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DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD OF GILTERITINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple, Précised, Accurate method was developed & validated for the evaluation of Gilteritinib by RP-HPLC technique. Optimized variables used are stationary phase Ascentis C_{18} (150mm*4.6mm5µm), Mobile phase 0.1% OPA: Acetonitrile (70:30) and at 1ml/min Flow rate, wave length was 214nm, column temperature was set to 30°C. System suitability variables were calculated by inject the sample six times and results were within limit. Linearity conducted between 25% to 150% levels, R^2 value was get to be as 0.999. Precision was found to be 0.7 for repeatability and 0.8 for intermediate precision. LOD and LOQ are 0.95µg/ml and 2.88µg/ml in that order. By using above method assay of marketed formulation was conducted, 99.77% was there. Stability studies of Gilteritinib were done, in all circumstances purity threshold was more than purity angle and within the limit.

KEYWORDS: Gilteritinib, RP-HPLC, ICH Guidelines.

INTRODUCTION

Gilteritinib, also known as ASP2215, is a small molecule part of the FLT3 tyrosine kinase inhibitors that presented a greater selectivity and potency when compared with other agents from this group.1 It is a pyrazine carboxamide derivative that showed high selectivity to FLT3 preventing the c-Kit -driven myeloid suppression observed in other therapies.5 Gilteritinib was developed by Astellas Pharma and FDA approved on November 28, 2018. This drug was approved after being designed as an orphan drug with a fast track and priority review status.

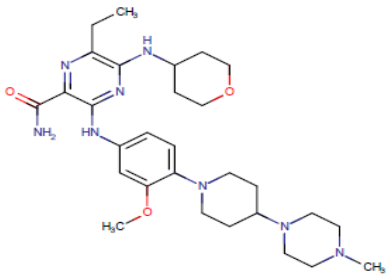

Figure 1: Structure of Gilteritinib.

A literature survey revealed that very few analytical methods for this drug are available in pharmaceutical formulations. These include Uv spectrophotometry, chiral chromatography. Hence the present work targeted to develop a new precise, accurate and sensitive RP-HPLC method for the determination of Gilteritinib in API and formulation. The developed method validated as per ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents used

Gliteritinib pure drugs (API), Combination Gliteritinib tablets (**Xospata)**, Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric. All the above chemicals and solvents are from Rankem.

Apparatus used

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 Gliteritinib solutions. Sonicator (Ultrasonic sonicator), P^H meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump.

Preparation of Standard stock solutions

Exactly weigh 10mg of Gilteritinib transferred to 25ml volumetric flasks, 5ml of diluents were added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as standard stock solution. 1ml of Gilteritinib from each stock solution was pipette out and taken into a final 10ml volumetric flask and made up with diluents (40µg/ml of Gilteritinib).

Preparation of Sample stock solutions
5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25minutes, further the volume was made wp with diluents and filterd by HPLC filters (400 µg/ml of gilteritinib). 1ml of filterd sample stock solution was transferred to 10ml of volumetric flask and made up with diluent (40µg/ml).

Preparation of buffer
0.1% OPA Buffer: 1ml of Perchloric was diluted to 1000ml with HPLC grade water.

Preparation of mobile phase
700 ml of 0.1% OPA and 300 ml of Acetonitrile were mixed and degassed in digital ultrasonicator for 10 minutes and then filtered through 0.45µ filter under vacuum filtration.

Experimental conditions
Chromatographic separation achieved using an analytical ascentis 150mm×4.6mm, 5µ. Mobile phase consisted of 0.1% OPA: Acetonitrile (70:30). The elution was achieved at a flow rate of 1.0ml/min with injection volume of 10µl. the column temperature was set at 30°C and chromatograph was recorded at wavelength 214nm.

Observation: Gliteritinib eluted with good peak shape and retention time and tailing was passed.

