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RP HPLC Method for Estimation of Dapoxetine Hydrochloride and Tadalafil Hydrochloride as API and in Tablet Dosage Form

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ABSTRACT

The article, for the first time, reports High performance liquid chromatography [HPLC] method for estimation of Dapoxetine hydrochloride [DAP] and Tadalafil hydrochloride [TAD]. The analytical method was developed for both the drugs as API and for tablet dosage form. The separation of two drugs was achieved on High quality octa decyl silane [C18 250x 4mm i.d] 5µ column. The mobile phase consists of Acetonitrile and phosphate buffer in the ratio of 45:55. Mobile phase flow rate adjusted at 1 ml/min and pH at 4.2 using ortho phosphoric acid. The detection was carried out at a wavelength 254 nm. Retention time was found 4.46 and 10.11 min respectively for TAD and DAP. The method was validated for system suitability, linearity, accuracy, and precision and solution stability as per ICH guidelines. Linearity was obeyed in the range of 8-48 µg/ml and 24- 144 µg/ml with correlation coefficient of 0.997 and 0.998 for TAD and DAP respectively. Recovery studies were found within prescribed limits that was 98.83 for TAD and 98.93 for DAP respectively. Detection and quantification limit were found to be 0.225µg/ml and 0.682µg/ml for TAD and 0.163 µg/ml and 0.494µg/ml for DAP respectively which expresses higher sensitivity of the method. Assay results were found to be 98.52 % and 98.26 % in generic brand whereas 99.03 % and 98.11 in other brand for TAD and DAP respectively.

Keywords: RP-HPLC, Dapoxetine Hydrochloride, Tadalafil Hydrochloride, Mobile phase, Retention time, Recovery

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INTRODUCTION

Dapoxetine Hydrochloride [Figure. 1] is chemically *N*,*N*-dimethyl-3-(naphthalen-1- yloxy)-1-phenylpropan-1-amine hydrochloride, is a Short acting serotonin reuptake inhibitor.[1, 2] It is widely used for premature ejaculation for 18 to 64 years of age.[5] Dapoxetine Hydrochloride [DAP] is not official in any pharmacopoeia. Tadalafil Hydrochloride [Figure. 2] is chemically 6-(1,3-benzodioxol-5-yl)- 2,3,6,7,12,12a-hexahydro- 2-methy pyrazino [1', 2':1,6] pyrido [3,4-b] indole - 1,4-dione. [3, 4] It belongs to PDE5 Inhibitor category and ensures an long time erection by increasing in blood flow in penile tissues so indicated in treatment of erectile dysfunction.[6, 7], [8] Tadalafil HCl [TAD] is official in European pharmacopoeia. A combination of Dapoxetine HCl with Tadalafil HCl as tablet dosage form has been approved [9] in India in 60:20 mg proportion[10]. Hence, a need was felt to develop a rapid analytical method for quantitative simultaneous estimation. The literature survey revealed that there are some analytical methods reported for Tadalafil HCl and its congeners like UV, HPTLC and HPLC and their add-ons either individually or in combination with other drug.[6-9] Many methods have been reported including UV and HPLC in literature for determination of Dapoxetine HCl as single component.[10-12] However, literature did not reveal any reported methods for the simultaneous estimation of Dapoxetine HCl with Tadalafil HCl in combined dosage form using liquid chromatography. Hence, aim of present work is to develop an analytical technique for estimation of the combination in tablet formulation.

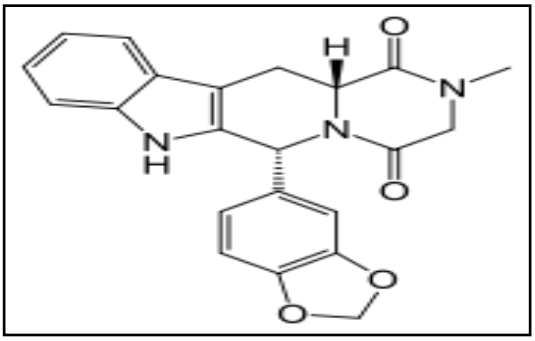


Figure 1:- Structure of Tadalafil

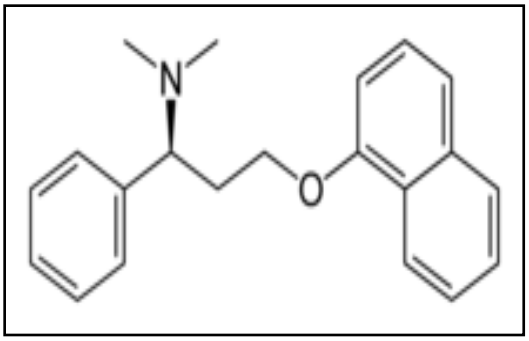


Figure 2:- Structure of Dapoxetine

MATERIAL AND METHODS

Equipments

A Jasco HPLC-1575 along with UV detector 1500 and a C_{18} column (Make Agela), connected to computer having application software "Borwin" was employed to obtain chromatogram and related parameters. All weights were measured on Digital balance GR-100 (A&D comp Ltd.).

