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Development and Validation of HPTLC Method for Gilteritinib in Tablet Dosage Form

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ABSTRACT

A simple, precise, accurate, high-performance thin-layer chromatography (HPTLC) method for the Gilteritinib tablet dosage form has been developed and validated. Gilteritinib from the formulation was separated on Silica gel 60 F_{254} HPTLC plates with a mobile phase containing toluene-ethyl acetate-methanol- DEA (5:5:1:0.01; V/V/V/V). Gilteritinib quantification was performed at 310 nm. Well-resolved bands were obtained with R_F values of 0.22. The method was validated for precision, accuracy, robustness, and specificity according to ICH guidelines. The calibration curve was found to be linear in the concentration range of 15-85 ng/band respectively. The correlation coefficient by area is 0.9999. The method is selective and specific with potential application in the pharmaceutical analysis of the drug in the tablet dosage form.

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1. INTRODUCTION:

Acute myeloid leukemia (AML) is deadly hematologic cancer [1]. 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 known as ASP2215 [2]. 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. Gilteritinib is a small-molecule tyrosine kinase inhibitor that inhibits multiple tyrosine kinase receptors, 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 is sold under the brand Xospata which is FDA approved. Which is 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 [1]. Gilteritinib Fumarate is also being used in the treatment of other types of cancer.

A literature survey reveals that the monograph is not official in any pharmacopeias USP, BP, or IP. There is one High-Performance Liquid chromatography [HPLC], a method for the estimation of Gilteritinib in the pharmaceutical formulation has been developed [3]. However, there is no HPTLC method has been reported so far in the pharmaceutical dosage form. Hence trials were made to develop a reliable and accurate HPTLC method for the Gilteritinib tablet dosage.

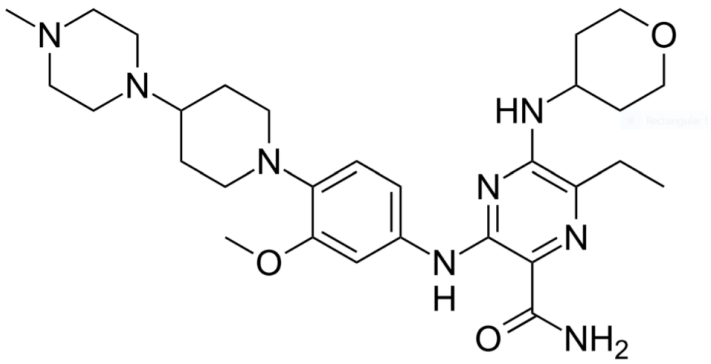

Fig. No. 1: Chemical Structure of Gilteritinib [4]

2. MATERIALS AND METHODS

2.1 Instrumentation

The HPTLC system comprises of CAMAG Linomat V sample applicator (CAMAG, Switzerland), coupled with Camag Hamilton Bonaduz Schweiz syringe (100µl), a UV chamber with dual-wavelength UV lamps, and CAMAG TLC scanner 4 controlled by vision CATS software (CAMAG) was used for the application and detection of spots respectively. The chromatographic separation of drugs was performed using pre-coated with HPTLC plates (silica gel 60 F_{254}). Selection of mobile phase and optimization of condition with 250 µm thickness; (Sigma-Aldrich) and a CAMAG twin-trough developing chamber were used for chromatographic method development.

2.2 Chemicals and reagents

Reference standards of Gilteritinib and their formulation (Xospata 40mg) are obtained from CDTL (Mumbai, India) whereas Analytical grade toluene, methanol, and DEA are from Finar Chemicals (Mumbai, India), and silica gel 60 F_{254} plates from Sigma- Aldrich (Germany).

2.3 Chromatographic conditions

Spotting was done using Camag Linomat 5 sample applicator (CAMAG, Switzerland) and Camag Hamilton Bonaduz microlitre syringe (100 µl) on HPTLC aluminum plates precoated with silica gel 60 F_{254} (20 cm × 10 cm with 250 µm thickness; Sigma-Aldrich). The plates were activated at 110^{O}C for 10 min. The samples were spotted in the form of narrow bands having a length of 8 mm. The application positions X and Y were kept at 8 mm and 20 mm, respectively, to avoid the edge effect. The distance between the two bands was 20 mm. Bands were applied at a constant rate of 15 nL/s using a nitrogen aspirator. Linear ascending development of chromatogram was carried out in a CAMAG twin trough glass chamber saturated with the mobile phase for 30 min and chromatogram run was kept up to 80 mm. Following the development, the HPTLC plates were dried in a stream of air with the help of an air dryer in a wooden chamber with adequate ventilation. Spectro densitometric analysis of the separated components was carried out using Camag TLC Scanner 4 in the reflectance–absorbance mode at 310 nm using a deuterium lamp. The slit dimension used was 6.0 mm × 0.3 mm and sensitivity was kept at auto mode. The scanning speed was 100 nm/s. The evaluation was achieved by linear regression of the peak area response against the amount of

drug by using vision CATS (CAMAG) software for peak area measurement and data processing.

