AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Tadalafil and Fluoxetine HCl in Synthetic Mixture

Radhika S. Patel[1*], Dilip G. Maheshwari[1]
1. L.J. Institute of pharmacy, S.G.Highway, Ahmedabad, Gujarat-382210.

ABSTRACT

Simple, accurate and precise and economical UV–spectroscopy method have been developed and validated for the estimation of Tadalafil and Fluoxetine HCl in a synthetic mixture. Tadalafil is used in treatment of Erectile Dysfunction and Fluoxetine HCl is used in depression. The Tadalafil and Fluoxetine HCl stock solutions are prepared in methanol solution. At zero crossing point (ZCP) of Tadalafil (230nm) Fluoxetine HCl showed a measurable derivative absorbance, whereas at zero crossing point (ZCP) of Fluoxetine HCl (235nm) Tadalafil showed a appreciable derivative absorbance value. The Tadalafil and Fluoxetine HCl are linear in concentration range of 5-25 µg/ml. Developed method was validated according to the ICH Q2 (R1) guidelines. The precision were found within limits (RSD< 2%). Accuracy were determined by recovery studies and showed % recovery between 97 to 100 % for both the drugs Tadalafil and Fluoxetine HCl. The LOD and LOQ values of Tadalafil at ZCP 230 nm were found to be 0.24 and 0.74 correspondingly and for Fluoxetine HCl at ZCP235nm were found to be 0.29 and 0.89 correspondingly.

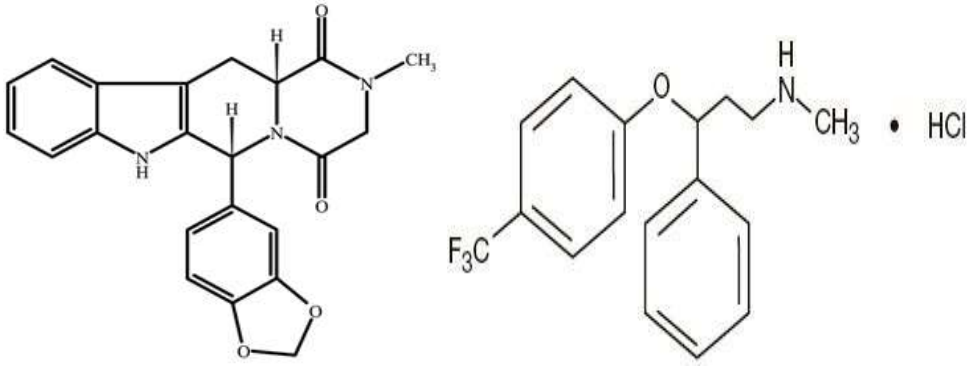
Keywords: Tadalafil, Fluoxetine HCl, UV Spectrophotometry, Second order Derivative Spectrophotometry.

*Corresponding Author Email: dgmaheshwari@gmail.com
Received 23 January 2015, Accepted13 2 February 2015

Please cite this article as: Patel RS *et al*., Development and Validation of Second Order Derivative Spectrophotometric Method for Simultaneous Estimation of Tadalafil and Fluoxetine HCl in Synthetic Mixture. American Journal of PharmTech Research 2015.

INTRODUCTION

Tadalafil is chemically (6R,12aR)-6-(1,3-Benzodioxol-5-yl)-2-methyl-2,3,6,7,12,12a Hexahydropyrazino [1'2'1,6] pyrido[3,4-b]indole-1,4-Dione is a phosphodiesterase 5 inhibitor used in the treatment of Erectile Dysfunction[1]. Fluoxetine HCl is chemically N-Methyl-3-[4-(trifluoromethyl) phenoxyl]-1- propanamine hydrochloride (1:1) is a serotonin uptake inhibitor used in the major depressive disorder[2]. Fluoxetine HCl is official in Indian Pharmacopoeia (IP) and United States Pharmacopoeia (USP)[3,4]. A Synthetic mixture is containing 20mg of Tadalafil and 20mg of Fluoxetine HCl. Tadalafil is used in Erectile Dysfunction (ED).Fluoxetine HCl is used in antidepressant drug. Mixture of Tadalafil with Fluoxetine HCl in patients effective in Premature Ejaculation gives Synergistic effect. If the association of a phosphodiesterase-5 inhibitor Tadalafil and a selective serotonin reuptake inhibitor Fluoxetine HCl can prolong the intravaginal ejaculatory latency time (IELT) in men. It can also decrease depression disorder of the premature ejaculation men and relieve anxiety status[5].The chemical structures of both drugs [1,2]were shown in figure 1.



