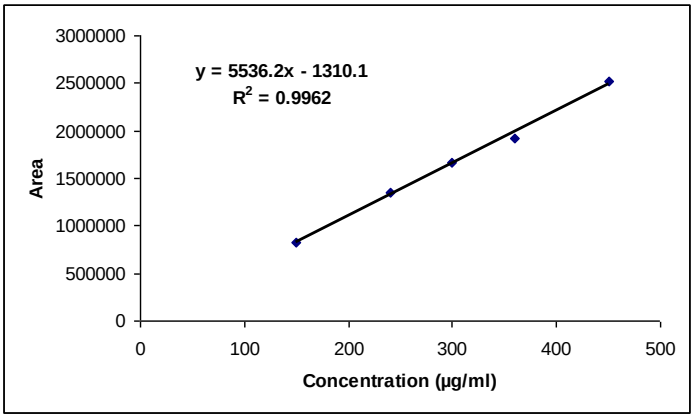


Figure 6: Linearity of response for Dapoxetine hydrochloride



Accuracy

Accuracy of the method was found out by recovery study by standard addition method. The known amounts of standards, tadalafil and dapoxetine hydrochloride were added to pre-analysed samples at a level from 80% up to 120% and then subjected to the proposed HPLC method individually. The results of recovery studies were shown in Table 7 and 8. It was observed that the mean percentage recoveries were found to be 100.11 and 99.97 for tadalafil and dapoxetine hydrochloride respectively which demonstrated that the method was highly accurate.

Table 7: Accuracy for Tadalafil

S.No.	Amount of Tadalafil spiked (mg)	Area	Amount recovered (mg)	Recovery (%)
1.	8.03	1033309.0	8.06	100.37
2.	8.09	1036121.5	8.10	100.12
3.	8.10	1039157.0	8.09	99.88
4.	10.01	1295962.0	10.02	100.10
5.	10.04	1304258.0	10.07	100.30
6.	10.06	1306953.0	10.05	99.90
7.	12.08	1567515.5	12.09	100.08
8.	12.10	1559884.5	12.10	100.00
9.	12.07	1565885.5	12.09	100.26
Mean				100.11
Standard deviation				0.1718
RSD in %				0.17

Table 8: Accuracy for Dapoxetine hydrochloride

S.No.	Amount of Dapoxetine hydrochloride spiked (mg)	Area	Amount recovered (mg)	Recovery (%)
1.	24.04	1309392.5	24.05	100.04
2.	24.06	1308047.5	24.08	100.08
3.	24.03	1309880.5	24.00	99.88
4.	30.03	1650178.0	30.04	100.03
5.	30.03	1651926.5	30.02	99.97
6.	30.04	1656876.5	30.00	99.87
7.	36.04	1984697.5	36.03	99.97
8.	36.19	1982813.5	36.20	100.03
9.	36.06	1982302.0	36.04	99.94
Mean				99.97
Standard deviation				0.0728
RSD in %				0.07

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the homogenous sample under the prescribed conditions.

Reproducibility

Examines the precision between laboratories and is often determined in collaborative studies. Reproducibility data for tadalafil and dapoxetine hydrochloride were shown in Table 9and 10. This indicated that method was highly precise.

Table 9: Precision - Reproducibility for Tadalafil

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	274.3	1302712.5	10.07
2.	Standard -2	275.3	1306904.5	10.07
3.	Standard -3	274.9	1298548.0	10.02
4.	Standard -4	274.5	1309124.5	10.11
5.	Standard -5	275.6	1302457.0	10.02
6.	Standard -6	275.5	1302722.0	10.03
Mean			1303744.75	10.05
Standard deviation			3733.67	0.0361
RSD in %			0.29	0.36

Table 10: Precision - Reproducibility for Dapoxetine hydrochloride

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	274.3	1651623.5	30.07
2.	Standard -2	275.3	1647787.5	29.89
3.	Standard -3	274.9	1656841.0	30.10
4.	Standard -4	274.5	1649205.0	30.00
5.	Standard -5	275.6	1653532.0	29.96
6.	Standard -6	275.5	1655599.5	30.01
Mean			1652431	30.00
Standard deviation			3558.67	0.0755
RSD in %			0.22	0.25

Intermediate precision

Intermediate precision expresses variations within laboratories such as different days, different analysts, different equipments used for its determination. The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same results once the development phase is over. Intermediate precision for tadalafil and dapoxetine hydrochloride were shown in Table 11 and 12 by using different analyst on different days. This indicated that method was highly precise.

