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DEVELOPMENT AND VALIDATION OF NEW UV METHOD FOR SIMULTANEOUS ESTIMATION OF TADALAFIL IN COMBINATION WITH DAPOXETINE HYDROCHLORIDE IN A PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

Objective: The present research studies describe development and validation for the simultaneous determination of Tadalafil and Dapoxetine hydrochloride in bulk and in tablet dosage form. **Method:** Simple UV spectrophotometric method has been developed for simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in bulk and in tablet dosage form. For the method, stock solutions were prepared by using acetonitrile: water (50:50) and the absorbance maxima for Tadalafil and Dapoxetine hydrochloride were found to be 284nm and 292nm respectively. **Results:** A linear response was absorbed in the range of 1 to 15ug/ml and 3 to 45ug/ml with correlation co-efficent of 0.9997 for Tadalafil and 0.9998 for Dapoxetine respectively. The percentage purity of the tablet formulation was found to be 103.4% and 99.99% for Tadalafil and Dapoxetine respectively. **Conclusion:** The method was validated for the different parameters like accuracy, precision, sensitivity and linearity as per ICH guidelines Q_2 (International Conference Harmonisation). This method can be used for the simultaneous estimation of Tadalafil and Dapoxetine hydrochloride in quality control of formulation without interference of excipients.

KEYWORDS: Simultaneous determination, Tadalafil, Dapoxetine, UV-spectrophotometer, λmax.

INTRODUCTION

Tadalafil (TAD)[1](fig.1) is chemically *(*(6r,12ar)-2,3,6,7,12,12a-hexahydro-2-methyl-6-(3,4Methylene dioxyphenyl) pyridine [1',2':1,6] pyrido [3,4-b] Indole-1,4-dione.

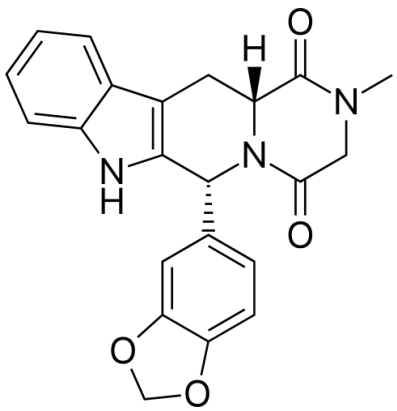

Fig. 1: Structure of Tadalafil.

Mechanism of action of TAD is classified as a phosphodiesterase-5 enzyme inhibitor the inhibition of phosphodiesterase type 5 (PDE5) enhances erectile function by increasing the amount of cGMP. It is used to Treat erectile dysfunction and Treat symptoms of benign prostatic hypertrophy.

Dapoxetine Hydrochloride (DAP)[2] (fig.2) is chemically ((S)-N, N-Dimethyl-3-(naphthalen-1-yloxy)-1-phenylpropan-1-amine).

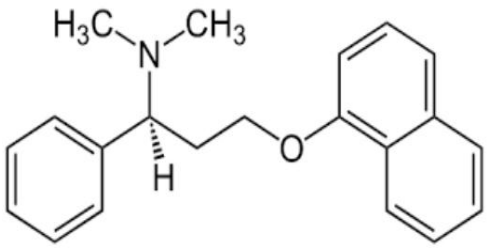

Fig. 2: Structure of Dapoxetine.

The mechanism action of Dapoxetine affects premature ejaculation is still unclear. However, it is presumed that Dapoxetine works by inhibiting serotonin transporter and subsequently increasing serotonin's action at pre and postsynaptic receptors. Human ejaculation is regulated by various areas in the central nervous system. The ejaculatory pathway originates from spinal reflex at the

thoracolumbar and lumbosacral level of spinal cord activated by stimuli from male genital. These signals are relayed to the brain stem, which then is influenced by a number of nuclei in the brain such as medial preoptic and para ventricular nuclei. It is used in the treatment of premature ejaculation in adult men and Antidepressant**.**

Literature revealed that some analytical methods have been reported for the estimation of TAD in pharmaceutical formulations like RP-HPLC method[3], UV- method[4], *in-vivo* dissolution studies and forced degradation study for UV-method[5]. Several methods are available for the individual determination of DAP in UV[6] related impurities determined by HPLC method[7], stability indicating HPLC method[8]. Some methods are reported for the simultaneous determination of Dapoxetine and Sildenafil by UV[9], stability indicating RP-HPLC method[10], RP-HPLC method for the simultaneous estimation of dapoxetine and tadalafil[11] and RP-UPLC.[12] First order derivative and Dual wavelength method for the determination of avanafil and dapoxetine[13] was reported. No spectrophotometric method or the HPLC method has been reported in the literature for the simultaneous determination of tadalafil and dapoxetine in their commercial formulations.

