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QUALITY BY DESIGN-BASED REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR FINERENONE IN TABLET DOSAGE FORM

SAKHARE RS[1*], BHATANE RB[2], MALWAD VB[2], MARSHIVANE PH[2], ZINGADE SG[3]

1: HOD & Professor, Department of Pharmaceutical Quality Assurance, Channabasweshwar Pharmacy College (Degree), Kava Road, Basweshwar Chowk, Latur – 413512, Maharashtra, India

2: Research Scholar Department of Pharmaceutical Quality Assurance, Channabasweshwar Pharmacy College (Degree), Kava Road, Basweshwar Chowk, Latur- 413512, Maharashtra, India

3: Assistant Professor Department of Pharmacognosy Channabasweshwar Pharmacy College (Degree), Kava Road, Basweshwar Chowk, Latur- 413512, Maharashtra, India

***Corresponding Author: Dr. Ram S. Sakhare: E Mail: ramsakhare85@gmail.com**

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1. ABSTRACT

The objective of this study is to develop Simple, rapid, and sensitive RP-HPLC for Finerenone in tablet dosage form by QbD approach. In this method, Finerenone was estimated using C18 Sunfire column (250×4.6 mm, 4.8 µm) and 0.01N KH_2:PO_4: methanol (61.2:38.8 % V/V) as mobile phase and flow rate 1 mL/min which are optimized using Design-Expert software. Method was developed at 272 nm detection wavelength. The retention time was found to be 3.051min. The proposed method was successfully applied to the determination of Finerenone in tablet dosage form. High linearity of developed method was confirmed over concentration range of (5-30) µg/mL and correlation co-efficient is 0.9994. The recovery was found to be 100.68 %; LOD was found to be 0.31 µg/mLand LOQ was found to be 0.95 µg/mL. There was no interference of excipient and degradation product in retention time, so the method was specific. Analytical parameters such as precision, accuracy, LOD, LOQ and robustness were determined according to ICH guidelines.

Keywords: Finerenone, QbD Approach, Method development, RP-HPLC, LOD, LOQ

2. INTRODUCTION:

The molecular name for finerenone is (4S)-(4)-(4-cyano-2-methoxyphenyl)-5-ethoxy-2,8-dimethyl-1,4-dihydro-1,6-naphthyridine-3-carboxamide **(Figure 1)** is a nonsteroidal mineralocorticoid receptor antagonist that is recommended to lower the risk of end-stage kidney disease, cardiovascular death, heart attacks, and hospitalization for heart failure in adults with chronic kidney disease associated with type II diabetes mellitus **[1]**. Finerenone (BAY94-8862) is a novel nonsteroidal MRA with more potential than spironolactone and greater affinity than eplerenone in vitro **[2, 3]**. It is generally formulated as immediate-release tablets with non-functional film coating under the brand name kerendia and generally this contains about 10 – 20 mg of Finerenone as an active ingredient **[4, 5]**.

Finerenone shows their action by preventing the binding of MR coactivators, preventing pro-inflammatory and pro-fibrotic gene transcription. Clinical trial data show that blocking the MR reduces mortality and morbidity in patients with chronic severe congestive heart failure, new onset atrial fibrillation or flutter, first onset of kidney failure, sustained eGFR decrease, and death from a renal cause. Cardiovascular outcomes are similar in patients with and without AFF history **[6]**.

Finerenone inhibits MR-mediated reabsorption of sodium as well as MR hyperactivity in both epithelial (kidney) and nonepithelial (heart and circulatory system) tissues. It is assumed that MR hyperactivation contributes to fibrosis and an allergic reaction. Finerenone has a high affinity for the MR and no affinity for androgen, progesterone, estrogen, or glucocorticoid receptors. Finerenone has been prescribed for the therapy of patients with type 2 diabetes-related persistent renal failure (stages 3 and 4 with albuminuria) in adults **[7]**. Until the development of the present method, there were no existing literature reports on reversed-phase high-performance liquid chromatography (RP-HPLC) for the determination of Finerenone by QbD approach.

A QbD framework is typically used to assess how important material and process variables affect the final quality of a processin this example, an analytical method. Multivariate regression models, like DoE, can be used to assess the influence of various independent input factors on a process's output **[8]**. DoE is a statistical and mathematical technique that has proven useful in process development and optimization. A popular method for studying several input factors on a response at once is response surface methodology, or RSM. The central composite (CCD) **[9]**, Box-Behnken **[10]**, Doehlert **[11]**, RSM, a full or fractional factorial, and QbD technique can be utilized to identify the experimental parameters for an optimal chromatographic separation. To guarantee that experiments are carried out in

accordance with and compliance with a well-defined experimental domain, it is required to identify the components that need to be explored and that affect critical responses in order to design an experimental matrix that is fit for purpose. Polynomial equations and mathematical techniques can be used to assess the model's fitness, and the last step of analysis involves confirming the ideal input region for each response under observation **[12, 13]**. The goal of this work was to create an RP-HPLC method that is simple, sensitive, selective, accurate, and precise for measuring finerenone in dosage forms. It also aimed to optimize the process through the use of RSM and validate the RP-HPLC method. As far as we are aware, this is the first publication on an RP-HPLC technique optimized with RSM• and QbD for Finerenone analysis.

