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Research Article

RP-HPLC Method Development and Validation for Simultaneous Estimation of Erlotinib and Ramucirumab in Bulk and Pharmaceutical Dosage Form and its Stability Studies

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ABSTRACT

The present study shows an easy, quick, and reliable reverse-phase high-performance liquid chromatography method for measuring both erlotinib and ramucirumab. For the separation and quantification, an Inertsil column (150 × 4.6 mm, 3.5 µ) is used and a mobile phase comprising acetonitrile and 0.1% formic acid (50:50) combination was utilized. A UV detector was used to locate the eluents at 236 nm, and the flow rate was 1.0 mL/min. The retention times were found to be 2.936 and 4.535 minutes, respectively. The suggested approach was validated in accordance with ICH guidelines Q2 (R1), and it was found that the linearity of the findings fell between 2.50 and 15.00 µg/mL for ramucirumab and between 37.50 and 225.00 µg/mL for erlotinib. Erlotinib and ramucirumab demonstrated recovery rates of 99.6 and 100.1%, respectively. The robustness, accuracy, precision, and linearity range of the proposed method were all validated.

Introduction

Erlotinib (Fig. 1) is used to treat a variety of cancers, including non-small cell lung cancer and pancreatic cancer.[1,2] It attaches itself reversibly to the receptor's adenosine triphosphate binding site. It has recently been demonstrated that erlotinib is a strong inhibitor of JAK2V617F activity.[3] Tyrosine kinase JAK2 mutation JAK2V617F is present in a significant fraction of individuals with essential thrombocythemia, idiopathic myelofibrosis, and polycythemia vera patients. JAK2V617F-positive PV and other myeloproliferative diseases can be treated with erlotinib.[4,5] Because of its promising effects in addressing several solid tumor types, it has garnered a great deal of interest and attention.[6,7] For the treatment of gastroesophageal junction adenocarcinoma, the FDA authorized ramucirumab on April 21, 2014, either alone or in combination with paclitaxel (Fig. 2). Numerous analytical techniques are available in the literature to determine erlotinib or ramucirumab separately.[8] So present study was done to develop and validate a simple reversed-phase high-performance liquid chromatography (RP-HPLC) method using a photodiode array (UV-PDA) detector to simultaneously quantify erlotinib and ramucirumab.

Materials And Methods

Chemicals and Reagents

For this work, a Waters Alliance HPLC system with a 2695 pump with Empower 2 software, an auto-injector, a UV detector, a Shimadzu UV-visible spectrophotometer, and a Phoenix 4.5 L digital ultrasonic cleaner was utilized.

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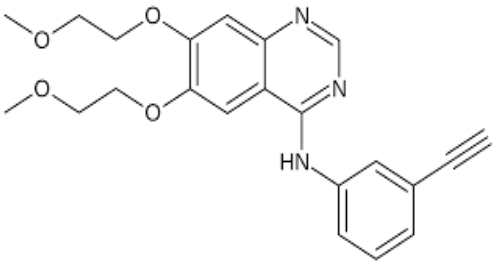

Fig. 1: Chemical structure of erlotinib

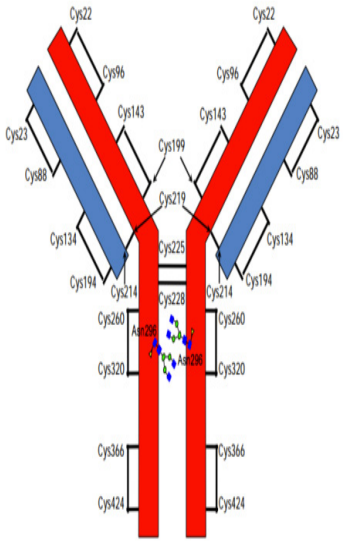

Fig. 2: Chemical structure of ramucirumab

The erlotinib and ramucirumab was procured from the Pharma Life Research facility located in Hyderabad, India. Additionally, the chemicals used were of AR-grade, as reported by Rankem Chemicals, India.

