International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 14, Issue 6, 2022

Original Article

DEVELOPMENT AND VALIDATION OF NOVEL RP-HPLC-DAD METHOD FOR QUANTIFICATION OF LAPATINIB DITOSYLATE IN NEWER NANO-LIPOSOME FORMULATION: A QUALITY BY DESIGN (QBD) APPROACH

PRIYANKA SONAR[1], KARIMUNNISA SHAIKH[1*], SUNIL HARER[2]

[1]Department of Pharmaceutics, PES's, Modern College of Pharmacy, Nigdi, Pune, Maharashtra, India, [2]Department of Pharmaceutical Chemistry, Dattakala College of Pharmacy, Pune, Maharashtra, India
Email: karima78@rediffmail.com

Received: 05 Jul 2022, Revised and Accepted: 25 Aug 2022

ABSTRACT

Objective: The current study entails quality by design (QbD) enabled the development of a simple, rapid, sensitive, and cost-effective RP-HPLC method for estimation of Lapatinib ditosylate (LPT) in a newly prepared nano-liposomal formulation which has not been reported earlier.

Methods: The chromatographic factors were screened using a fractional factorial design. A central composite design was employed as a response surface methodology. Mobile phase ratio, flow rate, and wavelength were identified as critical method parameters. To minimize retention time, peak area and theoretical plates were employed as critical analytical attributes. A novel nano-liposomal formulation of LPT was prepared by the film hydration method.

Results: The optimized chromatographic condition was obtained at a mobile phase composition of methanol and 0.05% v/v *o*-phosphoric acid in water (81:19 v/v), flow rate 0.7 ml/min, and peak detected at wavelength 261 nm using DAD detector. The retention time for Lapatinib was 3.702 min. The developed method was validated as per ICH guidelines ICH Q2 (R1). Linearity (R2= 0.999) was observed in the range of 10-50µg/ml. The limit of detection and limit of quantitation was found to be 0.6309µg/ml and 1.9120µg/ml, respectively. LPT containing liposome formulation assay was found to be 99.03% and %RSD was less than 1%.

Conclusion: The newly developed RP-HPLC method applying the QbD approach was found to be simple, specific, precise, accurate, linear, and rugged, with good recovery of LPT in the nano-liposome formulation in a cost-effective manner. Hence it can be employed for the quantification of LPT in bulk and pharmaceutical formulations.

Keywords: RP-HPLC, Quality by design, Validation, Lapatinib ditosylate, Liposome

DOI: https://dx.doi.org/10.22159/ijap.2022v14i6.45750. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

As per recommended ICH guidelines, validation of HPLC the analytical method does not provide much reliability concerning the reduction in method variability beyond the conventional robust testing. Thus, implementation of quality by design (QbD) principles for analytical method development has now been reported quite popularly for attaining high robustness and enhanced method performance [1]. The QbD method was based on the understanding and implementation of guidelines ICH Q8 Pharmaceutical Development, ICH Q9 Quality Risk Management, and ICH Q10 Pharmaceutical Quality System [2-4]. The QbD approach highlights product and process understanding with quality risk management and controls, resulting in higher product quality assurance, regulatory flexibility, and continual improvement.

Analytical QbD is defined as a science and risk-based paradigm for analytical method development, endeavoring to understand the predefined objectives to control the critical method variables affecting the critical method attributes (CMA) to accomplish enhanced method performance, high robustness, ruggedness, and flexibility for continual improvement [5, 6]. The result of analytical QbD is well known, fit for purpose, and robust method reliably delivers the intended output over its lifecycle, similar to the process QbD [7, 8]. QbD approach facilitates science and risk-based understanding of the major sources of variability, followed by the identification of critical method parameters (CMPs) using risk assessment and factor screening studies. The high-risk variables with a critical impact on the analytical method performance are screened and optimized using suitable experimental designs for augmenting method performance [9]. In the past few decades, literature reports on diverse drugs have vouched for the phenomenal benefits of the QbD approach for developing the analytical methods for drug substances, impurities, and degradation products effectively and cost-effectively [10].

LPT is an orally available selective dual tyrosine kinase inhibitor that inhibits both Epidermal Growth Factor Receptor (EGFR) and Human Epidermal Receptor (HER-2) receptors over-expressed in breast cancer cells [11]. LPT is designed chemically as *N*-{3-Chloro-4-[(3-fluorobenzyl)oxy]phenyl}-6-[5-({[2-(methylsulfonyl)ethyl]amino} methyl)-2-furyl]quinazolin-4-amine having molecular formula C29H26ClFN4O4S, the chemical structure is shown in fig. 1. LPT is recommended in the treatment guidelines of HER-2 positive breast tumors and also in metastatic breast cancer [12].

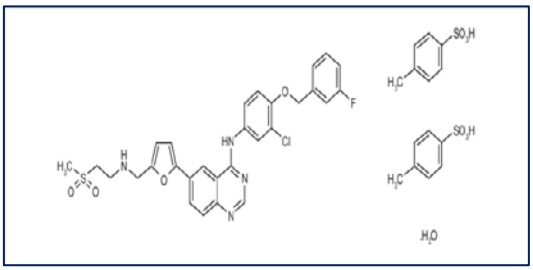


Fig. 1: Chemical structure of lapatinib ditosylate (LPT)

A literature survey reveals high-performance liquid chromatography (HPLC) [13, 14], liquid chromatography-mass spectrometry (LC-MS)/MS [15], and ultra-performance liquid chromatography (UPLC)/MS-MS methods have been developed for the estimation of LPT. Liquid chromatography electro-spray tandem mass [16] and liquid chromatography-mass spectrometry [17-21], were developed for quantitatively determining the LPT in biological samples. However, the reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of LPT in liposome formulation by

systematic quality by design approach has not yet been reported. Commonly, optimization of the RP-HPLC method is carried out by a trial and error approach where large numbers of experiments are required to perform the work. To overcome these pitfalls, RP-HPLC method development can be performed for the estimation of LPT using the QbD approach. Response surface methodology (RSM) is used after the preliminary screening of experimental factors that significantly affect the responses using fractional factorial designs [22-24].

In the present work, attempts were made for the development of a simple, rapid, sensitive, robust, effective, and economical RP-HPLC method by employing analytical QbD principles. The current research aims to design and validate an RP-HPLC method by Centre Composite Design (CCD) and quantify LPT in a newly prepared nano-liposome formulation. The rationale use of experimental design has been explored for a comprehensive understanding of the factor response relationship followed by the method validation studies ensuring robust performance [25]. The present analytical method can be used effectively for the quantification of Lapatinib in bulk and its formulations.

MATERIALS AND METHODS

Chemicals and reagents

Lapatinib ditosylate (CAS No 388082-78-8) was procured from Vedas laboratories, Ahmedabad, India. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was received as a generous gift sample from Lipoid GmbH. Frigenstrasse 4, Ludwigshafen, Germany DE. Cholesterol, Tris base, Sodium chloride, dichloromethane, and methanol was procured from Sigma Aldrich Co. (St. Louis, MO). Ultra-purified HPLC grade methanol, water, *o*-phosphoric acid (OPA), and all other solvents were procured from Merck, Ltd, Germany. The mobile phase was filtered using 0.45µ nylon filters made by Millipore (USA).

HPLC Instrumentation and conditions

The RP-HPLC method development and validation studies were performed using High-Performance Liquid Chromatographic Instrument, Agilent Model 1100 series with online degasser, column thermostat, autosampler and Diode Array detector (DAD) detector. An Agilent C-18 column (250 mm X i. d 4.6 mm, 5 µm particle size), at ambient temperature was used. The mobile phase was composed of methanol: 0.05%v/v *o*-phosphoric acid in water at the ratio of 81:19v/v. The chromatographic system operations like chromatogram output, integration of peaks and calculation of peak areas, retention times, system suitability, and recording of data were performed using the Chemstation software.

Software

Design Expert® 11.0 (Stat-Ease Inc., Minneapolis) was employed to set an experimental design and to perform data analysis and desirability function for optimizing the RP-HPLC method.

