Research Article

RP-HPLC Method for Determination of Gilteritinib in Tablet Dosage Form

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ABSTRACT

The development of pharmaceutical products brought a revolution in human health, but controlling the concentration of drugs in these products is essential to patient's safety. In this study, a simple, sensitive and specific HPLC method is developed and validated to quantitatively estimate Gilteritinib in pharmaceuticals dosage form. Chromatographic separation was performed on an Hemochrom Intsil C_{18} Column (250 x 4.6 mm; 5µm) via isocratic elution with mobile phase consisting of 25mm Sodium Perchlorate (pH = 2.0 adjusted with Orthophosphoric acid): acetonitrile (65:35) with a flow rate of 1mL/min. The detection was achieved with UV/Vis detector with a detection wavelength of 310 nm. The method was validated in terms of linearity, sensitivity, precision, accuracy and limit of quantification tests. Gilteritinib can be successfully separated with good linearity (the regression equation is y=41928x + 108090, R^2 = 0.9998 and perfect recovery with the results of accuracy were in the range 98-102%.

Keywords: Giteritinib, HPLC, Method Development, Validation.

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INTRODUCTION

Acute myeloid leukemia (AML) is deadly hematologic cancer[1-3]. Gilteritinib is the first nonchemotherapy drug that can be used as monotherapy for acute myeloid leukemia patients. Gilteritinib chemical structure $C_{29}H_{44}N_8O_3$, also referred to as ASP2215[3]. It acts as an inhibitor of FLT3 tyrosine kinase inhibitor. It is a pyrazine carboxamide derivative that showed high selectivity to FLT3 preventing the c-Kit -driven myelosuppression observed in other therapies. It is a small-molecule tyrosine kinase inhibitor that inhibits multiple tyrosine kinase receptor, including FLT3. In cells that exogenously express FLT3, with FLT3-ITD and tyrosine kinase domain mutations FLT3-D835Y and FLT3-ITD-D835Y, gilteritinib inhibits FLT3 receptor signaling and proliferation. Gilteritinib also induces cell death in leukemic cells.

Gilteritinib sold under the brand Xospata which is FDA approved. Which used to treat Acute myeloid leukemia that has relapsed (come back) or is refractory (does not respond to treatment). It is used in patients whose cancer has a mutation in the FLT3 gene[4]. Gilteritinib Fumarate is also being used in the treatment of other types of cancer.

A literature survey discovered that there is, High-Performance Liquid chromatography [HPLC], method for the estimation of Gilteritinib in pharmaceutical formulation have been developed[6].

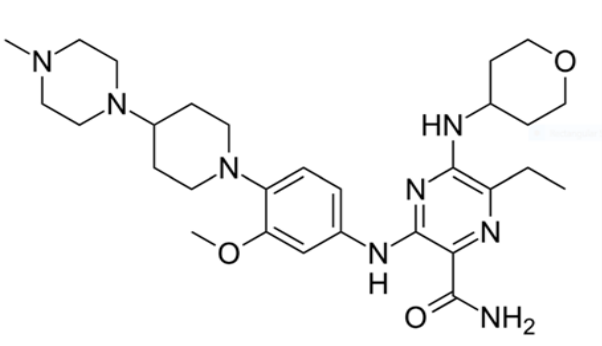

Figure 1: Chemical structure of Gilteritinib

MATERIALS AND METHODS

Chemicals and Reagents

Gilteritinib reference standard having defined potency of 99.3 % (on anhydrous basis) and Gilteritinib tablets (40 mg) were obtained from the Central Drugs Testing Laboratory, Mumbai. Potassium perchlorate (AR grade) obtained from Molychem and Acetonitrile of HPLC grade obtained from Merck life science Pvt. Ltd. were used. Ultra-purified HPLC grade distilled water from Milli-Q® system (Millipore, Milford, MA, USA) water purification unit was used. High flow nylon membrane filter (0.45 µm) was purchased from Axiva Sichem Pvt. Ltd.

