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Research Article

Analytical Evaluation of the Mobile Phase Variables by Factorial Design for Efavirenz Separation Using High Performance Liquid Chromatography

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Abstract
A statistical optimization strategy was effectively used to investigate the effect of system factors on the chromatographic analysis of Efavirenz. To improve the procedure for excellent chromatographic responses, the effect of altering the flow rate, temperature, and concentration of % Acetonitrile in buffer (phosphate buffer 25mM, pH 7.5) was investigated. Response surface methodology (RSM) was used with Plackett-Burman designs to discover the optimum circumstances. The optimal regions for flow rate (0.8 ml/min) and temperature (25◦C) were determined from the response surface graphs as -1, 0, and +1. According to the Pareto ranking, temperature was the most influential variable influencing the selected responses. Linearity was seen in the 30-70 ug/ml range, with a considerably high correlation coefficient (r2 = 0.999). LOD and LOQ values The concentrations were 0.30 and 0.81 µg/ml, respectively. The devised approach underwent validation for accuracy, precision, linearity, range, and specificity. The approach was successfully employed to examine the chromatographic performance of a tablet formulation, and it was determined to be 100.88% with a standard deviation of ±1.36.

Keywords: HPLC; Factorial design; Response surface methodology; Efavirenz.

INTRODUCTION

4S)-6-chloro-4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4-dihydro-1H-3,1-benzoxazin-2- one.1-2 It is used for the combination treatment of HIV infection (AIDS). Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used in HAART to treat HIV-1. The optimal NNRTI-based treatment for previously untreated HIV infection is efavirenz and lamivudine combined with zidovudine or tenofovir. It is recognized in the Indian Pharmacopeia. Although HPLC has various advantages, including accuracy, speed (min), high resolution, sensitivity (ng to fg), and reproducibility, it has several drawbacks, particularly in the composition of the mobile phase, which is optimized using response surfaces.

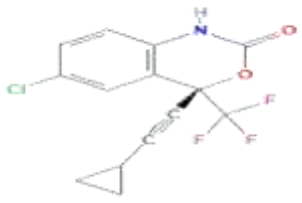

Fig 1 Molecular structure of efavirenz

The research focuses on enhancing the separation of efavirenz using high-performance liquid chromatography (HPLC) by combining factorial design and statistical screening techniques to discover the best mobile phase composition. Efavirenz, an important component of HIV/AIDS antiviral therapy, requiring precise chromatographic separation for reliable analysis and quantification in pharmaceutical formulations.

The paper begins by explaining the significance of efavirenz separation in pharmaceutical analysis, as well as the difficulties associated with attaining ideal chromatographic settings. It emphasizes the importance of mobile phase composition in HPLC, as well as the usefulness of factorial design in systematically assessing various components to understand how they affect the separation process. Factorial design is a statistical technique used to systematically modify the composition of the mobile phase, taking into account variables such as solvent type, solvent ratio, pH, and buffer concentration. The paper's goal is to discover the most influential elements and their appropriate levels for attaining successful efavirenz separation through tests using the specified factorial matrix.

The experimental data obtained from HPLC studies are statistically examined to determine the major effects of individual parameters and their combinations on efavirenz separation. The paper employs statistical software to assess experimental results, interpret data, and determine the ideal mobile phase settings for achieving the desired chromatographic performance.

The paper attempts to provide insights into the important parameters impacting efavirenz separation by HPLC using systematic testing and statistical analysis. By defining the best mobile phase composition, the study helps to develop robust analytical methods for accurately quantifying efavirenz in pharmaceutical formulations, improving the quality and reliability of HIV/AIDS treatment.
Overall, the paper advances the field of pharmaceutical analysis by demonstrating the use of factorial design and statistical screening techniques to optimize chromatographic conditions for efavirenz separation by HPLC, with implications for improving drug analysis and quality control in the pharmaceutical industry.

A literature search revealed that few analytical procedures have been documented that do not include force degradation studies. Furthermore, no one has investigated the effect of chromatographic conditions on drug resolution in the chromatographic conditions optimization process in HPLC by employing statistical design for the study of efavirenz in tablet form.

