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Statistical screening of mobile phase components by factorial design for efavirenz separation by high performance liquid chromatography

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Abstract

A statistical optimization method was successfully employed to study the effect of system variables on the chromatographic analysis of Efavirenz. The effect of simultaneously varying the flow rate, temperature and concentration of % Acetonitrile in buffer (phosphate buffer 25mM, pH 7.5) was studied to optimize the method to obtain excellent chromatographic responses. The optimum conditions were determined with the help of response surface methodology (RSM) using Plackett–Burman designs. From the response surface graphs, the optimum regions were selected to be −1, 0 and +1 for flow rate(0.8 ml/min), temperature (25◦C) Pareto ranking indicated that the most important variable affecting the selected responses was temperature. Linearity was found in the range of 30–70 ug/ml, with a significantly high correlation coefficient (r2= 0.999). The LOD and LOQ values were found to be 0.30 µg/ml and 0.81 µg/ml, respectively. The developed method was validated for accuracy, precision, linearity, range, and specificity. The method was successfully used to analyze a tablet formulation to assess the chromatographic performance, and it was found to be 100.88%,with a standard deviation of ±1.36

100.88 ± 1.36.

Keywords

HPLC; Factorial design; Response surface methodology; Efavirenz.

1. Introduction

4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2- one.1-2 It is used in the For use in combination treatment of HIV infection (AIDS) Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) and is used as part of highly active antiretroviral therapy (HAART) for the treatment of a human immunodeficiency virus (HIV) type 1 . For HIV infection that has not previously been treated, efavirenz and lamivudine in combination with zidovudine or tenofovir is the preferred NNRTI-based regimen. It is official in Indian Pharmacopeia. Although HPLC offers several advantages, such as accuracy, speed (min), high resolution, sensitivity (ng to fg), and reproducibility, it suffers from some disadvantages, in the composition of the mobile phase using response surface based optimization.

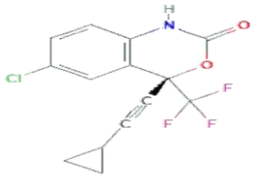


Fig 1 Molecular structure of efavirenz

The paper focuses on optimizing the separation of efavirenz using high-performance liquid chromatography (HPLC) by employing factorial design and statistical screening techniques to determine the optimal mobile phase composition. Efavirenz, a vital component in antiretroviral therapy for HIV/AIDS, requires precise chromatographic separation for accurate analysis and quantification in pharmaceutical formulations.

The study begins by outlining the importance of efavirenz separation in pharmaceutical analysis and the challenges associated with achieving optimal chromatographic conditions. It highlights the significance of mobile phase composition in HPLC and the role of factorial design in systematically screening multiple factors to determine their effects on the separation process. Factorial design, a statistical technique, is employed to systematically vary the composition of the mobile phase, considering factors such as solvent type, solvent ratio, pH, and buffer concentration. By conducting experiments according to the designed factorial matrix, the paper aims to identify the most influential factors and their optimal levels for achieving efficient efavirenz separation.

Experimental data generated from HPLC analyses are statistically analyzed to assess the main effects of individual factors, as well as their interactions, on efavirenz separation. The thesis

utilizes statistical software to analyze the experimental results, interpret the data, and identify the optimal mobile phase conditions that yield the desired chromatographic performance.

Through systematic experimentation and statistical analysis, the thesis aims to provide insights into the critical parameters affecting efavirenz separation by HPLC. By identifying the optimal mobile phase composition, the study contributes to the development of robust analytical methods for accurate quantification of efavirenz in pharmaceutical formulations, thereby enhancing the quality and reliability of HIV/AIDS treatment.

Overall, the paper contributes to the field of pharmaceutical analysis by demonstrating the application of factorial design and statistical screening techniques in optimizing chromatographic conditions for efavirenz separation by HPLC, with implications for improving drug analysis and quality control in pharmaceutical industries.

A literature search revealed that Few analytical methods were found to be reported which lack the force degradation study. In addition, nobody studied the effect of chromatographic conditions on resolution of drugs in chromatographic conditions optimization process in HPLC by using statistical design for the analysis of efavirenz from tablet dosage form.

