RESEARCH ARTICLE

QbD Driven Approach for Method Development and Validation for The Determination of Gilteritinib By RP-HPLC

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ABSTRACT
Quality by Design (QbD) assisted approach was used for development of robust and rugged RP-HPLC method and validated as per ICH guidelines. Methods developed using QbD approach are highly robust, cost effective, uses good experimental designs, have shorter run times, optimization can be done by statistical analysis and can be easily validated compared with the traditional methods developed by one-factor-at-a-time (OFAT) approach. Central-composite design (CCD) under Response Surface Methodology (RSM) was used for development of optimized method based on desirability functions approach. The factors selected for the present study were % organic composition in mobile phase, column temperature, flow rate and responses investigated were retention time of the drug and theoretical plate count. Chromatographic separation was achieved using Phenomenex C18 (150 mm x 4.6 mm, 5µ) column. Statistical analysis of CCD experimental data was done by applying ANOVA and the selected mathematical models for the responses were found to be significant with $p < 0.05$. The optimized conditions based on highest desirability value of 1 was achieved using Acetonitrile: Phosphate buffer (42.1: 57.9% v/v) mobile phase pumped at 0.93ml/min flow rate at 31.7^{0}C. The drug eluted at a retention time of 2.655 min. Finally the developed method was validated according to ICH Q2 (R1) guidelines. All the system suitability parameters were found to be within the limits. Forced degradation studies were performed according to ICH guidelines with notable degradation found in acidic condition.

Keywords: AQbD, CCD, Gilteritinib, Desirability function, ANOVA.
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INTRODUCTION

Gilteritinib (GTB) available under the brand name Xospata is an antineoplastic agent used in the treatment of acute myeloid leukemia (AML) with FMS-like tyrosine kinase 3 (FLT3) mutations.[1] It is an orally available small molecule inhibitor of FLT3 and it inhibits both wild and mutated forms of FLT3, AXL and ALK (Anaplastic lymphoma kinase) - mediated signal transduction pathways and reduces cancer cell proliferation.[2] All these three Receptor tyrosine kinases play a key role in cancer cell growth and survival. AML is a cancer that impacts the blood and bone marrow with a rapid progression and this condition produces lower number of normal blood cells which requires continuous need for blood transfusion.[3] The drug is soluble in organic solvents such as ethanol, DMSO and dimethyl formamide (DMF). The chemical structure of GTB was shown in figure 1.

The advantage in implementing QbD was ruggedness and robustness can be tested priorly in the method development stage rather than in validation part. Otherwise, if the concept of QbD is not adapted, significant time and resources are required to redevelop and revalidate robust and rugged method.[4] RSM represent superior category of Design of Experiments (DoE) where the factors are studied at more than 2 levels, and in order to determine the experimental error the replicated center points are included in the experimental domain.[5] CCD uses 5 levels for each input factor with reduced number of experiments when compared to other optimization designs.[6] Optimization of the chromatographic conditions can be done using Analysis of variance (ANOVA), multiple regression analysis and desirability functions approach. Factor-response relationships can be described using mathematical models, where optimal responses can be predicted.[7]

Extensive literature review reveals bioanalytical method based on UPLC-MS/MS[8] technique and HPLC method[9] for the determination of drug were reported. But no QbD approach has been reported for the determination of selected drug by RP-HPLC. Hence the objective of the current work was to develop and validate a novel Reverse

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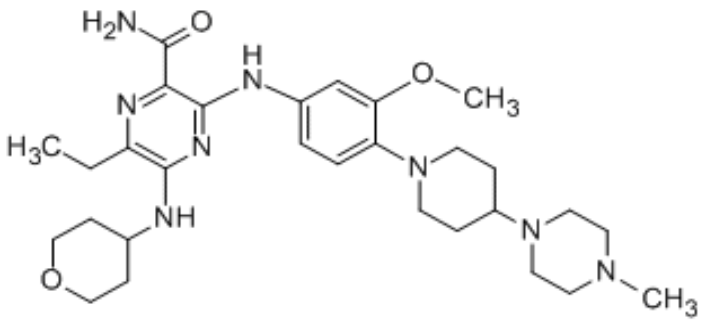

Figure 1: Chemical structure of GTB

phase-HPLC method for the estimation of Gilteritinib employing QbD principles.

MATERIALS AND METHOD

Authentication of drug was done using FT-IR/ATR (BRUKER ALFA) and UV-VIS spectrophotometer (Shimadzu -1800). Chromatographic separation was achieved on WATERS HPLC 2695 system with PDA