2. Theory

2.1. Retention Time (tR)

The retention time (tR) is the time between when the sample is injected and when the analyte reaches the detector. The intensity or polarity of the mobile phase has a significant impact on the analyte's retention period. The column temperature is a key regulator of retention time and also influences column selectivity. To improve retention time precision, most pharmaceutical tests require a column oven set to 30-50 ◦C. A temperature above 60 ◦C is uncommon because to concerns regarding heat degradation of analytes and column lifetime.

2.2. Peak Area (pA)

The peak size (area under the peak) is proportional to the concentration or quantity of that specific component in the sample. Both attributes can be used to do quantitative computations, but the peak area is more typically utilized since it offers a more accurate quantitative assessment and is less subject to flow changes. The peak areas of the standard components are used to calculate injection precision (reproducibility) and system linearity, whereas retention times are utilized to evaluate pump repeatability. To make this determination, HPLC methods leverage the relationship between the sample concentration and detector response (peak area).

2.3. Theoretical Plates (N).

Theoretical plates measure column efficiency and the number of peaks that can be found per unit run time of the chromatogram. The theoretical plates are computed using the formula N = 16(tR/W), where tR is the retention period and W is the peak width. This peak width, W, is calculated using the baseline intercepts of the tangent lines to a Gaussian peak, which corresponds to the peak width at 13.4% of the peak height. The flow rate of the mobile phase, column temperature peak position, particle size in column, mobile phase viscosity, and analyte molecular weight can all have an impact on theoretical plates.

2.4. Tailing Factor (TF)

Under ideal conditions, the chromatographic peaks will be Gaussian in shape and perfectly symmetrical. In actuality, most peaks are either slightly frontal or tailing. The tailing factor (Tf) is a measure of peak asymmetry [24] and is derived 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;

MATERIALS AND METHODS

3.1. Materials and instrumentation

Efavirenz medicines were gifted by Aurobindo Pharma Ltd, Hyderabad and utilized without further purification. VIRANZ tablets (Aurobindo Pharma Ltd) containing 200 mg of efavirenz, as stated on the label, were obtained at a local pharmacy. All chemicals, gradients, and reagents utilized in this procedure were HPLC grade. Mobile phase and solvents were filtered using 0.45µm Whatman filter paper before to use.The Agilent 1220 Infinity LC gradient pump with variable wavelength detector and Nucleosil C18 column (4.6mm I.D × 250mm) were utilized for chromatographic separation. A Rheodyne injector (manual loading) with a 20ul external loop was employed, and detection was done using a UV detector.

3.2. Selecting system variables

The experimental variables were chosen through trial and error, taking into account Efavirenz's physicochemical features such as pKa value, solvent solubility, and nature (acidic or basic). As Efavirenz is relatively weak acidic (close to 7) in nature,]. A flow rate of 1.5 ml/min and a temperature of 20-25 ◦C were utilized. Acetonitrile in buffer (phosphate buffer pH 7.5) is used as the mobile phase. A stock solution of the standard medication was produced in methanol with a final concentration of 100ug/ml. A column equilibration duration of 30 minutes between runs was maintained.

3.3. Factorial design and the effect of variables

The Plackett-Burman design 32 trial was performed to optimize the chromatographic conditions. This design used two variables, % acetonitrile in buffer (phosphate buffer 25 mM, pH 7.5) (X1) and flow rate (X2), as potential sources of changes in retention time (RT), theoretical plates (TP), and tailing factor (TF). The range of variables was determined by a trial and error investigation. The link between these characteristics and responses (retention time (RT), theoretical plates (TP), and tailing factor (TF) was statistically investigated. The response surface for each selected answer was plotted against two separate variables using the STATISTICA (Version 8.0.360.0 English, StatSoft Inc., Tulsa, OK, USA) program. The second-order quadratic equation is provided below.