Chemicals

Tadalafil hydrochloride was kindly provided by Cadila health care Dholka and Dapoxetine HCl was kindly provided by Sun Pharma, Silvassa. The purity of reference standards were more than 98.5%w/w. Starch, lactose, magnesium stearate and talc were obtained from Loba chem. and Merck Ltd, Maharashtra. Chemicals and solvents used were of HPLC grade. Distilled water was obtained from in house laboratory.

Preparation of standard stock solutions

Stock solution was prepared by transferring 10 mg of Tadalafil HCl [TAD] and Dapoxetine HCl [DAP] in a 100 ml volumetric flask separately. About 100 ml of acetonitrile was added and sonicated well. For determination of absorbance maxima in UV (Fig. 3, 4) it was further diluted, 5ml of TAD and 15 ml of DAP stock solution was transferred to 25 ml volumetric flask separately and volume was made up to mark. The final solution results in 20 µg/ml TAD and 60 µg/ml of DAP. Each of them was also scanned in UV range [Figure. 3 and 4]

Selection of detection wavelength

Both the drugs also showed absorbance at 275 and 242 nm in overlain spectra (Figure. 5), hence 254 nm was selected as common middle wavelength for detection and estimation, (254)

Preparation of mixture solution for chromatographic trials

The solution was prepared by transferring accurately about 10 mg of TAD and 30 mg of DAP in a 100 ml volumetric flask separately. About 70 ml of acetonitrile was added and sonicated well. The volume was made up to mark. Then 5 ml of stock solution was further diluted in 25 ml volumetric flask and make up the volume up to with same solvent. The final solution contained about 20 µg/ml of TAD and 60 µg/ml of DAP in 1:3 ratio. It was further filtered through 0.45µm membrane filter and sonicated under ultrasonic bath prior to inject.

Chromatographic trials

Different trials (Figure 6 to 12) were taken to reach the pin point target and optimized chromatographic condition for suitable quantitative estimation of both the drugs in tablet dosage form. Changes in flow rate, ratio of mobile phase and its pH and detection wavelength makes it feasible to find better to best separation condition as discussed in table 1. Ortho phosphoric acid is used to adjust pH of phosphate buffer (6.8).

Optimized mobile phase

Selected mobile phase is phosphate buffer and acetonitrile of pH 4.2 in ratio of 45:55 and conditions for run were flow rate of 1ml/min at detection wavelength of 254 nm as in trial 7 where resolution, shape of peak was found well with high number of theoretical plate counts and better symmetry (Figure 12).

Run the optimized mobile phase

The mobile phase was prepared as mentioned in section above and was injected into the HPLC system and the chromatogram was obtained. The chromatogram of baseline of optimized mobile phase has also taken [Figure. 13].

An Individual Run for Tadalafil HCl and Dapoxetine HCl

With the help of above prepared standard stock, 20 µg/ml of TAD and 60 µg/ml of DAP was prepared and injected separately into the HPLC system. Retention time and theoretical plates were calculated from system itself for the obtained chromatograms [Figure 14].

Method Validation

The developed methods have been validated in terms of linearity, range, detection and quantitation limits, specificity, accuracy, precision, assay, and system suitability as per ICH Q2(R1) 2010.[11, 54]

Linearity

Linearity of the method was determined by means of calibration curve using six different analyte concentration separately for TAD and DAP. At least three concentration levels were tested in agreement to ICH. Straight line equations were obtained from these calibration curves using MS-excel. The slope, intercept and correlation coefficient were calculated as required by ICH.

Range

Range of an analytical method is defined as the interval between upper and lower levels.

Working range: It begins from limit of quantitation to the maximum concentration used for the development of the analytical method.

Linearity range: It is the interval in which the response (peak area) is directly proportional to the concentration between the upper and lower levels.

Target concentration: It is defined as the concentration, which is equal to the midpoint of linearity range.

Target range: It is that concentration which is 80%, 100% and 120% of the target concentration.

Specificity

Commonly used excipients (lactose, starch, magnesium stearate and talc) were added into a pre-weighed quantity of standard drug synthetic mixture (1:13) and then it was injected into HPLC.

Accuracy

Accuracy of the method was measured by recovery studies and ascertained by standard addition method. In this standard drug mixture of TAD and DAP (1:3) to the pre-analyzed tablet mixture (1:3) preparation at three different levels i.e. 80 %, 100 %, and 120 % and total concentration

was determined using the proposed method.