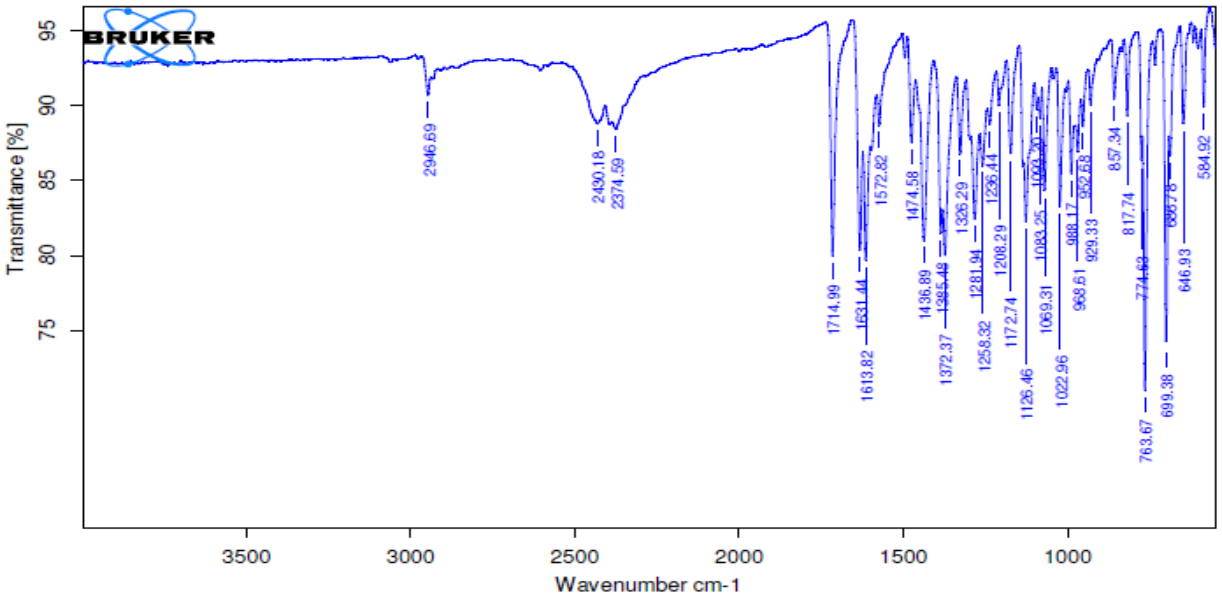

Figure 2: FT-IR Spectrum of GTB

Chemicals

HPLC grade ACN, Methanol and water were used. AR grade KH_2PO_4 and ortho phosphoric acid (OPA) were purchased from Merck India Pvt. Ltd. Spectrum laboratories, Hyderabad, India provided the gift sample of Gilteritinib for the present study.

Equipment

Detector equipped with Empower 2 software. Design Expert® (11.1.0.1) modelling software was used.

Preparation of reagents and solutions

Buffers preparation

Potassium dihydrogen ortho phosphate (0.01N): 1.36 g of precisely weighed Potassium dihyrogen ortho phosphate was added to a 1L volumetric flask and fill it to the brim

Table 1: Design summary of CCD for GTB

File version: DX 11.1.0.1	CQA: Retention time, Theoretical plates
Study Type: Response surface	Runs: 20
Design Type: CCD	Design model: Quadratic
Subtype: Randomized	

CMP	Unit	Type	Min.	Max.	Coded low	Coded High	Mean
A-% Organic content	%v/v	Numeric	29.91	50.09	-1 ↔ 34.00	+1 ↔ 46.00	40.00
B-Flow rate	mL/min	Numeric	0.8318	1.17	-1 ↔ 0.90	+1 ↔ 1.10	1.0000
C-Column temperature	^{0}C	Numeric	24.95	35.05	-1 ↔ 27.00	+1 ↔ 33.00	30.00

Table 2: Central-Composite experimental design matrix with responses for GTB

Run	Factor A(MP) %v/v	Factor B(FR) mL/min	Factor C(Temp) ^{0}C	Response 1(RT) (min)	Response 2(TP)
1	40	1	35.0454	2.516	3150.8
2	40	1	30	2.469	4899.3
3	40	1.16818	30	1.738	3041.2
4	40	1	24.9546	2.513	3339.4
5	40	1	30	2.421	4861.1
6	29.9092	1	30	3.252	3989.5
7	40	1	30	2.448	4621.4
8	46	0.9	27	2.57	3616.2
9	34	0.9	33	3.13	2547.1
10	40	1	30	2.423	4818.7
11	46	1.1	27	2.092	3447.5
12	34	1.1	27	2.563	3527.1
13	46	0.9	33	2.566	3994.9
14	40	1	30	2.434	4869.9
15	40	1	30	2.443	4734.7
16	34	0.9	27	3.143	2223.2
17	40	0.831821	30	3.106	2943.3
18	46	1.1	33	2.098	3810.4
19	34	1.1	33	2.598	3728.2
20	50.0908	1	30	2.229	4075.6

FR: Flow rate, MP: % Organic content in mobile phase, Temp: Temperature,
RT: Retention time, TP: Theoretical plates

using milli-Q water. The solution's pH was found to be 4.8. Using solutions of OPA and triethylamine, the pH was further adjusted.

0.1% OPA: 1 mL of OPA made upto 1L with milli-Q water.

Mobile phase

HPLC grade ACN and Phosphate buffer pH 4.4 in 40:60 ratio.