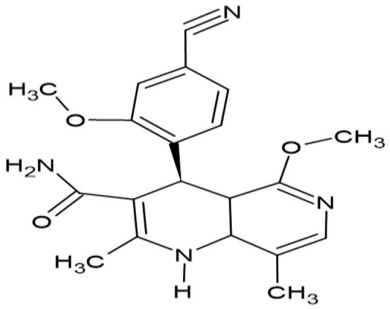

Figure 1: Structure of Finerenone

3. MATERIALS AND METHODS

3.1 Reagents and Chemicals:

API of Finerenone procured from Spectrum Lab Ltd., Hyderabad. HPLC grade of Methanol, Acetonitrile and water were obtained from Rankem, India. Ortho-phosphoric acid of HPLC grade were purchased from Merck Life Sciences Private Ltd., Mumbai, India.

3.2 Apparatus and Equipment:

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Finerenone solution. The chromatographic analysis was performed on C18 column (150 x 4.2 mm, 4.8 µm). Other Instruments include, Sonicator (Ultrasonic Sonicator), PH meter (Thermo scientific), Micro balance (Sartorius) Vacuum filter pump.

3.3 Preparation of solutions:

Preparation of Standard stock solutions:

Accurately weighed 10mg of Finerenone transferred to 50ml volumetric flask. 3/4th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/mL of Finerenone).

Preparation of Standard working solutions:

From the filtered solution 1 mL was pipetted out into a 10 mLvolumetric flask and made up to 10mLwith diluent. (20µg/mL of Finerenone).

Preparation of Sample stock solutions:

10 mg of tablets were taken in 10 quantity and average weight was calculated and then

Weight equivalent to 1 tablet(10mg) was transferred into a 100mLvolumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (100µg/mL of Finerenone).

Preparation of Sample working solutions:

From the filtered solution 2 mL was pippeted out into a 10 mL volumetric flask and made up to 10mLwith diluent. (20µg/mL of Finerenone).

3.4 Chromatographic conditions:

Separation of peaks was achieved using a Waters Alliance system fitted with a PDA detector set at 272 nm and a Sunfire C18 (150 x 4.2 mm, 4.8 µm) stationary phase and mobile phase consisting of a mixture of 0.01N KH_2PO_4: Methanol taken in the ratio 61.2: 38.8 (%V/V). The system was operated in gradient mode at flow rate of 1.0 mL/min and a column temperature of 27.4 °C.

3.5 Method Optimization by QbD Approach:

Software aided method development:

A QbD based RP-HPLC method was developed for the Determination of Finerenone in bulk form and dosage form. Two phases are involved in method development by QbD approach:

Screening Phase

Screening phase involves screening of major contributors of selectivity and peak shape which include Mobile Phase Composition, flow rate, and temperature. The screening phase was carried out using Design Expert 11 software as shown in **Table 1**. In this software, Central composite statistical screening design was used to optimize the critical process parameters (CPPs) or critical method parameters (CMPs) and to evaluate interaction effects of these parameters on the critical quality attributes (CQAs). This Central composite statistical screening design is a 3 factor-3 level design. All the parameters are varied simultaneously, unlike the conventional OFAT (one factor at a time) approach. The responses obtained after carrying out 20 experimental trials were fed back into DoE software.

Statistical Analysis and Final Optimization

The three responses-retention time, NTP, and Finerenone symmetry factor were determined by statistical analysis to determine the important chromatographic variables and the effects of their interactions.

Table 1: Coded Valued For Independent Variables

Factor	Coded values given factor	Levels		
		-1	0	1
Mobile Phase	A	56.2: 43.8	61.2: 38.8	66.2: 33.8
Flow rate	B	0.9	1	0.8
Temperature	C	22.4	27.4	32.4

3.6 Method Validation:

Specificity: It is the capacity to definitively evaluate the analyte in the existence of substances that might be anticipated to be present. The chromatograms of the test and standard solutions were compared in order to assess the method's specificity. There should not be any peaks that interfere with the analyte peak.

Linearity: Working standard solution aliquots (0.25, 0.50, 0.75, 1.0, 1.25, and 1.5mL) were put into a succession of volumetric flasks measuring 10 mL, and they were diluted with methanol and water (60: 40 % V/V) until they reached the mark. A solution containing 5, 10, 15, 20, 25, and 30 µg/mL was obtained from these. For each solution, an aliquot of 20µLwas introduced while the chromatography apparatus was in operation. The calibration curve for Finerenone was plotted against the corresponding concentrations, and the correlation coefficient and regression line equation were determined. Every response was the mean of three conclusions.