Establishment of a Standard Solution

Accurately weigh out 0.15 g of erlotinib and 0.01 g of ramucirumab into a 100 mL volumetric flask that has been kept dry and clean. After that, include the diluent and use sonication to ensure complete dissolution of the entire mixture. Finally, ensure that this is done correctly by using the same solvent. The stock solution that was talked about earlier should be in 5 mL of the 50 mL volumetric flask. This solution needs to be diluted with diluents until the desired final volume was reached. The final concentration of erlotinib is 150 ppm, while the concentration of ramucirumab is 10 ppm.

Preparation of Working Solution

Proceed to precisely quantify and transfer 224 mL of erlotinib and 1-mL of ramucirumab into a 100 mL dry volumetric flask. To make sure the solution dissolves completely, dilute it first, then sonicate it for up to 30 minutes. To help the mixture dissolve even more, centrifuge it for 30 minutes. Lastly, use the same solvent to raise the volume to the appropriate amount. It is then run through an injection filter (stock solution) with a 0.45-micron opening. Carefully transfer 5 mL of the stock solutions into a 50 mL volumetric flask, and then dilute it to the desired concentration using the diluents (150 ppm for erlotinib and 10 ppm for ramucirumab).

Method Validation[9-12]

The reproducibility of the chromatographic system is verified through system suitability tests. The effectiveness of the system was determined by checking specific parameters of the system suitability test. The drug solution was injected multiple times at specific concentrations to ensure the system's reproducibility. About 150 µg/mL of erlotinib and 50 µg/mL of ramucirumab were the concentrations utilized.

To investigate the linearity of erlotinib and ramucirumab at different concentrations, an investigation was carried out. Confirm that there is a linear connection between the response of the detector and the concentrations of erlotinib (37.50–225.00 µg/mL) and ramucirumab (2.50–15.00 µg/mL). Give duplicate doses of each preparation to the HPLC system. Create a graph showing the association between the concentration and the average area response of injections made in triplicate. Furthermore, ascertain the Y-intercept's percentage in relation to the response at the 100% level. [13,14]

The method's accuracy and recovery were assessed by determining the proportion of erlotinib and ramucirumab that were successfully recovered. Recovery tests were conducted for both medications using the usual addition approach. This involved applying the methodology to drug samples that had a known quantity of erlotinib and ramucirumab, equivalent to 50%, (75 ppm of erlotinib 5 ppm of ramucirumab) 100%, (150 ppm of erlotinib and 10 ppm of ramucirumab) and 150% (225 ppm of erlotinib 15 ppm of ramucirumab) of the label claim. At each level of the quantity, three determinations were conducted, and the acquired findings were compared. Research was conducted to determine the precision of erlotinib and ramucirumab, both interday and intraday. This was achieved by conducting three measurements of the pertinent responses on three separate days and on the same day, employing erlotinib at a concentration of 150 µg/mL and ramucirumab at 50 µg/mL.[15,16]

Limit of detection and quantitation

The lowest amount of analyte that the technology can dependably detect but which isn't always measured precisely. Looks like the lowest quantitatively measured analyte concentration that can be done with respectable accuracy and precision.

Assay

Measure the regions for the peaks of erlotinib and ramucirumab after injecting 10 µL of the standard and sample into the chromatographic apparatus.

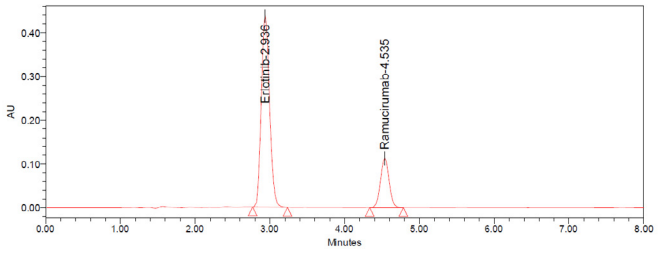

Fig. 3: Standard chromatogram of erlotinib and ramucirumab

Investigating forced degradation studies

The ICH recommendations suggest stress testing as a means of evaluating the intrinsic stability of medicinal ingredients. The solution of the standard underwent various degradation methods in these investigations, including "acid degradation, alkali degradation, peroxide degradation, thermal degradation, photolytic degradation, and hydrolysis".

