Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

Development and Validation of Stability - Indicating Rp - Hplc Method for Assessment of Lapatinib in Bulk and Marketed Formulation

Lokesh Thote* Jagdish Baheti

Corrosponding Author

Mr. Lokesh T. Thote, Kamla Nehru College of Pharmacy, Bitibori, Nagpur – 44108.

(Received: 27 October 2025 Revised: 05 November 2025 Accepted: 04 December 2025)

KEYWORDS

Lapatinib, stability indicating assay method, RP – HPLC, method development

ABSTRACT:

Introduction

Lapatinib is an anticancer agent requiring reliable analytical methods for quality control and stability assessment. The development of a stability-indicating RP-HPLC method is essential to ensure accurate quantification in bulk and marketed formulations, even in the presence of degradation products.

Objective

To develop and validate a simple, precise, and robust stability-indicating RP-HPLC method for the quantitative estimation of Lapatinib in bulk drug and tablet dosage forms, and to confirm its ability to separate the drug from its degradation products.

Method

Chromatographic separation was performed using a C18 column with an isocratic mobile phase consisting of Acetonitrile: Water (pH 2.6): TEA in the ratio 40:60:0.1 (v/v). The flow rate was maintained at 1.0 mL/min, and detection was carried out at 260 nm. The method was validated as per ICH Q2(R1) guidelines for specificity, linearity, precision, accuracy, robustness, LOD, and LOQ. Forced degradation studies were conducted under acidic, basic, oxidative, thermal, and photolytic conditions.

Results

The method produced a sharp, well-resolved peak for Lapatinib with a retention time of approximately 4.853 minutes. Linearity was achieved in the concentration range of 25–125 µg/mL, with a correlation coefficient (R^2) greater than 0.999, demonstrating excellent linearity. Forced degradation studies confirmed that the developed method could effectively separate Lapatinib from its degradation products, establishing its stability-indicating nature.

Conclusion

The developed RP-HPLC method is simple, sensitive, precise, and robust. Its proven ability to detect and separate degradation products makes it highly suitable for routine quality control and stability testing of Lapatinib in bulk and pharmaceutical formulations.

INTRODUCTION

Lapatinib is a potent and promising inhibitor of human epidermal growth factor receptor–2 or EGFR(epidermal growth factor receptor), which is involved in tumour growth and angiogenesis.

Lapatinib, a tyrosine kinase inhibitor known for cancer breast chemotherapy for the treatment of molecular abnormalities that occur in cancer cell

By preventing self-phosphorylation and the corresponding activation of the signal mechanism through ligand attachment to the ATP-binding pockets of the EGFR/HER-2 protein kinase domains, lapatinib

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

acts by inhibiting the receptor signal activation mechanism.

Chemically lapatinib is "N-{3-chloro-4[(3-fluorophenyl) methoxy] phenyl}-6(5-{[(2-methane sulfonyl ethyl) amino] methyl} furan-2-yl) quinazolin-4-amine, with molecular formula $C_{29}H_{26}ClFN_4O_4S$. The structure" of Lapatinib is shown in Figure 1.

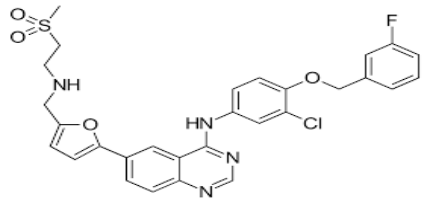

Figure1: Structure of lapatinib

Various analytical methods were published for the investigation of lapatinib in both pure form and marketed dose forms, according to a review of the scientific literature. The literature contains a variety of techniques for estimating lapatinib both singly and in various combinations. With full validation for the research of Lapatinib in bulk and marketed tablet dosage form, which is more affordable, precise, accurate, new, robust, and accurate than the early published methods, the current estimation was intended to produce stability indicating the HPLC approach.

1. OBJECTIVE

To develop and validate a simple, precise, and robust stability-indicating RP-HPLC method for the quantitative estimation of Lapatinib in bulk drug and tablet dosage forms, and to confirm its ability to separate the drug from its degradation products.

2. MATERIAL AND METHOD

Chemicals and reagents

The lapatinib bulk drug utilised for this research was kindly supplied as a sample by NATCO Pharma. Ltd. Hyderabad. Various chemicals like analytical grade methanol, ortho-phosphoric acid, dihydrogen phosphate and HPLC standard acetonitrile, water and tri - ethylamine amine were purchased from Loba Chemie Pvt. Ltd. Mumbai.