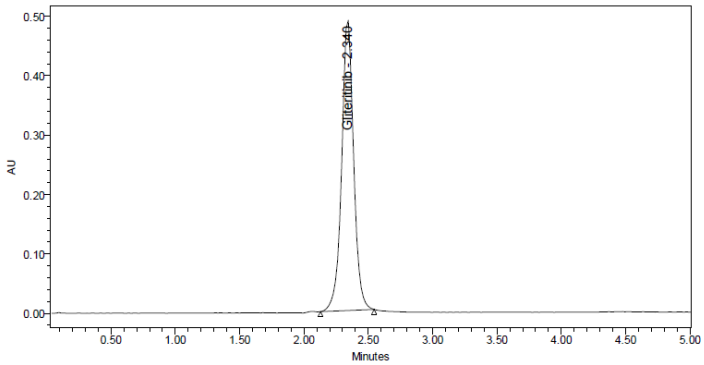

Fig. 2: Optimized chromatogram.

Trials showed that mobile phase with reverse phase ascentis 150mm×4.6mm 5µ column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Gilteritinib as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Gileritinib was recorded. Now the sample solution was injected separately and chromatogram (Figure 4) was recorded until the reproducibility of the peak areas were satisfactory.

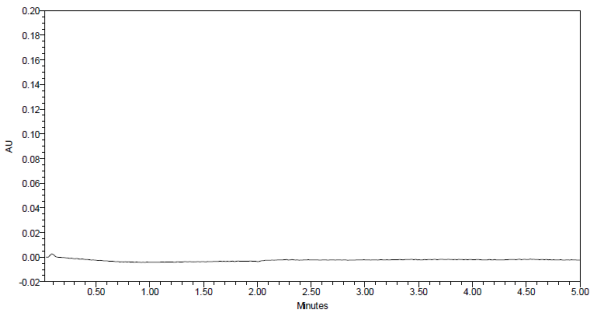

Figure 3: Blank chromatogram.

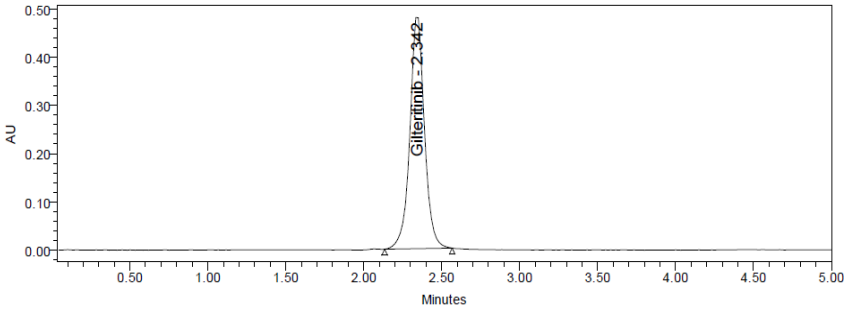

Figure 4: Sample chromatogram.

Analytical method validation
HPLC method was validated according to the International Conference on Harmonization guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as specificity, linearity, precision, accuracy, system

suitability limit of detection, limit of quantification and robustness.

Linearity: Linearity Inject each level (10, 20, 30,40,50 and 60µg/mL) solutions (prepared from standard stock solution) into HPLC system and observed the linear relationship between concentration and peak area. in the concentration range of 10 – 60µg/mL. Calibration curves were plotted with observed peak areas against concentration followed by the determination of regression equations and calculation of the correlation coefficients.

Specificity: Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Precision

Repeatability: The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was calculated.

Intermediate precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different analysts by maintaining same conditions. For intermediate precision % RSD was calculated from repeated studies.

Accuracy

Inject the three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Gilteritinib and calculate the individual recovery and mean recovery values.

Robustness

Robustness was performed by applying little deliberate changes of the following method conditions:
(i) pH of mobile phase: ±0.2
(ii) Temperature: ±5°C
(iii) Flow rate: ±0.1 mL/min
(iv) Wavelength: ±2 nm
(v) Mobile phase composition, organic composition ±5%

Sample and standard solutions were analyzed for each change. Change was made to evaluate its effect on the method. Obtained data for each case was evaluated by calculating % RSD and percent of recovery.