Standard stock solution preparation

To obtain 1000 µg/ml, 100 mg of GTB that had been precisely weighed was put into a 100 ml dry and clean

Table 3: ANOVA for RT of GTB

ANOVA for Response Surface Quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value	Inference
Model	2.66	9	0.2955	35.21	< 0.0001	Significant
A-MP	1.07	1	1.07	127.87	< 0.0001	Significant
B-FR	1.39	1	1.39	165.75	< 0.0001	Significant
C-Temp	0.0001	1	0.0001	0.0074	0.9333	
AB	0.0034	1	0.0034	0.4104	0.5362	
AC	0.0000	1	0.0000	0.0060	0.9400	
BC	0.0004	1	0.0004	0.0501	0.8274	
A^2	0.1822	1	0.1822	21.70	0.0009	Significant
B^2	4.505E-07	1	4.505E-07	0.0001	0.9943	
C^2	0.0152	1	0.0152	1.82	0.2075	
Residual	0.0839	10	0.0084			

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

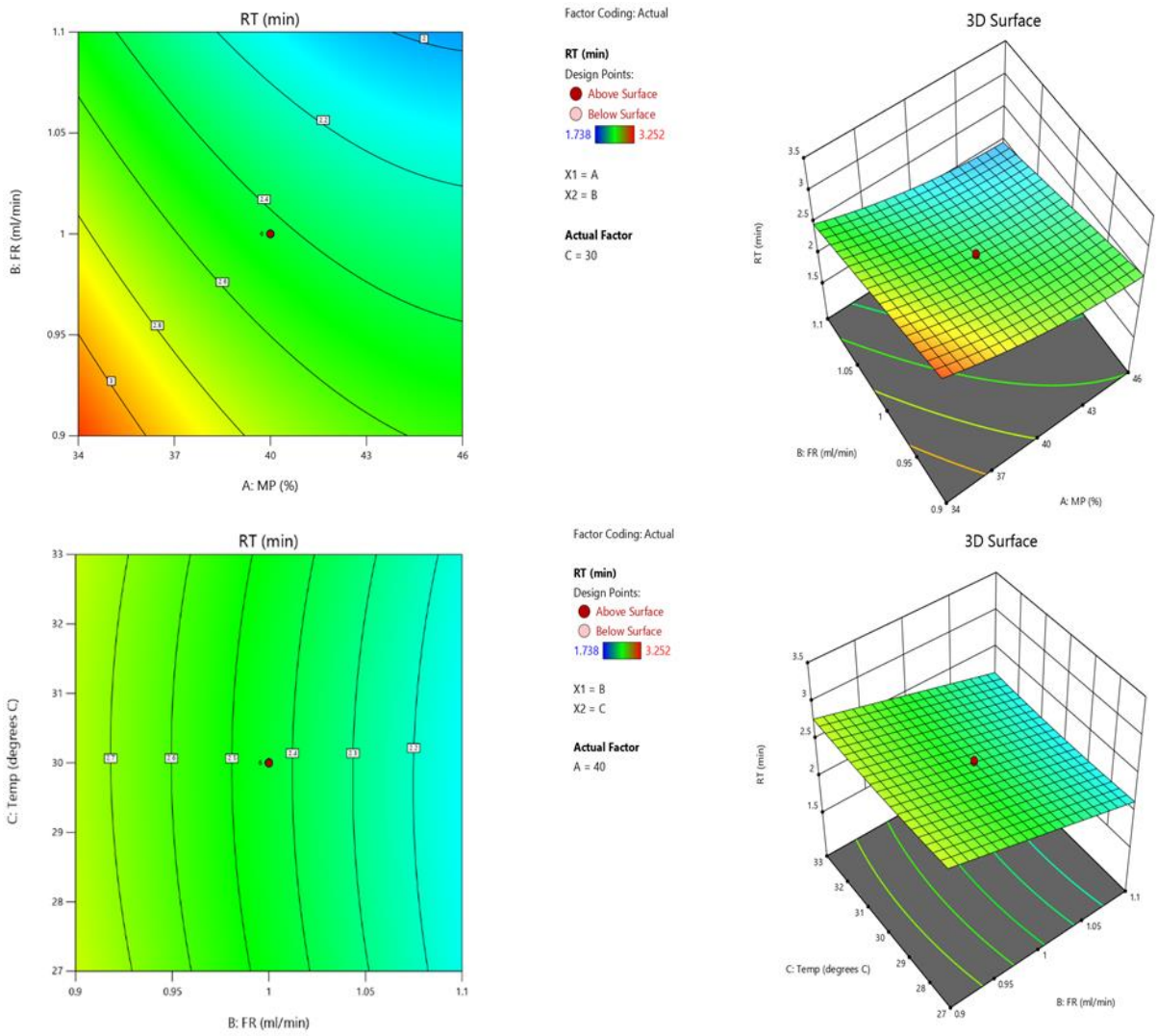

Figure 3: 2D Contour and 3D Surface plots of RT as a function of organic phase composition, flow rate & column temperature for GTB

volumetric flask, dissolved in a small amount of mobile phase, sonicated and then built up to the final volume. Next, using mobile phase, the final concentration was adjusted to 40µg/ml for use in subsequent research.

