RESEARCH ARTICLE

Development and Validation of HPTLC for the Determination of Gefitinib HCl in Tablet Dosage Form

Premakumari KB[1*], Meenashi Vanathi B[2], Ezhilarasan V[3], Harshal Tare[4]

[1]*College of Pharmaceutical Sciences, Dayananda Sagar University, Bengaluru, Karnataka, India.*
[2]*Karpagam College of Pharmacy, Coimbatore, Tamil Nadu, India.*
[3]*Lotus Labs, Bengaluru, Karnataka, India.*
[4]*Dr. Harshal Tare (OPC) Pvt. Ltd., Jalgaon, Maharashtra, India.*

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ABSTRACT

A simple, high-performance thin-layer chromatography (HPTLC) method of gefitinib HCl in pharmaceutical formulation was developed. Separation was done on TLC aluminum plates precoated by silica gel $60GF_{254}$ (20 × 20 cm) with 200 µm layer depth. With UV detection set to 254nm, chromatography was conceded out by the mobile phase of dichloromethane and methanol (9:1, v/v). Linearity was found to range of 50 to 300 ng/spot and, the correlation coefficient was found to be 0.99 and the R_f value was found to be 0.46. The system's suitability was considered to ascertain the performance of ascertaining quality performance of the developed chromatographic method. The method's accuracy, precision, and specificity were all verified against the International Council of Harmonization (ICH) standards. Application of the approach to the determination of gefitinib in tablet form was successful.

Keywords: Gefitinib, HPTLC, ICH guidelines.

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INTRODUCTION

Some forms of cancer can be treated with the medication gefitinib (GFT). Gefitinib, like erlotinib, is an EGFR inhibitor that blocks signaling *via* epidermal growth factor receptors in its intended cells.[1-3] Tyrosine kinase is an epidermal growth factor receptor (EGFR) domain selective inhibitor gefitinib HCl.[4-6] Greater than the appearance of EGFR has been observed in numerous solid tumors, with cancers of the lung, colon, breast, brain, and ovaries.[7-9] In 2015, the United States Food and Drug Administration (USFDA) approved GFT as the initial treatment for non-small cell lung cancer (NSCLC).[10-12] Some organic solvents and combinations are insoluble in water and aqueous buffers, whereas others are marginally soluble in isopropanol, 1-butanol, methanol, ethylene glycol, ethanol, and propylene glycol.[13,14] The purpose of this research was to establish and validate an reversed-phase high-performance liquid chromatography (RP-HPLC) method for precise and accurate quantification of gefitinib in tablet dosage form. In cancer medicine, gefitinib is used to treat specific cancers. The purpose of the research was to establish and validate an RP-HPLC technique for precise and accurate quantification of gefitinib in tablet dosage form. A literature search revealed many commercial formulations and biological samples demonstrating distinct analytical tests of gefitinib HCl estimation. Researchers have released visible spectrometry and derivative spectrometry tests that can be used to find gefitinib HCl in both bulk medication and dose forms.[15-18] Gefitinib HCl process-related impurities have also been estimated using HPLC.[19] Off-targets of gefitinib HCl were discovered using an in silico approach based on system biology.[20] GFT has been measured in mouse and human plasma samples using a variety of "liquid chromatography-mass spectrometry (LC-MS)" techniques.[21-23] GFT investigation in human plasma samples was additionally performed using ultra-performance liquid chromatography (UPLC) method.[24] Magnetic nanoparticles made of iron oxide were also used to measure GFT in water and human plasma.[25] Analytical approaches for detecting GFTs were found to span a wide spectrum in the literature. However, HPTLC has not been used for GFT estimation.[26-30]

This study set out to improve upon the current normal-phase high-performance thin-layer chromatography (HPTLC) assay for the measurement of GFT in commercially available

**Author for Correspondence: prematenny@gmail.com*

tablets by designing and validating a new approach that is more robust, sensitive, precise, and eco-friendly. Standard and environmentally friendly HPTLC assessments for GFT quantification were validated using the International Council for Harmonisation (ICH) Q2-R1 recommendations.[31-33] Our study's goal was to create an easy, quick, and accurate HPTLC method that can be used every day to check how much gefitinib is present in both bulk and set dose combos. Gefitinib's structure is shown in Figure 1.

MATERIALS AND METHODS

Materials

Study procured methanol, dichloromethane, and ammonium formate from Merck chemicals, Gefitinib from Hetero drugs, commercial tablets (Gefiticib 250 mg) from PharmaSave pharmacy, and from E-Merck precoated silica gel 60 F_{254} HPTLC plates.

Instrumentation

Study utilized the Camag HPTLC system through a CamagLinomat V sample applicator, Camag Plate heater, precoated plates, Camag TLC Scanner 3, Camagwin CATS software, UV lamp, Camag twin-trough glass chamber, Hamilton syringe, and Sartorius Analytical balance.