Precision: Two forms of precision were identified: intra- and inter-day. Finerenone standard solutions in the range of 20 ug/mL of it were analyzed in triplicate within a day to assess the intra-day precision; the percentage of the relative standard deviation (RSD) for Finerenone was computed. Analyzing Finerenone standard solutions in the Concentration of 20 ug/mL on three separate days allowed for the calculation of inter-day precision.

Accuracy: By estimating Finerenone recovery using the conventional addition approach, accuracy was ascertained. To a vial containing 0.5, 1.0, 1.5 mL of Finerenone test solution, known volumes of working standard solutions (1mL) were added. Every solution was injected three times, and the peak areas were measured to determine the recovery using the calibration curve's regression equation.

Limit of Detection and Limit of Quantification: As per ICH rules, the drug's LOD and LOQ were determined using following equations. The values for LOD and LOQ were 3.3 and 10 σ/S, respectively, where σ represents the standard deviation of the regression line and S is the slope of the calibration curve.

Robustness: The robustness study was conducted in order to assess the impact of a purposeful, little alteration in the chromatographic state. Three minor adjustments were made to the mobile phase (61.2±5 mL), flow rate (1±0.1 mL/min), and Temperature (27.4±5°C). The percentage assay and system appropriateness parameters were then verified after each sample solution was injected.

4. RESULTS AND DISCUSSION

The sample was scanned in UV spectrophotometer from a range of 400-200nm. λmax of Finerenone was found to be

272 nm. Which is shown in **Figure 2**. Chromatogram for standard and test Finerenone has been depicted in **Figure 3** and **Figure 4**.

Method optimization using the QbD technique was used to execute the experimental design, and the results were assessed **(Table 2)**. Analysis of model was carried out using Analysis of Variance (ANOVA) for Quadratic model for response of Rt of Finerenone was studied **(Table 3)**. The Lack of Fit F-value of 48.25 implies the Lack of Fit is significant. There is only a 0.03% chance that a Lack of Fit F-value this large could occur due to noise. Similarly, P-values less than 0.0500 indicate model terms are significant. In this case A, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. Further, ANOVA for quadratic model for response of Tf of Finerenone was studied **(Table 4)**. F-value of 7.08 implies the Lack of Fit is significant. There is only a 2.55% chance that a Lack of Fit F-value this large could occur due to noise. Similarly, $p < 0.05$ was found to be significant. In this case A, B, AB, BC, B^2, C^2 are significant model terms. Further, ANOVA for quadratic model for response of NTP of Finerenone was studied (Table V**)**. F-value of 195.60 implies the Lack of Fit is significant. There is only a 0.01% chance that a Lack of Fit F-value this large could occur due to noise. Then 3D plots of the effect of interaction terms A and B on response variable of Rt **(Figure 5, Figure 6)**, Tf **(Figure 7, Figure 8)** and NTP **(Figure 9, Figure 10)** were determined.

Optimization of model was carried out according to the solution suggested by design expert software, where the mobile phase composition included 61.2: 38.8(% V/V) and the flow rate was 1 mL/min. Temperature of Column is set to be 27.4 °C. The method's specificity was assessed by contrasting the chromatographs of the test and standard solutions. The chromatogram's test sample had no interfering peaks. It reveals that developed method is specific for Finerenone. Linearity and range of Finerenone were determined and was found to be in the range of 5-30 µg/mL **(Figure 11)**.