2.4 Preparation of solutions

2.4.1 Standard Preparation

10mg of Gilteritinib reference standard was accurately weighed and transferred into a 100-mL volumetric flask and the volume was made up of Methanol. Further dilutions were made from the above solutions to get a final concentration of 10 µg/mL with methanol.

2.4.2 Analysis of marketed formulation

20 tablets of Gilteritinib (Xospata 40mg) were weighed and the mean weight was calculated. The weight of fine powder equivalent to 40 mg of Gilteritinib was transferred into a 100 mL volumetric flask containing 50 mL of methanol and sonicated for 20 min, cooled and the volume was completed to 100 mL. Further dilutions were made from the above solution to get a final concentration of 10µg/mL. The analysis was repeated six times and the possibility of excipients interference with the analysis was examined.

2.5 Optimization of the HPTLC method

The HPTLC method was optimized to develop an assay method, respectively. Considering the chemical nature and polarity of the molecules to be separated, Silica gel F_{254} TLC plates were used. Initial trials were made using the different solvent systems with different ratios including toluene, ethyl acetate, methanol, and acetic acid. After trials, toluene- ethyl acetate-methanol-acetic acid (5:5:1:0.01; V/V/V/V) was used, but it was found that although the separation was achieved, peak shape was not good for the drug. Hence, DEA was added to the mobile phase to enhance the peak shape. Finally, good resolution, as well as a sharp and symmetrical peak with improved RF values, was obtained from the mobile phase containing toluene- ethyl acetate-methanol-DEA in the ratio of (5:5:1:0.01; V/V/V/V).

2.6 Selection of wavelength

UV spectra were taken by scanning the solutions of Gilteritinib between 200 to 400 nm and 310 nm was selected as the wavelength for the determination of Gilteritinib at this

wavelength, the drug showed maximum absorbance, and spectra of Gilteritinib were depicted in **Fig. No. 2.**

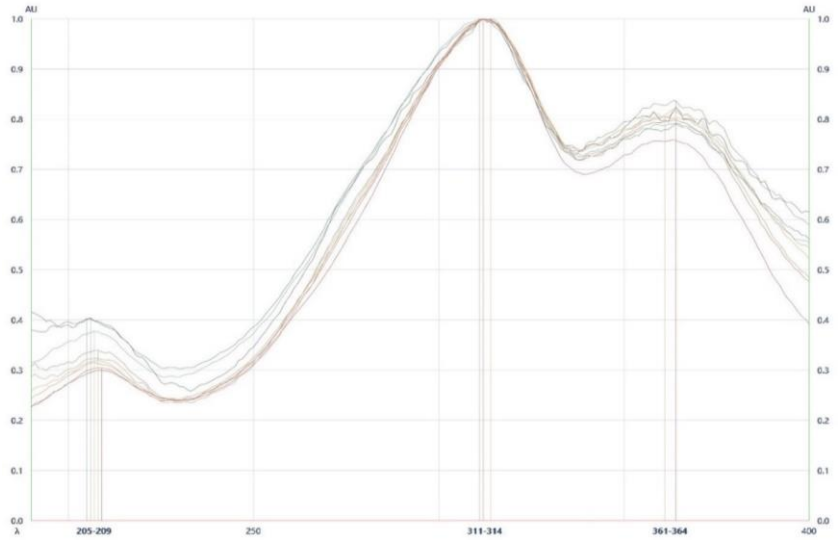

Fig. No. 2: UV Spectra of Gilteritinib

2.7 Method Validation

To fortify the suitability of the method for its intended purpose, the method was validated according to ICH guidelines Q2(R1) and validation of the optimized HPTLC method was carried out concerning specificity, linearity, precision, accuracy, and sensitivity.