A successful attempt has been proposed in present investigation to quantify the tadalafil and Dapoxetine simultaneously by spectrophotometer. The UV spectrophotometric analyses are often preferred in quality control testing and ordinary laboratories due to its broad availability and suitability. The objective of this study was to develop and validate a simple and specific UV spectrophotometric method for the simultaneous determination of tadalafil and dapoxetine in pure drug and marketed formulation. This method exhibits precise, accurate and cost effective assay for pure drug and marketed formulation.

MATERIALS AND METHODS

Dapoxetine and tadalafil were obtained as a gift sample from madras pharmaceutical, Chennai. Tablets formulation Uphold containing 30 mg of DAP and 10 mg of TAD also purchased from a local pharmacy, HPLC-grade acetonitrile and water from qualigens, Mumbai. Purified water obtained from triple distillation and filter through nylon 0.45 µg and 47mm member filter (German laboratory, Mumbai, India). Spectrophotometric analysis was performed on Shimadzu -1700 double beam UV-visible spectrophotometer using with a pair of 10mm quartz cells with slit width of 1nm and a scan speed of 60nm/minutes.

Preparation of standard stock solution

The solubility of drug was determined in a variety of solvent as per Indian pharmacopeia[14] standards. Solubility was carried out in non-polar to polar solvents. The common solvent was found to be acetonitrile and water in the ratio of (1:1) for the analysis of TAD and DAP for the proposed method. 25 mg of TAD and DAP raw material were weighed separately and transferred into 25ml standard flask, dissolved in acetonitrile and water (1:1) and made upto the volume with same solvent which contain 1000µg/ml. From that, the solution further dilutions were made by diluting 1ml to 100 ml with the same solvent acetonitrile and water (1:1) to obtain10µg/ml.

Spectral characteristics and Wave length selection

The absorption spectra 10µg/ml of each of TAD and DAP in the mix of solvent acetonitrile and water (1:1) were recorded over the range of 200-400nm using the above set solvent as a blank. The overlain spectra were observed for the selection of the suitable wavelength for each of the developed method.

Linearity and Range

The aliquots of stock solution of TAD (1, 3, 6, 9, 12 and15ml) of 100µg/ml and DAP (3, 9,18,27,36 and 45ml) of 300µg/ml were transferred into 100ml volumetric flask and made upto the volume to get concentrations with same solvent acetonitrile and water (1:1). The absorbance of different concentration solutions were measured at 284 nm and 292 nm in normal spectrum. The calibration curves were plotted using concentration against absorbance. Both drugs were linear with of 1-15µg/ml for TAD and 3-45µg/ml for DAP respectively at their respective wavelength.

Analysis of Marketed formulation

20 tablets of Uphold (containing 10 mg of TAD and 30 mg of DAP) were weighed accurately. The average weight of tablets were found and powdered. The tablet powder equivalent to 30 mg of DAP was weighed and transferred into 100 ml volumetric flask and a minimum quantity of mixture of solvent was added to dissolve the substance by using ultrasonicator form 15 minutes. Then the solution was made up to the volume with the same solvent (300µg/ml). The content was filtered through the whatmann filter paper No.41. From the clear solution further dilutions were made by diluting 6 ml to 100 ml volumetric flask with solvent to obtain 6µg/ml of TAD and DAP were determined by using simultaneous equation. The procedure was repeated for 6 times for each percentage. The Concentration of the samples was calculated by using following equation (Beckett and Stenlake. 2007).

$$C_x = A_2a_{y1}-A_1a_{y2} / a_{x2}a_{y1}-a_{x1}a_{y2}$$

$$C_y = A_1a_{x2}-A_2a_{x1} / a_{x2}a_{y2}-a_{x1}a_{y2}$$

A_1 and A_2 are absorbance of mixture at 292 and 284 nm.

a_{x1} and a_{x2} are the absorptivity of Tadalafil at λ_1 and λ_2 respectively.

a_{y1} and a_{y2} are absorptivity of Dapoxetine at λ_1 and λ_2 respectively.

C_X and C_Y are the concentration of Tadalafil and Dapoxetine respectively.