Limit of detection

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.
LOD= 3.3 × σ / s

Limit of quantification

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.
LOQ=10×σ/S

System suitability variables

This variable was calculated by preparing conventional Gilteritinib alternatives (40ppm) and the solutions were injected six times and the factors such as maximum tailing, resolution and USP plate count were determined.

The % RSD for the area of six standard injections results should not be > 2%.

STABILITY STUDIES

It is the ability of the analytical method to measure the active ingredient response in the presence of other excipients and its potential degradants. Forced degradation was carried out to evaluate the stability-indicating properties of the method, by exposing samples of the drug substance and drug product to stress conditions of hydrolysis, oxidation, photodegradation, and thermal degradation. The stress degradation studies were performed until about 5-20% degradation is achieved.

Oxidation

1ml of 20% hydrogen peroxide (H2O2) were added alone to 1 ml of stock solution and the solution was retained at 600c for 30 minutes and solution was diluted to achieve (40ppm) solution and 10 µl solutions were injected into the scheme and chromatogram were recorded. Giltertinib was found to be stable under oxidative hydrolysis condition with the degradation of about 4.85%.

Acid stability test

To 1ml of stock solution, 1ml of 1N Hcl was added and refluxed at 600c for 30mins and the solution was diluted to achieve (40ppm) solution and 10µl solutions were injected into the scheme and chromatograms were recorded. Gilteritinib was found to be labile under acidic hydrolysis condition with the presence of maximum degradation of about 6.59%.

Alkali stability test

To 1 ml of inventory solution, 1ml of 2 N NaoH was added and refluxed at 600c for 30mins and the solution was diluted to achieve (40ppm) relief and 10µl was pushed into the atmosphere and the chromatograms were reported. Giltritinib was found to be stable under basic hydrolysis condition as it was found to be degraded by approximately 4.95%.

Dry Heat stabilty Studies

The normal drug solution was put in the oven at 105^0C for 6 hours. The resulting solution was diluted to (40ppm) solution and 10µl was introduced into the structure and the chromatograms were registered to

evaluate the sample strength. It was found to be degradate by 1.81%.

Photo Stability studies

It is also researched by exposing the solution to UV light by maintaining the beaker in the UV chamber for 7days or 200 Watt hours / m2 in the picture stabilization chamber. The resulting solution was diluted to achieve (40ppm). It was found to be degradate by 1.85%.

RESULTS AND DISCUSSION

Linearity and range

Linearity and range estimated by constructing the calibration curve by taking concentration on X-axis and peak area on Y-axis of (10, 20, 30,40,50 and 60µg/mL) solutions (prepared from standard stock solution) and calculate the correlation coefficient. Correlation Coefficient (r) is 0.9993, and slope obtained was 81439Y- intercept 50789. These values meet the validation criteria as shown in Figure 4 and linearity values tabulated in Table 1.

Table 1: Linearity Concentration and Response.

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	10	932451
50	20	1672251
75	30	2482609
100	40	3293341
125	50	4184622
150	60	4892542

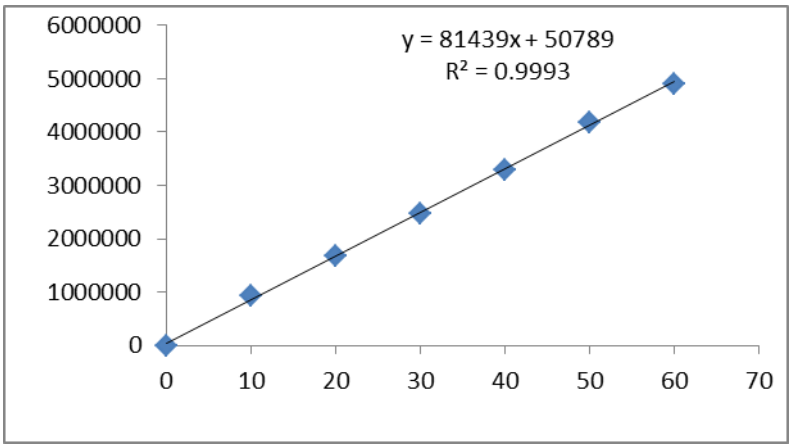

Fig. 18: Linearity graph.