Instruments, Make and Model

High-Performance Liquid Chromatography – Shimadzu, LC 20AD

UV – Visible spectrophotometer – Shimadzu 1800

Weighing balance – ATX – 225

Ultrasonicator - Mvtex

Formation of bulk drug stock solution

The stock solution of the bulk drug of lapatinib have been prepared by mixing 10mg accurately weight lapatinib with methanol in a 100ml volumetric flask up to the mark.

Identification of detection wavelength

From the aforementioned stock solution, 1ml of solution have been pipetted out as well as diluted to 10ml with methanol and scanned within the electromagnetic range of 200nm-400nm to observe absorption performance. It was observed drug shows maximum absorption at 260nm.

Mobile phase composition and chromatographic conditions

The optimisation of mobile phase and chromatographic condition

Several trials for the development of a chromatogram of lapatinib working solution (50µg/ml) were carried out. The optimisation of the chromatogram was carried out with acetonitrile and water in varying ratios and at different pH to get a good peak and satisfactory system suitability parameters. After different trials, acetonitrile: water (pH 2.6): TEA (40:60:0.1v/v) have been selected as mobile phase as it shows good peak and acceptable peak characteristics (Table No. 1)

Table 1: Optimisation of mobile phase and chromatographic condition

S. N.	Parameters	Specific conditions for analysis
	Mobile phase ratio	Acetonitrile: water (pH 2.6): TEA (40:60:0.1 v/v)
	Elution rate	1 ml/min
	Wavelength of detection	260 nm
	The volume of sample injection	20 µl
	Column	C18column(5µm, 4.6mmX250)
	Temperature of	Ambient temperature

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

	column	

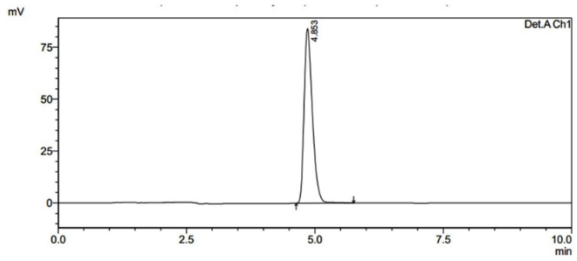

Figure 2: Lapatinib optimises chromatogram with a retention time of 4.853 min.

Chromatogram of bulk lapatinib drug and Parameters of System Suitability

Lapatinib bulk drug working solution (50µg/ml) was injected into the system. The retention period noted for each successive injection was found to be 4.213±0.067 minutes shown in Table 2. The chromatogram for the corresponding injection is provided in Figure 3.

Assay for marketed formulation

Twenty lapatinib tablets were accurately weighed and fully triturated, and then the tablet power proportionates to ten milligrams of lapatinib have been weighed & passed on into a 100 ml volumetric flask. Sixty millilitres of methanol was then added, and the mixture was agitated for fifteen minutes in order to provide a volume of 100millimetres with methanol. The flask was kept for sonication for 20minutes. After sonication, undissolved particles were removed by filtration through the Whatman filter paper 40. After filtration, the volume of the solution have been adjusted up to 100ml. Methanol was added to the aforementioned solution to bring the concentration of lapatinib down to 50µg/ml. The whole operation was repeated six times, and the assay of lapatinib was estimated from the graph of peak response and linearity equation.

Verification of the Analytical Process

System suitability

To assess the best constraints—such as resolution, tailing factors, and theoretical plate—on a freshly formulated standard working solution of lapatinib, system suitability constraints were applied.

Accuracy

The method's accuracy was assessed using the conventional standard addition technique. The mean of recovery for lapatinib was estimated using concentrations of lapatinib at different levels (80, 100 and 120%).

Precision

An intraday precision of lapatinib have been studied on the same day. Interday precision of lapatinib was studied at a similar concentration on 3 different days by different operators. The standard solution have been injected six times, along with using HPLC, the area of each injection was determined.

Intraday precision of lapatinib was carried out in a single day. On the other hand, interday precision was carried out on three different days by different operators on the same concentration. Six injections of the standard working solution were made, and the peak area of each sample was determined using RP-HPLC.

Linearity

The study of the linearity of lapatinib solution was carried out by preparing a stock solution of lapatinib in the mobile phase to obtain the range of concentration is 25-125µg/ml. The study of linearity was studied by linear regression analysis.

