# DEVELOPMENT AND VALIDATION OF TWO SPECTROPHOTOMETRIC METHODS FOR SIMULTANEOUS ESTIMATION OF FINASTERIDE AND TADALAFIL FROM THE COMBINED DOSAGE FORM

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*Abstract*

*Two simple, specific, accurate and precise UV spectrophotometric methods have been developed for simultaneous estimation of Finasteride and Tadalafil in pharmaceutical dosage form. Method I was based on simultaneous equation method while Method II depends on Q-absorbance ratio method. Method I was based on the measurement of absorbances at their respective absorbance maximas 217 nm and 282 nm, of Finasteride and Tadalafil in 0.1M NaoH Correspondingly. Calibration curve of Finasteride and Tadalafil were found to be linear in the concentration ranges of 10-50 µg/ml and 10-50 µg/ml, respectively. LOD and LOQ were 0.1 µg/ml and 0.5 µg/ml for Finasteride and 0.2 µg/ml and 0.7µg/ml for Tadalafil. Method II is the Q analysis or absorption ratio method. Absorbances were measures at 250 nm (Iso-absorptive point) and 282 nm (λ max of Tadalafil). Calibration curve of Finasteride and Tadalafil were found to be linear in the concentration ranges of 10-50 µg/ml and 10-50 µg/ml, respectively. LOD and LOQ were 0. 2 µg/ml and 0.6 µg/ml for Finasteride and 0.1 µg/ml and 0.5 µg/ml for Tadalafil. The methods were validated following ICH guidelines. Based on the proposed results, the described methods could be utilized as simple methods for the quality control of the studied drugs.*

**Keywords:** Finasteride (FIN), Tadalafil (TAD), Simultaneous equation, Q-Absorbance ratio, Validation.

## Introduction

Finasteride ((5α, 17β)-N-(1, 1-dimethylethyl)-3-oxo-4- azaandrost-1-ene-17-carboxamide) is a selective inhibitor of type II- 5 α- reductase.It is an intracellular enzyme that converts the androgen testosteroneinto 5α-dihydrotestosterone. It is commonly used to treat benign prostatic hyperplasia, prostate cancer, and androgenetic alopecia. The chemical structure of Finasteride is shown in (fig.1-A)[1]. Tadalafil, chemically pyrazino [1′,2′:1,6] pyrido [3,4-b] indole-1,4-dione,6-(1,3-benzodioxol5-yl)-2,3,6,7,12,12 a-hexahydro-2-methyl-, ( 6R,12aR )is an impotence agent. It is indicated for the treatment of erectile dysfunction. It is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). It is indicated for the treatment of erectile dysfunction and the signs and symptoms of benign prostatic hyperplasia. The chemical structure of Tadalafil is shown in (fig.1-B)[2].

Analytical method development and validation play important roles in the discovery, development and manufacture of pharmaceuticals. The goal of the analytical method is to separate, quantify the main active drug, any reaction impurities, all available synthetic intermediates and any degradants. Analytical methods are used to ensure the identity, purity, potency, and safety of drug products. In present study, all validation parameters for quantitative analysis of Finasteride and Tadalafil tablets were tested and data were evaluated according to their acceptance criteria[3-8].The UV absorption spectra of Finasteride and Tadalafil show sever overlap, which make the simultaneous determination extremely challenging. Spectrophotometry approach offers a powerful tool for resolving the overlapping spectra and enhancing spectral resolution and selectivity[9-11]. Selection of the wavelength at which the mixture is analyzed by spectrophotometry is the critical step and may include analysis of the spectra[12, 13].