2. Theory

2.1. Retention time (tR)

The time between the sample injection point and the analyte reaching a detector is called the retention time (tR). The retention time of the analyte is strongly influenced by the strength or polarity of the mobile phase (i.e., the % of organic solvent content). The column temperature is a strong determinant of the retention time and also affects the column selectivity. A column oven is therefore required for most pharmaceutical assays to improve the retention time precision, typically at temperatures of 30–50 ◦C. A temperature >60 ◦C is atypical due to concerns about the thermal degradation of the analytes and the column lifetime [23,24]. 2.2. Peak area (pA)

The peak area (area under peak) is proportional to the concentration or the amount of that particular component in the sample. Either attribute can be used to perform quantitative calculations, but the peak area is more commonly used because it provides a more accurate quantitative measurement, as it is less susceptible to flow variations. The standard component's peak areas are used for calculating the injection precision (reproducibility) and system linearity, whereas the retention times are used to calculate the pump repeatability. Therefore, in HPLC

methods, the relationship between the sample concentration and detector response (peak area) is used to make this determination [23,24].

2.3. Theoretical plates (N)

Theoretical plates are a measure of column efficiency and determine the number of peaks that can be located per unit run time of the chromatogram. The theoretical plates are calculated by

$$N = 16(tR/ W)$$

where tR is the retention time and W is the peak width. This peak width, W, is based on the baseline intercepts of the tangent lines to a Gaussian peak, which is equivalent to the peak width at 13.4% of the peak height. Parameters that can affect the theoretical plates include the flow rate of the mobile phase, column temperature peak position, particle size in column, viscosity of mobile phase and molecular weight of analyte [24,25].

2.4. Tailing factor (Tf)

Under ideal conditions, the chromatographic peaks will have Gaussian peak shapes with perfect symmetry. In reality, most peaks are either slightly fronting or tailing. The tailing factor (Tf) is a measure of the peak asymmetry [24] and is calculated by T f = wx 2f where wx is the width of the peak determined at either 5% or 10% from the baseline of the peak height and f is the distance between the peak maximum and peak front

Table 1 : *Study of variables on dependent by first factorial design*

Batch	Coded variables		Natural condition		RT (Y1)		TP (Y2)		TF (Y3)	
	% ACN (X1)	FR (X2)	X1	X2	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
L -1	-1	-1	25	1.3	11.97	11.65	7147	6802	0.81	0.77
L -2	-1	0	25	1.5	9.80	9.96	5917	6301	0.82	0.92

L -3	**-1**	**+1**	**25**	**1.8**	**7.72**	**7.87**	**7630**	**7591**	**1.25**	**1.19**
L -4	**0**	**-1**	**50**	**1.3**	**2.98**	**3.43**	**2776**	**2915**	**1.33**	**1.30**
L -5	**0**	**0**	**50**	**1.5**	**2.55**	**2.53**	**2028**	**2180**	**1.33**	**1.33**
L -6	**0**	**+1**	**50**	**1.8**	**2.07**	**1.63**	**3411**	**3120**	**1.38**	**1.42**
L -7	**+1**	**-1**	**75**	**1.3**	**1.47**	**1.33**	**1718**	**1924**	**1.25**	**1.33**
L -8	**+1**	**0**	**75**	**1.5**	**1.37**	**1.22**	**1491**	**956**	**1.33**	**1.24**
L -9	**+1**	**+1**	**75**	**1.8**	**1.23**	**1.51**	**1215**	**1544**	**1.13**	**1.14**
				Minimum	**1.23**	**1.22**	**1215**	**956**	**0.81**	**0.77**
				Maximum	**11.97**	**11.65**	**7630**	**7591**	**1.38**	**1.42**
				Mean	**4.94**	**4.91**	**3834**	**3807**	**1.16**	**1.16**

Where, % ACN- % Acetonitrile in buffer (phosphate buffer 25mM, pH 7.5) FR- Flow rate, ml/min; RT – Retention time, min; TP – Theoretical plate TF- Tailing factor; Exp. – Experimental result;

3. Materials and methods

3.1. Materials and instrumentation

Efavirenz drugs gifted by Aurobindo Pharma Ltd, Hyderabad and was used without further purification Tablets VIRANZ tablet (Aurobindo Pharma Ltd) containing 200 mg of efavirenz as per label claim were purchased from local pharmacy. All chemicals gradient and reagents used in method were of HPLC grade. Before use, mobile phase and other solvents were filtered through 0.45µm Whatman filter paper.The apparatus used was an Agilent 1220 Infinity LC gradient pump, with variable wavelength detector and Nucleosil (4.6mm I.D × 250mm) C18 column was used for chromatographic separation. A Rheodyne injector (manual loading) with a 20l external loop was used and detection was performed with a UV detector.