(a) (b)

Figure 1: Chemical structure of Tadalafil (a) and Fluoxetine HCl (b).

Derived from the literature Survey (UV[6-8],HPLC[9-13],HPTLC[14-15])method for the determination of Tadalafil and Fluoxetine HCl with additional new drugs combination. There is no any reported method is available for the estimation of Tadalafil and Fluoxetine HCl by UV Spectrophotometry method, So the research work describes the spectrophotometric method which is based on second order derivative for simultaneous estimation of Tadalafil and Fluoxetine HCl in synthetic mixture.

MATERIALS AND METHOD

Instrument

Double beam UV-visible spectro photometer SHIMADZU (model 1800) with 1 cm matched quartz cuvettes Which were used for all absorbance measurements. All the weighing were done

through electronic analytical balance (Wensar Dab220). Distilled water and Whatmann filter paper (no.41) were used the whole time in the experimental work.

Software: UV Probe Version 2.31

Reagents and Materials

Tadalafil and Fluoxetine HCl bulk powder were kindly gifted by reputed Pharmaceutical Company in Ahmedabad, India

Selection of Solvent

Methanol was used as a solvent as both the drugs shows good solubility in methanol.

Preparation of Standard Solutions

Preparation of Standard Solution of Tadalafil.

Accurately weighed10 mg Tadalafil was transferred in to 100 ml volumetric flask and diluted up to the mark with Methanol solvent to give a stock solution of 100µg/ml. From the above stock solution pipette out 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5ml of solution and transferred in to 10 ml volumetric flask and make up the volume up to 10 ml with the Methanol solvent to Produce concentration 5,10,15,20 and 25µg/ml respectively for Tadalafil.

Preparation of Standard Solution of Fluoxetine HCl

Accurately weighed 10 mg Fluoxetine HCl was transferred to 100 ml volumetric flask and diluted up to the mark with Methanol solvent to give a stock solution having strength of 100µg/ml. From the above stock solution pipette out 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml of solution and transferred in to 10 ml volumetric flask and make up the volume up to 10 ml with Methanol solvent to Produce concentration 5, 10, 15, 20 and25µg/ml respectively For Fluoxetine HCl.

Preparation of Sample Solution

From the Standard stock solution of Tadalafil and Fluoxetine HCl pipette out 1 ml of Tadalafil and 1 ml of Fluoxetine HCl in 10 ml volumetric flask respectively and make up the volume with the Methanol. The mixture contain 10ml Tadalafil and 10 ml of Fluoxetine HCl

Prepration of Sythetic mixture of Tadalafil and Fluoxetine HCl

Tadalafil and Fluoxetine HCl synthetic mixture was prepared in the ratio of 1:1.Here accurately weighed 20mg of Tadalafil and 20mg of Fluoxetine HCl. Common excipients like Talc (4mg), Magnesium stearate (4mg), Micro crystalline cellulose(25mg), Lactose monohydrate(32mg),HPMC(45mg), starch were added in this above mixture. Tadalafil and Fluoxetine HCl both drugs were transferred in 100ml volumetric flask and dissolved in methanol solvent then sonicate for 20-25 min. Filtered this solution with whatman filter paper. The filtrate was diluted up to the mark with Methanol. Tadalafil and Fluoxetine HCl mixture contains

200µg/ml of Tadalafil and 200µg/ml of Fluoxetine HCl .From this above solution pipette out 0.5ml of solution was transferred to a 10ml of volumetric flask and dilute up to the mark with methanol and make final concentration of Tadalafil 10µg/ml solution and Fluoxetine HCl 10µg/ml solution

Selection of Derivative method

Though second derivative spectra's showed zero crossing points in Methanol solvent and their absorbance's were considerably better. The second derivative method was selected because the spectral characteristics and resolution were good in the second derivative spectra.