Detection limit and quantitation limit

As recommended by ICH guidelines, the LoD as well as LoQ were determined. To estimate the result of LoD as well as LoQ, a graph of the calibration curve has been prepared by plotting the obtained value of area under every level's curve against the concentration.

Forced degradation studies

For analysis of the intrinsic stability of drug substances, ICH suggested and recommended stability testing of drug substances. In this research, many methods of degradation were applied to the standard solution, such as degradation by acid, photolytic degradation, hydrolysis, peroxide degradation, alkali degradation, and heat degradation. The areas of the peak of each stressed condition sample were estimated, and a comparison was done with the lapatinib peak areas of standard samples.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

3. RESULTS

Several trials for the development of a chromatogram of lapatinib working solution (50µg/ml) were carried out. The optimisation of the chromatogram for the stability-indicating assay method was carried out with acetonitrile and water in varying ration, flow rates and at different pH to get good peak and satisfactory system suitability parameters. After different trials acetonitrile: water (pH 2.6): TEA (40:60:0.1v/v) have been selected as mobile phase as it produced defined peak and acceptable peak characteristics. The well-defined resolved peak of lapatinib was eluted at a time of retention of 4.853 minutes.

Test for System Suitability

The test for system suitability is necessary for analysing analytical methods as well as verifying peak of interest resolution among other peaks. The tailing factor, as well as the number of theoretical plates under the circumstances listed in Table 1, indicate the method worked.

Table 2: System suitability parameters of lapatinib

Parameters	**Lapatinib**
Retention time	4.852
Plate count	4240.737
Tailing factor	1.417
% RSD	0.49

Specificity

Lapatinib had a retention time of 4.852 minutes. This approach did not reveal any interfering peaks in the drug substance's retention period, including those of the placebo and blank. This process has been recognised for being very particular. Figures 3 and 4 show the chromatograms for the sample and blank.

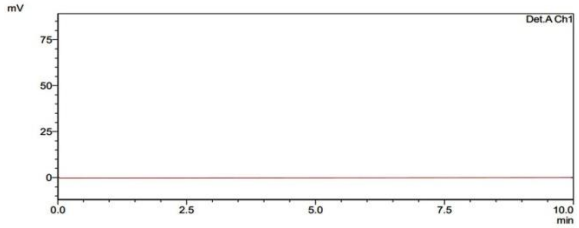

Figure 3: Blank chromatogram

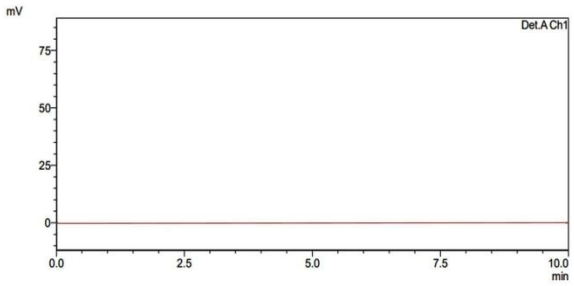

Figure 4: A chronogram of a placebo

LoD and LoQ

The values obtained for lapatinib's limit of quantification along with the limit of detection had been 1.35µg/ml & 0.45µg/ml, respectively.

Linearity

Lapatinib's linearity range was examined between 25 & 125µg/ml in concentration. The method's linearity within the examined range was demonstrated by the calibration curve data. Figure 5 illustrates a lapatinib linear response. Table 2 lists the lapatinib findings.

Table 3: Linearity response table for lapatinib

S. N.	**Lapatinib**	
	Concentration (µg/ml)	**Peak area**
1.	25	523106
2.	50	1025202
3.	75	1453918
4.	100	2045213
5.	125	2495350
Regression equation		y = 19858x + 19208
Slope		19857.996
Intercepts		19208.1
R^2		0.998

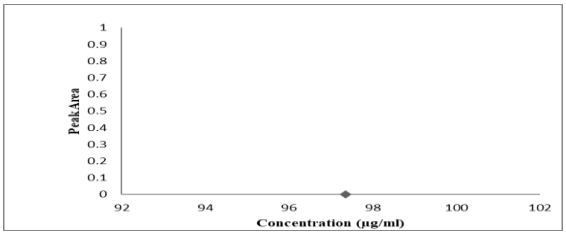

Figure 5: Lapatinib calibration curve at 260 nm

Precision

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

The total % RSD, i.e. one of the findings of the precision study, was below 2, indicating that the precision study was satisfactorily achieved within the given limit. The findings of method precision study and technique precision are presented in Table 3.