Experimental methods

Preparation of standard stock solution

A stock solution of LPT was prepared in solvent methanol having a concentration of 1 mg/ml. The stock solution was wrapped with an aluminum foil protected from light, followed by storage at 4 °C for further use. Aliquots of 0.1, 0.2, 0.3, 0.4, and 0.5 ml from standard stock solution were transferred into the 10 ml A-grade volumetric flasks using A-grade bulb pipettes, and volume was made up to the mark with methanol to get final concentrations of 10, 20, 30, 40 and 50µg/ml.

Selection of wavelength

Standard solutions of LPT were scanned in the UV spectral range of 200-400 nm individually. λmax of the UV spectra was determined and fixed as detection wavelength**.**

Formulation of nano-liposome

A weighed amount of 1, 2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC, 25 mg), cholesterol (5 mg), and LPT (5 mg) was dissolved in 5 ml of dichloromethane: methanol (2:1) in a pear-shaped flask. The solution was evaporated at a temperature of 33 °C at 50rpm under vacuum using a rotary vacuum evaporator (Superfit, VE115) to give a thin film. The trace amount of solvent was removed by placing the flask under a vacuum and kept overnight for dehydration of the film. The hydration of the dried film was performed with 10 ml of Tris buffer pH 7.4±0.1 (20 mmolTris+150 mmol NaCl). The resulting suspension was sonicated to get uniform small unilamellar vesicles (SUVs). Finally, the suspension containing LPT-loaded liposome was stored in a refrigerator at 2-8 °C for short-term use and lyophilized for further long-term use [26, 27].

Preparation of sample solution

Liposome formulation (1 ml) containing LPT was centrifuged at 7000rpm for 3 min using Micro-centrifuge (Brinkmann Inst. Inc., NY, USA) to form pellets. The further pellet was dissolved in methanol, allowed to sonicate and a final volume was adjusted up to 10 ml. From this solution, 0.5 ml was transferred to the 10 ml volumetric flask and a final volume was made up of methanol, providing a final concentration of 25µg/ml.

Factor screening using fractional factorial design

The preliminary studies were carried out as per the literature reports for developing the HPLC method for the estimation of LPT [28]. The majority of the reported HPLC methods describe the use of complex mobile phase composition with solvents as acetonitrile, phosphate buffer at a particular pH range between 3.0 and 5.0, the flow rate in the range of 0.5 and 2.0 ml/min, with isocratic/gradient elution type, specific column chemistry (C8 and C18), and variable temperature settings, etc. for chromatographic separation of LPT. The literature review suggested that methanol and 0.05% of *o*-phosphoric acid and pH 3.0 would be a suitable chromatographic condition, owing to faster chromatographic separation with lower RT values, along with adequate peak symmetry and lower peak tailing.

A two-level five-factor fractional factorial design was employed for factor screening studies to identify the critical method parameters/critical process parameters (CMPs/CPPs). These are considered as critically affecting analytical attributes (CAAs), like retention time (RT), peak area (PA), theoretical plates (TP), and tailing factor (TF). Table 1 depicts the design matrix enlisting the studied factors and the decoded translation of their respective low (-1) and high (+1) levels. A total of sixteen experimental runs were performed, and the design was analyzed for understanding the significance of factors on the CAAs. Mathematical data analysis was performed byfitting the obtained experimental data to the linear polynomial model by obviating the interaction term(s). As screening is primarily based on the principle of factor sparsity, the half-normal plot and Pareto charts were employed for quantitative identification of the effect of each factor on the selected CAAs [29-32].

Optimization using response surface methodology

Response surface methodology (RSM) is used for exploring problems where several independent variables, such as mobile phase composition, flow rate, and wavelength, affect critical responses like retention time, peak area, theoretical plates, and tailing factor. The levels of different variables are optimized to attain the best system performance. In the experimental design, quadratic models can accurately explain all the response values of the chromatographic conditions. For the calculation of quadratic regression model coefficients, each variable having at least distinct levels must be studied to calculate quadratic regression model coefficients, and thus a Central Composite Design (CCD) was employed for optimization.

A Central Composite Design for response surface methodology was applied for the optimization of analytical variables. Methanol composition (X_1), flow rate (X_2), and wavelength (X_3) were taken as independent variables, while Retention time (Y_1), Peak area (Y_2), and Theoretical plates (Y_3) as depicted in table 2 were taken as dependent variables to study 3D response surface and contour plots. Effects of independent variables on dependent variables were studied to evaluate the quality target method profiles (QTMP), which was relative resolution time (RRT). Centre composite design that involved 20 runs, these experiments were performed and the results are summarized in table 2.

Based on the factor screening studies, the selection of the CMPs affecting the method performance was optimized using Central

Composite Design (CCD) at three equidistant levels, as low (-1), and high (+1) levels. The design matrix as per the CCD with 20 experimental runs along with quintuplicate studies of the center point (0, 0) runs is summarized in table 2. A standard concentration of 20µg/ml was used for all experimental runs and analyzed for CAAs like peak area, retention time, theoretical plates, and peak tailing.

Table 1: Matrix and coded levels for fractional factorial screening designs

Independent variables		**Low level (-1)**		**High level (+1)**	
X_1-Methanol (%)		80		82	
X_2-Flow Rate (ml/min)		0.7		0.9	
X_3-Temp (°C)		25		26	
X_4-Wavelength (nm)		260		262	
X_5-pH		2.5		3	
	(X_1)	(X_2)	(X_3)	(X_4)	(X_5)
Std	Run	Methanol (%)	Flow rate (ml/min)	Temp (°C)	Wavelength (nm)	pH
8	1	82 (+1)	0.9 (+1)	26(+1)	260 (-1)	2.5 (-1)
3	2	80 (-1)	0.9 (+1)	25 (-1)	260 (-1)	2.5 (-1)
1	3	80 (-1)	0.7 (-1)	25 (-1)	260 (-1)	3 (+1)
15	4	80 (-1)	0.9 (+1)	26 (+1)	262 (+1)	2.5 (-1)
4	5	82 (+1)	0.9 (+1)	25 (-1)	260 (-1)	3 (+1)
11	6	80 (-1)	0.9 (+1)	25 (-1)	262 (+1)	3 (+1)
14	7	82 (+1)	0.7 (-1)	26 (+1)	262 (+1)	2.5 (-1)
10	8	82 (+1)	0.7(-1)	25 (-1)	262 (+1)	3 (+1)
5	9	80 (-1)	0.7 (-1)	26 (+1)	260 (-1)	2.5 (-1)
16	10	82 (+1)	0.9 (+1)	26 (+1)	262 (+1)	3 (+1)
6	11	82 (+1)	0.7 (-1)	26 (+1)	260 (-1)	3 (+1)
2	12	82 (+1)	0.7 (-1)	25 (-1)	260 (-1)	2.5 (-1)
7	13	80 (-1)	0.9 (+1)	26 (+1)	260 (-1)	3 (+1)
9	14	80 (-1)	0.7 (-1)	25 (-1)	262 (+1)	2.5 (-1)
13	15	80 (-1)	0.7(-1)	26 (+1)	262 (+1)	3 (+1)
12	16	82 (+1)	0.9 (+1)	25 (-1)	262 (+1)	2.5 (-1)

Table 2: Coded levels and matrix for central composite design (CCD)