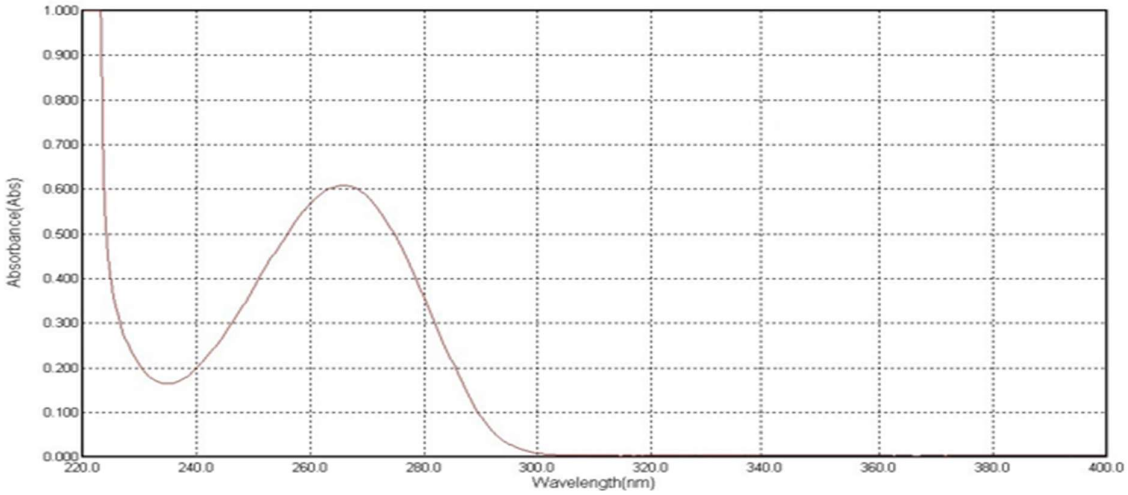

Figure 2: UV spectrum of Finerenone

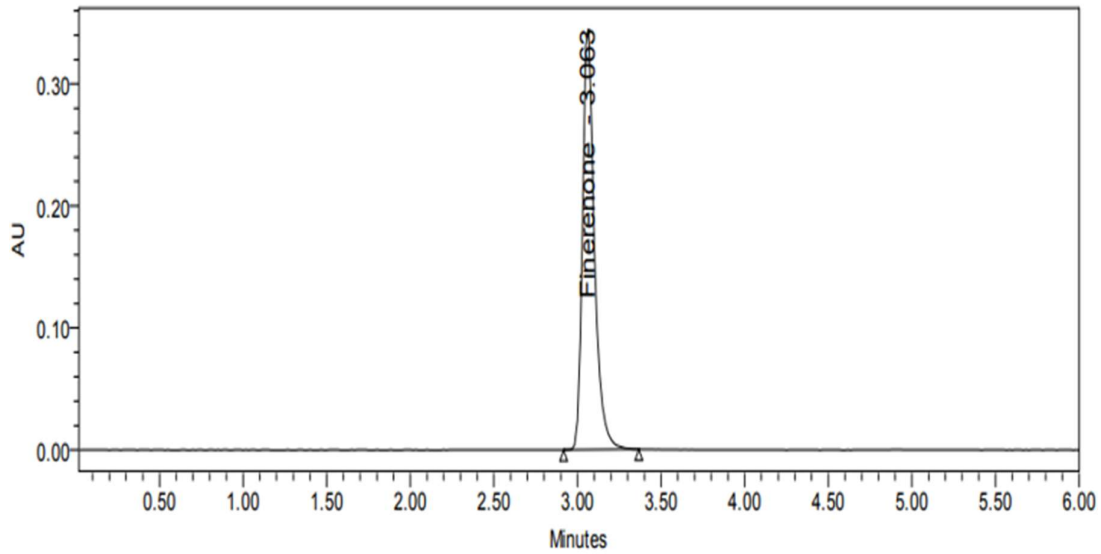

Figure 3: Chromatogram of Finerenone using 20 µg/mLof Standard solution

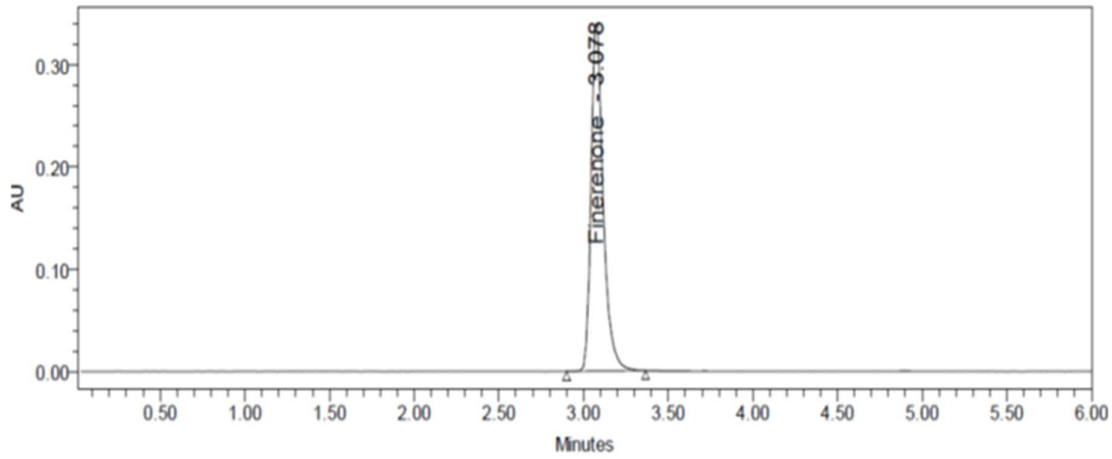

Figure 4: Chromatogram of Finerenone using 20 µg/mLof Test solution

Table 2: Execution of Experimental Design and Responses

Standard	Run	Factor 1 A:FR (mL/min)	Factor 2 B:MP (%V/V)	Factor 3 C: Temp (^{0}C)	RT (min.)	NTP (num.)	TF (num.)
1	19	0.9	35	27	3.309	4351	1.1
2	5	1.1	35	27	2.783	4519	1.07
3	18	0.9	45	27	3.255	5301	1.55
4	7	1.1	45	27	2.743	6735	1
5	13	0.9	35	33	3.309	5494	1.2
6	3	1.1	35	33	2.797	5390	1.19
7	16	0.9	45	33	3.355	8652	1.42
8	15	1.1	45	33	2.651	9034	0.9
9	2	0.83182	40	30	3.546	5203	1.36
10	14	1.16818	40	30	2.684	6690	0.92
11	10	1	31.591	30	2.997	5352	1.16
12	8	1	48.409	30	3.104	8353	1.2
13	11	1	40	24.9546	2.929	5694	1.19
14	12	1	40	35.0454	2.936	7932	1.18
15	4	1	40	30	3.03	5284	1.14
16	1	1	40	30	3.057	5294	1.12
17	6	1	40	30	3.046	5300	1.12
18	17	1	40	30	3.044	5353	1.13
19	20	1	40	30	3.043	5382	1.13
20	9	1	40	30	3.046	5301	1.14