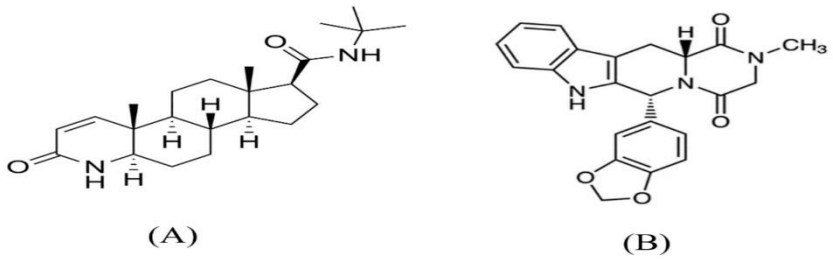



**Figure-1: Chemical structure of (A) Finasteride (B) Tadalafil**

## MATERIALS AND METHODS:

### Materials:

Finasteride and Tadalafil were obtained as gift sample from Scitus,Pharma Services Private Limited, Chennai, India. NaOH was procured from Loba chemie pvt. Ltd. Mumbai, India.

### Instruments:

All measurements were carried out with Shimadzu UV-Visible 1650 pc Spectrophotometer (Shimadzu Corp., Tokyo, Japan). A 10 mm quartz cuvettes were used to scan the samples absorption spectra. The manipulation of scanned spectra was performed using Shimadzu UV-Probe software version 2.43.

## SIMULTANEOUS EQUATION METHOD

If a sample contains two absorbing drugs, each of which absorbs at the λmax of the other, it may be possible to determine both drugs simultaneously using multicomponent analysis UV Spectrophotometric 'Simultaneous Equation Method.' Two wavelengths selected for the development of the simultaneous equations are 217nm and 282 nm. The absorptivity values determined for Finasteride are 0.121(ax1), 0.291

(ax2) and Tadalafil are 0.158 (ay1), 0.121 (ay2) at 217 nm and 282nm respectively. These values are means of six estimations. The absorbances and absorptivity at these wavelengths were substituted in equation 1 and 2 to obtain the concentration of both drugs

$$CX = \frac{A2\ ay1 - A1\ ay2}{ax2ay1 - aX1\ ay2}$$

$$CY = \frac{A2ax1 - A1ax\ 2}{Ax2ax1 - ax1ay2}$$

CX and cy are the concentrations of Xand Y.

ax1 and ax2 are absorptivities of X.

ay1 and ay2 are absorptivities of Y.

A1 and A2 absorbance of the mixture

Where Cx and Cy are concentration of Finasteride and Tadalafil respectively in µg/ml. 0.275(A1) and 0.853(A2) are the absorbances of the mixture at 217 nm and 282 nm respectively.

**Preparation of standard stock solution:**

Stock solution (100 µg/ml) of Finasteride and Tadalafil were prepared by accurately weighing 2.5 mg of the drug in minimum quantity and finally to make the volume up to 100 ml. A series of standard drug solution in concentration range of 10-50µg/ml were prepared by diluting appropriate volumes of standard stock solutions. The scanning for solution of Finasteride and Tadalafil were carried out in the range of 200-400 nm. The absorbance was noted at selected wavelength of 217nm and 282nm.The Linearity range 10-50 µg/mL of Finasteride and Tadalafil obeyed Beer -Lambert's law condition.

**Methodology:**

In the wavelength range of 200–400 nm, the absorption spectra of these dilutions were monitored and recorded against 0.1 M NaOH as a blank. The absorbance was noted at selected wavelength of 217nm for Finasteride and 282nm for Tadalafil.UV spectrum of Finasteride at 217nm with concentration of 10µg/ml is shown in (fig. 2). UV spectrum of Tadalafil at 282nm with concentration of 10µg/ml is shown in (fig. 3).Five samples were made by transferring aliquots of different concentrations from Finasteride and Tadalafil standard solutions into a set of 10-mL volumetric flasks and diluting to volume with 0.1 M NaOH. The samples were analyzed using the procedures outlined under the linearity and calibration graphs for each method, and the concentrations of each drug were calculated. UV spectrum of mixture is shown in (fig.4).Contents of 10 Entadfi™ capsules (5 mg Finasteride and 5 mg Tadalafil per capsule) were weighed and mixed well. A weighed powder equivalent to one capsule was accurately transferred to 10-mL volumetric flask with 0.1 M NaOH, shaken vigorously for 20 min, filtered, and adjusted to 10 mL with 0.1 M NaOH . Further dilution with 0.1 M NaOH was made to prepare five samples of different concentrations. The samples

were analyzed following the procedure mentioned for each method under the linearity and calibration graphs and the concentration of each drug was computed. UV spectrum of formulation is shown in (fig.5).The five concentration of Finasteride and Tadalafil, UV-spectrum were shown in (fig. 6 & 7).

