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Original Article

A NEW NP-UPLC METHOD FOR THE SEPARATION AND SIMULTANEOUS QUANTIFICATION OF RAMUCIRUMAB AND ERLOTINIB

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ABSTRACT

Objective: This investigation demonstrates a stability-indicating and reliable "normal phase ultra-performance liquid chromatography" method to simultaneously quantify Ramucirumab and Erlotinib in the pharmaceutical dosage form.

Methods: Successful separation was accomplished using Chiralcel-OD-3 column (50 mm x 4.6 mm, 3 µm) with an isocratic type of elution using a mobile phase containing n-hexane+isopropyl alcohol+methanol (89:10:1), respectively with 1.0 ml/min flow rate. The wavelength sensor was attuned at 266 nm to quantify Ramucirumab and Erlotinib.

Results: Erlotinib and Ramucirumab peaks were eluted with fine resolution at retention times 1.7807 min and 3.175 min, respectively. In the 10-150 µg/ml and 1-15 µg/ml concentration ranges for Erlotinib and Ramucirumab, the calibration graphs were linear, with regression coefficients of 0.99928 and 0.99976, respectively. The suggested ultra-performance liquid chromatography approach has been shown as sensitive, precise, robust, accurate, specific and stability indicating through the resolution of Erlotinib and Ramucirumab from its degradation-based compounds.

Conclusion: The established ultra-performance liquid chromatography technique was effectively extended to the evaluation of Erlotinib and Ramucirumab in the pharmaceutical dosage form and the test results appeared satisfactory.

Keywords: Erlotinib, Ramucirumab, Development, Validation, NP-UPLC

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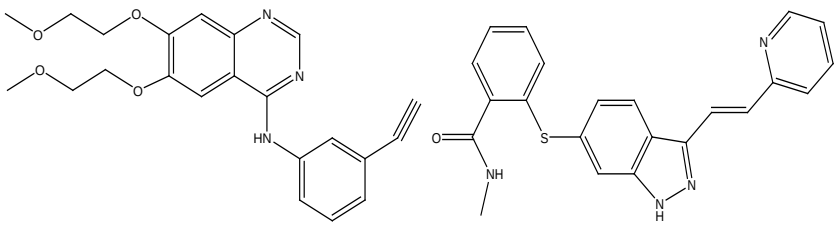
INTRODUCTION

Ramucirumab trade name Cyramza is a fully human monoclonal antibody [1, 2] (IgG1) developed for the treatment of solid tumors [3]. the US Food and Drug Administration (FDA) approved ramucirumab as a single-agent treatment for advanced gastric cancer [4, 5] or gastroesophageal junction [6] (GE) adenocarcinoma [7] after prior treatment with fluoropyrimidine-or platinum-containing chemotherapy [8, 9]. The approval was based on the results of the REGARD trial, a phase III, international, randomized, double-blind, placebo-controlled study that evaluated the safety and efficacy of ramucirumab combinated with best supportive care versus placebo [10]. This trial has been criticised for its use of a placebo control arm, which does not reflect standard of care in most Western countries. Ramucirumab is a direct VEGFR2 antagonist [11], that binds with high affinity to the extracellular domain of VEGFR2 and blocks the binding of natural VEGFR ligands (VEGF-A, VEGF-C and VEGF-D). These ligands are secreted by solid tumors to promote angiogenesis [12, 13] (formation of new blood vessels from pre-existing ones) and enhance tumor blood supply. Binding of ramucirumab to VEGFR2 leads to inhibition of VEGF-mediated tumor angiogenesis.