Results And Discussion

A 50:50 mixtures of 0.1% formic acid and acetonitrile made up the mobile phase, which was designed to flow at a rate of 1-mL per minute. This resulted in the appearance of two distinct and well-defined peaks for erlotinib and ramucirumab, as shown in Fig. 3. The peaks exhibited minimal tailing factor, indicating a precise and accurate separation. The retention times for erlotinib and ramucirumab are 2.936 and 4.535 minutes, respectively. The UV spectra have peaks at 2.936 and 4.535 minutes, respectively. The calibration curve for erlotinib and ramucirumab demonstrates a linear relationship over the range of 37.50 to 225.00 and 2.50 to 15.00 µg/mL, respectively.

System Suitability

Validating analytical techniques and confirming resolution among numerous peaks of interest requires the system suitability test. Given the conditions outlined in Table 1, a tailing factor of less than 2 and a theoretical plate of greater than 2000 indicate that the technique was effective.

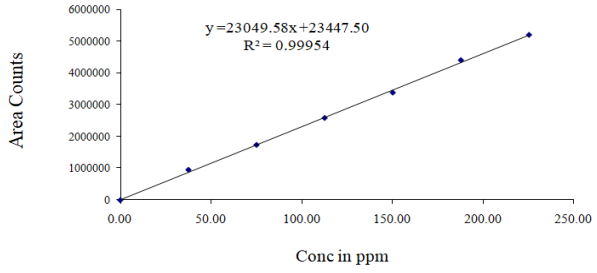

Fig. 4: Erlotinib calibration curve

Table 1: Parameters for evaluating the suitability of erlotinib and ramucirumab

Parameter	*Erlotinib 150 ppm*	*Ramucirumab 10 ppm*
Retention time	2.936	4.535
Plate count	3345	7245
Tailing factor	1.18	1.04
Resolution	----	7.56

Table 2: ETN and RAM results of linearity

ETN		*RAM*	
Conc. (µg/mL)	*Peak area*	*Conc. (µg/mL)*	*Peak area*
37.5	958085	2.5	69581
75	1744753	5	115651
112.5	2591560	7.5	178128
150	3392175	10	229473
187.5	4416503	12.5	295033
225.	5212600	15	349557

Limits on quantitation and detection

The limit of detection (LoD) and limit of quantitation (LoQ) values for erlotinib were 0.45 and 1.50 µg/mL, respectively. On the other hand, ramucirumab had a LoD of 0.03 µg/mL and a LoQ of 0.10 µg/mL.

Linearity

Linear response was obtained by preparing six different concentrations of erlotinib 37.50 to 225.00 µg/mL and ramucirumab 2.50 to 15.00 µg/mL. An erlotinib regression equation was determined to be y = 23049.58x + 23447.50, with an R2 value of 0.999. Ramucirumab regression equation was determined to be y = 23048.53x + 3910.75, with an R2 value of 0.999. Figs 4 and 5 provide graphs, and Table 2 displays the outcomes.

Precision

The precision investigation revealed that the total %RSD of system method precision was below 2, indicating effective achievement of precision within the specified limit. Tables 3 and 4 provide the outcomes of system precision and technique precision of erlotinib and ramucirumab.

Accuracy

Then recovery was estimated at 50% (75 ppm of erlotinib 5 ppm of ramucirumab) 100%, (150 ppm of erlotinib and 10 ppm of ramucirumab) and 150% (225 ppm of erlotinib 15 ppm of ramucirumab) of the selected concentrations. Erlotinib and ramucirumab recovery values were determined to be 99.6 and 100.1%, respectively, as stated in Table 5.