Independent variables		**Low level (-1)**		**High level (+1)**	
X_1-Methanol (%)		80		82	
X_2-Flow Rate (ml/min)		0.7		0.8	
X_3-Wavelength (nm)		260		261	
	Independent variables			Dependant variables		
	(X_1)	(X_2)	(X_2)	(Y_1)	(Y_2)	(Y_3)
Std	Run	A: Methanol (%)	B: Flow rate (ml/min)	C: Wavelength (nm)	Retention time (RT) (min)	Peak Area (PA) (mAU)	Theoretical plates (TP) (N per m)
14	1	81.0	0.8	261.4	3.5	156.2	4752
12	2	81.0	0.8	260.5	3.5	151.4	5033
16	3	81.0	0.8	260.5	3.6	154.2	6150
5	4	80.0	0.7	261.0	4.0	161.8	4717
20	5	81.0	0.8	260.5	3.6	154.5	6142
19	6	81.0	0.8	260.5	3.6	153.8	6162
10	7	82.7	0.8	260.5	3.7	152.4	5565
18	8	81.0	0.8	260.5	3.6	154.3	6138
6	9	82.0	0.7	261.0	3.7	159.6	5845
3	10	80.0	0.8	260.0	3.5	146.9	4419
7	11	80.0	0.8	261.0	3.5	152.3	4514
9	12	79.3	0.8	260.5	3.9	152.2	4680
13	13	81.0	0.8	259.6	3.7	156.1	5370
15	14	81.0	0.8	260.5	3.6	153.8	6145
8	15	82.0	0.8	261.0	3.5	150.3	5305
11	16	81.0	0.7	260.5	4.2	166.3	6186
4	17	82.0	0.8	260.0	3.6	148.8	5525
2	18	82.0	0.7	260.0	3.8	167.4	5530
1	19	80.0	0.7	260.0	3.9	165.0	4850
17	20	81.0	0.8	260.5	3.6	146.2	6135

Statistical data analysis and model validation

The optimization data analysis was carried out by multiple linear regression analysis (MLRA) using Design Expert® version11 software (M/s Stat-Ease Inc., MN, USA). The experimental data were fit to the second-order quadratic polynomial model and were analyzed statistically by analysis of variance (ANOVA). The model coefficients with statistical significance ($P<0.05$) were considered in framing the polynomial equation. The model aptness was ratified by analyzing various parameters like the coefficient of correlation (R^2), predicted error sum of squares (PRESS), and lack of fit analysis. Response surface analysis was carried out from the 3D-response and 2D-contour surface plots to discriminate the factor–response relationship and plausible interaction effect(s). A search for the optimum chromatographic solution was performed to obtain efficient method performance. Numerical and graphical optimization was carried out to embark upon the analytical design space and location of the optimized solution [33, 34].

RP-HPLC method validation

The newly developed HPLC method for the estimation of LPT was validated according to the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use [35]. This method was validated concerning to linearity, accuracy, precision, the limit of detection

(LOD), the limit of quantification (LOQ), ruggedness, robustness, and system suitability.

Linearity

Linearity is the ability of the analytical procedure to obtain a response that is directly proportional to the concentration (amount) of the analyte sample. The method is linear when test results show proportionality concerning the concentrations. The linearity was performed by preparing the five different concentrations of LPT as 10, 20, 30, 40, and 50µg/ml, respectively. Linearity arrangements were infused in triplicate. The test results obtained here are directly proportional to the analyte samples of LPT. Linear calibration curves were obtained by plotting mean peak areas of LPT on Y-axis versus the respective concentrations on X-axis at five levels each in triplicate. Linearity was determined by least squares linear regression analysis of obtained calibration curves for five points [36, 37]. The linearity was measured by linear regression analysis.

Accuracy

The accuracy of an analytical procedure reveals the closeness of experimental values to the reference. It is expressed as recovery (%) and determined by the standard addition method. It was analyzed against the standard and blank solutions to ensure that no interference exists. The accuracy of LPT assay was determined in triplicates at three levels of 80%, 100%, and 120% of the standard working concentrations of 20µg/ml of LPT. The solutions were then analyzed and the percentage recoveries were calculated.

Precision

The exactness of a logic method is the level of agreement among single test results obtained when the method is a reality of many sampling of a similar sample. It is a measure of the dependability of the whole analytical process. Repeatability or intra-day precision was evaluated by injection of LPT solutions at three different concentrations. Inter-day precision was carried out by introducing the same three samples over three consecutive days and % RSD values were calculated.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOQ was determined as a signal-to-noise ratio of 10 following triplicate injections of LPT. The LOD was determined as a signal-to-noise of 3 following injection of LPT. LOD and LOQ were determined based on the standard deviation of the response of the respective calibration curves using the formula given below;

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

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

Where σ is the average standard deviation of the peak, and S is a slope from the linearity plot.

Ruggedness and robustness

The robustness of an analytical method is to compute the capacity of the method to remain unaffected by small but deliberate variations in method parameters and indicates its reliability during normal usage. The robustness is an important component in any analytical method with regard to ICH guidelines [38]. The robustness of the method was determined by changing the condition of analysis parameters, including wavelength, flow rate, and mobile phase composition [39]. The reproducibility of the result due to the applied small changes indicates the robustness of the method. The ruggedness of the method is determined by a comparison of the results of the assay from two different laboratories and two analysts.

System suitability parameter

The system suitability parameter is the evaluation of a composition of an analytical system to show that the performance of the system meets the standards required by the method. The system suitability study was performed from three replicate injections of LPT. There was a measurement of the United States Pharmacopeia (USP) tailing factor, USP plate count for the peak of LPT, percentage relative standard deviations (RSDs) for the peak area of LPT, number of theoretical plates (efficacy) capacity factor, separation (relative retention), resolution, tailing factor, and relative standard deviation (precision).

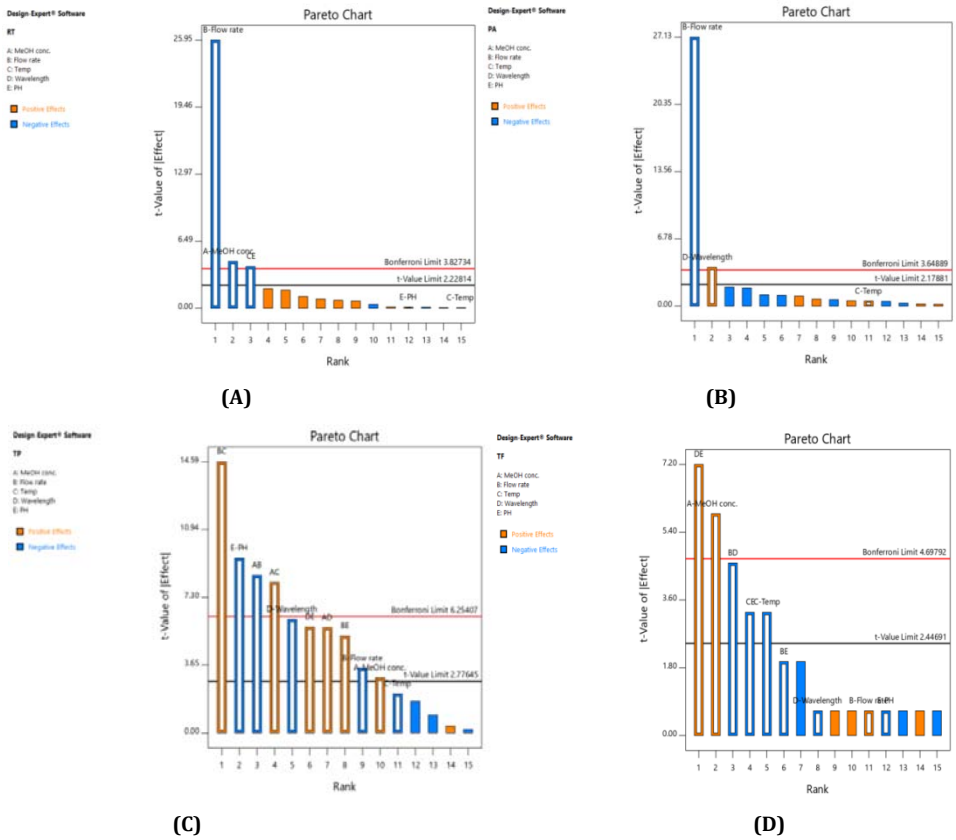


Fig. 2: Pareto charts portraying the effect of critical method parameters on A) Retention time; B) Peak area; C) Theoretical plate count, and D) Tailing factor during factor screening studies

RESULTS

Factor screening using fractional factorial design

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the variance inflation factor (vifs) is 1; however, when vifs is greater than 1, it indicates multi-colinearity. The higher the vifs, the more severe the correlation of factors. In broad meaning, vifs less than 10 are tolerable.

The Pareto charts in fig. 2 show the critical method parameters that were screened viz., mobile phase composition, wavelength, and flow rate. The t-values, being less than the demarcated value of 2, were found to be highly acceptable. The corresponding Pareto charts portrayed that the intermediate levels of flow rate and wavelength were ideal to attain the minimal t-value.