Selection of wavelengths (Zero crossing points)

Out of these wavelengths 230nm Tadalafil and 235nm for Fluoxetine HCl were selected as the Zero crossing point for the Method based on their linearity data. At 230nm Tadalafil showed zero absorbance but Fluoxetine HCl had considerable absorbance. Similarly at 235nm Fluoxetine HCl showed zero absorbance but Fluoxetine HCl had considerable amount of absorbance.

METHOD VALIDATION[16,17]

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines.

Linearity (n=5)

The linearity of the method is its ability to bring out the test results which are directly proportional to the concentration of analyte present in the samples. Tadalafil was linear with the concentration range of 5-25 µg/ml at ZCP 230 nm. Fluoxetine HCl showed the linearity range of 5-25 µg/ml at ZCP 235nm. Plot the calibration curve of absorbance Vs concentration and find out correlation coefficient for the Tadalafil and Fluoxetine HCl.

Precision (Repeatability) (n=6)

The repeatability method was established by the analysis of Tadalafil and Fluoxetine HCl were repeated for 6 times with the same concentration (5 µg/ml).

Intermediate precision (Reproducibility):(n=3)

The intraday and interday precision was determined by the analyzing the responses 3 times on the same day and 3 different days and 3 different concentrations of standard solutions of Tadalafil (10,15 and 20 µg/ml) and Fluoxetine HCl (10,15 and 20µg/ml) .

Accuracy (Recovery study) (n=3)

To check the accuracy of the proposed methods, recovery studies is carried out at 80%, 100%, and 120% of the test concentration as indicated by the ICH Guideline.

Procedure for accuracy

Tadalafil and Fluoxetine HCl synthetic mixture was prepared in the ratio of 1:1.Here known amount of Tadalafil (18, 20, 22 µg/ml) and Fluoxetine HCl (18,20,22 µg/ml) were added to a pre-quantified test solution of Tadalafil (10µg/ml) and Fluoxetine HCl (10µg/ml). Recovery study is done at 80%, 100%, and 120% Levels. Amount of Tadalafil and Fluoxetine HCl was estimated by applying these all value of Tadalafil and Fluoxetine HCl to these equation of calibration curve.

Limit of detection and Limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derivative resulting by the calculating signal-to-noise ratio using the following equations.

International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the Intercept of the 5 calibration curve and S = slope of the calibration curve.

RESULTS AND DISSCUSSION

Tadalafil and Fluoxetine HCl showed well defined wavelength at 230nm and 235nm respectively (Figure 2).The wavelengths 230 and 235 nm were considered for development of Derivative Method. The two drugs individually and in their mixture were found over the concentration range of 5-25µg/ml for Tadalafil and Fluoxetine HCl respectively (Figure 3, 4).The calibration plot for the method was linear over the concentration range of 5-25µg/ml for Tadalafil and Fluoxetine HCl (Figure 5). The determination of coefficients (r^2) was 0.9970 for Tadalafil and 0.9959 for Fluoxetine HCl (Table 7).The method was found to be precise and as the % RSD values for interday and intraday were found to be less than 2 % for Tadalafil and Fluoxetine HCl.% recovery(97- 100 %) was found to be good at each additional concentration, indicating that method was accurate(Table 5,6). The LOD and LOQ were found to be for Tadalafil at 230nm 0.24 and 0.74 . The LOD and LOQ were found to be for Fluoxetine HCl at 235 nm 0.29 and 0.89 (Table 7). The proposed method was specific for the estimation of Tadalafil and Fluoxetine HCl.