Precision in mixture

Repeatability

It indicates the precision under the same operating conditions over a short interval of time and inter-assay precision. Repeatability was performed for six times with single target concentration ratio (1:3).

Intermediate Precision

In intraday study concentration of drugs were calculated on the same day at an interval of two hour. In inter day study the drug contents were calculated on three different days at 80%, 100% and 120% of ratio (1:3)

Limit of Detection and Quantitation

LOD and LOQ were determined from the linearity data. This helps to determine sensitivity of the method. . LOD and LOQ were estimated from the standard deviation of the response and the slope of the calibration curve. The standard deviation can be determined from the standard deviation of the intercepts of the regression lines done in the range of the detection limit.

Assay

Ten tablets were taken and their average weight was calculated. An accurately weighed quantity of tablet powder equivalent to about 20 mg of Tadalafil HCl [TAD] and 60 mg of Dapoxetine HCl [DAP] were transferred to 100 ml volumetric flask. After certain dilutions, the average result of duplicate estimations of tablet mixture solution of Tadalafil HCl (20 μg/ml) and Dapoxetine HCl (60 μg/ml) was calculated. This procedure has been followed for both local generic and marketed brand other than generic. The results are shown in table.

System Suitability Tests (SST)

After the method validation the task of checking the suitability of the system. It allows comparison of the peak shape, peak width, and baseline resolution. Alternatively these test report includes number of theoretical plates (efficiency), capacity factor, separation or relative retention, resolution, tailing factor.

RESULTS AND DISCUSSIONS

Method Development [Figure 3 to14]

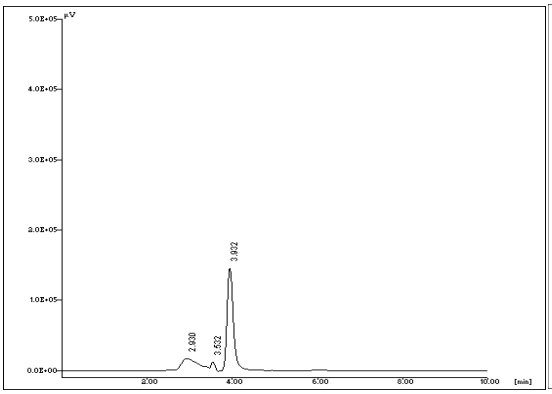
Octadecylsilane column using as stationary phases with different varieties of mobile phases were tried in aim of better separation and resolution named as chromatographic trials recorded in table 1. It was found that trial 7 having phosphate buffer (pH 4.2) and acetonitrile of in ratio of 45:55

as mobile phase with flow rate of 1ml/min at detection wavelength of 254 nm in which resolution, shape of peak was found good with high number of theoretical plate counts and better symmetry was selected as optimized separation condition. Individual drug solution and mixture sample solution was injected into column and elution pattern, theoretical plates, peak areas and resolution parameters were studied.UV spectra of individual drugs were recorded for selection of detection wavelength in HPLC. The choice of wavelength 254nm was considered best suitable for quantitatively detection of both drugs simultaneously with appropriate sensitivity.

Table 1: Different chromatographic trials

S. no	Trial-1	Trial-2	Trial-3	Trial-4	Trial-5	Trial-6	Trial-7
Flow-ml/min	0.8	0.8	0.8	0.8	1.0	0.8	1.0
Detector	275	275	254	254	254	254	254
Mobile phase	Methanol ACN	Buffer ACN	Buffer CAN	Buffer ACN	Buffer ACN	Buffer ACN	Buffer ACN
Ratio (v/v)	(50:50)	(50:50)	(35:65)	(30:70)	(40:60)	(40:60)	(45:55)
pH	NA	6.8	5.8	5.0	4.8	4.5	4.2
Run time	10 min	14 min	10 min	14 min	12 min	20 min	14 min

*ACN: - Acetonitrile; Buffer: - Phosphate buffer; ml: - milliliter; min:-minute; v/v:-volume by volume



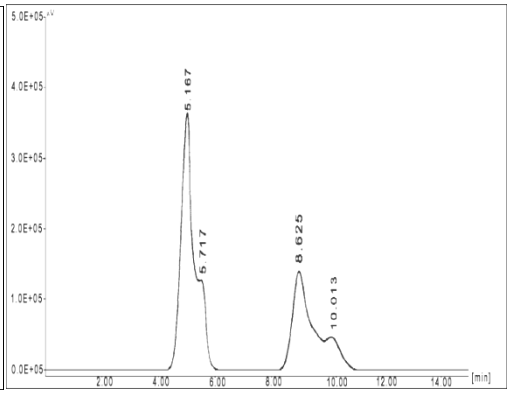
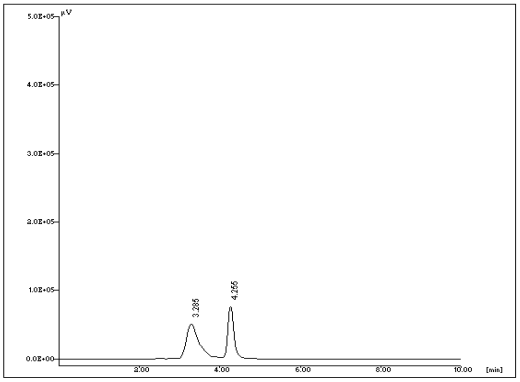