Optimization using central composite design

CCD model was designed to fit the quadratic model using multiple regression analysis according to the following equation (1).

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{12}X_1X_2+\varepsilon \ldots\ldots\ldots\ldots (1)$$

Where Y corresponds to the predicted response, X_1, X_2 and X_3 correspond to the studied factor, β0 is an intercept value, and β_1, β_2, β_{11}, β_{22}, and β_{12} are the regression coefficients.

The independent and dependent variables were related using the polynomial equation with statistical analysis through Design Expert® version 11 software. The approximation of the response values of Y_1, Y_2, Y_3, and Y_4 was based on the quadratic model and their PRESS was the smallest. The value of coefficients X_1, X_2, and X_3 were related to the effect of these variables on the responses as follows:

Effect of variables on retention time (Y_1)

$$RT=+3.61-0.0411X_1-0.1692X_2+0.0762X_1X_2+0.0494X_1^2+0.0779X_2^2 \ldots\ldots (2)$$

The negative sign represented an antagonistic effect upon response, while the positive sign indicated the synergistic effect upon response. The higher coefficient meant the independent variable has a higher prominent influence on the response.

As shown in table 2, Retention time ranged from 3.47 to 4.23 min. From equation (2) negative coefficients of X_1 and X_2 indicated an increase in % methanol and flow rate; there is a decrease in the retention time. This could be due to an increase in the polarity of the mobile phase.

Effect of variables on peak area (Y_2)

$$PA=+152.79+0.0279X_1-5.86X_2-0.2856X_3-0.0400X_1X_2-1.05X_1X_3+2.25X_2X_3-0.0443X_1^2+2.22X_2^2+1.29X_3^2 \ldots\ldots (3)$$

As shown in table 2, the Peak area ranged from 146.2 to 167.4 mAU. As per equation (3), the positive coefficient of X_1 indicated that an increase in % methanol, there is an increase in the peak area could be due to better resolution of the chromatogram, while the negative coefficient for X_2 and X_3 indicated that, a decrease in flow rate and wavelength; there is an increase in the peak area, this could be due to better elution of LPT with reduced flow rate.

Effect of variables on theoretical plates (Y_3)

$$TP=+6148.30+378.05X_1-227.80X_2-72.10X_3+11.12X_1X_2+16.62X_1X_3-38.38X_2X_3-382.97X_1^2-214.46X_2^2-404.25X_3^2 \ldots\ldots\ldots (4)$$

As shown in table 2, the Peak area ranged from 4419 to 6186 N per m. As per equation (4), the positive coefficient of X_1 indicated that with an increase in % methanol, there is an increase in the theoretical plates, while the negative coefficient for X_2 and X_3 indicated that decrease in flow rate and wavelength; there is an increase in the theoretical plates this indicates that better separation of LPT is achieved in the chromatographic system due to increase in methanol and decrease in flow rate and wavelength.

The statistical parameters for responses of retention time, peak area, and theoretical plates were obtained from ANOVA and are depicted in table 3. The model P-values of all response parameters were found less than 0.0001 i.e. P<0.05, showing that these models are significant. The Model F-value as depicted in table 3 implies the model is significant. Adjusted R^2 was found in an acceptable limit that indicates the experimental model is a good fit with polynomial equations. The difference between adjusted R^2 and predicted R^2 is less than 0.2, indicating reasonable agreement. The adequate precision value of all responses was found to be greater than 4, which indicates a sufficient signal and thus, the model is significant for the quantification process. The coefficient of variation (CV) indicates the reproducibility of the model found within the limit for all responses (% CV<10) [40]. Effects of variables on various responses were depicted by the 3D response surface plot in fig. 3 and the contour plot described in fig. 4.

Table 3: Statistical parameters obtained from ANOVA

Response	R^2	Adjusted R^2	Predicted R^2	Actual *P*-value	F value	% CV	Adequate precision
Retention time	0.8789	0.8357	0.6443	<0.05	20.33	2.05	14.22
Peak area	0.8893	0.7898	0.6265	<0.05	9.93	1.78	11.12
Theoretical plates	0.9327	0.8721	0.6749	<0.05	15.39	4.18	10.83

The chromatographic conditions were optimized for the determination of LPT within a lesser analysis time (<4 min). Predicted mean variables for optimized chromatographic conditions with the desirability of 1.000 as shown in fig. 5, that is consist of confirmation location Methanol 81%, a flow rate of 0.7 min, and wavelength of 261 nm. Statistical parameters are as shown in table 4 with 95% confidence. Predicted mean responses were validated with actual responses.

Table 4: Statistical parameters for predicted mean and 95% confidence values

Responses	Predicted mean	Observed mean	SD	df	SE predicted	95% PI low	95% PI high
RT	3.7	3.702	0.1	1	0.1	3.6	3.9
PA	155.6	165.4	2.8	1	3.1	148.7	162.5
TP	5323.94	5120.1	228.29	1	255.192	4755.34	5892.55

Where, df; degrees of freedom, SD; standard deviation, SE; standard error, PI; prediction interval

DISCUSSION

Linearity

HPLC chromatogram of LPT is shown in fig. 6(A) and an overlay of the chromatogram corresponding to the linearity concentration range of LPT is depicted in fig. 6(B). Linear calibration curves (n=3) were obtained by plotting peak areas of LPT versus concentration at five levels (10, 20, 30, 40, and 50µg/ml) each in triplicate, as shown in fig. 7. Three correlation coefficients of R^21 =0.9988, R^22 =0.9990 and R^23 = 0.9992 were obtained with relative standard deviation (RSD %) values between 0.58 and 0.59%. The regression equation for the calibration curve was typically calculated to be y = 13.07x+4.72 in which y is the peak area and x corresponds to the LPT concentration.

