Chemometric Access for RP-HPLC Simultaneous Estimation of Tadalafil and Dapoxetine Applying Response Surface Methodology

S. Jayaseelan¹*, N. Kannappan² and V. Ganesan³

¹Department of Pharmaceutical Analysis, the Erode College of Pharmacy, Veerampalayam, Erode, India.
²Department of Pharmacy, Annamalai University, Chidambaram, India.
³Department of Pharmaceutics, the Erode College of Pharmacy, Veerampalayam, Erode, Tamilnadu, India.

Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Aims: A RP-HPLC method was developed and validated for simultaneous estimation of Tadalafil and Dapoxetine applying statistical experimental design.

Methodology: Multivariate optimization of the experimental conditions of RP-HPLC method was using Design of experiments. Independent three factors like phosphate buffer pH, mobile phase composition and flow rate were applied to design mathematical models. To study the response surface methodology by using Central composite design (CCD). In depth the effects of these independent factors was studied using CCD. Simultaneously optimize the retention time and resolution of the analytes was applying Desirability function.

Results: The predicted and optimized data from contour picture containing phosphate buffer (pH 3.4) and acetonitrile in the ratio of 40:60%v/v respectively. Flow rate was found to be 0.8 ml/min. Baseline separation of both analytes with run time of less than 10.0 min and good resolution were achieved using these optimum conditions.

*Corresponding author: E-mail: jvchrsdy@yahoo.co.in, Jayanote4a@gmail.com;
Conclusion: Method was validated according to ICH guidelines by using optimized assay conditions. Therefore, the reports distinctly indicated that Quality by design access could be satisfactorily used to optimize RP-HPLC method for simultaneous estimation of Tadalafil and Dapoxetine.

Keywords: RP-HPLC; optimum conditions; central composite design; response surface methodology; tadalafil and dapoxetine.

1. INTRODUCTION

Tadalafil (Fig. 1a) is 2-(1,3-benzodioxol-5-yl)-6-methyl-3,6,17-triazatetracyclo heptadeca-1(10), 11,13,15-tetraene-4,7-dione. It is used for the treatment of erectile dysfunction. Dapoxetine (Fig. 1b) is (S)-N,N-Dimethyl-3-(naphthalene-1-yl oxy)-1-Phenylpropan-1-amine. It is prescribed for the treatment of premature ejaculation [1-4]. Extensive literature survey revealed no RP-HPLC method has been available for simultaneous determination of Tadalafil and Dapoxetine in bulk and its tablet dosage forms using experimental design access (Quality by Design). Some Analytical methods has been reported in the literature for the simultaneous determination of tadalafil and dapoxetine bulk and its dosage form by derivative UV, HPTLC, Spectrophotometric and RP-HPLC [5-8]. Estimation of these drugs combination with other drugs or alone has been reported. Derivative UV methods for tadalafil in pharmaceutical dosage form [9] and other methods like stability indicating HPLC [10] HPTLC [11], spectrofluorimetry [12], Human serum in HPLC [13], capillary electrophoresis [14], with other combinations [15,16], Estimation of dapoxetine by UV [17], related impurities in HPLC [18], rat plasma in UPLC/MS [19] and combined with other drugs [20-21] has been reported. Therefore, the aim of the present study for developing simple, rapid, economical and effective method for the simultaneous analysis of tadalafil and dapoxetine applied with DOE and CCD estimation of the developed method.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Pure Active pharmaceutical ingredients of Dimenhydrinate and Cinnarizine were obtained as a gift samples from Nebulae Hi- Tech Laboratories, Chennai, Tamilnadu, India. Combination tablet of uphold was procured from the local market. HPLC grade methanol, Acetonitrile, water and ammonium acetate were purchased from Merck Chemicals India Pvt. Limited, Mumbai, India.