Figure 3:- Wavelength scan of Tadalafil **Figure 4:- Wavelength scan of Dapoxetine**



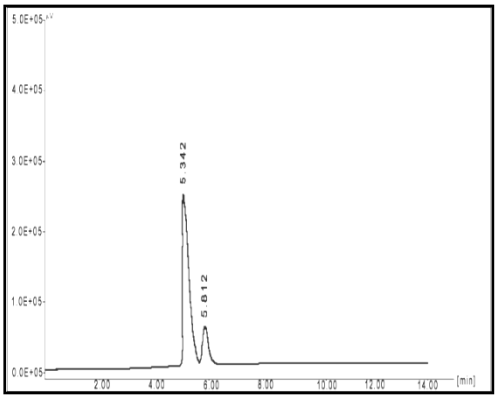


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Figure 6:- Chromatogram of trial 1 **Figure 7:- Chromatogram of trial 2**

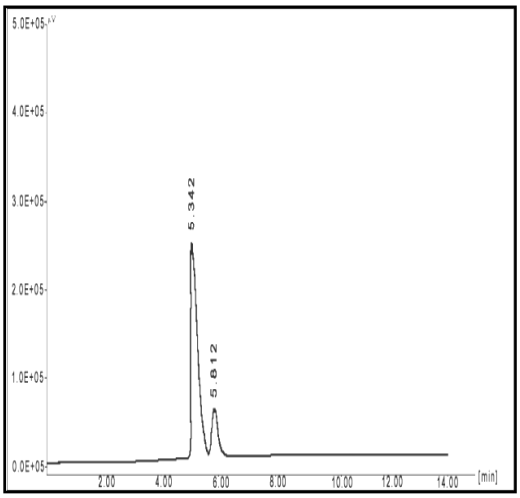


Figure 8:- Chromatogram of trial 3

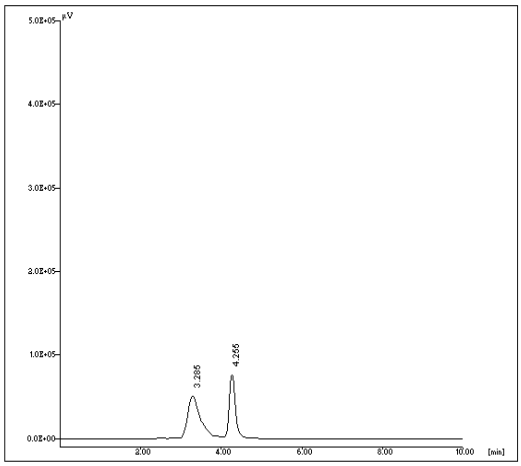