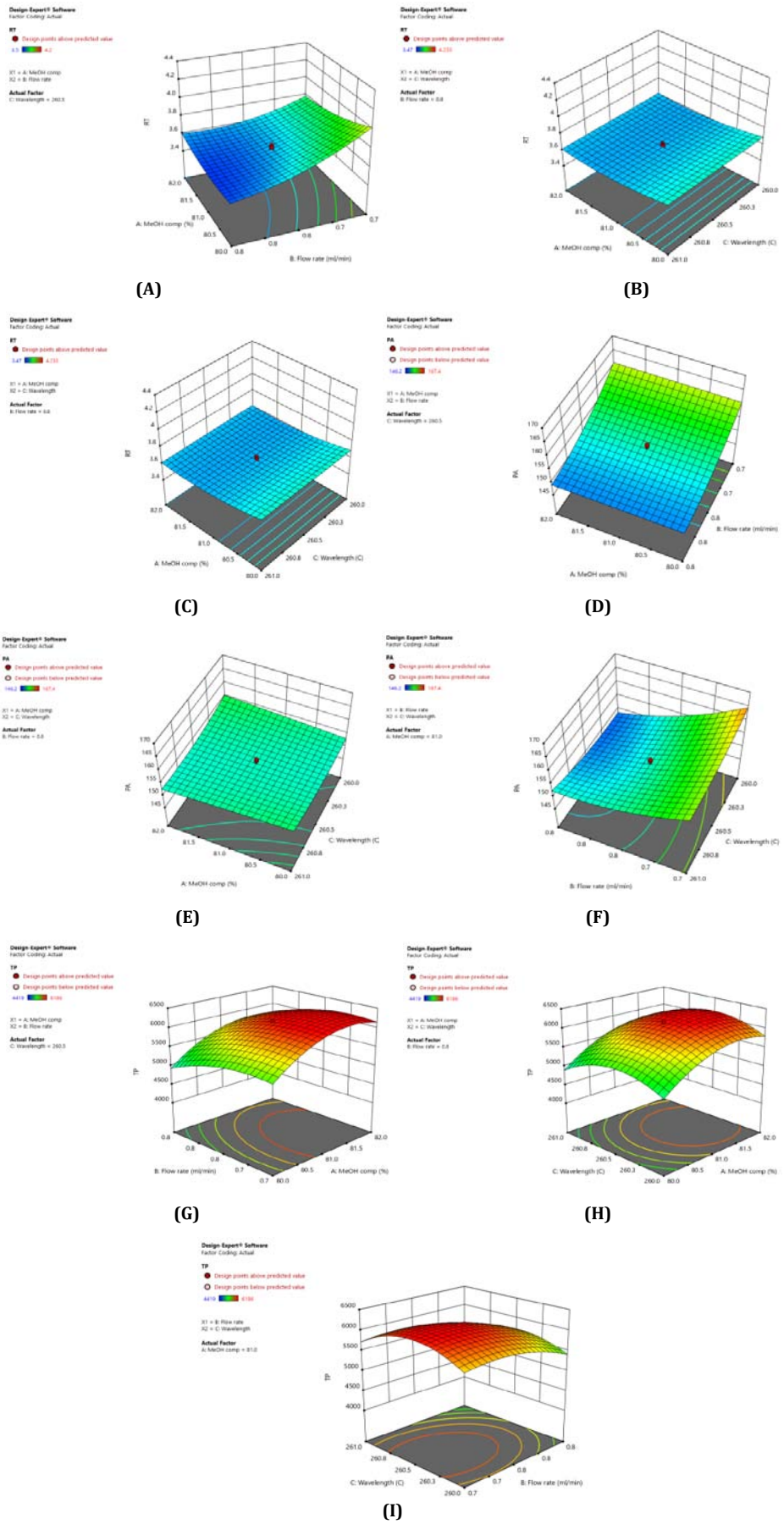


Fig. 3: 3D Response surface plots depicting the influence of independent variables on responses (Y1)-Retention time as shown (A) (B) and (C); Y2-Peak area as shown in (D) (E) and (F); and Y3-theoretical plate as shown in (G) (H) and (I)

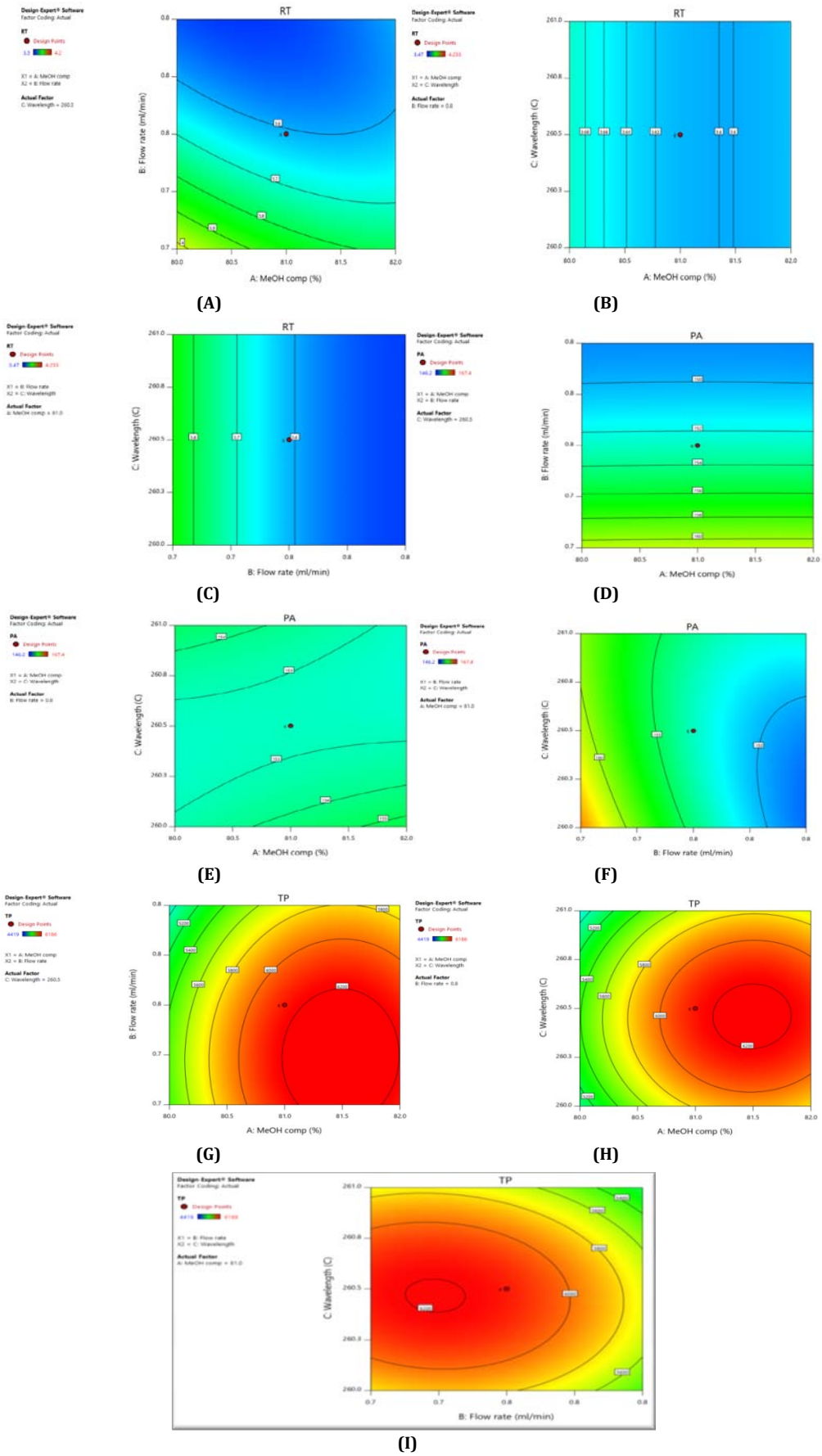


Fig. 4: Contour plots depicting the influence of independent variables on responses Y1-Retention time as shown in (A) (B) and (C); Y2-Peak area as shown in (D) (E) and (F); and Y3-theoretical plate as shown in (G) (H) and (I)