Robustness

The data on the resilience of both medications is shown in Table 6

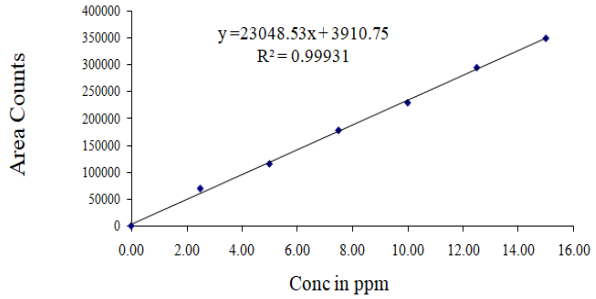

Fig. 5: Ramucirumab calibration curve

Table 3: Precision results of erlotinib (150 µg/mL)

Concentration erlotinib (150 µg/mL)			
Parameters	*System precision*	*Repeatability*	*Intermediate precision*
Mean area	2748189	3351468	3342005
S.D	5764.790	23174.702	24544.276
%RSD	0.21	0.69	0.73

Table 4: Precision results of ramucirumab (10 µg/mL)

Concentration ramucirumab (10 µg/mL)			
Parameters	*System precision*	*Repeatability*	*Intermediate precision*
Mean area	224434	224408	223991
S.D	3275.780	998.044	2450.928
%RSD	1.46	0.44	1.09

