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RESEARCH ARTICLE

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

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ABSTRACT

The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Ramucirumab and Paclitaxel in tablet dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer: Methanol 65:35 were set (Buffer P^H 2.45 adjusted with Triethylamine), Kromosil C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient, eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Ramucirumab and Paclitaxel got eluted with good peak symmetric properties. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between50% to150 % levels, R^2 value was found to be as 0.999.By using above method assay of marketed formulation was carried out, 100.7% was present. Full length method was not performed; if it is done this method can be used for routine analysis of Ramucirumab and Paclitaxel .

Keywords: HPLC, Paclitaxel, Ramucirumab, Ortho Phosphoric Acid Buffer: Methanol, Kromosil C 18.

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CONTENTS

1. Introduction

Paclitaxel (PTX), sold under the brand name Taxol among others, is a chemotherapy medication used to treat a number of types of cancer. This includes ovarian cancer, breast cancer, lung cancer, Kaposi sarcoma, cervical cancer, and pancreatic cancer. It is given by injection into a vein. There is also an albumin-bound formulation.

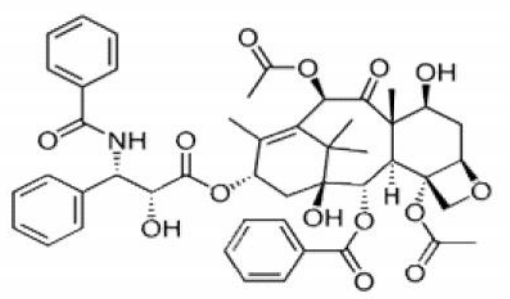

Fig 1: Structure of Paclitaxel

Ramucirumab is a fully human monoclonal antibody developed for the treatment of solid tumors. This drug was developed by ImClone Systems Inc. It was isolated from a native phage display library from Dyax.

2. Materials and Methods

Instrumentation:

System Alliance Waters 2690 separation module, Pump Analytical HPLC isocratic pump, Detector Photo diode array detector, Software Empower 2 software, Column Agilent (250×4.6mm, 5µ) C-18 RP-column, Sonicator, Analytical Technologies Limited- Ultrasonic cleaner. U.V double beam spectrophotometer LABINDIA, UV 3000^{+}pH meter, Weighing machine.

Chemicals:

Ramucirumab and Paclitaxel, KH_2PO_4, Water and Methanol for HPLC, Acetonitrile for HPLC, Ortho phosphoric Acid, Tri ethyl amine.

Table 1: Optimized chromatographic conditions

Parameters	Description
Flow rate	1ml min^{-1}
Column	Kromosil C_{18} Column (250mm x 4.6mm)5µg.
Mobile Phase	Phosphate buffer: Methanol P^H 2.5 (65:35 v/v)
Buffer	Potassium dihydrogen orthophosphate PH 2.5 adjusted with Orthophosphoric acid
Detector	PDA
Column temperature	Ambient
Type of elution	Isocratic
Wavelength	254 nm
Injection volume	20µl
Run time	10min

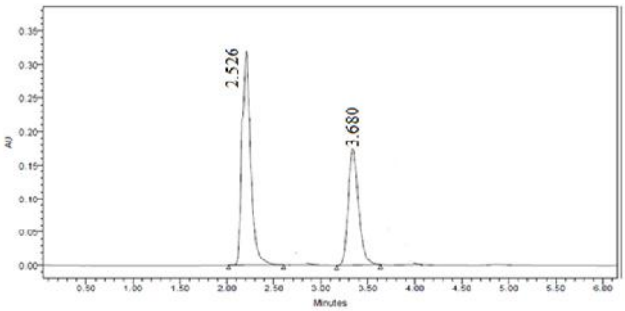

Fig 2: Chromatogram from optimized conditions

Observation: The separation of two analytical peaks was good. The plate count also above 2000, tailing factor below 2, and the resolution is above 2. The condition is taken as optimized method.

Sample solution preparation:

Weigh accurately 10mg Ramucirumab Working Reference Standard and 15mg of Levomisol Working Reference Standard is taken in to 100ml volumetric flask and then it was dissolved and diluted to volume with mobile phase up to the mark. After that 50ml of the above solution was taken into 100ml standard flask and made up with mobile phase. (Stock solution)Further pipette 0.5ml of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Standard solution preparation:

10 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 10 tablets was transferred into a 100ml standard flask. A volume of 70ml of mobile phase was added and sonicate for 30min.Then the solution was cooled and diluted to volume with mobile phase and filtered through 0.45µm membrane filter. (Stock solution)Further pipette 0.25ml of Ramucirumab and Paclitaxel of the above stock solution in to a 10ml volumetric flask and dilute up to the mark with diluent.