Method Validation

The developed method was validated according to ICH guidelines Q2A[16]. The validation parameters like

specificity, linearity and range, limit of detection, limit of quantitation, precision and accuracy.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. To check the interference between tablet excipients used in the formulation and drug substance, the specificity study was conducted. The tablet excipients (as per marketed formulation) were mixed in proportion, diluted by using selected solvent and filtered using whatman filter paper no 41. The prepared solutions (Placebo and standard) were scanned in the UV region. Then compare to assess the interference among excipients and drugs.

Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample. Serial dilutions of 1-15µg/ml for TAD and 3-45µg/ml for DAP were prepared separately from the standard stock solutions. Then measure the absorbance at 284 nm and 292 nm for TAD and DAP respectively. Calibration curves were constructed for both drugs. Regression analysis was performed by least squares method to determine the values of slope, intercept and correlation coefficient.

Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. The repeatability of the method was confirmed by the analysis of tablet formulation. 6µg/ml for TAD and 18 µg/ml for DAP solution concentration were prepared and measure the absorbance. The procedure was repeated for 6 times with the same concentration. The amount of each drug present in the tablet formulation was calculated. The percentage RSD was calculated.

Accuracy

The accuracy of the proposed method recovery studies were performed by standard addition method. The recovery experiment was done by adding known concentration of Tadalafil and Dapoxetine Hydrochloride raw material to pre-analysed formulation. The tablet powder equivalent to 30 mg of Dapoxetine Hydrochloride (10 mg of Tadalafil) was weighed accurately and transfer into a series of three 100ml standard flask. To that raw material Tadalafil and Dapoxetine Hydrochloride (25%, 50%, 75%) were added, dissolved with minimum quantity of solvent mixture acetonitrile and water (1:1) and made upto the mark with the same solvent. The content was kept in a sonicator for 15 minutes, after sonication the solutions were filtered through whatmann filter paper no.41 and measure the absorbance. The absorbance was used for the determination of Tadalafil and Dapoxetine respectively. The procedure was done for three times.

Limit of Detection and Limit of Quantitation

The limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy.

The detection limit (LOD) for the proposed method was calculated using the following equation:

$$LOD = 3.3\ S / K$$

Where "S" is the standard deviation of replicate determination values under the same conditions as for sample analysis in absence of the analyte and „K" is the sensitivity namely the slope of the calibration graph. The limit of quantification (LOQ) was defined as LOQ = 10 S / K.

Stability of the Solution

Stability of the solutions were checked by observing any changes in terms of absorbance and spectral pattern which was compared to freshly prepared solutions by keeping the solutions at room temperature and analyzing at frequent intervals.

RESULT AND DISCUSSION

A simple precise, rapid and accurate simulateneous method was developed and validated for DAP and TAD in bulk and in combine tablet dosage form. Development of accurate analytical method was used for routine quantitative determination of samples. It will reduce unnecessary tedious sample preparation cost materials and laboratories. Alternative method of HPLC is UV-spectrometric method of analysis offer cost effective and time saving.[17]

The solubility of the drug in various polar and non-polar solvents checked as per Indian pharmacopeia guidelines. Both drugs were exhibited different solubility character, from the solubility data, the common solvent was found to be acetonitrile and water (1:1). Hence, the solvent mixture was selected as the common solvent and it was used for the preparation of stock solution. 10 µg/ml concentration solutions of TAD and DAP were prepared and the spectra were recorded. The overlaid spectra were used by observing the spectral characters of TAD and DAP. The method was used for multi component analysis like simultaneous equation method. The overlaid spectrum was shown in (fig. 3).

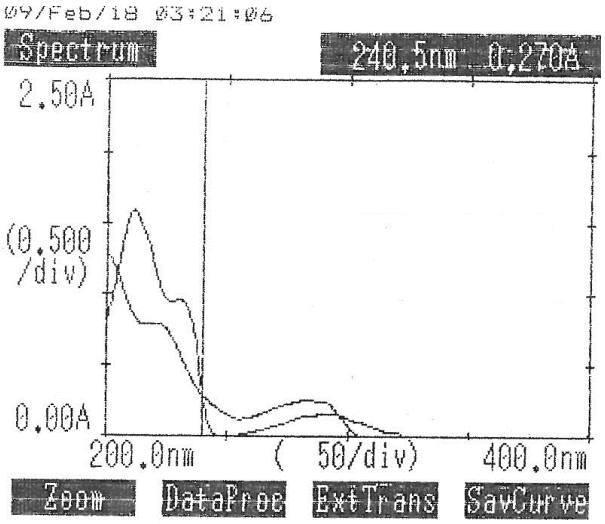

Fig. 3: Overlaid Spectrum of Tadalafil and Dapoxetine Hydrochloride.