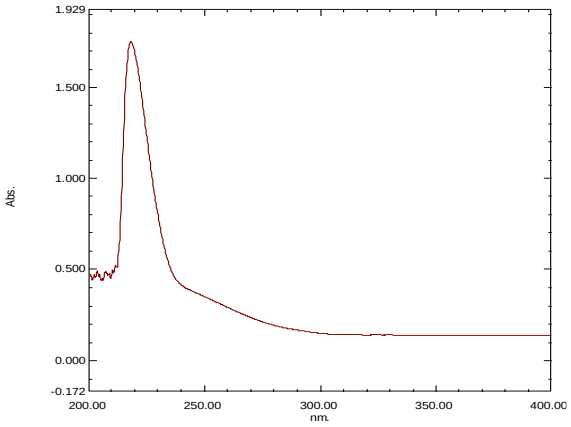



**Figure-2: UV Spectrum of Finasteride at 217nm (10 µg/ml)**

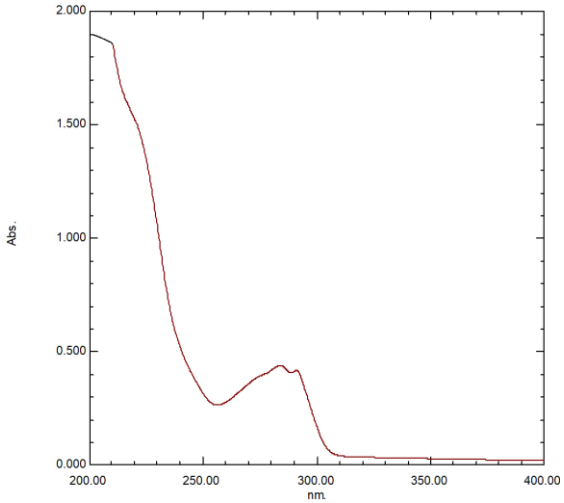



**Figure-3: UV Spectrum of Tadalafil at 282nm (10 µg/ml)**

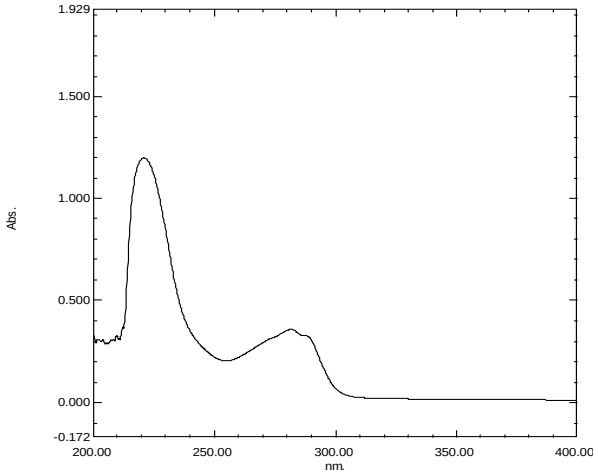



**Figure-4: Mixture of Finasteride and Tadalafil**

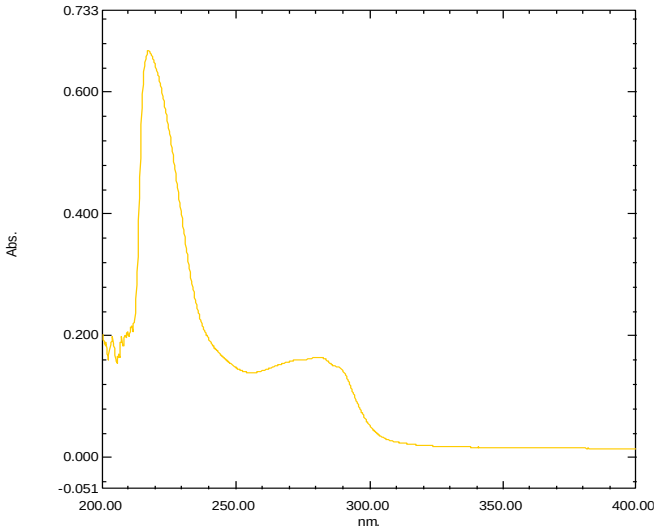



**Figure-5: Formulation of Finasteride and Tadalafil**

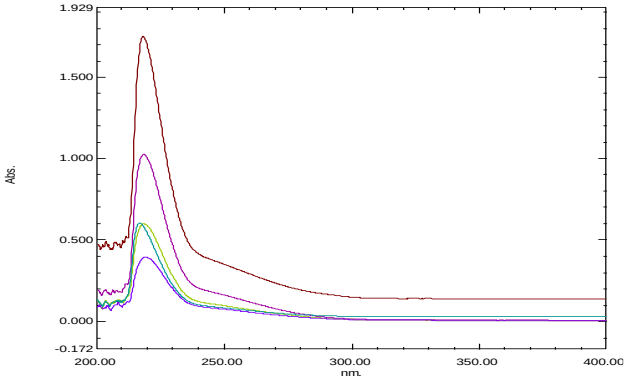



**Figure-6: UV Spectrum of Finasteride at 217nm (10-50 µg/ml)**

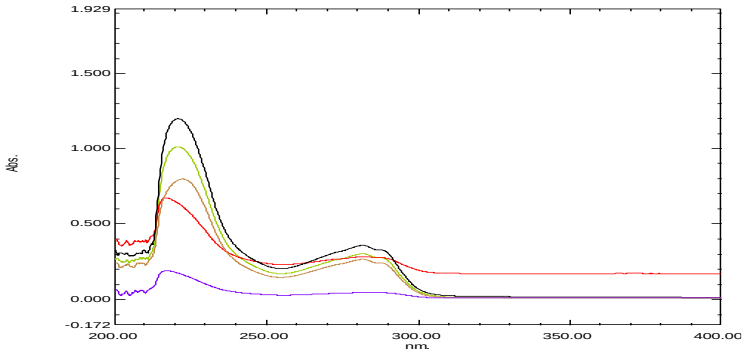



**Figure-7: UV Spectrum of Tadalafil at 282nm (10-50 µg/ml)**

## Q ABSORBANCE RATIO METHOD

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components.

The concentration of two drugs in the mixture can be calculated using following equations.

$$CX = [(QM – QY) / (QX - QY)] \times A1/ax1 \text{..... (1)}$$

$$CY = [(QM – QX) / (QY - QX)] \times A1/ay1 \text{ …… (2)}$$

Where, A1 and A2 are absorbances of mixture of drugs at isobestic point and selected wavelength

ax1 and ay1 are absorptivities of drug at isobestic point and selected wavelength

ax2 and ay2 are absorptivities of drug at isobestic point and selected wavelength

$QM = A2 / A1,$

$QX = ax2 / ax1$

$QY = ay2 / ay1.$

**Preparation of standard stock solution:**

Stock solution (100 µg/ml) of Finasteride and Tadalafil were prepared by accurately weighing 2.5 mg of the drug in minimum quantity and finally, to make the volume up to 100 ml. A series of standard drug solution in concentration range of 10-50µg/ml were prepared by diluting appropriate volumes of standard stock solutions. The scanning for solution of Finasteride and Tadalafil were carried out in the range of 200-400 nm. The maximum absorption (λmax) of Tadalafil was found at 282 nm and iso-absorptive point at 250 nm. Absorption and absorptivity for a series of standard solutions were recorded at selected wavelengths.

