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Research Article

Analytical Method Development and Validation for the Simultaneous Estimation of Ramucirumab and Paclitaxel by RP-HPLC Technique

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Ramucirumab and Paclitaxel was done by RP-HPLC. The Phosphate buffer was p^H 4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). Kromosil C_{18} Column (250mm x 4.6mm) 5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1 ml min^{-1}. The linearity range of Ramucirumab and Paclitaxel were found to be from 100-500 µg/ml of Ramucirumab and 1-5µg/ml of Paclitaxel. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. Ramucirumab %RSD 0.2 and Paclitaxel %RSD 0.6 Intermediate precision for Ramucirumab %RSD 0.2 and Paclitaxel %RSD 0.1. The percentage recovery varies from 98-102% of Ramucirumab and Paclitaxel. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Keywords: Kromosil C18, Ramucirumab and Paclitaxel, RP-HPLC

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CONTENTS

1. Introduction

Ramucirumab is a human monoclonal antibody (IgG1) against vascular endothelial growth factor receptor 2 (VEGFR2), a type II trans-membrane tyrosine kinase receptor expressed on endothelial cells. By binding to VEGFR2, ramucirumab prevents binding of its ligands (VEGF-A, VEGF-C, and VEGF-D), thereby preventing VEGF-stimulated receptor phosphorylation and downstream ligand-induced proliferation, permeability, and migration of human endothelial cells. VEGFR stimulation also mediates downstream signalling required for angiogenesis and is postulated to be heavily involved in cancer progression, making it a highly likely drug target. In contrast to other agents directed against VEGFR-2, ramucirumab binds a specific epitope on the extracellular domain of VEGFR-2, thereby blocking all VEGF ligands from binding to it. Ramucirumab is indicated for us in advanced gastric or gastro-esophageal junction adenocarcinoma as a single agent or in combination with paclitaxel after prior fluoropyrimidine- or platinum-containing chemotherapy. Paclitaxel is a chemotherapeutic agent marketed under the brand name Taxol among others. Used as a treatment for various cancers, paclitaxel is a mitotic inhibitor that was first isolated in 1971 from the bark of the Pacific yew tree which contains endophytic fungi that synthesize paclitaxel. It is available as an intravenous solution for injection and the newer formulation contains albumin-bound paclitaxel marketed under the brand name Abraxane.

2. Methodology

Instrumentation

The instrument used was HPLC Alliance Waters 2695, empower software. The stationary phase used was kromosil C_{18} Column (250mm x 4.6mm) 5µgDigital weighing balance-Model number BSA224SCW (Ascoset), Sonicator (Enertech)-SE60US, Ph meter Model number AD102U.

Materials and reagents

Ramucirumab And Paclitaxel were gift samples provided by Dr. Reddy's laboratory, Hyderabad, Potassium dihydrogen was supplied by Mumbai finer chemical LTD, Acetonitrile was supplied by Moly chem, Methanol and Water for HPLC were supplied by Merck India Ltd, Mumbai,

Method development

Initially the mobile phase tried was methanol: Ammonium acetate buffer and Methanol: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 4.5), Methanol in proportion 20: 80 v/v respectively.

Chromatographic conditions

kromosil C_{18} Column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}

Study Period: The Present study was conducted for a period of six months.

Study site:

The Present study was conducted in a Nephrology department in a tertiary care hospital.

Sample size: It was 210 Patients.

Inclusion criteria

- Patients with renal abnormalities.
- Patients of either sex, diagnosed with renal abnormalities.
- Patients who are willing to give consent.
- Patients receiving treatment for renal abnormalities.
- Patients with clinical profile of renal abnormalities.

Exclusion criteria

- Patients below 18 years.
- Patients who are not diagnosed with gastro intestinal abnormalities.
- Special population including pregnant women and lactating women.
- Psychiatric abnormalities.
- Patients who were not willing to join in the study.

Institutional ethics committee (IEC) consideration:

The research protocol was submitted to ethical committee and ethical Committee was permitted to perform the research work in the Nephrology department.

Patient data collection and management:

The data collection form contains information regarding age, sex, diagnosis, past medical history, laboratory data, and diagnostic results. The information about risk factors, clinical laboratory reports, treatment, dose and frequency of administration and duration of therapy was collected from the patients treatment chart.

Statistical analysis:

The data was represented as percentages. The $P<0.05$ was considered to indicate a statistically significant difference.