Instrumentation

PerkinElmer UV/VIS Spectrophotometer having PerkinElmer UV WinLab ES software/version 6.0.4.0738 was used for all spectrophotometric measurements. The chromatographic separation was achieved by using Thermo scientific chromatographic system (Dionex Ultimate 3000 series). All weighing was carried out using Sartorius Analytical Balance.

Selection of Wavelength

10 mg of Gilteritinib standard was weighed accurately and transferred to 100.0 ml volumetric flask and volume was made up to the mark with mobile phase (100 µg/ml). The aliquot portion of the standard stock solution of Gilteritinib was diluted appropriately with mobile phase so as to obtain a solution of 10 µg/ml concentration. The above solution was scanned in the range of 400.0 nm to 200.0 nm using UV/Vis spectrophotometer using mobile phase as a blank. Gilteritinib showed maximum absorbance at 310 nm as shown in Fig. 2. Hence, the same wavelength was selected for the analysis of the Gilteritinib.

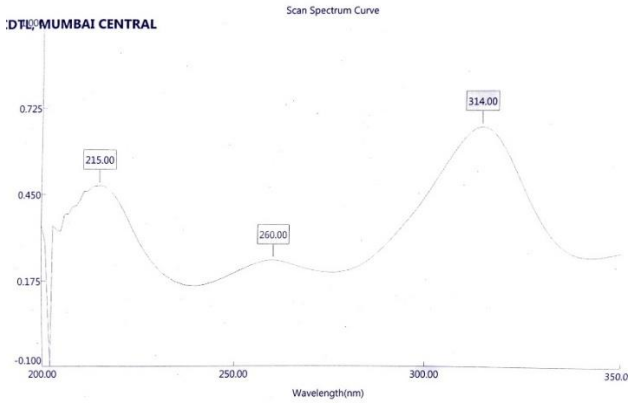

Figure 2: UV Scan of Gilteritinib

Preparation of Buffer for Mobile Phase

25 mm Sodium Perchlorate buffer (pH 2.0) was prepared by weighing 3.06 gm of Sodium Perchlorate, transferred to 1000 ml mobile phase bottle, added 1000 ml water, and sonicated for few minutes using an ultra sonicator. Further, pH was adjusted to 2.0 and vacuum filtered through 0.45 µm high flow nylon membrane filter and was ready for use.

Selection of Diluent

Considering the chemical nature of Gilteritinib, mobile phase was selected for all standard and sample preparations.

Preparation of Mobile Phase

Sodium Perchlorate buffer pH 2.0 and Acetonitrile in the ratio of 65:35 % v/v were used as a mobile phase for the present study. The mobile phase was sonicated and degassed using an ultra sonicator.

Preparation of standard solution

A standard solution of concentration 40 µg/ml of Gilteritinib was prepared using a mobile phase.

Analysis of Marketed Formulation

Twenty tablets of Gilteritinib (40 mg) were weighed and their average weight was determined. The tablets were then crushed to fine powder using mortar and pestle and powder equivalent to 40 mg of Gilteritinib was weighed and transferred to 100.0 ml volumetric flask and dissolved in sufficient quantity of mobile phase. The contents were sonicated for 10 minutes and the final volume was made up to the mark with diluent. Further dilutions were made to get 40 µg/ml of sample solution.

10 µl volumes of standard and sample solutions of Gilteritinib in triplicates were injected into the HPLC system for performing assay on the above tablets. Mean, SD, and % RSD of sample peak areas and % assay were calculated and reported. The results are depicted in Table 9 and the chromatogram of sample (40 µg/ml) solution of Gilteritinib is shown in Fig. 5.

RESULT AND DISCUSSIONS

Validation of developed RP-HPLC method was done for parameters such as specificity, linearity, precision, accuracy and recovery, LOD, LOQ and robustness as per ICH guidelines.