**Methodology:**

Absorption ratio method uses the ratio of absorptions of two selected wavelength, one of which is iso-absorptive point and other being the λ max of one of the two components. From the overlain spectra of two drugs as shown in (fig. 8), it shows that Finasteride and Tadalafil having iso-absorptive point at 250 nm. The second wavelength used is 282 nm, which is the λ max of Tadalafil. Working standard solutions having concentration 10-50µg/ml for Finasteride and Tadalafil were prepared and the absorbance at 250 nm (iso-absorptive point) and 282 nm (λ max of Tadalafil ) were measured and absorptivity coefficient were calculated using calibrations curve,

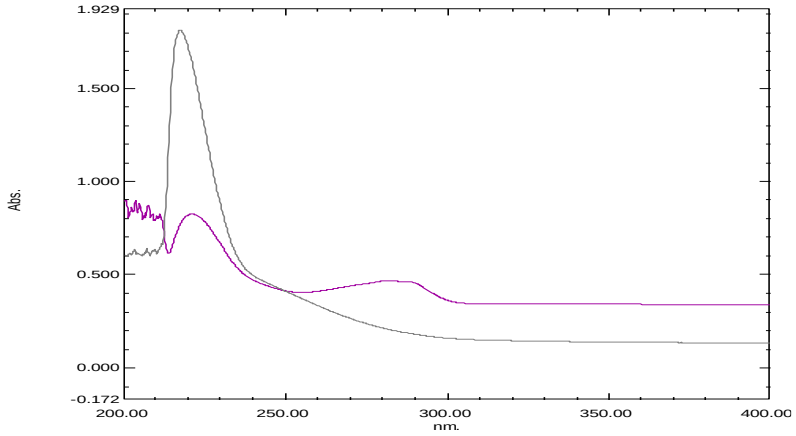


**Figure- 8: Overlay spectrum of Finasteride and Tadalafil**

## METHOD VALIDATION

### Linearity and Range:

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample within a given range. The linearity of method was established by analyzing different concentrations of Finasteride and Tadalafil at 217 nm and 282 nm. Finasteride in the concentration range 10-50 µg/ml obeyed Beer's Law at 217 nm and 282 nm. Linearity equation is $y = 0.0125x + 0.0128$ with $R^2 = 0.9983$. Tadalafil in the concentration range 10-50 µg/ml obeyed Beer's Law at 217nm and 282nm. Linearity equation is $y = 0.0188x + 0.1163$ with $R^2 = 0.9974$.

### Precision:

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as relative standard deviation (coefficient of variation).

### Procedure for the Determination of Intra-day Precision:

Intraday precision was found out by carrying out analysis of standard drug solution at three different concentrations (10 – 50µg/ml) for Finasteride and (10 – 50 µg/ml) for Tadalafil in the linearity range for three times on the same day and % RSD was calculated.

### Procedure for the Determination of Inter-day Precision:

Interday precision was found out by carrying out analysis of standard drug solution at three different concentrations (10 – 50µg/ml) for Finasteride and (10 – 50 µg/ml) for Tadalafil in the linearity range for three day over a period of one week and % RSD was calculated.

### Procedure for the Determination of Repeatability:

Standard solution of the same concentrations of (30µg/ml) for Finasteride and (40µg/ml) for Tadalafil were measured six times and its % RSD was calculated.

### Detection and quantification limit:

The limit of detection and limit of quantification was calculated by using the average value of standard deviation and slope. The LOD and LOQ were determined from the linearity studies and the values were tabulated.

$$LOD = 3.3*(SD/Slope)$$

$$LOQ = 10*(SD/slope)$$

# RESULTS & DISCUSSION

### Simultaneous Equation Method

Calibration curve of Finasteride and Tadalafil in 0.1M NaOH follows Beers Lambert's law. The graph of absorbance against concentration for Finasteride and Tadalafil were found to be linear in the concentration range of 10-50 µg/ml at 217nm and 282nm.The coefficient of regression of the calibration curve was found to be 0.9999 %. Regression Equation gives the difference between a true value and approximate value. Finasteride and Tadalafil in the concentration range 10-50 µg/ml obeyed Beer's Law at 217nm and 282nm. Linearity equation of Finasteride and Tadalafil were shown in ( fig. 9 & 10). The lower magnitude of error ( <2%) indicates a high prediction power of the regression equation. The results obtained for linearity are summarized in Table 1. Precision was calculated as repeatability for both the drugs as shown in Table 2 & 3.