Sample solution preparation

Mixed 40 mg of GTB and 60 mg of placebo (Hydroxy propyl methyl cellulose) to prepare synthetic mixture and transferred to 100 mL volumetric flask, dissolved in few ml of mobile phase, sonicated and built upto the final volume to get 400 µg/ml. After passing the solution through a 0.45

Table 4: ANOVA for TP of GTB

ANOVA for Response Surface Quadratic model

Source	Sum of Squares	df	Mean Square	F Value	p-value	Inference
Model	1.169E+07	9	1.299E+06	17.15	< 0.0001	Significant
A-MP	6.538E+05	1	6.538E+05	8.63	0.0148	Significant
B-FR	3.862E+05	1	3.862E+05	5.10	0.0475	Significant
C-Temp	66002.51	1	66002.51	0.8713	0.3726	
AB	1.007E+06	1	1.007E+06	13.29	0.0045	Significant
AC	5864.44	1	5864.44	0.0774	0.7865	
BC	2401.24	1	2401.24	0.0317	0.8622	
A^2	1.027E+06	1	1.027E+06	13.56	0.0042	Significant
B^2	5.807E+06	1	5.807E+06	76.66	< 0.0001	Significant
C^2	4.286E+06	1	4.286E+06	56.59	< 0.0001	Significant
Residual	7.575E+05	10	75748.65			

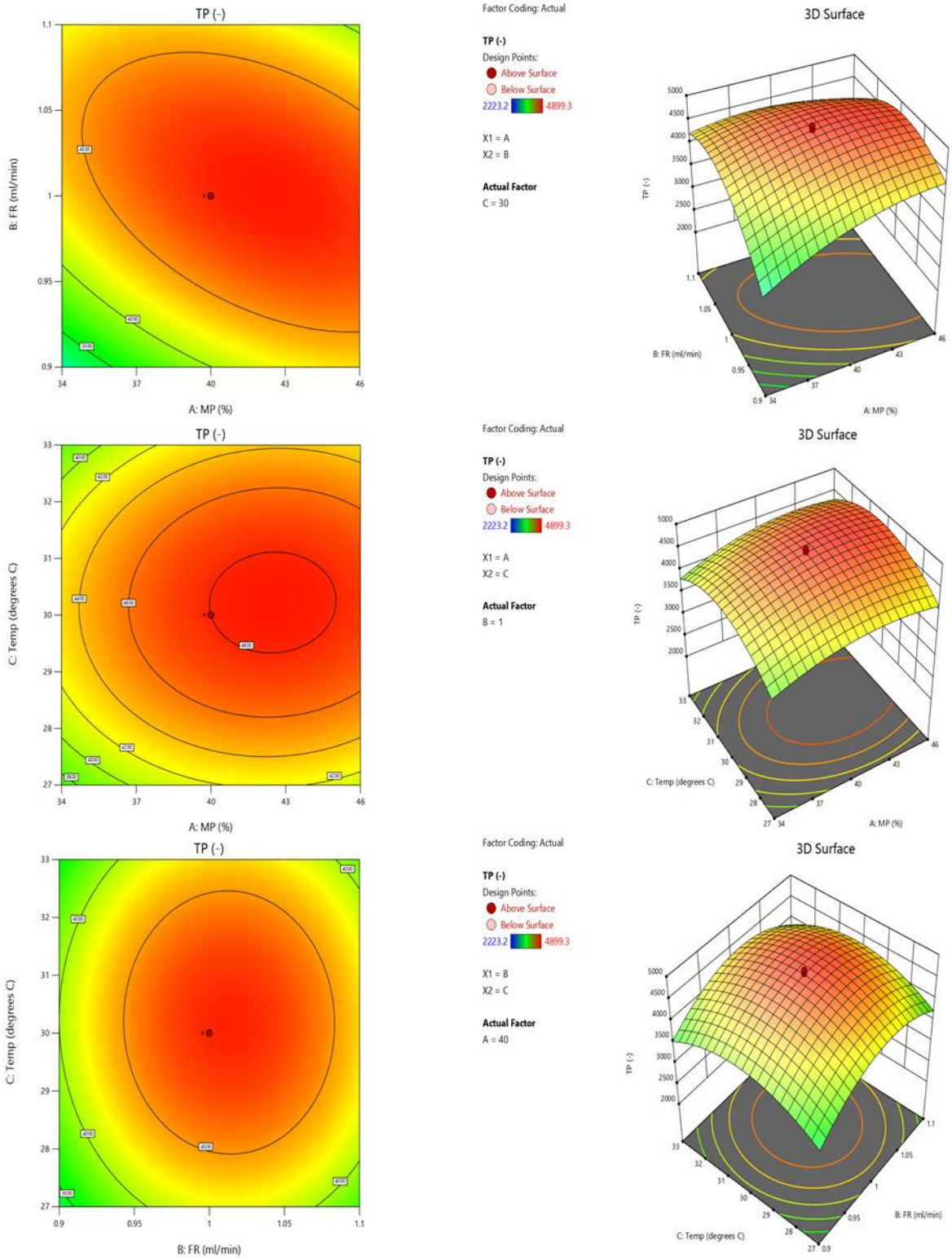

Figure 4: 2D Contour and 3D Surface plots of TP as a function of organic phase composition, flow rate & column temperature for GTB

µm membrane filter, a drug solution containing 40 µg/mL was prepared.