2.2 Instrumentation and Chromatographic Conditions

Analysis was performed with a Shimadzu LC2010 CHT separation module equipped with LC solution software, Pump LC2010 binary and UV detector set at 240 nm. Compounds were separated on an Intek chromasol column (250 × 4.6 mm i.d., 5μm particle size) under reversed phase partition conditions. The mobile phase was Acetonitrile and ammonium acetate buffer. The flow rate was 1.0 ml/min and the run time was set as 12 minutes. Samples were injected by using Rheodyne injector with 10 μL loop and detection was carried out at 290 nm. Prior to analysis mobile phase were degassed by the use of a sonicator (Ultrasonic Cleaner, Power Sonic 420) and filtered through a 0.45μ nylon filter. Chromatography was carrying out in column temperature maintained at 30 ± 5°C.

Fig. 1. Structure for analytes
2.3 Preparation of Working Standard Stock Solution

About 20.2 mg of Tadalafil and 67.3 mg of Dapoxetine were weighed accurately and transferred into a 50 ml volumetric flask. 10 ml of the mobile phase was added and sonicated for 15 min. and the volume was made up to 50 ml with the mobile phase. From this, pipette out 4.5 ml of the solution and transferred into 50 ml volumetric flask. Then it was made up to the volume with mobile phase to get a concentration of 36.36 µg/ml for Tadalafil and 121.14 µg/ml for Dapoxetine.

2.4 Preparation of Sample Solution

Ten tablets were weighed and powdered (Uphold tablets containing Tadalafil 10 mg and Dapoxetine 30 mg). The tablet powder equivalent to 67.3 mg was weighed accurately and transferred into 50 ml volumetric flask. 10 ml of the mobile phase was added and sonicated for 15 minutes and the volume was made up to 50ml with the mobile phase. 4.5 ml of the above solution was pipetted out and transferred into a 50 ml standard flask and made up to the volume with the same. Finally, it was filtered through a 0.45µ membrane filter. Hence, the final concentrations were attaining 36.36 µg/ml for Tadalafil & 121.14 µg/ml for Dapoxetine.

2.5 Method Validation

The developed RP-HPLC method was validated by using ICH guidelines [22-23] for validation of analytical procedure to determine the linearity, LOD, LOQ, Precision, accuracy, Robustness and Ruggedness.

2.5.1 System suitability

System suitability tests are an integral part of any chromatographic analysis method which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repeatedly injecting the drug solution at the concentrations of 36 µg /ml and 121 µg / ml respectively for Tadalafil and Dapoxetine to check the reproducibility of the system. 20 µl standard solutions were injected.

2.5.2 Linearity

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. The linearity study was conducted for standard stock solutions of Tadalafil and Dapoxetine. For the construction of calibration curves, five calibration standard solutions were prepared over the concentration range of 32-48 µg / ml for Tadalafil and 107-161 µg / ml for Dapoxetine.

2.5.3 Limit of detection

The limit of detection is the lowest level of analyte that can be detected, but not necessarily determined in a quantitative fashion, by using a specific method under the required experimental conditions. The lowest detection limit was calculated by using the following formula. LOQ = 3.3 x std. dev / slope. Preparation of calibration curve from the serial dilutions of standard was repeated for three times. Limit of quantification was calculated by using the value of the slope and the standard deviation of intercept.

2.5.4 Limit of quantification

The limit of quantification is the lowest concentration of analyte in a sample that may be determined with acceptable accuracy and precision when the required procedure is applied. The lowest detection limit was calculated by using the following formula. LOQ = 10 x std.dev / slope.

2.5.6 Content estimation (assay)

About 36 µg / ml of Tadalafil and 121 µg / ml of Dapoxetine standard and sample (Uphold tablets containing Tadalafil 10 mg and Dapoxetine 30 mg) solutions were prepared separately and 20 µl of each standard and sample solution were injected The percentage purity was calculated by using the peak area.

2.5.7 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple samples of the same homogeneous sample under prescribed conditions. Precision study was conducted by injecting standard solutions of Tadalafil and Dapoxetine five times at a concentration of 36 µg / ml and 121 µg / ml respectively. The peak area was used to determine standard deviation.
2.5.8 Accuracy

The accuracy of an analytical method may be defined as the closeness of the test results obtained by the method to the true value. It is the measure of the exactness of the analytical method developed. Accuracy may often express as percent recovery by the assay of a known amount of analyte added. The accuracy of the method was checked by spiking the sample with reference compound. It was evaluated in triplicate at the concentration levels (80%, 100% & 120%) of the target test concentrations (36 µg / ml of Tadalafil and 121 µg / ml of Dapoxetine). 20 µl solutions of each concentration were injected and the chromatograms were recorded.