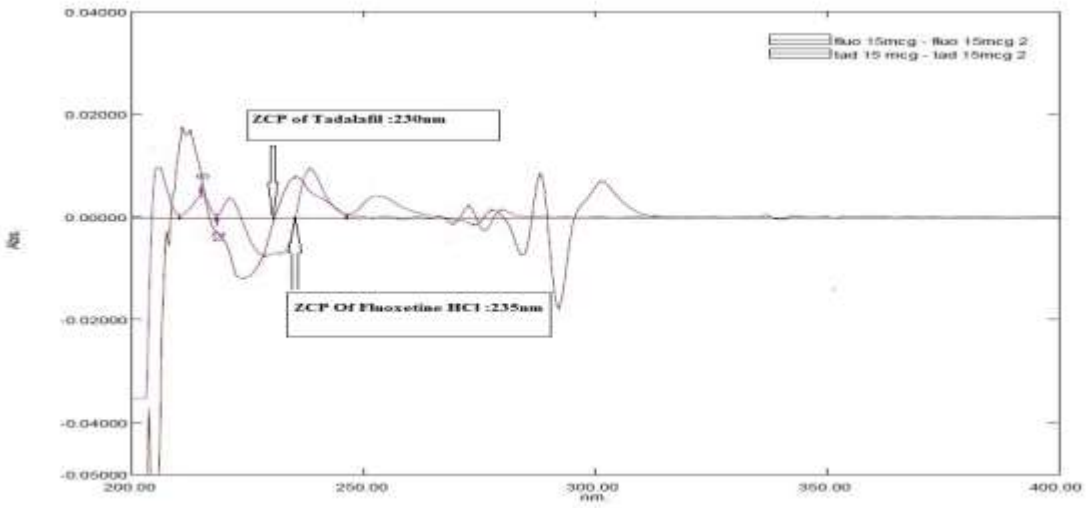


Figure 2: Overlain Second order spectra of Tadalafil (15µg/ml) and Fluoxetine HCl (15µg/ml) in Methanol

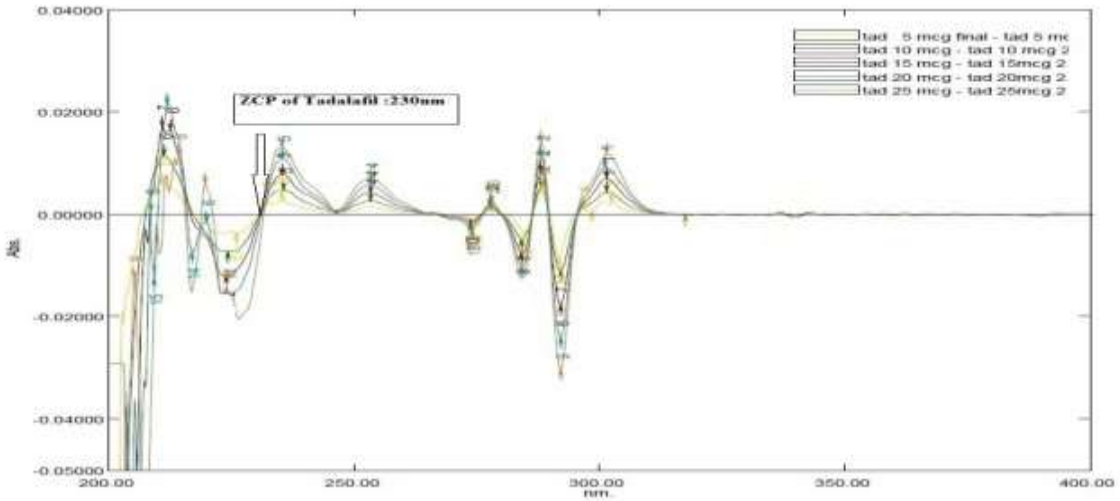


Figure 3: Overlay Second order derivative of Tadalafil at ZCP 230nm (5-25µg/ml)