Erlotinib, sold under the brand name Tarceva among others, is a medication used to treat non-small cell lung cancer [14, 15] (NSCLC) and pancreatic cancer [16, 17]. Specifically, it is used for NSCLC with mutations in the epidermal growth factor receptor [18, 19] (EGFR) either an exon 19 deletion (del19) or exon 21 (L858R) substitution mutation, which has spread to other parts of the body. It is taken by mouth. Common side effects include rash [20], diarrhea [21], muscle pain, joint pain, and cough [22]. Serious side effects may include lung problems, kidney problems [23], liver failure [24], gastrointestinal perforation [25], stroke, and corneal ulceration [26]. Use in pregnancy may harm the baby. It is a receptor tyrosine kinase inhibitor [27, 28], which acts on the epidermal growth factor receptor (EGFR). This paper proposes a novel sensitive stability-indicating NP-UPLC procedure for the assessment of Erlotinib and Ramucirumab combination. The proposed process enables the rapid assessment of the Erlotinib and Ramucirumab in bulk drugs and formulation preparations without sample pretreatment with high precision and specificity and with no excipient intervention.



A B

Fig. 1: Structure of (A) Erlotinib and (B) Ramucirumab

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade methanol, water, isopropyl alcohol and n-hexane were purchased from Merck India Ltd, Mumbai, India. APIs of Erlotinib, Ramucirumab standards were procured from Glenmark, Mumbai. Ramucirumab injection formulation from Euphoria India Pharma with a lable claim of 10 mg/ml and Erlotinib tablet formulation from Modern Agencies with a lable claim of 100 mg was used.

The Instrumentation

Waters Acquity UPLC with a quaternary pump, PDA detector with Empower 2.0 software was employed.

Method optimization

To optimize the chromatographic conditions, different ratios of phosphate buffer and the acetonitrile in the mobile phase with isocratic and gradient mode was tested. However, the mobile phase composition was modified at each trial to enhance the resolution and also to achieve acceptable retention times. Finally a mixture of n-hexane, isopropyl alcohol and methanol with isocractic elution was selected as mobile phase because it results in a greater response of active pharmacy ingredient. During the optimization of the method various stationary phases such as C_8, C_{18} and amino, phenyl columns were tested. From these trials the peak shapes were relatively good with Chiralcel-OD-3 column of 50 x 4.6 mm, 3 µ with a PDA detector. The mobile phase flow rate has been done at 266 nm in order to obtain enough sensitivity. By using the above conditions we get retention times of Erlotinib and Ramucirumab were about 1.7 min and 3.1 min with a tailing factor of 1.18 and 1.26. The number of theoretical plates for Erlotinib and Ramucirumab were 7654, 9253 which indicate the column's successful output the % RSD for six replicate injections was around 0.97% and 1.25%, the proposed approach suggests that it is extremely precise. According to ICH guidelines, the method established was validated.

Till today there are no UPLC and HPLC methods were reported in the literature. Hence we developed method for the simultaneous quantification of Erlotinib and Ramucirumab. The developed UPLC method was utilized for the estimation of the combined drugs by *in vitro* method. Different extractions were tried using acetonitrile, methanol, and dimethylformamide.

Validation procedure

The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [29, 30].

Preparation of buffer

Add 89 ml of n-hexane and 10 ml of isopropyl alcohol and 1 ml of methanol and filter through 0.45 µ filter paper.

Chromatographic conditions

The UPLC analysis was performed on a normal phase UPLC system with isocratic elution mode using a mobile phase of n-hexane, isopropyl alcohol and methanol (89:10:1) and Chiralcel-OD-3 (50x4.6 mm, 3 µ) column with a flow rate of 1 ml/min.

Diluent

Mobile phase was used as a diluent.

Preparation of the standard solution

Standard Erlotinib and Ramucirumab solution containing 100 µg/ml and 10 µg/ml was prepared by dissolving 100 mg of Erlotinib and 10 mg of Ramucirumab in 100 ml of mobile phase solvent blend. The standard Erlotinib and Ramucirumab solution were diluted employing mobile phase solvent blend further as needed.