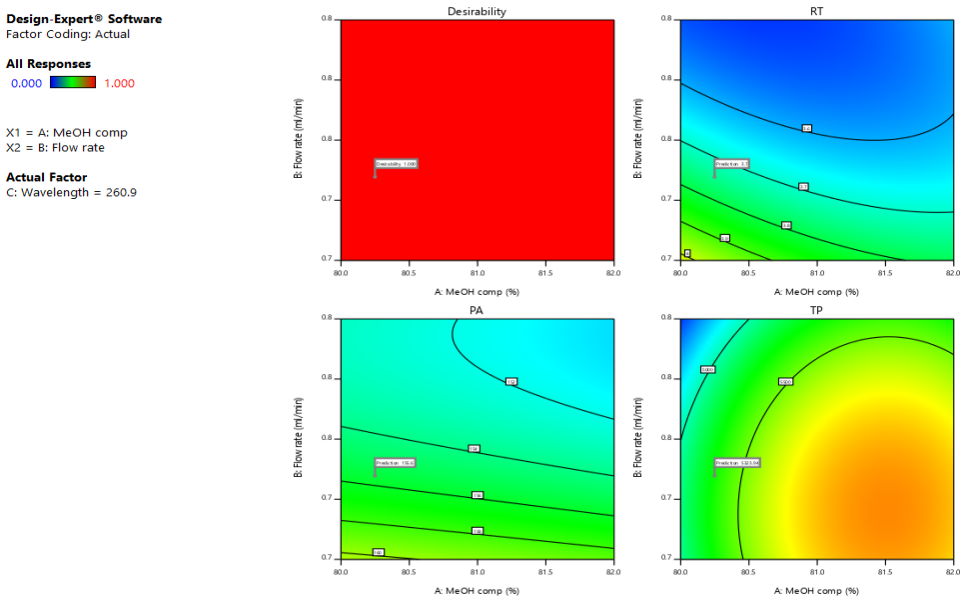
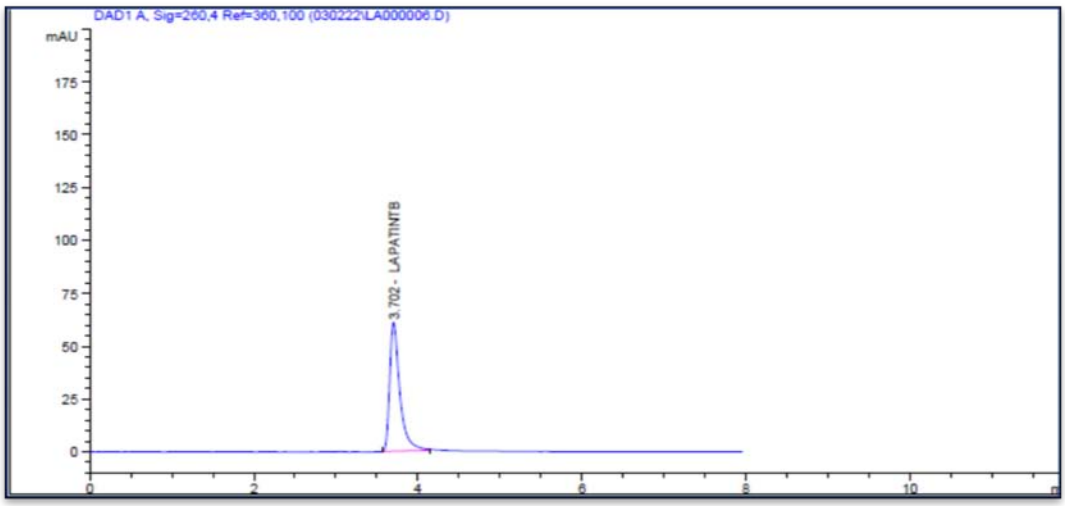
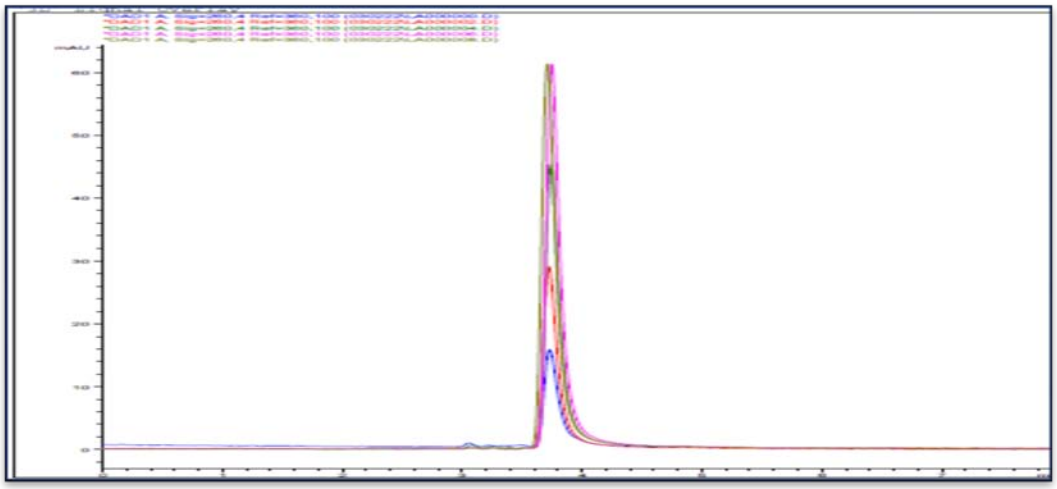


Fig. 5: Actual predicted values of variables with desirability value



(A)



(B)

Fig. 6: (A) Representative HPLC chromatogram of LPT and (B) Overlay of linearity HPLC chromatograms of LPT

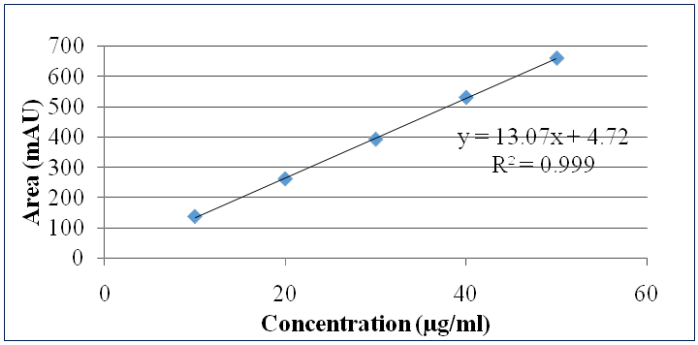


Fig. 7: Linearity curve of LPT

Accuracy

The accuracy of an analytical procedure reveals the closeness of experimental values to the reference. The accuracy of the LPT assay was determined in triplicates at three concentrations of 16, 20, and 24µg/ml. Table 5 summarizes the accuracy data of the developed method.

Precision

The precision or intra-and inter-day variability are summarized in table 6. It was found the %RSD was a maximum of 1.63% for intra-day and 1.92% for inter-day assay, respectively.

LOD and LOQ

The LOQ was determined as a signal-to-noise ratio of 10 following triplicate injections of LPT. The LOD was determined as a signal-to-noise of 3 following injection of LPT. From acceptable precision and accuracy data, LOQ was found to be 1.9120µg/ml and LOD was found to be 0.6309µg/ml. The average standard deviation (σ) was 2.50 and the slope of linearity plot S was 13.07, which was taken into consideration for the LOD and LOQ determination.

Ruggedness and robustness

The reproducibility of the result due to the slight modifications indicates the robustness of the method. The recoveries of LPT under newly deliberate changes are summarized in table 7, which indicates no significant changes under modified important analysis parameters ($P<0.05$). The ruggedness of the method is determined by the comparison of the results of the assay from two different laboratories and two analysts. The % RSD values on the assay of LPT from two different laboratories by two analysts were not more than 1.8%, which indicates the ruggedness of the developed method.

Table 5: Accuracy data of lapatinib (n=3)[a]

LPT concentration (µg/ml)	Mean interpolated concentration±SD (µg/ml)	% Recovery±SD	% RSD
16	16.35±0.042	102.16±0.26	0.256
20	19.90±0.100	99.51±0.5	0.501
24	23.97±0.094	99.87±0.39	0.394

(n=3)[a], Data expressed as mean for "measured concentration" values three times,±SD; designate measurement of results by application of standard deviation.

Table 6: Intra-day and Inter-day precision data (n=3)[a]

Actual concentrations	Intra-day		Inter-day	
(µg/ml)	Mean of measured concentration (µg/ml)	%RSD	Mean of measured concentration (µg/ml)	%RSD
10	9.97	1.63	9.97	1.57
30	29.53	0.18	29.46	1.92
50	50.83	0.08	50.97	1.08

(n=3)[a]; Data expressed as mean for "measured concentration" values for three times, %RSD; suggest measurement of LPT concentration with application of %RSD.

Table 7: Influence of changes in parameters on recovery of LPT (%)

Parameter	Modifications	%LPT recovery	%RSD
Flow rate (ml/min)	0.65	58.697±0.3869	0.2271
	0.85	45.37±0.2526	0.1146
Methanol (%)	80	52.03±0.0594	0.0303
	82	51.89±0.1120	0.0581
Wavelength (nm)	259	51.96±0.6664	0.3349
	261	51.93±0.0719	0.0379

%RSD; indicates measurement of recovery of LPT in the form of %RSD by changing parameters.

System suitability parameters

The system suitability parameters were theoretical plates per meter 5095, height equivalent to theoretical plates 0.0056, and tailing factor 0.95.

Assay of LPT-loaded liposome

The Validated HPLC method was used for the analysis of LPT-loaded liposomes. The percentage assay was found to be 99.03% and the concentration was found to be 24.75µg/ml for LPT-loaded

liposomes. The typical HPLC chromatogram of samples is shown in fig. 8. As no interfering peaks were observed, clearly indicating that there was no interference in the excipient used in liposome formulation. The RSD values were less than 1%.