2.7.1 Specificity

The specificity of the method was ascertained by analyzing the standard drug and test samples. The band for Gilteritinib in the sample was confirmed by comparing the R_F value and spectrum of the spot with that of a standard. The peak purity of Gilteritinib was determined by comparing the spectrum at three different regions of the spot, i.e. peak start (S), peak apex (M), and peak-end (E).

2.7.2 Linearity

The linearity of the method was studied by six concentrations of the drug. Calibration curves were plotted over a concentration range of 15–85 ng/band. The standard working solution was applied to the plate (15-85 ng/band). The calibration curves were developed by plotting peak area versus concentrations (n= 6) with the help of the vision CATS software. Three-dimensional densitogram for the linearity of Gilteritinib at 310 nm shown in Fig. No. 3.

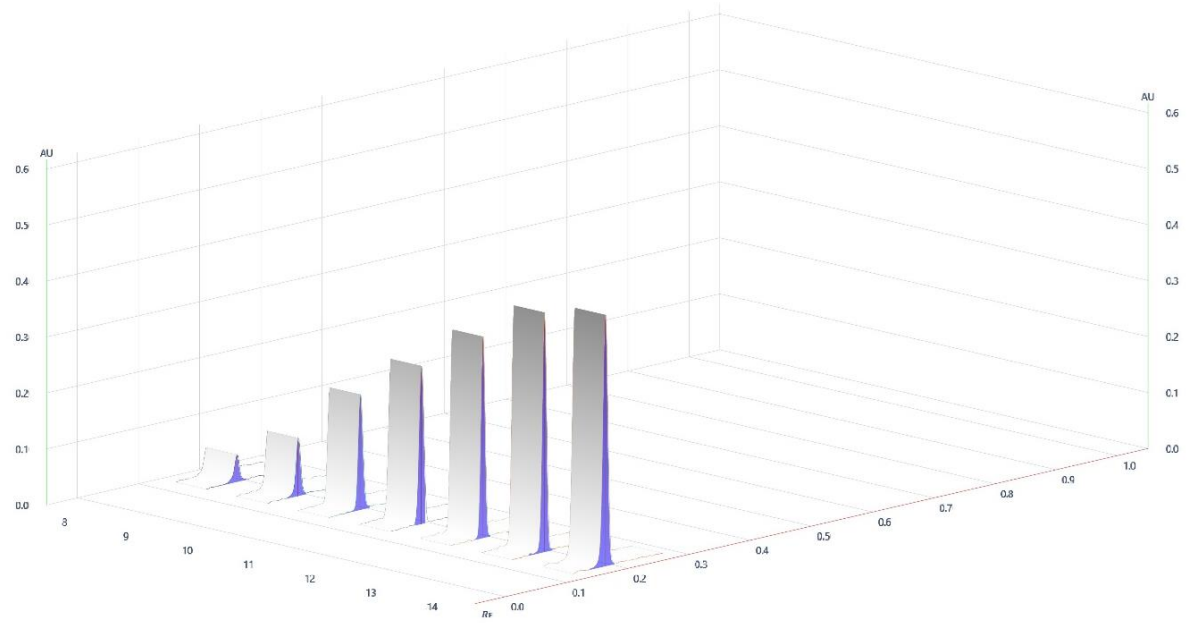

Fig. No. 3: Three-dimensional densitogram for the linearity of Gilteritinib at 310 nm

2.7.3 Precision

The precision of the method was demonstrated by inter-day, intraday, and repeatability precision studies. Repeatability (% RSD) was determined by analysis of Gilteritinib at the concentration of 50 ng/band, respectively. Intraday precision (% RSD) was determined by the analysis standard solution of Gilteritinib at three levels of the low, medium, and higher concentrations of 40, 50 and 60 ng/band for the drugs three times on the same day. Inter-day precision (% RSD) was determined by the analysis of the same solution on three completely different days over 1 week.

2.7.4 Accuracy

The accuracy of the method was determined by the analysis of standard addition at four different levels. Recovery experiments were performed by adding standard drug spiked with 100%, 110%, 120%, and 130% of the standard drug, and the mixture was re-analyzed by the proposed method. This was done to verify the recovery of the drug at different levels in the formulation. The results are given in Table 4.

2.7.5 Sensitivity

The sensitivity of the developed method is expressed as the limit of detection (LOD), the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as

an exact value under the experimental conditions, as well as the limit of quantification (LOQ), which is the lowest amount of analyte that can be detected and quantified with suitable precision, accuracy, and reproducibility. The LOD and LOQ are calculated based on the standard deviation of the regression lines and slope of the calibration curves using the below equations:

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ is the standard deviation of the regression line and S is the slope of the calibration curve.