3.2. Selection of system variables

Experimental variables were selected on a trial and error basis by considering the physicochemical properties of Efavirenz such as its pKa value, solubility in solvents, and nature (acidic or basic). As Efavirenz is slightly weak acidic (near to 7) in nature,]. A flow rate of 1.5 ml/min and temperature 20–25 ◦C was used . mobile phase acetonitrile in buffer (phosphate buffer pH 7. 5). A stock solution of the standard drug was prepared in methanol to obtain a final concentration of 100ug/ml. A column equilibration time of 30 min between each run was maintained.

3.3. Factorial design and effect of variables]

A Plackett-Burman design 3^2 trial was used for optimizing the chromatographic conditions. In this design selected two variables i.e.% acetonitrile in buffer (phosphate buffer 25 mM, pH 7.5) (X_1) and flow rate (X_2) as a possible causes for change in retention time (RT), theoretical plates (TP) and tailing factor (TF). The range of variables was chosen from the trial and error study. The correlation of these variables with responses (retention time (RT), theoretical plates (TP) and tailing factor (TF) was statistically studied. The response surface for each considered response was plotted against two different variables using STATISTICA (Version 8.0.360.0 English, StatSoft Inc., Tulsa, OK, USA) software. The second order quadratic equation is given bellow.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{22} x_2^2 \quad \text{..... (1)}$$

Where,y = chromatographic response; β_0 = constant (intercept); β_1= coefficient of X_1: β_2 = coefficient of X_2; β_{11}, β_{12}, β_{22} = inter-action coefficients; X_1= first experimental variable; X_2 = second experimental variable. According to this design, total 9 trial batches were formed. All the batches were named as L-1 to L-9. For investigating the effect, each independent variable was studied at three levels, namely, “low”, “middle” and “high”. These levels define the lower, middle and higher limits of the range covered by each variable. Mobile phase was degassed by ultrasonic vibrations prior use.

3.4. Analysis and confirmation of optimization Model

The responses (retention time, peak area, theoretical plates, tailing factor) were studied by a two-way ANOVA-based factorial analysis. An RSM calculation for the present optimization was performed using STA-TISTICA version 8 (Stat-soft, Inc., USA). The data were fitted to a second-order polynomial equation (Eq. (1)),and the adequacy of the fitted response was

tested by ANOVA. The statistical significance was set to $p < 0.05$,and the generated response surfaces.

3.5. Standard stock solution and calibration graph

A standard stock solution of Efavirenz was prepared in methanol (100 ug/ml). A calibration graph was obtained by injecting five concentrations (20 ul loop) of the drug in the range of 30-70 ug/ml into the HPLC system and plotting the peak areas against the corresponding concentrations.

3.6. Method validation

The intra-day and inter-day variations for the determination of efavirenz was carried out at three different concentration levels of 40, 50, 60 ug/ml using homogeneous authentic tablet samples (200 mg efavirenz per tablet; VIRANZ tablet (Aurobindo Pharma Ltd). The parameters for robustness include the variation of the Fig $\boldsymbol{y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{22} x_2^2}$ **(1)** flow rate of the mobile phase (ml/min), column temperature (◦C) and % acetonitrile in Phosphate buffer pH 7.5) of different lots. A signal-to-noise ratio between 3:1 and 10:1 is generally considered acceptable for estimating the limit of detection and limit of quantitation [31]. For the specificity study, the peak area for efavirenz in the sample was confirmed by comparing the retention time (RT) of the sample with those of a standard [31,32]. For an assay, Twenty tablets of drug efavirenz were weighed and average weight of tablet was determined. The tablets were then crushed to fine powder. On the basis of labelled claim, powder equivalent to 500 mg of efavirenz was taken in 100ml volumetric flask. To this flask 30ml of methanol was added, the flask was sonicated for 30 min for complete dissolution. Final volume was made up to 100ml with the same solvent (5000µg/ml). About 10 ml of this solution was diluted to 100ml with same solvent (500 µg/ml). Again, 10 ml of this solution was diluted to 100ml with same solvent (50 µg/ml). The solution was then filtered through Whatman filter paper (0.45mg/ml). A 20µl volume of solution was injected into HPLC system, six times under the conditions described above. The resulting solution was analyzed for drug content. The drug content in the sample solution was calculated from the regression equation of the standard calibration graph (Fig. 2).