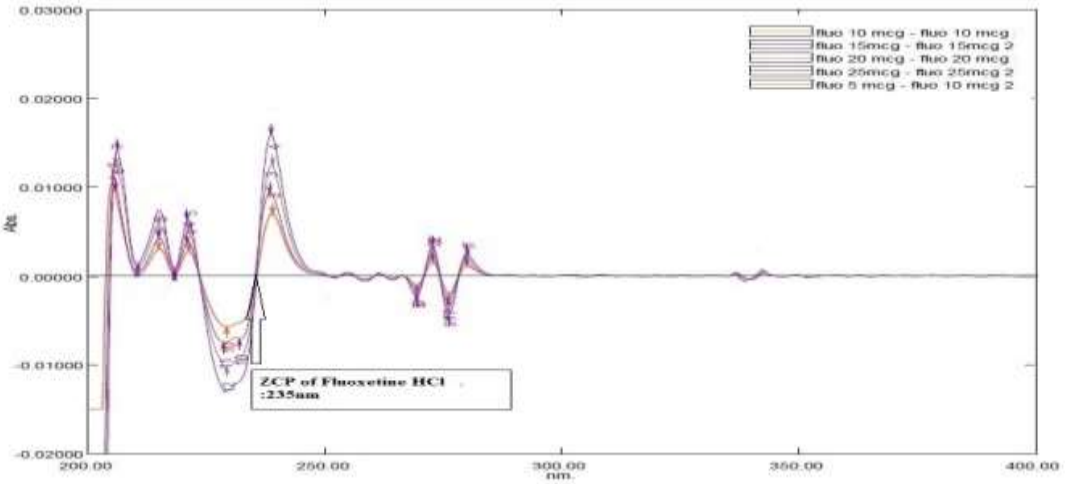
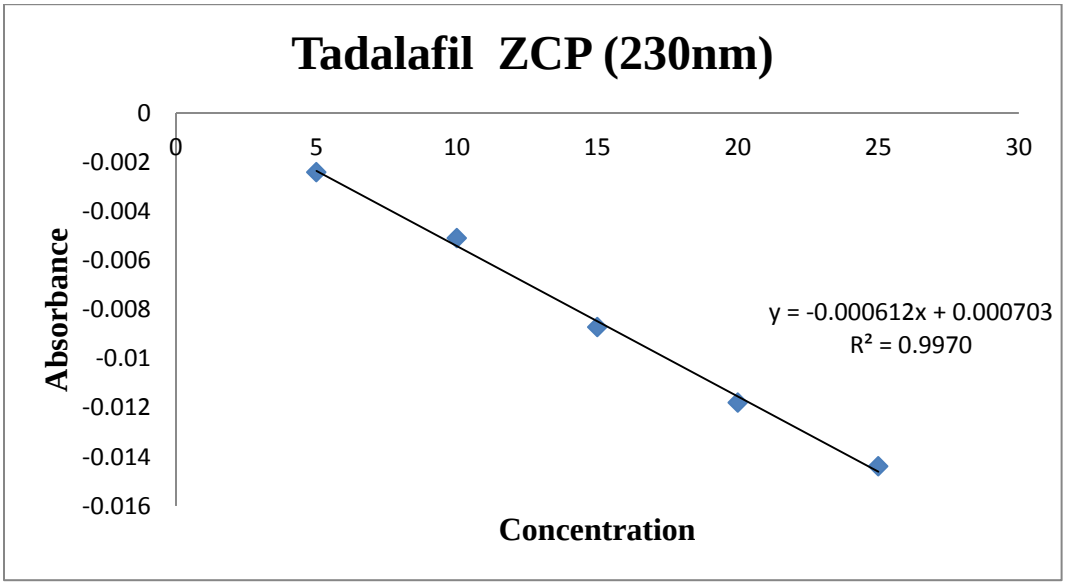
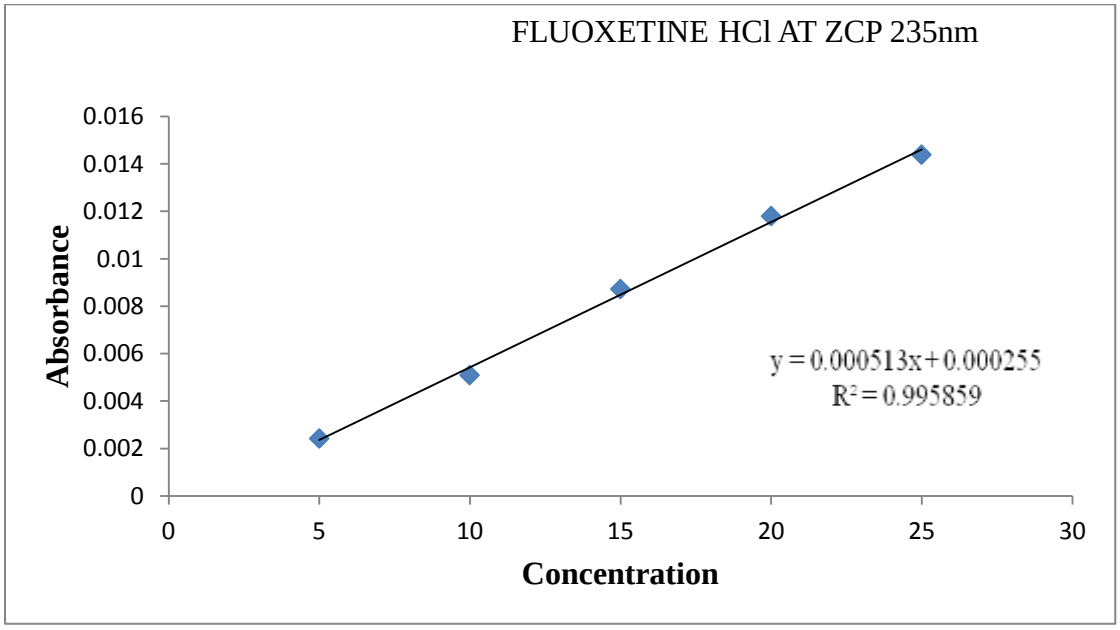