Preparation of reagents for stability studies

2N HCL, 2N NaOH, 20% H_2O_2 solutions were prepared and used for stress study.

Method development

Authentication of drug by FT-IR study

By using the process of KBr pressed- pellet technique, drug pellet was prepared and scanned between 4000 and 400 cm^{-1} using an FT-IR spectrophotometer. Functional groups distinctive absorption peaks were seen at 1714, 1631, 1613, 1126, and 1022 cm^{-1}. The FT-IR spectra of GTB was shown in figure 2.

Determination of absorption maxima

Table 5: Fit statistical parameters of CQA's obtained from ANOVA for GTB

Response & Model	Mean	SD	%CV	Press value	R^2	Adjust -ed R^2	Predic -ted R^2	Adequate precision
Retention time, Quadratic	2.54	0.0916	3.61	0.6279	0.9694	0.9419	0.7711	20.48
Theoretical plates, Quadratic	3811.98	275.22	7.22	5.418E+ 06	0.9392	0.8844	0.7148	11.55

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

10 µg/mL GTB was prepared in ACN and scanned in the range of 200-400 nm against the blank. From the UV spectrum recorded the absorption maxima was 240 nm.

Trials for selection of initial chromatographic conditions

Initial trials were carried out for the selection of column and mobile phase. Finally best chromatographic separation was accomplished on Phenomenex C18 (150 mm x 4.6 mm, 5µ) at 30^0C column temperature. The mobile phase consisted of mixture of ACN: 0.01N KH_2PO_4 buffer pH 4.8 (40:60% v/v) pumped at a flow rate of 1.0 ml /min and λ_{max} was set at 240nm.

QbD assisted method development

Critical method parameters or factors as well as Critical quality attributes or responses, are analyzed with risk assessment in the QbD method. The CMP's selected for the current study were flow rate, % organic content and column temperature. Retention time and theoretical plate count were selected as CQA's.

Optimization by response surface methodology

The CCD under the Response surface methodology (RSM) was employed for the optimization of experimental conditions of the method[10] over five levels as demonstrated in table 1.

A Three-factor five-level CCD design generating 20 experimental runs were performed and the obtained responses were given in the table 2.

Method optimization by desirability functions approach[11]

Desirability function was used to predict optimized chromatographic conditions based on required objectives for each response i.e retention time should be minimum and theoretical plates should be maximum. Desirability function range relys on a scale of d = 0 for a completely undesirable response, to d = 1 for a fully desirable response. Based on the specified goals and boundaries for the chosen CQA's, the set of CMP's which shows a maximum composite desirability was taken as optimized chromatographic conditions and evaluated for the responses.

Method validation

In accordance with ICH Q2 (R1) recommendations, the optimized method was verified for robustness, specificity, linearity, limit of detection and quantitation, accuracy, precision. [12]

Forced degradation studies[13]

Degradation studies were done as per ICH Q1A (R2) to demonstrate that the optimized method was stability suggesting.

RESULTS AND DISCUSSION

Statistical analysis of CCD data by design-expert software

According to evaluation of results from CCD experimental data, it had been possible to elaborate statistical models and to find out the relationship between them. The model significance obtained for the two responses were determined by applying the ANOVA[21] as shown in tables 3 &4. The quadratic model was significant with F-value of 35.21. A, B, A^2 are significant model terms with *p*-values less than 0.05. Design Expert software was used to analyze 2D contour and 3D surface plots[15] in order to visualize the impact of factors and their interactions on the responses.