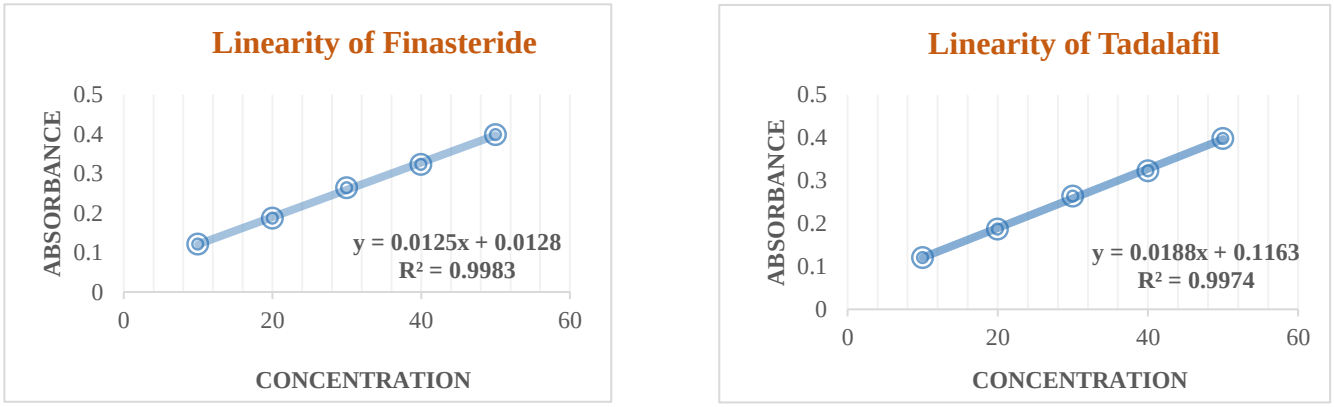



**Figure-9: Linearity of Finasteride at 217nm & Tadalafil at 217**

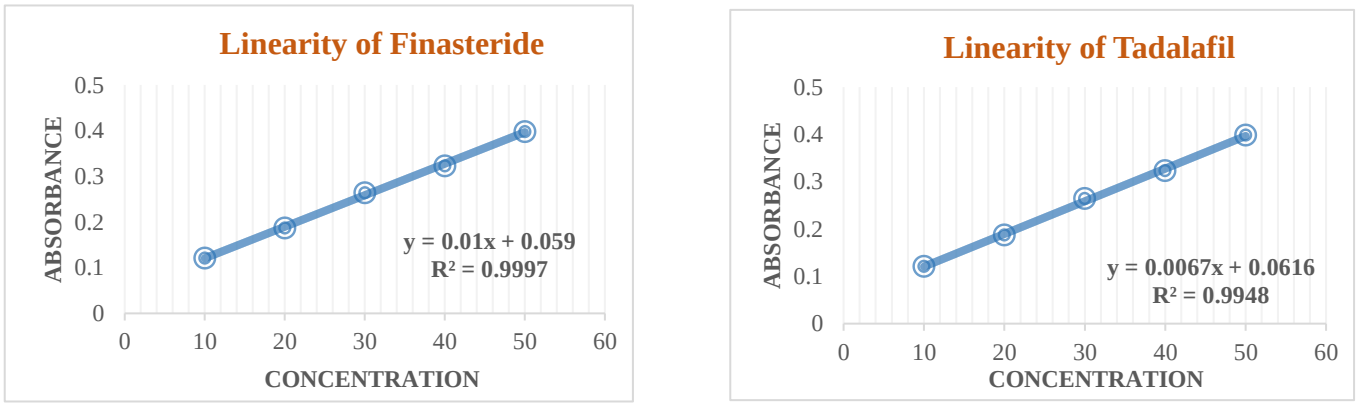



**Figure-10: Linearity of Finasteride and Tadalafil at 282nm**

**TABLE-1: RESULTS OF LINEARITY SIMULTANEOUS EQUATION METHOD**

| Conc(µg/ml) | Absorbance 217nm | | Absorbance 282nm | | Con.(µg/ml) | Absorbance Mixture | |
|---|---|---|---|---|---|---|---|
| | FIN | TAD | FIN | TAD | | FIN | TAD |
| 10 | 0.121 | 0.291 | 0.158 | 0.121 | 20 | 0.552 | 0.159 |
| 20 | 0.258 | 0.494 | 0.263 | 0.207 | 40 | 0.855 | 0.227 |
| 30 | 0.415 | 0.704 | 0.356 | 0.264 | 60 | 1.266 | 0.307 |
| 40 | 0.548 | 0.871 | 0.457 | 0.323 | 80 | 1.593 | 0.399 |
| 50 | 0.603 | 1.043 | 0.561 | 0.398 | 100 | 1.974 | 0.589 |