Preparation of the sample solution

Sample Erlotinib and Ramucirumab solution containing 100 µg/ml and 10 µg/ml was prepared by dissolving 154 mg of Erlotinib sample (lable claim 100 mg) and 10 mg of Ramucirumab (lable claim 10 mg/ml) in 100 ml of mobile phase solvent blend. The standard Erlotinib and Ramucirumab solution were diluted employing mobile phase solvent blend further as needed.

RESULTS AND DISCUSSION

In acquiescence with ICH recommendations, the validity parameters were established [31].

System suitability

In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1.

Table 1: Results of system suitability

System suitability parameter	Acceptance criteria	Drug name	
		Erlotinib	Ramucirumab
USP Plate Count	NLT 2000	7654	9253
USP Tailing	NMT 2.0	1.18	1.26
USP Resolution	NLT 2.0	-	8.42
% RSD	NMT 2.0	0.97	1.25

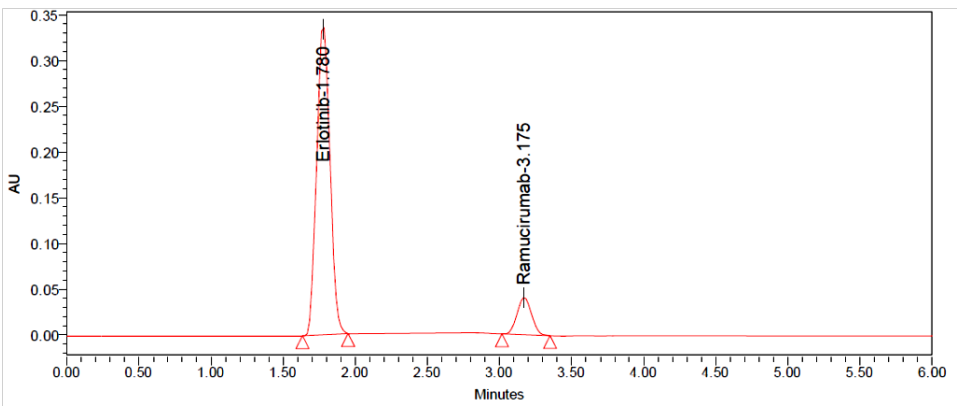


Fig. 2: Chromatogram of standard

Specificity

In this test method placebo, standard and sample solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredients were well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity

During this work, the linearity of area response was checked for both Erlotinib and Ramucirumab. Chromatographed solutions with concentrations of 10-150 µg/ml for Erlotinib and 1-15 µg/ml for Ramucirumab given linear peak response areas. The regression line equation, regression coefficient and Erlotinib and Ramucirumab calibration curves are shown in fig. 4.

Accuracy

The accuracy was determined by assay of Erlotinib and Ramucirumab in spiked Erlotinib and Ramucirumab samples according to the proposed method. Three diverse quantities (50% quantity degree, 100% quantity degree and 150% quantity degree) of Erlotinib and Ramucirumab standards were put into samples. The results are given in table 3.