The equation $y = \beta0 + \beta1x1 + \beta2x2 + \beta11x12 + \beta12x1x2 + \beta22x22$ (1)

y = chromatographic response; β0 = constant (intercept); β1 = coefficient of X1; β2 = coefficient of X2; β11, β12, β22 = interaction coefficients; X1 = first experimental variable; X2 = second experimental variable. According to this concept, nine testing batches were created. All of the batches were designated as

L-1 through L-9. Each independent variable was evaluated at three levels to investigate the effect: "low", "middle", and "high". These levels indicate the lower, middle, and upper bounds of the range covered by each variable. The mobile phase was degassed before to use using ultrasonic vibrations.

3.4. Analysis and Validation of the Optimization Model

The responses (retention duration, peak area, theoretical plates, tailing factor) were investigated using a two-way ANOVA-based factorial analysis. For the current optimization, an RSM calculation 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 appropriateness of the fitted answer was determined using ANOVA. Statistical significance was determined at $p < 0.05$ for the derived response surfaces.

3.5. Standard Stock Solution and Calibration Graph

Efavirenz was produced as a standard stock solution in methanol (100 ug/mL). A calibration graph was created by injecting five concentrations (20 ul loop) of the medication into the HPLC system and graphing the peak regions vs the corresponding concentrations.

3.6. Method Validation

The intra-day and inter-day variations for determining efavirenz were carried out at three distinct concentration levels of 40, 50, and 60 ug/ml using homogeneous authentic tablet samples (200 mg efavirenz per tablet; VIRANZ tablet (Aurobindo Pharma Ltd). The robustness parameters include the modification of the

Fig $y = \beta0 + \beta1x1 + \beta2x2 + \beta11x12 + \beta12x1x2 + \beta22x22$ (1) flow rate of the mobile phase (ml/min), column temperature (◦C), and% acetonitrile in Phosphate buffer pH 7.5) of various lots. A signal-to-noise ratio of 3:1 to 10:1 is often accepted for evaluating the limit of detection and limit of quantitation [31]. In the specificity research, the peak area for efavirenz in the sample was confirmed by comparing its retention time (RT) with Those of the standard. For an assay, twenty tablets of efavirenz were weighed, and the average weight of the pills was calculated. The tablets were then crushed into fine powder. Based on the labeled claim, a 100ml volumetric flask was filled with powder equivalent to 500 mg of efavirenz. 30ml of methanol was added to the flask, which was then sonicated for 30 minutes to ensure full dissolution. The final volume was created up to 100ml with the same solvent (5000µg/ml). The solution was diluted from 10 ml to 100 ml using the same solvent (500 µg/ml). The solution was diluted from 10 ml to 100 ml using the same solvent (50 µg/ml). The solution was then filtered using Whatman filter paper (0.45 mg/mL). A 20 µl solution was injected six times into the HPLC system under the above-mentioned circumstances. The resulting solution was tested for drug content. The drug content of the sample solution was estimated using the regression equation from the standard calibration graph (Fig. 2).

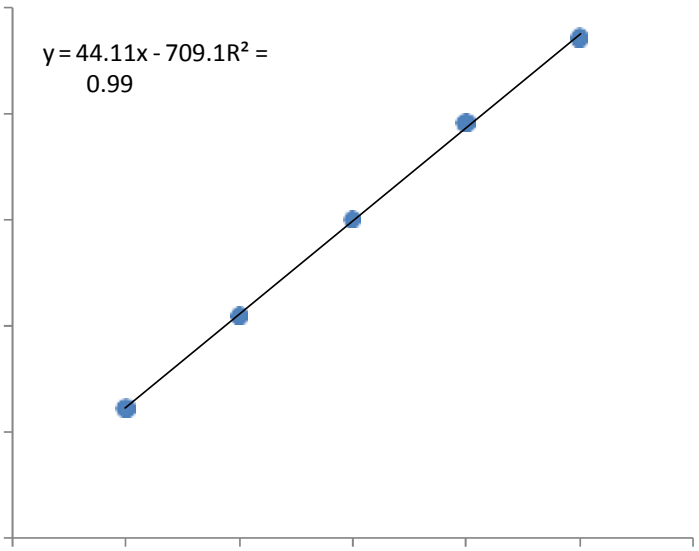

Fig. 11: Calibration curve for efavirenz by HPLC

3.7. Forced deterioration studies

To verify whether the method and assay indicate stability, pure medication was subjected to forced degradation tests under a variety of settings. A medication stock solution in methanol (50 ug/ml) was employed in the forced degradation to demonstrate the suggested method's stability-indicating property and specificity. In all degradation studies, the average peak area of the standard medication and degraded sample after six replicates was determined.