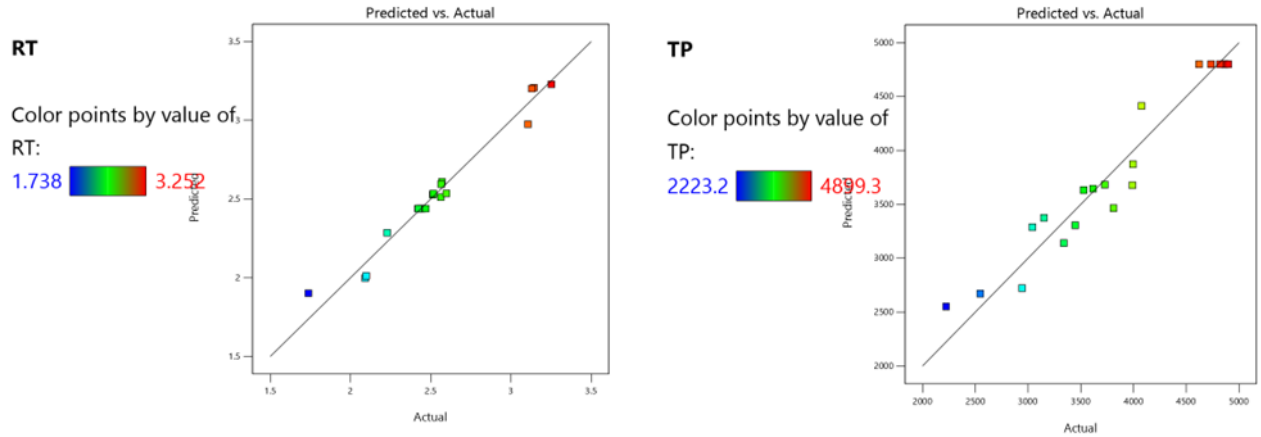

Figure 5: Predicted versus actual plots of RT and TP for GTB

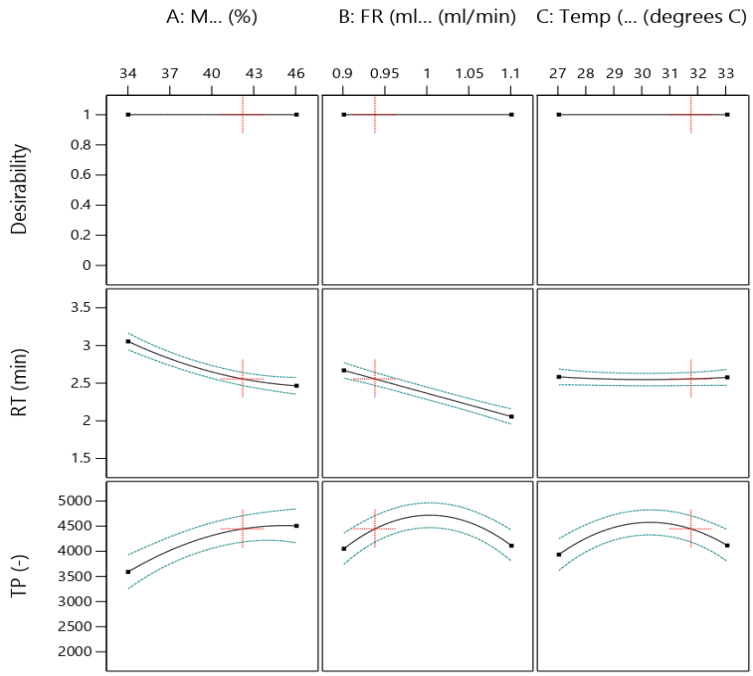

Figure 6: Desirability plot for GTB

Dark blue in the graphs denotes lower values, whereas dark red denotes greater values. Intermediate values are represented by the remaining areas. It was concluded from the aforementioned plots in figure 3 that, the value of retention time decreased with increasing flow rate and organic phase composition. Retention time is not much impacted by column temperature. The quadratic model was significant with F-value of 17.15. Significant model terms with *p*-values less than 0.05 are A, B, AB, A^2, B^2, and C^2. 2D contour and 3D surface plots[15] were analyzed in order to visualize the impact of factors and their interactions on the responses. From the above plots shown in the figure 4 it was found that, the value of theoretical plates are high at a higher organic phase composition, intermediate values of flow rate and column temperature.

The fit statistical results of responses obtained from ANOVA were given in table 5 and found to be within acceptable ranges.

Design validation

The suggested models for the responses were shown to be appropriate for CCD based on the predicted *vs* actual plots[16]