Table 4: Precision study result of lapatinib (50µg/ml)

Parameters	System precision	Repeatability	Intermediate precision
Mean	1028109	1024304	102100
SD	5763.680	12160.70	9827.55
% RSD	0.21	0.44	0.36

Table 5: Result of lapatinib accuracy determined using the HPLC method

% concentration	Average area	Average % recovery	Mean % recovery
80	2293.2467	99.81	100.19
	2274.6671	98.99	
	2359.1671	101.84	
100	2799.4381	99.25	99.72
	2785.6102	98.71	
	2854.7217	101.21	
120	3198.9381	101.73	99.96
	3128.8149	99.51	
	3101.7718	98.64	

Forced degradation studies

The stress conditions degradation approach was carried out in various stress conditions, for example, degradation under acidic conditions, degradation under basic conditions, etc. The peak of degradation products under different stress conditions and the findings of stability studies are shown in Table 5 and Figures 6 – 12, respectively.

In this research, separation and analysis of lapatinib were successfully achieved by the HPLC method. For this mobile phase containing a mixture of acetonitrile: water (pH 2.6): TEA (40:60:1v/v) was utilised. At these suppositions, lapatinib had a strong peak with a time of retention of 4.853 minutes. The lapatinib expressed linearity under the concentration series range of 25 to 125 µg/ml. The percentage assay of the marketed formulation of lapatinib was recovered to be 99.6%. Hence, an established HPLC procedure was claimed to routine deterioration behaviour study of lapatinib. It was also found that lapatinib was more easily degraded in stress conditions of alkaline, acidic thermal and peroxide environments.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

Table 6: Stress degradation study results for lapatinib

Degradation conditions	% Assay	% Degradation	Purity angle	Purity threshold
Acid	84.82	5.62	0.452	7.566
Alkali	80.21	4.19	0.445	7.639
Peroxide	88.54	2.54	0.462	7.661
Photolytic	99.87	3.96	0.412	7.665
Thermal	99.51	7.06	0.428	7.666