3.7.1. Oxidation.

Approximately 2 ml of 1% hydrogen peroxide was added to 2 ml of medication stock solution and stored at room temperature. After 30 minutes, the solution was diluted to yield a 50ug/ml solution. A 20ul 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 drug stock solution, which was then maintained at room temperature. After 15 minutes, the solution was diluted to 50 ug/mL. A 20-ul 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 drug stock solution, which was then maintained at room temperature. After 15 minutes, the solution was diluted to 50 ug/mL. 20ul of solution was injected, and chromatograms were obtained.

RESULTS AND DISCUSSION

4.1. Analysis of RSM curves and Validation of Optimization Model

All eight studies were conducted at a concentration of 50ug/ml. Table 1 shows the experimental plans and their corresponding responses. The experimental results were analyzed with STATISTICA software (v8.0.360.0 English, Stat Soft, Inc., Tulsa, OK, USA). The coefficients for the second-order polynomial model were determined using least squares regression. To determine the model's significance and validity, the regression model for each evaluated answer was tested using analysis of variance. The response surface plots were generated with the same software, and the fitted model's competence was tested using ANOVA [33, 34]. The equation model for the constants, regression coefficients, and statistical parameters for each response variable, such as retention duration, peak area, theoretical plates, and tailing factor, are given in Table 2.As illustrated in Fig. 3(A1-D3), the analysis generates 3D graphs by graphing the response model against two of the components while leaving the third constant. Based on the answer graphs, the following conclusions were reached:

4.1.1. Effect of variables on retention time

Response surface plots are used to quickly identify the link between retention time and various variables. Figure D shows that the effect of all variables on response is considerable. The following regression equation was developed to estimate retention time based on the outcomes of various experimental factors.

$$Y1 = 47.9709 - 0.9014X1 - 20.8121X2 + 0.0049X2 + 0.1582X1X2 + 3X2 \ldots (2)$$

X1 and X2 are independent variables, representing the percentage of ACN in buffer (phosphate buffer 25mM, pH 7.5) (v/v) and flow rate (ml/min), respectively.

As indicated in Table 7, the average retention time of several medication trials ranged from 1.23 to 11.97 minutes. Equation (2) displays the effect of variables on retention time and was found to be statistically significant ($P = 0.038902$). The equation indicates the effect of variables on retention time, which was shown to be statistically significant. It illustrates that as the concentration of ACN in the buffer (X1) and the flow rate of the mobile phase (X2) increased, the retention time decreased. However, a strong negative regression value for X2 revealed that the flow rate was a substantial factor contributing to the change in retention time. We can increase the flow rate to shorten the retention time, however this may reduce peak resolution. Backpressures may exceed the system's limits. By raising the temperature, we may accelerate the separation and reduce the viscosity of the mobile phase, lowering the backpressure.