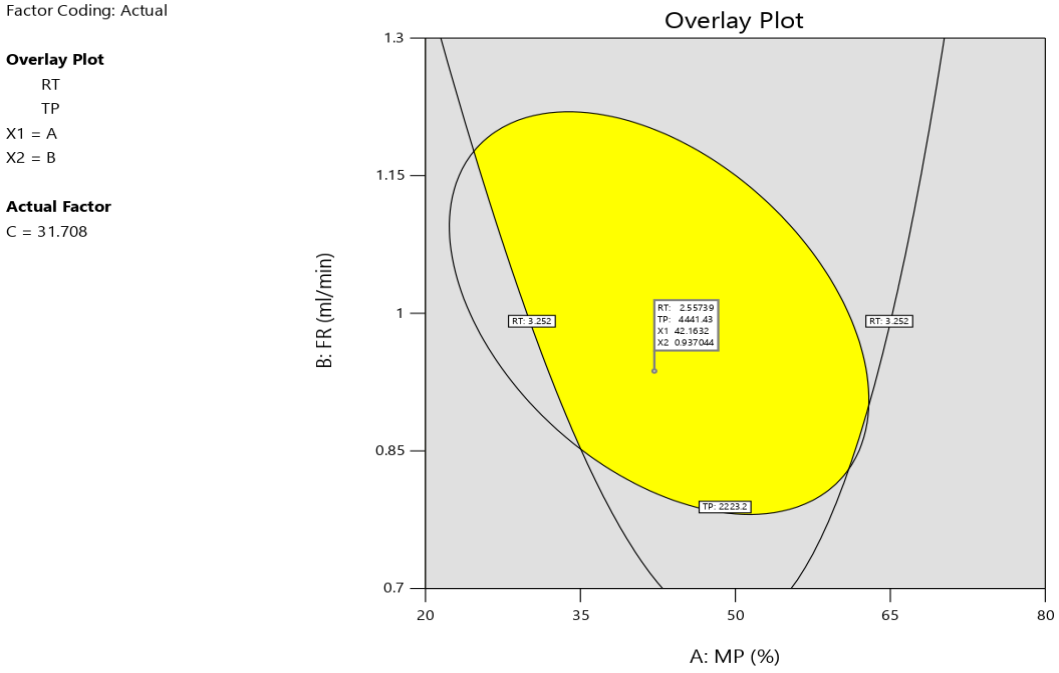

Figure 7: Overlay Contour plot for GTB

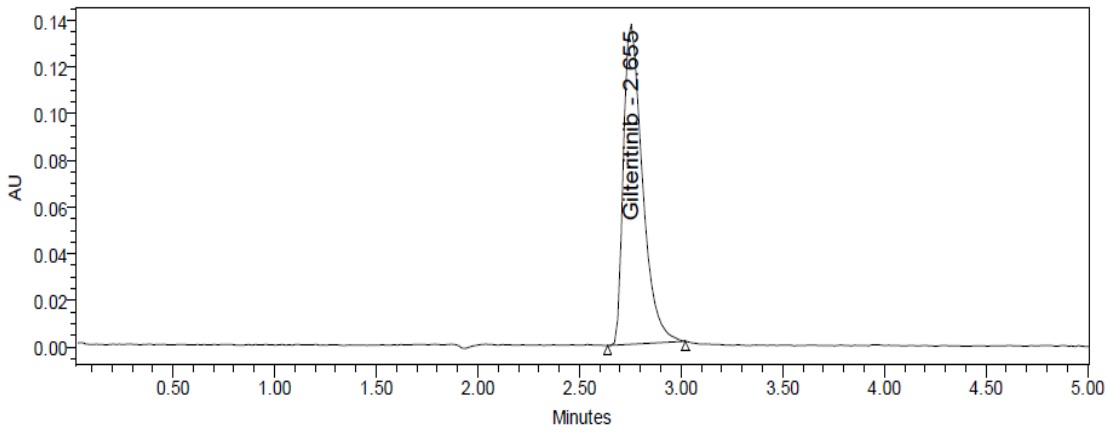

Figure 8: Chromatogram of the optimized method for GTB

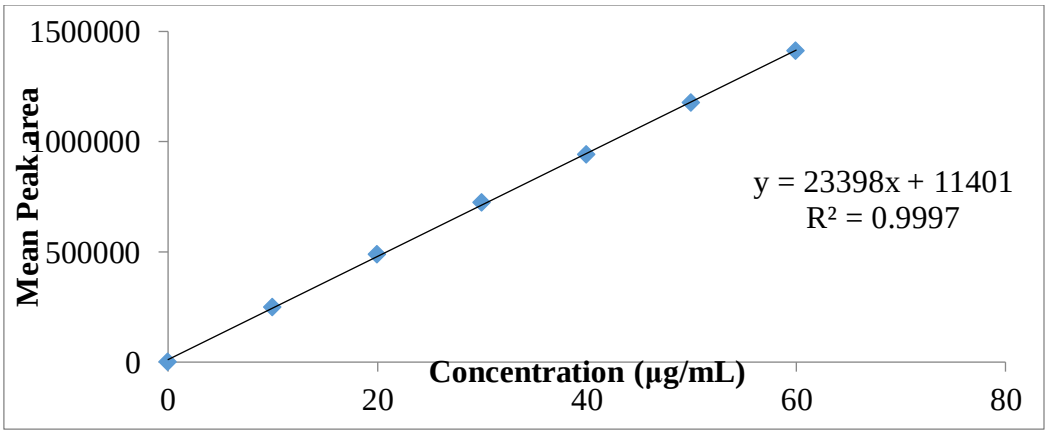

Figure 9: Linearity curve of GTB

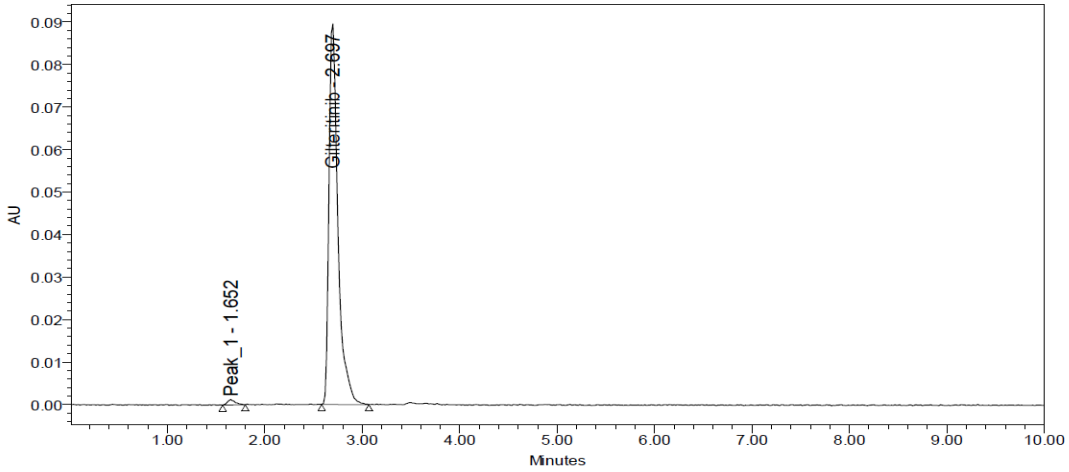

Figure 10: Degradation chromatogram under acidic condition for GTB

for the two responses displayed in Figure 5, which showed an equal distribution of the data points around the 45^0 line. Furthermore, ANOVA tables 3 and 4 showed that the selected models were significant with $p < 0.05$.