Specificity

Specificity of the method was carried out by injecting Blank, showed there was no interference and co-elution of any other peaks with the retention of Gilteritinib. The peak purity of gilteritinib in tablet dosage form was found within the limit which proved that there was no interference of the blank peaks and excipients peaks at the retention time of Gilteritinib fig. 3.

Linearity

Linearity studies on Gilteritinib standard solutions were performed in the concentration range of 5-70 µg/ml. Linearity of Gilteritinib was found to be linear with Correlation coefficient (r^2) value as 0.9996 and regression equation was found to be y=37465x-11690, having the slope 37465 and y-intercept 11690. The linearity data is shown in Table 2. The graph of peak areas obtained verses respective concentrations was plotted in terms of slope, intercept, and correlation coefficient value as shown in Fig. 6.

Precision

System Precision

Six replicate injections of a standard solution of Gilteritinib (40 µg/ml) were injected into HPLC while performing system precision studies. The mean, SD, and % RSD of peak areas of six replicate injections were calculated and reported and the results are shown in Table 3.

Method Precision (Assay Repeatability)

Six replicate injections of standard solution of Gilteritinib (40 µg/ml) and six sample solutions of Gilteritinib (40 µg/ml) in triplicates were injected into the HPLC system. Mean, SD, and % RSD of % Assay were calculated and reported. The mean assay percentage results of Gilteritinib sample solutions are shown in Table 4.

Intermediate Precision

This was performed on two different days. Six replicates of standard solution of Gilteritinib (40 µg/ml) and six sample solutions of Gilteritinib (40 µg/ml) in triplicates were injected into the HPLC system. Mean, SD, and % RSD of % Assay were calculated and reported for the same. The results are summarized in Table 5.

Accuracy and Recovery (Standard addition method)

Accuracy is the closeness of test results obtained by a particular method to the true value. Recovery studies was done by standard addition method by adding known amount of standard solution to the preanalyzed formulation at three different levels (110 %, 120 %, 130 %). At each level three determinations was performed and mean % recovery was calculated and reported. Results for accuracy studies at various concentration levels are shown in Table 6.

LOD and LOQ

Following formulae were used to estimate Limit of detection (LOD) and limit of quantification (LOQ) of Gilteritinib from calibration curve method:

$LOD = 3.3 \times \propto/s$, $LOQ = 10 \times \propto/s$

Where $\propto$ denotes the standard deviation of regression line response and s denotes the slope obtained from the calibration curve. Solutions of desired concentrations for LOD and LOQ were prepared and injected.

The sensitivity of measurement of Gilteritinib by the current method was estimated in terms of Limit of Detection and Limit of Quantitation. The results are summarized in Table 7.

Robustness

The Robustness of the method was performed by changing flow rate (± 2 %), Mobile Phase composition (± 2 %), temperature (± 2°C) and wavelength (± 2 nm). Under different chromatographic conditions, three sample solutions of Gilteritinib was prepared and injected in triplicates along with six replicate injections of a standard solution of Gilteritinib. Mean, SD, and % RSD of % estimation were calculated and reported for the same. The results are shown in Table 8 and no significant deviation was found in the results.

System Suitability Studies

Analysis and evaluation of System Suitability parameters was done to check the system performance by injecting standard solution of Gilteritinib (six replicates) of a working concentration of 40 µg/ml and a blank preparation (single injection) into the HPLC. The chromatograms were recorded and % RSD of parameters such as Area and retention time were evaluated. Tailing factor and Theoretical plates was also evaluated. In this method, all parameters were found to be within the acceptance limits. The results of system suitability studies are summarized in Table 1 and the chromatograms of blank and standard (40 µg/ml) solution of Gilteritinib are shown in Fig. 3, and 4 respectively.

METHOD VALIDATION

The proposed method for determination of Gilteritinib in tablets is specific. The method was found to have good linearity over the concentration range of 5-70 µg/ml with a correlation coefficient of 0.9998.

