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Research Article

Implementing Quality by Design approach in Analytical Reverse Phase High Performance Liquid Chromatography Method Development and Validation for the Determination of Fedratinib

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ABSTRACT

A novel, accurate, precise, specific, sensitive, and robust reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the determination of Fedratinib using the analytical quality by design (AQbD) approach mentioned in International Council for Harmonisation (ICH) Q8 (R2) guidelines. By implementing QbD in HPLC methods, ruggedness and robustness will be verified early in the stage of method development to ensure the method's performance over the product's lifetime. Design Expert® (12.0.12.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for response surface methodology (RSM). Plackett-Burman design was employed for the factor screening studies to identify the critical method parameters (CMP) affecting the critical quality attributes (CQA). The selected CMP's were systematically optimized using Central-composite design (CCD). Statistical analysis of the responses was done by applying analysis of variance. Chromatographic separation was accomplished on Agilent C18 (150×4.6 mm, 5 µm) column and PDA-UV detection was set at 268 nm. The optimized and predicted data from Design Expert software consisted of mobile phase Acetonitrile: 0.1% OPA buffer pH 4.18 (43: 57% v/v), pumped at a flow rate of 0.967 mL/min gave the highest desirability of 1. The developed chromatographic method was validated as per ICH Q2 (R1) guidelines and found to be linear over a concentration range of 15–90 µg/mL with a correlation coefficient of 0.999. Degradation studies were performed by exposing the drug to various stress conditions as per ICH Q1A (R2) guidelines, and significant degradation was found in acidic conditions.

INTRODUCTION

Fedratinib (FDB), chemically known as N-tert-butyl-3-[[5-methyl-2-[4-(2-pyrrolidin-1-ylethoxy) anilino] pyrimidin-4-yl] amino] benzenesulfonamide is an antineoplastic agent used to treat intermediate-2 and high risk primary and secondary myelofibrosis in adult patients.[1] It is available under the brand name Inrebic and is an orally bioavailable Adenosine triphosphate (ATP)-competitive inhibitor of Janus-associated kinase 2 (JAK2) and FMS-like tyrosine kinase 3(FLT3) with potential antineoplastic activity.[2-3] Upon oral administration, FDB competes with wild and mutated forms of JAK2 for ATP binding and inhibits cell proliferation and induces apoptosis. The drug is soluble in Dimethylsulfoxide (DMSO) and ethanol.[4] The drug was developed by Celgene Corporation and granted Food and Drug Administration (FDA) approval on August 16, 2019.[5] The chemical structure of FDB was given in the Fig. 1.

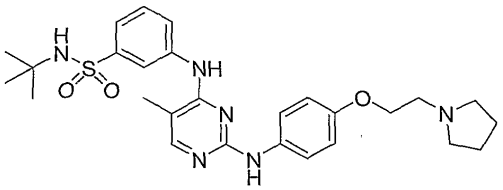

Fig. 1: Chemical structure of FDB

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An extensive literature survey disclosed that only Liquid chromatography-tandem mass spectrometry (LC-MS/MS) method[6] for estimating FDB in human plasma with pharmacokinetic study in healthy rabbits and phase studies[7-9] for determining the safety and efficacy of the drug were reported. No RP-HPLC methods were reported for the determination of FDB using AQbD approach. The traditional analytical method development is quite tedious and was based on one factor at a time (OFAT) approach, in which only one parameter is optimized to get the expected response while others remained constant. Though the OFAT approach is systematic, it is time-consuming. To eliminate the defects encountered during traditional method development, the systematic AQbD approach is considered, which uses good experimental designs, risk assessment, ruggedness and robustness testing.[10] Screening designs were used to identify the CMP's affecting the CQA's and the selected CMP's were optimized using CCD.[11] 2D contour and 3D surface plots were used for the geometrical representation of response variables plotted as a function of independent variables.[12] Statistical analysis of the results was done using analysis of variance (ANOVA). Predicted versus actual plots and normal plot of residuals were used for design validation. Optimization of the method was done by applying the Derringer's desirability functions approach.[13] Hence the present work is aimed at development and validation of RP-HPLC method for the determination of FDB using AQbD approach.

Materials and Method

Chemicals

The HPLC grade acetonitrile (ACN) and methanol were purchased from Fischer Scientific, HPLC grade water obtained from Merck milli-Q water purification unit. Orthophosphoric acid (OPA) was purchased from Merck India Pvt. Ltd, Mumbai, India. The other reagents used in this research were analytical grade. Active pharmaceutical ingredient (API) of FDB was obtained as a gift sample from BMR Pharma and Chemicals, Hyderabad, India.

Equipment

UV-VIS spectrophotometer (Shimadzu -1800, Japan) was used for the authentication of the drug sample. HPLC study was carried out on WATERS HPLC 2695 system with photodiode array (PDA) Detector. The software used is Empower 2 for HPLC method development and validation. Design Expert® (12.0.12.0) modeling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for RSM.

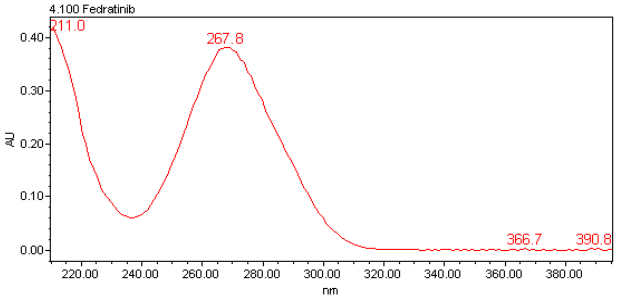

Fig. 2: UV Spectrum of FDB

Reagents and Solutions Preparation

Preparation of Buffer

0.1% Orthophosphoric acid: 1-mL of orthophosphoric acid diluted to 1000 mL with milli-Q water.