3. Results and Discussion

Table 1: Results of system suitability parameters for Ramucirumab and Paclitaxel

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate count
1	**Ramucirumab**	2.669	124505	223532	**1.2**	1.2	4523.3
2	**Ramucirumab**	2.5264	123442	134544	1.2	1.2	5020.2
3	**Ramucirumab**	2.5265	123431	124386	1.2	1.2	4061.2
4	**Ramucirumab**	2.5266	125432	134568	1.2	1.2	5032.4

5	**Ramucirumab**	2.5267	122434	146852	1.2	1.2	5076.4
6	**Ramucirumab**	2.5268	124438	145782	1.2	1.2	6024.8
7	**Paclitaxel**	3.855	1308495	154566	1.3	1.3	6090.3
8	**Paclitaxel**	3.902	1309496	156428	1.3	1.3	5023.2
9	**Paclitaxel**	3.903	1306498	152634	1.3	1.3	8060.7
10	**Paclitaxel**	3.904	1342499	158426	1.3	1.3	7080.1
11	**Paclitaxel**	3.905	1343451	158484	1.3	1.3	6054.4
12	**Paclitaxel**	3.906	1346455	158423	1.3	1.3	7080.6

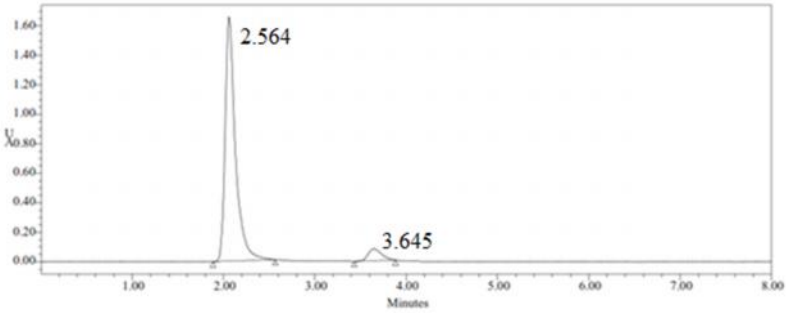

Figure 1: chromatogram for standard injection

Table 2: Showing % RSD results method precision for Ramucirumab

Injection	Peak Name	Rt	Area	Height
1	**Ramucirumab**	**3.699**	**1302729**	**341432.2**
2	**Ramucirumab**	**3.790**	**1302947**	**523341.4**
3	**Ramucirumab**	**3.663**	**1303236**	**374642.4**
4	**Ramucirumab**	**3.658**	**1303977**	**327514.3**
5	**Ramucirumab**	**3.647**	**1309759**	**374028.1**
6.	**Ramucirumab**	**3.645**	**1309789**	**346280.2**
Mean			**1304529.8**	
Std.dev			**2961.1**	
%RSD			**0.2**	

Table 3: Showing % RSD results method precision for Paclitaxel

Injection	Peak Name	Rt	Area	Height
1	**Paclitaxel**	**3.616**	**123149**	**248742.3**
2	**Paclitaxel**	**3.634**	**123766**	**281441.2**
3	**Paclitaxel**	**3.460**	**124271**	**271721.2**
4	**Paclitaxel**	**3.446**	**124691**	**284393.8**
5	**Paclitaxel**	**3.437**	**124956**	**256318.0**
6	**Paclitaxel**	**3.438**	**125845**	**226813.0**
Mean			**124162.7**	
Std.dev			**725.6**	
%RSD			**0.6**	

Table 4: Showing results for intermediate precision of Ramucirumab

Injection	Peak name	Rt	Area	Height
1	**Ramucirumab**	**2.554**	**1300148**	**438467.1**
2	**Ramucirumab**	**2.557**	**1304520**	**436873.3**
3	**Ramucirumab**	**2.563**	**1305937**	**438572.1**
4	**Ramucirumab**	**2.562**	**1306476**	**435587.5**
5	**Ramucirumab**	**2.561**	**130871**	**432826.4**
6	**Ramucirumab**	**2.561**	**130872**	**432838.3**
mean			**1305070.2**	
Std.dev			**3061.8**	
%RSD			**0.2**	

Table 5: Showing results for intermediate precision of Paclitaxel

Injection	Peak name	Rt	Area	Height
1	Paclitaxel	3.790	122487	241421.6
2	Paclitaxel	3.657	122626	233417.3
3	Paclitaxel	3.663	122632	281751.1
4	Paclitaxel	3.646	122702	241843.6
5	Paclitaxel	3.662	122962	281564.1
6	Paclitaxel	3.663	122972	284917.2
mean			122681.8	
Std.dev			174.8	
%RSD			0.1	

Table 6: Details of Accuracy 50%

Injection	Peak Name	RT	Area	Height
1	Ramucirumab	2.572	132457	86026
2	Ramucirumab	2.573	132458	85549
3	Ramucirumab	2.576	134242	84196
4	Paclitaxel	3.881	122487	21744
5	Paclitaxel	3.882	122489	21909
6	Paclitaxel	3.792	122392	21382
Mean			371513.5	
Std.Dev			253899.3	
% RSD			0.532	