Chromatographic Conditions

As a stationary phase, the experiment was done on 20 x 20 cm pieces of silica gel 60F $_{254}$ aluminum. Mobile phase Dichloromethane:Methanol (9:1 v/v). Using a CamagLinomat V automatic sample applicator and a stream of nitrogen gas, solutions were put on the TLC plate in bands that were 6 mm wide. There was 10 mm of room between each band. The development went up to 80 mm in a 10 x 10 cm Camag twin trough glass box that was filled through the mobile phase and left at room temperature for 30 minutes. The prepared TLC plate was left to dry in the air and then scan with a Camag TLC scanner 3 running WinCATS software between 200 and 400 nm. Good reaction from the part at 254 nm, as seen in Figure 2.

Preparation of Standard Solution

Gefitinib 10 mg by weight, dissolved in 10 mL volumetric flask. Solubility: 1000 µg/mL after dilution with methanol. The working stock was prepared to get the concentration of (10 µg/mL). Various volumes were spotted on the TLC plate to obtain concentrations of 300, 250, 200, 150, 100, and 50 ng/spot.

Preparation of Sample Solution

The formulation was analyzed by averaging the weight of 10 mg equivalents. A quantity of tablet powder equal to 10 mg was measured out and poured into a volumetric flask containing 10 mL of methanol to bring the volume up to the correct level. Concentrations of 100 ng/spot were obtained by spot-tracking the sample solution across a TLC plate.[34]

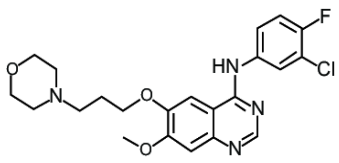


Figure 1: Structure of gefitinib HCl

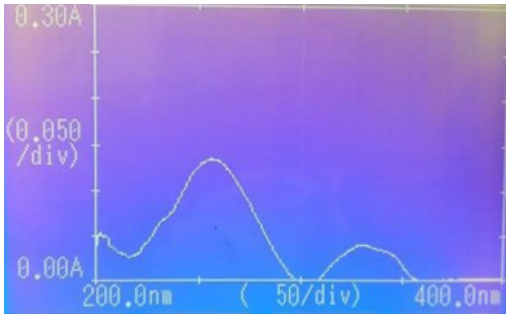


Figure 2: UV spectra of gefitinib at 254 nm

Validation of the Proposed Method

The ICH criteria were used to validate the proposed approach.

Linearity (Calibration curve)

A concentration range of 50 to 300 ng/spot was used to generate calibration curves for gefitinib. The TLC plate was treated with standard solutions of gefitinib. The TLC plate was processed in the same way as indicated for chromatographic separation, including development and photometric analysis. Peak area was compared to spot concentration (in ng/spot) to generate the calibration curve. Five separate measurements were averaged for each reading.

Accuracy (%Recovery)

Gefitinib recoveries were calculated using the conventional addition technique to confirm the method's precision. Standard solutions were added to a previously quantified sample solution at 80, 100, and 120% levels, respectively. Gefitinib dosage was calculated by plugging the observed data into the corresponding regression line equation.

Method Precision (%Repeatability)

Gefitinib solutions were injected six times without changing any of the parameters of the suggested method. This was done to check the accuracy of the instrument. The numbers were given in terms of %RSD.

Intermediate precision (Reproducibility)

We found out how accurate the suggested method is both within and between days by estimating the same responses three times on the same day and three times on three different days over the course of a week using a range of gefitinib standard solutions. %RSD was used to show the data.

LoQ and LoD

The following formulae were used to figure out the LoD and LoQ of the drug according to rules set by ICH.

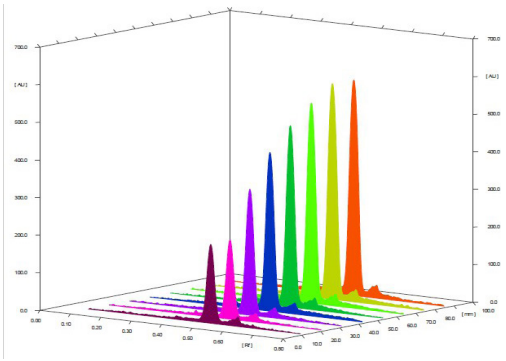
$LoD = 3.3 \times \sigma/S$

$LoQ = 10 \times \sigma/S$

Where

S = Slope of the calibration curve

σ = Standard deviation of response



Stationary phase: 20 X 20 cm HPTLC silica gel 60F254 aluminum plates, Mobile phase: Dichloromethane:Methanol (9:1 v/v), Detection:UV at 254 nm.