For system precision, the % RSD for peak areas of Gilteritinib standard solution was found to be 0.765 and the mean assay percentage results of Gilteritinib sample solutions was found to be within limits and % RSD was found to be 0.638, hence the method was found to be precise. The % RSD values of intermediate precision and assay was found to be within the acceptance limits.

The method was found to be accurate as the mean percent recovery of Gilteritinib sample solutions was found to be 100.19 % which was within limit i.e. between 98 %-102 %. The LOD and LOQ values were found to be 1.08 µg/ml and 3.26 µg/ml respectively for Gilteritinib. Lower values for LOD and LOQ demonstrate that the method developed is sensitive, accurate and precise as it can detect and quantify the analyte at very low concentration.

Reproducible results were obtained which proves the method to be robust. % RSD of % assay during changes in method parameters was less than 2.0 % and the results were not adversely affected by these changes. High percent recovery values and very low SD and % RSD values confirm that the current developed method is suitable for routine analysis of Gilteritinib in its pharmaceutical dosage form i.e. tablets.

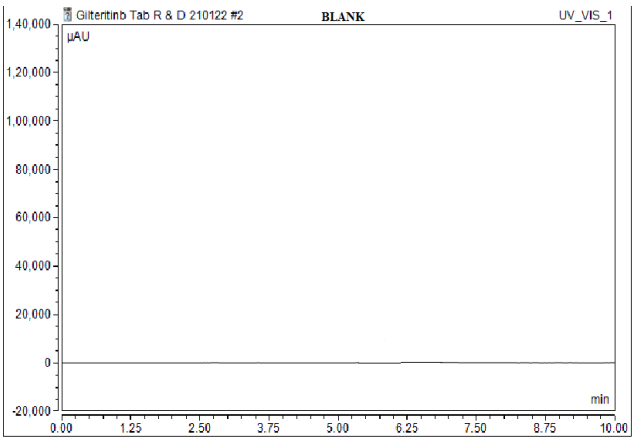

Figure 3: Chromatogram of Blank solution

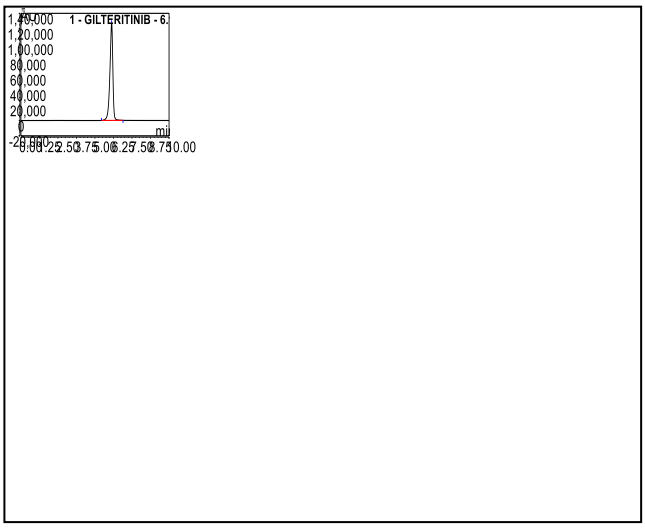

Figure 4: Chromatogram of Standard solution

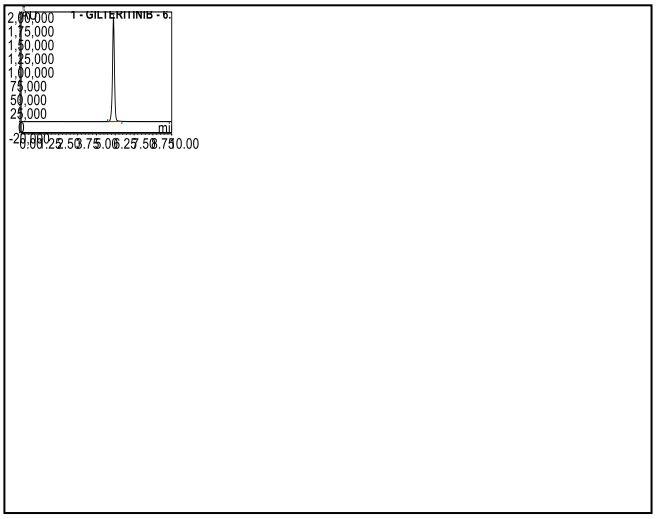