Method optimization by desirability functions approach

The highest desirability[17-20] of 1.0 shown in the figure 6 was achieved using mobile phase consisting of Acetonitrile: Phosphate buffer (42.1: 57.9% v/v) pumped at 0.93 mL/min flow rate. The CMP's which gave the maximum desirability was shown as a flag in the overlay contour plot represented in Figure 7. To confirm the optimized chromatographic conditions, three replicates of 40 µg/ml GTB were injected

Table 8: Forced degradation results for GTB

Stress condition	% Drug degraded	Purity angle	Purity threshold	Pass/Fail
Control	–	0.212	0.472	Pass
Acidic (2N HCl, 70^{0}C, 60 min)	6.82	0.312	0.489	Pass
Alkali (2N NaOH,70^{0}C, 60 min)	4.21	0.268	0.442	Pass
Neutral (H_2O, 70^{0}C, 4 hrs)	0.28	0.254	0.406	Pass
Oxidative (20% H_2O_2, 4 hrs)	5.45	0.235	0.369	Pass
UV radiation (24 hrs)	1.68	0.238	0.366	Pass
Thermal (70^{0}C, 60 min)	3.48	0.343	0.531	Pass

Acceptance limit: Percentage degradation should be not more than 20%.

into HPLC system in order to find out if the obtained values were in the desirable limit given in Table 6 and the respective chromatogram was depicted in Figure 8.

Optimized chromatographic conditions

Column: Phenomenex C18 (150 mm x 4.6 mm, 5µ)
Mobile phase: Acetonitrile: Phosphate buffer (42.1: 57.9% v/v)
pH of the buffer: 4.4
Flow rate of mobile phase: 0.93 mL/min
Wavelength: 240 nm

Table 6: Optimized method responses for GTB

S.No.	Responses	Predicted value	Actual value	Desirable range
1	Retention time (min)	2.557	2.655	2.335-2.779
2	Theoretical plates	4441.96	3989.3	3775.19-5108.73

Table 7: Validation results

S.No.	Parameter		Results
1	Linearity	Range(µg/ml)	10-60
		Correlation Coefficient	0.999
		Regression equation	y = 23398x + 11401
2	Accuracy (% Recovery)	50%, 100% &150% levels	Between 99.36-99.48
3	Precision (% RSD of peak area)	Intermediate precision	0.62
		Repeatability	0.27
4	Sensitivity	LOD (µg/ml)	0.17
		LOQ (µg/ml)	0.53
5	Robustness (% RSD of peak area)	Flow rate (±0.1ml/min)	0.8
		Organic phase (±5%)	0.6
		Temperature (±5ºC)	0.7
6	System suitability	Retention time (min)	2.62
		Tailing factor	1.25
		Plate count	3978

Temperature of the column: 31.7^{0}C
Injection volume: 10 µL

Validation

ICH Q2 (R1) guidelines were used to validate the final optimized method. The method developed was found to be linear over 10-60 µg/ml with 0.999 correlation coefficient shown in the figure 9. For accuracy studies the % recovery of the drug was found to be within 98-102%. The method was found to be precise with % RSD values <2%. The LOD & LOQ values were found to be 0.17 and 0.53 µg/ml respectively. The optimized method was found to be robust by making minute variations in the experimental parameters and the concise validation results were given in Table 9.

Forced degradation studies

The drug samples subjected to different stress conditions showed well separated peak of the analyte and degradation peaks at different retention times. In some conditions, separate degradation peaks are not observed, instead height and area of the analyte peak decrease was observed and the obtained degradation results were shown in table 8. From the results, it was found that GTB was more susceptible to acidic degradation than other applied stress conditions and the analogous degradation chromatogram was represented in figure 10.

CONCLUSION

Response surface methodology was used to develop and validate a new, accurate, robust, precise, specific and stability-indicating analytical method for the estimation of Gilteritinib. The chromatographic separation was achieved on Phenomenex C18 (150 mm x 4.6 mm, 5µ) column which gave the best peak shape and low baseline noise. The UV detection was set at 240 nm and the total run time was 5 min. Optimization was done using CCD under RSM for selection of CMP's and CQA's where factors are studied at five levels. The highest desirability of 1.0 was achieved using Acetonitrile: Phosphate buffer pH 4.4 (42.1: 57.9% v/v) which was delivered at a flow rate of 0.93 ml/min. The RT of GTB was 2.655 min. ICH Q2 (R1) guidelines were used to validate the final optimized method and all the validation parameters were found to be within the limits. Degradation studies were performed according to ICH Q1A(R2) guidelines in specified conditions where the drug was susceptible to acidic condition. RSM was considered as a tool for method development where robustness can be tested early in method development stage rather than in validation and statistical analysis of the responses can be done using ANOVA.

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