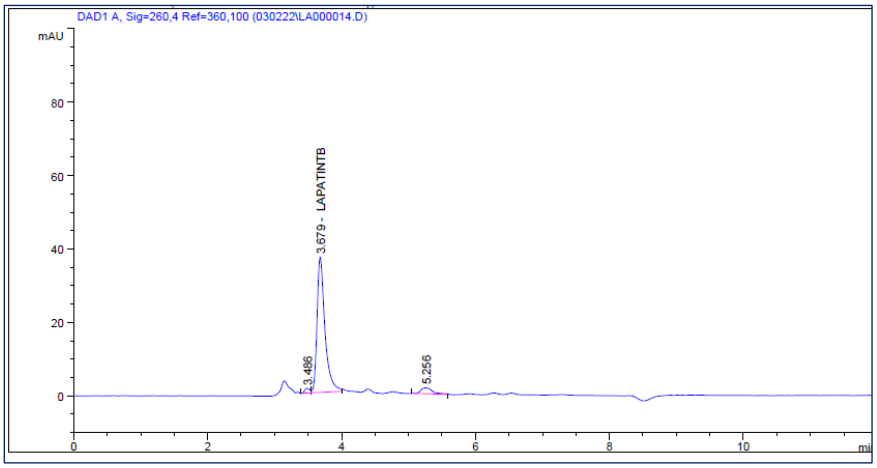


Fig. 8: HPLC Chromatogram of LPT loaded liposome formulation

A new RP-HPLC method was developed and validated for the quantification of LPT in which the DAD detection method is applied. The newness of this method is an estimation of drug LPT in liposome formulation, where liposome formulation of LPT has not been prepared previously as well as LPT was not estimated in liposome formulation as per previous study reports. The results of various validation parameters showed that the newly developed HPLC method is suitable for the quantitative determination of LPT in liposome formulation. QbD has been employed during the development of the method to minimize retention time and optimize peak area and peak asymmetry as compared to the previous reports [40]. The model equation showed a good agreement between predicted values and observed values. Hence the validated RP-HPLC method can be readily adapted for the estimation of LPT in liposome formulation. The new analytical method was linear in an almost wide concentration range with an accepted % relative standard deviation from 0.05% to 0.90%. Accuracy is another important validation factor, which was reported as percent recovery. The accuracy of the previous HPLC method for estimation of Lapatinib was reported between 99.85-100.8%, however, the present method was found in the range of 99.51% to 102.16% recovery and 0.39 to 0.26 % RSD, respectively [41]. The precision of the method was found satisfactory as compared to previously reported methods reported for the estimation of Lapatinib and degraded products using HPLC-MS [42]. In the earlier studies, performed by LC-MS/MS and UPLC-MS-MS, the RSD percentages varied from 3.9 to 8.1%, and 2.84% while in the present method the maximum RSD was calculated to be 1.63% intra-day and 1.92% inter-day precision respectively [43]. The % RSD values on the assay of LPT from two different laboratories by two analysts were not more than 1.8%, which indicates the ruggedness of the developed method. The system suitability parameters were theoretical plates per meter 5095, height equivalent to theoretical plates 0.0056, and tailing factor 0.95. The percentage assay for LPT-loaded liposomes was found to be 99.03%. Further, no interfering peaks were observed in the chromatogram of LPT-loaded liposome, clearly indicating that there was no interference in the excipient used in liposome formulation. The developed method is convenient and effective for quality control as well as routine analysis of LPT in the pharmaceutical dosage form.

CONCLUSION

A new chemometrics-assisted RP-HPLC method was developed and validated according to ICH guidelines for quantification of LPT in newly developed nano-liposome formulation using a Quality by Design approach. In the experimental design, Analysis of Variance (ANOVA) was performed to study the significance of independent factors like Mobile phase, flow rate, and wavelength on response or dependent factors such as retention time (Y_1), peak area (Y_2), and a number of theoretical plates (Y_3) in minimal experimental runs. The Design of Experiment (DOE) has been employed during the development of this method to minimize retention time (4 min) and optimize peak area and peak asymmetry. The model equation has shown good agreement between predicted values and observed values. The assay for the LPT-loaded liposome formulation was found to be more accurate and reliable. The polynomial regression data for the calibration plots exhibited a linear relationship (r^2 = 0.999) over a concentration range of 10-50µg/ml. The % RSD for both intra-day and inter-day precision was found to be less than 2%. All the validation parameters showed satisfactory results. The present method was found to be simple, rapid, linear, accurate, precise, rugged, robust, and effective for analyzing the LPT in its formulation. The study proved that chemometrics can be effectively coupled with chromatography to enhance the separation process. Hence the validated RP-HPLC method can be readily adapted for the estimation of LPT in formulations with good recoveries.

ACKNOWLEDGEMENT

All contributing authors are thankful and express gratitude to the respected Head of the Institution, for providing the necessary facilities to support the successful completion of this research work.

FUNDING

This research work is supported by the Department of Science and Technology (DST) New Delhi, India in the form of Women Scientist Scheme B under Grant [DST/WOSB/2018/1016/ETD/Priyanka (G), dated 17.10.2019].

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report that there is no conflict of interest

REFERENCES

1. Beg S, Sharma G, Katare OP, Lohan S, Singh B. Development and validation of a stability-indicating liquid chromatographic method for estimating olmesartan medoxomil using quality by design. J Chromatogr Sci. 2015;53(7):1048-59. doi: 10.1093/chromsci/bmu165, PMID 25583970.
2. The International Conference on Harmonization ICH technical requirements for registration of pharmaceuticals for human use on pharmaceutical. Development. 2009;Q8(R2). https://database.ich.org/sites/default/files/Q8%28R2%29%20Guideline.pdf