Preparation of Mobile Phase

Mobile phase was prepared using HPLC grade ACN and 0.1% OPA buffer pH 4.18 in 43: 57 ratio.

Preparation of Diluent

Diluent was prepared using ACN and milli-Q Water in 50:50 ratio.

Preparation of Standard Stock Solution

Accurately weighed, 100 mg of FDB was transferred to 100 mL volumetric flask, 3/4th of final volume was filled with diluent and sonicated to dissolve completely. Final volume was made up to 100 mL and labeled as a standard stock solution (1000 µg/mL of FDB). 0.6 mL of the above stock solution was pipetted into a 10 mL volumetric flask and made up to volume with diluent to get 60 µg/mL, and this concentration was used for further study.

Preparation of Sample Solution

The synthetic mixture was prepared by mixing 100 mg of FDB, 150 mg of microcrystalline cellulose, and 5 mg of sodium stearyl fumarate. The amount of drug equivalent to 10 mg was transferred to 10 mL clean dry volumetric flask, and a diluent was added to dissolve the drug and sonicated for 30 min. Then the volume was made up to the mark with diluent to get standard stock solution with a concentration of 1000 µg/mL of FDB. Then it was filtered through 0.45 µm membrane filter. Further 0.6 mL of above solution was pipetted into 10 mL volumetric flask and diluted up to the mark with diluent to get 60 µg/mL.

Preparation of Reagents for Stress Studies

1N HCl: 85 mL of hydrochloric acid diluted to 1000 mL with milli-Q water.

1N NaOH: 4 gm of NaOH dissolved in 1000 mL of milli-Q water.

20% H_2O_2: 33.32 mL of 30% H_2O_2 diluted to 50 mL with milli-Q water.

Method Development

Selection of Detection Wavelength

60 µg/mL concentration of FDB was prepared using ACN, and UV spectrum was recorded. The absorption maxima were found to be 267.8 nm, as shown in Fig. 2.

Screening Design for the Selection of CQA's

Plackett Burman's design (PBD) was employed for the factor screening studies to identify the CMP's affecting the CQA's. The selected screening design resulted in 12 trial runs suggesting various combinations for the factors chosen.

Optimization by RSM

Different types of response surface designs are used for optimization like CCD, Box-Behnken design (BBD), and Doehlert. The CMP's selected in the screening study were systematically optimized using CCD and is preferred over other designs because it contains points at the extremes of the cubic region and provides five levels for each chosen factor.

Method Validation

The final optimized analytical method was validated as per the ICH Q2 (R1) guidelines for system suitability, specificity, linearity, accuracy, precision, the limit of detection (LoD), the limit of quantitation (LoQ), and robustness.[14]

Linearity

The standard calibration curve was generated with six different concentrations over 15–90µg/mL. A linear calibration curve was generated between the mean peak area and drug concentration. The linearity was examined using linear regression, which was calculated by the least square regression method.

Accuracy

Accuracy was carried out by adding known amount of standard to the sample solution at 50, 100, 150% levels in triplicate and samples were analysed by the optimized method. Percentage recovery was calculated.

Precision

The precision of the optimized method was determined by studying the intermediate precision and repeatability. Six standard working solutions of 60 µg/mL are injected on the same day and next day of the preparation of samples, and the % RSD of the peak area was calculated.

Limits of Detection and Quantitation

LoD and LoQ were determined from the signal-to-noise ratio. The detection limit refers to the lowest concentration level resulting in a peak area of three times the baseline noise. The quantification limit refers to the lowest concentration level that provided a peak area with a signal-to-noise ratio higher than ten.

Robustness

Small deliberate changes in the method were made like flow rate (0.86–1.06 mL/min), the proportion of organic composition in the mobile phase (38–48%), and wavelength (263–273 nm). % RSD of the above conditions was calculated.

System Suitability

The system suitability was determined by taking six replicates of the drug at same concentration of 60 µg/mL. The acceptance criteria were ± 2% for the percent coefficient of variation (% CV) for the peak area, retention time of drug, USP plate count, and asymmetry.

Forced Degradation Studies[15-16]

Acid Hydrolysis

To 1-mL of stock solution, 1 mL of 1N HCl solution was added, and the degradation sample was kept for reflux in radley apparatus (Veego) with continuous stirring at 60°C for 30 minutes. The sample was neutralized with 1N NaOH, diluted to 60 µg/mL with mobile phase, and analyzed using HPLC system.

Base Hydrolysis

To 1-mL of stock solution, 1 mL of 1N NaOH solution was added, and the degradation sample was kept for reflux in radley apparatus with continuous stirring at 60°C for 30 minutes. The sample was neutralized with 1N HCl, diluted to 60 µg/mL with mobile phase, and analyzed using HPLC system.

Neutral Hydrolysis

1 mL of stock solution was diluted to 10 mL with HPLC grade water and the degradation sample was placed for reflux in radley apparatus with continuous stirring at 60°C for 30 minutes, diluted to 60 µg/mL with mobile phase and analyzed using HPLC system.