Figure 3: Chromatogram of gefitinib HCl with Rf values at 0.46

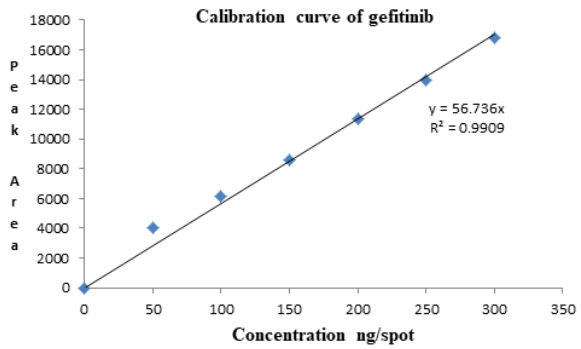


Figure 4: Calibration graph of gefitinib HCl

Table 1: Linearity of gefitinib

S. No	*Concentration (ng/spot)*	*Gefitinib*
1	50	4070.3
2	100	6158
3	150	8589.5
4	200	11341.1
5	250	13985
6	300	16784.2

Table 2: Intraday precision

Replicate	*Concentration (ng/spot)*	*Peak area*	*%RSD**
	Gefitinib	*Gefitinib*	*Gefitinib*
1	100	6158.0	0.19
2	100	6165.0	
3	100	6184.0	
1	150	8589.5	0.39
2	150	8515.5	
3	150	8568.0	

% RSD-Relative Standard Deviation
*Average of 3 determinations

Table 3: Interday precision

Replicate	*Concentration (µg/spot)*	*Peak area*	*%RSD**
	Gefitinib	*Gefitinib*	*Gefitinib*
1	100	6158	0.17
2	100	6149	
3	100	6173	
1	150	8463.5	0.95
2	150	8505.3	
3	150	8638.1	

% RSD-Relative Standard Deviation
*Average of 3 determinations

Table 4: Recovery study

S. No	*Level (%)*	**%Recovery*	*%RSD**
		Gefitinib	
1	80	99.65	0.67
2	100	99.42	0.93
3	120	100.25	0.36

*Average of three determinations

Table 5: Assay of gefitinib HCl

Brand name	*Label claim (mg)*	*Amount found (mg)*	**%Label Claim ± S.D**
Gefiticib	Gefitinib	Gefitinib	Gefitinib
	250 mg	248.85	99.54 ± 0.54

*Average of three determinations

Table 6: Summary of validation parameters

Validation parameters	*Gefitinib HCl*
Linearity	50–300 ng/spot
Correlation coefficient	0.9975
Regression equation	56.736x
Accuracy	99.42–100.25%
Precision (%RSD)	Less than 2%
Specificity	Specific
LoD	10 ng/spot
LoQ	50 ng/spot

Specificity

Standard and sample were looked at to see how exact the method was. Surely the spots for gefitinib were real by comparing Rf and spectra of spots to those of standards.[35,36]

RESULTS AND DISCUSSION

With the goal of creating an assay method for determining gefitinib concentration, the TLC approach was optimized. TLC plates were spotted with standard solutions of both medications and ran in a variety of solvent systems to separate them. The mobile phase is dichloromethane and methanol at a 9:1 volume ratio (v/v). Rf values of 0.46 produced peaks

that were both sharp and symmetrical. After 30 minutes of saturation with the mobile phase at room temperature (27 ± 30ºC), clearly defined spots were obtained. Figure 3 depicts a 3-dimensional chromatogram of gefitinibin at 254 nm, along with a densitogram of standard concentrations. Linearity, accuracy, LoD, precision, LoQ, and specificity were all verified for the proposed HPTLC approach. Gefitinib's linear calibration plot was determined to exist between 50 and 300 ng/spot, with r^2 of 0.997 (Figure 4 and Table 1). The method's sensitivity is demonstrated by the low LoD of 10 ng/spot and LoQ of 50 ng/spot for gefitinib. Tables 2 and 3 show that the intraday and interday RSD values are small, indicating that the proposed method is reliable. Recovery studies were conducted to examine the reliability of the approach. Average recoveries for gefitinib using the suggested HPTLC approach ranged from 99.42 to 100.25% (Table 4), demonstrating its great precision. Gefitinib tablet dosage forms were effectively determined using the proposed validated approach. Table 5 displays that the mean assay of gefitinib was 99.54 and 0.54%. The standard deviation values are low enough to suggest that this method might be used for routine testing of gefitinibin pharmaceutical dosage forms. To make sure that the suggested method would work, the formulation solution was put on a TLC plate, established, and scanned. It was found that the formulation's excipients did not obscure sample peak. Table 6 presents verified configurations.[37,38]

CONCLUSION

An HPTLC procedure for the determination of gefitinib HCl was developed and validated. The proposed method was effectively employed and it has numerous advantages, including a simple mobile phase, sample preparation, rapid analysis, and less consumption of solvent. It has been shown that this approach is suitable for the determination of gefitinib in tablet dosage form, and it satisfies all validation parameter requirements and reveals an accurate chromatographic system.

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