Figure 9:- Chromatogram of trial 4

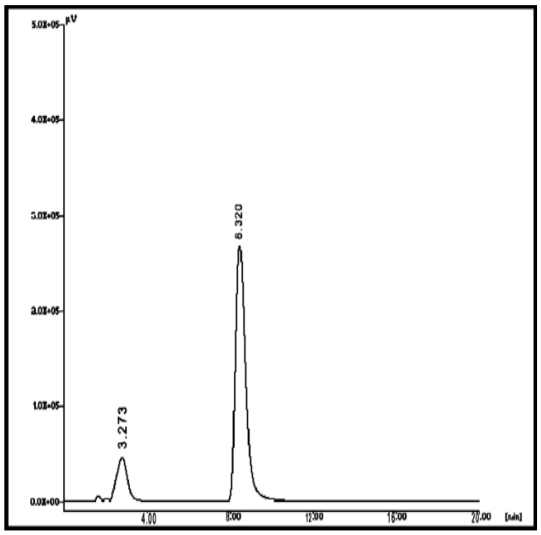


Figure 10:- Chromatogram of trial 5

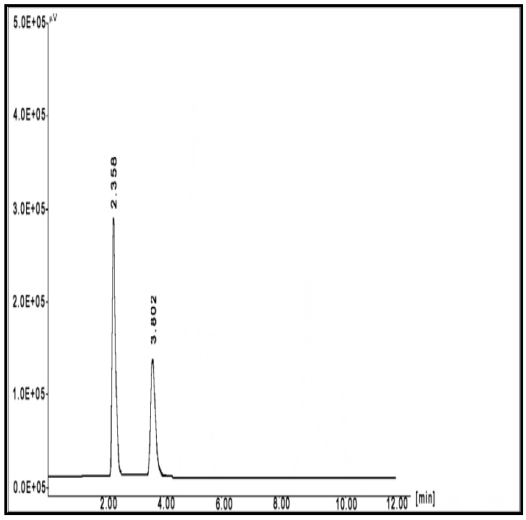


Figure 11:- Chromatogram of trial 6

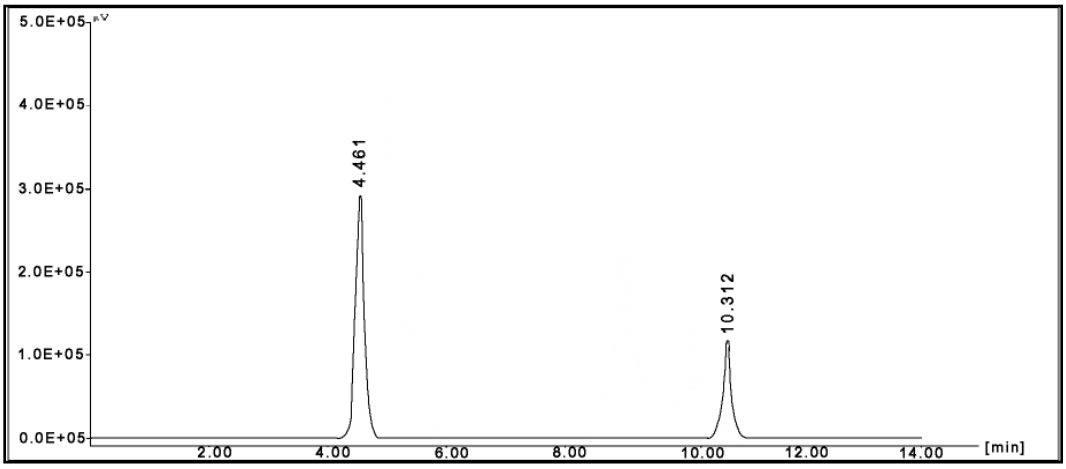


Figure 12:- chromatogram of trial 7 (optimized)

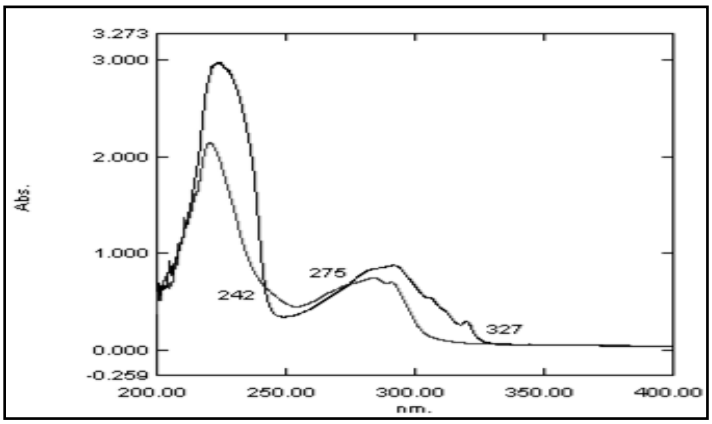
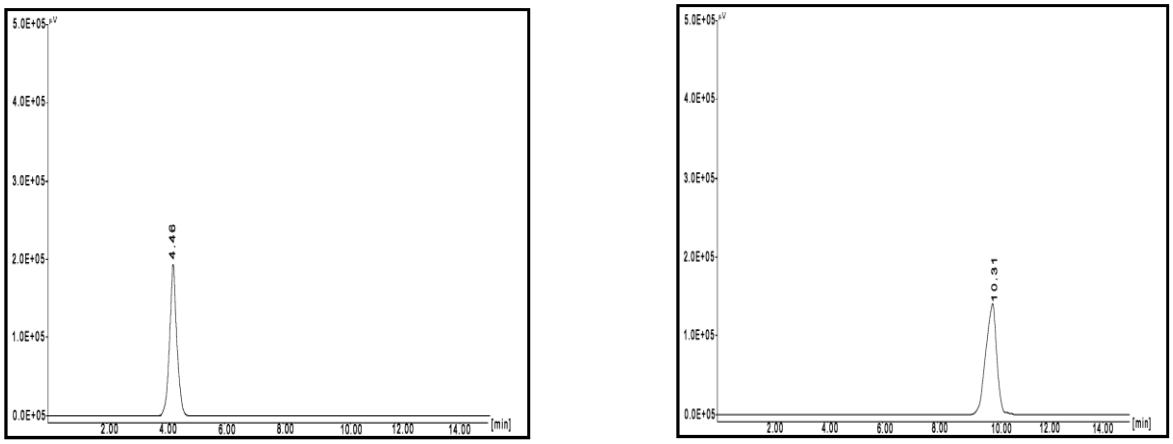