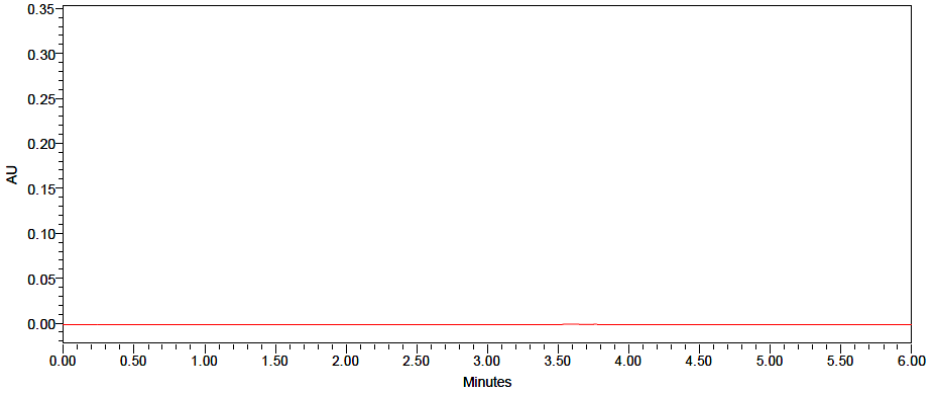
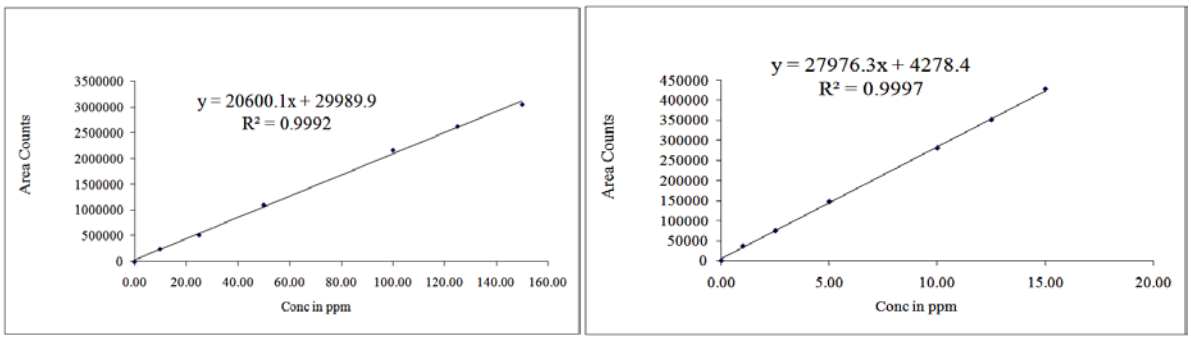


Fig. 3: Chromatogram of blank

Table 2: Linearity of erlotinib and ramucirumab

S. No.	Conc µg/ml	Erlotinib area count	Conc. µg/ml	Ramucirumab area count
1	10.00	241110	1.00	36383
2	25.00	514159	2.50	74638
3	50.00	1097769	5.00	146882
4	100.00	2158351	10.00	280360
5	125.00	2622646	12.50	350920
6	150.00	3051942	15.00	427679
Correl coef		0.99928		0.99976
Slope		20600.10		27976.37
intercept		29989.91		4278.44



Erlotinib **Ramucirumab**

Fig. 4: Calibration plots of (A) Erlotinib (B) Ramucirumab

Table 3: Results of accuracy

S. No.	% Level	Erlotinib % recovery	Ramucirumab % recovery
1	50	99.24	99.66
2	100	100.36	99.87
3	150	100.48	99.99
mean		100.03	99.84
SD		0.684	0.167

mean+SD (n=3)

Precision

The precision measurements were assessed using measurements of Erlotinib and Ramucirumab solution (100 µg/ml and 10 µg/ml) repeated six times within the day. The precision was validated by the RSD measurements of the Erlotinib and Ramucirumab peak areas, while the accuracy was validated by the Erlotinib and Ramucirumab percentage content assays. These results are given below table 4.

Intraday precision

Six replicates of a standard solution containing Erlotinib (100µg/ml) and Ramucirumab (10µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values.

Intermediate precision

Six replicates of the standard solution were studied by various researchers, and on separate days different instruments were tested. The peak regions used to determine mean percent RSD values have been calculated. The results are given in the following table.

Inter-day precision

Six replicates of a sample solution containing Erlotinib (100µg/ml) and Ramucirumab (10µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5.