2.7.6 Robustness

To evaluate the robustness of the developed method, deliberate variations were made in the method parameters such as changing the mobile phase ratio, chamber saturation period and distance traveled, and a slight change in the solvent phase distance that affects the results. The amount of mobile phase was varied over the range of ±5 ml, the chamber saturation period was varied over the range of ±5 min and the distance traveled was varied over the range of ±5 mm.

3. RESULTS AND DISCUSSION

Results of the validation studies of the method developed for Gilteritinib in the current study involving toluene-ethyl acetate-methanol–DEA (5:5:1:0.01; V/V/V/V) as the mobile phase for HPTLC is given as follows.

Linearity

Linear relationships were observed by plotting drug concentrations against peak areas for each compound. Gilteritinib showed a linear response in the concentration range of 15-85 ng/band, respectively. The corresponding linear regression equation was y = 5.5317x + 23.535 with the correlation coefficient of the calibration plot being 0.9999, respectively as shown in Fig. 7.

Repeatability

Results of the repeatability and intermediate precision experiments are shown in Tables 2 and 3. The developed method was found to be precise as the % RSD values for repeatability and intermediate precision studies were 1.74%, respectively as recommended by ICH guidelines.

LOD and LOQ

Using the trend line equations derived from the experiments, the sensitivity of the method in terms of LOD and LOQ was calculated based on the standard deviation of the regression lines and slope of calibration curves. The LOD and LOQ were found to be 3.51, and 10.63 respectively indicating the sensitivity of the proposed method Table 5.

Accuracy

When used to evaluate the recovery after spiking with four concentrations of standard, 100%, 110%, 120%, and 130%. The proposed method showed good percentage recovery rates between 99.01- 100.86. The results of the recovery studies and their statistical validation are given in Table 4.

Specificity

The chromatogram of the pharmaceutical formulation obtained using the developed method showed peaks at R_F for Gilteritinib, respectively. And was found to be at the same R_F as the standard drug. The peak purity of Gilteritinib was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the band. The results shown in Table 5 demonstrate that the purity exceeded 0.999 for all peaks, indicating the specificity of the method in the presence of various excipients. Overlain peak purity spectra of Gilteritinib are shown in Fig. 4.

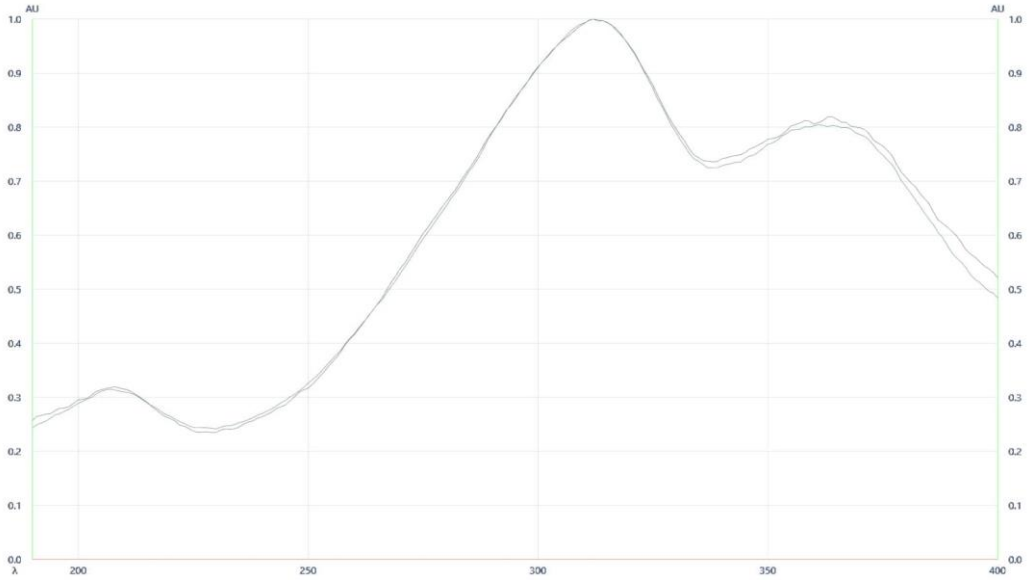

Fig. No. 4: Overlain peak purity spectra of Gilteritinib

The R_F and standard deviation of peak areas were calculated for each parameter and the % RSD was found to be <2%. The low values of the % RSD and no significant changes in the R_F, as shown in Table 6, indicate the robustness of the method.