Figure 5: Chromatogram of Sample solution

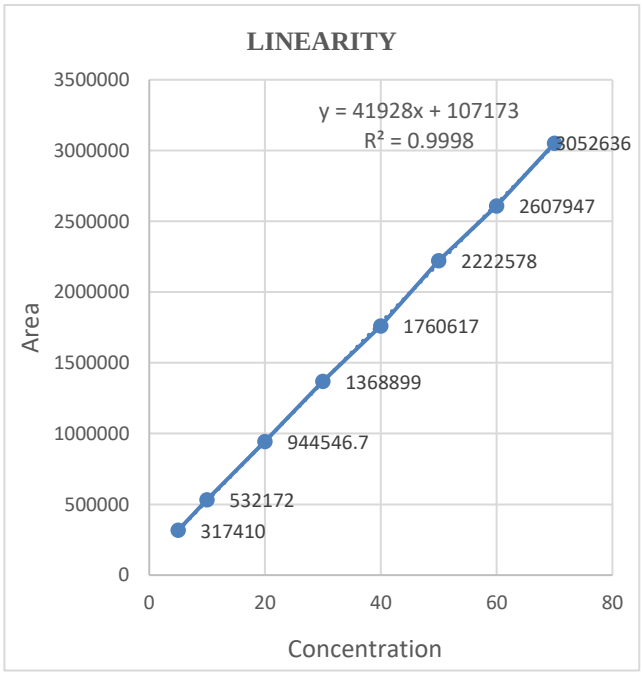

Figure 6: Linearity graph of Gilteritinib

Table 1: System suitability studies of Gilteritinib

Sr No	Peak Area	Retention Time
1	1746666	6.11
2	1767134	6.12
3	1754628	6.11
4	1765474	6.12
5	1759244	6.11
Average	1758629	6.114
SD	8346.985	0.005477
% RSD	0.47	0.09
Limit	NMT 2.0%	NMT 1.0%

SD= Standard Deviation.; %RSD= Percentage relative standard deviation.; NMT= Not more than

Table 2: Linearity data of Gilteritinib

Linearity level	Concentration (µg/ml)	Peak Area
1	5	317410
2	10	532172
3	20	944546.7
4	30	1368899
5	40	1760617
6	50	2222578
7	60	2607947
8	70	3052636

Table 3: System Precision (Standard)

Injection no.	Area at 310 nm	Limit
1	1746666	NMT 2.0 %
2	1767134	
3	1754628	
4	1765474	
5	1759244	
Mean	1758629	
SD	8346.985	
% RSD	0.47	
Limit	NMT 2.0%	

Table 4: Method Precision (Sample)

Sample no.	% Assay	Limit
1	101.28	NMT 2.0 %
2	101.14	
3	101.38	
4	101.14	
5	100.40	
6	101.57	
Mean	101.15	
SD	0.369	
% RSD	0.36	

Table 5: Intermediate Precision/ Interday Precision

Sample no.	Day 1	Day 2	Limit
1	101.28	99.7	NMT 2.0 %
2	101.14	98.8	
3	101.38	99.2	
Mean	101.27	99.23	
SD	0.12	0.46	
% RSD	0.119	0.466	

Table 7: LOD and LOQ data of Gilteritinib

Injection no.	Area at 310 nm
1	1746666
2	1767134
3	1754628
4	1765474
5	1759244
Mean	**1758629**
SD	**8346.985**
% RSD	**0.47**
Regression equation	y=41928x+107173
Slope (S)	41928
LOD=3.3σ/S (µg/ml)	0.53
LOQ=10σ/S (µg/ml)	1.62