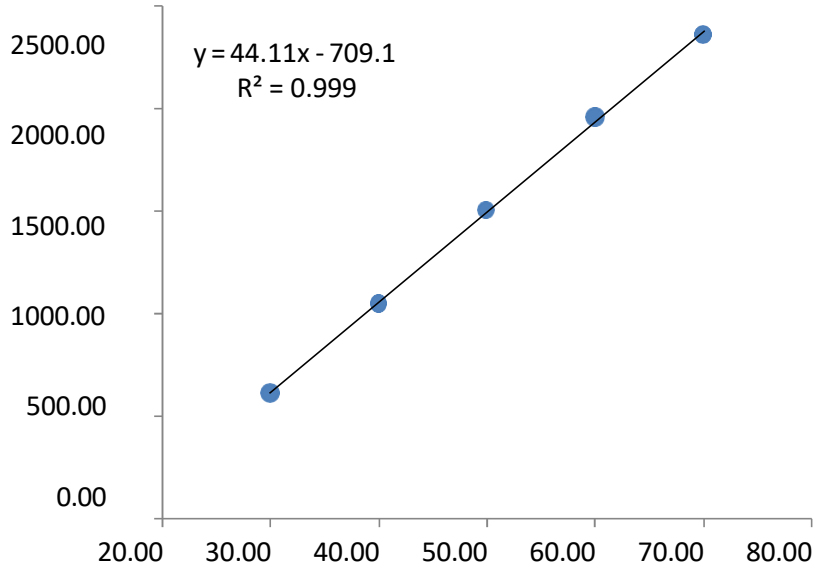


Fig. 11: *Calibration curve for efavirenz by HPLC*

3.7. Forced degradation studies

To determine whether the method and assay are stability-indicating, pure drug was stressed under various conditions to conduct forced degradation studies. A stock solution of drug in methanol (50 ug/ml) was used in the forced degradation to provide an indication of the stability-indicating property and specificity of the proposed method. In all degradation studies, the average peak area of the standard drug and degraded sample after six replicates were obtained.

3.7.1. Oxidation

Approximately 2 ml of 1% hydrogen peroxide was added to 2 ml of stock solution of drug, and the solution was kept at room temperature. After 30 min, the resultant solution was diluted to obtain a 50ug/ml solution. 20ul of solution was injected, and the chromatograms were recorded.

3.7.2. Acid degradation

Approximately 2 ml of 0.01 N hydrochloric acid was added to 2 ml of stock solution of drug, and the solution was kept at room temperature. After 15 min, the solution was diluted to 50 ug/ml. 20ul of the solution was injected, and chromatograms were recorded

.3.7.3. Base degradation

Approximately 2 ml of 0.01 N sodium hydroxide was added to 2 ml of stock solution of drug, and the solution was kept at room temperature. After 15 min, the solution was diluted to 50 ug/ml. 20ul of solution was injected, and chromatograms were recorded.

4. Results and discussion

4.1. Analysis of RSM curves and Validation of Optimization Model

All 8 experiments were run at a concentration 50ug/ml. The experimental plans and the respective responses are reported in Table 1. The experimental results were analysed using STATISTICA software (v8.0.360.0 English, Stat Soft, Inc., Tulsa, OK, USA). The coefficients of the second-order polynomial model were calculated by least squares regression. For the determination of the significance and validity of the model, the regression model for each considered response was tested by analysis of variance (ANOVA). The response surface plots were generated using the same software, and the capability of the fitted model was checked by ANOVA [33,34]. The equation model for the constants, the regression coefficients and the statistical parameters for each response variable, viz., retention time, peak area ,theoretical plates and tailing factor, are given in Table 2.As shown in Fig. 3(A1–D3), the analysis produces 3Dgraphs by plotting the response model against two of the factors, while the third was kept constant. From the response graphs, the following conclusions have been reached:

4.1.1. Effect of variables on retention time

Response surface plots are used to draw quick information about the relationship between retention time and individual variable. From **Fig. D**, it is clear that the effect of all variables on response is significant. From the results of retention time varying experimental variables, following regression equation was obtained to predict the retention time.

$$Y_1 = 47.9709 - 0.9014X_1 - 20.8121X_2 + 0.0049X_1^2 + 0.1582X_1X_2 + 3X_2^2 \quad \ldots.(2)$$

Where, X_1 and X_2 are the independent variables i.e. % ACN in buffer (phosphate buffer 25mM, pH 7.5) (v/v), and flow rate (ml/min), respectively.

As shown in **Table 7**, the average retention time of different trials of drug varied from 1.23 to 11.97 min. Equation (2) depicts the effect of variables on the retention time, and found to have statistical significance (P value 0.038902). Equation indicate the effect of variables on the retention time, and found to have statistical significant. It

indicates the retention time was decrease with increase in concentrations of ACN in buffer (X_1) and with increase in flow rate of mobile phase (X_2). How-ever, a high negative regression coefficient for X_2 indicated that the flow rate was a major factor responsible for the change in retention time. We can increase the flow rate to make retention time shorter, but it may drop some of peak resolution, and backpressures may be outside the limits of system. By increasing the temperature, we can also speed up a separation and reduce the viscosity of the mobile phase, thereby reducing the backpressure.

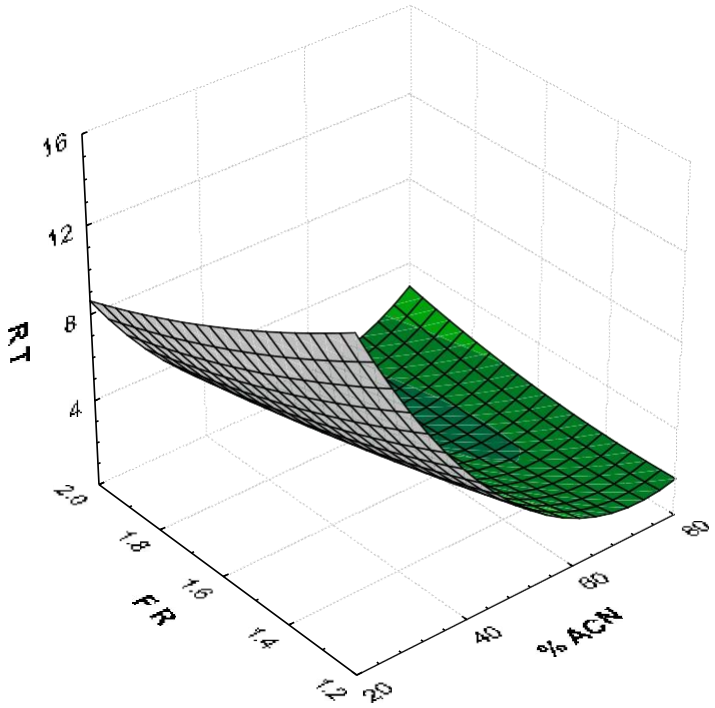


Fig.D: *3D Surface plots of Retention time against % ACN in buffer and flow rate*

Table 2: Effect estimates from first factorial design for retention time

	Regression co-eff.	Std.Err	t-value	p-value

Mean/Interact.	47.9709	13.62599	3.5205	0.038902
(1) % ACN in Buffer (L)	-0.9014	0.07810	-11.5415	0.001397
% ACN in Buffer (Q)	0.0049	0.00053	9.2526	0.002671
(2) Flow rate (L)	-20.8121	17.39509	-1.1964	0.317460
Flow rate (Q)	3.0000	5.54563	0.5410	0.626116
1L By 2L	0.1582	0.03715	4.2596	0.023728

*P<0.05 (significant for a 95% confidence level)

With increase in mobile phase modifier concentration, i.e. ACN retention time decreases and all other responses increases. This might be due to rising polarity of mobile phase resulting in establishment of faster equilibrium of analyte between stationary phase and mobile phase. Statistical data and results of work are given in **Table 2**.