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

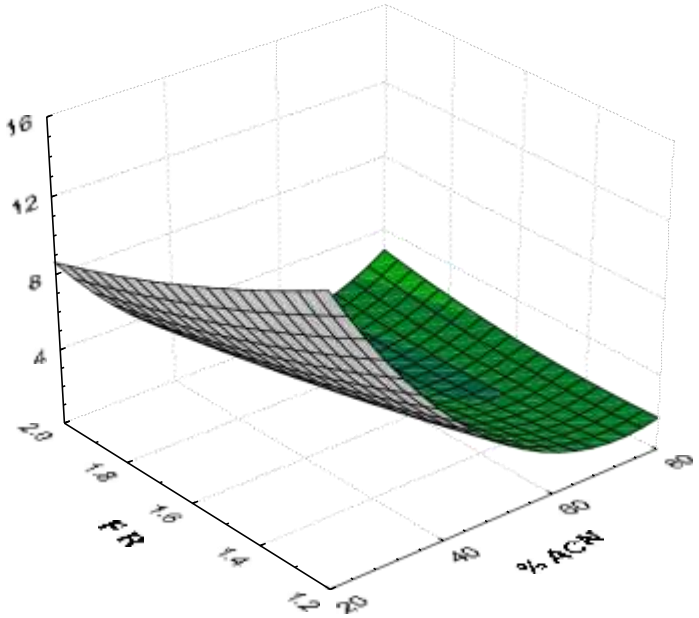

Table 2: Effect estimates from first factorial design for retention time

	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 a rise in mobile phase modifier concentration, ACN retention time falls while all other responses increase. This could be owing to the increasing polarity of the mobile phase, which results in a quicker equilibrium of the analyte between the stationary and mobile phases. Table 2 presents statistical data and work results.

4.1.2. Effect of variables on therotical plate

To quickly learn about the relationship between theoretical plates and individual variables, see Fig. E, which shows that the effect of all variables on reaction is considerable. The retention time was predicted using the following regression equation based on the results of theoretical plates.

Y2 = 41851.2526 - 268.3831X1 - 39433.5029X2 + 2.3166X1 2- 46.7911X1X2 + 13607.2222X2……. (3)

X1 and X2 are independent variables, representing the percentage of ACN in buffer (phosphate buffer 25mM, pH 7.5) (v/v) and flow rate (ml/min), respectively. Table 3 shows that the average theoretical plates for several pharmacological trials ranged from 1215 to 7630. Equation (3) displays the effect of variables on the theoretical plates and was determined to be statistically significant (P = 0.072256). Equation (3) shows that the theoretical Plates decreased with increasing ACN concentrations in buffer (X1) and flow rate (X2). However, a significant positive regression value for X2 revealed that the flow rate was a substantial factor influencing the change in theoretical plates. Table 3 presents statistical data and work results.

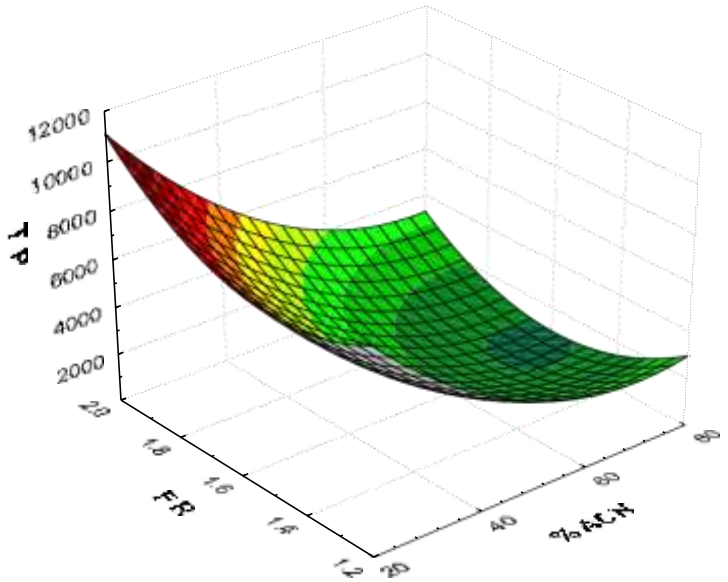

Fig. E: 3D Surface plots of theoretical plates against % ACN in buffer and flow rate

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 quickly determine the relationship between the tailing component and the individual variable. Figure F shows that the effect of all variables on response is considerable. Based on the results of tailing factor altering experimental variables, the following regression equation was developed to estimate retention time.

$$Y3 = -1.5023 + 0.0828X1 + 0.591X2 - 0.0004X2 - 0.0243X1X2 + 0.2789X2 \ldots. (4)$$

X1 and X2 are independent variables, representing the percentage of 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

Table 4 shows that the average tailing factor value of several pharmacological trials ranged between 0.81 and 1.38. Eq. (4) shows that the tailing factor increases with increasing methanol concentrations in the buffer (X1) and flow rate (X2).