Method Validation

- System Suitability
- Linearity
- Specificity
- Precision (Repeatability & Intermediate precision)
- Accuracy
- Limit of Detection and Limit of Quantification
- Robustness

3. Results and Discussion

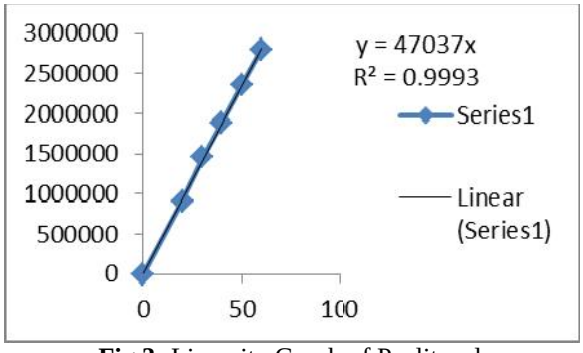

Fig 3: Linearity Graph of Paclitaxel

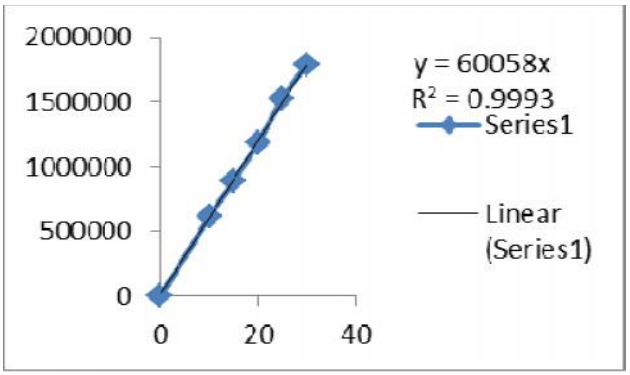

Fig 4: Linearity Graph of Ramucirumab

Table 2: Peak results of Standard & Test Chromatograms for Assay

Parameter	Standard		Test	
	Ramucirumab	**Paclitaxel**	**Ramucirumab**	**Paclitaxel**
Retention time	2.589	3.711	2.591	3.733
Peak Area	2008408	1185786	2005829	1189695
USP Plate Count	6167	6389	5752	7187
Tailing Factor	1.3	1.3	1.4	1.2
USP Resolution	-	6.6	-	9.3

Table 3: Results of Assay

Parameters	**Ramucirumab**	**Paclitaxel**
Standard peak area	2008408	1185786
Test peak area (mean)	2005829	1189695
Average Weight	694.2mg	694.2mg
Label claim	400 mg	150 mg
% Purity of Standard	99.50	99.58
Amt obtained	399.88 mg	150.10 mg
% Assay	99.77%	100.12%

Table 4: Results of System suitability Test for Paclitaxel

Injection	**Retention time (t_R)**	**Peak Area**	**Plate count**	**Tailing factor**
1	3.711	1185786	6389	1.3
2	3.702	1184759	6455	1.3
3	3.698	1187496	6234	1.6
4	3.708	1190478	6478	1.3
5	3.715	1183897	6502	1.30
6	3.714	1184759	6384	1.2
Mean	-	1186196	-	-
SD	-	2433.47	-	-
% RSD	-	0.20	-	-

Table 5: Results of System suitability Test for Ramucirumab

Injection	**Retention time (t_R)**	**Peak Area**	**Plate count**	**Tailing Factor**
1	2.589	2008408	5752	1.4
2	2.570	2008412	5758	1.3
3	2.572	2008357	5672	1.2
4	2.578	2007478	5674	1.4
5	2.582	2008475	5749	1.3
6	2.584	2008364	5843	1.4
Mean	-	2008249	-	-
SD	-	380.0	-	-

% RSD	-	0.01	-	-

Table 6: Preparation of working standard solutions for Linearity

Sample ID	Paclitaxel		Ramucirumab	
	Concentration (mcg/ml)	Area	Concentration (mcg/ml)	Area
20% of operating concentration	20	914140	10	610046
40% of operating concentration	30	1455681	15	890204
60% of operating concentration	**40***	**1892966**	**20***	**1183023**
80% of operating concentration	50	2356546	25	1529886
100% of operating concentration	60	2797214	30	1792302
Correlation Coefficient			0.999	

Table 7: Method Precision data for Ramucirumab &Paclitaxel

S.No.	Concentration (µg/ml)	Ramucirumab		Paclitaxel	
		Retention time(Rt)	Peak Area	Retention time(Rt)	Peak Area
1	40 & 20	2.586	2010800	3.713	1184689
2	40 & 20	2.588	2002956	3.714	1188199
3	40 & 20	2.590	2012800	3.734	1195842
4	40 & 20	2.590	2005243	3.737	1184210
5	40 & 20	2.591	2011092	3.741	1198327
6	40 & 20	2.589	2011098	3.740	1198320
Avg			2008998		1191598
SD			3920.9		6668.5
%RSD			**0.19**		**0.55**

Table 8: Intermediate Precision data for Ramucirumab and Paclitaxel

S.No.	Concentration (µg/ml)	Intermediate Precision			
		Day 1 Ramucirumab		Day 1 Paclitaxel	
		Retention time	Peak Area	Retention time	Peak Area
1	40&20	2.591	2005053	3.741	1183951
2	40&20	2.590	2007362	3.734	1184689
3	40&20	2.590	2007473	3.737	1186232
4	40&20	2.586	2009153	3.714	1186406
5	40&20	2.583	2012800	3.713	1188564
6	40&20	2.590	2012785	3.737	1187621
Avg			2009104		1186244
SD			3140.6		1730.9
%RSD			0.15		0.14