4.1.2. Effect of variables on therotical plate

In order to get quick information about the relationship between theoretical plates and individual variable, From **Fig. E**, it is clear that the effect of all variables on response is significant. From the results of theoretical plates, following regression equation was obtained to predict the retention time.

$$\mathbf{Y_2 = 41851.2526 - 268.3831X_1 - 39433.5029X_2 + 2.3166X_1^{\,2} - 46.7911X_1X_2 + 13607.2222X^{\,2}\ldots\ldots (3)}$$

2

Where, X_1 and X_2 are the independent variables i.e. % ACN in buffer (phosphate buffer 25mM, pH 7.5) (v/v), and flow rate (ml/min), respectively. As shown in **Table 3**, the average theoretical plates of different trials of drug varied from **1215** to **7630**. Equation (3) depicts the effect of variables on the theoretical plates, and found to have statistical significance (P value 0.072256). Eq. (3) indicates that the theoretical plates were decrease with increase in concentrations of ACN in buffer (X_1) and flow rate (X_2). How-ever, a high positive regression coefficient for X_2 indicated that the flow rate was a major factor responsible for the change in theoretical plates. Statistical data and results of work are given in **Table 3**

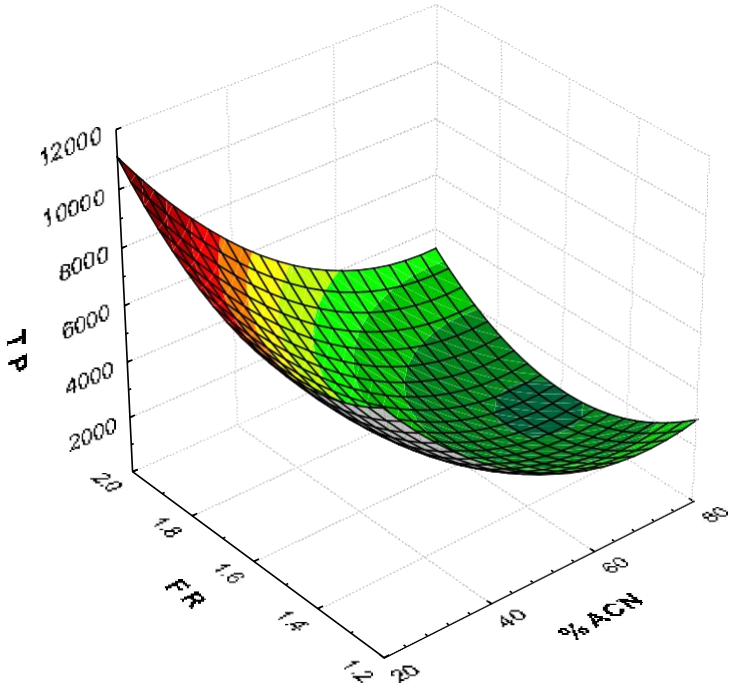


Fig. E: *3D Surface plots of theoretical plates against % ACN in buffer and flow rate*

Table 3: Effect estimates from first factorial design for theoretical plates

	Regression co-eff.	Std.Err	t-value	p-value
Mean/Interact.	41851.3	15359.34	2.72481	0.072256
(1) % ACN in Buffer (L)	-268.4	88.04	-3.04849	0.055494
% ACN in Buffer (Q)	2.3	0.60	3.88605	0.030201
(2) Flow rate (L)	-39433.5	19607.91	-2.01110	0.137836
Flow rate (Q)	13607.2	6251.08	2.17678	0.117704
1L By 2L	-46.8	41.88	-1.11739	0.345252

*P<0.05 (significant for a 95% confidence level)

4.1.3. Effect of variables on tailing factor

In order to get quick information about the relationship between tailing factor and individual

variable. From **Fig. F**, it is clear that the effect of all variables on response is significant. From the results of tailing factor varying experimental variables, following regression equation was obtained to predict the retention time.

$$Y_3 = -1.5023 + 0.0828X_1 + 0.591X_2 - 0.0004X_2 - 0.0243X_1X_2 + 0.2789X^2 \ldots\ldots (4)$$

Where, X_1 and X_2 are the independent variables i.e. % ACN in buffer (phosphate buffer 25mM, pH 7.5) (v/v), and flow rate (ml/min), respectively.