2.5.9 Robustness

The Robustness study indicated that the factors selected remained unaffected by small variation of flow rate and the organic composition of mobile phase. The condition studied were flow rate (± 0.2 ml/min) and composition of mobile phase (± 3%).

2.5.10 Ruggedness

Ruggeness is a measure of reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

3. RESULTS AND DISCUSSION

HPLC method of analysis of Tadalafil and Dapoxetine was carried out by means of a central composite design. CCD was selecting ample to its pliability. Optimize an HPLC partition by acquiring improved interpretation of the main factors by using CCD and their interconnection results. Previous theory from the article used to chosen the key factors. Acetonitrile concentration (A) (50-60%), Phosphate buffer pH (B) (3.0-3.4) and flow rate (C) (0.8-1.2 ml/min) were the factors chosen for optimization. The capacity factor for 1st peak i.e. Tadalafil (k1), the resolution between two pairs Tadalafil and Dapoxetine (Rs1, 2) and the retention time of last peak i.e. Dapoxetine (tR2), were chosen for response variables Table 1. Data was showed the ranges of the factors and response [2]. The effects of uncontrolled variables that may introduce a bias on the measurements randomized order to minimize all experiments were conducted. The estimation of experimental error replicates (n=6) of the central points were performed. Linear, quadratic and cross terms the model can be expressed as the following Y = β0 + β1X1 + β2X2 + β3X3 + β12X1X2 + β13X1X3 + β23X2X3 + β11X12 + β22X22 + β33X32 with three factors an experimental design

where Y is the response to be modeled, β is the regression coefficients and X1, X2 and X3 represents factors A, B and C respectively. ANOVA provides statistical parameters for the reduced models datas were shown in table 2. The pointless terms (p > 0.05) were removed from the model through reverse removing process to procure an easy and realistic model. Considering R2 always reduces when a regressor variable is removed from a regression model. In statistical modeling the adjusted R2 which precedes the number of regressor variables into report, is usually chosen [24]. Adjusted R2 values were found to be within the standard limits of R2 ≥ 0.81 [25], which declared that the experimental data indicated a fine suited with the second order polynomial equations. p value was procured > 0.05 for all the reduced models, suggested that these models were significant. The signal (response) to noise (deviation) ratio is given adequate precision value. A ratio more than 4 is advisable [26]. In the present study, the adequate precision value was found to be in the range of 9.696 – 13.494 which suggested an adequate signal. Hence the model was significant for the partition procedure. Measurement of reproducibility of the model is denoted as coefficient of variation (% C.V). As a common rule a model can be examined reasonably reproducible the % C.V value is less than 10%. Our study, the % C.V value was found to be for all models within the limit (1.01 – 5.87). The interconnection with the biggest perfect coefficients among the suited model was AC (+0.922AC) of tR2 model shown in Table 2 [27]. The interconnection between A and C positive sign was statistically significant (< 0.0001) for tR2.

The present study revealed that changing the concentration of acetonitrile from low to high reported in a fast decrease in the retention time Dapoxetine and big levels of flow rate. In addition at small level of factor A, an raise in the flow rate resulted in a decline in retention time. Hence, when the acetonitrile concentration was set at the smallest level, the flow rate had to be at its biggest level to reduce the run time. So that obtain good interpretation of the reports, the predicted models were represented in the form of perturbation plots 2 (Fig. a, b and c) and 3D
response surface plots 3 (Figs. a, b and c). Variables giving quadratic and interconnection terms with the biggest perfect coefficients in the suited models were selected for the axes of the response surface plots.