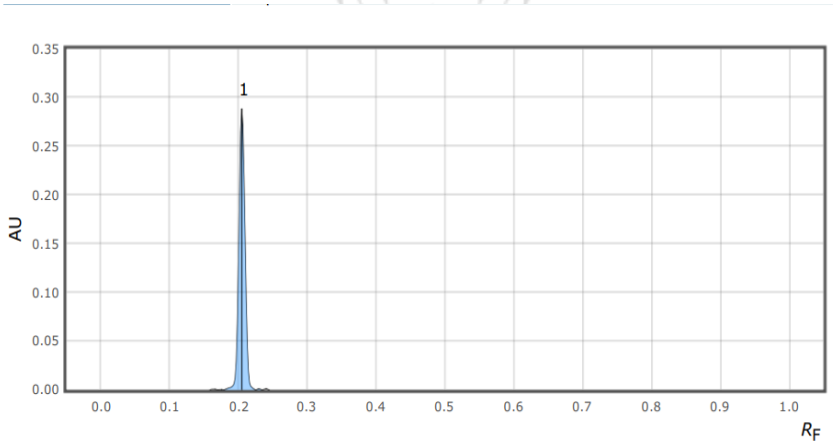

Fig. No. 5: HPTLC densitogram under optimized conditions showing R_F values of 0.2 for Gilteritinib (10 ng/band)