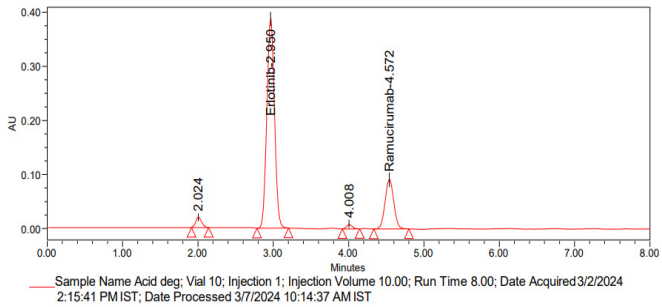

Fig. 6: Acid degradation chromatogram of erlotinib and ramucirumab

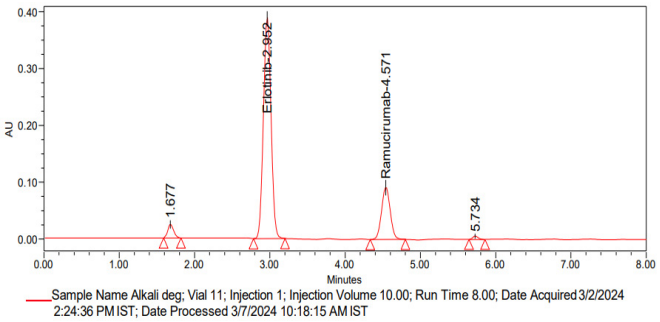

Fig. 7: Alkali degradation chromatogram of erlotinib and ramucirumab

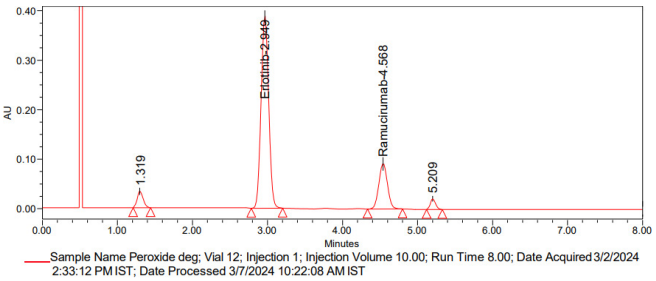

Fig. 8: Peroxide degradation chromatogram of erlotinib and ramucirumab

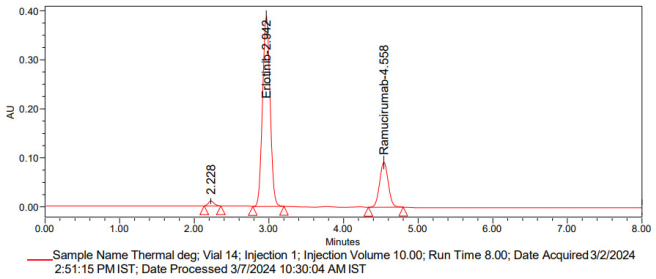

Fig. 9: Thermal degradation chromatogram of erlotinib and ramucirumab

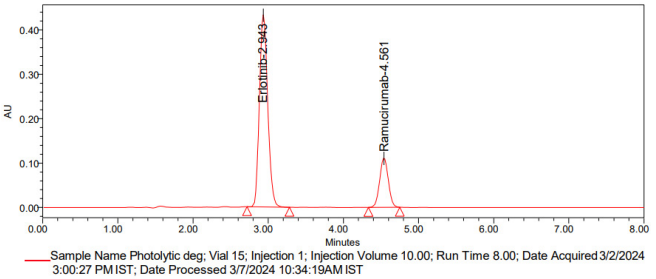

Fig. 10: Photolytic degradation chromatogram of erlotinib and ramucirumab

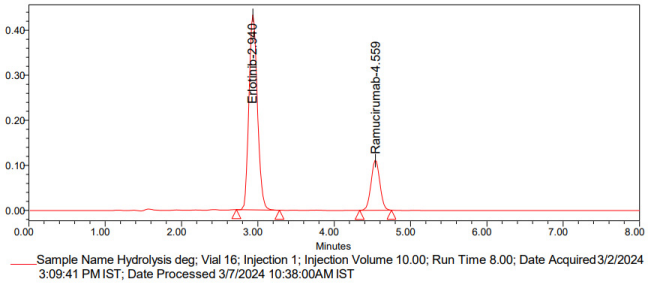

Fig. 11: Hydrolysis degradation chromatogram of erlotinib and ramucirumab

Forced degradation studies

The suggested HPLC approach was employed to periodically monitor the behavior of deterioration. Furthermore, the drug exhibited increased degradation when exposed to acidic, alkaline, peroxide, and thermal conditions. The elution of peaks of degradation occurred under various

Table 5: Accuracy data for erlotinib and ramucirumab

	Erlotinib		*Ramucirumab*	
%Concentration (at specification Level)	*%Mean Recovery*	*%RSD*	*%Mean Recovery*	*%RSD*
50	99.5	1.08	100.03	0.50
100	100.0	0.46	100.0	1.54
150	**99.2**	0.147	100.0	0.58

Table 6: ETN and RAM robustness results

		ETN		*RAM*	
Parameter	*Condition*	*Rt(min)*	*Area*	*Rt(min)*	*area*
Flow rate change (mL/min)	Less flow (0.9 mL)	3.664	3027452	5.747	208123
	Actual (1-mL)	2.936	3362885	4.535	224237
	More flow (1.1 mL)	2.464	3526947	3.854	249861
Organic phase change	Less Org (45:55)	3.434	2957416	6.829	191178
	Actual (50:50)	2.938	3353793	4.538	220356
	More Org (55:45)	2.673	3661411	3.489	233373

Table 7: Erlotinib with ramucirumab: Forced degradation results

	Erlotinib			*Ramucirumab*		
Results: %Deg. results	*Area*	*%Assay*	*%Deg*	*Area*	*%Assay*	*%Deg*
Acid	2889005	86.3	13.7	199267	88.7	11.3
Alkali	2862487	85.5	14.5	200180	89.1	10.9
Peroxide	2801366	83.6	16.4	194596	86.6	13.4
Reduction	3259487	97.3	2.7	197775	88.1	11.9
Thermal	3006043	89.8	10.2	215841	96.1	3.9
Photolytic	3319291	99.1	0.9	220365	98.1	1.9
Hydrolysis	3305108	98.7	1.3	219312	97.6	2.4

conditions, including acidic, thermal, peroxide and basic. Table 7 displays the findings of the stability investigation. Figs 6–11 show the chromatogram peaks of degradation research.

Conclusion

The method was designed to be sensitive and precise while also being able to detect any potential stability issues. The sample solutions were injected six times in different concentration ranges, and the drugs consistently showed the same retention times in all cases. The standard chromatograms showed no significant changes in system suitability parameters, indicating the robustness of the developed HPLC method. The specified concentration range was determined from the linearity studies. The linearity ranges for erlotinib and ramucirumab were found to be 37.50 to 225.00 and 2.50 to 15.00 µg/mL, respectively. The correlation coefficient between erlotinib and ramucirumab was determined to be 0.999. This method is a straightforward, precise, and reliable analytical procedure for estimating erlotinib and ramucirumab. It is cost-effective and can be easily reproduced with consistent results. The method provides accurate measurements with good precision and stability.

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