Table 3: Anova Table for Response RT

Source	Sum of squares	RT	Mean square	F	P
Model	1.05	9	0.1164	63.31	< 0.0001
A-flow rate	1.00	1	1.00	546.24	<0.0001
B-MP	0.0000	1	0.0000	0.0079	0.9311
C-Temp.	0.0001	1	0.0001	0.0454	0.8355

Table 4: Anova Table for Response TF

Source	Sum of squares	TF	Mean square	F	P
Model	0.4301	9	0.0478	147.95	<0.0001
A-flow rate	0.2506	1	0.2506	775.74	<0.0001
B-MP	0.0104	1	0.0104	32.26	0.0002
C-Temp.	0.0001	1	0.0001	0.1630	0.6949

Table 5: Anova Table for Response NTP

Source	Sum of squares	NTP	Mean square	F	P
Model	3.497E+07	9	3.885E+06	25.80	< 0.0001
A-flow rate	1.405E+06	1	1.405E+06	9.33	0.0122
B-MP	1.651E+07	1	1.651E+07	109.62	< 0.0001
C-Temp.	9.563E+06	1	9.563E+06	63.50	< 0.0001

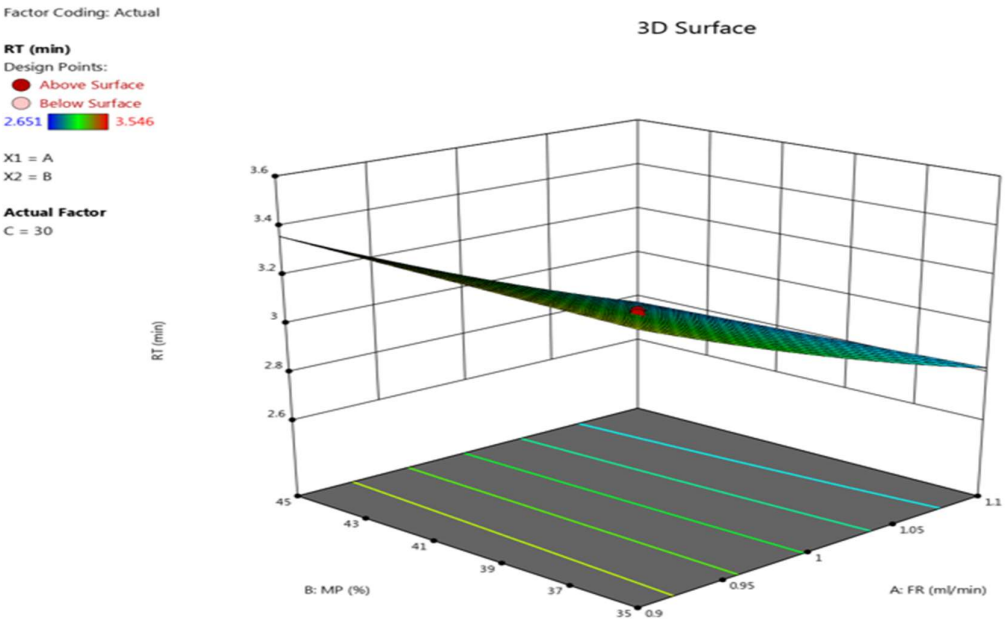

Figure 5: Effect of FR and MP on retention time of Finerenone

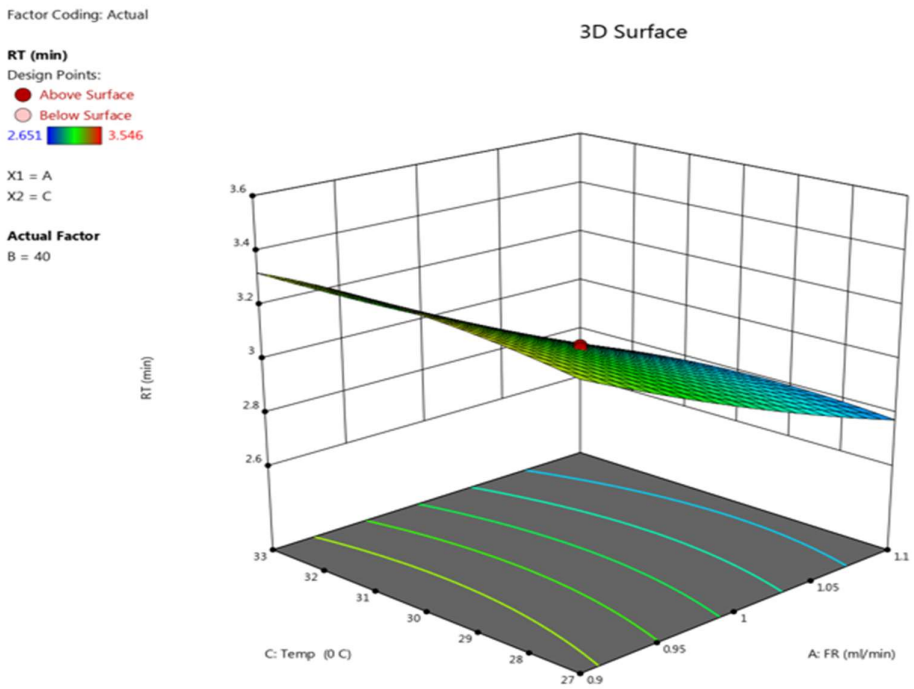

Figure 6: Effect of FR and Temp. on retention time of Finerenone

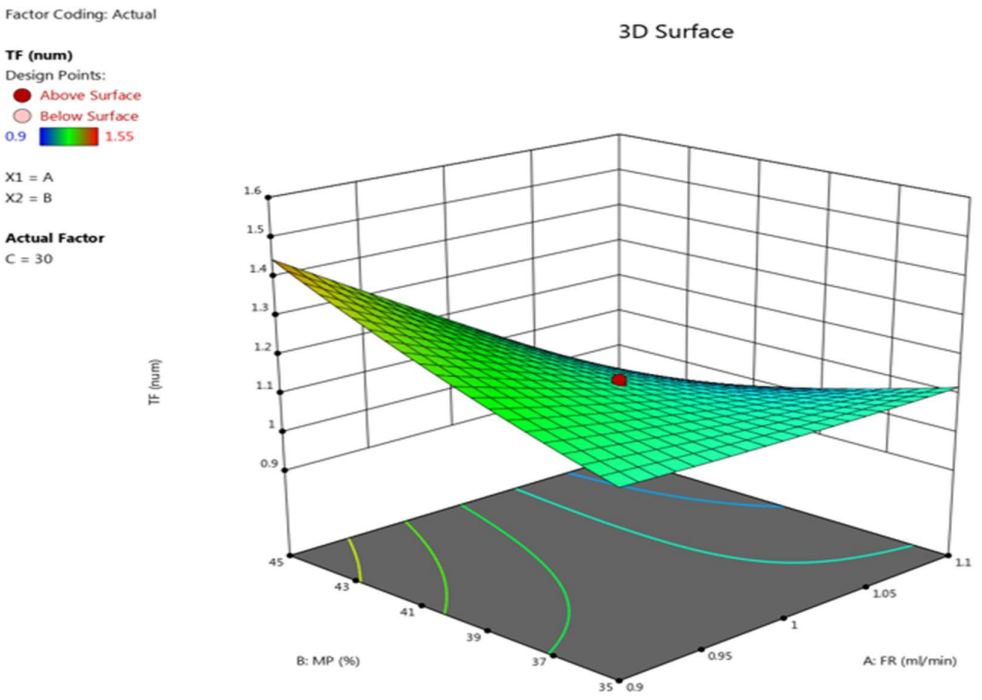

Figure 7: Effect of FR and MP on TF of Finerenone

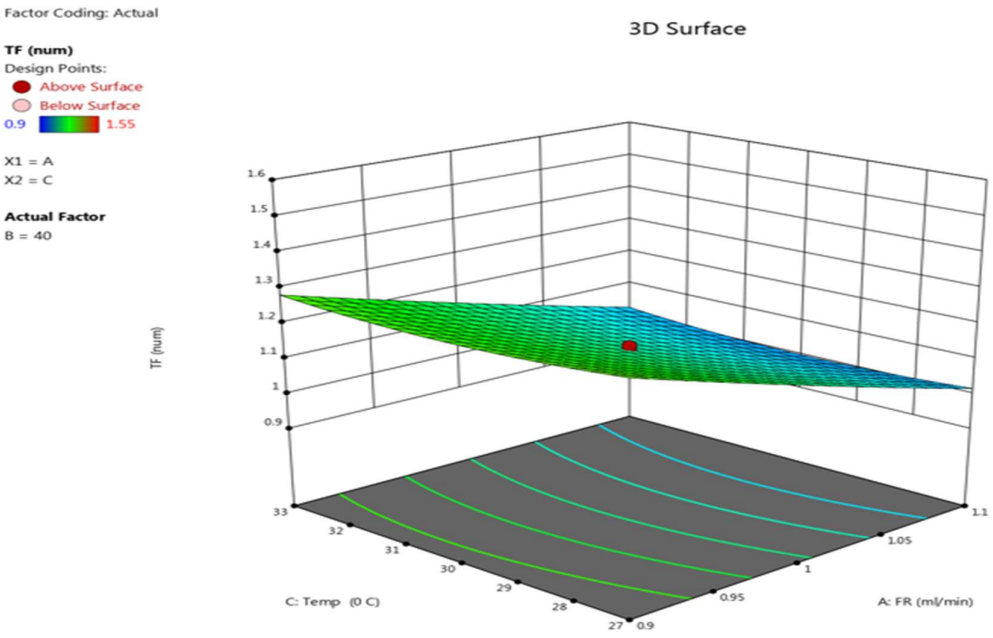