Precision: Repeatability: Six working sample solutions of 40ppm are injected and the %RSD was found to be 0.7 and chromatogram was shown in fig.

Table 2: Repeatability data.

S.No	Peak Area
1	3109464
2	3091776
3	3078051
4	3051122
5	3053867
6	3078329
AVG	3077102
Std dev	22270.4
%RSD	0.7

Intermediate precision: six working sample solutions of 10ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.8.

Table 3: Intermediate precision data.

S.No	Peak Area
1	3093343
2	3081212
3	3069004
4	3061833
5	3055563
6	3023395
AVG	3064058
Std dev	24111.9
%RSD	0.8

Accuracy

Table 4: Accuracy data.

% Level	Amount Spiked (µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	20	19.82	99.11	99.77%
	20	19.80	99.01	
	20	19.91	99.56	
100%	40	39.92	99.81	
	40	39.68	99.21	
	40	40.39	100.99	
150%	60	59.94	99.91	
	60	59.79	99.65	
	60	60.43	100.72	

LOD: Detection limit of the Gilteritinib in this method was found to be 0.95µg/ml.

Table 4: LOD Data.

Parameter	Obtained value	Limit
LOD	0.95µg/ml.	NMT 3

LOQ: Quantification limit of the Gilteritinib in this method was found to be 2.88µg/ml.

Table 5: LOQ Data.

Parameter	Obtained value	Limit
LOQ	2.88µg/ml.	NMT 3

Robustness

Little conscious shift in the method is produced like brief flow, moreover stream, less mobile stage, moreover mobile stage, less temperature, more temperature. The % RSD of the above conditions is resolute.

Table 5: Robustness Data.

Parameter	%RSD
Flow 1.2 ml/min	0.4
Flow 0.8 ml/min	0.9
Mobile phase (72:38)	0.4
Mobile phase (68:42)	0.5
Temperature 35	0.7
Temperature 25	0.7

System suitability

All the system suitability variables were within the limit and suitable according to ICH rules.

Table 6: System Suitabilityparameters.

S.No	Peak name	Retention time	Area	USP Plate count	USP tailing
1	Gilteritinib	2.332	3080740	3223	1.01
2	Gilteritinib	2.333	3052090	3195	1.02
3	Gilteritinib	2.339	3071465	3210	1.00
4	Gilteritinib	2.34	3086990	3245	1.00
5	Gilteritinib	2.342	3080394	3212	1.01
6	Gilteritinib	2.344	3028154	3275	0.99
Mean			3066639		
Std.Dev.			22442.2		
%RSD			0.7		

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected discretely into the system and chromatograms were record and drug present in sample was calculated using before mentioned formulation.

STABILITY STUDIES: Detailed solidity studies were conducted and corrupted examples were infused. Testing of the infused tests was determined and each of the examples passed the corruption breakpoints.

Table 7: Assay of Formulation.

Sample No.	%Assay
1	100.99
2	100.42
3.	99.97
4.	99.10
5.	99.19
6.	99.98
AVG	99.94
Std dev	0.72
%RSD	0.7

Table 8: Stability Data of Gilteritinib.

S.NO	Degradation Condition	%Drug Degraded	Purity Angle	Purity Threshold
1	Acidic	6.59	0.156	0.320
2	Alkali	4.95	0.158	0.323
3	Oxidation	4.85	0.257	0.321
4	Thermal	1.81	0.162	0.320
5	UV	1.85	0.156	0.309
6	Water	1.85	0.145	0.310

DISCUSSION

Regarding the pH adjustment in mobile phase for the Acid and base degradation studies have movement in retention time of drugs. But due to neutralized acid sample with 2N Base solution and base sample with 2N acid solution there will be no change in retention time.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Gilteritinib in bulk drug and pharmaceutical dosage forms. Gilteritinib was freely soluble in acetonitrile and sparingly soluble in water. acetonitrile: 0.1% OPA was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Gilteritinib in bulk drug and dosage form.

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