Oxidative Study

To 1-mL of stock solution, 1-mL of 20% H_2O_2 solution was added, and the degradation sample was kept in the dark area without disturbance at room temperature for 4 hours. The sample was diluted to 60 µg/mL with mobile phase and analyzed using HPLC system.

Thermal Degradation

100 mg of FDB was taken in a petri dish and placed in a hot air oven at 70°C for 60 minutes. The sample was diluted to 60 µg/mL with mobile phase and analyzed using HPLC system.

Photo Degradation

100 mg of FDB was uniformly spread in a petri dish and was exposed to UV light by placing in UV chamber for 24 hrs. The sample was diluted to 60 µg/mL with mobile phase and analysed using HPLC system.

Results and Discussion

Screening Design for Selecting the Critical Method Parameters

A five-factor twelve-run PBD was employed for the factor screening studies to identify the CMP's affecting the CQA's

(Retention time, theoretical plates, and tailing factor). The stationary phases selected were C8 and C18. Methanol and acetonitrile were chosen as organic solvents since they were most commonly used in RP-HPLC. Since the drug has high solubility at low pH the buffer selected was 0.1% OPA at pH 3 and 6. The factors and the levels selected for the screening design were given in Table 1.

The selected screening design resulted in 12 trial runs suggesting various combinations for the chosen factors presented in Table 2. The responses selected were retention time, theoretical plates, and tailing factor. The above factors were optimized using design expert software.

The responses obtained after carrying out the trial runs were fed back to the Design Expert software, and the pareto chart analysis[17] of the three responses was done, represented in Figs. 3 to 5. In the pareto chart analysis, the blue color represents the negative effect, and the light brown color represents the positive effect on responses.

From Fig. 3, it was observed that factors like buffer pH, % organic composition, and organic modifier ranked first, second and third, respectively, for retention time.

From Fig. 4, it was observed that factors like buffer pH, organic modifier, and % organic composition ranked first, second and third, respectively for theoretical plates.

From Fig. 5, it was observed that factors like flow rate, column, and buffer pH ranked first, second, and third, respectively, for the tailing factor.

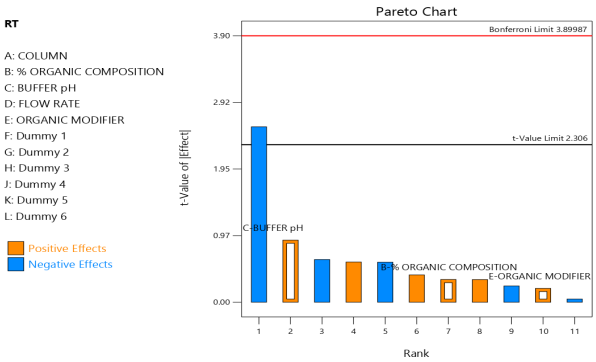

Fig. 3: Illustration showing the pareto chart ranking order of selected factors on RT for FDB

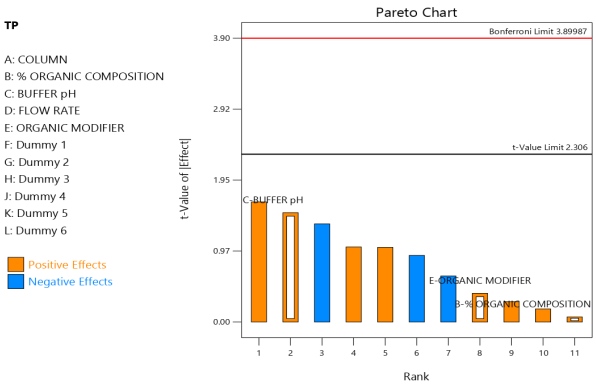

Fig. 4: Illustration showing the pareto chart ranking order of selected factors on TP for FDB

Table 1: Factors and levels selected for Plackett Burman design of FDB

Factor	*Name*	*Units*	*Type*	*Low level*	*High level*
A	Column	-	Categoric	C8	C18
B	% Organic composition	%v/v	Numeric	30	50
C	Buffer pH	-	Numeric	3	6
D	Flow rate	mL/min	Numeric	0.8	1.0
E	Organic modifier	-	Categoric	Methanol	ACN

Table 2: Trial runs with responses for Plackett Burman design of FDB

Trial Run	*Factor A*	*Factor B*	*Factor C*	*Factor D*	*Factor E*	*Response 1 (RT)*	*Response 2 (TP)*	*Response 3 (TF)*
1	C18	50	3	1	ACN	2.741	4189.1	1.2
2	C8	50	3	1	ACN	2.722	694.5	1.4
3	C8	50	6	0.8	ACN	5.4	2916.2	1.4
4	C18	30	6	1	Methanol	3.435	4154.3	1.5
5	C8	30	6	0.8	ACN	4.174	2878	1.3
6	C18	50	6	0.8	Methanol	4.042	2978.4	1.5
7	C8	50	6	1	Methanol	3.08	2359.3	1.4
8	C18	30	6	1	ACN	2.889	5152.2	1.1
9	C8	30	3	0.8	Methanol	3.94	1884.1	0.9
10	C18	50	3	0.8	Methanol	4.121	4165.9	1.3
11	C8	30	3	1	Methanol	2.64	3323.3	1.4
12	C18	30	3	0.8	ACN	3.973	2934	1.0

RT: Retention time, TP: Theoretical plates, TF: Tailing factor

Conclusion: From the PBD, based on the overall pareto chart ranking analysis of selected factors on three responses, the CMP's most affecting the CQA's considered for the optimization study included flow rate, % organic content in the mobile phase, and buffer pH. The others factors like column and organic modifier were fixed at constant levels. Based on the results shown in Table 2, the C18 column and ACN were selected at which theoretical plates are more, and the tailing factor is less compared to C8 column and methanol as organic modifiers.