Figure 13: Chromatogram of baseline of optimized mobile phase



(A) **(B)**

Figure 14:- Chromatogram of individual API (A) Tadalafil HCl (B) Dapoxetine HCl

Method Validation

Linearity and range

Linear calibration curves for the two drugs were obtained by plotting the concentrations with the mean peak areas in MS-excel [Figure. 15 and 16]. The linear ranges of Tadalafil HCl and Dapoxetine HCl are 8-48 µg/ml, 24-144µg/ml respectively with coefficient of correlation 0.997 and 0.998 respectively. Values of different types of ranges and linearity were recorded in table 2.

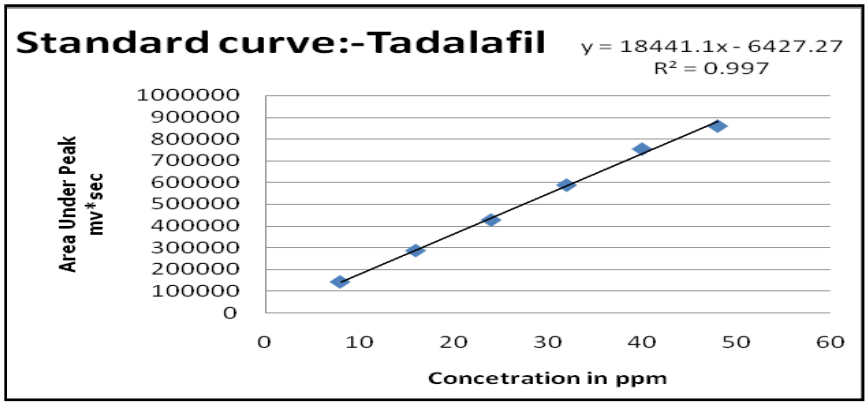


Figure 15: Calibration curve of Tadalafil HCl

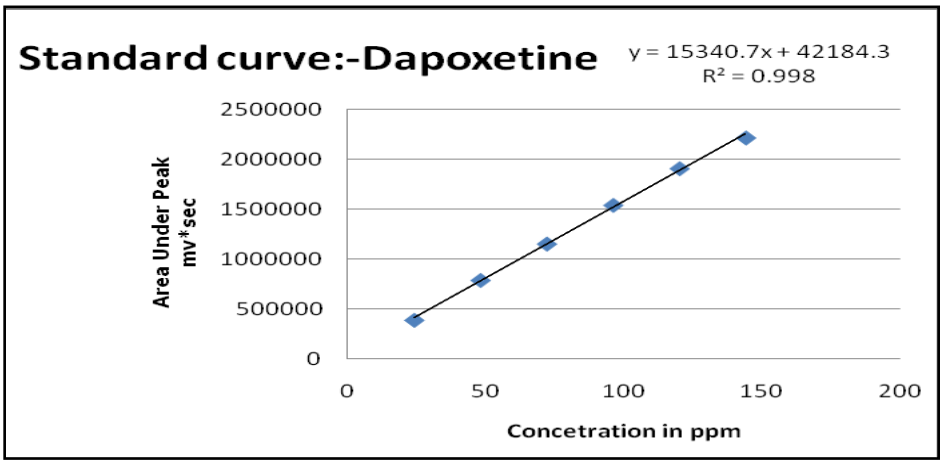


Figure 16: Calibration curve of Dapoxetine HCl

Specificity

Developed method has negligible excipients interference of less than 0.5%. Specificity study with excipients was come out with a conclusion that there are no ghost peaks and additional peaks at the time of retention of API. Two additional peaks earlier at start of run were elute out first but not observed at major retention time

Precision

Precision was determined by studying the repeatability and intermediate precision. Results indicate good repeatability and intermediate precision. Percentage RSD was found to be less than 2 as shown in table 3.