Perturbation plot produce silhouette scene of the response surface plots where it appears how the response replace as each factor moves from a selected reference point, with all factors held constant at the reference value. The steepest slope or inflection showed the sensitiveness of the response to a specific factor. Acetonitrile concentration (factor A) had the most important effect on retention time $t_R^2$ followed by factor C and then B was shown in Fig. 2c. The remaining factors (buffer concentration and flow rate) had important effect on $R_{s1,2}$ and $k_1$. In (Fig. 3a, 3b) $k_1$ and $R_{s1,2}$ values incremented as the level of buffer concentration incremented and $k_1$, $R_{s2,3}$ values declined as the level of flow rate incremented. Analysis of the perturbation plots and response plots of optimization models revealed that factor A and C had important effect on separation of analytes, whereas the factor B i.e. the buffer pH was of little important.

Table 1. Factors and responses for central composite arrangement

| Run | Space type | Factor 1 ACN con (%v/v) | Factor 2 PB pH | Factor 3 Flow rate (ml/min) | Response 1 ($k_1$) | Response 2 ($R_{s1,2}$) | Response 3 ($t_R^2$) |
|-----|------------|------------------------|----------------|-----------------------------|-------------------|------------------------|-------------------|
| 4   | Factorial  | 60                     | 3.4            | 0.8                         | 1.1               | 8.58                   | 7.87              |
| 6   | Factorial  | 50                     | 3.0            | 0.8                         | 1.12              | 9.52                   | 12.24             |
| 11  | Factorial  | 50                     | 3.4            | 0.8                         | 1.1               | 9.46                   | 11.56             |
| 13  | Factorial  | 50                     | 3.0            | 1.2                         | 1.09              | 8.76                   | 8.95              |
| 14  | Factorial  | 60                     | 3.0            | 0.8                         | 1.12              | 9.29                   | 10.94             |
| 17  | Factorial  | 60                     | 3.4            | 1.2                         | 1.11              | 9.21                   | 10.34             |
| 19  | Factorial  | 50                     | 3.4            | 1.2                         | 1.09              | 8.38                   | 7.63              |
| 20  | Factorial  | 60                     | 3.0            | 1.2                         | 1.09              | 8.65                   | 8.63              |
| 1   | Axial      | 55                     | 2.86           | 1.0                         | 1.09              | 8.65                   | 8.63              |
| 5   | Axial      | 55                     | 3.2            | 0.66                        | 1.1               | 8.40                   | 7.35              |
| 7   | Axial      | 55                     | 3.53           | 1.0                         | 1.1               | 8.80                   | 8.66              |
| 10  | Axial      | 63.40                  | 3.2            | 1.0                         | 1.1               | 8.09                   | 6.44              |
| 12  | Axial      | 55                     | 3.2            | 1.33                        | 1.12              | 10.10                  | 14.99             |
| 18  | Axial      | 46.59                  | 3.2            | 1.0                         | 1.13              | 9.70                   | 13.03             |
| 2   | center     | 55                     | 3.2            | 1.0                         | 1.1               | 8.93                   | 9.25              |
| 3   | center     | 55                     | 3.2            | 1.0                         | 1.1               | 8.93                   | 9.25              |
| 9   | center     | 55                     | 3.2            | 1.0                         | 1.1               | 8.93                   | 9.25              |
| 15  | center     | 55                     | 3.2            | 1.0                         | 1.1               | 8.93                   | 9.25              |
| 16  | center     | 55                     | 3.2            | 1.0                         | 1.1               | 8.93                   | 9.25              |

Table 2. Regression models and ANOVA provides statistical parameters

| Responses | Regression model                                                                 | Adjusted $R^2$ | Model p value | % C.V | Adequate Precision |
|-----------|-----------------------------------------------------------------------------------|----------------|----------------|-------|--------------------|
| $K_1$     | +1.10-0.0022A-0.0002B-0.0019C+0.0025AB+0.0025AC+0.0075BC+0.0043A^2-0.0027B^2+0.0026C^2 | 0.8208         | <0.0001        | 1.01  | 9.696              |
| $R_{s1,2}$| +8.93-0.227A-0.023B+0.074C+0.036AB+0.227AC+0.118BC-0.006A^2+0.067B^2+0.118C^2      | 0.9189         | <0.0001        | 5.87  | 10.204             |
| $t_R^2$   | +9.25-1.00A-0.217B+0.423C+0.080AB+0.922AC+0.517BC+0.139A^2-0.210B^2+0.646C^2       | 0.8086         | <0.0001        | 2.31  | 13.494             |
Fig. 2. Perturbation plots for responses