Optimization by Response Surface Methodology-CCD

AQbD method involves identifying CMP's and CQA's with risk assessment and generating design space. In the present study, CMP's selected were flow rate, % organic content in the mobile phase, and pH of the buffer. The CQA's selected were retention time, theoretical plates, and tailing factor. So CCD was used to optimize these parameters, which were varied over five levels. Different ranges of three parameters 23.12–56.82% acetonitrile, the flow rate of 0.73–1.07 mL/min, and pH of the buffer 1.98–7.02 were taken shown in Table 3.

A 3-factor 5-level CCD design was established. This study design of 20 experimental runs was generated and performed, and the obtained results of CQA's were analyzed by Design-expert software shown in Table 4.

Statistical Analysis of CCD Experimental Data by Design-Expert software

Based on the effects of three factors on responses and evaluation of these results, it was feasible to elaborate mathematical models that have been endeavored to determine the relationship between factors and responses. The significance of the models generated for the three responses retention time, theoretical plates, and tailing factor were studied by applying the ANOVA,[18] as shown in Tables 5-7.

From the ANOVA Table 5 for retention time, the Model F-value of 4.90 implies the model was significant. There was only a 1.33% chance that an F-value this large could occur due to noise. *p*-values less than 0.05 indicate model terms are significant. In this case B, C are significant model terms. The lack of fit was insignificant, with a *p*-value of 0.062. To study the effect of significant terms B and C on RT, 2D contour plot was analyzed using Design Expert® software. The regions shaded in dark blue represented lower values, and shaded in dark red represents higher values. The regions shaded in light blue, green, and yellow represents intermediate values.

From the above 2D contour plot shown in Fig. 6, it was found that at a higher organic phase content and lower pH the value of retention time was less.

From the ANOVA Table 6 for theoretical plates, the model F-value of 28.97 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. *p*-values less than 0.05 indicate

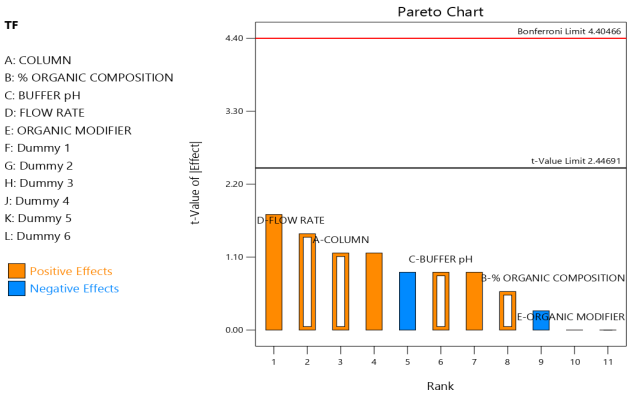

Fig. 5: Illustration showing the pareto chart ranking order of selected factors on TF for FDB

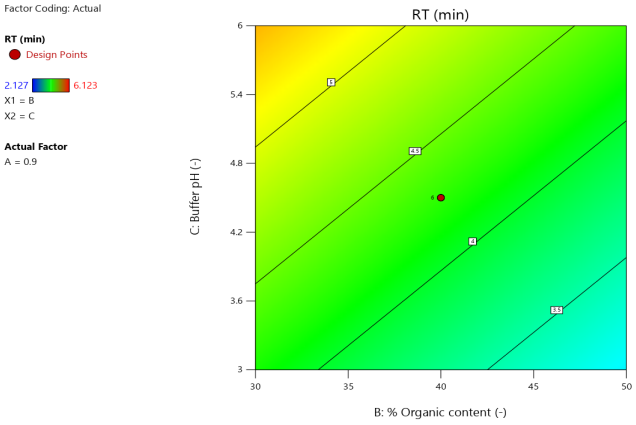

Fig. 6: 2D contour plot of retention time as a function of % organic content in mobile phase and buffer pH for FDB

Table 3: Design summary of CCD for FDB

Design Summary							
File version: DX 12.0.12.0 Study Type: Response surface Design Type: CCD Subtype: Randomized					CQA: Retention time, theoretical plates, tailing factor Runs: 20 Design model: Quadratic		
CMP/Factor	*Unit*	*Type*	*Min.*	*Max.*	*Coded low*	*Coded high*	*Mean*
A-Flow rate	mL/min	Numeric	0.73	1.07	-1 ↔ 0.80	+1 ↔ 1.0	0.90
B- % Organic content in mobile phase	%v/v	Numeric	23.12	56.82	-1 ↔ 30.00	+1 ↔ 50.00	40.00
C- Buffer pH	-	Numeric	1.97	7.02	-1 ↔ 3.0	+1 ↔ 6.0	4.50

model terms are significant. In this case B, C, BC, A^2, B^2, C^2 are significant model terms. The lack of fit was insignificant, with a *p*-value of 0.075. To study the effect of significant terms on TP, 2D contour plot was analysed using Design Expert® software.

From the above 2D contour plots shown in Fig. 7, it was found that at a higher organic content, lower pH, and intermediate flow rate, the value of theoretical plates is more.