Figure 8: Effect of FR and Temp. on TF of Finerenone

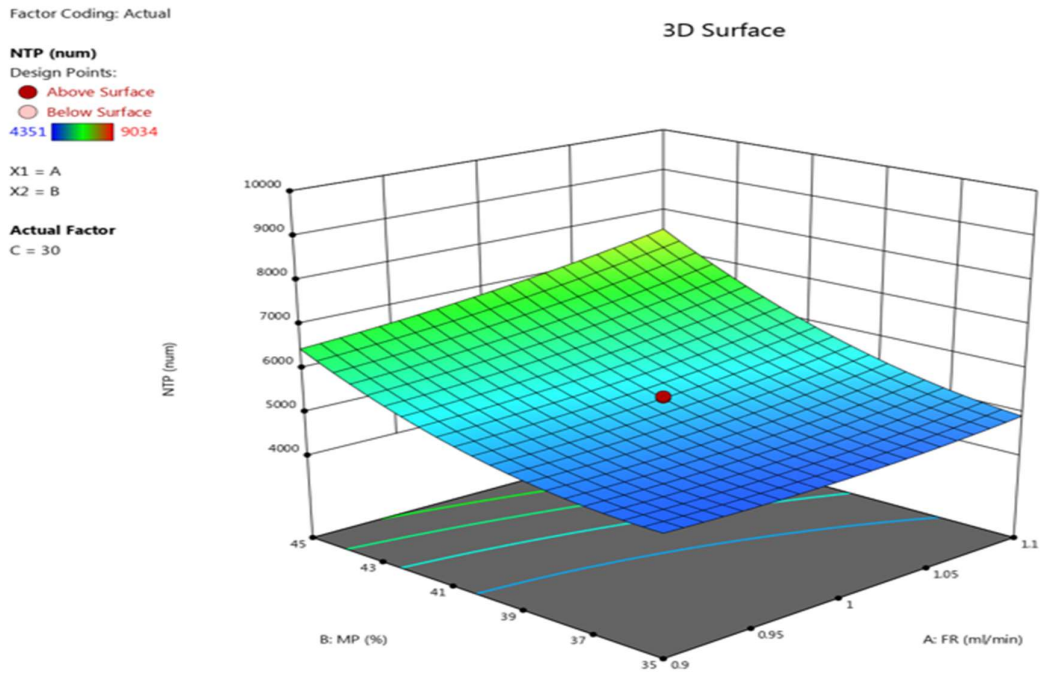

Figure 9: Effect of FR and MP on NTP of Finerenone

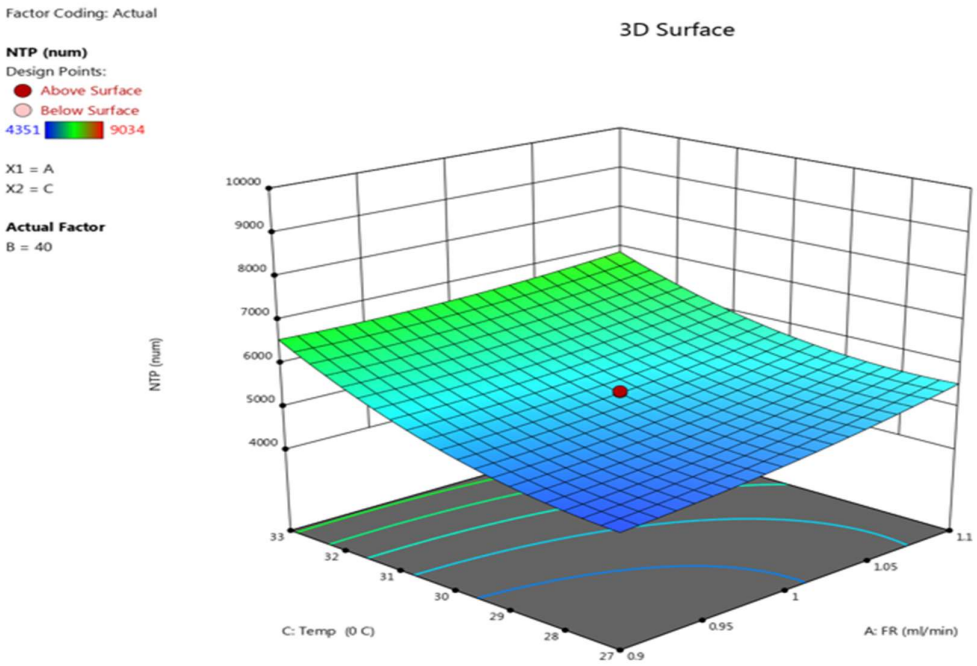

Figure 10: Effect of FR and Temp. on NTP of Finerenone

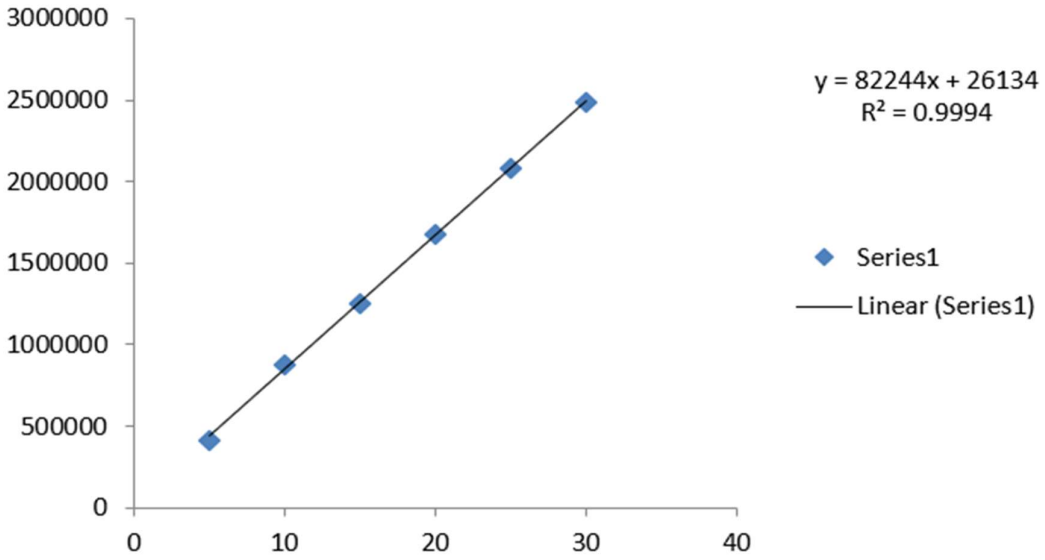

Figure 11: Calibration curve of Finerenone

Precision was calculated using % RSD; intra-day and inter-day % RSD of Finerenone was found to be 0.7 % and 1.1 %, respectively. Similarly, accuracy of the method was confirmed by recovery study from marketed formulation of Finerenone at three levels (50%-100%-150 %) of standard addition. Percentage recovery for Finerenone was found to be 100.68 % **(Table 6)**. LOD and LOQ was found to be 0.31 µg/mL and 0.95 µg/respectively. Robustness was determined using typical variations studied under these parameters such as flow rate, mobile phase composition and temperature. According to data comparison the developed method was estimated to be robust. Analysis of marketed formulation was studied and tested. Applicability