Table 3: Precision study and results

S.No	Repeatability (28:84)*		TAD:DAP	Mean Reproducibility			
	TAD 28µg/ml	DAP 84µg/ml	Concentration ratio 1:3	Intra-day		Inter-day	
				TAD	DAP	TAD	DAP
1	508088	1345260	22.4 : 67.24	9.6±0.11	6.5±	9.6±0.0	6.48±
2	513531	1351661	(80%)	5	0.022	47	0.032
3	515346	1350224	28 : 84	12.07±	8.02±	12.07±	8.06
4	510265	1350224	(100%)	0.113	0.109	0.081	±0.035
5	506273	1345260	33.6 : 100.8	14.4	9.67	14.42	9.58
6	519154	1348623	(120%)	±0.026	±0.041	±0.054	±0.042
Mean	512109.8 ± 4811.7	1348542 ± 2717.9					
	0.934	0.2015	Mean % RSD	0.71	1.05	0.513	0.461

* For target concentration only; TAD: - Tadalafil HCl, DAP: - Dapoxetine HCl, RSD: - Relative standard deviation

Sensitivity

The Detection limit (LOD) was found to be 0.225 µg/ml and 0.163 µg/ml and Quantitation limit (LOQ) was found to be 0.682 µg/ml and 0.494 µg/ml or Tadalafil HCl and Dapoxetine HCl

respectively which expresses higher sensitivity of the method as, given in table 2.

Table 2: Results of calibration curve's statistics

Parameters	Tadalafil	Dapoxetine HCl
Linearity Range	8.0 – 48 µg/ml	24-144 µg/ml
Working range	0.682 to 48 µg/ml	0.494 to 144 µg/ml
Target range	22.4, 28.0 and 33.6 µg/ml	67.2 84.0 and 100.8 µg/ml
Target Concentration	28.0 µg/ml	84.0 µg/ml
Linearity equation	y = 18207.22x-2843.33	y = 15340x +42184.33
Correlation coefficient	0.997	0.998
LOD	0.225 µg/ml	0.163 µg/ml
LOQ	0.682 µg/ml	0.494 µg/ml

**LOD: - limit of detection; LOQ: - limit of quantitation;* µg/ml: - microgram per milliliter

Accuracy

Accuracy was determined using recovery studies on tablet formulation. Recovery was found to be greater than 98% which showed good accuracy of method for estimation as show in table 4.

Table 4: Data for accuracy or recovery studies

Pre-analyzed tablet mixture TAD:DAP=20 : 60	Spiking concentration TAD:DAP=1:3	% Recovered concentration	Mean percent recovery	%RSD or %CV
Tadalafil HCl (TAD)	16, 20 and 24	15.8, 19.8, 23.7	98.83 ± 0.14	0.334
Dapoxetine HCl(DAP)	48, 60 and 72	47.41, 59.4, 71.3	98.93 ± 0.33	0.146

TAD: - Tadalafil HCl, DAP: - Dapoxetine HCl, RSD: - Relative standard deviation

Assay

Percentage assay of the drugs in local generic brand was found to be 98.52% and 98.56% for TAD and DAP whereas in other marketed brand it was found to be 99.03% and 98.11% respectively with Percentage RSD less than 2 respectively. The data are shown in table 5

Table 5: Assay determination data

Approved Brand Name	Actual Claimed weight (mg) 20 : 60	Conc. found (µg/ml)	Amount found (mg)	Percent Assay	% RSD
T-Ject-60	TAD	19.7	19.7 ± 0.0921	98.52 ± 0.451	0.457
(Generic)	DAP	58.45	58.45 ± 0.864	98.26 ± 0.813	0. 827
Super Tadarise	TAD	19.8	19.8 ± 0.01	99.03 ± 0.0364	0.367
	DAP	59.51	59.51 ± 0.048	98.11 ± 0.247	0.249

**Mean of duplicate readings; TAD: - Tadalafil HCl, DAP: - Dapoxetine HCl, mg: - milligram; µg/ml: - microgram per milliliter; RSD: - Relative standard deviation*

System Suitability

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), asymmetric factor or

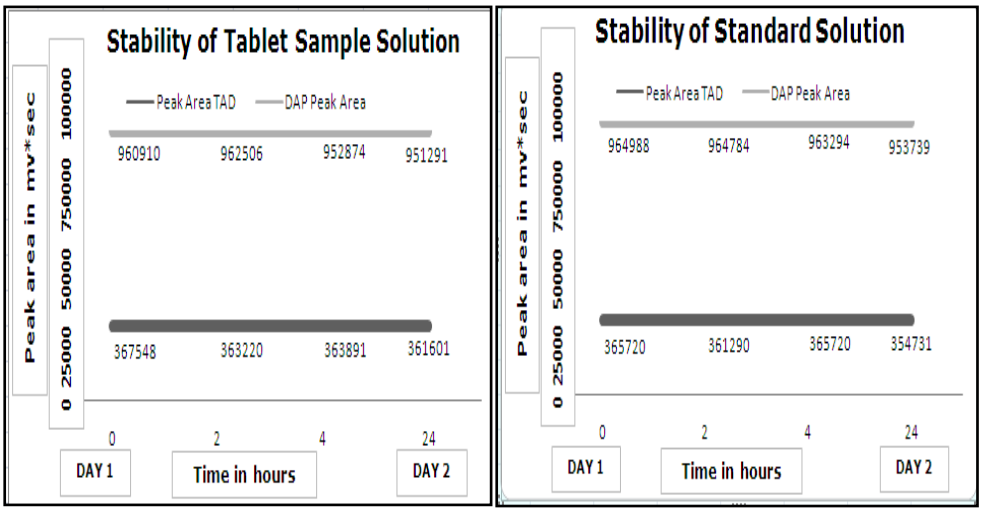
tailing factor (As), capacity factor (k^1), resolution (Rs), and retention time (Rt) were determined. The results are shown in Table 6. Data indicates good performance of system.