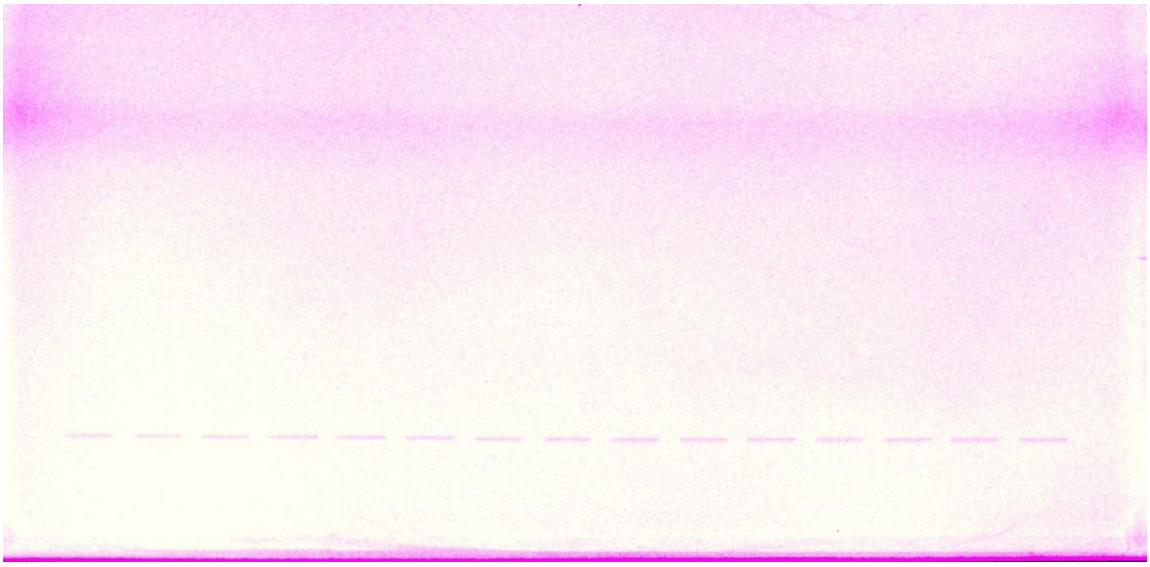

Fig. No. 6: Images of the HPTLC plates taken at 310 nm

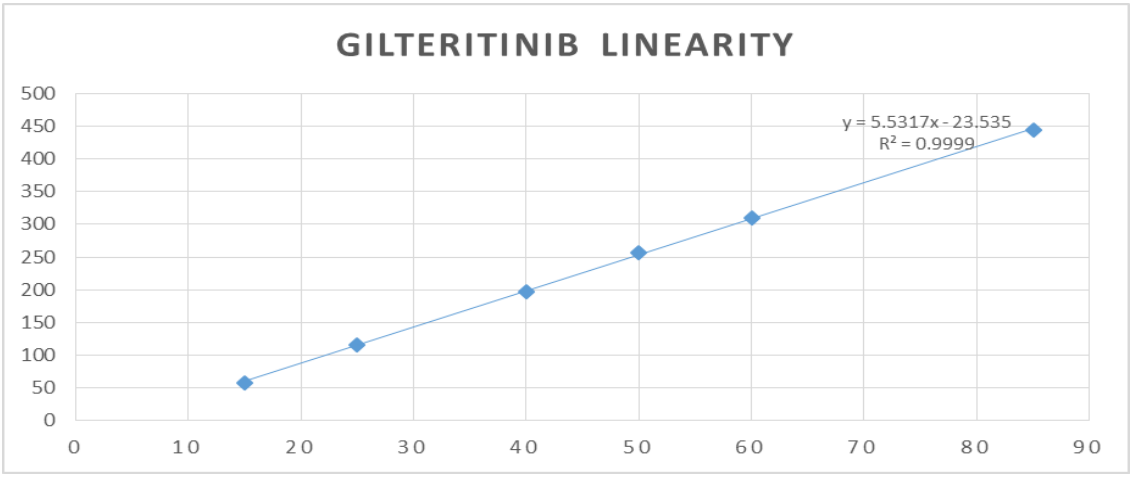

Fig. No. 7: Calibration plot for Gilteritinib

Table No. 1: Linearity data of Gilteritinib

Concentration (ng/band)	**Peak Area**
15	0.00058
25	0.00115
40	0.00197
50	0.00256
60	0.00309
85	0.00445

Table No. 2: Result from the determination of precision Gilteritinib as repeatability

Concentration (ng/band)	**Peak Area**
50	0.00291
50	0.00297
50	0.00302
50	0.00294
50	0.00301
50	0.00304
Average (n=6)	298.3333333
SD	5.20256
RSD (%)	1.743876024

Table No. 3: Result from the determination of precision Gilteritinib

Concentration (ng/band)	**Intra-day precision**		**Inter-day precision**	
	Peak area SD (n=3)	**%RSD**	**Peak area SD (n=3)**	**% RSD**
40	0.001754 ±0.000024	1.40	0.00232 ±0.000025	1.19
50	0.002374 ±0.000038	1.54	0.00215 ±0.000034	1.62
60	0.003015 ±0.000036	1.23	0.00240 ±0.000039	1.56

Table No. 4: Result from recovery study of Gilteritinib

% LEVEL	Amount Spiked (ng/band)	Amount Recovered (ng/band)	% Recovery	% Mean Recovery	% RSD
100 100 100	50 50 50	196 197 200	99.70 100.55 100.97	100.41	0.6454
110 110 110	55 55 55	216 216 215	99.89 100.28 100.28	100.15	0.2223
120 120 120	60 60 60	235 240 237	99.35 101.26 101.97	100.86	1.3458
130 130 130	65 65 65	264 260 256	99.34 98.03 99.66	99.01	0.8703

Table No. 5: Analysis of marketed formulation

Label claim (mg/tablet)	Amount found (mg)	Label claim estimated (%)	(%) RSD
40	39.81	99.54	1.68

Table No. 6: Results of robustness from the proposed HPTLC method

Parameter	Ratio	R_F	Area ± SD (ng/band)	% RSD
Change in the mobile phase ratio (±0.2 in toluene content)	4.8:5.2:1:0.01% 5.2:4.8:1:0.01%	0.198±0.02 0.197±0.02	0.00221±0.00003 0.00224±0.00004	1.53 1.34

Parameter	Saturation time (min)	R_F	Area ± SD (ng/band)	% RSD
Change in chamber saturation time (40 min ± 5)	35 45	0.200±0.02 0.196±0.02	0.00223±0.00003 0.00227±0.00003	1.58 1.00

Parameter	Distance travelled (mm)	R_F	Area ± SD (ng/band)	% RSD
Change in distance travelled (80mm ± 5)	75 85	0.190±0.02 0.187±0.02	0.00227±0.00002 0.00226±0.00002	1.74 1.23

4. DISCUSSION

This study reports a simple, fully validated HPTLC protocol for the quantification of Gilteritinib in pharmaceutical formulation. It demonstrates that the method can accurately quantify the drug content of the tablet formulation without excipient interference or the necessity of a drug extraction step before analysis. The data obtained from various parameters indicate the accuracy of the method, hence the method can be applied for the routine quality control of gilteritinib tablet dosage form. Given the ease of sample preparation, HPTLC's high capacity (with up to 15 samples per plate), and the flexibility of running qualitative and quantitative assays simultaneously, the approach should be considered.

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