Table 7: Details of Accuracy 100%

Injection	Peak Name	RT	Area	Height
1	Ramucirumab	2.306	132405	86096
2	Ramucirumab	2.243	132452	86549
3	Ramucirumab	2.223	133232	84176
4	Paclitaxel	3.546	124465	21784
5	Paclitaxel	3.542	122428	25909
6	Paclitaxel	3.546	124345	21372
Mean			372523.5	
Std.Dev			2508918.3	
% RSD			0.535	

Table 8: Details of Accuracy 150%

Injection	Peak name	Rt	Area	Height
1	Ramucirumab	2.592	142526	76083
2	Ramucirumab	2.573	142527	76348
3	Ramucirumab	2.223	143532	74275
4	Paclitaxel	3.841	135545	21682
5	Paclitaxel	3.882	132558	25508
6	Paclitaxel	3.842	134345	21476
Mean			338742.3	
Std.Dev			840776.2	
% RSD			0.575	

Table 9: accuracy (recovery) data for Ramucirumab

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 10: accuracy (recovery) data for Paclitaxel

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Table 11: Area of different concentration of Ramucirumab

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

Table 12: Area of different concentration of Paclitaxel

S.No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

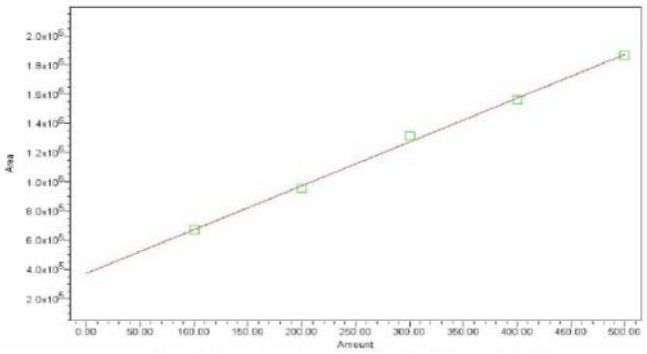

Figure 2 calibration graph for Ramucirumab at 254nm

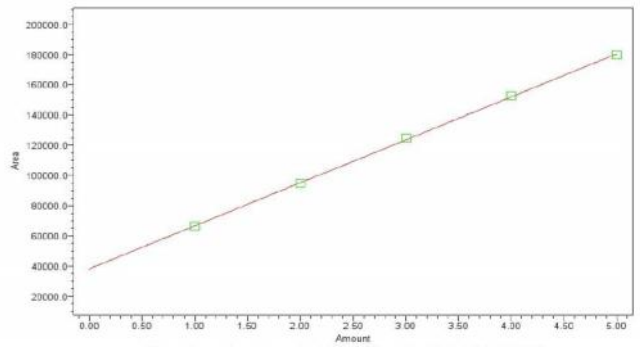

Figure 3 calibration graph for Paclitaxel at 254 nm

Table 13 Analytical performance parameters of Ramucirumab and Paclitaxel

Parameters	Ramucirumab	Paclitaxel
Slope (m)	66574	12529
Intercept (c)	53592	50245
Correlation coefficient (R^2)	0.999	0.999

Table 14 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ramucirumab	52	152	2.9
Paclitaxel	52	156	3

Table 15 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Ramucirumab	52	522	10.03
Paclitaxel	52	524	10.1

Table 16: Flow Rate (ml/min) data for Ramucirumab

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.6	5339.9	1.4
2	0.8	4673.4	1.3
3	1.0	5216.0	1.4

Table 17: flow rate (ml/min) data for Paclitaxel

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1.0	6090.3	1.2
3	1.2	6998.0	1.3

Table 18 Change in Organic Composition in the Mobile Phase for Ramucirumab

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	4508.4	1.3
2	*Actual	4673.4	1.4
3	10% more	4318.1	1.3

Table 19 Change in Organic Composition in the Mobile Phase for Paclitaxel

S.No	Change in Organic Composition in the Mobile Phase	System Suitability Results	
		USP Plate Count	USP Tailing
1	10% less	6387.7	1.2
2	*Actual	6090.3	1.2
3	10% more	6232.5	1.2

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Ramucirumab and Paclitaxel was done by RP-HPLC. The Phosphate buffer was p^H4.5 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of P^H 4.5(20:80 v/v). kromosil C_{18} column (250mm x 4.6mm)5µg or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 254 nm. The solutions were chromatographed at a constant flow rate of 1ml min^{-1}. The linearity range of Ramucirumab and Paclitaxel were found to be from 100-500 µg/ml of Ramucirumab and 1-5µg/ml of Paclitaxel. Linear regression coefficient was not more than 0.999.The values of % RSD are less than 2% indicating accuracy and precision of the method. Ramucirumab %RSD0.2 and Paclitaxel %RSD 0.6. Intermediate precision for Ramucirumab %RSD 0.2 and Paclitaxel %RSD 0.1. The percentage recovery varies from 98-102% of Ramucirumab and Paclitaxel. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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