Table 3. Optimimum individual reponse goal

| Response | low limit | high limit | Goal          |
|----------|-----------|------------|---------------|
| $k_1$    | 1.09      | 1.13       | -is in range  |
| $R_{S1,2}$ | 8.098    | 10.106     | minimize      |
| $R_{T2}$ | 6.44      | 14.99      | minimize      |

Table 4. Experimental and Predictive procedure values comparison under the optimal settings

| Optimal settings | ACN (%) v/v | Phosphate Buffer (pH) | Flow rate (ml/min) | $k_1$   | $R_{S1,2}$ | $R_{T2}$ |
|------------------|-------------|-----------------------|-------------------|---------|------------|----------|
| Predictive       | 60.00       | 3.40                  | 0.8               | 1.09    | 7.341      | 9.927    |
| Experimental     | 60.00       | 3.40                  | 0.8               | 1.13    | 7.189      | 9.740    |
| Average error    | 3.66        | 2.07                  | 1.88              |

Desirability value ($D$) = 0.921

3.1 Multi Criteria Decision Making

Global optimization of three responses and select different optimal conditions for the analysis of samples were using by Derringer’s desirability function.

3.2 Optimal Condition for Assay

To substantiate the flexibility of the development strategy and to search for evaluating analytes, standard was accepted by varying the response objective and their major values (Table-4). For illustration, the high value of $R_{T2}$ had to be chosen for the partition of Dapoxetine from the components. The desirability function was reduced at overall desirability of about $D= 0.921$. The global desirability function response surface was shown in Fig. 4. Each individual response goal was seen in Table 4.

The correlative producing the high value were acetonitrile concentration 60% v/v, Phosphate buffer pH 3.4 and flow rate 0.8 ml/min. Hence,
C_{18} column with Phosphate buffer (pH 3.4): acetonitrile 40: 60%v/v as mobile phase at a flow rate of 0.8 ml/min and UV detection at 288 nm using were the optimal assay condition. Between experimental and predicted responses agreement report was shown in Table 4. Fig. 5 was shown the corresponding chromatogram.

### 3.3 Validation Report of the Methods

The system suitability parameters results were within the limit. Linearity report showed good correlation between analytes peak area and concentration with $r^2 > 0.9998$ ($n = 6$). 0.0097 µg /ml and 0.0071 µg /ml for Tadalafil and Dapoxetine respectively for LOD value were found. 0.0294 µg /ml and 0.0215 µg /ml for Tadalafil and Dapoxetine respectively for LOQ values were found. Determination of purity of Tadalafil and Dapoxetine in tablet formulation was performed by assay (content determination) From the calibration curve nominal concentration was chosen and quantification of Tadalafil and Dapoxetine were performed. The tablet formulation Uphold contains (10 mg of Tadalafil and 30mg of Dapoxetine) was selected for the analysis. The % of drugs present in the tablet dosage form were found in the range of 99.84 - 100.72%. The assay percentage RSD values were found 0.9679 and 1.2999 for Tadalafil and Dapoxetine.