FIN - Finasteride : TAD -Tadalafil

**TABLE -2: RESULT OF PRECISION STUDIES**

| Drug | Amount taken (µg/ml) | Intra day | | Inter day | | Parameter | |
|---|---|---|---|---|---|---|---|
| | | Absorbance | %RSD | Absorbance | %RSD | LOD | LOQ |
| Finasteride | 30 | 0.919<br>0.919<br>0.915 | 0.25 | 0.951<br>0.952<br>0.954 | 0.13 | 0.1 | 0.5 |
| Tadalafil | 40 | 0.216<br>0.230<br>0.261 | 1.66 | 0.216<br>0.217<br>0.218 | 0.22 | 0.2 | 0.7 |

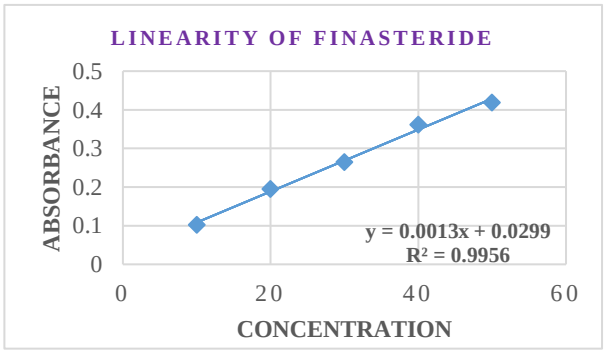
RSD – Relative Standard Deviation : LOD – Limit Of Detection : LOQ – Limit Of Quantification.

**TABLE -3: REPEATABILITY**

| | | No.of samples | Absorbance | | % RSD | |
|---|---|---|---|---|---|---|
| **Finasteride** | **Tadalafil** | | **Finasteride** | **Tadalafil** | **Finasteride** | **Tadalafil** |
| 30 | 40 | 1 | 0.873 | 0.168 | 0.127 | 0.281 |
| | | 2 | 0.875 | 0.167 | | |
| | | 3 | 0.875 | 0.168 | | |
| | | 4 | 0.873 | 0.168 | | |
| | | 5 | 0.874 | 0.168 | | |
| | | 6 | 0.875 | 0.167 | | |

**Q – Absorbance ratio Method:**

In absorbance ratio method (Q-analysis), the primary requirement for developing a method for analysis is that the entire spectra should follow the Beer's law at all the wavelength, which was fulfilled in case of both these drugs. The two wavelengths were used for the analysis of the drugs were 250 nm (iso-absorptive point) and 282nm (λ-max of Tadalafil) at which the calibration curves were prepared for both the drugs. The overlain UV absorption spectra of Finasteride (217 nm) and Tadalafil (282nm) showing iso-absorptive point (250 nm) in 0.1N NaOH. Linearity equation of Finasteride and Tadalafil were shown in (fig. 11 & 12). The results of linearity are depicted in Table 4. The validation parameters were studied at all the wavelengths for the proposed method. Precision was calculated as repeatability for both the drugs as shown in Table 5 & 6. Hence, the method can be employed for the routine analysis of these two drugs in combined dosage form.