Table 4: Intraday precision results of erlotinib and ramucirumab

Erlotinib				Ramucirumab		
S. No.	**Conc.(µg/ml)**	**Area counts**	**% assay as is**	**Conc.(µg/ml)**	**Area counts**	**% assay as is**
1		2154632	100.55		284567	100.32
2	100	2174583	101.24	10	283512	99.46
3		2153262	100.36		284531	100.33
4		2146398	100.17		281574	100.18
5		2147589	99.98		284453	100.21
6		2163215	99.67		285201	100.09
% RSD	0.494			0.456		
mean	100.33			100.10		
SD	0.539			0.325		

mean+SD (n=6)

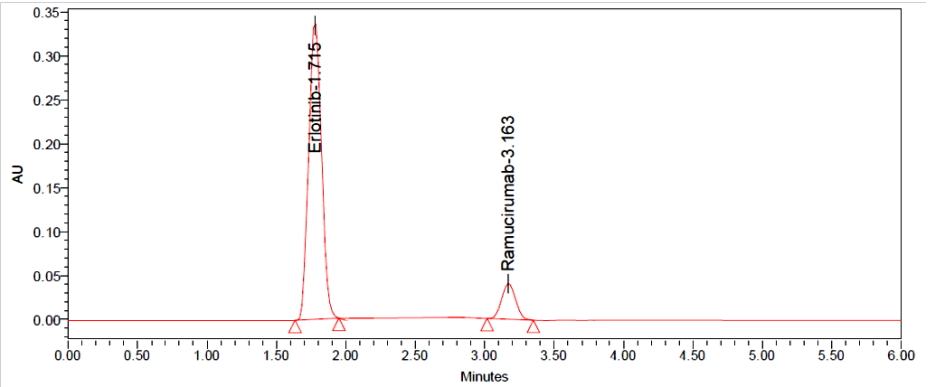


Fig. 5: Chromatogram of method precision

Table 5: Inter-day outcomes of accuracy of Erlotinib and Ramucirumab

Erlotinib				Ramucirumab		
S. No.	**Conc.(µg/ml)**	**Area counts**	**% assay as is**	**Conc.(µg/ml)**	**Area count**	**% assay as is**
1		2142301	100.22	10	285649	100.24
2	175	2129586	100.14		284732	98.34
3		2137458	100.36		289645	99.17
4		2146953	101.41		283541	98.52
5		2103569	100.08		282754	99.33
6		2121593	99.88		289362	99.94
%RSD	0.746			1.024		
Mean	100.35			99.26		
SD	0.544			0.752		

mean+SD (n=6)

Table 6: LOD and LOQ for erlotinib and ramucirumab

Erlotinib				Ramucirumab			
LOD		**LOQ**		**LOD**		**LOQ**	
Concentration	s/n	Concentration	s/n	concentration	s/n	Concentration	s/n
0.125µg/ml	8	0.413µg/ml	27	0.013µg/ml	4	0.041µg/ml	24

LOD and LOQ

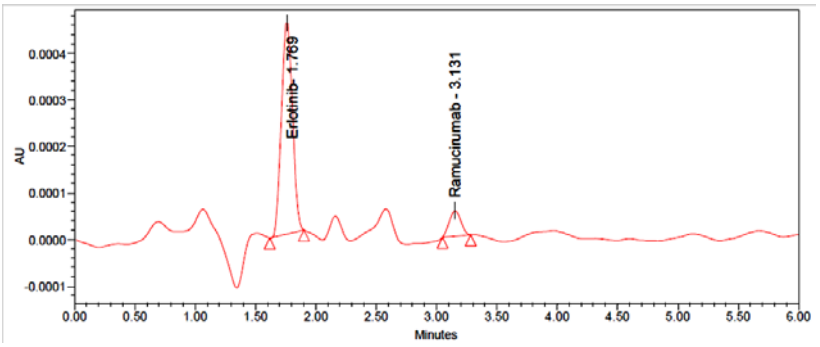
Both LOD and LOQ were measured utilizing a signal-to-noise methodology. LOQ and LOD were defined as the Erlotinib and Ramucirumab concentration levels that ensuing a peak height of 10 times and 3 times, respectively, the baseline noise.

Robustness

The robustness was measured using peak area measurements of Erlotinib and Ramucirumab solution (100 µg/ml and 10 µg/ml) with considerably changed parameters in UPLC assay operating conditions. The changed parameters and peak areas obtained were presented in table 7.