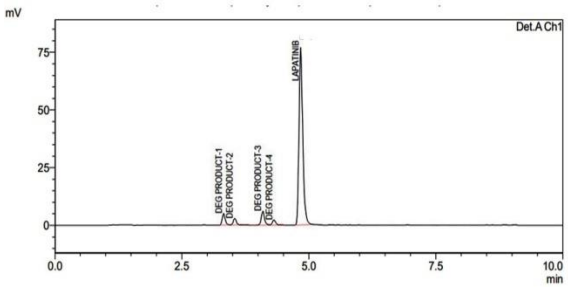

Figure 6: Acid degradation

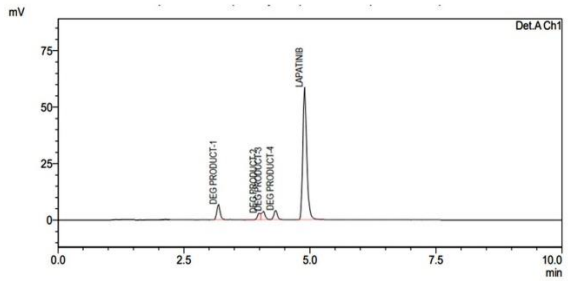

Figure 7: Alkali degradation

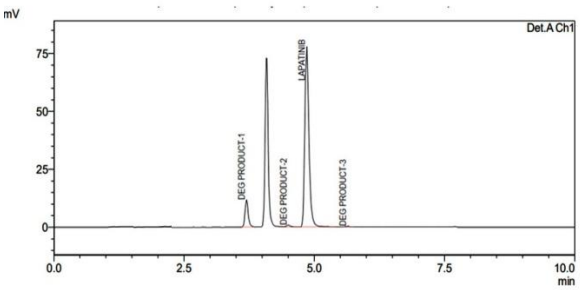

Figure 8: Peroxide degradation

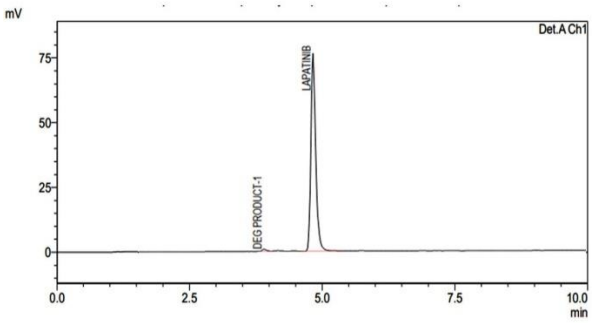

Figure 9: Photolytic degradation

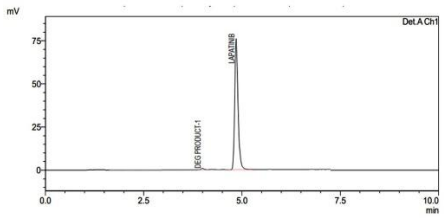

Figure 10: Thermal degradation

4. DISCUSSION

The validation of the proposed method has been successfully performed as per ICH guidelines as well as applicable to routine analysis procedures. The validation results of the established study indicate that the proposed degradation pathway was used to quantify lapatinib in both pharmaceutical dosage forms as well as bulk drugs. The proven technique is noted for its simplicity, accuracy, as well as precision. The statistical analysis of this technology demonstrates its acceptable linearity along with the validity of several factors.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(6), 2001-2007 | ISSN:2251-6727

REFERENCES

1. Konecny GE, Pegram MD. Lapatinib: A novel dual tyrosine kinase inhibitor of HER2 and EGFR. Expert Opin Pharmacother. 2009;10(11):1867-80. doi: 10.1517/14656566.10.11.1867.
2. Gianni L, Pienkowski T, Im YH, et al. Efficacy of lapatinib plus capecitabine in women with trastuzumab-resistant metastatic breast cancer: a randomized trial. Lancet Oncol. 2010;11(3):263-70. doi: 10.1016/S1470-2045(09)70332-0.
3. Arteaga CL, Sliwkowski MX. The epidermal growth factor receptor: a promising target for cancer therapy. Nat Rev Cancer. 2011;11(8):514-28. doi: 10.1038/nrc3088.
4. Sweeney C, Schmitt M. Lapatinib: a dual inhibitor of HER2 and EGFR. Curr Oncol Rep. 2009;11(3):208-13. doi: 10.1007/s11912-009-0027-6.
5. Roché J, Coates J. Lapatinib: Mechanisms of action and clinical efficacy. Cancer Treat Rev. 2011;37(3):215-22. doi: 10.1016/j.ctrv.2010.06.007.
6. Kumar M, Kumar R. Lapatinib: A potent dual inhibitor of HER2 and EGFR. Expert Opin Pharmacother. 2009;10(6):1057-70. doi: 10.1517/14656566.10.6.1057.
7. Sinha S, Tiwari A. Analytical methods for determination of lapatinib in pharmaceutical formulations. J Pharm Anal. 2014;4(3):169-77. doi: 10.1016/j.jpha.2014.05.001.
8. Kaur G, Arora A. Development and validation of a stability-indicating HPLC method for lapatinib in bulk and formulations. J Liq Chromatogr Relat Technol. 2016;39(12):617-25. doi: 10.1080/10826076.2016.1147928.
9. Pahlavan P, Mohammadi M. Recent advances in analytical methods for lapatinib determination in pharmaceutical formulations. Pharm Chem J. 2018;52(6):583-91. doi: 10.1007/s11094-018-0785-1.
10. Sahu SK, Sahu RK. System suitability testing and its significance in HPLC method development for the estimation of pharmaceutical formulations. J Pharm Sci Res. 2018;10(5):1223-30.
11. Tiwari A, Sinha S. Evaluation of accuracy and precision of HPLC method for the determination of lapatinib in pharmaceutical formulations. Asian J Chem. 2015;27(1):125-30. doi: 10.14233/ajchem.2015.17804.
12. Ghosh S, Ray S. Intraday and interday precision studies of lapatinib using RP-HPLC. Pharm Methods. 2016;7(2):62-7. doi: 10.4103/2229-4708.186036.
13. Patel KM, Patel MP. Establishment of linearity and validation of HPLC method for estimation of lapatinib in bulk and formulations. Int J Pharm Sci Res. 2017;8(8):3344-50. doi: 10.13040/IJPSR.0975-8232.8(8).3344-50.
14. Bhalerao S, Shinkar DM. Determination of limit of detection and limit of quantitation for lapatinib using HPLC. J Appl Pharm Sci. 2018;8(3):52-7. doi: 10.7324/JAPS.2018.8309.
15. Kaur G, Gupta R. Forced degradation studies of lapatinib: stability indicating method development. J Chem Pharm Res. 2015;7(10):129-36.