Table 9: Intermediate Precision data for Ramucirumab and Paclitaxel

S.No.	Concentration (µg/ml)	Day 2 Ramucirumab		Day 2 Paclitaxel	
		Retention time(Rt)	Peak Area	Retention time(Rt)	Peak Area
1	40 & 20	2.586	2010800	3.713	1184689
2	40 & 20	2.588	2002956	3.714	1188199

3	40 & 20	2.590	2012800	3.734	1195842
4	40 & 20	2.590	2005243	3.737	1184210
5	40 & 20	2.591	2011092	3.741	1198327
6	40 & 20	2.589	2011098	3.740	1198320
Avg			2008998		1191598
SD			3920.9		6668.5
%RSD			0.19		0.55

Table 10: System Precision data for Ramucirumab &Paclitaxel

S.No.	Ramucirumab		Paclitaxel	
	Retention time(Rt)	Area	Retention time(Rt)	Area
1	2.592	2025051	3.743	1175422
2	2.594	2026574	3.734	1175841
3	2.594	2026471	3.736	1175234
4	2.576	2026489	3.724	1174894
5	2.585	2026523	3.723	1175023
6	2.592	2026471	3.735	1175236
Avg		2026263		1175275
SD		595.1		332.8
%RSD		0.02		0.02

Table 11: Accuracy Study of Ramucirumab

Sample Id	Conc found (µg/ml)	Concn Obtained (µg/ml)	%Recovery	Mean recovery	Statistical Analysis
50%	5	5.01	100.2		
50%	5	4.96	99.2	99.73	
50%	5	4.99	99.8		%RSD= 0.505
100%	10	9.95	99.5		
100%	10	9.87	98.7	98.8	
100%	10	9.82	98.2		%RSD=0.66
150%	15	14.64	97.6		
150%	15	14.76	98.4	98.8	
150%	15	15.06	100.4		%RSD=1.45

Table 12: Accuracy Study of Paclitaxel

Conc (µg/ml)	Conc. Obtained(µg/ml)	%Recovery of drug	Mean accuracy	%RSD
5	4.92	98.0		
5	4.96	99.2		
5	5.02	100.4	99.2	1.2
10	9.95	99.5		
10	9.94	99.4		
10	9.98	99.8	99.5	0.2
15	14.78	98.6		
15	14.94	99.6	99.0	0.530
15	14.83	98.8		

Table 13: LOD and LOQ Data of Ramucirumab and Paclitaxel

Ramucirumab			Paclitaxel		
Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis	Conc.(x) (µg/ml)	Peak Areas (y)	Statistical Analysis
40	2004682	S = 39092 c = 618048	20	1184227	S = 39092 c =369381
40	2004587	LOD: 0.001µg/ml	20	1186425	LOD:0.005 µg/ml
		LOQ: 0.004µg/ml			LOQ: 0.015µg/ml

Table 14: Robustness data for Ramucirumab

Std. Replicate	Variation in flow rate		Variation in Mobile phase composition	
	Flow Rate 0.8ml/min	**Flow Rate 1.2ml/min**	**Buffer: Methanol (40:60)**	**Buffer: Methanol (30:70)**
1	2492492	1676589	1951632	1979168
2	2495874	1675428	1954783	1967452
Mean	2494183	1676009	1953208.0	1973310
SD	2391.4	820.9	2228.0	8284.46
%RSD	0.09	0.04	0.11	0.4
Retention time	3.150	2.168	2.618	2.572
Tailing factor	1.4	1.3	1.3	1.3
Theoretical plates	5752	4207	4577	4476

Table 15: Robustness data for Paclitaxel

Parameter	Variation in flow rate		Variation in Mobile phase composition	
Standard	Flow Rate 0.8ml/min	Flow Rate 1.2ml/min	Buffer: Methanol (40:60)	Buffer: Methanol (30:70)
1	1500192	100524	1196996	1153397
2	1500426	100468	1198547	1154782
Mean	1500309	100496	1197772	1154090
SD	165.5	39.59	1096.2	979.34
%RSD	0.01	0.03	0.09	0.08
Retention time	4.674	3.121	4.394	3.331
Tailing factor	1.2	1.2	1.2	1.2
Theoretical plates	7187	5412	6498	6471

4. Conclusion

The analytical method was developed by studying different parameters. Isobistic point of wavelength for both the drugs was set at 254nm and the peaks purity was excellent. Injection volume was selected to be 20 µl which gave a good peak area. The column used for study was Kromosil C 18 , chosen good peak shape. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1ml min^{-1} because of good peak area, satisfactory retention time and good resolution. Different ratios of mobile phase with ratio of buffer:Methanol 65:35 was fixed due to good symmetrical peaks and for good resolution. So this mobile phase was used for the proposed study. The result obtained in this study demonstrated that the HPLC method described is specific, accurate, precise, linear, rugged, robust and stability indicating for the determination of assay of Ramucirumab and Paclitaxel. Therefore, the method is suitable for intended uses.

5. Reference

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