Table :4 Effect estimates from first factorial design for tailing factor

	Regression co-eff.	Std.Err	t-value	p-value
Mean/Interact.	-1.50231	3.116347	-0.48207	0.662738
(1) % ACN in Buffer (L)	0.08277	0.017863	4.63355	0.018937
% ACN in Buffer (Q)	-0.00040	0.000121	-3.30261	0.045644
(2) Flow rate (L)	0.59102	3.978364	0.14856	0.891325
Flow rate (Q)	0.27889	1.268319	0.21989	0.840071
1L By 2L	-0.02431	0.008496	-2.86142	0.064502

As shown in **Table 4**, the average tailing factor value of different trials of drug varied from **0.81** to **1.38**. Eq. (4) indicates that the tailing factor was increase with increase in concentrations of methanol in buffer (X_1) and increase in flow rate (X_2).

4.2. Calibration curves

The linearity was studied by analysing the standard working solutions containing 30–70 ug/ml($r2$= 0.999, slope = 44.11 of standard in triplicate (Fig. 2). The standard deviation of the slope value was less than 2 (Table 3)

4.3. Validation of method

4.3.1. Precision of method

The means of the intra-day and inter-day precisions were found to be 0.91 and 1.02, respectively. The results indicate that the selected factors were unaffected by small variations I the parameters (Table 5).

***Table 5.** Intra- and inter-day precision (n=6)*

Concentration (µg/ml)	Assay of efavirenz as % labeled content			
	Inter-day	%R.S.D	Intra-day	%R.S.D
40	99.45	1.51	100.36	1.05
50	100.98	1.18	100.52	1.1
60	100.15	1.31	100.05	1.25
Mean	100.19	1.33	100.31	1.13

4.3.2. Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ were confirmed by diluting known concentrations of drug until the average responses were approximately 3 or 10 times the standard deviation of the responses of the blank for six replicate determinations. The signal/noise ratios 3:1 and 10:1 were taken as the LOD and LOQ, respectively. The LOD and LOQ values were found to be 0.30 µg/ml and 0.81 µg/ml, respectively.

4.3.3 Stability in sample solution

Three different concentrations of drug 40, 50 and 60 µg/ml were prepared from sample solution and stored at room temperature for 3 days. They were then injected into the HPLC system and no additional peak was found in the chromatogram indicating the stability of drug in the sample solution.

Table 6: *Stability of drug in Sample Solutions (n=6)*

Parameter	HPLC Data
SD of Area	1.27
%RSD	1.39

4.4. Analysis of the marketed formulation and recovery study

Proposed method when used for extraction and subsequent estimation of drug from pharmaceutical dosage form after spiking with additional drug, afforded recovery of 100.40 to 100.64 % and mean recovery of drug from the marketed formulation are listed in **Table 7**

Table 7: *Standard addition technique for determination of drug*

Level (%)	**Actual conc. (□g/ml)**	**Observed conc. (□g/ml)**	**% Recovery ± % RSD**
80	40	40.12	100.31 ± 1.92
100	50	49.88	99.77 ± 1.64
120	60	60.63	101.05 ± 1.13
		Mean	100.38 ± 1.56

4.5. Forced degradation studies

Forced degradation studies were performed by treating the sample under the following stress conditions

4.5.1. Oxidation studies

Under oxidative stress condition at 1% w/v H_2O_2 at R.T for 60 min, the compound was degraded at lesser extent i.e. 2.49 % of drug was degraded. The detail results are shown in (Table 18) and degraded chromatogram is shown in **(Fig. 12).**

Table 8: *Results of Oxidation studies*

Sr.No.	Name	R_T	T_P	T_F	Resolution
1	Impurity-01	4.18	2777	0.95	0.00
2	Drug	7.60	6100	1.29	9.67

4.5.2. Acid degradation

Under acidic stress condition of 0.01N HCL at R.T for 60 min, the compound was degraded at greater extent i.e. 23.84% of drug was degraded. The detail results are shown in **Table 9** and degraded chromatogram is shown in **(Fig.).**

9:*Results of acid degradation*

Sr.No.	Name	R_T	T_P	T_F	Resolution
1	Impurity-01	4.20	2662	0.99	0.00
2	Drug	7.52	5357	1.25	9.01
3	Impurity-02	10.73	7152	1.18	7.00

Efacirenz was found to undergo acid degradation very rapidly. Reaction in 0.01 N hydrochloric acid for 15 min resulted in the extensive degradation of the drug, with three additional peaks at *t*R values of 3.01, 3.83, and 5.23. Under this condition, approximately 79.52% of the drug was degraded.