![Capacity factor $k_1$](image1)

![Resolution $R_s_{1,2}$](image2)

![Retention time $tR_2$](image3)

**Fig. 3.** Response surface plots for responses
Fig. 4. Global desirability function graphical representation (D=0.921)

Fig. 5. Optimal conditions for corresponding Chromatogram

Table 5. Reports for validation parameters

| Parameters          | Tadalafil | Dapoxetine |
|---------------------|-----------|------------|
| Range (µg/ml)       | 12-20     | 6-10       |
| y = mx + c          | y = 27627x + 1131.1 | y = 164609x + 12713 |
| r²                  | 0.9990    | 0.9993     |
| Slope (m)           | 27627     | 164609     |
| Intercept (c)       | 1131.1    | 12713      |
| LOD (µg/ml)         | 0.0040    | 0.0050     |
| LOQ (µg/ml)         | 0.0122    | 0.0151     |
| Accuracy (%)        | 99.35     | 99.94      |
| Precision (%RSD)    | 0.3854    | 0.1541     |
| Ruggedness          | 0.9328    | 0.6343     |
| Analyst-I (%RSD)    | 1.4939    | 0.7594     |
| Analyst-II (%RSD)   |           |            |
The percentage RSD (1.0328 and 0.7641 for Tadalafil and Dapoxetine) for the area of five replicate injections was found less than 2%. It showed that the drug was having good precision. The amount of drug recovered was calculated. The % recovery of Tadalafil and Dapoxetine were found 99.48 and 99.94. The percentage RSD value for Tadalafil and Dapoxetine were report 0.9801 and 0.3487 %. The percentage RSD value was found less than 2%. The lower % RSD value suggested there was no intervention due to the excipients used in formulation. Therefore, the accuracy of the method was confirmed. System suitability parameters reports were within the standard. Therefore, the method was robust. The % RSD value for analyst I was found 1.1034 and 1.2093 % for Tadalafil and Dapoxetine. The % RSD value for analyst II was found to be 1.0012 and 1.2727 % for Tadalafil and Dapoxetine. The results are express in Table 5.

4. CONCLUSION

An easy and fast RP-HPLC method was developed and validated prosperously for the simultaneous estimation of tadalafil and dapoxetine. Design of Experiments and Central Composite Design were used successfully for the estimation. Statistical analysis reported that the model represents the circumstance completely great. The responses variations were correctly interact to the factors variations. This method can be validated for linearity, precision, accuracy and selectivity as per ICH guidelines. The method has verified to be suitable and successful for the quality control of tadalafil and dapoxetine in pharmaceutical dosage form.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

The authors are grateful to The Erode College of Pharmacy, Erode and Annamali University, Chittambaram, Taminadu the facilities provided to complete the Research work completely.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Maryadele J, Neil O. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals, Merck Research Laboratories, Division of Merck and Co., Whitehouse station, NJ, USA; 2006.
2. Sweetman SC. Martindale: The Complete Drug Reference, Pharmaceutical Press, London, UK; 2007.
3. Gupta M, Kovar A, Meibohm B. The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. J Clin Pharma. 2005;45(9):987–1003.
4. Dresser M, Kang D, Staehr P, Gidwani S, Guo C, Mulhall J, et al. Pharmacokinetics of dapoxetine, a new treatment for premature ejaculation: impact of age and effects of a high-fat meal. J Clin Pharmacol. 2006;46(9):1023–1029.
5. Sudha T, Bhuvaneswari N, Geetha S, Mohanapriya S, Nivedhitha S, Nanthini S. Development and validation of new UV method for simultaneous estimation of Tadalafil in combination with Dapoxetine Hydrochloride in a pharmaceutical dosage form. European J Pharma Med Res. 2019; 6(4):424-429.
6. Basma H. Anwar, Naguib IA, Magdy MA, Abdelhamid NS. A validated Green HPTLC method for quantitative determination of Dapoxetine Hydrochloride and Tadalafil in bulk and pharmaceutical formulations. J Chroma Sci. 2020;58(4):303–308.
7. Basma H. Anwar, Maimana A Magdy, Ibrahim A Naguib, Nessreen S Abdelhamid. Quantitative determination of Dapoxetine Hydrochloride and Tadalafil using different validated spectrophotometric methods. Spectrochimica Acta...
8. Rajeshwari M, Chenthilnathan A, Rama K. Validated RP-HPLC method for simultaneous estimation of Tadalafil and Dapoxetine Hydrochloride in combined pharmaceutical dosage forms. Int J Pharma Biological sciences. 2014;4(2):72-82.