Table 6: System suitability data

Sr. No.	Parameters	Tadalafil	Dapoxetine
1.	Retention Time (min)	4.46	10.31
2.	Theoretical Plates	4723	4534
3.	Asymmetry/Tailing factor	1.1	0.96
4	Capacity factor	2.23	4.155
5	Resolution	11.2	

Solution stability studies

Solution of standard API and tablet sample were prepared of random concentration (20 µg/ml of TAD and 60 µg/ml of DAP) and kept overnight for study. Peak area was observed with time and plotted in excel [Figure 17, 18] to find out effect on stability due to mobile phase.



(A) **(B)**

Figure 17: Stability study curve (A) Standard solutions (B) Tablet sample solutions

CONCLUSION

The proposed reverse phase high performance iqid chromatographic method provides quantitative estimation of Tadalafil HCl and Dapoxetine HCl simultaneously adhering with validation parameters like linearity, precision, accuracy, system suitability and stability in tablet or in solution alone. Newly developed method resides in prescribed limits as per ICH guidelines in terms of Relative standard deviation. In conclusion, the newly developed method is strongly recommended for the assay of two drugs in the locally available pharmaceutical dosage form.

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REFERENCES

1. http://en.wikipedia.org/wiki/Dapoxetine, Accessed online on 08.03.2013
2. http://www.calbiochem.com/ dapoxetine.html, Accessed online on 08.3.2013
3. http://www.nlm.nih.gov/medlineplus/druginfo/meds/a604008.html
4. http://www.cialis.com, NDA 21-468 (2003) 3, Accessed online on 07.03.2013
5. Kendirci M, Salem E, Hellstrom WJ. Dapoxetine, a novel selective serotonin transport inhibitor for the treatment of premature ejaculation. Therapeutic and Clinical Risk Management 2007;3(2):277–89.
6. Alfred Gilman and Louis Goodman. Manual of pharmacology and therapeutics. XI edition, 2008, The McGraw-Hill Companies, United States of America., 295,531,1111.
7. Montague DK, Jarow J, Broderick GA, et al, and the AUA Erectile Dysfunction Guideline Update Panel. AUA Guideline on the Pharmacologic Management of Premature Ejaculation. J Urol. 2004; 172:290–294.
8. Carson CC, Rajfer J, Eardley I, Carrier S, Dennes JS, Walker DJ, et al. The efficacy and safety of tadalafil: an update. BJU Int 2004; 93(9):1276.
9. European Pharmacopoeia 7.4, 2012, European Directorate for the Quality of Medicines & HealthCare, Europe, 1527
10. T. Rohith and S. Ananda. A Validated Chiral Liquid Chromatographic Method for the Enantiomeric Separation of Dapoxetine Hydrochloride. IJARPB, 2012; 2 (3):311-19
11. N.V.S. Ramakrishna, K.N. Vishwottam, S. Puran, M. Koteshwara, Quantitation of Tadalafil in human plasma by liquid chromatography–tandem mass spectrometry with electrospray ionization. *Journal of Chromatography B, Analytical Technologies in the Biomedical and Life Sciences*, 2004;809(2):243-249.
12. Mohammad Yunoos, D. Gowri Sankar, B. Pragati Kumar.UV Spectrophotometric Method for the Estimation of Tadalafil in Bulk and Tablet Dosage form. Eurasian J Chemistry. 2009; 7(3): 833-836
13. Y. J Yang, D. M. Song and B. R. Xiang. Rapid Resolution RP-HPLC-DAD Method for Simultaneous Determination of Sildenafil, Verdanafil, and Tadalafil in Pharmaceutical Preparations and Counterfeit Drugs. Analytical Letters 2010; 43(3):373-380
14. International Conference on Harmonization (ICH) (2005, Nov.), Harmonized tripartite guideline Q2(R1), Validation of analytical procedures: Text and methodology.
15. Nash RA, Watcher AH, Pharmaceutical Process Validation, Marcel Dekker Inc. New York; 2003:507-522.