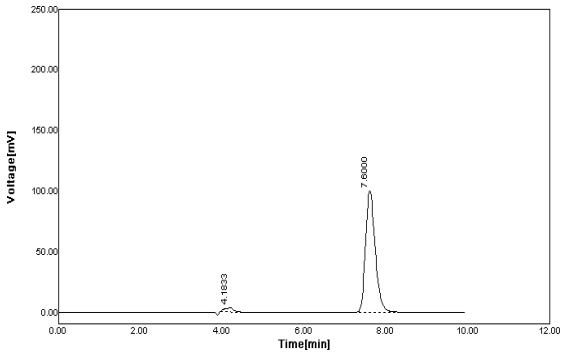
4.5.3. Base degradation

Under basic stress condition of 0.01N NaOH at R.T for 60 min, the compound was degraded at greater extent i.e. 91.02 % of drug was degraded. The detail results are shown in (Table 16) and degraded chromatogram is shown in **(Fig. 14).**

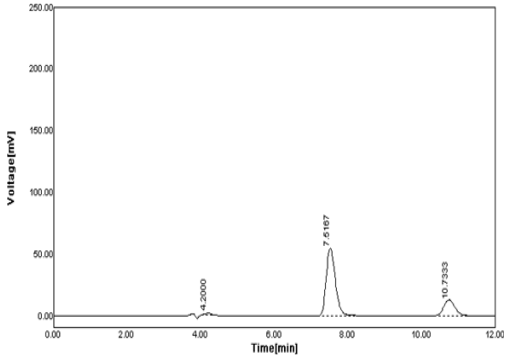
Table 10: *Results of basic degradation*

Sr.No.	Name	R_T	T_P	T_F	Resolution
1	Impurity-01	4.22	1659	1.46	0.00
2	Impurity-02	4.88	1200	1.68	1.35
3	Impurity-03	6.77	1696	0.93	2.95
4	Drug	7.50	3712	1.44	1.19

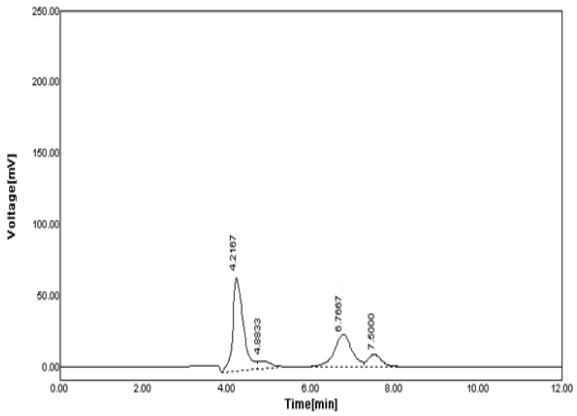
Thus, this indicates that the drug is susceptible to oxidation, acidic, and basic hydrolysis.



OXIDATION



ACID



BASE

Fig. 6. Chromatograms of efavirenz and its degradation products.

5. Conclusions

This work clearly reveals the usefulness of response surface methodology for the optimization of system variables in developing a HPLC method for the analysis of efacirenz in tablet formulation. The proposed HPLC methods provide simple, accurate, reproducible and stability indicating for quantitative analysis for determination of efavirenz from solid dosage form, without any interference from the excipients and in the presence of its acidic, alkaline, and oxidative degradation products. Statistical tests showed that the proposed HPLC methods reduce the duration of analysis and appear to be equally suitable for routine determination of drug in pharmaceutical formulation.

The method can be used to determine the purity of the drug available from various sources by detecting the related impurities and in stability studies. As the method separates the drug from its degradation products, it could be used as a stability indicating one.

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