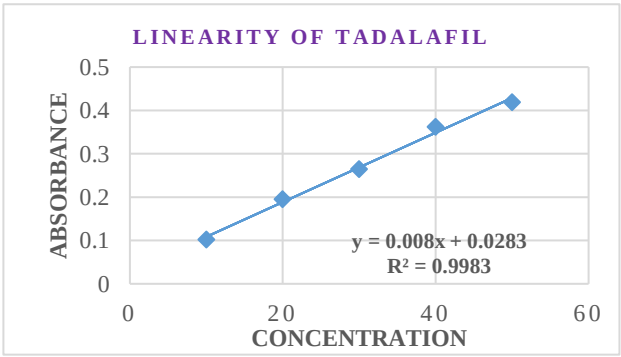
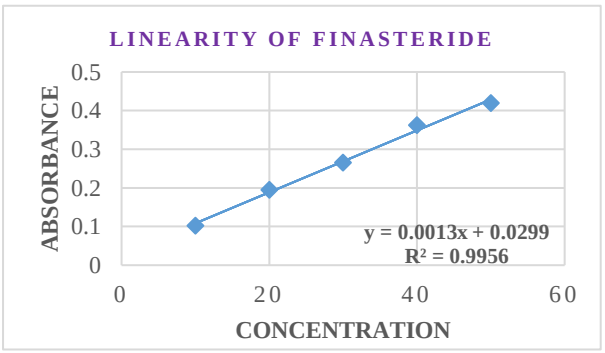



**Figure-11: Linearity graph of Finasteride and Tadalafil at 282nm**



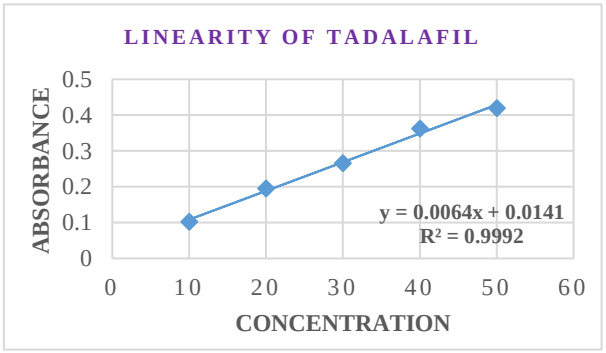




**Figure-12: Linearity graph of Finasteride and Tadalafil at 250nm**

**TABLE 4: RESULTS OF LINEARITY Q ABSORBANCE METHOD**

| Con.(µg/ml) | Absorbance 282nm | | Absorbance 250nm | |
|---|---|---|---|---|
| | FIN | TAD | FIN | TAD |
| 10 | 0.077 | 0.118 | 0.053 | 0.075 |
| 20 | 0.143 | 0.229 | 0.106 | 0.164 |
| 30 | 0.204 | 0.320 | 0.147 | 0.243 |
| 40 | 0.274 | 0.353 | 0.221 | 0.269 |
| 50 | 0.331 | 0.454 | 0.269 | 0.348 |

**TABLE 5: RESULT OF PRECISION STUDIES**

| Drug | Amount taken (µg/ml) | Intra day | | Inter day | | Parameter | |
|---|---|---|---|---|---|---|---|
| | | Absorbance | %RSD | Abs | %RSD | LOD | LOQ |
| FIN | 30 | 0.185<br>0.184<br>0.186 | 0.44 | 0.176<br>0.178<br>0.179 | 0.17 | 0.2 | 0.6 |
| TAD | 40 | 0.316<br>0.317<br>0.318 | 0.25 | 0.293<br>0.290<br>0.294 | 0.58 | 0.1 | 0.5 |

**TABLE 6: REPEATABILITY**

| Concentration ((µg/m) | | No.of samples | Absorbance | | % RSD | |
|---|---|---|---|---|---|---|
| FIN | TAD | | FIN | TAD | FIN | TAD |
| 30 | 40 | 1<br>2<br>3<br>4<br>5<br>6 | 0.300<br>0.301<br>0.301<br>0.301<br>0.301<br>0.304 | 0.378<br>0.376<br>0.375<br>0.376<br>0.378<br>0.378 | 0.41 | 0.32 |

## Conclusion

Two UV spectrophotometric procedures, Simultaneous equation method and Q absorbance ratio method, were found to accurately and precisely estimate Finasteride and Tadalafil in the pure and in the pharmaceutical formulation. The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity and range, precision, LOD and LOQ. This method can be successfully used for simultaneous estimation of Finasteride and Tadalafil.

## ACKNOWLEDGEMENTS

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