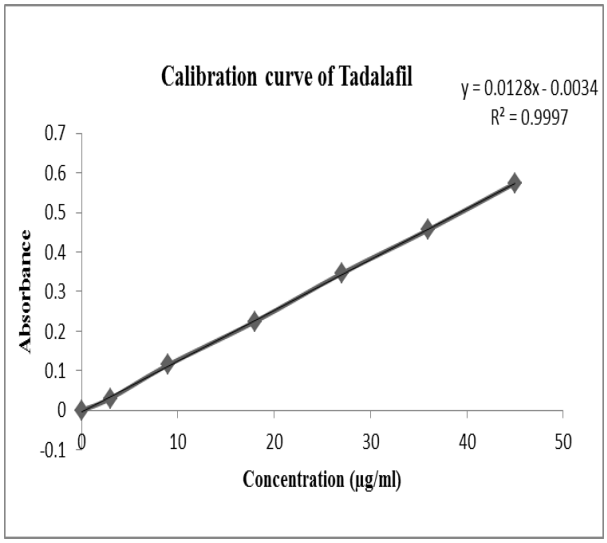

Fig. 4: Calibration Curve of Tadalafil.

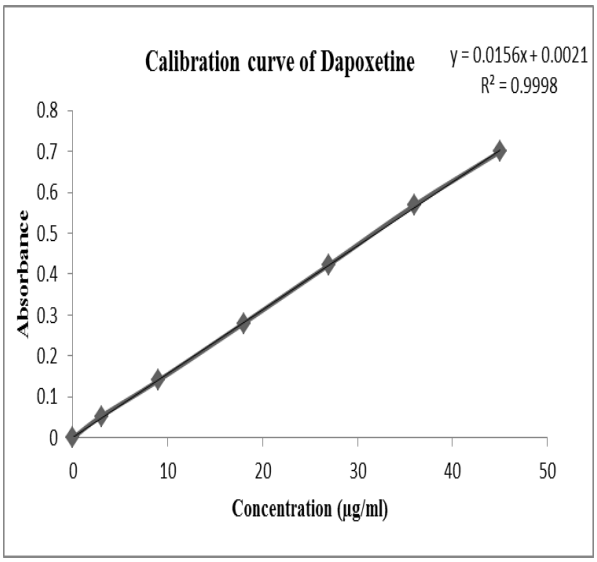

Fig. 5: Calibration Curve of Dapoxetine Hydrochloride.

The stability of TAD and DAP were checked at the selective wavelength TAD for 284 nm and DAP for 292 nm using acetonitrile and water as solvent. It was found that TAD stable for 3hours and DAP stable for about 2 hours 30 minutes.

Various aliquots of TAD and DAP were prepared in the concentration range from 1-15µg/ml for Tad and 3-45µg/ml for Dap respectively. The absorbance of these solutions was measured at the selected wavelengths. The calibration curve was constructed using concentration vs. absorbance. The linearity study was repeated for 5 times for each drug at their selected wavelengths. Regression analysis was performed by applying least square method for calculating values of slope, intercept and correlation coefficient for TAD and DAP at their relative wavelengths. The correlation co-efficient values for both drugs were found to be 0.999. This indicated both drugs obey Beer's law in the selected concentration ranges. Moreover, low LOD and LOQ values prove the sensitivity of the proposed methods. Hence, the concentration was found to be linear. The calibration curve for TAD at 284 nm and DAP at 292 nm were shown in (fig.4) and (fig.5). The optical characteristics of the drug were shown in Table 1.

Table 1: Linearity Data For Tadalafil And Dapoxetine.

Parameters (µG/ML)	Tadalafil	Dapoxetine
Beer's law limit(µg/ml)	1-15µg/ml	3-45µg/ml
Correlation coefficient	0.9997	0.9998
Regression equation	Y= 0.012X-0.003	Y= 0.015X+0.002
Slope (m)	0.012	0.015
Intercept (c)	0.003	0.002
LOD(µg/ml)	0.2094	0.2981
LOQ(µg/ml)	0.6344	0.9032

The developed method was successfully applied for the quantitative determination of TAD and DAP in tablet formulation (Uphold tablet: 10 mg of TAD and 30 mg of DAP). Sample solutions were analyzed six times and experimental values were found to be within 99 and 103 % for both the drugs. Hence the developed methods can be used for the simultaneous determination of TAD and DAP in bulk and in tablet formulation (Table 2).