Table 6: Accuracy studies of Gilteritinib

% level	STD spiked (µg/ml)	Amount recovered (mg)	% amount recovered	% recovery	Mean % recovery
100	0	39.29	98.2	98.2	99.23
110	4	43.99	110.00	100	
120	8	47.57	118.9	99.1	
130	12	51.79	129.5	99.6	

Table 8: Robustness studies of Gilteritinib

Parameter	Change in parameter (±)	% Estimation	Mean	SD	% RSD	Limit
Wavelength (± 2 nm)	308	98.77	98.72	0.05	0.05	NMT 2.0 %
	310	98.67				
	312	98.73				
Flow rate (± 0.2ml)	0.8	99.11	99.08	0.04	0.04	
	1.0	99.10				
	1.2	99.04				
Temperature (± 5°C)	30	99.44	99.3	0.19	0.19	
	35	99.45				
	40	99.12				

Table 9: Assay results of Gilteritinib

Sample no.	Weight of standard (mg)	Sample Weight (equivalent to 40 mg)	Mean Area of standard at 310 nm	Area of sample at 310 nm	% Assay
1	20.00	144.51	1758629	1747509	101.28
2		144.55		1745611	101.14
3		144.86		1753519	101.38
4		144.77		1748174	101.14
5		144.79		1735666	100.40
6		144.6		1753681	101.57
			Mean	**1747360**	**101.15**
Average weight	144.50 mg		**SD**	**6608.809**	**0.369**
Limit	NMT 2.0 %		**% RSD**	**0.37**	**0.36**

CONCLUSION

The proposed RP-HPLC method was successfully validated for parameters such as specificity, linearity, precision, accuracy and recovery, LOD, LOQ and robustness as per ICH guidelines. The method was found to be simple, rapid, accurate and precise. Only one method was reported for estimation of Gilteritinib in tablet dosage form before; hence this method development is worthwhile. All validation parameters were within the acceptance limit. Hence this method can be used for routine analysis and quality control of Gilteritinib in tablet dosage form in Pharmaceutical industry.

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REFERENCES

1. Einstein A. Wikipedia, the free encyclopedia https://en. wikipedia. org/wiki.
2. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 49803313, Gilteritinib. Retrieved July 18, 2022. https://pubchem.ncbi.nlm.nih.gov/compound/Gilteritinib#section=Drug-Indication.
3. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, Sajed T, Johnson D, Li C, Sayeeda Z, Assempour N. DrugBank 5.0: a major update to the DrugBank database for 2018. Nucleic acids research. 2018 Jan 4;46(D1):D1074-82.
4. Raedler LA. Xospata (Gilteritinib) First Drug Approved as Monotherapy for Adults with Relapsed or Refractory Acute Myeloid Leukemia with FLT3 Mutation. 2018.
5. Baer MR. FLT3 Inhibitors as Sensitizing Agents for Cancer Chemotherapy. InProtein Kinase Inhibitors as Sensitizing Agents for Chemotherapy 2019 Jan 1 (pp. 67-88). Academic Press.
6. Marjoncu D, Andrick B. Gilteritinib: a novel FLT3 inhibitor for relapsed/refractory acute myeloid leukemia. Journal of the advanced practitioner in oncology. 2020 Jan;11(1):104.
7. Othman J, Afzal U, Amofa R, Austin MJ, Bashford A, Belsham E, Byrne J, Coats T, Dang R, Dennis M, Dhawan S. Gilteritinib for Relapsed Acute Myeloid Leukaemia with FLT3 Mutation during the COVID-19 Pandemic: Real World Experience from the UK National Health Service. Blood. 2021 Nov 23;138:1254.
8. Levis M, Perl AE. Gilteritinib: potent targeting of FLT3 mutations in AML. Blood Advances. 2020 Mar 24;4(6):1178-91.
9. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, Montesinos P, Baer MR, Larson RA, Ustun C, Fabbiano F. Gilteritinib or chemotherapy for relapsed or refractory FLT3-mutated AML. New England Journal of Medicine. 2019 Oct 30.

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