Figure 4: Overlay Second order derivative of Fluoxetine HCl at ZCP 235nm (5-25µg/ml)



(a)Linearity graph for Tadalafil (5-25µg/ml)



(b)Linearity graph for Fluoxetine HCl(5-25µg/ml)

Figure 5: Linearity graph for Second order derivative of Tadalafil and Fluoxetine HCl

Table 1: Linearity Data of Tadalafil at ZCP (230 nm) and Fluoxetine HCl at ZCP(235 nm)

Tadalafil (230nm)			Fluoxetine HCl (235nm)		
Conc (µg/ml)	**Mean absorbance±SD (n=5)**	**% RSD**	**Conc (µg/ml)**	**Mean absorbance±SD (n=5)**	**% RSD**
5	0.002418±0.000008	0.33	5	0.002774±0.000005	0.18
10	0.005098±0.000010	0.19	10	0.005718±0.000007	0.12
15	0.008726±0.000008	0.09	15	0.007638±0.000007	0.09
20	0.011791±0.000012	0.10	20	0.010382±0.000008	0.07
25	0.014382±0.000010	0.06	25	0.013280±0.000007	0.05

Table 2: Repeatability Data of Tadalafil and Fluoxetine HCl

Tadalafil (230nm)			Fluoxetine HCl (235nm)		
Conc (µg/ml)	**Mean absorbance±SD (n=6)**	**% RSD**	**Conc (µg/ml)**	**Mean absorbance±SD (n=6)**	**% RSD**
15	0.008727±0.000008	0.09	**15**	0.007642±0.000007	0.09