Table 2: Quantification of Tablet Formulation (Uphold) By Simultaneous Equation Method.

Drug Name	Sample Number	Labeled Amount (mg/tablet)	Amount Found (mg)	Percentage Obtained (%)	Average (%)	SD	%RSD
Tadalafil	1	10	10.33	103.3	103.4	1.9374	1.8736
	2	10	10.5	105			
	3	10	10.5	105			
	4	10	10.16	101.6			
	5	10	10.06	100.6			
	6	10	10.50	105.0			
Dapoxetine Hydro-chloride	1	30	29.83	99.43	99.99	0.8279	0.8279
	2	30	30.16	100.5			
	3	30	30.16	100.5			
	4	30	30.16	100.5			
	5	30	29.56	98.55			
	6	30	30.16	100.5			

SD= Standard Deviation for n=6 observations, %RSD= Percentage Relative Standard Deviation

The precision of the method was confirmed by the repeated analysis of formulation for 6 times. The percentage relative standard deviation values were found to be 0.9289 and 0.8570 for TAD and DAP respectively. The results were shown in Table 3. The low percentage RSD values indicated that the precision of method was confirmed.

Table 3: Precision Study for Formulation (Uphold).

Drug Name	Labeled Amount (mg/tablet)	Amount Found (mg)	Percentage Obtained (%)	Average (%)	SD	%RSD
Tadalafil	10	10.5	105.0	105.83	0.9831	0.9289
	10	10.7	107.0			
	10	10.5	105.0			
	10	10.2	102.0			
	10	10.5	105.0			
	10	10.6	106.0			
Dapoxetine Hydro-chloride	30	30.15	100.5	101.36	0.8687	0.8570
	30	30.36	101.2			
	30	30.66	102.2			
	30	30.80	102.6			
	30	30.16	100.5			
	30	30.36	101.2			

SD= Standard Deviation for n=6 observations, %RSD= Percentage Relative Standard Deviation

The accuracy of the method was confirmed by recovery studies. To the pre-analyzed formulation, a known quantity of raw material was added 25%, 50% and 75% for TAD and DAP. The percentage recovery was found to be in the range from 99.3 to 100.6% and 98.8 to 100.7% for TAD and DAP respectively. The percentage RSD value was found to be 0.6799 and 0.9857 for TAD and DAP respectively. The low percentage RSD value indicated there was no interference due to excipients used in formulation. The results were shown in Table 4.

Table 4: Recovery Study Data Of Pre Analysed Formulation.

Drug name	%	Amount present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered	% Recovery	Average	SD	%RSD
Tadalafil	25	6.00	1.5	7.69	6.16	99.33	99.82	0.6787	0.6799
	50	6.00	3.0	9.32	6.22	100.6			
	75	6.00	4.5	10.68	6.18	99.55			
Dapoxetine	25	18.00	4.5	21.5	18.11	100.2	99.9	0.9848	0.9857
	50	18.00	9.00	26.8	18	98.8			
	75	18.00	13.5	31.6	18.2	100.7			

SD= Standard Deviation for n=3 observations, %RSD= Percentage Relative Standard Deviation

Table 4: Recovery Study Data Of Pre Analysed Formulation.

Drug name	%	Amount present (µg/ml)	Amount Added (µg/ml)	Amount Found (µg/ml)	Amount Recovered	% Recovery	Aver age	SD	%RSD
Tadalafil	25	6.00	1.5	7.69	6.16	99.33	99.82	0.6787	0.6799
	50	6.00	3.0	9.32	6.22	100.6			
	75	6.00	4.5	10.68	6.18	99.55			
Dapoxetine	25	18.00	4.5	21.5	18.11	100.2	99.9	0.9848	0.9857
	50	18.00	9.00	26.8	18	98.8			
	75	18.00	13.5	31.6	18.2	100.7			

SD= Standard Deviation for n=3 observations, %RSD= Percentage Relative Standard Deviation

CONCLUSION

The developed photometric method have been successfully applied for simultaneous determination of TAD and DAP in combined tablet dosage form. They were found to be simple .precise, rapid and accurate. The method were completely validated showing satisfactory dates for all the method validation parameters tested. Recovery studies indicated that practically there was no interference from tablet additives. So the method can be easily and conveniently adapted for routine quality control analysis of Tadalafil and Dapoxetine.