From the ANOVA Table 7 for the tailing factor, the Model F-value of 15.24 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise. *P*-values less than 0.05 indicate model terms are significant. In this case, A, B, C are significant model terms. The lack of fit was insignificant, with a *p*-value of 0.059. To study the effect of significant terms on TF, 2D contour plot was analyzed using Design Expert® software.

From the above 2D contour plot shown in Fig. 8, it was found that at a higher organic phase content, lower pH,

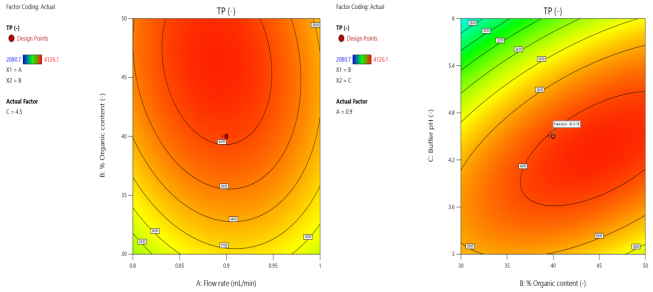

Fig. 7: 2D contour plots of theoretical plates as a function of % organic content in the mobile phase and buffer pH for FDB

Table 4: Central-composite experimental design matrix with responses for FDB

Run	*Flow rate (mL/min)*	*%Organic content in mobile phase*	*Buffer pH*	*Response 1(RT) (min)*	*Response 2(TP)*	*Response 3(TF)*
1	0.9	23.18	4.5	5.967	3099.2	1.1
2	0.9	40	4.5	4.876	4053.9	1.1
3	1.06	40	4.5	4.257	3363.2	1
4	0.9	40	4.5	4.874	3998.3	1.1
5	0.8	30	6	4.006	2436.9	1.1
6	0.9	40	7.02	6.123	2080.7	1.1
7	1	30	6	4.193	2606.7	0.9
8	0.9	40	4.5	4.862	4091.2	1.1
9	0.9	56.81	4.5	2.321	4126.1	1.2
10	0.9	40	4.5	4.863	3957.6	1.1
11	1	50	3	3.14	3008.5	1
12	0.8	50	6	3.986	3334.3	1.1
13	0.8	50	3	3.446	3257.8	1.3
14	0.9	40	4.5	4.841	4035.4	1.1
15	0.9	40	4.5	4.825	3937.9	1.1
16	0.73	40	4.5	5.287	3459.1	1.2
17	1	30	3	3.611	3581.3	1
18	0.8	30	3	3.922	3515.8	1.2
19	1	50	6	3.805	3376.8	1.1
20	0.9	40	1.97	2.127	3254.6	1.2

RT: Retention time, TP: Theoretical plates, TF: Tailing factor

Table 5: *ANOVA for a retention time of FDB*

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value*	*Inference*
Model	9.91	3	3.30	4.90	0.0133	significant
A- Flow rate	0.4021	1	0.4021	0.5968	0.4511	-
B- %Organic content in mobile phase	4.10	1	4.10	6.09	0.0252	significant
C-Buffer pH	5.40	1	5.40	8.02	0.0120	significant
Residual	10.78	16	0.6737	-	-	-

df: degrees of freedom, F: Fischer's ratio, *p*: Probability value

and higher flow rate, the value of the tailing factor was less.

From the fit statistical parameters obtained from ANOVA given in Table 8, it was found that the predicted R^2 values of retention time 0.765, theoretical plates 0.734, and tailing factor 0.818 were in reasonable agreement with the adjusted R^2 values of 0.781, 0.929, and 0.894 respectively i.e., the difference was less than 0.2. Adequate precision measures the signal-to-noise ratio. Therefore, a ratio greater than four is desirable, and the obtained values were 7.34, 16.64, and 13.12 for the responses RT, TP, and TF, respectively indicate an adequate signal, and these models can be used to navigate the design space.

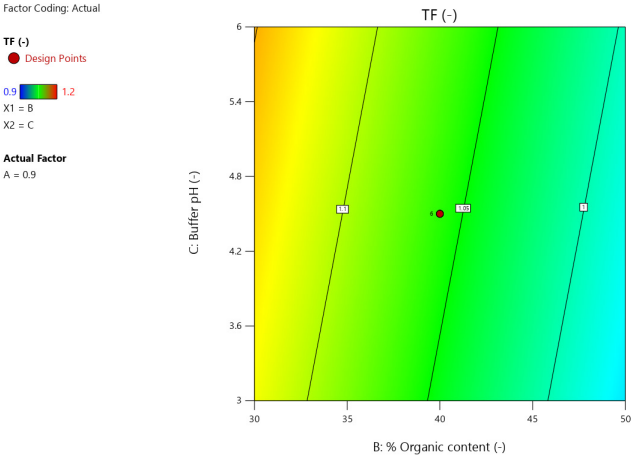

Fig. 8: 2D contour plot of tailing factor as a function of % organic content in mobile phase and buffer pH for FDB

Design Validation

From the normal plot of studentized residuals[19] for the three responses shown in Fig. 9, it was observed that the