3. The international Conference on Harmonization ICH technical requirements for registration of pharmaceuticals for human use on quality. Risk Manag. 2005;Q9. https://database.ich.org/sites/default/files/Q9%20Guideline.pdf.
4. The international Conference on Harmonization ICH technical requirements for registration of pharmaceuticals for human use on pharmaceutical quality system; 2008. Available from: https://database.ich.org/sites/default/files/Q10%20Guideline.pdf.
5. Borman P, Nethercote P, Chatfield M, Thompson D, Truman K. The application of quality by design to analytical methods. Pharm Technol. 2007;31:142-52.
6. Schweitzer M, Pohl M, Hanna BM, Nethercote P, Borman P, Hansen G. Implications and opportunities of applying QbD principles to analytical measurements. Pharm Technol. 2010;34:52-9.
7. Galen WE. Analytical instrumentation handbook. 2nd ed. New York: Marcel Dekker Inc; 2004.
8. Snyder L, Kirkland J, Glajch L. New York: John Wiley & Sons Inc. 2nd ed. Practical HPLC method development; 2014. p. 859. doi: 10.1002/978111.
9. Bossunia MTI, Urmi KF, Shaha CK. Quality-by-design approach to stability indicating RP-HPLC analytical method development for estimation of canagliflozin API and its validation. PHME. 2017;8(2):92-101. doi: 10.5530/phm.2017.8.15.
10. Sandhu PS, Beg S, Katare OP, Singh B. QbD-driven development and validation of a HPLC method for estimation of tamoxifen citrate with improved performance. J Chromatogr Sci. 2016;54(8):1373-84. doi: 10.1093/chromsci/bmw090.
11. Mondal S, Biswal S, Acharya T, Mondal P, Bhar K. Determination of lapatinib in bulk and tablet dosage form using ultraviolet spectrophotometric and RP-HPLC analytical methods. Int J Pharm Investig. 2021;11(2):208-13. doi: 10.5530/ijpi.2021.2.37.
12. Kumar KK, Nagoji KE, Nadh RV. A validated RP-HPLC method for the estimation of lapatinib in tablet dosage form using gemcitabine hydrochloride as an internal standard. Indian J Pharm Sci. 2012;74(6):580-3. doi: 10.4103/0250-474X.110621, PMID 23798787.
13. Haribabu B, BalaMurali KK, Rama KP. Development and validation of HPLC method for the estimation of lapatinib in bulk drugs and pharmaceutical formulations. Int J Res Rev Pharm Appl Sci. 2011;1:207-14.
14. Reehana SK, Sujana K. LC-MS/MS characterization of forced degradation products of tucatinib, a novel tyrosine kinase inhibitor: development and validation of RP-HPLC method. Int J Appl Pharm. 2022;14(1):58-66. doi: 10.22159/ijap.2022v14i1.43252.
15. Palnati N, Kotapati N, Vaidyanathan G. Liquid chromatography–mass spectrometry/mass spectrometry method for the determination of lapatinib in rat plasma: application to pharmacokinetic studies in wistar rats. Asian J Pharm Clin Res. 2021;14(2):74-7. doi: 10.22159/ajpcr.2021.v14i2.39660.
16. Bai F, Freeman BB 3rd, Fraga CH, Fouladi M, Stewart CF. Determination of lapatinib (GW572016) in human plasma by liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS). J Chromatogr B Anal Technol Biomed Life Sci. 2006;831(1-2):169-75. doi: 10.1016/j.jchromb.2005.11.044, PMID 16364699.
17. Haouala A, Zanolari B, Rochat B, Montemurro M, Zaman K, Duchosal MA. Therapeutic drug monitoring of the new targeted anticancer agents imatinib, nilotinib, dasatinib, sunitinib, sorafenib and lapatinib by LC tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877(22):1982-96. doi: 10.1016/j.jchromb.2009.04.045, PMID 19505856.
18. Sandra R, Gillian McM, Martin C, Robert OC. Development and application of novel analytical methods for molecularly targeted cancer therapeutics. J Chromatogr B. 2009;877:3982-90.
19. Götze L, Hegele A, Metzelder SK, Renz H, Nockher WA. Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma. Clin Chim Acta. 2012;413(1-2):143-9. doi: 10.1016/j.cca.2011.09.012, PMID 21945732.
20. Couchman L, Birch M, Ireland R, Corrigan A, Wickramasinghe S, Josephs D. An automated method for the measurement of a range of tyrosine kinase inhibitors in human plasma or serum using turbulent flow liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2012;403(6):1685-95. doi: 10.1007/s00216-012-5970-2, PMID 22526649.
21. Bouchet S, Chauzit E, Ducint D, Castaing N, Canal Raffin M, Moore N. Simultaneous determination of nine tyrosine kinase inhibitors by 96-well solid-phase extraction and ultra performance LC/MS-MS. Clin Chim Acta. 2011;412(11-12):1060-7. doi: 10.1016/j.cca.2011.02.023, PMID 21345336.
22. Rakic T, Kasagic Vujanovic I, Jovanovic M, Jancic Stojanovic B, Ivanovic D. Comparison of full factorial design, central composite design, and box-behnken design in chromatographic method development for the determination of fluconazole and its impurities. Anal Lett. 2014;47(8):1334-47. doi: 10.1080/00032719.2013.867503.
23. Bezerra MA, Santelli RE, Oliveira EP, Villar LS, Escaleira LA. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. Talanta. 2008;76(5):965-77. doi: 10.1016/j.talanta.2008.05.019, PMID 18761143.
24. Narahari P, Vijayaraj S, Lathasri K, Archana D, Rajavel P. Chemometric approach to develop and validate RP-HPLC method for estimation of erlotinib hydrochloride in nano structured lipid carriers. Curr Pharm Anal. 2019;15:1-6.
25. Ferreira SLC, Caires AO, Tda BS, Lima AMDS, Silva LOB, dos Santos WNL. Robustness evaluation in analytical methods optimized using experimental designs. Microchemical Journal. 2017;131:163-9. doi: 10.1016/j.microc.2016.12.004.
26. Shrivastava R, Trivedi S, Singh PK, Asif M, Chourasia MK, Khanna A. Design and development of pegylated liposomal formulation of HER2 blocker Lapatinib for enhanced anticancer activity and diminished cardiotoxicity. Biochem Biophys Res Commun. 2018;503(2):677-83. doi: 10.1016/j.bbrc.2018.06.060, PMID 29908185.
27. Charumathy A, Ubaidulla U, Sinha P, Rathnam G. Recent update on liposome-based drug delivery system. Int J Curr Pharm Sci. 2022;14(3):22-7. doi: 10.22159/ijcpr.2022v14i3.1991.
28. Kumar KK, Nagoji KEV, Nadh RV. A validated RP-HPLC method for the estimation of Lapatinib in tablet dosage form using gemcitabine hydrochloride as an internal standard. Indian J Pharm Sci. 2012;74(6):580-3. doi: 10.4103/0250-474X.110621, PMID 23798787.
29. Singh B, Kumar R, Ahuja N. Optimizing drug delivery systems using systematic "Design of Experiments." Part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst. 2005;22(1):27-105. doi: 10.1615/CritRevTherDrugCarrierSyst.v22.i1.20.
30. Beg S, Chaudhary V, Sharma G, Garg B, Panda SS, Singh B. QbD-oriented development and validation of a bioanalytical method for nevirapine with enhanced liquid–liquid extraction and chromatographic separation. Biomed Chromatogr. 2016;30(6):818-28. doi: 10.1002/bmc.3613, PMID 26352459.
31. Merey HA, Galal MM, Salem MY, Abdel-Moety EM. Novel stability indicating methods for the determination of certain synthetic estrogen level modifiers. Bull Fac Pharm Cairo Univ. 2013;51(1):69-79. doi: 10.1016/j.bfopcu.2012.11.001.
32. Bhoop BS, Beg S, Raza K. Developing "optimized" drug products employing "designed" experiments. Chemical Industry Digest. 2013;23:70-6.
33. Kaur R, Saini S, Sharma T, Katare OP, Kaushik A, Singh B. Implementation of analytical quality-by-design for developing a robust HPLC method for quantitative estimation of voriconazole: application in drug formulations. Anal Chem Lett. 2021;11(2):168-86. doi: 10.1080/22297928.2021.1888793.
34. Sandhu PS, Beg S, Katare OP, Singh B. QbD-driven development and validation of a HPLC method for estimation of tamoxifen citrate with improved performance. J Chromatogr Sci. 2016;54(8):1373-84. doi: 10.1093/chromsci/bmw090.
35. IHT Guideline. Validation of analytical procedures: text and methodology Q2 (R1); 2005. http://www.Ich.org/cache/compo/ 363-272-1 html.
36. Shimek JL, Rao NGS, Khalil SKW. High performance liquid chromatographic analysis of tolmetin, indomethacin and

sulindac in plasma. J Liq Chromatogr. 1981;4(11):1987-2013. doi: 10.1080/01483918108067556.

37. Muller IB, Windberg CN. Validation of an HPLC method for quantitation of MDMA in tablets. J Chromatogr Sci. 2005;43(8):434-37. doi: 10.1093/chromsci/43.8.434, PMID 16212786.
38. Vander Heyden Y, Nijhuis A, Smeyers Verbeke J, Vandeginste BG, Massart DL. Guidance for robustness/ruggedness tests in method validation. J Pharm Biomed Anal. 2001;24(5-6):723-53. doi: 10.1016/s0731-7085(00)00529-x, PMID 11248467.
39. Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. J Chromatogr A. 2003;987(1-2):57-66. doi: 10.1016/s0021-9673(02)01536-4, PMID 12613797.
40. Saadat E, Dehghan Kelishady P, Ravar F, Kobarfard F, Dorkoosh FA. Development and validation of rapid stability-indicating RP-HPLC-DAD method for the quantification of lapatinib and mass spectrometry analysis of degraded products. J Chromatogr Sci. 2015;53(6):932-9. doi: 10.1093/chromsci/bmu150, PMID 25491314.
41. Panda SS, Bera RKVV, Behera AK, Beg S. Chemometrics-assisted development of a liquid chromatography method for estimation of lapatinib in tablets: A case study on a novel quality concept. Sep Sci Plus. 2020;3(1-2):12-21. doi: 10.1002/sscp.201900096.
42. Saadat E, Ravar F, Dehghankelishadi P, Dorkoosh FA. Development and validation of a rapid RP-HPLC-DAD analysis method for the simultaneous quantitation of paclitaxel and lapatinib in a polymeric micelle formulation. Sci Pharm. 2016;84(2):333-45. doi: 10.3797/scipharm.1507-03, PMID 27222608.
43. Alrobaian M, Panda SS, Almalki WH, Afzal O, Kazmi I, Alossaimi MA. Development and validation of chemometrics-assisted green UPLC-MS/MS bioanalytical method for simultaneous estimation of capecitabine and lapatinib in rat plasma. J Chromatogr Sci. 2022;60(6):559-70. doi: 10.1093/chromsci/bmab094, PMID 34318311.