9. Zamir G. Khan, Amod S. Patil, Atul A. Shirkhedkar. Estimation of Tadalafil using derivative spectrophotometry in bulk material and in pharmaceutical formulation. Int J Spectros. 2014;2014:1-6.

10. Rao DVS, Radhakrishnanand P, Himabindu V. Stress degradation studies on tadalafil and development of a validated stability-indicating LC assay for bulk drug and pharmaceutical dosage form. Chromatographia, 2008;67(1-2):183–188.

11. Patel SA, Patel NJ. High performance thin layer chromatographic method for determination of Tadalafil in tablet dosage form. The American J Pharm Tech Research. 2011;1(3):138–146.

12. Kavitha AD, VijayaDurga DS, Himabindu K, Eshvendar, Khaleel NP, Ani Kumar D. Forced degradation studies, quantification and in-vitro dissolution studies of Tadalafil by spectrofluorimetry. Asian J Pharma Clin Res. 2013;6(2):326–329.

13. Khabbaz L, Daoud R. A sensitive and simple HPLC method for quantification of tadalafil in human serum. J App Res. 2006; 6(1):170-175.

14. Ali I, Enein H. Validated method for tadalafil analysis in pharmaceutical preparations by capillary electrophoresis. Chromatographia. 2004;60:187-191.

15. Kannappan N, Yada D, Yada D, Shashikanth MR. Method development and validation of stability indicating methods for assay of Tadalafil and Sildenafil citrate by HPLC. Int J Chem Tech Res. 2010;2(1):329–333.

16. Abdel-Hamid M. Determination of sildenafil, tadalafil and vardenaflu in tablets and adulterated herbal product by ESI-MS-MS. J liquid chromat related tech. 2006; 29(4):591-603.

17. Panchumary 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 Techn. 2015;6(11):1976-1980.

18. Rohith T, Ananda S. Development and validation of High performance liquid chromatography method for the determination of process related impurities in Dapoxetine Hydrochloride. Int J Res Pharma Chem. 2013;3(1):74-892.

19. Tae Kon Kina, In Sook Kimb, Seok Hyun Honga, Yun Kyoung Choic, Hohyun Kimc, Hye Hyun Yoob. Determination of dapoxetine in rat plasma by Ultra performance liquid chromatography-tandem spectrometry. J Chromatogra, B. 2013; 926:642-646.

20. Chapla B, Amin G, Pandya A, Prajapati CA, Patel BS, Badmanaban R. Development and validation of HPTLC method for simultaneous estimation of Sildenafil Citrate and Dapoxetine Hydrochloride in ocmbined dosage form. Pharma Tutor. 2014;2(10):142-152.

21. Chapla B, Amin G, Pandya A, Kakadiya J, Shah N. Simultaneous estimation and validation of Verdenafil and Dapoxitine HCl in pharmaceutical formulation by Thin Layer Chromatographic Densitometric method. Int Res J Pharma. 2012;3(5):480-483.

22. International conference on Harmonization guidance for Industry. Q2A Text on validation of Analytical methods. Switzerland, IFPMIA. 1994;1-4.

23. International conference on Harmonization guidance for Industry. Q2B Text on validation of Analytical methods. Switzerland, IFPMIA. 1996;1-8.

24. Parajo JC, Alonso JL, Lage MA and Vazquez D. Empirical modeling of eucalyptus wood processing, Bio Eng. 1992 May; 8: 129-136.

25. Lundstedt T, Seifert E, Abramo L, Thein B, Nystrom A, Pettersen J, Bergman R. Design and optimization, Chrometrics and Intelligent Laboratory Systems. 1998; 42(1-2):3–40.

26. Beg Q, Sahai V, Gupta R. Statistical media optimization and alkaline protease production from Bacillus mojavensis in a bioreactor, Process Biochemistry. 2003; 39(2):203-209.
27. Gundala A, Prasad KV, Koganti B. Application of quality by design approach in RP-HPLC method development for simultaneous estimation of saxagliptin and dapagliflozin in tablet dosage form. Brazilian Journal of Pharmaceutical Sciences. 2019;24;55.

© 2021 Jayaseelan et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/73369