Table 3: Precision Data of Tadalafil at ZCP (230 nm)

Parameters	Concentration µg/ml	Mean Absorbance ±S.D	% R.S.D
Intraday	10	0.00579±0.00004	0.69
	15	0.00773±0.00006	0.77
	20	0.01042±0.00001	0.09
Interday	10	0.00580±0.00005	0.86
	15	0.00775±0.00007	0.90
	20	0.01044±0.00003	0.28

Table 4: Precision Data of Fluoxetine HCl at ZCP (235 nm)

Parameters	Concentration µg/ml	Mean absorbance±SD (n=3)	% R.S.D
Intraday	10	0.00272±0.00001	0.36
	15	0.00726±0.00001	0.13
	20	0.01142±0.00001	0.08
Interday	10	0.00273±0.00002	0.73
	15	0.00728±0.00003	0.41
	20	0.01143±0.00002	0.17

Table 5: Recovery Studydataof Tadalafil at ZCP (230 nm)(n=3)

Level	Concentration (µg/ml)	Total amount found (µg/ml)	% Recovery	SD	RSD
80 %	18	17.60	97.81	0.525	0.53
100 %	20	19.83	99.15	0.857	0.86
120 %	22	22.16	100.74	0.750	0.74

Table 6: Recovery Studydata of Fluoxetine HCl at ZCP (235 nm) (n=3)

Level	Concentration (µg/ml)	Total amount found (µg/ml)	% Recovery	SD	RSD
80 %	18	17.99	99.97	1.918	1.91
100%	20	19.84	99.23	1.365	1.37
120%	22	21.93	99.99	0.905	0.90

Table 7: Assay data of Tadalafil and Fluoxetine HCl

Tadalafil (ZCP 230 nm)				Fluoxetine HCl (ZCP 235 nm)			
Amount Taken (µg/ml)	**Amount found** (µg/ml)	**% assay± SD (n=3)**	**%RSD**	**Amount Taken** (µg/ml)	**Amount found** (µg/ml)	**% assay ±SD (n=3)**	**%RSD**
10 µg/ml	9.94µg/ml	99.43 ±0.513	0.51	10µg/ml	9.96µg/ml	99.66 ±1.527	1.53

Table 8: Regression Analysis Data and Summary of Validation Parameter for the Proposed Method

Derivative Spectrophotometric Method		
Parameter	**Tadalafil**	**Fluoxetine HCl**
Analytical Wavelength	230nm	235nm

Linearity range(µg/mL)	5-25	5-25
Regression Equation	y = 0.000612x - 0.000703	y =0.000513x + 0.000255
Correlation coefficient (r^2)	0.9970	0.9959
Repeatability (%RSD, n=6)	0.09	0.09
Intraday Precision (%RSD, n=3)	0.69-0.09	0.36-0.08
Interday precision(%RSD, n=3)	0.86-0.28	0.73-0.17
LOD (µg/mL)	0.24	0.29
LOQ (µg/mL)	0.74	0.89

CONCLUSION

Derivative spectrophotometric method is a simple, fast, accurate and precise Derivative method for the estimation of Tadalafil and Fluoxetine HCl in Synthetic mixture. This method Developed in Methanol as diluent. Developed Derivative Spectrophotometric method for the estimation of Tadalafil and Fluoxetine HCl in synthetic mixture using linearity, range, accuracy and precision parameters. The RSD for all parameters was found to be less than two, which indicates the validity of Derivative method and assay results obtained by Derivative method are in fair agreement.

ACKNOWLEDGEMENTS

The authors are thankful to Intas Pharmaceutical. Ltd., Gujarat, India for providing the gift sample of Tadalafil and Fluoxetine HCl for the research work. The authors are highly thankful to the Director of L. J. Institute of Pharmacy for providing all the facilities to Carry out the Research work.

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