REFERENCES

1. https://www.rxlist.com/cialis-drug.htm
2. https://www.rxlist.com/paxil-drug.htm
3. Ali Al Kaf, Ayman A Gouda. Spectrophotometric determination of Tadalafil in pure and dosage forms. Chem Ind & Chem Eng Quart, 2011; 17(2): 125−132.
4. Alivelu Samala, Santhosh Pawar, Sowmya Manala, Sravanthi Chada, Nageshwar M. RP- HPLC method development and validation of Tadalafil in tablet dosage form. Journal of Chem Pharma Research, 2013; 5(4): 315-318.
5. Kavitha A, Vijaya durga D, Hima bindu S, Eshvendar K, Khaleel N, Pani kumar D, Anumolu. Forced degradation studies, quantification and *in-vitro* Dissolution studies of Tadalafil by spectrofluorimetry. Asian J Pharmac Clin Res., 2013; 6(2): 326-329.
6. Panchumarthy Ravisankar, Niharika A, Pavan G, Madhavi V, Shiny Susan T. Validated UV spectrophotometric method for quantitative analysis of Dapoxetine in pharmaceutical dosage form. Asian J Sci Tech., 2015; 06(11): 1976-1980.
7. Rohith T, Ananda S. Development and Validation of High performance liquid chromatography method for the determination of process related impurities in Dapoxetine Hydrochloride. Inter J Res in Pharmacy Chem., 2013; 3(1): 74-82.
8. Rajendra B, Patil, Tushar A, Deshmukh, Vijay R, Patil. Stability indicating HPLC method for Dapoxetine HCL in bulk and in formulation. Inter J Pharma Pharma Sci., 2014; 6(5): 687-690.
9. Anuruddha P, Chabukswar, Bhanudas S, Kuchekar, Sonali L, Patel, Seagate A, Moon, Subhash G, Chute, Bharat D. Pagare. Spectrophotometric Simultaneous Determination of Dapoxetine and Sildenafil in combined tablet dosage form by Absorbance Corrected method. Der Pharma Chemica, 2012; 4(4): 1404-1407.
10. Kalyani K, Anuradha A. A novel stability indicating RP-HPLC method for the simultaneous estimation of Sildenfil citrate and Dapoxetine hydrochloride in bulk and pharmaceutical formulations. Der Pharmacia Lettre, 2015; 7(10): 98-106.
11. Abha D, Giri, Vidhya K, Bursary Sunil R, Dhaneswar. Validated HPLC method for Simultaneous Quantization of Tadalafil and Dapoxetine Hydrochloride in Bulk drug and formulation. Inter J Pharma Pharma Sci., 2012: 4(2): 654-658.
12. Kiran Kumar A, Balakrishnan M, Chandrasekhar KB. A New validated simultaneous method development by RP-UPLC for the estimation of Tadalafil and Dapoxetine in bulk and pharmaceutical dosages. Inter J Pharmacy Bio Sci., 2018; 8(2): 65-73.
13. Raval Kashyap, Srinivasa U, Kalindi Badodaria. First order derivative and Dual wavelength spectrophotometry methods development and validation for simultaneous estimation of Avanafil and Dapoxetine Hydrochloride in bulk and dosage form. Inter J Pharma & Techn., 2014; 6(1): 6418-6438.
14. Indian pharmacopoeia, Controller of Publication, Govt. of India, Ministry of Health and Family Welfare, New Delhi, 2007; 1: 177-183.
15. ICH, Q2A, Hamonised Tripartite Guideline, Test on Validation of Analytical Procedures, IFPMA, in: Proceedings of the International Conference on Harmonization, Geneva, March, 1994.
16. Beckett AH, Stenlake JB. Practical Pharmaceutical Chemistry. Part B. 4th ed. New Delhi: CBS Publishers and Distributors, 2007; 157-166.
17. Laxman R, Acharya A, Jain V, Bhardwaj S, Jain D. Development and Validation of RP-HPLC and Spectrophotometric methods for simultaneous Estimation of Spironolactone and Torsemide in Pharmaceutical Dosage form. Inter J Res Ayurveda Pharmacy, 2010; 1: 459– 467.