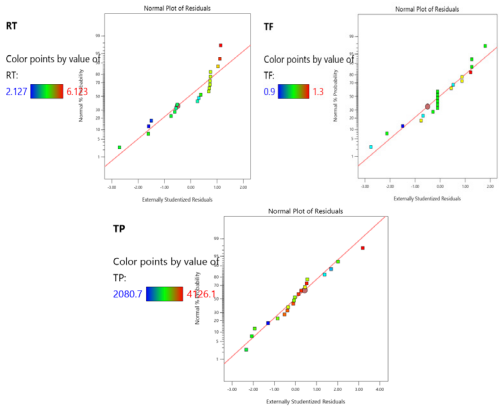

Fig. 9: Normal plot of studentized residuals of RT, TP and TF for FDB

Table 6: ANOVA for theoretical plates of FDB

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value*	*Inference*
Model	6.180E+05	9	6.866E+05	28.97	< 0.0001	significant
A- Flow rate	1.291E+05	1	1.291E+05	0.0545	0.8202	-
B- %Organic content in mobile phase	4.183E+05	1	4.183E+05	20.31	0.0011	significant
C-Buffer pH	9.400E+05	1	9.400E+05	39.66	< 0.0001	significant
AB	2.443E+05	1	2.443E+05	1.03	0.3339	-
AC	1.961E+05	1	1.961E+05	0.827	0.3844	-
BC	7.802E+05	1	7.802E+05	32.92	0.0002	significant
A^2	7.083E+05	1	7.083E+05	29.89	0.0003	significant
B^2	3.263E+05	1	3.263E+05	13.77	0.0040	significant
C^2	3.384E+05	1	3.384E+05	142.78	< 0.0001	significant
Residual	2.370E+05	10	2.569E+05	-	-	-

df: degrees of freedom, F: Fischer's ratio, *p*: Probability value

Table 7: ANOVA for tailing factor of FDB

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	*Sum of Squares*	*df*	*Mean Square*	*F Value*	*p-value*	*Inference*
Model	0.1107	3	0.0369	15.24	< 0.0001	significant
A- Flow rate	0.0786	1	0.0786	32.47	< 0.0001	significant
B- %Organic content in mobile phase	0.0160	1	0.0160	6.63	0.0204	significant
C-Buffer pH	0.0160	1	0.0160	6.63	0.0204	significant
Residual	0.0388	16	0.0024	-	-	-

df: degrees of freedom, F: Fischer's ratio, *p*: Probability value

selected models for the respective responses were suitable for the selected design as these plots indicated a straight line. It was further evidenced from the ANOVA Tables 5-7 that the selected models were significant with $p < 0.05$.

Optimization of the Method by Desirability Functions Approach

The optimized chromatographic conditions selected based on the desirability functions approach were mobile phase consisting of ACN: 0.1% OPA buffer pH 4.18 (42.9: 57.1 % v/v) pumped at a flow rate of 0.967 mL/min gave the highest desirability of 1 shown in Fig. 10. In the overlay contour plot shown in Fig. 11, the flag represents the optimized combination of the three selected independent factors, which gave the maximum desirability. To confirm these optimum conditions, three replicate injections of 60 µg/mL FDB were analyzed to determine if their observed responses were within the predicted range as shown in Table 9 and the corresponding optimized chromatogram as shown in Fig. 12.

Optimized Chromatographic Conditions

Column: Agilent C18 (150×4.6 mm, 5 µm)
Mobile phase: ACN: 0.1% OPA buffer pH 4.18 (42.9: 57.1 %v/v)
Buffer pH: 4.18
Flow rate: 0.967 mL/min
Wavelength: PDA-UV detection at 268 nm
Column temperature: Ambient
Injection volume: 10 µL
Run time: 9 min

Method Validation

The developed method was linear over the concentration range of 15–90 µg/mL with a correlation coefficient of 0.999 shown in Fig. 13. For the accuracy studies at 50, 100 and 150% levels, the % recovery of the drug was found to be within 98-102%. Intermediate precision and repeatability were carried out, and the % RSD values were less than 2%. LoD and LoQ values were found to be 0.081 µg/mL and 0.245 µg/mL. The robustness of the developed method was checked by making minor changes in the experimental conditions like flow rate, %organic composition, wavelength, and %RSD values for the peak area were found to be less than 2%. From the system suitability tests, the number of theoretical plates was found to be more than 2000, and the tailing factor was found to be less than 2. The summary of the method validation parameters was shown in Table 10.