4.2. Calibration Curves

The linearity was investigated by analyzing 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 mean intra-day and inter-day precisions were 0.91 and 1.02, respectively. The findings show that tiny modifications in the parameters had little effect on the selected criteria (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 drug concentrations until the average responses were approximately 3 or 10 times the standard deviation of the blank answers over six replicate measurements. The signal/noise ratios of 3:1 and 10:1 were used as the LOD and LOQ, respectively. The LOD and LOQ values were determined to be 0.30 µg/ml and 0.81 µg/ml, respectively.

4.3.3 Stability of sample solution.

Three drug concentrations (40, 50, and 60 µg/ml) were created from the sample solution and stored for three days at room temperature. They were then injected into the HPLC machine, and no new peak appeared in the chromatogram, indicating that the drug is stable 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

The proposed approach, when applied f

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 conditions of 1% w/v H2O2 at room temperature for 60 minutes, the molecule was destroyed to a reduced level, with 2.49% of the medication degraded. The detailed results are provided in Table 18, while the deteriorated chromatogram is displayed in Figure 12. or extraction and subsequent estimation of drug from pharmaceutical dosage form after spiking with additional drug, gave recovery of 100.40 to 100.64%. The mean recovery of drug from the marketed formulation is presented in Table 7.

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 conditions of 0.01N HCL at room temperature for 60 minutes, the compound was deteriorated to a larger extent, with 23.84% of the medication destroyed. The detailed results are provided in Table 9, and the deteriorated chromatogram is displayed in Figure.

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 degrade rapidly in acidic environments. Reaction in 0.01 N hydrochloric acid for 15 minutes resulted in substantial drug degradation, with three further peaks at tR values of 3.01, 3.83, and 5.23. Under these conditions, about 79.52% of the medication was degraded.

4.5.3. Base Degradation

Under basic stress conditions of 0.01N NaOH at room temperature for 60 minutes, the compound was degraded to a larger extent, with 91.02% of the drug destroyed. The detailed results are provided in Table 16, while the deteriorated chromatogram is displayed in Figure 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.

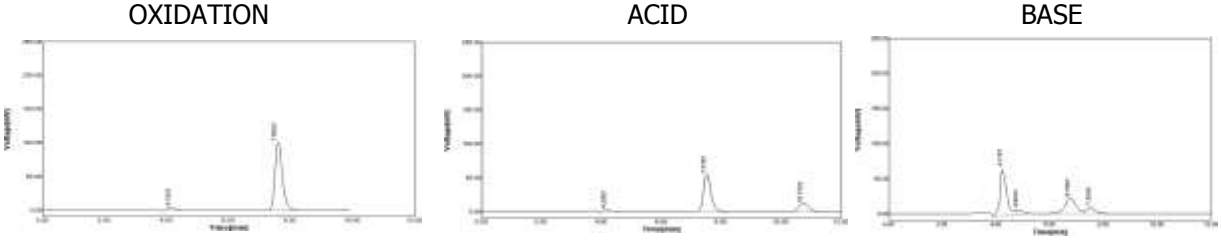

Fig. 6. Chromatograms of efavirenz and its degradation products.

CONCLUSIONS

This study clearly demonstrates the utility of response surface methodology for optimisation of system factors in establishing an HPLC method for the measurement of efacirenz in tablet formulations. The proposed HPLC procedures give simple, accurate, reproducible, and stable results for quantitative measurement of efavirenz from solid dosage form, free of excipient interference and in the presence of acidic, alkaline, and oxidative degradation products. Statistical studies revealed that the proposed HPLC methods reduce analysis time and appear to be equally appropriate for regular drug determination in pharmaceutical formulations.

The method can be used to determine the purity of drugs obtained from various sources by detecting associated contaminants, as well as for stability studies. As the process separates The drug's breakdown products could be utilized to indicate its stability.

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