of the proposed method was tested by analyzing the available tablet formulation Lyvelsa (10 mg). All validation parameters of Finerenone results have been presented in **Table 7**.

Table 6: Recovery Data for Finerenone

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
50%	10	10.07	100.73	100.68%
	10	10.09	100.89	
	10	10.04	100.36	
100%	20	20.01	100.06	
	20	20.15	100.74	
	20	19.97	99.86	
150%	30	30.24	100.81	
	30	30.38	101.27	
	30	30.43	101.44	

Table 7: Summary of Validation Parameters for RP-HPLC Method

Sr. No.	Parameters	Finerenone
1	Specificity	Specific
2	Linearity range	5-30 µg/mL
3	Regression line equation	y = 82244x +26134
4	Correlation co-efficient	0.9994
5	Precision(%RSD)	
	Intra-day precision	0.7 %
	Inter-day precision	1.1 %
6	% assay	99.96 %
7	LOD	0.31
8	LOQ	0.95

5. CONCLUSION

In conclusion, Statistical, analytical, and risk management methodologies are successfully used in the design, development, and production of Pharmaceuticals as part of the quality by design strategy, which attempts to guarantee the quality of medication. A description of the QbD approach's development of a validated RP-HPLC method has been given. With a greater understanding of the method variables and a lower risk of failure during method validation, the QbD approach to method development has proven beneficial. The mobile phase composition was tested via multiple repetitions to achieve optimal chromatographic conditions. Good resolution and symmetric peak morphologies of the analyte have been achieved by doing this. It was discovered that the RP-HPLC method for determining finerenone was straightforward, linear, accurate, and resilient; as such, it was a reliable and easy method that could be used in routine quality control testing in labs and businesses. The validation process revealed that every parameter fell inside the acceptable range. The method has been confirmed by validation parameters in accordance with the guidelines of ICH.

6. ACKNOWEDGEMENT:

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7. CONFLICT OF INTEREST: None

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