Stability

The sample solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [32]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Erlotinib and Ramucirumab drugs. The results are given below table 8.



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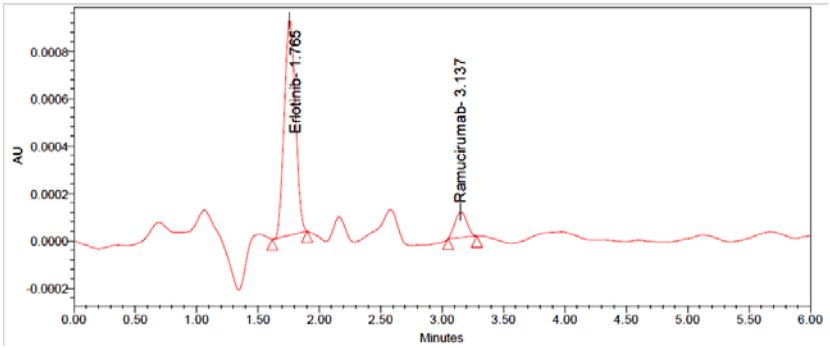

B

Fig. 6: Chromatogram of (A) LOD and (B) LOQ

Table 7: Robustness data of Erlotinib and Ramucirumab

Parameter name	% RSD	
	Erlotinib	Ramucirumab
Flow minus (0.8 ml/min	0.52	0.66
Flow plus (1.2 ml/min)	0.69	0.49
Organic minus (-10%)	1.42	0.99
Organic plus (+10%)	0.71	0.47

Table 8: Stability results of Erlotinib and Ramucirumab

Stability	Erlotinib		Ramucirumab	
	Purity	% of deviation	Purity	% of deviation
Initial	100.1	0.00	99.9	0.00
6 H	99.5	-0.60	99.7	-0.20
12 H	99	-1.10	99.6	-0.30
18 H	98.5	-1.60	98.9	-1.00
24 H	98.2	-1.90	98.4	-1.50

Degradation studies

The Erlotinib and Ramucirumab standard was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [33, 34]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [35].

Acid degradation

Acid degradation was done by using 1N HCl and 14.2% of Erlotinib and 15.8% of Ramucirumab degradation was observed.

Alkali degradation

Alkali degradation was done at 1N NaOH and 16.2% of Erlotinib and 15.1% of Ramucirumab degradation was observed.

Peroxide degradation

Peroxide degradation was performed with 30% hydrogen peroxide and 14.9% Erlotinib, 15.5% of Ramucirumab degradation was observed.

Reduction degradation

Reduction degradation was performed with 30% sodium bi sulphate solution, 15.9% Erlotinib and 14.4% Ramucirumab degradation was observed.

Thermal degradation

In thermal degradation, the standard was degraded to 15.6% of Erlotinib and 13.7% of Ramucirumab.

Hydrolysis degradation

In hydrolysis degradation the standard was degraded to 12.7% of Erlotinib and 12.0% of Ramucirumab.

All degradation results are tabulated in table 9.

CONCLUSION

An Ultra-performance liquid chromatography process for determining the combination of Erlotinib and Ramucirumab in individual formulation form and pure form has been described in the established method. The present Ultra-performance liquid chromatography process is exemplified by its speed, ease and relatively inexpensive. The successful validity criteria of the proposed approach permit its use in laboratories for quality control.

Table 9: Forced degradation results of Erlotinib and Ramucirumab

Degradation condition	Erlotinib		Ramucirumab	
	% assay	%Deg	% assay	% Deg
Acid degradation	85.9	14.2	84.3	15.8
Alkali degradation	83.8	16.2	84.9	15.1
Peroxide degradation	85.1	14.9	85.6	14.4
Reduction degradation	84.1	15.9	86.3	13.7
Thermal degradation	84.4	15.6	85.2	14.8
Hydrolysis degradation	87.3	12.7	88	12

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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