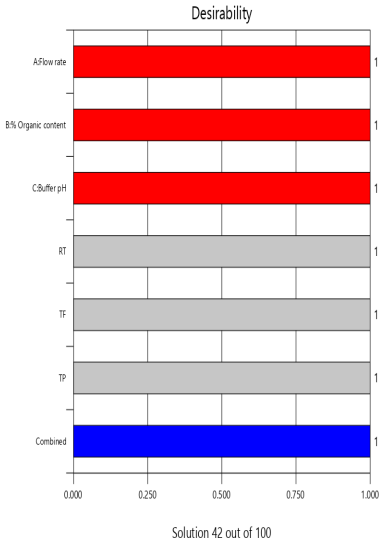

Fig. 10: Optimization by Desirability function for FDB

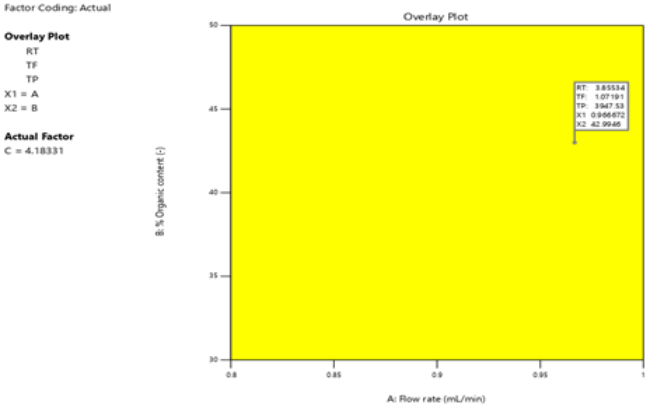

Fig. 11: Overlay contour plot supported by responses for FDB

Table 8: Fit statistical parameters of responses obtained from ANOVA for FDB

Response & Model	*Mean*	*SD*	*%CV*	*Press value*	*R^2*	*Adjust-ed R^2*	*Predic-ted R^2*	*Adequate precision*
Retention time, Linear	4.27	0.8208	19.24	0.183	0.889	0.781	0.765	7.34
Theoretical plates, Quadratic	3428.76	153.95	4.49	1.701E+06	0.963	0.929	0.734	16.64
Tailing factor, Linear	1.11	0.0492	4.45	0.072	0.940	0.894	0.818	13.12

SD: Standard deviation, CV: Coefficient of variation, R^2: Coefficient of regression

Table 9: Responses of the optimized method for FDB

S.No.	*Response variables*	*Predicted value*	*Actual value*	*Desirable range*
1	Retention time (min)	3.855	3.982	2.036–5.673
2	Theoretical plates	3947.52	4256	3577.09–4317.94
3	Tailing factor	1.07	1.15	0.962–1.180

Table 10: Results of the validation parameters

S.No.	*Parameter*		*Results*
1	Linearity	Linearity range(µg/mL)	15-90
		Correlation coefficient	0.999
		Regression equation	y = 58259x + 12656
2	Accuracy (% recovery)	50, 100, and 150% levels	Between 99.97-100.89
3	Precision(% RSD of peak area)	Intermediate precision	0.835
		Repeatability	0.707
4	Sensitivity	LOD(µg/mL)	0.081
		LOQ(µg/mL)	0.245
5	Robustness(% RSD of peak area)	Flow rate (±0.1 mL/min)	0.7
		Organic phase (± 5%)	0.8
		Wavelength(± 5 nm)	0.7
6	System suitability	Retention time(min)	3.976
		Tailing factor	1.14
		Plate count	4187

Table 11: Results of forced degradation studies for FDB

Stress condition	*% Drug degraded*	*Purity angle*	*Purity threshold*	*Pass/Fail*
Control	--	0.521	0.595	Pass
Acidic (1N HCl, 60^{0}C, 30 min)	5.40	0.546	0.651	Pass
Alkali (1N NaOH,60^{0}C, 30 min)	4.66	0.692	0.702	Pass
Neutral (H_2O, 60^{0}C, 30 min)	0.50	0.515	0.529	Pass
Oxidative (20% H_2O_2, RT, 30 min)	3.08	0.605	0.712	Pass
UV light (24 hrs)	1.28	0.533	0.560	Pass
Thermal(70^{0}C, 24 hrs)	1.95	0.505	0.527	Pass

Acceptance criteria: % Degradation should be NMT 20% according to ICH guidelines.

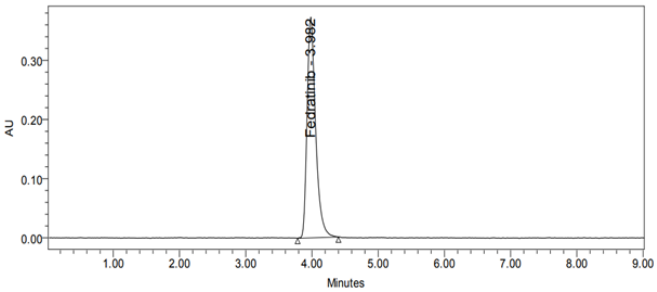

Fig. 12: Chromatogram of the optimized method for FDB

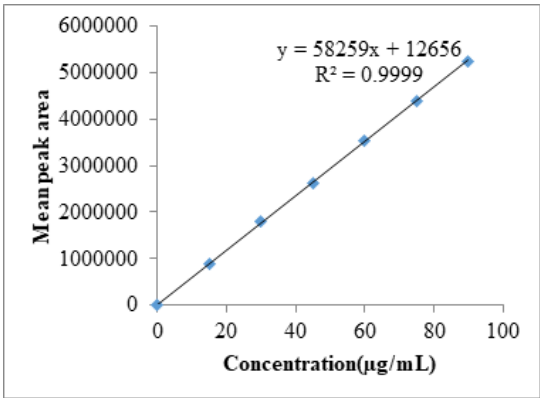

Fig. 13: Linearity curve of FDB

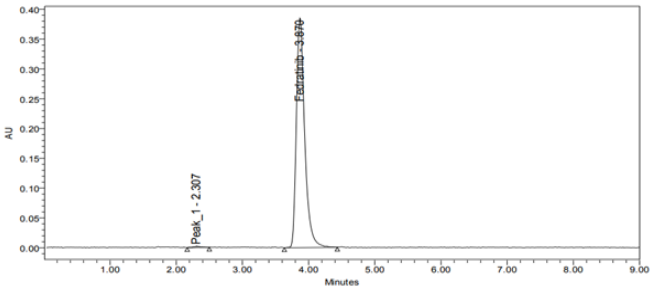

Fig. 14: Chromatogram of stability studies under acidic condition for FDB

Forced Degradation Studies

Forced degradation studies of FDB in various conditions like acidic, basic, peroxide, thermal, photolytic and hydrolytic were performed. The drug showed significant degradation in the acidic condition represented in Fig. 14. Results of forced degradation studies were presented in Table 11.