Table 11: Precision - Intermediate precision for Tadalafil

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	275.3	1302758.0	10.10
2.	Standard -2	274.6	1320267.0	10.26
3.	Standard -3	275.1	1299787.5	10.08
4.	Standard -4	274.9	1299337.0	10.09
5.	Standard -5	274.3	1299086.0	10.11
6.	Standard -6	275.8	1306250.0	10.11
Mean			1304580.91	10.12
Standard deviation			8158.81	0.067
RSD in %			0.63	0.66

Table 12: Precision - Intermediate precision for Dapoxetine hydrochloride

S.No.	Sample Name	Amount of preparation used (mg)	Area	Drug content (mg)
1.	Standard -1	274.3	1606219.0	30.05
2.	Standard -2	275.1	1605972.0	29.96
3.	Standard -3	276.3	1603735.0	29.79
4.	Standard -4	274.9	1606321.0	29.99
5.	Standard -5	274.1	1607958.5	30.11
6.	Standard -6	275.8	1607979.0	29.92
Mean			1606364.08	29.97
Standard deviation			1564.53	0.1108
RSD in %			0.10	0.37

Robustness

Measure of method's capacity to remain unaffected by small, but deliberate variations in method.

I. Change in the ratio of solvents in the mobile phase (±2.0)

Two sample preparations were analyzed as per methodology by changing the ratio of solvents in the mobile phase by means of by means of ±2.0. The robustness data was found for tadalafil and dapoxetine hydrochloride by changing the ratio of solvents in the mobile phase. It was shown in Table 13 and 14. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 13: Robustness - Change in the ratio of solvents in the mobile phase ±2.0 for Tadalafil

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (53:47)	
			Area	Amount (mg)
1.	Sample -1	274.3	1321506.5	10.19
2.	Sample -2	275.3	1319449.0	10.14
Mean				10.16
Standard deviation				0.0353
RSD in %				0.35

Table 14: Robustness - Change in the ratio of solvents in the mobile phase ±2.0 for Dapoxetine hydrochloride

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (53:47)	
			Area	Amount (mg)
1.	Sample -1	274.3	1673218.5	30.26
2.	Sample -2	275.3	1674725.0	30.17
Mean				30.21
Standard deviation				0.0636
RSD in %				0.21

II. Change in the pH of buffer in the mobile phase

Two sample preparations were analyzed as per methodology by changing the pH of the buffer in the mobile phase by means of ±2.0. The robustness data was found for tadalafil and dapoxetine hydrochloride by changing the pH of buffer in the mobile phase. It was shown in Table 15 and 16. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was rugged.

Table 15: Robustness - Change in the pH of buffer in the mobile phase (-0.2) for Tadalafil (pH 4.4)

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (55:45) [Buffer pH :4.4]	
			Area	Amount (mg)
1.	Sample -1	274.3	1304681.0	10.11
2.	Sample -2	275.3	1301491.5	10.05
Mean				10.08
Standard deviation				0.0424
RSD in %				0.42

Table 16: Robustness - Change in the pH of buffer in the mobile phase (-0.2) for Dapoxetine hydrochloride (pH 4.4)

S.No.	Sample Name	Wt. taken (mg)	Buffer: Acetonitrile (55:45) [Buffer pH :4.4]	
			Area	Amount (mg)
1.	Sample -1	274.3	1651074.5	30.02
2.	Sample -2	275.3	1649036.5	29.88
Mean				29.95
Standard deviation				0.0989
RSD in %				0.33

Ruggedness

Six sample preparations were analyzed as per the methodology by a different analyst on a different instrument on a different day. The robustness data for tadalafil and dapoxetine hydrochloride were shown in Table 17and 18. It was observed that there were no marked changes in the chromatograms, which demonstrated that the proposed method was robust.

Table 17: Ruggedness data for Tadalafil -Change of analyst

S.No.	Sample Name	Wt. taken (mg)	Area	Amount (mg)
1.	Sample -1	275.3	1302758.0	10.10
2.	Sample -2	274.6	1320267.0	10.26
3.	Sample -3	275.1	1299787.5	10.08
4.	Sample -4	274.9	1299337.0	10.09
5.	Sample -5	274.3	1299086.0	10.11
6.	Sample -6	275.8	1306250.0	10.11
Mean				10.12
Standard deviation				0.0671
RSD in %				0.66

Table 18: Ruggedness data for Dapoxetine hydrochloride -Change of analyst

S.No.	Sample Name	Wt. taken (mg)	Area	Amount (mg)
1.	Sample -1	275.3	1661153.5	30.04
2.	Sample -2	274.6	1658158.0	30.06
3.	Sample -3	275.1	1657861.0	30.00
4.	Sample -4	274.9	1656251.5	29.99
5.	Sample -5	274.3	1653395.5	30.01
6.	Sample -6	275.8	1669240.5	30.13
Mean				30.03
Standard deviation				0.0519
RSD in %				0.17

CONCLUSION

The Proposed study describes new and simple RP-HPLC method for the simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in combined solid dosage form. 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 hydrochloride in combined solid dosage form without interference.

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