Conclusion

A simple, accurate, precise, specific, and robust RP-HPLC method was developed to determine FDB by using the Quality by Design approach. PBD was employed for the

factor screening studies to identify the CMP's affecting the CQA's and optimization was done using Central-composite design. The critical method parameters selected for optimization were % organic content in the mobile phase, flow rate, and buffer pH. The critical quality attributes are retention time, theoretical plates, and tailing factor. Optimized chromatographic conditions suggested by the desirability functions approach consisted of mobile phase Acetonitrile: 0.1% OPA buffer pH 4.18 (42.9: 57.1 % v/v) pumped at a flow rate 0.967mL /min gave the highest desirability of one. The retention time of the drug was found to be 3.982 minutes. Theoretical plates and tailing factors were found to be within limits. The developed method was validated as per ICH Q2 (R1) guidelines. The utilization of RSM provides better insight for method development and robustness testing. Degradation studies were performed in various stress conditions, and the drug was found to be degraded more in acidic conditions.

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References

1. Blair HA. Fedratinib: First Approval. Drugs. 2019;79:1719-1725.
2. Talpaz M, Kiladjian JJ. Fedratinib, a newly approved treatment for patients with myeloproliferative neoplasm-associated myelofibrosis. Leukemia (2020). https://doi.org/10.1038/s41375-020-0954-2.
3. Jamieson C, Hasserjian R, Gotlib J, Cortes J, Stone R, Talpaz M *et al*. Effect of treatment with a JAK2-selective inhibitor, fedratinib, on bone marrow fibrosis in patients with myelofibrosis. J Transl Med. 2015; 13:294.
4. Pardanani A, Harrison C, Cortes JE. Safety and efficacy of fedratinib in patients with primary or secondary myelofibrosis: a randomized clinical trial. JAMA Oncol. 2015; 1(5):643-651.
5. Celgene Corporation. US FDA approves INREBIC® (fedratinib) as first new treatment in nearly a decade for patients with myelofibrosis [media release]; August 16 2019. http://ir.celgene.com.
6. Ayesha Begum K, Shiva Kumar G, Sandhya P, Bhikshapathi DVRN. A Highly Sensitive LC–MS/MS Method Development and Validation of Fedratinib in Human Plasma and Pharmacokinetic Evaluation in Healthy Rabbits. Curr. Pharm. Anal. 2020; 16: 1. https://doi.org/10.2174/1573412916999200512121023.
7. Pardanani A, Tefferi A, Jamieson C, Gabrail NY, Lebedinsky C, Gao G *et al*. A phase 2 randomized dose-ranging study of the JAK2-selective inhibitor fedratinib (SAR302503) in patients with myelofibrosis. Blood Cancer Journal. 2015;5:e335. https://doi.org/10.1038/bcj.2015.63.
8. Zhang M, Xu CR, Shamiyeh E, Feng Liu, Jian YY, Smith WB *et al*. A randomized, placebo-controlled study of the pharmacokinetics, pharmacodynamics, and tolerability of the oral JAK2 inhibitor fedratinib (SAR302503) in healthy volunteers. J Clin Pharmacol. 2014;54(4):415-421.
9. Harrison CN, Schaap N, Vannucchi AM, Kiladjian JJ, Ramon VT, Zachee P *et al*. Janus kinase-2 inhibitor fedratinib in patients with myelofibrosis previously treated with ruxolitinib (JAKARTA-2): a single-arm, open-label, non-randomised, phase 2, multicentre study. Lancet Haematol. 2017;4(7):e317-324.
10. Nishendu PN, Rakshit VK, Vidhi NK, Patel PB. Quality by Design: A complete review. Int J Pharm Sci Res. 2012;17(2):20-28.
11. Sahu PK, Ramisetti NR, Cecchi T, Swain S, Patro CS, Panda J. An Overview of Experimental Designs in HPLC Method Development and Validation. J Pharm Biomed Anal. 2018; 147: 590-611.
12. Politis SN, Colombo P, Colombo G, Rekkas DM. Design of experiments (DoE) in pharmaceutical development. Drug Develop Ind Pharm. 2017; 43(6):889-901.
13. Candioti LV, De Zan MM, Camara MS, Goichoechea HC. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta. 2014; 124:123-138.
14. ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2 (R1), current Step 4 version; International Conference on Harmonisation: Geneva, 2005.
15. ICH Harmonised Tripartite Guideline: Stability Testing of New Drug Substances and Products Q1A (R2), current Step 4 version; International Conference on Harmonisation: Geneva, 2003.
16. ICH Harmonised Tripartite Guideline: Photostability testing of New Drug Substances and Products Q1B, current Step 4 version; International Conference on Harmonisation: Geneva, 2003.
17. Suhkbir Singh, Yash Paul Singla, Sandeep Arora. Statistical, Diagnostic and Response surface analysis of Nefopam Hydrochloride Nanospheres using 3^5 Box-Behnken design. Int J Pharm Pharm Sci. 2015; 7(10): 89-101.
18. Montgomery DC. Design and analysis of experiments. Edn 7, John Wiley and Sons, New York, 2008, pp. 387-389.
19. Geoff Vining. Technical advice: Residual plots to check assumptions. Quality Engineering. 2011; 23(1):105-110.

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